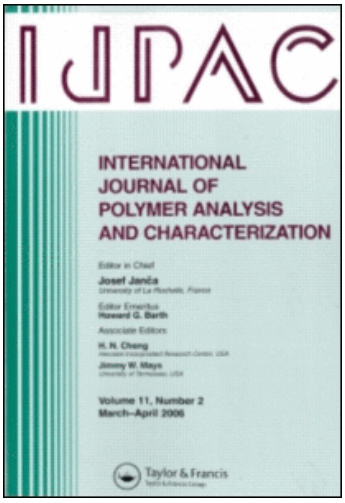

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Fractional Isolation and Spectroscopic Characterization of Sago Starch

RunCang Sun[a]; Jeremy Tomkinson[a]
[a] The BioComposites Centre, University of Wales, Bangor, United Kingdom

Online publication date: 27 October 2010

To cite this Article Sun, RunCang and Tomkinson, Jeremy(2003) 'Fractional Isolation and Spectroscopic Characterization of Sago Starch', International Journal of Polymer Analysis and Characterization, 8: 1, 29 — 46
To link to this Article: DOI: 10.1080/10236660304887
URL: http://dx.doi.org/10.1080/10236660304887

International Journal of Polymer Anal. Charact., 8: 29–46, 2003

1023-666X/03 $12.00 + .00
DOI: 10.1080/10236660390128256

Taylor & Francis
Taylor & Francis Group

Fractional Isolation and Spectroscopic Characterization of Sago Starch

RunCang Sun and Jeremy Tomkinson
The BioComposites Centre, University of Wales,
Bangor, United Kingdom

Successive extraction of sago pith with cold water, hot water, dimethylsulfoxide, and 5% NaOH yielded 48.9% cold-water-soluble starch, 11.5% hot-water-soluble starch, 21.9% DMSO-soluble starch, and 7.1% alkali-soluble starch, which contain 5.4%, 7.0%, 3.6%, and 16.2% non-starch polysaccharides, such as β-glucan and hemicelluloses, respectively. The total pure starch accounted for 83.9% of the dried sago pith. Cold- and hot-water-soluble starches contained noticeable amounts of amylose, whereas DMSO- and alkali-soluble starches predominated in amylopectin. All the four starch fractions also contained trace quantities of ash (0.05%–0.4%), crude protein (0.1%–0.15%), and lipids (0.1%–0.16%). The isolated straches were further analyzed by UV, FT-IR, and ^{13}C-NMR spectroscopies as well as thermal analysis, and the results are reported.

Keywords: Sago pith; Extraction; Starch; Thermal analysis

Starch is the carbohydrate polymer most widely produced by plants. It is deposited in the form of tiny granules that consist of amylose and amylopectin in variable proportions[1]. Amylose is a linear (1→4)-linked -α-*D*-glucan with a small number of α-(1→6)-linkage branches[2]. Amylopectin, however, is a highly branched molecule that consists of short chains (17–26 *D*-glucosyl units) of (1→4)-linked -α-*D*-glucose with (1→6)-α-linked branches. Chain lengths of amylose are commonly in

Received 23 February 2001; accepted 16 April 2001.
Address correspondence to RunCang Sun, The BioComposites Centre, University of Wales, Bangor, Gwynedd LL57 2UW, UK. E-mail: bcs00a@bangor.ac.uk

excess of 6000 D-glucopyranose units with molar mass between 150,000 and 600,000 Da. The molecular weight of amylopectin is almost too large to be determined accurately but light scattering studies indicate a value of 10^6 *D*-glucosyl resides per molecule, which is one of the largest naturally occurring macromolecules[2,3]. The ratio of amylose to amylopectin varies with the starch source but is typically 20:80[4].

In its native form, starch exists in relatively inert granular structures that are composed of macromolecules arranged in a polycrystalline state. These granules are insoluble in water and resistant to many chemicals and enzymes. Gelatinization by heating in water disrupts the granular structure and enhances its chemical reactivity[5]. In other words, when the cells of the plant are broken by mechanical means, the starch granules are suspended in the wash water and settle out. In a centrifugal separator the settling can be done very quickly[6]. Research on the structure and physicochemical characteristics of the main cereal starches of commerce such as rice, wheat, and corn has resulted in their extensive utilization in the food, paper, corrugating, chemical, building, and pharmaceutical industries[7].

Sago starch is obtained from the stems of sago palms (*Metroxylon sago rottboel*). It is estimated that about 60 million tons of sago starch are produced annually in Southeast Asia[8]. As a cheap and adequate source of energy, sago starch fulfills a vital need for the local people. Traditionally, it is processed into various products such as sago noodle, sago biscuit, and sago pearl[1]. Although sago starch is a useful resource for foodstuffs and industrial raw materials, it has not yet sufficiently been utilized by the textile, paper, and food industries because of its poor and varying quality[9,10]. The previous studies showed that raw sago starch differs in chemical composition and physical properties from potato, cassava, and amaranth starches with respect to fat, nitrogen, and carbohydrate contents, gelatinization temperature, swelling power, solubility, water-binding capacity, and amylograph consistency[11–13]. However, there has been much interest recently in enzymes capable of digesting raw starch granules. Use of these enzymes would bring about a reduction in the costs associated with the high temperatures required for gelatinization[8,14,15]. Furthermore, it has been reported that treatment of sago starch before incubation with the enzyme by heating to below gelatinization temperature at low pH condition is effective in improving hydrolysis[16]. A strong synergism between glucoamylase and α-amylase on the hydrolysis of both untreated and pretreated sago starch has also been observed in the studies of sago starch as a biomass source by Wang and coworkers[8].

The aim of this study is to isolate the sago starch fractions and partially analyze cold-water-soluble, hot-water-soluble, dimethylsulfoxide (DMSO)-soluble, and alkali-soluble starches from sago pith in order to obtain more detailed information on the physico-chemical properties and structural features.

EXPERIMENTAL

Materials

The meal of sago pith was kindly supplied from the Craun Research SDN BHD, Sarawak, Malaysia, and was ground to pass a 1.2-mm screen. The ground sago pith was dried in an oven with air circulation for 16 h at 60°C and stored at 5°C until use. Corn amylose, potato starch, α-amylase (EC 3.2.1.1, type II-A from *Bacillus* species, 1820 units/mg solid), amyloglucosidase (EC 3.2.1.3, from *Aspergillus niger*, 52 units/mg solid), and β-glucosidase (EC 3.2.1.21, from *Almonds*, 5.0 units/mg solid) were purchased from Sigma Chemical Co., St. Louis, MO. Lichenase, specific, endo-(1-3), (1-4)-β-D-glucan 4-glucanohydrolase EC 3.2.1.73 (>1000 units/mL) was purchased from Megazyme, Bray, Co. Wicklow, Ireland.

Fractional Isolation of Sago Starch

Figure 1 illustrates the scheme for fractional extraction of sago starch from sago palm pith. The ground sago palm pith (100 g) was steeped in 1200 mL water (at 19°C) for 3 h. A ratio of one part soaked grains to three parts distilled water was mixed in a Waring blender at low speed. The resultant slurry was then passed through a 20-μm nylon cloth. The residue was washed six times with distilled water and dried at 60°C in an oven for 16 h. The combined extractions were concentrated by a rotary vacuum evaporator at 40°C. The cold-water-soluble sago starch was obtained by precipitation of the concentrated filtrate with four volumes of ethanol. After being washed with 70% ethanol, the isolated cold-water-soluble sago starch was air-dried and kept at 5°C before analysis. The hot-water-soluble sago starch was isolated from the above residues by extraction with distilled water (1.0 g residue/22.4 mL water) at 70°C for 3 h and separated as described for cold-water-soluble sago starch. Next, the hot-water-extracted residues were dispersed in 90% dimethylsulfoxide (DMSO) and stirred for 12 h at 75°C. After exhaustive filtration, the DMSO-soluble sago starch was collected by evaporation of the solvent in a rotary vacuum evaporator and then precipitation with four volumes of ethanol. The resulting DMSO-soluble sago starch was washed with 70% ethanol and then air-dried. Finally, alkali-soluble sago starch was prepared by treatment of the above DMSO insoluble residues with 5% NaOH at 70°C for 5 h (1.0 g residue/32 mL alkaline solution). After steeping, the solution was screened by using a 20-μm nylon cloth to remove the alkali-insoluble materials. The solubles were recovered by precipitation of the neutralized hydrolysate (pH 5.5 adjusted by 20% HCl) in four volumes of ethanol. After filtration, the alkali-soluble sago

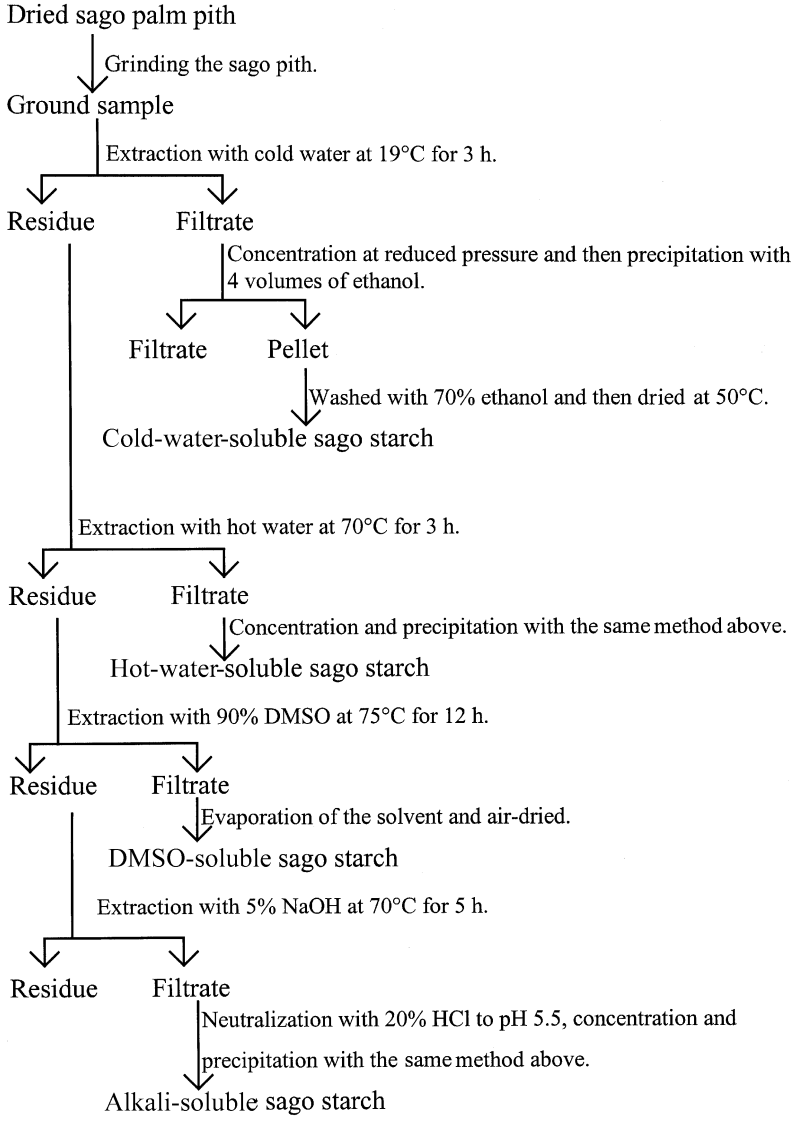

FIGURE 1 Scheme for fractional extraction of sago starch from sago palm pith.

starch was dried overnight on the table and then further dried in a forced-air oven at 50°C for 18 h.

Determination of Starch Purity by Enzymatic Hydrolysis

To determine purity of cold-water-soluble starch, the starch was first hydrolyzed using α-amylase and amyloglucosidase enzymes; its detailed method was described in a previous article[17]. The produced glucoses were monitored using gas chromatography (GC) as their alditol acetates[18] and then calculated as purity of cold-water-soluble starch. The content of β-glucan in cold-water-soluble sago starch was calculated by the corresponding glucose liberated during the sequential treatment of the above residue by Lichenase at 40°C for 4 h at pH 6.5 and β-glucosidase at 40°C for 48 h at pH 5.0. The insoluble residue was isolated by precipitation with four volumes of ethanol and washed with 70% ethanol. After being air-dried, the residue was named as cold-water-soluble hemicelluloses (Figure 2). To determine the purity of hot-water-soluble, DMSO-soluble, and 5% NaOH-soluble sago starch fractions, the soluble sago starch fractions were incubated with α-amylase and amyloglucosidase to hydrolyze them into glucose. The starch purity in each fraction is measured by GC as the glucose released.

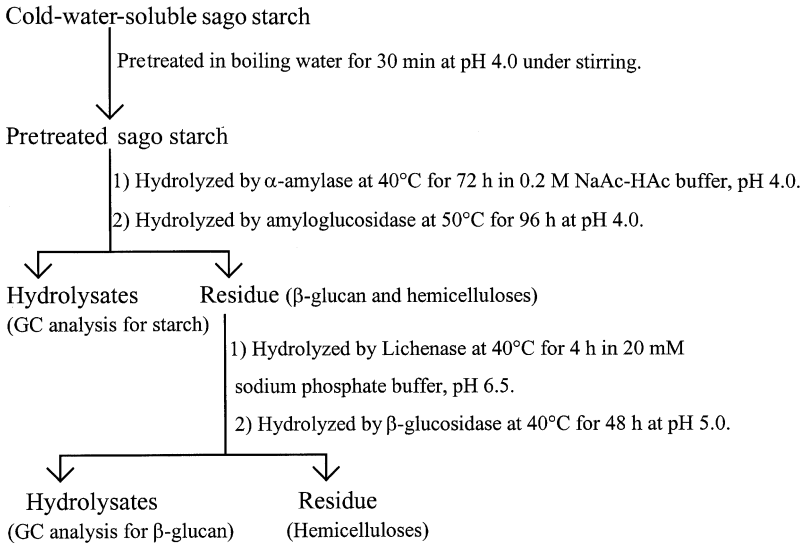

FIGURE 2 Scheme for enzyme hydrolysis of cold-water-soluble sago starch.

Iodine-Absorption Spectra

Slurries (0.8%, w/v) of cold-water-, hot-water-, DMSO-, and alkali-soluble sago starches, corn amylose, and potato starch in distilled water were heated in sealed tubes at 70°C for 30 min. The tubes were cooled to ambient temperature and centrifuged at 3000 rev/min for 2 min. The iodine-starch complexes were prepared by mixing 5 mL of the supernatant with suitable amounts of 0.0005 N I_2 and 0.005 N KI mixture until the solutions became blue or purple. The absorption was measured as a function of wavelength using a diode array spectrophotometer with water as a blank.

FT-IR and ^{13}C-NMR Spectroscopy

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. Thirty-two scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. The solution-state ^{13}C-NMR spectra were obtained on a Bruker 250 AC spectrometer at 62.4 MHz in D_2O. They were recorded at 25°C from 120 mg of sample dissolved in 1.0 mL D_2O after 15,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width, and a 0.85 s acquisition time were used.

Thermal Analysis

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of cold-water-soluble sago starch was performed with a Simultaneous Thermal Analyser (STA 625). This apparatus provides for a continuous measurement of sample weight at a range of temperatures between ambient and 600°C. A sample of approximately 10 mg was heated in a platinum crucible to 600°C at a heating rate of 10°C min^{-1}. Air was used as the purge gas, and a positive pressure was maintained through the weighing chamber in order to protect the balance mechanism from the condensables formed during pyrolysis.

RESULTS AND DISCUSSION

Fractional Yield and Purity of Sago Starch

The results of the analysis on the chemical composition of sago starch fractions are given in Table I. As can be seen from Table I, the cold-water-soluble, hot-water-soluble, DMSO-soluble, and 5% NaOH-soluble starch fractions contained 94.5%, 92.9%, 96.4%, and 83.4%

TABLE I Fractional yield (% dry weight of sago pith) and content of β-glucan, hemicelluloses, and ash in sago starch fractions (% dry weight of sago starch)

Fractions of sago starch	Yield/content
Cold-water-soluble sago starch	48.9 ± 0.5
purity	$94.5^{a} \pm 0.8$
β-glucan	$3.3^{b} \pm 0.2$
hemicelluloses	2.1 ± 0.3
ash	0.1 ± 0.02
Hot-water-soluble sago starch	11.5 ± 0.3
purity	$92.9^{c} \pm 0.8$
hemicelluloses	7.0 ± 0.4
ash	0.08 ± 0.01
DMSO-soluble sago starch	21.9 ± 0.4
purity	$96.4^{c} \pm 0.6$
hemicelluloses	3.6 ± 0.3
ash	0.05 ± 0.01
Alkali-soluble sago starch	7.7 ± 0.4
purity	$83.4^{c} \pm 0.6$
hemicelluloses	15.6 ± 0.5
ash	0.4 ± 0.03

[a]GC of glucose liberated during the treatment of cold-water-soluble sago starch with α-amylase in 0.2 M NaAc-HAc buffer, pH 4.0 at 40°C for 72 h, and then with amyloglucosidase in 0.2 M NaAc-HAc buffer, pH 4.0 at 50°C for 96 h, respectively.

[b]GC of glucose liberated during the treatment of the α-amylase and amyloglucosidase treated residue of cold-water-soluble sago starch with β-glucosidase in phosphate buffer pH 5.0 at 40°C for 48 h.

[c]GC of glucose liberated during the treatments of hot-water-soluble, DMSO-soluble, and 5% NaOH-soluble sago starch fractions with mixture of α-amylase and amyloglucosidase in 0.2 N NaAc-HAc buffer, pH 4.5 at 55°C for 40 h, respectively.

starch, respectively, with β-glucan, hemicelluloses, and ash being the principal minor constituents. In addition, the sago starches also contained 0.1%–0.15% crude protein determined by element analysis ($N \times 6.25$), and 0.1%–0.16% lipids determined by Soxhlet extraction using dichloromethane as a solvent (data not shown in Table 1). All commercial starches either from cereal or tuber sources contain minor or trace amounts of uncombined inorganic materials, which normally originate in the crop from which the starch is isolated and also from the

water used to process the starch. The lipid content for all four sago starches studied was low and comparable to crude fat content from other starches. Commercial starches normally contain trace amounts of fatty acid glycerides, usually less than 0.1%[3]. Furthermore, the commercial starches also contain about 0.5%–0.6% free fatty acids, which are complexed with the amylose[19]. These complexed fatty acids cannot be removed by normal lipid solvent such as hexane but need extended extraction by using hot methanol or ethanol to field lipid-free starches[3].

The above findings indicated that starch was the predominant constituent of sago pith, which yielded 83.9% dry weight of the palm pith. This result is in good agreement with the studies on sago starch by Fujii and coworkers[20]. The authors reported that the whole sago pith contained 83.5% starch (% dry weight). However, the results obtained here are in disagreement with a number of published data by Malaysian scientists. Eng-Tian et al.[21] reported that sago pith yielded 63.3% starch. These variations of starch yields in sago piths depend on palm age, growth stage, palm height, and soil type[22]. Immature sago pith contains a lower level of starch and higher level of impurities[20]. The outer zone has a significantly lower starch content than the inner part of the sago pith[22]. In other words, the yield of starch varies depending on both radial and vertical directions of the sago pith. A similar observation was also made by Eng-Tian et al.[21] They mentioned that the central portion of the pith showed higher starch distribution than the outer portion of the pith. In general, short stout trunks are expected to posses a higher starch density than tall, slender trunks[23]. In addition, the analysis methods also play a very important factor in determination of starch yield from sago pith. The routine methods, such as weight, bulk density, and starch concentration (weight basis) methods, use only water as a solvent for extraction of starches. The starch granules are suspended in the wash water as the cells of the plant are broken by mechanical means and can then be settled out by centrifugation.

Another method has been proposed and developed to extract starch, particularly for amylopectin, from cereal flours using 90% DMSO as a solvent[24]. Alkali has been used for isolation of starches from native corn flour. The starch extraction method developed by Dimler et al.[25] for wheat flour was the most effective method so far for obtaining starch with high yield and purity. They used 0.5%–1.0% alkali solutions to extract starch from wheat flour. This method also was applied to corn flour to extract cornstarch[26]. Further studies have shown that strong alkali solutions have varying effects on starch, such as granule swelling, increased viscosity, and gelatinization. As shown in Table I, the yield of pure cold-water-soluble starch, pure-hot water-soluble starch, pure DMSO-soluble starch, and pure 5% NaOH-soluble starch accounted for 46.2%, 10.7%, 21.1%, and 5.9% of the dried sago pith, respectively. Obviously, the higher total yield of sago starch (83.9% based on pure

starch weight) obtained in our studies is not surprising. This is due to the exhaustively successive extractions of the sago pith with cold water, hot water, DMSO, and 5% NaOH procedures in our experiments.

Enzyme hydrolysis of starch by α-amylase occurs in three successive steps: diffusion of the enzyme molecule toward its substrate, absorption of the enzyme on the substrate, and the catalytic event[27]. In general, freshly gelatinized starch, which is in a solubilized structure, is easily accessed by the enzyme[1]. In the case of sago starch, its hydrolysis is currently limited by granular resistance to commercial enzymes and the high paste viscosity of gelatinized starch pastes[28]. It has been found that raw sago starch is a poor substrate for enzyme action compared to corn and tapioca starches tested under the same conditions[8]. Enzymatic conversion of liquefied starch to glucose requires a long reaction time (24–96 h), and this period of hydrolysis is dependent on the desired concentration of glucose in the final product[29]. The use of high concentration of enzymes, prolonged reaction hydrolysis times, and relatively high concentration of substrates (30%–40% dry substance) in the industrial process can cause reversion reactions involving resynthesis of saccharides from glucose[30]. The main reversion products are isomaltose and higher branched polymers[31]. It has been demonstrated that at high enzyme concentration, maltose and isomaltose are produced during prolonged saccharification due to reversible reactions attributed to amyloglucosidase[32]. During the saccharification process, although the initial rate of saccharification is high with the increase of amyloglucosidase concentration and hydrolysis time, it subsequently decreases continuously during prolonged reaction, probably due to lower affinity of amyloglucosidase for α-1,6-linkages and occurrence of reversible reactions[31]. In addition, based on the study of the effect of retrogradation on enzyme susceptibility of sago starch, Cui and Oates[1] revealed that sago starch samples, native, gelatinized, and retrograded, were significantly different in their reactivity to enzyme hydrolysis by porcine pancreatic α-amylase, and freshly gelatinized sago starch displayed the greatest susceptibility to porcine pancreatic α-amylase. Within the first hour, 35.3% hydrolysis was achieved, and after 8 h incubation 78.3% hydrolysis was obtained. However, these hydrolysis rates are relatively low compared to those for cereal starches[33].

Interestingly, Wang et al.[5] found that treatment of all starch batches prior to the addition of the enzyme improved hydrolysis dramatically. For example, pretreatment of the sago starch granules before incubation with the enzyme by heating to below gelatinization temperature at low pH condition improved hydrolysis. Further studies showed that the major macromolecular compounds were apparently unaffected by the pretreatment. In the case pretreatment, the starch did not physically change the starch fractions or impose any observable effects on the granule morphology, but subsequently increased hydrolysis. The reason

for the poor hydrolysis of untreated sago starch is presumed to be the presence of substances inhibitory to the actions of the amylase not associate with or adhering to the starch granules, and the pretreatment inactivated such compounds[8]. Meanwhile, a strong synergism between glucoamylase and α-amylase on the hydrolysis of both untreated and pretreated sago starch was observed by Wang and coworkers[8] in the studies of sago starch hydrolysis by commercial enzymes. They found that single enzymes were not effective in producing soluble products from raw sago starch granules while the addition of amyloglucosidase to the enzyme mixtures in all cases resulted in glucose as the sole product. This synergism effect between α-amylase and glucoamylase has been explained as resulting from the action of α-amylase at the granule surface, which supplies new nonreducing groups for amyloglucosidase[8]. In our experiments, both pretreatments and two enzymes (α-amylase and amyloglucosidase) were used in the hydrolysis of sago starch fractions, which led to satisfactory results of enzyme hydrolysis.

Iodine-Absorption Spectra of Starch Fractions

Amylose is defined as the starch polysaccharide that binds 19.5% mg iodine per 100 mg of polysaccharide. It is commonly determined by the classical reaction between α-1,4-glucans and iodine to form a blue complex, which is measured either spectrophotometrically or potentiometrically. However, spectrophometric determinations are more widely used because of convenience and simplicity of use[34]. Amylopectin is defined as the polysaccharide that binds <0.5 mg iodine per 100 mg polysaccharide. In sago starch with lipid, amylose content ranged between 23.8% and 25.5%. Average unit chain length of the sago starch was found to be 23–20. The average chain length of sago starch appeared to be between that of wheat and potato[9]. As the length of the glucan chain, and hence the number of helical turns, increases, the number of iodine molecules that can be accommodated also increases, so that the iodine binding capacity is increased. This increased binding has been shown to result in a shift in the wavelength of maximum absorbance[34].

Figure 3 illustrates the spectra of amylose-iodine complexes of cold-water-soluble sago starch (spectrum 1), hot-water-soluble sago starch (spectrum 2), DMSO-soluble sago starch (spectrum 3), corn amylose (spectrum 4), and potato starch (spectrum 5). It has been reported that the wavelength of maximum absorption (λ_{max}) of pure amylose and pure amylopectin occurs at 640 and 550 nm, respectively[7]. As shown in Figure 3, a much higher absorption between 400 and 540 nm of potato starch (containing 73%–77% amylopectin) than of the corn amylose was observed in our experiments. From this point, the DMSO-soluble sago

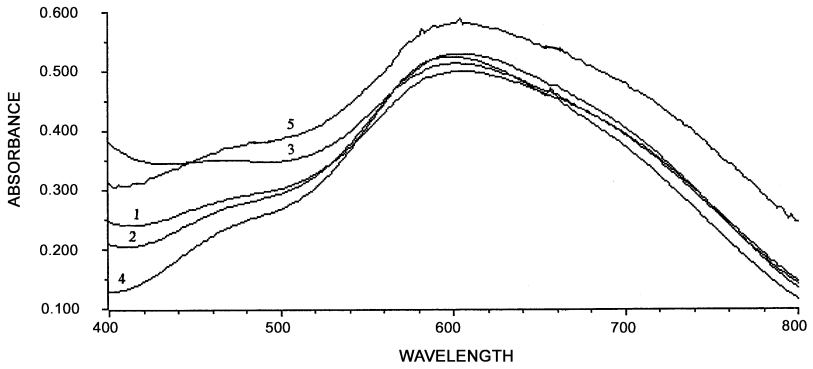

FIGURE 3 UV spectra of cold-water-soluble starch (spectrum 1), hot-water-soluble starch (spectrum 2), DMSO-soluble starch, (spectrum 3), corn amylose (spectrum 4), and potato starch (spectrum 5).

starch contained substantial quantities of amylopectin, whereas the cold- and hot-water-soluble sago starches contained noticeable amounts of amylose. These observations were consistent with the results obtained from the characterization of the solution behavior of starch polysaccharides from wheat starch granules by Anson et al.[24] It is very likely that amylose, and probably a molecule less branched than amylopectin, dissolves during water extraction of sago pith, and preferential dissolution of amylopectin occurs in the DMSO extraction. The fact that variation in spectra of amylose-iodine complexes from cold- and hot-water-soluble sago starches was not significant implied a similar content of amylose. In addition, these two spectra of water-soluble starch fractions, with λ_{max} around 600 nm, were nearly symmetrical, whereas the spectrum of DMSO-soluble starch was skewed. This observation revealed that the presence of amylopectin substantially increased the absorption values between 400 and 560 nm and had a small effect on λ_{max}, but did not significantly affect the absorbance values greater than 560 nm. In addition, variations in spectra of amylose-iodine complex can be simply and conveniently evaluated by determining the slopes of the sample absorbance curve below λ_{max} since these values can give additional information about the samples being assayed.

The previous studies demonstrated the existence of fractions intermediate in behavior to amylose and amylopectin. An amylopectin subfraction, termed anomalous amylopectin, was identified for all cereal starches. It has been reported that oat starch contains 56% amylopectin, 26% intermediate material, and 18% amylose. The corresponding values for wheat were 56%, 16%, and 28%[7]. It is probable that anomalous

amylopectin (in the intermediate material) may exist in sago starch. This hypothesis, however, needs further examination.

Color of the Sago Starch

The color of the sago starch, which turns brown in the process of separation, is one of the major factors responsible for low starch quality. When sago palm pith is chopped and ground into a powder, colored substances are formed and bind tightly to the starch granules even when the pith is stored at low temperature. It is well known that when plant tissues are infected and/or experience cut-injury, colored substances are formed in the tissues; generally, phenolic components and polyphenol oxidase are associated with browning. It is, therefore, considered that the browning of sago starch results from the presence of phenolic compounds and polyphenol oxidase in the pith[35].

Five kinds of phenolic compounds were isolated from the pith solvent extraction and column chromatography. They are identified as *DL*-epicatechin, *D*-catechin, proanthocyanidin, (2S)-4′-hydroxy-5,7-dimethoxyflavane, and (2*S*)-5-methoxy-7-hydroxyflavane. *DL*-epicatechin and *D*-catechin gave rise to colored substances as a result of oxidation by the enzyme isolated from sago palm pith[35,36]. That is, the browning of sago starch arises from the binding to starch granules of the colored substances produced by the enzymatic oxidation of *DL*-epicatechin and *D*-catechin. This enzymatic browning reaction requires the presence of three substances: polyphenol oxidase, oxygen, and polyphenol. Further studies showed that polyphenolic compounds such as tannin react with Fe ion to produce a dark blue complex called ink. This coloring matter could not be eliminated by washing or by bleaching with sulfite. Therefore, iron tools in the starch production should be avoided as much as possible.

For good quality starch production, the raw materials should be fresh and mature. Inferior starch can be obtained from immature material and material that stood for a long time after harvesting, barking, or rasping, since starch content is extremely high in parenchymas and fungus grows fast on the surface of the cross section of the trunks. Moreover, immature pith yields a very small amount of starch that settles slowly. When short-term storage of the cut pith is inevitable, some suitable methods to prevent exposure to air may be taken. The whiteness value of the sago starch could be greatly improved by using an oxidation treatment with hydrogen peroxide or sodium hypochlorite[20].

In this study, the cold- and hot-water-soluble starches were white, whereas the DMSO- and alkali-soluble sago starches were brown. Thus, the latter two fractional starch samples were tentatively treated with 0.5% H_2O_2 at 45°C for 2 h at pH 11.5, which improved the color significantly.

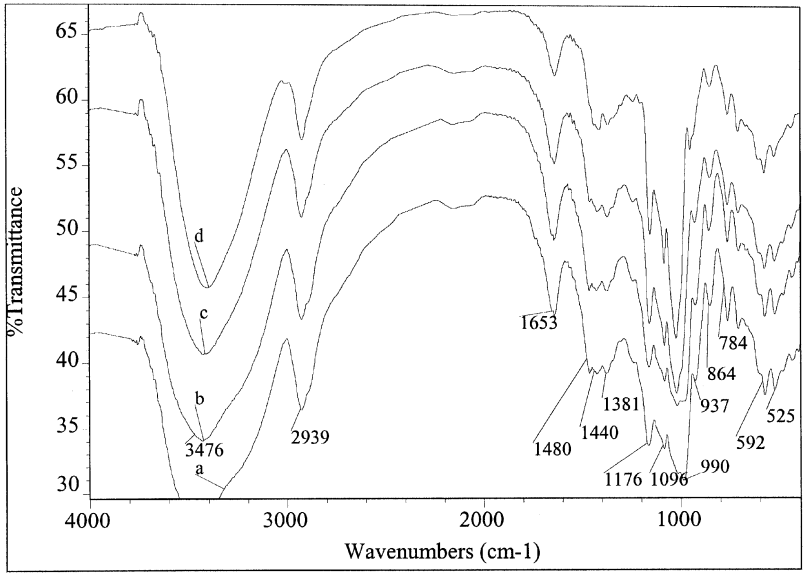

FIGURE 4 FT-IR spectra of potato starch (a), cold-water-soluble starch (b), hot-water-soluble starch (c), and DMSO-soluble starch (d) isolated from sago pith.

FT-IR Spectra

The FT-IR spectra of potato starch (spectrum a), cold-water-soluble starch (spectrum b), hot-water-soluble starch (spectrum c), and DMSO-soluble starch (spectrum d) are shown in Figure 4. As can be seen from Figure 4, all four spectra appeared to be rather similar, indicating that the "core" of the sago starch structure does not change significantly during the hot water and DMSO extraction processes. The strong broad band between 980 and 1200 cm^{-1} with three peaks at 990, 1096, and 1176 cm^{-1} is the most characteristic band for starch and is attributed to C-O stretching[37]. A small band at 1480 cm^{-1} originates from methylene bending vibrations. The absorption at 1653 cm^{-1} is principally associated with absorbed water[38]. Another characteristic band is the one between 3400–3480 cm^{-1}, due to hydroxyl bond stretching. The sharp band at 2939 cm^{-1} is indicative of methylene group deformation in sago starch.

^{13}C-NMR Spectra

Figure 5 illustrates two typical ^{13}C-NMR spectra of cold-water-soluble starch (spectrum a) and hot-water-soluble starch (spectrum b).

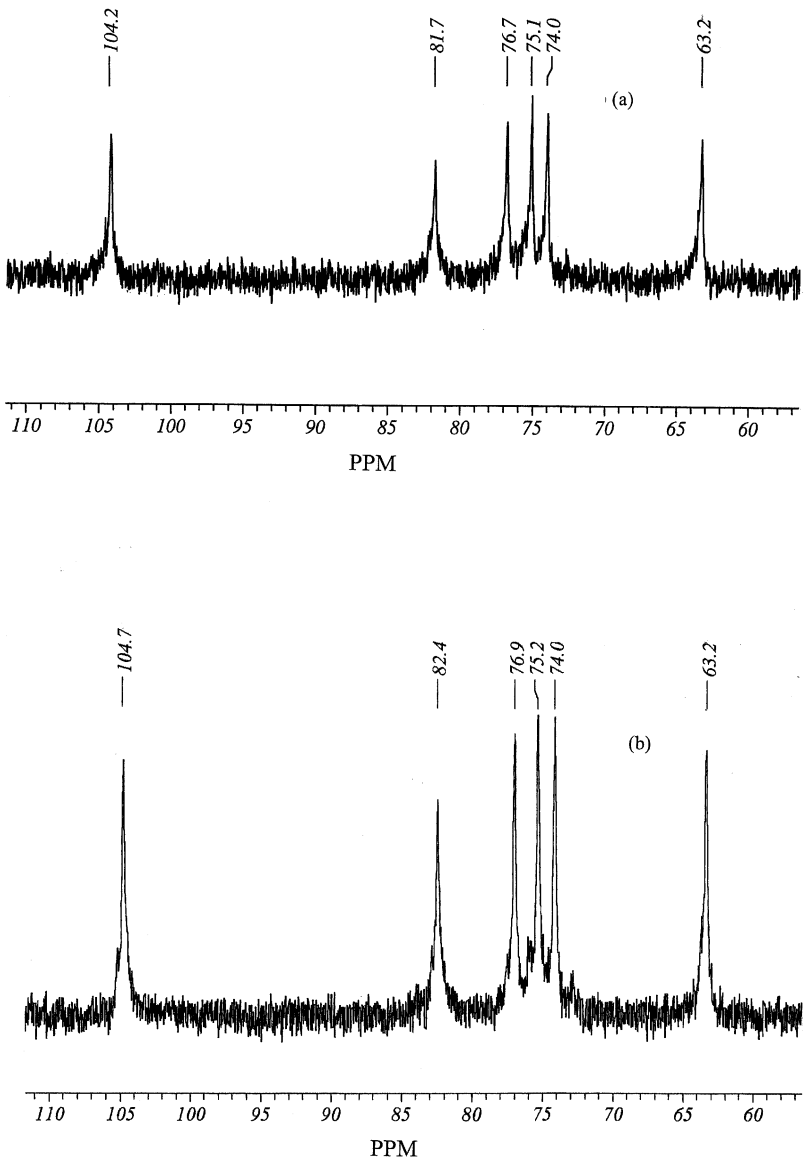

FIGURE 5 ^{13}C-NMR spectra of cold-water-soluble starch (spectrum a) and hot-water-soluble starch (spectrum b).

The similar C_1 and C_6 peaks can be unambiguously attributed on the spectra, again indicating a similar structure of the two starch fractions. The main 1,4-linked α-*D*-glucose units are obviously characterized by the signals at 104.2–104.7, 81.7–82.4, 76.7–76.9, 75.1–75.2, 74.0, and 63.2 ppm, which corresponds to C_1, C_4, C_3, C_2, C_5, and C_6 of the α-*D*-glucose units, respectively[39].

Thermal Analysis

When starch granules are heated under atmospheric conditions, they are thermally stable up to 200°–230°C. Thermal degradation occurs beyond 230°–250°C, depending mainly on the water content of the starch[37]. Pure amylose is stable up to 290°C, and its maximum decomposition rate appears at 330°C[37]. In this study, the thermal stability of the cold-water-soluble starch was examined by TGA, and its thermogram is shown in Figure 6. As observed, the starch has three weight loss stages. The main product in the first stage (25°–276°C) is water, formed by intermolecular or intramolecular condensation of starch hydroxyls[40]. The weight loss at this stage is 4.4% of the initial weight. The second stage starts at above 276°C and ends at about 400°C. The weight loss in the second and third stages is attributed to starch degradation.

Native granular starch contains crystalline areas within the amylopectin (branched) component but the linear amylose is largely amorphous

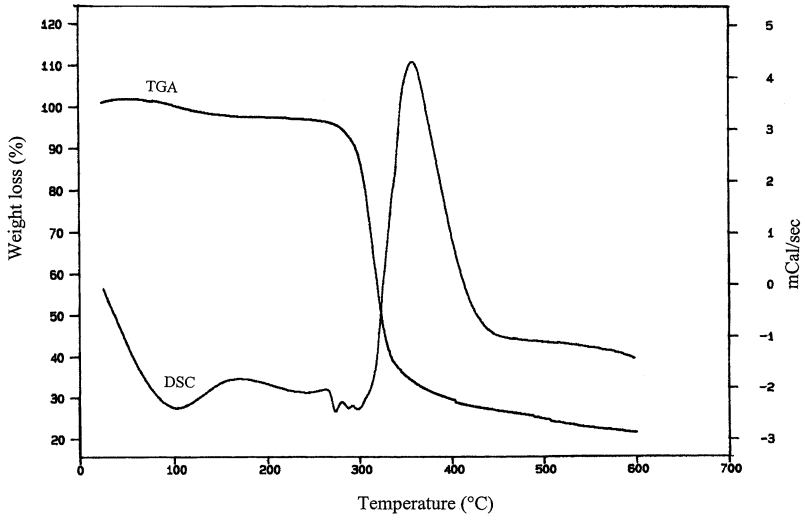

FIGURE 6 Thermogram of cold-water-soluble starch.

and can mostly be extracted in cold water[41]. Previous studies also found that amylose melts at a lower temperature compared to amylopectin and that the melting temperature depends on the water content of starch[37]. To determine the melting point, the DSC of cold-water-soluble starch was carried out and its curve is shown in Figure 6. A broad exothermic peak followed by a large and sharp endothermic one (maximum at 357°C) is observed on the thermogram of cold-water-soluble starch. Such endotherms are typical for transition from a crystallized to a more amorphous state, namely gelatinization[39]. The melting temperature of the starch occurs at 101°C, mainly due to the presence of lipids in starch, which are able to complex the amylose[3]. However, other studies of amylose, dextran, and pullulan have reported that the endothermic peaks above 100°C (about 120°–125°C) were caused by water evaporation[42]. In addition, studies on resistant starch have reported that the endothermic transition centered around 150°C corresponds to the reassociation of amylose[43], which is in accordance with the transition observed in this study.

CONCLUSIONS

The above results revealed that starch is a predominant constituent of sago pith, which represents 83.9% of the dried pith. Successive extraction with cold and hot water together resulted in a release of 67.9% of the original starch, containing noticeable amounts of amylose, whereas subsequent extraction with DMSO and 5% NaOH yielded 21.1% and 5.9% starch based on dried sago pith, respectively, enriched in amylopectin. Small amounts of other polysaccharides such as β-glucan and hemicelluloses were also co-extracted with the starch fractions. It should be noted that the values of starch differ from some of those already published, but this is not surprising in view of the difficulty in measuring these polysaccharides and the steady evolution of methods to deal with the various problems in their analysis. Another reason for these higher yields of starch is due to the extensively fractional extractions of sago pith with cold water, hot water, DMSO, and 5% NaOH in this study. Further work is now in progress on their physical-chemical properties, pasting behavior and gel characteristics, and the fine structures to obtain a more precise insight into the granular structure.

REFERENCES

[1] Cui, R. and C. G. Oates. (1997). *Carbohydr. Polym.* 32:65.
[2] Kennedy, J. F., A. J. Griffiths, and D. P. Atkins. (1983). In G. O. Phillips, D. J. Wedlock, and P. A. Williams, eds., *Gums and Stabilizers for Food Industrials 2*, p. 430. Oxford: Pergamon Press.

[3] Ahmad, F. B., P. A. Williams, J. L. Doublier, S. Durand, and A. Buleon. (1999). *Carbohydr. Polym.* 38:361.
[4] Oxford, P. D., S. G. Ring, V. Carroll, M. J. Miles, and V. J. Morris. (1987). *J. Sci. Food Agric.* 39:169.
[5] Wang, W. J., A. D. Powell, and C. G. Oates. (1995). *Carbohydr. Polym.* 26:91.
[6] Zachariassen, B. (1977). In *The First International Sago Symposium*, ed. K. Tan and K. Lumpur, p. 214.
[7] Hoover, R., and T. Vasanthan. (1992). *Carbohydr. Polym.* 19:285.
[8] Wang, W. J., A. D. Powell, and C. G. Oates. (1996). *Bioresource Technol.* 55:55.
[9] Sim, S. L., C. G. Oates, and H. A. Wong. (1991). *Starch/Stärke*. 43:459.
[10] Muhammad, K., F. Hussin, Y. C. Man, H. M. Ghazali, and J. F. Kennedy. (2000). *Carbohydr. Polym.* 42:85.
[11] Gorinstein, S., C. G. Oates, S. M. Chang, and C. Y. Lii. (1994). *Food Chem.* 49:411.
[12] Gorinstein, S., and C. Y. Lii. (1992). *Starch/Stärke*, 44:461.
[13] Govindasamy, S., C. G. Oates, and H. A. Wong. (1992). *Carbohydr. Polym.* 18:89.
[14] Fogarty, W. M., and C. T. Kelly. (1990). In W. M. Fogarty and T. Kelly, eds., *Microbial Enzymes and Biotechnology*, p. 71. London: Elsevier.
[15] Bergmann, F. W., J. Abe, and S. Hizikuri. (1988). *Appl. Microbiol. Biotechnol.* 27:443.
[16] Haska, N., and Y. Ohta. (1993). *Starch/Stärke*. 45:241.
[17] Sun, R. C., G. L. Jones, J. Tomkinson, and J. Bolton. (1999). *Ind. Crops Prod.* 19:211.
[18] Blakeney, A. B., P. J. Harris, R. J. Henry, and B. A. Stone. (1983). *Carbohydr. Res.* 113:291.
[19] Schoch, T. J. (1942). *J. Am. Chem. Soc.* 64:2954.
[20] Fujii, S., S. Kishihara, and M. Komoto. (1986). In *Proceedings of the Third International Sago Symposium*, p. 186. Tokyo.
[21] Eng-Tian, K. L., T. Yiu-Liong, and P. S. Chin-Yun. (1991). In *Proceedings of the Fourth International Sago Symposium*, p. 137. Kuching, Sarawak, Malaysia.
[22] Maeda, E. (1986). In *Proceedings of the Third International Sago Symposium*, p. 109. Tokyo.
[23] Flach, M., and D. L. Schuiling. (1989). *Agroforestry Systems*, 7:259.
[24] Anson, K. I., V. J. Morris, and S. G. Ring. (1985). In R. D. Hill and L. Munck, eds., *New Approaches to Research on Cereal Carbohydrates*, p. 115. Amsterdam: Elsevier.
[25] Dimler, R. J., H. A. Davis, C. E. Rist, and G. E. Hilbert. (1944). *Cereal Chem.* 21:430.
[26] Mistry, A. H., and S. R. Eckhoff. (1992). *Cereal Chem.* 69:296.
[27] Eerlingen, R. C., H. Jacobs, and J. A. Delcour. (1994). *Cereal Chem.* 71:351.
[28] Oates, C. G., W. J. Wang, and A. D. Powell. (1994). In *Trends in Biotechnology: Meeting the Challenges of the 21st Century. Proceedings of the 2nd Symposium*, eds. H. M. Ghazali, N. M. Salleh, and N. A. A. Rashid, p. 33.
[29] Sims, K. A., and M. Cheryan. (1992). *J. Food Sci.* 57:163.
[30] Labout, J. J. M. (1985). *Starch/Stärke*. 37:157.
[31] Govindasamy, S., O. H. Campanella, and C. G. Oates. (1995). *Food Chem.* 54:289.
[32] Fullbrook, P. D. (1984). In S. Z. Dziedzic and M. W. Kearsley, eds., *Glucose Syrups: Science and Technology*, p. 65. London: Elsevier.
[33] Holm, J., I. Lundquist, and I. Bjorck. (1988). *Am. J. Clin. Nutr.* 47:1010.
[34] Knutson, C. A. (1999). *Carbohydr. Polym.* 42:65.
[35] Ozawa, T. and Y. Arai. (1986). In *Proceedings of the Third International Sago Symposium*, p. 197. Tokyo.
[36] Okamoto, A., T. Ozawa, H. Imagawa, and Y. Arai. (1985). *Nippon Nogeikagaku Kaishi*. 59:1257.

[37] Aburto, J., I. Alric, S. Thiebaud, E. Borredon, D. Bikiaris, J. Prinos, and C. Panayiotou. (1999). *J. Appl. Polym. Sci.* 74:1440.
[38] Sun, R. C., J. Tomkinson, P. L. Ma, and S. F. Liang. (2000). *Carbohydr. Polym.* 42:111.
[39] Fringant, C., J. Desbrieres, and M. Rinaudo. (1996). *Polymer*, 37:2663.
[40] Morita, H. (1956). *Anal. Chem.* 28:64.
[41] Swanson, C. L., R. L. Shorgren, G. F. Fanta, and S. H. Imam. (1993). *J. Environ. Polym. Degrad.* 1:155.
[42] Aguilera, J. M., T. R. Cuadros, and J. M. del Valle. (1998). *Carbohydr. Polym.* 37:79.
[43] Billiaderis, C. G., C. M. Page, L. Slade, and R. R. Sirett. (1985). *Carbohydr. Polym.* 5:367.