POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

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Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 3, pp. 373-376

COMPARISON OF GLYCEMIC RESPONSES TO FROZEN AND NON-FROZEN WHEAT ROLLS IN HUMAN VOLUNTEERS – A SHORT REPORT

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Key words: wheat rolls, frozen storage, glycemic index

Glycemic responses to frozen and non-frozen wheat rolls were studied in humans. The subjects were served each four test wheat rolls (fully baked--non frozen – FBNF; fully baked and frozen – FBF; partly baked and frozen – PBF; and unfermented frozen dough, before proofing and baking – UFD). Blood glucose concentrations were measured at 0 min as well as at 15, 30, 45, 60, 90 and 120 min after the start of the meal. Generally, the frozen storage treatment resulted in lower GI values. In one case the difference reached statistical significance ($p<0.05$). Namely, compared to FBNF rolls (GI=83.03±5), the PBF rolls gave significantly ($p<0.05$) lower values of GI (GI=60.66±6). We suggest that gelatinized starch formed in frozen rolls retrograded during the cooling process into resistant starch (RS), thus resulting in lower GI values of frozen storage rolls.

ABBREVIATIONS

AOAC – Association of Official Analytical Chemists; BMI – body mass index; FAO – Food and Agriculture Organisation; FBF – fully baked and frozen; FBNF –fully baked–non frozen; GI – glycemic index; IAUC – incremental area under the curve; PBF – partially baked and frozen; RS – resistant starch; UFD – unfermented and frozen dough; WHO – World Health Organization.

INTRODUCTION

In 1981 Jenkins *et al*. [1998] introduced the glycemic index (GI) concept. It is defined as the incremental area under the blood glucose curve (IAUC) of 50 g available carbohydrate portion of a test food expressed as a percentage of the response to 50 g available carbohydrate of a reference food (glucose), taken by the same subject, on a different day [FAO/WHO, 1998]. Food with low GI value provides longer satiety sensation and insulin maintenance, as well as, protective effects against development of type 2 diabetes, cardiovascular diseases and obesity [Wolever, 1990; Jenkins *et al*., 1998; Morris & Zemel, 1999; Hallfrisch & Behall, 2000; Mennen *et al*., 2000; Pi-Sunyer, 2002; Bell, 2003; Brouns *et al*., 2005; Roder *et al*., 2005; Maki *et al*., 2007]. Impact of food on postprandial blood glucose level depends on several factors, especially: glucose, starch digestibility, amylose/amylopectin ratio (GI value decreases with the rise of amylose content), starch interaction with protein, the amount of fat, type of chemical bonds in carbohydrate moiety (GI value plunges with the rise of bonds others than α-1-4 and α-1-6), the presence of dietary fiber and the form of food (crude, processed, liquid, solid, *etc.*). What is more, the processing method of an individual food can greatly change its GI.

Nowadays, growing nutritional knowledge of consumers forces the food industry to manufacture products with lower GI values. At the same time, white bread is one of the major sources of carbohydrates in typical Western diets. Consequently, common consumption of white bread is responsible for high GI of European diets [Wolever, 1990; Powell *et al*., 2002; Chlup *et al*., 2004; Burton & Lightlower, 2007]. This adverse effect of white bread consumption can be alleviated by modification of the baking process. The conventional process of baking under conditions of high temperature and moderate water content leads to gelatinization of starch granules [Bárcenas *et al*., 2003; Niba, 2003; Selomulyo & Zhou, 2007] and results in a rapidly digestible product with high GI. However, cooling of gelatinized starch leads to its retrogradation, and resulting starch complexes, called resistant starch (RS), are insoluble and resistant to gastrointestinal enzymes, thus giving low GI values when consumed. Indeed, several research groups have reported a decline in postprandial glycemic or insulinemic responses to resistant starch ingestion [Liljeberg *et al*., 1999; Hoebler *et al*., 1999; Haralampu, 2000; Carreira *et al*., 2004; Selomulyo & Zhou, 2007]. Additionally, RS undergoes colonic fermentation and short-chain fatty acids are produced, including acetate, propionate and butyrate, which exert beneficial effects on human intestine [Mennen *et al*., 2000].

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More recently, the bakery industry has exploited the applications of freezing technology [Selomulyo & Zhou, 2007] to obtain the so-called "frozen bakery products". This approach gives the possibility of making "fresh" bread available at any time of the day, as well as, improve the nutritional quality of bread by lowering its GI [Burton & Lightlower, 2007].

In this context, the aim of this study was to evaluate the effect of freezing treatment of wheat rolls, applied during different stages of the baking process, on the impact of these rolls on glycemic responses (GI), when consumed by human volunteers.

MATERIALS AND METHODS

Wheat rolls

Four kinds of wheat rolls were tested. They were: (1) fully baked-non frozen – FBNF; (2) fully baked and frozen – FBF; (3) partly baked and frozen – PBF; and (4) unfermented frozen dough (before proofing and baking) – UFD.

The wheat rolls were prepared by an adaptation of recipes from a bakery ingredients company (PURATOS, Belgium) using the following ingredients for the dough: wheat flour, type 55 (Moulins Soufflet, Pornic, France), salt (Janikosoda S.A., Janikowo, Poland), yeast (SAF – Instant red – Lesaffre Group, Strasbourg, France), Freshbake improver (Puratos, Belgium) and fresh running water. The ingredients were mixed for 9 min in a mixer (DIOSNA SP-12, GETH, Germany), then underwent proofing (60 min, 35°C, 95% RH) and baking in an electric oven (Meteor, Viva, Italy).

The FBNF rolls were baked conventionally (20 min, 230°C). The FBF rolls were at first directly baked (20 min, 230°C), then frozen in a blast freezer (Frigor TLM 300, Biogenet, Denmark) for about 30 min at -30°C and subsequently stored in a freezer at -18°C in airtight containers, for 48 h. Just before the GI evaluation, the FBF rolls were defrosted at room temperature for about 1 h and intended for consumption. The PBF rolls were partly baked (3 min at 190°C, 14 min at 165°C), frozen in a blast freezer for about 30 min at -30°C and then stored in a freezer at -18°C, in airtight containers, for 48 h. At the end of the storage, the rolls were defrosted for about 10 min, put in the oven and fully baked (12 min, 230°C). The UFD rolls were prepared from the frozen dough, kept firstly in a blast freezer for 30 min, at -30°C and then stored in a freezer at -18°C, in airtight containers, for 48 h. Just before the GI evaluation, the dough was defrosted at room temperature for about 60 min, put in the proofing box and then fully baked (20 min, 230°C).

The weight of the rolls and the content of portion given to the participants is given in Table 1.

TABLE 1. Rolls weight and the amount of portion that contains 50 g of available carbohydrates.

	FBNF	FBF	PBF	UFD
Rolls weight (g)	56.63	57.98	59.20	57.54
Amount (g) of rolls that contains 50 g of available carbohydrate	107.71	108.49	101.39	104.06

All bakings were conducted at the Department of Carbohydrate Technology of the Agricultural University of Cracow.

Chemical analysis of fresh and frozen storage wheat rolls

Chemical analysis of wheat rolls was performed using AOAC [1995] standard methods. The content of available carbohydrates (total carbohydrates minus dietary fiber) was evaluated according to FAO/WHO [1998], (Table 2).

Subjects

Twelve, healthy volunteers, two men and ten women, at the age of 22.47±0.92 years, with average body mass index of 22.06±2.31 kg/m^2, took part in the GI study (Table 3). They were recruited among the students of Agricultural University of Cracow. The Regional Chamber of Bioethics Committee approved the experimental procedure and the participants signed their agreements to participate in the tests. Each volunteer was medically examined before the tests.

Evaluation of glycemic index (GI)

Subjects were asked to come six times in the morning during three weeks. In order to reduce the intra- and inter-individual variability, the volunteers were instructed to fast 10-12 h before the test, as well as, to avoid strong physical activity, alcohol and to restrict time spent ingesting the test food. Each participant tested four kinds of wheat rolls and twice the reference food. The rolls were given to the subjects in random order with, at least, two-days gap between the GI evaluation, to minimize carry-over effects. The rolls were served each time with 250 mL of low-mineralized water. Subjects were asked to eat the test wheat rolls within 10-15 min and to drink reference food in 5-10 min [Brouns *et al*., 2005]. Pure glucose was used as a reference food. The amount of 50 g of glucose was dissolved in 250 mL of low-mineralized water, just before GI evaluation, and served to the volunteers.

TABLE 2. Nutritional composition of fresh and frozen stored wheat rolls. Values are means of duplicate analysis on the sample and are expressed as g per 100 g of fresh sample.

	FBNF	FBF	PBF	UFD
Moisture	41.90	43.00	39.44	40.29
Proteins	7.04	6.83	6.93	6.77
Lipids	0.64	0.17	0.34	0.78
Carbohydrates				
total	48.76	48.37	51.59	50.43
available	46.42	46.11	49.30	48.06
Dietary fiber				
total	2.34	2.27	2.28	2.38
insoluble	1.62	1.26	1.11	1.33
soluble	0.72	1.01	1.17	1.05
Ash	1.66	1.64	1.71	1.74

TABLE 3. Characteristics of participants.

Characteristics	Mean ± S.D.
Age (years)	22.47±0.92
Height (m)	1.71±0.05
Weight (kg)	64.93±9.04
BMI (kg/m^2)	22.06±2.31

Blood glucose concentrations were measured at 0 min as well as at 15, 30, 45, 60, 90 and 120 min after the start of the meal. Finger-prick blood samples were taken for capillary blood glucose analysis. Glucose concentration was measured by a glucose hexokinase enzymatic assay (Olympus Glucose OSR6121). The results were given in mmol/L.

Calculation of glycemic index (GI)

The incremental area under the glycemic curve (IAUC) was measured using the trapezoidal method [FAO/WHO, 1998]. The glycemic index (GI) was calculated as the IAUC of the blood glucose response curve of 50 g carbohydrate amount of a test roll expressed as a percentage of the response to the same amount of carbohydrate from the reference food (glucose).

Statistical analysis

The GI was expressed as a mean ± SEM. Comparisons of the mean values were performed by Fisher test using STATISTICA (StatSoft Company, version 7.1.). Statistical significance was set at $p<0.05$. Levels of intra-individual variation of the two reference (glucose) tests were assessed by determining the coefficient of variation (CV%=100 × standard deviation / mean).

RESULTS AND DISCUSSION

The mean intra-individual variation in glycemic response to the two reference tests in the volunteers was 22% CV. This value is consistent with data in normal subjects [Brouns *et al*., 2005].

Figure 1 shows the incremental blood glucose response curves for the fresh and frozen stored wheat rolls. There was no effect of frozen storage on the peak rise in blood glucose response, except one case. Namely, blood glucose for FBF rolls was significantly lower than that for fully baked-non frozen rolls (FBNF) ($p<0.05$).

The GI values of fully baked-non frozen (FBNF) and frozen storage (PBF, FBF, UFD) wheat rolls are shown in Figure 2. Generally, the frozen storage resulted in lower GI values, compared to fresh (non-frozen) wheat rolls. In one case the difference reached statistical significance ($p<0.05$). Namely, compared to FBNF rolls (GI=83.03±5), the PBF rolls gave significantly ($p<0.05$) lower values of GI (GI=60.66±6). On the other hand, no significant differences were noted in GI values between FBNF, FBF (GI=73.00±8) and UFD rolls (GI=73.28±6).

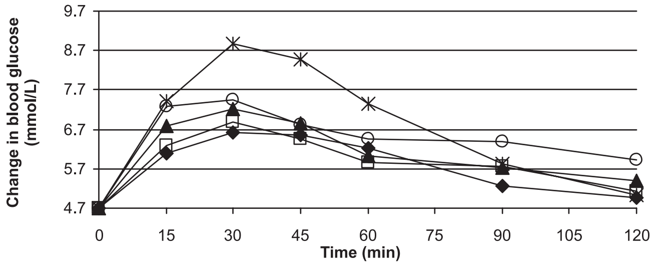

FIGURE 1. Blood glucose response curve for fresh- and frozen stored wheat rolls: Glucose (×); FBNF (○); FBF (♦); PBF (□); UFD (▲).

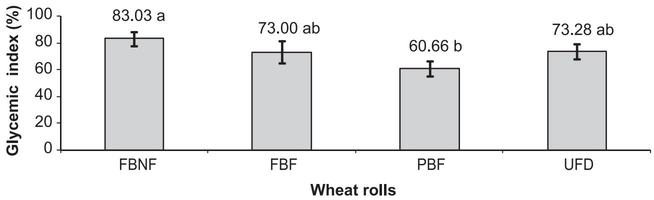

FIGURE 2. Glycemic index (%) of fresh (FBNF) and frozen storage wheat rolls (FBF, PBF, UFD). Data are shown as a mean ± SEM. Different letters show significantly different values at $p<0.05$.

The present study evaluated the effect of frozen storage of wheat rolls on their impact on glycemic responses (GI), when consumed by human volunteers.

The influence of food processing and cooking on glycemic response is well documented [Raben *et al*., 1994; Haralampu, 2000; Carreira *et al*., 2004; Burton & Lightlower, 2007]. Processing of starchy foods results in disruption of starch granules, further affecting susceptibility of the starch to enzymatic digestion, resulting in greater availability of glucose for absorption and increased glycemic response. Generally, wheat products, such as wheat bread and wheat rolls, give a high glycemic index. This has been shown in many studies [Holm & Björck, 1992; El Nehir, 1998; Powell *et al*., 2002; Chlup *et al*., 2004 Marques *et al*., 2007].

Frozen storage of starchy foods causes retrogradation of starch, which, from a technological point of view, is detrimental to the quality of bakery products, as resulting in staling. On the other hand, retrograded starch exerts a beneficial effect on the nutritional value of bread, delaying glucose absorption in the small intestine. As shown in our studies, the glycemic index of PBF wheat rolls has been decreased by frozen storage as compared with non-frozen wheat rolls FBNF ($p<0.05$). The possible explanation for our results may be the presence of resistant starch formed in wheat rolls during the heating-cooling cycles. Similarly, a recent study in humans [Burton & Lightlower, 2007], has demonstrated a decrease in glycemic response to frozen and defrosted homemade and commercial white bread. Interestingly, larger reduction in the glycemic response was demonstrated in homemade bread, which was made without addition of any baking improver, as compared to commercial bread. Consequently, the presence of improvers was indicated to be the main reason for smaller reduction in the glycemic response in commercial white bread. In our study, all kinds of rolls were prepared using baking improver. Nonetheless, the decline in the glycemic index was considerable, especially in PBF rolls (GI=60.66±6), compared to fresh-non frozen (FBNF, GI=83.03±5). An important factor required to obtain maximum retrogradation rate is water content in the range of 35-50% [León *et al*., 1997]. In our study, all frozen wheat rolls had the water content between 39.44 and 43.00% (Table 2).

In our study, the tested rolls were kept under frozen storage conditions for 48 h. Niba [2003] reported that the prolonged frozen storage of corn bread (over 4 days) results in decreased resistant starch content. Alternatively, Selomulyo & Zhou [2007] concluded that the longer dough remains in frozen conditions, the more pronounced is the degree of starch retrogradation. In the above study, bread made from frozen dough also exhibited faster starch retrogradation at low temperatures (4°C) when compared with bread made from non--frozen dough, causing an increase in bread firmness. This may explain the reduction in the glycemic index of frozen storage wheat rolls (UFD rolls), as observed in our study.

CONCLUSIONS

The results of this study suggest that the proposed processing of baking goods, *i.e.* frozen storage, may decrease the glycemic response to wheat rolls, usually considered as a product with high GI. Thus, lowering the GI of wheat rolls, resulting from frozen storage, increases their nutritional value. To our best knowledge, it is the first study to show reductions in glycemic index resulting from frozen storage conditions of wheat rolls before their consumption. Indeed, all three frozen stored wheat rolls, FBF, PBF and UFD, led to a reduced glycemic index. However, more detailed investigation is needed, including evaluation of the real effect of freezing treatment, applied during different stages of the baking process, on the content of resistant starch in wheat rolls, as well as, the relationship between the content of resistant starch in these rolls and the value of their GI.

ACKNOWLEDGEMENTS

This study has been carried out with financial support from the Commission of the European Communities, FP 6, Thematic Area "Food quality and safety", FOOD-2006-36302 EU-FRESH BAKE. It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area. This study was also supported by the Polish Ministry of Science and Higher Education, grant No. 162/6 PR UE/2007/7.

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Received December 2007. Revision received and accepted April 2008.