Glycaemic potency reduction by coarse grain structure in breads is largely eliminated during normal ingestion

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Short title: Glycaemic potency of kibbled-grain breads
Abstract

The hypothesis that coarse grain particles in breads reduce glycaemic response only if the particles remain intact during ingestion was tested. Three breads were formulated: (1) White bread (WB - reference), (2) 75% of kibbled purple wheat in 25% white bread matrix (PB), (3) a 1:1 mixture of 37.5% kibbled soy beans and 37.5% of kibble purple wheat in 25% white bread matrix (SPB). Each bread was ingested in three forms: unchewed (U), as customarily consumed (C), and homogenized (H). Twelve participants ingested 40 g available carbohydrate portions of each bread in each form, with post prandial blood glucose measured over 120 min. Glycaemic responses to WB were the same regardless of its form when ingested. Unchewed PB had significantly less glycaemic effect than WB, whereas the C and H forms were similar to WB. Based on a glycaemic index (GI) of 70 for WB the GI values for the C, U and H breads respectively were WB: 70.0, 70, 70, PB: 75, 42, 61, SPB: 57, 48, 55 (%) (Least significant difference = 17.43, p <0.05, bold numbers significantly different from WB). The similar glycaemic response to the H and C forms of the breads, and their difference from the U form, showed that the glycaemia-moderating effect of grain structure on starch digestion was lost during customary ingestion of bread. We conclude that kibbled grain structure may not effectively retard starch digestion in breads as normally consumed because it is largely eliminated by ingestive processes including chewing.

Keywords: Glycaemia, grain, structure, breads
1 Introduction

Glucose intolerance, involving both postprandial and chronic hyperglycaemia, is recognized as a global public health problem, associated with the metabolic syndrome, obesity, diabetes complex, and leading to multiple downstream medical complications\(^1\)\(^2\). The well established damage caused by hyperglycaemia has lead to a demand for foods formulated to reduce glycaemic impact.

One of the strategies used in the baking industry to reduce glycaemic impact is to include intact or partially intact grains, in products such as breads\(^3\)\(^4\). The assumption is that grain structure will impede digestive enzyme access to starch, reducing the rate of release of digestion products to the intestinal lumen, thus reducing the rate of glucose absorption and subsequent glycaemic response. However, initial oral and gastric processes associated with ingestion, including mastication, have evolved specifically to break down food structure and facilitate extraction of as much nutrient as possible from particulate foods such as grains. So, although particle size and intactness can have the clear effects of reducing starch digestion rate \textit{in vitro}\(^5\), inhibition of starch digestion is likely to be diminished by the processes of normal ingestion.

Ingestion is multifaceted. Chewing is a first step, when food is crushed while being moistened and mixed with saliva containing salivary amylase, as it is formed into a bolus\(^6\)\(^7\). Physical breakdown of food structure continues in the stomach while salivary amylase activity, which had initiated oral breakdown of starch, continues until reduced by low pH and pepsin activity in the gastric chyme\(^8\), as the bolus disintegrates. It has been estimated that as much as 59\% of starch in bread may be digested by the time food enters the small intestine\(^9\).

It is not only particle size that retards starch digestion, but also the cellular structure within the particles. The starch in graminaceous (cereal) grains, such as wheat, is typically stored in endosperm tissue consisting of thin-walled starch-filled cells with the whole surrounded by a resistant pericarp. Once the pericarp is ruptured during ingestion the starch is relatively accessible to digestive enzymes. In contrast, in legumes (pulses) the cell walls throughout the cotyledon tissue are robust, with a support function, and effectively encapsulate the starch within cells that restrict enzyme access until breached\(^10\). Therefore, the effectiveness of ingestive processes in making starch susceptible to digestion may differ between graminaceous and pulse grain particles.

The aim of the research reported here was to quantify the effects of normal ingestion on the glycaemic impact of foods that had been formulated on the basis of \textit{in vitro} digestion.
to contain enough cereal and/or pulse grain structure to reduce their glycaemic impact in vivo. We hypothesised that the processes that are part of normal ingestion and assimilation – chewing, swallowing, multienzyme digestion, gastric shear, and so on – will largely overcome the restrictions to starch digestion imposed by seed structure in kibbled grains and pulses. Breads with a low rate of in vitro starch digestion due to their content of kibbled cereal or pulse grains were prepared, and the glycaemic response to them when swallowed without chewing was compared with the glycaemic response when they were ingested normally, or after homogenising to completely eliminate coarse structure. The study tested the inclusion of coarse cereal and pulse grain particles as a reformulation strategy for lowering glycaemic impact of breads, given that the human ingestion process is designed to overcome the restrictions that food structure places on digestion.

2 Methods

2.1.1 Test foods

The test foods consisted of three breads:

1. White bread (WB, control) — Bread with a 100% white bread matrix and no kibbled grains.
2. Kibbled purple wheat (PB) — containing 75% of kibbled (> 2.8 mm) purple wheat and 25% of white bread matrix.
3. Kibbled soy-purple wheat (SPB) — containing 37.5% kibbled (> 2.8 mm) soy, 37.5% kibbled (> 2.8 mm) purple wheat and 25% of white bread matrix.

The kibbled soy and purple wheat grains had been prepared by passing the whole grains through a Kenwood grain mill (AT941A) with fluted rollers adjusted to give the desired particle size spectrum, and the particles winnowed with an air blower and finally sieved using a mechanical shaker with a 2.8 mm screen (Model RX-6-1, W.S Tyler, 8570 Tyler Blvd., Mentor, OH 44060, USA) to obtain the large kibble (> 2.8 mm) required for the experimental breads. The grain particles were soaked overnight and blotted dry before breadmaking. The formulation of the breads is given in Table 1 and full details of the bread preparation in Appendix A.

2.1.2 Digestive analysis of the test foods

Each of the test breads was subjected to an in vitro digestive analysis in both the intact and homogenised form, to determine the relative release of glucose equivalents with time, and the total potentially available carbohydrate, where “potentially available” means digested if unoccluded by particle mass, in each of the breads. Therefore it includes the Type 1 resistant
starch that would have been present in the coarse particles due to grain structure. An *in vitro* digestion procedure, using 5 g of the bread in a volume of 50 ml of digest was used\textsuperscript{11}. The samples were moistened and either gently crumbled or homogenized in 30 ml of deionised water before adjusting to pH 2.5 with 1 M hydrochloric acid (HCl). A volume of 1 ml of 10% pepsin (Sigma P-7125) solution in 0.05 M HCl was added and the pots incubated at 37°C for 30 min to simulate gastric digestion. The pH was then adjusted to 6.5 with 0.1 M sodium hydroxide (NaOH) and maleate buffer (5 ml, 0.2 M, pH 6.5) and made accurately to 50 ml with deionized water, followed by addition of pancreatin (Sigma P-7545) solution (5% w/v, 0.2 ml) and amyloglucosidase (Megazyme E-AMG, 0.1 ml). The pancreatic digestion was continued for 120 min with sampling (1.0 ml) at intervals of 0, 10, 20, 30, 40, 60 and 120 min. The 120 min sample of the homogenised breads was used to determine the content of potentially available carbohydrate in each bread. The 1 ml samples of digesta from each sampling time point were added to 4 ml ethanol and mixed. After centrifuging an aliquot of the ethanolic supernatant was subjected to an amyloglucosidase–invertase secondary digestion to convert maltose and limit dextrins to glucose. Free sugar was measured spectrophotometrically as glucose equivalents using the dinitrosalicylic acid method\textsuperscript{12}. As starch was the only digestion product no allowance was necessary for non-glucose sugars, so the glucose equivalents represented glycaemic glucose equivalents (GGE), defined as the amount of glucose of the same glycaemic load as a given carbohydrate source\textsuperscript{13}.

Digestograms of the release of glucose with time of digestion were plotted. Theoretical glucose disposal lines derived from clinical trials\textsuperscript{11} were inserted and the difference between glucose disposal and glucose release calculated. The resulting curves of net GGE accumulation with time gave simulated blood glucose responses curves, as previously described\textsuperscript{11}. The areas under these curves were determined by the trapezoid summation method routinely used in glycaemic index determination. By comparing the area under the net GGE curve of a bread with the area for homogenised white bread relative glycaemic potency (RGP) values were obtained for comparison with those calculated from clinical blood glucose responses. This comparison gave an indication of the correspondence between *in vitro* and *in vivo* starch digestion in support of basing the clinical trial on in vitro digestibility data.
2.1.3 Formulation of Meals

The nine test meals were each formulated to contain 40 g of potentially available carbohydrate, based on the in vitro digestive analysis of the available carbohydrate content of homogenized samples (Section 2.3.1) (WB, 38.1%; PB, 31.7%; SPB 24.6% available carbohydrate), and on moisture contents of the breads measured according to an official AOAC method \(^{14}\) (WB, 40.2%; PB, 47.2% and SPB, 49.1%). The breads were baked weekly, divided into the 40 g available carbohydrate portions for ingestion, and frozen. Prior to ingesting, the breads were thawed to room temperature overnight (14 h). Starch digestibility was determined to confirm that freezing, storing and thawing the breads had not altered the digestibility of the starch. Breads for treatment H were homogenized with 150 ml of water not more than 1 h before being consumed. Based on the analyses conducted all meals contained the same mass of potentially digestible carbohydrate and water (Table 1).

2.1.4 Trial procedure

The clinical trial was carried out in the New Zealand Institute for Plant and Food Research clinical suite. Ethical approval was obtained from the New Zealand Health and Disabilities Ethics Committee (HDEC, no. 18NTA\160), and the trial was registered with the Australia New Zealand Clinical Trials Registry (no. ACTRN12618001826235). The participant flowchart is given in Figure 1 and the CONSORT checklist in Appendix A3.

Twenty-four volunteers were recruited for the initial screening using flyers or emails that briefly described the study. Volunteers were pre-screened and asked initial recruitment questions to determine their suitability to participate in the study, and the nature of the study and their involvement and responsibilities described. Eligible volunteers willing to participate in the study were presented with an information sheet containing study details, and an informed consent form. Their fasting blood glucose concentration and glycated haemoglobin (HbA1c) were measured to ensure that they were within the normal (non-diabetic) range and to familiarise them with the blood sampling procedure to be used.

A total of 12 participants (five male and seven female) were selected as suitable for the final study. The characteristics (mean values±standard deviations) of the study group were: age 33.3 ± 11.7 years, BMI 23.6 ± 3.3 kg/m\(^2\), fasting glucose 4.4 ± 0.3 mmol/l and HbA1c 34.5 ± 4.5 mmol/mol. The participant number (n = 12) exceeded the minimum (n = 10) specified by the current ISO method (ISO 26642:2010) for determining glycaemic index and was typical of studies comparing foods. The 12 participants were from within Plant and Food Research and Massey University at Palmerston North, and satisfied the following exclusion criteria:
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- Age: Below 18 or above 65.
- Body Mass Index: BMI below 18 and above 35 kg/m².
- Glucose intolerance: History of diabetes or evidence of glucose intolerance in a preliminary test.
- Gluten and soy intolerance: History of intolerance to gluten, soy or bread products.
- Non-fasting: Unwilling to not consume anything apart from water in the 12 hours before the test.
- Recent ill health

A non-blinded randomised repeated measures design was used in which the order of the treatments was randomised by computer for each participant and each participant ingested each of nine treatments once. The treatments were the three breads - white bread (WB), kibbled purple wheat bread (PB), and soy/kibbled purple wheat bread - each ingested in three forms – as was customary for the participant (“chewed”) (C), unchewed (U), homogenized (H) (Table 1). It was not practicable to blind the subjects to the breads they were ingesting, but the data analysis was completed by an investigator who was blinded to the identity of samples and treatments.

2.1.5 Ingestion of test foods

Participants were asked to attend on weekday mornings. In preparation for each testing session participants were requested to:

- Avoid strenuous physical activity, smoking or consuming alcohol the evening before and the day of a test.
- Consume a similar carbohydrate-based meal the evening before each test.
- Fast from 9.00 p.m. the night before a test, with water consumption not restricted.
- Allow at least 48 h (wash-out time) between tests.

On each test day the volunteers were seated and asked to remain so for the duration of the test. Once each subject was relaxed and comfortable for 10 min, a baseline blood sugar measurement was taken in duplicate. Each subject was then given a test food and instructed to consume the whole amount within 10 minutes. The meals included enough water to enable swallowing without chewing in the unchewed treatments (Table 2). Blood glucose was measured in finger prick blood samples collected at 0 (baseline), 15, 30, 45, 60, 90 and 120 min using a HemoCue® blood glucose meter.
2.1.6 Analysis of glycaemic response data

All 12 of the subjects completed the trial, and no obvious outliers were detected, all results were included in the analysis. The blood glucose concentration changes from baseline were plotted against time to obtain blood glucose response curves. Each individual’s baseline fasted blood glucose value was subtracted from subsequent measurements to obtain the incremental blood glucose response from which the incremental area under the blood glucose response curve (iAUC) was derived by trapezoid summation. The highest postprandial blood glucose peak for each individual, irrespective of the time of occurrence (nearly all were at either 30 or 45 min), was used to determine the mean peak height for each meal.

2.1.7 Glycaemic index (GI).

As all bread meals had been formulated to supply the same (40 g) available carbohydrate the GI value for each treatment could be estimated from its iAUC value up to 120 min, relative to that of WB, which was used as the reference with an assumed GI of 70:

\[ GI_{\text{food}} = 70 \times \frac{i\text{AUC}_{\text{food}}}{i\text{AUC}_{\text{WB}}} \]

2.1.8 Relative glycaemic potency (RGP).

Relative glycaemic potency of the breads refers to the blood glucose-raising effect of ingesting 100 g whole bread relative to the effect of ingesting 100 g of glucose, expressed as grams of glucose equivalents (GGE). As a GI of 70% for available carbohydrate in white bread (WB) means that the carbohydrate has a relative glycaemic impact that is 70% that of glucose, or 70 GGE/100 g available carbohydrate, the RGP of any other food ingested at the same available carbohydrate intake may be calculated from the glycaemic response to the food carbohydrate relative to white bread carbohydrate (iAUC_{food}/iAUC_{WB}), adjusted by the percentage of available carbohydrate in the bread.

\[ \text{RGP}_{\text{food}} = \%\text{CHO}_{\text{food}}/100 \times \frac{i\text{AUC}_{\text{food}}}{i\text{AUC}_{\text{WB}}} \times 70 \]

Data were entered into a Microsoft® Excel spreadsheet for preliminary analysis. For statistical comparison of means (ANOVA), GenStat software was used (version 11.1; VSNi Ltd). Data were analysed using analysis of variance (ANOVA) blocked by individual, testing differences between foods and treatments. Statistical analysis described the differences between the foods in their effects on blood glucose concentrations at different postprandial time points and allowed the precision of the glycaemic potency values to be determined. P values ≤0.05 were considered significant.
3 Results

3.1 In vitro digestive analysis

The in vitro digestive analysis revealed large differences between the breads (Figure 2). Homogenizing to eliminate coarse grain structure had no effect on the rate or extent of white bread digestion. In contrast, the extent and rate of digestion of the kibbled grain breads was much lower for the intact than for the homogenised forms, and digestion of the kibbled grain breads tended to plateau well before digestion was complete. After 120 min the digestion of intact WB was 98% of homogenised WB, whereas the digestion of intact PB and SPB breads were both 76% of the homogenised sample, and remained at 76% after further digestion to 180 min (not shown).

Mean values for glucose equivalent release from the homogenised sample at 120 min, used as “available carbohydrate”, were 38.1 g/100 g for WB, 31.7 g/100 g for PB and 23.7 g/100 g for SPB.

RGP values calculated from the net area under the curves for glucose equivalent release during digestion in vitro (Figure 2), after subtracting theoretical blood glucose disposal, were (where I = intact and H = homogenised): WBI, 25.5; WBH, 26.6; PBI, 14.7; PBH, 21.4; SPBI, 10.4; SPBH 16.5 g glucose equivalents/100 g bread. The in vitro data therefore predicted a much higher glycaemic impact per given mass of bread for the white bread than for the kibbled breads.

3.2 Glycaemic response

Both blood glucose response amplitude and incremental area under the blood glucose response curves differed significantly between breads and treatments (Table 3) despite large between-subject variations in the blood glucose responses typical of such studies.

3.2.1 Response amplitude (peak height)

Plasma glucose concentrations reached peak values between 30 and 45 minutes after the ingestion of test breads and decreased thereafter as a result of metabolic glucose disposal (Figure 3). The mean peak values (mmol/L) for most treatments, except PB(U) (1.94 ± 0.21) and PB(C) (3.24 ± 0.42), were similar and not significantly different, falling with the range 2.41-3.03 (mean 2.71, SD 0.20). PB(U) was significantly (p <0.05) lower than PB(C), and SPB(U) was lower than SPB(C), but not significantly so (p <0.05). Peak heights for the three forms in which WB was ingested (C, U, H) were very similar to one another (Table 3).

For both the PB and SPB breads, chewing and homogenisation resulted in a peak amplitude similar to that of the WB. However, swallowing PB and SPB without chewing
caused a substantial and statistically significant reduction in the peak amplitudes of about 26% and 15% respectively of the WB treatments (Figure 2 and Table 3).

3.2.2 The area under the blood glucose response curve

The mean iAUC for all ingested forms (C, U, H) of the white bread (WB) – chewed, unchewed and homogenized were similar (range: 173.4-182.1 mmol L⁻¹ min⁻¹) differing from one another by less than 5% (Table 3). The purple wheat bread (PB) when ingested chewed (C) had a mean iAUC (194.5 mmol/L) which was also similar to that of WB, but the iAUC was 17% less than that of the WB when it was ingested homogenised, and 45% less when ingested unchewed. The C and H forms of the soy/purple wheat bread (SPB), also induced lower iAUCs than WB by 12% and 23% respectively (Table 3). The greatest mean reductions in iAUC compared with white bread occurred upon ingestion of the unchewed (U) forms of PB (45% and statistically significant, p < 0.05) and SPB (31%).

3.2.3 Glycaemic index (GI)

The GI values for the WB samples based on a reference value of 70 for the cutomarily ingested (therefore chewed) WB sample C, were unaffected by physical form in which they were ingested.

The unchewed PB and SPB breads had GI values of less than 55 so would be classified as “low GI”, while the homogenised PB and SPB samples both fell into the “medium GI” (55-69) category. GI was significantly lower than WB only for the unchewed PB(U) (-41%) and SPB(U) (-30%) samples, reflecting the protection of starch from digestion by kibbled-grain structure. The chewed PB had the highest GI value.

3.2.4 Relative glycaemic potency

While GI refers to the glycaemic impact of food available carbohydrate and is expressed on a carbohydrate only basis, RGP refers to the relative glycaemic potency of the whole food. The differences in RGP of the breads (Table 3) were more striking than the differences for other variables. All forms of the granular breads (PB and SPB) were substantially lower in glycaemic impact than the white bread, and all except the PB(C) significantly so (p < 0.05). The unchewed forms of PB and SPB had the lowest glycaemic impact. The glycaemic impact of the white bread was the same in all three forms.

The clinically determined relative glycaemic potencies of the unchewed breads PB(U) and SPB(U) were 69% and 86% respectively (average 77%) of the RGP values for the homogenised forms (PB(H)) and SPB(H). Differences between intact (I) and homogenised (H) forms in vitro were 69% for PB and 63% for SPB. The clinically determined RGP values for unchewed and homogenised breads (Table 3) correlated closely.
(R² = 0.93) with the RGP values based on in vitro digestion and adjusted for theoretical blood glucose disposal (Figure 4), as described in the methods.

4 Discussion

The close correspondence between the RGP values determined by in vitro digestion and those derived from true blood glucose responses to the breads in this study suggests that in vitro digestion can give an accurate indication of the impact of starch digestibility, as affected by food structure, on blood glucose responses. At the same time, because of the large in vivo differences in glycaemic impact between chewed and unchewed forms of the same breads (Table 3), the sensitivity of the in vitro method to physical structure suggests a need to account for the physical effects of normal ingestion, when assessing the benefit of grain particles, in reformulating for reduced glycaemic impact.

The hypothesis that retardation of starch digestion by coarse grain structure in breads would be largely removed by the processes of customary ingestion was confirmed. In the intervention study all treatments contained the same quantity of potentially available carbohydrate, allowing comparison with the white bread, which was free of coarse kernel structure. For both the kibbled wheat (PB) and the kibbled soy/kibbled wheat (SPB) breads the customarily consumed sample induced a blood glucose response amplitude, and an incremental area under the curve that was not significantly or sizeably less than that of white bread. In contrast, the samples in which the grain kibbles were swallowed intact (samples PB(U) and SPB(U)) gave the lowest peak heights and areas under the curve, while the white bread, which had no grain structure, was unaffected by the way in which it was ingested, consistent with the in vitro findings (Figure 2). Therefore, the limitations to starch digestion that were imposed by kibble structure, and exposed by in vitro digestion, had been eliminated by normal ingestive processes.

The finding that the C, U and H samples of WB were very similar in glycaemic response amplitude, iAUC and GI may reflect the fact that the white bread lacked any structural impediment to starch digestion that could be removed by homogenizing or chewing. Assuming that bolus formation would occur with the chewed form of white bread, the results suggest that bolus structure had little effect on glycaemic response, perhaps because of a lack of components, such as polysaccharides and remnants of structural elements present in whole kernels, that might prolong bolus cohesion and thus amylase activity within it.
The GIs (GGE per 100 g of carbohydrates) for the breads, followed the same trends reported for peak amplitude and iAUC (Table 3). For the homogenised forms of the PB and SPB breads peak amplitude and iAUC, and therefore GI was less than for the chewed samples. Although the difference was not statistically significant, it provides circumstantial evidence that homogenization was less effective in preparing particulate foods for starch digestion than chewing. That normal oral processing lead to the highest glycaemic impact in the whole grain breads is reasonable, since human dentition and mastication has evolved specifically to reduce food structure and convert potentially digestible food components to available nutrients.

A number of elements of the normal ingestion process that are missing when homogenised sample is ingested may explain why the homogenised samples did not have a higher glycaemic impact than the corresponding normally ingested (C) samples. They include crushing and mixing with more salivary amylase over a much greater time period than it would take to simply swallow the homogenised sample, and formation of a bolus. Bolus formation involving crushing and physical confinement within the protected amylolytic environment of the bolus, has been shown to be important for the oral digestion process, and is likely to be affected by the texture of the bread. The homogenised slurry could disperse more quickly than a bolus in the acidic gastric phase, reducing the possibility of salivary amylase activity persisting within the bolus or fragments of it. Crushing and mixing with salivary amylase during mastication appear to be a precondition for rapid starch availability and make an important contribution to digestion of starches in the immediate postprandial period of acute glycaemic response.

The sample in which ingesting bread intact gave the greatest depression of glycaemic response was PB, whereas one might expect the intact SPB bread to give the lowest response, because of the reported resilience of legume cotyledon cells encapsulating starch. It appears that the occlusive effect of a solid mass of gelatinised starch in intact cooked endosperm may have retarded digestive enzyme access more than cell walls in cotyledon fragments, in which the starch is separated into individual cell contents, and protein digestion coupled with cell wall changes may have facilitated digestive enzyme access to the starch. It is also possible that the texture imparted by soy bean grains in the SPB bread may have induced more crushing and more salivary amylase incorporation into SPB boli than into PB boli, reducing the difference in starch digestion between the U and H forms of the SPB bread. Further research, in which chewing characteristics are measured, could help resolve this question.
The glycaemic response data (peak height, iAUC and GI in Table 3) suggests that comparing kibbled grain and white breads in equal carbohydrate portions may not reveal significant differences in glycaemic potency that exist between breads consumed in customary portions, or servings. When equal weights of the breads were consumed the differences in glycaemic impact between the white and kibbled grain breads were quite substantial and statistically significant, because in equal bread weights carbohydrate intakes differed. For instance, the *in vitro* analysis showed the SPB bread, which contained high proportions of soy and purple wheat >2.8 mm had a carbohydrate content of 24.6% compared with a much higher available carbohydrate content of 38.1% for WB. In all forms (chewed, unchewed and homogenized) SPB bread had a low relative glycaemic potency (RGP) compared with white bread (WB).

The results indicate why GI, which is based on equal carbohydrate comparisons rather than customarily consumed portions, can be quite misleading as a guide to the relative glycaemic impact of food portions at point of sale. People eat foods and not only the carbohydrates in them. Glycaemic impact expressed as glycaemic glucose equivalents per weight of food may be a more understandable and practical estimate of glycaemic effect because it may be based on familiar or customarily consumed quantities, such as 100 g or a serving of bread.

Reducing the proportion of glycaemic material in the bread formulation could be a more effective and reliable strategy to decrease the glycaemic potency of breads than inclusion of whole grains alone, and could be a focus of future research for the development of breads for reduced glycaemic potency. However, it is important to ensure that any apparent gain that results from reformulation does not have negative outcomes, such as has been suggested when fructose (GI = 19) is substituted for glucose (GI = 100).19

In the present study all participants consumed all breads, and the focus was on comparison of breads rather than of individuals. However, it is worth noting that the degree to which ingestion alters starch digestibility is likely to differ between individuals, and depend on their characteristic eating behaviours. Fast eaters have been reported to exhibit a lower postprandial glycaemic response than slow eaters.20, 21 Differences in bolus particle size at point of swallowing, and effects of bolus properties on amylase activity and glucose release also depend on individual chewing characteristics.20, 22 Furthermore, large individual differences in human salivary amylase (HSA) activity associated with differences in HSA gene copy number have been shown.23 Thus, effects of coarse grain particles in breads that
were not large in the present study may emerge more strongly in a comparison of participants differing in chewing characteristics and/or HSA activity.

5 Conclusion
Kernel structure may play a significant role in reducing the digestibility of starch in bread, but this translates to a commensurate reduction in glycaemic potency only if the effects of grain structure survive the ingestion process. The results presented here suggest that grain structure will reduce glycaemic response if effects of ingestion, such as crushing, can be minimised. Very coarse grain particles may not, therefore, be most suitable in reformulating for reduced glycaemic impact if they induce a mastication response. The results also showed the importance of available carbohydrate content per customarily consumed portion in determining glycaemic impact. Reformulating breads for reduced glycaemic impact therefore needs to focus on substituting highly glycaemic components as well as minimising disintegration of starch-containing particles during ingestion, while maintaining organoleptic attributes.

6 Acknowledgements
We express our appreciation and sincere gratitude to Plant & Food Research and Massey University for the scientific and technical assistance.

Financial Support
We are grateful for the financial support from the Riddet Institute, Palmerston North, New Zealand (Contract no. 34194) and the Baking Industry Research Trust, Auckland, New Zealand (Contract no. 33875).

Conflict of Interest
Authors declare no conflict of interest.

Authorship
A.S, J.A.M and S.M were involved in the study conception, design and analysis. A.S, J.A.M and A.H wrote the manuscript; L.M, K.G and F.W provided advice and consultation.
7 References


**Table 1** Ingredients and formulations for white (WB) and kibbled-grain breads (g).

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>WB(^1)</th>
<th>75% PB(^2)</th>
<th>75% SPB(^3)</th>
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<tbody>
<tr>
<td>Flour</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.89</td>
<td>1.89</td>
<td>1.89</td>
</tr>
<tr>
<td>Salt</td>
<td>1.89</td>
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<tr>
<td>Gluten</td>
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<td>Yeast</td>
<td>3.21</td>
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<tr>
<td>Dough improver</td>
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</tr>
<tr>
<td>Oil</td>
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</tr>
<tr>
<td>Water</td>
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</tr>
<tr>
<td>Kibbled- grains</td>
<td>N/A</td>
<td>300 purple wheat(^4)</td>
<td>150 soy; 150 purple wheat(^4)</td>
</tr>
</tbody>
</table>

\(^1\)WB = white bread, \(^2\)PB = purple kibbled wheat bread, \(^3\)SPB = kibbled soy/kibbled purple wheat bread. \(^4\)Dry weight, but grains prehydrated prior to breadmaking.
**Table 2** Composition of test meals to ensure the same intake (40 g) of available carbohydrate and water in each meal.

<table>
<thead>
<tr>
<th>Test foods(^1)</th>
<th>Treatment(^2)</th>
<th>Weight of bread (g)</th>
<th>Water for drinking (ml)</th>
<th>Water for Homogenizing (ml)</th>
</tr>
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<tr>
<td>WB</td>
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<td>105</td>
<td>338.2</td>
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</tr>
<tr>
<td></td>
<td>U</td>
<td>105</td>
<td>338.2</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>105</td>
<td>188.2</td>
<td>150</td>
</tr>
<tr>
<td>PB</td>
<td>H</td>
<td>126</td>
<td>171</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>126</td>
<td>321</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>126</td>
<td>321</td>
<td>_</td>
</tr>
<tr>
<td>SPB</td>
<td>H</td>
<td>163</td>
<td>150</td>
<td>150</td>
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</table>

\(^1\)WB = white bread, PB = purple kibbled wheat bread, SPB = kibbled soy/kibbled purple wheat bread.  
\(^2\)C = Chewed, U = unchewed. H = homogenized.
Table 3 Characteristics of the blood glucose response curves during 0–120 min after ingestion of three breads\(^1\) in three forms\(^2\), and glycaemic indexes and relative glycaemic potency values derived from the blood glucose response data.

<table>
<thead>
<tr>
<th></th>
<th>WB</th>
<th></th>
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<tr>
<td>Amplitude (mmol/L)</td>
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</tr>
<tr>
<td>Mean</td>
<td>2.61(^{a})</td>
<td>2.76(^{abc})</td>
<td>3.03(^{ab})</td>
<td>3.24(^{a})</td>
<td>1.94(^{d})</td>
<td>2.72(^{abc})</td>
<td>2.73(^{abc})</td>
<td>2.23(^{bc})</td>
<td>2.41(^{bcd})</td>
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<td>1.6</td>
<td>4.3</td>
<td>2.6</td>
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<td>0.31</td>
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<td>0.31</td>
<td>0.24</td>
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<tr>
<td>iAUC (mmol/L x min)</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>182(^{ab})</td>
<td>173(^{ab})</td>
<td>176(^{ab})</td>
<td>194(^{a})</td>
<td>100(^{cd})</td>
<td>152(^{abc})</td>
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<td>125(^{cd})</td>
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<tr>
<td>SEM</td>
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<tr>
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<tr>
<td>Mean</td>
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<td>69.5(^{ab})</td>
<td>70.4(^{ab})</td>
<td>76.7(^{a})</td>
<td>41.5(^{c})</td>
<td>60.9(^{abc})</td>
<td>63.0(^{abc})</td>
<td>49.0(^{bc})</td>
<td>55.3(^{abc})</td>
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<td>*</td>
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</tr>
</tbody>
</table>

\(^{1}\) WB: White bread, PB: Partially bleached bread, SPB: Spanish bread. 
\(^{2}\) LSD: Least significant difference (p<0.05).

**Amplitude**

**Mean**

- WB: 2.61
- PB: 2.76
- SPB: 3.03

**SEM**

- WB: 0.20
- PB: 0.23
- SPB: 0.29

**%WB(C)**

- WB: 100
- PB: 106
- SPB: 116

**iAUC**

**Mean**

- WB: 182
- PB: 173
- SPB: 176

**SEM**

- WB: 13.3
- PB: 18.2
- SPB: 24.7

**%WB(C)**

- WB: 100
- PB: 95
- SPB: 97

**GI**

**Mean**

- WB: 70.0
- PB: 69.5
- SPB: 70.4

**SEM**

- WB: 0.0
- PB: 7.13
- SPB: 9.78
### %WB(C) Table

<table>
<thead>
<tr>
<th></th>
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<th>99</th>
<th>101</th>
<th>110</th>
<th>59</th>
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<tbody>
<tr>
<td><strong>RGP (GGE g/100 g bread)</strong></td>
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<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>26.7\textsuperscript{a}</td>
<td>26.5\textsuperscript{a}</td>
<td>26.8\textsuperscript{a}</td>
<td>23.8\textsuperscript{b}</td>
<td>13.2\textsuperscript{cd}</td>
<td>19.3\textsuperscript{bc}</td>
<td>14.0\textsuperscript{cd}</td>
<td>11.7\textsuperscript{d}</td>
<td>13.6\textsuperscript{cd}</td>
<td>5.7</td>
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<tr>
<td>SEM</td>
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<td>3.73</td>
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<tr>
<td>%WB(C)</td>
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<td>101</td>
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<td>50</td>
<td>72</td>
<td>53</td>
<td>44</td>
<td>51</td>
<td>21</td>
</tr>
</tbody>
</table>

1. WB = white bread, PB = kibbled purple wheat bread, SPB = kibbled soy/purple wheat bread.  
3. GI = glycaemic index based on response to 40 g available carbohydrate in all treatments with the normally consumed white bread (WB(C), GI = 70) used as the reference. %WB (C) = % of (WB(C) value. Values in a row with a common letter in the data label do not differ significantly (p < 0.05 based on least significant difference).  
4. GGE = glycaemic glucose equivalents (g)
Figure 1 Human intervention study flowchart showing ethical approval, recruitment and intervention processes for this trial. HbA1c, glycated Hb.
Figure 2 Sugar release as glucose equivalents (GE)/100 g bread during in vitro digestion of white bread (WB), purple wheat bread (PB) and soy/purple wheat bread (SPB), digested intact (●) or homogenised (○). The average precision of measurements was SD = 0.5 g and the CV 1.75%
Figure 3 Blood glucose responses to 40 g carbohydrate intakes of white bread (WB), purple wheat bread (PB) and soy/purple wheat bread (SPB) each ingested chewed (C) unchewed (U), or homogenised (H).
Figure 4 Correspondence between relative glycaemic potency determined in vitro and in vivo for homogenised and intact white and kibbled grain breads.
Appendix A

7.1.1 Grain Milling
The hard purple wheat was supplied de-hulled and uncooked or otherwise processed. The soybeans were passed through a Kenwood Grain Mill AT941A to coarsely grind the seed and facilitate separation of the seed coat. The seed coat was blown from the partially ground endosperm using a heat gun set to the coolest setting. The purple wheat and soy particles were then milled using a Kenwood Grain Mill AT941A with variable feed rate and two fluted rollers operating at different speeds. The gaps between the rollers was adjusted by screws to mill the gain to the desired particle sizes. The milled grain was sieved (> 2.8 aperture) using a mechanical shaker (Model RX-6-1, W.S Tyler, 8570 Tyler Blvd., Mentor, OH 44060, USA) for 5 minutes, the sample fraction remaining on the sieves was collected to make the breads.

7.1.2 Bread Baking
The kibbled-grain breads and reference white breads were prepared in the The New Zealand Institute of Plant and Food Research Limited, food safe laboratory. The standard white bread (control) was formulated with white flour plus (g/100g of wheat flour basis) 1.9g salt, 1.9g sugar, 3.8g gluten, 3.2g yeast, 0.6g dough improver, 3.4g oil to which water was added to 73.6% of the final dough. Variations to make the kibbled-grain breads are described below. The yeast was dissolved in warm water (33±2 °C) and left standing for 10 min. The remaining dry ingredients were placed in a bowl and mixed KitchenAid® Mixer (Model 5KSM150PS, KitchenAid, USA) fitted with a dough hook at speed level 2. The fresh yeast suspension, oil and water were then added, and mixing was continued for 12 min. The resulting dough was then divided into 400 g pieces, and shaped into loaves and placed into 27.6 x 14 x 6.5 cm silicone loaf pan and sealed tightly with metal foil wraps allowing space for leavening of the bread. The loaves were then placed in a standard bread proofer (Irinox MF 70.1) (30 °C, 100% humidity) for 60 min for the initial proofing. After 60 min, the dough was again kneaded in the mixer (KitchenAid®) for 5 min. The dough was again shaped into loaves and placed into the loaf tins and tightly covered with metal foil allowing space for the leavening of the breads. The breads were finally proofed at 30 °C, 100%RH (Irinox MF 70.1) for 60 min, after which they were baked in a conventional oven (Zanussi Professional) at 215 °C with the air vent shut to prevent moisture loss until the internal temperature of the loaves reached 90° C (Traceable® Food Thermometer). After baking, the loaves were cooled for 1 h until the internal temperature of the bread reached 30 °C, and
then cut into slices, packed and labelled in polyethylene ziplock bags and stored at -20°C until analysis.

For the breads containing 75% of kibbled-grain, the grain particles were hydrated by soaking for 16 hr at 20°C after which they were drained and blotted dry. The white bread matrix was prepared as above and kneaded for 10 min. After 10 min the appropriate quantity of the blotted grains Table 1 were added to the white dough mixture and kneaded for a further 2 mins. The dough was divided into 800 g pieces, shaped into loaves, and proofed and baked as for the white bread. The dough weight of kibbled-grain breads was double that of the white bread to give loaves of similar dimensions to the white bread when baked. After baking, the loaves were cooled, packed and stored as for the white breads. All the test breads were baked, sliced, packed and frozen before the human study.