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Relationship between α-amylase degradation and physico-chemical properties of sweet potato starches

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Abstract

Six varieties of sweet potatoes, grown under identical conditions, produced starches that displayed different characteristics. Susceptibility to pancreatic α-amylase varied between starches produced by the different clones. Structural characteristics at various levels, such as ratio of major fractions, size of amylose, gelatinization temperature and granule morphology, were also different between clones. Correlating structural attributes with susceptibility led to the suggestion that granule structure, including amylopectin: amylose ratio and molecular associations, were important critical factors in the hydrolysis of sweet potato starch granules. High amylopectin content of sweet potato starch was associated with a high gelatinization temperature and correspondingly less susceptibility to α-amylase attack. The hydrolysis pattern was correlated with degree of hydrolysis. Extensive surface erosion was shown to indicate a high degree of hydrolysis, whereas less surface erosion indicated less degradation.

1. Introduction

Sweet potato is an important economic crop that can adapt successfully to a wide range of habitats, including marginal regions. It is a dicotyledonous plant belonging to the family Convolvulace, in which there are approximately 50 genera and over 1000 species (Woolfe, 1991). Artificial selection of sweet potatoes, as well as the occurrence of natural hybrids and mutations, has resulted in the existence of a very large number of cultivars. These varieties differ in many of their properties, ranging from the physical appearance and texture of the tuber to structure–function properties of the starch. Sweet potato starch can be used as an ingredient in bread, biscuits, cakes, juices, ice cream and noodles, or converted to glucose and isomerized glucose syrup. Glucose syrup is utilised in a variety of foods, such as: candies, ice-cream and jams and isomerized glucose can be used in lactic acid beverages, soft drinks, bread and many other foods.

Characteristics of α-amylase action on sweet potato starch granules have been the subject of numerous investigations and reports (Noda et al., 1992; Chang Rupp & Schwartz, 1988; Ice et al., 1980). These studies have shown that starches vary in their resistance to the action of α-amylase. Furthermore, these differences were found not only in the degree of hydrolysis but also in the mode of attack on the starch granule and in the products of hydrolysis. Starch susceptibility to enzyme attack is influenced by several factors, such as amylose and amylopectin content (Dreher et al., 1984; Hoover & Sosulski, 1985; Holm & Bjorck, 1988; Ring et al., 1988), crystalline structure, particle size and the presence of enzyme inhibitors. Among these factors, granular structure is believed to be the most important. Some properties of hydrolysis are well understood, but detailed information on the mode of action of amylases is still incomplete and becomes complicated considering the variety of α-amylase sources and the complex structure of starch granules. In general, cereal starches are less resistant to enzymatic degradation than non-cereal starches (Fuwa et al., 1977). Among non-cereal starches, cassava starch has a relatively higher enzyme susceptibility than other starches (Rickard et al., 1991), whereas potato starch shows stronger resistance to enzyme attack. Sweet potato starch is more susceptible than potato starch but less susceptible than cassava starch to α-amylase and glycoamylase attack (Delpeuch & Favier, 1980; Hizukuri et al., 1988; Kainuma, 1988).

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The present investigation was undertaken to study the differences in the physico-chemical properties of six sweet potato starches and the relationship between these properties and susceptibility to enzyme attack. In this study, the macromolecular composition, molecular weight of amylose, morphology of the granule, pattern of hydrolysis and the intermolecular association were determined.

2. Materials and methods

2.1. Sweet potato starches

Sweet potato starch samples (Table 1) were kindly donated by International Potato Centre (CIP), West Java, Indonesia. Sweet potatoes were grown from cuttings and irrigated from first planting for 2 weeks. Urean, TSP and KCl (100:100:100 kg Ha^{-1}) were used as fertilizer. All varieties were grown under identical conditions.

Sweet potato starches were washed with distilled water three times to remove impurities. After washing, the starches were dried at 37°C in an oven.

2.2. 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 790 units/mg protein. One unit will liberate 1.0 mg of maltose from starch in 3 min (pH 6.9, 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.

2.3. Enzyme hydrolysis of sweet potato starches

Sweet potato starches (2.0 g) were weighed into a flask; 100 ml of distilled water and 20 ml 0.1 M phosphate buffer (pH 7.1) were added. The enzyme reaction was initiated by the addition of 2520 units of porcine pancreatic α-amylase/g of starch and samples incubated in an incubation hood (Braun, Germany) at 37°C, with a shaking rate of 150 times/min. Periodically, 4 ml of suspension were removed. The enzyme was inactivated by adding an equal volume of 0.4 mM $HgCl_2$ and the suspension was centrifuged (Jouan BR.3.11, USA) at 2000 rpm for 10 min. The supernatant was subsequently incubated at 90°C for 15 min to inactivate the enzyme further and the reducing sugar was determined as outlined by Dygert et al. (1965). The precipitate obtained from the degraded residue was washed with distilled water and dried at room temperature in preparation for scanning electron microscopy (SEM) and high performance size exclusion chromatography (HPSEC).

Degree of hydrolysis (D.H.%) was defined as:

$$\text{D.H.\%} = \frac{\text{Reduced sugar provided by enzyme hydrolysis}}{\text{Reduced sugar produced by acid hydrolyis}} \times 100\%$$

Reducing sugar was determined using D-glucose as standard. Acid hydrolysis was carried out by treating starch with HCl (1 g starch mixed with 20 ml 1 N HCl) at 100°C for 2 h.

2.4. High performance size exclusion chromatography (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). The columns and refractometer were maintained at 40°C. The columns were connected in the following series: Ultrahydrogel guard column followed by Ultrahydrogel Linear and two Ultrahydrogel 120 columns. Ultrahydrogel columns are packed with cross-linked methacrylate gel. Ultrahydrogel liner has a blend pore size with exclusion limit 7×10^6, Ultrahydrogel 120 column, pore size 120 Å, 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).

Table 1
Physical properties of sweet potato starches

No.	Variety	Yield (ton ha^{-1})	Dry matter (%)	Starch recovery (%)	Skin colour	Flesh colour
A	BOO55	11.80	26.98	14.23	white	white
B	SA-7A	9.50	30.28	14.83	orange	orange
C	BOO68.12	2.40	29.97	11.48	red	white
D	BOO88.11	2.2	31.45	12.93	yellow	pale yellow
E	SA-1A	13.40	28.82	13.75	red	pale yellow
F	SQ-27	32.00	21.00	10.00	cream	pale yellow

Note: Information provided by International Potato Centre, Indonesia.

2.5. Macromolecular composition

Native and degraded sweet potato starches were prepared for analysis using a modified Jackson method (Jackson et al., 1988). Distilled water (15 ml) was added to 20 mg sweet potato starch and gelatinised in a boiling water bath for 10 min, with subsequent stirring. After cooling to 40°C, the sample was dispersed with continuous sonication for 40 s using a Sonicator Ultrasonic processor, model XL (Heat System, NY, USA), with output maintained at 4.5. The well-dispersed solution was filtered through a Millipore filter (8 μm) prior to HPSEC analysis. The injection volume was 50 μl.

2.6. Scanning electron microscopy (SEM)

Native and dried degraded sweet potato starch granules were sprinkled onto double-sided tape attached to a SEM stub and coated with gold using Balzers SCD 004 sputter coater. Sweet potato starch granules were examined on a scanning electron microscope (Philips SEM 5300) at an accelerating voltage of 10 kV. Photomicrographs were taken on Agfapan-Apx 100 films.

2.7. Differential scanning calorimeter (DSC)

A differential scanning calorimeter (Perkin–Elmer, DSC-7. Norwalk, CT, USA), equipped with an intercooler, was used to determine the endothermic characteristics of native and degraded starch. Sweet potato starches (30% dry matter starch slurry) were accurately weighed into aluminium pans and hermetically sealed. Samples were analysed by heating from 35 to 110°C at a heating rate of 10°C/min. An empty pan served as reference. Enthalpy changes and onset temperatures were integrated using DSC 7 software, calibrated on the basis of the melting enthalpy of an indium standard. All pans were cooled and reweighed to ensure that no moisture was lost during the run.

2.8. Analysis of granule size

Sweet potato starch granule size and size distributions were measured using image analysis (Zeizz, Axiophot, Germany), at a magnification of 20×. The analysis system, using KS 400 VER 2 software, was connected to a light microscope (Axiophot, Germany). The sweet potato starch granules were suspended in the 80% sucrose solution as refractive index matching media.

3. Results and discussion

Granule size, amylopectin and amylose ratio, molecular weight of amylose and endothermic transition enthalpy of sweet potato starches were evaluated to ascertain the relationship between their physico-chemical properties and susceptibility to α-amylase attack.

3.1. Degree of hydrolysis

The time course of α-amylase hydrolysis of sweet potato starches is presented in Fig. 1. Hydrolysis occurred in the following phases: rapid hydrolysis (0–10 h), slow hydrolysis (10–26 h) leading to maximal hydrolysis after about 26 h in the case of sample B, the hydrolysis of the remaining samples after 26 h was increasing at a very low rate. Starches extracted from different sweet potatoes showed variable susceptibilities to porcine pancreatic α-amylase attack, degree of hydrolysis ranged from 48.8% (sample A) to 63.4% (sample F). The relative order of hydrolysis was F > E > D > C > B > A.

3.2. Macromolecular composition

Conversion factors required for calculating the concentration of amylose and amylopectin were obtained by suitable calibration. The amylose peak was calibrated using a suitable molecular weight dextrin standard, and the amylopectin was back-calculated using a known concentration of purified starch.

All intact sweet potato starch samples giving similar HPSEC chromatograms (Fig. 2) suggested similarity in their granule macromolecular composition. The amylopectin content of sweet potato starch ranged from 78.3% (sample E) to 83.7% (sample A) and amylose content from 16.3% (sample A) to 21.7% (sample E). The peak eluting between amylopectin and amylose was the intermediate fraction, and for the purpose of this study it is included as part of the amylopectin peak.

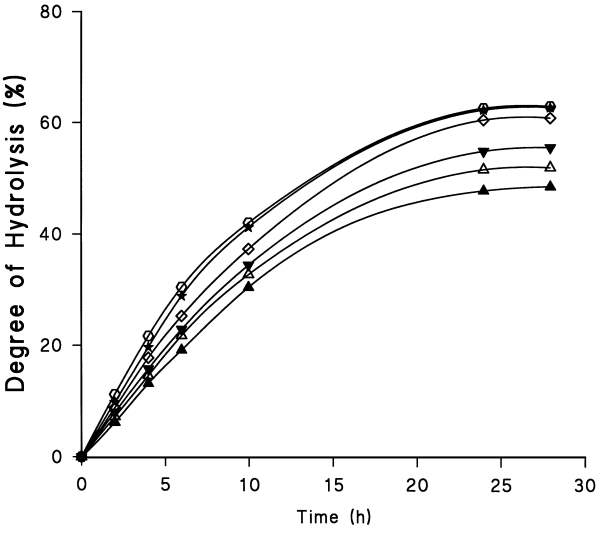

Fig. 1. Hydrolysis of different sweet potato starches by porcine pancreatic α-amylase at 37°C. ▲, sample A; ■, sample B; ▼, sample C; ◆, sample D; ⋆, sample E; ◆,: sample F.

Deviation in mean retention time was found to be no greater than ± 0.10 min across a concentration range of 200–800 $\mu g\,ml^{-1}$ (unpublished data).

The amylopectin content of the six native samples was ranked in the order of A > B > C > D > F > E, conversely, the order of hydrolysis was A < F (Table 2). This suggests that the amylopectin content was inversely related to susceptibility by α-amylase attack. This result is in accordance with the cluster and double helix structure model proposed by French (1984), in which the lightly bonded double helices offer resistance to enzyme or chemical attack. The amorphous regions, which can easily be degraded by enzyme, are present throughout the granule. Amylose, which has higher enzyme susceptibility, is present in the amorphous regions in the form of single helical structures. Branch points of amylopectin are thought to be located in the amorphous region, but poorly hydrolysed by α-amylase, due to the (1→6) configuration of the glucosidic bond. In this study, a higher proportion of amylopectin results in greater resistance to enzymatic degradation.

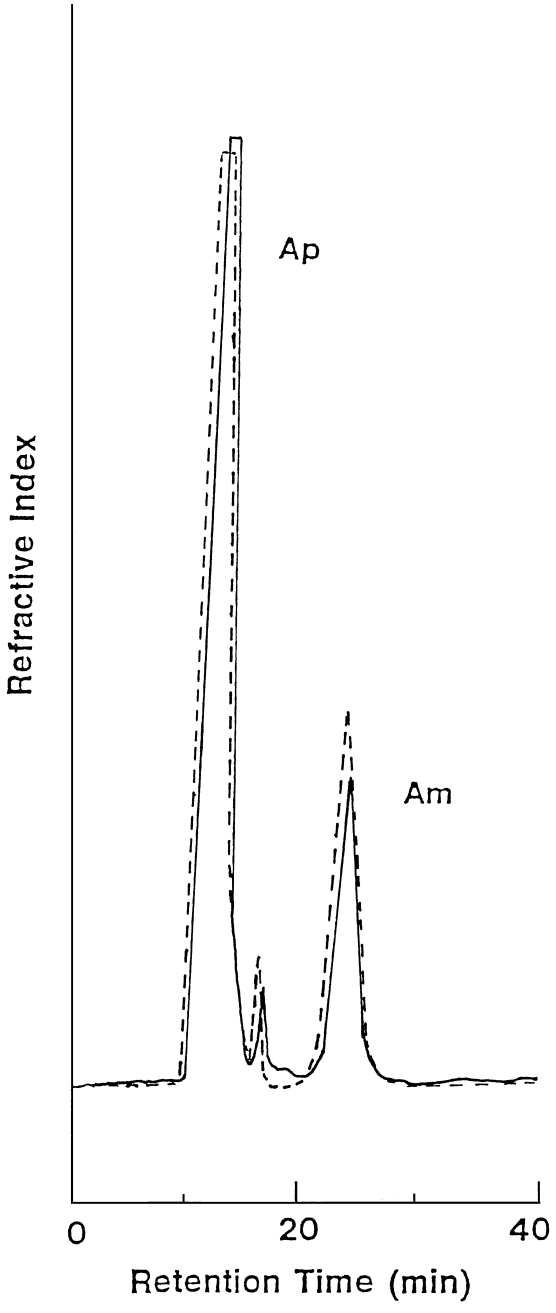

Fig. 2. HPSEC profiles of native and hydrolysed sweet potato starch sample A degraded by porcine pancreatic α-amylase (D.H. = 48.8%). (———), native starch; (- - - -), hydrolysed starch, degraded by porcine pancreatic alpha-amylase, D.H. = 48.8%.

α-Amylase degrades macromolecules in starch granules into low molecule weight dextrins, leaving a partially hydrolysed starch granule residue. After enzyme degradation, components of the hydrolysed residue display some differences from those of intact granules (Table 2). Samples A, B, C, D and E showed a decrease in their amylopectin content and an increase in amylose content; in contrast, sample F exhibited an increase in amylopectin content and a decrease in amylose content. High hydrolysis sample F seems to have a unique hydrolysis pattern, from observation of the hydrolysed residue (Fig. 4). After α-amylase degradation, sample F showed very significant erosion, with many small pieces peeling from the granule.

Compared with intact sweet potato starches, the molecular weight of the amylose remaining in the hydrolysed residue was different. After α-amylase attack, the molecular weight of amylose from the high hydrolysis samples E and F decreased. In contrast, in low hydrolysis samples A and C changes in the retention time and broadening of the peak associated with amylose suggest some breakdown of amylopectin. Samples B and D, which underwent a medium degree of hydrolysis, showed no difference in the molecular weight of their amylose after α-amylase attack (Table 2).

Table 2
Comparison table of macromolecule components and molecular weights of Am of intact and hydrolysed sweet potato starches

Variety		Ap (%)	Am (%)	Retention time of Am (min)	Apparent MW of Am	Degree of hydrolysis (%)
A	intact	79.1	18.6	23.44	104 712	
	hydrolysed	77.9	19.0	23.27	125 892	48.8
B	intact	79.5	18.0	23.32	120 226	
	hydrolysed	73.1	21.0	23.33	117 489	52.2
C	intact	76.4	19.8	23.58	89 125	
	hydrolysed	71.8	23.4	23.41	107 151	55.8
D	intact	74.7	19.6	23.59	83 176	
	hydrolysed	66.0	24.1	23.39	109 647	61.1
E	intact	74.3	20.2	23.39	109 647	
	hydrolysed	70.9	22.5	23.43	102 329	63.0
F	intact	74.9	19.1	23.20	141 253	
	hydrolysed	76.6	16.0	23.32	120 226	63.2

Table 3
Comparison table of granule size and hydrolyzed patterns of sweet potato starches

Variety		Granule shape	Size distribution		Hydrolysis pattern		Degree of hydrolysis (%)
			range (μm)	mean (μm)	erosion[a]	holes[b]	
A	intact	round oval	6.3–27.3	11.1			
	hydrolysed		5.04–27.7	11.1	1	2	48.8
B	intact	round oval	4.2–24.2	11.3			
	hydrolysed		4.0–24.4	10.9	0	2	52.2
C	intact	round oval	2.1–30.7	10.9			
	hydrolysed		4.6–25.8	10.9	2	1	55.8
D	intact	round	2.5–29.4	10.7			
	hydrolysed		2.5–23.1	10.1	2	1	61.1
E	intact	polygonal	5.5–23.9	9.7			
	hydrolysed		3.2–25.6	9.2	2	1	63.0
F	intact	round	2.1–29.4	12.4			
	hydrolysed		2.3–27.7	10.5	3	1	63.2

[a] erosion: 0: Surface clear, smooth basically, no small piece dropped from granule. 3: many small piece dropped from starch granule. 1,2: the degree of erosion is between 0 and 3.
[b] hole: 0: surface smooth, no hole in granule. 3: majority granule have holes. 1,2: the degree of having holes is between 0 and 3.

3.3. Morphological granular characteristics of intact and degraded starches

The different varieties of sweet potato starch granules were morphologically similar. The granules appeared to be round or oval, with characteristic dimensions in the range 2.1–30.7 μm (Table 3). The intact granule surfaces were smooth when viewed under SEM (Fig. 4).

The relative order of granule size was F > B > A > C > D > E. Sample F was the largest, mean 12.4 μm and size distribution from 2.1 to 29.4 μm. In comparison, sample E was the smallest, mean 9.7 μm and size distribution from 5.5 to 23.9 μm. After α-amylase digestion, samples A and C showed no differences in their granule size distributions, whereas, the mean granule sizes of samples F, E and D decreased. Samples F and E exhibited significant erosion after enzyme digestion (Fig. 4, Table 3).

Table 4
Thermal transition data of native and hydrolyzed sweet potato starches[a]

Variety	Transition temperatures (°C)			
	T_o	T_p	T_c	ΔH
A	81.08 ± 0.12	83.55 ± 0.14	92.82 ± 0.69	14.83 ± 0.47
B	79.26 ± 0.15	82.20 ± 0.07	93.40 ± 0.52	13.67 ± 0.25
C	80.28 ± 0.10	83.62 ± 0.09	94.17 ± 0.29	13.26 ± 0.37
D	78.9 ± 0.06	81.86 ± 0.08	91.27 ± 1.94	11.95 ± 0.24
E	78.54 ± 0.26	81.36 ± 0.27	90.99 ± 0.99	12.40 ± 0.87
F	75.67 ± 0.06	78.69 ± 0.20	90.61 ± 0.44	12.31 ± 0.57

[a] Mean ± SD ($n = 3$).
T_o: onset transition temperature (°C). T_p: peak transition temperature (°C). T_c: complete transition temperature (°C). DH: enthalpy, J/g.

3.4. Gelatinization parameters (DSC)

The gelatinization temperature of various native sweet potato starches was found to be different (Table 4). Thermograms of heated native sweet potato starch displayed a typical endothermic peak (Fig. 3). The temperature of gelatinization for the most resistant starch (sample A) was higher than that for the most susceptible (sample F). In sample A (Fig. 3A), the thermogram was centred at 83.6°C (T_p) ($T_o = 81.1$°C) enthalpy 14.8 J/g of starch (dry weight). Sample F (Fig. 3B) was centred at 78.7°C ($T_o = 75.7$°C) and enthalpy 12.3 J/g of starch (dry weight). Among the samples, onset transition temperature (T_o) ranged from 75.7°C (sample F) to 81.1°C (sample A); peak transition temperature (T_p) ranged from 78.7°C to 83.6°C (sample C) and enthalpy of gelatinization ranged from 12.3 J/g (sample F) to 14.8 J/g (sample A).

The gelatinization onset temperature (T_o) reflects heat–moisture induced transition from a crystalline to a more amorphous state. The gelatinization temperature of sweet potato starches ranked in the order A > C > B > D > E > F; however, the degree of hydrolysis was in the following order: F > E > D > C > B > A (Table 3). This indicates that a clear relationship exists between gelatinization temperature and susceptibilities to α-amylase attack. Crystalline arrangement of the starch granule plays an important role in its susceptibility to α-amylase attack, with the high gelatinization temperature sweet potato starches being less susceptible to enzyme attack.

The content of amylopectin was a critical factor in governing the gelatinization temperature. Low gelatinization temperature sweet potato starches had less amylopectin and more amylose than high gelatinization

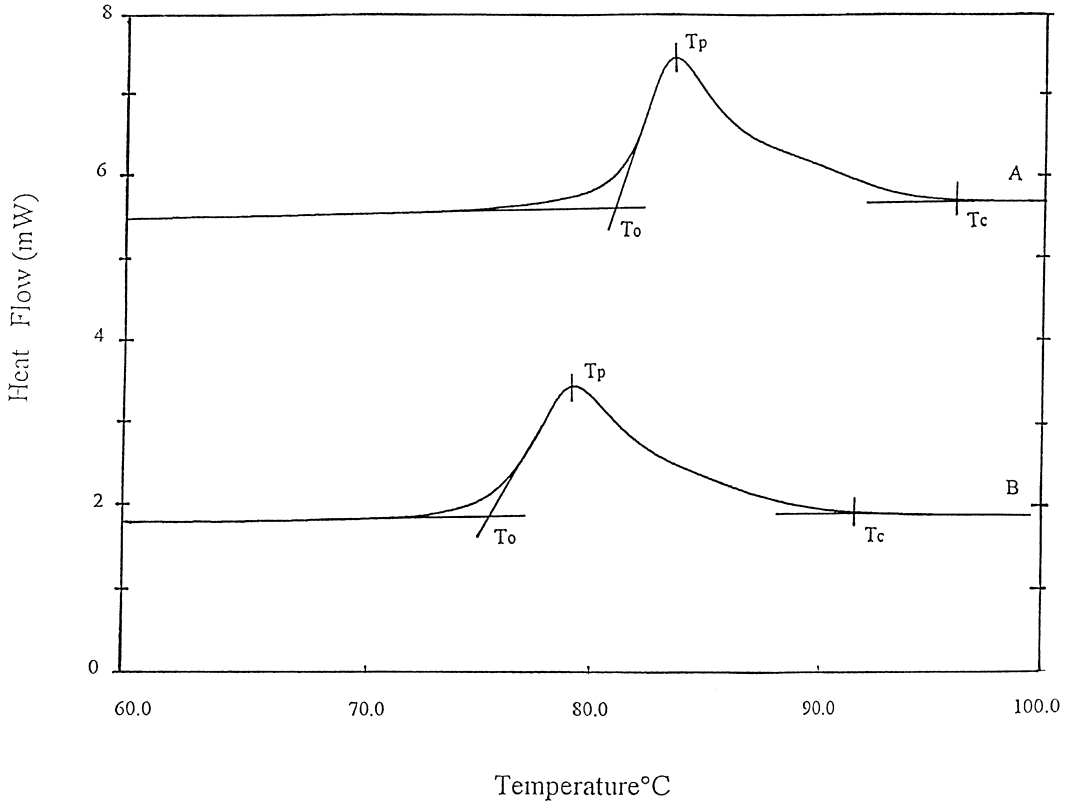

Fig. 3. DSC thermograms of various of sweet potato starches. (A), sample A; (B), sample F. T_o, onset transition temperature (°C). T_p, peak transition temperature (°C). T_c, complete transition temperature (°C).

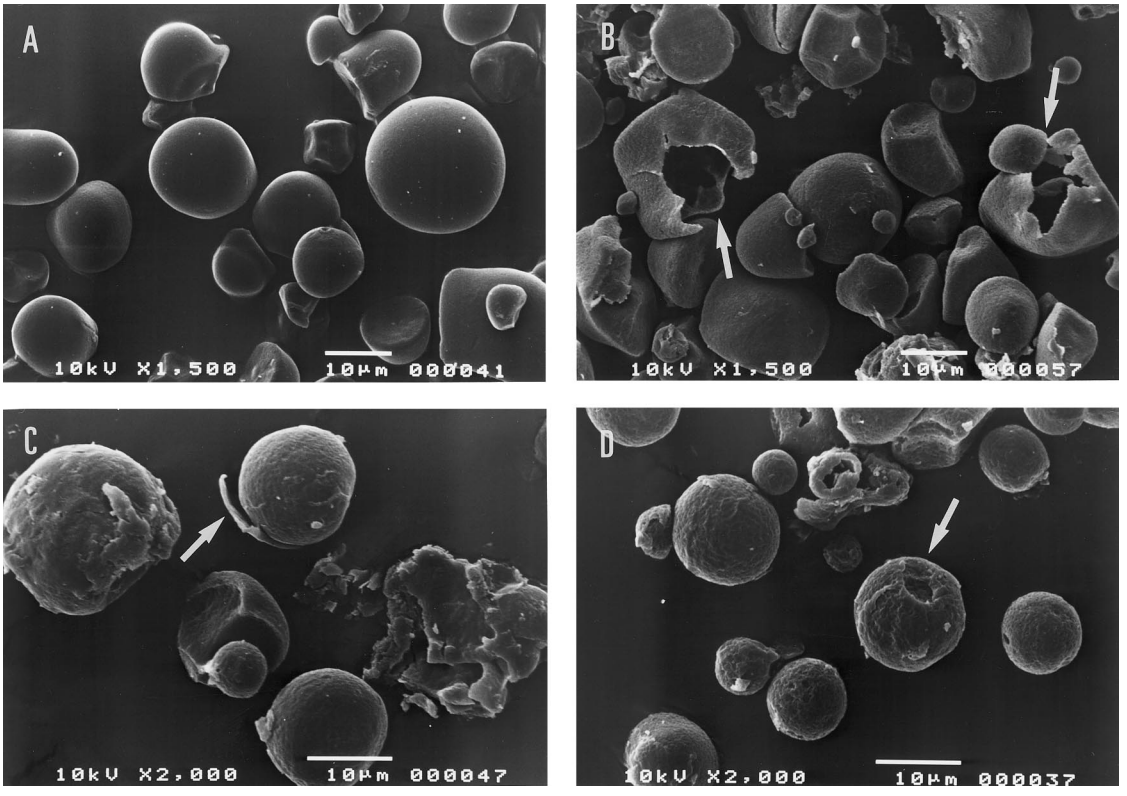

Fig. 4. SEM photomicrograph of native and degraded sweet potato starch granules. (A): sample B, native (B): sample B, D.H. = 52.2% (C): sample F, D.H. = 63.2% (D): sample D, D.H. = 61.1%.

temperature starches. Samples A, C and B were high gelatinization temperature starches and their amylopectin contents were higher than samples D, E and F (Table 2). These results were supported by the cluster model proposed by French.

3.5. Hydrolysis pattern

The modes of attack by α-amylase on varieties of sweet potato starches were investigated by SEM (Fig. 4 and Table 3) and the samples showed different hydrolysis patterns. The granule surface of the more extensively hydrolysed samples, E and F, exhibited significant erosion with many pieces peeling from the outer surface (Fig. 4C), some holes were also evident. The less extensively hydrolysed sweet potato starches A and B showed clean and rough surfaces with limited erosion and few holes. Sample A had more holes than sample B after α-amylase attack (Fig. 4B). α-Amylase apparently formed tunnels into the granule prior to hydrolysing the interior of the granule (Fig. 4). Samples C and D showed a very rough surface, with many craters but limited holes (Fig. 4D).

The enzymatic attack pattern could have an influence on the susceptibility of starch granules. Samples E and F, with greater susceptibility to enzyme attack and less intermolecular association, showed significant surface erosion. The low hydrolysis samples, A and B, displayed little erosion. This could be due to the differences in granule structure properties. Amylopectin and amylose ratio, crystalline structure and endothermic characteristic are critical factors for hydrolysis pattern and these factors act together to influence the susceptibility to enzyme attack.

4. Conclusion

This investigation suggested that amylopectin and amylose ratio, gelatinization temperature, granule size and mode of degradation of different sweet potato starch were different and depended on their varieties. The susceptibilities to α-amylase in sweet potato starch were primarily influenced by the granule structure, such as: amylopectin and amylose ratio, molecular association. High amylopectin content sweet potato starches showed high gelatinization temperature and less susceptibility to α-amylase attack. Hydrolysis pattern is an important factor for susceptibility to α-amylase attack.

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