

EFFECT OF CHEMICAL MODIFICATION ON THE PROXIMATE COMPOSITION OF *PLECTRANTHUS ESCULENTUS* STARCH AND CHARACTERIZATION USING FTIR SPECTROSCOPY

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ABSTRACT

Starch from tubers of *P.esculentus* was extracted and chemically modified using four different methods which includes HCl-ethanol treatment, dry heating or dextrinization at 200°C, xerogel formation, and gelatinization of the starch. Structural changes in native starch and the derivatives were elucidated using Fourier Transform Infrared Spectroscopy (FTIR). Also the effect of the chemical modifications on the proximate compositions of the starch was evaluated. The results showed that the crude protein content was lowest for the dextrinized starch (0.58%). while crude lipid was lowest (0.05%) in the HCl-ethanol hydrolysed starch derivative. The ash content was 1.5 and 2.0% in the xerogel and HCl-ethanol hydrolysed starch derivative respectively. FTIR spectroscopic analysis revealed substantial structural

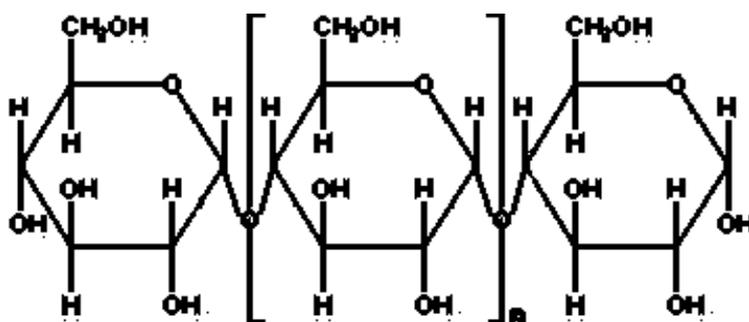
variations between the different derivatives of the native starch. Chemical modification of native *P. esculentus* starch resulted in derivatives which significantly differ from the native in proximate composition and structure. Significant variations in the functionality of the starch derivatives may be expected.

Keywords: *P.esculentus* starch, chemical modification, proximate composition, characterization, FTIR spectroscopy.

1. INTRODUCTION

Starch is a polysaccharide assembled from simple sugar glucose. It is chemically composed of two different molecules amylose and amylopectin. In amylose the glucose molecules are linked together in a linear fashion and it is made up of about 3000-6000 glucose molecules

joined together by α -1,4 -glycosidic linkages. It is however reported to contain few branch networks. Amylopectin on the other hand has a branched structure due to the different kinds of linkage between two glucose units. The α -1,4- linkages in amylopectin serves as the backbone while the α -1,6- serves as the branching point. In native (unmodified) starch, the amylose molecules and the amylopectin region with chain branching forms the amorphous region while the outer amylopectin linear chains coiled into double helices forms the crystalline region. The structure of starch is shown in fig. 1.



n = number of repeating glucose units.

Fig 1: Structure of starch

Native starch irrespective of their source are not desirable for many applications (Wang et al., 1993) because of their inability to withstand processing conditions such as high temperature, high shear rate, freeze-thaw variation, and different pH. They also have large molecular size, insolubility in water, instability in the viscous solution and are susceptible to microorganisms (Trease and Evans, 1983). In order to improve the desirable functional properties and broaden their range of physicochemical properties, modifications are often desired. Modification methods such as oxidation, acid treatment, cross-linking, methylation, and enzyme treatment have been used to overcome such problems.

Proximate composition is a simple and convenient way of illustrating the purity of starch whereby higher starch content and lower contents of other components (protein, fat, ash and fiber) are highly desirable. It has been speculated that high content of other components especially protein and fat influences the swelling power and pasting properties of starches (Schoch and Maywald, 1968).

Starch and protein interact due to attraction of their opposite charges and form complexes on the granule surface thereby preventing the escape of exudates from the granules during gelatinization and this restricts swelling (Olkku and Rha, 1978). Therefore protein removal

was reported to increase the paste viscosity of starch (Yang and Chang, 1991) but Liang and King (2003), observed the opposite effect. Hamaker and Griffin (1993), studied the effect of protein on rice starch and found that protein can alter the gelatinization and pasting properties of starch.

Detection of the structural changes in the starch due to chemical modification is important in order to determine quality of the modified starch. FTIR is a powerful technique for the elucidation of structural changes in samples, with the ability of discovering differences not seen by other physicochemical techniques because it has a unique region known as the fingerprint region where the position and intensity of bands is specific for every polysaccharides and it has been employed as probes to investigate process induced changes in samples and to measure the quality of food (Scotter, N.G.C 1997) due to its high resolution power compared to other IR spectroscopic techniques.

In this study, *P. esculentus* starch was modified using four different chemical methods viz: HCl-ethanol treatment, dry heating or dextrinization at 200°C, xerogel formation and gelatinization. Consequent upon modification, the proximate composition of the native and derivative starch samples were determined while structural changes in the derivatives as compared with the native starch were evaluated using FTIR spectroscopy.

2.0 MATERIALS AND METHODS

2.1 Materials

The tubers of *P.esculentus* were obtained from the local market in Plateau State, and the starch was extracted in our laboratory. Ethanol, sodium metabisulphite, sodium hydroxide and hydrochloric acid (Sigma-Aldrich, UK) were of analytical grade as procured from their manufacturer.

2.2 Methods

2.2.1 Extraction of *P.esculentus* starch from the tubers

The skin was peeled off to expose the inner white layer which was then sliced into several smaller pieces and washed in 0.1 % sodium metabisulphite before wet milling. The paste was sieved using muslin bag and allowed to settle overnight. Thereafter, the supernatant was decanted leaving the paste that has settled at the bottom of the container. The paste collected at the bottom was then washed thoroughly with 0.1% sodium metabisulphite which served as an antioxidant until the paste became white. The washed paste was then air- dried for 72 hours. Further drying was achieved in an air oven (GallenKamp, England) at 60°C for 48

hours. Thereupon the dried starch was size-reduced using a mortar and a pestle and thereafter passed through a sieve size 250 μm and stored in an air-tight container.

2.2.2 Preparation of HCl/ethanol hydrolyzed starch

The method of Chang *et al.*, (2006) was adopted without modification. Briefly, about 150 g of starch was suspended in 600 ml ethanol in a 1000 ml conical flask. A hydrolysis reaction was initiated by adding 50 ml of concentrated HCl and allowed to proceed for 1 hour at 50°C in a water bath. The reaction was stopped by neutralizing with 3.0 M NaOH. The sample was then filtered with a muslin bag. The supernatant was decanted and the precipitate was washed with 50% ethanol until neutral to litmus. The modified starch samples obtained were then filtered and dried in an oven at 40°C and weighed at room temperature.

2.2.3 Preparation of gelatinized starch

About 300 g of *P. esculentus* starch was suspended in 2.0 L of distilled water in a 500 ml beaker at room temperature. The suspension was heated at about 90°C in a water bath with continuous stirring until the starch gelatinized. The paste obtained was thinly spread on stainless steel trays and dried in an air oven (GallenKamp, England) at about 60°C for 48 hours. The flakes were powdered using a blender and then sieved.

2.2.4 Preparation of starch xerogel

A 150 g quantity of *P. esculentus* starch was suspended in 1.0 L of distilled water in a 500 ml beaker at room temperature. The suspension was then heated in a water bath at 90°C with continuous stirring until the starch gelatinized. The paste obtained was then allowed to cool for 1 hr then 600 ml of 95% ethanol was poured into the paste, stirred continuously and allowed to settle. The supernatant was decanted and sieved using a muslin bag. The precipitate obtained was dried in an air oven (GallenKamp, England) at 60°C for 45 min.

2.2.5 Dextrinization of starch

A 150 g quantity of *P. esculentus* starch was weighed and heated in an air oven (GallenKamp, England) at 200°C for 5 hr. The starch dextrin was then bottled and stored for FTIR analysis.

2.2.6 Photomicrograph of native *P. esculentus* and derivatives

Photomicrograph of starch was taken on Nikon (Japan) microscope fitted with a camera. Slide of the power in glycerol was prepared on the microscope slide and placed on the stage

and viewed with the aid of basic link system software connected to a computer.

2.2.7 Determination of Proximate Composition

Proximate analysis was carried out to determine moisture, crude protein, ash, crude lipid, crude fiber and total carbohydrate of *P.esculentus* starch and its derivatives. The methods recommended by the Association of Official Analytical Chemists (1984) were adopted for the determination of moisture, ash and crude lipid content. Moisture content was obtained by weighing 2.0 g of each sample in triplicates into previously dried and weighed crucibles. It was then dried to a constant weight in an air oven (Gallenkamp Ltd, England) at 105°C for 24 hr. The crucibles and content were allowed to cool in a desiccator and weighed. The moisture content was then calculated as the percentage loss in starch weight. Ash was determined by the incineration of three portions of a 2.0 g sample in a muffle furnace at 550°C for 8 hr. The ash was calculated as weight of ash obtained per weight of starch expressed in percentage. The crude protein was obtained by the Kjeldahl method in which nitrogen was determined and the % Nitrogen was multiplied by a factor of 6.25 to obtain % protein. The method involves the digestion of 1.5 g of the sample with concentrated sulphuric acid (H₂SO₄). The digest was distilled to liberate ammonia (NH₃) which was trapped into 2.0% boric acid solution. The distillate was titrated with 0.1M HCl and the titre value used to calculate percent nitrogen. Crude fat was obtained by completely extracting three portions of 10.0 g of starch samples in Soxhlet apparatus using petroleum ether (bp 40-60°C) as the extracting solvent. The crude fiber was obtained by the digestion of 2.0 g of starch sample with sulphuric acid. It was then boiled under reflux for 30 min. The flask was removed, cooled and the content was filtered through a Whatman filter paper. The residue was washed several times with hot water, dried to constant weight at 100°C, cooled for 3 min in a desiccator, weighed and ashed in a muffle furnace at 550°C for 2hr. The residue was cooled in a desiccator and reweighed. The crude fiber content was then calculated as the loss in weight on ashing expressed as a percentage of weight of starch sample. Total carbohydrate was determined by difference (summing the value of moisture, ash, fiber, crude protein, crude lipid, and subtracting the sum from 100).

2.2.8 FTIR Spectroscopy

Before mounting sample disc on a Nicolet 510 FTIR spectroscope (California, USA), sample discs were first prepared by mixing 2 mg of the dry sample with 300 mg of anhydrous finely

powdered KBr. Thereupon, the powder mix was poured into a die and compressed on a KBr press (Specac, Germany) under high pressure and vacuum to form a suitable disc.

2.2.9 Statistical Analysis

Statistical analysis was done using ANOVA (GraphPad Software Incorporation, San Diego, USA). At 95% confidence interval, p values of ≤ 0.05 were considered significant.

3.1 RESULTS AND DISCUSSION

3.1.1 Photomicrograph of native *P.esculentus* starch and derivatives

Figure 2A-D shows the photomicrograph of starch obtained from the tubers of *P. esculentus* and some of the derivatives. The particles of *P.esculentus* starch are more or less irregular in shape and chemical treatment was seen to have no effect on shapes of the starch granules. It has been reported that the shapes of starch particles may have an effect on the ability of the particles to rearrange themselves under compaction.

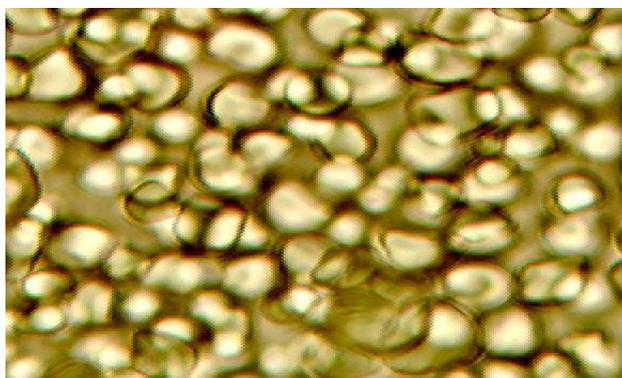


Figure 2A: Photomicrograph of native *P. esculentus* starch (X40)

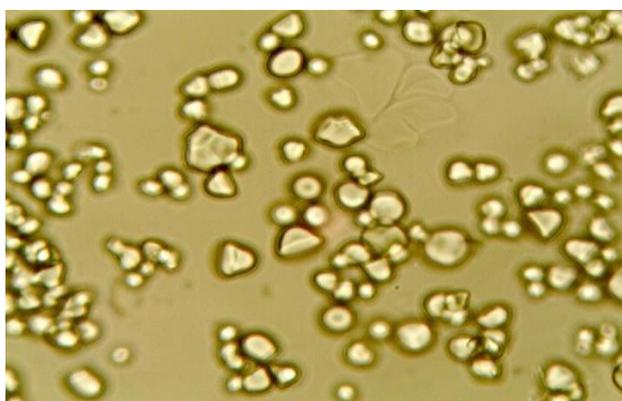


Figure 2B: Photomicrograph of *P. esculentus* starch dextrin (X40)

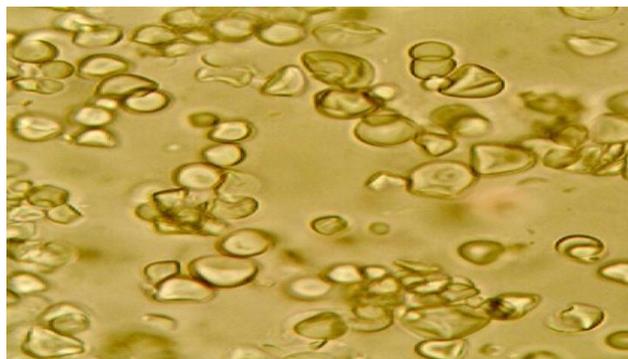


Figure 2C: Photomicrograph of HCl/ethanol hydrolysed *P. esculentus* starch derivative (X40)

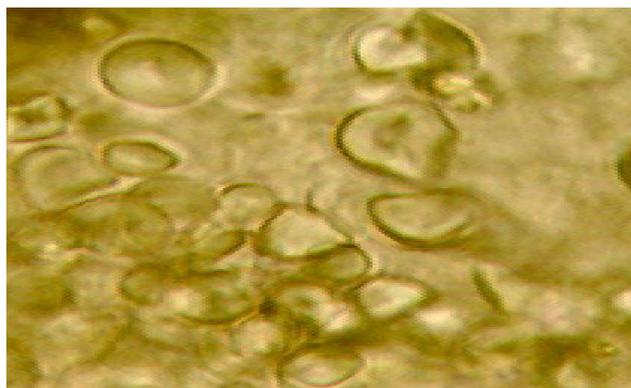


Figure 2D: Photomicrograph of *P. esculentus* starch xerogels (X40)

3.1.2 Proximate Composition

The proximate composition of *P. esculentus* starch and its derivatives are presented in table 1. Moisture was highest in the native starch sample followed by the gelatinized starch while the lowest value of 1.51 ± 0.53 % was recorded for the dextrinized starch derivative. Also the dextrinized starch sample recorded the lowest value of 0.58 ± 0.02 % for crude protein which was same as for HCl/ethanol modified derivative. The highest crude protein level was obtained for the native starch sample. HCl/ethanol modified derivative had the highest ash content of 2.01 ± 0.02 % followed by starch xerogel (1.5 ± 0.02 %). Crude fibre and total carbohydrate was highest in the starch dextrin compared to all the other samples.

TABLE 1: Effect of Modification on Proximate Composition of *P. esculentus* Starch and its Derivatives (n=3, mean \pm s.d)

	<i>P. esculentus</i> starch and derivatives				
	Native starch	Gelatinized	Xerogel	HCl/ethanol modified	Starch dextrin
Carbohydrate (%)	81.89 ± 0.01	-	$86.37 \pm 0.$	87.3 ± 0.00	92.91 ± 0.0

			0		1
Crude Fiber (%)	0.20±0.01	-	0.40±0.0	0.20±0.01	0.50±0.01
			1		
Crude Protein (%)	5.81±0.03	-	0.88±0.0	0.58±0.01	0.58±0.02
			1		
Crude Lipid (%)	0.50±0.01	-	2.0±0.01	0.05±0.03	4.00±0.03
Ash Content (%)	0.50±0.01	-	1.5±0.02	2.01±0.02	0.50±0.01
Moisture Content (%)	11.1±0.77	9.54±0.36	8.85±0.4	9.41±0.27	1.51±0.53
			9		

- Not determined

The moisture content of native *P.esculentus* starch (table 1) as determined was 11.1±0.77. This value is within the 10-20% moisture level recommended for commercial starches (Soni *et al.*, 1993). The derivatives of *P.esculentus* starch showed lower values of moisture content with the dextrinized sample having the least moisture content of 1.51%. This is significantly lower ($P<0.001$) than the moisture content of the gelatinized, xerogelized and HCl/ethanol hydrolyzed derivatives and can be attributed to loss of water molecules as a result of Maillard reaction. Reduction in moisture content reduces chances of oxidative deterioration thereby increasing the stability and shelf-life of the derivatives.

The native of *P.esculentus* starch had a crude protein content of 5.81%. However, Temple *et al.*, (1991) reported a crude protein content of 6.35-7.71%. The variation may be attributed to environmental factors such as soil type and weather. Derivatives of the native starch had significantly lower crude protein content ($P<0.001$) with a corresponding increase in carbohydrate content. This result is in agreement with the findings of Harrelson *et al.*, (2008). The observed decrease in the nitrogen and protein content of *P.esculentus* starch dextrinized at 200°C was possibly due to protein denaturation. It has been stated from literature (Nakia, 1983) that heating above 50°C denatures protein by breaking the non-covalent bonds such as hydrogen, hydrophobic and hydrostatic bonds involved in the stabilization of secondary and tertiary structure. This lead to the unfolding of protein and the exposed hydrophobic group interacts and reduce water binding as well as protein solubility.

Maillard browning reactions which involve the reaction of primary amines with reducing sugars could also be responsible for the loss in protein and changes in colour of foods as this

reaction depends on the intensity of heat and temperature. The reactive carbonyl group of the sugar reacts with the nucleophilic amino group of the primary amine. The amino acids are deprotonated and have an increased nucleophilicity, loss of water from this molecule produces an imine that is able to cyclise, resulting in the formation of an N-glycoside (a sugar attached to an NR_2 group), followed by an Amadori rearrangement to a compound called Ketosamine that is terminated by a dehydration step with the loss of water molecules. Hence, the greater reduction in protein content during dry heat processing is as a result of polymerization of amino acids (Kato *et al.*, 1985) or due to the formation of melanoidins (brown nitrogen containing pigments) by Aldol condensation and carbonyl-amine polymerization at high temperatures. Maillard reaction happens noticeably around 155°C , by this time the starch turns brown and almost all the water is vaporized. The results also showed a significant decrease ($P < 0.001$) in protein content upon xerogelization. The observed decrease in protein content may be attributed to precipitation and removal of depolymerize protein upon treatment of starch with ethanol.

The crude lipid value of native *P.esculentus* starch was 0.5% which fell within the range 0.27-2.63% reported by Temple *et al.*, (1991). The crude lipid value increased significantly ($P < 0.001$) in the dextrinized starch derivative, and this possibly suggests that dry heating of *P.esculentus* starch at higher temperature disrupted the lipid bodies of the starch allowing more oil to be expelled. Acid hydrolysis released bound lipids both polar and non-polar by dissociating lipid starch and lipid protein intermolecular forces. This explains why there was significant decrease ($P < 0.001$) in the value of crude fat and crude protein of the acid hydrolyzed starch derivative.

The total carbohydrate content of *P.esculentus* starch was 81.89% which fell within the range 29.32-85.40% reported by Temple *et al.* (1991). The total carbohydrate content increased significantly ($P < 0.001$) in dextrinized derivative possibly due to the heat involved which reduces some other components as a result of denaturation and leaching which leads to an increase in carbohydrate content. Also the total carbohydrate content of the HCl/Ethanol hydrolyzed *P.esculentus* starch and xerogelized derivatives increased significantly ($P < 0.001$). This shows the level of purity of the starch derivatives compared to the native.

The ash content represents the total mineral content present in the samples. The present result show no significant variation ($P > 0.05$) between ash content of the native starch and the starch

dextrin. There was however significant increase ($P < 0.001$) in the ash content values of the acid hydrolyzed and xerogel derivatives of the starch.

The crude fiber measures the cellulose, hemicelluloses and lignin content of the starch. Dextrinization increases the crude fiber content of *P.esculentus* starch significantly ($P < 0.001$). There was no significant variation ($P > 0.05$) in the crude fiber content of the native starch and the acid hydrolyzed starch on the one hand and the native starch and the starch xerogel on the other hand.

3.1.3 FTIR Analysis

The characteristic peaks and bands for the native starch and derivatives are summarized in table 2 and are here presented.

TABLE 2: Effect of Modification on Absorption bands of *P.esculentus* Starch and its Derivatives

	<i>P. esculentus</i> starch and derivatives				
	Native starch	Gelatinized	Xerogel	Acid/Alco modified	Heat modified
O-H Stretch (cm^{-1})	3215-3536	3010-3579	3390	3127-3559	3094-3428
C-H Stretch (cm^{-1})	2926	2886	2940	2938, 2832	2922
H₂O Absorbed (cm^{-1}) (amorphous region)	1644	1617	1644	-	1644
C-O-H Bending (cm^{-1})	1005	1085	1036	1051	1037
C-O, C-C Stretch (cm^{-1})	1142	1159	1143	1197	1172
C-O-C (cm^{-1})	926	946	924	926	925
Glucose pyranose ring (cm^{-1})	589, 461	631	523	673, 625, 578, 488	606, 479

The native starch showed the following absorption bands: The band between $3215-3536\text{cm}^{-1}$ represents O-H stretch; The absorption shoulder at 2926cm^{-1} represent C-H stretch; Aromatic absorption is absent in the diagnostic regions (1600cm^{-1} and 1500cm^{-1}) but medium intensity absorption at 1644cm^{-1} is due to water absorbed in the amorphous region of the starch. 1230cm^{-1}

represents CH_2OH ; The absorption peak at 1142 cm^{-1} represents coupling mode of C-C, C-O stretching vibrations while the band at 1091 cm^{-1} represent C-O-H bending vibration; The absorption at 926 cm^{-1} is typical of the system (C-O-C), skeletal mode vibration of α -1,4-glycosidic linkage while 589 and 461 cm^{-1} represent the skeletal mode of pyranose ring (See fig 3).

The starch dextrin (thermally treated at 200°C for 5hr) showed the following absorption bands: The broad band between 3094 and 3428 cm^{-1} represent (O-H stretch); 2922 cm^{-1} represent (C-H stretch); 1644 cm^{-1} is due to water absorbed in the amorphous region of starch. 1251 cm^{-1} represents (CH_2OH); 1172 cm^{-1} represents coupling mode of (C-C and C-O stretch); 1037 cm^{-1} represent (C-O-H bending); 925 cm^{-1} represent (C-O-C), skeletal mode vibration of α -1,4-glycosidic linkage. 606 and 479 cm^{-1} represent skeletal mode of pyranose ring.

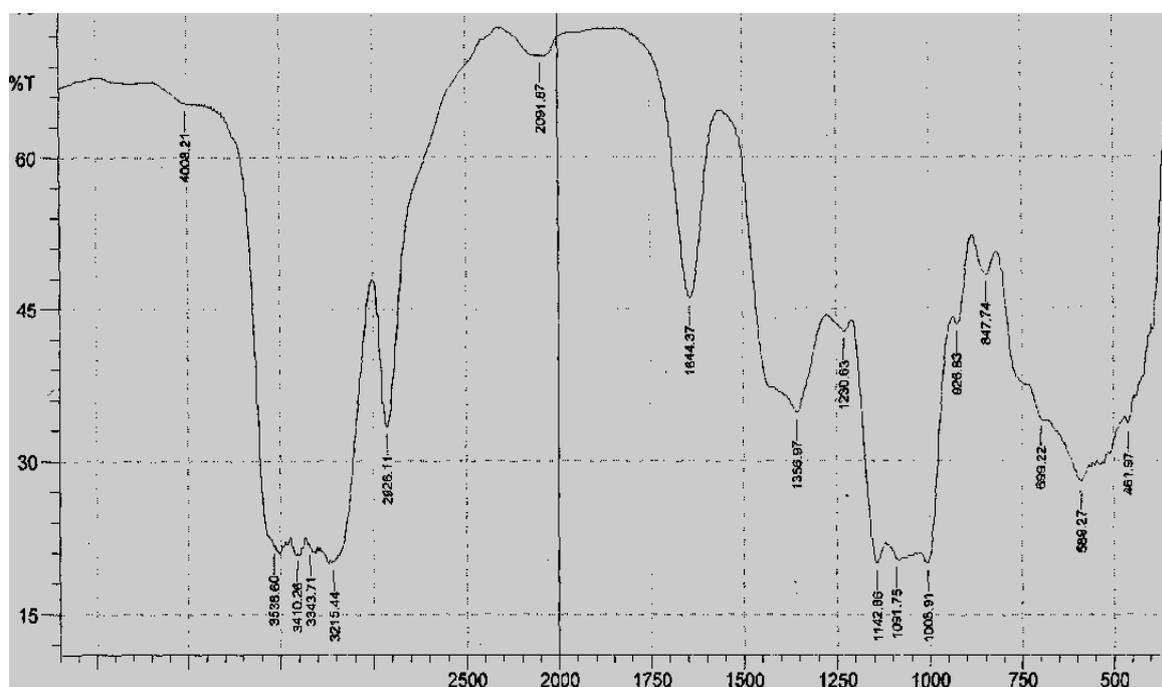


Fig 3: FTIR Spectrum of native *P.esculentus* starch

The FTIR spectrum of the starch xerogel showed the following absorption bands: 3144 - 3390 cm^{-1} represent (O-H stretch); 2940 cm^{-1} represent (C-H stretch); 1644 cm^{-1} is due to water in the amorphous region of starch; 1143 cm^{-1} represent coupling mode of (C-C, C-O stretch); 1036 cm^{-1} (C-O-H bending); 924 cm^{-1} represent (C-O-C), skeletal mode vibration of glycosidic linkage. 523 cm^{-1} and 641 cm^{-1} skeletal mode of pyranose ring.

The native starch of *P.esculentus* was gelatinized. The FTIR spectrum of the derivative gave the absorption bands here presented: 3010 - 3579 cm^{-1} represent (O-H stretch); 2886 cm^{-1} represent (C-

H stretch); 1617 cm^{-1} is due to water in the amorphous region of starch. The band at 1159 cm^{-1} represent coupling mode of (C-C, C-O stretch); 1085 cm^{-1} represent (C-O-H bending); 946 cm^{-1} represent (C-O-C), skeletal mode vibration of glycosidic linkage. 631 cm^{-1} skeletal mode of pyranose ring.

The *P.esculentus* starch was modified by HCl/ethanol hydrolysis. The FTIR spectrum of the resultant derivative showed the following absorption bands: $3127\text{-}3559\text{ cm}^{-1}$ represent (O-H stretch); 2938 and 2832 cm^{-1} represent (C-H stretch); 1251 cm^{-1} was attributed to (CH_2OH); 1197 cm^{-1} represent coupling mode vibration of (C-O, C-C); 1051 cm^{-1} represent (C-O-H bending) 926 cm^{-1} represent (C-O-C), skeletal mode vibration of glycosidic linkage. The bands at $673, 625, 578,$ and 488 cm^{-1} represent skeletal mode of pyranose ring.

FT-IR is a powerful technique for elucidation of structural changes in samples, with the ability of discovering differences not seen by certain other techniques because it has a unique region known as the finger print region where the position and intensity of bands is specific for every polysaccharide. The FTIR evaluation of starches in four main regions helps in the successive interpretation of the key bands. These regions are as follows; below 800 cm^{-1} , $800\text{-}1500\text{ cm}^{-1}$ (the finger print region), the region between 2800 and 3000 cm^{-1} (C-H stretch region), and finally the region between 3000 and 3600 cm^{-1} (O-H stretch region).

The spectra of starches show complex vibrational modes at low wave numbers below 800 cm^{-1} due to skeletal mode vibration of the glucose pyranose ring (Sekkal *et al.*, 1995). Therefore in this study, the major bands below 800 cm^{-1} from $625\text{-}581\text{ cm}^{-1}$ and minor bands between 560 and 400 cm^{-1} in the FTIR spectra of all the starches were attributed to the skeletal modes of the glucose pyranose ring.

The region between 800 and 1500 cm^{-1} (finger print region) is the empirical proof of identity of the samples and the pattern of vibration and band location is unique for each sample. Although it provides complex and overlapping spectra at this region making the exact assignment of band difficult. But the IR spectrum of polysaccharides in this region originates from the vibrational state of its monomer glucose (Cerna *et al.*, 2003). Therefore information obtained from glucose spectra is used in the assignments of wave numbers corresponding to the vibrational mode of starches. Since starches exhibit very similar spectra characteristics with glucose in this region.

In the FT-IR spectra of all the samples there was substantial chemical change within the starch structure which resulted in the change of intensity and shift of bands occurring in the 2800-3000 cm^{-1} range and 1000-1200 cm^{-1} where hydrogen bonding is apparent in the samples, and this could be attributed to the variations in the amount of amylose and amylopectin present in the starches. Also the C-OH band is moving to higher wave number in all the derivatives compared to the native starch, which means that the starch derivatives are moving from amorphous to semi crystalline state. (Van Soest *et al.*, 1995, Vicentini *et al.*, 2005).

However, very little changes in the peak location and intensity of the glycosidic linkage band occurring in the 900-950 cm^{-1} range was observed. This may be attributed to the presence of α -1, 6- linkage of the amylopectin that shift the band to higher wave number.

For the Gelatinized starch the band due to water absorbed in the amorphous region was observed at 1617 cm^{-1} because as the crystallinity of starch increases the band becomes weaker in infrared spectra. Also when starches are heated in hot water the starch granules swells as the water penetrates the granules and the hydrogen bonds weaken then they eventually burst releasing the amylose strain out of the granules into the water which thicken to form the gel. Addition of ethanol to the gel to form precipitate means exchanging the water in the gel with ethanol which has a lower surface tension compared to water. This reduces the capillary forces and makes the starch more hydrophobic by destroying the textural structure of the gel (Weissmuller *et al.*, 2010). This phenomenon explain why the starch xerogel was observed to dry within few minutes compared to the gelatinized starch which took days to dry even when placed in an air oven.

The HCl-Ethanol hydrolysed derivative showed four different bands representing the skeletal mode vibration of the glucose pyranose ring and this was due to the partial hydrolysis of the native starch which was expected since the partial hydrolysis of starches produces a disaccharide maltose together with low molecular weight dextrans.

The hydrolysis of the glycosidic linkage inside starch granules are influenced by three major factors; the availability of water molecules, the acid participating in the hydrolysis and the availability and reactivity of the glycosidic linkage. The mechanism for the acid hydrolysis of starch granules suspended in alcohol involves the hydrolysis of the glycosidic bond with the water originally inside the granules. It also degraded preferentially the amorphous regions of the starch during the hydrolysis which brings about the occurrence of cracks inside the granules and this explains the disappearance of the band representing water in the amorphous regions of the starch at

1644 cm^{-1} . Therefore the hydrolysis treatment significantly changed the inter and intra-molecular hydrogen bonding of the starch.

4.0 CONCLUSION

The present study suggests significant changes in proximate composition and structure of the native starch after chemical modifications. The starch derivatives had lower amounts of the starch contaminant (protein) which significantly affects starch functionality such as the swelling power and pasting properties. FTIR analysis also showed that there are substantial structural differences between the different derivatives of *P.esculentus* which may also affect the functional properties of the starch derivatives.

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