Original Article

Rising methods and leavening agents used in the production of bread do not impact the glycaemic index

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The aim of this study was to compare the glycaemic index of breads produced using different rising methods and leavening agents. Eleven bread varieties were selected based on method of production, and divided between three groups of ten participants (mean ± SD age 30.0 ± 10.7 years and BMI 22.9 ± 2.8). Standard glycaemic index testing protocol was implemented after an overnight fast, using glucose as the reference food, and collecting blood samples over a two-hour period. Glycemic index was calculated using the usual method. Additionally, incremental area under the curve data were log transformed and glycaemic index was calculated using regression analysis. Mean glycaemic index values of the breads in ascending order were as follows: Swiss Rye™; 60, Long oat; 68, Sourdough+oats; 71, Long rye; 76, Short oat; 77, Short whole meal; 78, Long whole meal; 80, Sourdough; 82, Short rye; 82, Yeast; 88, and Desem; 92. There were significant differences in mean glycaemic index values between Swiss Rye™ and Yeast ($p = 0.010$), Swiss Rye™ and Desem ($p = 0.007$) and Sourdough+oats and Desem ($p = 0.043$). The rising method and leavening agents used in this study did not impact on the glycaemic index of the breads tested. Other factors, such as increased bread density, and the addition of whole grains may be required to produce bread with a low glycaemic index.

Key Words: bread, glycaemic index, yeast, flour, fermentation

INTRODUCTION

Epidemiological studies demonstrate that health benefits of low-glycaemic index (GI) foods are not confined solely to people with type 2 diabetes. Diets characterized by a low GI are also associated with lower risk of developing cardiovascular disease and cancer.[1] Thus, the development of low-GI foods for consumption by the general population is recommended.[2] Recent reports indicate that some foods classified as low GI are energy dense and contain large amounts of sugar and fat, which lower glycaemic response, but do not contribute to better health outcomes associated with other high fiber or whole grain, low GI foods.[3] Glycaemic index does, however, remain a useful guide to food choice if a carbohydrate (CHO) rich, low GI food is used to replace a similar high GI food that is consumed regularly. This has the effect of lowering the overall glycaemic load of the diet, which may contribute to positive health outcomes.[3]

Bread is one such potentially nutritious, rich source of low GI CHO. As a staple of a westernised diet, lowering the GI of bread has the potential to positively affect the health of the population. Glycaemic index values have been determined for nearly 200 different breads.[4] Research suggests that increasing density,[5] adding parboiled or whole grains[6] or increasing the dietary fiber content can decrease the GI of bread.[7] Little is known about whether the rising methods used in the production of bread affect the GI. However, the organic acids used in the production of sourdough bread are thought to slow gastric emptying and reduce the rate of starch digestion resulting in a lower GI.[8] Therefore, the aim of this study was to compare the GI of breads produced specifically for this study using different rising methods and leavening agents.

MATERIALS AND METHODS

Study design and participants

To reduce participant burden associated with attending multiple testing sessions, the eleven test breads were divided between three groups of ten volunteers who met the study criteria: non-smoker, non-diabetic, 20-60 years of age, and body mass index (BMI) <28. In total, 10 men and 17 women participated in the study with three individuals participating in two of the study groups. All participants had normal fasting blood glucose concentrations according to the Diabetes New Zealand cut-off (6.1 mmol/L).

Height and weight measures were taken at a preliminary appointment prior to the first GI testing session using standardized methods, with all participants wearing light clothing and no shoes. BMI was calculated using the standard formula; weight (kg) divided by height (m) squared.

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Table 1. Participant characteristics at recruitment

Characteristic	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)
Age (years)	33.7 ± 14.3	30.4 ± 9.1	27.4 ± 6.6
Males (%)	50%	30%	30%
Body weight (kg)	72.9 ± 13.8	64.6 ± 9.9	64.2 ± 8.4
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
BMI (kg/m^2)	23.9 ± 2.6	22.8 ± 3.0	22.7 ± 2.6
Fasting glucose (mmol/L)	5.0 ± 0.5	4.7 ± 0.2	4.4 ± 0.2

Characteristics of study participants at recruitment are presented in Table 1. Ethical approval was obtained from the University of Otago ethics committee. All participants gave written informed consent before participating in the study.

Test breads

All breads except for the Swiss Rye bread were prepared specifically for this study by a local bakery. Groups one and two each tested three breads. Group one tested short rye, short oat and long whole meal (whole wheat). Group two tested long rye, long oat and short whole meal. These breads were made using three different types of flour (Rye, Oat and Whole meal) and two different rising methods: the no time method (short, 30 min prove) and the bulk fermentation method (long, 50 min rise and prove). The no time method requires the addition of a dough improver, which contains conditioners and oxidizing agents such as ascorbic acid, emulsifiers and enzymes.[9] This method is close to that used in the production of commercially available bread. In the bulk fermentation method, the dough is left to ferment once all ingredients are combined, and is then divided or scaled (see Table 2).

The breads tested by group three (Yeast, Desem, Sourdough, Sourdough + oats, and Swiss Rye™) were selected based on differing leavening agents used in the fermentation process. Four out of five of these breads were made with whole meal flour, but were leavened using yeast, yeast and desem, or sourdough. Yeast is the traditional rising agent used in the production of bread. The yeast organisms produce carbon dioxide, which is released when the dough is left to rise in a warm environment. The gas production helps the dough to rise, and also assists in the development of the gluten network. Desem (Dutch for leaven), is a fermented dough, which is added to standard bread ingredients to help the dough rise. The desem is usually made from flour, water, salt, yeast and sometimes honey that ferment to produce carbon dioxide. Sourdough is made from a starter similar to desem, but with a more porridge like consistency made from flour and water only.

Table 2. Ingredients and baking conditions of test breads

Bread	Flours used	Rising Agent	Rising time (mins)	Proving time (mins)	Baking temperature (°C)	Baking time (mins)
Short Whole meal	50% Whole meal wheat 50% White wheat	Bakers yeast (1.3% net flour weight)	-	~30	220	~30
Short Rye	50% Whole meal wheat 30% Rye 20% White wheat	Bakers yeast (1.3% net flour weight)	-	~30	220	~30
Short Oat	50 % Whole meal wheat 20% Rye 10% Oatmeal 20% White wheat	Bakers yeast (1.3% net flour weight)	-	~30	220	~30
Long Whole meal	50% Whole meal wheat 50% White wheat	Bakers yeast (13% net flour weight)	~20	~30	220	~30
Long Rye	50% Whole meal wheat 30% Rye 20% White wheat	Bakers yeast (13% net flour weight)	~20	~30	220	~30
Long Oat	50 % Whole meal wheat 20% Rye 10% Oatmeal 20% White wheat	Bakers yeast (13% net flour weight)	~20	~30	220	~30
Yeast	50% Whole meal wheat 50% White wheat	Bakers yeast (15% dry weight)	60	40	200	40-50
Desem	50% Whole meal wheat 50% White wheat	Bakers yeast (15% dry weight) Desem (15% wet weight)	60	40	200	40-50
Sourdough	50% Whole meal wheat 50% White wheat	Sourdough starter (70% wet weight)	80	60	200	60
Sourdough + oats	50% Whole meal wheat 50% White wheat 10% Rolled oats	Sourdough starter (70% wet weight)	80	60	200	60
Swiss Rye™†	30% Whole meal wheat 20% Kibbled Rye 30% Wheat Flour	Bakers Yeast‡	unknown	unknown	unknown	unknown

†Commercially produced
‡Percentage unknown

Table 3. Nutrient compositions of study breads

Bread	% Moisture	% Protein	Energy kJ/g	% Fat	% Dietary fibre	% AVL CHO	Portion size (g)[†]
Group One							
Short Rye	38.7	8.7	11.2	2.5	4.8	41.9	119
Short Oat	33.4	9.7	12.3	3.1	5.2	45.2	111
Long Whole meal	41.6	8.1	10.8	1.2	4.7	43.3	115
Group Two							
Long Rye	43.2	8.7	10.6	1.4	6.9	38.3	131
Long Oat	38.6	8.2	11.5	2.5	6.6	43.0	116
Short Whole meal	33.6	9.9	12.1	2.6	4.6	45.3	111
Group Three							
Yeast	40.6	7.0	11.0	1.5	7.5	41.9	119
Desem	40.9	6.9	10.7	1.2	7.3	42.2	119
Sourdough	39.2	7.1	11.2	1.3	7.8	43.3	116
Sourdough + oats	38.3	7.4	11.4	1.7	4.7	46.7	107
Swiss Rye™	47.5	10.5	10.1	1.9	5.5	32.5	154

[†] To provide 50 gm available CHO

Table 4. Glycaemic Index values and their 95% confidence intervals

Bread	Untransformed		Log transformed	
	Mean GI	95% CI	Mean GI	95% CI
Short Rye (n=10)	82	57-107	78	63-96
Short Oat (n=10)	77	63-92	76	62-94
Short Whole meal (n=10)	78	66-90	77	66-91
Long Rye (n=9)	76	63-30	76	64-90
Long Oat (n=8)	68	52-84	66	55-78
Long Whole meal (n=10)	80	48-112	75	61-94
Yeast (n=10)	88	70-107	86*	68-109
Desem (n=10)	92	69-116	88**	69-111
Sourdough (n=10)	82	53-111	73	58-93
Sourdough + oats (n=10)	71	48-94	65	52-82
Swiss Rye™ (n=10)	60	54-66	59	47-75

* Significantly different from *Swiss Rye™* (p=0.01)
**Significantly different from *Swiss Rye™* (p=0.007) and *Sourdough + oats* (p=0.043)

This slurry is left to capture wild yeasts from the environment. These yeasts breed in the slurry, which can then be used as a rising agent. The development of the sourdough starter can take up to two weeks, and extra ingredients must be added regularly to maintain the viability of the culture. Generally desem and sourdough are less potent rising agents than commercial baker's yeast. One commercially produced bread (Swiss Rye™) claiming to be "naturally fermented" was also tested by group three. The flour used in the production of all of the breads (except Swiss Rye™) was sourced from the same organic flourmill.

The available CHO contents of the breads were determined analytically by difference after moisture, ash, protein, energy, fat and total fibre content were determined.[10] The compositional breakdown and portion size required to provide 50 g available CHO for each bread is presented in Table 3. Further information about ingredients and baking conditions of the breads can be found in Table 2.

Experimental procedures

Standard GI testing protocol was used according to the recommendations of FAO/WHO.[2] The reference food was a 50 g glucose solution (Glucaid, Histolabs, Riverstone, Australia). Participants were asked to avoid alcohol and vigorous exercise the night before each trial and arrived at each morning testing session after a nine-hour overnight fast. In a randomised order, participants consumed three glucose reference tests, and their assigned test breads, on non-consecutive days. On arrival at the laboratory participants were given a hot wheat bag to warm their hands and ensure blood flow to the fingertips. Two fasting finger prick blood samples were taken using disposable lancets (Brand Safety Flow Lancet, Becton Dickinson), before the participant was provided with a glucose reference solution (50 g glucose in 300 mL of carbonated water) or one of their assigned test breads (in a portion that provided 50 g of available CHO) and 300 mL of water. Breads were consumed without the addition of spreads or fillings. Participants were instructed to consume all of the reference solution or bread within 10 minutes. Finger prick blood samples were repeated at 15, 30, 45, 60, 90 and 120 minutes after the participant first started to consume the bread or reference solution. For each finger prick blood sample, slightly less than 1.0 ml of blood was collected in 1.5 ml pre-heparinised eppendorf tubes. During the two-hour testing period all participants remained seated.

Blood glucose analysis

Capillary finger-prick blood samples were taken for plasma glucose analysis as this has been recommended to increase the reliability of GI testing.[11] Once the sample was collected each tube was inverted several times to ensure the heparin and blood were thoroughly mixed. Blood samples were then stored on ice for two to three hours until they were transported to the laboratory. Samples were then centrifuged at room temperature for 10 minutes at 3000 g. Plasma was removed and stored in cobas fara tubes at -80°C until analysis.

Blood glucose concentration was determined via the Glucose Hexokinase Assay on the Cobas II Fara Auto-analyser (Roche Diagnostica, Basle, Switzerland). Samples were run in batches that ensured all the samples from each bread were run together. The inter-assay co-efficient of variation was 1.5% for analysis of samples from groups one and two, and 3% for analysis of samples from group three.

Statistical analysis

The incremental area under the curve (IAUC) for both glucose and insulin response was calculated geometrically, excluding areas below baseline.[12] Data for three individuals (two for *Long Oat* and one for *Long Rye*) who did not consume all the test foods were excluded. Two other participants only completed two reference tests and their individual GI results were calculated using the mean of two rather than three reference tests.

Statistical analyses were performed using STATA version 8.0 (STATA Corporation, College Station, TX, USA). Glycaemic index values were calculated using the standard formula (see below):

$$\frac{\text{Glucose}_{\text{IAUC}}\text{b}\textit{read}}{\text{Glucose}_{\text{IAUC}}\text{reference}} \times 100 = \text{GI}$$

In addition, IAUC values were log transformed to adjust for skew, and to overcome the strong relationship between mean and standard deviation.[13] A regression analysis with a random effect for participant was used to compare each bread type with the mean glucose reference using the log transformed data. This model was used to analyze all observations in each experiment simultaneously, and provide estimates of the ratio of each bread to the reference food. When exponentiated, the results from the model provide an estimate of the GI of each bread and its 95% confidence interval. Level of significance was set at $p \leq 0.05$.

RESULTS

Participant characteristics at time of recruitment are presented in Table 1. The mean (± SD) age of participants was 30.0 ± 10.7 years (range 20-59). Mean BMI (± SD) was 22.9 ± 2.8 (range 17.4 - 28.2). Slightly more females than males participated in the study.

The GI values and their 95% confidence intervals are presented in Table 4. Logged GI values are also presented in Table 4 for comparison. When ranked by mean GI, all breads were classified as high GI (GI ≥ 70) except for *Long Oat* (GI = 68), and *Swiss Rye*™ (GI = 60), which were classified as moderate GI. Regression analysis revealed significant differences between the GI of *Swiss Rye*™ and *Yeast* ($p = 0.010$), *Swiss Rye*™ and *Desem* ($p = 0.007$) and *Sourdough+oats* and *Desem* ($p = 0.043$). Mean plasma glucose peaked at 30 minutes following ingestion of eight of the breads, and at 45 minutes for the remaining three breads (*Long rye, Short rye and Long whole meal*) (Figure 1). However, mean plasma glucose concentration had not returned to baseline on completion of testing at 120 minutes for any of the breads tested. The mean plasma glucose response to the glucose reference solution (based on three glucose tests) peaked at 30 minutes and returned to baseline values between 90 and 120 minutes.

DISCUSSION

This is the first study to determine and compare the GI of breads with the aim of determining whether rising methods and leavening agents influence GI values. Contrary to expectations, eight of the eleven breads tested in this study were found to have high GI values. None of the long fermentation breads had GI values that were significantly different from the GI values of the short fermentation breads. This result would suggest that rising methods do not influence GI, or that changing the rising method to increase the total fermentation or rising time by approximately 20 min is not enough to impact on the GI of bread.

In addition, no obvious trend arose from the data to suggest a relationship between leavening agent and GI. The finding that the plain sourdough bread (sourdough *without* oats) tested in this study produced a high glycaemic response was unexpected. The inorganic acids used in the production of sourdough bread have been proposed elsewhere to reduce the GI because they limit gastric emptying rate.[8] In addition, it has recently been reported that consumption of sourdough fermented bread results in a significantly lower glycaemic response than that found with the consumption of bread leavened with yeast.[14] The GI values of the sourdough breads measured in the current study are in contrast to the GI values for sourdough reported by Atkinson et al[7] of 54 for sourdough wheat bread (using glucose as the reference) and 48-66 for three other sourdough rye, barley and whole meal breads. Unfortunately, information regarding the production of these breads are not available for comparison to the current study.

The addition of whole grains to bread in the form of rye kernels or parboiled cracked wheat has been shown to produce a lower GI, when compared to similar breads made from whole meal flour.[6] *Swiss Rye*™, a commercially produced bread variant, was the only bread tested in the current study made with whole grains. It consisted of whole meal flour (30%), kibbled rye (20%) and white wheat flour (50%). The GI value obtained for *Swiss Rye*™ (GI = 60) places it above the range of values obtained for breads containing kibbled kernels (GI range 25 - 34, using a conversion factor of 0.7 to account for the use of white bread as the reference).[4] This may be attributable to the lower proportion of grains (20%) in *Swiss Rye*™ compared to other whole grain breads (50 - 80% grain).[4]

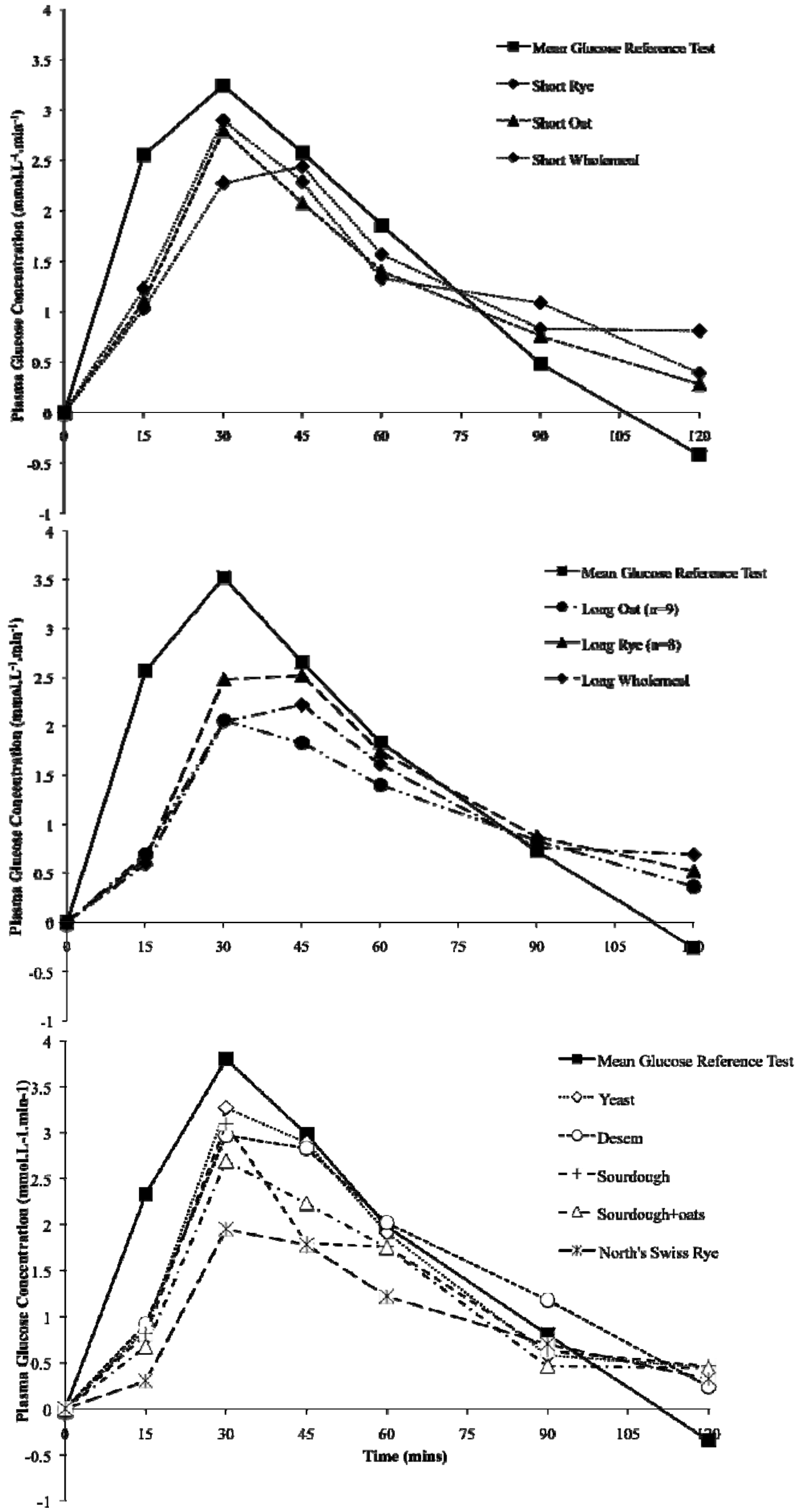

Figure 1. Mean plasma glucose responses to 11 selected breads tested in 3 groups of 10 participants

Despite the high percentage (80%) of whole grains used by Jenkins *et al*,[6] the GI of the breads with added rye kernels (GI = 55, using glucose as the reference) and par-boiled cracked wheat (GI = 46) are lower than the GI obtained for *Swiss Rye*™ in the current study. In a recent study, Ostman *et al*[10] developed two low GI breads (GI = 32 & 38, using glucose as the reference) by adding plant fibres, seeds and whole grains to a commercial blend containing white wheat flour. Unfortunately, the percentage of grains and other ingredients are not available for comparison to the breads used in the current study. When these low GI breads were consumed in exchange for their usual bread; women with impaired glucose tolerance experienced improved insulin economy.[7]

The glycaemic index values for bread range from 31-100, covering the full spectrum of GI categories from low to moderate to high.[4] The results from the current study add to the diversity of bread products tested worldwide. However, a recent review suggests that a much larger number of participants, combined with duplicate measures of test foods may be required in the measurement of GI to reduce intra-individual variation, and produce narrower confidence intervals.[3] These suggestions are not necessarily practical in terms of the cost involved in running a study, and the burden it would place on participants. Ideally, the breads tested in this study would have been tested in every participant in duplicate, however, this would have involved each participant attending at least 48 hours of testing, which was considered too burdensome. Comparison between other studies is also made difficult because of the use of different reference foods in the calculation of GI, as well as differences in the way GI values are calculated. The skewed nature of the GI data, combined with the strong relationship between mean and standard deviation indicates that the data should be log transformed.[13] Regression is, therefore, a sensible option for the calculation of GI. Clearly, there is a need for a re-examination of standardized GI methodology, to produce the most reliable and valid GI data possible.

The lower GI observed for the Swiss Rye™ bread in the current study may suggest that some component or combination of components in this bread play an important role in decreasing the GI value. However, consideration should also be given to the greater volume of Swiss Rye™ that was needed to provide 50 g of available CHO, which may have impacted on gastric emptying time, and thus glycaemic response.

The results of the current study, along with those from other studies,[4,5] suggest that the bread density, and the percentage of whole grains in bread may have more influence on GI than the rising methods, or leavening agents used in the current study.

Further investigation is needed into whether a higher density of bread, combined with more whole grains will produce low GI bread. That, when combined with carefully selected spreads and sandwich fillings may be effective in providing health benefits associated with lower glycaemic responses, to a wider population.

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AUTHOR DISCLOSURES

Tracy Perry manages Glycaemic Otago at the University of Otago, a commercial consultancy for determining the glycemic index of foods. None of the authors report any conflict of interest with respect to the current study.

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Original Article

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做麵包的發酵方法及發酵劑對升糖指數並無影響

本篇研究的目的是比較使用不同發酵方法及不同發酵劑所做出的麵包其升糖指數。依照不同生產方法選出 11 種麵包，並分配給 3 個試驗組，每組各 10 人；平均年齡為 30.0 ± 10.7 歲，平均 BMI 為 22.9 ± 2.8。執行標準升糖指數的試驗，即隔夜禁食，以葡萄糖為參考食物，蒐集給食後兩個小時內的血液樣本。升糖指數的計算使用的是一般慣用的方法。此外，增加的曲線下面積以對數轉換，而升糖指數使用回歸分析來計算。各式麵包的平均升糖指數，以遞增方式的排序如下：瑞士黑麥™ 60、長燕麥 68、酸麵糰加燕麥 71、長黑麥 76、短燕麥 77、短全麥 78、長全麥 80、酸麵糰 82、短黑麥 82、酵母 88、全麥發酵 92。平均升糖指數有顯著差異的麵包如下：瑞士黑麥與酵母(p = 0.010)、瑞士黑麥與全麥發酵(p = 0.007)及酸麵糰加燕麥與全麥發酵(p = 0.043)。本篇研究所使用的發酵方法和發酵劑對於測試用麵包的升糖指數並沒有影響。 其他的因素，如增加麵包的密度、添加全穀類，可能對於要生產低升糖指數的麵包來說是必需的。

關鍵字：麵包、升糖指數、酵母、麵粉、發酵