



Response Surface Method in Evaluating the Extrusion Effects on Molecular Degradation and Physical Properties of Sago Starch

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Authors' contributions

This work was carried out in collaboration among all authors. Author AA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SW, Tamrin and MN managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed to measure the effect of extrusion on the molecular degradation and physical characteristics of the sago starch by employing response surface method. The starch was extruded in a twin screw extruder with moisture contents of 25, 32.5, and 40%; melt temperature of 86, 95 and 104°C; and screw speed of 100, 150, and 200 rpm. The extruded products were then analyzed for degree of molecular degradation, reducing sugars of the water soluble materials, water solubility index (WSI), water absorption index (WAI), enzyme susceptibility, gelatinization endothermic energy (ΔH), and specific mechanical energy (SME). Increased mechanical and thermal energy input received by the products in the extruder gave rise to a significant degradation of the molecular weight of the macromolecules. It was believed that granule structures of the extruded starch have been reshaped. The extrusion process conditions did not significantly affect the WSI, WAI, reducing sugar content, and ΔH . All extruded samples had a much lower gelatinization endothermic energy than native starch.

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1. INTRODUCTION

Extrusion is a means of modifying starch to extent its usefulness in a variety of prepared and convenience food products. Twin screw extrusion has become an important process in the food processing to produce modified starch for various purposes [1,2,3,4]. In this process, there are certainly substantial changes in the molecules, structures and physicochemical properties. The extent of modifications in these properties is largely dependent upon the extrusion parameters used [5]. In addition, the botanical sources of the starch might also affect these modifications. The effects of extrusion on the physicochemical properties as well as molecular changes of various starches have been reported [6,7,8,9].

Sago starch, which is extracted from the trunk of the sago palm, has increasingly become one of the important starch sources in the Asia Pacific regions. Sago starch has a versatile functions in many applications, both in food and non-food industries [10]. With extrusion technology, sago starch may be processed into various products, which in turn would add to its economic value [11].

The objective of this study was to examine the influence of the various extrusion parameters, namely feed moisture content, melt temperature, and screw speed on the molecular and structural changes, as well as physical properties.

2. MATERIALS AND METHODS

2.1 Materials

The native sago starch was extracted from the palm of *Metroxylon sagu*, obtained from Southeast Sulawesi, Indonesia. The starch was freshly prepared by obtaining from the palm pith, separating the fibers and foreign matters, suspending it in the cold water, sun-dried for 16 h and subsequently milled and sieved of 80 mesh. The moisture content of the starch was 17.55% (wet basis). All chemical reagents used in this experiment were of analytical grade unless otherwise specified.

2.2 Extrusion Trials

A 40 min-APV Baker twin screw extruder was used in the experiment. The extruder (25 L/D) was configured without using a die plate nor die was used. The starch (5 kg/h) plus a variable amount of water were fed into the extruder in

which the barrel was cooled by water circulation to avoid steam loss from the starch water mix. The last barrel section was heated to various temperatures. The product temperature in the barrel, moisture content of the feed and screw speed are listed in Table 1.

The extruded starches were collected when steady-state conditions were achieved (defined as no visible drift in the % torque for at least 5 min). The barrel temperature, product temperature, screw speed, and % torque were automatically recorded from the extruder. The extruded were directly frozen in dry ice, then vacuum-dried at 60°C for 12 h, milled and sieved through 80 mesh size screen, stored in plastic bags and kept in the freezer for further analysis.

2.3 High Performance Size Exclusion Chromatography (HPSEC) Analysis

A modified method of Simsek et. al [12] was used in the analysis of chromatographic profiles of the native sago starch and its extrudates. The samples (10 mg) were solubilized with 1 mL dimethyl sulfoxide (DMSO) in a 4-mL screw-cap test tube by heating the dispersion for 5 min in a boiling water bath. The tubes were then incubated overnight at 80°C and centrifuged at 3000xg before chromatographic analysis.

An aliquot (50 μ L) of the sample solution was injected into a two column HPSEC system using DMSO as the eluent at a flow rate of 1.0 mL/min. The waters liquid chromatographic system was used including an automatic sample injection module (WISP model 712), a pump (model 510), and a differential refractometer (model 410). The refractive index detector sensitivity was set to 32. The software used for the acquisition, storage and processing of data was the Millennium 2010 Chromatography Manager. The two columns, 1000A and 10,000A (Polymer Laboratories, Ltd, Stow, OH) with dimensions of 300 x 7.5 mm were connected in series. The columns were maintained at 80°C, and calibrated using molecular weight (MW) of 853,000 to 5,800 pullulan standards. The log MW vs elution time plot for the pullulan standards was found to be linear ($r = -0.995$) at the lower MW range (5,800 to 186,000).

It was important to develop some measures of molecular degradation. The degree of apparent molecular degradation was developed for this study as a comparison of the one major peak of each chromatogram, and calculated as follows:

$$\% \text{ Apparent Degradation} = \frac{\text{Peak Time of Extrudates} - \text{Peak Time of Native Starch} \times 100}{\text{Peak Time of Native Starch}}$$

2.4 Water Soluble Reducing Sugars

A modified method of Al-kayyis and Susanti [13] was employed in measuring the amount of the reducing sugars. To 100 mg of extrudates in the test tube, 0.1 mL of absolute ethanol was added and agitated using a vortex mixer for about 5 min at ambient temperature. The mixture was then centrifuged at 1500xg for 5 min, and filtered using Whatman No. 1 filter paper. The reducing sugar content of the filtrate was measured using Somogyi-Nelson method. A standard curve was constructed using anhydrous-glucose (Sigma Chemical).

2.5 Water Absorption Index (WAI) and Water Solubility Index (WSI)

A modified method of Yagci and Gogus [14] was employed in determining WAI and WSI. About 0.5 g sample was suspended with 6 mL of distilled water in a 50-mL tarred centrifuged tube.

The samples were incubated in a 30°C water bath, vortex-mixed intermittently over a 30 min period, and centrifuged at 3000xg for 10 min. The supernatant was carefully poured into a pre-weighed evaporating dish. The remaining gel was weighed, and WAI was calculated as:

$$\text{WAI} = \frac{\text{Weight of gel} - \text{Weight of dry sample}}{\text{Weight of dry sample}}$$

The supernatant liquid from WAI study was vacuum-dried at 70°C until constant weight was reached. The WSI was calculated as the amount of dried solid recovered from evaporating the supernatant and expressed as dry solid in 0.5 g sample.

2.6 Enzyme Susceptibility

Stock solution of the enzyme was obtained by diluting a thermostable α -amylase, Termamyl 120L in the potassium dihydrogen phosphate buffer of pH 6.9. Working solution was prepared by adjusting/diluting the enzyme stock solution to 1.0 μg per mL. To 100 mg of extrudates in the test tube, 0.1 mL of absolute ethanol was added to disperse the samples. Then 10 mL of enzyme solution in phosphate buffer (containing 70 ppm Ca^{++}) was added, incubated at 37°C for another 20 min with intermittent shaking. The hydrolysis was stopped by adding 0.1 mL of 0.1 M HCl and immediately boiling for 5 min. The mix was centrifuged at 1500xg for 5 min and filtered using

a Whatman No. 1 filter paper. The reducing sugar content of the filtrate was measured using Somogyi-Nelson method against a blank lacking enzyme. As a control, the un-extruded sago starch sample was also analyzed for its enzyme susceptibility. A standard curve was constructed using anhydrous-glucose.

2.7 Gelatinization Endothermic Energy

Differential Scanning Calorimeter (DSC-7 Perkin Elmer), calibrated with indium (ΔH 28.45 J/g, melting point 156.6°C), was used to determine the thermal transition temperature and endothermic energy (ΔH) of the extrudates. Sample (100 mg) was mixed well with 200 μL of deionized water. Then 40 mg of the mix was placed in a 40 μL aluminium pan. After sealing, the pan was left for about 3 h for equilibration. The samples were scanned from 20 to 100°C at a heating rate of 10°C per min. An empty pan was used as a reference, and endothermic energy (ΔH) in J/g was determined by integrating the area of the DSC endotherm. The thermogram was analyzed with Perkin-Elmer DSC-7 software.

2.8 Specified Mechanical Energy (SME)

Percentage of the torque was recorded from the gauge in the extruder. As indicated in the manual of the extruder, SME was calculated by multiplying the % torque with angular velocity in rpm and a factor of 0.028, and the result was divided by the feed rate in kg/h. The unit of SME was kWh/kg.

2.9 Experimental Design and Statistical Analysis

A response surface method with the Box-Behnken design was adopted to collect the data [14]. This particular design was chosen to avoid the use of the extreme conditions of independent variables, which may not be possible in the extrusion process. Fifteen conditions of experiments were carried out in a random order, including three replications at the center points. Combinations of the coded and process variables are listed in Table 1.

A second order polynomial model, which may contain linear, interaction, and quadratic terms, and three-dimensional surface curves were generated was fitted using a Minitab 18 statistical package.

Table 1. The coded and actual units of the independent variables employed in the extrusion of sago starch

Run No.	Coded			Actual Unit		
	X ₁	X ₂	X ₃	X ₁ (%)	X ₂ (°C)	X ₃ (rpm)
1	-1	0	-1	25	95	100
2	-1	0	1	25	95	200
3	-1	-1	0	25	86	150
4	-1	1	0	25	104	150
5	0	-1	1	32.5	86	200
6	0	-1	-1	32.5	86	100
7	0	1	-1	32.5	104	100
8	0	1	1	32.5	104	200
9	0	0	0	32.5	95	150
10	0	0	0	32.5	95	150
11	0	0	0	32.5	95	150
12	1	0	1	40	95	200
13	1	-1	0	40	98	150
14	1	1	0	40	104	150
15	1	0	-1	40	95	100

X₁ = feed moisture; X₂ = melt temperature; and X₃ = screw speed

3. RESULTS AND DISCUSSION

3.1 The Effect of Extrusion on the Apparent Molecular Degradation

Chromatographic profiles of the sago starch extruded at 25% moisture content is shown in Fig. 1. Comparing to its native starch, all extrudates had peaks shifting to the lower molecular weight (MW), indicating molecular degradation had taken place during the extrusion. Similar phenomena were also found on the native corn starch [15].

It was evident from Fig. 1 that there was at least three peaks (A, B and C) on the native starch. Probably, peak A was attributed to the amylopectin fraction, which was eluted earlier due to its higher MW; peak C was attributed to the amylose fraction; and peak B was amylopectin having an intermediate MW. Similar peaks were also found on the native sago starch [16], and on the rice starch [17]. Using pullulan as standards, apparent MW of the amylopectin fraction (peak A) was estimated to be greater than 2,000,000, and lower for those of amylose fraction (peak C).

The relative peak areas for amylopectin (peaks A and B) were calculated to be 71.14%, and those for amylose (peak C) was 28.86%. This figure was close to the amylose content of 26.85% measured by the colorimetric method [18]. Abdorreza et al. [19] also reported that native sago starch solubilized in water contained 30.6 % amylose.

Considering the shifting of the major peaks of the extruded products, it was obvious from Fig. 1 that the amylopectin fraction (peak A) was affected by the extrusion process. The second major peak (peak B) was presumably representing the degraded amylopectin. It seems that the amylose fraction has not been affected.

The samples extruded at 25% moisture, 95°C melt temperature, and 200 rpm screw speed appeared to be the most highly degraded macromolecules in this study, indicated by the elution of the major peak in the range of MW between 380,000 and 853,000. It was most likely that the amylose fraction would have been also modified in this extrusion condition.

The extent of molecular degradation was defined by the percentage of the time difference between the first major peak of the native starch and that of the extrudates. Compared to that of the native starch, the apparent molecular degradation of the extrudates shifted in the ranges of 5.33 to 23.67%, as shown in Table 2. Statistical analysis (Table 3) suggested that the linear and quadratic effects of moisture ($p < 0.001$), as well as the interaction effect of moisture-temperature and moisture-screw speed ($p < 0.05$) had significantly influenced the molecular degradation and accounted for most variation (99.6%). The surface plot of the influence of moisture content and screw speed on the degree of molecular degradation is given in Fig. 2. It was indicated that decreasing moisture content during extrusion resulted in a more extensive molecular degradation.

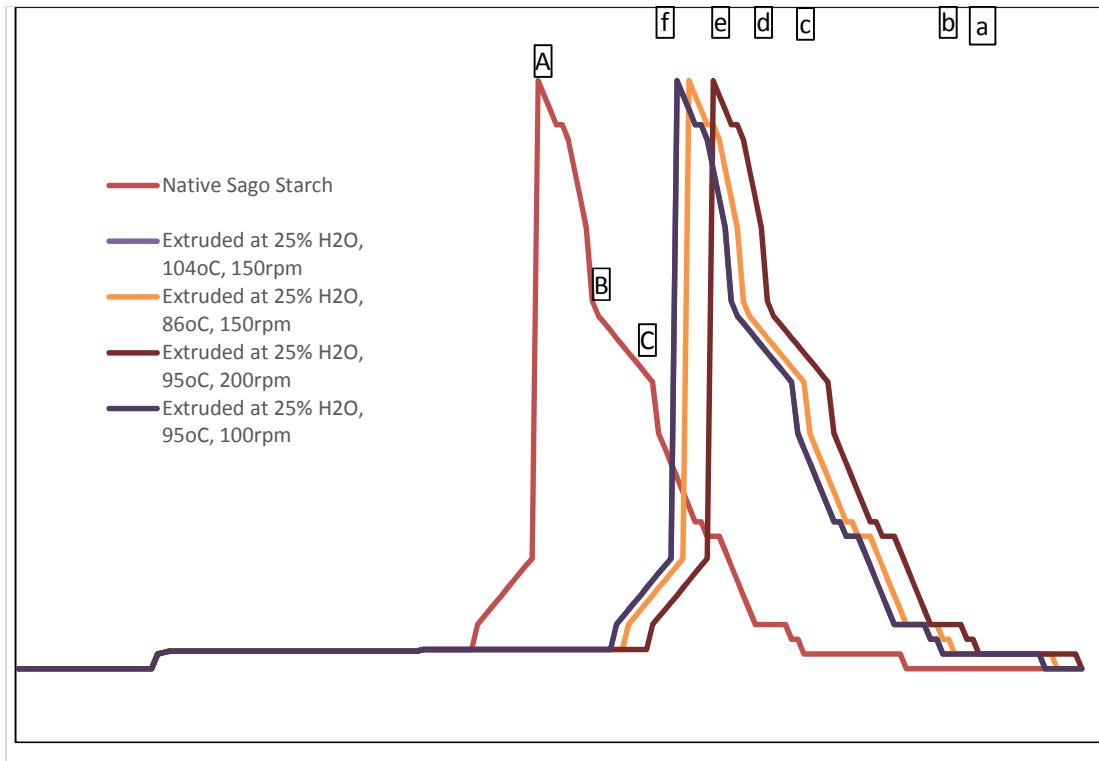


Fig. 1. Chromatographic profile of native and sago starch extruded at 25% moisture in three different temperatures (86, 95, 104°C) and three different screw speed (100, 150, 200 rpm). In native starch, peak A, B, and C denoted amylopectin, lower molecular weight (MW) amylopectin and amylose, respectively. The average MW of pullulan standards are denoted by: a= 5,800; b= 12,200; c=100,000, d= 186,000, e=380,000, f= 853,000

Table 2. Apparent molecular degradation, reducing sugar content (RS), water absorption index (WAI), water solubility index (WSI), enzyme susceptibility (ES), gelatinization endothermic energy (ΔH) and specific mechanical energy (SME) of the extruded sago starch*

Run No.	Apparent Molecular degradation	RS	WAI	WSI	ES	ΔH	SME
1	19.09	4.34	3.16	48.36	32.17	0.007	0.18
2	23.67	3.86	2.88	47.12	33.72	0.002	0.54
3	18.54	4.45	3.15	54.98	30.52	0.008	0.34
4	21.66	3.98	2.41	59.60	32.30	0.001	0.31
5	8.44	3.78	4.12	33.24	33.94	0.010	0.25
6	6.98	3.02	5.66	27.75	37.33	0.030	0.12
7	7.34	4.23	2.98	47.96	34.82	0.006	0.13
8	12.11	3.91	2.40	49.69	33.18	0.004	0.34
9	7.71	2.84	3.81	39.43	33.36	0.004	0.18
10	8.26	5.00	3.37	42.79	38.88	0.003	0.17
11	8.26	4.62	3.54	39.59	34.05	0.004	0.17
12	5.50	1.72	4.48	16.90	35.61	0.003	0.15
13	6.06	2.46	6.20	15.37	36.47	0.012	0.11
14	5.50	2.92	4.18	23.91	41.34	0.003	0.12
15	5.33	1.43	6.07	13.12	40.59	0.028	0.08

Apparent molecular degradation in %, reducing sugar content (RS) in mg/g sample, water absorption index (WAI) in g water absorbed/g sample, water solubility index (WSI) in %, enzyme susceptibility (ES) in mg RS/g sample, gelatinization endothermic energy (ΔH) in J/g and specific mechanical energy (SME) in kWh/kg

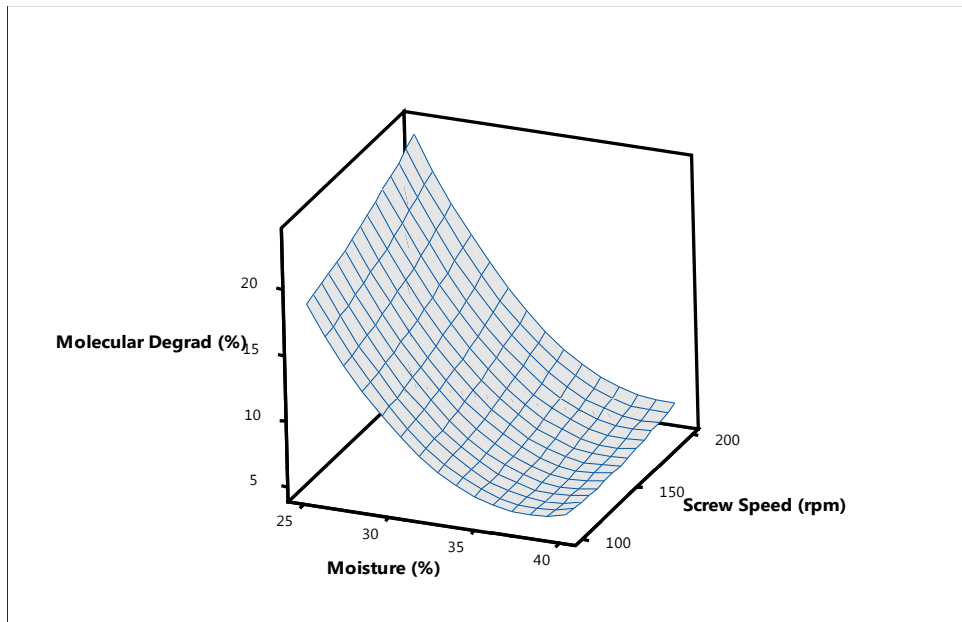


Fig. 2. The surface plot of the effect of feed moisture (%) and screw speed (rpm) on the apparent molecular degradation of extruded sago starch

Table 3. The R² value and regression coefficients for the apparent molecular degradation, reducing sugar, WAI, WSI, enzyme susceptibility, gelatinization endothermic energy (ΔH) and SME of the extruded sago starch

Dependent variables	Intercept values	R ²	Independent variables ^a	Regression coefficient	p-level	Significance ^b
Apparent molecular degradation	102.92	0.99	X ₁	-4.7940	0.001	***
			X ₁ ²	0.0851	0.000	***
			X ₁ X ₂	-0.0141	0.037	*
			X ₁ X ₃	-0.0032	0.019	*
Reducing sugar	-26.10	0.75	X ₃ ²	0.0002	0.118	ns
WAI	10.11	0.98	X ₁ ²	0.0068	0.058	ns
WSI	300.17	0.97	X ₁ ²	-0.0951	0.062	ns
Enzyme susceptibility	-3.10	0.76	X ₁ X ₃	-0.0042	0.268	ns
Endothermic energy (ΔH)	0.65	0.93	X ₃	-0.0019	0.091	ns
SME	2.30	0.98	X ₁ X ₃	-0.0002	0.003	**

^aX₁ = feed moisture (%); X₂ = melt temperature (°C); and X₃ = Screw speed (rpm).

Only independent variables having the lowest p-level value from statistical analysis were selected for presentation in this Table.

^bns = not significant; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

Li et al. [20] found that susceptibility to shear degradation was dependent upon the crystalline conditions, where the rigid crystallites of amylopectin was more susceptible to shear degradation than the flexible amorphous amylose. Meanwhile, in another study, Liu et al [15] found that the susceptibility of starch molecules to shear degradation was dependent on its size and branching structure. The more branches would give rise to the more

susceptibility to shear degradation. Considering those studies used various starch sources, these differences could be due to the differences in the botanical and native structures of the starch granules.

3.2 Water Soluble Reducing Sugars

Results of the measurements of the reducing sugar contents of the cold water soluble fraction

of the extrudates suggested that the starch has not been hydrolysed in the extruder. The amount of the reducing sugar of the extrudate (ranging from 1.43 to 5 mg/g sample), as indicated in Table 2, was not significantly different with those of the native starch, which was also measured and found in this study as 1.44 mg/g sample. As shown in Table 4, the reducing sugar content was positively correlated with the WSI ($r=0.83$). This might be explained by considering the hydroxyl sites of the reducing sugars which would tend to interact with its surrounding water leading to a greater solubility.

The extrusion conditions did significantly influence the content of the reducing sugars [21]. Dalbhagat et al [22] also reviewed that there was an increase in reducing sugars content because of the severe shearing during extrusion process.

3.3 Water Absorption Index (WAI) and Water Solubility Index (WSI)

The values of WAI of extruded sago starch ranged from 2.40 to 6.20 g water absorbed per g sample, while the values of WSI varied from 13.12 to 59.60 % (Table 2).

The decrease of WAI and increase of WSI might be associated with the increase in specific mechanical energy (SME) received by the product. This is supported by the fact that the SME was correlated with those parameters ($r = -0.64$ with WAI, and $r = 0.64$ with WSI) as indicated in Table 4.

A review by Ye et al [23] revealed that the WAI of extruded starch would be higher than that of native starch, except if there was a dextrinization or starch melting, in which WAI would decrease after extrusion.

The water solubility index (WSI) indicated the quantity of soluble components released from starch after extrusion. It was found that WSI of extruded starch would also be much higher than that of native starch in many cases. WSI was dependent upon the amount of soluble material, and it might be related to the degree of molecular breakdown. The results of this study suggested that a more extensive damage to the physical structure of the starch granules resulted in a higher WSI. This was indicated by the presence of a correlation between WSI and degree of molecular degradation, with a coefficient (r) of 0.67, as indicated in Table 4.

Regression analyses showed that most of the variations (97.9% for WAI and 97.1% for WSI) were accounted for. However, there was no significant difference amongst the sago extrudates for either WAI or WSI (Table 3).

3.4 Enzyme Susceptibility

The enzyme susceptibility (ES) of the sago starch extrudates ranged from 30.52 to 41.34, measured as g reducing sugars/g sample (Table 2). It was observed that the ES values of the extrudates tended to be lower for the samples having a more extensive molecular degradation e.g. those extruded at 25% moisture, with a correlation coefficient of -0.67 (Table 4). This may be explained by a possibility that the physical structure of those samples might have been made a more compact, leading to a more restricted penetration of the enzyme solution into the product. Conversely, the samples having a lesser molecular degradation (e.g. those extruded at 40% moisture) would have more porous structures, giving rise to an easier penetration of the enzyme.

Table 4. Summary of correlation coefficients of the relationship between properties of the sago extrudates investigated in this study*

	Reducing sugar content	Water absorption index (WAI)	Water solubility index (WSI)	Enzyme susceptibility (ES)	Specific mechanical energy (SME)
Apparent molecular degradation	-	-	0.67	-0.67	0.82
Reducing sugar content	-	-	0.83	-	-
Specific mechanical energy (SME)	-	-0.64	0.64	-	-

**Only values close to 1.00 were presented*

In a study by Yan et al [24], it was found that the combined process of extrusion and heat moisture treatment of corn starch had increased its enzyme digestibility. However, Lim [25] found small ES values of the sago starch processed in a single screw extruder with low moisture content. She postulated that this could be due to: (1) incomplete destruction of the crystalline structure; (2) new bonds formed upon cooling; and (3) compact structure of the resulting fragments which were not readily accessible to the α -amylase.

3.5 Gelatinization Endothermic Energy

All extruded samples had much lower endothermic energy (ranging from 0.001 to 0.030 J/g, as indicated in Table 2) compared to the raw material, which was also measured and found in this study with a gelatinization endothermic energy of 11.470 J/g. This suggested the extrudates have been gelatinized during extrusion. The condition might have also been worsened by a low feed rate of 5 kg/h, resulted in a more severe shearing of the products inside the barrel.

The results of the gelatinization endothermic energy indicated that all the extruded samples had very small gelatinization endotherms, and there were no significant differences between the samples extruded at various extrusion parameters (Table 3).

3.6 Specific Mechanical Energy (SME)

SME was the energy input received by the products and was provided by the extruder motor [22]. The range of SME values obtained in this experiment was from 0.08 to 0.54 kWh/kg (Table 2). SME was significantly influenced by the interaction between feed moisture and screw speed ($p < 0.01$), as indicated in Table 3. Decreasing feed moisture and screw speed would give rise to an increase of SME. The increase of energy input was probably caused by a more viscous feed dough with the lower moisture samples. Similar findings were also observed by Likimani et al. [26], who extruded the corn/soybean mix in the presence of thermo-stable α -amylase in a single screw extruder.

SME was found to be positively correlated with the degree of molecular degradation of the sago starch ($r = 0.82$), as indicated in Table 4. This suggested that a relatively higher mechanical energy might have caused more damage to the granules of the starch.

4. CONCLUSION

The results of this study indicated that within the experimental conditions employed, the extrusion had modified the physical structure of the sago starch. The extent of the modification seemed mostly to depend upon the feed moisture, with a lower moisture condition promoting a higher shear and friction, leading to a more severe degradation. The other properties, such as WAI, WSI, reducing sugar content and enzyme susceptibility, were apparently not affected by the process conditions applied.

The results of this study, especially the process for gelatinization and enzyme susceptibility, were essential in designing the two-stage extrusion process for the production of maltodextrin or any other starch based food ingredients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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