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# Effect of bread incorporated with flaxseed powder on postprandial glycemia and palatability in healthy individuals

## *Efeito do pão incorporado com pó de linhaça na glicemia pós-prandial e na palatabilidade em indivíduos saudáveis*

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## ABSTRACT

### Objective

The objective of this study was to investigate the effect of bread incorporated with flaxseed powder on glycemic response and palatability.

### Methods

In a cross-over study, 20 healthy participants were randomly assigned to consume one of two iso-caloric test foods: control bread, made from 100% plain wheat flour or bread containing flaxseed powder (10% w/w), as breakfast, 1-2 weeks apart. The portion size of bread consumed was calculated based on providing 50 g available carbohydrates. Capillary blood samples were collected to determine blood glucose at fasting and postprandially for 2 hours. Palatability of the test bread was assessed using a 9-point hedonic scale.

### Results

Consumption of FSB resulted in a significant decrease in blood glucose at 30 min ( $p=0.003$ ) and 45 min ( $p=0.004$ ) compared to control bread. The incremental area under the curve (iAUC) for blood glucose was also decreased ( $p=0.006$ ) after consumption of FSB compared to control bread. The glycemic index of flaxseed powder (57.79), falling within the medium category, was significantly lower ( $p<0.05$ ) compared to control bread (71). Palatability parameters did not differ between the test breads ( $p<0.05$ ).

### Conclusion

In conclusion, the consumption of bread incorporated with flaxseed powder decreased blood glucose level, suggesting that adding flaxseed to staple foods could help in better glycemic control.

**Keywords:** Bread. Flaxseed. Glycemic Control. Perception.



## RESUMO

### **Objetivo**

O objetivo deste estudo foi investigar o efeito do pão incorporado com pó de linhaça na resposta glicêmica e palatabilidade.

### **Métodos**

Em um estudo cruzado, 20 participantes saudáveis foram aleatoriamente designados para consumir um dos dois alimentos testados iso-calóricos: pão controle, feito de 100% farinha de trigo comum, ou pão contendo pó de linhaça (10% p/p), como café da manhã, com 1-2 semanas de intervalo. O tamanho da porção de pão consumido foi calculado com base em fornecer 50 g de carboidratos disponíveis. Amostras de sangue capilar foram coletadas para determinar a glicose no sangue em jejum e pós-prandial por 2 horas. A palatabilidade do pão de teste foi avaliada usando uma escala hedônica de 9 pontos.

### **Resultados**

O consumo de FSB resultou em uma diminuição significativa na glicose sanguínea aos 30 min ( $p=0.003$ ) e 45 min ( $p=0.004$ ) em comparação com pão controle. A área incremental sob a curva (iAUC) para a glicose no sangue também foi diminuída ( $p=0.006$ ) após o consumo de pó de linhaça em comparação com pão controle. O índice glicêmico do pó de linhaça (57.79), que se enquadra na categoria média, foi significativamente menor ( $p<0.05$ ) em comparação com o pão controle (71). Os parâmetros de palatabilidade não diferiram entre os pães testados ( $p<0.05$ ).

### **Conclusão**

Em conclusão, o consumo de pão incorporado com pó de linhaça diminuiu o nível de glicose no sangue, sugerindo que adicionar linhaça aos alimentos básicos poderia ajudar no melhor controle glicêmico.

**Palavras-chave:** Pão. Óleo de linhaça. Controle Glicêmico. Percepção.

## INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from either inadequate insulin secretion, insulin resistance or both [1]. Presently, diabetes affects more than 537 million people globally, and this figure is estimated to rise substantially to 783 million by 2045, particularly in middle-income countries [2].

Diet plays a vital role in the development of type 2 diabetes; hence, changing the diet may help to prevent its development. More specifically, the quality and quality of dietary carbohydrates have been shown to play a key role in developing impaired glucose tolerance and insulin resistance and increase the risk of type 2 diabetes [3]. Foods with high glycemic index (GI) have been shown to increase postprandial blood glucose and insulin demand and could possibly contribute to insulin resistance in the long term [4].

White bread is the most frequently consumed food all over the world, particularly in developing countries. It is considered a major source of rapidly digestible carbohydrates, contributing to high postprandial blood glucose and hence its frequent consumption increases the risk of developing chronic diseases, particularly type 2 diabetes [5,6]. In addition, the consumption of ultra-processed foods has been shown to be strongly associated with a higher risk of type 2 diabetes [7,8]. Therefore, different sources of functional food ingredients such as herbs, seeds, and underutilized cereal have been incorporated into bread to reduce its glycemic impact [5,9,10].

Flaxseed (*Linum usitatissimum L.*), also known as linseed, is a member of the Linaceae family. It is considered a potential functional food ingredient due to the presence of high amounts of alpha-linolenic acid, dietary fiber, phenolic compounds, high-quality protein and phytoestrogens [11]. The potential of flaxseed in reducing the glycemic response has been highlighted in numerous studies. For example, an in vitro study revealed that polyphenolic compounds in flaxseed may inhibit

the activities of starch hydrolyzing enzymes like  $\alpha$ -amylase and  $\beta$ -glucosidase, resulting in notable effects on reducing fasting blood levels [12]. Another study in broiler chicken demonstrated that the linoleic component of flaxseed regulates the  $\alpha$ -amylase activity and linolenic and oleic components inhibit the  $\beta$ -glucosidase activity in the experimental group compared to the control group [13]. In a systematic review, the consumption of ground flaxseed was shown to improve glycemic control and insulin resistance in individuals with prediabetes and type 2 diabetes [14]. In a randomized controlled study, consumption of 30 g/day milled flaxseed powder together with lifestyle modification for 12 weeks in women with polycystic ovary syndrome improved the glycemic indices, lipid profile and inflammatory markers in treatment group compared to control group [15].

Although several clinical studies have been conducted on investigating the impact of flaxseed on glycemic control and related parameters [16-19], no study has yet determined the acute effect of bread incorporating brown flaxseed powder on blood glucose in healthy individuals. Therefore, the objective of the current study was to determine the acute effect of incorporating flaxseed into bread on postprandial glycemic response whilst maintaining product palatability. It was hypothesized that bread incorporated with flaxseed powder would reduce postprandial glycemic response in healthy individuals.

## METHODS

### **Study participants**

Healthy individuals of both genders aged 18-60 years were recruited through posted flyers and direct personal communication. After obtaining written informed consent, a health questionnaire was used to assess individuals' eligibility for inclusion in the study. Participants with a history of diabetes, cardiovascular diseases, gluten allergy, respiratory problems, and major gastrointestinal problems, breakfast skipping, or being currently on weight loss were excluded. In addition, participants using medications with effects on the metabolism of glucose, satiety sensation, body weight, and smoking were also excluded. In addition, participants with a blood glucose concentration of  $\geq 5.6$  mmol/l were also excluded. The study was performed on twenty healthy individuals. The sample size was calculated based on a previous study of Khan et al. [20] using blood glucose as the main outcome variable. It was found that 20 subjects were sufficient to detect a 20% reduction in blood glucose, with a power of 80%, and at a 5% confidence level. This study was approved by the Human Research Ethics Committee of the department of Human Nutrition (HN-HREC/2018-0027), The University of Agriculture Peshawar and was conducted according to the guidelines of Helsinki Declaration.

### **Test breads**

Brown flaxseed was obtained in bulk (5 kg) from the Department of Horticulture, the University of Agriculture, Peshawar, Pakistan. The seeds had 6.8 % moisture content, with a smooth glossy texture and light brown colour. The seeds were cleaned to discard stones and foreign objects. Afterwards, the seeds were ground into powder using a knife grinder (Grindomix GM-200, Retsch, Germany; Haan, 1000 W, 3000 rpm) and stored in polythene bags until utilization. In the current study, there were two types of breads. Formulations consisted of 100% all-purpose wheat flour for Control Bread (CB) or replacement of wheat flour with flaxseed powder at 10% (w/w) for preparation of Flaxseed Bread (FSB). This incorporation level of flaxseed was selected based on a pilot consumer evaluation study (data not shown). The bread was prepared in the department of Human Nutrition laboratory following a standard procedure as described by Marpalé et al. [21]. First the wheat flour

and flaxseed powder were extensively mixed to obtain a homogenous mixture. All other ingredients used in the preparation of bread included 6 g sugar, 1 g salt, 5 g canola oil, 70 ml water and 2 g instant dry yeast per 100 g. The mixture was kneaded manually for 10 to 15 min to obtain a homogenous dough. The dough was placed in a baking pan and fermented for 40 min in a slightly warm room (30 °C) until it doubled in size. Subsequently, the bread was baked in an electric oven (Panasonic digital oven) at 220 °C for 30 min. After baking, the bread was cooled and stored in a refrigerator until it was required to be consumed by the participants.

### **Study design and protocol**

A randomized, controlled cross-over trial was used for the current study. Each participant attended two testing sessions with a washout period of about 1-2 weeks apart, to minimize the carryover effects. At each testing session, participants were served either control or treatment bread as breakfast. Composition of test meals is given in Table 1. Drinking water was used to equalize the weight of the test breakfasts. Participants were instructed to avoid strenuous physical activity and to record their evening meal the day before each testing session. After fasting for a period of 10-12 hours, participants arrived in the laboratory at 8:00 am to attend the study session. Baseline measurements including weight, height, and waist circumference were recorded before starting the session. After 5 minutes of rest, the fasting blood glucose was measured using finger prick method. Afterward, participants consumed the test bread within 10 minutes, during which time they rated the palatability of the bread using a 9-hedonic scale. Blood glucose was measured again at 15, 30, 45, 60, 90, and 120 minutes after eating the test bread. The participants were prohibited from drinking, eating, or performing any other activity apart from reading or using a laptop.

**Table 1** – Composition of test meals.

Ingredients	Control bread	Flaxseed bread
Bread (g)	103	109
Water (g)	247	241
Total weight (g)	350	350
Energy (Kcal)	260.46	284.13
Available carbohydrates (g)	50	50
Protein (g)	7.96	9.41
Fats (g)	3.18	5.17
Dietary fiber (g)	1.21	3.85

### **Baseline measurements**

Baseline measurements such as weight, height, and waist circumference were assessed using standard scales. A stadiometer was used to record height. A digital laboratory scale was used to record weight. Subjects were asked to remove their shoes and heavy clothing before weighing. Height and weight were used to calculate Body Mass Index (BMI). Waist circumference was measured using non-stretchable measuring tape between the lower rib and crest sections and was measured to the nearest 0.1 cm. Blood pressure was assessed using a portable manual mercurial monitor.

### **Blood glucose analysis**

Blood glucose was analyzed using the finger-prick method. Before pricking, the participant's finger was cleaned with the help of alcoholic swabs. The finger was allowed to dry before pricking to

avoid contamination of blood with alcohol. Then, the finger was pricked with an automatic lancet device. Finally, blood glucose was measured using a glucometer (Accu check Performa Nano, Roche, São Paulo, Brazil). A single glucometer was used throughout the study to avoid variations.

### **Palatability of test bread**

The palatability of the bread was tested in terms of appearance, flavor, texture, and overall acceptability using a 9-point hedonic scale [22]. The subjects filled the scale anywhere from extremely disliked to extremely like, representing their perception of the bread.

### **Calculation and statistical analysis**

Incremental blood glucose values for each test bread were calculated by subtracting fasting value of each participant from the corresponding postprandial value. Blood glucose response curves were constructed using the incremental blood glucose values. Trapezoidal method was used to calculate incremental area under the curves (iAUCs, incremental Area Under the Curves) for blood glucose. Peak time taken as the time during which blood glucose was highest. Incremental peak glucose was the highest glucose value during the 2 hours time. Glycemic profile was obtained by dividing the total time in minutes during which blood glucose concentration was higher than baseline by IPG value. Glycemic index was calculated using the formula; iAUCs of flaxseed bread divided by iAUCs of control bread × 100. Statistical software IBM®SPSS® (SPSS Inc., Chicago, IL) was used for data analysis. The effect of time, treatment, and their interaction (time x treatment) on blood glucose was determined by two-way repeated Analysis of Variance (ANOVA) with Bonferroni adjustment. Differences between treatments at each point were assessed using paired *t*-test. Peak time, incremental peak glucose, glycemic profile and glycemic index between the breads were compared using paired *t*-test. Palatability parameters between the two breads were compared by paired *t*-test. Statistical significance was set at *p*<0.05.

## **RESULTS**

### **Baseline characteristics**

All the twenty subjects completed the two testing sessions. Their baseline characteristics are presented in Table 2.

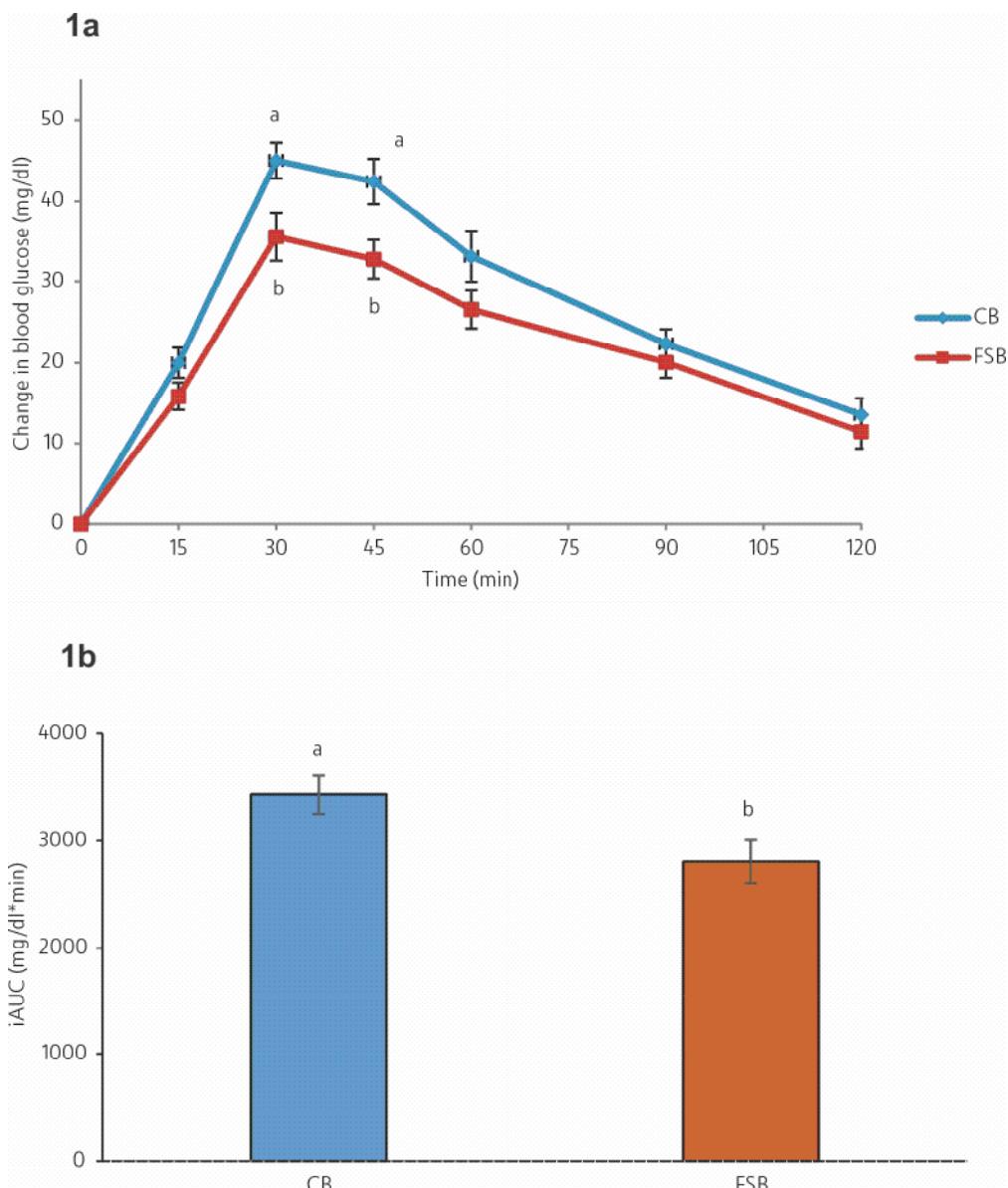
**Table 2** – Baseline characteristics of study subjects (n=20)\*.

Characteristics	N/ Mean±SEM
Male/Female	10/10
Age (years)	23.25±0.33
Weight (kg)	60.45±2.35
Height (m)	160.10±1.59
Body mass index (kg/m <sup>2</sup> )	21.95±0.88
Waist circumference (cm)	76.00±1.75
Fasting plasma glucose (mg/dl)	90.02±1.84
Systolic blood pressure (mm Hg)	112.75±2.32
Diastolic blood pressure (mm Hg)	71.20±2.13

Note: \*Values are means±SEM.

## Blood glucose response

The postprandial blood glucose changes after test bread consumption with the corresponding iAUC are shown in Figure 1 (a and b). There was a significant effect of time ( $p<0.001$ ) and treatment ( $p=0.002$ ) on postprandial blood glucose after consumption of FSB, while their interaction (time  $\times$  treatment) was non-significant ( $p=0.108$ ). Post-hoc analysis revealed that FSB significantly reduced blood glucose level at time points 30 min ( $p=0.003$ ) and 45 min ( $p=0.004$ ) compared to CB. In addition, the blood glucose iAUC was significantly lowered for FSB compared to CB ( $p=0.006$ ).



**Figure 1** – Blood glucose response.

Note: (a & b): Mean ( $\pm$ SEM) changes from baseline in blood glucose and incremental areas under the curves (iAUCs) in healthy individuals ( $n=20$ ) after consumption of test breads (CB: Control Bread; FSB: Flaxseed Bread). Values with different superscript letters are significantly different at each time point: two-way repeated measure ANOVA with Bonferroni adjustment, followed by paired *t*-test ( $p<0.05$ ). Vertical bars with different letters are significantly different,  $p<0.05$  (paired *t*-test).

## Peak time, incremental peak glucose, glycemic profile and glycemic index of test breads

Peak time, peak incremental glucose, glycemic profile and glycemic index of test breads are shown in Table 3. Peak time was not significantly different between FSB and CB ( $p=0.412$ ), whereas incremental peak glucose level was significantly lower after consumption of FSB compared to CB ( $p<0.001$ ). The glycemic profile was significantly higher after consumption of FSB compared to CB ( $p=0.001$ ). The glycemic index of FSB on both bread and glucose scale was significantly lower compared to CB ( $p=0.029$ ).

**Table 3** – Peak time, incremental peak glucose, glycemic profile and glycemic index of test breads\*.

Test bread	Peak time	IPG	GP	GI (bread scale)	GI (glucose scale)
	Mean±SEM				
Control bread	40.50±2.45	48.80±2.24 <sup>a</sup>	2.37±0.12 <sup>b</sup>	100.00±0.00 <sup>a</sup>	71.00±0.00 <sup>a</sup>
Flaxseed bread	44.25±4.28	39.05±2.50 <sup>b</sup>	3.36±0.25 <sup>a</sup>	81.65±6.77 <sup>b</sup>	57.79±4.81 <sup>b</sup>

Note: \*All values are mean±SEMs (n=20). Values in the same column with different superscript letters are significantly different from each other,  $p<0.05$  (paired t-test). GI: Glycemic Index; GP: Glycemic Profile; IPG: Incremental Peak Glucose.

## Palatability of test breads

The palatability of test bread measured in terms of appearance, texture, flavor and overall acceptability is shown in Table 4. No significant differences were observed between the test breads in terms of appearance ( $p=0.470$ ), texture ( $p=0.577$ ), flavor ( $p=0.603$ ), and overall acceptability ( $p=0.776$ ).

**Table 4** – Test breads' palatability\*.

Test bread	Appearance	Texture	Flavor	Overall acceptance
	Mean±SEM			
Control bread	6.40±0.22	6.00±0.19	6.10±0.17	6.35±0.22
Flaxseed bread	6.65±0.27	6.15±0.28	5.95±0.25	6.25±0.40

Note: \*All values are mean±SEMs (n=20).  $p<0.05$  (paired t-test).

## DISCUSSION

The current study provides evidence that FSB has the potential to significantly reduce postprandial blood glucose compared to CB. There was a mean difference of 13.5 mg/dl in peak blood glucose values between FSB and CB. Both the breads were liked equally by the study individuals.

The potential of flaxseed in lowering glycemia has been reported in a recent study in which flaxseed supplementation for 4-12 weeks improved glycemic response and insulin sensitivity in healthy and diabetic individuals [23]. However, limited studies have examined the acute response of flaxseed as the first meal of the day. For example, acute postprandial blood glucose response has been investigated in randomized controlled trials in healthy adults, in which consumption of flaxseed enriched bars (comprising 50 g available carbohydrate) significantly reduced postprandial blood glucose level compared to glucose solution (comprising 250 ml of water with 50 g of dissolved sugar) while simultaneously raised satiety level to a greater extent compared to saltine crackers

[18]. In another randomized cross-over trial, muffins containing 10 g flaxseed as three meals a day provided promising results in reducing and maintaining blood glucose levels related to control muffins [24]. In a recent systematic review and meta-analysis comprising of seven randomized control trials, flaxseed supplementation exhibited a considerable reduction in fasting blood glucose variables with an improvement in insulin sensitivity in pre-diabetic and type-2 diabetic adults [14]. Similarly, ingestion of 15 g raw flaxseed powder before breakfast by type-2 diabetic males significantly reduced the acute postprandial blood glucose level compared to control breakfast [25]. Furthermore, the consumption of cookies containing 10 g of flaxseed by type-2 diabetic patients with constipation revealed a significant reduction in fasting blood glucose response compared to the placebo group [26]. Most of the clinical trials utilized 30 g of flaxseed to demonstrate the acute hypoglycemic response of ground flaxseed in healthy subjects [27]. However, in our study, bread containing 10% flaxseed powder significantly reduced postprandial blood glucose levels in healthy subjects, and had a significant impact on the incremental peak, glycemic profile and glycemic index. The glycemic index of bread decreased from 71.0 to 57.79 on glucose scale, which reveals that glycemic index was reduced from high-category to low-category with the incorporation of flaxseed.

The reduction in postprandial blood glucose concentration after FSB intake may be attributed to the presence of bioactive components in flaxseed, such as  $\alpha$ -linolenic acid, linoleic acid, mucilage, phytoestrogenic lignans, cyclic peptides and dietary fibers [28]. For instance, foods with high dietary fiber content, primarily soluble dietary fiber, have been shown to reduce blood glucose levels in diabetic and healthy adults [29]. In this context, it has been reported that flaxseed mucilage or flaxseed gum has the ability to delay the digestion of food, increase food viscosity, slow the nutrient and digestive enzyme interactions, decrease absorption from the gastrointestinal tract, and reduce the permeability of the intestine, which results in lowered glycemic response [30]. The reduction in postprandial blood glucose level after the ingestion of FSB may also be due to the presence of polyphenols in flaxseed, which have been shown to inhibit the activities of carbohydrate digesting enzymes including  $\alpha$ -amylase and  $\alpha$ -glucosidase [12]. Furthermore, alpha-linolenic acid, a polyphenol in flaxseed, has shown the capability to improve insulin sensitivity by controlling intestinal hormone secretion, thus elevating insulin signal and producing insulin secretion from beta cells to upgrade glucose utilization [12].

The current study has certain limitations, including its acute experimental design and the inclusion of healthy subjects only. Therefore, more extensive studies are required to verify the effect of FSB on postprandial blood glucose levels in both obese and/or diabetic individuals. Further, the effect of FSB consumption on satiety, energy intake, gastric emptying rate, satiety-related hormones, colonic fermentation and oral exposure time were not measured and should be investigated in future studies to better understand the mechanisms underlying the hypoglycemic effect of FSB.

## CONCLUSION

In conclusion, the present study showed that the consumption of bread incorporated with flaxseed powder resulted in a lower glycemic response, which may offer potential benefits for type 2 diabetes, obesity and cardiovascular diseases. Long-term mechanistic studies are now needed to further investigate the effects of flaxseed on blood glucose levels, insulin sensitivity and energy homeostasis in both diabetic/obese and healthy individuals.

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