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PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERIZATION OF NATIVE AND STARCH-FREE *Cissus populnea*

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ABSTRACT

This current study is aimed at determining the effect of starch extraction on the physicochemical and rheological properties of *Cissus populnea* polymer and its suitability as a Pharmaceutical excipient. *Cissus populnea* polymer was obtained from the fresh inner bark of the stem of *Cissus populnea* Guill and Perr. Gum samples extracted were labelled native *Cissus populnea* polymer (nCPP). The starch content of nCPP was digested and subsequently labelled starch free *Cissus populnea* polymer (sfCPP). The yield for both extracts of the gum was high, with the sfCPP extract being a bit higher. The swelling index of sfCPP was higher than that of nCPP whereas moisture content and moisture sorption capacity of nCPP were higher. The sfCPP possessed higher fundamental characteristics that would make it a more suitable choice as a Pharmaceutical excipient when compared to nCPP. It is therefore recommended as a suitable Pharmaceutical excipient in the formulation of solid, semi-solid and liquid dosage forms.

Keywords: *Cissus populnea* polymer, pharmaceutical excipient, native and starch free polymer

INTRODUCTION

Pharmaceutical excipients are components other than the active pharmaceutical ingredient(s) which have been appropriately evaluated for safety and intentionally added to the formulation of a dosage form in order to achieve certain desired characteristics which make the dosage form suitable for administration to the patients (Patel *et al*., 2007). Excipients play a wide variety of functional roles which are crucial in the design of drug delivery systems, determining its quality and performance. Pharmaceutical excipients control the physicochemical properties as well as release profiles and availability of drugs from their formulated products. (Oyi *et al*., 2007). They must be non-toxic, free of any unacceptable microbial load and must be compatible with active pharmaceutical ingredients (Anekant *et al*., 2007).

Research into plant based pharmaceutical excipients is on the increase since plant products have been found to serve as an alternative to synthetic products because of its biocompatibility, non-toxicity, biodegradability, environmental-friendly nature and low prices compared to synthetic products (Patel et al., 2007; Meimei *et al*., 2007). Natural gums have been employed as disintegrants (Somboonpanyakul *et al.,* 2006), emulsifying agents (Nasipuri *et al*., 1997), suspending agents (Nasipuri *et al*., 1999), binders (Sinha and Kumria 2002) and sustained release formulations (Onunkwo and Udeala, 2003). Excipient characterization is a pre-formulation study which is an essential step in establishing the suitability of such excipient in dosage form design. It is a quantitative analysis which gives an insight into excipient behaviour and the key to successful formulation and processing is

identifying and controlling the parameters that define performance for any given application. Such parameters include optimization of flowability and compressibility. Some of the parameters usually characterized include particle packing, particle size, particle shape, surface area, density, porosity, flowability and compressibility.

Cissus populnea is a tropical plant belonging to the family Vitaceae. The plant is a tall woody climber of up to eight meters high, it is semi-circular and grows mainly in tropical regions of Africa, Asia, Australia, Central and South America, and North Mexico (Ojekale *et al*., 2006). It has a natural tendency of retaining water; thus it remains fresh almost throughout the season. It is gel forming and the gum is hydrocolloid and forms mucilage. Some of the common/local names of this plant include: 'Okoho' (Idoma and Igala tribes of Nigeria), 'Dafaaraa' (Hausa) 'Orogbolo ajara' (Yoruba).

Some drug formulation studies have been done on this plant by some researchers in Nigeria. Ibrahim *et al* (2002) investigated the mucilage obtained as a pharmaceutical excipient in tablet formulation Abioye *et al*. (2000; 2001; 2000), studied the emulsifying properties of *Cissus populnea* gum, invitro release kinetics of salicylic acid from C*issus populnea* gel and the stability effects of *Cissus populnea* gum in oil-in-water extemporaneous emulsions while Adeleye *et al*. (2011) evaluated the binding property of the gum in paracetamol tablet. However, there exists no information on the evaluation studies of the starch free *Cissus populnea* polymer. This study is aimed at determining the physicochemical properties of the starch free gum and its potential as a polymeric excipient in pharmaceutical formulations.

MATERIALS AND METHODS

Materials

The materials used in the investigation were C*issus* gum extracted in Pharmaceutical Laboratory, University of Jos, Nigeria. All other solvents and chemicals used were of analytical-reagent grade.

Extraction of Gum

The fresh inner bark from the stem of *Cissus populnea* was washed thoroughly with distilled water and then shredded. The shredded material was macerated under ambient conditions in 0.1 % w/v sodium metabisulphite for 24h. The swollen gum was separated from the residue by filtration through a muslin bag. The filtrate was precipitated from solution using absolute ethanol. The precipitated polymer was washed repeatedly with more ethanol to remove all water content until the gum became brittle. It was then dried in a hot air oven at 60^0C for 1 h, the dried mass was blended to fine powder, passed through sieve number 250 (Fisher-brand test sieve UK) and stored in an air tight amber coloured bottle and labelled nCPP.

Starch Digestion

The starch content in nCPP was digested according to the method of Nep *et al*., (2016). Briefly, 3000 ml of 1 % w/v dispersion of nCPP were treated with termamyl 120 Litres (1 % v/v) (sigma Life Sciences) while stirring constantly at 70^0C for 4 h. The termamyl was pre-treated by heating at 70^0C for 30 min to deactivate any pectinoses and arabinoxylanases. Every 1 h an aliquot of the dispersion was removed and tested for the presence of starch. Starch digestion was complete in 3 h after which the sample did not test positive for starch. Subsequently, protein from the sample was precipitated by adjusting the pH to 4.5 with 2 M HCl and centrifuging at 4400 revolutions per minute (rpm) for 20 min. The recovered starch free nCPP was

further washed with absolute ethanol to get a brittle polymer precipitate. The precipitate was oven dried for 1h at 60^0C. This sample was named sfCPP.

Evaluation of Physicochemical properties of the *Cissus* gum

Moisture content

The moisture content of the nCPP and sfCPP were determined by weighing accurately 5 g each in a tarred evaporated dish on a mettler AB54 Electronic balance (Mettler, A.G., Switzerland). This was then dried in a Gallenkamp size two oven BS at 105^0C for 5h and the final weight noted (Malviya, 2011). The percentage weight loss was calculated using the following equation:

Percentage moisture content = weight of wet sample/weight of dry sample x 100% [1]

Moisture Sorption Capacity of Cissus Gum Extract

Two (2) g each of nCPP and sfCPP were weighed and evenly distributed over the surface of a 70 mm tarred petri dish and placed in a large desiccator containing distilled water in its reservoir. The desiccator was stored at room temperature at various time intervals over a period of five days. The weight gained by the exposed sample was recorded and the amount of water sorbed was calculated from weight difference (Ohwoavworhua *et al.,* 2004).

Swelling Capacity

The swelling capacity was determined by weighing accurately 1 g each of nCPP and sfCPP into a 25 mL glass-stoppered graduated measuring cylinder and the volume occupied, Vo, was noted. About 20 mL of distilled water was added and the cylinder closed. This was shaken vigorously every 10 minutes for 1h and then allowed to stand for 6h at room temperature (Odeniyi, *et* al; 2011). The volume, v_s, occupied by the sample including any adhering mucilage was noted and the swelling capacity was calculated using Equation (2).

Swelling capacity = V_s/V_o X 100 [2]

Evaluation of Bulk and Tapped Densities of the Powders

The volumes of known quantities of nCPP and sfCP polymers were obtained before and after tapping. The volume before tapping was used to determine the bulk density while the volume after tapping was employed to determine the tap density mathematically. Furthermore, Hausner's quotient and Carr's compressibility Index properties of the polymers were obtained from the equations:

Hausner's quotient = Tapped density/Bulk [3]

Carr's compressibility = Tapped density – Bulk density/Tapped density [4]

Particle Density

The particle densities of the nCPP and sfCPP were determined by the pycnometer method using liquid immersion technique with xylene as the displacement liquid (Odeniyi, *et al.,* 2011). A 50ml pycnometer bottle was weighed when empty (W) with the stopper. This was filled with xylene to the brim till it overflows and excess was wiped off, and the weight with the stopper was noted as (W_1). The difference between this weight and the first was recorded as (W_2). A 2 g quantity of

the *Cissus* gum was weighed (W_3) and quantitatively transferred into the pycnometer bottle and filled with the solvent to the brim. The excess solvent was wiped off and the bottle weighed again with the stopper (W_4). The particle density, P_t, was calculated from the following Equation:

$P_t = W_2.W_3/50(W_3-W_4+W_2+W)$ [5]

Angle of Repose

The angle of repose was determined by using the method adapted by Iwuagwu and Onyekweli (2002). The *Cissus* gum powders were allowed to fall freely through a funnel onto a plain white sheet of paper, placed on a flat surface until the apex of the cone formed by the powder just touched the tip of the funnel clamped to a retort stand with its tip 2cm above the paper. The diameter of the base of the powder cone was obtained and the angle of repose was calculated using the Equation:

$\text{Tan } \Theta = h/r$ [6]

Where h is the height of the heap of powder, r is the radius of the cone and Θ is the angle made by the heap with the base.

Phytochemical Screening of *Cissus* Gum

Phytochemical screening tests were performed on the *Cissus* gum to determine the presence or absence of starch, sugar, saponin and some other secondary metabolites such as alkaloids, glycosides, tannins, quinones etc., according to the method described by Trease and Evans (2005).

Microscopy

The gum samples were spread over a glass slide placed under a light microscope (Model BH-2, Olympus Optical Co). Micrographs of the shapes of the gums (sfCPP and nCPP) were taken.

Fourier Transform Infra-Red (FT-IR) Spectroscopy

FT-IR spectroscopy of the gum samples (sfCPP and nCPP) were taken using the appropriate instrument (Perkin Elmer Spectrum version 10.03.09).

Statistical Methods

We used descriptive statistics of mean and standard deviation to describe the distribution of the variable after an initial exploratory analysis. The mean of the different parameters was then compared within different measurements and between different binder types using the one-way analysis of variance method (ANOVA). F-Statistics and P-values were reported for the comparison of means. The mean of various parameters was visualized using appropriate charts. All the statistical analyses conducted on the data gathered from this research were performed using the IBM SPSS statistics for windows, version 20 (IBM Corp, Armonk, New York, USA). The alpha (α) level was set at 0.05 and P-value < 0.05 were considered statistically significant.

RESULTS

The physicochemical properties of native *Cissus populnea* (nCPP) and starch free *Cissus populnea* (sfCPP) polymers are presented in Table 1. The colour of the gum ranges from brown to dark brown. The percentage yield of nCPP was 65 % w/v while that of sfCPP was 66.52 % w/v.

Table 1: Physicochemical and Rheological Characteristics of nCPP and sfCPP

Descriptive	Binder Type	Mean ± Std. Dev (n = 10)
Moisture content (%w/w)	nCPP	10.73 ± 0.04
	sfCPP	9.64 ± 0.24
Moisture Sorption capacity(%w/w)	nCPP	54.47 ± 0.14
	sfCPP	42.14 ± 0.11
Angle of repose (^{0})	nCPP	38.66 ± 0.09
	sfCPP	29.71 ± 0.01
Bulk density (g/cm3)	nCPP	0.24 ± 0.001
	sfCPP	0.38 ± 0.004
	sfCPP	0.45 ± 0.003
Particle density (g/cm3)	nCPP	2.10 ± 0.002
	sfCPP	1.91± 0.01
Hausner's ratio	nCPP	1.28± 0.001
	sfCPP	1.18 ± 0.001
Carr's Index (%)	nCPP	21.88 ± 0.01
	sfCPP	15.39 ± 0.01
Swelling ratio in 0.1N HCl	nCPP	3.57 ± 0.13
	sfCPP	4.01± 0.01
Swelling ratio in phosphate buffer 7.4	nCPP	4.01 ± 0.01
	sfCPP	5.01± 0.01
Swelling ratio in Water	nCPP	4.50 ± 0.07
	sfCPP	6.09 ± 0.11
Loss on drying (%)	nCPP	0.91 ± 0.01
	sfCPP	0.71± 0.01
Total ash (%)	nCPP	3.10 ± 0.11
	sfCPP	2.08 ± 0.08
Acid insoluble ash (%)	nCPP	1.08 ± 0.10
	sfCPP	1.08 ± 0.09
pH	nCPP	5.24 ± 0.12
	sfCPP	7.63 ± 0.08

Solubility: sfCPP and nCPP, slightly soluble in water, practically insoluble in ethanol, acetone and chloroform.

Table 2: Phytochemical screening of *Cissus populnea* gum extracts

Phytochemical	ncpp	sfcpp
Molisch test	Present	Absent
Iodine test	Present	Absent
Ferric Chloride test	Absent	Absent
Fehling's solution test	Absent	Absent
Shinoda test	Absent	Absent
Wagner's test	Present	Absent
Keller-Kiliani test	Absent	Absent
Ruthenium red test	Present	Present
Ninhydrin test	Absent	Absent

Figures 1 and 2 show the micrographs of nCPP and sfCPP respectively. Figures 3 and 4 are FT-IR spectrums of nCPP and sfCPP respectively.

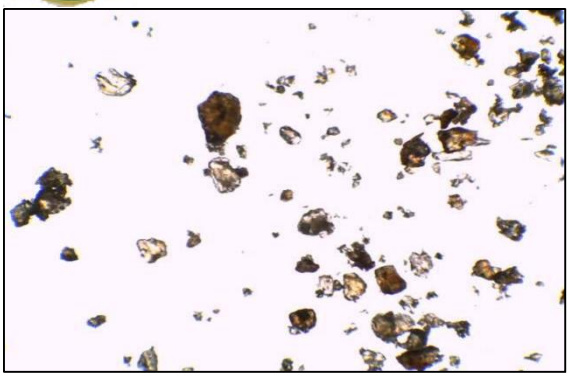

Figure 1: Micrograph of nCPP

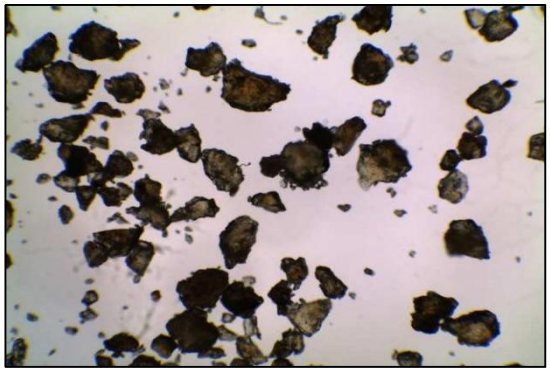

Figure 2: Micrograph of sfCPP.

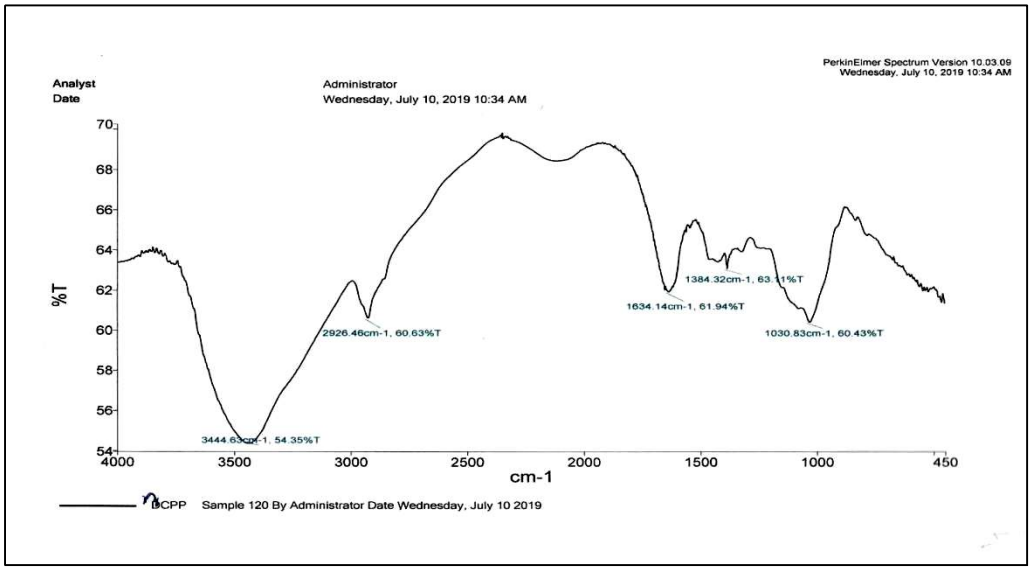

Figure 3: FT-IR Spectrum of nCPP

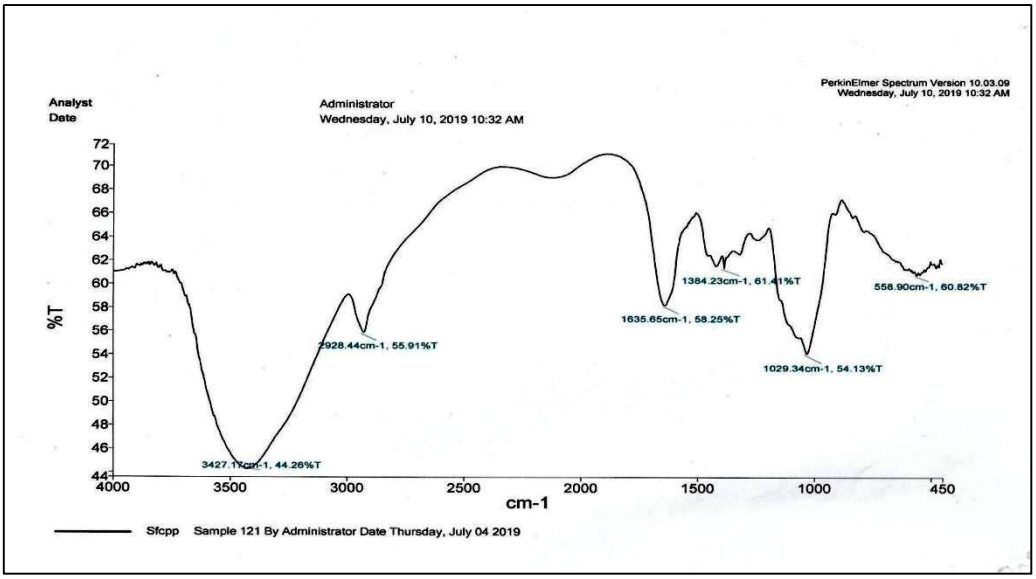

Figure 4: FT-IR Spectrum of sfCPP

DISCUSSION

This study is specific for extraction of gum from *Cissus populnea*, here referred to as the native *Cissus populnea* polymer (nCPP). The native gum further digested to remove the intracellular starch from the native polymer here referred to as starch free *Cissus populnea* (sfCPP). The yield for both gums is considered high enough for natural products (Olutayo *et al*., 2005) and thus desirable for use as excipient in pharmaceutical industries. The further digestion is aimed at presenting more functional and efficient gum since their features were found to vary significantly from each other. The viscous solution obtained from the incised stem of *Cissus populnea* is colourless while after precipitation and drying, the native polymer appeared brown while the starch free polymer was dark brown.

The gum extracts of nCPP and sfCPP are slightly soluble in water, and practically insoluble in ethanol, acetone and chloroform. The swelling characteristic of the extracts in different media; 0.1N hydrochloric acid, phosphate buffer (pH 7.4) and water. The swelling was highest in water followed by phosphate buffer and least in 0.1N HCl. Generally, sfCPP shows higher swelling index suggesting that the gum may perform better as binder/disintegrate/matrixing agent (Emeje et al., 2009). The gum is a pH responsive polymer; it is therefore a "smart polymer" and may find application in controlled release dosage formulation (Emeje et al., 2009). Swelling is a primary mechanism in diffusion controlled release dosage form (Jain *et al.,* 2004)

The total ash and acid insoluble ash value of sfCPP was found to be 2.080 and 1.080%w/w respectively. Ash values reflect the level of adulteration or handling of the drug. Adulteration by sand or earth is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. Therefore, the low values obtained in this study indicate low levels of contamination during gathering and handling of crude *Cissus populnea* (BP, 2004). The results show that sfCPP which gives lower values of total ash and acid insoluble ash is purer than the nCPP.

The angles of repose of both nCPP and sfCPP extracts were 38.6630 and 29.7090 respectively. This is an indication that sfCPP has excellent flow while nCPP has fair flow (Olorunsola, 2021). The angle of repose is usually affected by particle shape, particle size and size distribution among other factors. The angle of repose can indicate the cohesiveness of a powder material (Al-Hashemi *et al.,* 2018).

The percentage compressibility (Carr's Index) is a quantitative descriptive assessment of the compressibility and flowability of a powder while Hausner's ratio is indicative of interparticle friction. As the values of these two parameters increase, the flow of the powder decreases. The sfCPP having Carr's Index of 15.3920% and Hausner's ratio of 1.1808 indicates good flow whereas; nCPP with an index above 21% and Hausner's ratio of 2.0954 shows poor flow (OLorunsola, 2021).

The moisture content in a powder may affect the frictional properties of the compact formed. The formation of moisture film may reduce friction at the die wall by acting as a lubricant thus decreasing tablet adhesion to the die wall. Starch free (sfCPP) when used in tablet formulation will ease tablet ejection better than when nCPP is used. The low moisture content of sfCPP suggests its suitability in formulations containing moisture sensitive drugs. Given suitable temperature, moisture will lead to the activation of enzymes and the proliferation of microorganisms thereby affecting the shelf life of most routine formulations. It is important to investigate the moisture content of a material because the economic importance of an excipient for industrial

application lies not only on the cheap and ready availability of the biomaterial but the optimization of production process such as drying, packaging and storage (Emeje *et al.,* 2008).

Moisture sorption capacity is a reflection of the relative physical stability of tablets made from the polymer when stored under humid conditions. The high value of the moisture sorption capacity of *Cissus* gum is an indication that they are sensitive to atmospheric moisture which suggests that this may undermine the stability of hydrolysable constituents of a solid dosage form if used as excipient in that formulation. *Cissus* gum should be stored in air tight containers since they are susceptible to moisture sorption at atmospheric condition.

A 1 % w/v suspension of sfCPP in water gave a pH of 7.7; the near neutral pH implies that when used in uncoated tablets, it may be less irritating to the gastro intestinal tract. It may also find useful application in formulation of acidic, basic and neutral drugs. Knowledge of the pH of an excipient is an important parameter in determining its suitability in formulations since the stability and physiological activity of most preparations depends on pH (Nasipuri *et al.,*1996).

The phytochemical screening revealed absence of starch in the starch free polymer while the same test showed presence of starch in the native polymer. This indicates that the process of starch digestion was complete.

The micrograph of both sfCPP and nCPP extracts show that the shape of the gum is polygonal. It has been shown that the compaction characteristics of powders are affected by the particle shape of powders (Ohwoauworhua *et al*., 2004). Since the particle shape of *Cissus* gum is polygonal, it shows that it has a higher tendency to fragment during compaction thus better compatibility.

Fourier Transform Infra-Red (FT-IR) spectroscopy is an important tool for obtaining information about the crystallinity, organization and structural arrangement of a polymeric material from analysis of its IR spectrum. Generally, spectral changes can be classified into changes in the intensity of absorption of specific bands and band narrowing [Sun et al., 2014a]. Band narrowing can be caused by changes in the ordering/arrangement of the polymers and a reduction in the number of conformations while changes in band intensity usually result from changes in specific conformations, such as long-range ordering and crystallinity [Sun et al., 2014b]. In this study, the FT-IR spectra of the native and enzyme hydrolyzed starch had similar appearances which indicates that no additional groups emerged after enzymatic treatment. The broad bands from 3100–3700 cm^{-1} represent the vibrational stretches of intermolecular hydroxyl groups [Jiang *et al*, 2011; Mathew and Abraham, 2008]. The native *Cissus populnae* starch (nCPP) and the α-amylase enzyme hydrolyzed *Cissus populnae* starch (sfCPP) showed the same broad bands from 3100–3700 cm^{-1} (see FT-IR spectra), but the hydrolyzed *Cissus populnae* starch (sfCPP) showed a greater absorption intensity as compared to the native starch (nCPP). This could be attributed to changes in conformation and crystallinity. The sharp bands at 2926 and 2928 cm^{-1} are due to the C-H asymmetric stretching vibration [Fang et al, 2002] for the native and hydrolyzed starch. The band at approximately 1635 cm^{-1} is a sign of firmly bound water present in the native and enzyme hydrolyzed starches. The absorption peaks in the 820–1280 cm^{-1} region can be attributed to highly coupled C-O and C-C stretching vibration (Likhitkar and Bajpai 2012). Comparing the native starch to the enzyme hydrolyzed starch, band intensity was increased but there were no significant changes in band width after hydrolysis. The

band at 1030 cm^{-1} is characteristic of the anhydrous glucose ring C-O stretch [Mu et al., 2015]. The observation that the enzyme hydrolyzed starch tested negative to Iodine indicates the complete absence of amylose which has been completely hydrolyzed by the alpha amylase enzyme. Reduction in the linear chains present in the structure of starch usually results in reduced crystallinity. This is supported by the observation in the FT-IR spectrum of sfCPP which shows more intense absorbance at 1030 cm^{-1} as compared to nCPP. Previous reports indicate that the infrared (IR) absorbance band of starch around this region is related to the structural feature of the crystalline region and represents the ordered structure of short-range molecules (Wu et al., 2020). FT-IR absorption bands at 928 - 930 cm^{-1} are attributed to the glycosidic linkages in starches while the absorption bands at 1020 - 1250 cm^{-1} are related to the CH_2OH function as well as the C–O–H deformation mode.

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