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Production of resistant starch from taro (*Colocasia esculenta* L. Schott) corm and determination of its effects on health by *in vitro* methods

Sebnem Simsek*, Sedef Nehir El

Ege University, Engineering Faculty, Food Engineering Department, 35100 Bornova-Izmir, Turkey

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ABSTRACT

The aim of the study was the production of resistant starch from taro (*Colocasia esculenta* L. Schott) corm and determination of its effects on health by *in vitro* methods. Starch was isolated from taro corms with 98% purity, and 10.4 ± 0.5% amylose content. By application of heating, autoclaving, enzymatic debranching, retrogradation, and drying processes to taro starch for two times, resistant starch (RS) content was increased 16 fold (35.1 ± 1.9%, dry basis). The expected glycemic index (eGI) of taro starch and taro resistant starch was determined as 60.6 ± 0.5 and 51.9 ± 0.9, respectively and the decrease in the glycemic index of taro resistant starch was found as statistically significant ($P < 0.05$). The *in vitro* binding of bile acids by taro starch and taro resistant starch relative to cholesterol decreasing drug cholestyramine were 5.2 ± 0.2% and 7.6 ± 1.7%, respectively.

1. Introduction

In the FAO/WHO Expert Consultation report related to diet, nutrition, and the prevention of chronic diseases, some recommendations have been made for carbohydrate consumption. According to the report, the acceptable macronutrient distribution range (AMDR) for carbohydrates is 55–75% of total energy intake, added sugars should be limited to 10% of total energy intake, and adequate intake (AI) for total dietary fiber is 38 g for men and 25 g for women (FAO/WHO Expert Consultation, 2002).

Starch is the main carbohydrate source in human nutrition and classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), according to the rate and extent of digestion (Englyst, Kingman, & Cummings, 1992). Rapidly digestible starch is the starch fraction that causes an increase in blood glucose level after ingestion immediately, whereas SDS is the starch fraction that is digested completely in the small intestine at a lower rate as compared to RDS. Resistant starch is the portion of starch and/or starch hydrolysis products that escape digestion in the small intestine, and enters the colon for fermentation (Sajilata, Singhal, & Kulkarni, 2006).

The demand of consumers for healthier food products has forced the food industry to produce high-quality products with functional properties. As a functional ingredient, RS has gained importance as a new source of dietary fiber (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). As a food ingredient, RS has desirable physicochemical properties such as white appearance, bland flavor, swelling, gel formation, viscosity increase, and water binding capacity. Resistant starch also has beneficial physiological effects such as improving colonic health, increasing absorption of minerals, and lowering plasma triglyceride and cholesterol levels (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009; Sajilata et al., 2006).

The glycemic index (GI) is a scale that has been introduced to enable comparison of carbohydrate-rich foods based on their glycemic response (Jenkins, 2007). FAO/WHO Expert Consultation suggested the use of the GI concept for classifying carbohydrate-rich foods to provide a useful means of helping people select the most appropriate carbohydrate containing foods for the maintenance of health and treatment of several diseases (Foster-Powell, Holt, & Brand-Miller, 2002). Low GI foods, due to the slow digestion and absorption of their carbohydrates, produce a more gradual rise in blood glucose and insulin levels, and are therefore associated with reduced incidence and prevalence of diabetes, heart disease, and some cancers. Resistant starch containing food products have lower GI values and, therefore, can be used for controlled glucose release applications (Sajilata et al., 2006).

Bile acids are synthesized in liver from cholesterol and following the conjunction with glycine or taurine; they are secreted into duodenum for lipid digestion. After the emulsification of bile with fat in the small intestine, most of the bile acids are actively reabsorbed by the terminal ileum and undergo enterohepatic circulation after digestion. The other possibility is the entrapment of bile by dietary fiber in the large intestine, which is then and carried out of the body with the feces. The excretion of bile in this way results in a reduction in blood cholesterol level. Bile acid binding is well correlated with insoluble fiber and total dietary fiber content (Kahlon & Smith,

* Corresponding author. Tel.: +90 2323111921; fax: +90 2323427592.
E-mail address: sebnem.simsek@ege.edu.tr (S. Simsek).

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2007a,b). Resistant starch also has a bile acid binding capacity and has been shown to be effective in lowering plasma cholesterol levels in genetically obese and lean rats as well as in diabetic rats (Sajilata et al., 2006).

Resistant starch can be found in foods naturally and can also be formed *via* processing at home and/or in a factory. There are four types of RS present: physically inaccessible starch locked within the cell walls (RS_1), ungelatinized native granular starch with type B crystallinity (RS_2), retrograded starch (RS_3), and chemically modified starch due to cross-linking with chemical reagents (RS_4) (Sajilata et al., 2006). The amount of RS in foods is related to the type and amount of starch (crystal and granular structure, amylose:amylopectin ratio, the chain length of amylose, and linearization of amylopectin), along with the processing, cooking, and storage conditions of food (heat and moisture conditions) (Fuentes-Zaragoza et al., 2010; Sajilata et al., 2006). The RS_3 form is mainly associated with the retrogradation of amylose fraction rather than with amylopectin, due to its linear-chain polymers whose degree of polymerization can be optimized (Onyango & Mutungi, 2008). Therefore, in RS production, the current tendency is to look for alternative plant sources for obtaining starch with a different crystal structure and/or to modify the structure of starch by hydrolyzing it with enzymes like pullulanase or isoamylase to convert branched chains into linear chains *via* hydrolysis of the amylopectin fraction through the elimination of α-D-(1 → 6) glycosidic linkages (Gonzalez-Soto, Mora-Escobedo, Hernandez-Sanchez, Sanchez-Rivera, & Bello-Perez, 2007; Onyango & Mutungi, 2008; Ozturk, Koksel, Kahraman, & Ng, 2009).

Taro (*Colocasia esculenta* L. Schott) is known as gölevez in Turkey and is called eddo, old cocoyam, and dasheen in other countries of the world. Taro is a starchy root crop with wide leaves, which are also edible. The most frequently eaten part of the taro plant is the corms and cormels, which are formed underground by a thickening of the base of the stem. Taro is the main food source for about 500 million people living in Asia, Africa, Middle America, and the Pacific Islands. It has also been grown and is consumed more frequently than potato in the southern Mediterranean region of Turkey. In addition to its local consumption, taro is also exported to England and Cyprus (Sen, Akgul, & Ozcan, 2001; Yemenicioglu, Ozkan, & Cemeroglu, 1999).

The aim of this study was to produce RS_3 from taro starch and to determine its starch hydrolysis rate (expected glycemic index, eGI) and bile acid binding capacity by *in vitro* methods to evaluate its effect on health.

2. Materials and methods

2.1. Materials

Taro (*Colocasia esculenta* L. Schott) corms were kindly supplied by the Bozyazi Agriculture Ministry of Turkey and stored at +6 to 8 °C with 85–90% relative humidity until the time of analysis. Amyloglucosidase (AMG 300 L), α-amylase (Termamyl 120 L), invertase, and pullulanase (Promozyme 400 L) were kindly supplied by Novozymes (Bagsvaerd, Denmark). Pancreatic α-amylase (A 3176), pancreatin (P 7545), pepsin (P 7000), cellulose (C 6288), cholic acid (C 1129), deoxycholic acid (D 2510), glycocholic acid (G 2878), taurocholic acid sodium salt hydrate (T 4009), cholestyramine (C 4650), DMSO (154839), amylose (10130), amylopectin (10118), KOH (P 5958), I_2 (20-777-2), glucose (G 5400), and guar gum (G 4129) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Glucose assay kit (K-GLUC) was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Bile Acid Diagnostic Kits (No. 450-11 and 450-100) were purchased from Trinity Biotech plc, Bray Co. (Wicklow, Ireland).

2.2. Starch Isolation

The starch was isolated using a laboratory scale procedure, according to the method of Perez, Schultz, and de Delahaye (2005) with some modifications. The corms were peeled, cut into small pieces and washed under tap water several times to remove dirt and mucilage. Corms were rasped and homogenized with 0.015% $Na_2S_3O_5$ at high speed using a Waring blender (1:1 tuber/solution) for 1.5 min. The homogenate was filtered through cheesecloth and the residue on the cloth was collected and homogenized one more time with water to increase the extraction performance. The filtrate was consecutively sieved through screens (0.075 and 0.034 mm) and then centrifuged in a Thermo Scientific CL31R (Germany) centrifuge at 8000 × *g* for 10 min. Following centrifugation, the supernatant was discarded, and the pellets were mixed with water and then centrifuged. This washing and centrifugation step was repeated two times and then the obtained pellet was dried in an oven at 40 °C.

For purification, the crude starch was mixed with 0.05 M NaOH and centrifuged at 10,000 × *g* for 10 min for the removal of proteins. The supernatant was discarded; the dark colored insoluble components (*e.g.* protein, fine fiber) on top of the starch layer were scraped with a spatula. Then the starch was washed with 0.1 M HCl for partial neutralization. Finally, to remove the fat, the starch was washed with a chloroform:methanol (2:1) solution and then with water for the removal of the aforementioned chemicals (Vasanthan & Hoover, 1992). The obtained purified starch was dried in an oven at 40 °C, ground, and stored in glass jars at room temperature until the analysis. This starch was called "taro starch" in the study.

2.3. Amylose content

The total amylose content of the starch was determined using the colorimetric method suggested by McGrance, Cornell, and Rix (1998). Total amylose content was given as a percentage through the calibration curve, which was prepared as a mixture of amylose and amylopectin (0, 10, 25, 50, and 75% amylose, respectively).

2.4. Resistant starch production

Resistant starch was produced according to the method suggested by Gonzalez-Soto et al. (2007) with some modifications. A taro starch suspension (8%, w/v) was heated in a boiling water bath for 10 min by stirring. This gel was gelatinized by autoclaving (NUVE OT 032, Turkey) at 121 °C for 15 min, cooled to 60 °C and 10 ml 0.5 M acetate buffer (pH 5.0) and pullulanase (80 U/g of starch) were added. The mixture was incubated in a shaking water bath (Memmert, WNB 14, Germany) at 60 °C for 8 h. At the end of the incubation period, the mixture was incubated in a boiling water bath for 10 min for enzyme inactivation.

The mixture was transferred to dishes and held at room temperature through the night for retrogradation. The retrograded starch was dried at 60 °C for 8 h and ground. The heating–autoclaving-storage at room temperature, as well as the drying and grinding, steps were applied one more time to this starch. The obtained RS_3-rich starch was called "taro resistant starch" in the study.

2.5. Resistant starch content

Isolation and determination of the amount of RS was applied according to the Anon (2007). Samples were incubated with pancreatic α-amylase and amyloglucosidase for 16 h at 37 °C in a shaking water bath. At the end of the incubation period, ethanol was added to terminate the reaction and RS was recovered by centrifugation as a pellet. This pellet was dissolved in 2 M KOH by vigorously stirring in an ice-water bath over a magnetic stirrer. This

solution was neutralized with acetate buffer, starch was hydrolyzed to glucose, and the total amount of glucose was measured with a glucose oxidase/peroxidase assay kit. Three ml of GOPOD reagent was added to aliquots (0.1 ml) of the sample, and the mixture was incubated at 50 °C for 20 min. Absorbance was measured using a spectrophotometer (Varian, Cary 50 Scan, Australia) at 510 nm. Resistant starch was calculated as the amount of glucose × 0.9.

2.6. X-ray diffraction (XRD)

X-ray diffraction patterns of starch samples were obtained with copper K_2 radiation ($\lambda = 1.544$) using a diffractometer (Phillips X'Pert Pro, The Netherlands). Diffractometer was operated at 40 mA and 45 kV, 2θ range from 5° to 50°.

2.7. Scanning electron microscopy (SEM)

The granule morphology and particle size of the starch granules were determined by SEM. The samples were fixed to a conductive, double-sided carbon tape, which was covered with a 20 nm-thick layer of gold in the sputter coater, Emitech K550X (England). Films were mounted on aluminum stubs using double-sided tape and then coated with a layer of gold (20–30 nm), allowing surface and cross-section visualization. All samples were examined using an accelerating voltage of 1.50 kV. Samples were analyzed and photographed with an FEI Quanta250 FEG scanning electron microscope. The samples were imaged at 20,000× magnification.

2.8. In vitro starch digestibility and expected glycemic index (eGI)

Digestibility of starch samples was determined according to the *in vitro* method suggested by Englyst, Hudson, and Englyst (2000). Enzymatic hydrolysis was applied to the starch samples and the rate of starch digestion was expressed as the percentage of total starch hydrolyzed at different times (20, 60, 90, 120, and 180 min). An equation $C = C_\infty(1 - e^{-kt})$ was used to describe the kinetics of starch hydrolysis where C, C_∞, and k were the hydrolysis degree at each time, the maximum hydrolysis extent, and the kinetic constant, respectively.

Hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the starch samples by the area obtained for white bread. The estimated glycemic index (eGI) was calculated using the equation described by Goni, Garcia-Alonso, and Saura-Calixto (1997): eGI = 39.71 + 0.549 (HI).

2.9. In vitro binding of bile acids

The bile acid binding capacity of starch samples were determined with the *in vitro* method developed by Kahlon and Smith (2007a,b) with some modifications. Fifty milligram taro starch, taro resistant starch, cellulose (negative control) and cholestyramine (positive control) were put into 15 ml centrifuge tubes. Samples were digested in 1 ml 0.01 M HCl for 1 h in a shaking water bath at 37 °C. After this incubation, 4 ml 1.8 μmol/ml bile acid solution (glycocholic acid 35%, deoxycholic acid 15%, taurocholic acid 15%, and cholic acid 15%) and 5 ml pancreatin (10 mg/ml, pH 6.9, prepared in 0.1 M phosphate buffer) were added and this mixture was incubated for 1 h in a 37 °C shaking water bath. After the incubation period, tubes were centrifuged at 9000 × *g* for 10 min. Bile acids in the supernatant were analyzed using Trinity Biotech bile acids procedure No. 450 and a spectrophotometer at 530 nm. Values were determined from the standard using a Trinity Biotech bile acid calibrator (No. 450-11) as described elsewhere.

2.10. Statistical analysis

The data reported were the mean of duplicate measurements. Statistical analysis was carried out using statistical software (SPSS version 13.0). Differences among samples were compared by using ANOVA or independent sample T test. C, C_∞, and k values in the eGI analysis were determined with the GraphPad Prism 5.04 program.

3. Results and discussion

3.1. Composition of taro starch

After the extraction and purification steps, a white starch was obtained with 98% purity. The obtained starch has approximately 14% moisture, which is in the range of moisture generally accepted for dry products to obtain a desirable shelf life (Perez et al., 2005) (Table 1).

Perez et al. (2005) extracted starch from taro corms only through homogenization with water, screening, and centrifugation steps. They purified the starch by washing it only with water for several cycles. However, in our study, after the centrifugation step, the water-insoluble components (*e.g.*, protein, fine fiber) formed a brown layer at the top of the starch layer. Although removal of this layer was accomplished by scraping it off with the aid of a spatula, some proteins, mucilage, and fiber co-sedimented with the starch granules. Therefore an extra purification step was applied by addition of NaOH to remove proteins. The tubers contain very little lipid (<1%, w/w, dry weight) compared to legume and cereal grains; however, the formed amylose–lipid complex can strongly affect the functional properties of starches (Vasanthan & Hoover, 1992). Therefore the starch slurry was once more treated with chloroform:methanol (1:2) for the removal of lipids.

The amylose content of taro starch was found to be 10.4 ± 0.5% (Table 1). There are different results in the past literature about the amylose content of taro starch, which has generally been found to be in the range of 5–28% (Moorthy, 2004). Aboubakar, Njintang, and Mboufung (2008) determined the amylose content of flour and starches of six different taro genera to be between 14.7% and 30.85%. The amylose content of taro starch changes according to the variety, harvest time, and the place where the taro was grown. For example, the amylose content of taro starches obtained in Taiwan were between 8.7% and 14.9%, and the amylose content was higher during the winter harvest than the spring harvest (Agama-Acevedo et al., 2011).

3.2. Formation of RS by the action of autoclaving and pullulanase debranching

Production of RS_3 is a two-step process that begins with gelatinization of hydrated starch during which amylose is leached from the granules into solution as a random-coil polymer. The second step involves retrogradation of starch during which the flexible linear amylose polymers recrystallize as double helices and form tightly packed helical or spherical structures stabilized

Table 1
Chemical composition and resistant starch (RS) content of taro starch and taro resistant starch.[a]

Sample	Moisture (%)	Total starch (%)	Amylose (%)	RS (%)[**]
Taro starch	13.6 ± 0.1	84.2 ± 5.0	10.4 ± 0.5	2.2 ± 0.2[b]
Taro resistant starch	6.8 ± 0.2	70.0 ± 1.4	nd[*]	35.1 ± 1.9[c]

[a] Mean (±standard deviation) of duplicate analysis. Values followed by a different superscript in each column are significantly different ($P < 0.05$).
[*] Not determined.
[**] On dry basis.

by hydrogen bonds that are resistant to enzymes. Resistant starch production can be enhanced by debranching enzymes (*e.g.*, pullulanase, isoamylase) to eliminate α-D-(1 → 6) glycosidic linkages of amylopectin, resulting with the increase of amylose fraction that favors RS formation (Onyango & Mutungi, 2008). Many factors may influence the formation of RS, such as the type of starch, its amylose content and chain length, autoclaving temperature, the number of autoclaving-cooling cycles, and the storage time and temperature. In particular, the amylose content and its degree of polymerization significantly influence the yield of RS production (Ozturk, Koksel, & Ng, 2011; Sajilata et al., 2006).

The RS content of taro starch and taro resistant starch on a dry basis is given in Table 1. The RS content of taro starch was 2.2 ± 0.2%. In the study, through the application of two cycles of heating, autoclaving, enzymatic debranching (with pullulanase), retrogradation, and drying processes to taro starch, the level of RS in the taro resistant starch increased significantly to 35.1 ± 1.9% ($P < 0.05$). Evidently this 16-fold increase in the RS content was associated with the combined action of the autoclaving, pullulanase debranching and retrogradation processes applied as two cycles.

By achieving broad application areas of RS as a functional component, different commercially produced and patented RS forms have been developed. In the commercial production of RS, starches with high amylose content have fundamentally been used. However, in recent years, substantial progress has been made to obtain and characterize starches from non-conventional sources.

In literature there are different studies on the production of RS and, in most of these studies, high amylose-content corn starch was selected as the starch source. Koksel, Masatcioglu, Kahraman, Ozturk, and Basman (2008) reported an RS content of 8.1% after application of gelatinization, autoclaving, and drying to corn starch, whereas the native starch did not contain any RS. Onyango and Mutungi (2008) increased the RS content of tapioca starch to 30–35% by application of enzymatic debranching and 2 h crystallization at 90 °C. With high amylose corn starch, a RS content of 48% was obtained by application of 48 h pullulanase incubation and three cycles autoclaving (133 °C) and storage (4 °C) (Ozturk et al., 2009).

In our study, it was the first time in literature that taro starch was used for RS production. When considering the 30% and 53% RS content of commercial RS samples, Novelose 300 and C*Actistar, respectively, the RS content obtained in our study (35.1%) is also very important.

3.3. X-ray diffraction pattern

X-ray diffraction patterns of taro starch and taro resistant starch are given in Fig. 1. Taro starch in its native form provides an X-ray pattern of A type with the characteristic $2\theta = 15°$ along with 23° strong peaks and in-between peaks at 17° and 18°. Although tuber-type starches display a B-type pattern, this result was compatible with the literature for taro starch. The taro resistant starch displayed a different pattern than the native form with loss of the peaks at 15° and 23°, and formation of in-between peaks at $2\theta = 14°$, 15°, 22°, and 24°. This was a result of the reorganization of the starch chain due to retrogradation. This type of X-ray pattern is related to the B-type pattern and agreed with the RS content, since the observed peaks were associated with the retrogradation phenomena. This type of XRD pattern was observed in RS_3 containing banana starch (Gonzalez-Soto et al., 2007), maize starch (Miao, Jiang, & Zhang, 2009), and also in a commercial RS product, Hi-maize (Bird et al., 2009).

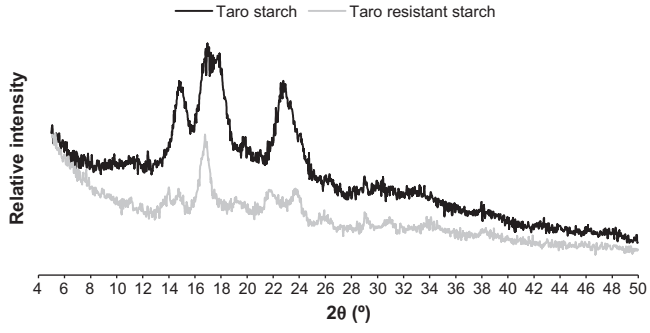

Fig. 1. X-ray diffraction patterns of taro starch and taro resistant starch.

3.4. Scanning electron microscopy

The microphotographs of taro starch and taro resistant starch are shown in Fig. 2. Taro starch granules have irregular and polygonal shapes of diameter lower than 2.5 μm. This result agreed with the granule size of taro starches reported in literature. Moorthy (2004) reported the taro starch granules among the smallest ones observed in the plant kingdom and the average granule size of ten cultivars ranged from 2.96 to 5.15 μm. Other studies also observed the size of taro starch granules smaller than 5 μm (Aboubakar et al., 2008; Perez et al., 2005).

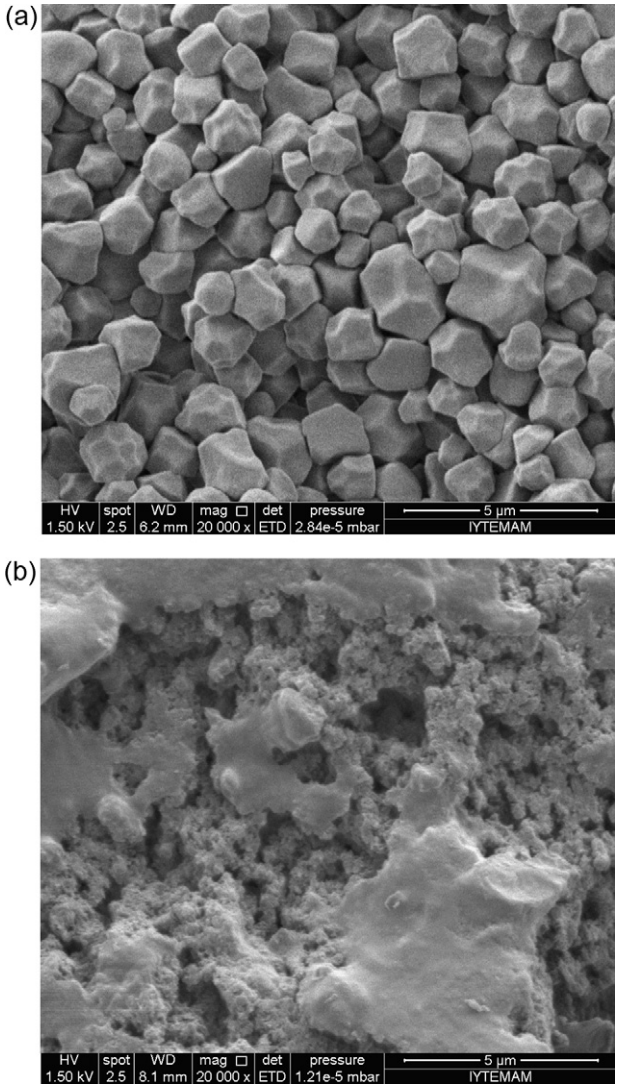

Fig. 2. Scanning electron microscopy of taro starch (a) and taro resistant starch (b).

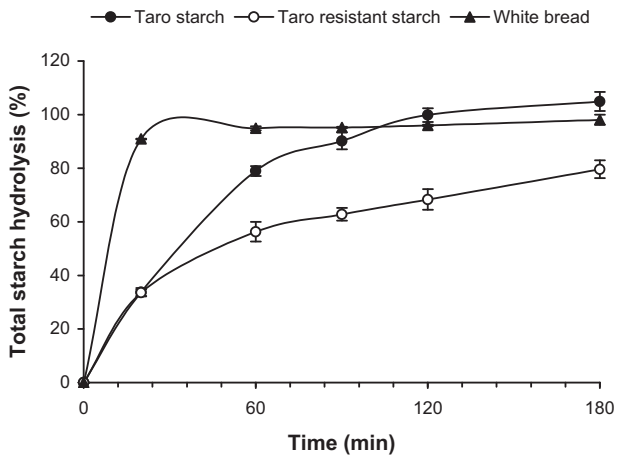

Fig. 3. Starch hydrolysis curves of white bread, taro starch and taro resistant starch.

The structure of taro resistant starch was different from the taro starch in that, due to retrogradation the granular structure disappeared and a continuous network with irregular shape was formed. This increased density of the crystal structure greatly increased its resistance to enzyme attack.

3.5. Expected glycemic index (eGI)

Starch that is not degraded rapidly by human digestive enzymes in the upper gut has been associated with health benefits due to a slower release of glucose into the blood stream, resulting in reduced postprandial glycemic and insulin responses. Therefore the rate and extent of starch digestion is a major factor for controlling blood glucose and insulin levels and *in vitro* starch digestibility methods are relatively successful at predicting the GI of high starchy foods (Dona, Pages, Gilbert, & Kuchel, 2010).

The hydrolysis curves of white bread, taro starch, and taro resistant starch are given in Fig. 3. According to these hydrolysis curves, equilibrium glucose concentration (C_∞), kinetic constant (k), hydrolysis index (HI), and expected glycemic index (eGI) are calculated and shown in Table 2. The C_∞ value of the taro starch and taro resistant starch were 108.7 ± 4.3 and 75.7 ± 3.5, respectively, indicating approximately 30% reduction of the hydrolysis extent for taro resistant starch. The eGI of taro starch and taro resistant starch were calculated according to the reference white bread (GI = 100) as 86.6 ± 0.8 and 74.1 ± 1.3, respectively.

Researchers classify high, medium, and low glycemic index foods as good, better, and the best choices for nutrition. According to this approach, foods are classified as: low glycemic index foods (GI ≤ 55), medium glycemic index foods (56 < GI < 69), and high glycemic index foods (GI ≥ 70). In this numerical classification, the reference material was taken as glucose (GI = 100) (Schakel, Schauer, Himes, Harnack, & Heel, 2008). The values obtained in this research were multiplied by 0.7 to obtain estimated GI values as suggested by Foster-Powell et al. (2002). According to this calculation, the eGI of taro starch and taro resistant starch were 60.6 ± 0.5 and 51.9 ± 0.9, respectively, and the decrease in the eGI of taro resistant starch was found to be statistically significant ($P < 0.05$).

In literature, there is no study related to the GI of taro starch. However, the lower hydrolysis rate (low GI) of taro resistant starch compared to the taro starch makes this product an alternative source that can be used in product formulations prepared for diabetic patients and for weight management purpose.

Table 2
Hydrolysis kinetics and expected glycemic index (eGI) of taro starch and taro resistant starch.[a,b]

Sample	C_∞	k	HI	eGI
Taro starch	108.7 ± 4.3^{c}	0.020 ± 0.002^{e}	85.3 ± 1.4^{g}	86.6 ± 0.8^{k}
Taro resistant starch	75.7 ± 3.5^{d}	0.024 ± 0.002^{f}	62.6 ± 2.3^{h}	74.1 ± 1.3^{l}

[a] Mean (±standard deviation) of duplicate analysis. Values followed by a different superscript in each column are significantly different ($P < 0.05$).
[b] C_∞, equilibrium hydrolysis extent; k, kinetic constant; HI, hydrolysis index; eGI, expected glycemic index.

Table 3
In vitro bile acid binding of taro starch and taro resistant starch on equal weight.[a]

Treatment	Bile acid binding relative to cholestyramine (%)
Cholestyramine	100
Cellulose	2.3 ± 0.8^{b}
Taro starch	5.2 ± 0.2^{c}
Taro resistant starch	7.6 ± 1.7^{d}

[a] Mean (±standard deviation) of duplicate analysis. Values followed by a different superscripts in each column are significantly different ($P < 0.05$).

3.6. In vitro binding of bile acids

The bile acid binding capacity of foods is mainly associated with their nutrient and bioactive compound composition, such as antioxidants and polyphenols. Studies also showed a significant correlation between bile acid binding capacity and total dietary fiber, especially non-soluble dietary fiber (Kahlon & Smith, 2007a; Sayar, Jannink, & White, 2005).

Considering the cholestyramine as 100% bound, the relative bile acid-binding capacities of cellulose, taro starch, and taro resistant starch are given in Table 3. The *in vitro* binding of bile acids by cellulose (negative control) was $2.3 \pm 0.8\%$. Bile acid binding capacity of cellulose was found to be in the range of 0.2–1.6% during previous studies (Kahlon & Smith, 2007a,b; Kim & White, 2010). Our result was higher than these results, which may be related to the structure of cellulose (*e.g.* size of fiber) used in the study.

The *in vitro* binding of bile acids by taro starch and taro resistant starch relative to cholestyramine were $5.2 \pm 0.2\%$ and $7.6 \pm 1.7\%$, respectively. Kahlon and Smith (2007a) reported the bile acid binding capacity of banana, peach, pineapple, grape, and plum in the range of 5–9% on a dry basis and concluded that this was an indicator of the positive potential effect of these fruits on health. In another study the same authors also examined the effects of different vegetables (spinach, cabbage, cauliflower, broccoli, and leek) and found the bile acid-binding capacity as 2–9% (Kahlon, Chapman, & Smith, 2007). Sayar et al. (2005) found the bile acid-binding capacity of different oat flours to be in the range of 7.5–14.8%. Comparison of the bile acid-binding capacity of taro starch and taro resistant starch with results from previous studies indicates that both of the starches have health-protecting potential due to their possible cholesterol-lowering effect.

4. Conclusions

The starch isolated from taro corms was used as an alternative source for RS production for the first time in literature. RS_3-rich starch was produced from taro starch by application of heating, autoclaving, enzymatic debranching (with pullulanase), retrogradation, and drying processes for two times. The *in vitro* starch hydrolysis rate of taro resistant starch was lower than that of native taro starch, indicating a lower eGI. The lower eGI value of taro resistant starch makes it suitable for formulation of foods, especially for diabetic people and those who are interested in weight management. The *in vitro* bile acid binding capacity of taro resistant

starch was noted as a health-promoting potential due to its possible potential cholesterol-lowering effect.

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