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Okra (*Abelmoschus esculentus* L.) pectin yield as influenced by particle size and extraction solvent

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ABSTRACT

Pectin extractable from okra (*Abelmoschus esculentus* L.) is known to have various food and non-food applications. The objective of this work was to investigate the effect of particle size of milled samples on okra pectin yield using two different extraction solvents. Phosphate buffer (PB) and citric acid solution (CAS) (at pH 6, temperature of 80°C and for 1 h) were used to extract pectin from 10 g of milled okra sample with varying particle sizes (0.5, 1 and 2 mm). Ethanol was added to the aqueous extract to precipitate the pectin. The crude pectin obtained was then freeze-dried and the % pectin yield was calculated. The average okra pectin yield when PB was used for extraction were 19.6±4.0%, 15.8±1.0% and 11.9±1.5% for 0.5 mm, 1 mm and 2 mm particle sizes, whereas that of the CAS counterparts were 32.7±8.1%, 25.6±0.8% and 35.6±5.5%, respectively. However, considering purity of the pectin extracts (PB > CAS), the present findings indicated that a higher pectin yield is achievable with 0.5 mm as the optimal particle size of okra pod powder using phosphate buffer as extraction solvent.

1. Introduction

Pectins are polysaccharides extracted from plant sources; they are heterogeneous, hygroscopic and soluble in acids and water, with properties of gelling, stabilization of emulsions and nutritional fiber supply (de la Hoz Vega et al., 2018; Kpodo et al., 2021). It is considered an essential ingredient in the food and chemical industries. It is used for the manufacture of jellies, ice cream, sauces, and cheeses, among others. Also, it is employed in the pharmaceutical industries when it is desired to modify the viscosity of their products and in the plastics industry for the manufacture of foam products (de la Hoz Vega et al., 2018; Agbenorhevi et al., 2020; Abe-Inge et al. 2020; Owusu et al., 2021; Boakye-Gyasi et al., 2021). Commercial sources of pectin are from apple pulp (15-18%) and citrus peel, 20-30% (de la Hoz Vega et al., 2018, Vanitha & Khan, 2019; Masmoudi et al 2008). In recent times, companies and micro-companies processing natural juices of oranges, pomelo and lemons produce large amounts of waste (albedo) which potentially could lead to environmental and health problems, while promoting the proliferation of insects, fungi, bacteria and odors by decomposition (de la Hoz Vega et al., 2018). One solution to this problem is that a potentially marketable byproduct like pectin is extracted from the peels of these fruits (Yeoh et al., 2008; de la Hoz Vega et al., 2018).

A fruit that is underutilized for pectin extraction in the food industry is okra (*Abelmoschus esculentus L*), formerly known as *Hibiscus esculentus*, which is a plant of the Malvacae family (Kontogiorgos et al 2012). It is an integral part of the diet of several Africa and India as well as of other countries with the worldwide gross production value of okra estimated to be over 725 million US$ and that of Ghana over 47 million US$ (FAO, 2019; Karakoltsidis & Constantinides, 1975; Woolfe et al., 1977; Whistler & Conrad, 1954). The fruit is cultivated throughout the tropical, subtropical and temperate regions of the world including the shores of the Mediterranean Sea owing to its high economic and nutritional values (Kpodo et al., 2017; Agbenorhevi et al., 2020). Okra flour has been found to possess antioxidant activity which increased by roasting, while in vitro digestibility studies showed that most of its antioxidant activity is available in the intestinal phase of gastrointestinal tracts (Adelakun et al., 2009). Okra seeds are considered a high-protein oilseed crop that could be used to complement other protein sources (Bryant et al., 1988; Ofori et al., 2020). Okra pods are rich in phenolic compounds mainly composed of oligomeric catechins and flavonol derivatives, while the polyphenolic profile of the epidermis is composed principally of 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 techno-

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logical interest for food and non-food applications, and major applications have been previously reviewed (Whistler & BeMiller, 1993; Ghori et al., 2014). Such extracts can be used as natural food-grade emulsifiers or thickeners and emulsion stabilizers (Ndjouenkeu et al., 1997; Georgiadis et al., 2011; Kpodo et al., 2018; Bawa et al., 2020; Datsomor et al., 2019; Kissiedu et al., 2020; Tobil et al., 2020), suggesting that they could be a promising source of texture modifiers for complex food matrices.

Although many researchers have studied okra in terms of their flocculating abilities, the effect of extraction conditions including temperature and time on pectin yields (Mao et al., 2020; Chan & Choo, 2013), to the best of the knowledge of authors, no research to date has studied the effect of particle size on okra pectin yield. The objective of the present work, therefore, was to investigate the effect of particle size on okra pectin yield using two different extraction solvents.

2. Materials and methods

2.1. Materials

Agbagoma and Balabi okra pods/samples (~ 3 months old) were obtained from Ho, Volta Region, Ghana. The Nigeria genotype (Jokoso variety) was obtained from Ibadan, Nigeria. The Nigeria genotype has similar phenotype just as Agbagoma. Thus the intention was to compare the results of the two common Ghanaian okra genotypes to the Nigerian counterpart. The okra pod was dried and milled to varying particles sizes of 0.5 mm, 1 mm and 2 mm using a Retsch sieve shaker (AS 200, Haan, Germany). All chemicals used were of analytical grade. Deionized water was used throughout the extraction experiment.

2.2. Preparation of extraction solvents

Preparation of 5000 mL phosphate buffer solution (0.1 molL^{-1}) for the experiments: 11.496 g of K_2HPO_4 and 59.063 g of KH_2PO_4 were dissolved with a small amount of distilled water in separate beakers. The solutions were then transferred into a 5000 mL round bottom flask and topped up with distilled water to the 5000 mL mark, making sure a pH of 6 was maintained by adjusting the final solution with HCl.

Preparation of 5000 mL citric acid solution (0.1 molL^{-1}): 96.062 g of $C_6H_8O_7$ was weighed and dissolved with a small amount of distilled water in a beaker. The solution was stirred and transferred into a 5000 mL round bottom flask. It was then topped up with distilled water to the 5000 mL mark while maintaining a pH of 6, by adjusting the solution with NaOH.

2.3. Pectin extraction and chemical analysis

Okra pectin extraction using phosphate buffer was performed as previously reported (Kpodo et al. 2017; Kpodo et al., 2021). A 10 g of milled

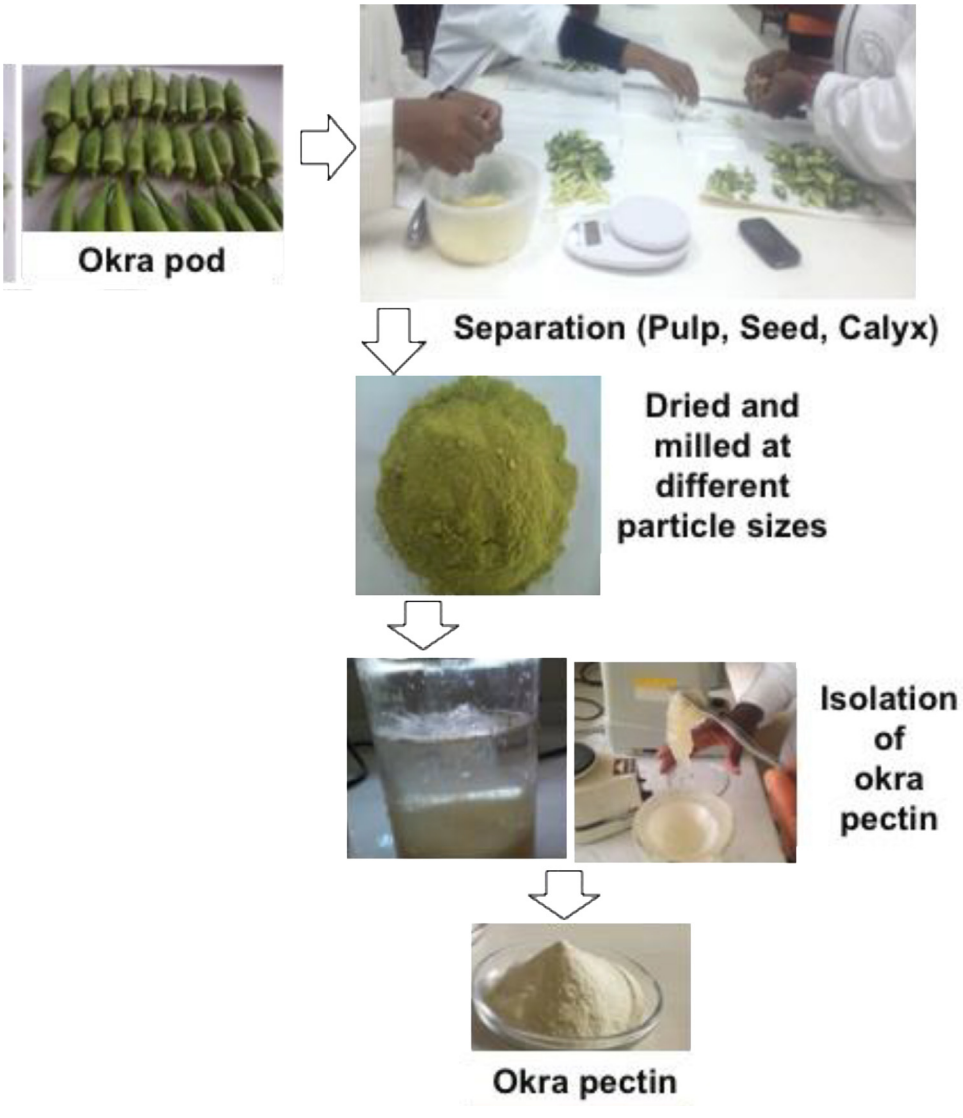

Fig. 1. Extraction of okra pectin.

okra of particle size 2 mm was weighed and added to a 300 mL of phosphate buffer solution, stirred and pectin extracted at a temperature of ~80°C for 1 h. The solution was then filtered using a muslin cloth to separate the aqueous extract from the okra residue. The volume of the aqueous extract was measured and precipitated with ethanol using a ratio of 1:2 respectively. The mixture was then placed on an electric heater at 40°C for 1 h and stirred to allow precipitation to complete. The slimy precipitate was collected, washed with isopropanol and placed in a ziplock bag. The process was repeated two more times for the consistency of the results. The experiment was then repeated for okra particle sizes of 1 mm and 0.5 mm.

A similar experimental process was conducted using the citric acid solution for 2 mm, 1 mm and 0.5 mm particle sizes. The extraction process is illustrated in Fig. 1, and presented in Table 1. The % pectin yield was calculated using the Equation 1:

$$\text{Pectin yield (\%)} = \frac{Weight\ of\ dried\ pectin}{Weight\ of\ dried\ okra\ powder} \times 100 \quad (1)$$

The chemical analysis (total carbohydrate, protein and ash contents) of the isolated okra pectin were done as previously reported (Kpodo et al., 2017, 2018, 2021).

2.4. Spectroscopy

The Nuclear Magnetic Resonance (NMR) spectroscopy was performed using 10 mg of okra pectin sample which was dissolved in 90 % H_2O + 10 % D_2O. A Bruker Avance IV 500MHz NMR spectrometer (Ettlingen, Germany) was used to acquire proton NMR. All experiments were performed at 300K The ^1H (proton) spectra were measured at 500.03 MHz using the "zg30" pulse for proton. The acquisition parameters for this experiment were as follows; time-domain (number of data points 65K; acquisition time 3.2767999s; delay (relaxation) 1.00s number of scans 64; spectral width 10,000Hz; sweep width 19.9987ppm; total acquisition time was 1min 7s.

Fourier Transform Infra-Red (FT-IR) spectra were obtained over a wavelength of 4000 cm^{-1} to 400 cm^{-1} for all the okra pectin samples at a resolution of 4 using 24 scans (Nicolet 38, Thermo Scientific, UK) equipped with spectral smoothing instrument software (NIOS2 Main 00.02.0009). 2 mg of sample was mixed with spectroscopic grade KBr powder and pressed into thin slices under optimal conditions.

2.5. Statistical analysis

Data obtained were analyzed statistically by using SPSS version 20 (IBM SPSS Statistics, US) using two-way ANOVA followed by LSD post hoc tests. Results were analyzed at a 5% significance level (95% confidence interval; at $p < 0.05$).

3. Results and discussion

Table 1 shows the results of pectin yield from okra samples of varying particles sizes using phosphate buffer (PB) and citric acid solution (CAS) as extraction solvents. In this experiment, the same Agbagoma genotype was used to investigate the particle size and solvent factors on the pectin yield and chemical characteristics. The results showed that the pectin yield was higher when the okra particle size was smaller. This indicates that a larger surface area promotes higher yield. For the

Table 1
Pectin yield from okra (Agbagoma) sample of varying particle sizes and extraction solvents.

Extraction solvent	Sample size (mm)	Volume of solvent (mL)	Test	Volume of aqueous extract (mL)	Volume of ethanol used. (mL)	*Pectin yield (%)
Phosphate Buffer	2.0	1st Extraction = 300	1	200	400	11.9±1.5[a]
			2	210	420	
			3	220	440	
		2nd Extraction = 150	1	110	220	
			2	120	240	
			3	110	220	
	1.0	1st Extraction = 300	1	210	420	
			2	200	400	15.8±1.0[a]
			3	190	380	
		2nd Extraction = 150	1	100	200	
			2	105	210	
			3	115	230	
	<0.5	1st Extraction = 300	1	170	340	19.6±4.0[a]
			2	180	360	
			3	190	380	
		2nd Extraction = 150	1	90	180	
			2	95	190	
			3	100	200	
Citric acid solution	2.0	1st Extraction = 300	1	200	400	35.6±5.5[b]
			2	190	380	
			3	195	390	
		2nd Extraction = 150	1	100	200	
			2	105	210	
			3	100	200	
	1.0	1st Extraction = 300	1	190	380	25.6±0.8[b]
			2	195	390	
			3	180	360	
		2nd Extraction =150	1	90	180	
			2	100	200	
			3	80	160	
	<0.5	1st Extraction = 300	1	170	340	32.7+8.1[b]
			2	160	320	
			3	175	350	
		2nd Extraction =150	1	80	160	
			2	85	170	
			3	70	140	

*Values are mean ± SD. [a-b]Values with different superscript letters are significantly different ($p < 0.05$).

Table 2
Total carbohydrate and protein contents of the extracted okra pectin (% dry basis).

Sample	Total Carbohydrate (%)	Protein (%)
Agbagoma	78.2±2.7	9.8±0.1
Nigeria	56.9±1.1	14.5±0.1
Balabi	87.5±3.5	2.0±0.0
*PB 0.5	88.8±1.6	7.6±0.2
*PB 1.0	95.4±0.5	2.0±0.0
*PB 2.0	58.7±0.7	14.3±0.3
*CAS 0.5	59.2±0.0	24.5±0.1
*CAS 1.0	55.3±0.0	9.5±0.3
*CAS 2.0	56.3±1.0	19.7±0.1

*Note: PB and CAS pectin samples of varying particle size were obtained from the Agbagoma genotype.

three different particle sizes (2 mm, 1 mm and 0.5 mm) considered during the experiment, the 0.5 mm okra particle size gave the highest yield of pectin using the PB.

According to previous work (Kpodo *et al.,* 2017), pectin was isolated from six okra genotypes and it was found that the highest pectin yield (14.6%) was recorded in Asha and Agbagoma (14.2%). However, the pectin yield reported was not based on screened particle sizes of the okra pod powder used. Pectin was extracted just after grinding which resulted in a mixture of all sizes as in the case of Agbagoma, Balabi and Nigeria genotypes in the present work. This affected the overall pectin yield just as those of the previous works (Kpodo *et al.,* 2017) which also varied depending on the okra genotype.

The results also showed that citric acid solution (CAS) as extraction solvent gave a higher pectin yield than the phosphate buffer (Table 1). There was a significant difference ($p < 0.05$) between the two extraction solvents at all particle sizes studied. However, the results of citric acid extraction were not so consistent (with a higher standard deviation) compared to phosphate buffer. The data from the present work was subjected to a two-way ANOVA which revealed that the intercept and the extraction solvent were significant ($p < 0.05$) whereas the particle size alone and its interaction with the extraction solvent were not significant ($p > 0.05$).

The purity of the pectin extracts also varied among the samples (Table 2). The ash contents of the samples ranged between 0.1-1.3 % . The total carbohydrate content (58-95%) of the PB samples were higher than those of the CAS counterparts (56-59%) whereas the protein content was higher in the respective CAS samples than that of PB (Table 2). Thus the PB extracted pectin samples had higher purity as compared to the CAS counterparts.

The ^{1}H-NMR spectra (Fig. 2) data show similar structural patterns between the okra pectin samples, which indicates similarities in the compositional characteristics among the okra pectin samples. The same Agbagoma genotype was used for the different particle size and solvent factor experiments and they showed no difference in the ^{1}H-NMR spectra obtained. From the ^{1}H-NMR spectra data, the highest peak from the various okra pectin samples was around 3.2 to 2.0 ppm which shows greater association with the groups of methyl linking to carboxyl groups of D-Galacturonic acid (Wang *et al.,* 2016). Agbagoma had the highest RG-I as compared to the other okra pectin sample analysed. The results however obtained indicate assurance of the existence of the structure of pectin analysed which were in line with previous findings (Kpodo *et al.,* 2017, 2021).

Fig. 3 shows typical FT-IR spectra of the okra pectin samples analysed which also correspond to those previously reported (Kpodo *et al.,* 2017, 2021). The shifting of the bands for the various okra samples is attributed to the degree and strength of the hydrogen bonding or hydroxyl association (Coates, 2000).

The superimposable FT-IR spectra obtained for okra pectin samples of different particle sizes (0.5 mm, 1.0 mm and 2.0 mm) extracted with

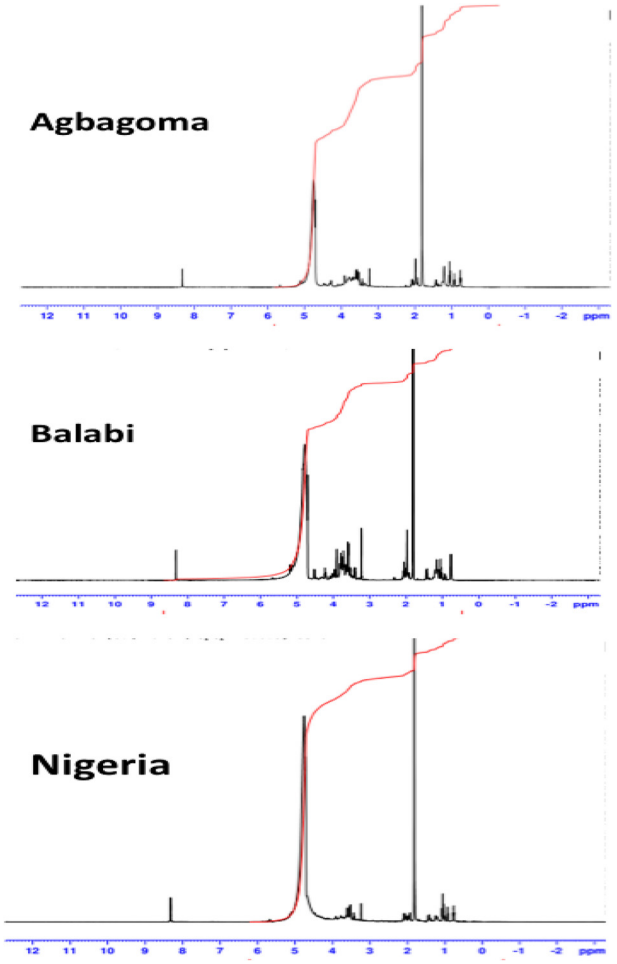

Fig. 2. ^{1}H- NMR spectra of okra genotypes.

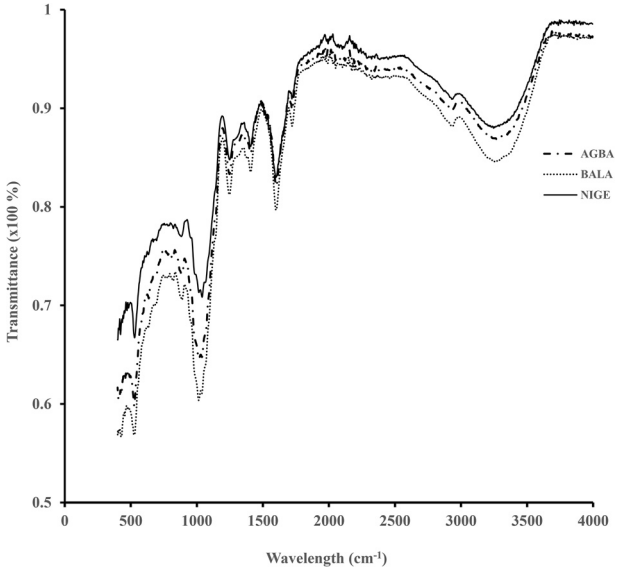

Fig. 3. FT-IR spectra of okra genotypes.

phosphate buffer (PB) and citric acid solution (CAS) showed similarity in structure/functional groups with some degree variation especially those of CAS 0.5 and CAS 1.0 which had lower transmittance in the region of 500-1750 cm^{-1} and 3000-3500 cm^{-1} wavelength (Fig. 4). The lower transmittance values could be attributable to the lower purity of the CAS pectin extracts in comparison to the PB counterparts. This

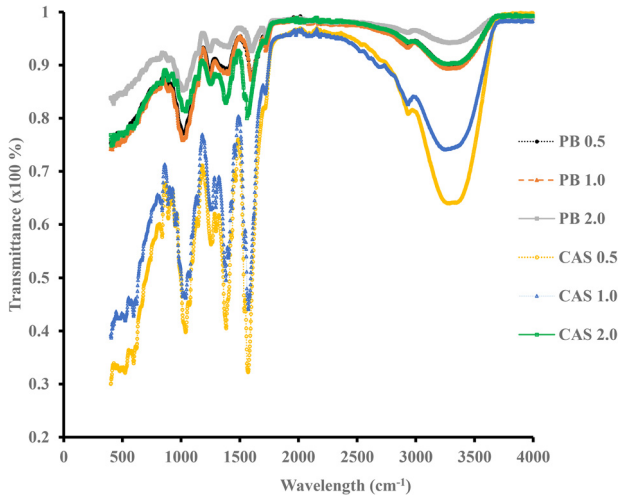

Fig. 4. FT-IR spectra of okra (Agbagoma) pectin samples of different particle sizes (0.5 mm, 1.0 mm and 2.0 mm) extracted with phosphate buffer (PB) and citric acid solution (CAS).

is possibly due to CAS having greater interactions (such as hydrogen bonding or hydroxyl associations) with the okra powder components (proteins/carbohydrates) thereby resulting in higher contaminants or impurity of the extracts.

The carbohydrate fingerprint is usually exhibited in the region from 900 to 1200 cm^{-1}. Peaks/bands associated with the degree of esterification occur in the region of 1610-1630 cm^{-1} which correspond to the symmetrical stretching vibration of the carboxylic group (COO^{-}) whereas those in the region of 1720-1730 cm^{-1} correspond to the methyl esterified group ($COOCH_3$). Peaks in the region of 3000-3500 cm^{-1} correspond to the O-H stretching absorption and have been attributed to the inter- and intra- molecular hydrogen bonding of the galacturonic acid (GalA) backbone of okra pectins (Kpodo et al., 2017, 2021).

Conclusion

In the present work, the optimal particle size for higher yield of pectin from okra was 0.5 mm using phosphate buffer as extraction solvent. Results however showed that extraction with citric acid solution gave higher yield but with lower purity as compared with the phosphate buffer counterparts. Extraction with citric acid solution was our first attempt and thus further studies to exploit its impact on the structure and functionality of okra pectin would be useful adventure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Benjamin Afotey: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jacob K. Agbenorhevi:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Leonard D.K. De-Souza:** Conceptualization, Funding acquisition, Resources, Project administration, Supervision, Writing – review & editing. **John K. Logosu:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Fidelis M. Kpodo:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. **Kolawole O. Falade:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

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