Effect of Resistant Starch Type 4 Addition on the In Vivo and In Vitro Glycemic Index of Turkish Noodle

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Summary. Background: Resistant starch (RS) is known to improve glucose tolerance and its intake with foods is low. The objective of the study is to enriching Turkish noodles with type 4 RS and determine the effect on the glycemic index (GI). Methods: After preliminary trials and sensory analyses, noodle formulations including 20% and 35% RS were determined and the noodles were produced accordingly. Reference foods (white bread, glucose) and noodles were used in GI test conducted on 15 healthy women. The participants consumed 25 g available carbohydrate amounts of food once a week for 11 weeks. The available carbohydrate amounts of the noodles with RS were calculated in two different ways and two different GI tests were performed for each. The noodle group for which the RS is evaluated in the total dietary fiber is called "TDF" and the noodle group for which it is evaluated in available carbohydrate amount is called "AC". Blood glucose values were measured before consumption and at 15, 30, 45, 60, 90 and 120 min postprandially. GI values were determined by calculating the incremental area under the curve (IAUC). Results: It was determined that the RS noodles in the AC group and the control noodles had low GI. [control noodles: 54.8, noodles with 20% RS(AC): 51.8, noodles with 35% RS(AC): 49.1] As the RS amount increased in the AC group, GI decreased (p<0.05). In vitro GI values were found to be similar to in vivo GI values in the AC group. Conclusions: The addition of type 4 RS reduced the glycemic response of Turkish noodles. The studies examining the effect of RS types on glycemic response should be increased.

Key words: Resistant starch, Glycemic index, Glycemic response, Available carbohydrates, Noodles.

Introduction

The glycemic index (GI) is an extension of the fiber hypothesis stating that dietary fiber diminishes the intestinal absorption speed of foods (1). GI is the percentage of the blood glucose incremental area formed over two hours by a test food containing 25 g or 50 g available carbohydrates consumed by the same individual compared to the blood glucose incremental area formed by the reference food containing an equal amount of available carbohydrates. In 1997, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) expert committee stated that GI was a good classification method that could be used to protect health and select appropriate carbohydrate sources for the treatment of certain diseases (2). Diets based on low GI foods proved to be effective in the prevention of many chronic diseases. Such diets ensured glycemic control in Type 2 diabetes patients and reduced LDL cholesterol level (3-5). They are also known to balance insulin level and proinflammatory parameters such as C reactive protein (CRP) and reduce the risk of obesity and obesity-related diseases (6, 7).

Although there are many factors affecting GI of foods, the starch contents of foods are one of the important ones. This is because the starch is the most

common carbohydrate type in a diet (3, 8, 9). Since the starches in foods are not digested at the same speed, their effects on blood glucose level differ (1, 3). The interest in resistant starch (RS) increased after the starch was discovered to have indigestible fractions and these fractions to be identified as RS (10). RS goes into the large intestine without being digested in the small intestines and it is fermented by bacteria in here. Short chain fatty acids formed by fermenting RS in the colon positively affect intestinal microbiota and digestive system functions. Since it has a low energy value, it supports the prevention of obesity (11). RS supports the reduction of cholesterol levels by increasing the excretion of bile acids and provides a protective effect against heart diseases (12). Also RS has a lower digestion rate than starch and it increases the glucose level in the blood in a more controlled and slowly. In this way, it provides a decrease in glycemic and insulinemic response. There are many studies indicating that it decreases particularly the glycemic response (13-19).

Although RS and GI concepts have gained much importance over the last 10 years, *in vivo* studies to determine GI of foods are limited. Due to high cost of *in vivo* tests and challenging procedures in the testing process, *in vitro* GI determination is generally applied. However, *in vitro* tests are not so reliable as *in vivo* tests (20, 21). This study aims to use type 4 RS which is known to have many benefits for health but cannot be taken in the recommended amount in diets, in enriching Turkish noodles that are frequently consumed in Turkish cuisine and determine its *in vivo* and *in vitro* GI.

Materials and Methods

Plan of the study

The general plan of the study is schematized in figure 1.

Materials

Formulation of the noodle included Fibersym[®] RW (MGP Ingredients, USA) wheat-based crosslinked type 4 RS. Fibersym[®] RW included 91.9% dietary fiber and 83.3% RS (22). The data from this study were used to calculate approximate RS amount of the noodles.

Wheat flour (Selva Food Industry, Inc., for baklava and pastry, TR) and eggs used in noodles were bought from a local grocery considering the expiration date. Using RS instead of some of the wheat flour causes protein loss and spoils the consistency of the noodle dough. Vital gluten was used (Havancızade, TR) to replace the declining protein and reach the ideal noodle dough consistency in noodles with RS.

Test foods

The control noodles and noodles with RS were used as test foods. To determine the control noodle formulation, TS 12950 Turkish noodle standard was used (23). For 100 g wheat flour, 30 g egg and 0.5 g salt was used. The visual of Turkish noodles is shown in figure 2. An expert food engineer was consulted to determine the formulation of RS added noodle. Formulations including RS at different rates were tried, sensory analyses were made and the appropriate formulations were determined.

Thirty and fifty percent Fibersym[®] RW was added to the noodle formulation in noodles with RS. However, since Fibersym[®] RW is not entirely composed of resistant starch and due to other ingredients added to the noodle formulation, these ratios have decreased. The RS content of the noodles with 30% Fibersym RW added is 20% and the RS content of the noodles with 50% Fibersym RW added is 35%.

Reference foods

White bread and glucose were used as reference foods. Pure glucose (Galenik, TR) was used in the powder form. White fresh baked breads were bought from the same local bakery every morning at the same time to minimize the variations in GI tests. (24).

Determination of available carbohydrate amount of the reference and test foods

The moisture, ash, protein and total fat analyses were carried out in Selcuk University Natural Products Application and Research Center and the total dietary

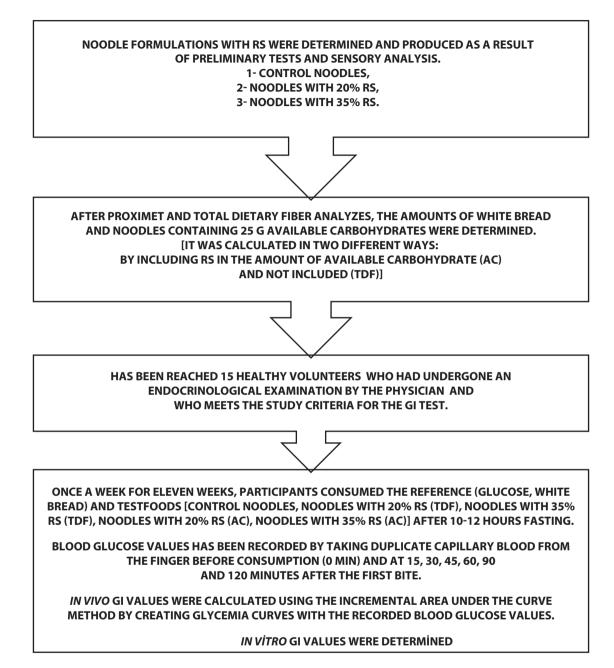


Figure 1. The general plan of the study

fiber analyses were conducted in TUBITAK Marmara Research Center laboratories. The total dietary fiber was determined using the Megazyme Total Dietary Fiber kit with AOAC 991.43 method (25). The available carbohydrate (CHO) amounts were determined by subtracting the sum of moisture, ash, protein, total fat and total dietary fiber values of white bread and noodles from the value of 100 (Table 1) (20). It is recommended to make evaluation over 25 g or 50 g available CHO amounts in GI studies (20, 26). In this study, the 25 g available carbohydrate amounts of reference and test foods were used.

Since RS is a dietary fiber, is excluded from available CHO in GI tests and is consumed in addition to the stable amount of available CHO consumed (2). However, European Food Safety Authority (EFSA)



Figure 2. Turkish noodles

states that the effect of RS on the improvement of glycemia can be achieved when it is replaced by available CHO (27). This creates contrast when calculating foods' GI containing RS. In this study, calculation was made in two different ways while determining the amounts including 25 g available CHO of noodles with RS to shed light on this contrast. The GI values were determined with and without RS in available CHO (with the inclusion of total dietary fiber). The noodle group for which the RS is evaluated in the total dietary fiber is called "TDF" and the noodle group for which it is evaluated in available carbohydrate amount is called "AC". Formulations of the noodles in the TDF and AC groups are the same. The difference between the two groups is the way in which the available CHO amount of the noodles is calculated. Thus, their consumption levels in the test are different and given in Table 2.

Participants

Thirteen healthy adult women (mean age of 23.13±2.74 years) who had undergone endocrinologist examination and biochemical tests were conducted in Selcuk University Medical Faculty Hospital were included in the study. Although gender had no effect on the results in GI studies (26), individuals who volunteered to take part in the study were women, so the study was conducted on women. The following individuals were not included in the study; those who had body mass index (BMI) of 25 kg/m2 and above, professional or amateur athletes, regular medication takers, smoking, pregnant and breastfeeding women, those

Table 1. Proximate analysis (%), total dietary fiber (%), available CHO (%) and total CHO (%) values of reference and test foods

Food	Moisture (%)	Total fat (%)	Protein (%)	Total dietary fiber (%)	Ash (%)	Available CHO [°] (%)	Total CHO°(%)
White bread	27.98	0.48	8.96	5.25	1.58	55.75	61
Control noodles	7.23	2.9	11.51	5.05	1.39	71.92	76.97
Noodles with 20% RS	7.98	2.79	10.59	26.09	1.47	51.08	77.17
Noodles with 35% RS	8.4	2.48	9.24	41.06	1.47	37.35	78.41

*CHO: carbohydrate

Table 2. The 25 g available CHO amounts of reference and test foods

Foods	Available CHO (%)	The amount (g) including 25 g available CHO
Glucose	100.0	25.0
White bread	55.75	44.8
Control noodles	71.92	34.7
Noodles with 20% RS (TDF)	51.08	48.9
Noodles with 35% RS (TDF)	37.35	66.9
Noodles with 20% RS (AC)	71.66	34.9
Noodles with 35% RS (AC)	72.51	34.5

AC: RS was evaluated in the available carbohydrate.

TDF: RS was evaluated in the dietary fiber.

outside the age range of 18-35 years, those with insulin resistance and diagnosed with type 1 or type 2 diabetes having undergone endocrinological examination and biochemical tests, those with a chronic disease and diagnosed by a physician, and those having impaired glucose tolerance according to the oral glucose tolerance test. The study was conducted after the approval of Selcuk University Clinical Research Ethics Committee (approval number: 40209705-050.01.04/53295) and the participants signed the informed consent form after being informed about the study.

Measurement of capillary blood glucose

The capillary blood samples at fasting and post prandial 15th, 30th, 45th, 60th, 90th, 120th minutes were taken from both hands and different fingers. The average blood glucose values from both hands were recorded (20). For each GI test, capillary blood samples were collected 14 times from every individual for 2 hours. Accu-Chek Performa Nano[®] measuring device and Accu-Chek Softclick[®] finger drill were used for measurements. The devices were calibrated by a control strips before using.

Glycemic index test plan

The participants were invited to the Nutrition and Dietetics laboratory after 10-12 hours of hunger. They were advised not to change their nutrition behavior, not to use alcohol, not to take caffeine, to limit consumption of foods including CHO and not to do much exercise the day before the test.

While cooking the noodles offered to the participants, no oil or salt was added as not to influence glycemic response. To ensure comfortable consumption of noodles in terms of sensory flavor, 25 g available carbohydrate amounts were served to the participants.

Before the reference and test foods consumption, capillary blood samples were taken from the fingers of both hands, blood glucose levels were measured and recorded. Afterwards, the participants were asked to consume the foods within 10 minutes. The foods were accompanied by 250 ml water. No additional water was allowed, since the glucose had been diluted with 250 ml water. Duplicate capillary blood glucose

measurements were taken at 15th, 30th, 45th, 60th, 90th and 120th minutes after the first bite. During those 2 hours, a special attention was paid to keep the participants in a sitting position.

The International Organization for Standardization (ISO) 26642 test protocol suggests that reference foods should be consume three times to minimize individual variation (20). The participants consumed glucose and white bread 3 times in one-week intervals during 6 weeks in total. They consumed the test foods once in one-week intervals during 5 weeks in total.

Calculation of glycemic index value of foods

GI values of foods were calculated for everyone. Glucose values changing against time recorded during test were shown in graphics for each individual and blood glucose level curve was obtained for every reference and the test food. The calculations were made through calculation of the incremental area under the curve recommended by FAO/WHO expert committee and included in ISO 26642 standard (20). Since reference foods were consumed three times, the average of these three area values was calculated. The ratio of the area under the curve obtained from test food to the area under the curved obtained from reference food gives GI of that food. The average of fifteen GI values calculated for each participant was determined.

Determination of the test foods' in vitro glycemic index values

The AOAC 996.11 method (megazyme amyloglucosidase/alpha amylase) was used for the starch determination of the samples. The rate of starch hydrolyzed was determined and plotted based on time. The hydrolysis index (HI), namely, *in vitro* (GI) value defined as the ratio of the area under the hydrolysis curve of noodles to the area under the hydrolysis curve of white bread was calculated. The area under the white bread was calculated 100 and was proportioned to the area under the sample whose *in vitro* GI value would be determined. The result was multiplied by 0.7. GI of the white bread was taken as 70. The GI=39.71+(0.549x HI) formula was used to calculate the *in vitro* starch hydrolysis rate of noodles (28).

Statistical analyses

The data obtained were evaluated using the SPSS 20.0 statistical software package. The qualitative variables were provided as number (n) and percentage (%) and the quantitative variables were given as mean (\bar{X}) , standard deviation (SD) as well as minimum and maximum values. The Kolmogorov-Smirnov test was used to determine whether quantitative data were normally distributed. For the data normally distributed between the groups, two groups were compared using dependent two-sample t-tests. For situations based on data collection from the participants in more than one point of time, repeated measures ANOVA was used to compare means of three or more groups. In case of any difference between the groups was used and paired comparisons were evaluated by post-hoc tests to determine subscales causing the difference. P<0.05 was considered statistically significant.

Results

In the comparison of the initial and two-hour blood glucose measurement values of the reference and test foods according to food and time, a statistically significant difference (p<0.05) was found between blood glucose levels measured at 15th, 30th, 45th, 60th, 90th and 120th minutes after the reference and test foods were consumed (Table 3). The highest blood glucose value was at 30th minute after the consumption of glucose and control noodles and at 45th minute after the consumption of white bread atd noodles with RS. The lowest blood glucose level mean was in the white bread at 15th minute, in the noodles with 35% RS (AC) at 35th minute, and noodles with 20% RS (AC) at 45th minute.

GI values of the control and test foods according to glucose and white bread are given in Table 4. The noodles with the lowest GI value according to glucose and white bread were noodles with 35% RS (AC) and control noodles with 20% RS (AC), respectively.

Considering GI of control noodles, noodles with 20% RS (AC) and noodles with 35% RS (AC) according to glucose and white bread, the difference between all foods was found statistically significant (p<0.05). It was found that the lowest GI belongs to noodles with 35% RS (AC).

The starch hydrolysis rates are given in figure 3 according to the time used to determine *in vitro* GI values of noodles. The total starch hydrolysis of noodles realized at low and high levels in regards to white bread. The starch hydrolysis of the control noodles is faster than the noodles with resistant RS. The lowest starch hydrolysis is of noodles with 35% RS.

Table 3. Comparison of the initial and two-hour blood glucose measurement values of the reference and test foods according to food and time (mg/dl)

			Noodles					
Time	Glucose	White bread	Control	20% RS (AC)	35% RS (AC)	20% RS (TDF)	35% RS (TDF)	
(min)	X ±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	р
0.	86.5±4.0 ^{a,1}	87.8±4.5 ^{a,1}	87.6±3.2 ^{a,1}	$86.3 \pm 4.7^{a,h}$	86.9±5.2 ^{a,1}	87.3±4.7 ^{a,h}	86.9±5.3ª,h	0.969
15.	111.1±10.8 ^{a,g}	$93.4 \pm 7.6^{d,i}$	$99.4 \pm 4.9^{bc,gh}$	$95.1 \pm 4.6^{\text{cd,g}}$	$96.3 \pm 4.9^{\text{bcd,h}}$	$98.8{\pm}5.8^{\rm bcd,g}$	$101.1 \pm 8.3^{b,g}$	0,000
30.	$139.2 \pm 11.0^{a,f}$	$114.5{\pm}10.3^{\rm de,fg}$	$116.9{\pm}8.0^{\rm dc,f}$	$108.3 \pm 11.5^{e,f}$	$108.2 \pm 11.1^{e,fg}$	$122.7{\pm}9.8^{\rm bc,f}$	$127.2{\pm}10.8^{\rm b,f}$	0,000
45.	$134.1 \pm 14.1^{a,f}$	$120.7{\pm}10.2^{\rm bc,f}$	$110.9{\pm}12.4^{\rm cd,f}$	$108.5 \pm 13.3^{\rm d,f}$	$110.4 \pm 12.3^{cd,f}$	$126.7 \pm 19.0^{\rm ab,f}$	$130.9{\pm}20.7^{\rm ab,f}$	0,000
60.	$119.5 \pm 17.6^{ab,g}$	$111.1{\pm}10.6^{\rm bc,g}$	101.9±13.8 ^{c,g}	$104.5 \pm 9.6^{c,f}$	102.8±9.6 ^{c,g}	$119.5{\pm}20.0^{\rm ab,f}$	$123.8 \pm 20.6^{a,f}$	0.000
90.	$98.3 \pm 16.1^{d,h}$	$100.2{\pm}10.5^{\rm bc,h}$	$96.2{\pm}5.7^{\rm bc,gh}$	93.9±5.9 ^{c,g}	$93.3\pm6.6^{\mathrm{c,h}}$	$103.7 \pm 12.4^{ab,g}$	$108.8 \pm 17.5^{a,g}$	0,004
120.	$84.7\pm6.4^{d,i}$	$92.7 \pm 6.9^{bc,i}$	$93.1{\pm}3.0^{\rm bc,hi}$	$90.2\pm3.6^{c,gh}$	$90.5 \pm 3.1^{c,hi}$	$97.2 \pm 8.5^{ab,g}$	98.1±10.0ª,g	0,000
Р	0,000	0,000	0,000	0,000	0,000	0,000	0,000	

AC: RS was evaluated in the available carbohydrate. TDF: RS was evaluated in the dietary fiber.

ANOVA test in repeated measures

a-e The difference is significant between those indicated with different exponential letters in the same line p<0.05

f-1 The difference is significant between those indicated with different exponential letters in the same column p<0.05

	GI value according to glucose		GI value according to white bread		
Foods	X ±SD	min-max	X ±SD	min-max	
White bread/Glucose	66.9±11.3	45.1-83.3	153,8±29,4	119,9-221,5	
Control noodles*	54.8±11.5 ª	27.5-69.0	83,2±18,1	45,2-110,1	
Noodles with 20% RS (AC)*	51.8±11.2 ^b	26.0-66.1	79,0±19,9	42,6-116,8	
Noodles with 35% RS (AC)*	49.1±9.9 °	26.3-64.0	74,5±15,5	43,1-98,2	
Noodles with 20% RS (TDF)	90.8±20.8	53.1-126.7	138,6±36,1	79,5-205,1	
Noodles with 35% RS (TDF)	107.5±29.6	62.6-180.4	164,6±50,3	99,2-246,8	

AC: RS was evaluated in the available carbohydrate.

TDF: RS was evaluated in the dietary fiber.

* Evaluated using ANOVA test in repeated measures.

^{a-c} The difference is significant between those indicated with different exponential letters in the same column p<0.05.

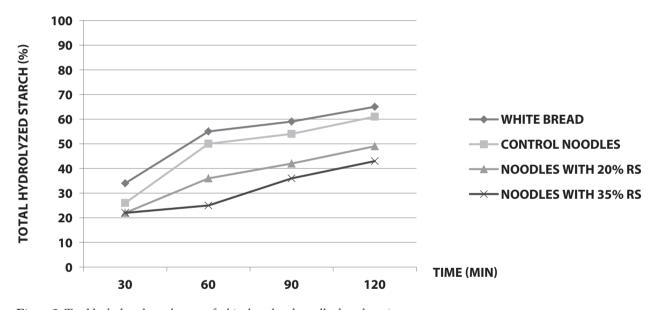


Figure 3. Total hydrolyzed starch rates of white bread and noodles based on time

Comparison of *in vivo* and *in vitro* GI values of white bread and test foods according to glucose shows that *in vitro* GI results are included in available carbohydrate of RS and are similar to *in vivo* GI results in the AC group (Table 5).

Discussion

This study aimed to determine the glycemic effect of the RS addition to the Turkish noodles., which has an important in Turkish culture and known to have many benefits for health yet cannot be taken at the suggested amounts with diet. While determining the available carbohydrate amount to be consumed in the GI test, the dietary fiber is subtracted from the total carbohydrate amount and there is no recommendation that the amount of RS should be calculated separately (2). However, with the increased interest in determining the GI values of foods containing high RS, which diet should RS be included is being discussed (29). Some researchers discuss that RS can decrease glycemic only when it is replaced by available carbohydrate, while others state that the same effect can be ensured when the present carbohydrate is fixed without any replacement (30, 31).

	GI value according to glucose				
Method	White bread	Control noodles		Noodles with 20% RS	Noodles with 35% RS
In vitro	70.0	61.4		54.1	50.3
			AC	51.8	49.1
In vivo	66.9	54.8	TDF	90.8	107.5

Table 5. In vivo and in vitro GI values of white bread and test foods according to glucose

AC: RS was evaluated in the available carbohydrate.

TDF: RS was evaluated in the dietary fiber.

FAO, in its explanation on GI in 1998, considered RS one of the components affecting glycemic response. Since RS analysis methods were not developed in those years, the RS types had to be included in available carbohydrate (2). In 2005, the British Nutrition Foundation (BNF) stated that decreasing GI value of RS-enriched foods could be possible by replacing it with available carbohydrate (29). Brouns et al. published a GI method pointing that RS could decrease glycemia when replaced by available carbohydrate in food. More comprehensive studies are needed for the protocols that are necessary to determine GI value of foods enriched with RS (26).

The GI test protocol published by ISO in 2010 stated that only foods digested in the small intestine were appropriate for GI test. However, RS is fermented by bacteria in the large intestine. Because of the difficulties of analyzing the exact amount of RS, inclusion of low amounts of RS in the carbohydrate portion in the GI test can be ignored (20).

EFSA stated in 2011 that RS decreased blood glucose response only when consumed instead of available carbohydrate and there was no sufficient evidence to prove its effect when available carbohydrate remained stable (27). The glycemic carbohydrate should remain stable in GI tests. Excluding RS from the amount of available carbohydrates leads to the increase in consumable amount of food in GI study. However, the aim to enrich a food with RS is to make that food consumed at the same amount healthier rather than increase portion of the food. While determining the portion of the food consumed in GI tests, RS is not replaced by available carbohydrate amount, but is added on it. This situation prevents the demonstration of the glycemic healing effect of resistant starch in GI tests. There are different applications in the literature for the components in which RS is included (31-35). This study investigated the glycemic response when included in and excluded from available carbohydrate. When RS was included in available carbohydrate, the control noodles, noodles with 20% RS and 35% RS were consumed in close amounts of 34.7 g, 34.9 g and 34.5 g, respectively. However, the amount of consumption also increased much with the increase in RS amount of noodles when calculated by excluding RS from the amount of available carbohydrates, namely, by counting dietary fiber. These amounts are 34.7 g, 48.9 g and 66.9 g for the control noodles, noodles with 20% RS and noodles with 35% RS, respectively.

The food with the lowest GI was noodles with 35% RS (AC), GI values according to glucose and white bread were 49.1 and 74.5 respectively. GI according to white bread was 74.5. The subsequent low GI value was found in noodles with 20% RS (AC). GI values of the control noodles, noodles with 20% RS (AC) and 35% RS (AC) according to glucose were found to be low. GI values decreased in the AC group where RS was included in available carbohydrate amount with the increase of RS in noodles. The difference between the means of GI values of the control noodles, noodles with 20% RS (AC) and 35% RS (AC) was found to be statistically significant. The GI values of noodles with 20% and 35% RS (TDF) were found to be quite high because the amount of noodles consumed during the test was very high when the RS was not included in the available carbohydrate and was assessed as dietary fiber. These findings support the thesis of EFSA suggesting that only when RS is replaced by available carbohydrate, then it decreases the blood glucose response (27).

Although RS was not included in available carbohydrate in a study investigating GI of bread enriched with Type 3 RS, a significant decrease was observed in the GI value (31). Another study investigating the effect of Type 2 RS amounts in bananas on their GI did not include RS in available carbohydrate amounts. The increase in the RS content significantly decreased GI value of the food (21). The difference between these studies and our study may be due to different RS used and the GI test protocol.

Since there is no absolute recommendation in the literature about including RS in available carbohydrate in GI test, many researchers discussed equal portions of the foods to be tested and evaluated glycemic responses instead of GI value (16, 17, 36-38). In a study comparing glycemic responses of bar prepared with wheat starch and bar prepared with Type 4 RS, available carbohydrate amounts of the bars were equated and glycemic response of the ones enriched with RS was significantly lower (16). In three other studies investigating the effect of adding Type 4 RS in muffin, cookies and cakes on glycemic response, enriched foods with RS decreased glycemic response significantly when consumption amounts were equal (17, 36, 37). Similar results were obtained from a study investigating the effect of Type 2 RS addition on glycemic response (38). Many studies conducted besides this current study show that RS has a glycemic response-reducing effect. However, new regulations for RS are needed in GI test as the GI results show major changes according to the component to which RS is added and there is no precise recommendation.

This study calculated GI value of the control noodles as 54.8 according to glucose. GI value of Australian noodles is 62 and GI value of instant noodles is 47 according to glucose, as shown in the international GI table (39). This difference may have stemmed from the lack of egg in the formulation.

GI values of the test foods used in this study were also identified as estimated values (*in vitro*). The starch hydrolysis ratio and *in vitro* GI decreased with the increase in the RS ratio of noodles. The *in vitro* GI values for the control noodles, noodles with 20% RS and 35% RS were 61.4, 54.1, and 50.3 respectively. The *in vitro* results were similar to those of *in vivo* GI results of the AC group where RS was taken as available carbohydrate. In a study examining the effect of noodle RS addition on *in vitro* GI values, the *in vitro* GI value of 15% RS noodles was found to be 552, which is close to the results of our study (40).

Conclusion

The study found that type 4 RS, which is added into Turkish noodles decreased glycemic response of noodles. However, when RS is added in available carbohydrate only, a significant decrease was observed in GI value of noodles. Since GI is based on the amount of equal available carbohydrate, it has no curative effect for glycemia when the RS is assessed in fiber component. There are no precise procedures for foods enriched with RS in GI tests. In this sense, there is a need to create new procedures regarding RS in GI test protocols. Furthermore, although its amount of intake with a diet is low, the RS which functions as soluble and fermentable dietary fiber has many benefits on health. The RS should be used more to enrich foods and the studies comparing its sub types should be increased.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical standard: All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

References

1. Frost G, Dornhorst A. Glycemic Index. In: Caballero B, Allen L, Prentice A (ed) Encyclopedia of Human Nutrition, 2nd edn. Elsevier Academic Press, Oxford, UK, 2005; pp 413-419.

- FAO. Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 1998; 66:1-140 Food and Agriculture Organization of the United Nations: Rome.
- Jenkins DJ, Kendall CW, Augustin LS, Franceschi S, Hamidi M, Marchie A, Jenkins AL, Axelsen M. Glycemic index: overview of implications in health and disease. Am J Clin Nutr 2002; 76 (1):266-273.
- Goff LM, Cowland DE, Hooper L, Frost GS. Low glycaemic index diets and blood lipids: a systematic review and meta-analysis of randomised controlled trials. Nutr Metab Cardiovasc Dis 2013; 23 (1):1-10.
- 5. Livesey G, Taylor R, Hulshof T, Howlett J. Glycemic response and health—a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. Am J Clin Nutr 2008; 87 (1):258-268.
- Schwingshackl L, Hoffmann G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. Nutr Metab Cardiovasc Dis 2013;23 (8):699-706.
- 7. Juanola-Falgarona M, Salas-Salvado J, Ibarrola-Jurado N, et al. Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial. Am J Clin Nutr 2014; 100 (1):27-35.
- Memiş E, Şanlıer N. Glisemik indeks ve sağlık ilişkisi. Gazi Üniversitesi Endüstriyel Sanatlar Eğitim Fakültesi Dergisi 2009;24:17-27.
- Güler MS, Bilici S. Besinin içeriği, işleme ve pişirme yöntemlerinin glisemik indeks üzerine etkisi. Gazi Sağlık Bilimleri Dergisi 2017; 2 (3):1-12.
- Kotancılar HG, Gerçekaslan KE, Karaoğlu MM, Boz H. Besinsel lif kaynağı olarak enzime dirençli nişasta. Journal of the Faculty of Agriculture 2009; 40 (1):103-107.
- Demirekin A. Enzime Dirençli Nişasta ve Sağlık Üzerindeki Etkileri. Journal of Agricultural Faculty 2016;30 (2):71-78.
- Dongowski G, Jacobasch G, Schmiedl D. Structural stability and prebiotic properties of resistant starch type 3 increase bile acid turnover and lower secondary bile acid formation. J Agric Food Chem. 2005; 16;53(23):9257-67.
- Luhovyy BL, Mollard RC, Yurchenko S, et al. The effects of whole grain high-amylose maize flour as a source of resistant starch on blood glucose, satiety, and food intake in young men. J Food Sci 2014; 79 (12):H2550-2556.
- 14. Li M, Piao J-H, Tian Y, Li W-D, Li K-J, Yang X-G. Postprandial glycaemic and insulinaemic responses to GM-resistant starch-enriched rice and the production of fermentation-related H 2 in healthy Chinese adults. Br J Nutr 2010; 103 (7):1029-1034.
- 15. Klosterbuer AS, Thomas W, Slavin JL. Resistant starch and pullulan reduce postprandial glucose, insulin, and GLP-1, but have no effect on satiety in healthy humans. J Agr Food Chem 2012; 60 (48):11928-11934.

- Al-Tamimi E, Seib P, Snyder B, Haub M. Consumption of Cross-Linked Resistant Starch (RS4XL) on Glucose and Insulin Responses in Humans. J Nutr Metab 2010; 1-6.
- Stewart ML, Wilcox ML, Bell M, Buggia MA, Maki KC. Type-4 Resistant Starch in Substitution for Available Carbohydrate Reduces Postprandial Glycemic Response and Hunger in Acute, Randomized, Double-Blind, Controlled Study. Nutrients 2018;10 (2).
- Maziarz MP, Preisendanz S, Juma S, Imrhan V, Prasad C, Vijayagopal P. Resistant starch lowers postprandial glucose and leptin in overweight adults consuming a moderate-tohigh-fat diet: a randomized-controlled trial. Nutrition journal 2017;16 (1):14.
- 19. Alfa MJ, Strang D, Tappia PS, et al. A randomized placebo controlled clinical trial to determine the impact of digestion resistant starch msprebiotic((r)) on glucose, insulin, and insulin resistance in elderly and mid-age adults. Frontiers in medicine 2017;4:260.
- 20. ISO (International Standards Organisation). ISO 26642:2010 Food products – determination of the glycaemic index (GI) and recommendation for food classification, 2010;1-14.
- 21. Oladele E-O, Williamson G. Impact of resistant starch in three plantain (Musa AAB) products on glycaemic response of healthy volunteers. Eur J Nutr 2016;55 (1):75-81.
- 22. Lee Yeo L, Seib P. White Pan Bread and Sugar-Snap Cookies Containing Wheat Starch Phosphate, A Cross-Linked Resistant Starch. Cereal Chem 2009;86(2):210-220.
- 23. TSE. TS12950 Erişte. Türk Standartları Enstitüsü. 2003; 11, Ankara.
- Venter C, Slabber M, Vorster H. Labelling of foods for glycaemic index—advantages and problems. South African Journal of Clinical Nutrition 2003; 16(4): 118-126.
- 25. AOAC (Association of Analytical Communities). Official methods of analysis of AOAC. International 17 th edn, USA Association of Analytical Communities, Gaithersburg, Maryland, 2000.
- Brouns F, Bjorck I, Frayn KN, et al. Glycaemic index methodology. Nutr Res Rev. 2005;18 (1):145-171.
- 27. EFSA. Scientifc Opinion on the substantiation of health claims related to resistant starch and reduction of postprandial glycaemic responses (ID 681), "digestive health benefts" (ID 682) and "favours a normal colon metabolism" (ID 783) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 2011;9:2024–2041.
- 28. Goñi I, Garcia-Alonso A, Saura-Calixto F. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 1997;17(3), 427-437.
- 29. Nugent AP. Health properties of resistant starch. Nutrition Bulletin 2005; 30(1):27-54.
- Robertson MD. Dietary-resistant starch and glucose metabolism. Curr Opin Clin Nutr Metab Care. 2012;15 (4):362-367.
- Lin MHA, Shyr CR, Lin J. Bread containing type 3 resistant starch reduced glycemic index and glycemic response in healthy young adults. Current Topics in Nutraceutical Research, 2012;10(3); 143-150.

- 32. Ferrer-Mairal A, Penalva-Lapuente C, Iglesia I, et al. In vitro and in vivo assessment of the glycemic index of bakery products: influence of the reformulation of ingredients. Eur J Nutr 2012; 51 (8):947-954.
- 33. Jenkins DJ, Vuksan V, Kendall CW, et al. Physiological effects of resistant starches on fecal bulk, short chain fatty acids, blood lipids and glycemic index. J Am Coll Nutr 1998;17 (6):609-616.
- 34. Hoebler C, Karinthi A, Chiron H, Champ M, Barry JL. Bioavailability of starch in bread rich in amylose: metabolic responses in healthy subjects and starch structure. Eur J Clin Nutr. 1999;53 (5):360-366.
- Çiftçi S. Different cooking methods impcat on the value of the glycemic index of potatoes. Dissertation, University of Hacettepe, 2015.
- 36. Stewart ML, Zimmer JP. A high fiber cookie made with resistant starch type 4 reduces post-prandial glucose and insulin responses in healthy adults. Nutrients, 2017; 9 (3):237.
- 37. Stewart ML, Zimmer JP. Postprandial glucose and insulin response to a high-fiber muffin top containing resistant starch type 4 in healthy adults: a double-blind, randomized, controlled trial. Nutrition 2018;53:59-63.

- 38. MacNeil S, Rebry RM, Tetlow IJ, Emes MJ, McKeown B, Graham TE. Resistant starch intake at breakfast affects postprandial responses in type 2 diabetics and enhances the glucose-dependent insulinotropic polypeptide–insulin relationship following a second meal. Appl Physiol Nutr Metabol 2013;38 (12):1187-1195.
- Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. Am J Clin Nutr 2002;76 (1):5-56.
- 40. Menon R, Padmaja G, Sajeev MS. Cooking behavior and starch digestibility of NUTRIOSE[®] (resistant starch) enriched noodles from sweet potato flour and starch. Food Chem. 2015;182:217-223.

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