In Vitro Digestibility and Thermal Properties of Native and Modified Sago (*Metroxylon Sagu*) Starch

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Abstract. In recent years, there is growing interest in the nutritional implications of resistant starch in foods due to its functional properties and health benefits. This has resulted to the development of various modification techniques to induce the formation of resistant starch. This study aimed to elucidate the effect of different modifications on digestibility and thermal properties of sago starch. Sago starch was treated with hydrothermal treatment [heat moisture treatment (HMT) and annealing (ANN)] as well as combined modification of acid methanol treatment (AMT) and hydrothermal treatment (HMT and ANN), respectively. Combined modification (AMT-HMT and AMT-ANN) had more pronounced effect in increasing the resistant starch (RS) content and lowering the glycemic index (GI) of sago starch, especially in gelatinized form. Strong negative correlation was obtained between RS and GI in both raw and gelatinised starch. Combination of acid methanol treatment with annealing caused the highest increment in gelatinization temperatures of sago starch.

1. Introduction

Starch has been obtained from various botanical sources such as maize, wheat, rice, potato for application in food and non-food industries. Sago, which has changed its role from merely a staple food to an income generating source [1] has the potential to be one of the important socioeconomic crops in Southeast Asia owing to its versatility and wide application in local food delicacies and industrial applications [2]. Malaysia is the third leading world producers of sago starch, in which continuous improvement in functional properties and nutritional benefits of sago starch are much needed to compete in the current world starch market [3].

Starch-based foods are often classified as high glycemic index food, which may constitute a public health problem such as obesity and diabetes [4]. Starch can be classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) based on the rate and extent of digestion [5]. RS is a portion of starch that is not hydrolysed by enzymes in the small intestine but passes to the large intestine for fermentation. RS, which is physiologically similar to dietary fibre has received much attention in recent years for its potential health benefits, such as increase the faecal bulk, lower the colonic pH and show improvement in control of glycemic response [6]. The formation of RS is dependent on the botanical source as well as the processing condition [7]. Extensive studies also evident that various modification methods can increase the RS content in starch [8]. Annealing (ANN) and heat-moisture treatment (HMT) are among the treatments used for modification of starch digestibility [9]. These treatments were found to enhance the RS content without destroying the granule structure [10]. There is a growing trend in combined modification to increase the RS content of starch [11]. Hydrolysis
of starch using acid prior to hydrothermal treatment to enhance the RS content was previously examined in several studies [12-15].

Some of the RS fractions in raw starch is highly susceptible to heating [16] and becoming very digestible after cooking. Since starchy foods is generally subjected to cooking before consumption, the knowledge of digestibility of a modified starch in gelatinised (cooked) form is more meaningful than its raw form. However, most of the abovementioned studies determined the digestibility of raw starches. Therefore, this study was attempted to investigate the effect of combined modification of acid methanol treatment with heat moisture treatment or annealing on the digestibility of raw and gelatinitized sago starch. Thermal behaviour of sago starch was also determined as it is one of the important parameters in determining the suitability for food applications.

2. Materials and Methods

2.1. Materials
Sago starch was purchased from Nee Seng Ngeng and Sons Sago Industries Sdn. Bhd. (Malaysia). Pancreatin from porcine pancreas, 8xUSP (P-7545); Amylogluosidase from Aspergillus niger, 260 U/ml (A-7095); Invertase from baker's yeast (S. cerevisiae), 300 U/mg (I-4504) were purchased from Sigma-Aldrich and used in in vitro digestibility test. Glucose oxidase-peroxidase (GOPOD) assay kit (K-GLUC) was purchased from Megazyme International Ireland Ltd. Other chemicals used were of analytical grades.

2.2. Starch modifications

2.2.1. Heat Moisture Treatment (HMT). Heat moisture treatment was conducted following Li et al. [17]. Starch samples were weighed into glass containers where moisture level was adjusted (15%). The sealed containers were equilibrated at ambient temperature for 24 h before heating in a forced air oven at 110 °C for 12 h. The samples were dried at 40 °C.

2.2.2. Annealing (ANN). Annealing was performed according to the procedure described by Chung et al. [9]. Starch slurries (70% moisture) were incubated in a water bath at 10 °C below the onset temperature (T_o) of gelatinization for 72 hours. At the end of the incubation period, samples were centrifuged for 10 min and supernatant was decanted. The annealed starches were washed once with deionized water and oven dried at 40 °C.

2.2.3. Acid Methanol Treatment (AMT). Acid methanol treatment was carried out following [18]. Starch (25 g) was suspended in 100 ml methanol and the reaction was started by adding 1 ml of concentrated hydrochloric acid (36%, v/v) into the starch suspension. The suspension was heated at 35 °C for 24 h. The reaction was stopped by adding 1 M sodium hydrogen carbonate (NaHCO₃) followed by cooling in an ice-bath. The starch was centrifuged and washed four times with 50% ethanol. The starch was air-oven dried at 40 °C. Acid methanol treated starch was then subjected to heat moisture treatment (as outlined in section 2.2.1) or annealing (as describe in section 2.2.2), which was abbreviated as AMT-HMT and AMT-ANN, respectively.

2.3. In vitro digestibility
The in vitro starch digestibility was determined by following the method of [19]. Starch (0.6 g) and 0.1 M sodium acetate buffer (pH 5.2) were added to test tubes and incubated in shaking water bath (37 °C) after the addition of enzyme solution (5 ml). The aliquots (0.5 ml) were taken at 20 and 120 min time intervals and mixed with 66 % ethanol. The solution was centrifuged at 3000x g for 10 min and the glucose content was measured using the glucose oxidase-peroxidase (GOPOD) assay kit (K-GLUC, Megazyme). Based on the hydrolysis rate, starch was classified as RDS (digested within 20 min), SDS
(digested between 20 and 120 min) and RS (undigested within 120 min) respectively. Raw starch was analysed directly while for cooked starch, starch suspension was first heated in a boiling water bath (30 min) prior to analysis. RS was calculated as the difference between TS and digestible starch

\[ RS = TS - RDS - SDS \] (1)

2.4. Estimated glycemic index (GI)
Estimated Glycemic Index of raw and gelatinised starch was determined in accordance with the procedure described by Goni et al. [20]. Starch sample (50 mg, dry basis) was first mixed with HCl-KCl buffer (10 mL, pH = 1.5) before the addition of pepsin solution (1 g of pepsin in 10 mL of HCl-KCl buffer) and subsequent incubation at 40 °C for 1 h. Upon incubation, the solution was topped up to 25 mL with tris-maleate buffer (pH = 6.9) before 5 mL of tris-maleate solution containing pancreatic α-amylase (3 U/mg) being added. The solution was kept at 37 °C using a shaking water bath. Sampling (1 mL of aliquot) was carried out every 30 min over a duration of 3 h. Each aliquot (1 mL) was heated in a test tube at 100 °C to inactivate the enzyme. The aliquot was then added with 3 mL of 0.4 M sodium acetate buffer (pH = 4.75) and amyloglucosidase (60 µL) before being heated at 60 °C in a shaking water bath for 40 min. The solution was then topped up to 100 mL with distilled water. The glucose content was measured using GOPOD reagent. The hydrolysis index (HI) was calculated based on the starch hydrolysis curve (0–3 h) as the ratio of glucose content of the sample to the glucose content of reference food (glucose) released over 3 h. The glycemic index (GI) was then estimated by using the

\[ GI = 39.71 + 0.549HI \] (2)

2.5. Thermal Properties
Thermal properties of sago starches were determined according to the method of [21], using Differential Scanning Calorimeter (Diamond DSC, Perkin Elmer). Starch (2.0 mg) and distilled water (6.0 mg) were weighed into an aluminium pan, sealed and equilibrate for 24 hours before scanning from 30 to 110 °C at a heating rate of 10 °C/min. The gelatinization onset (\( T_o \)), peak (\( T_p \)), conclusion (\( T_c \)) temperatures and gelatinization enthalpy (\( \Delta H \)) were quantified.

3. Results and Discussion
3.1. In Vitro Digestibility
The results for RDS, SDS and RS content of native and modified sago starches in both raw and gelatinized state are summarised in Table 1. In raw starch, the SDS content of heat moisture treated sago starch (HMT) was significantly higher than native (P < 0.05) while the RDS and RS content were similar to the native counterpart (P > 0.05). Annealing however was found to increase the digestibility of sago starch. The amount of RDS in annealed starch (ANN) was twice the amount of native starch while the RS content was reduced by half after annealing (P < 0.05). The increase in RDS content and reduction in RS content could be due to the formation of porou structure after annealing that allowed greater accessibility of enzyme into the interior of granules [22]. Acid methanol treatment coupled with heat moisture treatment (AMT-HMT) caused no significant changes to the digestibility of sago starch. Similar observation was found when acid methanol treatment was combined with annealing (AMT-ANN). Combined modified starches (AMT-HMT and AMT-ANN) were found to have lower SDS content but higher RS content than their single modified counterparts (P < 0.05). Degradation of starch molecules by acid alcohol treatment produce short and linear chains which facilitate the molecular rearrangement during hydrothermal treatment, hence contributed to the formation of enzyme resistant fraction [14].
Table 1. RDS, SDS and RS content of native and modified sago starches in raw and gelatinized state.

| Treatment          | RDS (%)   | SDS (%)   | RS (%)   |
|--------------------|-----------|-----------|----------|
| **Raw starch**     |           |           |          |
| Native             | 6.4 ± 0.3<sup>ab</sup> | 31.7 ± 1.4<sup>a</sup> | 61.9 ± 1.4<sup>bc</sup> |
| HMT                | 4.9 ± 0.2<sup>a</sup>  | 35.5 ± 0.3<sup>b</sup> | 59.6 ± 0.3<sup>b</sup> |
| ANN                | 13.1 ± 0.7<sup>c</sup> | 55.6 ± 0.3<sup>c</sup> | 31.3 ± 1.0<sup>c</sup> |
| AMT-HMT            | 6.3 ± 1.0<sup>ab</sup> | 28.8 ± 0.7<sup>a</sup> | 64.8 ± 1.7<sup>c</sup> |
| AMT-ANN            | 6.7 ± 0.2<sup>b</sup>  | 32.2 ± 2.7<sup>ab</sup> | 61.2 ± 2.6<sup>bc</sup> |
| **Gelatinized starch** |           |           |          |
| Native             | 75.8 ± 1.0<sup>c</sup> | 18.5 ± 1.8<sup>a</sup> | 5.7 ± 1.3<sup>a</sup> |
| HMT                | 71.8 ± 0.7<sup>b</sup> | 21.7 ± 1.7<sup>a</sup> | 6.5 ± 1.8<sup>a</sup> |
| ANN                | 69.0 ± 0.7<sup>b</sup> | 25.8 ± 1.1<sup>b</sup> | 5.1 ± 1.5<sup>a</sup> |
| AMT-HMT            | 51.2 ± 1.8<sup>a</sup> | 34.1 ± 1.3<sup>c</sup> | 14.7 ± 1.5<sup>b</sup> |
| AMT-ANN            | 48.3 ± 1.3<sup>a</sup> | 35.9 ± 0.4<sup>c</sup> | 15.7 ± 0.9<sup>b</sup> |

Values with different superscripts are significantly different (P < 0.05).
HMT: Heat Moisture Treatment; ANN: Annealing; AMT: Acid Methanol Treatment.
Dual modification is indicated with abbreviation AMT-HMT and AMT-ANN.

The digestibility of starch was greatly increased after gelatinisation, in which the RDS content was drastically increased while the RS content was greatly reduced. Heat moisture treatment reduced the RDS content of starch while annealed starch (ANN) had lower RDS content and higher SDS content than native starch. Both HMT and ANN had similar RS content as gelatinised native starch (P > 0.05). Combined modification on the other hand had more pronounced effect in lowering the digestibility of gelatinised starch. The SDS content of AMT-HMT and AMT-ANN was two times higher than native starch while there was a threefold increase in RS content after combined modification. Both AMT-HMT and AMT-ANN recorded lower RDS content than native counterpart (P < 0.05). Lin et al. [14] mentioned that combination of acid alcohol treatment and HMT enhanced the thermal stability of RS. The reduction in molecular size of starch by acid modification increased the mobility of starch molecules, which enhanced the reorganisation of starch chains during annealing, and thus increased the RS content [13].

3.2. Estimated Glycemic Index (GI)
Heat moisture treatment was found to increase the GI of starch in the raw state (P < 0.05). Similar observation was reported on HMT treated corn, pea and lentil starches [9]. Disruption of crystalline and dissociation of double helical structures by HMT allowed the enzymatic accessibility to the amorphous regions [10], which enhanced the digestibility of starch and subsequently causing higher GI. Annealing also increased the GI of raw starch, in which ANN recorded the highest GI among all the samples. This was associated with its highest RDS and SDS contents but lowest RS content, which was supported by the strong positive correlation between GI and RDS content (r = 0.875, P < 0.01) and (r = 0.969, P < 0.01) while negative correlation was found between GI and RS content (r = -0.964, P < 0.01). This was also in agreement with Simsek and El [23] that greater RDS content will lead to higher GI. The combination of acid methanol treatment with annealing (AMT-ANN) had significantly lower GI than native counterpart (P < 0.05). According to Miao et al. [24], the cleavage of starch chains by acid hydrolysis induced higher perfection of crystallites and ordered domains during annealing which might contribute to lower GI.
Table 2. Estimated glycemic index (GI) of native and modified sago starches in raw and gelatinized state.

| Treatment | Raw starch       | Gelatinized starch |
|-----------|------------------|--------------------|
| Glucose   | 100.0 ± 0.0^e    | 100.0 ± 0.0^d      |
| Native    | 57.1 ± 0.3^b     | 92.4 ± 0.1^c       |
| HMT       | 61.5 ± 0.4^c     | 90.2 ± 0.5^b       |
| ANN       | 74.1 ± 0.4^d     | 91.9 ± 0.2          |
| AMT - HMT | 58.0 ± 0.4^b     | 89.1 ± 0.5^ab      |
| AMT - ANN | 56.2 ± 0.2^a     | 88.0 ± 0.8^a       |

Values with different superscripts are significantly different (P < 0.05).

HMT: Heat Moisture Treatment; ANN: Annealing; AMT: Acid Methanol Treatment.
Dual modification is indicated with abbreviation AMT-HMT and AMT-ANN.

After gelatinisation, GI of all modified starches were significantly lower than native counterpart, except annealed starch (ANN). In this study, HMT was found to have significantly lower GI than ANN (P < 0.05). This is in line with Chung et al. [9] that GI of gelatinized HMT starch was lower than the gelatinized ANN starch. The authors explained the interactions between amylose–amylose chains that formed during HMT were not disrupted when gelatinization occurred. This would restrict the accessibility of digestive enzymes to starch molecules, causing the reduction in GI. Acid methanol treatment coupled with HMT was found to decrease the GI in gelatinized starch. This was in line with Kim and Huber [25] that HMT under mild acidic condition generated higher amount of heat stable RS, consequently causing lower GI. However, GI of AMT-HMT was not significantly different from the HMT counterpart in gelatinised form (P > 0.05). Similar to AMT-HMT, acid methanol treatment prior to annealing also significantly reduced the GI of gelatinised starch. AMT-HMT and AMT-ANN which showed low RDS content but high SDS and RS content had lower GI than native counterpart (P < 0.05). This was supported by the strong positive correlation between GI and RDS content (r = 0.867, P < 0.01) as well as negative correlation between GI with SDS content (r = -0.813, P < 0.01) and RS content (r = -0.870, P < 0.01). In this study, all the raw starches except ANN fall into the category of medium GI food (56 < GI < 69) whereas all the gelatinized starches are categorized as high GI food (GI > 70).

3.3. Thermal Properties

The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and gelatinisation enthalpy (ΔH) of native and modified sago starches are presented in Table 3. Gelatinization temperatures (T_o, T_p, T_c) of sago starch were significantly increased after heat moisture treatment (P < 0.05). The amylose-amylose or amylose-amylopectin interactions resulted from HMT restricted the chain mobility in amorphous regions, thus required a higher temperature to incur swelling [9]. Annealing had also significantly increased the T_o, T_p, T_c, and the increment was found to be much higher than heat moisture treatment (P < 0.05). Such phenomenon might be related to the perfection of less stable crystallites or improved ordering of starch chains within amorphous regions [26]. Acid methanol treatment prior to HMT did not induce significant changes in thermal behaviour to heat moisture treated starch. The combination of acid methanol treatment with annealing (AMT-ANN) on the other hand had shown higher T_o, T_p, T_c as compared to its annealed counterpart (P < 0.05), which was also the highest among the modified samples. Lin et al. [13] stated that reduction in molecule size of starch enhances the efficiency of annealing, which further increase the gelatinisation temperatures in comparison with annealing alone. Furthermore, AMT-ANN dual modified starches recorded higher ΔH than native and annealed counterpart (P < 0.05). Similar finding was reported in acid alcohol treated-annealed corn starch [13].
Table 3. Gelatinization properties of native and modified sago starches.

| Treatment       | Gelatinization Temperature (°C) | Enthalpy (ΔH) (J/g) |
|-----------------|---------------------------------|---------------------|
|                 | Onset (T_o) | Peak (T_p) | Conclusion (T_c) |
| Native          | 68.71 ± 0.20^a | 74.06 ± 0.10^a | 78.69 ± 0.21^a | 3.26 ± 0.20^ab |
| HMT             | 70.33 ± 0.16^b | 75.15 ± 0.24^b | 80.20 ± 0.40^b | 2.89 ± 0.23^c |
| ANN             | 76.32 ± 0.09^c | 78.71 ± 0.00^d | 81.17 ± 0.09^c | 3.42 ± 0.07^bc |
| AMT-HMT         | 70.40 ± 0.29^b | 75.93 ± 0.26^c | 81.72 ± 0.40^c | 3.18 ± 0.05^ab |
| AMT-ANN         | 77.28 ± 0.25^d | 81.01 ± 0.26^e | 85.17 ± 0.54^d | 3.86 ± 0.29^c |

Values with different superscripts are significantly different (P < 0.05).
HMT: Heat Moisture Treatment; ANN: Annealing; AMT: Acid Methanol Treatment.
Dual modification is indicated with abbreviation AMT-HMT and AMT-ANN.

4. Conclusion
Starch treated with acid methanol treatment (AMT) coupled with hydrothermal treatment (HMT and ANN) had more pronounced effect in reducing the digestibility of sago starch. Both AMT-HMT and AMT-ANN recorded higher resistant starch (RS) content and lower glycemic index (GI) than single modification in raw and gelatinised state. Heat moisture treatment, annealing and combined modification (AMT-ANN and AMT-HMT) all increased the gelatinization temperatures of sago starch, particularly the combination of AMT-ANN which had the highest gelatinization temperatures. This study demonstrated that combination of acid methanol treatment with annealing could be employed to enhance the RS content as well as the thermal stability of sago starch. The relationship between RS and GI in both raw and gelatinised starch was successfully identified in this study. Strong negative correlation was obtained between RS content and GI in raw (r = -0.964, P < 0.01) and gelatinised form (r = -0.870, P < 0.01), indicating the reduction in glycemic index was associated with high RS content.

5. References
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