

# Physicochemical and structural characterization of yam starch modified by potassium dihydrogen phosphate treatment in aqueous glycerol

A. M. Ogunmolasuyi, E. C. Egwim, M. A. Adewoyin, E. J. Nkop

## ABSTRACT

**Aims:** The effect of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) and  $\text{KH}_2\text{PO}_4$  in the presence of aqueous glycerol on selected physicochemical properties of yam starch was analyzed in this work. **Methods:** The physicochemical properties such as swelling capacity, paste clarity, blue value and amylose content of native yam starch were evaluated whereas optical microscopy and FTIR spectroscopy were used to determine the structure. **Results:** The result indicated that, swelling capacity, paste clarity, blue value and amylose content of native yam starch were 3.84 g, 44%, 0.528 and 25.96 respectively, whereas starch-phosphate and glycerol-phosphate starch showed 0.94g and 1.22 g; 32% and 54%; 0.320 and 0.352 and 11.08 and 13.46 accordingly. From the result, there was a decrease in swelling capacity, blue value and amylose content of the modified starch when compared to the native starch. **Withal,** the paste clarity of glycerol-phosphate,

modified starch was higher than the native starch. Also, swelling capacity, paste clarity, blue value and amylose content were higher in glycerol-phosphate starch compared to starch phosphate. The various physicochemical characteristics indicated in the modified starch could be linked up with the essence of the modifying agents. FTIR spectroscopy and optical microscopy results further supported the structural alterations of functional groups in the modified yam starch at  $-\text{OH}$  stretch ( $\text{cm}^{-1}$ ),  $\text{CH}_2\text{O}$  and  $\text{C-O-H}$  and morphology of the granules after treatment with potassium dihydrogen phosphate and glycerol-phosphate, respectively. **Conclusion:** Therefore, yam, besides being a staple food has a portion of potential industrial applications and commercialization if processed into value added products.

**Keywords:** Fourier transform infrared spectrometry (FTIR), Modification, Physicochemical and structural, Yam starch

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## INTRODUCTION

Yam (*Dioscorea* spp) is a monocotyledons' tuber bearing plant, belonging to the family Dioscoreaceae within the genus *Dioscorea* [1]. It is a major staple food for an estimated 60 million people in the region stretching from Ivory Coast to Cameroon, and having Nigeria as the largest producer. This region is usually referred to as “yam zone” of West Africa [2, 3]. Yams are high in starch and also take the enzyme  $\alpha$ -amylase which converts starch to sugars as the tuber matures in storage [4].

Starch is the major storage form of glucose in plants, and has gained wide application in food, textile, pharmaceutical and other manufactures. The polymeric constituents of starch are the amylose and amylopectin. Amylose is the linear chain, typically representing up to 3000 glucose units interconnected primarily by  $\alpha$ -1, 4 glycosidic bonds and is reported to contain a few branched networks [5]. Amylopectin is a bulky and large branched polymer having  $\alpha$ -1, 4 glycosidic bonds as the backbone and  $\alpha$ -1, 6 bridges as branching points. These networked linkages are ordered systematically to form semi-crystalline granules that possess a degree of crystallinity of 15–45% [6].

As a result of the semi-crystalline nature and structure of native starch granules, three categories of polymorphisms; A, B and C was obtained by X-ray diffraction patterns. Thus, depending on the starch source, they are grouped into A-, B- and C- polymorphs [7]. In a universal term, cereal starches belong to A-polymorphs, tuber and amylase-rich starch belongs to B-polymorph and legumes and some tuber starches are categorized as C-polymorphs [6].

During product development, quality and process control, the crystallinity of starch granules is usually an important component for consideration because of the consequence it has on the physical, mechanical and technological characteristics of much starches-based merchandise. In preparation of starchy foods, loss of native crystallinity through starch gelatinization influences gelation, apparent viscosity and matrix forming characteristics, whereas structural rearrangement in starch during processing or storage can influence functional qualities. Likewise, in pharmaceutical industries, starch can be utilized as an exponent. As such, a certain degree of crystallinity should be maintained in order to achieve desired and specific drug release by the starch [8].

Modification of starches leading to structural alterations includes; succinylation [9], phosphorylation [10], acetylation [11] and maleination [12]. These methods of chemical modification can promote functional value and broaden range of physicochemical properties of starch.

Phosphorylation is the oldest method of starch modification and is recognized to enhance sound quality in starch [13]. Interestingly, the only naturally occurring covalent modification of starch is phosphorylation, which

is starting point of the glycolytic pathway. The products of starch and phosphate reactions can either be mono or di-phosphate starch. These derivatives are a function of reactants concentration and reaction conditions [13]. Phosphate starches possess characteristic resistance to an increased temperature and acidic pH, which could result into higher stability of the swollen starch granules. Phosphorylation of starch could yield starch paste with ability to absorb large amount of water beyond gelatinization. This features link starch phosphate content to starch paste peak viscosity, prevention of crystallization and gel-formation [13]. These new properties of starch, obtained through phosphorylation makes them a very useful pharmaceutical raw materials–tableting.

Structural and morphological changes in starch due to chemical modifications can be detected by FTIR [14] and it is an important industrial necessity in order to regulate quality of modified starches and degree of modification [9]. In this sense, the purpose of this work was to partially characterize the physicochemical properties and structure of yam starch modified by potassium dihydrogen phosphate in aqueous glycerol using optical microscopy and Fourier transform infrared spectrometry (FTIR).

## MATERIALS AND METHODS

### Materials

The starch was extracted from the yam purchased from a local market of Omoku, Rivers State Nigeria. All reagents used were of analytical grade.

### Methods

#### Preparation of starch phosphate and glycerol-phosphate

The method of Prasanthi et al. [15] was adopted in the preparation of potassium dihydrogen phosphate starch. The same procedure was repeated for glycerol-phosphate modified starch.

#### Determination of Swelling Power

The swelling capacity was evaluated using the method of [16].

#### Paste clarity

The paste clarity was determined according to the method of [17]. The paste clarity of starches was determined by measuring percentage transmittance at 600 nm wavelength against water blank on a colorimeter (JENWAY 6051 model).

## Determination of blue value of native and modified yam starch

The blue value of yam starch was determined according to the method of [18]. The color of starch–iodine complex developed was read in a colorimeter (JENWAY 6051 model) at 600 nm.

The blue value was calculated according to the following formula

$$\text{Blue value} = \frac{\text{Absorbance} \times 4}{\text{Concentration (mg/dl)}}$$

## Determination of amylose content of native and modified yam starch

The amylose content of native and modified yam starch was found utilizing the standard curve designed by [19].

$$Y = 0.0168x + 0.2138 \quad (i)$$

Where, Y = absorbance at 600 nm and X = %amylose

## Optical Microscopy

The native or modified starch sample solution was placed on a glass slide and stain with 0.2% iodine solution. Observation and imaging were taken at x100 and x200 magnification lens of light microscope connected to a computer.

## FTIR Spectroscopy

The FTIR spectrum of native or modified yam starch was acquired on a PerkinElmer FTIR spectrophotometer (PerkinElmer, Inc., MA, USA) using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr. Spectrum was recorded (16 scans) in the transparent mode from 4000 to 400  $\text{cm}^{-1}$ , at 4  $\text{cm}^{-1}$  resolution.

## RESULTS

### Swelling capacity

The results of swelling capacity and paste clarity of native and modified starch are presented in Table 1.

### Paste clarity

The result of paste clarity of starch showed that, native yam starch, phosphate and glycerol-phosphate starch pastes were 44%, 32% and 54% at T600 nm respectively.

## Blue value and amylose content of native and modified starch

The results of blue value and amylose content of native and modified starch are presented in Table 2.

## Optical microscopy

The result of optical microscopy of native, phosphate and glycerol-phosphate starch granules is presented in Figures 1–3. Light microscopy revealed that all the modified starches retained their granular appearance when compared to the native yam starch (Figure 1–3).

Table 1: Swelling capacity and paste clarity of modified yam starch

Sample	Swelling of Starch (g)	Paste clarity (%)
Native Starch	3.84	44
Starch-PO4	0.94	32
Starch-Gly-PO4	1.22	54

Table 2: Blue value and amylose content of native and modified starch

Sample	Absorbance ( $\lambda = 600 \text{ nm}$ )	Amylose content	Blue value
Native Starch	0.66	25.96	0.528
Starch-PO4	0.40	11.08	0.320
Starch-Gly-PO4	0.44	13.46	0.352

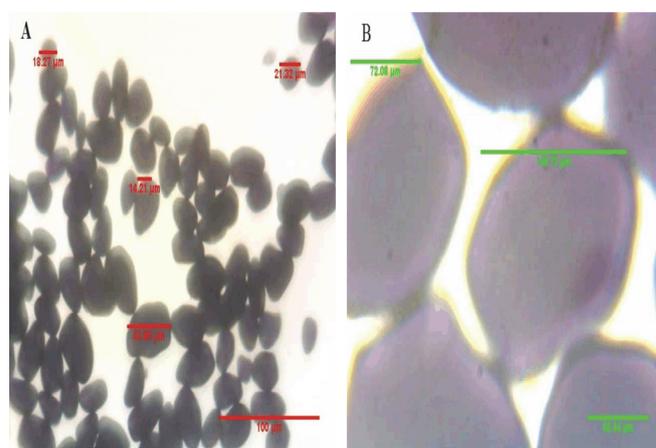


Figure 1: Calibration curve for various amylose ratios of standard samples.

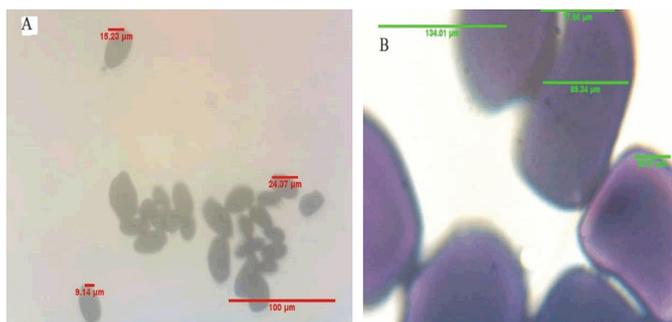


Figure 2: Possible reactions between starch, potassium dihydrogen phosphate and glycerol.

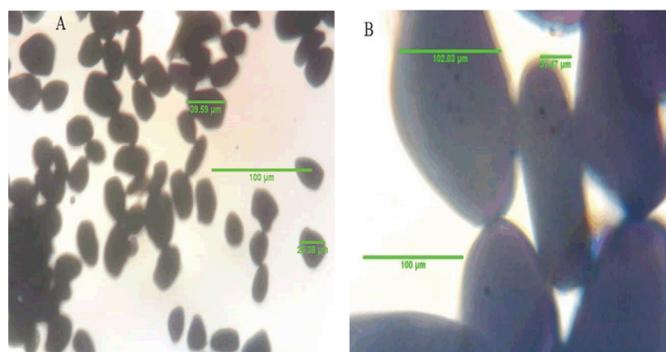


Figure 3: (A, B) Photomicrograph of native yam starch. (Magnification of (A) is x200, and (B) is x1000).

Table 3: Absorption bands of native and modified yam starch

Absorption band	Starch		
	Native	PO <sub>4</sub>	Glycerol-PO <sub>4</sub>
O–H Stretch (cm-1)	3176.45	---	3367.99
C–H Stretch (cm-1)	2923.34, 2853.76	2951.6-2853.84	2951.6-2853.4
H <sub>2</sub> O Absorbed (cm-1)	1643.49	1643.58	1643.76
(Amorphous region)			
–CH bend (cm-1)	1461.52, 1376.82	1462.08, 1376.89	1455.92, 1376
CH <sub>2</sub> OH (cm-1)	1252.8	---	1243
C–O, C–C Stretch (cm-1)	1155.43,	1155.7	1150.9
C–O–H Bending (cm-1)	1078.36	1078	---
C–O–C (cm-1)	982.18, 930.99	961.38, 922.74	992.22, 927.6
C (1)–H, CH <sub>2</sub>	860.26	860.18	854.79 deformation (cm-1)
Glucose pyranose ring (cm-1)	757.71, 720.00	720.92, 665.5	665.5

## Spectroscopy

The FTIR spectra of native starch, starch phosphate and glycerol-phosphate starch polymers are presented in Figures 4–6 and summarized in Table 3. The result indicated the various points of interaction of modifying agent with the starch.

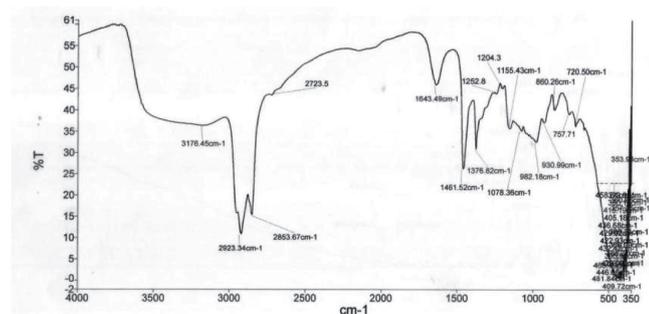


Figure 4: (A, B) Photomicrograph of phosphate modified starch. (Magnification of (A) is x200, and (B) is x1000).

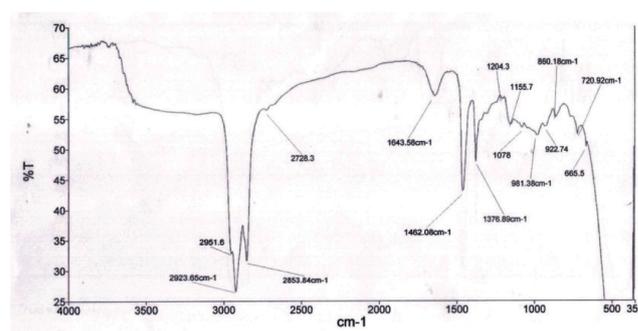


Figure 5: (A, B) Photomicrograph of glycerol-phosphate modified starch. (Magnification of (A) is x200, and (B) is x1000).

## DISCUSSION

### Swelling capacity

Swelling power is a measure of hydration ability of starch granule and it is determined by weighing the

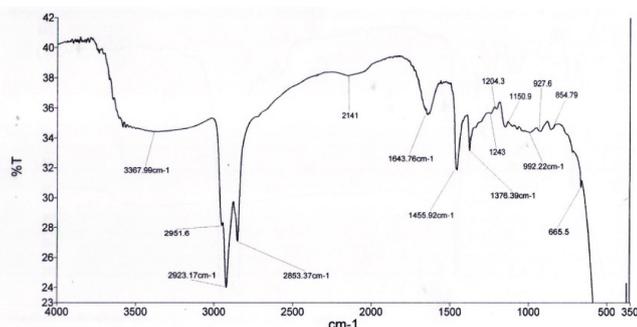


Figure 6: The FTIR spectrum of native yam (*Dioscorea rotundata*).

swelled starch and retained water. Such property is very important for certain starch applications like those from food industry where the quality of starch-based products is strongly related to the capacity of starch granule to retain water and swell [20].

From the result, native starch exhibited the highest swelling capacity followed by starch glycerol-phosphate, and starch phosphate has the lowest swelling capacity. Starch phosphate is a class of cross-linked starch derivatives, which contains mono-, di- and triester starch phosphate [21]. This is obtained when phosphate containing compound such as mono- or disodium orthophosphates or sodium hexametaphosphate, etc, reacts with the hydroxyl groups of two different molecules within the starch granule. The reaction between starch and potassium dihydrogen phosphate compound (Figure 7) forms a synthetic bridge that strengthens the natural hydrogen bonds and thereby causing a delay in the rate at which starch granules swell and rupture of the swollen granule [22]. Nutan et al. [13], reported that, phosphate cross-linked starches showed significant resistance to high temperature, which strengthened and stabilized the swollen starch granule. The presence of a phosphate group in starch, therefore increased the hydration capacity of starch pastes after gelatinization, prevents crystallization and gel-forming capacity.

Nevertheless, the introduction of 20% aqueous glycerol improved on the swelling capacity of the starch phosphate. This could be associated with the fact that, intermolecular and intramolecular hydrogen bonds in starch, which is very strong coupled with the synthetic bridge formed by phosphate reaction, are weakened by addition effect of water and glycerol plasticizers [23]. Additionally, although the phosphate functional group formed a cross-link between the hydrogen bonds by reacting with the hydroxyl group (-OH) of starch, thereby decreasing the swelling capacity of the phosphate modified starch, the presence of glycerol increased it by introducing -OH group. As a result, the reaction of glycerol with starch phosphate relaxed the synthetic bridge that reinforced the hydrogen bond in phosphate starch. Thus, glycerol could be an important reagent for moderating swelling if the cross-linker is used for modification of starch.

## Paste clarity

Paste clarity of starch is one of the prominent attributes usually considered in the food industry because of its influence on products' brightness and opacity. The physical orderliness of starch molecule is a key factor for determining the clarity of starch paste [18]. Light transmittance of starch paste offers information about its behavior when light passes through it, thus the higher the transmittance value of the starch paste the more transparent it is.

Cross-linking of starch has been reported to reduce paste clarity [24]. From the result, modification of starch with potassium dihydrogen phosphate reduced the clarity of yam starch paste by 12% when compared to the native yam starch. However, upon addition of 20% aqueous glycerol to starch phosphate, there was a meaningful improvement of 10% in the paste clarity when compared to native starch. Such behavior appeared due to the modifications induced by potassium dihydrogen phosphate and glycerol treatment (Figure 7) in starch structure, as reported by [18]. Nemțanu et al. [25, 26], showed that corn starch had a low clarity ( $T_{620\text{ nm}} < 10\%$ ) which increased spectacularly ( $T_{620\text{ nm}} \approx 85\%$ ) after 24 hour of storage. Regarding the present work using glycerol to improve starch clarity could save time and toil. Glycerol has been proven to be a good plasticizer [24]. Starch phosphate is an important starch derivative used as excipient in the pharmaceutical and as stabilizer in food industry [27]. Thus, the paste clarity of starch phosphate and perhaps other starches can be improved using glycerol plasticizer.

## Blue value and amylose content of native and modified starch

Blue value is used as an indicator of amylose content, which is considered as the absorbance of color produced as a result of iodine interaction with a dry mass of starch diluted with the appropriate amount of distilled water [28].

Amylose-amylopectin ratio is one of the criteria for good textural properties of starch extracted from root and tuber crops [29]. The result of this study showed that,

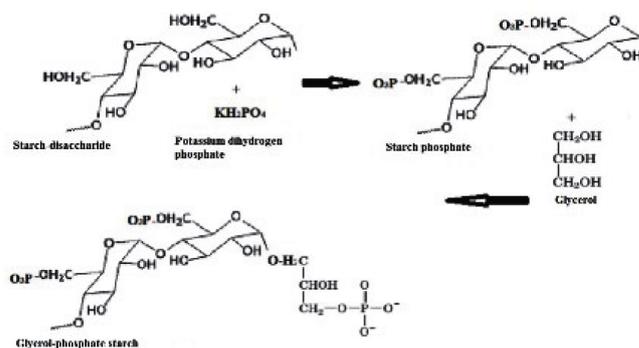


Figure 7: The FTIR Spectrum of phosphate modified yam starch.

potassium dihydrogen phosphate reduced the amylose content (Figure 8) when compared to native yam starch. However, addition of glycerol increased the amylose content (Table 2).

On a general note, a low amylose content of starch is proportionate to the higher swelling capacity of a starch incorporated into food products [30]. However, modification of starch with potassium dihydrogen phosphate reduced amylose content. This is because; the increased substitution of functional group ( $\text{PO}_4$ ) causes the starch to be highly cross-linked to form a synthetic bridge in the granular structure [24]. Thus, the increase in amylose content of the glycerol-phosphate, modified starch only showed the effect of glycerol on the intermolecular and intramolecular bonds in starch structure.

### Optical Microscopy

Starches exhibit spherical, elliptical to oval shaped granules [31] with smooth surface and no obvious fissures or cavities. However, the granule of the modified starches (Figures 2 and 3) showed smaller size at x200 and x1000 magnifications when compared to native starch (Figure 3). Starch granule shape and size are factors that contribute to the rate at which starch gelatinizes at its gelatinization temperature, swelling power and viscosity (flow rate) [32, 33].

### Spectroscopy

The FTIR is a powerful technique for identifying types of chemical bonds and functional groups of polymers. The broad band appeared at 3176.45 and 3367.99  $\text{cm}^{-1}$  of the native and glycerol-phosphate starch respectively and was attributed to  $-\text{OH}$  stretching. Vibrational bands from native, phosphate and glycerol-phosphate starch component were found in the wavenumber range of 2853–2951  $\text{cm}^{-1}$  and were assigned from C–H asymmetric

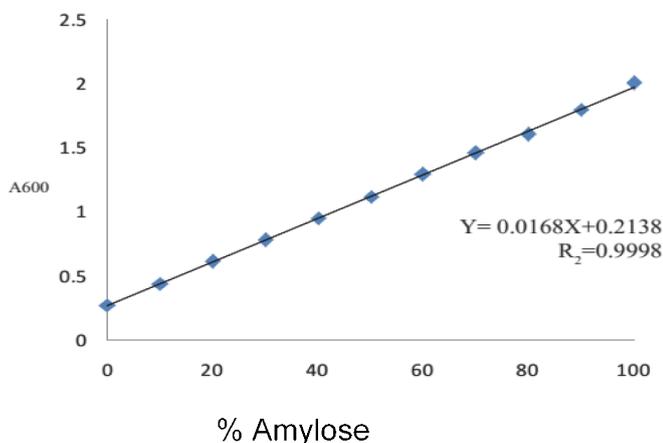


Figure 8: The FTIR Spectrum of glycerol-phosphate modified starch.

stretching of  $-\text{CH}_2-$  from glucose. The peak at 1643  $\text{cm}^{-1}$  was attributed to scissors vibrations of  $-\text{OH}$  coming from water of hydration in starch. Peaks in the region of 1462, 1455 and 1376  $\text{cm}^{-1}$  was characteristic for  $-\text{CH}_2-$  folding. The peaks at 1252.8 and 1243 represent  $\text{CH}_2\text{OH}$  side chain related mode. The peaks at 1155  $\text{cm}^{-1}$  and 1078  $\text{cm}^{-1}$  are related to the stretching vibration of C–O, C–C stretching and C–O–H bending respectively. Absorption bands at 982, 961 and 992 are assigned to skeletal mode vibrations of  $\alpha$ -1, 4 glycosidic linkage characteristic of polysaccharides. Moreover, the wavenumber in the range of 860.24, 860.18 and 854.79  $\text{cm}^{-1}$  were designed for C–H bending and the band at 757.71, 720 and 665.5  $\text{cm}^{-1}$  are assigned to glucose pyranose ring.

These peak positions were due to the functional groups of native, phosphate and glycerol-phosphate starch [34]. The result in Figures 4–6 showed that all the modified starch showed the same peak positions as the native, except the band representing  $-\text{OH}$  stretching and  $\text{CH}_2\text{OH}$  side chain which was not represented in potassium dihydrogen phosphate modified starch. This is an indication that there were structural changes in phosphate starch as confirmed by the previous analysis. In addition, the broad band observed in glycerol-phosphate modified starch showed that the presence of glycerol introduced  $-\text{OH}$  functional group into the phosphate modified starch in comparison to ordinary phosphate modified starch.

### CONCLUSION

This study evaluated the structural and physicochemical characteristics of yam starch modified with potassium dihydrogen phosphate and glycerol. The swelling capacity, paste clarity, blue value and amylose content as well as FTIR spectroscopy and optical microscopy of the modified starch showed the effect of both potassium dihydrogen phosphate and glycerol on the structural and physicochemical properties of the native and modified starch. The potassium dihydrogen phosphate reduced the swelling capacity, paste clarity, blue value and amylose content of the modified starch, whereas the introduction of glycerol in the presence of potassium dihydrogen phosphate increased the various physicochemical properties. In addition, the FTIR and optical microscopy analysis confirmed the structural and physicochemical properties of the modified starch. Yam could be cheap source of starch for industrial application if optimized for value added products, however, glycerol could be used to control of physicochemical properties of native and modified starches.

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### Author Contributions

A. M. Ogunmolayuyi – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

E. C. Egwim – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

M. A. Adewoyin – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

E. J. Nkop – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

### Guarantor

The corresponding author is the guarantor of submission.

### Conflict of Interest

Authors declare no conflict of interest.

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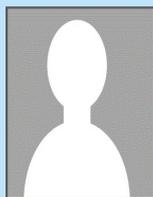
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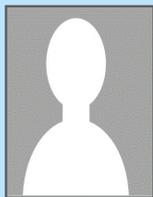
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