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The Effect of Bread with and without Cumin on Glycemic Index, Glycemic Load and Glycemic Response in Healthy People: A Randomized Clinical Trial

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ABSTRACT

Background: This study aimed to evaluate the effects of bread with and without cumin on glycemic index (GI), glycemic load (GL), and postprandial glycemic response in healthy adults. **Methods:** In this randomized clinical trial, 27 healthy participants were assigned to consume either cumin bread (made with 98% Setareh flour and 2% cumin powder) or ordinary bread (made with Setareh flour). Both breads were baked under identical conditions. Capillary blood glucose was measured at fasting and at 15, 30, 45, 60, 90, and 120 minutes after consumption. Then, GI and GL were calculated using standard methods based on glucose response and carbohydrate content. **Results:** Twenty participants completed the study. Cumin bread significantly reduced both GI and GL compared to ordinary bread (P<0.001). However, no significant differences were found between the groups in blood glucose levels at any measured time points. No adverse effects were reported following consumption of either bread. **Conclusion:** Incorporating cumin into wheat bread significantly lowers its GI and GL without affecting short-term glycemic response or causing side effects in healthy individuals.

Introduction

After food consumption, glucose is released into the bloodstream; a physiological phenomenon known as the glycemic response (Salmerón *et al.*). This response depends on the rate of glucose entry, the quantity of glucose ingested, and the rate at which it is cleared from the bloodstream (Triplitt, 2012). Evidence suggests that elevated levels of insulin and blood glucose increase

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the risk of diet-related diseases, including cardiovascular disorders, certain cancers, and particularly type 2 diabetes (Lin *et al.*, 2019, Törrönen *et al.*, 2010).

Accordingly, the Food and Agriculture Organization/World Health Organization (FAO/WHO) introduced the glycemic index (GI) as a tool to guide healthier food choices (Jenkins *et al.*, 1981). GI measures the incremental area under the blood glucose response curve after consuming a food containing a fixed amount of carbohydrates (typically 50 g), in comparison with a standard reference food (Sievert and Buzzard, 1988). This index reflects the blood glucose response and the insulin demand associated with a given carbohydrate load. While the total amount of carbohydrate consumed plays a key role in determining the glycemic response, GI is independent of this quantity. For example, although pasta has a low GI, a large portion may elevate blood glucose more significantly than a smaller serving. This led to the concept of glycemic load (GL), which accounts for both the quality and quantity of carbohydrates in a portion of food (Penlioglou *et al.*).

Bread is one of the most widely consumed staple foods globally. However, certain types of bread possess high GI and GL values due to their substantial carbohydrate content. As mentioned earlier, considering the impact of high-GI foods on metabolic risk factors, reducing the GI and GL of bread could have significant preventive and therapeutic benefits for chronic diseases (Krawęcka *et al.*, 2021, Maehre *et al.*, 2021).

In response, researchers in food industry are increasingly focused on developing new products aimed at enhancing public health (Mann *et al.*, 2010). The addition of medicinal herbs to bread and other food items has shown promise in improving nutritional quality and health outcomes (Aderonke, 2021, Kurek *et al.*, 2018, Masood *et al.*, 2021). In Iranian culinary traditions, a variety of additives such as herbs, cumin, and black cumin are commonly incorporated into bread dough. According to Persian medicine texts, these ingredients enhance the nutritional and functional quality of bread (Emami and Sobhani, 2020) . Moreover, some herbal additives have been shown to lower the GI of bread (Borczak *et al.*, 2018).

Recent research indicates that incorporating spices into whole-grain bread may improve glycemic outcomes in healthy individuals (Shakib and Gabrial, 2010). One commonly used additive is cumin, a spice with diverse therapeutic properties and a long history of use in traditional medicine (Allaq *et al.*, 2020, Thippeswamy and Naidu, 2005). Scientific evidence suggests that cumin has a low GI and GL, likely due to its bioactive compounds (e.g., cuminaldehyde), which inhibit carbohydrate-digesting enzymes such as alpha-amylase, thereby slowing glucose absorption (Dinesh *et al.*, 2024, Jafari *et al.*, 2018, Kaur *et al.*, 2019). Studies have demonstrated that cumin consumption can lead to moderated postprandial glucose levels and a smaller area under the curve (AUC) for blood glucose response (Cindya Zanzer, 2018). It is hypothesized that the synergistic effects between the fiber content of whole grains and the phytochemicals in cumin further enhance glycemic regulation. Notably, optimal efficacy appears to be dose-dependent, with 3–5% cumin by weight, showing the most consistent benefits (Srinivasan, 2018). However, current research is limited by small sample sizes, individual variability in metabolic responses, and a focus on acute rather than long-term effects. Therefore, further studies are needed to standardize formulations, elucidate mechanisms of action, and evaluate sustained impacts on metabolic health. Thus, the aim of this study was to investigate the effect of wheat bread, with and without cumin, on the glycemic index, glycemic load, and postprandial glycemic response in healthy individuals.

Materials and Methods

Study design and participants

This randomized clinical trial was conducted from January to February 2022 at Yazd Diabetes Research Center. The participants were selected via purposive sampling and stratified randomization based on age, gender and body mass index (BMI). The sample size was

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estimated through comparing the two means for the clinical trials using $\alpha=0.05$ and $\beta=20\%$ (Brouns *et al.*, 2005, Lin *et al.*, 2010) . Accordingly, 10 people were selected for each group. The inclusion criteria were: age between 20-45 years old and body mass index (BMI) in the range of 18-24.9 kg/m^2. The exclusion criteria were fasting blood glucose (FBG) more than 100 mg/dl, smoking or opioid addiction, pregnancy and lactation, a special diet and professional exercise, metabolic or any chronic diseases, and medications that affect glucose metabolism. According to the inclusion and exclusion criteria, at first, there were 27 candidates for this clinical trial, five of them were excluded from the study. Also, one candidate was excluded due to non-cooperation and one more due to glucose intolerance. Finally, 20 people were left for the analyses (**Figure 1**).

The reference food was 50 g of glucose powder (glucose dextrose monohydrate) dissolved in 200 ml of water. The test meals (100 g of cumin bread and 100 g of ordinary bread) were equivalent to 50 g of carbohydrates and were used with 200 ml of water. After written consent was received from the participants, they went to a laboratory in the morning while fasting for 10-12 hours on four different days with at least a one-day gap between the measurements. They were asked to preserve their regular physical activity levels and avoid having heavy meals on the nights before the trial. First, on three separate days with at least one day apart, everyone received 50 g of glucose dissolved in a 200-ml glass of water as standard food. On the fourth day of the study, the individuals were randomly divided into 1:1groups. The first group ate 100 g of ordinary bread, and the second group had 100 g of cumin bread. In this study, only one researcher who was in charge of distributing the bread was not blind to the type of the bread, but those involved including the testers, statistical analysts and the other researchers were blind.

Bread preparation

All the research materials were obtained from a local market in Tehran, Iran. Cumin seeds were authenticated by Dr. Mohammad Kamalinejad and a voucher specimen was deposited with number SBMU-8035 at Herbarium of the School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The cumin bread was made in the laboratory and then adapted for production in a commercial bakery. The flour composition includes 98% wheat flour (with commercial name Setareh flour) and 2% cumin powder. The flour was supplied by SETAREH FLOUR MILL Company, Alborz, Iran. Also, the ordinary bread was produced with the same basic ingredients except for cumin. Each bread sample was turned into bulky bread under the same conditions at the "Golnan Puratos" factory in Karaj, Iran. In addition, the bread preparation formulation was standardized based on the measurement of total phenol.

Chemical analyses

Proximate analysis was carried out for the test food at "Behesht Aein" Laboratory Complex in Tehran. This analysis was done for both bread types using the standard method proposed by the association of official analytical chemists (Association of Official Analytical Chemists (AOAC), 2003). The analysis was performed in duplicate, and then, the carbohydrate, protein, crude fiber, fat, moisture and ash contents as well as the energy (kcal) were recorded.

To measure the moisture content of the bread, 5 g of the sample was weighed and poured into a container, and then it was placed in an oven at the temperature of 105 °C for 120 minutes. At the end, the sample was weighed again (Iran National Standard Organization (INSO), 2010). To determine the ash content, 2 g of the sample was put in a china cup and heated in the oven at 550 °C until it completely turns into white ash (Iran National Standard Organization (INSO), 2010) . To measure the protein content, the sample was digested using sulfuric acid. The result of the alkaline digestion reaction was then distilled. The ammonia released in a boric acid solution was collected and titrated with sulfuric acid. After nitrogen was measured, the amount of protein was calculated (Iran National Standard Organization (INSO), 2010). To calculate

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the amount of fat, the sample was boiled with dilute hydrochloric acid to release non-free and combined fats. As filtering and drying were performed and the fat remaining on the filter paper was extracted with light petroleum ether in a Soxhlet machine, the residue was dried in the oven at 103 °C and the amount of fat was calculated (Iran National Standard Organization (INSO), 2003). In order to calculate the raw fiber in each bread sample, the sample was crushed and boiled with sulfuric acid to remove fat, and then, the insoluble residue was separated and washed. The residue was boiled with a sodium hydroxide solution. Following that, the insoluble residue was separated, washed, dried and weighed, finally the mass loss due to burning was measured (Iran National Standard Organization (INSO), 2010). To calculate carbohydrates, first, they were fully hydrolyzed as a portion of the sample was heated with hydrochloric acid. After it was cooled, the precipitating materials were added, the suspension was smoothed, and its glucose was measured with Fehling's solution (Iran National Standard Organization (INSO), 2006). Finally, the amount of energy was calculated via summing the energy of carbohydrates, protein, and fat in 100 g of the sample (Food and Agriculture Organization, 2003).

Fasting blood measurement

On the sampling days, fasting blood glucose concentrations were measured immediately before the start of the study (0 min) using a calibrated finger-stick blood glucose meter (GLUCOCARD™ 01-mini, ARKRY, Japan). Then, the reference bread or the test were taken within 10-12 minutes. The participants were encouraged to avoid eating and drinking for two hours, the blood glucose levels were measured 15, 30, 45, 60, 90 and 120 minutes after eating.

Calculation of GI and GL

A blood sugar (BS) curve was plotted with connection points to measure BS at specified times. The incremental area under the curve (IAUC) was calculated after the tested bread was eaten. This was done through a trapezoidal formula with a possible fasting fraction (Matthews *et al.*, 1990). The procedure for the measurement of GI was adopted from Wolever *et al.* (Wolever *et al.*, 1991) and Brouns *et al.* (Brouns *et al.*, 2005). It was based on the flowing formula:

$$GI = \frac{\text{IAUC test bread}}{\text{IAUC glucose}} * 100$$

Foods are classified into those with low GI (GI ≤ 55), medium GI (GI of 56-69) and high GI (GI ≥70) categories.

The formula to measure GL was as follows:

$$GL = (GI\ of\ test\ bread \times available\ carbohydrate\ in\ a\ serving\ of\ test\ bread\ (g))/100$$

The GL of foods may be low (≤10), medium (11 19), or high (≥20) (Vega-López *et al.*, 2018).

Ethical considerations

Informed consent to enter the study was received from the individual participants. They incurred no costs and were free to leave the study at any stage. This study was approved by the ethics committee of Yazd Shahid Sadoughi University of Medical Sciences (IR.SSU.REC.1400.239 IRCT code: IRCT20160221026684N3, https://irct.behdasht.gov.ir/trial/62258). In the initial assessment of the research team, the estimated date for the proposal registration, approval of the code of ethics, and registration was February 30, 2022.

Data analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 26 (SPSS Inc., Chicago, USA). Data were presented as the mean±standard deviation (SD) for continuous variables. Given the number of participants and the non-normal distribution of the data, nonparametric tests, including the Mann-Whitney and Kruskal-Wallis tests, were conducted. Also, one sample t-test was carried out. P-values<0.05 were considered statistically significant. In normal distribution, independent t-test was done, and GraphPad.Prism 8.0 was used.

Results

At first, there were 27 candidates for this clinical

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trial. According to the inclusion and exclusion criteria, five of them were excluded from the study. Moreover, one candidate was excluded due to non-cooperation and one more due to glucose intolerance. Finally, 20 people were left for the analyses (**Figure 1**).

The people in the two study groups had no statistical difference in terms of age, sex, BMI and FBG at baseline (**Table 1**).

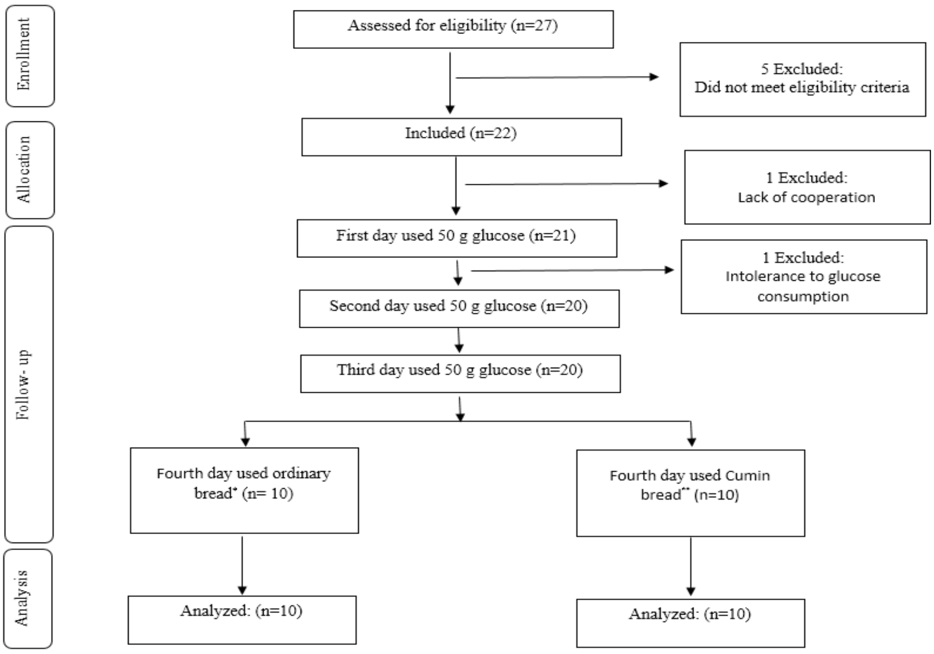

Figure 1. Flow diagram of study.
*:Bread made with Setareh flour; **: Bread designed on a mixture of Setareh flour and cumin powder 2%

Table 1. Characteristics of subject at the beginning of study.

Variables	Ordinary bread group (n=10)	Cumin bread group (n=10)	P-value[c]
Age (y)	30.75 ± 5.09[a]	30.25 ± 6.43	0.86
Body mass index (kg/m^{2})	23.69 ± 2.40	24.80 ±2.38	0.37
Fasting blood glucose (mg/dl)	94.38 ± 7.42	87.25 ± 8.43	0.09
Gender			
Male	5 (50)[b]	5 (50)	1.00
Female	5 (50)	5 (50)	

[a]: Mean±SD; [b]: n (%); [c]: Was obtained from Mann-Whitney for quantitative and Fisher's Exact test for qualitative variables.

The compositions of the bread types are presented in **Table 2**. The proportion of carbohydrate in the cumin bread was lower than ordinary bread (33.9 g/100 g vs. 46.7 g/100 g). Furthermore, the amount of energy in the cumin bread was lower than ordinary bread (206.4 kcal vs. 254.9 kcal). As for the crude fiber content, the cumin bread was almost twice as rich as the ordinary bread (0.54 g/100 g vs. 0.32 g/100 g).

As shown in **Table 3**, no significant difference was observed in BS (at baseline until 120 minutes) after consuming cumin bread and ordinary bread.

The mean postprandial changes in the blood glucose level after eating the test breads are shown in **Figure. 2**. The GI and GL of cumin bread was significantly lower than ordinary bread. **Table 4** shows GI and GL values and a classification of the test bread. The GI values for the cumin bread and

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the ordinary bread were 48.80 and 54.72, respectively; thus, they were classified as low-GI bread. The GL values for the two tested bread (cumin bread and ordinary bread) were 16.54 and 25.55, respectively; therefore, cumin bread had a medium GL, and ordinary bread had a high GL.

Table 2. Chemical composition of test bread types (g/100 g).

Components	Ordinary bread	Cumin bread	P-value[a]
Carbohydrate	46.7	33.9	<0.001
Protein	8.33	8.7	<0.001
Crude Fiber	0.32	0.54	<0.001
Fat	3.8	3.9	0.143
Moisture	32.04	32.51	0.214
Ash	1.8	1.9	0.145
Energy (kcal)	254.9	206.4	<0.001

[a]: *Was obtained from independent t-test.*

Discussion

Postprandial hyperglycemia is a major pathophysiological condition. One of the main tools for managing postprandial hyperglycemia is the use of foods with low GI and GL, which can have effects such as weight loss, lowered FBG, increased insulin levels, lowered plasma triglyceride levels, and improved blood pressure (Penlioglou *et al.*, 2021). Bread is a common food used with different flavors in many countries, but some of breads have high glycemic indices. In the present research, the effect of bread combined with cumin based on a certain formula is investigated in comparison with ordinary bread. According to the results, although both tested bread samples are classified in low-GI, the GI of cumin bread is significantly lower than that of ordinary bread (P<0.001). In addition, this study shows that the GL between cumin bread and ordinary bread is significantly different (P<0.001); cumin bread is placed in the group of foods with medium GL, while ordinary bread is of high GL. As the literature in the field suggests, no study has ever examined the effect of adding cumin to bread on glycemic index and glycemic load.

Compared to previous studies, the findings of this research indicate that the addition of cumin to bread can significantly reduce the GI and GL; a topic that has not been directly addressed in the scientific literature to date. The results of this study are consistent with previous reports suggesting that low-carbohydrate diets and increased protein intake contribute to postprandial glucose reduction and improved insulin secretion (Samkani *et al.*, 2018, Wang *et al.*, 2018).

Based on the analysis of the chemical compounds, the carbohydrates, protein, fiber and calories (P<0.001) of cumin bread are significantly different from those in ordinary bread. According to the formulation of cumin bread, if 2 g of regular bakery flour is reduced in favor of 2 g of cumin, there is a reduction of 12.8 g of carbohydrates per 100 g of the bread. A significant reduction in the carbohydrate content of the diet is an effective way to improve postprandial glucose and average glucose concentration daily (Rasmussen *et al.*, 2020). Only a few clinical trials have compared low-carbohydrate and high-carbohydrate diets. In a randomized cross-over study, a diet consisting of 40% carbohydrates and 60% carbohydrates has been compared. The results of this study indicate a decrease in postprandial glucose, the average concentration of glucose during the day, the level under the glucose curve 2 hours after a meal; and 24-hour total glucose levels were below the curve in the 40% carbohydrate diet group compared to the 60% carbohydrate diet group (Hernandez *et al.*, 2014). Cumin also increases the fiber by 0.22 g per 100 g of bread. In fact, this seed has relatively high fiber; as mentioned in some studies, the amount of fiber in cumin is up to 62% (Sowbhagya *et al.*, 2007). Increasing the intake of dietary fiber is the main way to reduce the sticky mucus in the intestines as a result of the reduced absorption of carbohydrates in food, which reduces GI. The increased fiber content in cumin-enriched bread, consistent with findings from several other studies, has facilitated reduced carbohydrate absorption, and consequently, a decrease in the GI. However, in some other investigations, particularly those involving patients with type 2 diabetes; the addition of dietary fiber alone has not led to significant improvements in glycemic control,

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which may be attributed to the type and structural characteristics of the fiber consumed (Ames *et al.*, 2015, Jenkins *et al.*, 2002, Scazzina *et al.*, 2013).

Table 3. Blood sugar in two groups before and after receiving breads.

Variables	Ordinary bread (n=10)	Cumin bread (n=10)	P-value[b]
Fasting blood glucose at base (mg/dl)	94.38 ± 7.42[a]	87.25 ± 8.43	0.09
Blood glucose (mg/dl) after….min.			
15	93.75 ± 7.08	97.50 ± 14.95	0.53
30	106.75 ± 12.18	122.88 ± 18.93	0.06
45	122.75 ± 15.20	137.13 ± 27.31	0.21
60	124.38 ± 16.28	129.75 ± 28.10	0.64
90	107.00 ±12.67	119.25 ± 32.07	0.33
120	101.00 ±11.14	101.63 ± 23.85	0.94

[a]: Mean±SD; [b]: Was obtained from Mann-Whitney.

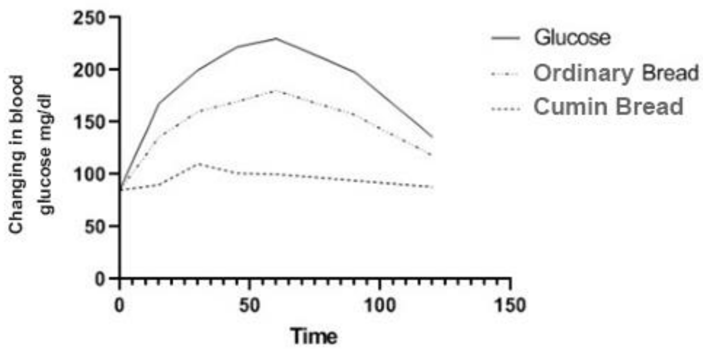

Figure 2. Glucose response curve of the ordinary bread, cumin bread, and glucose (reference).

Table 4. Glycemic index and Glycemic load values.

Variables	Cumin bread	Ordinary bread	P-value[a]
Glycemic index	48.80	54.72	<0.001
Glycemic index classification	Low	Low	-
Glycemic load	16.54	25.55	<0.001
Glycemic load classification	Medium	High	-

[a]: Was obtained from one sample t-test.

In this study, the addition of cumin to bread raised the protein by 0.37 g per 100 g of the bread. The increase of protein in foods containing carbohydrates can decrease GI (Flint *et al.*, 2004, Henry *et al.*, 2006). In this regard, the mechanism reported in studies points to the role of protein in increasing the production of gastric inhibitory peptide (GIP) and increases the amount of insulin secreted, which can lead to blood glucose levels to be less affected (Hätönen *et al.*, 2011). In addition, protein can around the carbohydrate molecule, build protective network, and prevent glycolytic enzyme activity (Bornet *et al.*, 1987). In a study on patients with type 2 diabetes 50 g of protein, 50 g of glucose, or 50 g of glucose with 50 g of protein as a meal were given in random order. Data suggest that protein given with glucose increases insulin secretion and reduces the rise in plasma glucose in at least some type 2 diabetic subjects (Nuttall *et al.*, 1984).

In this study, by keeping the bread baking method and the time constant, the effects of chemical components, particularly carbohydrates, proteins, and fiber, on GI and GL were examined more precisely. A significant reduction in caloric intake observed in cumin-enriched bread is also noteworthy, aligning with the goals of weight-loss and metabolic control diets. Although numerous studies have highlighted the antidiabetic properties

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of cumin and its role in enhancing insulin sensitivity, this research provides a more practical application of this herbal compound by focusing on its incorporation into a widely consumed product such as bread, thereby contributing to the regulation of postprandial glucose response. On the other hand, Cumin (*Cuminum cyminum* L from the Apiaceae family) is a biennial plant which is native to East Mediterranean up to South Asia and is used as an important species in the world, especially in Asian countries (Srinivasan, 2018). Various studies have shown that cumin has antibacterial, antifungal, anti-inflammatory, antioxidant and anti-diabetic effects as well as immune system-modulating activities (Allaq *et al.*, 2020). So that, in a study, it was shown that the consumption of 75 mg of cumin per day for two months caused a significant reduction in BMI, plasma triglyceride level and hemoglobin A1c (Jafari *et al.*, 2017). In one study, it was shown it was shown on overweight participants that the consumption of cumin for eight weeks had similar effects to Orlistat 120 on weight and BMI and beneficial effects on insulin metabolism compared to Orlistat 120 and placebo (Taghizadeh *et al.*, 2015). Among the effects mentioned in the literature, one may refer to the inhibitory effect of cumin on two enzymes, alpha-glycosidase and aldose reductase, in the carbohydrate pathway (Lee, 2005). Also, by maintaining the integrity of β-cells, cumin can cause a significant increase in insulin secretion and the sensitivity of hepatocytes to insulin (El-Dakhakhny *et al.*, 2002). Moreover, by increasing the secretion of testosterone, it may somehow cause an increase in glucose transporter-4 (Sacks and Campos, 2006).

The present study has several notable strengths. First, it is the first investigation to explore the effects of incorporating cumin into bread on GI and GL, thereby addressing a gap in previous research. The use of a control group (bread without cumin) as a baseline strengthened the validity of the findings. A precise and controlled formulation was employed, in which a portion of wheat flour was substituted with cumin. This enabled a detailed assessment of the nutritional and glycemic consequences of the modification. The chemical composition of both types of bread including carbohydrate, protein, fiber, and caloric content was thoroughly analyzed, and statistically significant differences were observed ($P<0.001$). The study also considered physiological mechanisms through which the protein, fiber, and bioactive compounds in cumin may influence glycemic responses. Moreover, baking conditions (method and duration) were standardized across both bread types, eliminating a potential confounding factor.

Nonetheless, the study has certain limitations. A larger sample size may be necessary to achieve sufficient statistical power for group comparisons. Furthermore, the study was conducted exclusively on healthy individuals, limiting the generalizability of the results to populations with metabolic disorders such as diabetes. Long-term effects of cumin-enriched bread on glycemic control and metabolic health were not assessed. Additionally, sensory evaluation and consumer acceptability as critical aspects in the practical application of functional foods were not included in the study. Lastly, uncontrolled dietary variations among participants may have influenced the outcomes.

Conclusion

In summary, the addition of cumin significantly reduces the GI and GL of bread. Hence, cumin bread may be a more effective option for managing diabetes mellitus, obesity, and metabolic syndromes. Further studies are needed to confirm the veracity of our results.

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Authors' contributions

Namiranian N generated the random allocation sequence, statistical analysis, and manuscript edition; Nikkhah H, Razavi R, and Khodaie SA

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enrolled participants, and assigned participants to interventions. Study design was done by Kamalinejad M, Safari AA and Mozaffari-Khosravi H. Nikkhah H, Razavi R and Khodaie SA wrote the manuscript. All authors read the final draft of manuscript and approved it.

Conflict of interest

The authors declared no conflict of interest.

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