Journal Name:	**Asian Journal of Biology**
Manuscript Number:	**Ms_AJOB_91085**
Title of the Manuscript:	**Evaluation Of Cassava (Manihot Esculenta) And Sweet Potato (Ipomea Batata) Starch From South East Nigeria In The Separation Of Deoxyribonucleic Acids As Alternative To Agarose Gel.**
Type of the Article	**Original Research Article**

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PART 1: Review Comments

<table>
<tr><td></td><td colspan="3">Reviewer's comment</td><td>Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</td></tr>
<tr><td><u>Compulsory</u>
REVISION comments</td><td colspan="3"><table>
<tr><td>ABSTRACT</td><td>selected cassava (RC and OP) and sweet potato (WSP and YSP)</td><td>Comment: the abstract should not contain abbreviations or terms that may be confusing to readers, so write the full name before the local name</td></tr>
<tr><td></td><td>The sample pH, gelling temperature and time were determined by standard procedures. Standard electrophoresis procedure was used for the starch gel electrophoresis.</td><td>Comment: name the standard procedures and show the relevant citation</td></tr>
<tr><td></td><td>The result showed that composite starch gelled within 18-21 minutes while agaraose</td><td>Comment: agaraose spelling mistake</td></tr>
<tr><td>INTRODUCTION</td><td>However, agarose gels are not suitable for separation of low molecular weight DNA and also produce poor band resolution (Barril and Nates, 2012). 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 (Barril and Nates, 2012). High cost of both agarose and acrylamide impose serious hindrance to molecular studies not only in Nigeria but also in limited</td><td>The word (Therefore) when read after the preceding sentences gives a sense of rationale rather than the objective. (This is the reasoning for your objective)
My opinion:
The objectives should reflect the main thrust</td></tr>
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			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 investigate the ability of cassava and sweet potato starch from South East of Nigeria in the separation of DNA molecules.	of your paper. There is no need for the nitty gritty details	
		MATERIALS AND METHODS:	2.1 Collection of Cassava and Sweet potato Tubers Two fresh cultivars of cassava (*Manihot esculenta*) and two sweet potato (*Ipomea 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) are the varieties of cassava mostly cultivated within the area. White sweet potato (WSP) and yellow sweet potato (YSP) were randomly selected.	Comment: when and where the study was carried out?	
			2.5 Starch Clarity and Impurity Level Determination About 20 - 100 µm of pure glucose was used as control. Spectrophotometer was used and	Comment: is it µm or µL?	

			the absorbance of the solutions were recorded at 490 nm. The amount of sugar present in the starch samples were calculated by multiplying the total sugar by 0.9.		
			2.7 Gel Preparation of Unblended Cassava and sweet potato Starch **To determine the gelling ability of the samples, the cassava (RC and OP) and sweet potato (WSP and YSP) starch were weighed in different quantities (1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, 5 g, 5.5 g and 6 g respectively)**	**comment:** 1) Place the unit after the last in a ***series of numbers*** all having the same unit. Then **(1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, 5 g, 5.5 g and 6 g)** changes to **(1 , 1.5 , 2 , 2.5 , 3 , 3.5 , 4 , 4.5 , 5 , 5.5 and 6 g).** **2)The word respectively is used to emphasize the relationship between two parallel lists that contain the same number of items. cassava (RC and OP) and sweet potato (WSP and YSP)= 4 items. (1 , 1.5 , 2 , 2.5 , 3 , 3.5 , 4 , 4.5 , 5 , 5.5 and 6 g)= 11 items** **Correction: 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)	
		2.7 Gel Preparation of Unblended Cassava and sweet potato Starch 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 shaking at 30 seconds to 1-minute interval.	Comment: Is it manual or automatic shaking?	
		2.8 Gel Formation of Starch-Agar Mixtures This experiment was carried out in triplicate by blending different concentrations of cassava (RC and OP) and sweet potato (WSP and YSP) starch (1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, 5 g, 5.5 g and 6 g) with different concentrations of (0.1g, 0.2g, 0.3g, 0.4g, 0.5g, 0.6g, 0.7g, 0.75g 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 measured into 250 ml capacity beakers containing 100 ml of 1x TBE (Tris-Borate-EDTA) buffer.	Comment: A) quantities instead of concentrations B) Also place the unit after the last in a *series of numbers* all having the same unit.	
		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, no literature containing standard protocol suit for our purpose was found.	Comment: are you sure? Say as far as we know no literature containing standard protocol suit for our purpose was found	

		2.13 Loading of PCR Product (CMLA Gene) and DNA Ladder into Blended Starch Gel Five microliters (5 µg/ml) of 1kb (Quick Load Purple DNA ladder, NO551S, New England BioLab) was carefully pipetted into the third (3rd) well of 3% RCagar gel and 1kb DNA ladder into the fiveth (5th)	Comment: fifth	
		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.	Comment: put the gel thickness	
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		2.15 STATISTICAL ANALYSIS Data collected were analyzed using Microsoft Excel and SPSS (version 23). Results are presented as mean ± SD from One-way ANOVA.	A) Were B) SAY one way analysis of variance (ANOVA)	
	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 observed. Most starch granules studied were round or oval, but asymmetrical granules were also seen.	Comment: repetion Most starch granules studied were round or oval, but asymmetrical granules were also observed. Most starch granules studied were round or oval, but asymmetrical granules were also seen.	

3.2 Starch Clarity and Impurity Level

Table 2 Shows the clarity, purity and pH value of agarose, agar-agar, unblended, blended starch samples studied. The study reveled that there was significant difference ($p \leq 0.05$) between clarity of the samples studied. RC agar (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 ($p \leq 0.05$) between the purity of the sample showed the least contamination levels. Rubber Cassava (RC) (36.089 ± 0.116 ml) followed by RC agar (40.087 ± 1.112 ml) while YSP agar (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 ($P \geq 0.05$) between the pH of the starch samples and agarose.

A) Revealed
B) STD = 0?

Table 5. Gel Formation on Composite Starch Blended with Agar-agar and Agarose

					Conc				

Comment: 0.2 ag and 0.4 ag were not found in this table

					(%)				
Samples	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)
RC agarose	++	+++							
WSP agarose	++	+++							
RC agar	+	+	+	++	+++	+++	++++	+++++	++++
OPagar	+	+	+	++	+++	+++	++++	+++++	++++
WS Pagar	+	+	+	++	+++	+++	++++	+++++	++++
YSP agar	+	+	+	++	+++	+++	++++	+++++	++++

Key: (0.1ag, 0.2ag, 0.3ag, 0.4ag, 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. RC agarose = Rubber Cassava blended with Agarose, WSP agarose = White sweet potato blended with Agarose, RC agar = Rubber Cassava blended with Agar-agar, OP agar = Opokopo Cassava blended with Agar-agar, WSP agar = White sweet potato blended with Agar-agar, YSP agar = Yellow sweet potato blended with Agar-agar.		
Minor REVISION comments					
Optional/General comments					

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

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