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Exploring the Role of Cereal Dietary Fiber in Digestion

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ABSTRACT: Increasing the dietary fiber of staple foods such as bread is an attractive way to promote healthy eating in a large part of the population, where dietary fiber consumption is reportedly below the recommended values. However, many consumers prefer white breads, which are typically low in dietary fiber. In this work, white bread was made from two wheat cultivars with differing fiber contents. The resulting breads showed similar quality parameters (volume, specific volume, firmness, inner structure characteristics) with any differences maintained below 7%. Bread digestibility was evaluated using a novel dynamic in vitro digestion model. Reduced digestion rates of 30% were estimated for the high-fiber white bread compared to that in the control. Overall, this work demonstrates the potential to produce healthy, high-fiber white breads that are acceptable to consumers, with a reduced rate of starch digestion, by exploiting a genetic variation in the dietary fiber content of wheat cultivars.

KEYWORDS: *arabinoxylan, wheat, high fiber bread, in vitro dynamic digestion*

INTRODUCTION

Dietary fiber (DF) is an essential part of the human diet. The majority of DF is derived from plant foods, in particular, from nondigestible carbohydrates which form the plant cell walls.[1,2] The most widely accepted definition of DF is that proposed by the EU (Commission Directive 2008/100/EC, 28 October 2018): "carbohydrate polymers with three or more monomeric units (to exclude mono- and disaccharides, simple sugars of one or two molecules) which are neither digested nor absorbed in the small intestine". Although the health benefits of DF were first recognized for toxaemia in pregnant women,[3] research over the past few decades has demonstrated a range of health benefits,[4−6] including reduced risk of obesity[7] and a range of chronic diseases such as cardiovascular disease,[7] type II diabetes,[8] and colo-rectal cancer.[9] In particular, soluble forms of fiber, which confer high viscosity to aqueous solutions, have been associated with improved blood glucose levels[10] and reduced blood cholesterol concentrations,[11] prolonged gastric emptying,[12] and reduced starch digestion/glucose absorption in the small intestine.[12,13] However, despite these widely recognized benefits the consumption of dietary fiber in many countries, in particular in developed countries in Europe and North America remains below the recommended levels. Indeed, DF is now considered as one of the five "nutrients of concern" in the U.S.A.[14]

Bread is widely consumed globally. The National Diet and Nutrition Survey (NDNS) estimates that adults in the U.K. consume 90 g of bread a day on average, which accounts for between 10 and 13% of their total energy intake,[4] while in places such as Central Asia, energy intake from bread can reach 60%.[15] Cereals are major sources of dietary fiber,[6] and it has been estimated that up to 20% of the daily fiber intake in the U.K. diet comes from consumption of bread alone.[4] Therefore, an increase in the dietary fiber content of bread is an attractive target to help attain health benefits in large sections of the population and indeed globally.

Wholemeal products are higher in fiber content compared to those made from white flour;[6] however, their taste and texture are less popular with consumers.[4,16] The National Association of British and Irish Flour Millers (NABIM) estimates that wholemeal flour production in the U.K. is only 7% of total flour production. Bread consumption shows similar trends, with white and wholemeal breads contributing to 65% and 17% of the total U.K. bread consumption, respectively.[4] In addition, socio-economic factors are likely to influence food choices.[17,18] People with higher educational levels and/or income tend to be more health aware and consume increased amounts of fiber-rich and wholemeal products, while those with lower educational levels prefer low-fiber foods.[17,18] Wholemeal/whole grain and white breads therefore appeal to very different consumer segments.[16] Increasing the dietary fiber content of white bread is of particular interest as it has the advantage that it promotes healthy eating without having to

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change eating habits. To avoid substantial effects on the palatability of products, most efforts to increase the dietary fiber content of breads and bakery products typically involve supplementation of the dough with fiber or partial substitution of flour with fiber-rich flours.[19,20] For example, addition of hydrocolloids[21] or fruit pomace[22,23] has been considered in wheat breads, while hydrocolloid supplementation has also been considered in gluten-free baking.[24,25] However, these processes have two drawbacks. First, they increase the cost of products, which may limit their consumption and second, the dietary effect of exogenously added fiber in breads may differ from that of the endogenously present wholegrain wheat fiber (which has established health benefits), and it is still not well understood. Consequently, increasing the content of endogenous fiber in white flour is a more attractive and sustainable option. A typical white wheat flour contains only about 2−3% dry wt cell wall polysaccharides, of which about 70% is arabinoxylan (AX) and 20% (1 → 3,1 → 4)-β-D-glucan (β-glucan). From a technological viewpoint, arabinoxylans found in wheat grains have the additional advantage of improving the bread-making quality of wheat flours.[26]

We have therefore screened a range of wheat genotypes in order to identify types of wheat which are rich in fiber in order to incorporate the high fiber trait into modern commercial cultivars.[26,27] The identification of cultivars, which vary by 2-fold or more in their content of AX in white flour, also provides an opportunity to explore the effects of fiber content on food digestion, and we have therefore compared the behavior of high fiber and normal white breads using a novel in vitro small intestinal digestion system. The aim of this study was to establish the methodology for the determination of duodenal digestion using a new model in vitro system and to demonstrate its application to high fiber white bread.

MATERIALS AND METHODS

Grains. Field plots (1.8 m × 10 m) of wheat cv. Yumai 34 and Hereward were grown at Rothamsted Research in 2011/12. Standard agronomy was used with 200 kg N/Ha. Grains were milled using a Buhler laboratory mill at Campden BRI to produce white flour (extraction rate 81.4%) and bread prepared as described below.

Bread Preparation. Bread was prepared at Campden BRI using the Chorleywood Bread Process (CBP). A 100 g portion of flour was added to a high-speed mixer together with 1.5 g of table salt, 2 g of yeast, 1 g of Bako fat emulsion, and 0.01 g of ascorbic acid. Bakezyme P180 (DSM, Delft, Netherlands) was added to 80 Farrad Units (based on HFN), and water was added based on the Brabender Farinograph (600 line) water absorption value (for the breadmaking process; see also reference 27). The materials were mixed for 3 min, and the dough was proofed for 45 min (40 °C, 70% RH) before baking (230 °C) for 20 min. The Hereward bread comprised of 42.1% carbohydrate, 7.1% protein, and 42% water. The Yumai-34 bread contained 4% more water due to increased water absorption by the fiber. As a result, the Yumai-34 bread comprised of 39.2% carbohydrate, 6.6% protein, and 48% water.

In Vitro Digestion. Two dynamic models were used in the in vitro digestion experiments: the dynamic gastric model (DGM) and the dynamic duodenal model (DDM), both with the ability to replicate real-time changes in mixing, shearing, pH, enzyme addition, and retention time. The DGM mimics gastric motility by squeezing of a conical tube acting as the gastric wall (detailed description of the DGM can be found elsewhere, e.g., Vardakou et al. (2011)[28]). The DDM was developed as part of this project, and it has been previously described.[29] In brief, it consists of an inner dialysis membrane (Spectre/Por 7, MWCO 8 kDa, diameter of 32 mm, cut in 250 mm long pieces) that simulates the luminal space and an outer nonpermeable silicone tube. The annular space between the membrane and the tube is initially filled with distilled water. The chyme and digestive fluids pass through the inner membrane. While the membrane is impermeable to macromolecules, the products of amylolysis pass through the membrane's pores and into the annular side. Chemically, these products are reducing sugars, and therefore as they pass to the annular space of the in vitro intestine, which was initially filled with distilled water, they increase its reducing potential. Sampling from the annular side with time therefore provides a way to determine how fast the food is being digested and estimate the simulated sugar absorption rate by measuring the increase of the concentration of those reducing sugars. Intestinal contractions in the DDM are simulated by squeezing of the tube and membrane.

Preliminary in Vitro Digestions. The DGM has been widely used and shown to have good reproducibility.[28,30] However, because the DDM has not been widely used or evaluated for reproducibility it was initially tested using (i) glucose solutions with and without guar gum and (ii) commercial white and wholegrain breads. Glucose solutions were prepared by dissolving the appropriate amount of glucose in distilled water to produce 500 mL of 1% by weight glucose. To these, guar gum was added with mixing to prepare solutions with 0, 1, and 2% by weight guar gum. A 700 mL portion of distilled water was added into the annular space of the DDM. Glucose solutions were then placed in the lumen, contractions were set to 2 per minute, and samples were taken from the annular side every 5 min for 90 min. Glucose was determined using the dinitrosalicylic acid (DNS) method (see Preliminary in Vitro Digestions). A typical example graph of glucose increase in the annular side over time is shown in Figure 1.

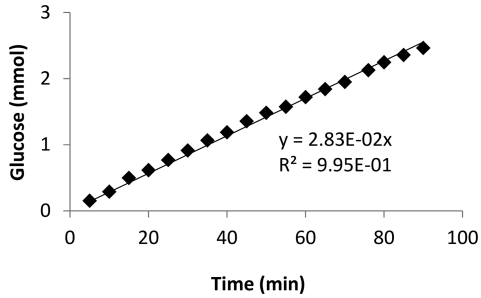

Figure 1. Example graph of glucose present in the annular side of the DDM with time showing linear increase.

The slope of the glucose curve is reported as the simulated glucose absorption rate in the results. White and wholegrain breads were digested using the same procedure as the Hereward and Yumai 34 (see In Vitro Digestion), except for gastric digestion, which was performed in a shaking incubator (30 rpm, 37 °C) in place of the DGM. Preliminary experiments were carried out with and without the application of simulated segmentation contractions, although detailed examination of how motility affects digestion rates is out of the scope of the current study; they have been reported elsewhere by the authors.[29] Average values of triplicate experiments and one standard deviation are reported in the results.

Solution Preparation. Digestive fluids were prepared based on literature[31,32] with some modifications. All digestive fluids used were prepared the previous day using distilled water except the enzymes which were prepared and added on the day of the digestion. All solutions were used at 37 °C.

Simulated salivary fluid (SSF) contained 0.15 M NaCl, 6 μg/mL lysozyme, and 29.7 U of porcine amylase/g carbohydrate in the sample; pH was adjusted to 6.9 with 1.0 M HCl.

Simulated gastric fluid (SGF) contained 0.9 mM NaH_2PO_4, 3 mM $CaCl_2{\cdot}2H2O$, 0.1 M HCl, 0.15 M NaCl, 16 mM KCl, and 63 U/mg protein in the sample of pepsin from porcine gastric mucosa; pH was adjusted to 2.5 using 1.0 M HCl.

Simulated intestinal fluid (SIF) contained 130 mM NaCl, 0.6 mM $CaCl_2{\cdot}2H_2O$, 0.2 mM $MgCl_2{\cdot}6H2O$, 1.22 mM KCl, 34.5 BAEE units/mg protein in the sample of trypsin from porcine pancreas, 0.4 BTEE units/mg protein in the sample of α-chymotrypsin from bovine

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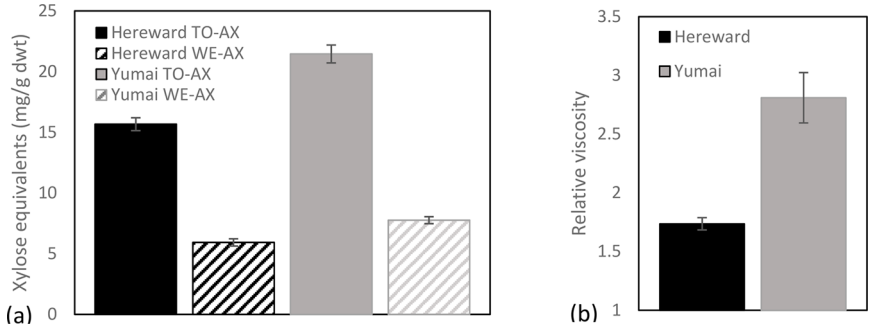

Figure 2. (a) Total (solid bars) and water extractable (hashed bars) arabinoxylan content (as xylose equivalents) of white flour from Hereward (black bars) and Yumai 34 (gray bars) wheat lines. (b) Relative viscosity of water extracts from the same flours.

pancreas, and 1.7 U/mg carbohydrate in the sample of porcine amylase; pH was adjusted to 6.5 with 0.1M HCl.

Modified Krebs-ringer buffer (MKRB) contained 0.7 mM $Na_2HP0_4 \cdot 12H_2O$; 4.56 mM KCl; 0.49 mM $MgCl_2 \cdot 6H_2O$; 1.5 mM $NaH_2P0_4 \cdot 2H_2O$; 80 mM $NaHCO_3$; and 54.5 mM NaCl,; pH was adjusted to 9 with 0.5 M $NaHCO_3$.

In Vitro Digestion Process. Digestion protocol was based on literature[28,31,32] with some modifications. For oral digestion, a 320 g portion of bread samples was cut into 4 cm^3 cubes and passed through a mincer with 112 mL SSF. To this, 224 mL of distilled water was added, and the mixture was mixed by hand for 1 min. The quantities of material used for oral digestion were based on the amounts required to pass through the mincer. This process was identical for all bread digestions.

Gastric digestion was performed at the Quadram Institute Bioscience (QIB) using the DGM. As the Hereward and Yumai 34 breads had slightly different compositions (see Bread preparation), amounts of breads and fluids were adjusted so as to ensure that the same amount of starch was digested in the gastrointestinal in vitro system. Similar adjustments were made in the digestions of the commercial breads. The following amounts refer to the Hereward bread digestion. The simulated stomach was initially conditioned with 20 mL of 0.1 M HCl prior to bolus addition, which is representative of the mean residual gastric volume of a fasted stomach.[33−35] A 150 g portion of bolus (containing about 75g of bread or the equivalent of approximately two slices) was added into the DGM together with 150 mL of SGF. Samples (40 mL each) of chyme were collected during gastric digestion at 6, 12, 18, 24, 30, and 36 min to mimic dynamic gastric emptying. For digestion of white bread, it was reported that at 36 min, nearly all of the liquid phase and 20% of the solid phase were emptied from the stomach.[36] The pH of the chyme samples was then increased to 6.5 using 0.5 M $NaHCO_3$, and the neutralized samples were immediately frozen to −80 °C. Frozen samples were transferred to the University of Birmingham (UoB) to undergo dynamic duodenal digestion.

Small intestinal digestion was carried out at UoB using the DDM. The inner membrane of the model was initially conditioned with 100 mL of MKRB, and a sample was taken from the annular side as a reference. A 80 mL portion of chyme (taken equally from samples digested in the stomach for up to 24 min) was thawed, the pH was adjusted to 2.5 with 1.0 M HCl, and the mixture was added to the rig. A sample was taken from the annular side, and 80 mL of SIF was added. This initiated the 2.5 h simulated intestinal digestion in which samples were collected from the outer tube every 5 min to determine reducing sugars. Simulated intestinal contractions were set at 2 cpm (this essentially translates to two contractions per minute; see Gouseti et al.[31] for more details). After 30 min, the remaining 80 mL of chyme was thawed, the pH was adjusted to 2.5 (with 1.0 M HCl), and the mixture was added to the DDM together with 80 mL of SIF. The intestinal digestion continued for a further 120 min.

Analyses. *Characterization of Flours and Breads.* Flours and breads were analyzed for pentosan content using the methods of Douglas (1981)[37] and Finnie et al. (2006).[38] The relative viscosity of aqueous extracts of white flour samples was determined using a micro-Ostwald capillary viscometer (AVS 370, SI Analytics Germany) and the method of Saulnier et al. (1995).[39] Relative viscosity was determined as the ratio of the sample viscosity over water viscosity. Pentosan and viscosity analyses were performed in triplicates. Average values are presented; error bars depict one standard deviation.

Doughs were scored for softness (scale 1−5 with 1 being tight, 3 optimum, 5 soft) and stickiness (A = sticky, B = slightly sticky, C = not sticky/optimum) by experienced bakers. Bread volume was measured by laser topography following the AACCI Method 10-14.01 Determination of Bread Volume by Laser Topography: BVM Method using a TexVol. Specific volume was determined by dividing the laser volume with the mass of loaves. Internal structure features such as cell diameter and wall thickness are C-Cell parameters that were measured following the AACCI Method 10-18.01 Measurement of Crumb Structure of Baked Products by C-Cell. Firmness was measured with a Stable Micro Systems TA-XT2 Plus texture analyzer following AACCI Method 74-09.01 Measurement of Bread Firmness by Universal Testing Machine.

Bread Digestion. The digestibility of the different breads was determined by measuring the rates of release of reducing sugars during simulated intestinal digestion using a modified DNS method as described in reference 40. The DNS reagent (0.1% DNS; 0.4 M NaOH; 30% potassium sodium tartrate) and sample were added in 1:1 volume ratio (1 mL each) in a test tube; they were vortex mixed and placed in boiling water for 5 min. Once cooled (under cold running water) to room temperature (20 °C), absorbance of the cooled product at 540 nm was measured and compared with those of standard solutions.

RESULTS AND DISCUSSION

Characterization of Wheat Flours. The wheat varieties Hereward and Yumai 34 were selected for the comparison of a standard and a high fiber white flour and white bread, respectively. Hereward was released in the U.K. as a hard winter wheat in 1992 and rapidly became accepted as the "gold standard" for bread making wheats, showing high and consistent quality over years and environments. It remained widely grown for about 15 years but is now outclassed by more modern cultivars in terms of yield and pathogen resistance. Yumai 34 is a Chinese breadmaking cultivar which was also highly successful in China, being described as a "landmark variety".[41] It was identified as having the highest contents of TOT-AX and WE-AX in a screen of 150 wheat genotypes grown together as part of the EU HEALTHGRAIN project,[42] but is not well-adapted to U.K. climatic conditions.

White flours produced by Buhler test milling contained different amounts of both total (TOT) and water extractable (WE) arabinoxylans (AX) (determined as pentosans, Figure 2a). The TOT-AX and WE-AX contents of the Yumai 34 flour were 21 and 7.8 mg xylose equivalents/g of dry-weight flour,

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respectively, which were 27% and 23% higher than those in Hereward (Figure 2a).

The WE-AX fraction is the major determinant of the viscosity of aqueous extracts of white flour,[43,44] and the relative viscosity (RV) of an aqueous extract of Yumai 34 was about 30% higher than that of a similar extract from Hereward flour (Figure 2b). De Santis et al.[43] have reported a linear relationship between the content of WE-AX content and the RV of the aqueous extracts of flours of 30 durum wheat cultivars.

Bread Formulations. White flours from the two wheat lines were used to produce white breads (see Bread Preparation). Images of slices are shown in Figure 3, and

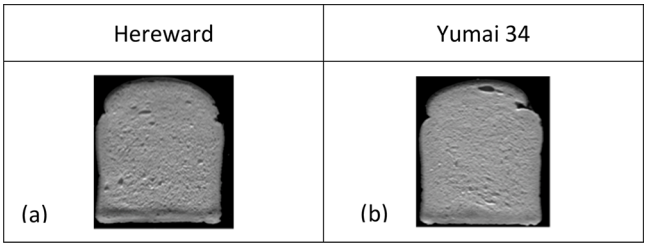

Figure 3. Images of bread slices prepared with white flours from Hereward and Yumai 34.

comparison of quality parameters (dough score, volume, specific volume, inner structural characteristics (cell diameter and wall thickness), and firmness) for the two investigated bread varieties are summarized in Table 1. The two breads are

Table 1. Comparison of the Dough and Bread Quality Parameters for Breads Prepared Using Hereward (Control) and Yumai 34 Wheat Grains

Parameter	Hereward	Yumai 34
Dough score	3C (optimum, not sticky)	3B/C (optimum, slightly sticky)
Volume (cm^3)	3430 ± 55	3186 ± 89
Specific volume (cm^3/g)	4.33 ± 0.13	4.00 ± 0.33
Cell diameter (mm)	1.341 ± 0.021	1.326 ± 0.045
Wall thickness (mm)	0.380 ± 0.004	0.382 ± 0.007
Firmness (g)	237 ± 45	255 ± 72

comparable in appearance, with similar, homogeneous inner structures (similar cell diameters and wall thickness). Although the Yumai-34 bread was lower in volume and firmer in texture, the differences with the standard Hereward bread were low, at 7%. The observed stickiness of the Yumai 34 dough is probably due to the higher fiber content compared to the standard Hereward flour.

The data presented demonstrate the potential to produce healthy white breads that are rich in dietary fiber without having to compromise the processing quality and palatability of the product, by using flour with inherent high fiber content. The white breads showed acceptable sensory attributes, comparable to those of a standard white bread loaf, and have the potential to make a meaningful contribution to the fiber deficit, as white bread remains high in consumer choices.

The contents of TOT-AX and WE-AX (determined as pentose in Figure 4) were higher in the Yumai 34 breads, by 12% and 39% higher, respectively, than in the Hereward breads. It was not possible to determine the relative viscosities

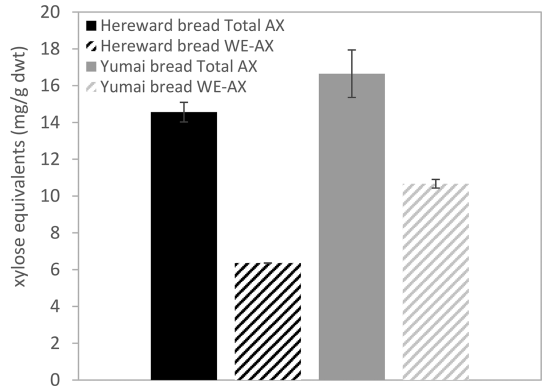

Figure 4. Pentose content (Total: solid bars; and WE-AX (soluble AX): hashed bars) (as xylose equivalents) of bread made from white flours of Hereward (black bars) and Yumai 34 (gray bars).

of aqueous extracts of the breads due to interference from gelatinized starch, which greatly increases the water absorption of bread compared to flour.

In Vitro Digestion. As the Hereward and Yumai 34 breads were only digested once, the reproducibility of the experimental setup was tested first using glucose solutions and commercial breads with and without the application of simulated segmentation contractions (see In Vitro Digestion). Without contractions, mass transfer in the gut principally occurs through diffusion.

Figure 5 shows the average simulated glucose absorption rates, with and without segmentation contractions, and one

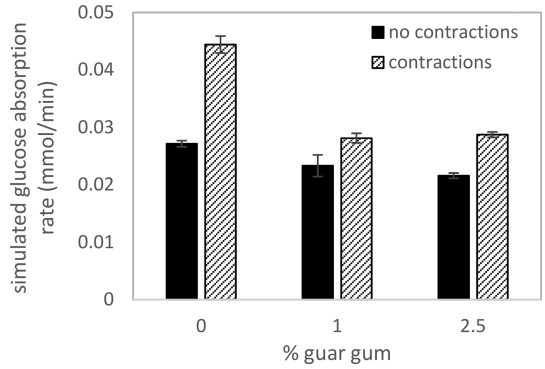

Figure 5. Simulated glucose absorption rate in the DDM for glucose solutions without and with guar gum and segmentation contraction.

standard deviation (error bars) from triplicate experiments using 1% w/w glucose solutions with and without guar gum (at concentrations 1% and 2% w/w). All error bars are ⩽8%, indicating that the setup was reproducible under the experimental conditions. The application of segmentation contractions resulted in faster glucose absorption, in particular for the pure glucose system (40% higher absorption rate) compared to the guar gum solutions (20% higher absorption rate on application of segmentation constrictions). This is because segmentations increase the rate of transfer of glucose molecules from the luminal space toward and through the intestinal wall for absorption (see also reference 31). As the viscosity of the system increased (e.g., by the addition of guar gum), the mass transfer due to segmentations was restricted and the glucose absorption rate decreased. The effect was similar when guar gum was added in 1 or 2% w/w

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concentrations. Without the segmentation contractions, all systems showed similar sugar absorption rates.

Digestion curves of commercial breads (white and multiseed) further confirmed the reproducibility of the method (average standard deviation <10%; see Figure 6) for bread

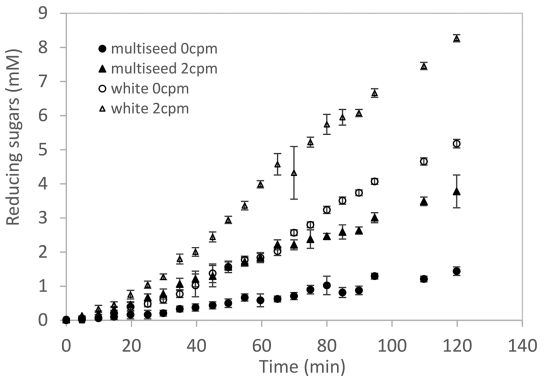

Figure 6. Simulated sugar absorption from commercial white and multiseed breads, with and without segmentation contraction, used to test reproducibility of the methods. Average values and one standard deviation from triplicates are shown.

samples. The shapes of all curves revealed an initial "lag" phase of about 10 min before sugar absorption was observed. This delay probably corresponds to the time required for luminal mass transfer and hydrolysis to take place. Following this lag phase, linear sugar absorption was observed, the slope of which is indicative of the digestion rate of each product and the associated bioaccessibility of the starch. The application of segmentation contractions increased the mass transfer and resulted in higher apparent digestion rates by >50% for both multiseed and white breads, the effect being more evident in the multiseed product. The white breads digested faster than the multiseed bread and showed more rapid glucose transfer to the annular side of the in vitro small intestinal model.

The digestion curves of the Hereward and Yumai 34 breads (Figure 7) show similar shapes as those of the commercial

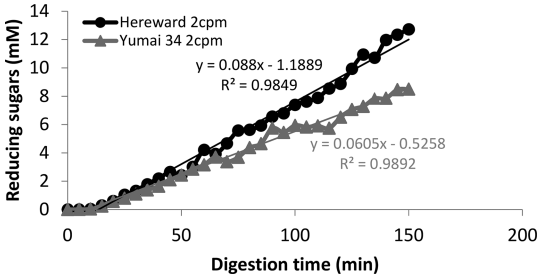

Figure 7. Simulated absorption of reducing sugars from a model small intestine digestion system (DDM) of Hereward (circles) and Yumai 34 (squares) white breads.

breads, featuring an initial lag phase (of about 10−15 min) followed by linear absorption kinetics. Although direct comparisons between the tested commercial breads and the breads developed in this work are limited within the space of the present work, it appears that both the Hereward and Yumai 34 loaves digested more slowly than the white commercial bread. After the initial lag phase, the high fiber Yumai 34 bread showed a reduced digestion rate by about 30% compared to the digestion rate of the Hereward bread, indicating slower digestion kinetics.

The slower digestion of the Yumai 34 bread is consistent with its higher content of TOT-AX and WE-AX (see Figure 4). It has been suggested that one mechanism by which increased fiber content may reduce the rate of digestion and absorption in the gut is by increasing the viscosity of the digesta.[29,45,46] This is consistent with the analysis of the guar gum solutions that is shown in Figure 5, but it is not possible to conclude whether this mechanism also contributed to the differences between the Yumai 34 and Hereward breads.

Overall, the present work demonstrates the potential to produce healthy, acceptable, and high-fiber white breads by exploiting genetic variation in the content of fiber (arabinoxylan) in white flour. This indicates the potential to develop white breads that are digested slower than the standard white breads and to contribute to increase in global fiber intake that is currently low.

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Notes

The authors declare no competing financial interest.

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