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Research Article

ENZYMATIC HYDROLYSIS AND GLYCEMIC ASSESSMENT OF THE CARBOHYDRATES IN SOME NATURAL TURKISH INDIGENOUS FOODSTUFFS IN SIMULATED SYSTEM

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ABSTRACT

It is important to understand the benefits of an indigenous foodstuff containing carbohydrate in terms of glycemic. The glycemic parameters are the glycemic index (GI) and glycemic load (GL). Turkish Kahramanmaraş tarhana and Beypazarı cookie were used as indigenous foodstuffs. Because of delicious and high protein content, these foods are commonly used in Turkey. In this study, we realized the glycemic assessment of these foodstuffs by carrying out enzymatic hydrolysis of the carbohydrates containing in our simulated *in vitro* system. It was found that Kahramanmaraş Tarhana as used snack and soup have a low-glycemic index (<55) using Turkish white bread (TWB) as a reference carbohydrate. The GI values of Beypazarı Cookie and Turkish Kahramanmaraş Tarhana were determined as 62.8±2.8 and 53.1±2.4, respectively. The glycemic load (GL) values of both samples were bigger than 20. The values of these species will increase if their consumption is high.
Keywords: Glycemic assessment, indigenous foodstuff, simulated digestion, Kahramanmaraş tarhana, Beypazarı cookie.

1. INTRODUCTION

There are some traditional foodstuffs unique to each country that is important to nutrition. TKT and BC are indigenous products unique to our culture. Tarhana or snack (crispy) tarhana, which is increasingly important among our indigenous products; is a fermented food having high nutritive value, produced by using lactic acid fermentation. In this study, we used one of the best sources Tarhana that including vegetables and animal origins protein including mineral-rich flour spice. Tarhana is very rich in terms of nutritional values such as protein, some minerals, and water-soluble vitamins. Tarhana is an appetizing, digestive-promoting, intestinal flora-regulating nutrient as well as has high nutritional value. Nowadays the glycemic index is very important because it changes lifestyle. So, we need to learn how can protect our body and especially blood glucose levels [1].

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The Glycemic Index (GI) is a relative ranking of carbohydrate in foods according to how they affect blood glucose levels. Carbohydrates with a low GI value (55 or less) are more slowly digested, absorbed, and metabolized and cause a lower and slower rise in blood glucose and, therefore usually, insulin levels [2].

For each gram of carbohydrate, foods having a high glycemic index (GI) yield a high peak in postprandial blood glucose and a great whole blood glucose reply during the first 2 h after intake than do foods having a low GI [3].

The glycemic load of a specific food -calculated by multiplying of that food's carbohydrate content and its glycemic index value has direct physiologic meaning in that each unit can be interpreted as the equivalent of 1 g carbohydrate from White bread (or glucose depending on the reference used in determining the glycemic index). The concept of glycemic load addresses the concern about rating foods as good or bad solely based on their glycemic index [4].

The dietary carbohydrate in humans and omnivorous animals is a major nutrient and the alimentary tract is well adapted for its digestion and subsequent absorption. Initially, polysaccharides are broken down by the enzymatic hydrolysis of salivary and pancreatic amylase mainly in the upper small bowel. Only a small amount is hydrolyzed in the stomach. The carbohydrates are built into the surface membranes of the mature enterocyte's microvilli and are in juxtaposition to the transport sites for the released monosaccharides. This chose integration of digestive breakdown with transport prompted Crane to describe the brush border of the enterocytes as a "digestive-absorptive interface. Dietary molecules as large as disaccharides do not cross the small intestinal epithelium although larger molecules such as polyethylene glycols can be absorbed and excreted into the urine. It is the high concentration and hydrolytic efficiency of the disaccharides in the brush border that effectively hydrolyzed all the disaccharides, leaving none to pass across intact [5].

Numerous *in vitro* carbohydrate digestion methods exist for the analysis of the likely glycaemic properties of foods. Generally, these methods encompass simulations of oral, gastric, and intestinal digestion processes, but how physiological conditions are implemented across methods differs considerably. Some differences are in the mode of commination, inclusion, and duration of gastric digestion, and choice of amylolitic enzyme. Incubation temperature, pH, duration, and stirring mode also differ between methods. Such differences, particularly the method used to mimic chewing, can have a substantial influence on the relative estimate of glycaemic potency for a given food. To achieve estimates of high predictive power, and global relevance, a validated, standardized *in vitro* digestion method must be developed. Methodological discrepancies between protocols are identified thus defining the route a systematic standardization investigation should take [6].

In this paper, the value of the glycemic index (GI) and glycemic load (GL) of both Kahramanmaraş Tarhana and Beypazarı cookie produced in Turkey were determined using *in vitro* conditions through spectrophotometric based methods. Flour, milk, butter, yeast, and cinnamon are used in the Beypazarı cookie called "Beypazarı Kurusu" manufactured in Beypazarı located in the capital of Ankara. Beypazarı cookie is the most preferred and delicious foodstuff because it can be store long-term, its structure is hard and dry. It is dried in the bakery for 12 to 24 hours. The famous Beypazarı cookie can be stored for 12 months preserving its freshness when stored under suitable conditions [7].

Kahramanmaraş is a province in the Mediterranean Region, famous for its ice cream, pepper, and tarhana. Kahramanmaraş tarhana is manufactured by using the wheat cut (wheat splitting), yoghurt, and the flavor and nutritional increased with the auxiliary elements such as oregano and black cumin. In the production method; the wheat cut is cooked with boiled water and added to salt.After the mixture has cooled down, yogurt, oregano, and black cumin are added and the resulting mixture is left to ferment. After the fermentation has been completed, the mixture is laid on the mesh floor and dried. After drying process the tarhana is packed. It can be used snack and soup [8], [9], [10].

In this study, Turkish Kahramanmaraş Tarhana and Beypazarı Cookie were evaluated in terms of glycemic. With this aim, we calculated the glycemic index and glycemic load values of these products by using hydrolyze index procedure after enzymatic hydrolysis that carried out in our constructed *in vitro* simulated system.

2. MATERIALS AND METHOD

2.1. Reagents and Apparatus

Haşiroğlu brand Turkish Kahramanmaraş Tarhana (TKT) and Buget brand Beypazarı Cookie (BC) used as indigenous foodstuff samples and Ekmecik brand Turkish white bread (TWB) used as reference carbohydrate to determine GI and GL values of TKT and BC were purchased from a local market in Istanbul, Turkey. The nutrition facts of Beypazarı cookie and Kahramanmaraş tarhana without a glycemic index (GI) and glycemic load (GL) values written on their package was presented in Table 1.

Table 1. The amount of carbohydrate, fat, protein and diet fibre of Turkish white bread (TWB), Turkish Kahramanmaraş tarhana (TKT) and Beypazarı cookie (BC)

	Carbohydrate (g)	**Fat (g)**	**Protein (g)**	**Diet Fibre (g)**	**Digestible Carbohyrate (g)**
TWB*	50.97	2.28	8.66	2.9	48.07
TKT	68.80	6.39	15.0	0.0	68.80
BC	42.00	9.50	1.90	6.9	35.10

*TWB was used as reference carbohydrate

Pepsin, guar gum, pancreatin, invertase, α-amylase, amyloglucosidase (AMG) used for *in vitro* digestion were obtained from Sigma Chem. Co. (St. Louis, MO). Glucose oxidase/peroxidase D-glucose assay kit (GOPOD format) used for determination of glucose in hydrolysate composed after intestinal digestion was purchased Megazyme International Ireland, Bray Business Park, Bray, Co. (Wicklow, IRELAND). Sodium acetate, hydrochloric acid, ethanol was purchased from Merck (Schuchardt OHG, Hohenbrunn, Germany).

All chemicals used in this study were of analytical grade and were used without further purification. The ethyl alcohol, buffer solutions, and all other solutions used for all experiments were prepared with distilled water obtained with a water purification system (Human Power).

All solutions throughout the experiments are mixed with Velp Scientifica Magnetic Stirrers with Hot Plates (India) brand magnetic stirrer and Heidolph Rax model vortex. The absorbance and pH measurements were carried out with Shimadzu UVmini-1240 model spectrophotometer and Thermo Scientific 3112000 Star LogR model pH meter (United Kingdom), respectively. We also used a shaking water bath (Nüve ST30, Germany), refrigerator (Samsung Electronics Co., Ltd., Korean), grinder (Sinbo), incubator (Wiseven), precision scales ATX224 Uni Bloc Series (Shimadzu), automatic pipettes (Eppendorf Research) and centrifuge (EBA 20 Hettich Zentrifugen).

2.2. Calculating of Amount Digestible Carbohaydrate of Samples

Goni`s method [11] was used with slight modifications to construct the simulation of mouth, stomach, and small intestine digestion in a laboratory medium for both samples (Figure 1).

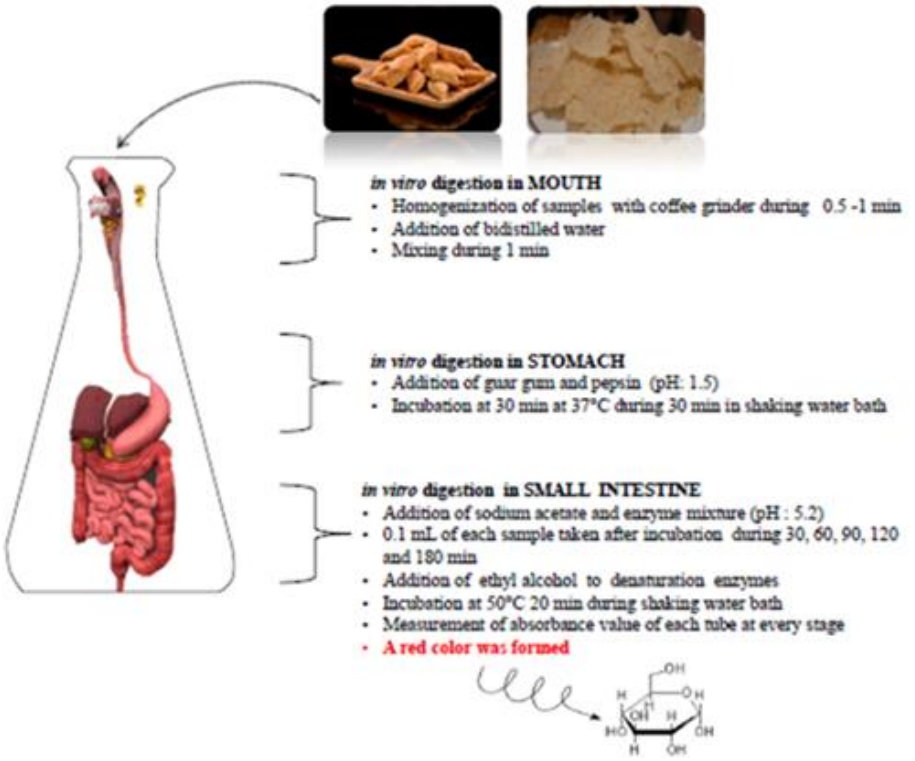

Figure 1. *in vitro* digestion system

Before *in vitro* hydrolysis of the samples, the digestible carbohydrate amount (DC) in the foodstuffs was determined. The contents of indigenous foodstuffs used in this study are given per 100 gram on the packages of products. The carbohydrate (C), diet fibre (DF), and calculated digestible carbohydrate (DC) values for each indigenous foodstuffs were given in Table 1. We were calculated how much bread as gram we have to use according to Equation 1 and 2. [15].

$$DC = C - DF \quad \text{Equation 1}$$

$$S = [0.5 / DC] \times 100 \quad \text{Equation 2}$$

DC: The digestible carbohydrate amount as gram
DF: The diet fibre (indigestible carbohydrate) amount of 100gram bread sample written on the package as gram
C: The carbohydrate amount of 100 gram bread sample written on the package as gram
S: The bread sample amount used as gram

2.3. *in vitro* Enzymatic Carbohydrate Hydroxylation of Indigenous Foodstuff

After calculated DC for each indigenous foodstuffs, we determined the sample used as 1.02g of TWB, 0.72g of TKT, 1.42g of BC, separately. 0.5g of maltose was used as reference carbohydrate for determining of the glycemic index of TWB by means of from Equation 1 and 2. Maltose was used as a reference carbohydrate to determine the glycemic index of TWB. On the other hand, TWB was used as a reference carbohydrate to determine the glycemic index values of

the two indigenous foodstuffs. *in vitro* mouth digestion was carried out after determining of each sample amount. In this study, we used a coffee grinder instead of mouth chewing for *in vitro* mouth digestion.

The two shredded of each bread sample and 5mL of distilled water were grinded and homogenized during 0.5-1 minutes in the coffee grinder at room temperature. We took grinded 0.72g of TKT and 1.42g of BC separately. All bread samples were provided to contain 0.5gram of DC by calculating from Equation 1 and 2.

To simulate *in vitro* digestion in the stomach, each bread sample containing 0.5 g of digestible carbohydrate was put 50 ml of falcon tube, added 5 mL of distilled water, and vortexed during 1 minute. Pepsin-guar gum solution was prepared by adding 100 mL of 0.05N HCl on 0.5 g pepsin and 0.5 g guar gum. 10 mL of pepsin–guar gum solution was added separately to each sample and adjusted pH to 1.5 and incubated at 37^{o}C for 30 min in a shaking water bath.

In the stage *of in vitro* digestion in the small intestine, we used 136 mg/mL of pancreatin, 13.4U/mL of aminoglucosides and 25.43 U/mL of invertase enzymes. To prepare triple enzyme mixture, after the mixture containing 5.44g pancreatin and 36.28 mL of distilled water was centrifuged during 5 minutes at 3000 rpm, 1.78 mL of amyloglucosidase and 0.00034g invertase were added on the supernatant of this mixture.

5 mL of sodium acetate and 5mL of triple enzyme mixture was added on each sample digested in the stomach at 30, 60, 90, 120 and 180 minutes and incubated at 37^{o}C during 30 min by shaking in a shaking water bath, respectively.

It was taken 0.5 mL from each sample, added 2 mL of ethanol, and distilled water was added until the final volume is 10 mL. We denatured the triple enzymes used in the digestion of the small intestine by adding ethanol.

2.4. Determination of Glucose Produced from *in vitro* Simulated Digestion System

After *in vitro* intestinal enzymatic digestion, the formed D-glucose amount was determined by using commercially available glucose oxidase/peroxidase D-glucose assay kit (GOPOD) that based on a simple enzymatic colorimetric method.

$$HI = \frac{AUHC\ (Reference\ Carbohydrate)^{*}}{AUHC\ (Bread\ Sample)} \times 100 \qquad \text{Equation 3}$$

*****While calculating HI (Hydrolysis Index) and AUHC (Areas Under the Hydrolysis Curves) values of postprandial D-glucose formation after digestion of carbohydrate in TWB and each indigenous foodstuff, it was used maltose and TWB as reference carbohydrate, respectively.

The calculated HI values of commercial glucose, the formed D-glucose obtained from the result of *in vitro* carbohydrate digestion of TWB, TKT, and BC were compared with each other.

2.5. Calculation of Glycemic Index (GI) Value

The glycemic index values (GI) of TKT and BC samples were calculated according to the following formula obtained by Goni et al. [11].

$$GI = 0.7 \times [39.71 + (0.559 \times HI)] \qquad \text{Equation 4}$$

2.6. Calculation of Glycemic Load (GL) Value

The glycemic load values (GL) of TKT and BC samples were calculated according to the following formula [11].

$$GL = [DC / 100] \times GI \qquad \text{Equation 5}$$

DC: Digestible carbohydrate amount as gram

3. RESULTS AND DISCUSSION

Although a lot of studies have been performed to determine *in vivo* GI and GL values of foods in Turkey and the world, *in vivo* determination of them have many disadvantages such as human factors, prolonged determination, inconvenience, and high cost. It has not seen any study done GI and GL determination of TKT and BC *in vitro* conditions. *in vitro* determination of GI and GL values has advantages in terms of being able to determine the GI values of multiple nutrients and a large number of bread samples in a much faster and shorter time [12].

To determine *in vivo* or *in vitro* GI value of the food, we should use reference carbohydrates such as maltose, glucose, white bread [12]. In this study, we used as reference carbohydrate maltose for TWB, TWB for TKT, and BC, respectively.

3.1. Calculation of GI and GL values of TWB, TKT and BC

GI and GL values of Turkish White bread were determined by using maltose as a reference carbohydrate. After Turkish white bread was digested *in vitro* in the mouth, stomach, and small intestine, the glucose levels formed after digestion were determined at each stage and the hydrolysis curve was drawn (Figure 2).

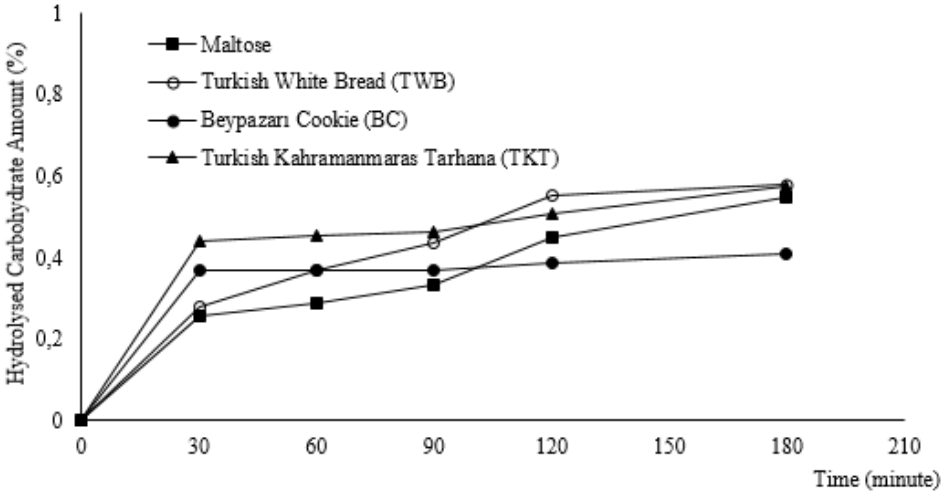

Figure 2. The concentration over-time curves used for the determination of the area under the curve (AUHC) of Maltose, Turkish white bread (TWB), Beypazarı Cookie (BC) and Turkish Kahramanmaraş Tarhana (TKT).

As it was shown in Table 2, the GI value of Turkish white bread (82.0±3.7) was found to be similar to the value stated for Turkish Bread in the Glycemic Index Database of the University of Sydney [14].

Table 2. The calculated AUHC, HI, GI, and GL values of maltose and Turkish white bread

Carbohydrate Type	AUHC	HI	GI	GL
Maltose	42.2±1.9	99.7± 4.5	99.7± 4.5	39.1±1.8
Turkish white bread	59.8±2.7	140.5±6.4	82.0±3.7	41.4±1.9

*TWB was used as reference carbohydrate for determining the GI and GL values of postprandial D-glucose after enzymatic gastrointestinal hydrolyzing for Turkish Kahramanmaraş Tarhana (TKT) and Beypazarı cookie (BC).

In this way, we have determined the GI value of Turkish white bread which we will use as a reference carbohydrate to obtain the GI and GL values of TKT and BC.
TWB was used as a reference carbohydrate to determine GI and GL values of Turkish Kahramanmaraş Tarhana (TKT) and Beypazarı Cookie (BC).

The glucose composition was tracked with respect to time in order to determine the hydrolysed carbohydrate amounts and results are given in Figure 2. The digestion increased rapidly in the first 30 minutes for all samples and continued to increase steadily in the range of 30 to 180 minutes. In the simulated system, maximum carbohydrate hydrolysis was achieved in 30 minutes for BK and 180 minutes for TWB and TKT.

In this study, firstly, the hydrolysis of the starch in indigenous TKT and BC foodstuff samples was carried out and the amount of glucose formed as a result of hydrolysis was determined with the colorimetric method at the end of 30, 60, 90, 120, and 180 minutes. After plotting the hydrolysis curves for each bread sample, the area under each curve (AUHC) was calculated.

Foods are categorized based on their GI values that imply the blood glucose-raising potential of carbohydrate foods as low GI foods (GI <55), intermediate GI foods (55<GI<70), and high GI foods (GI>70). In this study hydrolysis index (HI) procedure was used for the determination of GI and GL values. HI, GI and GL values of each bread sample were calculated from AUHC values and the results are shown in Table 3.

Table 3. The calculated AUHC, HI, GI and GL values of maltose and TWB, TKT and BC

	AUHC	HI	GI	GL
TWB*	42.2±1.9	99.7±4.5	99.7±4.5^{a}	39.1±1.8^{a}
TKT	39.6±1.8	65.8±3.0	53.1±2.4^{c}	37.2±1.7^{a}
BC	54.8±2.5	91.4±4.1	62.8±2.8^{b}	28.2±1.3^{b}

*TWB was used as reference carbohydrate. All analyses were performed three times and the average value was used. Significant differences between the applications were statistically evaluated using one-way analysis of variance (ANOVA, $p< 0.05$, Tukey's test).

As can be seen from Table 3 and Figure 2, the GI value of Beypazarı Cookie was determined as 62.8±2.8. Although it could be thought that it is not healthy for human body because of its higher fat content, it was found that the BC has an intermediate GI value.

It is known that high glycemic index is associated with especially diabetes and other diseases such as cardiovascular disease, colon cancer, and obesity.

Kahramanmaraş tarhana is also a protein-rich product because yogurt is used in its production. Although it has not contain diet fiber, it was found that Kahramanmaraş tarhana as used snack and soup has a low-glycemic index (<55) using Turkish white bread (TWB) as a reference carbohydrate. Yogurt is a fermented milk product. During the fermentation, the lactose in the milk is converted to lactic acid by lactic acid bacteria. Therefore the lactose content of the tarhana is low compared to the milk. The glycemic response to an ingested food was found to depend not only on the GI but also on the total amount of carbohydrates ingested, and this led to the concept of GL. GL accounts for how much carbohydrate is in the food and how each gram of carbohydrate in the food raises blood glucose levels. GL is classified as: low (< 10), intermediate

(11-19) and high (> 20). GL is a metric used as a basis for weight loss, or diabetes control [12]. The glycemic load (GL) values of both samples were bigger than 20. The values of these species will increase blood glucose levels if their consumption is high. One unit of GL approximates the glycemic effect of 1 g of glucose. Typical diets contain from 60-180 GL units per day. A dietary glycemic overload could eventually result in increased risk of diabetes and obesity [12]. The GL of a food is depending on 2 factors: the GI of the food and the serving size and as such, increases or decreases in GL can be achieved by varying either or both terms. Therefore, low GL food can be achieved by either decreasing the GI of the food or by eliminating most of the carbohydrates from the diet [15].

4. CONCLUSION

The study was conducted to investigate the glycemic index and glycemic load levels in some indigenous foodstuffs. A higher glycemic index value was obtained for BC as compared with the value obtained for TKT. As the glycemic index of TKT is relatively low its consumption by individuals who have diabetes and cardiovascular disorders is appropriate. This study is the first to report on GI and GL profile of BC, and TKT in Turkey.

5. SIGNIFICANCE STATEMENT

This study has become a reference for determining the glycemic responses of other indigenous food products. In this way, by learning the blood glucose response of indigenous foods consumed too much, it causes attention to use and increases the standard of living. In conclusion, people with diabetes, should consume low glycemic index foods. The study was conducted to determine the glycemic index and glycemic load levels in indigenous products such as Beypazarı Cookie and Turkish Kahramanmaraş Tarhana *in vitro* conditions, rapidly. We aim to develop a reference for indigenous foods in terms of glycemic response profiles.

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