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PREPARATION, FUNCTIONAL AND PASTING BEHAVIOR OF STARCH PHOSPHATES OF RED COCOYAM (*COLOCOSIA ESCULENTA*) AND WHITE COCOYAM (*COLOCOSIA ANTIQUORUM*): A COMPARATIVE STUDY

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ABSTRACT

A contemporary strategy in the starch industries is the diversification of raw materials. However, the hydrophilic nature of starch limits its use as a raw material for food and packaging products, resulting in a low level of biodegradation. To address this issue, it is imperative that starch be chemically modified. Starch phosphates are among the most common and commonly used chemically modified starches. In this study, native red cocoyam starch (NRCS) and native white cocoyam starch (NWCS) were chemically modified with di-sodium hydrogen orthophosphate as phosphorylating agent to produce red cocoyam starch phosphate (PRCS) and white cocoyam starch phosphate (PWCS). The functional and pasting properties of the native and starch phosphates of both cocoyam species were evaluated and compared. The starch yield increased from ~ 17% for NRCS to ~ 25% for PRCS and from ~ 12% for NWCS to ~ 20% for PWCS. The water and oil absorption capacities were 97.00 & 143.00% for NRCS and 78.00 & 164.00% for NWCS, whereas for PRCS it was 160.00 & 153.00% and for PWCS it was 127.00 & 173.00% respectively. NRCS and PRCS had a foam capacity of 8.80 and 9.00% while NWCS and PWCS had 8.00 and 10.00% respectively. Phosphorylation had a significant impact on the pasting properties leading to a reduction in the peak, breakdown, set back, and final viscosities of the native cocoyam starches. In conclusion, phosphorylation considerably improved the functional and pasting qualities of red and white cocoyam starch, revealing its tremendous application potential in the formulation of food components, as a stabilizer, tablet binders in pharmaceutical industries, as well as in the packaging industries.

Keywords: Dispersibility, Functional properties, Phosphorylation, Starch, Starch phosphates.

INTRODUCTION

Starch is the most abundant carbohydrate reserve in plants which can be found in seeds, flowers, fruits, leaves, different types of roots and stem (Alcázar-Alay & Meireles, 2015). Starch is a white, odourless, semi-crystalline, neutral powder that comes in the form of granules that are insoluble in organic solvents or water (Visakh & Thomas, 2010)**.** Starch contains small amounts of non-carbohydrate components like ash, proteins, lipid, and phosphate monoesters, as well as two major glucose polymers, amylopectin and amylose. The physical properties are greatly influenced by the contents and structures of amylopectin and amylose, as well as other minor components present (Apriyanto *et al*., 2022; Bashir & Aggarwal, 2019; Santana & Meireles, 2014). Starches have numerous applications due to their renewability, biodegradability, vast availability, and low cost (Ramadan & Sitohy, 2020).

However, due to their poor functional properties (poor thermal, shear, and acid stability, as well as high rates and extent of retrogradation), starches are not widely used in the food industry in their natural state and must be chemically or physically modified to enhance their positive attributes and minimize their defects. Increased stability against excessive heat, acid, shear, time, cooling or freezing, changing the native starch texture, decreasing viscosity, lengthening or shortening gelatinization time, improving adhesiveness, and increasing the transparency and texture of gels and paste are all reasons why starches are modified (Alcázar-Alay & Meireles, 2015)**.** Starch functional properties are greatly varied, thanks to botanical sources, physical and chemical modifications, all of which contribute to its utility in food and non-food applications. Through the processes of oxidation, acetylation, crosslinking, and phosphorylation, chemically modified starches have recently been created. Almost all native starches in plants are phosphorylated to some extent, with potato starch being more than cereal starch. The degree of phosphorylation in starch determines its physicochemical properties, which is critical for industrial applications like paper, adhesives, textiles, and meals. By increasing the hydration capacity of starch pastes, phosphorylation affects peak viscosity, gel-performing capability, swelling power, and paste stability (Carpenter *et al*., 2015)**.** Starch phosphates are phosphoric acid ester derivatives (Muhammad *et al.,* 2000; Solarek, 1986). The addition of negatively charged phosphate groups lowers interchain interactions and aids in starch hydration. It is produced when free hydroxyl groups on anhydroglucose units of starch molecules are phosphorylated (Liu *et al.,* 2022; Nabeshima *et al.,* 2010). Monostarch (where one starch hydroxyl group is esterified to phosphate) and distarch phosphates (where two starch hydroxyl group is esterified to phosphate) are the two types of starch phosphates. Monostarch phosphate is made by esterifying starch with sodium tripolyphosphate (STPP) or mixtures of sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dihydrate, whereas distarch phosphate is made with phosphorus oxychloride and sodium trimetaphosphate (STMP) (Lim & Seib, 1993; Nabeshima *et al.,* 2010; Ramadan & Sitohy, 2020). Starch phosphates produced chemically have been shown to produce clear pastes of excellent consistency, increased viscosity and water binding capacity, improve solubility, swelling power, retrogradation, freeze-thaw stability, and emulsifying capabilities (Liu *et al.,* 2022). In the food industry, starch phosphates are widely used in canned foods, frozen foods, food packaging biofilms, dairy and meat products and syrup (Leonel *et al.,* 2021). In the textile industry, it is used as additives to reduce environmental pollution and costs, as thickener and in printing and dyeing of fabrics (Leonel *et al.,* 2021). In paper industry as anti-wetting agents, as adhesives for coated cardboards and in gloss and finishes (Leonel *et al.,* 2021). The broad range of applications for starch phosphates suggests that research in phosphate starches will be continuous. Sweet potato (*Ipoemea batatas*), cassava (*Manihot esculenta*), yam (*Dioscorea sp*), red cocoyam (*Colocasia esculenta*), and white cocoyam (*Colocasia antiquorum*) are important household food security and income crops in many developing countries (Apata & Babalola, 2012)**.** The physicochemical, structural and pasting features of modified cocoyam starch have been widely researched. However, there are no studies in the literature that compared the properties of red and white cocoyam starch phosphates. The goal of this

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study is to compare the proximate composition, physicochemical properties, and pasting behavior of native and starch phosphates from red and white cocoyam respectively.

MATERIALS AND METHODS

Starch Extraction

Tubers of white and red cocoyam (*Colocasia antiquorum* and *Colocasia esculenta*) were purchased at a local market in Ago-Iwoye, Ogun State, Nigeria. Peeled red and white cocoyam tubers were cut into small pieces, washed thoroughly, and then blended with enough water. After that, the blended cocoyam was placed in a bucket with enough water. A muslin cloth was then used to filter it (shaft is obtained). The shaft is then filled with water, which is filtered once more. However, it is filtered again to remove any remaining particles. The filtrate was left for about 8 h to settle. Starch rapidly settled at the bottom; therefore, the starch free supernatant was decanted carefully. It was washed 3 to 4 times and the supernatant was decanted. The large mass of the starch was collected and allowed to dry for 48 h at 30 °C. It was then grinded so as to obtain its powdered form (Awokoya *et al.*, 2011).

Preparation of Starch Phosphates

Sung et al. (2005) method was used to prepare starch phosphates. In 100 ml of water, 100 g of starch and 30 g of di-sodium hydrogen orthophosphate anhydrous were suspended and stirred continuously for 20 min. To enhance phosphorylation, the starch slurry was conditioned for 12 h at room temperature (28 ^{o}C), then heated for 3 h in a forced air oven at 130 ^{o}C. The final product was ground and sized.

Determination of Starch Yield (%)

The starch yield was determined using Eqn. (1).

$$Starch\ (\%) = \frac{weight\ of\ starch\ (g)}{Weight\ of\ edible\ portion} \times 100 \qquad (1)$$

2.3.2 Determination of Moisture Content

In an automatic moisture analyser, 5 g of native and modified starch samples were weighed and the temperature was set to 105 °C for 30 min or more. The moisture percentage of the starch was then displayed on the analyser's screen**.**

$$Moisture\ content = \frac{Average\ change\ in\ volume}{Initial\ volume} \times 100 \qquad (2)$$

Physicochemical Properties

Determination of pH

Starch (10 g) native and modified starch samples were weighed in triplicate into a beaker and mixed with 40 ml distilled water. The resulting suspension was stirred for 5 min before being allowed to settle for 10 min. A calibrated pH meter was used to determine the pH of the supernatant (Yussuf *et al.*, 2018)**.**

Determination of Bulk Density

About 2 g of the powdered sample was placed in a 10 ml measuring cylinder and the volume (V0) occupied by the sample without tapping was noted. After 100 taps on the table, the filled volume (V100) was read. The bulk loose and tapped densities were calculated as the ratio of weight to volume (V0 and V100, respectively) (Emeje *et al.*, 2012).

$$Bulk\ density\left(\frac{g}{ml}\right) = \frac{weight\ (g)}{volume\ (ml)} \qquad (3)$$

Determination of Dispersibility

In a 10 ml measuring cylinder, 1 g of native and modified starch samples were placed and distilled water was added to reach the 10 ml mark. It was vigorously stirred and allowed to settle for 3 h. The volume of settled particles was recorded and subtracted from 10. The difference was reported as % Dispersibility according to Eqn. (4) (Ofoefule & Emeje, 2012)**.**

$$\%\,Dispersibility = 10 - volume\ of\ settled\ particle \qquad (4)$$

Determination of Water and Oil Absorption Capacity

10 ml of distilled water or oil (Power Oil, Raffles Oil LFTZ Enterprise, Lagos, Nigeria) was added to 1 g of sample. The mixture was mixed thoroughly with a glass rod for 5 min and allowed to stand for 30 min. Then, the volume of the supernatant was recorded (Yussuf *et al.,* 2018)**.**

Determination of Foam Capacity

A magnetic stirrer was used to homogenize 2 g of starch samples in 100 ml of distilled water for 5 minutes. After 30 s, the homogenate was poured into a 250 ml measuring cylinder, and the volume occupied was recorded. The foam capacity is measured in terms of volume increase as a percentage (Isah *et al.,* 2015).

Pasting Properties

3.5 g of starch samples were weighed, 25 ml of distilled water was dispensed in a canister, a paddle was placed in the canister and joggled for a few seconds, and the canister was then inserted into the Rapid Viscosity Analyzer (New port Scientific RVA super 4). The measurement cycle was started by pressing the instrument's motor tower. On the monitor of the computer connected to the instrument, the profile can be seen as it runs. The 13 min profile was used. The temperature regime was as follows: idle temperature at 50 °C for 1 min, heated from 50 °C to 95 °C in 3 min 45 s, then held at 95 °C for 2 min 30 s, the sample was then cooled at 50 °C for 3 min 45 s, followed by a 2 min period where the temperature was controlled at 50 °C. Following that, the starch sample's pasting properties were plotted on a graph.

FTIR Determination

About 200 mg of potassium bromide (KBr) was added to 2 mg of starch sample and the mixture was crushed together. The mixture was transferred into a pellet forming-die, and then compressed using a pressing machine to form a pellet. After forming a pellet, it was transferred into a cell holder which was then inserted in a FTIR (Perkin-Elmer Spectrum 100 FT-IR spectrometer Walth man, MA, USA) machine, and the results of the graph showed on the monitor.

RESULTS AND DISCUSSION

Starch Yield (%) and Moisture Content (%) of Native and Starch Phosphates of Cocoyam

The percentage yield obtained for the native starch samples (native red cocoyam starch - NRCS - 17.48% & native white cocoyam starch - NWCS - 12.20%) is less than that of the phosphorylated starch samples (phosphorylated red cocoyam starch - PRCS - 24.66% & phosphorylated white cocoyam starch - PWCS - 20.38%), this may be due to the addition of the phosphate group during the phosphorylation process. These values are higher than 11.47% reported for cocoyam starch (Jacob & Asogbon, 2019). Any product loss could have been caused by inaccurate measurement or wastage during the extraction process (Oladebeye *et al.,* 2009)**.** The percentage moisture content of starch

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determines if the water content of starch would volatilize under the same physical conditions as water, and lower moisture content assures a long shelf life. NRCS, PRCS, NWCS, and PWCS samples have moisture content of 14.49%, 22.38%, 14.27%, and 14.79%, respectively. For good quality starch, the moisture value obtained for cocoyam starches is within the acceptable range of 12 to 15.5 percent**.** The moisture content of NRCS and PWCS is higher than the values reported for sweet potato starch (8.72%) and red cocoyam (9.02%) (Oladebeye *et al.,* 2009)**.** Lower moisture content suggests higher microbial resistance, inhibit microbial growth and it is an important factor in food preservation while higher levels of moisture can lead to microbial damage and subsequent deterioration in quality (Weinberg *et al.,* 2008).

Functional Properties of Native and Starch Phosphates of Cocoyam

The functional properties of native and phosphorylated starches are shown in **Table 1.** The pH value of NRCS, NWCS, PRCS and PWCS are within the range of 8.20 - 9.56, which is higher than 7.19 reported for cocoyam starch by Jacob & Ashogbon, (2019) but within the range 7.23 - 9.84 reported for cassava and red cocoyam starch (Sanyaolu *et al.,* 2021). pH is an essential property in starch applications and it is generally used to indicate the acidic or alkaline properties of liquid media (Yussuf *et al.,* 2018). The modified starch samples have slightly higher pH values than the native starch samples. However, the pH for both the native and phosphorylated starches is similar to that of sago starch, which is used in the cosmetics, pharmaceutical, and food industries (Bhat *et al.,* 2014) indicating that native and starch phosphates of cocoyam could find application in the above industries**.** NRCS, NWCS, PRCS and PWCS have a bulk density of 0.71, 0.70, 0.59, and 0.60 g/ml respectively. These values are lower than 0.88 g/ml reported for cocoyam starch by Jacob & Asogbon, (2019), within 0.71 - 0.75 g/ml reported for white and red cocoyam cormel by Oladebeye *et al*, (2010) and 0.71 g/ml reported for cocoyam by Oladeji *et al,* (2013)**.** Bulk density is a metric for the heaviness of solid samples that is useful in determining the type of packaging material needed, as well as material handling and application in food processing (Ibikunle *et al.,* 2019). The bulk density (Loose and tapped) of NRCS is higher than that of PRCS and the bulk density of NWCS is higher than those of PWCS. As a result, the modified starch could be used in the food industry (Ibikunle *et al.,* 2019). The particles of native starches are coarser than that of the modified starches as a result of the moisture absorption of the starch samples, chemical reactions with disodium hydrogen orthophosphate anhydrous and mechanical attritions (Oladeji, 2013)**.** The higher value of bulk density of the native starch suggests its suitability as drug binder and suitability in pharmaceuticals. The starch dispersibilities of the native and phosphorylated starch samples ranges from 86 to 90%. PRCS (88.80%) had a higher dispersibility than NRCS (86.20%) while NWCS (89.46%) had higher starch dispersibility than PWCS (88.00%). The higher the dispersibility, the better the flour reconstitutes in water; the lower the dispersibility, the worse the flour reconstitutes in water (Olu *et al.,* 2013)**.** These values fall within the range reported by Jacob & Asogbon (2019) for cocoyam starch (86.50%)**,** 83.00 and 87.00% reported for Bambara groundnut starch and cassava starch blends (Asogbon, 2014)**,** and 85.83% reported by Yussuf *et al.* (2018) for *Chrysophyllum albidum* kernel starch. Starches with high dispersibilities are used in the food industry as well as for adsorptive ion removal from contaminated water systems (Ihegwuagu *et al.,* 2009). Water absorption capacity is the ability of a substance to associate with water under a limited water condition, while oil absorption capacity is the ability of the dry starch to physically bind fat by capillary attraction and it is of great importance, as fat is used as a flavor retainer and also increases the mouth feel of foods (Yussuf *et al.,* 2018). As shown in **Table 1**, the water and oil absorption capabilities of red and white cocoyam improved after phosphorylation. Sanyaolu *et al.* (2021) also reported an increase in water and oil absorption capacities for cassava and red cocoyam starch after modification**.** NRCS and NWCS had a lower water and oil absorption capacity than PRCS and PWCS. This suggests that hydrophobicity can help with phosphorylation (Polyansky & Zagrovic, 2012). The addition of a phosphorus group to starch molecules improved water and oil absorption thereby, allowing for a higher absorption capacity. The results showed that red and white cocoyam starches had higher oil absorption capacities than water absorption capacities. Water percolation and absorption within starch matrices were aided by the introduction of bulky functional groups and their electrostatic repulsion (Ihegwuagu *et al.,* 2009). PRCS has a higher foam capacity than NRCS, as shown in **Table 1**. In addition, PWCS has a greater foam capacity than NWCS. PRCS and PWCS have a foam capacity of 9.00% and 10.00%, respectively, whereas NRCS and NWCS have a foam capacity of 8.80% and 8.00% respectively. Because foam capacity is a direct measure of fat content, the results indicate that NRCS and NWCS have a lower fat content than PRCS and PWCS. An increase in foam capacity as a result of phosphorylation could find application as an emulsifier in food industries (Ihegwuagu *et al.*, 2009).

Table 1: Physicochemical and proximate analysis results of both native and phosphorylated Cocoyam starches

PARAMETERS	NRCS	PRCS	NWCS	PWCS
Moisture content (%)	14.49±0.00	22.38±0.00	14.27±0.00	14.79±0.00
Starch yield (%)	17.48	24.66	12.20	20.38
pH	8.37±0.07	9.56±0.01	8.20±0.03	8.48±0.07
Loose density (g/ml)	0.52±0.00	0.43±0.01	0.48±0.01	0.43±0.37
Tapped density (g/ml)	0.71±0.02	0.59±0.017	0.70±0.01	0.60±0.45
Dispersibility (%)	86.2±0.24	88.8±0.86	89.46 ±0.68	88.0±0.47
Water absorption capacity (%)	97.0±0.32	160.0±0.43	78.0±0.02	127±0.09
Oil absorption capacity (%)	143.0±0.05	153.0 ± 0.45	164±0.04	173±0.45
Foam capacity (%)	8.80±0.24	9.00±0.24	8.00 ± 1.63	10.00 ± 1.63

NB: Values are means of triplicates expressed on dry weight basis ± standard deviation

Pasting properties

The pasting properties of NRCS, PRCS, NWCS and PWCS are presented in **Table 2**. NWCS had a peak viscosity of 396.92 RVU, while NRCS had a peak viscosity of 330.25 RVU, whereas PRCS and PWCS had lower peak viscosity values of 170.833 RVU and 154.58 RVU, respectively. Phosphate intermolecular linkage in starch molecules resulted in a significant reduction in peak viscosity, i.e. modification significantly reduced peak viscosity (Shimelis *et al.,* 2006; Sanyaolu *et al.,* 2021)**.** Peak viscosity, or the maximum viscosity reached during or shortly after cooking, corresponds to the point when the numbers of swollen but still intact starch granules is at maximum, indicating a measure of the starch's ability to bind water and it is also frequently correlated to the quality of the finished product**.** It denotes the tensile strength of pastes formed during food processing as a result of gelatinization (Shimelis *et al.,* 2006; Sanyaolu *et al.,* 2021). Other pasting properties, such as trough, breakdown viscosity, and final viscosity, decreased after phosphorylation. The breakdown viscosity indicates the stability of starch pastes and their ability to resist shear force during the heating process by measuring the fragility of the granules. A decrease in the peak and breakdown viscosity may be that phosphorylation made the swollen granules more resistant to breaking under high shear and temperature conditions (Leonel *et al.,* 2021). The phosphorylation reduced the final viscosity of the starch paste, which represents an increase in the viscosity of the starch paste during cooling. The amount of phosphorylating agent used determines the final viscosity reduction (Sanyaolu *et al.,* 2021). The re-ordering or polymerization of leached amylose and long linear amylopectin is largely responsible for the final viscosity. The NRCS setback values were significantly lower than the PRCS setback values, while the NWCS setback values were significantly higher than the PWCS setback values. The lower the rate of weeping and syneresis, the higher the setback value (Sechi & Marques, 2017)**.** Phosphorylation increased the NRCS setback value, and a high PRCS setback value indicates a low retrogradation value. Cooking time commonly referred to as peak time (Maziya-Dixon *et al.*, 2007) is the amount of time it takes for starch samples to reach their maximum viscosity. The peak time of the red cocoyam starch sample ranges from 4.5 to 5.2 minutes, while the peak time of the white cocoyam starch sample ranges from 4.60 to 5.13 minutes. Pasting temperature is the temperature at which the first detectable viscosity is measured by the amylograph. The pasting temperature increases after phosphorylation for both red and white cocoyam starches. A lower pasting temperature indicates granular fragility implying that it will be easier to cook and will require less heat for gelatinization to start (Afoakwa & Sefa-Dedeh, 2002; Mélo *et al.,* 2003).

Table 2: Pasting characteristics of native and modified starch samples

STARCH SAMPLES	PEAK VISCOSITY	TROUGH	BREAKDOWN	FINAL VISCOSITY	SETBACK	PEAK TIME	PASTING TEMP
NRCS	330.25	197.41	132.83	297.00	99.58	4.60	84.10
PRCS	170.83	140.58	30.23	243.25	102.67	5.20	89.50
NWCS	396.92	219.25	177.67	303.42	84.12	4.60	84.90
PWCS	154.58	123.58	31.00	205.58	82.00	5.13	88.80

Fourier Transform Infrared Spectroscopy (FTIR)

Figure 1 and **Figure 2** depict the FTIR spectra of NRCS, PRCS, NWCS and PWCS samples. The phosphorylated starches showed changes in the intensity of some bands, as well as the emergence of new bands. The FTIR spectra of NRCS and PRCS were found to have similar overall characteristic absorption peak, whereas NWCS and PWCS spectra were also similar with a sharp decline in the overall intensity. The IR Spectrum of the NRCS revealed characteristic broad peaks at 3100 - 3600 cm^{-1} (O–H Stretch), assigned to alcohol, 2932 cm^{-1} (C–H Stretch) indicating an asymmetric stretch, strong absorption peaks in the region 1654 cm^{-1} were attributed to C=O stretching mode, 1432 cm^{-1} CH (bending), and the peak at 1088 cm^{-1} to (C–OH) stretching (Sechi & Marques, 2017). The hydroxyl peaks in the starch spectrum have been shifted to higher frequencies between (3200 - 3700 cm^{-1}) and their intensity has decreased, indicating phosphorylation of the free hydroxyl groups of starch in the PRCS spectrum (García-Gurrola *et al.,* 2019). The presence of a new characteristic absorption peak at 998 cm^{-1} (C–O–P) in the IR spectrum of the PRCS sample indicates the presence of phosphates (Meifang *et al.,* 2019). Asymmetric stretch was observed at 2942 cm^{-1} (C–H Stretch), 1660 cm^{-1} (C=O Stretch), 1462 cm^{-1} – CH (bending), and 1162 cm^{-1} attributed to C–OH bond stretching. The FTIR of NWCS, on the other hand, revealed a broad band between 3600 cm^{-1} and 3000 cm^{-1}, which is attributed to O-H stretching and is caused by hydrogen bonding involving the hydroxyl groups on the starch molecules. The band at 2953 cm^{-1} is assigned to C-H symmetrical stretching vibrations between a saturated carbon and attached hydrogen, the band at 2373cm^{-1} is assigned to the C-C stretch of alkyne, absorption peaks between 1450cm^{-1} and 1474cm^{-1} are due to stretching vibrations of C-C bonds in an aromatic ring, 1678cm^{-1} indicate a C-O unsaturated ketone (Sechi & Marques, 2017). PWCS shows that alcohol peak in the starch spectrum has been shifted (3100 – 3700 cm^{-1}) and the intensity has drastically decreased indicating a possible substitution of the hydroxyl groups by phosphate groups (Liu *et al.,* 2022). Peaks at 2960 cm^{-1}(C-H stretch) assigned to asymmetric stretch, 2396cm^{-1} assigned to C≡C bond, 1710cm^{-1} (C=O stretch) assigned to aliphatic ketones have all decreased in intensity as a result of phosphorylation (Meifang *et al.,* 2019).

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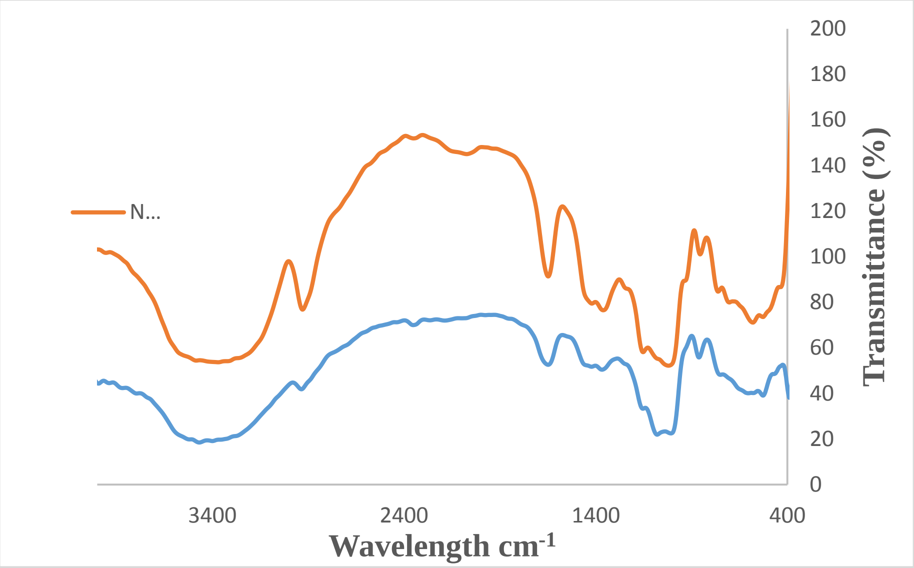

Figure 1.0: Infrared spectra of native and phosphorylated red cocoyam starch

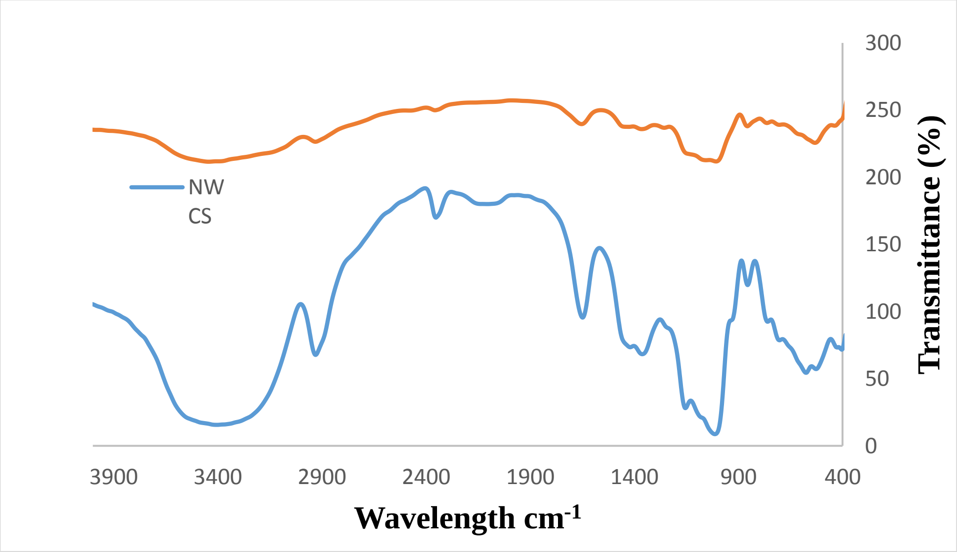

Figure 2.0: Infrared spectra of native and phosphorylated white cocoyam starch

CONCLUSION

This study found out that phosphorylation significantly influences the functional and pasting properties of cocoyam starches. The moisture content, water and oil absorption capacities, dispersibilities and foaming capacities improved following phosphorylation. The low moisture content of both native and starch phosphates of cocoyam would play an important role in the shelf life of dried products since the lower the product's initial moisture content, the better the product's storage stability. The improvement in the foam capacity of cocoyam starch phosphates shows that they could find application as emulsifier in the food industries. As a result, this study concludes that native and starch phosphates of cocoyam can be used as an alternative for industrial products in the starch, food, and packaging industries. It could also be used as a replacement for cereal and root crop starches in foods like bread and pie due to their high final viscosities.

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