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EFFECTS OF ACETYLATION, CARBOXYMETHYLATION AND CROSSLINKING ON SOME PHYSICOCHEMICAL PROPERTIES OF STARCH FROM TUBERS OF *ICACINA SENEGALENSIS* AND *CYRTOSPERMA SENEGALENSE*

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ABSTRACT

Starch extraction from Icacina senegalensis (IS) and Cyrtosperma senegalense (CS) tubers was carried out with water at room temperature. The tubers of Icacina senegalensis and Cyrtosperma senegalense gave a starch yield of 40.9% and 25.5% respectively. The amylose, amylopectin composition of starches from IS and CS tubers were determined to be 10.8%, 89.2% and 24.6%, 75.4 % respectively. The starch extracts were chemically modified to produce cross-linked, carboxymethylated and acetylated starch derivatives. The formation of new chemical bonds in the starch derivatives was confirmed with Fourier Transform Infra-red spectroscopy (FTIR). Both swelling power and gelatinization temperature of IS starch and CS starch increased with acetylation, crosslinking and carboxymethylation while the degrees of substitution for the starch derivatives ranged between 0.33 and 1.45 making the native and modified starches suitable for many industrial applications.

Keywords: Acetylation; Carboxymethylation; Crosslinking; Icacina senegalensis; Cyrtosperma senegalense; amylose; amylopectin

INTRODUCTION

For many years, starch has been widely used as a bio polymeric raw material for many industries because it is green, cheap, ubiquitous and renewable. The increasing application of starch as food and in several other industrial processes has brought about an increased interest in starch as a source of polysaccharide (Otache, 2021)

Fears about food security makes researches on non-food starch sources to be highly welcomed. Non-edible tubers like *Icacina senegalensis* and *Cyrtosperma senegalense* readily comes to mind as good candidates (Ogunwa et al., 2016)

Even though starch in its native form serves well as food, it is limited in its application as industrial raw material due to some undesirable characteristics that are directly linked to its physicochemical properties. One of these characteristics is temperature and pressure instability. The desirable qualities and properties of starch can be achieved through the physical or chemical modification of native starch depending on the intended use. Starches can be modified to improve their functionality in a variety of applications such as to alter their resistance to heat, acid, shear, time, cooling, or freezing, alter their texture, reduce or increase their viscosity, extend or shorten the gelatinization process, or improve their visco-stability The physicochemical properties of starch determine its characteristics and subsequent industrial use as coatings, sizing materials in paper and textiles, as well as binders, adhesives, absorbents, as encapsulants in bone replacement implants,

bone cements, drug delivery systems and tissue engineering scaffold (Kiatkamjornwong *et al*., 2000; Ogunwa et al., 2022).

The chemical modification of starch involves the introduction of functional groups other than hydroxyl group into the starch molecules, resulting in changed behavior of the starch molecules. Chemical modification changes the functional group of starch from hydrophilic hydroxyl groups to other functional groups (Kavlani *et al*., 2012; Otache et al., 2021). The sites for starch chemical modification are the three reactive hydroxyl groups on each of the glucose units in amylose and amylopectin molecules (Zanella et al., 2019). The most common ways to modify starch chemically involve oxidation with various oxidizing agents, etherification (with the addition of alkyl, hydroxyalkyl and carboxyalkyl groups) esterification, (acylation, succinylation, phosphorylation) cationization with the introduction of cationic molecules, cross-linking with the addition of various cross-linkers and copolymerization (Nadir et al., 2015; Chen et al., 2020).

The current study will be focused on the evaluation of the physicochemical characteristics and industrial potentials of chemically modified and unmodified starches from *Icacina senegalensis* and *Crytosperma senegalense* tubers which are non-conventional sources of starch.

MATERIALS AND METHODS

Materials

The *Icancina senegalensis* tuber was harvested from a farmland in Umulogho Obowo in Imo State. The *Cyrtosperma senegalense* corms were harvested from a farmland in Umunze Obowo in Imo State. The plants were identified in the herbarium unit of the Department of Plant Science and Biotechnology, University of Port Harcourt.

Sample Preparation

Freshly harvested *Icacina senegalensis* tuber and *Cyrtosperma senegalense* tuber weighing 3.2kg and 2.3kg respectively, were washed, peeled and washed again. The *Icancina senegalensis* tuber and the *Cyrtosperma senegalense* tuber were milled separately into pulpy slurries, sieved and left to stand for about 12hours; after which the supernatant liquid was decanted. The starch was collected, air-dried for about 24hours, and reduced to powder by trituration.

Acetylation

The ratio variations between starch and acetic acid (g/ml) are 1:1, 1:2, 1:3 and 1:4. The reaction mixture was stirred for about 50 minutes and then allowed to stand for about 12 hours at room temperature. Concentrated H_2SO_4 was added to the mixture as a catalyst and then the reaction mixture was stirred at about 80°C for about an hour. The suspension obtained was kept at 70°C and stirred again for about 55 minutes. The ideal ratio of starch-to-acetic acid for the esterification is 1:2 (g/ml), as proved by the extent of substitution in accordance with the method of Xu et al. (2004) with slight modification. 4.0g of unmodified *Icacina senegalensis* starch was measured and mixed with 80 ml of glacial acetic acid. The mixture was stirred for about 12 hours at room temperature. Then a drop of concentrated H_2SO_4 was added as a catalyst to the starch-acetic acid mixture and the reaction mixture was stirred at 80°C for about 55 minutes. At the end of the reaction, the mixture was washed repeatedly with water at about 25°C to afford the starch- acetate which was air-dried for about 48 hours.

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Carboxymethylation

Carboxymethylation was achieved with the modified method of Nattapulwat *et al.* (2009) where 8g of the starch sample was dispersed in 240ml of distilled water and 14ml of 11.5M sodium hydroxide solution was added to the starch sample. Continuous stirring was maintained for 60 minutes after which solution of sodium monochloroacatate (180g/dm^3), was added to the reactant mixture which was heated at 50°C for about 2 hours. The pH of the reaction mixture was reduced to 5 using 50% acetic acid. The resulting carboxymethylated starch was recovered, washed with 80% ethanol and air-dried for 48 hours.

Starch Crosslinking

4g of each of the unmodified starch sample was dispersed in 125mL of distilled water. The cross-linking agent, sodiumtrimetaphosphate (3%) was added to the starch-water mixture. A pH value of 10.5 was maintained using sodium hydroxide solution (5%) (Kavlani *et al.*, 2012). The mixture is gradually but continuously stirred and heated at about 50^{o}C for 1.5 hours. The reaction was stopped by the addition of 1M HCl to reduce the pH of the reaction mixture to 5.5. Then the modified starch slurry was mixed with large amount of water. The 1M HCl(aq) was neutralized with 1M sodium hydroxide.

pH

About 0.5g of the starch sample was dispersed in 100ml of boiling water and thoroughly mixed. The starch paste is allowed to cool and its pH determined with a digital pH meter.

Water Absorption Capacity

4% w/v of the starch was dispersed in a centrifuge tube. The tube was agitated for three minutes in a centrifuge after which the supernatant was removed. The weight of the tube and hydrated sample was determined. The increase in weight was calculated and expressed as:

WAC = weight gained by water absorption x100/4

Temperature of Gelatinization

The method of Attama *et al*. (2003) was used where the temperature of gelatinization was read with the help of a thermometer

IR spectroscopy

The IR spectra of the starch were obtained using an FTIR spectrometer, SHIMADZU FTIR-84005 model

Amylose and Amylopectin Content

A 0.2g of each of the starches from the two tubers were weighed into separate test-tubes and 1.5ml of dimethyl sulfoxide (DMSO) was added following the methods of Yun and Matheson (1990). The test tubes were put in a steaming water bath to form starch dispersion. 5ml of ethanol was added to precipitate the starch. The test-tube was allowed to stay for 24 hours. The supernatant was thrown away, but the pellets were kept for further use. 3ml of DMSO was added to the starch pellets in a test-tube, and the test-tube was placed in a steaming water bath for 20 minutes. 5ml of Concanavalin A solvent was added to the test-tube once it was brought out of the steaming water. The test-tube contents were transferred to a 25.0 ml volumetric flask, and diluted to volume with Concanavalin A solvent. 0.lml of amyloglucosidase/α-amylase enzyme mixture was added to the reaction mixture.

5ml of glucose oxidase/peroxidase reagent was added to 20.0ml portion of the supernatant. The supernatant, the reagent

blank and the D-glucose controls were incubated at 41°C for 25 minutes simultaneously. The absorbance of the starch sample and the D-glucose controls at 510nm against the reagent blank was read off.

$$Amylose, \left(\%\frac{w}{w}\right) = \frac{\text{Concanavalin A Supernatant Absorbance}}{\text{Total Starch Absorbance}} \times 66.8$$

Amylopectin=100%-Amylose

RESULTS AND DISCUSSION

Amylose and Amylopectin Composition

The amylose and amylopectin content of *Icacina senegalensis* tuber starch were 10.8% and 89.2% respectively, while those of *Cyrtosperma senegalense* were 24.6% and 75.4 % respectively. Omojola *et al*, (2010) obtained similar results of 9.98% and 90.02% respectively for *Icacina trichantha* tuber starch. Erega *et al* (2014) explained that the amylose content of starch controls the gelling property of starch. Starches that contain low percentage of amylose thicken at lower temperatures than starches higher in amylose. This explains why *Cyrtosperma senegalense* starch (amylose 24.6%) had a higher gelatinization temperature (76°C) than *Icacina senegalensis* (amylose 10.8%) and gelatinization temperature 74°C. Therefore, *Cyrtosperma senegalense* starch with amylose content of 24.6% can also be used as a suitable substitute for maize starch as a binder in drug formulation.

Granule Size

The granule size range of *Icacina senegalensis* tuber starch was 10-50µm and that of C*yrtosperma senegalens*e was 5-15µm. These results are similar to the results obtained by Eraga *et al.*, (2014). They observed a size range of 10-20μm for maize starch with polyhedral or spherical shape. Similarly, Omojola *et al*., (2010) observed a size range of 10-15μm for *Icacina trichantha* starch granules which were oblong in shape. These observations explain why *Icacina senegalense* starch settled at a faster rate than *Cyrtosperma senegalense* starch. Therefore, *Icacina senegalensis* starch (10-50μm) can be used as a suitable substitute for maize starch (10-20μm) and rice starch (5-10μm) in pharmaceutical drug formulation as disintegrant, while *Cyrtosperma senegalense* tuber starch (5-15μm) can be used as a suitable substitute for maize and rice starches in pharmaceutical drug formulation as binder.

Swelling Power

The swelling power of unmodified, acetylated, cross-linked and carboxymethylated *Icacina senegalensis* tuber starch were 8.4, 9.2, 10.2 and 14.5 respectively. The swelling power of unmodified, acetylated, cross-linked and carboxymethylated *Cyrtosperma senegalense* starch were 5.1, 8.8, 10.8 and 12.5 respectively. This shows that acetylation, crosslinking and carboxymethylation all improved the swelling power of the two starch samples. However, it must be pointed out that the swelling power of starch decreases as the degree of crosslinking increases. Berry *et al*., (1998) reported reduced swelling power of cross-linked Oak starch as the degree of cross-linking increased. The decrease in the swelling power of starch as the degree of cross-linking increases is due to the formation of inter-molecular bridges within the starch chains by the cross-linking reagent (Bos, 1992). According to Singh *et al*, (2007) this effect becomes more evident in starches that have higher degree of cross- linking. The acetylated l*cacina senegalensis* tuber starch and acetylated *Cyrtosperma senegalense* tuber starch showed higher swelling power than the

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unmodified starches. Hence, acetylation and cross-linking increased the swelling power of the starches. Chowdary (2011) explained that higher swelling occurs in acetylated starches because the acetyl groups facilitate water absorption into the amylose region due to the intra-granular structural disorganization caused by steric effect.

Cross-Linking

The degree of substitution obtained for cross-linked *Icacina senegalensis* starch and cross-linked *Cyrtosperma senegalense* starch were 1.45 and 1.30 respectively. The IR spectra of the modified starches show new peaks occurring between approximately 900cm^{-1} and 1100cm^{-1}. These new peaks are due to PO_4^{3-}, P-O-C, and P=O which absorb IR within these regions The cross-linked starch samples could not form suspension in water at room temperature, but they were dispersed in 1:1 mixture of acetone and trichloromethane.

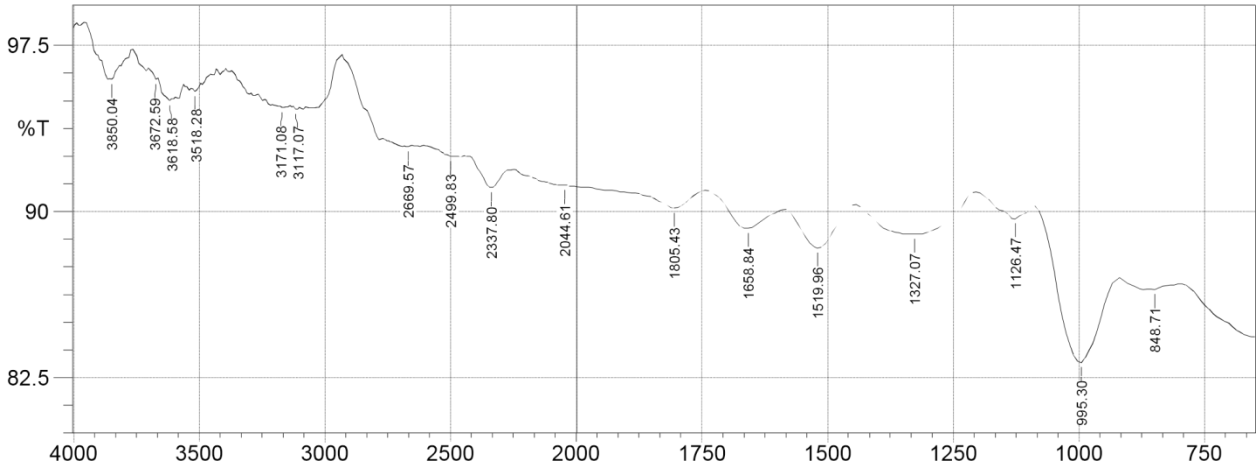

Fig. 1: IR spectrum of unmodified *Icacina senegalensis* tuber starch

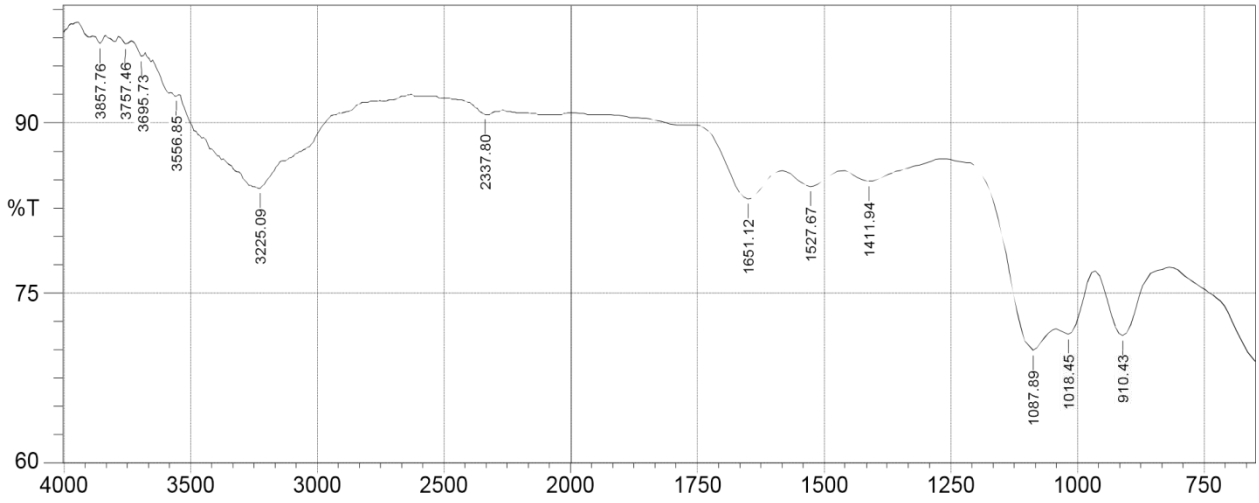

Fig. 2: IR Spectrum of Cross-linked *Icacina senegalensis* tuber starch

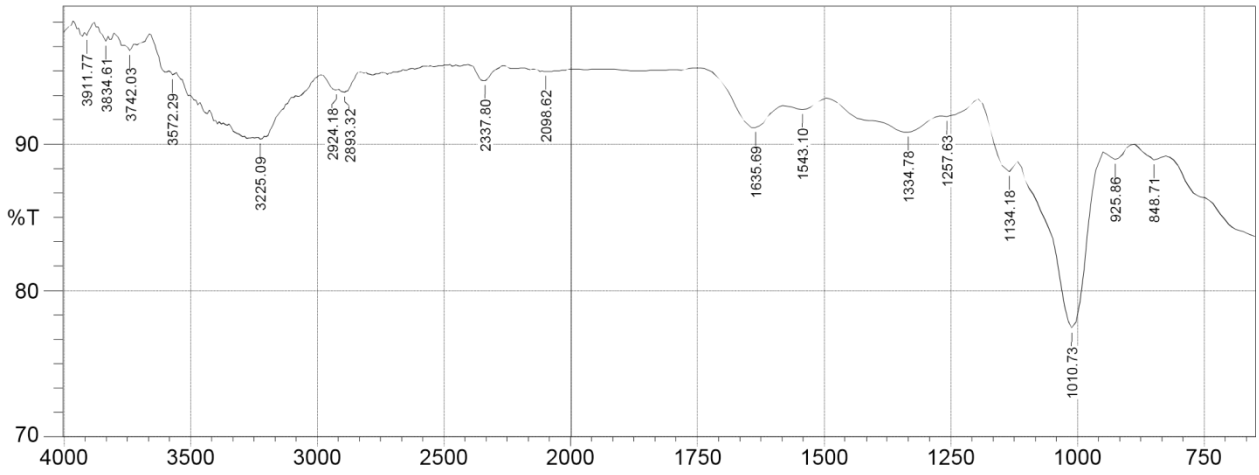

Fig. 3: IR spectrum of unmodified *Cyrtosperma senegalense* tuber starch

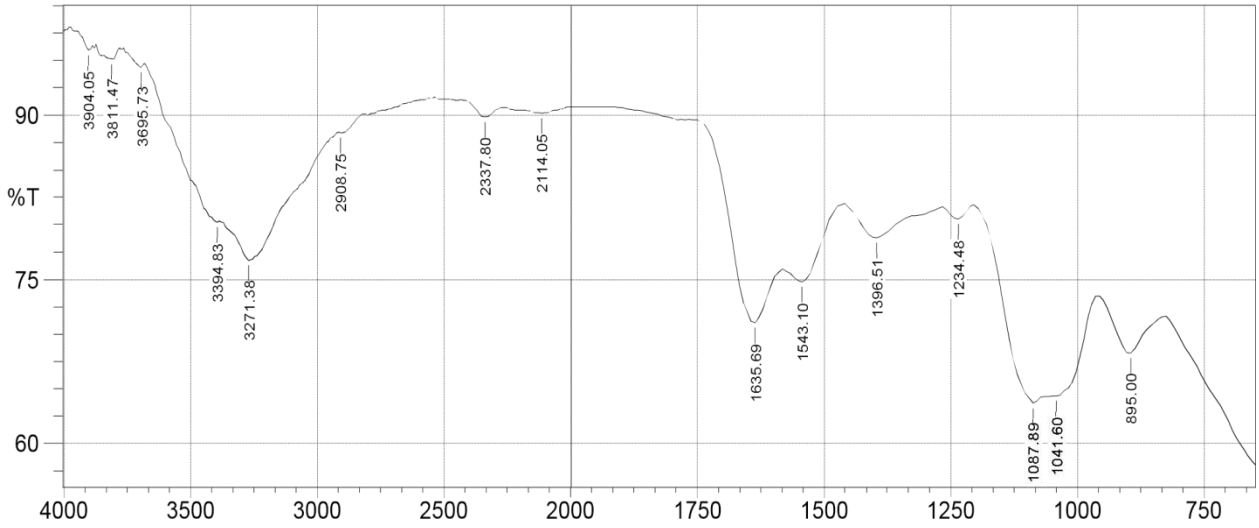

Fig. 4: IR spectrum of cross-linked *Crytosperma senegalense* tuber starch

The cross-linked starch from *Icacina senegalensis* had a swelling power of 10.2, which was higher than that of the unmodified starch which was 8.4. Hence, cross-linking increase the swelling power of starch. This is in line with the observation of Omojola *et al*, (2010). The cross-linked starch from *Cyrtosperma senegalense* had a swelling power of 10.8, against that of the unmodified starch which was 5.1. Chowdary *et al*. (2011) explained that the ability of a starch to be used as a disintegrant is determined by its swelling power. This shows that the cross-linked starch can be used as a better pharmaceutical disintegrant than the unmodified starch.

Acetylation

The degree of substitution obtained with the *Icacina senegalensis* starch was 0.3265, and 1.39 with *Cyrtosperma senegalense* starch. The acetylated *Icacina senegalensis* was slightly soluble in water but the acetylated *Cyrtosperma senegalense* starch was not soluble in water to any appreciable degree. Instead, it was able to form suspension with 1:1 mixture of acetone and chloroform with much stirring. The IR spectra of the acetylated starch samples showed new peaks at about 1643.41cm^{-1} for *Icacina senegalensis*, and 1651.l2cm^{-1} *for Cyrtosperma senegalense*. These new peaks are due to the stretching vibrations of the acetyl carbonyl group. The

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variation in the stretching and bending vibrations of O-H, C-O, and C-C bonds, as well as the absence of the 1651.12cm^{-1} and 1643.41cm^{-1} peaks in the FT-IR spectrum of the unmodified starch samples shows that the acetylation of unmodified starches actually occurred. The acetylated starches have higher swelling power because the acetyl groups facilitate water absorption into the amylose region due to the intra-granular structural disorganization which is caused by steric effect.

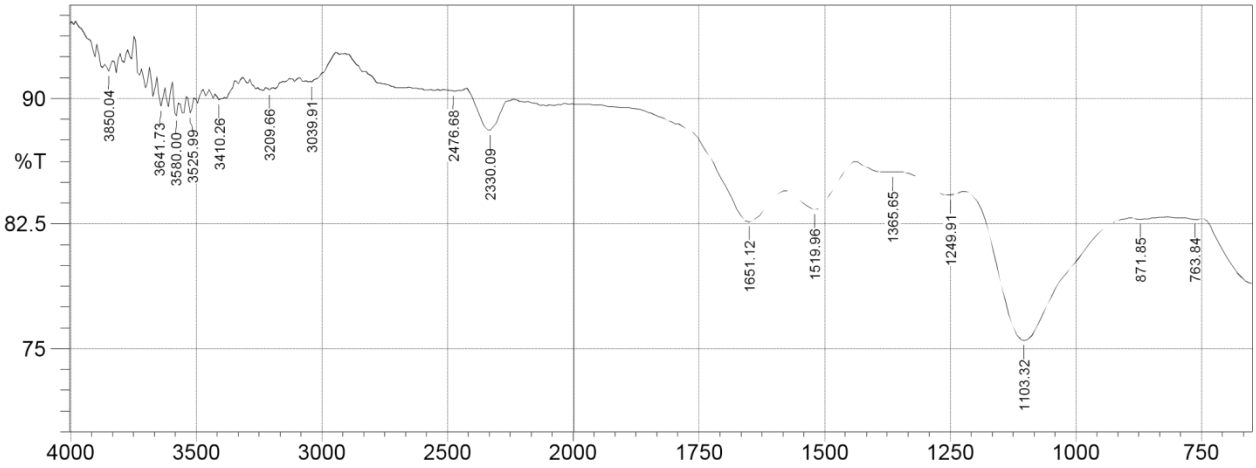

Fig. 5: IR spectrum of acetylated *Cyrtosperma senegalense* tuber starch

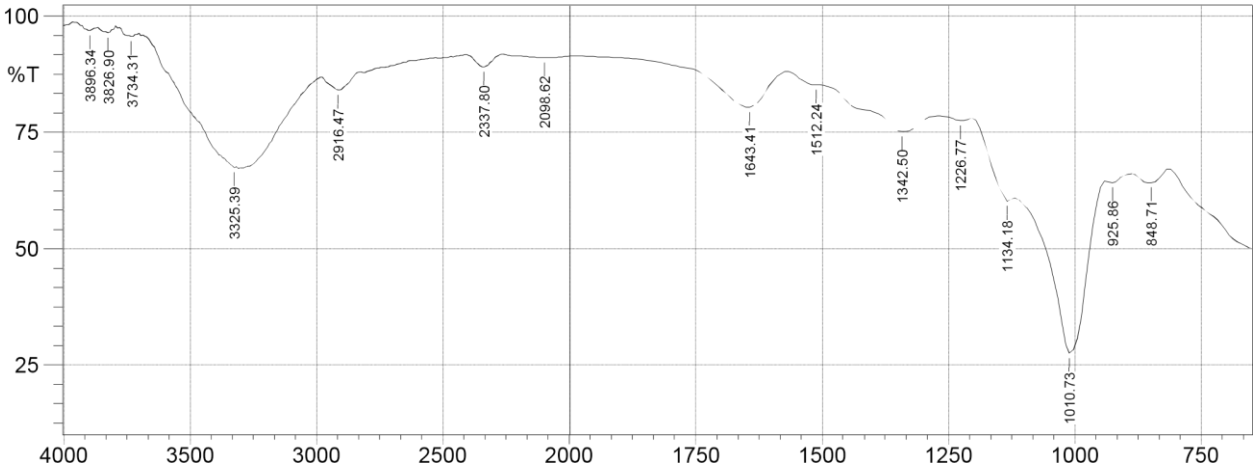

Fig. 6: IR spectrum of acetylated *Icacina senegalensis* tuber starch

The acetylated starch from *Icacina senegalensis* had a swelling power of 9.2, which was more than that of the unmodified starch, 8.4. This observation is similar to what was reported by Agbo and Odo (2010).

Carboxymethylation

The degree of substitution obtained in the carboxymethylation of *Cyrtosperma senegalensis* 0.70. The appearance of a new peak at 1026.16cm^{-1} and a strong absorption band at 1643.41cm^{-1} are due

to the carboxylic group, COO^-. A similar result was obtained for carboxymethylated starch from yarn (Nathapulwat *et al*, 2009).

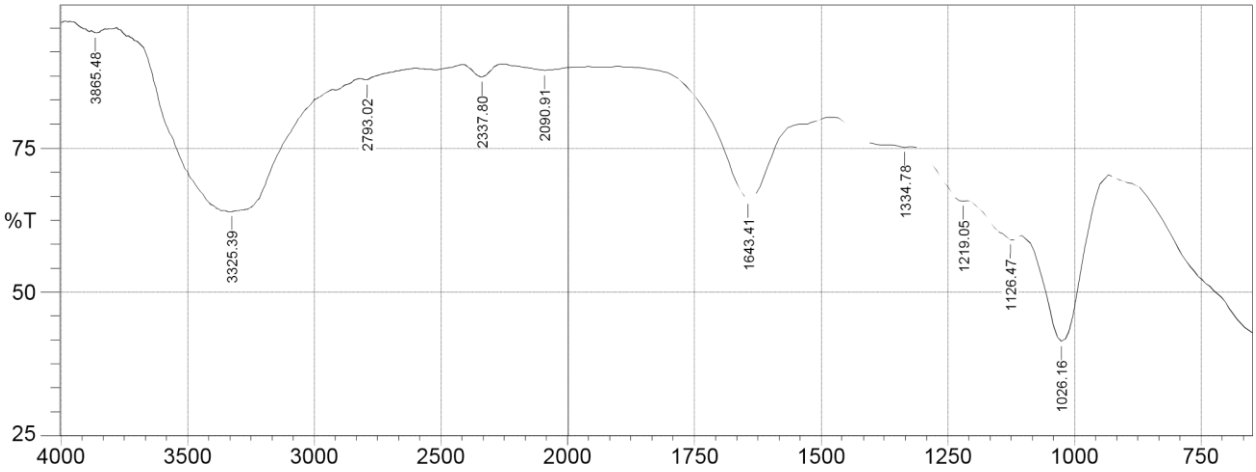

Fig. 7: IR spectrum of carboxymethylated *Cyrtosperma senegalense* tuber starch

The swelling power of carboxymethylated starch from *Icacina senegalensis* was 14.5 against that of the unmodified starch which was 8.4.

The swelling power of carboxymethylated *Cyrtosperma senegalense* tuber starch was 12.5, against that of the unmodified starch which was 5.1. The carboxymethyl substitution of the OH groups produces products which are cold-water soluble. Therefore, the carboxymethylated starches from tubers of *Icacina senegalensis* and *Cyrtosperma senegalense* obtained in this research can serve as suitable substitute for maize starch in producing cold-water soluble starch.

CONCLUSION

The amylose content of starch from cyrstosperma senegalense shows that it is a good candidate for use as binder for drugs. Their carboxymethylated, acetylated and crosslinked derivatives can serve as alternatives to starches that currently serve as binders, excipients and disintegrants in pharmaceutical and drug formulation industries. Acetylation and carboxymethylation improved the swelling power of the unmodified starch samples making them viable alternatives for use as cold water-soluble starches.

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