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The single screw extruder as a bioreactor for sago starch hydrolysis

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Simultaneous pregelatinization and preliquefaction of sago starch with a thermostable α-amylase was carried out in a Brabender single screw extruder. Response surface methodology was employed to study the effects of processing conditions; feed moisture content (21–38%), enzyme concentrations (1.48–6.52%) and mass temperatures in the compression and die zones (70.5–97.5°C), on the properties of the extrudates. Twenty runs were performed based on a multifactorial composite rotatable design. Changes in the dextrose equivalent (DE), water solubility index (WSI), water absorption index (WAI), degree of gelatinization (DG) and high performance size exclusion chromatography (HPSEC) profiles were assessed. From the HPSEC profiles, the degree of degradation (DGR) and oligosaccharide content were calculated. Feed moisture content and enzyme concentration were found to be the most significant variables affecting most of the measured physicochemical properties. Hydrolysis of starch granules was the fundamental reaction occurring during the extrusion process as indicated by the higher DE, WSI, oligosaccharide content and correspondingly lower WAI, DG and DGR. Product spectra from HPSEC showed that the amylopectin (Ap) was preferentially degraded and amylose (Am) was apparently protected. The predominant oligosaccharide species were G3, G5 and G6.

INTRODUCTION

Sago palm is a potentially agronomically important indigenous crop of the south-east Asian and Oceanic regions which has remained relatively unexploited (Flach, 1983). Increased production coupled with improved quality has stimulated interest in the use of this material in industrial processes. Products of starch hydrolysis, such as maltodextrins, corn, glucose and high fructose syrups, have wide application in the food, textile, brewing and pharmaceutical industries (Griffin & Brooke, 1989). Commercially, these products are derived mainly from corn, barley, wheat or potato but it should be possible to obtain similar and other functionally important products from sago starch.

Hydrolysis products of starch are conventionally produced following degradation by acid, enzymes or their combination (Fullbrook, 1984). Each procedure is generally associated with its own unique problems. Relatively low product yield and the excessive formation of by-products limits the use of acid hydrolysis (Linko, 1992). In contrast, the use of enzymes involves the handling of starch slurries and, frequently, relatively long reaction times must be employed (Linko, 1992). Processing of starch in an extruder has been suggested as a novel approach to circumvent many of the drawbacks of conventional hydrolysis procedures. The use of the extruder as a continuous bioreactor for pregelatinization and liquefaction has been described by a number of authors (Linko *et al.*, 1979, 1983, 1984; Korn & Harper, 1982; Ben-Gera *et al.*, 1983; Chouvel *et al.*, 1983; Hakulin *et al.*, 1983; Chay *et al.*, 1984; Likimani *et al.*, 1991; Roussel *et al.*, 1991).

Sago starch was chosen as a model because the native granules, though poor substrates for the action of thermostable α-amylase (Govindasamy *et al.*, 1992; Kainuma, 1986), can be converted to a susceptible form following mild heat treatment at temperatures below the gelatinization temperature (Wang *et al.*, 1995). In addition, granules of this starch are also easily fragmented on exposure to even relatively mild extrusion conditions (Lim & Oates, submitted).

This paper explores the feasibility of utilizing a single screw extruder as a bioreactor for the thermomechanical gelatinization and liquefaction of sago starch using a thermostable α-amylase. Multifactorial experimental

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design and response surface analysis are used to determine the influence of the process parameters on the properties of the liquefied extrudates.

MATERIALS AND METHODS

Starch

Sago starch isolated from the palm *Metroxylon sagu* was obtained from a commercial producer, Wah Chang International. This material was used in all experiments. Average moisture content of the raw material was determined by drying to constant weight in a convection oven at 120°C for 2 h. The moisture content of the sago starch was 11% (weight basis). It contained 25.5% amylose and 0.015% protein, respectively (Sim *et al.*, 1991).

Enzyme preparation

A commercial preparation of thermostable α-amylase from *Bacillus licheniformis* (Termamyl 120L) was purchased from Novo Laboratories, Kuala Lumpur. The enzymatic preparation was reported to have an activity of 120 Kilo Novo α-amylase (KNU) with one KNU being defined as the amount of enzyme necessary to break down 5.26 g of starch (Merck, Amylum solable Erg.B.6., Batch 9947275) per hour to a non-iodine polymer under Novo's standard conditions (Anonymous, 1985).

Materials

Malto-oligosaccharide and dextran standards used in high performance liquid chromatography (HPLC) calibration were purchased from both Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan) and Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of at least AR grade and obtained either from Merck, Darmstadt Co. or Sigma Chemical Co. (St. Louis, MO, USA).

Extruder

A single screw Brabender laboratory extruder 20DN, Model No. 8325 (C.W. Brabender Instruments, Inc.) was used throughout this study. The extruder was equipped with a grooved barrel 19 mm in diameter. The barrel, 38 cm long, was divided into three modules, feed, compression and die, each with a length to diameter ratio (L/D) of 20:1. Each module was equipped with heating and cooling systems. Heat was provided by contact of the barrel with three electrical elements and steady temperature was maintained by compressed air cooling. Temperature was monitored by a microprocessor-based digital thermometer (John Fluke Mfg. Co.) connected to two external J-type thermocouples (iron–constantan) as temperature sensors. The thermocouples were inserted at the metering zone and die, respectively, where both temperature probes were flush with the barrel wall. The extruder was driven by a Brabender DO-corder DCE 330 drive of 3.3 kW (Model E) and was operable over the range 0 to 250 rpm.

Samples were introduced into the extruder via a feed hopper controlled in the speed range 5–150 rpm. Starch was prevented from gelatinizing at the neck of the feed hopper by cooling this region with running water. Feed rate of the starch was maintained at half of the extruder screw speed. This was found to provide smooth feeding of material into the extruder.

A uniformly tapered screw with a 3:1 screw compression ratio was used. The die plate was 11 cm long with a die cap mount which has an orifice 6 mm in diameter.

Start-up was achieved with a starch sample of 30% feed moisture and 4% enzyme concentration (grams per 100 g of starch). After attaining constant running conditions at the desired settings, 10 min was allowed to pass before samples or data were collected.

Sample preparation

A distilled water suspension of thermostable α-amylase was added to sago starch (Table 1) to obtain the desired levels of enzyme concentration and feed moisture. All ingredients, in 1 kg batches, were blended at low speed (1) at 4°C in a Kenwood mixer (Model Major) attached with a K beater. Enzyme solutions were added gradually in 2 ml aliquots during mixing to ensure homogeneous distribution. All samples were mixed for a further 5 min after addition of the enzyme suspension to ensure uniform moisture and enzyme distribution. Feed mixtures were stored at 4°C overnight in sealed plastic bags but were equilibrated at room temperature 2 h prior to extrusion; hydrolysis of the starch granules did not occur during this time (unpublished results). Sample moisture levels were confirmed by oven drying at 120°C for 2 h to a constant weight.

Extrusion

Preliminary trials were used to select extruder conditions and reduce the number of variables employed in the generation of the final response surface methodology (RSM). Conditions were maintained at a constant value were: screw speed, 80 rpm; feeder rate, 40 rpm; temperature of the first barrel section, 60°C. Mass temperatures of the second and third barrel sections were varied between 60–120°C with both zones maintained at the same temperature relative to each other. Samples were extruded in a random order and, after steady state was attained, experimental samples collected for 30 min. Extrudates were collected, dried in a convection oven (40°C) to constant weight before grinding in a laboratory cross beater mill, type SKI (F. Kurt Retsh Gmbh and KG, Germany) fitted with a 0.5 mm screen. Ground

Table 1. Independent variables and experimental design levels for extrusion of sago starch with a thermostable α-amylase, Termamyl 120L

Variable	Codes	Levels				
		$-\alpha$	-1	0	$+1$	$+\alpha$
Feed moisture (M, %)	X_1	21.6	25.0	30.0	35.0	38.4
Enzyme concentration (E, %)	X_2	1.48	2.50	4.00	5.50	6.52
Mass temperature (T, °C)	X_3	70.5	76.0	84.0	92.0	97.5

extrudates were stored in air-tight plastic containers and held at 4°C until analysis.

Response surface methodology and experimental design

A multifactorial composite rotatable design (Mullen & Ennis, 1979) based on five levels and three variables was used to assess the effects of extrusion conditions on the physicochemical properties of the extrudates (Table 1). Three process variables were selected: total moisture content of the mass feed (%, g per 100 g of starch), mass temperatures in the compression and die zones and enzyme concentration (%, grams per 100 g of starch) (Tables 1 and 2). The design consisted of 20 experiments with eight (2^3) factorial points, eight axial points to form a central composite design with $\alpha = 1.682$ and six centre points for replication. Axial points represented the extreme high and low values of the independent variables (Table 2) under which the extruder could be operated.

Data obtained was processed using Statgraphics version 6.0 (Mannugistics, Inc., Rockville, USA). Second-order response surface predictions were fitted for each dependent response characteristic using the statistical package. The second order polynomial equations, which may contain linear, quadratic and interaction terms, were used to develop response surfaces in the optimum region. Surface plots were generated by showing the effects of two independent variables, while the other variable was maintained at a constant value in each response. Independent variables were located along the horizontal plane and the dependent or response variable on the vertical axis. After analysing the original data, each equation was statistically validated by analysis of variance.

Analytical methods

The reducing sugar was assayed according to a modified Park–Johnson method (Hizukuri *et al.*, 1981) using dextrose as the standard.

Water solubility and water absorption indices

The water solubility index (WSI) and water absorption index (WAI) were determined as described by Anderson *et al.* (1969), with one minor modification (Likimani *et al.*, 1991). A 0.5 g sample of ground product (sieved through 60 mesh size or 250 μm screen) was suspended in 6 ml of 0.1 M acetate buffer, pH 3.5, instead of distilled water, to inactivate residual α-amylase activity. The sample was incubated for 30 min at 30°C in a shaking water bath and then centrifuged at 2500 rpm for 15 min. The supernatant was transferred quantitatively to a weighed evaporating dish and dried over a boiling water bath. The amount of soluble material was expressed as a percentage of the original dry sample weight (WSI). The ratio of the total weight of the pellet to the dry weight of the samples is the calculated WAI.

Degree of gelatinization

The degree of gelatinized material was determined using a modified method originally outlined by Wootton *et al.* (1971). Two 0.1 g samples of ground extrudate were dispersed in 5 ml of 0.25 M KOH and 0.70 M KOH, respectively. The samples were shaken for 15 min in a shaking water bath with intermittent agitation with a vortex mixer, followed by centrifugation at 2500 rpm for 15 min. The supernatant was neutralized with equal volumes of the respective molarities of HCl (0.25 M and 0.70 M). The degree of gelatinization was assessed on each diluted supernatant according to the spectrocolorimetric method of Wootton *et al.* (1971) and expressed as the iodine responses of the samples divided by the iodine response of the samples totally solubilized in 0.70 M KOH (%).

Table 2. Values of independent variables of each experiment in dimensionless coded (x_i) and physical (X_i) units for the 2^3-second order factorial central composite experimental design

x_1	x_2	x_3	$X_1(M, \%)$	$X_2(E, \%)$	$X_3(T, °C)$
-1	-1	-1	25.0	2.50	76.0
-1	-1	$+1$	25.0	2.50	92.0
-1	$+1$	-1	25.0	5.50	76.0
-1	$+1$	$+1$	25.0	5.50	92.0
$+1$	-1	-1	35.0	2.50	76.0
$+1$	-1	$+1$	35.0	2.50	92.0
$+1$	$+1$	-1	35.0	5.50	76.0
$+1$	$+1$	$+1$	35.0	5.50	92.0
$+\alpha$	0	0	38.4	4.00	84.0
$-\alpha$	0	0	21.6	4.00	84.0
0	$+\alpha$	0	30.0	6.52	84.0
0	$-\alpha$	0	30.0	1.48	84.0
0	0	$+\alpha$	30.0	4.00	97.5
0	0	$-\alpha$	30.0	4.00	70.5
0	0	0	30.0	4.00	84.0
0	0	0	30.0	4.00	84.0
0	0	0	30.0	4.00	84.0
0	0	0	30.0	4.00	84.0
0	0	0	30.0	4.00	84.0
0	0	0	30.0	4.00	84.0

Sample preparation for high performance size exclusion chromatography (HPSEC) and dextrose equivalent (DE) analyses

The samples for HPSEC and DE were prepared using a modified method of Jackson *et al.* (1988) as outlined by Govindasamy *et al.* (1992). The ground extrudate sample (0.1 g) was solubilized with 1M NaOH (2 ml). Following purging with nitrogen, all tubes were subsequently stirred continuously at 25°C for 30 min. The pH was adjusted to 6.0 using 3 M acetic acid and the solution made to 5 ml with 100 mM acetate buffer (pH 6.0). To minimize microbial degradation during storage prior to injection into the HPLC, 5 ml of 0.4 mM $HgCl_2$ was added to the sample solutions.

For HPSEC analysis, an aliquot of this starch suspension was dispersed by an ultrasonic processor (ULTRA sonik® 300, Ney Co.) for 400 s. The supernatant was filtered through a millipore filter (8.0 μm). The filtrate was allowed to equilibrate at 40°C in an incubator oven prior to injection into the HPLC system.

HPLC equipment

The molecular weight profiles of the extrudates were established using high performance size exclusion chromatography. A Waters Associate (Milford, MA) 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 integrator was programmed to relate the response factors of the different oligosaccharides to their concentrations. The refractive index detector was set at a sensitivity of 128 and a scale factor 30. The detector cell temperature was maintained at 40°C.

The WISP model 712 injector was preprogrammed to perform three injections of 50 μl for each sample. Three Ultrahydrogel HPSEC columns (dimensions 7.8 mm i.d.×30 cm) were connected in series in descending order; an Ultrahydrogel linear column of mixed pore size and exclusion limit (polyethylene oxide, PEO as a standard) of 7×10^6 Da, and two Ultrahydrogel 120 columns with a pore size of 120 Å and exclusion limit (PEO) of 5×10^3 Da. The columns were maintained at 40°C. Samples were injected and eluted using a mobile phase of deionized water at a flow rate of 0.8 ml min^{-1}. The deionized water had been filtered with a millipore filter of 0.22 μm pore size and degassed at room temperature with ULTRAsonik® (Ney, Co.) before use.

Oligosaccharide and dextran molecular weight standards (162 to 2×10^6) were dissolved in water and injected into the HPSEC system. A calibration plot was made using the relationship between retention time and log molecular weight.

Dextrose equivalent determination

The DEs of the extrudates were assessed by measuring both the total dry weight and reducing sugar contents of the suspension. DE is defined as total reducing sugars expressed as dextrose and calculated as a percentage of the total dry weight.

RESULTS AND DISCUSSION

The second order polynomials fitted to the measured data were computed by stepwise regression analysis using coded values. Coded values ranged from −1.682 to +1.682 across the response surface tested. Formulae for converting actual values to coded values are given in Table 3.

Feed moisture content and enzyme concentration were found to be the most significant variables in the simultaneous pregelatinization and preliquefaction of sago starch in the single screw extruder (Table 4).

Effects of extrusion variables on DE

The DE values (data not shown) of all samples ranged from 0.3 to 10.4. This is comparable to those reported for other starches, DE values of 3.5–4.5 for barley extrudates (Linko *et al.*, 1983), 2.7–6.4 for corn/soybean extrudates (Likimani *et al.*, 1991) and 9–20 and 7–13 for corn extrudates obtained using two different methods (Roussel *et al.*, 1991).

Enzyme concentration had the greatest effect on DE of the extrudates (Table 4). Feed moisture content was also found to be significant. Degradation of starch in the extruder was promoted in the presence of higher enzyme concentrations and was markedly dependent on feed moisture (Fig. 1a). This dependence on feed moisture content is demonstrated at the lower hydration levels. Below 30%, the degradation of the starch molecules is reduced (Fig. 1a). The relatively high feed moisture contents required for enzymatic hydrolysis of sago starch in the extruder is not unique to sago starch. Other authors have reported similar relationships for a variety of starches. Reinikainen *et al.* (1986) reported that the degree of hydrolysis of wheat starch in the extruder, monitored as an increase in DE and release of oligosaccharides, was dependent on feed moisture, enzyme concentration and mass temperature. However, DE values of corn/soybean extrudates did not correlate with any system variables as the degradation was only at the macromolecular level (Likimani *et al.*, 1991).

Higher processing temperatures led to the formation of extrudates with lower DEs throughout the whole range of temperatures explored. However, this effect

Table 3. Formulae for converting actual values to coded values

$$\text{Coded feed moisture content} = \frac{\text{feed moisture content (\%)} - 30}{5}$$

$$\text{Coded mass temperature} = \frac{\text{mass temperature (°C)} - 100}{10}$$

$$\text{Coded enzyme concentration} = \frac{\text{enzyme concentration(\%)} - 4}{1.5}$$

was observable only at moisture levels above 26.5%. This phenomenon could not be attributed to the thermal inactivation of Termamyl 120L as the mass temperatures were maintained in the range 70.5–97.5°C, below the temperature at which the enzyme is deactivated (105°C). Reinikainen *et al.* (1986) concluded that relatively high extrusion moisture levels are needed for good and rapid conversion of wheat starch in a twin screw extruder and that even relatively low enzyme levels may be employed if the extrusion feed moisture is high and mass temperature relatively low. Previous studies have demonstrated the stability of this enzyme in the extruder; Roussel *et al.* (1991) reported that inactivation of Termamyl 120L occurred at barrel temperatures above 105°C; Likimani *et al.* (1991) observed maximum residual α-amylase activities at mass temperatures below 106°C (16.4–18.8% feed moisture content).

Interactive terms mass temperature*feed moisture and mass temperature*enzyme concentration, made significant contributions. At constant feed moisture (30%), DE declined as mass temperature was increased at a Termamyl concentration of 1.5% up to 99°C, beyond which there was no further change in DE with increasing temperature (Fig. 1b). However, at higher enzyme content (6.5%) an increase in mass temperature led to a corresponding increase in DE. Larger DE values recorded at the higher enzyme concentration can be attributed to the combined effects of starch gelatinization and dextrinization with increasing temperature in the presence of the enzyme. Maintaining the enzyme concentration at a constant value but increasing the thermal energy input at the highest moisture content caused a reduction in DE.

Water solubility index (WSI)

Water solubility indices (WSI) for liquefied extrudates were in the range 6.3–33.2%; native starch in comparison had a negligible WSI (less than 0.2%). WSI of the extrudates was dependent on the enzyme concentration and interaction of mass temperature with either feed moisture content or enzyme concentration (Table 4).

Table 4. Prediction equations for the dependent variables

Dependent variables	Independent variables	Coefficient	R^2 (adj.)	p value
DE	constant	5.269	0.96	0.0000
	M^{***a}	1.832		
	E^{***}	2.046		
	T^{**}	−0.653		
	E^{2***}	−0.734		
	$M*E^{***}$	1.038		
	$M*T^{***}$	−0.913		
	$E*T^{**}$	0.738		
WSI	constant	22.154	0.87	0.0000
	E^{***}	6.788		
	E^{2*}	−1.609		
	$M*T^{*}$	−1.995		
	$E*T^{*}$	2.063		
WAI	constant	3.068	0.72	0.0001
	M^{***}	−0.187		
	E^{**}	−0.110		
	$E*T^{*}$	−0.108		
Degree of degradation	constant	3.436	0.76	0.0001
	M^{***}	0.532		
	E^{*}	0.224		
	M^{2**}	0.346		
	T^{2*}	0.277		
Oligosaccharide content	constant	9.909	0.97	0.0000
	M^{***}	4.905		
	E^{***}	4.221		
	T^{***}	−2.439		
	$M2^{***}$	1.766		
	E^{2*}	0.736		
	$M*E^{***}$	3.708		
	$E*T^{***}$	−1.880		
Percent gelatinization	constant	26.419	0.67	0.0005
	M^{**}	−6.697		
	E^{*}	−5.847		
	E^{2*}	5.680		
	$M*T^{**}$	8.878		

***: $p < 0.001$.
**: $p < 0.01$.
*: $p < 0.05$.

Enzyme concentration displayed a strong positive correlation with WSI (Fig. 2a). This effect was also dependent on mass temperature; greater amounts of soluble material were released at the higher mass temperature (97.5°C). Apparent increased efficiency for hydrolysis would tend to support further the assumption that little or no enzyme inactivation occurred in the mass temperature ranges investigated. As simultaneous gelatinization and liquefaction are allowed to proceed in the extruder, a low thermal energy input into this high moisture system may also prevent proper gelatinization thereby impeding accessibility of glycosidic bonds to the enzyme. Maximum WSI was obtained at both high mass temperature (97.5°C) and enzyme concentration (6.5%) at a constant feed moisture of 30%. The higher amount of solubles released accounts for the maximum DE (Fig. 1a).

Mass temperature was dependent on feed moisture content, the positive influence of mass temperature on WSI being more critical at lower hydration levels. Increasing the thermal energy input under these conditions resulted in extrudates with a higher WSI, whilst extrusion at the higher moisture content (38.4%) resulted in extrudates with a lowered WSI. Extruding under the following conditions: lowest mass temperature and highest feed moisture content (70.5°C, 38.4%) or highest

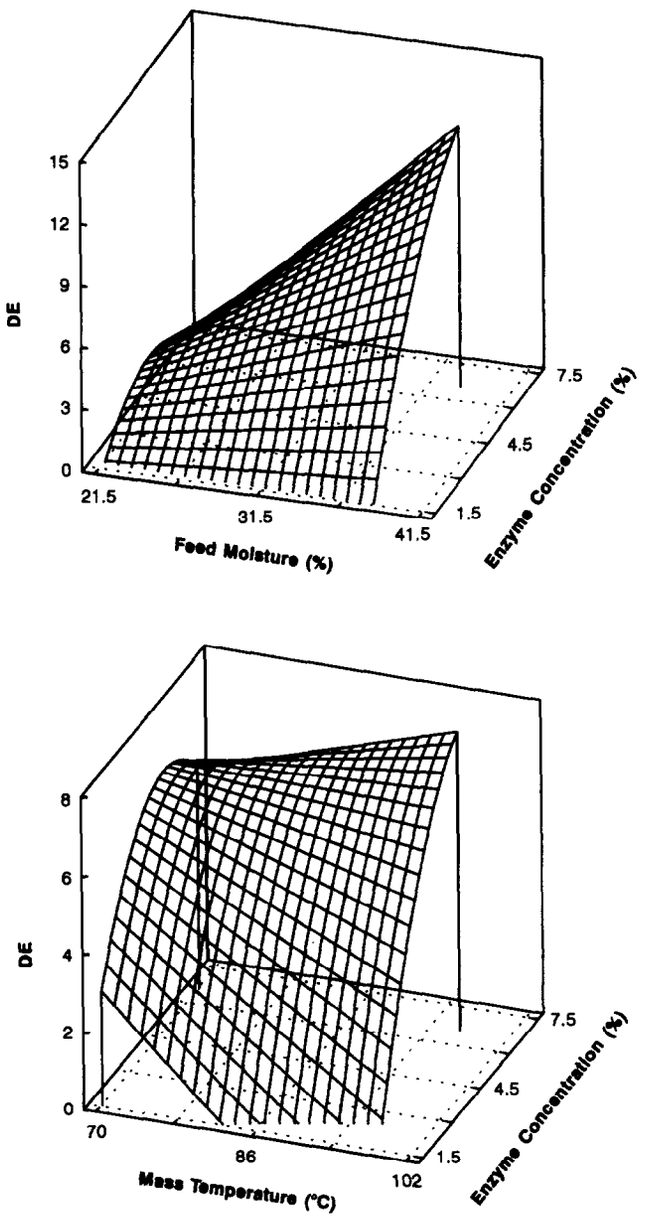

Fig. 1. (a: top) Influence of feed moisture content and enzyme concentration on DE of extrudates at mass temperature 84°C. (b: bottom) Influence of mass temperature and enzyme concentration on DE of extrudates at feed moisture content 30%.

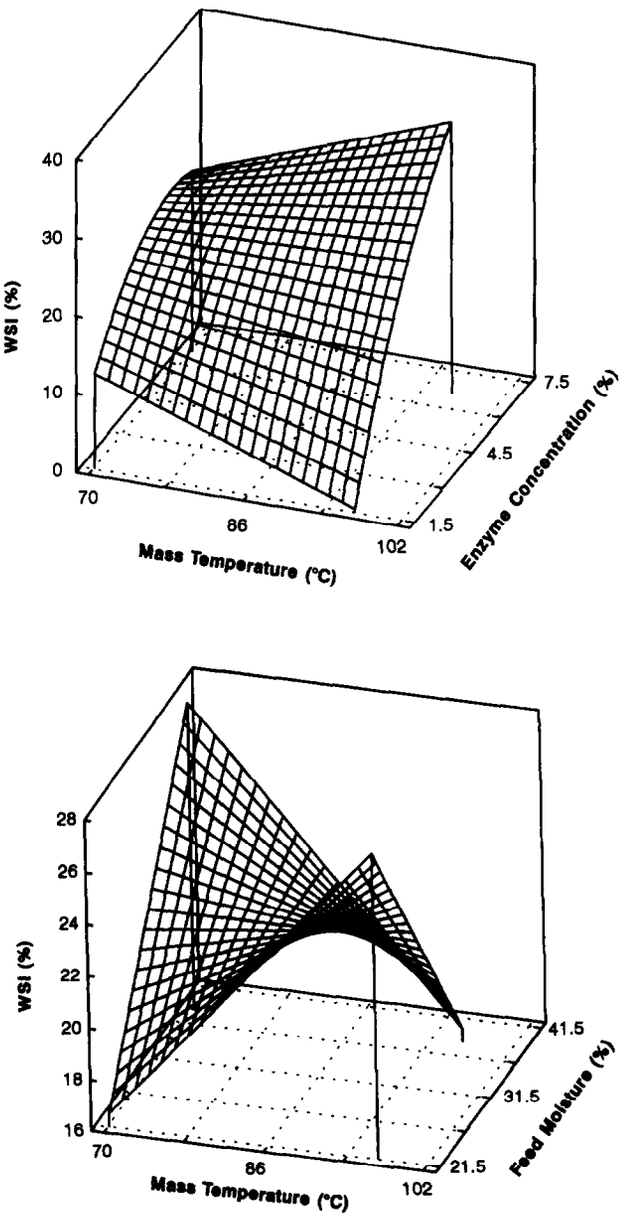

Fig. 2. (a: top) Influence of mass temperature and enzyme concentration on WSI of extrudates at feed moisture content 30%. (b: bottom) Influence of mass temperature and feed moisture content on WSI of extrudates at enzyme concentration of 4.0%.

mass temperature and lowest feed moisture content (97.5°C, 21.6%) results in products with a low WSI.

The range of WSI of extrudates obtained by extrusion of sago starch at high moisture and low mass temperatures (22–38%, 76.5–103.5°C) without enzyme using an identical extruder system and raw material (Lim & Oates, submitted), were similar to those attained by coprocessing with an α-amylase (3.68–30.24%). Despite similarities in WSI, extrusion in the absence of enzyme did not result in any apparent macromolecular degradation in comparison to the highly degraded macromolecules resulting from extrusion in the presence of enzyme.

Water absorption index (WAI)

The WAI of the samples ranged from 2.73–3.61 g g^{-1}. Differences in the WAI of the extrudates were negatively correlated to feed moisture content and enzyme concentration ($p < 0.001$) (Table 4). WAI increased linearly with reduction in enzyme concentration and feed moisture content; onset of dextrinization could clearly be seen.

Dextrinization occurred at higher enzyme concentrations and was dependent on mass temperature (Table 4). The influence of mass temperature can be demonstrated at a low enzyme concentration (1.5%); increasing mass temperature resulted in enhanced WAI, suggesting that gelatinization of starch was the fundamental mechanism under these conditions and that enzymatic degradation was minimal due to relatively low amounts of enzyme used (Fig. 3). The compact granular starch structure was loosened to some extent as a consequence of increasing the input of thermal energy, facilitating WAI. At higher concentrations (6.5%), a distinct decline in WAI was observed as a result of the onset of dextrinization with rising mass temperature. Furthermore, assessment of the results attained for DE and WSI show that relatively high DE and WSI are obtained when

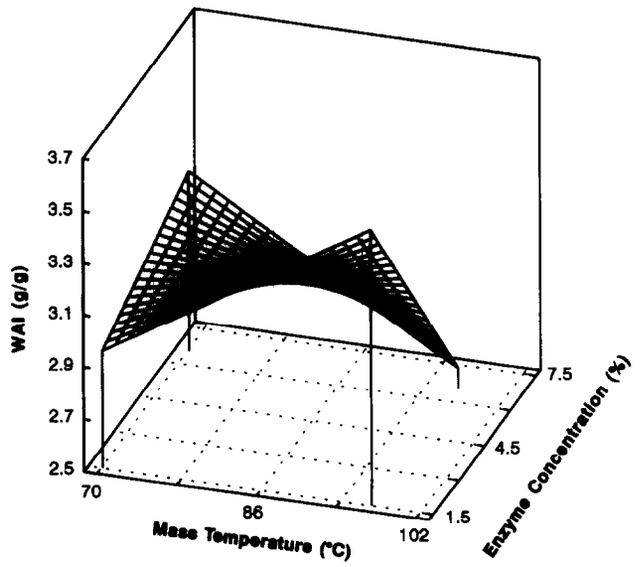

Fig. 3. Influence of mass temperature and enzyme concentration on WAI of extrudates at feed moisture content 30%.

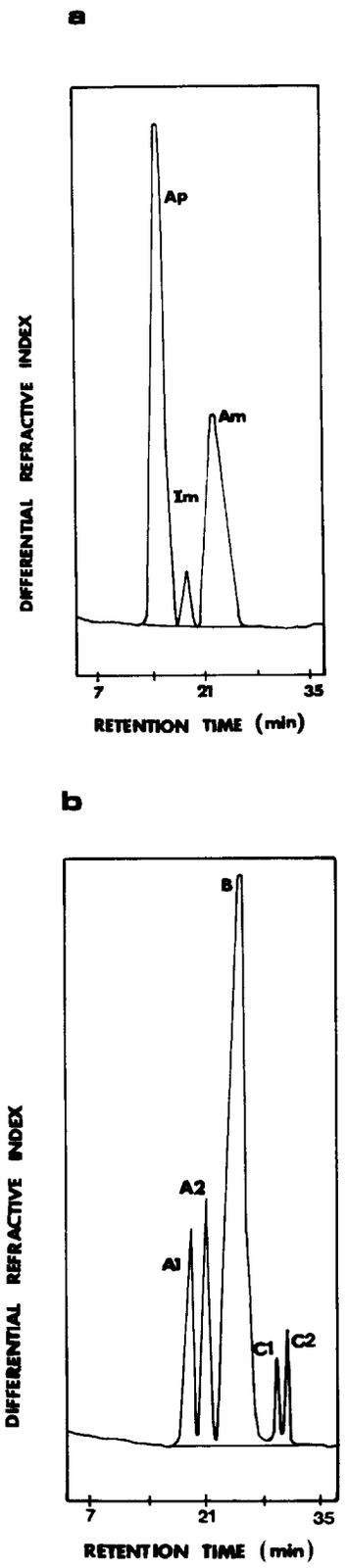

Fig. 4. HPSEC chromatogram of 0.5% (w/v), 1 N NaOH solubilized native and extruded sago starch. (a) Native starch: amylopectin (Ap), intermediate (Im) and amylose (Am); (b) starch extruded at 25% moisture content, 92°C mass temperature, 5.5% enzyme concentration: molecules with apparent molecular weight (i) smaller than amylopectin (A_1 and A_2), (ii) in the range 10^4–10^6 and (iii) oligosaccharide species (C_1 and C_2).

extruded at 97.5°C with 6.5% Termamyl 120L at 30% feed moisture (Figs 1b and 2a). Conversely, this extrudate had the lowest WAI, clearly indicating occurrence of dextrinization with formation of soluble material.

Chromatographic profiles

HPSEC profiles of native starch (Fig. 4a) show intact macromolecular fractions and these served as the reference throughout this investigation. Relative peak areas for each component were calculated at 70% for amylopectin (Ap) and 27% for amylose (Am). This ratio is very close to that reported by Ito *et al.* (1979) and is within the range of values reported for other starches. The small peak eluting between Ap and Am, comprising 3% of the total starch content, is believed to be an intermediate fraction. The structure of the intermediate material (Im) has been described as Am with a certain degree of branching (Hizukuri *et al.*, 1981; Takeda *et al.*, 1989). The apparent molecular weight of Ap was estimated to be greater than 10^8 Da and that of Am was about 8×10^5 Da compared with dextran standards.

Three populations of molecules could be detected in solubilized extrudates (Fig. 4b); peaks A_1 and A_2 had apparent molecular weights smaller than Ap; peak B was in the range 10^4 to 10^6 ($RT=22.63$ to 24.63 min) and peak C (C_1 and C_2) corresponded to oligosaccharide species (from G1 to G10). HPSEC profiles of all the solubilized extruded sago starch strongly suggested that Ap was relatively more susceptible to the α-amylase activity. Hence Ap was preferentially degraded to produce a range of smaller molecular weight macromolecules. On the other hand, critical examination of the profiles revealed that Am seemed little affected. A similar trend was demonstrated when sago starch was extruded under high moisture, low temperature conditions without enzyme (Lim & Oates, submitted). HPSEC profiles of the products of native starch hydrolysis carried out at 90°C revealed a similar trend; Ap was rapidly degraded within the first hour, resulting in the formation of intermediate products larger than Am and oligosaccharide, G7 (Govindasamy *et al.*, 1992). In contrast, Am was hydrolyzed at a much slower rate and was completely degraded only after 2 h of incubation (Govindasamy *et al.*, 1992). This suggests that amylose is apparently 'protected' in some way.

The degree of degradation (DGR) of the starch, illustrated by the molecular weight distribution ratio $M_w<2\times10^6/M_w>2\times10^6$, during extrusion cooking with Termamyl 120L, was influenced by moisture content and enzyme concentration (Table 4). Degradation was influenced predominantly by feed moisture content ($p<0.001$) with an apparent threshold moisture content around 26.5% (Fig. 5a) below which little degradation was evident. Increasing the feed moisture content above this value resulted in a sharp increase in degradation. Enzyme concentration exerted a positive effect at all moisture levels although enzyme concentration was

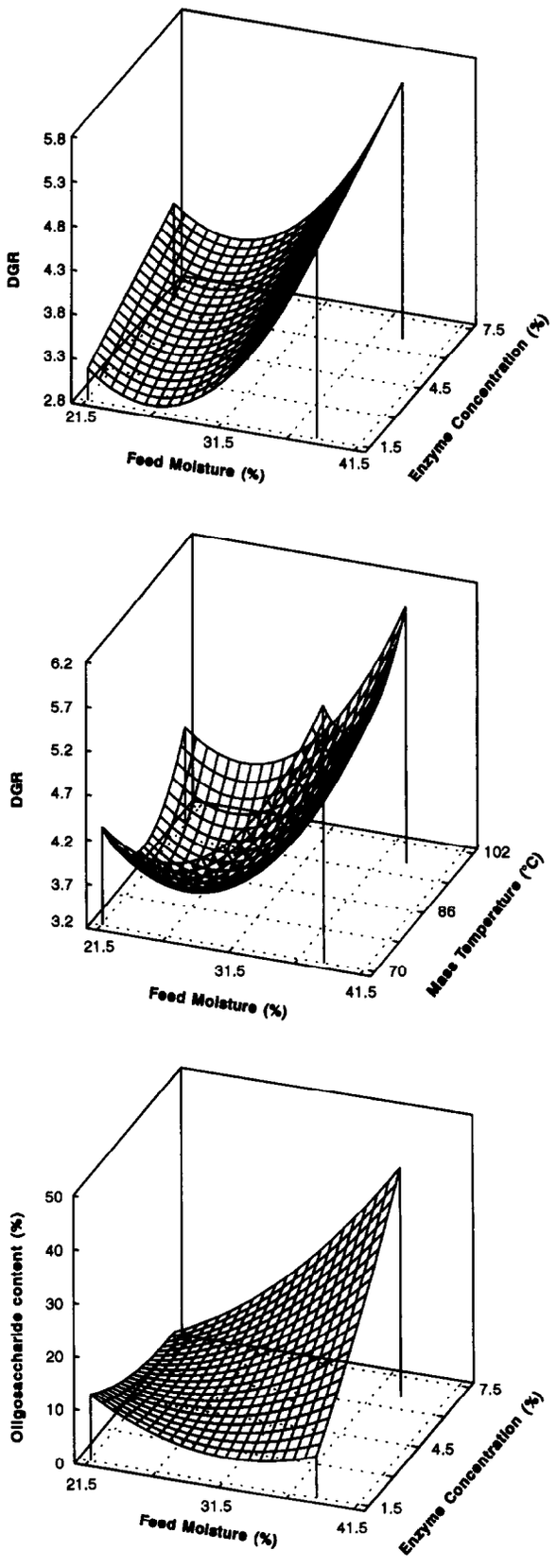

Fig. 5. (a: top) Influence of feed moisture content and enzyme concentration on degree of degradation at a mass temperature of 84°C. (b: middle) Influence of feed moisture content and mass temperature on degree of degradation at an enzyme concentration 4.0%. (c: bottom) Influence of feed moisture content and enzyme concentration on the amount of oligosaccharides formed in the extrudates at mass temperature 84°C.

more critical at lower moisture content. Lower moisture levels during twin-screw extrusion cooking of wheat starch have been demonstrated to be more destructive to the enzyme (Reinikainen *et al.*, 1986). At feed moisture contents below 26.5%, the degree of degradation could be due to mechanical and thermal effects while at higher feed moisture contents (>26.5%), the effects of enzyme are more prevalent (Fig. 5a).

The effects of temperature illustrated that degradation displayed a minimum value for mass temperature around 81–83°C (Fig. 5b). Departure from this mass temperature caused an elevation in the degree of degradation, with the breakdown being more extensive at the higher temperature range.

Oligosaccharide content

Examination of oligosaccharide components in HPSEC profiles for solubilized sago starch extrudates showed that G3, G5 and G6 were the predominant oligosaccharide species. Similar chromatographic profiles were previously observed by Linko *et al.* (1980), Chouvel *et al.* (1983) and Roussel *et al.* (1991). The amount of G1–G10 present in each sample, calculated from the HPSEC profiles, was denoted as percent oligosaccharides. Regression analysis showed that feed moisture content and enzyme concentration had the most pronounced effects on the amount of oligosaccharides produced (Table 4). Increasing the feed moisture or enzyme content resulted in the formation of higher amounts of oligosaccharides due to the combined action of enzyme and water on the rate of the hydrolytic reaction (Fig. 5c). The effect of feed moisture is more conspicuous at the highest enzyme concentration (6.5%). In addition, the influence of enzyme concentration on oligosaccharide formation is greater at the highest feed moisture content (38.4%). Investigators (Linko *et al.*, 1984; Chouvel *et al.*, 1983; Hakulin *et al.*, 1983) have demonstrated that extrusion conditions optimal for hydrolysis were low feed rate, high moisture content and high consumption of enzyme. This suggests that enzyme concentration is more critical at the lower moisture levels. In particular, high moisture content would prevent the formation of a thin layer on the internal surface of the extruder (one of the basic characteristics of extrusion processes) and therefore could protect the enzyme against denaturation by shearing (Colonna *et al.*, 1987).

Oligosaccharide formation was dependent on the interactive terms, feed moisture content and mass temperature. The positive effect of feed moisture content (at enzyme concentration of 4%) is more prominent at the lowest mass temperature (70.5°C) than at the highest (97.5°C) (Fig. 6).

Degree of gelatinization

Variables affecting the extent of gelatinization of extrudates were feed moisture content, enzyme concentration and the interaction term, mass temperature*feed moisture content (Table 4). The response surface plot (Fig. 5) generated from the regression equations at an enzyme concentration of 4% shows that processing at high mass temperature (97.5°C) led to less gelatinization. Introduction of more water into the system had an analogous effect. These results are anomalous because gelatinization would be expected to increase at higher levels of mass temperatures and feed moisture. This discrepancy may involve the action of α-amylase, which at increased moisture content and temperature should have been

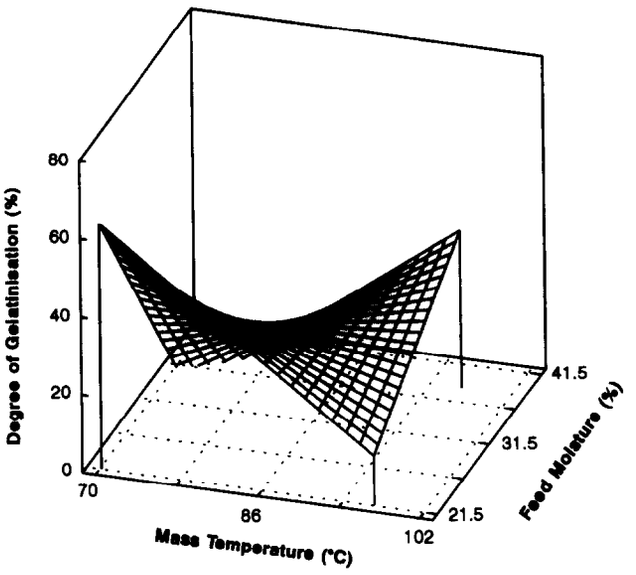

Fig. 6. Influence of feed moisture and mass temperature on degree of gelatinization at enzyme concentration 4.0%.

Table 5. Correlation coefficients between dependent variables

	WSI	WAI	DG	DE	Oligo
WAI	−0.4600*	ns	ns	ns	ns
DG	−0.5505*	+0.7879***	ns	ns	ns
DE	+0.7634***	−0.8469***	−0.7892***	ns	ns
Oligo[a]	+0.5586*	−0.7497***	−0.6009**	+0.9061***	ns
DGR	+0.3528	−0.5241*	−0.2245	+0.5824**	+0.6148**

***: $p < 0.001$.
**: $p < 0.01$.
*: $p < 0.05$.
ns: not significant.
[a]:Oligosaccharide content.

enhanced, resulting in increased solubility of the product. Since measurement of gelatinization was based on amylose–iodine complex formation, the production of lower molecular weight carbohydrates would cause a corresponding decrease in the absorbance ratio, and hence a reduction in the apparent degree of gelatinization. This could be responsible for the low R^2 values obtained for this regression equation.

Increasing feed moisture content or enzyme concentration caused a lowering in the degree of gelatinization of the product; the effect is more noticeable at highest moisture content (38.4%) (Table 4). Contrasting results were observed when the enzyme concentration was above 4.8%; the degree of gelatinization of the extrudates was elevated. This anomaly could be attributed to formation of linear 'amylose' like hydrolysates which probably complex with iodine, resulting in a correspondingly higher absorbance ratio, and hence a rise in the apparent degree of gelatinization.

Correlation analysis

A summary of the correlation between the different responses is presented in Table 5. Degradation of starch granules is the fundamental reaction occurring during extrusion; most of the measured physicochemical properties were found to be related to each other, especially DE and the amount of oligosaccharide released. The extensiveness of the enzymatic degradation (dextrinization) was represented by higher DE, WSI and oligosaccharide content and correspondingly lower WAI, DG and DGR. Solubility was seen to increase at the expense of water absorption index as dextrinization was the predominant mechanism taking place. Poor correlation between WSI and oligosaccharide content suggests that oligosaccharides formed during extrusion were not the exclusive contributor to solubility. In addition, it indicates the presence of other soluble but relatively higher molecular weight components. WAI was positively correlated with degree of gelatinization but showed a negative correlation towards DE, oligosaccharide content and DGR. This further emphasizes the onset of dextrinization.

CONCLUSION

The reported results indicate that combined gelatinization and enzymatic liquefaction of raw sago starch can be achieved in a single-screw extruder. Feed moisture content and enzyme concentration were found to be the most significant variables. Increased apparent breakdown of the macromolecule components and improved water solubility indicate that hydrolysis of starch granules was the fundamental reaction occurring during extrusion.

Data concerning how readily these samples are saccharified, the DEs and the HPSEC profiles of these saccharified samples will be discussed in a subsequent paper.

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