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Original article

In vitro enzymatic carbohydrate digestion and spectrophotometric glycemic indexes and glycemic loads determination of some turkish breads

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Summary. *Objective:* Carbohydrates are the most abundant and common organic sources in nature. They are vital substances of whole living organisms. Bread is the main food source of people all over the world and very important issue in human diet as a source of carbohydrate. Bread is the principal food of Turkish people and daily average consumption is around 400 g per person. More than half of the caloric intake is supplied by bread. Besides protein and some vitamins, it's a basic source of mineral supply in Turkish diet. Glycemic Index (GI) is glucose response of food as a percentage and it is generally made by in *vivo* methods and expressed as a value between 0 and 100. Foods with GI value more than 70 are (bad) carbohydrates with high glycemic index. Glycemic load (GL), measures carbonhydrate amount in one portion of one food and gives truer results and is expressed this formula: GL=(GIxconsumed amount of carbonhydrates as gram)/100. *Method:* In this study, after digestion the variety of some breads commercially sold in our country in *vitro* conditions, the assays of GI and GL of these products were carried out with spectrophotometric based methods. *Result:* Whole wheat bread, wheat germ bread, hazelnut-grape bread, gluten-free bread and village bread were calculated using the values of GI and GL as reference carbohydrates using white bread. As a result, it was found that high wheat bread, wheat germ bread and hazelnut-grape bread have higher GI values than 70 (>70), gluten-free bread medium, and village bread was low glycemic index (<55). *Conclusion:* After that directly we determined glycemic load values of different types of breads such as wheat bread, wheat germ bread and hazelnut-grape bread have higher GL values than (> 20).

Keywords: carbohydrate digestion, bread, Turkish bread, glycemic index, glycemic load

Introduction

Carbohydrates are an essential part of our diets, but not whole carbohydrate foods are same by far the most important sources of carbohydrate is bread in the human diet (1). The major energy carrying components in the human diet are starches, sugars, fats and proteins, often referred to as macronutrients. These components need to be hydrolyzed into smaller molecules in the human gastrointestinal systems (GIT) before they can be absorbed and further metabolized in the rest of the human body. The main regions in this GIT are the mouth, the stomach, the small intestine, the large intestine and the rectum. The digestion of carbohydrates starts with the hydrolysis of the glycosidic bonds between saccharide units making up the carbohydrate, to liberate small oligosaccharides, and free mono- and disaccharides which can migrate through the wall of the small intestine. The enzymes which catalyze the hydrolysis of starch are amylases, which are secreted in both the saliva and the pancreatic juices (in the small intestine) The main functions

of the mouth, in terms of digestion and absorption of carbohydrates, are chewing to increase the surface area of the molecules, and the initiation of starch hydrolysis catalyzed by amylase enzymes in saliva. Food particles are swallowed after chewing and propelled to the stomach via the oesophagus. In the stomach proteins are hydrolyzed via pepsin enzymes, a phase which is often called the gastric phase. The acidic conditions in the stomach denature the proteins, increasing the surface area which protolithic enzymes can hydrolyzed. The acidic conditions also denature the amylase enzymes which are introduced in the mouth, yielding them inactive. Various vitamins, minerals and even some carbohydrates are released during this phase as proteins are denatured and uncoiled, which disrupt various bonds. Once the food exits the stomach, it enters the small intestine which is responsible for most of the starch hydrolysis by amylase enzymes secreted by the pancreas. The acids from the stomach are buffered back to a neutral pH. Most of the products of digestion are absorbed through the intestinal walls of the small intestine into the rest of the body. Undigested food particles continue to the large intestine where water is absorbed and bacteria metabolize some undigested carbohydrates. The undigested gut contents are stored in the rectum prior to evacuation as faces (2).

Bread is formed that certain amount flour, water, salt and yeast are mixed by kneading dough and cooked. Classic bread has very simple formulation that doesn't contain sugar and oil. Its formulation is over 100 kg flour 55-60 % water, 3-4 % of dry yeast, 0.5-1 % of bread additive and 1.5 - 1.75 % of salt (3).

The report for carbohydrate consumption related to diet, nutrition and prevention of chronic diseases have been made by Food and Agriculture Organization (FAO)/ World Health Organization (WHO) Expert Consultation. FAO/WHO Expert Consultation suggested to use glycemic index concept for classifying carbohydrate-rich foods to provide a useful means of helping people to select the most appropriate carbohydrate containing foods for the maintenance of health and treatment of several diseases (4).

In the literature, it was reported breads prepared locally without food additives are healthier. However, nowadays, there are a lot of bread species in market containing many additives (5).

In Turkish food culture, white breads are main nutritional ingredient that comes first. They take different names because of their different shapes, flavors and cooking styles.

White bread differs in region to region in terms of materials, additives and cooking methods used in making dough in Turkey. Each region has its own climate with breads with special grains. Turkey has a rich bread culture (5). Therefore, some region have different special breads such as village bread, sac bread, lavash, bazlama, etc (6). In European countries, it is reported that the estimated bread intake per person have changed. These values have been 46 kg for Sweden, Great Britain, Finland, Austria and 100 kg for Greece, Portugal, Spain, Italy (7). The daily and annually standard bread consumptions in Turkey are 21.496 tons and 31.4 billion of 250 g amount per unit, respectively. The daily and annually standard bread productions in Turkey are 22719 tons and 33.2 billion of 250 g amount per unit, respectively. According to 2010 Turkey Nutrition and Health Survey, the consuming white bread types ratio are 85.4% every day and this ratio is increasing up from village to city (5).

According to the 2007 Edition of the Guinness Records Book Turkey has been involved as "the most consuming country bread" with "200 kilogram bread consumption per person". Even though daily bread consumption varies according to individual characteristics, habits, life-working patterns and the composition of their diet, bread appears to be the basic nutrient (3).

The glycemic index (GI) is a scale that has been introduced to enable comparison of carbohydrate-rich foods based on their glycemic response. Carbohydrates with 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. Researchers classify high, medium, and low glycemic index foods as good, better, and the best choices for nutrition. According to this approach, foods are classified as: low glycemic index foods (GI≤55), medium glycemic index foods (56 < GI < 69), and high glycemic index foods (GI≥70). Blood glucose levels rise and fall when you eat a meal containing carbohydrates. How high it rises and how long it stays high depends on the quality of the carbohydrates (the GI) as well as the quantity (4). FAO and WHO endorsed

GI method for classifying carbohydrate rich foods in 1997. Nutrition's with high glycemic foods (GI ≥ 70) are generally cause overweight and obesity and some diseases such as type 2 diabetes, certain cardiovascular diseases and cancer. Additionally, they recommended that GI values together with other food composition data be used in the guiding of healthy food choices. The foods with low GI (GI ≤ 55), such as low fat animal products and legumes have been recommended as part of many lose weight strategies target improving health (8).

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 (9). According to The University of Sydney Database, *in vivo* GI and GL values of Turkish white bread is 87 and 15, respectively compare with Turkey and other countries in terms of amount diet fiber, breads of Turkey has a lowest diet fiber so, GI value is highest then others (10).

In literature, although GI and GL assay were made after *in vivo* digestion, there is no study related to *in vitro* glycemic index procedure and glycemic load of some types of breads in Turkey. In this study, the evaluation of glycemic index and glycemic load of some white bread species consumed in Turkey was carried out by spectrophotometric method after in *vitro* enzymatic digestion of the samples with our constructed carbohydrate digestion system.

Materials and methods

Reagents and apparatus

Ekmecik brand Turkish white bread and village bread Halk Ekmek brand whole wheat bread, hazelnut grape bread and gluten-free bread were used as bread samples. Breads used in this study were purchased from a local market in Istanbul, Turkey.

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 hydrolase composed after intestinal digestion was purchased Megazyme International Ireland, Bray Business Park, Bray, Co. (Wicklow, IRELAND). Sodium acetate, hydrocloric acid, ethanol was purchased Merck (Schuchardt OHG, Hohenbrunn, Germany).

All chemicals used in this study were of analytical grade and were used without further purification. Ethyl alcohol, the buffer solutions and all the other solutions used for all experiments were prepared with bidistilled water obtained from the water purification system (Human Power/Seoul/Republic of Korea). All solutions throughout the experiments were 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 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 centrifugate (EBA 20 Hettich Zentrifugen).

Methods

In vitro mouth, stomach and intestinal digestion of each type bread sample was carried out according to our modified Goni`s method (11).

Preparation of bread samples

Determination of digestible carbohydrate amount in bread samples

The contents of bread packages used in this study are given in 100 gram. The samples have to contain 0.5 gram of digestible carbohydrate in each sample as reference (11). It was calculated how much bread as gram we have to use according to Equation 1 and 2.

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

$$\mathbf{S = [0.5 / DC] \, x100} \qquad \text{Equation 2}$$

DC: The digestible carbohydrate amount as gram

DF: The diet fibre (undigestible carbohydrate) amount of 100gram bread sample written on the package as gram

C: The carbohydrate amount of 100gram bread sample written on the package as gram

S: The bread sample amount used as gram

First of all, we calculated the amount of each Turkish bread sample that we used for all of the study containing 0.5 gram of DC by using from Equation 1 and 2. The each bread amounts used during all experiment were calculated as 1.02 g of Turkish white bread (TWB), 1.28 g of whole wheat bread (WWB), 1.42 g of wheat germ bread (WGB), 1.26g of hazelnut and grape bread (HGB), 1.01 g of gluten free bread (GFB) and 1.20 g of village bread (VB). We used TWB as reference carbohydrate in investigation of hydrolysis of the carbohydrate in all other type breads.

0.58 g of maltose calculated from Equation 1 and 2 was used as reference carbohydrate for determining of glycemic index of white bread. All other type breads, we used white bread as reference carbohydrate.

In vitro digestion in mouth

In this study, a coffee grinder instead of mouth chewing was used as simulating system *in vitro* mouth digestion medium. The two shredded of each bread sample and 5 mL of distilled water were grinded and homogenized separately during 0.5-1 minutes in the coffee grinder at room temperature separately. The grinded 1.02 g of TWB, 1.42 g of AWB, 1.27 g of SWB, 1.01 g of GWB were contain 0.5 g of DC by calculating from Equation 1 and 2 separately (Figure 1).

In vitro Digestion in Stomach

The each bread sample containing 0.5g of digestible carbohydrate was put 50 mL of falcon tube, added 5 mL of bidistilled water and vortexed during 1 minute. 10 mL of pepsin–guar gum solution prepared by adding 100 mL of 0.05N HCl on 0.5g pepsin and 0.5g guar gum was added separately to each sample and adjusted pH to 1.5 and incubated at 37°C during 30 min in shaking water bath (Figure 1).

In vitro digestion in small intestine

To carry out *in vitro* digestion of each samples in small intestine, we used 136 mg/mL of pancreatin, 13.4 U/mL of aminoglucosidase 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 bidistilled water was centrifuged during 5 minute at 3000 rpm, 1,78mL of amyloglucosidase and 0.00034 g invertase were added on the supernatant of this mixture. 5 mL of sodium acetate and 5mL of triple enzyme mixture was added on the each sample digested in stomach at 30, 60, 90, 120 and 180 minutes and incubated at 37°C during 30 min by shaking in 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. We denatured the triple enzymes (pancreatin, aminoglucosidase, invertase) used in digestion of small intestine by adding ethanol. We aimed to stop reaction, because we needed to measure response of glucose (Figure 1).

Determination of glucose produced after *in vitro* digestion

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

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

For this aim, 3 mL of GOPOD reagent containing glucose oxidase and peroxidase was added to 0.1 mL of each sample taken after incubated during 30, 60, 90, 120 and 180 min and incubated at 50°C during 20 min. After incubation, the absorbance reading was carried out at 510 nm via UV spectrophotometer

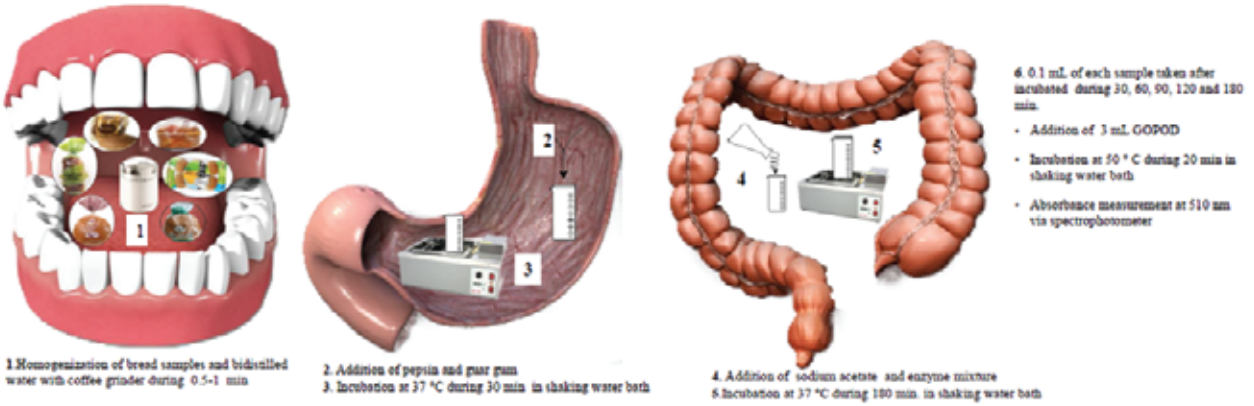

Figure 1. *In vitro* enzymatic carbohydrate digestion of some Turkish bread species.

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 hydrolyzing of each bread sample.

Calculation of hydrolyzed index value (HI)

The hydrolyzed index (HI) value of glucose obtained from the result of *in vitro* carbohydrate digestion of each bread sample was calculated from Hydrolyzed Curve between 0 and 180 min at 5 different times via excel. HI value of commercial glucose was also calculated by applying same procedures. Both HI values were calculated according to the following formula: (12)

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

*While calculating HI values of D-glucose formation after digestion of carbohydrate in TWB and the other bread species, it was used maltose and white bread from Turkey as reference carbohydrate, respectively.

The HI values of commercial glucose, and D-glucose formed from the result of *in vitro* carbohydrate digestion of TWB, WWB, WGB, HGB, GFB, VB were calculated separately.

Calculation of glycemic index (GI) value

In this paper, the glycemic index value (GI) of all bread samples used was calculated according to the following formula obtained by Goni (11).

$$GI = 0.7 \times [39.71 + (0.559 \times HI)]$$

Calculation of Glycemic Load (GL) Value

In this paper, the glycemic load value (GL) of all bread samples used was calculated according to the following formula (11).

$$GL = [DC / 100] \times GI$$

DC: Digestible carbohydrate amount as gram

Results and discussion

250 g of standard bread is the most widely used food in Turkey. The daily bread production is 22.719 tons and 90.9 million units, these values are 8.29 million tons and 33.2 billion units per year. The daily

bread consumption is 21.496 tons and 86 million pieces, these values are 7.85million tons and 31.4 billion units per year. World consumption of bread per person is 51 kg in Finland, 71 kg in Denmark, 68 kg in Italy, 62 kg in Germany, 58.5 kg in Spain, 60 kg in Netherlands and 128 kg in Turkey (5).

South Africa is the country with the highest label information written on the package of the foodstuff in the World. Although there is no GI label on the foodstuff package, continuous work is being carried out through brochures and media for the consumer's conscientious consumption in the United Kingdom and Canada (2).

In Turkey, food package doesn`t have GI values as label information. GI values of foods should be found as label information because the consumer will decide which product is healthier for them to be more conscious. Another parameter which is as important as GI values is glycemic load (GL) because the amount of food consumed is important as much as the GI value. In this study, it was aimed GI and GL values of different types of packaged bread consumed in Turkey determine 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 (8). It has not seen any study done *in vitro* GI and GL determination. 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 bread samples in a much faster and shorter time. To determine *in vivo* or *in vitro* GI value of the food, we should be use reference carbohydrate such as maltose, glucose, white bread (12).

In this study, we used to determine the GI value of Turkish white bread and other kinds of breads (whole wheat bread, wheat germ bread, gluten-free bread, nuts, raisin bread and village bread) by using reference carbohydrate maltose and Turkish white bread, respectively.

Calculation of GI and GL values of turkish white bread

GI and GL values of Turkish white bread was determined by using maltose as reference carbohydrate.

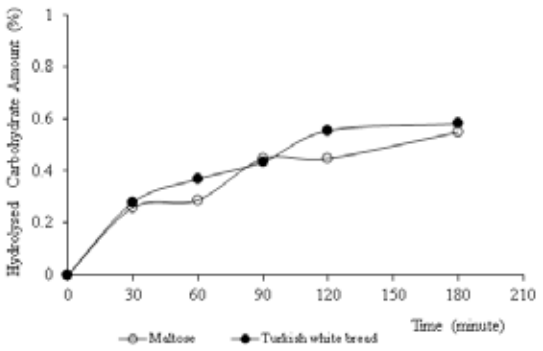

Figure 2. The over-time curve of in vitro enzymatic carbohydrate digestion our constructed for the determination of the area under the curve (AUHC) for TWB

Table 1. The calculated AUHC, HI, GI and GL values of maltose and Turkish white bread

Carbohydrate Type	Sample Used (g)	AUHC	HI	GI	GL
Maltose	0.58	42.35	100	100	85.00
Turkish white bread	1.02	60.00	141	82.24	41.58

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).

The time-varying curve of carbohydrate amount using maltose as reference carbohydrate during enzymatic hydrolysis was called "hydrolysis curve" for Turkish white bread (TWB) (Figure 2). The areas under the hydrolysis curves (AUHC) were calculated separately via excel in both maltose and Turkish white bread. Then, the areas under the hydrolysis curve of white bread (AUHC), hydrolysis index (HI), glycemic index (GI) and glycemic load (GL) values were calculated (Table 1).

As it was shown in Table 1, the GI value of Turkish white bread (82.24) was found to be similar the value stated by the Turkish bread in the Glycemic Index Database of the University of Sydney (87) (10). In this way, we have determined the GI value of Turkish white bread which we will use as reference carbohydrates to obtain other Turkish bread species.

Calculation of GI and GL values of turkish bread species

It was used Turkish white bread as reference carbohydrate to determine GI and GL values of whole wheat bread, wheat germ bread, whole wheat bread, nut grape bread, gluten free bread and village bread. The amount of carbohydrate, fat, protein and diet fibre of all kind of bread species used in the study was presented in Table 2.

The concentration over-time curves were used for the determination of the area under the curve (AUHC) of wheat germ bread (WGB), hazelnut grape bread (HGB), whole wheat bread (WWB), gluten free bread (GFB) and village bread (VB). Turkish white bread (TWB) was used as reference carbohydrate for calculating GI and GL values of five different bread species (Figure 3).

The concentration over-time curves were used for the determination of the area under the curve (AUHC) of wheat germ bread (WGB), hazelnut grape bread (HGB), whole wheat bread (WWB), gluten free bread (GFB) and village bread (VB). Turkish white bread (TWB) was used as reference carbohydrate for calculating GI and GL values of five different bread species (Figure 3).

The starch digestion of all kind of packaged bread species sold in Turkey is shown in Figure 3 until 180 minute. The reaction medium didn't contain glucose because of that bread species have no any digestion

Table 2. The amount of carbohydrate, fat, protein and diet fibre of all kind of bread species

Bread Sample	Amount of Carbohydrate (g)	Amount of Fat (g)	Amount of Protein (g)	Amount of Diet Fibre (g)
White bread*	50.97	2.28	8.66	2.9
WWB	45.20	2.98	8.57	6.20
WGB	42.00	9.50	1.90	6.90
HGB	45.41	12.05	9.63	5.89
GFB	49.20	0.59	2.30	0.00
VB	44.91	1.14	9.61	5.60

*White bread was used as reference carbohydrate

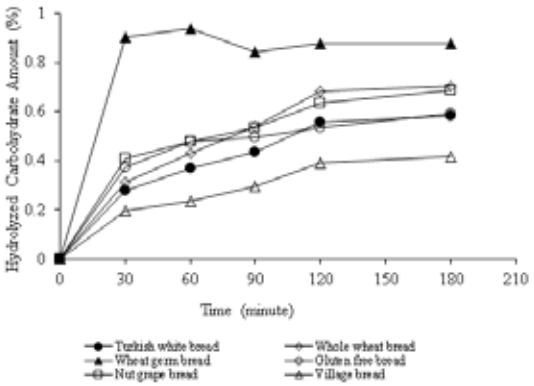

Figure 3. The concentration over-time curves used for the determination of the area under the curve (AUHC) of Turkish wheat bread (TWB), wheat germ bread (WGB), hazelnut grape bread (HGB), whole wheat bread (WWB), gluten free bread (GFB) and village bread (VB).

of carbohydrate at 0 minute. The highest digestion of carbohydrate digestion occurred at 30 minute for all of breads samples. When digestion was continued range from 30 minute to 180 minute during 1.5 hours, it was shown that digestion of carbohdyrate and hydrolyzed glucose amount were continue regularly increase in all samples. At the end of 1.5 hours, it is thought that the decrease in digestion may be due to lack of substrate in the reaction medium.

In this study, firstly, hydrolysis of the starch in each bread 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 was determined as colorimetric. After plotting the hydrolysis curves for each bread sample, the area under each hydrolysis curve (AUHC) was calculated separately. HI, GI and GL values of each bread sample were calculated from AUHC values (Table 3)

As can be seen in Table 2, Table 3 and Figure 4, GI values of bread types with high dietary fiber were observed to be low. It is seen that the dietary fiber amounts of the bread types used are shown as 2.39% of Turkish white bread, 6.20% of whole wheat bread, 6.90% of wheat germ bread, 5.89% of nut grape bread, 5.60% of village bread and 0.0 % gluten-free bread. GI values of each species decreases with the increase of dietary fiber amount.

Gluten-free bread with lower protein and gluten-free that can be used in diseases caused by nutritional

Table 3. The calculated AUHC, HI, GI and GL values of maltose and of wheat bread, wheat germ bread, hazelnut grape bread, whole wheat bread, gluten free bread and village bread used Turkish white bread as reference carbohydrate.

Bread Sample	Sample Used (g)	AUHC	HI	GI	GL
TWB*	1.02	42.35	100	100	39.20
WWB	1.28	60.00	100	82.24	28.32
WGB	1.42	70.00	116	72.63	33.61
HGB	1.26	106.0	176	95.78	32.50
GFB	1.01	60.00	100	82.24	32.97
VB	1.20	62.50	104	67.03	19.99

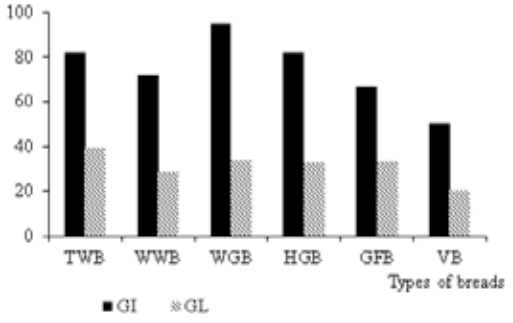

Figure 4. GI and GL values of WWB, WGB, HGB, GFB and VB by using TWB as reference carbohydrate.

disorders such as celiac, phenyl ketonuria (PKU), various liver, kidney, trozinemia, etc. It doesn't contain dietary fiber. Since dietary fiber is not present in gluten-free bread used in our study, GI value is moderate value (5).

The glycemic index of wheat germ bread with a high nutritional value is 95. The oil contained in the wheat germ bread reduces the level of cholesterol in the blood, prevents blood clotting, strengthens the muscles and improves the reflex by increasing performance and durability These states are said to enhance the immune system by increasing the ability to overcome stress (5).

Dietary fiber amount of nut grape bread is high, but the amount of fat in 100g. The presence of grapes in it cause high glycemic index. The GI value of village bread is 67.03. Village bread has low glycemic index value compared to other species because it contains whole wheat flour and very high dietary fiber.

GI values of foods were generally determined as *in vivo* which has difficulties in working on people. That *in vivo* is also long and laborious is disadvantage of it. Instead of *in vitro* analysis, *in vitro* assays can be performed to determine the concentration of multiple nutrients at one time. A reference carbohydrate should be used during *in vivo* or *in vitro* analysis of GI values. This reference carbohydrate can be maltose, glucose and white bread. The aim is to determine the GI value of the food we want by using these references (5).

In this paper, we used maltose and Turkish white bread to calculate GI value of Turkish white bread and the other bread species used as reference carbohydrate, respectively.

Bread is the most widely used food in Turkey. GI value of white bread is higher than the other type of breads there. Recently, with the increasing importance given to healthy nutrition, the use of other types of bread has increased instead of white bread. In order to increase the use of these breads, it is easier to digest it with a high amount of dietary fiber. Whole grain bread should be consumed to prevent cardiovascular diseases, obesity, diabetes and some types of cancer (5).

Another parameter that is as important as the GI values is the glycemic load (GL). The GI value of a food is important as well as its consumption is important. GI values of foods should be found as label information on its package in terms of more conscious of consumers. Thus, the consumer will decide which product is healthier for him/her (2).

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Conflicts of Interest: The authors declare no potential conflicts of interests.

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