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Influence of extrusion variables on subsequent saccharification behaviour of sago starch

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This study reports the effects of substrate concentrations (21·6–38·4 g/100 ml), amyloglucosidase (AMG) concentrations (0·66–2·34 U/g) and hydrolysis time (14·8–65·2 h) on the saccharification of sago starch extrudates. Regression analysis established AMG concentration and hydrolysis time as the most significant factors; a generated regression equation adequately predicted the outcome of saccharification. Using 30 g/100 ml solid content, 65·2 h and 2·34 U/g of AMG, syrups of highest glucose content (87·9%) were attained. Effects of extrusion conditions, i.e. feed moisture content (21·6–38·4), enzyme (Termamyl 120L) concentrations (1·48–6·52%) and mass temperatures in the compression and die zones (70·5–97·5°C) during the combined gelatinisation and liquefaction of sago starch, on the amount of glucose produced were evaluated. Enzyme concentrations and feed moisture contents had the most pronounced effects on saccharification, which ranged from 47·6 to 73·3% (DE = 85–94) and 54·5–93·8% (DE = 90–98) after 24 and 65 h, respectively. Response surface plots suggest that a higher percent saccharification can be achieved when sago starch is extruded at high Termamyl concentrations but low moisture contents.

INTRODUCTION

Production of glucose syrup from corn starch by enzymatic hydrolysis is a well-established industrial process (Sims & Cheryan, 1992). Commonly produced syrups, containing about 95% glucose (dry basis), are ideal substrates for the manufacture of high fructose corn syrups (HFCS) and as fermentation media (Sims & Cheryan, 1992). The industrial manufacture of dextrose hydrolysates involves two successive steps: liquefaction, carried out after gelatinisation by the action of α-amylase; and saccharification, resulting in further transformation of maltodextrins into glucose (Slominska, 1993). Enzymatic conversion of liquefied starch to glucose requires a long reaction time (24–96 h), the length of which is dependent on the desired concentration of glucose in the final product (Sims & Cheryan, 1992). The use of high concentrations of enzymes, prolonged reaction hydrolysis times and relatively high concentration of substrates (30–40% dry substance (ds), w/w) in the industrial process can cause reversion reactions involving resynthesis of saccharides from glucose (Labout, 1985). The main reversion product is isomaltose but higher branched polymers are also formed after prolonged incubation (Labout, 1985).

Saccharification time is reduced by the application of extrusion–liquefaction technology. Linko *et al.* (1983) have demonstrated that barley starch liquefied by *Bacillus licheniformis* α-amylase in a twin-screw extruder requires 24 h for subsequent saccharification to 94–95 DE (dextrose equivalent) with *Aspergillus niger* glucoamylase. Semi-continuous saccharification to 85–89 DE, immediately after extrusion by recycling the extrudate through a scraped-surface heat exchanger, can be achieved in only 4 h (Linko *et al.*, 1983). A rapid saccharification, 2·5 h, can be achieved by continuous hydrolysis of extruded cassava starch with glucoamylase in an ultrafiltration reactor (Darnoko *et al.*, 1989). Hydrolysates with a DE of 8–20, collected at the extruder outlet, can be transformed within 10–24 h to high glucose syrups (DE > 92) in the presence of glucoamylase (Chouvel *et al.*, 1983; Linko *et al.*, 1983). Little or no starch retrogradation occurs if the liquefied extrudates are processed immediately with glucoamylase and are not cooled (Linko *et al.*, 1983, 1984). Syrups produced by such technology can be efficiently converted during fermentation (Linko *et al.*, 1983, 1984; Chay *et al.*, 1984). Extrusion-liquefied starches

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can also be used as a starting material for the production of high maltose syrups, in the presence of β-amylase plus pullulanase. In this application, it is preferable to use liquefied extrudates of low DE (<2) (Linko *et al.*, 1983, 1984).

Generic differences between starches from different botanical sources necessitates use of a particular liquefaction technique appropriate to individual requirements (Knudsen & Karkalas, 1969). Most research on extrusion cooking technology has focused on starches produced in Western countries, such as corn, wheat and potato. Very few reports are available on extrusion processing of starches derived from crops indigenous to the tropical regions, including sago starch. Sago starch is an agronomically important indigenous crop of South East Asia, utilisation of which can provide high yields of starch and lead to conservation of agriculturally marginal areas of land (Oates *et al.*, 1994). Use of sago starch for hydrolysis is currently limited by granular resistance to commercial enzymes and the high paste viscosity of gelatinised starch pastes (Oates *et al.*, 1994). In our previous work, we have used a single-screw extruder (Brabender 20DN) as a bioreactor to carry out the combined gelatinisation and liquefaction of sago starch (Govindasamy *et al.*, submitted) Many of the physicochemical properties, e.g. DE, WSI, WAI, degree of degradation (DGR), oligosaccharide content and degree of gelatinisation (DG) of liquefied extrudates, have been significantly influenced by enzyme concentration and feed moisture content. In previous studies using twin-screw extrusion cookers it has been demonstrated that both processing conditions and the DE values before saccharification affect the rate of hydrolysis and the final glucose yield (Hakulin *et al.*, 1983; Linko *et al.*, 1983).

This study reports the use of response surface methodology (RSM) to optimise saccharification conditions, i.e. amount of substrate (extrudate), amyloglucosidase concentrations and hydrolysis time, for conversion of sago starch to glucose syrups. The feasibility of utilising a single-screw extruder for the simultaneous gelatinisation and liquefaction of sago starch using a thermostable α-amylase for subsequent saccharification is also explored. Based on a multifactorial experimental design, extrusion conditions were identified and suitable experimental trials carried out. Suitability of the samples as substrates for saccharification was assessed by comparing the amounts of glucose released. DE and oligosaccharide profiles of the saccharified samples were also determined.

MATERIALS AND METHODS

Sago starch (*Metroxylon sagu*), purchased from a commercial producer (Wah Chang International Group of Companies), was used as the feed material for all the experiments. Proximate analysis indicated that the starch contained 0·015% protein, 25·5% amylose (Sim *et al.*, 1991) and about 11% moisture (weight basis, wb).

Enzyme preparation

Bacillus licheniformis α-amylase Termamyl 120L (EC 3.2.1.1) was obtained from Novo Industri A/S (Kuala Lumpur, Malaysia). Amyloglucosidase (AMG, EC 3.2.1.3) from *Aspergillus niger* in 1 M glucose containing 0·5% sodium benzoate as preservative was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The enzymatic preparation was reported to have an activity of 6100 units/ml with one unit being defined as the amount of enzyme necessary to liberate 1·0 mg of glucose from starch in 3 min at pH 4·5 at 55°C.

Extrusion cooking

Pregelatinisation and preliquefaction of sago starch was performed in a Brabender single-screw extruder 20DN (Model No. 8325, C.W. Brabender Instruments, Inc.) based on a central composite rotatable experimental design (three-variable, five-level) (Mullen & Ennis, 1979) as described previously (Govindasamy *et al.*, submitted). Three process variables were selected: total moisture content of the mass feed (%, g/100 g of starch), mass temperature in the compression and die zones (°C) and enzyme concentration (%, g/100 g of starch). The design consisted of 20 experiments with eight (2^3) factorial points, eight axial points to form a central composite design with $\alpha = 1{\cdot}682$ and six points for replications. The extruder was driven by a Brabender DO-corder DCE 330 drive of 3·3 kW (Model E), and was equipped with a 38-cm-long grooved barrel of 19 mm diameter. A uniformly tapered screw with 3 : 1 screw compression ratio was used. The die was a round orifice of 6 mm diameter. The screw speed, feed-rate and first barrel section temperature were held constant at 80 rpm, 40 rpm and 60°C, respectively. The premixed starch mixture (starch, water and enzyme) was introduced into the extruder using a feed-hopper and the material was force-fed into the barrel by an auger. The mass temperatures (T) in the compression and the die zones varied from 60 to 120°C, the addition of thermostable α-amylase Termamyl 120L (E) from 1·48 to 6·52% (g/100 g of starch) and the moisture content (M) of the feed material from 21·6 to 38·4% (g/100 g of starch). A 10 min minimum running time was allowed for equilibration before samples were taken. All samples collected were oven-dried for 2 days before grinding in a laboratory cross beater mill (type, SKI, F. Kurt Retsh GmbH & KG, Germany). Ground extrudates were stored in air-tight plastic containers and held at 4°C until analysis.

Determination of conditions for saccharification

Slurries containing 21·6, 25·0, 30·0, 35·0, 38·4 g/100 ml ground sago extrudate (sample 8) were prepared with 0·1 M acetate buffer, pH 4·5. Sample flasks were equilibrated in an orbital benchtop shaker (Certomat R M, B.Braun Diessel Biotech GmbH) in an incubator (Inkubation hood, HK, B.Braun Diessel Biotech

Table 1. Independent variables and experimental design levels for saccharification of sago extrudate (sample 8) with AMG

Variables	Code	$-\alpha$	-1	0	$+1$	$+\alpha$
(1) Substrate concentration (g/100 ml)	S	21·6	25·0	30·0	35·0	38·4
(2) AMG concentration (U/g of extrudate)	A	0·66	1·00	1·50	2·00	2·34
(3) Hydrolysis time (h)	H	14·8	25·0	40·0	55·0	65·2

GmbH) set at 60°C. Enzymatic hydrolysis was initiated following addition of appropriate volumes of amyloglucosidase to the suspensions and kept at the desired conditions for a predetermined time (14·8, 25, 40, 55, 65·2 h). The enzyme concentration corresponded to the following total activities: 0·66, 1·00, 1·50, 2·00, 2·34 U/g of extrudate, respectively. After hydrolysis, 1 ml aliquots of the samples were withdrawn and added to chilled microfuge tubes containing 0·1 ml of 30% TCA. Samples were centrifuged at 10000 rpm for 15 min at 4°C and the glucose concentration in the supernatant was determined using a glucose oxidase–peroxidase system (Glucose Diagnostic Kit, Sigma Diagnostics, St. Louis, MO, USA, cat no. 510-A).

EXPERIMENTAL DESIGN AND DATA ANALYSIS

A multifactorial composite rotatable design (Mullen & Ennis, 1979) with six replicates at the centre point, eight (2^3) factorial points and eight axial points (with α = 1·682) was chosen to study the contribution of the three independent variables: amount of substrate (extrudate) (S), amyloglucosidase concentration (A) and hydrolysis time (H). Ranges of the selected independent variables are shown in Table 1. Glucose content in the supernatant was measured. Preliminary experiments indicated that these variables had measurable effects on the saccharification of sago starch extrudates and that the levels selected were attainable.

Data were treated by a multiple regression analysis using a commercial statistical package, Statgraphics version 6·0 (Mannugistics, Inc., Rockville, USA). Response surface methodology was applied for the defined experimental region. A second-order response surface prediction was fitted for each dependent response characteristic using the statistical package. Surface plots were generated by showing the effects of two independent variables, while the other variable was maintained constant, on each response.

Determination of DE of saccharified samples

DE values were determined using a modified osmometry method outlined by Fitton (1979). Osmolalities of samples were determined with a calibrated osmometer (model 5004 from Precision Systems Inc., Natick, MA, USA) using triplicate aliquots of 50 μl. Sample DE was defined as the osmolality of the sample at 15% solids divided by the osmolality of the dextrose at the same ds × 100 (Henderson & Teague, 1988). A calibration plot was constructed to relate osmolality with DE at 15% dry substance. For samples at concentration above 15% ds, suitable dilution was made and the osmolality measured. The corresponding DE was determined from the calibration plot. Total solid content of the samples was determined using a digital refractometer (PR-100 Atago, Palette).

Determination of percentage saccharification of extrudates

Percentage saccharifications (g of glucose/100 g of extrudate) of the extrudates were determined by assaying released glucose after treating 20% extrudate suspensions in 0·1 M acetate buffer, pH 4·5 with 1 ml of 2·34 U/g AMG for 24 h and 65·2 h at 60°C. DE of the supernatant and the oligosaccharide profiles were analysed.

High-performance size exclusion chromatography (HPSEC) analyses

The saccharified samples were filtered through a millipore filter (8·0 μm). The filtrates were allowed to equilibrate at 40°C in an incubator oven prior to injection and the molecular weight profiles established by HPSEC (Govindasamy *et al.*, 1992).

RESULTS AND DISCUSSION

Determination of conditions for saccharification

Percentage saccharifications resulting from the different treatments of 'sample 8' (randomly chosen) are presented in Table 2 and the regression equation for the response variables is shown in Table 3.

Samples were subsequently saccharified over a wide range (40·2–87·9%), the extent of hydrolysis corresponding to time of reaction (14·8 and 65·2 h, respectively) with 30 g/100 ml extrudate content and 1·5 U/g of AMG. The R^2 obtained for the model was 0·90 indicating that the model is well described by the significant variables. Saccharification was significantly affected ($P < 0·01$) by AMG concentration and incubation time; the positive values of the regression coefficients for both the variables indicate that saccharification increases with increasing AMG concentration and hydrolysis time. Quadratic terms for enzyme concentration and hydrolysis time had significant effects on saccharification as well. The net effect of the linear and the quadratic terms implies that saccharification stops increasing at an optimal point. The response surface for saccharification as a function of AMG concentration

Table 2. Independent variable level and resulting percent saccharification of sago starch extrudates (sample 8) with AMG

	Independent variables		Dependent variables	
Design point	Substrate concentration (%)	AMG concentration (U/g)	Hydrolysis Time (h)	%Saccharification (DX) (%)
1	−1	−1	−1	51·0
2	−1	−1	+1	66·5
3	−1	+1	−1	68·0
4	−1	+1	+1	79·2
5	+1	−1	−1	44·9
6	+1	−1	+1	60·7
7	+1	+1	−1	64·3
8	+1	+1	+1	81·5
9	+α	0	0	71·4
10	−α	0	0	72·5
11	0	+α	0	87·4
12	0	−α	0	40·8
13	0	0	+α	87·9
14	0	0	−α	40·2
15	0	0	0	71·9
16	0	0	0	72·7
17	0	0	0	72·7
18	0	0	0	71·7
19	0	0	0	72·7
20	0	0	0	72·5

and hydrolysis time (Fig. 1) indicates that enhanced saccharification was achieved by increasing AMG concentration and hydrolysis time. Although the initial rate of saccharification is high, it subsequently decreases continuously during prolonged reaction, probably due to lower affinity of amyloglucosidase for α-1,6-linkages and occurrence of reversible reactions.

Based on the results obtained from our model, the following conditions were selected for subsequent saccharification: 20 g/100 ml of extrudate, 2·34 U/g AMG and 65·2 h. Although extrudate concentration has no significant influence on the saccharification process, high suspension viscosity of two of the extrudates (samples 2 and 12)

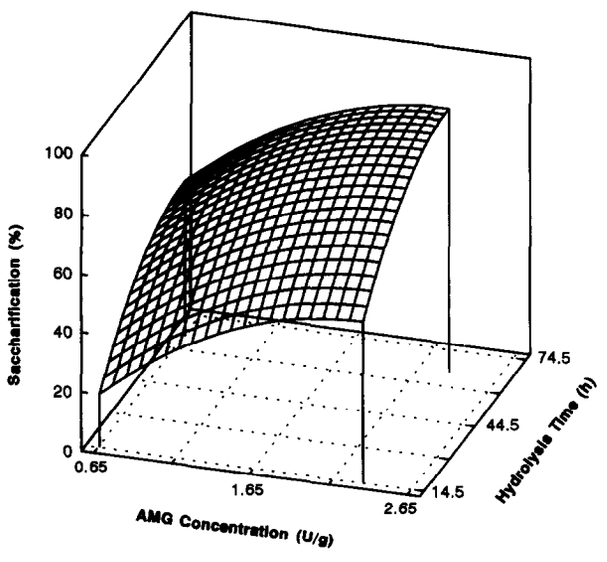

Fig. 1. Influence of amyloglucosidase concentration and hydrolysis time on saccharification at 30 g/100 ml of extrudate.

Table 3. Selected prediction equation for the saccharification of sago starch extrudate (sample 8) with AMG

Dependent variables	Independent variables[a]	Coefficient	R^2 (adjusted)	*P*–value
% Saccharification	Constant	71·989	0·90	0·0000
	A***	10·857		
	H***	10·258		
	A^2**	−3·255		
	H^2**	−3·290		

[a]*, **, ***, significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. A, AMG concentration (U/g); H, hydrolysis time (h).

limited the use of a solid content greater than 20 g/100 ml for subsequent hydrolysis. It has been demonstrated that at high enzyme concentration, maltulose and isomaltose are produced during prolonged saccharification due to reversible reactions attributed to AMG (Fullbrook, 1984; Dias & Panchal, 1987). As the enzyme concentration utilised was in the high range (2·34 U/g), saccharification was allowed to proceed for both short and long reaction times (24 h and 65·2 h, respectively) to monitor the rate of reaction as well as the reaction products.

The strength of the model was tested by selecting conditions that led to the highest degree of saccharification (Table 4). These conditions were not part of the original experimental design but in the range studied. Predicted values for % saccharification were very close to the experimental data and no significant disparity was observed between the predicted and the experimental results ($P < 0.01$).

Effects of extrusion variables on saccharification

Regression equations for the various responses, their respective R^2 and *P*-values are given in Table 5. Examination of the regression coefficients for the dependent response suggests that extruder enzyme concentration was the most significant variable affecting subsequent saccharification of sago starch.

Extent of saccharification achieved after 24 h hydrolysis was in the range 47·6–73·3% and after 65 h in the range 54·4–93·8%. Enzyme concentration, feed moisture content and the quadratic terms for enzyme concentration were significant variables for saccharification. Feed moisture content was a more significant contributor to

Table 4. Comparison of saccharification procedure responses predicted by the least square model for the % saccharification, with the experimental results

Treatments			Responses	
			% Saccharification	
S	A	H	Predicted[a]	Experimental[b]
0	+α	+α	69·0	68·5 ± 0·65
0	+α	−1·1	82·0	81·9 ± 0·98

[a]95% interval.
[b]Average of triplicate determinations from different experiments.

Table 5. Best selected prediction equations for the dependent variables (saccharification after 24 h and 65 h) for the extrusion of sago starch with Termamyl 120L

Dependent variables	Independent variables[a]	Coefficient	R^2 (adjusted)	*P*-value
% Saccharification (24 h)	Constant	65·310	0·90	0·0000
	M***	−3·306		
	E***	4·375		
	M^2**	1·606		
	E^2***	−3·201		
	M × E**	2·275		
% Saccharification (65 h)	Constant	85·490	0.80	0·0000
	M*	−2·639		
	E***	6·205		
	E^2***	−5·345		
	M × T*	3·013		

[a]*, **, ***, significant at $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$. M, feed moisture content (%); E, extruder enzyme concentrations (%); T, mass temperature at zones 2 and 3 of the extruder (°C).

saccharification, over the shorter reaction period (24 h), but not as important if saccharification was carried out for a longer period of time. This may reject the fact that saccharification was nearing completion before the end of 65 h.

24 h saccharification

Saccharification performed over a 24 h period was affected by the extruder enzyme (Termamyl 120L) concentration. This may reflect the fact that during the limited time available for saccharification, the greater availability of shorter chain length material, resulting from more extensive breakdown in the extruder at higher enzyme concentration, could enhance saccharification. Starch hydrolysis in the extruder has been demonstrated to increase with increasing enzyme content (Govindasamy *et al.*, submitted). However, the effect of enzyme concentration was found to be markedly dependent on feed moisture (Fig. 2). Dependence on feed moisture is demonstrated at lower hydration levels. Extruding at feed moisture levels below 30% causes a 'dip' in the response surface plot (Fig. 2) at 4·75% enzyme concentration, suggesting that moisture content is a limiting factor for the hydrolytic reaction in the extruder at these conditions (constant mass temperature of 84°C). Conversely, increasing the moisture content above 30%, and increasing the enzyme content beyond 4·75%, led only to slight increase in saccharification. This may imply that enzyme is present in excess.

Feed moisture content has a negative effect on the 24 h saccharification. The relative importance of the quadratic term implies that percentage saccharification stops decreasing at a minimal point and is dependent on enzyme concentration. At lowest enzyme content (1·5%), increasing the feed moisture results in decreased saccharification. Our previous work (Govindasamy *et al.*, submitted) indicates that, at this condition, there was degradation of the molecular components at the macromolecular level, as there was no increase in DE. This observation implies that there has to be a certain amount of degradation of the substrate for it to be easily saccharified. Though high moisture and enzyme contents promote hydrolysis of sago starch in the extruder, resulting in extrudates with higher DEs (Govindasamy *et al.*, submitted), these substrates are in turn not readily saccharified (Fig. 2).

65 h sacharification

Extruder enzyme concentration had the most significant effect on saccharification (Table 5). Increasing enzyme concentration above 4·45%, however, had only a marginal effect on saccharification; this effect was observed within the range of feed moisture 21–38% (Fig. 3). Feed moisture content exerted a negative effect at all enzyme concentrations.

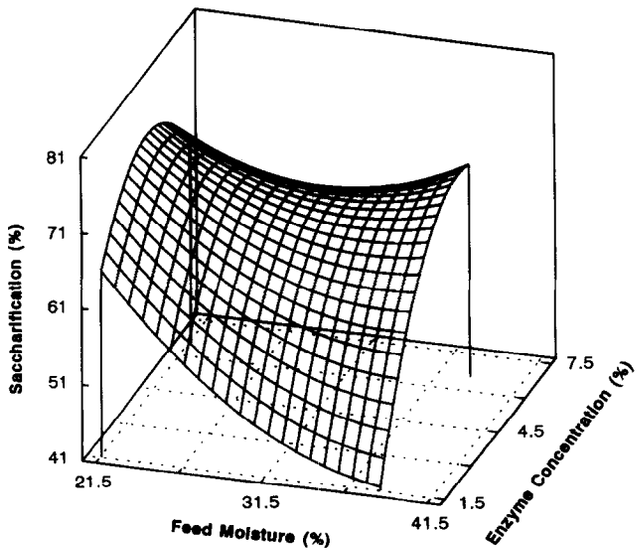

Fig. 2. Influence of feed moisture content and enzyme (Termamyl 120L) concentration on 24 h saccharification at mass temperature 84°C.

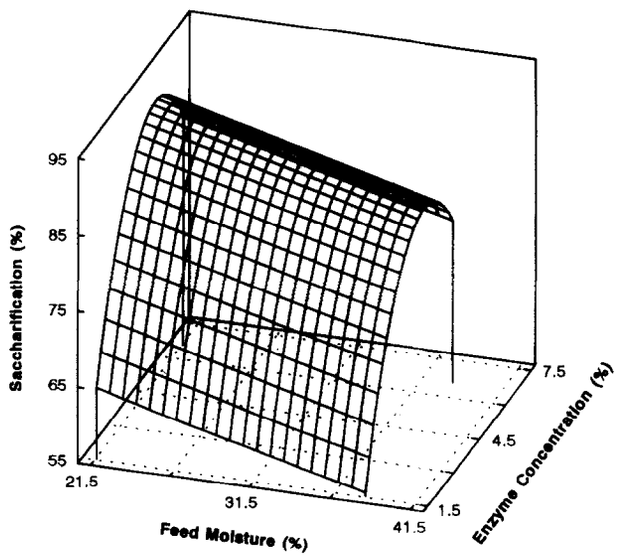

Fig. 3. Influence of feed moisture content and enzyme (Termamyl 120L) concentration on 65 h saccharification at mass temperature 84°C.

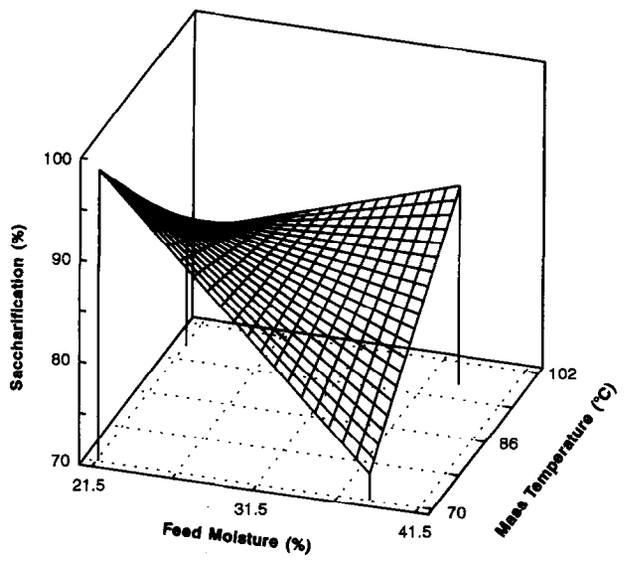

Fig. 4. Influence of feed moisture content and mass temperature on 65 h saccharification at enzyme (Termamyl 120L) concentration of 4·0%.

The relationship between mass temperature and feed moisture was found to be important, exemplified by the significance of the interactive term 'mass temperature × feed moisture' (Table 5). At the highest moisture content and enzyme concentration, increasing thermal energy input led to a reduction in percentage saccharification (Fig. 4).

Response surface plots (Figs 2 and 3) generated for both 24 h and 65 h saccharification suggest that high amounts of glucose can be produced when sago starch is co-extruded with a high enzyme concentration, but relatively low moisture content.

Residual analysis

Absence of outlying points was confirmed by residual analysis. Residuals for the fitted regression models are assumed to be normally distributed. Serious violation of the assumption is not apparent since the cumulative distribution plot for each fitted equation is approximately a straight line (Fig. 5a). Furthermore, the raw residuals are structureless, indicated by a random scatter of the points in residual plots (Fig. 5b).

DE and oligosaccharide profiles of the saccharified samples

DE values (data not shown) of all samples ranged from 85 to 94 and from 90 to 98 for the 24 h (DE_{24}) and 65 h (DE_{65}) samples, respectively. DE values did not correlate with any of the extrusion variables. The highest DE_{24} and DE_{65} of 94 and 99, respectively, did not correspond to those samples producing the highest amount of glucose. This may be a reflection of the presence of other oligosaccharides in these samples as DE is an indication of the oligosaccharide profile. Oligosaccharide species (Gl to G12) were resolved by HPSEC and the relative proportions were calculated. A discernible difference existed between the 24 h and 65 h

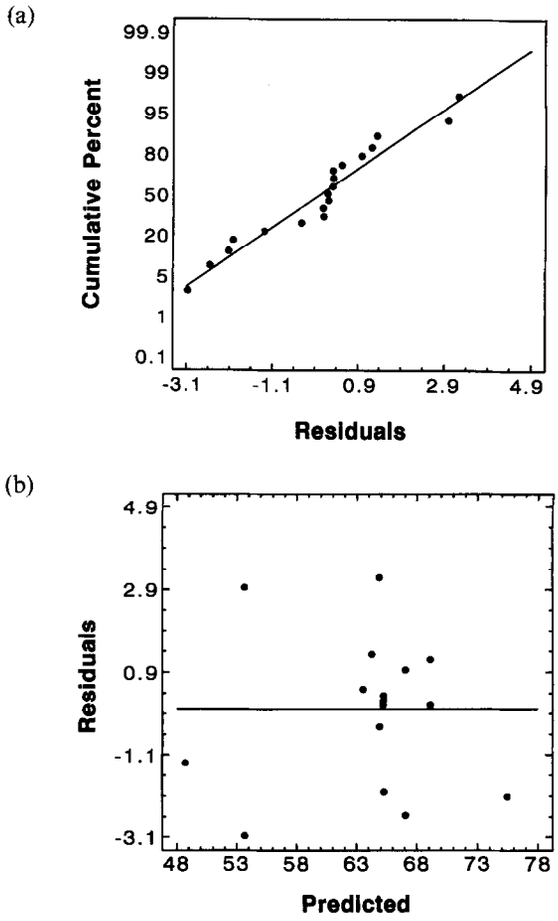

Fig. 5. (a) Normal probability plot for 24 h saccharification; (b) Residual plot for 24 h saccharification.

samples in terms of oligosaccharide profiles (Table 6). In the 24 h samples, the predominant oligosaccharide species were Gl, G4 and G8 for all samples except two (samples '6' and '12'):

Sample 6: G12, G9 and G1 were detected;
Sample 12: the predominant species were G12, G4 and G1.

Maltose and isomaltose could not be detected in any of the samples.

A peak eluting close to that for G7 was assumed to represent an oligosaccharide with some α-1,6-linkages. This species has been denoted as $G7^{+}$. $G7^{+}$, G4 and G1 were the only oligosaccharide species detected in the 65 h samples. G8 was absent in all samples. In samples 6 and 12, G12 was also detected. The amounts of the oligosaccharides G4 and $G7^{+}$ were relatively lower than in the 24 h samples, implying that these have been utilised as substrates for enzyme reaction.

Correlation analysis

Although a number of authors have demonstrated that DE values before saccharification can affect both the rate of hydrolysis and final glucose yield (Hakulin *et al.*, 1983; Linko *et al.*, 1983; Nebesny, 1992; Slominska, 1993), this is not the case for this study. A poor positive correlation (r = 0·44) was observed between DE of

Table 6. Predominant oligosaccharide species detected in the 24 h and 65 h saccharified samples using HPSEC

Extrudates	Oligosaccharide profile after	
	24 h saccharification	65 h saccharification
Sample 6	G1, G9, G12	G1, G4, G7$^+$, G12
Sample 12	G1, G4, G12	G1, G4, G7$^+$, G12
Sample E[a]	G1, G4, G8	G1, G4, G7$^+$

[a]E: samples 1–5, 7–11, 13–20.

the liquefied samples and percentage saccharification. However, good correlation (r = 0·74 and r = 0·80) was observed between WSI and % saccharification (24 h and 65 h samples) for liquefied starches (Table 7). Our previous work showed that there exists a strong positive correlation between DE and WSI (r = 0·76) (Govindasamy *et al.*, submitted). Initial DE of the liquefied products is not an indicator for the extent of starch hydrolysis but rather expresses both carbohydrate composition in the hydrolysate and dextrin molecular structure (Nebensy, 1992). Only the latter determines the usefulness of the hydrolysate in the successive saccharification with a chosen enzyme (Nebensy, 1992). The ease with which these dextrins go into solution would then affect the saccharification process. Increased water solubility of the liquefied samples, most likely promotes the action of the amyloglucosidase. This is further supported by the suggestion that oligosaccharides formed during extrusion were not the exclusive contributor to solubility as shown by the correlation between WSI and oligosaccharide content (r = 0·56) (Govindasamy *et al.*, submitted). The presence of other soluble but relatively higher molecular weight components would also seem plausible. Examination of the response surface plots for both percentage saccharification and DE suggests that the optimum starting DE is about 6, beyond which there is a reduction in percentage saccharification. This value is lower than that reported by Nebensy (1992) (optimum starting DE = 11) for potato starch hydrolysis.

CONCLUSION

This study investigated the feasibility of utilising a single-screw extruder as a bioreactor for the pretreatment of sago starch for subsequent saccharification. Enzyme concentration and feed moisture content had the most pronounced effects on saccharification. Higher percent saccharification is achieved when starch is co-extruded with high Termamyl 120L concentrations but at lower moisture content. Water solubility index rather than the DE values before saccharification seems to affect glucose yield. This is probably related to the ease at which the dextrin molecular structure goes into solution. As this pretreatment process requires a high concentration of α-amylase, studies are currently underway to explore the possibility of employing a lower enzyme/substrate ratio using a twin-screw extruder. Subsequent studies also deal with the utilisation of amyloglucosidase from a different source for the improvement of the glucose yield. Judicious selection of the appropriate enzyme parameters, together with the blending of the hydrolysates obtained under various conditions, could provide desirable substrates with specific characteristics.

Table 7. Correlation coefficients between dependent variables[a]

	Saccharification after 24 h	Saccharification after 65 h
WSI	0·7368***	0·8034***
WAI	–0·1660^{NS}	–0·1756^{NS}
DE	0·3598^{NS}	–0·4446*
Degree of gelatinisation	–0·2996^{NS}	–0·4496*
Oligosaccharide content	0·1571^{NS}	–0·1707^{NS}
Degree of degradation	–0·0738^{NS}	0·1134^{NS}
Saccharification after 65 h	0·9907***	

[a]*, **, ***, significant at $P < 0·05$, $P < 0·01$ and $P < 0·001$; NS, not significant

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REFERENCES

Chay, P. B., Chouvel, H., Cheftel, J. C., Ghommidh, G. & Navaroo, J. M. (1984). Extrusion-hydrolysed starch and flours as fermentation substrates for ethanol production. *Lebensm. Wiss. Technol.*, **17**, 257–67.

Chouvel, H., Chay, P. B. & Cheftel, J. C. (1983). Enzymatic hydrolysis of starch and cereal flours and intermediate moisture contents in a continuous extrusion-reactor. *Lebensm. Wiss. Technol.*, **16**, 346–53.

Darnoko, D., Cheryan, M. & Artz, W. E. (1989). Saccharification of cassava starch in an ultrafiltration reactor. *Enzyme Microb. Technol.*, **11**, 154–9.

Dias, F. F. & Panchal, D. C. (1987). Maltulose formation during saccharification of starch. *Starch/Stärke*, **39**(2), 64–6.

Fitton, M. G. (1979). Rapid determination of dextrose equivalent by cryscopy. *Starch/Stärke*, **31**(11), 381–4.

Fullbrook, P. D. (1984). The enzymatic production of glucose syrups. In *Glucose Syrups: Science and Technology*, ed. S. Z. Dziedzic & M. W. Kearsley. Elsevier Applied Publishers, London, UK, pp. 65–115.

Govindasamy, S., Oates, C. G. & Wong, H. A. (1992). Characterisation of changes of sago starch components during hydrolysis by a thermostable α-amylase. *Carbohydr. Polym.*, **18**, 89–100.

Hakulin, S., Linko, P., Seiler, K. & Seibel, W. (1983). Enzymatic conversion of starch in twin-screw HTST extruder. *Starch/Stärke*, **35**, 411–14.

Henderson, W. E. & Teague, W. M. (1988). A kinetic model of *Bacillus stearothermophilus* α-amylase under process conditions. *Starch/Stärke*, **40**(11), 412–18.

Knudsen, F. E. & Karkalas, J. (1969). Enzymic hydrolysis of starch hydrolysates. *Starch/Stärke*, **21**(11), 284–91.

Labout, J. J. M. (1985). Conversion of liquefied starch into glucose using a novel glucoamylase system. *Starch/Stärke*, **37**(5), 157–61.

Linko, P., Hakulin, S. & Linko, Y. Y. (1983). Extrusion cooking of barley starch for the production of glucose syrup and ethanol. *J. Cereal Sci.*, **1**, 275–84.

Linko, P., Hakulin, S. & Linko, Y. Y. (1984). HTST-extrusion in ethanol production from starchy materials. *Enzyme Microb. Technol.*, **6**, 457–61.

Mullen, K. & Ennis, D. M. (1979). Rotatable designs in product development. *Food Technol.*, **July**, 74–80.

Nebesny, E. (1992). Changes of carbohydrates and molecular structure of dextrins during enzymatic liquefaction of starch. *Starch/Stärke*, **44**, 398–401.

Oates, C. G., Wang, W. J. & Powell, A. D. (1994). Hydrolysis of sago starch: Do you need to know the processing history? *Proc. of the 2nd Symposium on 'Trends in Biotechnology: Meeting the Challenges of the 21st Century'*, ed. H. M. Ghazali, N. M. Salleh & N. A. A. Rashid. Universiti Pertanian Malaysia (UPM), Serdang, Selangor, Malaysia, pp. 33–6.

Sim, S. L., Oates, C. G. & Wong, H. A. (1991). Studies on sago starch. Part 1: Characterisation and comparison of sago starches obtained from *Metroxylon sagu* processed at different times. *Starch/Stärke*, **43**(12), 459–66.

Sims, K. A. & Cheryan, M. (1992). Continuous production of glucose syrup in an ultrafiltration reactor. *J. Food Sci.*, **57**(1), 163–6.

Slominska, L. (1993). Studies on modification of enzymatic saccharification process. *Starch/Stärke*, **45**(3), 88–90.