

Resistant Starch Content, Starch Digestibility and the Fermentation of Some Tropical Starches In Vitro

Alfonso Martín Bernabé · Khongsak Sriakao ·
Marina Schlüter

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Abstract Tropical starches including cereal starches (waxy, non-waxy rice and maize), legume starch (mung beans), tuber starches (potatoes, edible cannas, taros and tapiocas) and starchy fruit (unripe bananas) were investigated for their resistant starch (RS) content, thermal properties by differential scanning calorimetry, starch digestibility and the production of short chain fatty acids (SCFAs) through fermentation processes in the colon by an in vitro method. Two commercial high-fibre modified starches from corn and tapioca were also investigated for comparison. It was found that RS content of the samples varied from 10 to 52 g/100 g dry sample. All starch samples showed a single endothermic peak located broadly around 60–99 °C except both the commercial modified starches. The starch samples also exhibited as the very rapidly digested starch resulting in high values of predicted glycaemic index, ranging from 53 to 94. In vitro fermentation of the starch samples showed that SCFA production increased as the RS content of the samples increased.

Keywords Starch · Resistant starch · Starch digestibility · Starch fermentation · Short chain fatty acid

Introduction

Starch is the major source of carbohydrate in human diets [1]. Dietary starches are important sources of energy and they can also make quite specific contributions to health [2]. Starch researches have grown enormously in recent years. Many studies have reported differences in composition, structure and properties among starches from different sources [3–8]. Recently, resistant starch (RS) has received much attention for both its potential health benefits (similar to soluble fibre) and functional properties. RS positively influences the functioning of the digestive tract, microbial flora, the blood cholesterol level, the glycaemic index (GI) and assists in the control of diabetes [2]. RS escapes digestion in the human small intestine and is fermented in the colon with production of metabolically active short chain fatty acids (SCFAs) [9]. RS is an extremely broad and diverse range of materials and a number of different types exist. At present, these are mostly defined according to physical and chemical characteristics [10]. The four distinct classes of RS in foods are: (1) RS1—physically inaccessible starch, which is entrapped within whole or partly milled grains or seeds; (2) RS2—some types of raw starch granules (such as banana and potato) and high-amylose (high-amylose corn) starches; (3) RS3—retrograded starch (either processed from unmodified starch or resulting from food processing applications); (4) RS4—starches that are chemically modified to obtain resistance to enzymatic digestion (such as some starch ethers, starch esters and cross-linked starches) [1]. The natural types of RS are frequently destroyed when processed. Modern milling

A. Martín Bernabé
Faculty of Bioscience, Autonomous University of Barcelona,
08193 Bellaterra, Barcelona, Spain

K. Sriakao (✉)
Faculty of Food and Agricultural Technology,
Pibulsongkram Rajabhat University,
Muang, Phitsanulok 65000, Thailand
e-mail: khongsak@live.psu.ac.th

M. Schlüter
Faculty of Agriculture,
Rheinische Friedrich-Wilhelms Universität Bonn,
Meckenheimer Allee 174,
53115 Bonn, Germany

technology produces fine flours with very small particle size. The products contain more starch but lose a substantial amount of dietary fibre and RS [11]. In view of the importance of RS in dietary starches, this research investigated the RS content of 11 commercially available starches with an emphasis on tropical plant starches. Their functional properties and nutritional qualities as defined by starch digestibility and SCFA fermentation were also examined.

Materials and Methods

Starch Samples

This study examined 11 tropical starches which have been extracted from various sources and widely used as food ingredients. They included cereal starches (waxy, non-waxy rice and maize), legume starch (mung beans), tuber starches (potatoes, edible canna, taros and tapiocas) and starchy fruit (unripe bananas, *Musa sapientum* Linn.). As this study focused on the commercial tropical starches, all samples were carefully selected from the products which are produced in Thailand using local raw materials. To ensure that the sample is the representative of the commercially available products, at least three different brands for each sample were purchased and they were mixed thoroughly prior to analysis. Two commercial high-fibre modified starches from corns (A) and tapiocas (B) were also examined for comparison. Modified starch (A) was purchased from National Starch (Thailand) Co., Ltd. This product is derived from high-amylose corn starch. The supplier labelled a minimum 60 g/100 g dry sample total dietary fibre, pI 4.5–7.5 and particle size 10–15 μm . Modified starch (B), was purchased from T-fibre Innovation Co., Ltd. (Thailand). The supplier labelled a minimum of 40 g/100 g dry sample total dietary fibre. All samples were sieved through 100-mesh screen prior to analysis.

RS Content

RS, non-RS and total starch (TS) fractions were determined using the Megazyme RS assay kit (Megazyme International, Ireland), following the approved AACC Method 32–40 [12]. Briefly, ground sample (100 ± 5 mg) was accurately weighted into a screw cap tube. Non-RS fraction in the sample was solubilised and hydrolysed to glucose by the combined action of pancreatic α -amylase and amyloglucosidase (AMG) for 16 h at 37 °C in a shaking water bath. The reaction was stopped by the addition of ethanol and RS was recovered as a pellet by centrifugation. RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice-water bath. This solution was neutralised with acetate buffer and

the starch was quantitatively hydrolysed to glucose with AMG. Glucose released, which is a measure of RS content and non-RS (solubilised starch), was measured spectrophotometrically at 510 nm with glucose oxidase–peroxidase reagent (GOPOD). TS content was then calculated as the summation of RS and non-RS.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was conducted using the DSC 1 (Mettler-Toledo, US) equipped with a Huber TC100 (Germany) cooling device. To avoid the interference from different moisture content, all samples were adjusted to reach 70% (w/w) of moisture content by adding distilled water and kept overnight to ensure they were completely equilibrated prior to analysis [13, 14]. The hydrated samples (25 ± 5 mg) were weighed accurately into aluminium DSC pans and hermetically sealed. A sealed empty pan was used as a reference. Thermal scans were performed from 30 to 120 °C at a heating rate of 10 °C/min. DSC parameters including onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and transition enthalpy (ΔH) were quantified using the Star^c Software (Mettler-Toledo, USA). All measurements were performed in duplicate.

In Vitro Starch Digestion and Modelling of Starch Digestograms

Time-course starch digestion in the samples was determined using a rapid in vitro digestibility assay based on glucometry [15]. About 0.5 g of ground sample was treated with artificial saliva containing porcine α -amylase (Sigma A-3176 Type VI-B) before pepsin (Sigma P-6887; pH 2.0) was added and incubated at 37 °C for 30 min in a reciprocating water bath (85 rpm). The digesta was neutralised with NaOH before adjusting the pH to 6 (sodium acetate buffer) prior to the addition of pancreatin (Sigma P1750) and AMG (Sigma A-7420). The mixture was incubated for 4 h, during which the glucose concentration in the digesta was measured with an Accu-Check[®] Performa[®] glucometer at specific periods (0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min). Digested starch was calculated and the digestogram (digested starch at a specific time period) was modelled as described in details previously [13].

The hydrolysis index (HI) of each sample was calculated by dividing the area under its digestogram by the area under the digestogram of a fresh white bread [16], which was calculated to be about 13,000 min g/100 g dry starch from 0 to 240 min. The single-point measurement of starch digestion at 90 min (H_{90}) in the samples was also used to calculate predicted GI. The average predicted GI (GI_{AVG}) for each sample was defined as the average of GI_{H90} and GI_{HI} .

In Vitro Fermentation

Prior to the in vitro fermentation, the samples were digested by in vitro enzymatic digestion mimicking the upper intestine according to the method from previous studies [17, 18]. Briefly, 30 g of each sample was suspended in phosphate buffer and incubated stepwise with (a) 0.5 mL α -amylase solution (Sigma A1031; pH 6.9) for 15 min, (b) 1.25 mL porcine pepsin suspension (Sigma P7012; pH 2.0) for 30 min and (c) 10 mL porcine pancreatin solution (Sigma P7545; pH 6.9) for 3 h in the presence of 12 g bovine bile (Sigma B8381). The degradation products were removed by dialysis tubing (Cellu-Sep T4, MW cut-off 12,000–14,000, Membrane Filtration Products Ltd. USA) overnight against running distilled water. After dialysis, the retentates were dried. In vitro fermentation of the digested starch was conducted by a modified batch fermentation system under anaerobic conditions with fresh human faeces as inoculums [19]. Fresh human faeces were collected from three to four healthy volunteers, who usually consumed a normal diet, had no digestive disease and had not received antibiotics for at least 3 months. The faeces was diluted with anaerobic phosphate buffer to working concentration (208 g/L), filtered through surgical gauze and used immediately. In duplicate, the digested starch (100 mg) was weighed into fermentation tubes with 2 mL of carbonate-phosphate buffer and the same amount of faeces slurry. The tubes were fluxed with nitrogen gas to maintain anaerobic conditions. The fermentation was performed at 37 °C for 24 h. The tubes were vigorously mixed and the mixtures were removed for SCFA analysis.

Analysis of SCFAs

Gas chromatography (GC) was used for analysis of SCFAs [20]. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. Then 280 μ L of 0.36 M perchloric acid solution, 270 μ L of 1 M NaOH solution and 50 μ L of 5 M

phosphoric acid solution were added to 100 μ L of the supernatant. All tubes were vigorously mixed and then 300 μ L of methanol containing 1% concentrated sulfuric acid was added. The mixtures were incubated at 70 °C for 30 min before 300 μ L of cold hexane was added. All tubes were vigorously mixed. Meanwhile, SCFA standard solutions containing 20 mM formic acid, 20 mM acetic acid, 10 mM propionic acid and 10 mM butyric acid were prepared. Two microlitres of hexane supernatant from each tube was injected into GC with a split ratio of 50 on a HP-5 capillary column (30 m $\times$ 0.32 mm $\times$ 0.25 μ m). The injector temperature was set at 250 °C and column temperature was set at 150 °C. N₂ at the rate of 0.6 mL/min was used as carrier gas.

Statistical Analysis

Analysis of variance (ANOVA) and test of significance were performed using SPSS[®] ver. 17 with confidence level of 95%. The samples were randomised for all the analytes described above.

Results and Discussions

RS Content

Table 1 shows the RS, non-RS and TS content of the samples. All samples contained substantial amount of total starch ranging from 53 to 92 g/100 g dry solids, to suggest the quality of the samples obtained locally from commercial products. As some samples contained less than 70 g/100 g dry solids of total starch (Table 1), these samples were not pure starches. They might have been adulterated. However, as this study focused on commercial starches to reflect the commercial use, the samples were not re-isolated.

Some natural starches such as unripe banana, taro, canna and potato starches were found to contain significant

Table 1 Resistant starch, non-resistant starch and total starch of the starch samples

Starch samples	Resistant starch (g/100 g dry sample)	Non-resistant starch (g/100 g dry sample)	Total starch (g/100 g dry sample)
Unripe banana	36.26 ^b ±7.05	24.79 ^c ±0.17	61.04 ^c ±6.93
Taro	51.60 ^a ±4.10	19.94 ^f ±0.48	71.53 ^d ±4.20
Canna	46.18 ^a ±8.44	7.61 ^g ±1.41	53.79 ^f ±9.82
Waxy rice	9.76 ^{d,e} ±0.22	79.38 ^a ±1.23	89.14 ^{a,b,c} ±1.39
Non-waxy rice	10.11 ^{d,e} ±0.70	76.21 ^b ±1.37	86.38 ^{b,c} ±0.70
Mung bean	14.29 ^{c,d} ±0.29	79.39 ^a ±0.58	93.68 ^a ±0.82
Potato	39.32 ^b ±3.05	23.42 ^e ±0.38	62.74 ^e ±2.93
Maize	12.22 ^{c,d} ±1.84	79.29 ^a ±1.06	91.51 ^{a,b} ±2.47
Tapioca	16.75 ^c ±3.30	72.92 ^a ±0.85	89.67 ^{a,b} ±2.57
Modified starch (A)	34.84 ^b ±3.24	47.81 ^d ±1.46	82.59 ^c ±3.84
Modified starch (B)	6.00 ^e ±0.93	47.10 ^d ±1.32	53.10 ^f ±2.19

Values are means $\pm$ standard deviations. Values followed by a different superscript in each column are significantly different ($P < 0.05$). These apply to all tables where they appear

amount of RS. Unripe banana is considered the RS-richest non-processed food with more than 10% RS, which could vary with genotype and environment [2, 21]. Unripe or green banana could represent an alternative source of indigestible carbohydrates. Edible canna, taro and native potato starches were also reported to contain high RS [22–24], probably because of their large starch granules or presence of starch–protein interactions. Tubers and legumes generally tend to have high RS, but plant sources can vary (0–80%) in their RS contents [22, 25]. Moreover, hydro-thermal processing can cause an increase or decrease in the RS fraction depending on the process conditions and post-process handling [22]. For example, RS content in native potato starches at the level of 68–73% w/w was reduced to 4–6% w/w in gelatinised starch after 20 min incubation time with enzymes [23]. Notably, the commercial high-fibre modified starch (B) contained low RS content. The declared high fibre could be from non-RS type dietary fibre.

Gelatinisation Behaviours by DSC

All starch samples exhibited a single endothermic peak located broadly around 60–99 °C (Table 2). This confirms the remaining native raw starches in the samples. However, both the commercial modified starches (A and B) did not show any transition energy change. This suggests changes in starch granule molecular order and structures by the modification process. In this study, the gelatinisation temperatures and enthalpy of the rice (both waxy and non-waxy types) and unripe banana starches were found to be higher than those of the other samples. Gelatinisation temperatures of the starchy samples can vary due to factors that include genetic origin, environmental conditions and age of the parent plant [26–28].

In Vitro Starch Digestion

Table 3 summarises the model parameters of all the samples. It was found that the model was suitable to describe the

digestograms, providing $r^2=0.82\text{--}0.99$; MRDM=1–24%; SUMSQ=4–142. Most samples exhibited as the very rapidly digested starch (VRDS) resulting in high predicted GI values. Generally, based on the average predicted GI values in this study, the samples may be grouped into three groups, high, middle and low predicted GI. The high predicted GI group consists of taro and modified starch B. The majority of the samples are in the middle group which includes unripe banana, edible canna, waxy and non-waxy rice, mung bean and maize starches. The low predicted GI group consists of potato, tapioca and modified starch A. It was expected that modified starch A which has been formulated as the high-fibre starch would give low predicted GI values. Generally, the higher resistant starch content, the lower was the GI. Thus, modified starch B which contained less RS provided high predicted GI values. However, taro starch which contained high amount of RS but it provided high predicted GI values. This is probably influenced by starch structure. Taro starch had irregular, polygonal shapes and small granular sizes which promoted rapid digestibility [29]. The present study found that native unripe banana, edible canna and tapioca starches provided considerably acceptable predicted GI values. This appears to support the conclusions of Moorthy [7] that some tropical tuber and root crop starches have the potential to be used in low GI foods. The predicted GI value of potato starch in this study was found to be relatively low while most published data generally showed high GI values. Native potato starch is an enzyme-resistant starch which is associated with the large granule size and B-type crystalline. However, when potatoes are cooked for consumption, starch is gelatinised and becomes susceptible to hydrolysis by enzymes [23]. It should be noted that there are many factors that determine the GI of a food. Foods in which starch is easily broken down by digestive enzymes, liberating its glucose molecules for rapid absorption, generally have a high GI. For example, refining grain or reducing particle size by grinding, extruding or flaking exposes the starch to these enzymes, increasing GI. Cooking

Table 2 DSC parameters, onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and transition enthalpy (ΔH) of the starch samples

Starch samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g dry sample)
Unripe banana	79.28 ^b ±0.35	83.15 ^b ±0.76	88.52 ^c ±2.13	10.25 ^{c,d} ±0.17
Taro	67.25 ^f ±0.36	70.30 ^d ±0.14	74.50 ^d ±0.42	12.85 ^{b,c} ±0.21
Canna	67.18 ^f ±0.43	70.44 ^d ±0.39	75.51 ^d ±2.14	14.25 ^{b,c} ±5.47
Waxy rice	71.72 ^e ±0.77	84.40 ^b ±1.77	93.77 ^b ±2.42	10.40 ^{c,d} ±0.55
Non-waxy rice	81.71 ^a ±0.16	92.44 ^a ±0.50	99.12 ^a ±2.23	5.24 ^d ±1.79
Mung bean	69.96 ^d ±0.30	72.92 ^c ±0.17	77.00 ^d ±0.01	6.79 ^d ±0.04
Potato	59.87 ^h ±0.20	63.00 ^e ±0.12	69.05 ^e ±0.05	17.43 ^a ±1.69
Maize	68.72 ^e ±0.29	72.54 ^c ±0.31	77.22 ^d ±0.35	16.87 ^b ±1.32
Tapioca	65.82 ^e ±0.78	69.79 ^d ±0.23	74.25 ^d ±0.38	13.81 ^{b,c} ±1.54
Modified starch (A)	nd	nd	nd	nd
Modified starch (B)	nd	nd	nd	nd

nd not detectable

Table 3 Model parameters^a, hydrolysis index (HI) and glycaemic index (GI) of the starch samples

Starch samples	D_{∞} (g/100 g dry starch)	$K \times 10^{-3}$ (min ⁻¹)	HI	GI ₁₀₀	GI _{AVG}
Unripe banana	52.73±0.49	2.37±0.00	112.35±0.71	57.41±0.40	80.48 ^b ±0.40
Taro	36.02±2.83	4.05±0.10	144.24±7.80	74.83±4.23	98.28 ^a ±4.34
Canna	53.63±1.74	1.34±0.08	101.73±3.63	52.47±1.91	74.98 ^b ±1.99
Waxy rice	88.18±0.50	4.89±0.00	28.14±7.82	98.92±0.01	77.24 ^b ±2.24
Non-waxy rice	81.71±4.84	2.16±1.16	64.22±5.43	74.16±8.81	75.14 ^b ±2.86
Mung bean	89.71±1.18	0.09±0.01	42.57±1.38	87.11±1.51	75.44 ^b ±1.15
Potato	75.28±0.90	1.01±0.10	60.71±2.42	30.59±1.21	52.35 ^c ±1.30
Maize	90.56±0.02	1.11±0.93	81.97±4.06	63.40±3.53	74.82 ^b ±2.92
Tapioca	88.57±3.26	0.52±0.12	92.80±7.22	44.61±3.84	68.51 ^c ±3.98
Modified starch (A)	74.22±0.39	2.84±0.05	86.80±1.10	42.53±0.58	65.76 ^c ±0.60
Modified starch (B)	66.75±0.32	9.15±1.04	136.98±4.45	70.63±2.90	94.11 ^a ±2.72

^aModel parameters: D_{∞} (digestion at infinite time) and K (rate constant)

foods by moist heat gelatinises starch making it more readily hydrolysed and digested. Type of starch is also a factor. Amylopectin with its branched structure is more easily digested than is amylose [30]. Low GI foods are valuable for use in controlled glucose release applications and in lowering insulin response, and greater access to the use of stored fat is expected [10, 31]. This is important for diabetes and its dietary management.

SCFAs

Analysis results of SCFA production are shown in Table 4. Generally, the higher RS content of the samples, the higher was the SCFA content. Based on their concentrations, formic acid and acetic acid are the major SCFAs found in the fermentation products. In this study, it was found that RS level of the samples seemed to significantly affect the SCFA production, regardless of the starch digestibility of the samples. For example, taro starch contained high level

of RS but it was found to be rapidly digested, resulting in its high predicted GI value. However, taro starch after fermentation still yielded considerably high level of SCFAs. Previous comparative study of unripe banana mass and banana starch found that banana starch contained higher amount of RS and thus yielded higher level of total SCFAs [32]. Also, SCFA production was found to increase as the addition level of RS prepared from high-amylose maize starch increased [20]. Notably, several factors such as intestinal microflora, types of human faeces (adult/infant) and fermentation methods (in vivo/in vitro) could provide different responses to the fermentation of carbohydrates [33].

Conclusions

Tropical starches including cereal starches (waxy, non-waxy rice and maize), legume starch (mung beans), tuber

Table 4 Short chain fatty acid (SCFA) production from the fermentation of the starch samples

Starch samples	Formic acid (mmol/L)	Acetic acid (mmol/L)	Propionic acid (mmol/L)	Butyric acid (mmol/L)	Total SCFAs (mmol/L)
Unripe banana	24.05 ^c ±0.72	20.13 ^d ±0.33	15.72 ^d ±0.41	10.40 ^c ±0.21	70.30 ^d ±1.01
Taro	35.38 ^b ±0.72	31.68 ^b ±0.77	22.88 ^c ±0.42	15.44 ^d ±0.14	105.37 ^c ±2.05
Canna	39.84 ^a ±0.40	33.40 ^a ±0.88	28.92 ^a ±0.18	22.90 ^a ±0.16	125.65 ^a ±0.83
Waxy rice	10.00 ^f ±0.32	6.97 ^e ±0.27	5.13 ^{gh} ±0.77	2.82 ^h ±0.05	24.92 ^e ±1.31
Non-waxy rice	12.31 ^e ±1.56	8.58 ^f ±0.13	4.98 ^h ±0.16	2.56 ^{hi} ±0.16	28.43 ^f ±1.99
Mung bean	12.62 ^e ±0.68	8.82 ^f ±0.21	5.94 ^f ±0.10	3.38 ^g ±0.52	30.76 ^f ±1.32
Potato	35.09 ^b ±0.73	30.63 ^c ±0.19	22.92 ^c ±0.19	19.07 ^c ±0.39	107.69 ^c ±1.50
Maize	12.04 ^e ±0.08	8.02 ^f ±0.45	5.73 ^g ±0.07	3.48 ^g ±0.04	29.46 ^f ±0.33
Tapioca	15.17 ^d ±0.39	11.35 ^c ±0.33	6.93 ^c ±0.08	4.15 ^f ±0.12	37.59 ^c ±0.14
Modified starch (A)	35.04 ^b ±0.36	30.84 ^{bc} ±0.07	24.79 ^b ±0.09	20.22 ^b ±0.17	110.88 ^b ±0.17
Modified starch (B)	9.00 ^f ±0.05	4.30 ^h ±0.40	3.06 ^h ±0.01	2.2 ⁱ ±0.14	18.56 ^h ±0.61

starches (potatoes, edible cannas, taros and tapiocas) and starchy fruit (unripe bananas) contained substantial amount of TS. However, RS content of those starches varied depending on their types. Some natural starch sources such as taro and edible canna were either comparable or better than commercial modified starches in terms of RS level. All natural starches contained native starch granules which gelatinised during DSC heating. Most starch samples exhibited VRDS types resulting in their high predicted GI values. RS could lower starch digestion rate but this phenomenon was influenced by various factors such as starch granule morphology, amylose to amylopectin ratio, molecular structure, degree of branching in terms of steric hindrance etc. In vitro fermentation of the starch samples showed that SCFAs production increased as the RS content of the samples increased.

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