International Food Research Journal 28(5): 1057 - 1066 (October 2021)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of sago starch on body weight, food intake, caecum short chain fatty acids, adipose tissue, and hepatic lipid content of fat-induced Sprague Dawley rats

[1]Thompson, M. S., [1]Dahari, S. I., [2]Shamsuddin, M. S., [3]Rashed, A. and [1,4]*Sarbini, S. R.

[1]*Department of Crop Science, Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, Jalan Nyabau, 97008 Bintulu, Sarawak, Malaysia*
[2]*Department of Veterinary Services, Ministry of Agriculture and Food Industries, Wisma Tani, Blok Podium, Lot 4G1, Precinct 4, Federal Government Administrative Centre, 62624 Putrajaya, Malaysia*
[3]*Cardiovascular, Diabetes and Nutrition Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia*
[4]*Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Article history

Received: 30 October 2019
Received in revised form: 25 October 2020
Accepted: 5 April 2021

Keywords

sago starch, resistant starch, obesity, prebiotic, satiety

Abstract

Sago starch which naturally contains high amount of resistant starch, comes to the attention due to its ability to confer health benefits as functional food *i.e.,* prebiotic. The present work aimed to investigate the effects of sago starch consumption on body weight, satiation, caecum short chain fatty acids body, and hepatic lipid content on diet-induced obese rats for obesity management. A total of 36 male Sprague Dawley rats were fat-induced and divided into the obesity-prone and obesity-resistant groups. Eight percent and sixteen percent resistant starch from sago and Hi-maize260 were incorporated into the standardised feed formulation. Food intake was weighed throughout the intervention period. The caecum sample was subjected to short chain fatty acids analysis using HPLC. Hepatic lipid content was measured using the Folch method. Both dosages of sago starch (8 and 16% SRS) promoted body weight loss with a reduction of food intake, which suggested satiety. No significant differences was observed in the production of lactate, acetate, propionate, and butyrate from the caecum sample. Both dosages of sago starch (8 and 16% SRS) also showed lower hepatic lipid content and visceral adipose tissue than the baseline and control groups. However, 8% sago starch showed the lowest hepatic lipid content in obesity-prone and obesity-resistant groups. Overall results demonstrated that sago starch has the potential as an obesity and overweightness control regime as it promotes satiety, lowers visceral adipose tissue, and reduces hepatic lipid content. Consumers should consider adding sago starch in their daily meals.

Introduction

Obesity is turning into a common condition among adults and children. Worldwide statistics showed nearly 39% of adults (age 18 and above) and 26.5% of children (age 9 years old below) were overweight in 2019 (Elflien, 2019). Obesity and overweightness are primary public concerns as these conditions are associated with one's reduced quality of life and poor mental health (Kim *et al.,* 2020). Obesity poses health risks as it is commonly associated with comorbidities such as cancer, type-2 diabetes, dyslipidaemia, hypertension, and cardiovascular diseases (Chu *et al.,* 2018). The major causes of obesity are modern lifestyles that lack in physical activity, high calorie diet, endocrine disorder, and genetic inheritance (Nijhawan and Behl, 2020). Obesity and overweightness are illnesses preventable through diet and exercise.

As the result of high costs and concern on the possibility of hazardous side effects using medical approach, the demand for therapeutically potent and safe anti-obesity agents derived from natural products has increased (Choi *et al.,* 2007; Yun, 2010). In the present work, sago starch (*Metroxylon sagu*) is used as the test substrate. Sago starch is one of Malaysia's most important agricultural commodities, particularly in Sarawak, where 96% of the yields are (Uthumporn *et al.,* 2014; Zi-Ni *et al.,* 2015). Currently, Malaysia is the largest sago starch exporter with annual extracted starch stands at 40,000 to 51,000 metric tonnes, from 2004 to 2013 (Uthumporn *et al.,* 2014). Sago starch extracted per unit area is significantly higher as compared to other starch resources such as rice, corn,

*Corresponding author.
Email: s.r.sarbini@gmail.com

DOI: https://doi.org/10.47836/ifrj.28.5.19

wheat, and cassava. Sago starch could produce up to 25 tonnes per hectare per year (Silvi *et al.,* 1999). However, the consumption of sago takes up only 3% of starch resources as compared to the other dominated starch such as tapioca, potato, and corn, which can total up to 300,000 tonnes annually. To increase the competitiveness with other sources, a functional value should be added as a marketing strategy, and this can indirectly contribute to the growth of sago-farming.

In the present work, we are focusing on resistant starch as the main food ingredient. Previous study reported that sago starch has high resistant starch (RS), as high as 69% (Zaman, 2015). Resistant starch is the starch fraction residue that can resist enzyme hydrolysis prior to entering the colon, and has been gaining attention as prebiotic due to its ability to confer health benefits (Zaman and Sarbini, 2016). Prebiotic is defined as "a substrate that is selectively utilised by microorganisms conferring a health benefit" (Gibson *et al.,* 2017). It is proposed that upon the consumption of prebiotic, it modulates the gut microbiota which produce gut fermentation products that will be absorbed by the colonocytes.

Several mechanisms have been proposed to account for the potential influences of the microbiota on obesity. It has been determined that obesity and insulin resistance are associated with low-grade chronic systemic inflammation by the action of bacterial lipopolysaccharide (LPS) (Kallus and Brandt, 2012). These bacterial LPS are found on the outer membrane of bacterial cells, particularly the Gram-negative bacteria. The bacterial LPS are physiologically translocated into intestinal capillaries, and trigger the secretion of pro-inflammatory cytokines in response to a high-fat diet (Wright *et al.,* 1990; Sweet and Hume, 1996; Neal *et al.,* 2006). These cytokines, which are the key inducers to insulin resistance, will promote excessive hepatic and adipose tissue lipid storage, thus leading to weight gain and obesity (Cani, 2007; Kallus and Brandt, 2012).

The beneficial effects of RS have been extensively reviewed. The benefits include improved insulin sensitivity (Robertson *et al.,* 2003; Belobrajdic *et al.,* 2012; Bindels *et al.,* 2017), fat oxidation (So *et al.,* 2007; Shen *et al.,* 2015), and satiety (Belobrajdic *et al.,* 2012; Sardá *et al.,* 2016; Ble-Castillo *et al.,* 2017). However, there has been no study conducted on the anti-obesity effects of sago starch on animals or humans. The present work was therefore designed to investigate the anti-obesity properties of sago starch on the body weight and food intake of obesity-prone and obesity-resistant rats fed with sago starch.

In the present work, the rats were fed with different dosages of sago starch, and the effects of the intake on gut fermentation products from caecum (lactate, acetate, propionate, and butyrate) and hepatic lipid content were evaluated. Liver was chosen as one of the parameters because obesity is related to an increase number of non-alcoholic fatty liver diseases (Sarwar *et al.,* 2018). Caecum content was used instead of the whole gut content because it has been proposed to serve as a reservoir of anaerobic bacteria that populate the colon (Brown *et al.,* 2018). Mouse's or rat's caecum is the major site for gut fermentation. The gut fermentation decreases along the colon as a function of distance from the caecum due to absorption by epithelial cells lining of colon for energy (*e.g.,* butyrate), or for cholesterol, fat, and sugar metabolisms (*e.g.,* acetate and propionate) (Tremaroli and Bäckhed, 2012). The diet-induced obese rats closely mimic obese humans in developing insulin resistance and dyslipidaemia (Levin et al., 1989). Thus, the resulting changes can be comparable to the human body rather than the genetically modified obese rats.

Materials and methods

Materials

Unless otherwise stated, all chemicals used in the present work were purchased from Sigma (Gillingham, Dorset, UK). The commercial Hi-maize® 260 (Ingredion, USA) was used as positive control, while the sago starch (purchased from the local market) was used as the test substrate.

Animal study

All experimental protocols were approved by the Animal Care and Use Committee, National Institute of Health, Ministry of Health Malaysia (No. ACUC/KKM/02(6/2014)). A total of 36 male Sprague Dawley rats, seven weeks old, were purchased from the Animal Research Centre, Australia. The rats were housed in individual cages in a room with controlled heating and lighting (23°C with a 12-h light/dark cycle). Each cage was ventilated individually using the Techniplast individual ventilation cage system (Techniplast, Buguggiate, Italy). Food and water supply were provided *ad libitum*. At the age of nine weeks, all rats were given a fat-induced diet (containing 10% of fats) (Table 1). After five weeks, the rats that had gained the most weight (40% and above of the original weight) were classified as obesity-prone (OP), while the rats with the least weight (did not

Table 1. The composition of feed treatment based on AIN-93G formulation.

	Fat-induced	0% RS[1]	8% HRS[1]	16% HRS[1]	8% SRS[1]	16% SRS[1]
Composition (g/kg)						
Corn starch	200.0	530.0	400.0	270.0	365.0	400.0
Hi-maize® 260	0.0	0.0	130	260.0	0.0	0.0
Sago starch	0.00	0.0	0.00	0.0	65.0	130.0
Wheat bran	0.0	50.0	50.0	50.0	50.0	50.0
Maltodextrin	75.0	0.0	0.0	0.0	0.0	0.0
Cellulose	30.0	0.0	0.0	0.0	0.0	0.0
Sucrose	240.0	100.0	100.0	100.0	100.0	100.0
Anhydrous milk fat	100.0	0.0	0.0	0.0	0.0	0.0
Sunflower seed oil	100.0	70.0	70.0	70.0	70.0	70.0
L-cystine	3.0	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Casein	190.0	200.0	200.0	200.0	200.0	200.0
Mineral mix[2]	40.0	35.0	35.0	35.0	35.0	35.0
Vitamin mix[2]	10.0	10.0	10.0	10.0	10.0	10.0
TBHQ (antioxidant)	0.008	0.008	0.008	0.008	0.008	0.008
Composite analysis						
Carbohydrate (%)	63.84	67.18	65.55	66.23	67.90	65.08
Protein (%)	17.99	18.98	20.03	20.13	18.82	21.07
Fats (%)	6.07	1.3	1.59	1.50	1.53	1.70
Ash (%)	3.47	2.81	3.18	2.70	2.83	2.69
Fibre (%)	1.26	0.1	0.06	0.17	0.05	0.15
Moisture content (%)	7.38	9.63	9.61	9.29	9.24	9.33
Energy intake (kcal/100 g)[3]	366.07	342.76	341.10	343.46	347.29	342.77

[1]RS = resistant starch, HRS = Hi-maize group, and SRS = sago starch group. The amounts of RS in the diets as fed were 0, 4, 8 and 16 g/100 g of the diet. These levels were added based on RS percentage in Hi-maize (total starch = 91; RS = 28 - 31%) and sago starch (total starch 80; RS = 58 - 60%), based on its dry weight basis using resistant starch assay kit following the method of AOAC 2002.02 and AACC 38-40.01 (Megazyme, Wicklow, Ireland). [2]Listed items were of analytical grade from MPBioMed. [3]Energy intake was calculated based on the following formula described by Crisan and Sands (1978): Energy value (kcal/100 g) = (2.62 × % protein) + (8.37 × % fat) + (4.2 × % carbohydrate).

reach the 40% of the gained weight) were classified as obesity-resistant (OR) (Belobrajdic *et al.,* 2012). After one more week, six rats were randomly selected [baseline group containing OP and OR rats (n = 3, respectively)] for baseline hepatic lipid content, caecum short chain fatty acids profiling, and visceral adipose tissue weight. The remaining rats were allocated randomly into one of five dietary treatment groups (n = 6) containing equal numbers (n = 3) of OP and OR rats.

At the age of 15 weeks, the rats were treated with RS-enriched diets: 0% RS (control), 8% Hi Maize RS (HRS), 16% HRS, 8% sago RS (SRS), and 16% SRS. The body weight trend was recorded at weeks 0, 2, 4, 6, and 7. Monitoring was conducted twice a day; in the morning and evening. The feeding

treatment lasted for eight weeks. At the end of the RS intervention period, the rats were fasted overnight. On the following day, the rats were humanely anaesthetised using an inhalant anaesthetic agent, 5% isoflurane in oxygen, and confirmed unconscious by a veterinary officer prior to necropsy. The liver, caecum, and visceral adipose tissue were collected and weighed. The caecum contents were removed, weighed, and stored at -20°C for short chain fatty acids (SCFA) analysis.

Short chain fatty acids analysis

The caecum samples were diluted to 1:20 (w/v) in 1X phosphate buffered saline solution. The suspension was pipetted into a 1.5 mL centrifuge tube, vortexed for 1 min, and then centrifuged at 13,000 rpm for 10 min. The resulting supernatant was then filtered through a 0.22 µM filter unit (Millipore, France).

The analysis of caecum sample was performed using an ion exclusion HPLC system (Allianze HPLC, Waters Corporation, Massachusetts, US) equipped with a pump (WATERS 600), a UV detector (WATERS 2998), and an autosampler (WATERS 2707). Data were collected using Empower 2 Feature Release 5 (WATERS, Milford, Ireland). The column used was Rezex™ ROA-Organic Acid H+ (8%) (300 × 7.8 mm) (Phenomenex, US). The Rezex ROA Organic Acid precolumn (50 × 7.8 mm) (Phenomenex, New Jersey, US) guard column was used to protect the column from any particles that might have been injected together with the samples. The mobile phase used was 0.0025 M sulphuric acids in ultrapure water. An amount of 20 µL filtered caecum sample was injected into the HPLC, operating at a flow rate of 0.8 mL/min, with a heated column at 62°C. The sample run time was 35 min. Sample quantification was carried out using calibration curves of the external standard mixture of lactate, acetate, propionate, and butyrate at the concentrations of 12.5, 25, 50, 75, and 100 mM.

Hepatic lipid determination

Hepatic lipids were extracted following the procedures prescribed by Folch *et al.* (1957). The liver tissue was washed briefly with saline solution to eliminate any traces of blood. Then, the liver sample was homogenised with a 2:1 chloroform:methanol solution. Samples were then incubated at 50°C for 30 min, with 2 mL of KCl 0.1 M to quicken the phase separation process. The mixture was vortexed for 1 min.

The samples were kept for 2 h at 4°C, and then centrifuged at 2,500 rpm for 20 min to facilitate the separation of the upper phase (aqueous methanol dragging) and the lower phase (chloroform phase) containing the lipids. Most of the aqueous phase was removed, and the chloroform phase was adjusted to a known final volume with chloroform. The chloroform phase was dried under a nitrogen gas stream. The tube was weighed again, and the amount of fat was calculated using the gravimetric method. The following formula was used to calculate the total fats in the liver sample:

Lipid content = [Extraction of dried fats (g) / Weight of sample (g)] / 10

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS) version 9.3 (Cary, NC). Univariate analysis of variance (ANOVA) and *post hoc* Tukey's test were used to determine the significant difference of substrate on body weight, organ weight, adipose tissue, weight loss, weight loss percentage, cumulative feed intake, SCFA production, and liver fats content. Differences were deemed significant when $p < 0.05$. Pearson correlation analysis was used to evaluate the functional correlation between feed intake and body weight. Correlations were deemed significant at $p < 0.05$.

Results and discussion

The present work showed that sago starch works similarly with commercial resistant starch, Hi-maize®260, as a weight loss compound. It was also showed that sago starch (8 and 16% SRS) positively promotes weight loss (Table 2). A decrease in cumulative food intake (Figure 1) was detected with an increment of RS dosage from 8 to 16% SRS. Tables 3 and 4 display a strong correlation between cumulative food intake and body weight loss. These patterns may suggest satiety. Satiety is a full sensation in the gut following food consumption (Blundell *et al.,* 2010). Previous studies have also shown that the digestibility of starch significantly influenced the satiation in pre-school children (Alvina and Araya, 2004). This may suggest that sago starches can trigger a stimulus that promotes satiation within the host, thus suppressing further food consumption. The gut hormone signalling was not observed in the present work. However, a RS-enriched diet has been reported to induce the gut signalling hormone such as peptide YY and glucagon-like peptide-1, which reduced the appetite

Table 2. Growth performance of Sprague Dawley rats during eight weeks of RS-enriched treatment.

	Obesity-prone					Obesity-resistant				
	0% RS[1]	8% SRS[1]	16% SRS[1]	8% HRS[1]	16% HRS[1]	0% RS[1]	8% SRS[1]	16% SRS[1]	8% HRS[1]	16% HRS[1]
Initial weight (g)	602.99	559.18	546.24	557.22	580.56	515.32	505.31	533.01	518.42	531.04
Final weight (g)	582.65	464.25	434.56	467.27	484.75	490.45	430.03	450.50	449.40	452.32
Weight loss/gain (g)	32.00^{a**}	94.93^{b**}	111.68^{b**}	89.95^{b**}	95.80^{b**}	24.87^{a*}	75.28^{b*}	82.51^{b*}	69.02^{ab*}	78.73^{b*}
Weight loss (%BW)	5.26^{a*}	16.90^{ab*}	19.97^{b*}	16.17^{ab*}	16.50^{ab*}	4.83^{a*}	15.06^{b*}	15.463^{b*}	13.27^{ab*}	14.82^{b*}

[1]RS = resistant starch, SRS = sago starch group, and HRS = Hi-maize group. Values are mean ± SE of triplicates (n = 3). Means followed by different lowercase superscripts within the same row indicate significant difference at $p < 0.05$ among treatment within the phenotype. *correlation significant at $p < 0.05$, and **correlation significant at $p < 0.0001$.

Table 3. Pearson correlation coefficient between feed intake (FI) and body weight (BW) of Sprague Dawley rats fed with different levels of resistant starch from sago starch and Hi-maize, as shown in the parentheses (n = 3) in obesity-prone rats.

	FI (0% RS)	FI (8% SRS)	FI (16% SRS)	FI (8% HRS)	FI (16% HRS)
BW (0% RS)	0.2803*				
BW (8% SRS)		0.8399**			
BW (16% SRS)			0.9527***		
BW (8% HRS)				0.9483***	
BW (16% HRS)					0.7984**

RS = resistant starch, SRS = sago starch group, and HRS = Hi-maize group. *correlation significant at $p < 0.05$, **correlation significant at $p < 0.001$, and ***correlation significant at $p < 0.0001$.

Table 4. Pearson correlation coefficient between feed intake (FI) and body weight (BW) of Sprague Dawley rats fed with different levels of resistant starch from sago starch and Hi-maize as shown in the parentheses (n = 3) in obesity-resistant rats.

	FI (0% RS)	FI (8% SRS)	FI (16% SRS)	FI (8% HRS)	FI (16% HRS)
BW (0% RS)	0.2689*				
BW (8% SRS)		0.9352**			
BW (16% SRS)			0.9405**		
BW (8% HRS)				0.9058**	
BW (16% HRS)					0.6421*

RS = resistant starch, SRS = sago starch group, and HRS = Hi-maize group. *correlation significant at $p < 0.05$, and **correlation significant at $p < 0.0001$.

and food intake in both animal and human studies (Manz *et al.,* 1996; Raman *et al.,* 2016). Apart from that, satiety was also postulated due to the concentration of gastric inhibitor polypeptide (Al-Lahham *et al.,* 2010; Van Kleef *et al.,* 2012). Nevertheless, the measurement of a true value of satiety is complicated when conducted in animal studies as the subjects may be influenced by other

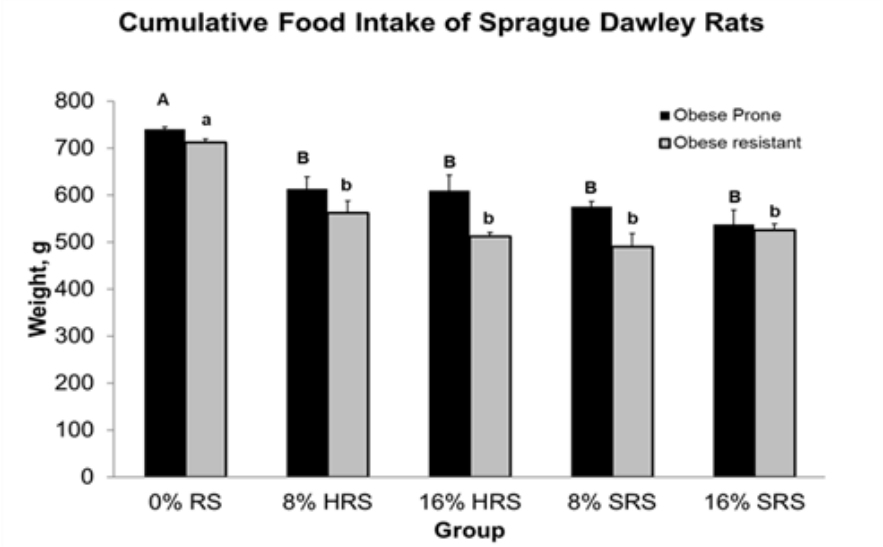

Figure 1. Cumulative food intake for eight weeks of RS enriched diet in obesity-prone and obesity-resistant (n = 3) groups. RS = resistant starch, HRS = Hi-maize group, and SRS = sago starch group. Analysis of variance (ANOVA) and *post hoc* Tukey's test were used to determine the significant difference of substrate. Different letters indicate significant difference at $p < 0.001$ within phenotype group.

factors such as physiological, *i.e.,* homeostatic appetite control, environmental factors or food appearance, and palatability (Benelam, 2009; Amin and Mercer, 2016). Thus, introducing a food ingredient that does not only suppresses appetite but also desirable to eat is needed to facilitate healthier food choices, especially when the ingredient is introduced for human consumption.

Table 5 shows no significant difference between the experimental diet in the production of lactate, acetate, propionate, and butyrate for OR and OP groups. Sago starch exhibited similar effects as the Hi-maize® 260 in lactate and SCFA profile. Many past studies have reported that acetate is the highest SCFA produced, followed by propionate and butyrate (Isken *et al.,* 2010). In the present work, a plausible factor causing the highest acetate production was the RS structure which is made of compact linear structure with α-1,4-linked glucose units within the starch granules. This renders it indigestible by enzymatic digestion in the large intestine (Nishina and Freedland, 1990). Resistant starch consumption can alter the abundance of intestinal bacterial genera and species. The *Ruminococcus* genus can elevate the supplementation of RS2 in the diet in several human and animal studies (Kieffer *et al.,* 2016; Maier *et al.,* 2017; Goldsmith *et al.,* 2017). It was also reported as one of acetate producers (Cummings *et al.,* 1987; Isken *et al.,* 2010). This could explain the high acetate concentration observed in the present work. *In vitro* analysis could be conducted to provide a more

Table 5. Mean value of organic acids (mM) between obesity-prone (n = 3) and obesity-resistant (n = 3) in the caecum sample of Sprague Dawley rats.

Organic acid (mM)	Obesity-prone						Obesity-resistant					
	Fat-induced	0% RS	8% SRS	16% SRS	8% HRS	16% HRS	Fats induced	0% RS	8% SRS	16% SRS	8% HRS	16% HRS
Lactate	14.95	27.85	34.87	24.86	9.98	30.49	51.42	15.14	30.07	20.04	41.87	28.84
Acetate	74.26	78.02	84.89	75.92	69.78	60.46	31.73	67.06	81.3	63.64	53.97	73.63
Propionate	7.29	9.59	14.17	14.47	15.13	15.28	11.83	9.35	17.4	20.46	15.41	18.77
Butyrate	11.96	9.75	20.2	19.8	14.36	17.27	7.12	6.95	18.73	17.13	14.41	16.59
Total SCFA	93.52	92.57	119.26	110.2	99.71	92.86	50.67^{c}	86.49^{ab}	117.43^{a}	97.87^{ab}	78.80^{ab}	108.99^{ab}

RS = resistant starch, SRS = sago starch group, and HRS = Hi-maize group. Values are mean ± SE of triplicates (n = 3). Means followed by different lowercase superscripts within the same row indicate significant difference at $p < 0.05$ among treatment within the phenotype.

detailed SCFA production pattern. Bacterial enumeration should also accompany the SCFA analysis to further understand the relationship between gut microbiota composition and the production of SCFA.

In the present work, the RS-enriched diet significantly influenced liver organ weight in the OP group but not in the OR group (Table 6). Table 6 also shows that RS-enriched diet reduced the hepatic lipid content. This effect can be observed in the OP group but not in the OR group when compared with fat-induced group (baseline). Additionally, the highest visceral adipose tissue was observed in 0% RS for both OP and OR groups. The least hepatic lipid content was observed in both OP (18.68%) and OR (18.51%) groups which were supplemented with 8% SRS diet. The RS-enriched diet had significantly reduced visceral adipose tissue in the OP group, but not in the OR group. The highest visceral adipose tissue was observed in 0% RS diet group with 29.82 g for OP and fat-induced groups, and 30.21 g for OR group.

The SCFA production from colonic fermentation may have a link with visceral adipose tissue and hepatic lipid content. It is well known that SCFA such as acetate, propionate, and butyrate have their own distinctive functions. Acetate is known for contributing to lipid and cholesterol synthesis in the kidney and liver (Mookerjea and Sadhu, 1955). In the present work, however, the 8% sago starch in both phenotypes showed the highest acetate concentration with the lowest hepatic lipid content (18.65% OP; 18.51% OR) and low visceral adipose tissue (14.04 g OP; 11.32 g OR). Previous studies conducted on high fat diet rats reported that acetate yielded the best body weight gain suppression effect among the three SCFA (Lu *et al.,* 2016). Similar studies also reported on the reduced size of adipose tissue when genetically-modified obese rats were treated with acetate (Yamashita *et al.,* 2009). This result may suggest that it is possible that acetate affects lipid oxidation instead of promoting lipid synthesis. Furthermore, propionate has been reported to reduce the development of white fats tissue (Cummings *et al.,* 1987). Propionate is absorbed and metabolised by the liver for gluconeogenesis, a process of glucose synthesis by breaking down lipids and proteins (Byrne *et al.,* 2015). The inconsistency of hepatic lipid content and the accumulation of visceral adipose tissue may be influenced by SCFA translocated into the colonic epithelial cells of every rat.

Nevertheless, the effectiveness of RS in reducing body fat does not solely depend on the food ingredient. Adding RS may alter the physiological responses such as glycaemic response, which reduces fat synthesis (Shen *et al.,* 2015). The glycaemic response can be regulated with a low glycaemic

Table 6. Total hepatic lipid content and abdominal fat tissue in Sprague Dawley rats.

Phenotype	Diet	Abdominal fat tissue (g)	Liver (g)	Hepatic lipid content (%)
Obesity-prone	Fats induced	29.59 ± 5.96^{a}	16.48 ± 0.55^{abc}	39.25^{a}
	0% RS	29.82 ± 4.40^{a}	17.51 ± 0.36^{ab}	40.48^{a}
	8% SRS	14.04 ± 2.61^{b}	16.93 ± 1.11^{ab}	18.68^{b}
	16% SRS	13.59 ± 3.42^{b}	12.71 ± 0.51^{c}	19.40^{b}
	8% HRS	13.05 ± 2.62^{b}	14.85 ± 0.83^{bc}	20.08^{b}
	16% HRS	15.12 ± 1.18^{b}	16.81 ± 0.29^{ab}	20.59^{b}
Obesity-resistant	Fats induced	30.21 ± 8.95	14.83 ± 1.90	35.89
	0% RS	23.94 ± 4.35	15.28 ± 1.36	37.33
	8% SRS	11.32 ± 2.03	15.50 ± 2.03	18.51
	16% SRS	11.34 ± 1.69	13.92 ± 0.06	20.58
	8% HRS	10.20 ± 1.87	15.73 ± 0.34	19.15
	16% HRS	16.55 ± 1.41	13.76 ± 1.88	20.55

RS = resistant starch, SRS = sago starch group, and HRS = Hi-maize group. Values are mean ± SE of triplicates (n = 3). Means followed by different lowercase superscripts within the same column within phenotype group indicate significant difference at $p < 0.05$.

index food such as RS (Al-Lahham *et al.,* 2010). Sago starch with 12.95% RS content was reported to have a GI value of 40.95 (Wahjuningsih *et al.,* 2016). Foods are ranked based on their GI values; GI < 55 is low; 56 - 75 GI is moderate; and 76 - 100 GI is high. Low GI denotes the slowest time taken to increase the blood glucose. Low GI food improves insulin sensitivity. Insulin is the hormone responsible to metabolise glucose to be used as fuel or stored as fats. Thus, this explains the lowest body fat content observed in 16% sago starch treatment; due to its low GI value.

Conclusion

Studies on indigestibility of sago starch, especially on its health benefits, are relatively new as it is underexploited and only consumed by the people in Southeast Asia. The results obtained in the present work provide evidence that the supplementation of RS produced from sago starch in diet improves the overall growth performance of fat-induced Sprague Dawley rats, especially in feed intake, body weight pattern, the production of lactate, and SCFA, specifically acetate. Additionally, sago starch was showed to have satiety effects. The 16% RS content, regardless of the sources, led to the lowest food intake and the highest percentage of weight loss. The reduction of body weight and food intake upon the consumption of sago starch confirms that sago starch is suitable as an anti-obesity ingredient. Moreover, the foods were given *ad libitum*; this suggests that RS may influence the food termination signal, one of the markers when finding therapeutic approach to combat overweight and obesity development.

Apart from that, body fat content showed that the higher the RS was from sago starch, the lower the RS accumulation in the body was. Hepatic lipid content revealed that a higher percentage of RS in diet does not further reduce the accumulation of visceral adipose tissue and hepatic lipid content. The 8% SRS led to lower hepatic lipid content as compared to 16% SRS which may indicate the threshold of dosage to combat hepatic lipid accumulation within eight weeks study on animals.

Acknowledgement

The authors would like to thank the Institute of Medical Research staff, especially from the Nutrition Unit and Animal House Unit, for their assistance. The Malaysian Ministry of Education financially supported the present work through the Fundamental Research Grant Scheme (grant no.: FRGS/2/2013/SG05/UPM/02/23).

References

Al-Lahham, S., Peppelenbosch, M., Roelofsen, H., Vonk, R. and Venema, K. 2010. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochimica et Biophysica Acta 1801: 1175-1183.

Alvina, M. and Araya, H. 2004. Rapid carbohydrate digestion rate produced lesser short-term satiety in obese preschool children. European Journal of Clinical Nutrition 58(4): 637-642.

Amin, T. and Mercer, J. 2016. Hunger and satiety mechanisms and their potential exploitation in the regulation of food intake. Current Obesity Reports 5(1): 106-112.

Belobrajdic, D., King, R., Christophersen, C. and Bird, A. 2012. Dietary resistant starch dose-dependently reduces adiposity in obesity-prone and obesity-resistant male rats. Nutrition and Metabolism 9(1): article no. 93.

Benelam, B. 2009. Satiation, satiety and their effects on eating behaviour. Nutrition Bulletin 34(2): 126-173.

Bindels, L. B., Munoz, R. R. S., Gomes-Neto, J. C., Mutemberezi, V., Martínez, I., Salazar, N., … and de Los Reyes-Gavilán, C. G. 2017. Resistant starch can improve insulin sensitivity independently of the gut microbiota. Microbiome 5(1): article no. 12.

Ble-Castillo, J. L., Juárez-Rojop, I. E., Tovilla-Zárate, C. A., García-Vázquez, C., Servin-Cruz, M. Z., Rodríguez-Hernández, A., ... and Díaz-Zagoya, J. C. 2017. Acute consumption of resistant starch reduces food intake but has no effect on appetite ratings in healthy subjects. Nutrients 9(7): article no. 696.

Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., ... and Van Der Knaap, H. 2010. Appetite control: methodological aspects of the evaluation of foods. Obesity Reviews 11(3): 251-270.

Brown, K., Abbott, D. W., Uwiera, R. R. and Inglis, G. D. 2018. Removal of the cecum affects intestinal fermentation, enteric bacterial community structure, and acute colitis in mice. Gut Microbes 9(3): 218-235.

Byrne, C., Chambers, E., Morrison, D. and Frost, G. 2015. The role of short chain fatty acids in appetite regulation and energy homeostasis. International Journal of Obesity 39(9): 1331-1338.

Cani, P. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes

56: 1761-1772.

Choi, H., Eo, H., Park, K., Jin, M., Park, E.-J., Kim, S.-H., ... and Kim, S. 2007. A water-soluble extract from *Cucurbita moschata* shows anti-obesity effects by controlling lipid metabolism in a high fat diet-induced obesity mouse model. Biochemical and Biophysical Research Communications 359(3): 419-425.

Chu, D.-T., Nguyet, N. T. M., Dinh, T. C., Lien, N. V. T., Nguyen, K.-H., Ngoc, V. T. N., ... and Jurgoński, A. 2018. An update on physical health and economic consequences of overweight and obesity. Diabetes and Metabolic Syndrome - Clinical Research and Reviews 12(6): 1095-1100.

Crisan, E. and Sands, A. 1978. Nutritional value. New York: Academic Press.

Cummings, J., Pomare, E., Branch, W., Naylor, C. and Macfarlane, G. 1987. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28: 1221-1227.

Elflien, J. 2019. Overweight prevalence by age worldwide 2019. Retrieved from website: https://www.statista.com/statistics/1065605/prevalence-overweight-people-worldwide-by-age/ 2020

Folch, J., Lees, M. and Sloane-Stanley, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226(1): 497-509.

Gibson, G., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., ... and Cani, P. D. 2017. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology and Hepatology 14(8): article no. 491.

Goldsmith, F., Guice, J., Page, R., Welsh, D. A., Taylor, C. M., Blanchard, E. E., ... and Carvajal-Aldaz, D. 2017. Obese ZDF rats fermented resistant starch with effects on gut microbiota but no reduction in abdominal fat. Molecular Nutrition and Food Research 61(1): article ID 1501025.

Isken, F., Klaus, S., Petzke, K., Loddenkemper, C., Pfeiffer, A. and Weickert, M. 2010. Impairment of fat oxidation under high- vs. low-glycemic index diet occurs before the development of an obese phenotype. American Journal of Physiology - Endocrinology and Metabolism 298: E287-E295.

Kallus, S. J. and Brandt, L. J. 2012. The intestinal microbiota and obesity. Journal of Clinical Gastroenterology 46(1): 16-24.

Kieffer, D. A., Piccolo, B. D., Vaziri, N. D., Liu, S., Lau, W. L., Khazaeli, M., ... and Martin, R. J. 2016. Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. American Journal of Physiology - Renal Physiology 310(9): F857-F871.

Kim, S.-R., Kim, H.-N. and Song, S.-W. 2020. Associations between mental health, quality of life, and obesity/metabolic risk phenotypes. Metabolic Syndrome and Related Disorders 18(7): 347-352.

Levin, B. E., Hogan, S. and Sullivan, A. 1989. Initiation and perpetuation of obesity and obesity resistance in rats. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 256(3): R766-R771.

Lu, Y., Fan, C., Li, P., Lu, Y., Chang, X. and Qi, K. 2016. Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating G protein-coupled receptors and gut microbiota. Scientific Reports 6: article ID 37589.

Maier, T. V., Lucio, M., Lee, L. H., VerBerkmoes, N. C., Brislawn, C. J., Bernhardt, J., ... and Heinzmann, S. S. 2017. Impact of dietary resistant starch on the human gut microbiome, metaproteome, and metabolome. mBio 8: article ID e01343-17.

Manz, W., Amann, R., Ludwig, W., Vancanneyt, M. and Schleifer, K.-H. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum Cytophaga-Flavobacter-Bacteroides in the natural environment. Microbiology 142(5): 1097-1106.

Mookerjea, S. and Sadhu, D. 1955. Metabolism of acetic acid in kidney and liver slices of the hypothyroid rats endocrinology 56(5): 507-510.

Neal, M. D., Leaphart, C., Levy, R., Prince, J., Billiar, T. R., Watkins, S., ... and Schreiber, A. 2006. Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. Journal of Immunology 176(5): 3070-3079.

Nijhawan, P. and Behl, T. 2020. Role of sirtuins in obesity. Obesity Medicine 17: article ID 100156.

Nishina, P. and Freedland, R. 1990. Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. Journal of Nutrition 120: 668-673.

Raman, M., Ambalam, P. and Doble, M. 2016. Short-chain fatty acids. In Raman, M., Ambalam, P. and Doble, M. (eds). Probiotics and Bioactive Carbohydrates in Colon Cancer

Management, p. 97-115. India: Springer.

Robertson, M., Currie, J., Morgan, L., Jewell, D. and Frayn, K. 2003. Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects. Diabetologia 46: 659-665.

Sardá, F. A. H., Giuntini, E. B., Gomez, M. L. P., Lui, M. C. Y., Negrini, J. A., Tadini, C. C., ... and Menezes, E. W. 2016. Impact of resistant starch from unripe banana flour on hunger, satiety, and glucose homeostasis in healthy volunteers. Journal of Functional Foods 24: 63-74.

Sarwar, R., Pierce, N. and Koppe, S. 2018. Obesity and nonalcoholic fatty liver disease: current perspectives. Diabetes, Metabolic Syndrome and Obesity - Targets and Therapy 11: 533-542.

Shen, R.-L., Zhang, W.-L., Dong, J.-L., Ren, G.-X. and Chen, M. 2015. Sorghum resistant starch reduces adiposity in high-fat diet-induced overweight and obese rats via mechanisms involving adipokines and intestinal flora. Food and Agricultural Immunology 26(1): 120-130.

Silvi, S., Rumney, C., Cresci, A. and Rowland, I. 1999. Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with faeces from Italian and UK donors. Journal of Applied Microbiology 86(3): 521-530.

So, P.-W., Yu, W.-S., Kuo, Y.-T., Wasserfall, C., Goldstone, A. P., Bell, J. D. and Frost, G. 2007. Impact of resistant starch on body fat patterning and central appetite regulation. PLoS One 2(12): article ID e1309.

Sweet, M. J. and Hume, D. A. 1996. Endotoxin signal transduction in macrophages. Journal of Leukocyte Biology 60(1): 8-26.

Tremaroli, V. and Bäckhed, F. 2012. Functional interactions between the gut microbiota and host metabolism. Nature 489: 242-249.

Uthumporn, U., Wahidah, N. and Karim, A. 2014. Physicochemical properties of starch from sago (*Metroxylon sagu*) palm grown in mineral soil at different growth stages. IOP Conference Series - Materials Science and Engineering 62: article ID 012026.

Van Kleef, E., Van Trijp, J., Van Den Borne, J. and Zondervan, C. 2012. Successful development of satiety enhancing food products: towards a multidisciplinary agenda of research challenges. Critical Reviews in Food Science and Nutrition 52(7): 611-628.

Wahjuningsih, S. B., Marsono, Y., Praseptiangga, D. and Haryanto, B. 2016. Resistant starch content and glycaemic index of sago (*Metroxylon* spp.) starch and red bean (*Phaseolus vulgaris*) based analogue ric. Pakistan Journal of Nutrition 15(7): 667-672.

Wright, S. D., Ramos, R. A., Tobias, P. S., Ulevitch, R. J. and Mathison, J. C. 1990. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 249: 1431-1433.

Yamashita, H., Maruta, H., Jozuka, M., Kimura, R., Iwabuchi, H., Yamato, M., ... and Kimoto, M. 2009. Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Bioscience, Biotechnology, and Biochemistry 73(3): 570-576.

Yun, J. W. 2010. Possible anti-obesity therapeutics from nature - a review. Phytochemistry 71(14-15): 1625-1641.

Zaman, S. A. 2015. Production of resistant starch type III and IV and their *in-vivo* characterisation using balb/c mice model. Malaysia: Universiti Putra Malaysia, MSc thesis.

Zaman, S. A. and Sarbini, S. R. 2016. The potential of resistant starch as a prebiotic. Critical Reviews in Biotechnology 36(3): 578-584.

Zi-Ni, T., Rosma, A., Karim, A. and Liong, M. 2015. Functional properties of resistant starch type-III from *Metroxylon sagu* as affected by processing conditions. Pertanika Journal of Tropical Agricultural Science 38(3): 399-412.