In vitro digestibility, glycaemic index and bile acid–binding capacity of foods containing different types of resistant starch in comparison with the commercial resistant starches

Seher Serin*, Sedat Sayar

Department of Food Engineering, University of Mersin, Mersin, Turkey

*Corresponding Author: Seher Serin, Department of Food Engineering, University of Mersin, Mersin 33343, Turkey. Email: seherserin@yahoo.com

OPEN ACCESS **RESEARCH ARTICLE**

Abstract

This study investigated the *in vitro* digestibility, glycaemic index (GI) and bile acid–binding capacity (BABC) of some potential resistant starch source food products (PRSF). Commercially available resistant starch (RS) samples, Hylon VII (RS2), Novelose 330 (RS3) and Fibersym (RS4) were also included in the study. The RS content of the PRSF used in this study was in the range of 25–77%. Standardised static *in vitro* digestion processes were applied, and the total digestibility, GI and BABC of the samples were determined. The digestibility of commercial RS samples was lower than the PRSF samples. No significant correlation was found between digestibility and RS or total dietary fibre (TDF) contents of the samples. A statistically significant positive correlation was obtained between GI and in vitro digestibility values. In addition, there was a statistically significant negative correlation between the GI and TDF content of the PRSF samples. In addition, it was observed that neither RS content nor RS type had a significant effect on BABC.

Keywords: bile acid–binding capacity; glycemic index; *in vitro* digestion; resistant starch

Introduction

There are many studies on the correlation between health and nutrition worldwide. Nutritional preferences play a positive role in the prevention of common diseases such as cardiovascular diseases, diabetes and obesity. The most important method for treating such diseases is to balance blood glucose levels. The quantity and type of carbohydrates found in foods are the most significant parameters affecting blood glucose levels (Ergun, 2014). Foods with a low glycemic index (GI) improve blood glucose control in patients with diabetes mellitus (Wolever *et al.*, 2003). In addition, the effects of specific functional food components in the structure of foods on these conditions have been investigated, and several *in vitro* studies have emphasized that dietary fibre effectively lowers cholesterol levels by binding bile acids (Sayar *et al.*, 2007). Therefore, it is recommended that the consumption of

foods with a low GI-containing high dietary fibre and resistant starch (RS) should be increased for managing such chronic metabolic conditions. RS is a type of starch and a starch hydrolysis product that is not digested or partially digested in the small intestine and fermented in the colon. A type of RS, RS1, is physically inaccessible starch, and RS2 is non-gelatinized starch. RS3 is retrograded starch formed in starchy foods during the cooling of gelatinized starch in moist and hot foods. It is the most resistant starch and is entirely resistant to the activity of pancreatic enzymes. RS4 is a modified (chemically) starch that is resistant to hydrolysis by enzymes. New bonds other than α-(1-4) or α-(1-6) are formed in this case. In addition to these four typical forms, a novel form, RS5, has recently been recognized. This has been achieved by the complexation of amylose with lipids (Hasjim *et al.*, 2013). Researchers have also focused on improving the content of RS in natural starch in different

foods via various modifications. Different cooking processes have a distinct impact on the RS content of foods. Nowadays, researchers are interested in RS owing to its health benefits and functional characteristics.

Bile acids are the main compounds of bile, and acidic steroids are synthesized from cholesterol in the liver, reabsorbed by the terminal ileum and stored in the gallbladder. Accumulating bile acids in body systems is not desirable because it may lead to hypercholesterolemia and high levels of total cholesterol in the bloodstream. A possible mechanism of lowering plasma cholesterol levels is to bind bile acids by forming insoluble complexes in the intestines and excreting them in faeces (Ngoh *et al.*, 2017). Cholesterol is a lipid that plays a vital role in forming cell membranes and synthesizing bile acids, vitamin D and steroid hormones. Despite its physiological function, abnormal cholesterol levels cause damage to the coronary arteries, thus leading to the development of various cardiovascular diseases. Overall, 30–60% of total cholesterol reaching the intestines is absorbed by entering the bloodstream. Dietary cholesterol is de-esterified by cholesterol esterases, which form micelles containing plant sterols that can be absorbed through the intestinal walls (Pérez-Gálvez *et al.*, 2015). There is a close correlation between bile acid metabolism and glucose and lipid metabolism, which indicates the potential use of bile acid–binding agents for the treatment of hypercholesterolemia, obesity, insulin resistance and diabetes mellitus (Siow *et al.*, 2016). Such continued depletion of bile acids reduces serum cholesterol levels by directing cholesterol to the production of bile acids (Camire and Dougherty, 2003). Another study has reported that polysaccharides or dietary fibre, owing to their high bile acid–binding capacity (BABC), can significantly decrease blood lipid levels in patients with hyperlipidaemia.

Different types of resistant starch may differ from each other chemically, physically and nutritionally. In the literature, there are studies examining many properties of resistant starches. However, studies examining the effects of resistant starch types on digestibility, cholesterol metabolism and effects on the GI are limited. Therefore, in this study, foods containing different types of resistant starch were selected and subjected to in vitro digestion, and as a result of these processes, the effects of resistant starch type on digestibility, BABC and GI were investigated. Results were also compared with commercial pure resistant starches.

Material and Methods

Materials

Pepsin (P7012, ≥2.500 U/mg protein), pancreatin (P7545, 8 × U.S.P. specification), bile extract (B8631),

cholestyramine (C4650), cellulose (435236), TAME (T4626), potassium chloride (P9541), potassium dihydrogen phosphate (1.04877), sodium bicarbonate (S5761), sodium chloride (S9888), magnesium chloride hexahydrate (M8266), ammonium carbonate (207861), calcium chloride hydrate (C8106), sodium acetate (S2889), potassium hydroxide (P5958), 2(N-morfolin) ethane sulfonic acid (MES) (1.06126), tris (hydroxymethyl) aminomethane (TRIS) (1.08382) and other chemicals and consumables used in the study were obtained from Sigma Aldrich (St. Luis, MO, USA). Bile-acid analysis kits and standards were purchased from Trinity Biotech (Bray Co., Wicklow, Ireland). The chemicals used in GI test, the glucose analysis kit, the total starch analysis kit and the dietary fibre analysis kit were purchased from Megazyme International Ireland Ltd (Wicklow, Ireland).

Two groups of food materials were used in this study. The first group was the commercially available resistant starch (RS) samples, high amylose corn starch-Hylon VII (RS2) (Ingredion, IL, USA), Novelose 330 (RS3) (Ingredion, IL, USA) and Fibersym (RS4) (MGP Ingredients, KS). The second group was the potential source of the different types of RS. For this purpose, white beans, green lentils, chickpeas, pasta, potatoes and green banana flour were chosen. All these food samples were obtained from various local companies and processed in different ways.

Preparation of samples

Commercial resistant starch samples were analyzed as is. The pasta sample was vermicelli obtained from durum wheat. The pasta with a total cooking time of 11 min was subjected to a cooking time of 8 min. The pasta samples were put into boiling water, and then they were removed from boiling water at the end of cooking time and washed with cold water. After draining, the samples were passed through the mincer twice. This fresh minced sample was used for the analysis. Green banana samples were peeled and sliced into rings. They were then lyophilized and ground and passed through a 250 μ sieve. They were used as dry flour in all analyses. Potato samples were peeled, cut into 2.5 × 2.5 cm cubes, and boiled in water for 20 min (shorter than the optimum cooking time). They were washed with cold water after the boiling time was complete. The boiled and cooled potato slices were mashed with a spatula. The mashed sample was subjected to analysis. While green lentil was boiled in water for 40 min with a lentil/water ratio of 1/4 (v/v), white beans were boiled in water for 110 min with a bean/water ratio of 1/6 (v/v) and chickpeas were boiled in water for 150 min with a chickpea/water ratio of 1/6 (v/v). The samples were filtered after the boiling time and cooled to room temperature. They were then stored overnight at +4°C. This storage process was chosen as it altered the resistant

starch formation in these samples (Ozturk *et al.*, 2011). They were thawed at room temperature on the following day. The legume samples thawed before analysis were passed through the mincer and used for the analysis.

Determination of proximate composition

The moisture contents of the samples were determined by an infrared moisture analyzer (Denver Instrument Moisture Analyzer, IR-200, USA). The amount of protein was determined according to Kjeldahl's method and based on a nitrogen–protein conversion factor of 6.25 (Method 46-13, AACC International, 1995). The total lipid content was gravimetrically determined by extracting samples with petroleum ether in the Soxhlet system (Method 30-20, AACC International, 1995). The results of the analyses were calculated on a dry matter basis.

Determination of total and resistant starch content, total dietary fibre content and glycaemic index

The total starch (TS) analysis of the samples was performed according to the KOH Format-AOAC 996.11 method (Anonymous, 2011). Englyst *et al.* (1992) method was used for the determination of resistant starch (RS) content. In this method, RS was taken as the unhydrolyzed starch remaining after 120 min of *in vitro* starch digestion. Total dietary fibre analyses were performed according to Prosky *et al.* (1988) using Megazyme Kits (K-TDFR; Megazyme Ltd, Co.Wicklow, Ireland), where the food samples (duplicate) were subjected to sequential enzymatic digestion by heat-stable α-amylase (30 min at 98–100°C), protease (30 min at 60°C) and amyloglucosidase (30 min at 60°C, pH = 4.5).

The estimated GI values of samples were based on the methods of Englyst *et al.* (1992) and Regand *et al.* (2011) under *in vitro* conditions. The hydrolysis index (HI) was calculated from the starch hydrolysis rate versus time curves. The area under the curve (0–180 min) for the test product is expressed as a percentage of the corresponding area for white bread. The estimated GI was calculated by using the following equation (Eq.1) given by Goni *et al.* (1997).

$$GI = 0.549\,HI + 39.71 \qquad (1)$$

In vitro digestion and bile acid–binding capacity

In vitro digestion analyses were performed according to the procedure prepared by Minekus *et al.* in 2014. The practical static digestion method was prepared by Minekus *et al.* (2014) based on human gastrointestinal

physiology. Saliva amylase hydrolysis method was not used in this study. Furthermore, pancreatin was used instead of individual enzymes such as trypsin and chymotrypsin for intestinal phase digestion. The mouth, gastric and intestinal phases used in the analysis are presented in Table 1. 5 g (dry basis) of each minced sample was dissolved in 3.5 mL of salivary solution (SSF, pH 7.0) to simulate mouth condition and incubated at 37°C for 2 min with continuous shaking. Subsequently, the pH of the mixture was adjusted to 3.0 (by 1M HCl), followed by the addition of 7.5 mL of gastric solution (SGF) and 1.6 mL pepsin solution (2000 U/mL) to simulate gastric condition. After incubation at 37°C for 2 h, the pH of the mixture was adjusted to 7.0 (by 1M NaOH). Then, 11 mL of intestinal solution (SIF), 5 mL pancreatin solution (based on trypsin activity at 100 U/mL) and 2.5 mL bile acid solution (1.6 mM) were added to simulate intestinal condition. The mixture was incubated at 37°C for 2 h with continuous shaking. The digested samples were centrifuged for 5 min at 5000 rpm. The supernatant was used for BABC analyses, and the pellets were freeze-dried for other studies. The digestibility of the samples was calculated from the following equation:

$$\text{Digestibility (\%)} = \frac{M_S - M_P}{M_S} \times 100 \qquad (2)$$

Where, M_S is the dry weight of the sample taken for the *in vitro* digestion process; and M_P is the dry weight of the pellets (indigestible part).

The amount of bound bile acid was determined from the difference between the total amount of bile acids added during the *in vitro* digestion step and the amount measured in the supernatant. BABC tests were performed in three parallels and three replicates for each sample.

Bile acid–binding tests of the samples were performed based on the method developed by Sayar *et al.* (2005).

Table 1. Composition of the stock solutions of simulated digestive fluids.

Salt solution added	Final salt concentration in the fluid, mM		
	SSF (pH 7)	SGF (pH 3)	SIF (pH 7)
KCl	15.1	6.9	6.8
KH$_2$PO$_4$	1.35	0.9	0.8
NaHCO$_3$	13.68	25	85
NaCl	-	47.2	38.4
MgCl$_2$(H$_2$O)$_6$	0.15	0.12	0.33
NH$_4$(CO$_3$)$_2$	0.06	0.5	-
CaCl$_2$(H$_2$O)$_2$	1.5	0.15	0.6

SSF, simulated salivary fluid; SGF, simulated gastric fluid; SIF, simulated intestinal fluid

The BABC was calculated as mmol/100 dry samples using commercial kit from Trinity Biontech Plc., Bray Co. Wicklow, Ireland. The absorbance of each mixture was measured at 530 nm, and cholestramine was also included as a positive control for each test.

Statistical evaluation

The SPSS version 16 (SPSS Inc., Chicago, IL) statistical analysis package programme was used to evaluate the data. The one-way ANOVA analysis was performed to determine whether there was a difference between the means in cases where more than two groups should be compared to each other in terms of one variable examined. The difference was evaluated by Duncan's test at a confidence interval of 95%. All procedures and analyses were performed repeatedly. However, the parallel number (at least two) varied according to the sensitivity of the analysis.

Results and Discussion

Chemical composition and resistant starch content

Results of the general composition analysis of the potential resistant starch source food samples are presented in Table 2. The values given for each food sample in this table agree with those given in the literature before (Brummer *et al.*, 2015; Ghribi *et al.*, 2015; Hoover and Ratnayake, 2002; Osorio-Diaz *et al.*, 2002; Ramdath *et al.*, 2020; Zhang *et al.*, 2005). Protein and lipid contents of potential RS2 source food samples are lower than potential RS3 source food samples. However, starch contents of RS2 source food samples are significantly higher. TDF results showed that potential RS3 source food legume samples contain higher amounts of TDF than green

banana flour, boiled potato and spaghetti samples. This can be explained by the presence of non-starch polysaccharides, which are more common in legume samples.

Resistant starch and TDF contents of the commercial resistant starch samples used in the study are presented in Table 2. TDF contents of Hylon VII, Novelose 330, and Fibersym found in this study were in the range of the results obtained in different previous studies (Kahraman *et al.*, 2019; Le Leu *et al.*, 2009; McCleary *et al.*, 2013; Sang and Seib, 2006). The RS values seen in Table 2 were determined by direct *in vitro* digestion of the samples as they were eaten, as stated in the "Material and Methods" section above. Therefore, as expected, the RS values were higher than the TDF results for most of the samples studied. Although resistant starch is considered a dietary fibre, the methods used for both components greatly affect the results obtained. In TDF analysis, there is a heating process at high temperatures at the thermostable alpha-amylase hydrolysis stage. Therefore, starch fraction resistant to digestion in the resistant starch analysis could be hydrolyzed in the TDF analysis because it was exposed to heating. It was evaluated that this situation caused the high difference between TDF and RS results. Similar discussions can be seen in McCleary *et al.* (2013).

Estimated glycaemic index

Hydrolysis index and estimated GI values of the samples used in this study are shown in Table 3. The HI expresses the ratio of the rate of digestion of starch in a food sample to the digestibility of starch in a reference food (mostly white bread) (Goni *et al.*, 1997). The GI value is calculated using the HI values from the widely accepted Equation 1 (Goni *et al.*, 1997).

Table 2. Protein, lipid, total starch (TS) and total dietary fibre (TDF) content of the potential resistant starch source food samples and the commercial resistant starch samples, % (d.b.).*

RS type	Sample	Protein	Lipid	TS	RS	TDF
Potential RS2 source	Green banana flour	6.0 ± 0.2[d]	0.8 ± 0.2[c]	82.5 ± 1.1[a,b]	76.7 ± 2.5[a,b]	19.4 ± 5.3[c,d]
	Boiled potato	14.4 ± 0.5[c]	1.6 ± 0.5[b]	87.1 ± 2.4[a]	24.8 ± 1.3[e]	5.0 ± 1.1[e]
	Al dente pasta	12.9 ± 0.5[c]	0.3 ± 0.0[d]	72.7 ± 2.6[c]	43.6 ± 3.6[c]	16.2 ± 3.0[d]
Potential RS3 source	Common bean	20.9 ± 0.8[b]	1.7 ± 0.4[b]	33.3 ± 2.1[e]	25.4 ± 1.2[e]	39.3 ± 3.8[b]
	Green lentil	23.2 ± 0.9[a]	1.6 ± 0.8[b]	40.3 ± 3.7[d,e]	31.0 ± 0.4[d]	36.6 ± 4.9[b]
	Chickpea	21.2 ± 0.8[b]	6.6 ± 0.2[a]	44.4 ± 1.7[d]	32.7 ± 1.1[d]	25.9 ± 3.4[c]
RS2	Hylon VII	-	-	-	86.1 ± 5.3[a]	16.3 ± 2.3[d]
RS3	Novelose 330	-	-	-	70.8 ± 2.0[b]	40.1 ± 2.6[b]
RS4	Fibersym	-	-	-	80.2 ± 3.5[a]	70.0 ± 2.7[a]

*Values are expressed as mean ± standard deviation of triplicate analysis. Values within a column followed by a common letter are not significantly different (P > 0.05).

It was found that the starch digestibility of the samples containing different types of RS was different (Figure 1). As expected, the white bread (control sample) exhibited the highest starch hydrolysis, followed by boiled potatoes and *Al dente* pasta. Green banana flour and Hylon VII exhibited the lowest starch hydrolysis rates. These differences are considered to be due to the amount of RS contained in the samples and their structure and interactions with other components. Generally, the GI value is greatly affected by starch digestibility in the food system. It was observed that the GI values of the samples used in this study ranged from 42.2 to 86.6. The WHO and United Nations Food and Agriculture Organization divided foods into the following three groups: those with low, moderate and high GI. Foods with a GI value of <55 are defined as those with a low GI, those with a value of 56–69 are defined as those with a moderate GI and those with a value of >70 are defined as those with a high GI (Henry *et al.*, 2005).

The evaluation of the results showed that the boiled potatoes had the highest GI value among the samples. *Al dente* pasta and commercial RS3 samples have moderate GI values. As expected, other legumes, green bananas and RS4 samples were found to have low GI values. Studies on the parameters affecting GI values have revealed a strong correlation between the GI values and *in vitro* digestibility of starchy foods. Resistant starch decreases the GI value because it passes through the small intestine to the large intestine without being digested.

Potato starch is digested faster because it contains less amylose than other foods. Potato starch contains approximately 18% amylose and 82% amylopectin (Ergun, 2014). Some studies comparing the GI values of various foods have shown that the highest blood glucose response is achieved in potatoes, and the lowest blood glucose response is achieved in legumes (Ergun, 2014). The results obtained from this study confirm these results. The GI values of the three different legume samples were lower than those of others. Furthermore, the GI values of
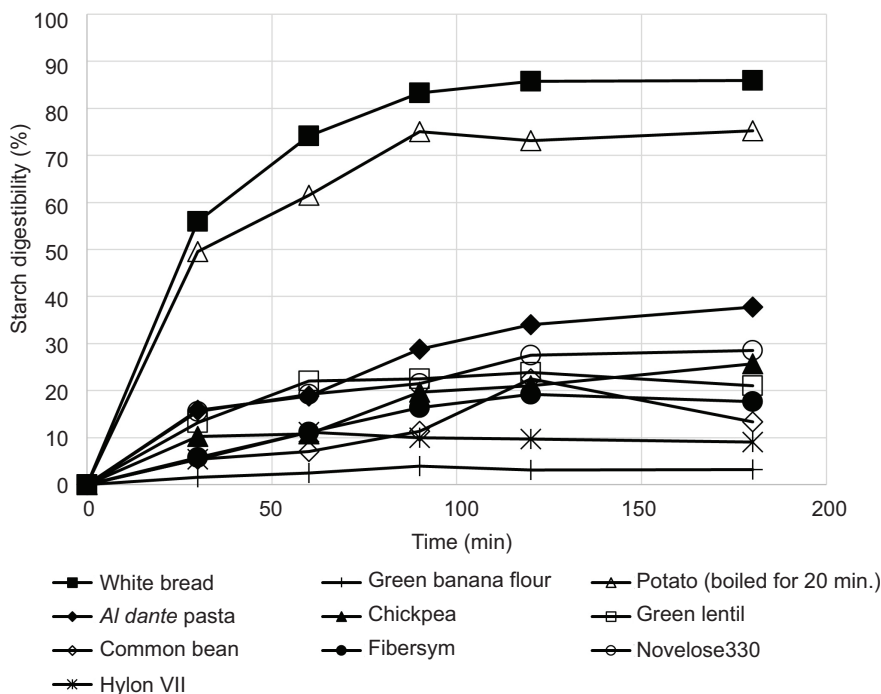
Table 3. Hydrolysis index (HI) and estimated glycaemic index (GI) of the potential resistant starch source food samples and the commercial resistant starch samples.*

RS type	Sample	HI	GI
	White bread (reference)	100 ± 0.0^a	92.3 ± 3.2^a
Potential RS2 source	Green banana flour	4.6 ± 1.3^g	42.2 ± 0.7^g
	Boiled potato	85.4 ± 8.9^b	86.6 ± 4.9^b
	Al dente pasta	44.3 ± 2.8^d	64.0 ± 1.5^d
Potential RS3 source	Common bean	$13.3 \pm 1.9^{f,g}$	$47.1 \pm 1.1^{f,g}$
	Green lentil	$22.0 \pm 8.3^{e,f}$	$51.8 \pm 4.6^{e,f}$
	Chickpea	$21.2 \pm 6.6^{e,f}$	$51.4 \pm 3.6^{e,f}$
RS2	Hylon VII	$21.9 \pm 5.3^{e,f}$	$49.7 \pm 1.0^{e,f}$
RS3	Novelose 330	42.8 ± 4.1^d	63.2 ± 2.2^d
RS4	Fibersym	$13.6 \pm 9.7^{f,g}$	$47.2 \pm 5.3^{f,g}$

*Values are expressed as mean ± standard deviation of triplicate analysis. Values within a column followed by a common letter are not significantly different (P > 0.05).

Figure 1. Hydrolysis curve of the samples containing resistant starch.

the legume samples are close to others. Low blood glucose levels of legumes have been correlated with higher levels of protein and amylose than those in other foods. Fat and protein contents of foods play significant roles in the GI value of foods. Fats also prolong food passage time through the stomach into the intestines and reduce the GI value by forming complexes with starch (Ergun, 2014). Green banana flour has the lowest GI value among the samples used in this study. Agama-Acevedo *et al.* (2012) observed that adding different amounts of unripe green banana flour to the biscuit formulation increased the RS and slowly digestible starch content and decreased the GI values in the products. In unripe banana starch, a large amount of RS is correlated with the type B crystal structure (Srikaeo *et al.*, 2011). In addition, the use of green banana flour without heating, unlike that carried out for other samples, is considered a significant parameter of the low GI value. In the case of *al dente* pasta, the GI value was obtained as 64, which belongs to the group of foods with moderate GI. Pasta is vital in the human diet because it is easy to prepare, economical and can be consumed in different ways based on cooking time. The cooking time for pasta has a great impact on starch gelatinization. Pasta with a short cooking time is called *al dente*. Because not all the starch in *Al dente* pasta is gelatinized, the RS in this sample was accepted as RS2 in this study. A similar approach is also seen in Serin *et al.* (2016).

In vitro digestibility (IVD) and bile acid–binding capacity

The *in vitro* digestibility of the samples used in this study is presented in Table 4. The results showed that the commercial RS samples have a lower digestibility than the potential RS-containing food samples. Fibersym (RS4) has the lowest digestibility rate among the commercial RS samples used. When the digestibility rates are examined, it is seen that they are tended to be inversely proportional to the RS contents. However, there was no significant correlation between digestibility and RS or TDF contents of the samples (P > 0.05). The enzyme systems used in the *in vitro* digestion method simulate the processes in the human digestive system.

The results of the BABC values obtained in this study are provided in Table 4. BABC of the positive control cholestyramine sample, known for its bile acid–binding property, 1.10 µmol/g, which is close to 84% of the total amount of added bile acids. This amount is similar to the values obtained for cholestyramine in the literature (Sayar *et al.*, 2005). Although the BABC of the samples used in this study were not significantly different, green lentil and common bean tend to have higher binding capacity. A comparison of these results with those from previous studies was made based on binding to cholestyramine (assigning BA binding to cholestyramine as 100%) to eliminate methodological implications. BABC of the commercial RS2, RS3 and RS4 samples were between 6.3 and 10.0% relative to cholestyramine. The relative BABC of the potential RS2 and RS3 source food samples were 2.7–5.4% and 10.0–11.8%, respectively. Except for the potential RS3 source food samples, the BABC values of the samples used in this study were lower than those found for various food components in the literature (Table 5). Although the mechanism by which hydrocolloids bind bile acids remains unclear, some possible mechanisms have been suggested. First, hydrocolloids form a physical barrier that prevents bile acid micelles from reaching

Table 4. In vitro digestibility and bile acid–binding capacity of the potential resistant starch source food samples and the commercial resistant starch samples.*

RS type	Samples	Digestibility (%)	BABC (µmol/g)	BABC, % (relative to cholestyramine)**
	Cholestyramine	-	1.10 ± 0.33[a]	100.00
Potential RS2 source	Green banana flour	15.7 ± 3.6[e]	0.03 ± 0.01[b]	2.7
	Boiled potato	77.9 ± 2.7[a]	0.06 ± 0.02[b]	5.4
	Al dente pasta	59.8 ± 0.4[b]	0.05 ± 0.05[b]	4.5
Potential RS3 source	Common bean	49.2 ± 2.6[c]	0.13 ± 0.01[b]	11.8
	Green lentil	27.1 ± 0.1[d]	0.13 ± 0.01[b]	11.8
	Chickpea	29.1 ± 2.5[d]	0.11 ± 0.01[b]	10.0
RS2	HylonVII	13.3 ± 1.1[e]	0.11 ± 0.01[b]	10.0
RS3	Novelose 330	25.6 ± 1.6[d]	0.07 ± 0.00[b]	6.3
RS4	Fibersym	5.4 ± 1.4[f]	0.07 ± 0.01[b]	6.3

*Values are expressed as mean ± standard deviation of triplicate analysis. Values within a column followed by a common letter are not significantly different (P > 0.05).
**In order to compare the results given in different units in various studies in the literature, the amount of bile acid binding was calculated as relative to cholestyramine as the amount bound by cholestyramine was taken as 100%.

Table 5. The amounts of bile acids binding capacities of different food samples.

Sample	BABC, % (relative to cholestramine)*	Reference
Modern and old bread wheat varieties and landraces	5.2–14.0	Cetiner et al., 2022
Lobster shell–derived chitin, chitosan and chitooligosaccharides	10–50	Xu et al., 2020
Spaghetti supplemented with 15–25% RS4	8.4–8.9	Aribas et al., 2020
Fava bean (whole)	14.62	Karatas et al., 2017
Different types of peptides of kidney beans	17.50–70.54	Ngoh et al., 2017
Different types of tea	13.20–45.83	Wu et al., 2015
Broccoli	4.6	Kahlon et al., 2007
Spinach	8.6	
Oat flours	7.5–14.8	Sayar et al., 2005

*In order to compare the results given in different units in various studies in the literature, the amount of bile acid binding was calculated as relative to cholestyramine as the amount bound by cholestyramine was taken as 100%.

intestinal absorptive cells; second, hydrocolloids interact directly with bile acid at the molecular level; and third, hydrocolloids form a viscous structure or gelatinous network that holds bile acids (Gannasin et al., 2016).

Based on Table 5, dietary fibre-rich foods or food components bind a significant amount of bile acids. Some of them have a BABC value of approximately 50–70% relative to cholestyramine. However, the BABC values of the samples in this study were 4.5–11% relative to cholestyramine. These results indicate that RS has a lower BABC value than the other types of dietary fibres. When all samples used in the study (commercial resistant starches and potential resistant starch source food samples) were included in the evaluation, no statistically significant correlation was found between BABC and RS or TDF (P > 0.05). However, if only food samples were considered, then there was no statistically significant correlation between BABC and RS (P > 0.05). At the same time, the correlation between BABC and TDF was significant (P < 0.05). According to the results obtained in this study, neither the amount nor the type of resistant starch had a significant effect on the BABC. However, it can be said that dietary fibres other than RS are more effective.

The existing studies have indicated that specific food components, such as insoluble dietary fibres, lignin and tannins, are relatively more effective in binding bile acids (Karatas et al., 2017; Sayar et al., 2005). Bile acids are complex amphipathic structures with both hydrophobic and hydrophilic groups. It has been stated that the carboxyl group and steroid rings of bile acids are important structural features for binding bile acid to ligands (Ngoh et al., 2017). The process of excretion of bile acids from the human digestive system by different mechanisms with the effect of various food components has not yet been fully elucidated. Although many factors affecting this mechanism have been identified, more in-depth studies are needed.

Conclusions

The effects of the amounts and the types of RS on digestibility, GI and BABC capacity were investigated by using different RS samples commercially available in the market and food samples containing different RS types. The digestibility values of commercial RS samples were found to be relatively low. There were significant differences between the digestibility values of the food samples containing different types of RS. GI values of the samples used in this study were determined to have "low" and "moderate" GIs, except boiled potato sample. A positive correlation trend between the *in vitro* digestibility and GI values of the samples was obtained. However, TDF values tend to have a negative correlation with the digestibility. Similarly, negative correlation between RS and digestibility was also found. It was found that types of RS did not have any correlation with digestibility or GI values. The most notable findings in this study are related to the bile acid–binding properties. According to the findings, it was seen that RS content or RS type did not affect BABC. The BABC values of both commercial resistant starch samples and food samples containing RS were relatively low. Although, most of the dietary fibres are known to increase BABC values, RS has no effect on BABC.

Acknowledgement

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

Author Contributions

Seher Serin contributed to the methodology, software and the original draft. Sedat Sayar contributed to the

conceptualization, software, validation and study super-vision. Both authors were involved in the review and editing of the manuscript.

References

AACC, 1995. "American Association of Cereal Chemists. Approved methods of the AACC" 9th -15 A., and Method 46-13 A., The Association, St. Paul, MN.

Agama-Acevedo, E., Islas-Hernández, J.J., Pacheco-Vargas, G., Osorio-Díaz, P. and Bello-Pérez, L.A., 2012. Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. LWT–Food Science and Technology 46(1): 177–182. https://doi.org/10.1016/j.lwt.2011.10.010

Anonymous, 2011. Total starch assay procedure online, AOCC Official Method 996.11, Megazyme. Available at: https://secure.megazyme.com/files/Booklet/K-TSTA-DATA.pdf Accessed 13 January 2014.

Aribas, M., Kahraman, K. and Koksel, H., 2020. *In vitro* glycemic index, bile acid binding capacity and mineral bioavailability of spaghetti supplemented with resistant starch type 4 and wheat bran. Journal of Functional Foods 65: 103778. https://doi.org/10.1016/j.jff.2020.103778

Brummer, Y., Kaviani, M., and Tosh, S.M., 2015. Structural and functional characteristics of dietary fibre in beans, lentils, peas and chickpeas. Food Research International 67: 117–125. https://doi.org/10.1016/j.foodres.2014.11.009

Camire, M.E. and Dougherty, M.P., 2003. Raisin dietary fiber composition and *in vitro* bile acid binding. Journal of Agricultural and Food Chemistry 51(3): 834–837. https://doi.org/10.1021/jf025923n

Cetiner, B., Tömösközi, S., Schall, E., Salantur, A. and Koksel, H., 2022. Bile acid binding capacity, dietary fibre and phenolic contents of modern and old bread wheat varieties and landraces: a comparison over the course of around one century. European Food Research and Technology 248(2): 589–598. https://doi.org/10.1007/s00217-021-03906-8

Englyst, H.N., Kingman, S.M. and Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46: 33–50.

Ergun, R., 2014. Türkiye'ye Özgü Bazı Ekmek Türlerinin Glisemik İndeks Değerlerinin Saptanması, Master Thesis, Hacettepe University Institute of Health Sciences, Ankara.

Gannasin, S.P., Adzahan, N.M., Mustafa, S. and Muhammad, K., 2016. Techno-functional properties and *in vitro* bile acid-binding capacities of tamarillo (*Solanum betaceum* Cav.) hydrocolloids. Food Chemistry 196: 903–909. https://doi.org/10.1016/j.foodchem.2015.09.081

Ghribi, A.M., Sila, A., Gafsi, I.M., Blecker, C., Danthine, S., Attia, H., *et al.*, 2015. Structural, functional, and ACE inhibitory properties of water-soluble polysaccharides from chickpea flours. International Journal of Biological Macromolecules 75: 276–282. https://doi.org/10.1016/j.ijbiomac.2015.01.037

Goni, I., Garcia-Alonso, A. and Saura-Calixto, F., 1997. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 17(3): 427–437. https://doi.org/10.1016/S0271-5317(97)00010-9

Hasjim, J., Ai, Y. and Jane, J.L., 2013. Novel applications of amylose-lipid complex as resistant starch type 5. In: Shi, Y.C. and Maningat, C.C. (eds.) Resistant starch: sources, applications and health benefits. Chapter 4, John Wiley & Sons, Hoboken, New Jersey, pp 79–94. https://doi.org/10.1002/9781118528723.ch4

Henry, C.J.K., Lightowler, H.J., Strik, C.M., Renton, H. and Hails, S., 2005. Glycemic index and glycemic load values of commercially available products in the UK. British Journal of Nutrition 94: 922–930. https://doi.org/10.1079/BJN20051594

Hoover, R. and Ratnayake, W.S., 2002. Starch characteristics of black bean, chickpea, lentil, navy bean and pinto bean cultivars grown in Canada. Food Chemistry 78(4): 489–498. https://doi.org/10.1016/S0308-8146(02)00163-2\

Kahlon, T.S., Chapman, M.H. and Smith, G.E., 2007. *In vitro* binding of bile acids by spinach, kale, brussels sprouts, broccoli, mustard greens, green bell pepper, cabbage and collards. Food Chemistry 100(4): 1531–1536. https://doi.org/10.1016/j.foodchem.2005.12.020

Kahraman, K., Aktas-Akyildiz, E., Ozturk, S. and Koksel, H., 2019. Effect of different resistant starch sources and wheat bran on dietary fibre content and *in vitro* glycemic index values of cookies. Journal of Cereal Science 90: 102851. https://doi.org/10.1016/j.jcs.2019.102851

Karatas, S.Ç., Günay, D. and Sayar, S., 2017. *In vitro* evaluation of whole faba bean and its seed coat as a potential source of functional food components. Food Chemistry 230: 182–188. https://doi.org/10.1016/j.foodchem.2017.03.037

Le Leu, R.K., Hu, Y., Brown, I.L. and Young, G.P., 2009. Effect of high amylose maize starches on colonic fermentation and apoptotic response to DNA-damage in the colon of rats. Nutrition & Metabolism 6(1): 1–9. https://doi:10.1186/1743-7075-6-11

McCleary, B.V., Sloane, N., Draga, A. and Lazewska, I., 2013. Measurement of total dietary fiber using AOAC Method 2009.01 (AACC International Approved Method 32-45.01): evaluation and updates. Cereal Chemistry 90(4): 396–414. https://doi.org/10.1094/CCHEM-10-12-0135-FI

Minekus, M., Alminger, M., Alvito, P., Ballence, S., Bohn, T., Bourlieu, C., *et al.*, 2014. A standardised static *in vitro* digestion method suitable for food-an international consensus. Food Function 5: 1113–1124. https://doi.org/10.1039/C3FO60702J

Ngoh, Y.Y., Choi, S.B. and Gan, C.Y., 2017. The potential roles of Pinto bean (*Phaseolus vulgaris* cv. Pinto) bioactive peptides in regulating physiological functions: protease activating, lipase inhibiting and bile acid binding activities. Journal of Functional Foods 33: 67–75. https://doi.org/10.1016/j.jff.2017.03.029

Osorio-Dıaz, P., Bello-Perez, L.A., Agama-Acevedo, E., Vargas-Torres, A., Tovar, J. and Paredes-López, O., 2002. *In vitro* digestibility and resistant starch content of some industrialized commercial beans (*Phaseolus vulgaris* L.). Food Chemistry 78(3): 333–337. https://doi.org/10.1016/S0308-8146(02)00117-6

Ozturk, S., Koksel, H. and Ng, P.K., 2011. Production of resistant starch from acid-modified amylotype starches with enhanced functional properties. Journal of Food Engineering 103(2): 156–164. https://doi.org/10.1016/j.jfoodeng.2010.10.011

Pérez-Gálvez, R., García-Moreno, P.J., Morales-Medina, R., Guadix, A. and Guadix, E.M., 2015. Bile acid binding capacity

of fish protein hydrolysates from discard species of the West Mediterranean Sea. Food & Function 6(4): 126–1267. https://doi.org/10.1039/c4fo01171f

Prosky, L., Asp, N.G., Schweizer, T.F., DeVries, J.W. and Furda, I., 1988. Determination of insoluble, soluble and total dietary fiber in foods and food products: interlaboratory study. Journal of AOAC International 71: 1017–1023. https://doi.org/10.1093/jaoac/71.5.1017

Ramdath, D.D., Lu, Z.H., Maharaj, P.L., Winberg, J., Brummer, Y. and Hawke, A., 2020. Proximate analysis and nutritional evaluation of twenty Canadian lentils by principal component and cluster analyses. Foods 9(2): 175. https://doi.org/10.3390/foods9020175

Regand, A., Chowdhury, Z., Tosh, S.M., Wolever, T.M.S. and Wood, P., 2011. The molecular weight, solubility and viscosity of oat beta-glukan affect human Glycemic response by modifying starch digestibility. Food Chemistry 129: 297–304. https://doi.org/10.1016/j.foodchem.2011.04.053

Sang, Y. and Seib, P.A., 2006. Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation. Carbohydrate Polymers 63(2): 167–175. https://doi.org/10.1016/j.carbpol.2005.07.022

Sayar, S., Jannink, J.L. and White, P.J., 2005. *In vitro* bile acid binding of flours from oat lines varying in percentage and molecular weight distribution on β-glucan. Journal of Agricultural and Food Chemistry 53: 8797–8803. https://doi.org/10.1021/jf051380g

Sayar, S., Jannink, J.L. and White, P.J., 2007. Digestion residues of typical and high-β-glucan oat flours provide substrates for *in vitro* fermentation. Journal of Agricultural and Food Chemistry 55(13): 5306–5311. https://doi.org/10.1021/jf070240z

Serin, S., Durkan, Ö. and Sayar, S., 2016. Effect of cooking time on *in vitro* starch digestibility and bile acid binding capacity of pasta, 15th European Young Cereal Scientists and Technologists Workshop, University of Milan, April 26–29.

Siow, H.L., Choi, S.B. and Gan, C.Y., 2016. Structure–activity studies of protease activating, lipase inhibiting, bile acid binding and cholesterol-lowering effects of pre-screened cumin seed bioactive peptides. Journal of Functional Foods 27: 600–611. https://doi.org/10.1016/j.jff.2016.10.013

Srikaeo, K., Mingyai, S. and Sopade, P.A., 2011. Physicochemical properties, resistant starch content and enzymatic digestibility of unripe banana, edible canna, taro flours and their rice noodle products. International Journal of Food Science & Technology 46(10): 2111–2117. https://doi.org/10.1111/j.1365-2621.2011.02724.x

Wolever, T.M.S., Vorster, H.H., Björck, I., Brand-Miller, J., Brighenti, F., Mann, J.I., *et al.*, 2003. Determination of the glycemic index of foods: interlaboratory study. European Journal of Clinical Nutrition 57(3): 475–482. https://doi:10.1038=sj.ejcn.1601551

Wu, Z., Teng, J., Huang, L., Xia, N. and Wei, B., 2015. Stability, antioxidant activity and *in vitro* bile acid-binding of green, black and dark tea polyphenols during simulated *in vitro* gastrointestinal digestion. RSC Advances 5(112): 92089–92095. https://doi.org/10.1039/C5RA18784B

Xu, W., Mohan, A., Pitts, N.L., Udenigwe, C. and Mason, B., 2020. Bile acid-binding capacity of lobster shell-derived chitin, chitosan and chitooligosaccharides. Food Bioscience 33: 100476. https://doi.org/10.1016/j.fbio.2019.100476

Zhang, P., Whistler, R.L., BeMiller, J.N. and Hamaker, B.R., 2005. Banana starch: production, physicochemical properties, and digestibility—a review. Carbohydrate Polymers 59(4): 443–458. https://doi.org/10.1016/j.carbpol.2004.10.014