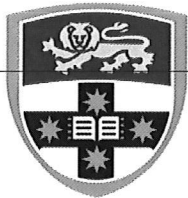


Glycemic Index Research Report #2018

For Holista Foods, Inc.

July 2020



Sydney University's

Glycemic Index Research Service (SUGiRS)

School of Life and Environmental Sciences

Charles Perkins Centre, D17

University of Sydney, NSW, 2006

AUSTRALIA

A study to measure the Glycemic Index value of one white bread

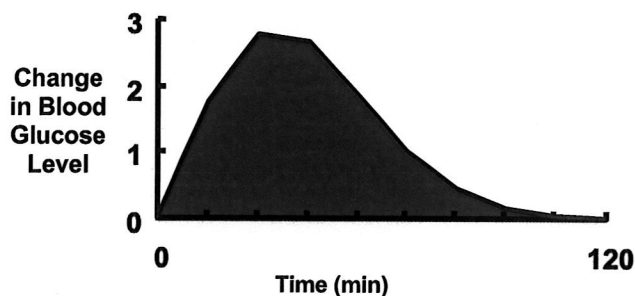
Background Information: The Glycemic Index

Nutrition research conducted in the 1970's showed that different carbohydrates did not have the same effects on blood glucose (sugar) levels after eating. These findings challenged the general assumption that all 'complex' carbohydrates (starches) produce lower blood glucose responses than 'simple' sugars, and questioned the clinical significance of carbohydrate exchange lists that have regulated the diets of people with diabetes for over three decades. These exchange lists are based on the assumption that portions of different foods containing equal amounts of carbohydrate will produce the same blood glucose response.

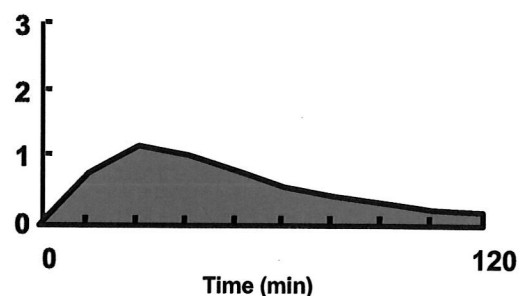
Consequently, the **glycemic index (GI)** was developed in order to rank equal carbohydrate portions of different foods according to the extent to which they increase blood glucose levels after being eaten (1). Foods with a high GI value contain rapidly digested carbohydrate, which produces a rapid and large rise and fall in the level of blood glucose. In contrast, foods with a low GI value contain slowly digested carbohydrate, which produces a gradual, relatively low rise in the level of blood glucose (Figure 1).

Figure 1. The 2-hour blood glucose response curves for a high-GI food (white bread: GI value = 70) and a low-GI food (lentils: GI value = 30).

a. High GI - White bread



b. Low GI - Lentils



Over two decades of research has confirmed that a food's glycemic effect cannot be accurately predicted from the type and amount of carbohydrate it contains. This is because the rate at which carbohydrate is digested and released into the bloodstream is influenced by many factors, such as the food's physical form, its fat, protein and fibre content, and the chemical structure of its carbohydrate (2). For these reasons, apparently similar foods within the same food group and different flavours of the same food can have quite different effects on blood glucose levels.

GI research has important implications for the food industry and people's health. Scientists now agree that the terms 'complex carbohydrate' and 'sugars', which commonly appear on food labels, have little nutritional or physiological significance. The World Health Organisation released a consensus report stating that these terms should be removed from food labels and replaced with the food's total digestible carbohydrate content and its GI value, in order to help people select foods that will reduce the overall glycemic impact of their diet (3). Currently, many dietitians refer to the glycemic index when planning more flexible diets for people with diabetes. In addition, GI values are being used in scientific research studies to examine the relationship between the overall glycemic effect of people's habitual diets and their risk of developing certain diseases over time. Results from large-scale epidemiological studies have shown that the long-term consumption of a diet with a high glycemic impact, which induces high and recurrent surges in blood glucose and insulin levels, increases the risk of developing diabetes, heart disease and certain cancers (3, 4). In contrast, results from both epidemiological and experimental studies show that low-GI diets can reduce the risk of these diseases, improve blood glucose control and insulin sensitivity in people with diabetes, reduce high blood fat levels, and can be useful for weight control (3, 5-7). Recently, high-GI diets have been shown to enhance body fat storage to a greater extent than equal-calorie low-GI diets in healthy people, which is likely to reflect the greater insulin secretion and lower satiety associated with high-GI foods (8).

Type 2 diabetes and coronary heart disease continue to be the major causes of illness and death in industrialised countries. Therefore, food manufacturers should be encouraged to develop more low-GI foods to assist with the prevention and treatment of these diseases.

Aim of the study

The aim of this study was to measure the glycemic index (GI) value of the white dinner rolls, using glucose sugar as the reference food (GI of glucose sugar fixed at 100).

Methods

This study was conducted using internationally recognised GI methodology (3, 9, 10), which has been validated by results obtained from small experimental studies and large multi-centre research trials (11). The experimental procedures used in this study were in accordance with international standards for conducting ethical research with humans and were approved by the Human Research Ethics Committee of the University of Sydney.

Participants

A power-based (90%) sample size calculation using data from many published GI studies indicated that a group of at least 10 people would be needed for this study in order to find a significant difference among the GI values of the reference and test foods, if a significant difference truly exists (a difference of 1.0 standard deviation units in GI). A group of 10 healthy, non-smoking people, aged between 18-65 years, were recruited from the staff and student population of the University of Sydney.

People volunteering to participate in the study were excluded if they: were over- or underweight; were dieting; had impaired glucose tolerance; were suffering from any illness or food allergy; or were regularly taking prescription medication other than standard contraceptive medication. The group that participated in the study consisted of six males and four females. Their average age was 28.4 years (range: 24.8 – 35.6 years) and the group’s average body mass index (BMI) was 22.5 kg/m² (range: 20.1 – 24.9 kg/m²). The BMI score is a measure of a person’s weight in relation to their height, values between 18 – 25 kg/m² are within the healthy weight range.

Test foods

The reference food and the test bread roll were served to the participants in fixed test portions containing 50-grams of digestible (available) carbohydrate. Pure glucose sugar (Glucodin[®] powder, Valeant Pharmaceuticals, NSW) dissolved in water was used as the reference food and was consumed by each participant on three separate occasions. The participants consumed the test product on one occasion. The nutritional contents of the equal-carbohydrate portions of the reference food and the white bread rolls are listed in Table 1 below, and were calculated using data supplied by the manufacturers.

Table 1. The weights and carbohydrate contents of the test portions of the reference food and the test product, calculated using manufacturers’ data.

Test food	Portion Size (g)	Energy (kJ)	Protein (g)	Fat (g)	Available Carbohydrate (g)	Sugar (g)	Fibre (g)
Reference food (glucose sugar)	51.4 g glucose 250 g water	850	0.0	0.0	50.0	50.0	0.0
White dinner roll	113.6 g	1188	10.2	3.4	50.0	2.3	4.5

Each reference food portion was prepared the day before required by dissolving 51.4 grams glucose in 250 grams warm water in a glass, which was covered with airtight wrap, labelled and stored overnight in a refrigerator. The next morning, a reference food portion was taken from the refrigerator shortly before being served with 250 grams plain water. The reference food was served to the participants at a cool temperature to improve its palatability. The test product was delivered in standard bread rolls to the research centre. Individual test portions were then prepared, wrapped in airtight plastic wrap, labelled and stored in the freezer. The day before a test session, a prepared bread roll test portion was left to defrost in the refrigerator overnight. The next morning, a bread roll portion was unwrapped immediately before it was served with 250 grams plain water.

Experimental procedures

Using standard GI methodology, a portion containing 25 or 50 grams of available carbohydrate is fed to at least 10 healthy people the morning after they have fasted overnight. A fasting blood sample is obtained and then the test food is consumed, after which additional blood samples are collected at regular intervals during the next 2 hours. In this way, it's possible to measure the total increase in blood sugar (glucose) produced by that product over a 2-hour period.

The same procedure is repeated in the same group of people on another day after they have consumed an equal-available carbohydrate portion of the reference food (pure glucose sugar dissolved in water). A GI value for the test food can then be calculated by expressing the 2-hour blood glucose response to the test food as a percentage of the response produced by the reference food (GI value of glucose = 100). Therefore, GI values for foods are relative measures. They indicate how high blood sugar levels rise after eating a particular food compared to the high response produced by the same amount of carbohydrate from glucose sugar. Equal-available carbohydrate portions are used in GI studies, as carbohydrate is the main nutrient that directly causes glucose levels to rise.

In this study, 10 healthy people consumed the reference food on three separate occasions and the test product on one occasion only. Therefore, each participant completed four test sessions. The reference food was consumed on the first, third and fourth test sessions, and the test portion of white dinner rolls was consumed on the second test session. Each test session was completed on a separate morning with at least a day in between consecutive sessions.

The night before each test session, the participants ate a regular evening meal based on a carbohydrate-rich food, other than legumes, and then fasted for at least 10 hours overnight. The participants were also required to avoid alcohol and unusual levels of food intake and physical activity for the whole day before each session. The next morning, the participants reported to the research centre in a fasting condition. On arrival, the investigators checked that the participants had complied with the preceding experimental conditions. The participants then warmed a hand in hot water, after which two fasting finger-prick blood samples (-5 and 0 min) were obtained (≥ 0.5 mL blood) using a non-reusable lancet (Accu-Chek[®] Safe-T-Pro Plus, Roche Diabetes Care GmbH, Germany). After the second fasting sample (0 min) was obtained, the participants were given a fixed portion of the test food or reference food, which they consumed with 250 grams water within 12 minutes. A stopwatch was started for each participant once they began eating.

The participants remained at the research centre for the next 2 hours during which additional blood samples were collected at 15, 30, 45, 60, 90 and 120 minutes after eating had commenced. Therefore, a total of eight blood samples were collected from each participant during each 2-hour test session. The participants were required to remain seated during their test sessions and only minimal movement was allowed. Each blood sample was centrifuged for 45 seconds immediately after collection. The plasma layer of the sample was then transferred into a labelled, uncoated tube, and was then immediately placed in a freezer. All plasma samples were stored in the freezer until their glucose concentrations were analysed.

Measurement of plasma glucose concentrations and GI values

The glucose concentration of each participant's eight plasma samples collected during each 2-hour test session was analysed in duplicate using a glucose hexokinase enzymatic assay (Beckman Coulter Inc.) and an automatic centrifugal spectrophotometric clinical chemistry analyser (Beckman Coulter AU480®, Beckman Instruments Inc., USA) with internal controls. A 2-hour plasma glucose response curve was constructed for each participant's test sessions using the average glucose concentrations for each of their plasma samples. The two fasting plasma samples of each test session were averaged to provide one baseline glucose concentration.

The incremental area under each 2-hour plasma glucose response curve (iAUC) was then calculated in order to obtain a single number, which expresses the total increase in blood glucose in that participant as a result of ingesting that food or drink during the 2-hour test session. A glycemic index (GI) value for the white dinner rolls was then calculated for each participant by dividing their 2-hour glucose iAUC value for the test product by their average 2-hour plasma glucose iAUC value for the reference food and multiplying by 100.

$$\text{GI value for test product} = \frac{\text{Plasma glucose iAUC value for test product}}{\text{Average iAUC value for the equal-carbohydrate portion of the reference food}} \times 100$$

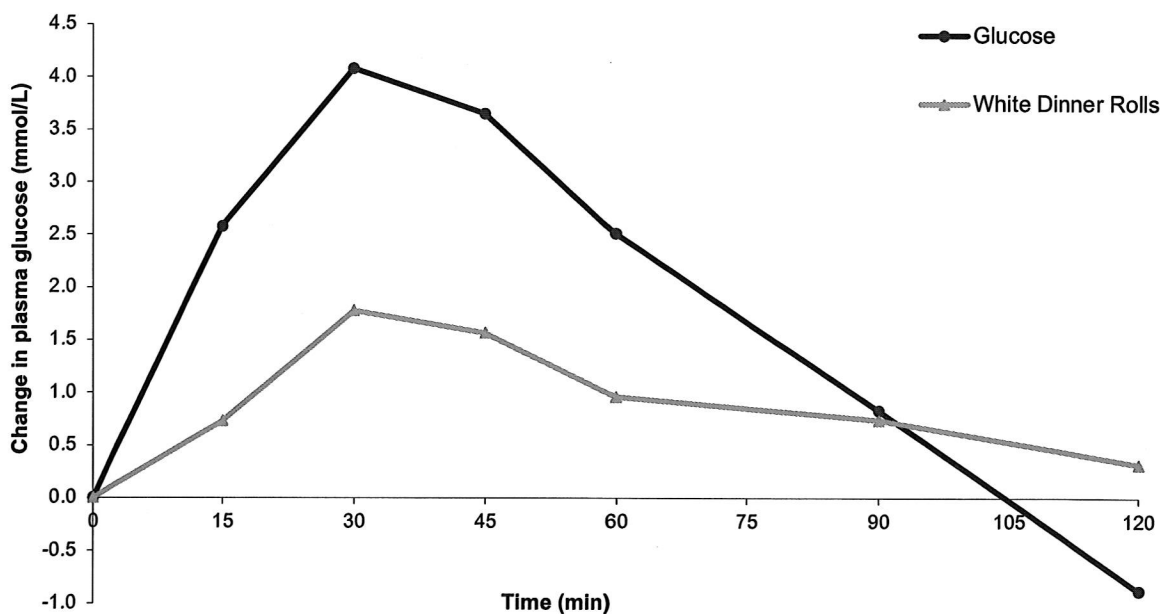
Due to differences in body weight and metabolism, blood glucose responses to the same food can vary between different people. The use of the reference food to calculate GI values reduces the variation between the participants' blood glucose results to the same food arising from these natural differences. Therefore, the GI value for the same food varies less between participants than their glucose iAUC values for this food. The participants' average plasma glucose concentrations for the reference food and the test product are shown in Appendix A.

Results

The average glycemic response curves for the reference food and the test product

The average 2-hour plasma glucose response curves for the 50-gram available carbohydrate portions of the reference food and the white dinner rolls are shown in Figure 2 below. The reference food (glucose solution) produced a rapid rise in plasma glucose to a high peak glucose concentration at 30 minutes and the greater overall glycemic response. The test bread produced a steady rise in glycemia to a peak response at 30 minutes followed by a gradual decline in glucose concentration between 45 – 120 minutes. The plasma glucose response produced by the test bread remained above the baseline, fasting concentration at the completion of the experimental period.

Figure 2. The average plasma glucose response curves for the equal-available carbohydrate portions of the reference food and the test product, shown as the change in plasma glucose from the fasting baseline level.



The foods' glycemic index values

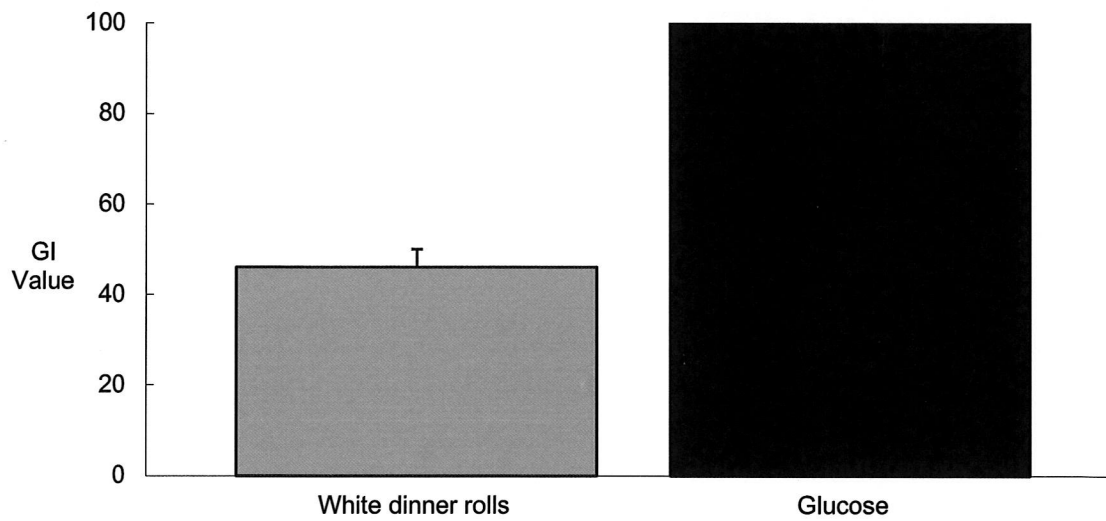
The differences in the glycemic responses produced by the reference food and the test product are more clearly reflected by their GI values than their plasma glucose response curves. The GI methodology helps to manage both day-to-day and person-to-person variability. Variation between responses to the same food is normal and is due to a number of factors, such as different rates at which the participants ingested the food, differences in the participants' carbohydrate metabolism, and lifestyle and genetic factors.

It is standard scientific practice that if any individual participant's GI value for a particular food is either greater than the group mean (average) value plus two standard deviations or less than the group mean value minus two standard deviations then that value is classified as an outlier and is removed from the dataset. No outlier GI values were observed amongst the participants' individual responses for the test product. Therefore, the final GI value for the white dinner rolls is the average of the entire group of 10 participants' data. The mean \pm standard error of the mean (SEM) GI values for the test food and the reference food are listed in Table 2 and illustrated in Figure 3 on the following page.

Table 2. The mean \pm SEM GI values for the test product and the reference food.

Test Food	GI value	GI Category
White dinner rolls	46 \pm 4	Low GI
Reference food (glucose sugar)	100 \pm 0	High GI

Figure 3. The mean GI values for the test food and the reference food.



Significant differences among the foods' average GI values

Standard parametric statistical tests (Analysis of Variance and T-test) performed using IBM® SPSS® Statistics software (version 24) were used to determine whether there was a significant difference between the GI values of the test product and the reference food. The smaller the p value, the more significant the difference, with $p < 0.001$ being the most significant difference. The results of these statistical analyses are shown in Appendix B. The reference food's GI value was significantly greater than the average GI value produced by the white dinner rolls ($p < 0.001$).

Conclusions

Using glucose as the reference food (GI = 100), foods with a GI value less than 55 are currently considered to be low-GI foods (12). Foods with a GI value between 56-69 are medium- or moderate-GI foods, and foods with a GI value of 70 or more are high-GI foods. The white dinner rolls tested in this study produced an average GI value of 46, which places this product within the low GI category. The GI value observed for this bread product is only valid as long as the ingredients and processing methods remain the same. Any change made to the product is likely to influence the GI, and therefore any modified formulation may need to be retested.

GI values are measured using portions of foods and drinks that contain between either 25 or 50 grams of digestible carbohydrate, but these may not be similar to the amounts of these products typically consumed by people in normal environments. It is possible to calculate a glycemic load (GL) value for any sized portion of a carbohydrate-containing food, as long as you know its GI value. The GL value for a food or drink is calculated by multiplying the amount of available carbohydrate in the portion of the food or drink by its GI value and then dividing by 100.

Similar to GI values, GL values are useful for helping people identify which types and amounts of foods will produce relatively lower blood glucose responses after consumption. A standard serve (i.e. 50 grams/1 bread roll) of the white dinner rolls contains a total of 22.0 grams of digestible carbohydrate. Therefore, the GL of an average serve of the white dinner rolls is $(22.0 \times 46)/100 = 10$. Currently, the consensus is that GL values of 10 or less are low GL; GL values between 11 – 19 are medium GL values; and GL values of 20 or more are high GL values (12).

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Sydney University's Glycemic Index Research Service

SUGiRS

The GI values of foods must be tested scientifically. At this stage, only a few research groups around the world currently provide a legitimate testing service. The University of Sydney has been at the forefront of glycemic index research for over a decade and has determined GI values for more than 2500 foods. In 1999, the Human Nutrition Unit established a commercial GI testing unit called 'Sydney University's Glycemic Index Research Service' (SUGiRS) to meet the increasing demand for GI research by local and international food manufacturers and pharmaceutical companies.

Fiona Atkinson and Jennie Brand-Miller are co-authors of *The International Tables of Glycemic Index* published by the scientific journal, *Diabetes Care*, in 2008. Previous editions of the *International Tables* (published in 1995 and 2002) have proven to be an important reference for health professionals when planning therapeutic diets for people with diabetes. Professor Brand-Miller's books, *The GI Factor* and related pocket books on diabetes, heart disease and weight reduction, are aimed at lay people and health professionals, and have sold more than 150,000 copies in Australia since 1996. A British edition of *The GI Factor* was released in 1997 and a North American edition (*The Glucose Revolution*) was released in July 1999. Each edition of the book includes tables listing the GI values of more than 350 different foods, many of which were tested at the University of Sydney. The glycemic index has been discussed in a number of best-selling books and in magazine articles in relation to a range of health topics such as diabetes, breast cancer and weight control. Publications such as these and ongoing research promoting the healthy nature of low-GI foods have generated an increasing demand for GI research.

Appendix A

The individual participants' plasma glucose results

Holista Bread Roll Study 2020

Reference Food: Glucodin glucose solution - 50 grams

Time (min)	Subjects										MEAN
	1 S1420	2 S1355	3 S1422	4 S1430	5 S1423	6 S1429	7 S1425	8 S1421	9 S1428	10 S1401	
0	5.36	5.42	5.40	4.93	5.27	5.32	4.53	5.31	5.39	5.35	5.23
15	7.01	8.45	7.52	7.68	8.25	7.65	7.07	7.91	7.61	7.48	7.66
30	8.24	10.59	8.62	9.41	10.37	8.97	8.60	9.69	9.34	10.18	9.40
45	8.14	10.03	8.19	8.86	9.75	8.87	7.19	8.30	10.05	10.24	8.96
60	7.10	8.32	7.81	7.06	8.75	7.27	6.19	7.26	9.78	9.42	7.89
90	5.26	6.28	5.95	5.22	7.00	6.44	5.73	5.06	6.49	7.05	6.05
120	4.61	3.23	5.40	4.66	3.62	4.34	4.36	4.31	4.55	4.25	4.33
iAUC	147	274	192	222	305	213	210	190	287	310	235
Mean iAUC of reference foods	149	249	167	229	308	194	189	197	289	364	

Holista Bread Roll Study 2020

Reference Food: Glucodin glucose solution - 50 grams

Subjects												MEAN
Time (min)	1 S1420	2 S1355	3 S1422	4 S1430	5 S1423	6 S1429	7 S1425	8 S1421	9 S1428	10 S1401		
0	5.39	5.11	5.06	5.39	5.11	5.40	5.04	5.44	5.38	5.22	5.25	
15	6.94	7.08	7.61	9.21	8.41	7.97	7.61	7.97	7.04	9.90	7.97	
30	7.19	8.95	9.02	10.45	10.02	8.80	8.60	9.64	9.66	10.95	9.33	
45	7.46	9.18	7.06	9.85	9.33	8.72	7.58	9.39	10.54	10.70	8.98	
60	6.20	8.03	6.17	7.64	8.63	7.07	6.94	7.65	9.50	8.22	7.60	
90	6.51	5.84	5.64	4.99	7.91	6.38	6.03	5.73	5.41	6.23	6.06	
120	4.67	3.68	3.90	4.65	4.66	5.56	4.40	4.13	3.93	3.69	4.32	
iAUC	126	228	164	246	344	208	196	215	260	327	231	
Mean iAUC of reference foods	149	249	167	229	308	194	189	197	289	364		

Holista Bread Roll Study 2020

Reference Food: Glucodin glucose solution - 50 grams

Subjects												MEAN
Time (min)	1	2	3	4	5	6	7	8	9	10		
	S1420	S1355	S1422	S1430	S1423	S1429	S1425	S1421	S1428	S1401		
0	5.54	5.49	4.81	5.30	4.74	5.27	4.94	5.40	5.37	5.02	5.19	
15	9.10	7.56	5.82	7.38	8.06	7.24	7.61	8.43	6.97	9.61	7.78	
30	9.08	10.44	7.16	9.84	9.51	7.45	8.04	9.99	9.68	10.74	9.19	
45	7.49	10.00	6.67	10.04	8.62	7.18	6.73	8.42	10.71	11.10	8.69	
60	6.62	8.98	6.30	7.01	7.43	6.69	6.13	6.94	10.16	10.88	7.71	
90	6.13	5.10	5.86	5.82	6.30	6.38	5.86	4.41	7.00	7.63	6.05	
120	4.50	3.70	4.93	4.62	3.21	5.54	3.59	3.73	5.11	4.95	4.39	
iAUC	172	246	145	220	275	160	159	185	322	455	234	
Mean iAUC of reference foods	149	249	167	229	308	194	189	197	289	364		

Holista Bread Roll Study 2020

Holista White dinner rolls

Subjects		1	2	3	4	5	6	7	8	9	10	MEAN
Time (min)		S1420	S1355	S1422	S1430	S1423	S1429	S1425	S1421	S1428	S1401	
0		5.40	5.03	4.96	5.49	5.27	5.24	4.86	5.07	5.47	5.47	5.23
15		6.11	5.60	5.41	5.93	6.05	5.64	5.16	5.81	5.75	8.14	5.96
30		6.64	6.49	6.39	7.18	7.53	6.51	6.64	6.60	6.42	9.70	7.01
45		6.14	6.64	5.36	7.86	7.81	6.21	5.99	6.47	7.50	8.03	6.80
60		5.54	5.90	5.15	7.74	7.36	5.64	5.10	6.04	7.19	6.25	6.19
90		5.96	5.84	5.27	6.64	6.76	5.47	5.25	5.59	7.10	5.84	5.97
120		5.45	5.73	5.30	5.35	6.20	5.58	5.41	5.21	5.94	5.31	5.55
iAUC		61	109	53	151	190	61	73	94	143	169	110
(by subject)		41	44	32	66	62	31	39	48	49	46	46

FINAL GI = 46 n = 10 SEM = 4

Holista Bread Roll Study 2020

Participant Characteristics

Subject	Gender	Age	BMI	Ethnicity
S1420	M	35.6	22.8	Caucasian
S1355	M	25.5	24.1	Indonesian
S1422	F	26.6	21.7	Caucasian
S1430	M	28.5	23.4	Caucasian
S1423	F	30.3	24.9	Caucasian
S1429	F	24.8	22.1	Caucasian
S1425	F	27.1	20.2	Filipino
S1421	M	28.9	20.1	Chinese
S1428	M	27.9	24.5	Caucasian
S1401	M	28.3	21.4	Indonesian
MEAN	4 F	28.4	22.5	
StDev	6 M	3.0	1.7	
min		24.8	20.1	
max		35.6	24.9	

Appendix B

Statistical analyses of the foods' GI values

These analyses were performed using IBM® SPSS® Statistics software (version 24). The analysis indicated that a significant difference existed between the GI values of the reference food and the test product. A value of $p < 0.05$ indicates a significant difference.

Oneway

Descriptives

GI

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
Reference Food	10	100.000	.0000	.0000	100.000	100.000	100.0	100.0
White Dinner Rolls	10	45.750	11.2849	3.5686	37.677	53.823	31.3	65.7
Total	20	72.875	28.8931	6.4607	59.353	86.397	31.3	100.0

ANOVA

GI

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14715.313	1	14715.313	231.101	.000
Within Groups	1146.145	18	63.675		
Total	15861.458	19			

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	RefFood	100.000	10	.0000	.0000
	BreadRolls	45.750	10	11.2849	3.5686

Paired Samples Test

Paired Differences

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	RefFood - BreadRolls	54.250	11.285	3.5686	46.1773	62.3227	15.202	9	.000