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The effect of amylose–lipid complex formation on enzyme susceptibility of sago starch

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Abstract

Native sago starch was incubated at 60°C with lysophosphatidylcholine, monomyristin, monopalmitin, and monostearin. Differential scanning calorimetry peaks centred at 100–120°C indicated formation of amylose–lipid complexes. Among the four lipids, lysophosphatidylcholine showed the highest complexing ability, while that of the monoglycerides decreased with the increasing chain length. Part of the amylose leached during the incubation, and the amount of leached material decreased in the presence of lipids. Starch–lipid samples were subjected to enzyme hydrolysis by porcine pancreatic α-amylase. The bioavailability of native and freshly gelatinised sago starch was decreased in the presence of lipids, while retrograded starch–lipid samples showed higher digestibility than starch control.

1. Introduction

Starch is the major form of carbohydrate storage in plants and is considered the second largest biomass, next to cellulose. In virtually all countries, starch is a major component of the diet. In the West, it comprises some 27% of the food energy sources, and in Southeast Asia, starch represents an even greater proportion of the diet. About 50% of food energy is provided from starch (FAO, 1991). In these countries, sago is an important starch source for indigenous populations; in contrast, world wide, maize is the major commercial source of starch, supplying more than 78% of all starch. Sago palm was probably one of the first plants used by man in Southeast Asia (Avé, 1977), distributed in the large tracts of swampy land, providing the only source of starch. Sago flour is obtained from the stem of sago palms, and in production of food energy, it out-yields any other flour. It has a high carbohydrate content, 95% (dry weight basis), compared with maize, 64%, and rice, 89% (Soerjono, 1980). Sago flour has a minimum of fat and protein in comparison with cereal flours; traditional sago-consuming communities normally supplement their diet with fish. With suitable supplementary foods, the sago diet can have the same quality as cereal diets (Flach, 1984).

Studies on the digestion of starch in humans suggest that digestion begins in the mouth with the action of salivary α-amylase, but the action of this enzyme is limited as it is quickly inactivated by the low stomach pH. In the small intestine, the vast majority of starch is digested by pancreatic α-amylase, resulting in production of maltose, maltotriose and α-limit-dextrin. A large number of these disaccharides and oligosaccharides are then hydrolysed by enzymes present in the brush border of the small intestine mucosa to yield glucose (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.

Starch consists of two main macromolecules, amylose and amylopectin. Amylose is an essentially linear polymer which consists of 1,4-linked-α-D-anhydroglucose units, while amylopectin is highly α-1,6-branched. It is generally agreed that these macromolecules are assembled in a cluster structure, 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 (Blanshard, 1987). One character of amylose is its ability to form an inclusion complex with a variety of ligands. In starch granules, the amylose chain displays a natural twist in a helical conformation with six anhydroglucose units

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per turn (Zobel, 1988). Hydroxyl groups of glucosyl residues are located on the outer surface of the helix, while the internal cavity is a hydrophobic tube. Therefore, the hydrophobic group of complexing agents can lie within the amylose helix and is stabilised by van der Waal contacts with adjacent C-hydrogens of amylose (Godet et al., 1993), while hydrophilic ends of the ligand are outside the helix. In food systems, complexes with monoglycerides, free fatty acids, lysophospholipids and surfactants are of interest because they can affect the functional properties of food products (Karkalas et al., 1995). For example, lipids or surfactants are used as modifiers in starch-containing foods, demonstrated by monoacyl lipids which are exploited in bread-making to retard staling (Krog and Jensen, 1970). Amylose–lipid complexes decrease susceptibility of amylose to amylolysis (Holm et al., 1983; Eliasson and Krog, 1985); it is suggested that there is a competitive mechanism between amylose retrogradation and formation of amylose–lipid complexes, in which crystallisation of amylose–lipid complexes is favoured (Slade and Levine, 1987). Eliasson has reported that formation of complexes prevents leaching of amylose during gelatinisation, inhibits swelling of starch granules heated in water, and reduces the water-binding capacity of starch.

Sago starch (as previously studied) is a nutritionally poor food, which must be supplemented with a source of protein and lipid. Traditionally, it is processed into various products such as sago noodle, sago biscuit, sago cake and sago pearl (a kind of partly gelatinised sago starch, which is an indispensable component of soups and puddings for local populations). Studies on sago starch–lipid interaction are therefore of importance. Dietary fats can be classified into two separate groups: storage fats, mainly triglycerides, and structural fats, mainly phospholipids and cholesterol. Diglycerides and monoglycerides are intermediate products of triglyceride hydrolysis in physiological conditions. Monoglycerides can change the starch granule structure by forming inclusion complexes with amylose, whereas di- or tri-acyl lipids do not form such complexes. Although triglycerides are normally the main components of dietary fats, in low-fat diets, phospholipids may constitute a relatively high proportion of the fat as they are components of all vegetable and animal cells (FAO, 1977). According to the FAO Food Balance Sheet (1991), in Malaysia, only 15.5% of food energy derives from fats (14.2% from vegetable oils and 1.3% from animal fats). Phospholipid is therefore an important component in Southeast Asian diets.

In the present study, we investigated interaction between lipids and native sago starch (amylose content approx. 30%). Lipids chosen in this study are lysophosphatidylcholine (LPC), 1-monomyristoyl-rac-glycerol (GMM), 1-monopalmitol-rac-glycerol (GMP), and 1-monostearoyl-rac-glycerol (GMS). Differential scanning calorimetry (DSC) was used to determine the amylose–lipid complexe formation. The soluble components of the incubated starch samples (leached material during complex formation) were determined by HPSEC analysis. Enzyme susceptibilities of variously processed lipid-complexed sago starch samples were assessed and compared. Morphological changes of the hydrolysed native starch–lipid residues were observed under scanning electron microscope (SEM).

The purpose of this study was to investigate the effects of amylose–monoglyceride and amylose–LPC complex formation on the properties of native sago starch, and to compare the different complexing ability of LPC, GMM, GMP and GMS.

2. Materials and methods

2.1. Starch

Sago starch (*Metroxylon* spp.) from Sarawak, Malaysia, was supplied by the Wah Chang International Group of Companies (Singapore); proximate composition of the sago starch is presented in Table 1.

2.2. Lipid

The following lipids were used: (i) lysophosphatidylcholine (LPC) from egg yolk (L-4129, Sigma Chemical Co., St Louis, MO, USA); (ii) 1-monomyristoyl-rac-glycerol (GMM) (M-1890, Sigma); (iii) 1-monopalmitoyl-rac-glycerol (GMP) (M-1640, Sigma); and (iv) 1-monostearoyl-rac-glycerol (GMS) (M-2015, Sigma).

2.3. Enzyme

Alpha-amylase (EC 3.2.1.1) was extracted from porcine pancreas (A6255, Sigma Chemical Co., St Louis, MO, USA), with an α-amylase activity of 1330 U mg^{-1} protein. One unit was 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-crystallised α-amylase in 2.9 M NaCl solution containing 3 mM $CaCl_2$ to a concentration of 1 mg ml^{-1}.

Table 1
Proximate analysis of sago starch

	Percentage content (%)
Moisture[a]	9.0
Lipid[b]	0.23
Amylose[c]	27.2
Protein[b]	0.012
Ash[b]	0.17

[a] Calculated from weight loss after oven drying, 120°C for 2 h.
[b] Sim et al. (1992).
[c] Govindasamy et al. (1992).

2.4. Preparation of amylose–lipid complexes

The procedure of Eliasson et al. (1988) was followed for complexing lipids with starch. LPC, GMM, GMP and GMS were not readily dispersible in water at room temperature. They were mixed with water at a ratio of 1:10, heated at 70°C to produce a uniform suspension, then allowed to cool to 60°C and kept at that temperature before adding to starch. All lipids were added at a concentration of 2% (w/w) calculated on dry starch basis. For DSC analysis, the ratio of starch:lipid suspension:water was 1:0.2:1.5; for enzymatic analysis, the ratio was 1:0.2:10. The control had the same amount of water in place of the lipid suspension. All mixtures were mixed thoroughly, and incubated at 60°C for 1 h prior to analysis.

2.5. Differential scanning calorimetry

Differential scanning calorimetry (Perkin Elmer, DSC-7. Norwork, CT, USA) was used to determine the thermal characteristics of lipid-complexed starch. An aliquot of the starch suspensions was accurately weighed into aluminium pans and hermetically sealed. Samples were analysed by heating from 20 to 160°C at 10°C min^{-1}. An empty pan served as reference. Enthalpy changes and onset temperatures, integrated using DC-7 software, were calibrated on the basis of an indium standard. T_O, T_P and T_C denote onset, peak and complete transition temperatures, respectively; ΔH, transition enthalpy, was computed in joules per gram. All pans were cooled and reweighed after analysis to ensure that no moisture was lost.

2.6. 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 refractometer 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 cross-linked 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 deionised water at a flow rate of 0.8 ml min^{-1}.

After incubating at 60°C, the starch–lipid samples prepared for enzyme analysis were centrifuged. The supernatant was passed through a 8.0-μm SC Millipore filter (0.04% of the sample is lost during filtration), and then analysed by HPSEC. These profiles are compared to that of native sago starch to determine the components of the leached materials during the complex formation process.

2.7. Enzyme hydrolysis of complexed native sago starch

Following incubation, an aliquot of the dispersion (granules + leached material) was hydrolysed and the remainder separated into non-soluble (starch granules) and soluble (leached material) fractions. These fractions were subsequently subjected separately to enzyme hydrolysis.

Incubated native sago starch–lipid suspensions (1 ml) were diluted by adding 4 ml distilled water in a 125-ml Erlenmeyer flask followed by 1 ml 0.1 M phosphate buffer (pH 7.1). Enzyme reaction was initiated by addition of 0.1% (based on the weight of starch) porcine pancreatic α-amylase solution. The enzyme added was in excess as determined by prior investigation (not reported). Following addition of the enzyme, samples were covered with sealing film and incubated in a B. Braun Incubation Hood (Germany) for 1, 2, 4 and 8 h at 37°C; 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, while the sediments were dried at room temperature for SEM observation. Starch granules and leached material were analysed separately using the same enzymatic procedure.

2.8. Enzyme hydrolysis of gelatinized and retrograded complexed starch

Complexed starch suspensions (starch:lipid suspension:water ratio 1:0.2:10) were autoclaved at 125°C for 20 min to obtain gelatinised starch. The samples were then allowed to cool to room temperature, and stored at 5°C for 1 day followed by storage at 34°C for 4 days to obtain retrograded starch. Both freshly gelatinised and stored starch samples were subjected to enzyme analysis as described above. Stored starch was physically cut into small pieces of similar size to those created during chewing of a starch gel (Cui and Oates, 1997) before subjecting to enzyme hydrolysis.

2.9. Analytical methods

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

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

Table 2
Comparison of the complexing ability of different lipids with 40% native sago starch at 60°C

Sample	ΔH_G[a]	$\Delta H_{complex}$[b]
Starch	16.02 ± 2.31	—
Starch–LPC[c]	13.84 ± 0.83	3.56 ± 0.12
Starch–GMM[c]	14.62 ± 2.20	2.20 ± 0.21
Starch–GMP	15.37 ± 1.78	1.38 ± 0.34
Starch–GMS	15.87 ± 2.15	0.31 ± 0.12

[ab] Enthalpy (ΔH, J g^{-1}); mean ± SD ($n = 4$).
[c] Significantly ($P < 0.05$) different from the control (starch). Student's t-test.

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

2.10. Scanning electron microscopy

Hydrolysed granule residues were sprinkled onto double-sided adhesive tape attached to a circle specimen stub and coated with gold using a Balzers SCD 004 sputter coater. The samples were viewed and photographed using a Philips SEM 515 scanning electron microscope on AGFAPAN-APX 100 film.

2.11. Statistical analysis

Student's t-test was used to compare the effect of added lipid on gelatinisation properties (Table 2) and influence on leached material (Table 3). Significance was determined after invoking Bonferroni's inequality.

3. Results and discussion

3.1. Complex formation ability

The complexing abilities of different lipids (LPC, GMM, GMP, GMS) with sago starch were investigated by analysing the gelatinisation properties of the lipid-incubated starch samples using DSC. Representative thermograms are shown in Fig. 1. A large peak centred at ~75°C (G) was observed with each gelatinisation curve, while it did not appear on immediate rescan. This peak is due to starch gelatinisation as identified from the starch control. Absence of a second peak in samples

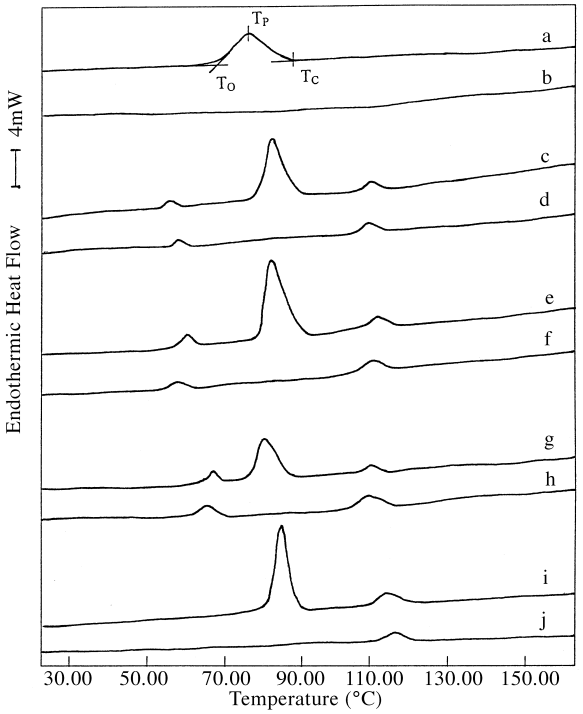

Fig. 1. DSC thermograms showing gelatinisation properties of starch and starch–lipid samples. (a) Sago starch, 3.364 mg; (b) rescan of (a); (c) starch–GMM, 2.645 mg; (d) rescan of (c); (e) starch–GMP, 3.018 mg; (f) rescan of (e); (g) starch–GMS, 2.987 mg; (h) rescan of (g); (i) starch–LPC, 3.937 mg; (j) rescan of (i). G, gelatinisation endotherm; M_1, endotherm of free lipid melting; M_2, endotherm of amylose–lipid complex melting; T_P, transition peak temperature; T_O, transition onset temperature; T_C, transition completion temperature.

heated in the absence of added lipid (Fig. 1a,b) reflects the relatively low amount of lipid (0.2%) in sago starch and the moisture content during analysis. Peak temperature of sago starch gelatinisation in the presence of lipid did not change, but the gelatinisation enthalpies (ΔH_G) of the LPC- and GMM-incubated samples were statistically lower ($P < 0.05$) than that of the control (Table 2). There was no significant difference between the ΔH_G of GMP-, GMS-incubated samples and that of the control. Two other peaks at lower and higher temperatures were observed on starch monoglyceride thermograms. They were also found on rescanned curves. The small peak centred at the lower temperature (M_1) was explained as the melting peak of free monoglyceride, confirmed by DSC analysis of monoglyceride–water suspensions. The melting temperatures of monoglycerides increase with increased chain length (Fig. 1).

Table 3
Amount of carbohydrates leached during incubation at 60°C

	Starch	Starch–LPC	Starch–GMM	Starch–GMP	Starch–GMS
Soluble[a] (%)	0.40 ± 0.05	0.21 ± 0.04[b]	0.23 ± 0.02[b]	0.25 ± 0.05[b]	0.36 ± 0.04

[a] Percentage total carbohydrates of the samples; mean ± SD ($n = 3$).
[b] Significantly ($P < 0.05$) different from the mean. Student's t-test.

The relatively broad peak centred at a higher temperature (M_2, T_P 95–111°C) was assumed to be due to amylose–lipid complex melting. The enthalpies of amylose–monoglyceride complex peaks decreased with an increase in monoglyceride chain length: GMM > GMP > GMS, 2.20, 1.38, and 0.31 J g^{-1}, respectively. The gelatinisation curve of starch–LPC showed only two peaks. One centred at 74°C, the starch gelatinisation peak; the other centred at ~100°C, the amylose–LPC complex peak. The melting enthalpy of the complex is 3.56 J g^{-1}, which is significantly higher than those of amylose–monoglycerides. No melting peak of free LPC was observed, since the melting temperature of LPC is well below 20°C.

A number of studies indicated similar changes in starch gelatinisation behaviour which were affected by the presence of monoglyceride or lysophosphatidylcholine (Eliasson et al., 1988; Huang and White, 1993). The cause of such phenomena may lie in structural changes of starch granules when complexed with lipids. The length of a GMP molecule is about 22 Å; thus an amylose helix will require 16.5 glucose units to accommodate it (Huang and White, 1993). In contrast, smaller monoglyceride molecules are more easily accommodated into the amylose helix. The melting enthalpy of amylose–monoglyceride complex is therefore influenced by the ease with which the monoglyceride can be accommodated into the helix. The significant difference in melting behaviour of amylose–LPC and amylose–monoglyceride is probably due to structural differences. LPC has both hydrophobic and hydrophilic portions and, as such, it can pass through the surface of granules more easily than the hydrophobic monoglyceride molecules. Theoretically, soluble compounds with molecular weights of up to 1000 can penetrate into the matrix of the starch granule, but in fact lipids (most of which have a molecular weight lower than 1000) are unlikely to enter intact granules; they are usually present in micellar form. Possessing both polar and non-polar groups, LPC presumably has less tendency to form micelles. The significant low gelatinisation enthalpies of starch–LPC and starch–GMM, 13.84, 14.62 J g^{-1}, respectively (ΔH_G of starch control was 16.02 J g^{-1}), might be due to further complex formation during the starch gelatinisation process. Morrison et al. (1994) noted that if free lipids capable of forming complexes with amylose are present during starch gelatinisation, the exothermic heat of complex formation partially offsets the endothermic heat of starch gelatinisation. In this study, LPC and GMM form complexes with greater ease with starch amylose.

3.2. Leached material

Soluble components of native starch, incubated with lipids at 60°C, were analysed by HPSEC (Fig. 2). Amylose was found to have leached from the granule, detected by HPSEC and confirmed by the profile of native sago starch (Govindasamy et al., 1992). This supports the assumption that part of the amylose leaches out at 60°C. The comparative amount of the leached amylose, determined by total sugar analysis, is shown in Table 3. Starch samples complexed with LPC displayed the least amylose leaching (0.21%), but all complexed samples displayed less amylose leaching than the control (0.40%). The presence of complexing lipids obviously has a negative effect on amylose leaching from the granule at 60°C and is related to the complexing ability. The formation of amylose–lipid complexes will inhibit granule swelling, the consequence of which is reduced amylose leaching. Similar phenomena for wheat and potato starch were also reported by Eliasson (1985) and Holm et al. (1983).

3.3. Enzyme hydrolysis of complexed starch

3.3.1. Native starch

The susceptibility of complexed native sago starch to porcine pancreatic α-amylase is shown in Table 4. Monoglycerides and lysophosphatidylcholine-complexed starch samples were more resistant to α-amylase, compared to free starch. After 1 h, the degrees of

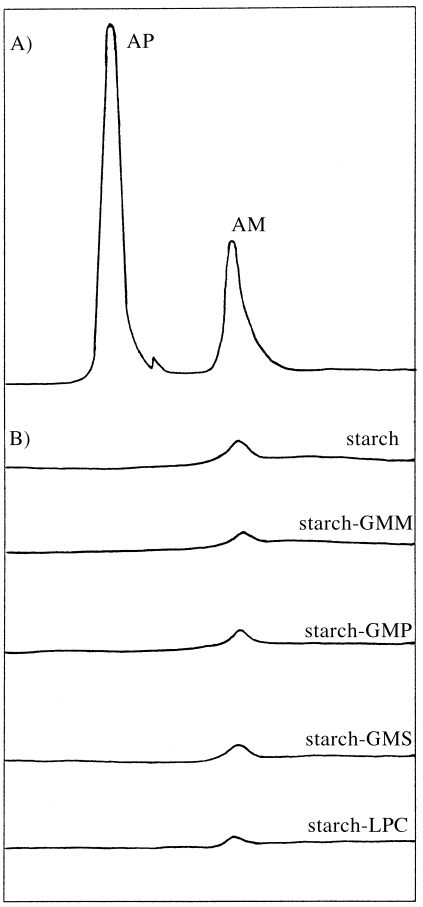

Fig. 2. HPSEC chromatograms of: (A) native sago starch; (B) leached material during incubation at 60°C.

hydrolysis of starch–LPC, starch–GMM were only 5.9 and 6.1%, respectively, lower than free starch, 8.8%. After 8 h, 32.2% of free starch was hydrolysed, while only 15.1% of starch–LPC and 21.1% of starch–GMM was hydrolysed. Enzyme susceptibilities of these samples rank in the order: free starch > starch–GMS > starch–GMP > starch–GMM > starch–LPC. The digestibility of starch–monoglycerides was significantly higher than that of starch–LPC, though lower than free starch. Apparent susceptibility to enzyme hydrolysis followed a similar pattern to that of amylose leaching during incubation at 60°C, which was inversely proportional to complex-forming ability. To investigate the separate influence on total starch hydrolysis, free amylose and complexed starch granules were subjected to enzyme hydrolysis separately (Table 4). The starch granules demonstrated greater influence on hydrolysis, and the combination of the degree hydrolysis of the two parts is equal to that of the total starch.

The enzyme susceptibility of starch granules was very close to that of whole starch samples. After 8 h, 31.7% degree of hydrolysis (based on a whole starch sample) was achieved from free starch granules. The hydrolysis rate for starch–LPC, starch–GMM, starch–GMP, and starch–GMS granules was 15.2, 20.9, 26.7, and 31.2% respectively. These results are in agreement with those of Holm et al. (1983). Complexed amylose displayed a substantially reduced susceptibility to α-amylase. The leached amylose was easily digested; the digest rates (based on whole starch sample) of the five samples were similar to their leaching rate.

Table 4
Degrees of hydrolysis (%) of lipid-complexed starch samples at 37°C by porcine pancreatic α-amylase

Starch	Incubation time (h)			
	1	2	4	8
Whole sample				
Starch	8.8 ± 1.0	16.8 ± 3.2	21.1 ± 2.4	32.2 ± 3.7
Starch–LPC	5.9 ± 0.4	9.2 ± 0.8	13.2 ± 1.1	15.1 ± 1.0
Starch–GMM	6.1 ± 0.4	10.9 ± 1.1	14.4 ± 0.9	21.1 ± 1.4
Starch–GMP	6.7 ± 0.7	13.1 ± 1.2	15.9 ± 1.6	26.6 ± 2.1
Starch–GMS	7.2 ± 0.3	14.6 ± 1.0	17.3 ± 1.2	31.2 ± 2.3
Starch granules				
Starch	8.5 ± 0.8	15.9 ± 0.9	20.9 ± 0.9	31.7 ± 2.2
Starch–LPC	5.7 ± 0.4	8.6 ± 0.3	13.1 ± 0.7	15.2 ± 0.5
Starch–GMM	6.0 ± 0.6	10.7 ± 0.5	14.5 ± 0.8	20.9 ± 1.2
Starch–GMP	6.8 ± 1.1	12.7 ± 1.5	15.8 ± 1.3	26.7 ± 2.1
Starch–GMS	7.0 ± 1.4	14.2 ± 2.4	17.2 ± 2.3	31.2 ± 2.6
Free amylose				
Starch	0.26 ± 0.02	0.32 ± 0.03	0.33 ± 0.02	0.34 ± 0.01
Starch–LPC	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	0.18 ± 0.01
Starch–GMM	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.19 ± 0.02
Starch–GMP	0.18 ± 0.02	0.18 ± 0.02	0.20 ± 0.02	0.21 ± 0.01
Starch–GMS	0.18 ± 0.01	0.19 ± 0.03	0.20 ± 0.02	0.23 ± 0.01

[a] Mean ± SD ($n = 3$).

It is apparent that amylose–lipid complex formation affects enzyme susceptibility of native sago starch at two levels: first, it restricts the swelling of the starch granule during incubation at 60°C; second, amylose–lipid complexes are more resistant to enzyme digestion than free amylose. Native sago starch is highly resistant to endo-acting enzymes (Sakano et al., 1986). Such behaviour is assumed to be due to structural characteristics which inhibit access of amylase to glycosidic bonds (Wang et al., 1998). It has been suggested that enzyme susceptibility is related to granule surface characteristics which can effect the accessibility of enzymes. Resistant wheat granules are assumed to possess a more perfect crystalline layer at the granule surface, the external chains of amylopectin, such that only the terminal glucosyl residues are accessible (Morrison et al., 1994). Under mild heating in water, sago starch granules swell to some extent due to solubilisation of amorphous zones and pores on the granule surface. Subsequently, amylose leaches from starch granules, and enzyme is able to gain access to the granule interior. In the presence of lipids, amylose–lipid complexes form, which restrict the swelling of starch granules during heating. Subsequently less amylose leaches out compared to free starch. Amylose–lipid complexes are much more resistant to amylolysis than uncomplexed amylose, although they can be degraded by thermostable α-amylase at high temperatures (Czuchajowska et al., 1991). Therefore, the ranking of enzyme susceptibility of lipid-complexed starch is opposite to that of the complex-forming ability of these lipids.

3.3.2. Freshly gelatinised and retrograded starch

The susceptibility of freshly gelatinised and retrograded starch–lipid samples to porcine pancreatic α-amylase is shown in Tables 5 and 6. Freshly gelatinised starch was hydrolysed readily, but the extent of hydrolysis was significantly decreased following starch–lipid interaction. The maximum differences were found in the last 4 h. After the first 4 h, 60.6% starch control was hydrolysed. The hydrolysis rates of starch–LPC, starch–GMM, starch–GMP, and starch–GMS were 49.8, 49.8, 52.7, and 60.1%, respectively, and at completion of the

Table 5
Degrees of hydrolysis (%) of freshly gelatinised starch–lipid samples at 37°C by porcine pancreatic α-amylase

Starch	Incubation time (h)			
	1	2	4	8
Starch	54.7 ± 1.5	55.4 ± 6.1	60.6 ± 6.4	78.3 ± 5.2
Starch–LPC	45.8 ± 2.1	48.4 ± 1.4	49.8 ± 1.9	53.5 ± 0.7
Starch–GMM	46.0 ± 2.7	47.7 ± 2.7	49.8 ± 2.8	53.5 ± 3.0
Starch–GMP	48.6 ± 1.3	49.5 ± 2.1	52.7 ± 3.8	53.3 ± 4.2
Starch–GMS	53.7 ± 2.3	54.1 ± 1.7	60.1 ± 3.7	63.1 ± 2.4

[a] Mean ± SD ($n = 3$).

Table 6
Degrees of hydrolysis (%) of retrograded starch–lipid samples at 37°C by porcine pancreatic α-amylase

Starch	Incubation time (h)			
	1	2	4	8
Starch	13.6 ± 0.3	20.7 ± 0.7	30.6 ± 0.4	41.5 ± 0.6
Starch–LPC	31.8 ± 1.4	38.4 ± 2.1	42.8 ± 1.7	46.7 ± 2.3
Starch–GMM	29.1 ± 1.8	33.5 ± 2.6	40.8 ± 2.7	45.3 ± 2.1
Starch–GMP	26.0 ± 1.6	32.2 ± 3.4	38.8 ± 2.7	45.1 ± 2.2
Starch–GMS	21.2 ± 3.7	30.3 ± 4.5	34.1 ± 2.8	43.6 ± 2.1

[a] Mean ± SD ($n = 3$).

trial (total 8 h), the degree of hydrolysis of starch was 78.3%, while those of starch–LPC and starch–GMS were 53.5 and 63.1%, respectively. Similar results have been reported by other researchers (Holm et al., 1983; Eliasson and Krog, 1985), indicating that amylose–lipid complexes decrease the susceptibility of amylose to amylolysis. The enzyme analysis data are in accord with DSC analysis of the complexing ability of different lipids.

After 5 days of storage, significant differences in speed and extent of enzyme hydrolysis were also found between starch–lipid samples and the starch control group. Enzyme susceptibility of retrograded starch was altered (Table 6). After 1 h, the degree of hydrolysis of retrograded free starch was as low as 13.6%, while those of retrograded starch–LPC, starch–GMM, starch–GMP, and starch–GMS were 31.8, 29.1, 26.0, and 21.2%, respectively. A competitive mechanism exists between amylose retrogradation and formation of amylose–lipid complexes which favours complex formation (Sarko and Wu, 1978; Czuchajowska et al., 1991). By competing with amylose retrogradation, lipid complexes can increase digestibility of stored starch.

3.4. Scanning electron microscopy

Residues of enzyme-hydrolysed native starch and starch–lipid samples were observed by scanning electron microscope (Fig. 3). The initially smooth starch granule surface was rough after 8 h hydrolysis, and some granules displayed deep round cavities (Fig. 1a). Shell residues, resulting from total digestion of the inner parts of granules, could be observed for some of the material. Some granules even lost their structure. However, the progress of degradation was not homogeneous. Smooth granules and disrupted granules were observed in the same sample. Approximately 30% of granules in hydrolysed free starch samples were visibly attacked by enzyme. Complexed with lipids, native starch became

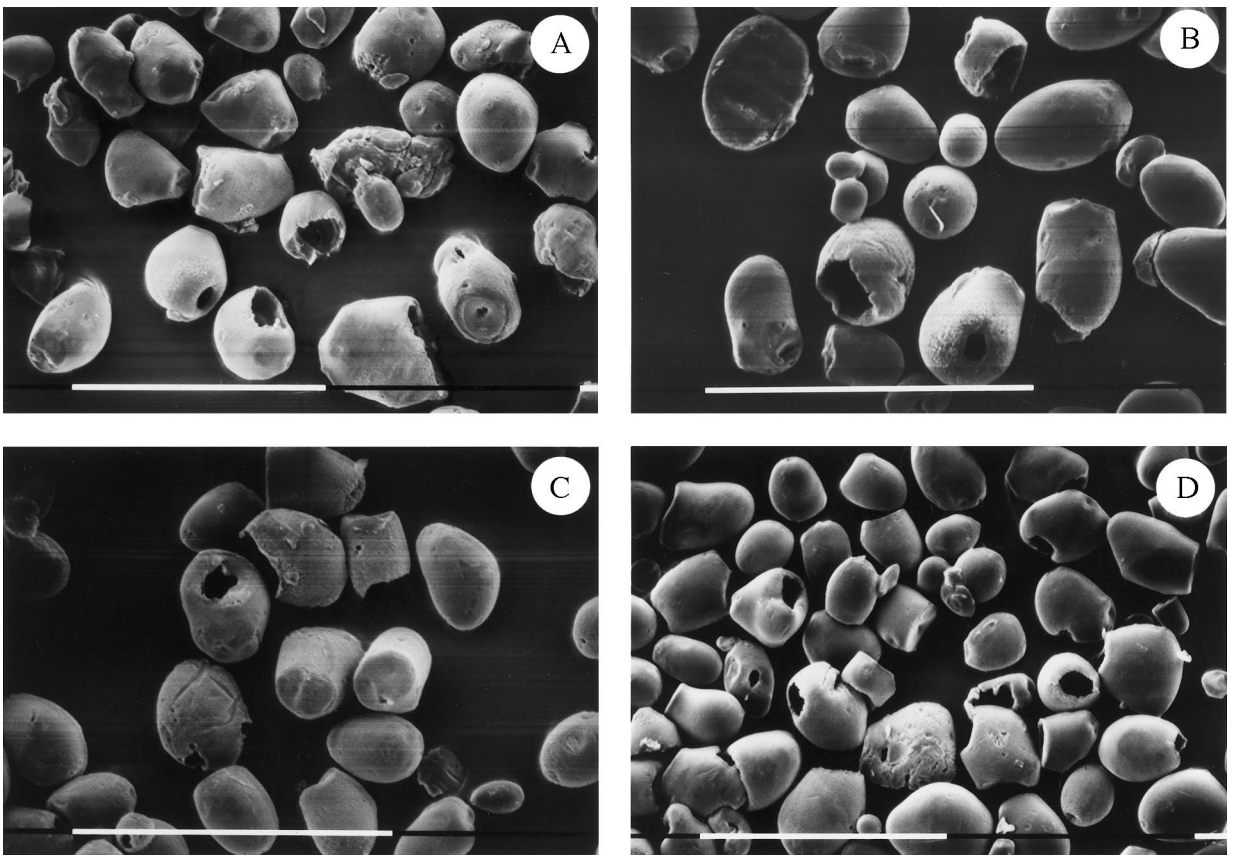

Fig. 3. SEM photographs of 60°C-incubated sago starch samples hydrolysed by enzyme for 8 h. (A) Starch; (B) starch–LPC; (C) starch–GMM; (D) starch–GMS. Scale bars = 0.1 mm.

less susceptible to enzyme attack. The percentage of attacked granules differed between the four starch–lipid samples. There were 30.4% attacked granules in the starch–GMS sample, similar to free starch. In contrast, attacked granules of starch–LPC and starch–GMM were 21.1 and 23.0%, respectively. Enzyme susceptibility of the samples ranked in the following order: starch $\cong$starch–GMS > starch–GMP > starch–GMM > starch–LPC. These results were in agreement with those from enzymatic analysis. No significant difference in the hole size was found between the samples. The average hole size (minor axis length) of the starch control is $8.1 \pm 0.7\,\mu m$, and those of starch–LPC, starch–GMM, starch–GMP, and starch–GMS are 8.2 ± 1.0, 8.1 ± 0.5, 8.3 ± 0.7, and $8.2 \pm 0.6\,\mu m$, respectively ($X \pm SD$, $n = 200$). The reason for such phenomena might lie in the negative effect of the amylose–lipid complex on granule swelling during heating in water. Incubating at 60°C, starch granules may swell to some extent and form pores on the granule surface. Some of these pores are of sufficient size that enzymes can gain entry into the granule interior, thereby increasing the rate of reaction. Since amylose–lipid complex formation decreased the swelling of starch granules, there were less holes formed on the granule surface. The hydrolysis pattern of starch granules observed in this study more closely resembles annealed starch than that of raw starch. It has been reported (Wang et al., 1995) that the degradation pattern of raw sago starch is mainly surface errosion and crevassing while, after annealing (pH 3.5, at 60°C for 2 h), a single deep hole develops on the granule surface during enzyme hydrolysis. In this study, the incubation conditions were not optimum for starch annealing, but rearrangement of granule components still occurred. Single cavity was the main pattern of granule degradation, while scribbled surface was found on a small portion of the starch granules.

4. Conclusions

Amylose–lipid complex formation affects enzyme susceptibility of sago starch in two aspects: first, it decreases the swelling of starch granules; thus there is less opportunity for enzyme to gain access to the granule interior and less amylose leaches from the granules; second, amylose–lipid complexes are more resistant to digestive enzymes than amylose. The complex formation reduced the digestibility of native and freshly gelatinised starch but, by competing with amylose retrogradation, it also increased enzyme susceptibility of stored starch. Thus, complexing with lipids will retard the staling process of cooked starchy food. For the sago-consuming communities, adding fats and oils to starchy food will not generally lower the nutritional value of starch, but complement other essential nutrients.

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