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Characteristics of Starch Extracted from the Stem of Pineapple Plant *(Ananas comosus)* - an Agro Waste from Pineapple Farms

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HIGHLIGHTS

- Extracts and characterizes the starch from the stem of the pineapple plant.
- Pineapple stem starch shows small granule size and high gelatinization temperature.
- It possesses A-type crystals and is more viscous compared to corn starch.
- Results indicate the usefulness of this agro-waste starch in food industries.

Abstract: The present study focused on the use of pineapple plant stem, which is an agro-waste, for the production of starch (11.08 % ± 0.77). Characters were studied using X-ray diffraction, nuclear magnetic resonance spectroscopy (NMR), fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and rheological methods. The granular size of stem starch was comparatively smaller than corn starch granules. The X-ray diffraction data revealed that stem starch has an A-type crystal structure. The molecular structure was similar to those obtained for native starches, which is confirmed by NMR and FTIR. The gelatinization temperature was observed to be higher than corn starch and rheological studies revealed; stem starch is more viscous than corn starch. The purity analysis showed that the harmful heavy metals were in negligible quantity and the tested pesticides were absent. This could make this a good source of starch for food industries. Results revealed that this agro-waste has a high potential for the production of good quality starch.

Keywords: pineapple plant stem; unconventional starch; agro-waste; characterization; applications.

INTRODUCTION

Starch is the primary source of carbohydrates in the human diet which is made up of amylose and amylopectin. Amylopectin (70–80 %) is a semi-crystalline, highly branched polysaccharide with an α-1,4 linked glucose units and 4–5 % α-1,6 branch points, while amylose (20–30 %) is amorphous in the native

starch granule and is composed of a single or a few long chains of α-1,4 linked glucose units. The major starch sources are corn, wheat, rice, potato, tapioca, etc. Properties of starch and its uses depend on its biological origin, and its compositions are unique for each botanical source [1,2]. Native starches are highly variable in their structure and properties [3]. Characterization of starch from new sources has much importance as it helps to find out their specific application in various industries. New food materials from starches are produced as a result of several characterization studies [4].

To make new food products, there are several challenges for manufacturers, as the chemistry and textural characteristics of starches are to be considered. The microstructures, mechanical properties and the nutritional qualities of starch-based materials strongly depend on the structure and properties of row starch and the processing methods used [5,6]. There are increasing trends towards the production of biodegradable starch derived products [7]. To meet these demands, many industries depend on food crops. This is not a sustainable solution because this may lead to the overexploitation of food crops, which will affect food security. Hence the sustainable alternatives like food extracting from agro-wastes will be one of the appreciable solutions. This study was designed to isolate starch from the stem of pineapple plant, which is an agro-waste and to investigate its properties to provide scientific inputs aiming their effective utilization.

The pineapple plant is a herbaceous perennial plant and cultivated, 909840 hectares in the world. The major pineapple producing countries are India (89000 hectares), Brazil (54070 hectares), Thailand (93310 hectares), Philippines (58550 hectares), Costa Rica (45000 hectares) and China (57300 hectares). In Kerala, 10200 hectares of land is utilized for its cultivation [8]. To make maximum use of forage from pineapple plant including stem and to explore its functional properties suitable for specific applications, we have characterized starch from this plant stem. The objective of this study was to isolate and characterize starch from the stem of the pineapple plant that may provide an insight into its usefulness in human nutrition.

MATERIALS AND METHODS

Isolation of starch

The pineapple plant stems were collected after the harvest from pineapple farms, washed with water and mild acid to remove soil and other debris. The stem was then ground in a mixer grinder with distilled water and filtered through a double-layered cloth. The steps were repeated for several times until the milkiness of the slurry disappeared or became minimal, then slurry was centrifuged and the supernatant was discarded. The residues obtained were washed with 60 % alcohol, 0.1 N NaOH, and distilled water. The centrifuged residues were dried at 40 ºC, powdered and passed through a standard sieve (75 µm), collected and stored in desiccators.

Fractionation based on solubility

Fractionation of stem starch in four different solvents (hot water, cold water, alkali and DMSO) was done by the method explained by RunCang Sun and Jeremy Tomkinson. Iodine-absorption spectra of fractioned sample were also observed [9].

Turbidity measurement

Turbidity development in pineapple stem starch and corn starch were observed by the method explained by Kaur and coauthors 2004 [10].

X-ray diffraction (XRD) study

X-ray diffraction (XRD) studies were carried out by an X-ray diffractometer (XRD-RigakuMiniflex 600) operating at 40 mV, and 15 mA. CuKα radiation (1.54 Å) was used. The radiation angle, two θ was set from 5 º to 60 º at a scanning rate of 10 deg/min. The relative crystallinity of samples was measured using the method of Nara and Komiya [11].

Nuclear magnetic resonance spectroscopic (NMR) study

Solid-state ^{13}C CP/MAS spectra were collected at x frequency of 100.5 MHz on a DELTA2_NMR spectrometer (JNM-ECX400II) operating at 25 ºC. 9.38976 [T] field strength was used and a spin set at 15

Hz, x 90 pulse width was 2.8 µs with a recycle time of 5 s. A contact time of 3500 µs was used for all samples; the filter width was 18 kHz. Total scans were 1028 with dimension 1.

Amylose estimation

It was done by the iodine method using standard amylose solution [12].

Fourier transform infrared spectroscopic analysis (FTIR)

FTIR spectra were recorded using an FTIR 4100 JASCO model instrument (FT/IR-4100typeA and serial number-B076161016) and compared with corn starch. 5 mg of powdered sample was blended with potassium bromide (KBr) and made a pellet and used for this study. The resolution was 8 cm^{-1}, and the range of wavenumber 4000-400 cm^{-1} was used [13].

Scanning electron microscopy (SEM)

The surface and structure of native starch were characterized using a scanning electron microscope (Carl-ZEISS Gemini SEM 300), using a secondary electron detector with 2.00 kV of acceleration. (Magnification 10.00 K X).

Thermal characterization

Thermal properties were studied by a differential scanning calorimeter (DSC) (Perkin Elmer DSC 4000). The sample (5 mg) and the water (50 µL) was directly weighed into the aluminum pan and sealed with its lid. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), the enthalpy change of gelatinization (ΔH) and the transition temperature interval (ΔT) were calculated with the scanning temperature range of 40–120 ºC (heating rate-10 ºC/min) [14, 15]. An empty pan with lid was used as a reference [16]. Data collection and analysis were performed using the Perkin Elmer Pyris software.

Rheological studies

A dynamic rheological measurement was made with a rotational rheometer (Physica MCR 51). 1g (10 %) of the sample was used, the frequency was set from 0.1 to 10 Hz and the dynamic rheological properties, such as storage modulus (G'), loss modulus (G''), complex viscosity (η*) and phase angle (δ) were determined and compared with commercial corn starch.

Heavy metal and pesticide analysis

The heavy metal analysis was performed by an Energy Dispersive X-ray Fluorescence Spectrometer (ED-XRF) Model No.XEP05, AMETEK-SPECTRO, and pesticide analysis was by gas chromatography (GC-Model No. Agilent 7890A). GC-FPD (gas chromatography with flame photometric detector) is used for organophosphate pesticides, and GC-ECD (gas chromatography with electron capture detector) is used for organochlorine pesticides.

Statistical analysis

IBM SPSS Software v 21, Microsoft Office Excel 2007 and OriginPro 8.0 were used to analyse the experimental data.

RESULTS AND DISCUSSION

Starch yield

Starches from different botanical origins vary in their physicochemical properties. The most important factor differentiating the physicochemical properties of starches is their unique chemical and physical structure [17]. Starch yield from the pineapple stem was 11.08 % ± 0.77 on a wet basis. The stem has an average weight of 500 g, and from one plant the yield will be 55 g. This is appreciable when considering the enormous amount of pineapple agro-waste accumulating every year. Bello-Perez and coauthors reported that one variety of banana starch contains 11.8 % of starch [18]. One study from Thailand reported that the pineapple stem contains 9 % of starch in wet basis [19].

If we develop an economically feasible small scale non-polluting technology for the extraction of starch from this agro-waste, we can produce at least 3 tonnes of starch per hectare of pineapple farm (considering the plant density of 63400 plants/ha.).

Fractionation based on solubility

Fractional isolation revealed that the stem has 4.75 % ± 0.41 of cold water-soluble fraction, 9.00 % ± 1.78 of hot-water-soluble fraction, 75.00 % ± 1.26 of DMSO soluble fraction and 6.00 % ± 2.00 of alkali soluble fraction. Cold water soluble fraction showed maximum iodine absorption at 640 nm, hot water soluble fraction at 600 nm, alkali soluble fraction at the range of 400-460 nm and DMSO soluble fraction showed a maximum at the range of 500-600 nm (Figure 1). The study of Sun and Tomkinson on sago pith starch reported that the absorption spectra of water-soluble starch fractions showed maximum absorption around 600 nm, is the absorption range of amylose, whereas the spectrum of DMSO-soluble starch showed higher absorption between 400 and 560 nm, it is the absorption range of amylopectin [9]. It has been reported that amylopectin shows maximum iodine binding capacity at the range of 525-595 nm [20]. The results indicated that the pineapple stem starch is highly composed by amylopectin, which is desirable for fruit fillings and gellies.

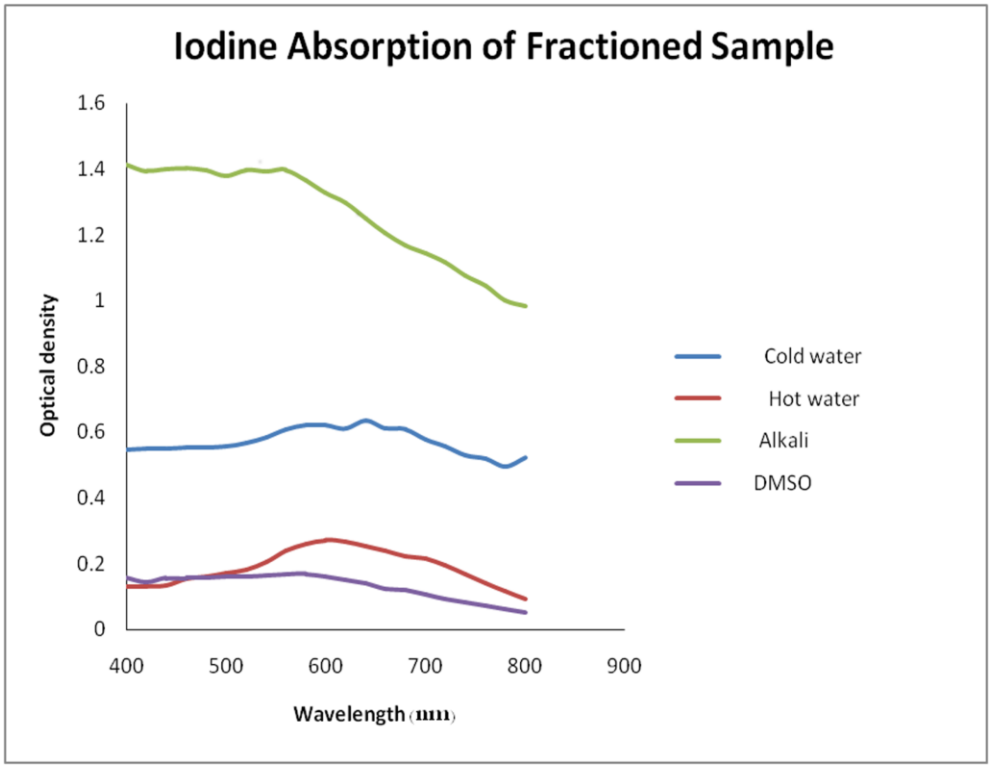

Figure 1. Iodine absorption spectra of fractioned pineapple stem starch

Turbidity measurement

When gelatinized starches are cooled, the disrupted chains of amylose and amylopectin get reassociated into ordered structures which phenomenon is known as retrogradation. The retrograded starches are resistant to enzymatic digestion and show a slower release of glucose into the bloodstream. Retrogradation is related to the turbidity of starch which is commonly used to study the physical changes that occur during the retrogradation process [21]. Stem starch showed comparatively higher turbidity values than the corn starch paste. The turbidity values of both starch suspensions showed significant increase during storage from 0 h to 120 h (Table 1). It has been reported that the starch granule size, granule swelling, granule remnants, molecular weight and chain-lengths of amylose and amylopectin are responsible for the turbidity development in starches [10, 22]. The change in turbidity during storage is due to the interaction between leached amylose and amylopectin chains [23].

Table 1. Turbidity measurement

Turbidity (absorbance at 640 nm)		
Storage Time	**Stem starch**	**Corn starch**
0 h	2.75 ± 0.01	1.56 ± 0.01
24 h	2.78 ± 0.02	1.63 ± 0.01
48 h	2.8 ± 0.02	1.81 ± 0.02
72 h	2.81 ± 0.02	1.95 ± 0.01
96 h	2.83 ± 0.02	2.03 ± 0.01
120 h	2.83 ± 0.02	2.09 ± 0.01

Molecular properties

Amylose content has an important role in determining starch quality [24]. The amylose content obtained was 23.86 % ± 2.56 which is higher than the amylose content in the cassava stem starch (20.8 %) [7]. Nakthong and coauthors reported that the pineapple plant stem contains 34.37% ± 2.04 of amylose [19]. Starch properties are highly variable, and it mainly depends on the environment, genotype and botanical sources [7].

In XRD studies, stem starch showed main peaks at 2θ angles 15.4º, 17.26º and 23.21º and corn starch showed at 14.73º, 17.85º and 23º (Figure 2). It was reported that A-type starch granule has reflections around 15º, 17º, 18º, 20º and 23º 2θ angles, B-type has 5º, 15º, 17º, 20º, 22º and 24º 2θ angles [25, 26]. Stem starch showed the peaks of A-type granules, a characteristic feature of cereal starches. The result was in agreement with those reported by Nakthong and coauthors [19]. The relative crystallinity of stem starch was found to be 25.12 % ± 0.50, and that of corn starch was 22.34 % ± 0.67. Higher crystallinity is the indication of higher structural stability of stem starch than corn starch [19].

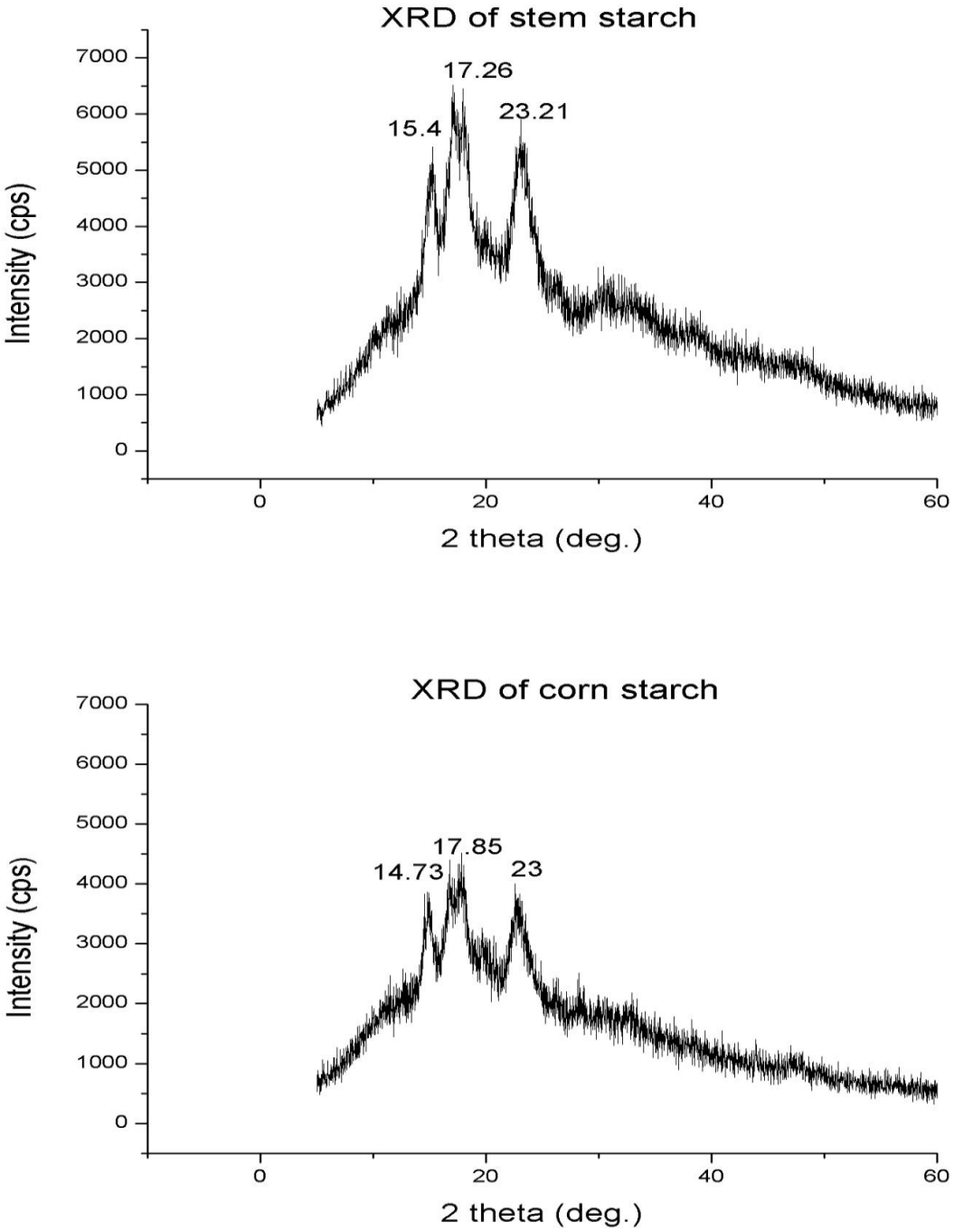

Figure 2. The X-ray diffractogram

NMR studies allow the quantitative analysis of molecular structures within the starch granules. It depends on the degree of branching, orientations of molecules and the crystallinity of starch granules [27,28]. Stem starch showed a chemical shift of 102.222 ppm, 100.256 ppm (C1 position [29], 82.653 ppm (C4 position [30], 73.252 ppm (C-3 of oligosaccharides and large amylopectin fragments [27], C2 and C5 positions [30], 63.083 ppm C-6 position [30] (Figure 3). The result can be successfully used for further modification studies on stem starch.

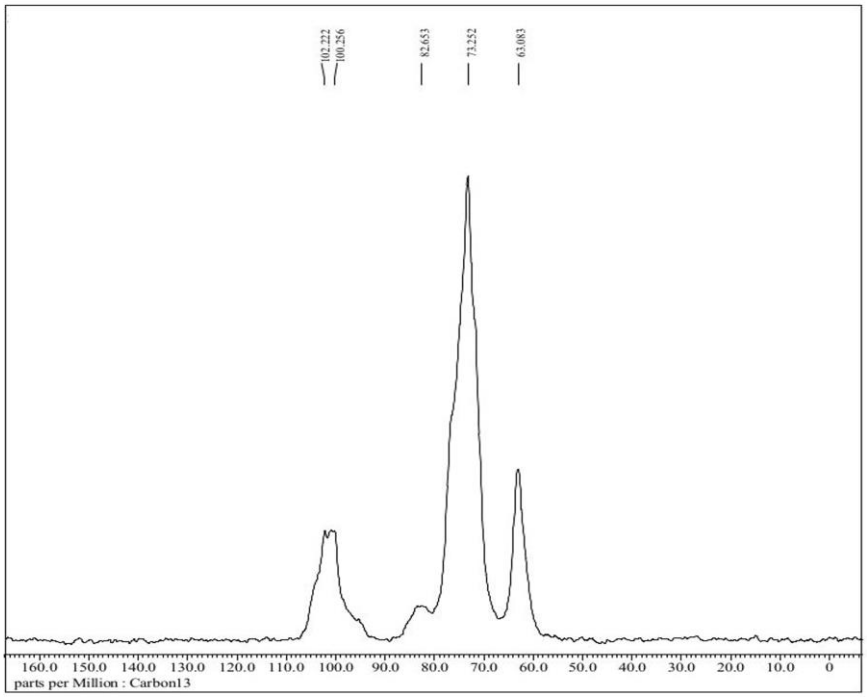

Figure 3. NMR spectra obtained for pineapple plant stem starch

FTIR spectroscopy is a valuable tool for starch characterization as it creates a molecular fingerprint of that molecule. This technique helps us to identify the primary functional group present in the extracted starch sample [31]. Stem starch showed a broad, large band at 3386 cm^{-1} and a small peak at 2929 cm^{-1}. Peak around 2929 cm^{-1} is due to the stretching of the CH bond present in the glucose molecules. Peak obtained at 1640 cm^{-1} is due to the –OH group vibration of water molecules present. Other peaks around 1157 cm^{-1}, 1082 cm^{-1} and 1017 cm^{-1}, are reported to be the characteristic feature of polysaccharides [31]. Warren and coauthors reported that the major peaks from the starch molecules can be observed in 1200-1000 cm^{-1} region [32]. Peak assignments are summarised in Table 2. FTIR spectra obtained for stem starch were characteristic of native starches and comparable with that of corn starch (Figure 4).

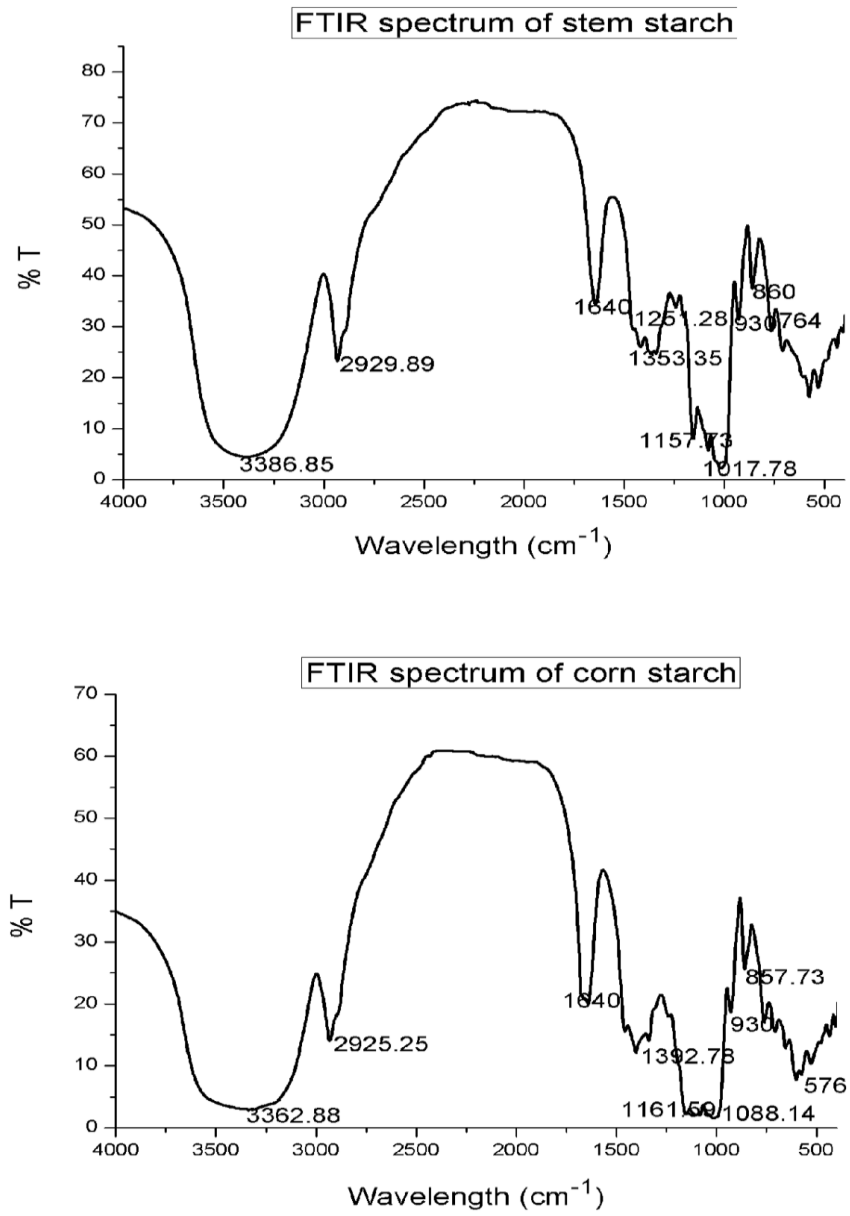

Figure 4. FTIR spectra of pineapple stem starch and corn starch

Table 2. FTIR peak assignments of native pineapple stem starch

Wavelength (cm^{-1})	**Peak assignment**
3386	Water content (peaks around 3300 cm^{-1}) [33]
2929	CH—stretch [32] (Approx.2900 cm^{-1})
1640	Water content [33, 13] (approximately 1640 cm^{-1})
1353	Near 1353 cm-1 (CH2 twisting, C-O-H bending [34]
1251	Near 1251 cm-1 (CH2-OH (side chain) related mode [34]
1157, 1082, 1017	C-O, C-C and C-O-H stretching and C-O-H bending [32]
930	Skeletal mode vibrations of α(1,4) glycosidic linkage,(C-O-C) [34]
860	C(1)-H, CH2 deformation [34]
764	C-C stretching [34]

Granular properties

SEM (Scanning Electron Microscopy) studies revealed that the granular size of stem starch was comparatively smaller than corn starch and was mainly polyhedral with sharp angles and edges and surfaces were smooth with no surface pores (Figure 5). Results are in agreement with that reported by Nakthong and coauthors [19]. Small and medium-sized starch can be used as a fat substituent, stabilizers in baking powder, stiffening agent in laundry and in the manufacture of biodegradable plastics [35, 36].

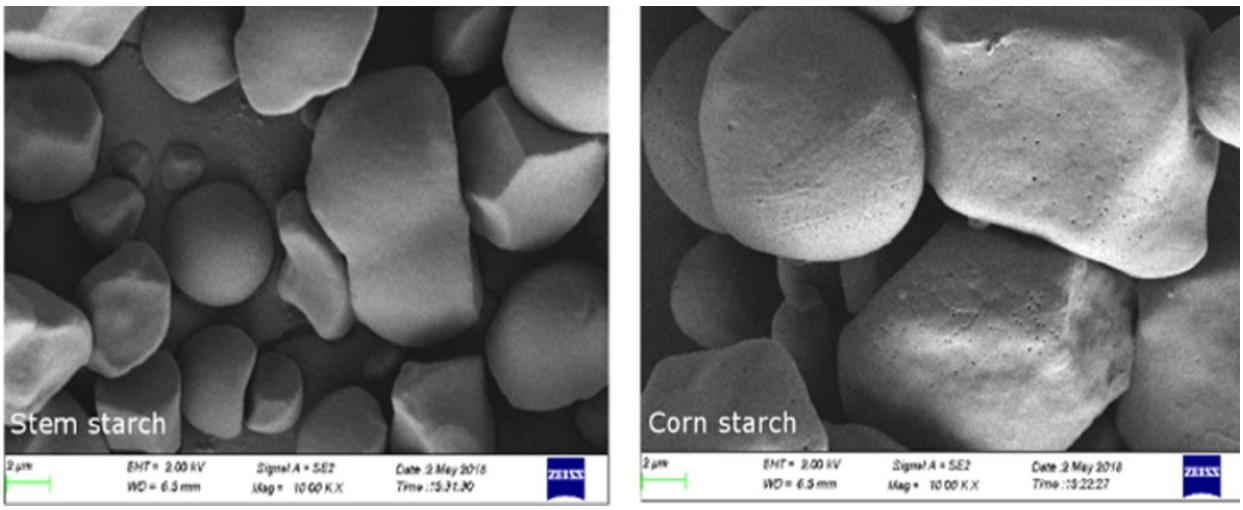

Figure 5. Scanning electron microscopic image of pineapple stem starch and corn starch.

Differential scanning colorimetric (DSC) analysis

Gelatinization is an irreversible change (mainly the loss of crystalline structure) that occurs in starch granules in the presence of water and heat. DSC is widely used to study starch gelatinization. DSC data revealed that the stem starch had onset temperature (T_o) 84.00 ºC ± 2.05, peak temperature (T_p) 89.45 ºC ± 0.41, conclusion temperature (T_c), 99.51 ºC ± 2.83 and enthalpy change of gelatinization (ΔH) 15.45 J/g ± 0.43. Corn starch had T_o, 55.96 ºC ± 1.42, T_p, 66.73 ºC ± 1.36, T_c, 74.08 ºC ± 1.69 and ΔH, 36.55 J/g ± 1.15 (Figure 6). Stem starch had higher gelatinization temperature than corn starch. Higher gelatinization temperature indicating higher starch crystal stability [37] and the gelatinization enthalpy (ΔH) related to the amount of starch in amorphous phase [38]. The gelatinization transition interval (ΔT) of stem starch was 15.51 ºC, and that of corn starch was 18.12 ºC. ΔT is dependent on heating rate used, and low ΔT is the indication of high homogeneity and purity of the extracted material [31,37]. It was also reported that A-type crystals have higher thermal transition temperatures [3]. Starches with high gelatinization temperatures have comparatively stable starch gels and are resistant to acid and enzymatic hydrolysis and can be used in the preparation of gums [39,3].

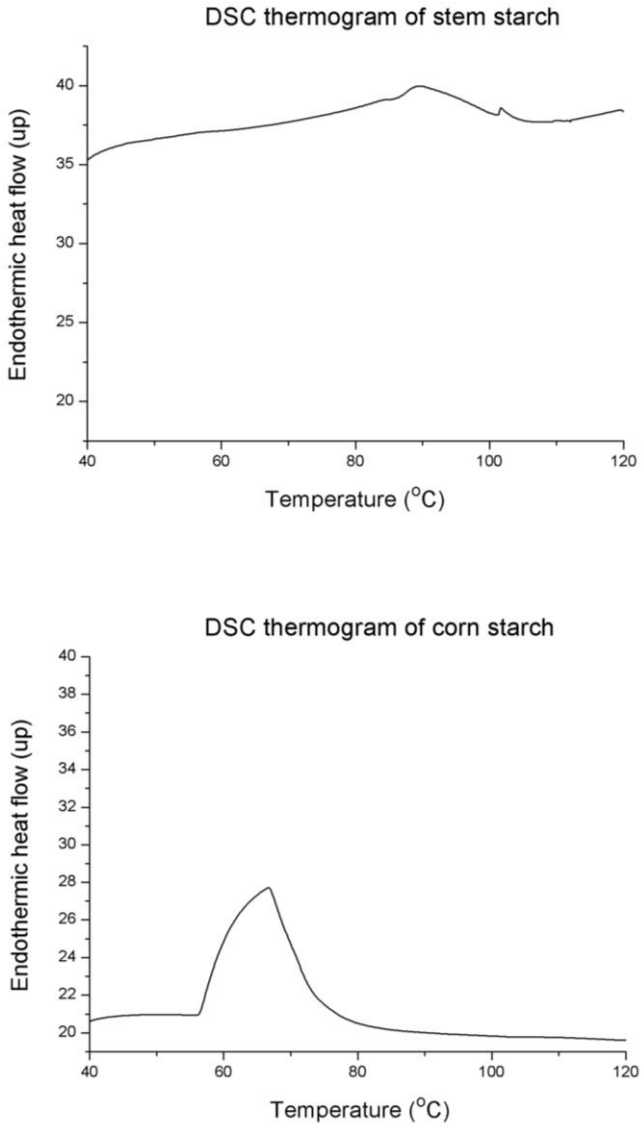

Figure 6. DSC thermogram obtained for pineapple stem starch and corn starch.

Rheological studies

Rheological studies can be used to describe the consistency of different food products, by measuring viscosity and elasticity. It was reported that the rheological properties of starch mainly depends on its granule size, shape, rigidity, swelling pattern, amount and type of amylose/amylopectin and complexes with other components [40]. The rheological parameters such as storage modulus (G'), loss modulus (G''), complex viscosity (η*) and phase angle (δ) were studied against frequency change. G' is the measure of energy stored in a material, which describes the elastic properties of that material [40]. G' of both starches were increasing, and corn starch showed higher G' values than that of stem starch as it is directly proportional to the amylose content and gel stiffness [10,41]. During frequency variation, G' of both starches showed almost a steady state until 3.56 Hz, and after that increased markedly (Figure 7). It was reported that the plateau-like graph is the indication of the presence of network structures in the starch gels [42].

G'' describes the viscous properties of a material. Stem starch showed slightly higher G'' value than corn starch. Data indicated that stem starch was more viscous (more liquid-like) than corn starch. Complex viscosity (η*) of both starches decreased markedly (shear thinning behaviour) with frequency change. A similar result has earlier been reported for lentil starch by Ahmed and Auras [43]. The lower viscosity at lower frequencies resulted in the reduction of energetic interactions [42]. During the same frequency change, the native stem starch showed a low η* values than corn starch. It was reported that the amylose content is directly proportional to shear viscosity and shear thinning behaviour [44]. The phase angle (δ) of stem starch was comparatively higher than corn starch, and the lower value of corn starch indicated the higher solid-like behaviour of corn starch than stem starch [43]. There was a significant difference observed

for all rheological properties for both starches (Figure 7). It is concluded that the stem starch was comparatively more viscous and presented a lesser stiff gel than corn starch. These specific properties could be useful in many industries where viscous starch is preferred.

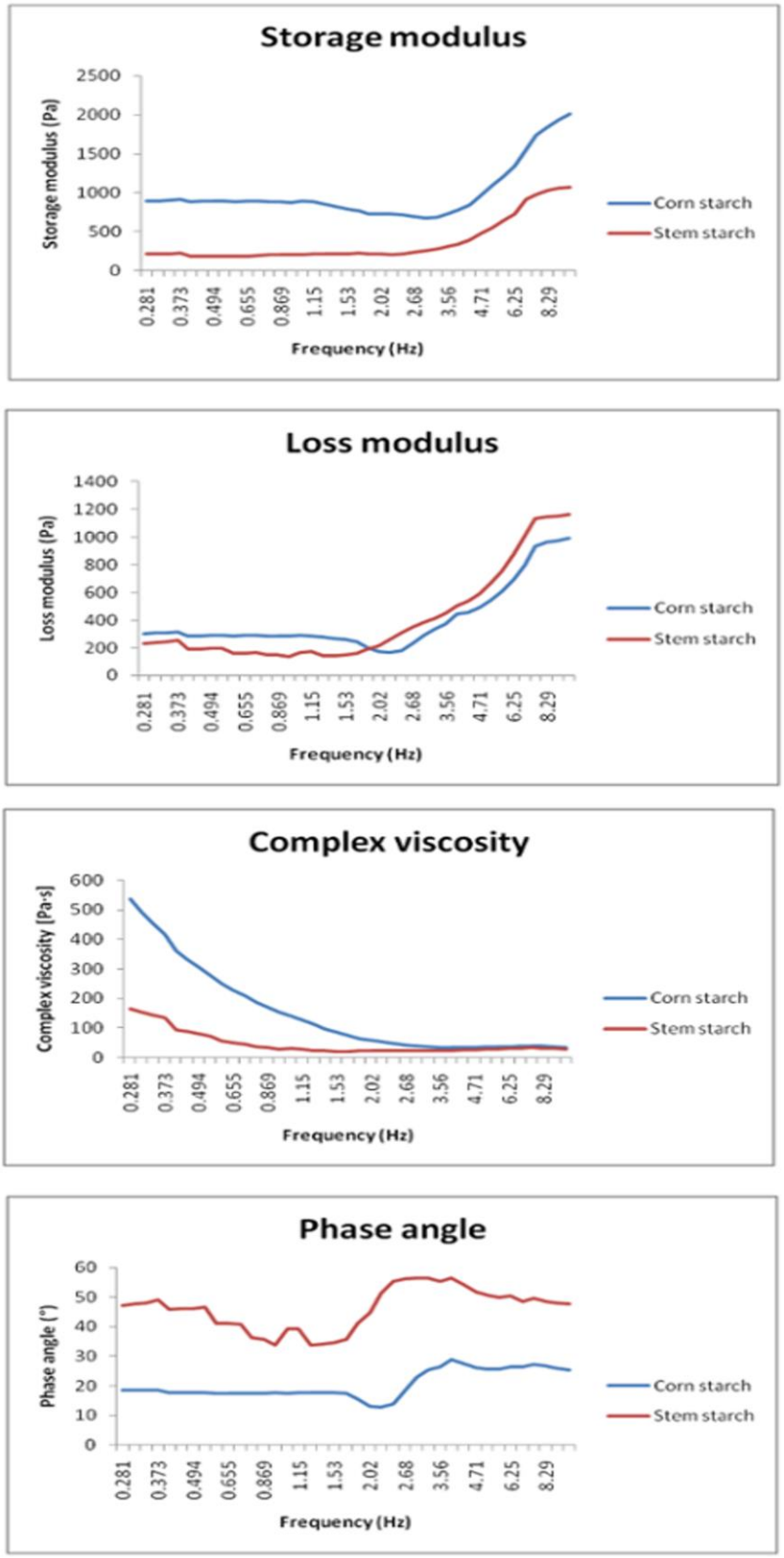

Figure 7. Rheological parameters of pineapple plant stem starch and corn starch.

Heavy metal and Pesticide analysis

The concern about the metal toxicity has been increased nowadays as a result of overpopulation and expansion of industrial activities [45]. Thus the screening of the toxic metals in food materials is essential. The heavy metals like cadmium, chromium, copper, mercury, lead zinc, iron, manganese, cobalt, nickel, arsenic are commonly found in the environment. Generally, heavy metals in small amount are beneficial and become dangerous in a large amount [46]. The heavy metal analysis revealed that there was a negligible concentration of heavy metals present in the extracted starch of pineapple stem and the most toxic mercury is observed to be absent (Table 3). The pesticide analysis of parathion-methyl, malathion, chlorpyrifos, dichlorvos, ethoprophos and heptachlor has been done to assess the purity of the extracted starch from pineapple stem which was collected from the pineapple farm. The results showed that there was no pesticide content in the extracted starch which is a positive indication for using this starch in food preparations.

Table 3. Heavy metal analysis

Elements	**%**
Cadmium	0.02 ± 0.01
Chromium	0.59 ± 0.05
Copper	0.49 ± 0.06
Mercury	-
Lead	0.03 ± 0.00
Zinc	0.13 ± 0.01
Iron	3.10 ± 0.31
Manganese	0.66 ± 0.05
Cobalt	0.35 ± 0.18
Nickel	0.30 ± 0.03
Arsenic	< 0.00006

CONCLUSIONS

The crystal type (A-type) and FTIR patterns of pineapple stem starch were comparable to that of corn starch. The turbidity values and gelatinization temperature were observed to be higher than the corn starch. Stem starch possesses comparatively smaller granules and is more viscous than the corn starch. Starches with unique characters are always appreciated as the starch industries require new food formulations. Utilization of this agro-waste starch helps to provide an extra income to pineapple farmers. This type of technologies promises the maximum utilization of arable land, reduction of agro-wastes and sustainable agriculture practices.

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Conflicts of Interest: "The authors declare no conflict of interest."

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