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# Evaluation of Cassava (*Manihot esculenta*) and Sweet Potato (*Ipomea batatas*) Starch from South East Nigeria in the Separation of Deoxyribonucleic Acids as Alternative to Agarose Gel

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## ABSTRACT

Gel electrophoresis technique is an indispensable tool in biotechnology and among other related fields for separation of nucleic acids and proteins. This study determined the potential of selected cassava and sweet potato starch in the separation of DNA as alternative to agarose gel. The sample pH, gelling temperature and time were determined by Light transmittance method proposed by Craig et al. [1] Standard electrophoresis procedure was used for the starch gel electrophoresis. The result showed that composite starch gelled within 18-21 minutes while agarose and agar-agar gelled after 12 minutes. Cassava starch blended with agar-agar gelled at a temperature of 58$^{o}$C while sweet potato starch blended with agar-agar gelled between 35$^{o}$C - 47$^{o}$C. Agarose and agar-agar maintained 54$^{o}$C and 53$^{o}$C respectively. There was no significant difference (P > 0.05) in pH value of the composite starch when compared to 1% agarose gel. Unblended starch samples did not form

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solid gel except when blended with some amount of agar-agar or agarose powder. Cassava and sweet potato composite starch formed good gel strength at 3% (2.2 g of starch and 0.8 g of agar-agar) but solid gel at 4% (3.6 g of starch and 0.8 g of agar-agar). This study demonstrated the possibilities for agarose cost reduction by 60% when cassava starch (3.6 g) was blended with 0.4 g of agarose. The cassava composite starch (4%) separated DNA molecules comparably to that of 1% agarose. Therefore, the use of these cheaper, accessible and readily available blended starch sources is highly recommended for separation of biomolecules such as DNA.

*Keywords: Cassava starch; potato starch; agarose; electrophoresis; DNA.*

## 1. INTRODUCTION

Starch has been considered the most essential polysaccharide and major profitable and nutritional composition of many carbohydrate sources (wheat, corn, rice, barley, sweet potato and cassava) [2]. It is commonly used industrially as emulsion stabilizer, water binder, a thickener, and gelling (pasting) agent, it is therefore a very good ingredient for the manufacturing of various products [3].

Recent study showed that cassava can grow well in low rainfall areas and unfertile soil thus making it a good source of starch [4]. Undoubtedly, Nigeria has been known to be the highest producer of cassava in the world with about 59 million tonnes in 2017 [5] which accounted for 20.4% world share of cassava production in 2017 [6]. Sweet potato on the other hand, is the 4$^{th}$ most important food crop in the world after rice, maize and wheat [7,8]. Tuberous root of cassava contains 25 - 30% of starch and 30 - 40% of dry matter [9] while freshly harvested tubers of sweet potato contain about 80% water and 20% dry matter [10]. Cassava and sweet potato starches are good sources of soft and transparent gel despite their low protein content [11]. Appearance, grade and shape of the products of cassava and sweet potato starch depend largely on the starch viscosity attributes, retrogradation and pasting elements, thereby making starch the main functional components in food products [12]. Pfister and Zeeman, [13] reported that the paste and stickiness as well as clarity of paste and odourless properties of cassava starch enhanced its attractiveness in food industry.

Generally, pastes of tapioca and waxy maize starches are transparent. Wheat and corn starches invariably give opaque pastes [14]. Many factors for example, sugars, can greatly enhance clarity of pastes of cereal starches, such as corn starch whereas glyceryl-monostearate increases the opaque nature of the pastes [15]. However, potato starch contains high amount of non-starch impurities such as polyphenols, ascorbic acids and carotene which may have negative effects on the starch quality and the final functionality [16-18]. Our previous study on the physiochemical characterization of cassava (*Manihot esculenta*) and potato (*I. batatas)* varieties in South East Nigeria in comparison with agarose for use in the separation of deoxyribonucleic acid revealed that cassava and sweet potato starch consists of amylose and amylopectin molecules in molar ratios of 24 - 27% and 75% - 77% respectively [19], contrary to pure agarose which is made up of the repeating units of agarobiose (a disaccharide consisting of one hundred and twenty thousand alternating D-galactose and 3,6-anhydro-L-galactopyranose (L-galactose units) linked by α-(1→3) and β-(1→4) glycosidic bonds [20]. 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 [21] (Martinez-Garcia et al., 2017). Chen et al. [14] noted that amylose (Am) in starch leads to tougher film whereas amylopectin (Ap) generally produces lesser mechanical properties. Physical and chemical properties of starch films can be controlled by adjusting Am:Ap ratio [14]. Egbe et al. [19] also established that the granule distributions of cassava and potato starch are within the range of 5 - 8 µm which is comparatively within the pore size of agarose (6 - 35 µm) [22]. More also, Jiménez et al. [7] stated that when starch constituents are in solution, its 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 and according to Šubarić et al. (2012), starch functionality is directly related to gelatinization and the properties of the paste.

The importance of gel electrophoresis technique in molecular biology and other related fields in the separation of nucleic acids and proteins can not be overemphasized. The standard use and popularity of agarose gels as medium for electrophoresis depends largely on its advantages such as formation of gel that is easy to cast [23], wide separation of both moderately-sized and large DNA molecules as well as provision of a non-toxic gel medium [24]. However, agarose gels are not suitable for separation of low molecular weight DNA and also produce poor band resolution [25]. Polyacrylamide (acrylamide) gels which may be used to remedy the disadvantages of agarose gels also produce toxic and fragile gels which are tedious and difficult to handle [24]. High cost of both agarose and acrylamide impose serious hindrance to molecular studies not only in Nigeria but also in limited resource-laboratories of other developing countries. In early 1960s before the emergence of agarose, cheap and available materials such as starch (though not popular) and agar were used as electrophoretic medium. Unfortunately, the use of starch has fallen out in modern science. However, cassava and sweet potato have been reported to contain polysaccharides with similar chemical properties as agarose (D-galactose and agarobiose) and these crops are locally abundant and affordable; yet little or no effort has been put to develop them as an alternative to agarose. Therefore, the objective of this study was to proffer lasting solutions to lacks of local content development surrounding molecular analysis in developing country like Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Cassava and Sweet Potato Tubers

Two fresh cultivars of cassava (*Manihot esculenta*) and two sweet potato (*Ipomae batatas)* tubers were obtained from farm in Obomanagu Ukwuagba Ngbo, Ohaukwu Local Government Area, Ebonyi State, Nigeria. The cassava species locally identified as Rubber Cassava (RC) and Opokopo Cassava (OP) were the varieties of cassava mostly cultivated within the area. White sweet potato (WSP) and yellow sweet potato (YSP) were randomly selected. Starch of the cassava and sweet potato samples were extracted from fresh root tubers of each cultivar. The research was conducted at Biotechnology laboratory of Nigerian Defense Academy, Kaduna, Nigeria, in September, 2021.

### 2.2 Extraction of Starch

Starch extraction was carried out within two hours of harvesting based on the method of Brabet et al. [24] and Kamaljit et al. [26]. Fresh tubers of each cultivar were washed, peeled, chopped into approximately 1 cm cubes and were ground in a high-speed blender for 5 minutes. The pulp was suspended in ten times its volume of water, stirred for 5 minutes and filtered using double fold cheese cloth. 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 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 further analysis.

### 2.3 Determination of Starch Granules Morphology (Pore Sizes and Shapes)

Method described by Fitt and Snyder [27], was adopted for determining the granule morphology of RC, OP, WSP and YSP starch. A drop of distilled water was placed on one side of a standard microscope slide. Each starch sample (5 mg) was weighed and transferred into the water and a narrow-pointed spatula was used to mix and homogenize the starch. The suspension was covered with a cover slip. Air bubbles were not entrapped and to obtain a thin film, a tissue paper held at the edge of the cover slip was used to wick off excess water. 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 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 Gelatinization Temperature and pH Value

To determine the gelling temperature of each sample, 2.2 g of RC, OP, WSP and YSP was mixed with 0.8 g agar-agar (3% composite starch), 1 g of agar-agar and 1 g of agarose were transferred into 250 ml capacity beakers containing 100 ml of 1x TBE (Tris-Borate-EDTA) buffer. The solution was stirred thoroughly and then solubilized by heating in a microwave oven 60$^{o}$C for 7 - 10 minutes intermittently until the mixture became clear. An electronic thermometer

and pH meter (model HI 8424 with pH buffer 7) was inserted immediately into the solution. The melting and gelling temperature and the pH values of each sample were recorded.

## 2.5 Starch Clarity and Impurity Level Determination

The clarity and purity of the samples were determined by Spectrophotometer Light Transmittance (%T) Method. The reconstituted solutions of starch (1% w/w) were used to determine the clarity and purity of the starch (RC, OP, WSP, YSP and pure agarose) following the method for measuring light transmittance in starch solution as described by Craig et al. [1]. To determine the starch clarity, 0.9 g each of agarose, RC, OP, WSP and YSP were weighed into different 250 ml conical flask capacity and dissolved with 100 ml of distilled water. Each solution was heated in water bath at 90 $^{o}$C for 50 minutes and allowed to cool to room temperature (the flasks were shaken at a minute interval to avoid formation of lumps). The transmittance was measured at 610 nm using spectrophotometer (Spectrumlab 23A No. 23A08182). Method of Bhagya et al. [28] was used to determine the starch purity. A 100 mg of each starch was boiled in 3 ml of 80% ethanol for 10 minutes and the residue collected was dried for 4 hours at 70 $^{o}$C. After drying the starch residue, 10 ml of 52% $HClO_4$ was added to the dried residue. To digest the dried starch residue, the flask containing the starch was placed in water bath (28$^{o}$C) for 15 minutes and made up to 25 ml with distilled water, then filtered with Whatman No. 1. After filtration, 20 µl of the filtrate was made up to 1 ml with distilled water before addition of 1 ml of 5% phenol and 5 ml 36N $H_2SO_4$. About 20 - 100 µL of pure glucose was used as control. Spectrophotometer was used and the absorbance of the solutions was recorded at 490 nm. The amount of sugar present in the starch samples were calculated by multiplying the total sugar by 0.9.

## 2.6 Preparation of 1 Litre of 1X TBE Bufer Stock Solution

A working solution of TBE (tris–boric EDTA (Ethylenediaminetetraacetic Acid)) was prepared by measuring 10.8 g of Tris, 5.5 g Boric acid into a beaker and dissolved with 100 ml of distilled water using magnetic stirrer. A volume of 4 ml of $Na_2EDTA$ at pH 8.0 was dispensed into the solution. It was topped to the 1 L mark with distilled water to obtain a working solution of 1 x.

## 2.7 Gel Preparation of Unblended Cassava and sweet potato Starch

To determine the gelling ability of the samples, each of the cassava (RC and OP) and sweet potato (WSP and YSP) starch were weighed in different quantities (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 g) into 250 ml capacity beakers and each were dissolved in 100 ml of 1x TBE (0.13 M tris (pH 7.6), 45 mM boric acid, and 2.5 mM EDTA) buffer. The solutions were solubilized by heating in a microwave oven (74HW-1 Temp., microwave. Search Tech) at 60 $^{o}$C for 7 to 10 minutes using stop watch intermittently until transparent mixture was observed under constant manual shaking at 30 seconds to 1-minute interval. The dissolved solutions were separately plate poured into separate petri-dishes and allowed to gel under close observation.

## 2.8 Gel Formation of Starch-agar Mixtures

This experiment was carried out in triplicate by blending different quantities of cassava (RC and OP) and sweet potato (WSP and YSP) starch (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 g) with different quantities of (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.75 and 0.8g) of agar-agar to determine the concentration of the composite starch that would form firm gel suitable for gel electrophoresis. Each composite starch was weighed into 250 ml capacity beakers containing 100 ml of 1x TBE (Tris-Borate-EDTA) buffer. The solutions were solubilized by heating in a microwave oven (74HW-1 Temp., microwave. Search Tech) at 60$^{o}$C for 7 - 10 minutes using stop watch intermittently until transparent mixture was observed under constant shaking at 30 seconds to 1 min interval. Each solution was plate poured separately into petri-dishes and allowed to gel under close observation. The concentrations of the starch, agar-agar concentration, melting time (min), pH, gelling time (min) and gelling temperature (°C) were recorded.

## 2.9 Gel Formation of Starch-agarose Mixtures

In our previous study [19], Rubber Cassava (RC) starch proved to possess physiochemical properties comparable to that of agarose gel more than other starch studied. Therefore, different quantities (0.1, 0.2, 0.3, 0.4g, 0.5 and 0.6g) of RC and agarose powder (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6g) were blended to determine the best quantities of gel for gel electrophoresis.

Several quantities of the starch were checked against each agarose quantity. The composite starch was weighed into 250 ml capacity beakers containing 100 ml of 1x TBE (Tris-Borate-EDTA) buffer and the mixture was solubilized by heating in a microwave oven 60$^{o}$C for 7 - 10 minutes intermittently until transparent mixture was observed under constant shaking at 30 seconds to 1-minute interval. The dissolved solutions were separately plate poured into petri-dishes and allowed to gel under close observation.

## 2.10 Preparation of 1% agarose gel (Control)

An amount of 1 g of agarose powder was weighed and transferred into 100 ml of TBE buffer. It was gently stirred and initially microwaved for 1 minute, and subsequently for about 20 seconds until clear solution was seen. After heating, the solution was allowed to cool for 5 minutes before 10 µl of DNA stain (Apex safe DNA gel stain) was pipetted into the agarose solution. The solution was gently stirred and carefully poured into the gel tray having comb inserted appropriately and allowed to solidify for about 12 minutes before the comb was carefully removed and the gel was horizontally placed into a gel tank. The 1x TBE buffer was poured into the gel tank until it reached the maximum mark.

## 2.11 Loading of PCR Product (CMLA Gene) and DNA Ladder into Agarose Gel

Five microliters (5µg/ml) 100bp DNA ladder (100bp Quick Load Purple DNA ladder, NO551S, New England BioLab) was pipetted first into the well followed by the CMLA Gene (mixture of 15µl of CMLA and 5 µl of loading dye) and electrophoresis was run at 100 V and 400 Watts for 30 minutes. The gel containing DNA bands was transferred to UV trans-illuminator/ gel documentation (Azure Biosystems) system. The gel was photographed and saved in the computer.

## 2.12 Starch Gel Preparation for Electrophoresis

The Rubber Cassava (RC) and White Sweet Potato (WSP) were modified with agar-agar as well as agarose and then used for gel preparation. As at the time this study was carried out as far as we know, no literature containing standard protocol suit for our purpose was found. We therefore adopted try and error method to determine concentration of starch, agar-agar and agarose that can be combined to form good gel for electrophoresis. The quantity of composite starch selected out of other concentrations considered are 3% RCagar (2.2 g of RC and 0.8 g of agar-agar), 4% RCagar (3.2 g of RC and 0.8 g of agar-agar), 4% WSPagar (3.2 g of RC and 0.8 g of agar-agar), 4% RCagarose (3.6 g of RC and 0.4 g of agarose), 4% WSPagarose (3.6 g of RC and 0.4 g of agarose). The 3% RCagar was dissolved in 100 ml of 1x TBE buffer while 4% RCagar, 4% WSPagar, 4% RCagarose and 4% WSPagarose were dissolved in 100 ml of 1x TBE buffer respectively. Each of the composite starch was stirred for 4 - 5 minutes to properly homogenize the mixture and then, initially microwaved and stirred for 2 minute and subsequently for about 15 - 20 seconds until transparent solution was observed. The mixture was allowed to cool for 2 minutes before dispensing 10 µl of DNA stain (Apex safe DNA gel stain) into the solution and stirred immediately, then carefully poured into the gel tray with comb inserted appropriately and the gel was allowed to solidify for about 12 minutes before the comb was carefully removed and the gel was horizontally placed into a gel tank. The 1x TBE buffer was poured into the gel tank until it reached the maximum mark.

## 2.13 Loading of PCR Product (CMLA Gene) and DNA Ladder into Blended Starch Gel

Five microliters of 5 µg 1kb /ml of 1kb (Quick Load Purple DNA ladder, NO551S, New England BioLab) was carefully pipetted into the third (3$^{rd}$) well of 3% RCagar gel and 1kb DNA ladder into the fifth (5$^{th}$) well. DNA ladder (1kb) was pipetted into 4% RCagar and WSPagar gel. Similarly, 5 µl of 1kb and 100kb DNA ladder were pipetted into second (2$^{nd}$) and ninth (9$^{th}$) well of 4% RCagarose. 20 µl PCR amplicon (mixture of 15µl of CMLA and 5 µl of loading dye) was also loaded in 4% RCagarose. The gel was run at 100V for 30 minutes. The power source was switched off and the gel tray containing the gel removed when the dye front had travelled approximately 80% of the length.

## 2.14 Visualization under UV trans-illuminator / Gel Documentation Machine

The gel containing DNA bands was transferred to UV trans-illuminator/ gel documentation (Azure

Biosystems) system. The gel was photographed and saved in the computer.

### 2.15 Statistical Analysis

Data collected were analyzed using Microsoft Excel and SPSS (Version 23). Results were presented as mean ± SD from one way analysis of variance (ANOVA). 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. RESULTS

### 3.1 Starch Granules Morphology

Table 1 shows the result of the granule morphology (pore sizes and shapes) of cassava and sweet potato starch samples studied. The results revealed that the pore size of starch for RC and OP were 5.525 ± 1.9346 µm and 5.650 ± 1.0472 µm respectively. Similarly, WSP and YSP pore sizes were 6.075 ± 2.3838 µm and 8.275 ± 1.5064 µm respectively. The results revealed that YSP 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 seen.

### 3.2 Starch Clarity and Impurity Level

Table 2 showed the clarity, purity and pH of agarose, agar-agar, unblended, blended starch samples studied. The study revealed that there was significant difference (p ≤ 0.05) between clarity of the samples studied. RCagar (0.346 ± 0.002 ml) was clearer than other blended starch samples studied though not comparable to that of Agarose (0.008 ± 0.000 ml). Blended cassava and sweet potato starches were more turbid than unblended samples. The result also established that there was a significant difference (p ≤ 0.05) between the purity of the sample showed the least contamination levels. Rubber Cassava (RC) (36.089 ± 0.116 ml) followed by RCagar (40.087 ± 1.112 ml) while YSPagar (129.400 ± 0.000 ml) and YSP (124.4 ± .000 ml) had higher contamination level. The results of the study as presented in Table 2 also established that the pH values of cassava and sweet potato starch studied ranged from 7.7 to 7.8 while agarose and agar-agar were 7.5 and 7.9. Hence, there was no significant difference (P ≥ 0.05) between the pH of the starch samples and agarose.

**Table 1. Cassava and sweet potatoes starch granule morphology**

| Common name | Starch pore size (µm) | Shape |
|---|---|---|
| RC | 5.525 ± 1.9346[a] | Oval, round |
| OP | 5.650 ± 1.0472[a] | Oval, asymmetrical |
| YSP | 6.075 ± 2.3838[b] | Oval, round |
| WSP | 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. Results in this table are the same with the result in our previous publication [19]*
*Key: OP = Opokopo Cassava, WSP = white sweet potato, YSP = Yellow sweet potato*

**Table 2. Clarity, Purity and pH Values of the Samples**

| Samples | Clarity ± S.D (nm) | Purity ± S. D | pH ± S. D |
|---|---|---|---|
| Agarose | 0.008 ± 0.000[a] | 0.000 ± 0.000[a] | 7.51± 1.57[a] |
| Agar-agar | 0.269 ± 0.000[b] | 5.269 ± 0.000[b] | 7.93± 2.30[a] |
| RC | 0.265 ± 0.004[b] | 36.089 ± 0.116[c] | 7.95 ± 0.38[a] |
| OP | 0.686 ± 0.037[c] | 108.834 ± 2.722[d] | 8.62 ± 0.15[a] |
| WSP | 0.798 ± 0.000[c] | 121.1 ± .057[d] | 7.77 ± 0.25[a] |
| YSP | 0.753 ± 0.001[c] | 124.4 ± .000[d] | 7.72 ± 0.16[a] |
| RCagar | 0.346 ± 0.002[b] | 40.087 ± 1.112[c] | 7.84 ± 0.09[a] |
| OPagar | 0.867 ± 0.001[c] | 114.833 ± 4.722[d] | 7.76 ± 0.12[a] |
| WSPagar | 0.886 ± 0.001[c] | 123.367 ± .0578[d] | 7.89 ± 0.30[a] |
| YSPagar | 0.884 ± 0.002[c] | 129.400 ± 0.000[d] | 7.71 ± 0.36[a] |
| RCagarose | 0.850 ± 0.002[c] | 40.087 ± 1.116[c] | 7.79 ± 0.84[a] |

*Results are presented as mean ± SD. Values with different superscripts down the column significantly different from each other at P ≤ 0.05. Tukey's-HSD Post Hoc test*
*Key: OP = Opokopo Cassava, WSP = white sweet potato, YSP = Yellow sweet potato, RCagarose = Rubber cassava blended with Agarose, RCagar = Rubber Cassava blended with Agar-agar, OPagar = Opokopo Cassava blended with Agar-agar, WSPagar = White sweet potato blended with Agar-agar, YSP agar = Yellow sweet potato blended with Agar-agar*

### 3.3 Properties of Gels Formed from Agarose, Agar-agar, Blended and Unblended Starch

Table 3 presents the result of the properties of gels formed from agarose, agar-agar, blended and unblended starch. The result revealed that RC starch could not form a strong gel except when blended with some amount of agarose or agar-agar. Hence, best gel for the purpose of this study was formed when 3.2 g of each starch sample was blended with 0.8 g of agar-agar (4% composite starch). Meanwhile, 4% of RCagarose (3.6g of starch and 0.4 g of agarose) formed the gel comparable to that of pure agarose. The melting temperature (Tm) and gelling temperature (Tg) of the starch samples studied ranged from 74 - 85$^{o}$C and 51 - 58$^{o}$C respectively. While the melting and gelling time (Min) ranged from 4 - 6 minutes and 12 - 21 minutes respectively depending on the amount of agarose or agar-agar used. The result also showed that the sweet potato composite starch (WSPagar and YSPagar) formed a cloudy gel.

### 3.4 Gel Formation of Unblended Cassava and Sweet Potato Starch

Table 4 shows the result of gel formation of unblended cassava (RC and OP) and Sweet potato (WSP and YSP) starch. The result revealed that none of the starch concentrations (1% to 6%) starch could form a strong gel. However, RC and OP formed lumps at 5.5% and 6%.

**Table 3. Properties of gels formed from agarose, agar-agar, blended and unblended starch**

| Sample | Conc. (%) | Starch (g) | Agar-agar (g) | $M_{time}$ (Min) | Tm ($^{o}$C) | Tg ($^{o}$C) | $G_{time}$(Min) | GS | Clarity |
|---|---|---|---|---|---|---|---|---|---|
| Agarose | 1 | - | - | 5.15 | 83.4 | 54 | 12 | ++++ | clear |
| Agar-Agar | 1 | - | - | 6.0 | 79.6 | 53 | 12 | +++ | clear |
| RC | 1 | - | - | 5.55 | 65.0 | - | - | - | clear |
| RCagar | 2.5 | 0.6 | 0.6 | 5.10 | 79.5 | 57 | 18 | + | clear |
| | 3 | 0.9 | 0.6 | 6.0 | 67.4 | 56 | 15 | ++ | clear |
| | 4 | 3.2 | 0.8 | 6.05 | 78.43 | 58 | 16 | +++ | clear |
| OPagar | 2.5 | 0.6 | 0.6 | 5.15 | 75.2 | 55 | 21 | + | clear |
| | 3 | 0.9 | 0.6 | 6.10 | 82.4 | 54 | 18 | ++ | clear |
| | 4 | 3.2 | 0.8 | 6.15 | 86.5 | 58 | 15 | +++ | clear |
| YSPagar | 2.5 | 0.6 | 0.6 | 5.25 | 61.0 | 31 | 15 | + | clear |
| | 3 | 0.9 | 0.6 | 5.55 | 77.1 | 40 | 15 | ++ | clear |
| | 4 | 3.2 | 0.8 | 6.00 | 85.8 | 47 | 14 | +++ | cloudy |
| WSPagar | 2.5 | 0.6 | 0.6 | 5.55 | 82.0 | 35 | 18 | + | clear |
| | 3 | 0.9 | 0.6 | 6.00 | 80.8 | 37 | 16 | ++ | clear |
| | 4 | 3.2 | 0.8 | 6.15 | 80.6 | 35 | 15 | | cloudy |
| | 4 | 3.6 | 0.4 | 4.10 | 85.3 | 51 | 16 | ++++ | clear |

*Key: Conc = Starch Concentrations (%); $M_{time}$ = Melting Time (Minutes); Tm = Melting Temperature ($^{o}$C); agar-agar = agar-agar concentration; Tg = Gelling Temperature; $G_{time}$ = Gelling Time (Minutes).- = Neither gelled nor solidified; + = lumps; ++ = soft gel, +++ = semi-solid gel; ++++ = solid gel; RC = Rubber Cassava, RCagar = Rubber Cassava blended with Agar-agar, OPagar = Opokopo Cassava blended with Agar-agar, WSPagar = White sweet potato blended with Agar-agar, YSP agar = Yellow sweet potato blended with Agar-agar*

**Table 4. Gel Formation on unblended cassava and sweet potato starch**

| Samples | Concentration (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 |
| RC | - | - | - | - | - | - | - | - | - | + | + |
| OP | - | - | - | - | - | - | - | - | - | + | + |
| YSP | - | - | - | - | - | - | - | - | - | - | - |
| WSP | - | - | - | - | - | - | - | - | - | - | - |

*Key: - = Neither gelled nor solidified, + = Lumps were formed.*
*RC =Rubber Cassava, OP = Opokopo Cassava, WSP = white sweet potato, YSP = Yellow sweet potato*

### 3.5 Gel Formation of Composite Starch Blended with Agar-agar and Agarose

Table 5 presents the results of gel formed by starch blended with agar-agar and agarose. The result revealed that the amount of agar-agar or agarose mixed with either cassava or sweet potato starch determines how solid the gel becomes. Lumps and soft gels were formed at lower concentration (1 - 2%) of RCagar, OPagar, WSPagar and YSPagar. Semi-solid but sticky gel was formed when 2.2 g of starch was blended with 0.8 g of agar-agar. By varying both the concentration of starch and agar-agar, gel suitable for electrophoresis formed at 4% (3.2 g of starch and 0.8 g of agar-agar). Meanwhile, 5% -6% concentration formed solid gel. However, WSPagar and YSPagar formed cloudy gel formed when compared to that of RCagar and pure agarose as shown in Fig. 1. Agarose (0.4 g) was also mixed with 3. 6 g of RCagar and 3.6 g of WSP (4% RCagarose and WSPagarose) and solid gel suitable for gel electrophoresis was formed. However, WSPagarose formed an opaque gel.

### 3.6 Electropherogram of DNA Ladders on Starch-Agar Gel

igure 1 is the electropherogram of DNA ladders on cassava and sweet potato composite starch gel. Migration of DNA ladders (1kb and 100bp) occurred through the blended cassava (Figs. 1B and C) and sweet potato (Fig. 1D) starch gel. However, the DNA bands on 3% and 4% RCagar did not appear as clear as that of 1% agarose (Fig. 1A). Gel formed by 4% WSPagar was cloudy, therefore, the DNA bands were not clear when visualized in blue light and ultra violet light of gel documentation (Azure Biosystems) system as shown in Fig. 1D.

### 3.7 Electropherogram of DNA Ladder on Starch-agarose Gel

Figs. 2 A, B and C are the electropherograms of DNA ladders resolved on starch-agarose gel. Migration of DNA ladders (1kb and 100bp) occurred through the gel formed by cassava and sweet potato starch blended with agarose. The bands of DNA ladder resolved by 4% RCagarose gel (Fig. 2B) was as clear as the bands on 1% agarose gel (Fig. 2A). However, 4% WSPagarose gel appeared cloudy and the resolved bands of 100bp DNA ladder on it (Fig. 2C) was not as visible as that of 4% RCagarose gel and 1% agarose gel.

### 3.8 Electropherogram of DNA Ladder and PCR Product on Starch-agarose Gel

Fig. 3 shows the electropherogram of DNA ladders and PCR product (*CLMA* gene) on starch-agarose gel. Migration of DNA ladders (100bp) and *Cł.MA* gene (amplicon of Antimicrobial-resistant genes associated with *Salmonella* species) occurred through the gel formed by 1% agarose gel (Fig. 3A) and 4% RCagarose gel (Fig. 3B). DNA bands resolution on 4% RCagarose (Fig. 3B) was comparable to that of 1% agarose gel (Fig. 3A).

**Table 5. Gel Formation on composite starch blended with agar-agar and agarose**

| Samples | Concentrations (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1(0.1ag) | 2(0.0.3ag) | 3(0.5ag) | 3.5(0.6ag) | 4(0.7ag) | 4.5(0.75ag) | 5(0.8ag) | 5.5(0.8ag) | 6(0.8ag) |
| RCagarose | ++ | +++ | | | | | | | |
| WSPagarose | ++ | +++ | | | | | | | |
| RCagar | + | + | + | ++ | +++ | +++ | ++++ | ++++ | ++++ |
| OPagar | + | + | + | ++ | +++ | +++ | ++++ | ++++ | ++++ |
| WSPagar | + | + | + | ++ | +++ | +++ | ++++ | ++++ | ++++ |
| YSPagar | + | + | + | ++ | +++ | +++ | ++++ | ++++ | ++++ |

*Key: (0.1ag, 0.3ag, 0.5ag, 0.6ag, 0.7ag, 0.75ag, and 0.8ag) = agar-agar concentration. 0.4g of agarose formed strong gel with 3.6g of starch, + = lumps, ++ = soft gel, +++ = semi-solid gel, ++++ = solid gel. RCagarose = Rubber Cassava blended with Agarose, WSPagarose = White sweet potato blended with Agarose, RCagar = Rubber Cassava blended with Agar-agar, OPagar = Opokopo Cassava blended with Agar-agar, WSPagar = White sweet potato blended with Agar-agar, YSP agar = Yellow sweet potato blended with Agar-agar*

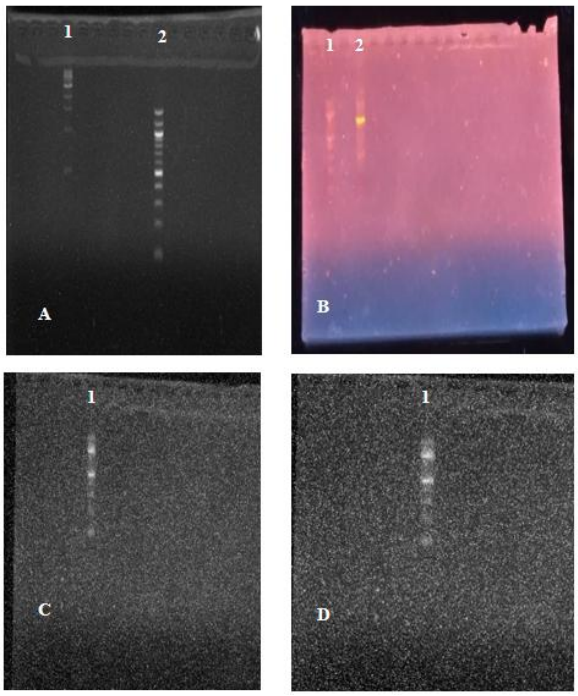


**Fig. 1. Electropherogram of DNA Ladders on Starch-Agar Gel. A= 1% Agarose gel, B= 3% RCagar gel (2.2g rubber cassava starch and 0.8g agar-agar), C= 4% RCagar gel (3.2g rubber cassava starch and 0.8g agar-agar) and D = 4% WSPagar gel (3.2g white Sweet potato starch and 0.8g agar-agar). Well 1 contains 1kb DNA Ladder and Well 2 contains 100bp ladder DNA**

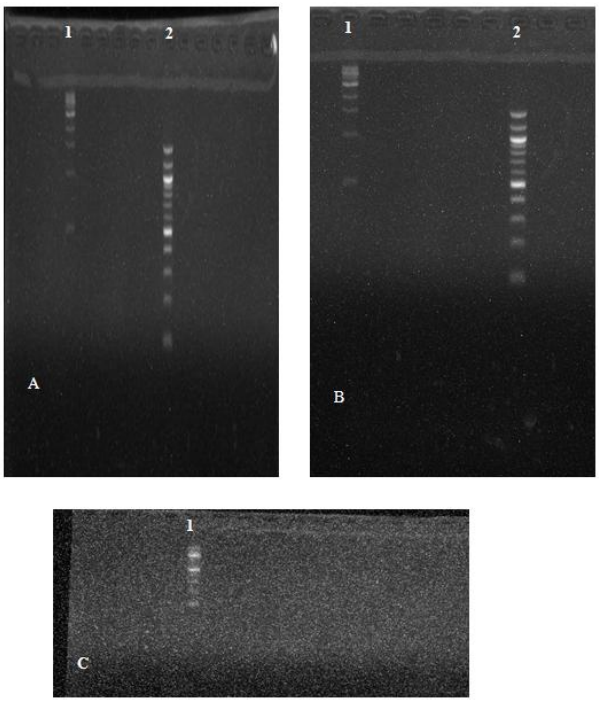


**Fig. 2. Electropherogram of DNA Ladder on Starch-Agarose Gel. Well 1 contains 1kb DNA ladder, Well 2 contains 100bp DNA ladder, 2A= 1% agarose gel (Control), 2B= 4% RCagarose gel (3.6 g of rubber cassava starch and 0.4 g of agarose) and 2C= WSPagarose (3.6 g of white sweet potato starch and 0.4 g of agarose)**

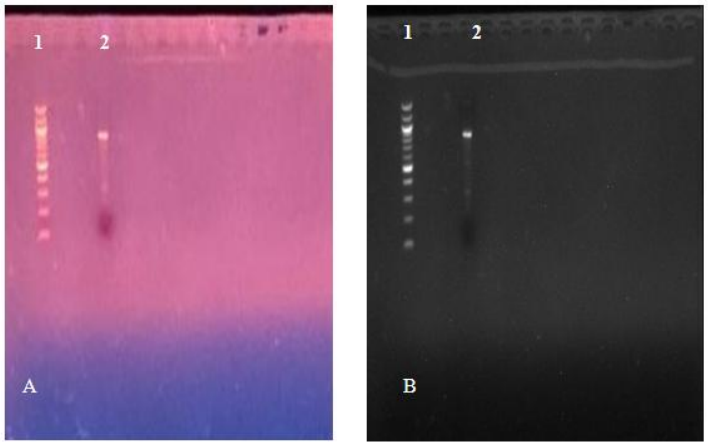


**Fig. 3. Electropherogram of DNA ladder and CMLA Gene on Agarose and Composite Starch Gel. Well 1 contains 100bp ladder DNA while well 2 contains CMLA gene. A= 4% RCagarose gel (3.6 g of rubber cassava starch and 0.4 g of agarose) gel, B= 1% Agarose (Control)**

## 4. DISCUSSION

Starch consists of two glucose-containing polymers such as α-linked large amylose and highly branched amylopectin [29] whereas agarose is made up of repeated residues of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose [20]. Polysaccharides present in starch are also held together by hydrogen-bond [30]. A report on gel formation by starch established that starch granules swell, gelatinize and hydrate easily when heated in water [30]. However, the functional properties and applications of starch depend on the crop source [30].

The migration of DNA molecules through agarose gel largely depends on the pore size of the matrix of agarose gel, molecular size and charges of the biomolecules [22]. The results (Table 1) showed starch granule distribution in the range of 5.525 ± 1.9346 µm to 8.275 ± 1.5064 µm for Rubber Cassava (RC), Opokopo Cassava (OP), white sweet potato (WSP) and yellow sweet potato (YSP). Comparatively, Altland and Hackler, [22] reported the pore size of agarose to be within the range of 6 to 35µm. The values of starch pore sizes obtained from this study are also in line with literature figures which have been reported to fall within the ranges of 2 to 42µm [31,32].

Study on the clarity of starch gel samples revealed that RCagar and RCagarose (Table 2) formed the most transparent gel (Figs. 2B, C and 3B) compared to other starch composite studied. The purity level of cassava starch in this study (Table 2) was higher than those of sweet potato starch which could also account for cassava starch yielding better separation of DNA biomolecule. Independent researchers reported that the presence of non-starch impurities such as polyphenols, ascorbic acids and carotene may have negative effects on the starch quality and the final functionality of starch [16,18].

The pH values (Table 2) of cassava and sweet potato starch studied ranged from 7.7 to 7.9. Hence, there was no significant difference ($p \geq 0.05$) in the pH of the starch when compared with that of pure agarose (7.5). Chen et al. [32] speculated that pH level in starch has some effects on the functionality of starch. Adebowale et al. [33] reported increase in the solubility of high pH starches as a result of increase in the hydrophilic characteristics of the starch at these pH values.

The melting temperature (Tm) and gelling temperature (Tg) of the starch samples studied ranged from 74 - 85$^{o}$C and 51 - 58$^{o}$C respectively. While the melting and gelling time (Min) ranged from 4 - 6 minutes and 12 - 21 minutes respectively depending on the amount of agarose or agar-agar used. Table 3 shows that the melting temperature (Tm) and gelling temperature (Tg) of the starch samples studied ranged from 74 - 85$^{o}$C and 51 - 58$^{o}$C respectively. While the melting and gelling time (Min) ranged from 4 - 6 minutes and 12 - 21 minutes respectively depending on the amount of agarose or agar-agar used. Values obtained in this study are in line with the literature report by Chen et al. [14] who noted that the melting temperature on heat moisture treatment for most starch is within 70 - 130°C and gelling

temperature of 15-35°C comparable to the melting temperature (85 - 95°C) and gelling temperature range (35-42°C) of agarose gel [34].

The result also showed that the sweet potato composite starch (WSPagar and YSPagar) formed a cloudy gel which can be attributed to the level of opacity of the starch gel. The DNA samples (100bp and 1kb ladder as well as CLMA gene) migrated clearly through the starch composite gel at different times throughout the electrophoresis (30 minutes to 1 hour) and 30 - 45 minutes for 1% agarose. The differences in the gelatinization temperature could be as a result of branch chain lengths of amylopectin distributions. Meyer et al. [35] reported that starch containing longer chain lenghts of branched amylopectin void of other structural differences such as phosphate monoester contents show higher gelatinization temperature and formation of strong gel.

None of the concentrations of the starch studied (Table 4) formed strong and solid gel suitable for gel electrophoresis, hence, the need for blending with little amount of either agar-agar or agarose before gelling (Table 5). This is in agreement with the report of Meyer et al. [35] who noted that starch paste would produce a highly viscous liquid without addition of amylose. Agarose gel electrophoresis was carried out as a positive control to blended cassava and sweet potato starch gel electrophoresis. Table 2 provided the choice of using RCagar, RCagarose, WSPagar and WSPagarose over OPagar, OPagarose, YSPagar and YSPagarose on gel electrophoresis. The DNA ladders were separated as expected. Cassava starch blended with agar-agar resolved DNA ladder but the DNA bands were not as clear as that of pure agarose due to high level of contamination in the composite starch gel. Table 2 provided the evidence that both agar-agar (5.269 ± 0.000) and cassava (RC) starch (36.089 ± 0.116) contained high amount of impurity while agarose had no contamination (0.000 ± 0.000). This is also in line with some research literatures which established that there is presence of non-starch impurities like ascorbic acids, polyphenols and carotene which may have negative effects on the starch quality and the final functionality of starch [16-18].

However, DNA bands found in RCagarose were clear and comparable to that of pure agarose gel (Figs. 2A and B) but the electropherogram of DNA ladder found on white sweet potato blended with agarose (4% WSPagarose) was cloudy. This could also be attributed to the higher level of contamination in sweet potato starch samples as shown in Table 2. Generally, sweet potato starch blended with either agar-agar or agarose formed cloudy gel and as a result, the DNA molecules resolved were not visible enough (Figs. 1D and 2C). We also investigated the possibility of reducing the cost of agarose by blending cassava starch (3.6 g of RC) with little amount of agarose (0.4 g). The composite starch (4% RCagarose) resolved DNA molecules (100kb DNA ladders and CLMA genes (Antimicrobial-resistant genes associated with *Salmonella* species)) were separated as expected (Fig. 3B). The result was comparable to that of 1% agarose gel (Fig. 3A). Therefore, these results, do not only demonstrate the possibility of replacing agarose with cheaper and available local resources like cassava but also proved that the cost of molecular analysis can be reduced to a significant amount by combining cassava starch with little amount of agarose [36-42].

## 5. CONCLUSION

The importance of starch as a biopolymer continues to be on the upward trend due to its versatility. The use of cassava starch (RCagar and RCagarose) gel to separate DNA molecules offers huge hope for future studies. We therefore recommend that further research should be carried out on the optimization of cassava starch gel in DNA electrophoresis.

## 6. LIMITATIONS

None of the starch concentrations gelled except when mixed with agar-agar or pure agarose powder which increased the cost of the study. The disparity in the clarity between composite starch and agarose could be attributed to the high level of contamination in the starch used which would have reduced to minimal level if highly purified starch was used.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Craig SAS, Manifigat CC, Seib PA, Hoseney RC. Starch paste clarity. Cereal Chem. 1989;66(3):173-82.
2. Naz MY, Sulaiman SA, Ariwahjoedi B, Shaari KZK. Characterization of blended tapioca starch solutions and their sprays for high temperature coating applications. Sci World J. 2014;7:585-90.
3. Gilbert RG, Witt T, Hasjim J. What is being learned about starch properties from multiple-level characterization. Cereal Chem. 2013;90(4):312-25.
   DOI: 10.1094/CCHEM-11-12-0141-FI.
4. Singh A, Geveke DJ, Yadav MP. Improvement of rheological, thermal and functional properties of tapioca starch by using gum arabic. LWT Food Sci Technol. 2017;80:155-62.
   DOI: 10.1016/j.lwt.2016.07.059.
5. IITA. An annual report on cassava production; 2017. p. 4-6.
6. Food and Agriculture Organization of the United Nations (FAO). Rome: FAOSTAT Statistical Database, Statistical Division; 2019.
7. Jiménez A, Fabra MJ, Talens P, Chiralt A. Edible and biodegradable starch films: a review. Food Bioprocess Technol. 2012; 5(6):2058-76.
   DOI: 10.1007/s11947-012-0835-4.
8. Spooner DM, Ghislain M, Simon R, Jansky SH, Gavrilenko T. Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. The botanical review. 2014;80(4):283-383.
9. Fakir MSA. Effect of branch number on growth and yield in two cassava morphotypes. Bangladesh J Agric. 2012;1:1-4.
10. Alvarado PM, Grosmaire L, Dufour D, Toro AG, Sánchez T, Calle F et al. Combined Effect of Fermentation, Sun Drying and Genotype on Bread making Ability of Sour Cassava Starch. Carbohydr Polym. 2013;98(1):1137-46.
    DOI: 10.1016/j.carbpol.2013.07.012
11. Ogunmuyiwa OH, Adebowale AA, Sobukola OP, Onabanjo OO, Obadina AO, Adegunwa MO et al. Production and quality evaluation of extruded snack from blends of Bambara groundnut flour, cassava starch, and Corn Bran flour. J Food Process Preserv. 2017;41(5): 1-11.
    DOI: 10.1111/jfpp.13183.
12. Ahmed Z, Tetlow IJ, Falk DE, Liu Q, Emes MJ. Resistant starch content is related to granule size in barley. Cereal Chem. 2016;96(6):618-30.
    DOI: 10.1016/B978-0-12-811971-6.00011-5.
13. Pfister B, Zeeman SC. Formation of starch in plant cells. Cell Mol Life Sci. 2016;73(14):2781-807. Electrophor–Princ Basics.
    DOI: 10.1007/s00018-016-2250-x
14. Chen P, Liu X, Zhang X, Sangwan P, Yu L. Phase transition of waxy and normal wheat starch granules during gelatinization. Int J Polym Sci. 2015;2015:1-7.
    DOI: 10.1155/2015/397128.
15. Osman EM 1967. Starch in the food industry. In: R. L. pp. 163-215.
16. Spingo G, de Faveri DM. Gelatinization kinetics of rice starch studies by non-isothermal calometric technique; influence of extraction method, water concentration and heating rate. J Food Eng. 2004;64(4):337-44.
17. Sasaki T. Effect of wheat starch characteristics on the gelatinisation, retrogation and gelatinisation properties. Jpn Agric Res Quarters. 2005;39(4): 253-60.
18. Shimelis EA, Meaza M, Rakshhit S. Physicochemical properties, pasting behaviour and functional characteristics of flour and starches from improved bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. Agric Res. 2006;5:34-7.
19. Egbe NE, Ume AO, Vantsawa PA, Nwankwo HC, Effiong TE, Eze GO et al. 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. J Adv Biol Biotechnol. 2022;25(3): 28-35.
20. Araki C. Structure of the agarose constituent of agar-agar. Bull Chem Soc Jpn. 1956;29(4):543-4.
    DOI: 10.1246/bcsj.29.543.
21. Gao J, Sun SP, Zhu WP, Chung TS. Chelating polymer modified P84 nanofiltration (NF) hollow fiber membranes for high efficient heavy metal removal. Water research. 2014;63:252-61.

22. Altland K, Hackler R. In: Neuhoff V, editor. Electrophoresis' 84. Vol. 1984. Weinheim: Verlag Chemie; 1984;362-78.
23. Chawla HS. Introduction to Plant Biotechnology (3/e). Boca Raton: CRC Press; 2011.
DOI: 10.1201/9781315275369.
24. Barril P, Nates S 2012. Introduction to agarose and polyacrylamide gel electrophoresis matrices with respect to their detection sensitivities. Gel.
25. Brabet T, Deshmukh R, Annapure U. 'Modification of Starch using Low Pressure Radio Frequency Air Plasma'inLWT, 89:719-724; 1998.
26. Kaur K, Ahluwalia P, Singh H. Cassava: extraction of starch and utilization of flour in bakery products. Int J Food Ferment Technol. 2016;6(2):351-5.
DOI: 10.5958/2277-9396.2016.00059.3.
27. Fitt LE, Snyder EM. Photomicrographs of starches, industrial microscopy of starches. In: Whistler RL, BeMiller JN, Paschall EF, editors, Academic Press Starch: chemistry and technology. 2nd ed. NY: New York; 1984. p. 675, P. 661.
28. Bhagya B, Sridhar KR, Seena S, Young CC, Arun A, Nagaraja K. Nutritional qualities and in vitro starch digestibility of ripened Canavalia cathartica beans of coastal sand dunes of southern India. Electr J Environ Food Chem. 2006;5:25-6.
29. Hamaker BR, Tuncil YE, Shen X. Carbohydrates of the kernel. In: Corn, editor. Serna-sal-divar SO. 3rd Ed. AACC International Press. 2019;305-18
30. Pan SC, Rickard TC. Transfer of test-enhanced learning: Meta-analytic review and synthesis. Psychological bulletin. 2018;144(7):710.
31. Woolfe IA, Hofreiter BT, Watson PR, Deatherage WL, MacHasters MM. The structure of a new starch of high amylose content. J Am Chernistry Soc. 1992;77:1654-9.
32. Chen Z, Schols HA, Voragen AGJ. Physicochemical properties of starches obtained from three varieties of Chinese sweet potatoes. J Food Sci. 2003a;68(2): 431-7.
DOI: 10.1111/j.1365-2621.2003.tb05690.x.
33. Adebowale K, Owolabi B, Olayinka O, Lawal O. Effect of heat-moisture treatment and annealing on physicochemical properties of red sorghum starch. Afr J Biotechnol. 2005;4:928-33.
34. Samar J, Chutia BS, Yashwanth A, Kashyap RK, Pranab D. Fundamentals of Agar-Agarose Gel Electrophoresis, Kuntal Krishna Bera. 2019;34-6.
35. Meyer KH, Bernfeld P, Wolf E. Recherches sur l'amidon III. Fractionnement et purifcation de l'amylose de maïs naturel. Helv Chim Acta. 1940;23(1): 854-64.
DOI: 10.1002/hlca.194002301110.
36. Abraham TE. Stabilization of paste viscosity of cassava starch by heat moisture treatment. Starch/Stärke. 1993; 45(4):131-5.
DOI: 10.1002/star.19930450404.
37. Getrudis DJM, Wattimena DIG. The effect of agar-agar substitution on micro shoot production of two sweet potato cultivars (*Solanum tuberosum* L.). Acta Hortic. 1994;369:447-50.
38. Henry OE. Chemical properties of starch and its application in the food industry, chemical properties of starch. Intech Open; 2019.
DOI: 10.5772/intechopen.87777.
39. Jan P. Electrophoresisin biochemistry. Institute of Medical Biochemistryfirst faculty of Medicine, Charles University in Prague. 2009;2.
40. Kasanadze AK. Replacement of Agar by cassava flour in microbial media [BSc dissertation] University of Malawi. Lilongwe: Bunda College of Agriculture; 2000.
41. Maliro MFA, Lameck G. Potential of cassava flour as a gelling agent in media for plant tissue cultures. Afr J Biotechnol. 2004;3:244-74.
42. McCrone WC, Delly JG. 2nd Ed, Ann Arbor Science Publishers, Ann Arbor, Michigan. Starch Identification (Microscopy); The Particle Atlas. 1973;2: 457-62.



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