

Functional Properties of Resistant Starch Type-III from *Metroxylon sagu* as Affected by Processing Conditions

Zi-Ni, T., Rosma, A.*, Karim, A. A. and Liong, M. T.

School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

ABSTRACT

Type III resistant starch (RS₃) was produced from native sago starch using different processing conditions. Native sago starch contained 93.5% total starch, of which was 25.8% amylose and 67.7% amylopectin. A sample with the highest RS₃ content (35.7%) was produced when the native sago starch was suspended in distilled water, gelatinised by autoclaving at 121°C for 1 h, followed by debranching with 20U pullulanase per g starch at 60°C for 24 h, autoclaved again at 121°C for 1 h before storage at 4°C for 24 h. The sago RS₃ sample contained 54.0% amylose and 38.8% amylopectin. The powder had solubility, swelling power, water-holding and oil-holding capacity of 27.4%, 2.8g/g, 1.7g/g and 1.1g/g, respectively. Treatment of the sago RS₃ with 0.5M HCl acid at 60°C for 24 h produced HCl-sago RS₃ with 68.30% RS₃ content. The solubility and swelling power of HCl-sago RS₃ was 14.9% and 1.9g/g, respectively. Different processing conditions had significantly influenced the amount and properties of RS₃ produced from sago starch.

Keywords: Resistant starch, *Metroxylon sagu*, sago, amylose, amylopectin, starch property

INTRODUCTION

Consumption of resistant starch in daily meals has captured increasing worldwide attention owing to its health-promoting benefits.

Resistant starch refers to the nondigestible starch fraction that resists absorption and digestion along the gastrointestinal tract and may be completely or partially fermented in the colon (Englyst *et al.*, 1992). The beneficial physiological effects of resistant starch have been extensively reviewed, and these include prevention of colonic cancer, hypoglycaemic effects, hypercholesterolemia effects, prebiotic function, inhibition of fat accumulation, reduction of gall stone formation, and

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E-mail addresses:

camillia_zntan713@yahoo.com (Zi-Ni, T.),

rosmah@usm.my (Rosma, A.),

akarim@usm.my (Karim, A. A.),

mintze.liong@usm.my (Liong, M. T.)

* Corresponding author

increased absorption of minerals (Fuentes-Zaragoza *et al.*, 2011).

Adequate intake of resistant starch is necessary to exert its health benefit effects. The Joint Food and Agricultural Organisation of the United Nations or World Health Organisation Expert Consultation on Human Nutrition have not yet recommended levels for resistant starch consumption. However, the recommended daily consumption of resistant starch by Australia's Commonwealth Scientific and Industrial Research Organisation is approximately 20g (Baghurst *et al.*, 2001). This intake level can only be achieved by consuming foods with added resistant starches as food ingredient because foods generally contain low resistant starch (per 100g food): breakfast cereals, 0-3.6g (Alsaffar, 2011); white bread, 0.9g (Brown, 2004); cooked white rice, 7.1g (Vatanasuchart *et al.*, 2009) and starchy foods, 0.2-10g (Liljeberg, 2002). Hence, it is suggested that resistant starch be added to foods to increase its amount.

Resistant starch is described as a linear molecule of α -1,4-D-glucan derived from the retrograded amylose fraction of starch that has a relatively low molecular weight of 1.2×10^5 a (Fuentes-Zaragoza *et al.*, 2011). There are five different types of resistant starch: RS₁, RS₂, RS₃, RS₄ and RS₅. A description and the food sources of each type of resistant starches may be found in Fuentes-Zaragoza *et al.* (2011). RS₃, which was produced in this research, is preferred from among the resistant starches as a functional food ingredient due to its thermal

stability high melting temperature at the range of 140°C to 160°C (Shamaia *et al.*, 2003). On the other hand, RS₁ and RS₂ are thermally instable, causing them to lose their functional benefits after food processing (Zhao & Lin, 2009), while the legality of RS₄ being used in food production is a major concern (Lunn & Buttriss, 2007). RS₅ is an amylose-lipid complex starch formed from high amylose starches that require a high gelatinisation temperature (Jiang *et al.*, 2010). The thermal stability characteristic allows food with added RS₃ to retain its functional benefits even after cooking. Research has also shown that RS₃ can be incorporated into batter without compromising consumer acceptability (Sanz *et al.*, 2008).

Production of RS₃ involves four sequential processing steps: disruption of starch granules, enzymatic debranching of starch polymer, starch retrogradation and drying. Every processing step has its own influencing factors in addition to the starch botanical sources, ratio of amylose and amylopectin content and the presence of other components in the starch (Sajilata *et al.*, 2006). Previous research has focused on the production of RS₃ from readily accessible starch sources such as maize (Zhao & Lin, 2009), wheat, rice and potato (Garcia-Alonso *et al.*, 1998). Less research has been reported on the production of RS₃ from sago (*Metroxylon sagu*) except for our two previous research studies (Leong *et al.*, 2007; Siew-Wai *et al.*, 2012).

Sago starch is one of the major export commodities for Malaysia, with an increased

output from 44,448.84 metric tonnes in the year 2010 to 50,965.39 metric tonnes in 2011, with an increase in revenue from USD19.1 million to USD27.8 million (Department of Agriculture Sarawak, 2014). Moreover, sago palm produces a relatively higher starch yield, 3 to 4 times more than rice, corn and wheat and 17 times more than cassava in per unit plantation area (Karim *et al.*, 2008). Therefore, sago starch was chosen as the raw material to produce RS₃ in this work.

Our previous research produced sago RS₃ with resistant starch content of 11.5% (Leong *et al.*, 2007) and 12.2% (Siew-Wai *et al.*, 2010). This research was aimed to investigate different processing conditions in increasing the resistant starch content and to investigate the effects of these conditions on functional properties of the resistant starch produced from sago.

MATERIALS AND METHODS

Materials

Native sago starch (Soon Huat Moh Trading Co.) was purchased from a local grocery in Kuching, Sarawak, Malaysia. Pullulanase, a debranching enzyme (Promozyme D2), was purchased from Novozymes (Bagsvaerd, Denmark) and used upon arrival. The enzyme was a technical grade enzyme with a specific activity of 1350 PUN/g (one Pullulanase Unit Novo) and a density of 1.20g/mL. All other chemicals used were of analytical grade and purchased from Sigma Chemicals Ltd. (St. Louis, Missouri, USA).

Production of Sago RS₃

In every processing step, factors that were thought to influence the amount of RS₃ produced were individually assessed. The experiments were conducted in triplicate. Fig.1 summarises the flow of the processing conditions. There were four main processing steps involved in the production of sago RS₃:

Step 1: disruption of starch granules. Two disruption methods were investigated in this step as follows:

Gelatinization by heat treatment. Twenty gram of native sago starch (20%, w/v) was suspended in 100mL of 0.1M acetate buffer, pH5 or distilled water and subjected to heat treatment; boiling for 10 min and/or autoclaving at 121°C for 1 h. The starch gel was cooled to 60°C prior to the enzymatic debranching step (Step 2).

Partial acid hydrolysis. Native sago starch was suspended with continuous stirring at ambient temperature (25°C) in 1M hydrochloric acid (HCl) at a ratio of 1 g starch to 3.5mL acid for 24 h. The pH of starch-acid suspension was then adjusted to pH7.0 with 2M of NaOH and centrifuged (2330×g, 15 min). The pellet was washed three times with distilled water. It was then oven-dried at 40°C until its moisture content was less than 13% and ground to fine powder with particles of less than 180µm. This acid-treated powder (20%, w/v) was then suspended in 0.1M acetate buffer (pH5.0), and subjected to enzymatic debranching and further processing steps.

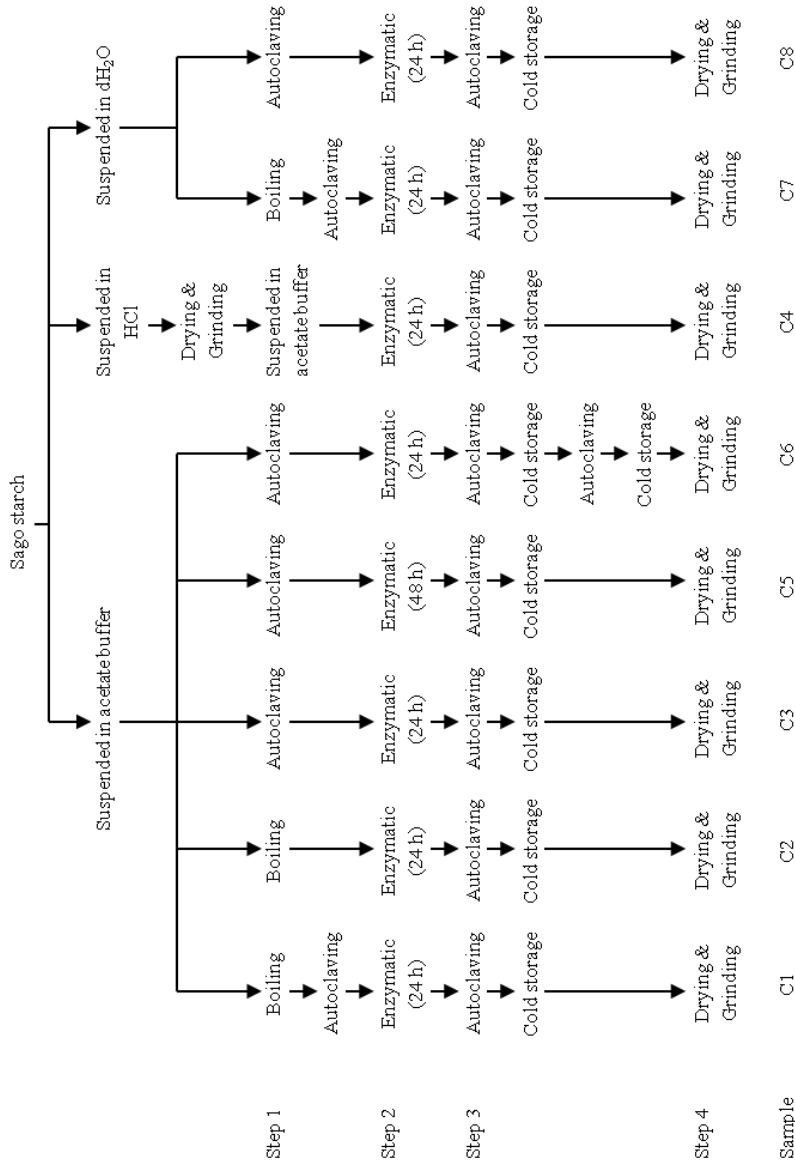


Fig. 1: Different processing conditions in the production of sago RS₃.

Step 2: Enzymatic debranching.

Pullulanase enzyme was added at 20 PUN/g starch, and the starch-enzyme suspension was incubated in an orbital incubator shaker (Certomart SII, Sartorius, Melsungen, Germany) at 60°C for 24 h or 48 h. The reaction was stopped by heating the starch-enzyme suspension in a water bath (80°C) for 15 min.

Step 3: Thermal processing and cold storage.

Starch-enzyme suspensions from Step 2 was autoclaved at 121°C for 1 h and cooled to ambient temperature (25°C) before storing in a refrigerator at 4°C for 24 h. Some sample suspensions were subjected to few cycles of thermal processing and cold storage.

Step 4: Drying and grinding. The resulting starch was oven-dried at 40°C until its moisture content was less than 13%, and finally ground to fine powder with particles of less than 180µm. The ground powder was used as sago RS₃ samples.

Acid hydrolysis of sago RS₃ to produce HCl-sago RS₃

Sago resistant starch sample (sago RS₃ from Step 4) with the highest resistant starch content was further subjected to hydrolysis with 0.5M HCl at a ratio of 1:3.5 (sago RS₃:HCl) at 60°C for 24 h with continuous shaking (150rpm) to produce HCl-sago RS₃. The starch slurry was centrifuged at 2330×g for 15 min. The starch pellet was washed with distilled water several times and dried

in an oven at 40°C until its moisture content was less than 13%. Dried starch was then ground to fine particles of less than 180µm.

Chemical Analyses

Native sago starch was analysed for its moisture, ash, crude protein, crude fat and crude fibre contents (AACC, 2000). Native sago starch was also analysed for total starch (Goni *et al.*, 1997) and amylose contents (Hoover & Ratnayake, 2001). Amylopectin content was determined by the difference of total starch and amylose contents.

Resistant starch content was determined according to Goni *et al.*, (1996) and calculated as follows:

Resistant starch (%)

$$= \frac{\text{mg glucose} \times \text{dilution factor} \times 0.9}{\text{sample weight (mg, dry basis)}} \times 100\% \quad [1]$$

The method involved the removal of protein from samples with pepsin (HiMedia RM084, 400U/mg sample, 40°C, pH 1.5, 60 min), hydrolysis of digestible starch with pancreatic α -amylase (Sigma A-3176, 4 U/mg sample, 37°C, pH6.9, 16 h), solubilisation of precipitates with 4M KOH and hydrolysis of samples with amyloglucosidase (Sigma 10115, 0.12U/mg sample, 60°C, 45 min, pH4.8). Finally, the liberated glucose in the sample was determined by using the glucose oxidase assay.

Physical characterisation of sago RS₃ and HCl-sago RS₃

Swelling power and solubility of samples were determined (Chan *et al.*, 2010) whereby 100mg of sample was accurately weighed in a pre-weighed 50mL centrifuge tube and 10mL of distilled water was added. The tube was placed in a water bath at 90°C for 30 min and centrifuged at 2330×g for 15 min. Then, 5mL of the supernatant was carefully pipetted to a pre-weighed moisture dish and dried in an oven at 110°C overnight. The moisture dish was then cooled in a dessicator and weighed. The wet sediment in the centrifuge tube was also weighed. The swelling power and solubility were calculated as follows:

Swelling power (g/g)

$$= \frac{\text{weight of wet sediment (g)}}{\text{weight of sample used (g)}} \times 100\%$$

[2]

Solubility (%)

$$= \frac{\text{weight of wet supernatant (g)}}{\text{weight of sample used (g)}} \times 100\%$$

[3]

Water-holding capacity (WHC) of samples was determined (Chau *et al.*, 1997) whereby 1g of sample was vortexed in 10mL of distilled water at an ambient temperature (25°C) for 1 min and followed by centrifugation at 2200×g for 30 min. The supernatant was removed and the wet sediment was weighed. Oil-holding capacity (OHC) was determined by replacing the distilled water with corn oil (Yee Lee Edible

Oils Pvt. Ltd., Malaysia). The water-holding and oil-holding capacities were expressed as weight of water or oil held per gram of sample, and was calculated as follows:

WHC or OHC (g/g)

$$= \frac{\text{Weight of wet sediment} - \text{weight of sample used (g)}}{\text{weight of sample used (g)}} \times 100\%$$

[4]

Statistical Analysis

All the data from triplicate experiments with triplicate analyses were subjected to one-way ANOVA using a computer software, SPSS version 14.0 (Illinois, USA) and the significance of difference between means was determined by the Duncan test at 5% probability level. Pearson’s correlation coefficients between the amount of RS₃ produced with amylose content, solubility, water-holding capacity and oil-holding capacity were analysed and considered significantly different at a 1% probability level.

RESULTS AND DISCUSSION

Chemical Analysis of Native Sago Starch

Table 1 shows the chemical composition of native sago starch. Ash, crude protein, crude fat and crude fibre content of the native sago starch in dry basis were 0.17%, 0.18%, 0.13% and 0.64%, respectively. The native sago starch contained 93.45% total starch of which was 25.77% amylose and 67.68% amylopectin. The crude fibre content in the sago starch was higher than that of

sago starch from various manufacturers as determined by Ahmad *et al.* (1999) whereas other values were similar. The native sago starch contained 34.38% of resistant starch. Fuentes-Zaragoza *et al.* (2011) had reported that the resistant starch of various food sources including native starches from corn, wheat rice and potato ranged from 1.6 to 11.0%. However, a comparison of resistant starch content of native starch in this study could not be made with other native starches because of the difference in analysis protocol. Furthermore, the flours were subjected to processing stages of grinding, fine milling, sieving and steam processing (Fuentes-Zaragoza *et al.*, 2011), and this processing method had converted the native starches into RS₃. Considering all the factors influencing the resistant starch content determination, Leong *et al.* (2007) had reported that native sago starch contained 41.8% RS, which is higher than what was obtained from this research.

Resistant Starch, Amylose and Amylopectin Content of Samples

Table 2 shows the resistant starch, amylose and amylopectin contents in samples obtained from eight different processing conditions. A decreasing order of resistant starch content from 35.71% to 12.34% was obtained: C8 > C5 ≥ C3 > C7 > C1 > C6 ≥ C2 > C4. A similar decreasing order of amylose content in the samples from 54.05% to 3.84% was also observed.

Production of RS₃ is dependent on amylose content of the starting starchy materials. This was demonstrated by Sievert and Pomeranz (1989) whereby seven different types of starch with different amylose contents ranging from less than 1% to 70% were used to produce RS₃. It was found that the highest RS₃ yield (21.3%) was from amylo maize VII, which contained initial amylose content of 70%. Resistant starch content produced from sago starch in this research was higher (35.71%) with a lower initial amylose content of 24.08%

TABLE 1
Chemical Composition of Native Sago Starch

Analysis	Content (% , dry basis)
Ash	0.17±0.06
Crude fat	0.13±0.02
Crude protein	0.18±0.02
Crude fibre	0.64 ± 0.02
Total starch	93.45±1.19
Resistant starch	34.38 ± 0.22
Digestible starch (by difference)*	59.07 ± 0.22
Amylose	25.77±0.20
Amylopectin (by difference)#	67.68 ±0.20

Results are expressed as means ± standard deviation (N=3).

* Digestible starch = Total starch – Resistant starch; # Amylopectin = Total starch – Amylose.

than amylo maize. This indicated that the production of RS₃ was influenced not only by the initial amylose content of the starchy materials but also by the processing conditions.

Apparently, suspending the sago starch in 0.1M acetate buffer, pH 5 did not significantly improve the production of sago RS₃ as compared to suspending the starch in distilled water (comparing samples C3 and C8 and samples C1 and C7). Debranching α -1,6-glucosidic linkages in sago starch polymer with pullulanase enzyme for a longer time (48 h) also did not significantly improve the RS₃ content (comparing samples C3 and C5). A longer enzymatic reaction time probably produced debranched samples with an abundance of short chain amylose chains that were unable to form RS structure. Consequently, the yield of RS, which varied with average chain length of amylose would be low. It is generally recognised that RS is a 20-25 glucose residue long, retrograded or recrystallised and hydrogen-bonded, polydisperse linear oligosaccharide (Eerlingen *et al.*, 1993).

The most apparent reason for high production of sago RS₃ was the gelatinisation step. Gelatinisation of sago starch by autoclaving for 1 h had significantly produced higher RS₃ content than by boiling (comparing samples C2 and C3, and samples C7 and C8). Samples C1 and C7 that were boiled and subsequently autoclaved also contained lower RS₃ content than that of samples C3 and C8, respectively. This is because during boiling, formation of starch gel in samples was observed. The

TABLE 2
Resistant Starch, Amylose and Amylopectin Contents, and Swelling Power and Solubility of Samples Obtained from different processing Conditions

Sample	Resistant Starch (%)	Amylose (%)	Amylopectin (%)	Swelling at 90°C (g/g, dry basis)	Solubility at 90°C (%; dry basis)
C8	35.71 ± 0.59 ^a	54.05 ± 0.53 ^a	38.79 ± 0.53 ^g	2.82 ± 0.05 ^f	27.36 ± 0.80 ^h
C5	33.00 ± 0.09 ^b	49.97 ± 0.60 ^b	42.87 ± 0.60 ^f	4.01 ± 0.22 ^d	21.64 ± 0.03 ^b
C3	32.99 ± 0.41 ^b	46.88 ± 0.83 ^c	45.96 ± 0.83 ^e	3.49 ± 0.06 ^e	19.15 ± 0.42 ^c
C7	31.00 ± 0.36 ^c	43.32 ± 0.38 ^d	49.52 ± 0.38 ^d	3.52 ± 0.08 ^e	21.45 ± 1.05 ^b
C1	28.42 ± 0.57 ^d	37.11 ± 0.23 ^c	55.73 ± 0.23 ^c	4.37 ± 0.11 ^c	17.00 ± 0.03 ^d
C6	26.47 ± 0.34 ^e	25.42 ± 0.39 ^f	67.42 ± 0.39 ^b	5.04 ± 0.07 ^{ab}	16.56 ± 0.36 ^d
C2	26.31 ± 0.84 ^e	24.47 ± 1.17 ^f	68.37 ± 1.17 ^b	4.81 ± 0.05 ^b	16.74 ± 0.38 ^d
C4	12.34 ± 1.63 ^f	3.84 ± 0.19 ^g	89.00 ± 0.19 ^a	5.15 ± 0.18 ^a	9.93 ± 0.38 ^c

Results are expressed as means ± standard deviation (N=3).

Mean values in the same column followed by different superscript lower case letters^{abcdeefg} are significantly different at 5% probability level.

subsequent autoclaving process did not affect sample gelatinisation as much as during boiling because the starch was in gel form with less available water. It was shown that gelatinisation of wheat and corn starches at 120°C had increased RS₃ yield more than gelatinisation at 100°C (Garcia-Alonso *et al.*, 1998). One research study had also proved that increasing the heating temperature of locust bean starch from 50°C to 110°C increased the yield of RS₃ from 39% to 45% (Sankhon *et al.*, 2012). However, the effect of gelatinisation temperature on RS₃ yield was also dependent on the botanical source of starches. It was reported that autoclaving had decreased the RS₃ content of rice and potato starches (Garcia-Alonso *et al.*, 1998).

RS₃ content in this research was very much improved than in our two previous research studies (Leong *et al.*, 2007 and Siew-Wai *et al.*, 2010). It was noted that in those two previous studies, sago starch was subgelatinised at 60°C prior to enzymatic debranching. The extent of starch gelatinisation affects the degree of solubilisation of amylose chains and the available amount of amylopectin for the subsequent enzymatic debranching process. A higher temperature applied ensures that starch granules are fully gelatinised (Yao *et al.*, 2010) while pressure enhances the diffusion of water molecules into starch granules (Liu *et al.*, 2009). During the gelatinisation process, the heating of starch suspension in excessive water raises its temperature progressively, allowing starch molecules to absorb heat

energy and increasing the vibration causing the breakage of hydrogen bonds among the starch molecules (Bryksa & Yada, 2009). Meanwhile, hydrogen bonds are formed between water molecules and starch molecules, allowing water to penetrate into the starch granules to such an extent that the irreversible swelling of starch granules occurs (Vaclavik & Christian, 2014). Swelling causes starch granules to lose their birefringence and their ordered crystalline structure. Eventually, they are disrupted, allowing polymer chains to leach out from the starch granules (Vaclavik & Christian, 2014).

Pullulanase enzyme hydrolyses α -1,6-glucosidic linkages of amylopectin in sago starch polymer, releasing short and long linear chains of amylose molecules (Leong *et al.*, 2007). Therefore, together with the starch gelatinisation steps, availability of amylose molecules increased and recrystallisation of amylose polymers formed resistant starch easily (Zhao & Lin, 2009). It was shown that the RS₃ content of samples C1 to C8 were positively correlated ($r=0.97$) with amylose content ($p<0.01$).

Two cycles of heat treatment and cooling (comparing samples C3 and C6) did not improve the RS₃ content in this present research. The effect of the cycle was pronounced with a higher number of cycles (up to 20 cycles), which could raise the RS₃ yield of corn starch from 21.3% to over 40% (Sievert & Pomeranz, 1989).

We also hydrolysed the sago starch polymer with 1M HCl for 24 h to replace starch gelatinisation by heat treatment

while maintaining the other processing steps, producing sample C4 with 12.34% RS₃ content. Although this was an energy-saving approach that utilised only one cycle of autoclaving, the condition had produced the lowest RS₃ content among the samples. It was also noted that sample C4 contained low amounts of amylose (only 3.84%) and 89% amylopectin. It was thought that the hydroxonium ion (H₃O⁺) from acid easily attacks the glycosidic linkages of branched polymers in the amorphous region to allow increased polymer mobility for molecular rearrangement and produce short linear chains of amylose (Thompson, 2000). The short linear chains appeared to participate in the rearrangement and recrystallisation of starch during autoclaving and the cooling treatment for the formation of RS₃. However, without starch gelatinisation by heat treatment, a high amount of amylopectin still remained in the crystalline region of the starch granules, and this limited the access of the pullulanase enzyme.

Swelling Power and Solubility

The amount of amylose and amylopectin content in a starchy sample is important, as it does not only influence the amount of RS₃ content of the samples, but also influences the physical characteristics of the samples. Table 2 shows the swelling power and solubility of the RS₃ samples from different processing conditions. One research study showed that swelling power of a product was positively correlated with amylopectin content (Tester & Morrison, 1990). In this research, sample C4 contained

the highest amylopectin content ($p < 0.05$). Consequently, sample C4 had the highest swelling power than other samples because amylopectin can swell freely without restriction by amylose. In contrast, sample C8 with the lowest amylopectin content had the lowest swelling power ($p < 0.05$).

Generally, samples with a higher amount of resistant starch, regardless of the type of resistant starch, have lower solubility. This is demonstrated in research by Shin *et al.* (2003) and Ozturk *et al.* (2009). Similarly, gelatinised and retrograded banana starch with higher RS₃ content also exhibited lower solubility of the sample at 90°C (Aparicio-Saguilan *et al.*, 2005). However, processing conditions to produce resistant starch had influenced the solubility of the samples. As noted in this research, the solubility of samples C1 to C8 was positively correlated with the RS₃ content ($r = 0.91$). A similar trend was also observed in the research by Ozturk *et al.* (2009) whereby the sample with the highest RS₃ content from corn starch demonstrated the highest solubility. The main factor that is thought to have contributed to this observation was the use of enzymatic debranching in the processing steps. RS₃ samples that were produced without going through the enzymatic debranching process, as in the case of banana RS₃ (Aparicio-Saguilan *et al.*, 2005), had negative correlation with their solubility. As mentioned earlier, pullulanase enzyme cleaves α -1,6 glycosidic linkages of amylopectin to release branching chains and generates a mixture of long and short units of amylose for the production of

resistant starch (Leong *et al.*, 2007). These linear chains of amylose unit cause the RS₃ samples to solubilise more. Significant increases in solubility were observed in samples hydrolysed longer with pullulanase (Ozturk *et al.*, 2009).

Water-Holding and Oil-Holding Capacity

Water-holding capacity (WHC) measures the interaction magnitude of samples with water molecules. It was noted that WHC of sample was negatively correlated with RS₃ content ($r=0.97$). Table 3 shows that the sample with the highest RS₃ content (35.71%) had the lowest WHC (1.66 g/g) while the sample with the lowest RS₃ content (12.34%) had the highest WHC (2.45 g/g).

RS₃ mainly comprises retrograded amylose that is joined tightly by a hydrogen bond. Due to the tight bonding within the insoluble crystalline structure, RS₃ is not able to form a hydrogen bond with water molecules. Hence, increasing the RS₃

content in the formulation of processed food can lower the food's WHC. In the baking industry, resistant starch-containing bread with a lower WHC provides a better texture and is more easily handled during processing (Sajilata *et al.*, 2006). It also decreases the loaf volume (Sajilata *et al.*, 2006) and maintains the structure of the crumb (Ranhotra *et al.*, 1999). Hence, RS₃ in food can exert both nutritional benefit and improve the property of the food.

Table 3 shows the oil-holding capacity (OHC) property of the sago RS₃ samples produced from different conditions. The OHC was used to measure the ability of starches to hold the oil. Sample C8 with the highest resistant starch content (35.71%) had the highest capacity in holding oil (1.04 g/g) while sample C4 and C1 had the lowest OHC although RS₃ content of sample C1 was double that of sample C4. Statistical analysis showed that RS₃ content of sample C1 to C8 was not significantly ($p>0.01$) correlated with the OHC. Previous research

TABLE 3
Water-holding (WHC) and Oil-holding Capacity (OHC) of Samples Obtained from Different Processing Conditions

Condition	Resistant Starch (%)	WHC (g/g)	OHC (g/g)
C4	12.34 ± 1.63 ^f	2.45 ± 0.01 ^a	0.85 ± 0.02 ^c
C2	26.31 ± 0.84 ^e	1.85 ± 0.06 ^b	0.88 ± 0.03 ^{cde}
C1	28.42 ± 0.57 ^d	1.85 ± 0.05 ^b	0.82 ± 0.03 ^e
C6	26.47 ± 0.34 ^e	1.79 ± 0.05 ^{bc}	0.87 ± 0.04 ^{de}
C7	31.00 ± 0.36 ^c	1.78 ± 0.01 ^{bc}	0.94 ± 0.03 ^{bc}
C3	32.99 ± 0.41 ^b	1.71 ± 0.09 ^c	0.93 ± 0.01 ^{bcd}
C5	33.00 ± 0.09 ^b	1.69 ± 0.01 ^c	0.96 ± 0.03 ^b
C8	35.71 ± 0.59 ^a	1.66 ± 0.07 ^c	1.04 ± 0.04 ^a

Results are expressed as means ± standard deviation (N=3).

Mean values in the same column followed by different superscript lower case letters^{abcde} are significantly different at 5% probability level.

has shown that the OHC levels of lentil and chickpea flours were not dependent on the amount of RS₃ (Aguilera *et al.*, 2009). Further research is anticipated to study the functionality and suitability of this sago RS₃ to be incorporated in food formulation especially in fried foods.

RS₃ content was further increased with the treatment of sago RS (sample C8) with 0.5M HCl (sample designated HCl-sago RS3) to 63.80%. It was thought that the hydroxonium ion (H₃O⁺) of acid hydrolysed the digestible portion of the sago RS₃ sample, contributing to the enhanced level and purity of the RS₃ content in the HCl-sago RS₃ sample. Native sago starch, sago RS and HCl-sago RS samples were subjected to further analyses. It was found that the solubility and the swelling power of HCl-sago RS was 14.90% and 1.94 g/g, respectively, which was lower than sago RS due to higher RS₃ content.

CONCLUSION

Processing conditions influenced the amount of sago RS₃ and its functional properties. Processing condition with gelatinisation of starch suspension in distilled water by autoclaving at 121°C for 1 h, followed by pullulanase debranching of the starch polymers at 60°C for 24 h prior to autoclaving and cold storage at 4°C for 24 h produced a sago RS sample with 35.71% RS₃ content. Among the sago RS samples, sample C8 had the highest amylose content, highest solubility and oil-holding capacity while it was the lowest in amylopectin

content, swelling power and water-holding capacity. Hydrolysis of this sago RS with 0.5M HCl acid at 60°C for 24 h produced HCl-sago RS with 68.30% RS₃ content. The solubility and swelling power of HCl-sago RS were lower than those of sago RS.

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