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RESEARCH ARTICLE

In vitro Glycemic Response Determination Based on Digestion Enzymes in Some Snacks via Hydrolysis Index Procedure

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HIGHLIGHTS

> Glycemic Index (GI) and Glycemic Load (GL) are defined and their values in snack foods are given in this study.
> The study presents an accurate spectrophotometer method to determine glycemic response.

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ABSTRACT

The glycemic response to an ingested food was found to depend not merely on the glycemic index (GI) but also on the total amount of carbohydrates ingested, and this led to the concept of glycemic load (GL). GL, accounts for how much of carbohydrate is in the food and how each gram of carbohydrate in the food raises blood glucose levels. GI and GL are determined in vivo and in vitro. However, in vivo method is required long time, low speed and addition of scientific ethics board approval, we proposed in vitro hydrolyzed index procedure. On the other hand, in vitro methods have many advantages such as time, speed, cost and no scientific ethics board approval. In this paper we evaluated, in terms of glycemic parameters some types of salty and sweet snacks such as whole wheat snack, light biscuit, bread chips with pepper, bread chips with basil and apple pie respectively. We have shown an in vitro analytical hydrolysis index procedure based on digestion enzymes that allows the prediction of the GI of each snack type. Herein, it was aimed to determine the glycemic response of some snacks types commonly consumed in Turkey by using our method.

Contents

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1. Introduction

The carbohydrate-rich nutrition is an important energy source for a person. Besides carbohydrates, a foodstuff also contains fiber, protein, and fat [1]. Foods containing carbohydrate differ considerably in their effects on formed glucose after digestion. On the other hand, qualitative differences among starchy foods are particularly interesting owing to the dominance of starch in human diets [2]. Dietary carbohydrates are digested and absorbed at different rates and to different extents in the human small intestine. It has been suggested that diets that contain large amounts of rapidly digested carbohydrates, which elevate blood glucose and insulin responses, may be detrimental to health. According to some studies, an increased relative risk of Type 2 diabetes mellitus due to high glycemic load and low cereal fiber intake. It has been suggested also that diets rich in slowly digested carbohydrates may protect against chronic disease. The rate of starch digestion and absorption seems to be a determinant of the metabolic response to a meal. There are evidences that slowly digested and absorbed carbohydrates are favorable in the dietary management of metabolic disorders, such as diabetes and hyperlipidemia [3]. The concept of glycemic response is important both in diabetes hyperlipidemia and in healthy subjects [4]. The interest in *in vitro* methodology to estimate this glycemic response has recently increased. Taking into account the lack of a common *in vitro* starch hydrolysis procedure to estimate the glycemic response [5, 6].

In *in vivo* digestion conditions, determination of glycemic index (GI) has disadvantages with respect to long time, high cost and slow response. The improved *in vitro* method standardized in our laboratory was used to measure the ratio of glucose in sample hydrolysis at different times. Because of the advantage of *in vitro* methods such as easier application, lower cost and quick response, we carried out GI assay with *in vitro* method by using digestion enzymes [6].

Glycemic load (GL) combines both the quantity and quality of carbohydrates. It is also the best way to compare blood glucose values of different types and amounts of foods. GL has been proposed as a global indicator of the glucose response for food portions [6].

Some salty and sweet snacks consumption has increased significantly in recent years. Given that snack foods tend to be high in fat and sugar, this has implications for weight gain and obesity. Cutting back on snacks may therefore help with weight loss [7].

in vitro digestion system has three steps, oral, stomach and intestine respectively. By imitating the human digestive system, we used digestive enzymes such as α-amylase, pepsin, pancreatin, invertase and amyloglucosidase.

The amount of glucose after carbohydrate hydrolysis was measured using digestive enzymes by means of the hydrolysis index procedure. The rate of glucose digestion was expressed as the percentage of hydrolysed at different times (30, 60, 90, 120 and 180 min). The glycemic response of whole wheat snack (WWS), light biscuit (LB), bread chips with pepper (BCP), bread chips with basil (BCB) and apple pie (AP) were separately determined by using hydrolysis index procedure.

2. Materials and methods

2.1. Chemicals and Equipment

All bread samples were purchased from a local market in Istanbul, Turkey. Herein, all enzymes used for in vitro digestion were obtained from Sigma Chem. Co. (St. Louis, MO). Pepsin guar gum, pancreatin, invertase, α-amylase, amyloglucosidase (AMG,) were used during in vitro digestion and their cas numbers are 9001-75-6, 9000-30-0, 8049-47-6, 9001-57-4, 9001-19-8, 9032-08-0 respectively. 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). And other chemicals were obtained by Merck (Schuchardt OHG, Hohenbrunn, Germany).

2.2. Preparation of Snacks Samples

In order to use hydrolysis index procedure, we prepared all snack samples containing 0.5 gram of digestible carbohydrate (DC) in each sample as reference and calculated how much gram of sample we have to take according to Eq. (1) and Eq. (2) [5].

$$DC = C - DF \tag{1}$$

$$S = \frac{0.5}{DC} * 100 \tag{2}$$

Where **DC** is the digestible carbohydrate amount in grams, **DF** is the diet fiber (indigestible carbohydrate) amount of 100 g bread sample written on the package in grams, **C** is the carbohydrate amount of 100 g bread sample written on the package in grams, and **S** is the bread sample amount in grams.

First of all, we determined the glycemic index of Turkish white bread (TWB) by using 0.58 g of maltose as reference carbohydrate calculated from Equation 1 and 2. In all types of snacks our used, TWB was used as reference carbohydrate. The samples were taken containing 0.5 g of its DC as 1.02 g TWB, 0.81 g of WWS, 0.69 g of LB, 1.54 g of BCP, 1.54 g of BCB and 1.20 g of AP was used.

2.3. Steps of Glycemic Response Determination System

Glycemic response determination was carried out after *in vitro* simulated three-stage digestion system named mouth, stomach and small intestine [5].

Step 1: Mouth digestion

In this study, a coffee grinder instead of mouth chewing was used for in vitro mouth digestion. Each of two shredded bread samples was grinded and homogenized separately during 0.5-1 minutes with the coffee grinder at room temperature.

Step 2: Stomach digestion

All samples containing 0.5 g of DC were 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 0.05 N 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°C during 30 min in a shaking water bath.

Step 3: Small intestine digestion

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

5 mL of sodium acetate and 5 mL 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°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 to distilled water until the final volume is 10 mL.

2.4. Determination of Glucose Produced after *in-Vitro* Digestion

D-Glucose was measured in the final reaction medium after in vitro intestinal enzymatic digestion using commercially available glucose oxidase/peroxidase (GOPOD) D-glucose assay kit (GOPOD format) based on enzymatic procedures as colorimetric.

$$\text{D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{D-Gluconate} + H_2O_2$$

For this aim, 3 mL of GOPOD reagent was added to 0.1mL of each sample taken after incubating during 30, 60, 90, 120 and 180 min and added and incubated at 50°C during 20 min. After incubation, the absorbance reading was carried out at 510 nm via UV spectrophotometer for each sample, separately. The amount of glucose obtained from the final step of in vitro digestion was graph between 0 and 180 min. This curve is defined as "Hydrolyzed Curve". The area under Hydrolyzed Curve (AUHC) was calculated via excel program.

In this stage, standard glucose assay was also carried out by using 0.1 mL of D - Glucose instead of bread sample with GOPOD assay kit. Hydrolyzed curve and the area under hydrolyzed curve (AUHC) of standard D-glucose were also determined by applying all procedures like hydrolysing of each bread sample.

2.5. Hydrolysis Index (HI) Procedure and Expression of HI Value

The Hydrolyzed Index (HI) value of glucose performed from the result of *in vitro* digestion of each bread sample was calculated from Hydrolyzed Curve between 0 and 180 min at 5 different times by excel program. HI value of commercial glucose was also calculated by applying the same procedures. Both HI values were calculated according to the following formula: Eq. (3) [8].

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

***While calculating HI 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.*

After *in vitro* digestion, the HI values were calculated from hydrolyzed curve for glucose formed and compared with that of commercial glucose. To determine *in vitro* glycemic response estimation by using digestion enzyme, the glucose formed after *in vitro* carbohydrate digestion was assayed as spectrophotometric method.

2.6. Expression of GI and GL Values

In this paper, the glycemic index value of all snacks types used were calculated according to the following formula obtained by Goni {GI = 0.7 x [39.71 + (0.559 x HI)]} [5]. In this paper, the glycemic load value of all snacks types used was calculated according to the following formula. Eq. (4)

$$\text{GL} = \frac{\text{DC}}{100} \text{XGI} \qquad (4)$$

***DC:** Digestible carbohydrate amount in grams*

3. Results

The hydrolyzed index of snacks ranged from 99.7±4.5 to 155.5±7.0, whereas the GI ranged from 67.5±3.1 to 78.7±3.6. In this paper, we used maltose and TWB to calculate GI value of TWB and the other snacks types used as reference carbohydrate, respectively. We found GI WWS of 78.7±3.6, LB of 70.0±3.2, BCP of 86.7±3.9, BCB of 67.5±3.1 and, AP of 70.0±3.2, respectively. It was found GL values >20 for all types.

Consequently, these snacks types can be used for people under the condition of controlled consumption. In a study, they examined the amount of GI different frequently consumed products such as red lentils, spaghetti and rice flour. As a result of this research paper, they found GIs range of 33.3-51.7. According to literature, GI levels depending on many parameters such as of fiber, fat, starch, and oat [9]. In another study has been demonstrated GIs samples containing oats were lower than those containing rice and corn by using breakfast cereal products. According to this research, breakfast cereals aren't suitable for healthy life because of that GI values relatively high and may bring about many diseases such as obesity, Type 2 diabetes, and cardiovascular diseases [10].

In this study, it was aimed GI and GL values of some salty and sweet snack types in Turkey are determined by using maltose and Turkish white bread as reference carbohydrate. 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 (Yaman *et al.*, 2019).

It has not seen any study done *in vitro* determination of GI and GL of Turkish salty and sweet snack. *in vitro* determination of GI and GL values has advantages of being able to determine the GI values of multiple nutrients and to make a large number of samples in a much faster and shorter

time. So as to determine *in vivo* or *in vitro* GI value of the food, we should use reference carbohydrates such as maltose, glucose, white bread [11]. In this study, we used to determine the GI value of TWB and snack samples (whole wheat snack, light biscuit, bread chips with pepper, bread chips with pepper and apple pie) maltose and TWB as reference carbohydrate, respectively.

It was used Turkish white bread as reference carbohydrate to determine GI and GL values of whole wheat snack, light biscuit, bread chips with pepper, bread chips with basil and apple pie. The types of snacks used in this study were produced and used in Turkey. The amount of carbohydrate, fat, protein and diet fiber of all kind of bread species used in the study was presented in Table 1.

Table 1 The amount of carbohydrate, fat, protein and diet fiber of all kind of snacks

Samples	Carbohydrate	Fat (g)	Protein (g)	Fiber (g)
TWB*	50.97	2.28	8.66	2.90
WWS	65.70	4.74	11.50	4.26
AP	53.00	11.70	4.01	4.22
LB	77.20	12.5	6.40	5.10
BCP	35.70	2.38	5.85	3.31
BCB	35.70	2.38	5.85	3.31

*TWB was used as reference carbohydrate for determining the GI and GL values of postprandial D-glucose after enzymatic gastrointestinal hydrolyzing for snacks

GI and GL values of TWB were determined by using maltose as reference carbohydrate. After TWB 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 plotted (Figure 1).

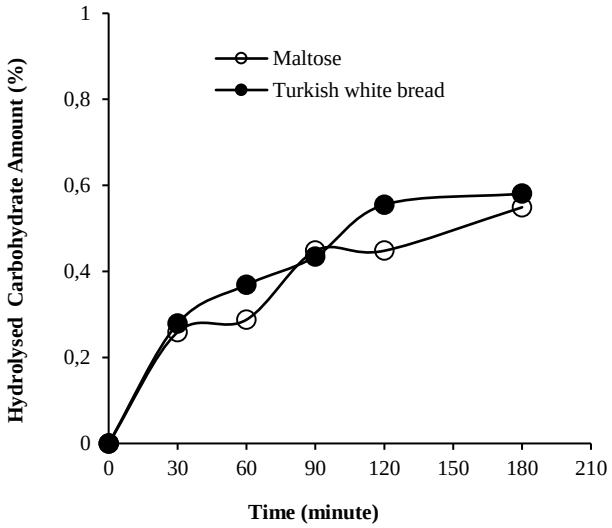

Figure 1 Hydrolysed over-time curve of in vitro carbohydrate hydrolysis using biochemical hydrolysis system our constructed for the determination of the area under the curve (AUHC) for TWB

The time-varying curve of carbohydrate amount using maltose as reference carbohydrate during gastrointestinal hydrolysis was called “hydrolysis curve” for TWB (Figure 1). The areas under the hydrolysis curves (AUHC) were calculated separately via excel in both maltose and TWB.

Then, the areas under the hydrolysis curve of TWB, AUHC, HI, GI, GL values of maltose and TWB were calculated separately (Table 2).

Table 2 The calculated AUHC, HI, GI and GL values of maltose and of WWS, LB, BCP, BCB, and AP used Turkish white bread as reference carbohydrate.

	Sample Used (g)	AUHC	HI	GI	GL
Maltose*	0.58	42.2±1.9	99.7± 4.5	99.7± 4.5	84.7±3.8
TWB	1.02	59.8±2.7	99.7±4.5	82.0±3.7a	41.4±1.9b
WWS	0.81	80.7±3.7	134.6±6.1	78.7±3.6ab	51.7±2.3a
AP	1.02	66.6±3.0	111.0±5.0	70.3±3.2bc	37.3±1.7b
LB	1.38	66.1±3.0	109.6±5.0	70.0±3.2bc	54.1±2.4a
BCP	1.54	93.3±4.2	155.5±7.0	86.7±3.9a	30.9±1.4c
BCB	1.54	62.2±2.8	103.7±4.7	67.5±3.1c	24.1±1.1d

*Reference carbohydrate used for determining the GI and GL values of the hydroxylation of amyloglucose formed from enzymatic digestion for each snack sample. ANOVA $p < 0.05$, Tukey’s test).

In this study, firstly, hydrolysis of the starch in each sample used was carried out separately and the amount of glucose formed as a result of hydrolysis in 30, 60, 90, 120, and 180th minutes were determined as colorimetric. The digestion of carbohydrates in snack samples during 180 minute is shown in Figure 2.

As can be seen in Table 2 and Figure 2, GI values of bread types with high dietary fiber were observed to be low. As it was shown in Table 2, the GI value of TWB (82.0±3.7) was found to be similar the value stated by the Turkish bread in the Glycemic Index Database of the University of Sydney [12].

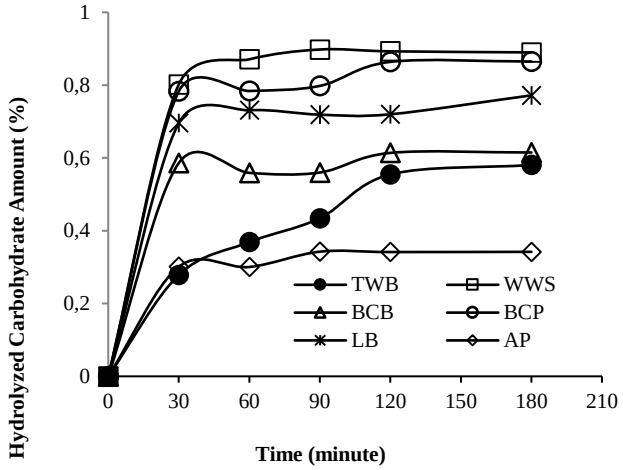

Figure 2 The concentration over-time curves used for the determination of the area under the curve (AUHC) of TWB, WWS, LB, BCP, BCB, and AP Turkish white bread was used as reference carbohydrate for calculating GI and GL values of five different snacks types.

When the GI and GL amounts in Table 2 were compared, this study have shown that the amount of consumption during daily diets were important. Therefore, a food with a high GI value may not rapidly increase blood glucose if consumed in small amounts. In this way, we have determined the GI value of Turkish white bread which we will use as reference carbohydrates to obtain other samples. Several studies found correlation between the decrease of GI and the amount of fiber, protein, and fat. This work is based just on one big analysis, *in vitro* digestibility. It is fundamental to understand the digestibility of the starch. The glycemic index (GI) is the concept used to classify foods on the basis of their postprandial blood glucose response.

Foods with high-GI are those rapidly digested and absorbed resulting in marked fluctuations in blood glucose levels and glucose response demand. Low-GI foods are the ones slowly digested and absorbed, resulting in gradual rise in blood glucose and insulin levels. There is increasing evidence that a low GI diet provides potential beneficial health effects by improving glucose and lipid levels in people with diabetes (Type 1 and 2), also reducing insulin levels and insulin resistance. A reduction in the GI of starch-based foods can be obtained with the use of fibers.

This study investigated the GI of different salty and sweet snacks, produced and sold in Turkey. The aim of this study is to use the *in vitro* digestion to investigate the GI of commercial snacks, and draw at the end a conclusion, based on these data, about their healthiness. Consumers demand nutritious, convenient, tasty snacks that satisfy their hunger momentarily until the next meal [13]. Although GI values of foods should be found as label information because the consumer will decide which product is healthier for him/her to be more conscious, the food package doesn't have GI values as label information in Turkey. Another important parameter as glycemic response is glycemic load (GL) because the amount of food consumed is important as much as the GI value.

4. Conclusions

In accordance with World Health Organization (WHO), approximately 422 million people with diabetes, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year all of the world. Glycemic control management is important prevent and treatment of diabetes. Therefore, it also thought GI and GL values should be given on packaged foods. For this aim, researchers need to further detailed study this method, for presenting consumed packaged products to public. Moreover, some organizations such as The Food and Agriculture Organization FAO and WHO have standardized the analysis of GI values in foods using *in vivo* methods. However, *in vivo* methods are disadvantageous in terms of time, cost, and ethical problems. The aim of this study was to determine predicted GI of different types of some snacks commonly consumed in Turkey. Today, modern diets contain some types of snacks that food products that are frequently consumed because they are a good alternative in terms of being easily available, cheap and practical. For these reasons, the variety of snacks, which have an important place in recently nutrition, is gradually increasing. Busy lifestyles and the increasing demand from consumers for meals and snacks that are quick sources of good nutrition have prompted the food industry to develop foods like ready-to-eat snacks that combine convenience and nutrition. Due to the high consumption rates of these products in our country, studies on their effects on glycemic index and blood sugar are needed due to the need to determine their role in healthy nutrition.

Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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