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The effect of retrogradation on enzyme susceptibility of sago starch

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Gelatinized sago starch was stored for different times and under different temperature conditions for the investigation of retrogradation. DSC was used to monitor the thermal properties of retrograded starch. Bioavailability of starch samples was determined by porcine pancreatic α-amylase at 37°C. Amylose retrograded rapidly at 5°C, whereas amylopectin recrystallization was enhanced by sequential storage at 5°C followed by 30°C. Increased extent of retrogradation (high melting enthalpy values and melting temperature) caused reduced enzyme susceptibility of sago starch and sago products at 37°C. © 1997 Elsevier Science Ltd

INTRODUCTION

Studies (Englyst *et al.*, 1992; Lintas & Cappelloni, 1992; Muir & O'Dea, 1992) have shown that many carbohydrate-rich foods contain variable amounts of starch that escape digestion in the small intestine, this fraction of starch has been called resistant starch. There are three kinds of resistant starch: (a) starch granules physically inaccessible to digestive enzymes, such as partly milled grains and seeds (Type I); (b) starch in a certain granular form which is particularly resistant to enzyme digestion, such as raw potato and banana (Type II); (c) retrograded starch (TYPE III) (Englyst *et al.*, 1992). Many studies (Siljestrom *et al.*, 1989; Sievert & Pomeranz, 1989; Sievert & Pomeranz, 1990; Czuchajowska *et al.*, 1991) conclude that resistant starch (Type III), which is formed during storage of gelatinized starch, consists mainly of retrograded amylose. However, Eerlingen's recent work on waxy maize starch shows that highly retrograded amylopectin was also resistant to digestive enzymes, but to a lower extent than retrograded amylose.

Starch is the most widely produced carbohydrate by plants. It is deposited in the form of tiny granules which consist of amylose and amylopectin in variable proportions. Amylose is an essentially linear polymer composed mostly of α-1,4-linked D-glucose with a low degree of α-1,6-linkage branch points (French, 1984). Amylopectin is highly α-1,6-branched. The generally accepted model of the branching system is the cluster model suggested by Kainuma & French (1972), in which the starch granule is composed of crystalline and amorphous regions. Linear portions of amylopectin constitute the crystalline region, whereas the branch points and amylose are the main components of the amorphous portion. Amylose is believed to fill the spaces between the clusters (Blanshard, 1987). Starch granules are insoluble in cold water, but on heating above 50°C in excess water, granule structure is altered by swelling, hydration and solubilization. This process, called gelatinization, involves the melting of the crystallites (Colonna *et al.*, 1992). On cooling gelatinized starch, retrogradation, involving amylose and amylopectin, may occur. Recent studies (Miles *et al.*, 1984; Eerlingen *et al.*, 1994) have shown that retrogradation consists of two processes: (a) gelation of amylose-formation of a gel network which happens in hours; (b) recrystallization of amylopectin—a much slower process influenced by storage temperature. Amylose gelation involves chain entanglement, which is thermally irreversible (at temperatures < 120°C), while amylopectin recrystallization is thermally reversible.

Differential scanning calorimetry (DSC) is a thermal analysis method widely used to monitor starch retrogradation. Controlled heating of a suspension of native starch is characterized by an endotherm, reflecting the melting transition of starch (Biliaderis, 1990). Freshly gelatinized starch does not give any melting peak on reheating. Disassociation of retrograded amylopectin is also an endothermic process, but occurs at a lower temperature than that of native starch. The more closely bonded nature of retrograded amylose is reflected by a

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higher melting temperature, 150°C for wheat starch (Sievert & Pomeranz, 1990). Melting temperatures and enthalpies of these endothermic transitions can be used to deduce the extent of starch retrogradation.

Digestion of starch, under physiological conditions, begins in the mouth with the action of salivary α-amylase. The action of salivary α-amylase is limited, as this enzyme is quickly inactivated by the low stomach pH. The small intestine is the site for the majority of starch breakdown, principally by pancreatic α-amylase, to maltose, maltotriose and α-limit-dextrins. Disaccharides and oligosaccharides are hydrolysed by enzymes present in the brush border of the small intestine mucosa. These enzymes, including glucoamylase and sucraseisomaltase, are complementary in specificity: sucrase can initiate α-limit-dextrin hydrolysis by removing the nonreducing α-1,4-linkages until it is stopped by a α-1,6-linkage, which will, in turn, be cleaved by isomaltase. The liberated maltose and maltotriose are then cleaved by sucrase, isomaltase and glucoamylase to yield glucose (Flourié, 1989; Würsch, 1989). In this study, porcine pancreatic α-amylase was chosen in an *in vitro* test to grossly mimic starch digestion in the human intestine.

Enzyme susceptibility of starchy foods is affected by several factors, such as botanical structure, physical texture and granular structure. Among these factors, granular structure is believed to be the most important (Granfeldt *et al.*, 1995; Holm *et al.*, 1988). Heating and mixing during cooking disrupts the granular structure, thus increasing enzymatic availability (Snow & O'Dea, 1981), but on cooling gelatinized starch, amylose retrogradation again reduces the digestibility of starch, which is further complicated by the recrystallization of amylopectin (Eerlingen *et al.*, 1994).

Sago palm is a once-flowering perennial palm commonly distributed in tropical swampy areas such as southeast Asia (Corbishley & Miller, 1984). Sago starch is obtained from the stems of sago palms. Since sago palm thrives well in swampy areas and on peat soils, which would require extensive engineering and costly maintenance to exploit for other plantations and annual crops, it has long been a staple food in these areas (Tan, 1980). Sago starch can be used for the same purpose as any other kind of starch. 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. Sago pearl is a starch product for preservation. In processing, slightly wet flour is pressed through a sieve and dried in a pan over a fire while being stirred continuously. The small sago pearls become rounded and the outer portion gelatinized, the network so formed on the outer surface thus holds the pearls intact. These pearls are an indispensable part of soups and puddings in sago consuming areas (Flach, 1984).

In this study, bioavailability of sago starch processed and stored under a variety of conditions was determined. Extent of gelatinization and retrogradation of the starch was assessed and compared with enzyme susceptibility, by porcine pancreatic α-amylase, crudely mimicking starch digestion under physiological conditions. Retrograded starch gel was cut into small particles before being subjected to enzyme hydrolysis. The particle sizes were similar to those swallowed following mastication of gels by human subjects (Diaz-Tay & Lucas, personal communication). Sago pearls were also similarly investigated.

The aim of this study was to observe the retrogradation process of gelatinized sago starch stored under different conditions, to investigate the influence of retrogradation on enzyme susceptibility of sago starch and starch products at 37°C, and to provide recommendations on increasing the nutritional value of sago products through the control of their storage and processing.

MATERIALS AND METHODS

Starch

Sago starch (*Metroxylon* spp.) from Sarawak, Malaysia, was supplied by Wah Chang International Group of Companies (Singapore). It contained 9.0% moisture (calculated from weight-loss after drying in an oven at 12°C for 2 h) and was used, as such, in the experiments. Its total lipids content was 0.23% (Lim, personal communication).

Sago pearl (product of Malaysia, moisture content 11.4%) was obtained from a commercial source. It was ground in a pestle and mortar before being subjected to DSC analysis.

Enzyme

The enzyme used in this experiment was α-amylase (EC 3.2.1.1) from porcine pancreas (A6255, Sigma Chemical Co., St. Louis, MO, USA), with an α-amylase activity of 1260 units/mg prot. One unit is defined as the amount of enzyme liberating 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20°C. A working solution was prepared by diluting a suspension of twice crystallized α-amylase in 2.9 M NaCl solution containing 3 mM $CaCl_2$ to a concentration of 1 mg/ml.

Gel preparation for enzyme hydrolysis

The 40% dry matter gels were prepared by the method of Roulet *et al.* (1990). Sago starch (0.2%) suspensions were gelatinized by incubating for 15 min in a boiling water bath, then cooled to 30°C. By that stage, more starch was added to obtain the 40% dry matter suspension which was then homogenized for 2 min, with a

Ultra-Turrax T25 homogenizer, at a speed of 8000 rev min^{-1}. The suspension was transferred to screw bottles for subsequent gelatinization in a ventilated oven at 95°C for 110 min without mixing. After cooling to ambient temperature, the capped screw bottles were sealed with sealing film and stored at 5°C, or 5°C followed by 34°C, for up to 5 days.

Enzyme hydrolysis of sago starch

Hydrolysis was performed with freshly gelatinized and retrograded sago starch. Sago starch (0.1 g) was weighed into a 125 ml Erlenmeyer flask and 5 ml distilled water added. This dispersion was gelatinized in a boiling water bath for 15 min and cooled to 37°C before being subjected to enzyme hydrolysis. Stored, processed starch (0.25 g) was cut into small particles, dispersed in distilled water (4.85 ml) and made up to a final weight of 6.0 g by addition of 0.1 M phosphate buffer (pH 7.1). Enzyme reaction was initiated by the addition of 0.1% (% based on the weight of starch) porcine pancreatic α-amylase solution. The enzyme added was in excess, determined by prior investigation (not reported). Following the addition of the enzyme, samples were covered with sealing film and incubated in B.Braun Incubation Hood (Germany) for 1, 2, 4 and 8 h at 37°C, and the shaking rate was 150 min^{-1}. Each sample was subsequently mixed with 0.6 ml, 0.4 mM $HgCl_2$ and incubated at 90°C for 15 min to inactivate the enzyme (Govindasamy *et al.*, 1992). Samples were centrifuged at 2500 rpm for 10 min (Jouan BR. A 3.11), the supernatants were used for reducing sugar determination.

Analytical methods

The degree of hydrolysis (D.H.) was defined as follows:

$$\text{D.H.}(\%) = \frac{\text{Reducing sugar produced by enzyme hydrolysis}}{\text{Reducing sugar produced by acid hydrolysis}} \times 100\%$$

Reducing sugar was determined by the method of Dygert *et al.* (1965) using glucose as a standard. Acid hydrolysis was carried out by incubating starch granules with 1 M HCl at 100°C for 2 h.

Differential scanning calorimetry

Differential scanning calorimetry (Perkin-Elmer, DSC-7, Newark, CT, USA) was used to determine the thermal characteristics of native, freshly gelatinized and retrograded starch. Starch samples (40% dry matter starch slurry, volume fraction of water = 0.69) were accurately weighed into aluminum pans and hermetically sealed. The samples, native starch or gelatinized starch, were analyzed by heating them from 20 to 160°C at 10°C/min. After heating, pans were reweighed to check for leakage, and stored at 5 or 34°C for up to 5 days. The samples were reheated from 20 to 160°C at a scanning rate of 10°C/min, with an empty pan serving as a reference. The upper temperature limit was determined by failure of the hermetic pans. Enthalpy changes and onset temperatures, integrated using DC-7 software, were calibrated on the basis of the indium standard. T_o, T_p and T_e denote onset, peak and complete transition temperatures, respectively; ΔH is transition enthalpy computed in J/g. All pans were cooled and reweighed after the run to ensure that no moisture was lost during the run.

HPSEC

A Waters Associates (Milford, MA, USA) series liquid chromatography system, with a model 510 pump, WISP model 712 injector and a model 410 differential refractometer detector, was used. The detector signal was electronically recorded and integrated by a Data Module Integrator Waters 746. Columns and refractmeter were maintained at 40°C. Columns were connected in the following order: Ultrahydrogel guard column followed by Ultrahydrogel linear and two Ultrahydrogel 120. Ultrahydrogel columns are packed with crosslinked methacrylate gel. Ultrahydrogel linear has a blend pore size with exclusion limit 7×10^6, Ultrahydrogel 120 is 120 Å in pore size, 5×10^3 exclusion limit. The mobile phase was deionized water at a flow rate of 0.8 ml/min. The analysis procedure was performed following the method of Govindasamy *et al.* (1992).

Raw sago starch and soluble components of 5 days, 5°C stored sago starch gel were analyzed by HPSEC. Raw starch stored starch gel samples were dissolved by a modified Jackson method (Jackson *et al.*, 1988) that resulted in 95.5% of intact sago starch solubilised. Twenty milliliters distilled water was added to 30 mg (dry matter) starch sample and the suspension heated in a boiling water bath for 20 min. When cooled, the suspension was dispersed further by sonication (Heat System Inc., New York, USA) for 50 s. After centrifuging 10 min at 2500 and 10 000 rpm (for native starch and starch gel, respectively), the supernatant passed through a 8.0-μm SC Milipore filter prior to HPSEC analysis (filtration loss is 0.04%).

RESULTS AND CONCLUSION

Differential scanning calorimetry

The thermal characteristics of the native, freshly gelatinized and retrograded starches were found to be different (Table 1). Representative DSC thermograms are shown in Fig. 1.

Thermograms of heated native sago starch (volume

Table 1. Thermal transition data[a] of native, freshly gelatinized and retrograded sago starch

Starch	T_o[b]	T_p[c]	T_c[d]	ΔH[e]	T_o[b]	T_p[c]	T_c[d]	ΔH[e]
Native	67.2±3.1	74.0±0.8	88.2±0.7	15.6±2.4	—[f]	—	—	—
Gelatinized	—	—	—	—	—	—	—	—
Retrograded								
1 h 5°C	—	—	—	—	142.0±3.4	145.2±1.2	147.7±1.6	1.7±0.2
3 h 5°C	—	—	—	—	147.3±5.7	150.4±3.6	153.2±3.4	1.8±0.3
6 h 5°C	41.5±0.6	58.4±1.6	69.9±0.3	3.7±0.4	147.4±3.0	152.4±1.8	158.5±1.8	2.1±0.2
12 h 5°C	45.3±0.3	61.4±1.4	72.2±1.4	4.4±0.8	147.5±2.5	150.2±3.8	159.5±5.5	2.5±0.4
24 h 5°C	46.2±0.7	59.7±1.2	72.8±0.9	5.1±0.7	152.0±2.1	155.5±1.9	158.5±2.3	2.6±0.3
2 days 5°C	45.9±0.8	57.4±0.2	71.0±0.9	6.6±0.8	148.3±0.9	149.1±2.1	158.1±2.0	1.9±0.4
5 days 5°C	46.4±0.3	55.5±0.2	70.2±1.6	6.8±0.4	139.6±3.2	144.9±7.6	149.7±4.3	1.8±0.1
1 day 5°C, 4 days 34°C	65.5±0.2	70.2±0.4	83.9±0.6	6.8±0.8	154.4±1.4	157.5±1.8	159.4±2.2	1.9±0.2

[a]Mean ± SD ($n=4$).
[b]Onset transition temperature (°C).
[c]Peak transition temperature (°C).
[d]Complete transition temperature (°C).
[e]Enthalpy (ΔH, J/g).
[f]None detected.

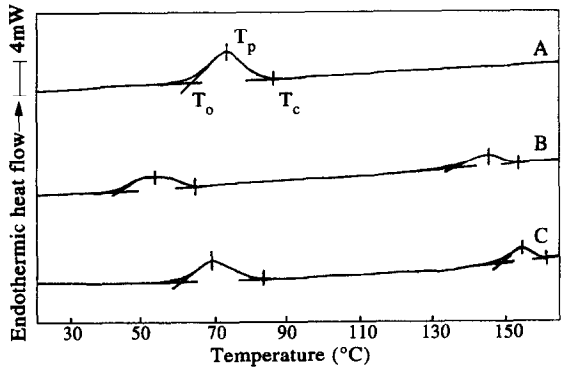

Fig. 1. Representative DSC thermograms of sago starch samples. Treatments of starch: (A) native; (B) 5 days, 5°C stored; (C) 1 day, 5°C, followed by 4 days, 34°C stored. Weight of starch (dry basis), from top to bottom: 3.531. 3,538 and 2.821 mg.

fraction of water, 0.69) revealed one endothermic peak (Fig. 1A), centered at (T_p) 74°C and enthalpy 15.6 J/g of starch (dry weight). Such endotherms are typical for heated starch slurries, reflecting heat–moisture induced transition from a crystallized to a more amorphous state, namely gelatinization. Heating freshly gelatinized sago starch, 20–100°C, did not produce any thermal transitions (Table 1), indicating that gelatinization under the experimental conditions was complete and retrogradation had not occurred at this stage. Gelatinized starch samples on storage at 5°C recrystallized, indicated by the appearance of two endothermic transitions, attributed to the retrogradation of both amylose and amylopectin (Fig. 1B). Amylose retrogradation occurred rapidly, within 1 h storage at 5°C, and was characterized by an endothermic transition centered at 145.2°C (T_p), enthalpy 1.7 J/g. Extent of retrogradation increased rapidly with storage time, enthalpy of the endotherm increasing to 1.8 J/g after 3 h and reaching a maximum after 6 h (ΔH_R 2.1 J/g). Studies on resistant starch have reported that the endothermic transition centered around 150°C corresponds to the reassociation of amylose (Biliaderis *et al.*, 1985; Szczodrak & Pomeranz, 1991; Gruchala & Pomeranz, 1993) which is consistent with the transition reported in this work. Comparing the HPSEC profiles of native sago starch and that of solublised stored starch gels (Fig. 3), the absence of an amylose peak in the latter also confirms complete amylose retrogradation. Increasing time of storage was characterized by appearance of an endothermic transition centered at 55–70°C, attributed to retrograded amylopectin. This transition was absent during the first 3 h of storage (Table 1). After storing at 5°C for 6 h, a broad transition peak was observed, centered around 58.4°C (T_p), enthalpy 3.9 J/g. Storage for 12 h, 24 h, 2 days and 5 days led to structural changes, expressed as an increase in enthalpy with time (4.4, 5.1, 6.6 and 6.8 J/g, respectively). There was no change in the transition temperature (Table 1). Significant increase in the onset, peak and completion temperature were observed when starch samples were stored for 24 h at 5°C followed by 4 days at 34°C (Table 1 and Fig. 1C), the transition peak of retrograded amylopectin (T_p 70.2°C) approaching that of native starch. The endothermic transition was sharper compared with starch samples stored at 5°C, suggesting that a more perfect recrystallization was obtained at 34°C than at 5°C. Such results are in accord with Eerlingen's study on waxy maize starch (100% amylopectin) and Levine and Slade's study on wheat starch. Many studies on starch retrogradation (Roulet *et al.*, 1990; Levine & Slade, 1990; White *et al.*, 1989; Russell, 1987) have not reported the amylose transition peak, because their scanning ranges were lower than 130°C.

Comparing amylopectin retrogradation under different temperatures, significant difference could be observed. The amylopectin transition temperature for stored sago starch gels was 5°C, 15–20°C lower than that of native sago starch. Whereas, in material stored for 24 h at 5°C, followed by 4 days at 34°C, slightly lower transition temperatures were evident compared to native sago starch (Table 1). The recrystallization process strongly depends on temperature. Similar phenomena were also found by Slade & Levine (1987) and Wunderlich (1976) which suggests that recrystallization can only proceed within the temperature range of T_g–T_m (from glass transition temperature, T_g, to crystalline melting temperature, T_m) via a three-step sequential mechanism: (1) nucleation, formation of critical nuclei; (2) propagation crystal growth; and (3) maturation, crystal perfection. Within the range T_g–T_m, nucleation rate increases with decreasing temperature, down to T_g, while propagation rate increases with increasing temperature, up to T_m. Levine & Slade (1990) have shown that starch gels initially held at 6°C, to promote nucleation, then at 40°C to allow rapid propagation, undergo the greatest overall rate and extent of amylopectin recrystallization. The endothermic transition of the recrystallized amylopectin occurs at a lower temperature than that of gelatinization. When gelatinized starch is stored at a low temperature (0°C to room temperature), less perfect crystallites (lower T_p and broader melting transitions) are formed than at a higher temperature (room temperature to T_{in}) (White *et al.*, 1989).

Enzyme hydrolysis

Sago starch samples, native, gelatinized and retrograded, were significantly different in their reactivity to enzyme hydrolysis by porcine pancreatic α-amylase (Table 2 and Fig. 2). This enzyme was chosen so as to mimic starch digestion in the human small intestine.

Freshly gelatinized sago starch displayed the greatest susceptibility to porcine pancreatic α-amylase (added at a concentration of 0.1% starch weight, i.e. 1260 units/g starch). Within the first hour, 35.3% hydrolysis was achieved; a greater extent of hydrolysis (78.3%) was obtained after 8 h incubation. These hydrolysis rates are relatively low compared to other starches. Holm *et al.* (1988) has reported that freshly gelatinized wheat starch could be hydrolysed to 65% within 30 min by porcine pancreatic α-amylase (at a concentration of 200 units/g starch), reaching a plateau at 70% after 1 h.

Retrogradation significantly lowered enzymatic susceptibility of gelatinized starch. In this experiment, retrograded starch gel had been cut into small particles before subjected to enzyme hydrolysis. The size (minor axis length) of these particles range from fine size to

Table 2. Degree of hydrolysis (D.H.,%)[a] of sago starch samples at 37°C by porcine pancreatic α-amylase

Starch	Incubation time (h)			
	1	2	4	8
Gelatinized	54.7±1.5	55.4±6.1	60.0±6.4	78.3±5.2
Retrograded				
1 h 5°C	28.2±1.2	32.8±2.4	43.4±2.3	45.4±3.1
3 h 5°C	24.0±1.3	27.1±1.5	34.3±2.1	48.4±3.0
6 h 5°C	19.3±2.1	26.6±1.4	36.1±2.5	49.8±2.7
12 h 5°C	20.5±1.7	30.8±4.2	34.6±2.0	44.8±1.4
24 h 5°C	20.4±2.2	27.6±7.1	34.4±1.9	44.9±1.3
2 days 5°C	17.0±0.8	26.8±1.3	38.2±3.4	44.4±0.5
5 days 5°C	15.4±1.1	27.9±2.3	35.6±1.8	49.9±5.1
1 day 5°C, 4 days 34°C	13.6±0.3	20.7±0.7	30.6±0.4	41.5±0.2

[a]Mean±SD (n=3).

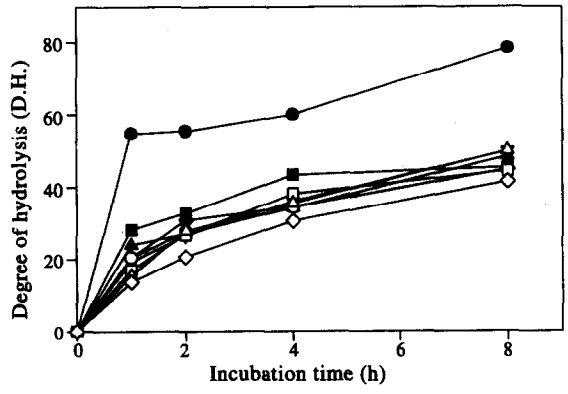

Fig. 2. Hydrolysis of starch samples at 37°C by porcine pancreatic α-amylase: (●) freshly gelatinized; (■) 1 h, 5°C stored; (▲) 3 h, 5°C stored; (▼) 6 h, 5°C stored; (◆) 12 h, 5°C stored; (○) 24 h, 5°C stored; (□) 2 days, 5°C stored; (△) 5 days, 5°C stored; (◇) 1 day, 5°C, followed by 4 days, 34°C stored.

4.1 mm, 50% of particles are under 2.2 mm. This size range falls into the tested particle size range (fine size to 11.2 mm) of similar gel chewed by human subjects (Diaz-Tay & Lucas, personal communication). The enzyme hydrolysis results of these gel samples showed, after 1 h storage at 5°C, that the digestion rate of sago starch gel was much lower than that of freshly gelatinized samples (Table 2). Within the first hour, the degree of hydrolysis decreased to 28.2% and at 8 h, the degree of hydrolysis was 45.4%. The degree of hydrolysis of starch samples decreased further with continued storage. After 6 h of storage at 5°C, the 1 and 8 h degree of hydrolysis were 19.3 and 49.8%, respectively. Storage time longer than 6 h at 5°C did not seem to have further influence on the gel's enzyme susceptibility. Samples stored for 12 h, 24 h, 2 days and 5 days shared similar digestion rates to that of 6 h stored starch samples, but samples stored for 24 h at 5°C followed by 4 days at 34°C showed higher enzyme resistance. A relatively low degree of hydrolysis (13.41%) was achieved in the first

hour of incubation and 41.3% degree of hydrolysis was obtained after 8 h incubation. These results were in accord with the DSC profiles of gelatinized sago starch stored under the same conditions (Table 1, Fig. 1). Amylose retrogradation rapidly developed to full scale in the first 6 h of storage at 5°C. It is reasonable to assume that amylose retrogradation contributed to the decrease in enzyme availability of sago starch samples in the first few hours of storage. This assumption is supported by recent work of Wang *et al.* (1995) on raw sago starch, which shows that enthalpy of gelatinization remained constant during the course of enzyme activity upto 85% hydrolysis, suggesting that the amorphous regions were preferentially hydrolysed. Retrogradation of amylose, namely network formation in amorphous regions, can significantly reduce enzyme susceptibility of starch samples. Recrystallization of amylopectin, a much slower process, resulted in the formation of less perfect crystallites. The DSC gelatinization endotherm gives a measure of crystallite quality from peak temperature. The low-gelatinization temperature starches have more amorphous and less crystalline material than the high-gelatinization temperature starches (Tester & Morrison, 1990). The peak temperatures of the melting transition of these crystallites were about 15°C lower than that of native sago starch (Table 1). Onset temperatures were in the range of 41–46°C. Since the onset temperatures were close to incubation temperature, amylopectin retrogradation at 5°C did not show any significant effect on enzyme susceptibility of starch samples. At a higher storage temperature (34°C), starch samples, with increased melting temperature and melting enthalpy values, showed lower enzyme availability.

Enzyme hydrolysis of starch by α-amylase occurs in three successive steps: diffusion of the enzyme molecule towards its substrate, absorption of the enzyme on the substrate and the catalytic event (Colonna *et al.*, 1992). Diffusion is considered the limiting step in hydrolysis of retrograded starch (Eerlingen *et al.*, 1994). Freshly gelatinized starch, which is in a solubilized structure, is easily accessed by the enzyme. On cooling this starch at 5° and 34°C, retrogradation occurs. This rapid development of a network via amylose chain entanglement followed by a slow amylopectin recrystallization in the polymer-rich regions (Ring *et al.*, 1987; Miles *et al.*, 1984) makes it increasingly difficult for the enzyme to access the substrate. In this experiment, not only amylose gelation, but also the high extent of amylopectin recrystallization (1 day 5°C followed by 4 days 34°C stored) played a role in decreasing enzyme susceptibility of starch. It is reasonable to assume that both the development of a gel network and increase in molecular order are responsible for the decrease of enzyme susceptibility. Eerlingen *et al.* (1994) also found a decrease in porcine pancreatic α-amylase susceptibility in stored waxy maize starch (100% amylopectin).

HPSEC analysis of the gels

Native sago starch and soluble components of sago starch gel stored at 5°C for 5 days were analyzed by HPSEC (Fig. 3). Comparing the two HPSEC profiles, significant differences were observed. According to the work of Govindasamy *et al.* (1992), Fig. 3A illustrates a typical native sago starch HPSEC profile showing amylopectin and amylose peaks, with an amylopectin/amylose ratio (peak area ratio) of 70.3/29.7. However, only an amylopectin peak was seen in the chromatogram of dissolved starch gels (Fig. 3B). Dissolved in 0.1 M sodium hydroxide and then also analyzed by HPSEC, the sediment of starch gel proved to be exclusively amylose (centrifuge and filtration loss of carbohydrate material was 5.2%). This suggests that amylose in the gel was completely retrograded, as amylose retrogradation is thermally irreversible, while retrograded amylopectin can be disrupted by gentle heating (Flourié, 1989). Such results are consistent with DSC

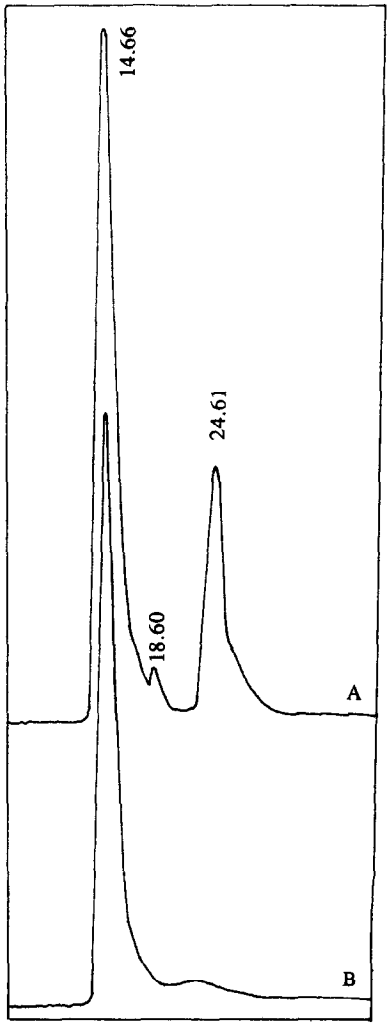

Fig. 3. HPSEC chromatograms of sago starch: (A) native starch; (B) supernatant of dissolved starch gel, which has been stored for 5 days at 5°C.

Table 3. Degree of hydrolysis (D.H., %)[a] of sago pearl samples at 37°C by porcine pancreatic α-amylase

Sago pearl	Incubation time (h)			
	1	2	4	8
Gelatinized	59.4±0.6	58.3±1.0	59.8±0.1	62.0±3.4
Stored	18.5±0.4	29.4±0.6	44.2±0.5	54.8±2.1

[a]Mean±SD ($n=3$).

analysis of 5°C stored starch gels. Stored samples exhibit a characteristic amylose retrograded melting peak at −150°C. After 6 h storage at 5°C, the transition enthalpy of retrograded amylose almost reached its maximum and did not change much with prolonged storage time.

Sago pearl

Thermal analysis of sago pearl-derived material, resulted in an endothermic peak centered at 77.6°C (T_p), with lower enthalpy (ΔH_G, 2.5 J/g) than that of native sago starch (ΔH_G 15.6 J/g). Since this peak appeared at the same temperature as that of native sago starch and no other peaks at lower temperature were detected, it is reasonable to suggest that the sago pearls consisted of partially gelatinized starch which had not retrograded. Freshly gelatinized (15 min in a boiling water bath) sago pearls were readily digested by porcine pancreatic α-amylase (Table 3). After 1 h of incubation at 37°C, the degree of hydrolysis was 59.4% (on 2 h acid hydrolysis basis). This is similar to the rate of hydrolysis of freshly gelatinized sago starch digested under similar conditions. When gelatinized sago pearls were cooled and stored, retrogradation occurred. Thermoanalysis of material stored for 1 day at 5°C followed by 4 days at 34°C revealed two endothermic peaks centered at 70.2 and 150.7°C (T_p), nearly the same as that of stored sago starch, suggesting that both amylose and amylopectin retrogradation had occurred. The enthalpy (ΔH_R) of the two peaks were 2.6 and 1.6 J/g, respectively. Stored sago pearl gel was also subjected to enzyme hydrolysis, enzyme susceptibility of which was significantly decreased, compared to freshly gelatinized sago pearl. After 1 h of incubation, the degree of hydrolysis of the sago pearl gel was only 18.5%, much lower than that of freshly cooked samples. As a result, the nutritional value of sago pearl was decreased during storage.

CONCLUSIONS

Sago starch retrogradation consists of two separate processes: gelation of amylose and; recrystallization of amylopectin.

Under storage at 5°C, amylose retrogradation was rapid and significantly reduced enzyme susceptibility of the starch. However, amylopectin recrystallization was not promoted at 5°C. Broad transition peaks at much lower temperatures than that of native sago starch were found for amylopectin retrogradation which had little influence on enzyme hydrolysis. Extensive retrogradation of amylopectin was achieved by sequential storage at 5°C followed by 34°C. Enzyme susceptibility of sago starch was further decreased at this stage. Based on these conclusions, several recommendations can be given on the consumption of sago foods. In general, as a staple food in swampy areas, sago foods should be well cooked (fully gelatinized) before they are served, and served soon after cooking. If there is a need for storage, refrigerated storage is highly recommended. For sago pearl, since it is usually added to soup and puddings, the boiling time should be sufficiently long (i.e. not shorter than 15 min). Immediate eating is also recommended. In the food industry, physical and/or chemical modifications are considered as practical ways to maintain the nutritional value of sago products during food processing. Further studies are needed to find the influence of these modifications on the hydrolysis behavior of sago starch.

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