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Original Article

Influence of Freezing-Thawing Cycles on the Glycemic Index of the Iraqi White Bread after Oral Ingestion

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Abstract

Background: The food storage at low temperature showed a significant drop down in its glycemic index. ***Aims***: The present study aims to investigate the impact of freezing and thawing processes and duration on the glycemic response of Iraqi local white bread. ***Methods***: In this prospective longitudinal study, twelve healthy subjects (seven males, five females), aged 21-53 years, were recruited from Al-Rafidain University College campus and the local community. After overnight fasting, commercial Iraqi local white bread (200 g) was administered as fresh bread, following 1-week or 2-week freezing and thawing. Peak glucose response, 2 hr incremental area under the glucose response curve (AUC_{0-120}) was evaluated as an outcome. ***Results***: The freezing and thawing processes and duration for the white bread resulted in a significant lowering blood glucose AUC values compared to AUC values of the fresh one ($P< 0.05$), AUC values of the fresh bread (14176±1134 mg min/dl), after 1-week of freezing and thawing (13205±660 mg min/dl), and after 2-weeks (12828±642 mg min/dl). ***Conclusion***: freezing and thawing processes and duration significantly decreased the glycemic index of the fresh Iraqi local white bread.

Keywords: Carbohydrate absorption, freezing-thawing, glycemic index, white bread.

تأثير دورات التجميد والذوبان على مؤشر نسبة السكر في الدم للخبز الأبيض العراقي بعد تناوله عن طريق الفم

الخلاصة

الخلفية: أن تخزين المواد الغذائية في درجة حرارة منخفضة يتسبب في خفض مؤشر نسبة السكر في الدم. الأهداف: دراسة تأثير عمليات التجميد والذوبان وفترتها على نسبة السكر في الدم للخبز الأبيض المحلي العراقي. **الطرائق**: أجريت الدراسة على اثني عشر شخصا من الأصحاء (سبعة ذكور وخمس إناث)، تتراوح أعمارهم بين 21 و53 عاما، من حرم كلية الرافدين الجامعة والمجتمع المحلي. وبعد الصيام الليلي، تناولوا 200 غم من الخبز الأبيض العراقي المحلي الطازج، ثم بعد أسبوع أو أسبوعين من التجميد والذوبان. تم تقييم ذروة استجابة الجلوكوز، ومستوى الأرتفاع خلال 2 ساعة و منحنى استجابة الجلوكوز AUC_{-120} . **النتائج**: أدت عمليات التجميد والذوبان إلى انخفاض كبير في قيم السكر في الدم مقارنة بقيم AUC للخبز الطازج بعد أسبوع من التجميد والذوبان (13205±660 ملغ.دقيقة/دل), وبعد أسبوعين (12828±642 ملغ.دقيقة/دل). **الأستنتاجات**: إن عمليات التجميد والذوبان قد خفضت بشكل ملحوظ مؤشر نسبة السكر في الدم للخبز الأبيض العراقي المحلي الطازج.

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INTRODUCTION

Utilization of freezing techniques to elongate the life cycle of food has gained widespread interest. The decrease in water contents due to the subzero temperatures provides a favorable condition that reduced chemical reactions and improved storage stability [1]. Freezing starch-containing forms ice crystals and a liquid-solid phase separation process happen, which predisposes physical stress to the contents [2,3]. Freezing and thawing cycles are processes of heat transfer that involve chemical modifications which may greatly influence the product quality [4,5]. Moreover, freezing produces resistant carbohydrates that resist intestinal digestion and ferment in the large intestine [6]. This means that the biological system gets fewer calories from frozen bread [7]. Meanwhile, more than 60% of human food includes carbohydrates which should be hydrolyzed before absorption to monosaccharides [8]. Glycemic Index (GI) is a relative ranking of the carbohydrate contents based on the relative rise in the blood glucose level two hours after ingesting that food [9]. Low-GI carbohydrates showed a slower rate of digestion, absorption, and biotransformation; they cause a slower rise in blood glucose and insulin levels. Accordingly, they may have preventive potential against the development of type 2 diabetes and obesity [10,11]. The quality of bread depends on many factors such as its ingredients, preparation techniques, and storage which influence the rate of staling [12,13]. Freezing bread may halt staling since extremely low temperatures may inhibit the degradation of carbohydrates and maintain the bread in a stable state [13]. Although freezing has been used globally, many types of research are conducted to optimize the freezing and thawing conditions of the bread [14]. It has been reported that relatively small differences in the glycemic response of regularly consumed complex carbohydrates have shown beneficial health effects, including reduced cardiovascular disease risk and glycemic control [15]. Decreasing the glycemic response to Iraqi white bread represents an application of the above idea. The present study aims to identify the changes in the glycemic response after two freezing-thawing cycles of traditional Iraqi white bread.

MATERIALS AND METHODS

Study design

A prospective longitudinal study was conducted at Al-Rafidain University College campus and the local community.

Subjects

Twelve healthy subjects (7 males and 5 females) were recruited to participate in the study. Subjects were required to be between 19 and 55 years of age, with a body mass index (BMI) <25 kg/m2. The recruited subjects were asked to complete a health screening questionnaire to detect health problems such as abnormal glucose metabolism (fasting blood glucose 125 mg/dl). Medical conditions or medications that affect glucose regulation, gastric emptying, body weight, appetite, or energy expenditure are also inspected. On the day of the first test, anthropometric measurements were performed in the fasting state, using standardized methods. Height was recorded to the nearest centimeter using a stadiometer, with subjects standing erect and without shoes. Bodyweight was recorded to the nearest 0.1 kg, with subjects wearing light clothing and no shoes. BMI was calculated using the standard formula weight (kg)/height (m)2. The local Scientific Committee of the Faculty of Pharmacy, Al-Rafidain University College approved the research protocol. The participants have briefed about the full details of the study protocol and the opportunity to ask questions. All subjects gave verbal consent before participation.

Test bread

Commercial Iraqi local white bread was tested for the glycemic response. This commercial white bread was made from white wheat flour mixed with NaCl, tap water, and dehydrated yeast without additives. Details of the contents such as macronutrient, dietary fiber, and sodium content of the test bread are not reported. This is due to a lack of analytical methods or the unavailability of an official content list. The bread was tested either as a freshly prepared sample or following two different freezing storage conditions: (1) frozen for one week or (2) frozen for 2 weeks. Frozen bread was defrosted at room temperature before the time of consumption. It has been suggested that further retrogradation during freezing following temperature reduction to the frozen state does not occur [16].

Blood glucose measurements

Finger-prick blood samples were taken for capillary blood glucose analysis. Recent reports suggest that capillary blood sampling is preferred for reliable glycemic index testing [17]. To establish blood glucose stability at the start of the blood glucose response curve, fasting blood samples were taken at 5 and 0 min before consuming the bread, and the baseline value was taken as a mean of the two results. The fresh bread was ingested immediately after the zero-time sampling, and further blood samples were taken at 15, 30, 60, 90, and 120 min after eating the tested bread sample. Blood was obtained by finger prick using the Accu-Chek glucometer system (Bayer HealthCare, Newbury, UK). Where necessary, before a finger prick, subjects were encouraged to warm their hand under running warm water to increase blood flow. Fingers were not squeezed to extract blood from the fingertip as this may dilute with plasma.

Statistical analysis

Statistical analysis was performed using the Graph Pad Prism software (GraphPad Prism version 5.1, Chicago, IL, USA). Data are presented as means and standard deviations. Before statistical analysis, the normality of the data was tested using the Shapiro–Wilk statistic. Intra-individual variations of the three tests were assessed using the coefficient of variation (CV%¼100 x standard deviation/mean). Repeated measures analysis of variance (ANOVA), with Bonferroni's post hoc correction, was used to compare glycemic response between test bread stored in different ways. Statistical significance was set at $P<0.05$.

RESULTS

Table 1 shows the characteristics of the healthy subjects involved in the present study. Figure 1 shows the incremental blood glucose response for the fresh bread.

Table 1: Characteristic features of the volunteers (n=12)

Parameters	
Age (year)	28.67±10.5
BMI (kg/m2)	22.0±1.95
FBS (mg/dl)	86.91±14.58
Gender n(%)	
Male	7(58.3)
Education n(%)	
BSc	10(83.3)
Diploma	2(16.7)
Smoking habit n(%)	4(33.3)
Dietary restriction n(%)	5(41.6)
Physical Exercise n(%)	4(33.3)

Values expressed as frequency and percentage or mean±SD; n: number of subjects; BMI: Body mass index.

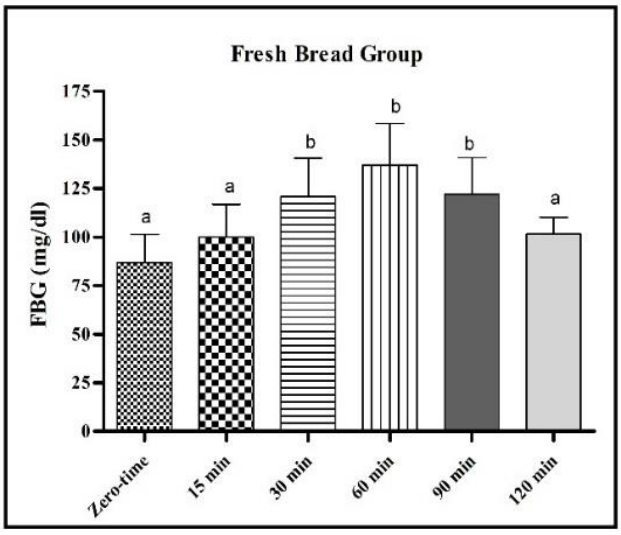

Figure 1: The incremental blood glucose response with time for the fresh local Iraqi commercial bread. Values are expressed as mean±SD; n= 12 volunteers; values with non-identical letters (a,b) are significantly different (P<0.05, ANOVA).

After ingestion, the blood glucose peaks to the maximum level after 60 min and is significantly different from the baseline value; the peak level returns to around the baseline value after 120 min. Figure 2 shows the incremental blood glucose response for 1-week frozen bread. After ingestion, the blood glucose peaks to maximum level after 60 min, and significantly different from both the baseline and 120-min values; the peak level returns after 120 min to a level higher than that reported at baseline (P<0.05). Figure 3 shows the incremental blood glucose response for the 2-week frozen bread.

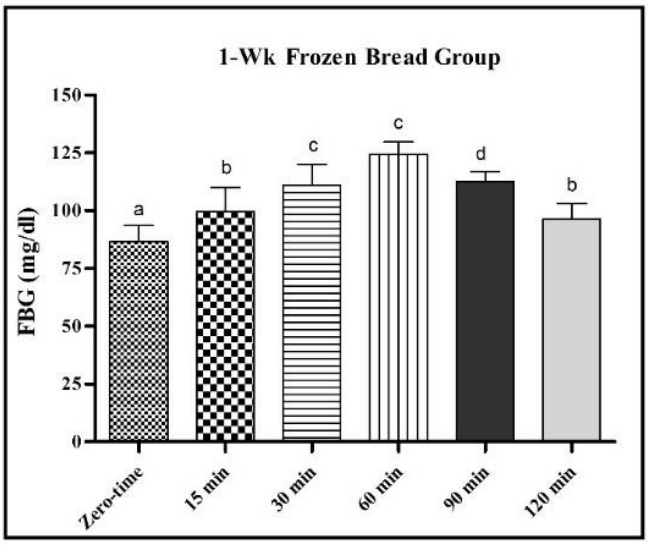

Figure 2: The incremental blood glucose response with time for the 1-week frozen local Iraqi commercial bread. Values are expressed as mean±SD; n= 12 volunteers; values with non-identical letters (a,b,c,d) are significantly different (P<0.05, ANOVA).

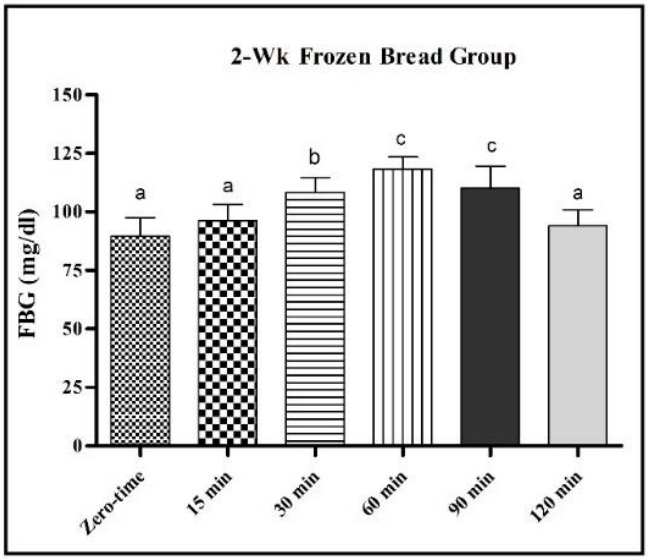

Figure 3: The incremental blood glucose response with time for the 2-week frozen local Iraqi commercial bread. Values are expressed as mean±SD; n= 12 volunteers; values with non-identical letters (a,b,c) are significantly different (P<0.05, ANOVA).

After ingestion, the blood glucose peaks to the maximum level after 60 min and is significantly different from a baseline value; the peak level returns to around the baseline value after 120 min. Figure 4 shows the incremental blood glucose

response curves for the fresh bread, 1-week frozen bread, and 2-week frozen brad.

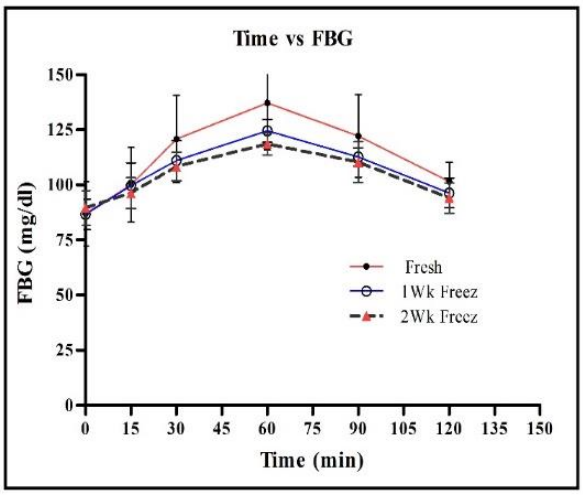

Figure 4: The incremental blood glucose response with time after ingestion of fresh bread, 1-week frozen bread, or the 2-week frozen local Iraqi commercial bread. Values expressed as mean±SD; n= 12 volunteers.

There was a recognized overall effect of freezing and thawing on the peak rise in blood glucose response compared with the freshly consumed bread. Figure 5 showed that a comparison between the blood glucose response after 60 min of ingesting bread with different storage conditions (1-week and 2-week freezing) result in significantly lower blood glucose values (P<0.05), compared with that after ingestion of fresh bread. Meanwhile, the two freezing conditions showed non-significant differences in this respect.

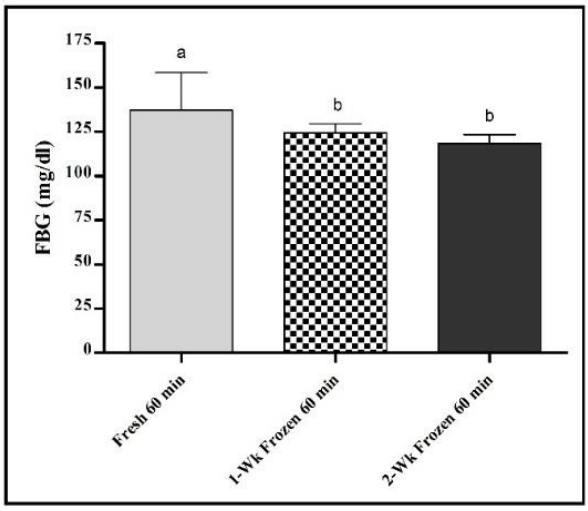

Figure 5: Comparison between blood glucose response 60 min after ingestion of fresh bread, 1-week frozen bread, or the 2-week frozen local Iraqi commercial bread. Values are expressed as mean±SD; n= 12 volunteers; values with non-identical letters (a,b) are significantly different (P<0.05, ANOVA).

Table 2 demonstrates the AUC0-120 of changes in blood glucose across the different storage conditions for the local Iraqi white bread.

Table 2: Area Under the Curve (AUC0-120) of the change in blood glucose with time (n=12)

Group	FBG AUC_{0-120} (mg.min/dl)	% Change (AUC)
Fresh Bread	14176±1134[a]	----
1-Wk Frozen Bread	13205±660[b]	6.8
2-Wk Frozen Bread	12828±642[b]	9.5

Values are expressed as mean±SD; n: number of subjects; values with non-identical superscripts (a,b) are significantly different (P<0.05, ANOVA).

Compared to the fresh bread, AUC_{0-120} was significantly lower for 1-week and 2-weel frozen bread (P<0.01); however, the AUC_{0-120} values after ingestion of the two types of frozen brad are not significantly different when compared with each other (P>0.05).

DISCUSSION

According to our knowledge, this is the first study conducted in Iraq to detect the impact of freezing conditions on the glycemic response to commercial local Iraqi white bread. Evaluating the effects of freezing and thawing predisposed to reduced glycemic response and decrease in the AUC values; meanwhile, the impacts of food processing and cooking techniques on the glycemic response are well reported [18, 19]. Treatments that include extreme heat changes during bread storage increased gelatinization, which results in the breakdown of the starch granules. Thus, decreasing storage heat down to freezing point levels modifies the structure of the starch granules and the consequent sensitivity to enzymatic salivary and pancreatic amylases following consumption; this may lead to lower availability of glucose for absorption and decreased glycemic response [6]. Retrograded starch (RS3) represents a form of resistant starch, and there is sufficient evidence of RS3 formation during the processes of starch freezing [15]. Currently, there has been an increasing interest in resistant starch. Its positive health benefits have been also demonstrated, and known to be mediated through the effects on colonic fermentation and both postprandial glucose and lipid profile [20,21]. This study may present two implications. First, it suggests that the freezing and thawing procedure may alter the glycemic response to white bread; thus, white bread needs not always be a high glycemic index food. However, there remains a preference for white bread in many countries; therefore, the current study will be informative to consumers for optimal storage of white bread to favorably alter the glycemic response. Second, the present study highlights the need to reconsider using white bread as a reference food in glycemic index methodology. Using white bread as a reference food, the determination of GI values can refer to blood glucose levels [18]. However, the current study showed that the use of white bread as reference food could be inadequate and hard to represent a comparative indicator of blood glucose levels. A limitation of this study was the indirect estimation of the available carbohydrate content of the white bread samples. It is recommended that

future studies utilizing such measurements have to include both resistant and retrograded starch [22] both before and after storage conditions. Other methods have been proposed to classify the glycemic impact of foods based on total carbohydrate rather than the available type, such as relative glycemic effect [23]. However, further research is needed regarding the alternative indices to measure the glycemic response of stored food under freezing conditions.

Conclusion

The present study shows reductions in glycemic response following freezing and thawing of local Iraqi white bread; it highlights the need to define and maintain storage conditions of food products when the glycemic response of foods is determined. High consumption of white bread is well-recognized. Exploring ways to reduce the glycemic response of white bread and other foods are critical to prevent and manage chronic diseases.

Acknowledgement

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Conflicting interest

The authors declared no conflicts of interest.

Data sharing statement

The datasets analyzed during the current study will be available from the corresponding author on a reasonable request.

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