COMPARATIVE STUDY OF THE EFFECTS OF PHOSPHORYLATION ON CASSAVA AND RED COCOYAM STARCHES

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Abstract: Native starches isolated from cassava cultivar (TME 305) and red cocoyam were chemically modified by phosphorylation using sodium tripolyphosphate (STPP). Proximate analysis, some functional properties, pasting properties and FTIR of the native and modified starches were determined. The proximate analyses revealed that the fats and crude protein contents reduced after phosphorylation with the red cocoyam starch showing a greater reduction while the moisture content, water and oil absorption capacity, swelling power and solubility increased after phosphorylation with the red cocoyam starch showing the greater increase when compared to the cassava starch. Pasting temperature, peak viscosity, final viscosity, breakdown and setback viscosity increases following chemical modification while the trough viscosity decreases after phosphorylation. Phosphorylation increased the setback values in both starches indicating that chemical modification increased starch retrogradation. The chemical modification on the native cassava and red cocoyam starches through phosphorylation had improved the solubility, texture, adhesion and tolerance to the kind of temperature used in industrial processes.

Keywords: Phosphorylation, starch modification, viscosity, gelatinization

Introduction

Starch is the most abundant carbohydrate reserve in plants and can be gotten majorly from sources such as; cereals (40 to 90%), roots (30 to 70%), tubers (65 to 85%), legumes (25 to 50%) and some immature fruits like bananas or mangoes, which contain approximately 70 per cent of starch by dry weight (Santana and Meireles, 2014). Amylopectin and amylose are the two types of starch polymers synthesized by plant cells. Amylopectin constitutes a larger starch percentage; between 70 to 80 of common starches. The sizes of starch granules are microscopic with diameters ranging from 0.1 to 200 μm, and its morphology varies between different shapes, such as oval, ellipsoidal, spherical, smooth, angular and lenticular, depending on the botanical source (Hoover, 2001; Singh *et al*., 2003; Grace, 1977). Each botanical species is characterized by a unique accumulation pattern of starch granules in each plant tissue, shape, size, structure and composition (Smith, 2001).

The industrial utilization of starch in its native form has been found to be rare as well as its consumption. The instability of most native starches to changes such as, temperature, pH and shear forces are partly responsible for their limited applications. Native starches show a strong ability for decomposition and retro-gradation (Singh *et al*., 2007). Additionally, some starch granules are inert, insoluble in water at room temperature, highly resistant to enzymatic hydrolysis and consequently lacking in functional properties. Native starches are mostly modified to develop specific properties such as solubility, texture, adhesion and tolerance to the kind of temperatures used in industrial processes (Sweedman *et al*., 2013; Lopez *et al*., 2010). Several methods have been developed to produce modified starches with a variety of characteristics and applications. These techniques alter the granular structure of starch polymer, making it highly flexible and changing its physicochemical properties and structural attributes to increase its applicability in food and non-food industries (Yadav *et al*., 2013).

Modifications of starch include; physical, chemical and enzymatic methods (Wursburg, 1986). Heat and moisture are use in bringing about physical modification while the chemical modification involves the introduction of functional groups into the starch molecule (for example etherification, esterification, crosslinking) or involve breakdown reactions (for example hydrolysis and oxidation) (Sweedman *et al*., 2013; Seker and Hanna, 2006). Chemical modification involves the introduction of functional groups on the starch molecule without affecting the morphology or size distribution of the granules. It also generates significant changes in starch behaviour, retro-gradation, gelatinization capacity and paste properties (Yadav *et al*., 2013).

Phosphorylation is another method of modifying starches chemically. It involves the formation of either ether or ester intermolecular lingkages between the hydroxyl groups of starch molecules by the treatment of the granular starch with multifuncional reagents . There are different reagents that can be used for phosphorylation aome of them include, sodium trimetaphosphate, mono-sodium phosphate, sodium tripolyphosphate (STPP), phosphoryl chloride, a mixture of adipic acid, acetic anhydride, and vinyl chloride (Sweedman *et al*., 2013). The molecular structures of the phosphorylated starch systems is affected by the type of phosphorylation agent used therefore, the type of phosphorylation agents used determines the functional properties of treated starch (Moorthy *et al*., 1999). Pastes of phosphorylated starch offer higher qualities than the native in terms of its resistance to shear and acidic conditions. This study aims to compare the effect of phosphorylation on cassava and red cocoyam starch.

Materials and Methods

Experimental

The cassava tubers (*Manihot esculenta* Crantz) and red cocoyam (*Colocasia esculenta*) were purchased from a local market in September, 2017 at Atan Ota, Ado-Odo Local Government area of Ogun State, Nigeria.

Apparatus

Analytical weighing balance, beakers, funnels, measuring cylinders, pH meter, centrifuge, spatula, water baths, oven, thermometer, standard flasks, desiccator, petri dishes, and glass rod.

Reagents

All reagents used in this work were of analytical grade. The reagents include sodium tripolyphosphate (STPP-$Na_5P_3O_{10}$), de-ionized water, potassium hydroxide (KOH), hydrochloric acid and buffer solution (buffer 4 and 9).

Methods

Extraction of cassava starch

The cassava tubers were taken to the International Institutes of Tropical Agriculture (IITA), Moniya, Ibadan for identification in November, 2017. The method Lim and Seib (1993) was followed for the preparation of the ccassava starches. Cassava tubers were peeled, washed and disintegrated in a grating machine. The cassava mesh thus formed was reground and

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mixed with water in the ratio 1:5 (w/v %). A double layer nylon cloth was used together with the mesh to filter the mixture to obtain starch solution. Sedimentation and decantation methods were used to separate the starch from the starch solution. The starch was set in an oven to dry and labelled NCS (native cassava starch).

Extraction of red cocoyam
The method described by Garcia *et al*. (2015) was followed for the extraction of red cocoyam starch. The red cocoyam tubers were washed to remove soil and dirt from the skin and then peeled using a kitchen knife. The peeled tubers were washed, grated and sieved by washing off in a basin of water. The mixture was filtered through a fine mesh sieve (Muslin cloth), filtrated and allowed to settle, after which the supernatant was decanted and sediment was collected in order to obtain the wet starch. The red cocoyam wet starch was air dried at room temperature for 48 h and grinded to powder form using blender. The powdery starch was stored in polythene bag until further use and labelled NRS (native red cocoyam starch).

Modification of starch samples
Preparation of phosphorylated starches
Phosphorylated starches were prepared using the method of AOAC (2000) with slight modification. The native starches; NCS and NRS (75 g) were phosphorylated with STPP (5 g) in the presence of Na_2SO_4 (5 g); an aqueous starch slurry was prepared and STTP was impregnated for 1 h at 25°C and dried to 10-15 per cent moisture at 40°C in a forced-air oven. At 130°C in a forced-convention oven, the dried starch cake was heated for 2 h. After being cooled to 27°C, the reaction mixture was dispersed in distilled water (350 ml). The starch samples were recovered by centrifugation (2,000 rpm for 10 min) and re-dispersed in 500 ml of distilled water. The dispersion was adjusted to pH 6.5. The starch samples were washed with distilled water, centrifuged and dried at 40°C and the resulting materials are regarded as phosphorylated starches. Phosphorylated NCS and NRS were labelled PCS and PRS, respectively.

Proximate analysis
Standard Association of Official Analtical Chemistry Methods (AOAC) were used for estimating moisture content, total ash,crude protein crude fat and amylose were determined (Yussuf *et al*., 2018).

Physicochemical properties
Oil and water absorption capacity
The methods of Hirsch and Kokini (2002) was used with slight modification to determine the oil and water absorption capacities of the starch samples. The starch samples (1 g) each were placed in four 15 ml plastic centrifuge tubes and 10 ml distilled water were added and then covered. The contents were mixed on a vortex mixer for 2 min, allowed to stand for 10min and immediately centrifuged. The supernatant was carefully decanted and the sediment weighed. The ratio of the weight of the sediment to the dry sample weight was taken as the hydration capacity. For the oil absorption capacities, the procedure above were repeated by replacing the water with oil (density of 0.95 gcm^{-3}).

Determination of pH
The pH was obtained according to the methods of Association of Official Analytical Chemists (AOAC, 2000). This was determined by using a pH meter (model HI 8424 with pH buffer 9). The starch samples (1.5 g) were dissolved in beaker containing 10 ml of distilled water and stirred properly. An already caliberated pH meter was inserted into the supernatant and the readings were taken.

Determination of swelling power and solubility
The swelling power and solubility were determined in accordance with the methods described by Gbenga *et al*. (2014); 2 g of the samples were poured into graduated centrifuge tubes containing water and appropriately labeled. On a water bath with heating facility, the solution was stirred and heated to 95°C while shaking the sample gently to ensure that the starch granules remain in suspension until gelatinization occurs. The gelatinized samples were held at 95°C in the water bath for 1 h. The samples were cooled to room temperature under running water and centrifuged for 30 min at 2000 rpm. The swelling volume was obtained directly by reading the volume of swollen sediment in the tube after centrifugation. The supernatant was separated from the sediment, put in a metal dish, weighed, dried at 105°C for 1 h, weighed and dried again. The starch swelling power and solubility were determined according to equations 5 and 6, respectively;

$$\text{Swelling power} = \frac{\text{Weight of swollen sediment}}{\textit{Weight of starch sample}} \qquad \text{Eqn 1}$$

$$\text{Solubility} = \frac{\text{Weight of dry supernatant}}{\text{W eight of starch sample}} \text{ x 100} \qquad \text{Eqn 2}$$

Infra-red spectral analysis
The IR spectra of starches were run as KBR pellets on FTIR system (Spectrum BX Perkin Elmer, England) in the frequency range 350 – 4000 cm^{-1} or more according to method of British Pharmacopoeia (2010) with slight modifications. 2 mg of the samples were grounded and mixed uniformly with 200 mg pure KBr powder. This mixture was next placed in pellet forming-die and then pressed in a hydraulic press to form a KBr pellet. The pelletized sample was placed in a cell holder and then inserted into the FTIR equipment and scanned at a range of 350 – 4000 nm or more. The spectrum and suspected compound were displayed and recorded on the computer screen.

Pasting properties of starch samples
Starch pasting properties were evaluated using Rapid Viscosity Analyzer (New port Scientific RVA super 4, Central Laboratory, University of Ibadan, Oyo State). The sample (3.5 g) was weighed and 25 ml of distilled water was dispensed into a canister and Paddle was placed inside the canister. This was placed centrally onto the paddle coupling and then inserted into the RVA machine. The measurement cycle was initiated by pressing the motor tower of the instrument. The profile can be seen as it is running on the computer monitor connected to the instrument. The 13 min profile was used, the time-temperature regime used was: ideal temperature of 50°C for 1 min, heated from 50 C to 95°C in 3 min 45 seconds, then held at 95°C for 2 min 30 seconds, the sample was subsequently cooled to 50°C over a period of 3 min 45 seconds, followed by a period of 2 min where the temperature was controlled at 50°C. Initial viscosity, peak viscosity (PV), time to peak viscosity (PT), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV) were recorded by rapid viscosity analyzer (RVA).

Results and Discussion
Proximate composition
Table 1 shows the results of the proximate analysis of the native and modified starch samples. The moisture content in the samples ranges between 10.09 and 13.22% with NRS and PRS having the lowest and highest values respectively. The values fall within the maximum allowable limit for moisture in starch flour which is about 14 per cent (Mahmut and Sosulski, 2004) as higher values will promote the growth of organisms which causes odour and off-flavour. It can also be deduced from the table that modification by phosphorylation lead to an increases in the moisture content of the starches with a greater increase in phosphorylated red cocoyam starch. This is in line with the submission of Lawal and Adebowale (Lawal and Adebowale, 2005) who had earlier reported an increased in water absorption ability of modified starches.

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Low Moisture content defines the stability of a drug when starch is used as an excipient by decreasing the rate of deterioration of the drug. The ash contents in the samples ranged from 0.02 – 0.04% with red cocoyam showing the greater effect upon modification than the cassava species. The ash contents of the two starches were within the specification of the British Pharmacopoeia (Adebowale and Lawal, 2003) which specifies that starch samples should have ash contents less than 0.6 per cent. The fat contents in the sample range from 0.09 – 0.14% with native red cocoyam starch having the highest value. The value decreases upon chemical modification which shows that products made from PCS and PRS will be resistant to quick rancidity (Yussuf *et al*., 2018). The protein contents ranged from 0.24 – 1.085 per cent with the NCS having the highest value and PRS having the lowest value. There was an observed increase in crude protein contents due to phosphorylation with the red cocoyam having a higher increase of 1.77%. The fat contents ranged from 0.09-0.14 per cent with PRS having the lowest and the NRS having the highest. It can be showed from Table 1 that the fat content values decreased upon modification but a greater effect (0.05%) was observed in red cocoyam starch. These reductions were due to the various degradation that took place during the modification processes, these were in agreement with reports by Kemas *et al*. (2012).

Table 1: The Proximate analysis of the starch samples

Starch Samples	Ash Content (%)	Fat content (%)	Crude Protein (%)	Moisture Content (%)
NCS	0.02±0.000	0.12±0.007	1.085±0.007	10.54±0.020
NRS	0.03±0.000	0.14±0.007	2.005±0.007	10.09±0.007
PCS	0.02±0.000	0.10±0.007	0.24±0.030	12.91±0.020
PRS	0.04±0.007	0.09±0.007	1.13±0.040	13.22±0.020

All values are means of duplicate determinations + or – standard deviation

Table 2 Physiochemical properties of native and modified starch samples

Starch Samples	Swelling Power (g/g)	Solubility (%)	Oil absorption	Water absorption	pH
NCS	0.55	55	0.80	0.75	9.51
NRS	0.48	48	0.93	0.91	9.84
PCS	0.75	75	0.90	0.80	7.46
PRS	0.69	69	0.80	0.72	7.23

All values are means of duplicate determinations

Oil and water absorption capacity
From the results obtained from Table 2, the water and oil absorption capacity of the modified starches were higher compared to the native starches. The values obtained for the water absorption ability of the modified starches showed that the modified starches absorbed more water compared to oil. The water and oil absorption values obtained from Table 2 showed that modification improves the absorption properties of the starch samples with the cassava sample showing greater absorption. The improved absorbtion for water and oil observed in the phosphorylated starches compared to the native starches is in accordance with the submission of (Awokoya *et al*., 2011). The introduction of bulky functional groups and their electrostatic repulsion facilitate percolation and absorption of water within the starch matrices. Increase in water absorption capacities following etherification reaction have also been reported by Gunarantne and Corke (Gunarantine and Corke, 2007).

Swelling power and solubility of samples
The results obtained from the table also showed that phosphorylation increased the swelling power and solubility compared to the native starches. The swelling power and solubility of the red cocoyam starch upon phosphorylation is higher than those observed in cassava; the former increased by 44% while the latter by 37% for both. When an aqueous suspension of starch is heated, as the temperature increases and exceeds gelatinization temperature, the starch granules become weakened and the intermolecular bonds of the starch molecule become distorted. This gives room for more water molecules to become attached to the starch molecules. As the absorbed water molecules increases, the granules continue to swell (Akanbi *et al*., 2009). High swelling power of starches will enhance its dispersilbility and ability to be used in solution suggesting improved dietary properties and the use of starch in a range of dietary applications. Phosphorylated starches had higher swelling indicating the formation of mono-starch phosphate, due to the fact that phosphate groups diminished the hydrogen bonding among adjacent molecules, which allow greater water penetration and swelling.

pH
The degree of acidity or alkalinity of any substance is the measure of its pH**.** The pH of all the samples fall within the alkaline range (7.23-9.84) with NRS being the most alkaline and PRS with the least alkalinity. So also, the native starches show a higher pH than the modified starches. It is necessary that the pH of starch tends towards neutrality so that it can be used in industries where a change in the pH of products is not desired. Modification by phosphorylation improved the pH values of the native starch to desirable limits of neutrality with PRS having the nearest to neutrality pH. From the results obtained from Table 2, it showed that Modification by phosphorylation reduced the pH of the native starches towards neutral. This is in agreement with (Yixiang and Vesselin, 2004).

Figures 1 and 2 show the IR spectra of native and phosphorylated red cocoyam and cassavea starches, respectively. In both spectra, the absorption bands are similar. The absorption bands for O-H stretch in the samples were within the range of 3490 cm^{-1} and this decreased can be due to weakening effects on the O-H bond from the hydrogen bond in the glycosidic ring thereby shifting the absorption band from the usual 3700-3500 to 3400-3200 cm^{-1}. The C-H stretching of the CH$_2$ symetric vibration is observed on both spectra at 2900 cm^{-1} but on the contrary, the intensity of the hydroxyl groups peaks decreased in the PCS suggesting the replacement of the hydroxyl group by the phosphate group as suggested by previous studies (Xie and Shao, 2009). However, the O-H absorption of the native and the phosphorylated starches was similar but differs in intensity as a result of the abundant hydroxyl group being the crosslinking sites (Zhang *et al*., 2007). The C-H absorption of the phosphorylated starches at 2968 cm^{-1} is common. The absorption bands for the carbonyl group were within the range of 1664-1676 cm^{-1}. There is an observable shift in the C=O absorption band of the phosphorylated starch to 1676 cm^{-1} from the 1664 cm^{-1} in the native starch as a result of the modification. The peaks generated at 1635 cm^{-1} indicates the vibration of water molecules adsorbed by the starch as reported by Ibrahim *et al*. (2013); Kweon *et al*. (2001). The absorptions at 1350 cm^{-1} is also responsible for C-H bending vibration (Kweon *et al*., 2001; Fang *et al*., 2002).

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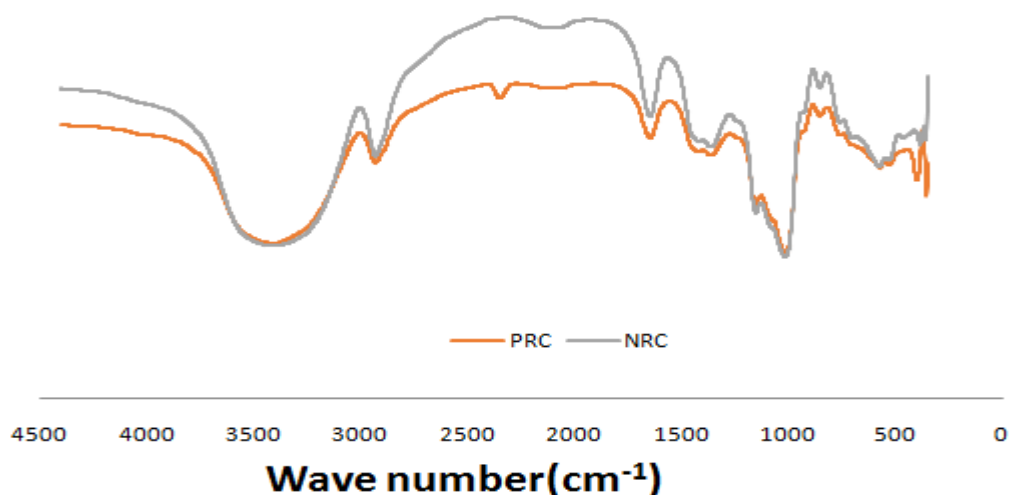
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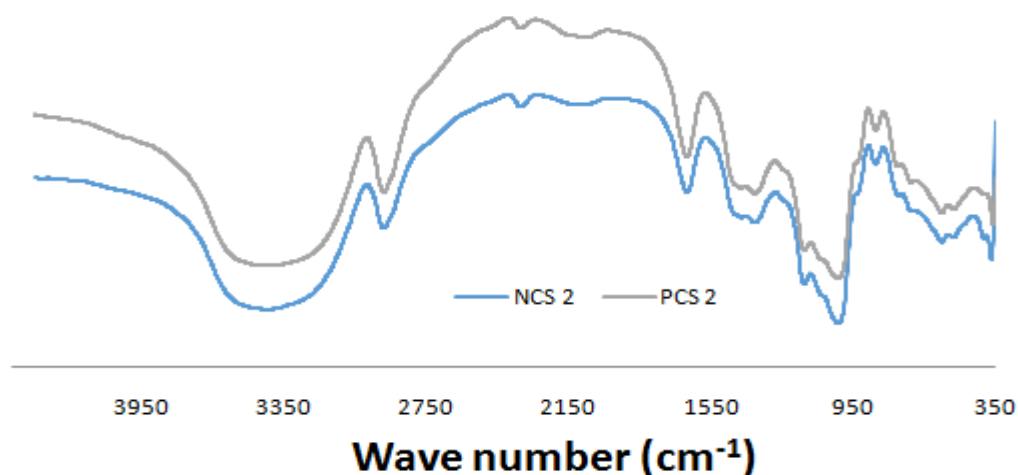**Fig. 1: FT-IR spectral of the native and the modified red cocoyam**

Fig. 2: FT-IR spectral of the native and the modified cassava starches

Table 3: pasting properties of the native and the modified starch samples

Starch Samples	Peak Viscosity (RVU)	Trough Viscosity (RVU)	Breakdown Viscosity (RVU)	Final Viscosity (RVU)	Set back Viscosity (RVU)	Peak time (Min)	Pasting temp (^{0}C)
NCS	454.0000	202.9167	251.0800	251.0833	48.1600	3.9333	73.5000
NRC	273.4167	171.2500	102.1667	275.8330	104.5830	4.5333	83.1500
PCS	485.6667	151.1667	334.5000	270.5833	119.4167	3.8000	74.5000
PRC	306.6667	164.3333	142.3333	281.5000	117.1667	4.6000	86.5005

Results of proximate analysis were the average of duplicate analysis

Pasting properties of starch samples

Table 3 showed the pasting properties of the native and phosphorylated starches. Peak viscosity ranged from 306.67 to 485.67 RVU with the modified samples having higher values. Greater effects of phosphorylation was felt on the peak viscosity value of red cocoyam than the cassava starch. Peak viscosity is an indication of the strength of the pastes which are formed from gelatinization. Peak viscosity is the maximum viscosity developed during heating. Trough viscosity ranged from 151.17 to 202.9 RVU, the native starch samples shows higher values than the modified samples (Sweedman *et al*., 2013). Cassava starch on modification shows a more significant decrease in value of trough viscosity than the red cocoyam starch. The trough viscosity for phosphorylated starches decreases, indicating that phosphorylation of starch reduced the intermolecular bonding strength of starch. There is an observed increase in final

viscousity upon modification, the values ranged from 251.08 to 281.5 RVU. The cassava starch shows higher response to modification in terms of final viscosity than the red cocoyam starch (6 RVU). Set back viscosity ranged from 71.500-119.4167 RVU, the cassava starch showing greater effect (48 RVU) than the red cocoyam starch (13 RVU). The phosphorylated cassava starch had the highest set back viscousity value while the native cassava starch had the lowest. A higher value of set-back viscosity indicated a greater degree of retro-gradation and vice versa (Sweedman *et al*., 2013). Breakdown viscosity ranged from 102.17 to 334.5 RVU with a general increase on modification and this can be attributed to the prevention of linearity of molecular chains due to steric hindrance resulting from the introduction of phophate substitution (Neelam *et al*., 2012).

The cassava starch (132 RVU) shows higher response to modification in terms of breakdown viscousity than the red

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cocoyam starch (40 RVU). Breakdown viscosities reflect the stability of the peak viscosity during processing (Liang and King, 2003). Pasting time ranged from 3.8 to 4.6 min; PCS had the lowest and PRS had the highest values. Pasting temperature ranged from 73.50 to 86.55°C with a general increase in temperature as a result of modification and this can be traced to the absence of interaction between individual chain segment mnot close enough to establish intermolecular interaction(Neelam *et al*., 2012). NCS had the lowest and PRS had the highest value. During processing, the pasting temperature needs to be attained to ensure the swelling, gelatinization and subsequent gel formation. The temperature at which viscousity start to rise is regarded as the pasting temperature (Austin, 1984).

Conclussion
The chemical modification via phosphorylation showed a significant difference in the two starch sources. Phosphorylation causes a reduction in the fats and crude protein and the reduction was more pronounced in the red cocoyam. However, there were observable increased in moisture content, water and oil absorption capacity, swelling power and solubility after the phosphorylation process and the increasement was more in the red cocoyam. Phophate cross-linked starches are resistant to low pH, possess better textural properties, improved viscosity and solubility, high shear and with increased stability of swollen starch granule. These makes phosphorylated starches more superior to native in terms of usage as thickners and stabilizers.

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