Research Article

Physicochemical, rheological and functional properties of modified starches from the tubers of *Plectranthus esculentus*

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ABSTRACT

Native starch extracted from the tubers of *Plectranthus esculentus* (Family: *Lamiaceae*) was modified by acetylation, oxidation, carboxymethylation, xerogel formation, acetylation/xerogel formation, and acetylation/oxidation. Starch syneresis, swelling power and solubility were determined by gravimetric techniques at 10% $^w/_v$ of starch dispersion. Rheological properties were determined on a Bohlin Gemini HR Nano Rotonetic drive 2 rheometer while the structural properties were evaluated using Fourier transform infra-red (FTIR) spectroscopy and x-ray diffractometry (XRD). FTIR confirmed the presence of acetyl groups at 1700 cm^{-1} and carboxymethyl groups at 1579 cm^{-1}. The acetylated derivatives were resistant to syneresis. XRD displayed a crystallized region with three prominent peaks, centred on 2θ = 15.1, 17.2 and 23.2°, for the native starch, acetylated and oxidized starches while the carboxymethylated, xerogelized, and acetylated/xerogelized derivatives were typically amorphous. The derivatives (carboxymethylated and acetylated/xerogelyzed) were thermally stable and formed viscoelastic gel at room temperature. Conversely, dispersions of the native starch and the derivatives (acetylated, oxidized and acetylated/oxidized) exhibited thermal transitions due to gelatinization. The acetylated derivatives have potential in terms of shelf-life, stability, and diverse opportunities for multiple applications in pharmaceutical and food industries.

INTRODUCTION

Native starches are well explored as binders and disintegrants in solid dosage formulations, but due to limitations such as poor flow, poor solubility in cold water, syneresis and loss of viscosity after heating at high temperature, their utilization is highly restricted (Samutsri & Suphantharika, 2012). These limitations of the native starch can be overcome by modifying the starch (Sherry et al., 2005).

Modifications alter properties such as solution viscosity, association behaviour, syneresis and shelf-life stability in final products (Kemas, Ngwuluka, Ochekpe & Nep, 2017; Nep, Ngwuluka, Kemas, & Ochekpe, 2016; Korma, Alahmad, Niazi, Ammar, Zaaboul & Zhang, 2016). A number of chemical modifications have been used to produce many different functional characteristics. These include etherification, esterification, cross-linking, oxidation, cationization, and grafting. However, because of the dearth of new methods in chemical modifications, there has been a trend to combine different kinds of chemical treatments to create new kinds of modified products with improved and enhanced physicochemical properties. The chemical and functional properties achieved following chemical modification, depends largely on the botanical or biological source of the starch, reaction conditions (reactant concentration, reaction time, pH and the presence of catalyst), type of substituent, extent of substitution (degree of substitution, or molar substitution), and the distribution of the substituent in the starch molecule (Singh, Kaur & McCarthy, 2007).

Underutilized starches that could be modified and explored further for food and pharmaceutical applications are those from the edible tubers of *Plectranthus esculentus* (Family, Lamiaceae) (Ochekpe, Kemas, & Nep, 2013). They are wide spread throughout Africa, and in Nigeria, they are widely cultivated in the northern parts notably Kaduna, Gombe, Bauchi and Plateau states (Muazu, Musa, Isah, Bhatia & Tom, 2011). Tubers of *P. esculentus* have three known different varieties that contain starch, namely *P. esculentus* 'B'bot, *P. esculentus* "Riyom", and *P. esculentus* "Long at" which is the most common variety (Agyeno, Jayeola, Ajala & Mamman, 2014).

The potential of starch from the plant for pharmaceutical applications as binder/disintegrant in tablets have been investigated (Kemas, Nep & Ochekpe, 2013; Ochekpe et al., 2013). The studies suggest that the native starch from *P. esculentus* tubers have limitations such as syneresis, poor solubility, and poor binding properties. Also no work has been reported on the rheological properties of modified starches from *P. esculentus* tubers. We hypothesize that modification will result in derivatives devoid of these limitations. In view of these, the present study aims at investigating the functional, physicochemical and rheological properties of modified starches from the tubers of the "Long at" variety of *P. esculentus*. Modification of the native starch will add value in terms of shelf-life and stability when utilized in both pharmaceutical and food industry.

MATERIALS AND METHODS

The "Long at" variety of *P. esculentus* tubers were purchased from the local market (Heipang in Barkin Ladi, Plateau state, Nigeria) and extracted in our laboratory. All chemical reagents used were of analytical grade and include: sodium metabisulphite, sodium hydroxide, acetic anhydride, ethanol, hydroxylamine reagent, hydrochloric acid, sodium hypochlorite, sulphuric acid, methanol (BHD chemicals, England), iodine, monochloroacetic acid, and glacial acetic acid (Sigma-Aldrich, UK).

Starch Isolation from P. esculentus Tubers
Extraction of the starch was done according to previous report (Kemas et al., 2013) with slight modification. Tubers were peeled, cut into small pieces, washed, and 12.2 kg was wet milled (Corona, Columbia). The paste was sieved and the pH of the filtrate was noted. Sodium metabisulphite (2.0 g) was added to the filtrate and allowed to settle overnight. The pH of the supernatant was noted again before decanting the supernatant leaving the paste that had

settled at the bottom of the container. Fresh water (5.0 L) was added to the paste and the dispersion was stirred and left overnight again to settle. This was done consecutively for three days until the paste turns pure white with monitoring of the pH of the supernatant to rule out starch fermentation. Thereafter, the paste was air-dried and stored in an air-tight bottle. The native starch as extracted was code named native- Pesc. The confirmation test for starch was done using BP (2002) procedure for starch identification test.

Starch Oxidation
Oxidation of native Pesc was done according to the method of Parovouri, Hamunen, Forssell, Autio & Poutanen, (1995). Briefly, 100.0 g of native Pesc was dispersed in distilled water. The slurry was oxidized using 5.0 g of NaOCl at room temperature within a pH range of 9.0 - 9.5 for 30 min, after which the sample was neutralized using H_2SO_4 to pH 7.0, before it was centrifuged at 2000 rpm for 30 min. Thereafter, the starch (sediment) was washed 3 times with distilled water, and then dried in a cabinet dryer (30.0 ± 2.0 °C) for 8 h. This modified sample was code named Pesc-OXI.

Starch Acetylation
Acetylation of native Pesc was done as described by Phillips, Huijum, Duohai & Harold (1999). Native Pesc (100.0 g) was dispersed in 225 mL of distilled water, stirring for 60 min at 25 °C. The suspension was adjusted to pH 8.0 with 3.0% NaOH. Acetic anhydride (6.0 g) was added drop wise to the slurry over 30 min, while maintaining pH within the range 8.0 – 8.4 using 3.0% NaOH solution. The slurry was then adjusted to pH 4.5 with 0.4 N HCl and allowed to sediment. The sediment was washed free of acid, using distilled water and then with 95% ethanol, before oven-drying at 45 °C for 1 h. This sample was code named Pesc-ACS.

Acetylation followed by Oxidation
First acetylation of the native Pesc was done as reported in previous section. Thereafter, the Pesc-ACS was oxidized as in previous section. This sample was code named Pesc-ACS-OXI

Carboxymethylation
This was done according to the method of Khalil, Hashem & Nebeish, (1990). Monochloroacetic acid (40.0 g) was dissolved in 200.0 mL of methanol, and 140.0 g of native Pesc was added into the solution followed by 80.0 mL of 12.5 M NaOH. The mixture was then heated to 50 °C with continuous stirring for 60 min. The reaction was terminated by neutralizing with 5.0 mL glacial acetic acid. The starch slurry was precipitated with 100 % methanol. The sample was oven-dried at 50 °C for 24 h and then passed through

sieve size 355 µm. This derivative was code named Pesc-CMS.

Formation of starch xerogels

Starch xerogel was prepared following the modified methods of Ohwoavworhua & Osinowo, (2010). It involves dispersing 150.0 g of native Pesc in 1.0 L of distilled water. The dispersion was heated in a water bath at 90 °C with continuous stirring until the starch gelatinized. The paste obtained was rapidly cooled in an ice bath and then 600 mL of 95 % ethanol added and stirred continuously. The supernatant was decanted and the precipitate obtained was dried in an air oven at 50 °C for 30 min and then passed through sieve size 355 µm. This was code named Pesc-XG.

Starch acetylation followed by xerogel formation

First, the native Pesc was acetylated as reported in previous section. Thereafter, the resultant Pesc-ACS was treated to form xerogels as also reported in previous section. This was code named Pesc-ACS-XG.

Fourier Transform Infrared Spectroscopy Analysis

The Fourier transform infrared (FT-IR) spectroscopy was carried out on the native Pesc and the derivatives using a Nicolet 380 FTIR Spectrometer (Thermo-Electron Corporation, USA) over the range between 4000 and 400 cm^{-1} at 2 cm^{-1} resolution averaging 100 scans.

X-ray Diffraction Analysis

The x-ray diffractometry (XRD) was performed on a Bruker D2 Phaser (Bruker, UK). The starch samples were packed tightly in a circular aluminium cell before exposure to the x-ray beam from an x-ray generator running continuously at 40 kV and 30 mA for 4 min and scanning regions of the diffraction angle, 2θ.

Starch Syneresis

Syneresis was determined according to the method of Singh et al., (2006). Dispersion of the sample (10 %w/v) was heated at 90 °C for 30 min in a water bath followed by rapid cooling to room temperature in an ice bath. The starch sample was then stored for 24, 72, and 168 h at 4 °C. Syneresis was measured gravimetrically as the amount of water (%) released after centrifugation at 4000 rpm for 15 min.

Starch morphology

Starch morphology was done according to pervious methods (Kemas et al., 2017), using light microscope (Nikkon, Japan) fitted with a camera. Slides of the samples in glycerol were viewed using a magnification of X40 with the aid of basic link system software connected to a computer.

Swelling power and Solubility of native Pesc and derivatives

The swelling power and solubility of samples were determined using previous method (Kemas et al., 2017). Exactly 0.5 g of sample was weighed into a pre-weighed test tube containing 25 mL of distilled water and heated in a water bath with continuous stirring at 30, 50 or 80 °C for 30 min. Thereafter, the test tube was cooled to room temperature and centrifuged at 4000 rpm for 15 min. The supernatant was decanted and the weight of starch paste noted. The swelling power was calculated using equation 1. The supernatant (8.0 mL) was dried to constant weight at 80 °C and solubility calculated using equation 2.

$$Swelling\ power = \frac{Weight\ of\ starch\ paste}{Weight\ of\ dry\ starch\ sample} \qquad (1)$$

$$Solubility\ (\%) = \frac{Weight\ of\ starch\ dissolved}{Initial\ weight\ of\ starch\ sample}\ x\ 100 \quad (2)$$

Rheological Evaluation of native Pesc and derivatives

Small deformation oscillatory measurements of elastic (G′) and viscous modulus (G″) were done on 10 %$^w/_v$ dispersions of the starch samples using a Bohlin Gemini HR Nano Rotonetic drive 2 rheometer (Malvern Instruments, UK) employing a Bohlin software (Gemini 200HR Nano) fitted with a 55 mm and 2° cone and plate geometry with gap of 70. Measurements of G′, G″ and complex dynamic viscosity (η*) were taken over a frequency range of 0.1 to 100 rad/s at 1 % strain at 25 °C. Shear sweep was measured over a shear rate range of 0.1-1000/sec. Heating and cooling scans were also performed by measuring G′ and G″ (10 rad/s, 1% strain) between 25 – 80 °C at a rate of 2 °C/min. All samples were heated to 80 °C followed by cooling to 25 °C prior to shear and frequency sweeps. Evaporation of moisture from the samples was minimized by using a solvent trap.

RESULTS AND DISCUSSION

FTIR of Native Starch and Derivatives

The FTIR spectra of the native Pesc and derivatives are presented in Figure 1. The peaks have been previously assigned (Kemas et al., 2012; Nep et al., 2016). The broad band at 3253 cm^{-1} represents -OH stretch region due to associated moisture or intramolecular hydroxyl groups of the glucose monomers. The peak at 2890 cm^{-1} was assigned to stretching vibration of C-H bond from glucose (Yaacob et al., 2011) while the peak at about 1640 cm^{-1} is attributed to scissoring of two O–H bonds of water molecules of hydration held tightly in the amorphous region of starch (Yaacob et al., 2011; Nep et al., 2016). The peaks at about 1138 cm^{-1}, 1051 cm^{-1}

and 1000 cm⁻¹ are related to the stretching vibration of C-O-C and C-O-H from glycosidic bonds typical of polysaccharides (Nikonenko et al., 2000)
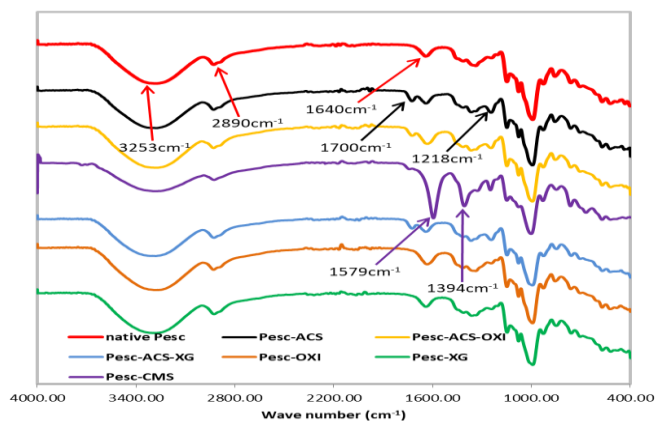

Fig. 1. *FTIR spectra of native P. esculentus starch and its derivatives*

Acetylation of the starch results in the new peak at 1700 cm⁻¹ which corresponds to the stretching of ester carbonyl (C=O), and this was observed for Pesc-ACS-OXI, with an associated increase in intensity of the peak at 1218 cm⁻¹ which corresponds specifically to the C-O stretching of acetyl groups (Kemas et al., 2017; Nep et al., 2016; Sodhi & Singh, 2005; Chi, Xu, Wu, Chen, Xue, et al., 2008). However, oxidation of Pesc-OXI and Pesc-ACS-OXI only resulted in the slight increase in the intensity of peak at 1640 cm⁻¹ and this is consistent with previous report (Nep et al., 2016). Carboxymethylation is accountable for the increasing intensity of the bands at 1579 cm⁻¹ due to the overlapped signal of water in the amorphous regions of starch and the asymmetric stretching vibration of C=O bond of carboxylate groups (Simón, Jesús, Francisco & Alejandro, 2012). The band at 1394 cm⁻¹ corresponds to the symmetric stretching of carboxylate groups (-COO-) (Simón et al., 2012)

X-ray Diffractometry of Native Starch and Derivatives
The XRD spectra of the native Pesc and its derivatives are presented in Fig 2. The native Pesc, Pesc-ACS, Pesc-ACS-OXI and Pesc-OXI) showed a crystalline region embedded within the amorphous region of the material with three prominent peaks, centered at 2Θ = 15.1, 17.2 and 23.2°. In line with previous reports (Nep et al., 2016; Utrilla-Coello, Rodríguez-Huezo, Utrilla-Coello et al., 2014; Faisant, Buleon, Colonna, Molis, Lartigue, Galmiche, et al., 1995), this diffraction pattern is consistent with B-type crystallinity.

Starch oxidation occurs mainly in the amorphous regions of the starch (Kuakpetoon & Wang, 2001), hence there is no observed change in the X-ray

pattern and intensity of oxidized starches. This was also corroborated by Lawal et al., (2005), who observed no differences in the X-ray pattern of native hybrid maize starch and its oxidized derivatives. Also other studies reported that acetylated maize starch with low degree of substitution have a crystallinity profile that are similar to that of native starch, and diminishes as the degree of substitution increases (Xu et al., 2004). This is consistent with the result obtained in this study.
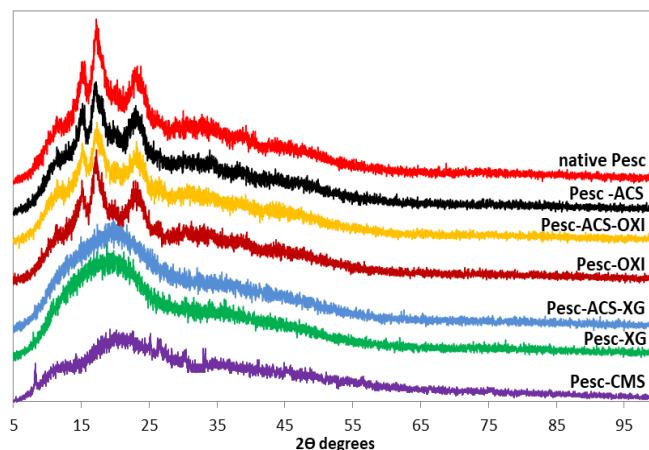

Fig. 2. XRD spectra of native P. esculentus starch and its derivatives

The degree of crystallinity for the starch derivatives – Pesc-ACS-*XG*, Pesc-XG and Pesc-CMS decreased in comparison with the native-Pesc. Pesc-CMS exhibited total loss of the crystalline region within the starch granules and consequently was typically amorphous in nature. The amorphous nature is due to alkaline gelatinization of the starch granules which occurred during during the carboxymethylation process (Fang, Fowler, Sayers & Williams, 2004). On the other hand thermal gelatinization accounts for the loss of crystalinity of Pesc-ACS-XG and Pesc-XG.

Syneresis of Native Starch and Derivatives
The syneresis of native Pesc and its derivatives was measured as the amount of water released from pastes during storage for up to 7 days (Table 1). Native Pesc exhibited 28.5% syneresis after 7 days of storage. Increase syneresis of the native Pesc during refrigerated storage is due to increased molecular association between glycosidic bonds at reduced temperature, which excludes water from the paste structure (Ferrero et al., 1994; Wang et al., 2015; Nep et al., 2016).

Syneresis was reduced upon modification due to the introduction of various functional groups that hinders double helix formation by the amylopectin chains during storage (Kawaljit, & Narpinder, 2007).

Acetylation leads to the formation of stable pastes as the acetyl groups on the starch molecules are able to increase water retention capacity of stored pastes (Nep et al., 2016; Kemas et al., 2017). It also minimizes association of the outer branches of amylopectin which causes syneresis in aqueous starch dispersions (Kemas et al., 2017; Bentacur, Chel & Canizares, 1997). Consequently, Pesc-ACS did not undergo syneresis. For the same reason, Pesc-ACS-XG formed stable pastes at refrigerated storage (Sodhi & Singh, 2005; Henry, 2007). In a similar manner bulky carboxymethyl groups on Pesc-CMS effectively retards syneresis by reducing association in starch molecules to form stable pastes (Thomas & Atwell, 1999; Yeh & Yeh, 1993.

Table 1. *Syneresis of 10 % Paste of Native P. esculentus starch (Native Pesc), P. esculentus oxidized (Pesc-OXI), P. esculentus acetylated-oxidized (Pes-ACS-OXI) and P. esculentus acetylated (Pesc-ACS) during storage time (n=2, mean)*

	24 h	72 h	168 h
Native Pesc	9.0%	28.0%	28.5%
Pesc - OXI	4.6%	4.6%	4.6%
Pesc-ACS-OXI	ND	ND	ND
Pesc-ACS	-	-	-
Pesc-CMS	-	-	-
Pesc-ACS-XG	-	-	-
Pesc-XG	-	-	-

ND: Not be determined
- : No water released

Syneresis in Pesc-OXI was only 4.6%. Oxidation prevents hydrogen bonding of hydroxyl groups on starch molecules. It also causes depolymerization of starch molecules and the degraded long chain amylopectin or amylose molecules, produces dextrins with inappropriate length for syneresis (Kaukpetoon & Wang, 2006). This implies that during storage of oxidized starch pastes, double helix formation between adjacent amylopectin chains is slower and less extensive than in the native starches.

During the process of starch xerogelization, gelatinization of the starch occurs, which promotes the release of amylose from the starch granules, and the higher the amylose content of the starch the faster the syneresis. However, precipitation of the starch paste with ethanol leads to a more extensive shrinkage of the paste to form precipitate which gelatinizes at lower temperature with decreasing content of amylose (Mehling, Smirnova, Guenther & Neubert, 2009). This explains why the Pesc-XG retarded syneresis. Syneresis in the Pesc-ACS-OXI starch paste could not be determined due to low dispersion viscosity of the starch paste on storage at refrigerated temperature.

Effect of temperature on swelling power and solubility

The effect of temperature on swelling power and solubility of the native starch and derivatives is presented in Table 2. Swelling power and solubility of starch samples are temperature dependent (Kemas, Ngwuluka, Ochekpe, & Nep, 2017; Olu-Owolabi, Olayinka, Adegbemile, & Adebowale, 2014; Gebre-Mariam, & Schmidt, 1996). The native Pesc exhibited the lowest solubility at all temperatures when compared. The sharp increase in the swelling power and solubility of native Pesc, Pesc-ACS, Pesc-OXI and Pesc-ACS-OXI at 80 °C, is attributed to gelatinization (Kemas, Ngwuluka, Ochekpe, & Nep, 2017). Introduction of acetyl groups reduces the bond strength between starch molecules thereby increasing the ingress of water into starch granules with consequent increase in swelling power and solubility of the acetylated starch (Ashogbon & Akintayo, 2014). The swelling power of Pesc-OXI is lower than that of the native starch at all temperatures due to structural disintegration within starch granules upon oxidation. Conversely, the solubility of the Pesc-OXI is much higher than native Pesc due to oxidative depolymerisation of amylose and amylopectin which causes weakening of starch granule structure (Adebowale, Afolabi, & Lawal, 2002). The case is the same for Pesc-ACS-OXI.

The derivatives (Pesc-CMS, Pesc-ACS-XG and Pesc-XG) dissolve when dispersed in cold water (30 °C) to form fluid gels. The increase in swelling power and solubility of these derivatives at low temperature is attributed to the loss of granular structure upon modification as evident from the photomicrographs, as well as the decreased crystallinity of these derivatives as evident from the XRD. Furthermore, the presence of bulky groups in Pesc-CMS and Pesc-ACS-XG decreases inter-granular bond strength between starch molecules thereby facilitating the uptake of water (Kemas, Ngwuluka, Ochekpe, & Nep, 2017; Henry, 2007).

Photomicrograph of native-Pesc and derivatives
The photomicrographs of the starch samples at X 40 magnification are presented in Figure 3(A-G). The granules of native-Pesc were mostly half-moon, spherical, sub-spherical and round especially the small ones with an aggregate of 2-4. The sizes of the granules were small, medium and large but mostly small with an actual size range of between 3 μm: 12 μm: 26 μm. The hilum is present mostly as linear, cleft and dot but striations are not visible. The granules of Pesc-ACS-OXI, Pesc-OXI and Pesc-ACS presented similar morphology as the native Pesc. However, they appear to have more definite shapes with more aggregates (Pesc-ACS-OXI and Pesc-OXI).

Table 2: Effect of temperature on solubility and swelling of native-Pesc and derivatives (n=3, mean ± standard deviation)

	Solubility %			Swelling power		
	30°C	50°C	80°C	30°C	50°C	80°C
Native Pesc	0.52±0.64	1.60±1.01	4.48±0.48	1.27±0.37	2.04±0.09	13.53±0.50
Pesc-ACS	0.80±0.41	2.22±0.36	7.94±0.60	2.19±1.00	2.71±0.82	17.45±0.43
Pesc-OXI	6.57±0.05	7.31±0.11	13.82±0.84	0.98±0.62	1.24±0.46	8.51±0.28
Pesc-ACS-OXI	7.45±0.63	8.14±0.52	14.01±0.22	0.76±0.82	0.95±0.31	6.26±0.04
Pesc-CMS	11.64±0.51	13.70±0.65	14.22±0.71	12.18±1.03	12.97±0.42	13.21±0.33
Pesc-XG	4.74±1.21	6.10±0.09	10.50±0.25	10.17±0.25	11.99±0.12	14.17±0.30
Pesc-ACS-XG	12.54±0.31	14.42±0.43	27.46±0.19	15.19±0.09	16.01±0.11	16.22±0.05

Conversely, the granules of Pesc-ACS-XG and Pesc-XG appear to be ruptured and distorted. This is attributed to the elevated temperature of the reaction process resulting in swelling and subsequent rupture of the granules (McGinity, 1994; Kwon, Auh, Kim, Park & Ko, 1997). Furthermore, the loss of granular morphology for Pesc-CMS is attributed to alkali gelatinization of the starch granules which occurred during the carboxymethylation process (Cardoso, Putaux, Samios & da Silveira, (2007).
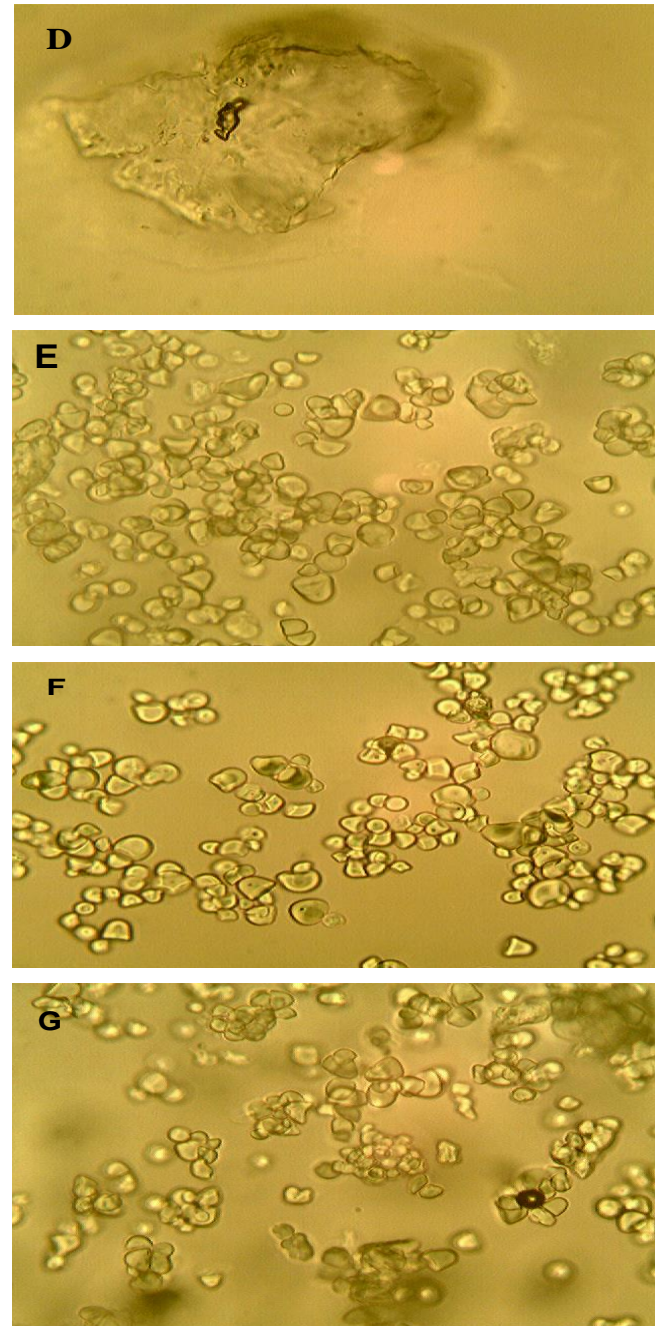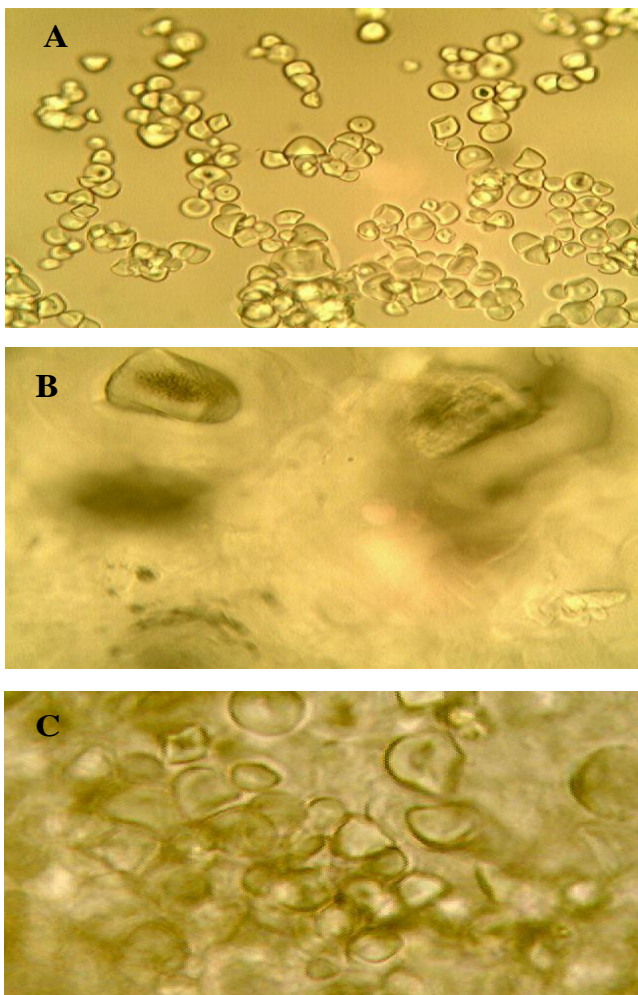

Figure 3: Photomicrograph of A) native-Pesc B) Pesc-CMS, C) Pesc-XG, D) Pesc-ACS-XG, (E) Pesc- OXI, (F) Pesc- ACS, (G) Pesc- ACS-OXI

Temperature Sweep of Native and Derivative Starch Dispersions

The dependence of elastic (storage) modulus (G') and viscous (loss) modulus (G'') on temperature for the native Pesc and modified starches (Pesc-ACS, Pesc-OXI and Pesc-ACS-OXI) was investigated between 25 °C and 80 °C, and the results are presented in Figure 4(A-D). During the first stage of heating, starch granules swell and amylose leaches from granules leading to rise in G' and G'' to a maximum. The G' increases progressively to a maximum (Peak G') at various temperatures for the native Pesc, and Pesc-ACS, Pesc-OXI and Pesc-ACS-OXI. This initial increase in G' and G'' is caused by the formation of three-dimensional gel network developed by leached out amylose and reinforced by strong interactions among swollen starch particles (Eliasson, 1986; Nep et al., 2016). Granule swelling destabilizes the amylopectin crystallites within the crystalline lamellae (Eliasson, 1986) resulting in a rapid rise in the elastic modulus for the native Pesc between 63 °C and 78 °C, and between 54 °C and 60 °C (Pesc-ACS), 63 °C and 67 °C (Pesc-OXI) and 51 °C and 60 °C (Pesc-ACS-OXI). With further increase in temperature a plateau was reached due to irreversible swelling and solubilisation of leached out amylose (Nep et al., 2016; Ahmed et al., 2008), followed by a sudden decrease in G' above 80 °C (Native-Pesc & Pesc-ACS), 69 °C (Pesc-OXI), and 63 °C (Pesc-ACS-OXI) indicating that the gel structure is destroyed. This destruction is due to deformation of granules as a result of melting of the crystalline regions of the amylopectin molecules remaining in the swollen granules (Tsai et al., 1997; Ahmed et al., 2008). These events were similarly observed for G'' with a predominance of G' over G'' indicating viscoelastic behaviour for the native Pesc and the derivatives (Pesc-ACS, Pesc-OXI and Pesc-ACS-OXI).
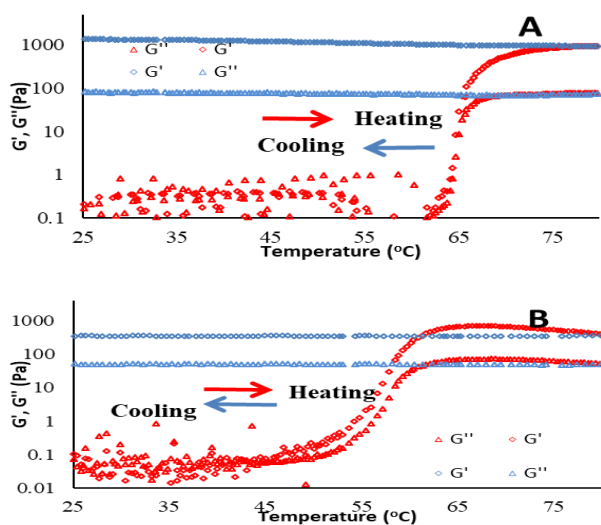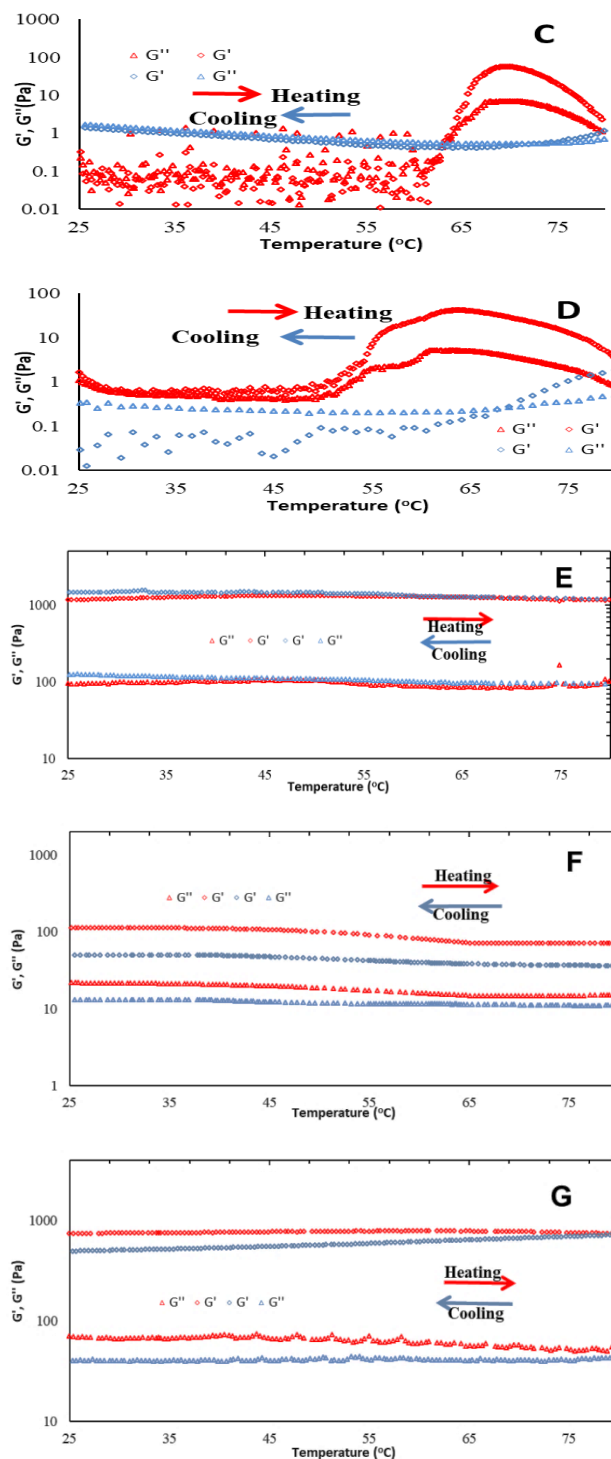

Fig. 4. Dependence of elastic (G') and viscous (G'') moduli on temperature for 10% w/v dispersions of (A) native Pesc (B) Pesc-ACS (C) Pesc-OXI (D) Pesc-ACS-OXI (E) Pesc-XG (F) Pesc-ACS-XG and (G) Pesc-CMS

During cooling of starch paste from 80 °C to 25 °C, both G' and G'' increased, and this increase indicates that the gel become firmer. The increase in G' and G'' with decreasing temperature is reflective of retrogradation in starches (Nep et al., 2016; Kaur et al., 2008; Kong et al., 2012), and this was observed for the native Pesc and Pesc-OXI. The derivatives, Pesc-OXI and Pesc-ACS-OXI showed a rapid decrease of

peak and final viscosity during heating and upon cooling of the starch gels from 80 °C to 25 °C. There was a crossover point of storage and loss moduli at 74 °C (Pesc-OXI in Fig 4C), and 69 °C (Pesc-ACS-OXI in Fig 4D) when *tan δ* approaches unity. Beyond these temperatures the values of the loss moduli (G'') became higher than the storage moduli (G') for both Pesc-OXI and Pesc-ACS-OXI indicating that viscous flow predominates over elastic flow and the material behaves more like a liquid with lower dispersion viscosity (Ngwuluka et al., 2014). This is as a result of higher depolymerization of starch during the oxidation process (Kuakpetoon & Wang, 2001). Pesc-ACS was highly stable upon cooling with both G' and G'' consistently parallel to each other inicating stability to syneresis, and corroborates the data from starch syneresis. The temperature dependence of Pesc-XG, Pesc-ACS-XG and Pesc-CMS are presented in Figure 4(E-G). No themal transition was observed indicating these derivatives did not gelatinize. This observation corroborates the XRD results which show that these derivatives were typically amorphous having no crystalline region accountable for the gelatinization of starch (Kemas et al., 2017; Lawal et al., 2011). Consequently, Pesc-XG, Pesc-ACS-XG and Pesc-CMS dissolve in water at room temperature forming fluid gels. Upon cooling of these derivatives from 80 °C to 25 °C, again no thermal transitions were observed. The G' & G'' remained thermally stable with G' being consistently higher than G'' indicating viscoelastic behaviour (Kemas et al., 2017).

Frequency Sweep of Native and Derivative Starches Dispersions
The frequency sweep (mechanical spectra) was determined after heating (to 80 °C) and cooling (to 20 °C). The mechanical spectrum is used to tell the gel structure of a material (Mandala, 2012). The dynamic mechanical spectra of native Pesc and the derivatives are presented in Figure 5 (A-G). The native-Pesc and all derivatives (except Pesc-OXI and Pesc-ACS-OXI) showed similar spectra and had values of G' > G'' throughout the frequency range covered with varying degree of G' prevailing over G'', indicating viscoelastic nature of the starch pastes (Nep et al., 2016; Kemas, et al., 2017). Pesc-OXI and Pesc-ACS-OXI both showed lower G' values than that of G'' at higher frequency. The ratio of G'' to G' is defined by *tan δ*, a parameter that indicates the physical behaviour of a system. The *tan δ* the samples at angular frequency of 20 rads/s are presented in Table 3. The *tan δ* approaches 1 when the degree of elastic and viscous behaviour of a material is the same. Lower values of *tan δ* (<1), is indicative of a more solid-like (elastic) behaviour while higher values of *tan δ* (>1), describes viscous behaviour and

the material is more liquid in nature (Kemas et al., 2017; Li et al., 2011).

Shear Sweep of 10%$^w/_v$ Dispersions of Native Starch and Derivatives
The shear profiles of the starch samples after heating (to 80 °C) and cooling (to 25 °C) are presented in Figure 6. The native Pesc, and all the derivatives exhibited shear-thinning (Kemas et al., 2017). Native Pesc displayed higher viscosity profile at lower values of shear rate while *Pesc*-ACS-OXI exhibited the lowest viscosity profile across all shear rates. Shear thinning is also known as pseudoplastic flow behaviour and it is produced when the applied stress causes the macromolecules inside the starch matrix to align in the direction of flow (Talou et al., 2011). The degree of shear-thinning is related to the morphology and rigidity of the swollen granules (Ellis et al., 1989). This type of flow behaviour has been reported for starch dispersions from other botanical sources (Guerra et al., 2009; Nep et al., 2016; Kemas et al., 2017). Shear-thinning is a rheological parameter for predicting product end user performance in liquid formulations as it relates flow property of formulation (Trivedi, 2008).

Table 3. *Frequency Dependence of Tan δ of Native P.esculentus starch (Native Pesc), P.esculentus acetylated (Pesc-ACS), P.esculentus oxidized (Pesc-OXI) and P.esculentus acetylated-oxidized (Pesc-ACS-OXI) at 20 rads/seconds*

Starch & Derivatives	Tan δ (°) at 20 rads/s
Native Pesc	0.083
Pesc-CMS	0.085
Pesc-XG	0.094
Pesc-ACS-XG	0.255
Pesc-ACS	0.149
Pesc-OXI	0.719
Pesc-ACS-OXI	0.593
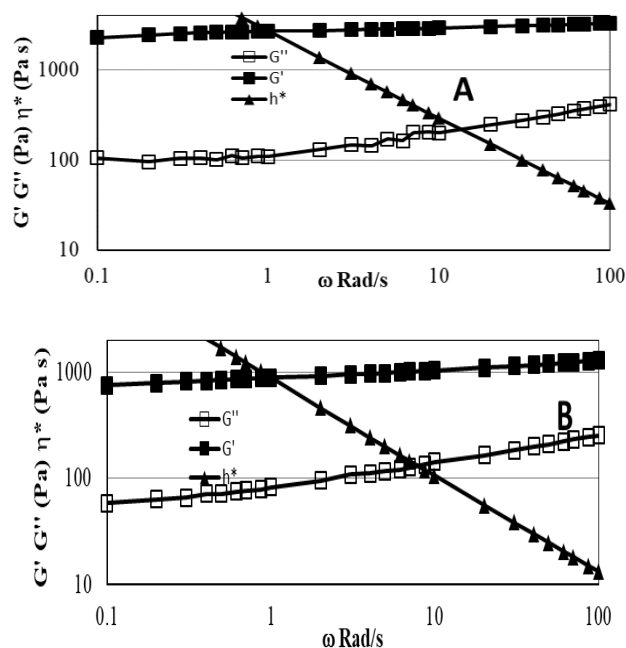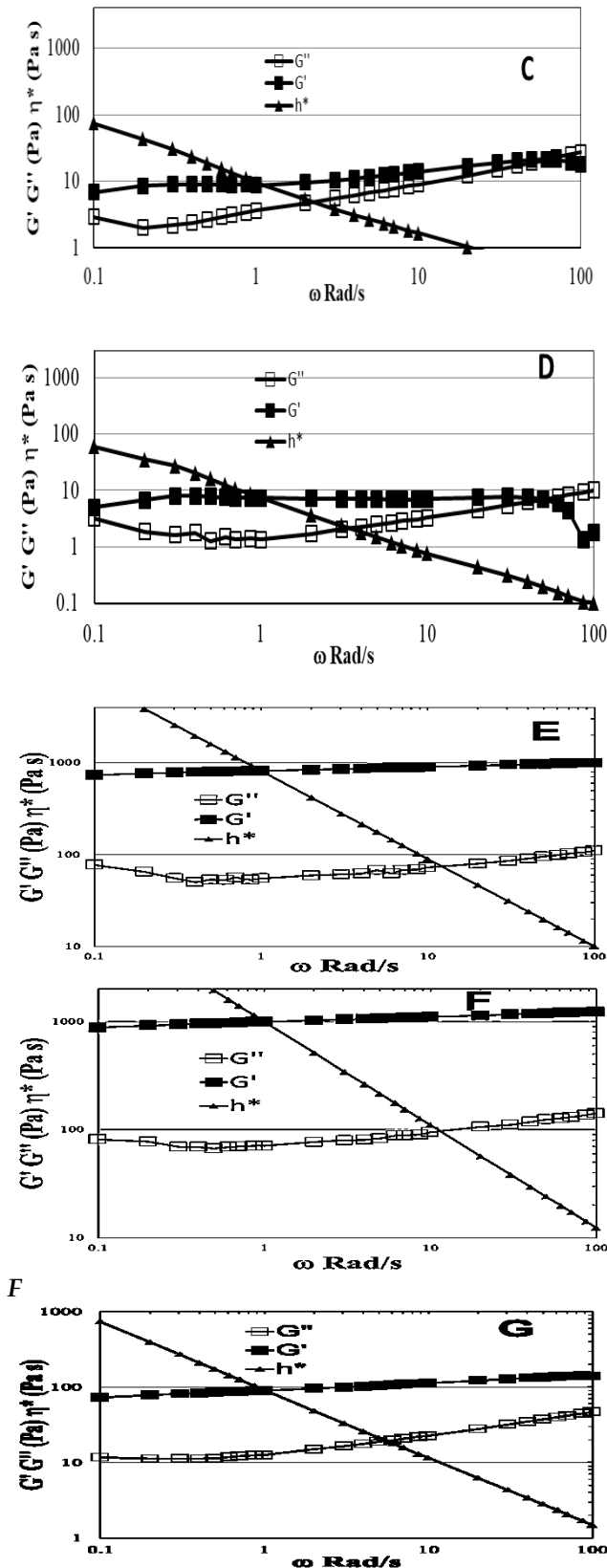
Fig. 6. *Shear-thinning behaviour of 10% w/v dispersion of native P. esculentus starch and derivatives after heating to 80 °C followed by cooling to 20 °C*

CONCLUSIONS

In this study, the effects of modification on the structural and rheological properties of starch from the tubers of *P. esculentus* were evaluated. The starches were modified by a combination of techniques which includes acetylation, oxidation, carboxymethylation, xerogel formation and dual modification (acetylation followed by oxidation and acetylation followed by xerogel formation). FTIR analysis provided evidence of chemical modification. The XRD analysis revealed that the native Pesc, Pesc-ACS, Pesc-OXI and Pesc-ACS-OXI exhibited a crystalline region embedded within the amorphous region of the material. Also rheological studies showed that the native Pesc, Pesc-ACS, Pesc-OXI and Pesc-ACS-OXI exhibited thermal transition due to gelatinization. Conversely, Pesc-XG, Pesc-ACS-XG and Pesc-CMS were typically amorphous and dissolve in water at room temperature to form gels. They were thermally stable upon heating and cooling. The native starch and all derivatives displayed shear thinning behaviour. Although native Pesc formed the strongest pastes it exhibited high degree syneresis upon storage which was significantly overcome by acetylation. This practical value of the acetylated starch derivative is advantageous in terms of shelf-life and stability when utilized in pharmaceutical and food industry.

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Fig. 5. *Mechanical spectra of 10% w/v dispersions of (A) native Pesc (B) Pesc-ACS (C) Pesc-OXI (D) Pesc-ACS-OXI (E) Pesc-CMS (F) Pesc-XG (G) Pesc-ACS-XG, after heating to 80 °C followed by cooling to 25 °C*
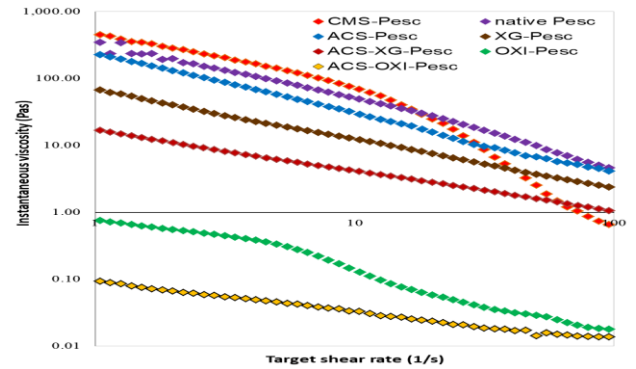
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