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Challenges to Increasing Dietary Fiber in White Flour and Bread

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ABSTRACT: Increasing the intake of dietary fiber from staple foods is a key strategy to improve the health of consumers. White bread is an attractive vehicle to deliver increased fiber as it is widely consumed and available to all socio-economic groups. However, fiber only accounts for about 4% of the dry weight of white flour and bread compared to 10–15% in whole grain bread and flour. We therefore discuss the challenges and barriers to developing and exploiting new types of wheat with high fiber content in white flour. These include defining and quantifying individual fiber components and understanding how they are affected by genetic and environmental factors. Rapid high throughput assays suitable for determining fiber content during plant breeding and in grain-utilizing industries are urgently required, while the impact of fiber amount and composition on flour processing quality needs to be understood. Overcoming these challenges should have significant effects on human health.

KEYWORDS: *Wheat, fiber, health benefits, analysis*

■ INTRODUCTION

The importance of adequate intake of fiber in the human diet has become increasingly apparent over the past few decades, with a number of studies showing that fiber intake is associated with reduced risk of a range of chronic diseases, including cardio-vascular disease, type 2 diabetes and several types of cancer.^{1–3} Consequently, regulatory authorities in many countries have set targets for the daily intake of dietary fiber which are generally within the range 25–30 g/day for adults.⁴ However, these targets are rarely met; for example, the average adult intake of dietary fiber in the USA is about 15g and in the UK about 20 g.⁵

Almost all of the fiber in the human diet comes from plants, with the most important sources being cereal grains, fruits, and vegetables. In particular, cereal grains provide between about 25% and 55% of total fiber intake in different countries,⁶ for example, about 40% of the total intake in the UK.⁷ Bread is the most widely consumed cereal product in many cultures, such as the UK where it provides about 20% of total fiber intake.⁸

A number of individual studies and meta-analyses^{2,9,10} have shown that diets rich in whole grains are particularly effective in reducing disease risks leading to the approval in the USA and Europe of health claims for either whole grains or grain fiber (<https://www.fda.gov/food/food-labeling-nutrition/health-claim-notification-whole-grain-foods>; <https://ec.europa.eu/food-feed-portal/screen/health-claims/eu-register>). These beneficial effects have not been demonstrated for products made from refined cereal grains, such as white bread, noodles, pasta and white rice,⁶ which have lower contents of a range of beneficial components including fiber. For example, white bread may contain about 4% fiber on a dry weight basis compared to up to 15% dry weight in some whole grain breads. The replacement of refined cereals with whole grains has therefore been widely promoted in dietary guidelines, including in the USA to make “half the grains whole”.⁶

However, such attempts to change dietary habits have largely failed, for example, the proportion of whole grain and brown wheat flours produced in the UK has actually declined over the past 20 years.¹¹ This is perhaps not surprising because eating habits have a strong cultural basis and are notoriously resistant to change.

An alternative strategy is to increase the fiber contents of cereal-based foods which are culturally accepted and widely consumed, which in many cultures are bread, pasta and noodles made with white flour.^{11,12} However, it has become clear that there are a number of challenges to achieving high and stable fiber contents in white flour and food products. The most important of these are discussed here.

■ DEFINING DIETARY FIBER IN WHITE FLOUR

Dietary fiber is defined by the Cereals and Grains Association (<https://www.cerealsgrains.org>) as comprising “carbohydrate oligomers and polymers that are not digested and absorbed in the small intestine and are partially or completely fermented in the colon. It also includes associated components such as lignin”. This definition includes fructo-oligosaccharides (fructans), which may comprise less than 10 monomeric units. However, the inclusion of carbohydrates comprising 3 to 9 monomers is optional under the Codex Alimentarius definition and it has been argued that fructans should be placed in a separate category of “prebiotics”.¹³

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White wheat flour is derived from the starchy endosperm tissue of the grain. This tissue accounts for over 80% of the dry weight of the mature grain and itself comprises about 80% starch and about 10% protein.¹⁴ The dietary fiber components in white flour can be classified into three groups: cell wall polysaccharides, other fermentable oligosaccharides and polysaccharides, and resistant starch, each of which poses challenges for characterization and quantification.

Cell Wall Polysaccharides. The cell walls of the starchy endosperm are defined as “primary” walls: this means that they do not undergo the secondary thickening, notably lignification, which occurs in most other plant tissues, including the pericarp (bran) of the grain. The major primary cell wall components in most plant tissues are insoluble microfibrils of cellulose (unbranched chains of β 1,4-linked glucose units) embedded in a matrix of pectin (a heterogeneous group of branched polysaccharides that contain negatively charged galacturonic acid units), the latter being abundant in the middle lamella which “glues” the cells together. However, none of these polysaccharides are particularly abundant in wheat flour and their actual amounts are still not conclusively established.¹⁵

In fact, the major polysaccharides in white flour are neither cellulose nor pectins but other types, which are often together called “hemicelluloses”.

The most widely quoted composition of cell wall polysaccharides in white flour dates from 1980 and lists 70% heteroxylan, 20% β -1,3;1,4-glucan (usually called β -glucan or mixed linkage β -glucan), 7% glucomannan and 2% cellulose.^{16,17} Heteroxylan corresponds to arabinoxylan (AX) which comprises a backbone of β -1,4 linked xylose some of which are substituted with arabinose residues at either position O-3 or positions O-2 and O-3.¹⁷

Most more recent studies have focused on AX and β -glucan, showing 1.35 to 2.75% dry weight AX and about 0.2% dry weight β -glucan.^{17–21} These two components therefore together account for between 2% and 3% of the flour dry weight, although this amount varies widely (as discussed below).

Less is known about the amounts and properties of other cell wall polysaccharides in white flour but a recent study showed 1.7 mol % arabinan, 64.3 mol % AX, 5.2 mol % β -glucan, 3.4 mol % heteromannan, 1.7 mol % xyloglucan and 23.7 mol % cellulose,²² the latter value being over 10-fold greater than the previously reported proportion of cellulose (as discussed below).

Verhertbruggen et al.²³ showed that white flour contained 0.2% dry weight unsubstituted β -1,4 linked mannan, but not glucomannan as reported previously, while mannan, xyloglucan and callose (β -1,3-glucan) have been identified in the starchy endosperm cell walls of developing grain by immunocytochemistry with specific antibodies.^{24,25}

In addition to cellulose and hemicelluloses, immunocytochemistry shows that pectic polysaccharides (rhamnogalacturonan-I (RG-I) and homogalacturonan) are also present in the cell walls of the starchy endosperm of developing and mature grain.^{25,26} However, these components have not been quantified.

Our knowledge of the composition of cell wall polysaccharides and other dietary fiber components in white flour is summarized in Table 1, while Table 2 and Figure 1 provide details of their structures.

It is clear that our understanding of the cell wall polysaccharides of the starchy endosperm cells of the wheat

Table 1. Summary of the Contents of Dietary Fiber Components in White Wheat Flour

| | | total cell wall polysaccharides (mol %) | white flour (% dry weight) |
|--|--|---|----------------------------|
| Cell wall polysaccharides | arabinoxylan/arabinan | 66 ^a | 1.35–2.7 ^b |
| | β -glucan (β -1,3;1,4-glucan) | 5.2 ^a | 0.2 ^c |
| | cellulose (β -1,4-glucan) | 23.7 ^a | nd |
| | heteromannan | 3.4 ^a | nd |
| | mannan | nd | 0.2 ^d |
| | xyloglucan | 1.7 ^a | nd |
| | callose (β -1,3-glucan) | nd | nd |
| | pectins | nd | nd |
| Fermentable oligosaccharides and polysaccharides | fructans | na | 0.5–2.0 ^e |
| | arabinogalactan peptide | na | 0.24–0.33 ^f |
| Resistant starch | | na | 0.4/0.5 ^g |

^aData from ref 22. ^bData from ref 18. ^cData from ref 20. ^dData from ref 23. ^eData from authors' unpublished results. ^fData from ref 28. ^gData from ref 29.

grain is still far from complete, with limited information about any components except arabinoxylans. One challenge is that most reports describe analyses of purified cell wall fractions rather than white flour. Furthermore, the compositions may be expressed either as mol % or weight %: these values are broadly similar but are not equivalent because the polysaccharides comprise both pentose (five carbon) sugars (xylose, arabinose) and hexose (six carbon) sugars (glucose, mannose).

Although the content of AX in white flour is known to vary by 2-fold (as discussed below), it is not known whether this variation is associated with changes in the contents of other cell wall polysaccharides, either positively (resulting in higher total fiber) or negatively (resulting in no change or even a decrease in total fiber).

Fermentable Oligosaccharides and Polysaccharides.

Fructans (fructo-oligosaccharides) and arabinogalactan peptide (AGP) are soluble non-cell wall components which contribute significantly to the dietary fiber (DF) fraction of white flour.^{27,28}

Wheat fructans are of the graminan (branched)-type, comprising mainly fructose residues but with a single glucose residue and both β -2,1-linkages and branches through β -2,6-linkages.³⁰ Their average degree of polymerization (DP) is 4, with the major components being DP 3, 4, or 5. A number analyses of fructans in whole wheat grain have been reported, showing concentrations ranging from below 1% to almost 3% dry weight.³¹ Little work has been carried out on white flour but Haska et al.²⁷ reported 1.5 to 1.7% dry weight while our analyses of a diverse set of samples shows variation between about 0.5% and 2% dry weight (authors' unpublished results).

AGP accounts for 0.24 to 0.33% dry weight of white flour and consists of a short (15 amino acid) peptide with three hydroxyproline residues which are *o*-glycosylated with branched arabinogalactan chains which comprise about 90% of the total mass.²⁸ AGP is not considered to be a cell wall component and its function is not known.³² Nevertheless, it represents a type of dietary fiber and is fermented by the colonic microbiota.³³

Table 2. Structural Characteristics of DF Components in White Flour and Methods for Routine Analysis

| Group | Component | Structure | Routine method |
|---------------------------------------|-----------------------------|--|--|
| cellulose | cellulose | β -1,4-D-glucose | as glucose after hydrolysis |
| hemicelluloses | arabinoxylan | Backbone of β -1,4-D-xylose residues with some residues substituted with α -L-arabinose residues at either position O-3 or positions O-2 and O-3. Some arabinofuranosyl residues at position O-3 of the xylan residues may be substituted with ferulic acid at the O-5 position which allows the formation of diferulate cross-links between adjacent AX chains | as xylose equivalents by phloroglucinol assay as arabinose and xylose after hydrolysis |
| | β -glucan | Polymer of D-glucose with single β -1,3 linkages which are usually separated by two or three β -1,4-linkages | as glucose after hydrolysis Megazyme β -glucan kit K-BGLU |
| | callose | β -1,3-D-glucose | as glucose after hydrolysis |
| | xyloglucan | β -1,4-D-glucan with three out of four residues substituted with α -D-xylose at the O-6 position. | as xylose and glucose after hydrolysis |
| | mannan | short unsubstituted chains of β -1,4-D-mannose residues with low acetylation | as mannose after hydrolysis |
| pectins | rhamnogalacturonan-I (RG-I) | Complex branched structure with backbone of repeated disaccharides galacturonic acid-rhamnose with arabinan and/or galactan and/or arabinogalactan side chains | as monosaccharides after hydrolysis |
| | homogalacturonan | linear homopolymer of α -1,4-D-galacturonic acid which is partially methyl-esterified at the C-6 carboxyl. | |
| resistant starch | | About 25% amylose, which is a mainly linear α -1,4-D-glucan with about 1% α 1,6-D-linkages forming branches, and 75% amylopectin which is more highly branched with about 5% α -1,6-linkages. | as glucose after hydrolysis |
| fermentable oligo and polysaccharides | arabinogalactan peptide | 15 amino acid peptide with three hydroxyproline residues which are α -glycosylated with β -1,3-D-galactan backbone with β -1,6-D-galactan side chains | as arabinose and galactose after hydrolysis |
| | fructans | β -2,1-D-fructose residues with a single glucose residue and branches through β -2,6 linkages. Most comprise 3, 4, or 5 units. | Megazyme fructan kit K-FRUC as fructose after hydrolysis (corrected for free fructose, raffinose and sucrose) |

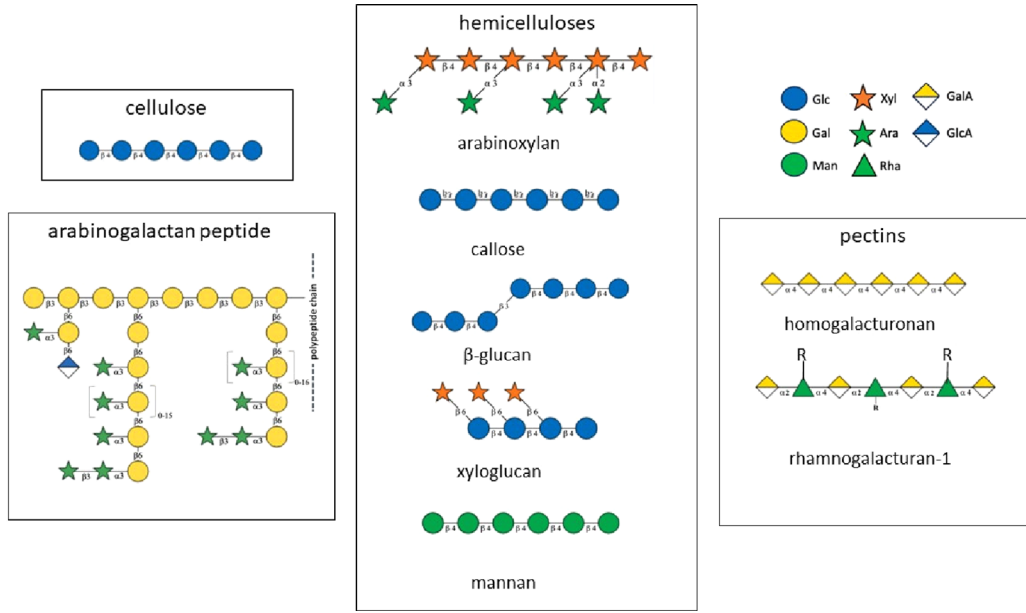


Figure 1. Schematic structures of major cell wall polysaccharides and AGP in white flour. Key: Glc, glucose; Gal, galactose; Man, mannose; Xyl, xylose; Ara, arabinose; Rha, rhamnose; GalA, galacturonic acid; GlcA, glucuronic acid. Circles are hexose (6 carbon) sugars; stars are pentose (5 carbon) sugars; diamonds are sugar acids (uronic acids). The structures are based on reported analyses of components from wheat except for pectins and xyloglucan. The homogalacturonan structure shown is a generic structure based on pectins from other sources. The structure of RG-I is based on ref 24 which detected RG-I in wheat using an antibody which recognized the repeating GalA-Rha epitope. This repeated disaccharide is typical of RG-I from other sources where the rhamnose residues may be decorated with arabinan and/or galactan and/or arabinogalactan side chains (indicated as R). The xyloglucan structure is based on the heptasaccharide unit present in xyloglucan from tamarind where one or two of the xylose residues may be decorated with galactose residues to give octasaccharides and nonasaccharides.⁸⁶

Resistant Starch. Resistant starch (RS) is defined as starch and partial digestion products of starch which escape digestion in the small intestine and pass into the colon. It is classified

into several types,³⁴ two of which are relevant to wheat flour and bread. RS2 is resistant to digestion in its native state and includes high amylose starches in cereal grains. RS3 is formed

when starch is heated and then recrystallizes (retrogradation) and is again higher in high amylose starches.³⁵ For example, Schonhofen et al.²⁹ reported mean values of 0.4 and 0.5% dry weight for RS in flours of two bread wheat cultivars grown in multiple environments and 3.9 and 6.0% dry weight, respectively, in flours of high amylose genotypes (with mutations in the starch branching enzyme II (SBEII)).

■ DETERMINATION OF FIBER IN WHITE FLOUR

As discussed above, the DF fraction of white flour is a complex mixture of carbohydrates that differ widely in their mass and properties, from soluble trisaccharides and oligosaccharides to large soluble and insoluble polymers. Consequently, DF analysis is challenging, with no single methodology providing a complete picture of amount and composition.

Several methods of analysis of dietary fiber in foods are recommended by the Codex Committee on Methods of Analysis and Sampling (Codex Alimentarius Recommended Methods of Analysis and Sampling, CXS no. 234-1999. Amendments adopted by the 44th Session of the Codex Alimentarius Commission in 2021). While these methods measure different classes of polysaccharide, their accuracy and specificity can vary³⁶ and most do not distinguish between different types of polysaccharides. Specific methods are only recommended for β 1,3;1,4-glucan, fructans, trans-galactooligosaccharides, and resistant starch (recommended only for RS3).

Determining Polysaccharides. Cell wall polysaccharides and AGP can be determined together, with distinctions sometimes being made between soluble and insoluble components. However, the solubility of any given polysaccharide is determined by the extraction method used.^{37–39} Aqueous ethanol (76%) is routinely used to precipitate high molecular weight soluble fiber, separating it from lower molecular weight soluble fiber and non-digestible oligosaccharides, and it has been suggested⁴⁰ that soluble fiber should be divided into two types, which are soluble either in water but not 76% aqueous ethanol or in both in water and 76% aqueous ethanol. However, other methods that simply incubate samples in aqueous solutions or water before the determination of fiber content⁴¹ are also widely used, where temperature plays a crucial role in determining the amount of fiber extracted from samples.³⁷ There is practical interest in quantifying soluble fiber as it determines the viscosity of solutions which has been associated with specific health outcomes.^{42,43}

The most accurate method to measure polysaccharides is to determine the monosaccharides released by acidic hydrolysis after enzymic digestion of the starch in the sample. However, because starch accounts for 80% of flour and is composed of glucose (which is also present in several cell wall polysaccharides: cellulose, glucomannan, β -glucan, callose, and xyloglucan), it is crucial that starch digestion is complete. Monosaccharide analysis is the basis of the Englyst⁴⁴ and Uppsala⁴⁵ methods which are accepted as the gold standard by many cereal scientists.

However, whereas hydrolysis provides accurate data on the sugars present in DF it does not quantify the individual components. Notably, glucose may be derived from cellulose, β -glucan, callose, xyloglucan, and any starch which is present (whether as genuine RS or due to insufficient destarching of the samples). Similarly, arabinose is derived from AX, AGP, and RGI, xylose is derived from AX and xyloglucan, and galactose is derived from AGP and RG-I.

The amount of AX is often calculated by combining the amounts of arabinose and xylose after adjusting the amount of arabinose for that present in AGP (assuming that all of the galactose is present in AGP which has an arabinose:galactose ratio of 0.69).⁴⁶

Both AX and β -glucan are routinely determined using specific assays. The Megazyme β -Glucan Assay Kit (Mixed Linkage) (K-BGLU, Neogen/Megazyme Ltd., Bray, Ireland)⁴⁷ is widely used and regarded as highly specific. Similarly, the phloroglucinol colorimetric assay⁴⁸ is specific for pentose sugars and is widely used to determine AX. The assay is specific and accurate but AX is determined as “xylose equivalents” which cannot be precisely converted to % dry weight. Other cell wall polysaccharides (xyloglucan, mannan, callose, and pectins) are minor components and their contributions are often ignored when calculating DF “balance sheets”; either because they do not contribute significantly to the total dietary fiber in a sample or because the techniques used to quantify them lack sufficient sensitivity.

Fructans require specific analytical methods because of the presence of glucose and/or fructose in several polysaccharides, which may interfere with fructan quantification after acid hydrolysis. Such methods include the Megazyme K-FRUC kit (Neogen/Megazyme Ltd., Bray, Ireland) which removes interfering sugars through stepwise enzymatic degradation and chemical reduction⁴⁹ and the HPAEC-based method of Verspreet et al.⁵⁰ which corrects for free fructose in the sample, as well as fructose derived from hydrolyzed raffinose and sucrose. Similarly, RS can be determined using the Megazyme K-RSTAR kit (Neogen/Megazyme Ltd., Bray, Ireland) which specifically removes nonresistant starch, leaving RS for enzymatic quantification.

Methods for analysis of fiber components in white flour are summarized in Table 2.

Do Analyses of White Flour Predict the DF Contents of Foods? It is often assumed that the fiber content of white flour is an accurate predictor of the fiber content of foods made from the flour. However, this is not necessarily true.

Fermentation during breadmaking, using either yeast or sourdough systems, results in significant changes in composition, particularly in the concentrations of soluble sugars, amino acids, organic acids and sugar alcohols.⁵¹ Limited effects on the contents of cell wall polysaccharides may also occur, but these are generally too small to have significant effects on total dietary fiber.

The most significant effect of fermentation is on the fructan content. There is extensive literature on the effects of sourdough systems on fructan content showing that long fermentation can result in almost complete elimination.⁵² However, the extent of reduction depends on the sourdough system that is used, and fructans are also fermented by conventional yeast systems. For example, a recent comparison of breads made from whole meal flour showed that the content of fructans fell from 1.23% in flour to 0.56% in sourdough bread and 0.41% in yeast bread.⁵¹

Pasta and noodles are not fermented, but water-soluble dietary fiber components may be lost during cooking. For example, Gelinis et al.⁵³ reported that 40–50% of fructans and 25% of AGP are lost when pasta is boiled.

The relationships between the types and contents of RS in flours and wheat-based foods are incompletely understood. RS2 is present in flour, the proportion being greater in high amylose wheats. However, starch gelatinizes during cooking

and recrystallizes (retrogradation) during cooling to give RS3. The concentration of RS3 in cooked foods is generally higher than that of RS2 in flours and is also higher in high amylose wheat. However, the precise stoichiometric relationships between the contents of RS2 in flour and RS3 in cooked foods, and their resistance to digestion *in vivo* (as opposed to during *in vitro* digestion), are incompletely understood. Consequently, it is not possible to accurately predict the amounts of RS in cooked products from the analyses of white flours.

■ STABILITY OF FIBER CONTENT IN WHITE FLOUR

Variation in crop composition and properties is a major challenge to food processors because it may have significant effects on processing quality and, in the case of proposed health benefits, affect the validity of labeling (such as for “high fiber”) and health claims.

However, in a broader context, variation between crop genotypes is the basis for crop improvement as it allows the selection of new cultivars by plant breeders. The real challenge is therefore to minimize the variation between samples of the same cultivar. This variation results from the effects of environmental factors, including weather conditions, soil types and properties, agronomic treatments, and farming systems, and the interactions of these factors with the crop genotype. For example, the quality of breadmaking wheat may be reduced if it is grown with insufficient nitrogen or if the weather conditions during grain development and maturation are too cool and wet, but the extent of these effects varies between genotypes.

Analyses of collections of genotypes grown in field trials in multiple environments allows the variation in grain composition to be partitioned between the effects of three factors: the crop genotype, the environment, and the interactions between genotype and environment. The proportion of the total variation controlled by the genotype is often called the “broad sense heritability”.

Several studies of the heritability of AX in white flour have been reported with varying results. Martinant et al.⁵⁴ calculated the broad sense heritability of soluble AX in 19 cultivars grown in France as 0.75 while Shewry et al.⁵⁵ reported 0.60 heritability for soluble AX and 0.72 heritability for total AX based on multisite trials of 26 lines. Similarly, Hernandez-Espinosa et al.⁵⁶ reported heritabilities of 0.70 for total AX and 0.81 for soluble AX in 193 wheat lines grown in Mexico and Tremmel-Bede et al.⁵⁷ 0.516 for total AX and 0.840 for soluble AX in Hungarian lines which had been selected for high AX in white flour. However, two studies of wheat grown in the USA showed stronger effects of environment. Thus, Finnie et al.⁴¹ showed that whereas genotype was the major determinant of variation in the amounts of total and soluble AX, environment had a greater effect on the amount of insoluble AX, while Li et al.⁵⁸ reported that environment had much greater effects than genotype on both soluble AX and total AX.

The heritabilities of other fiber components in white flour have not been determined, but Veenstra et al.⁵⁹ showed that genotype, environment, and genotype \times environment accounted for 53.4%, 19.4%, and 24.5%, respectively, of the variation in fructan content of whole grains of 288 genotypes grown on three sites for two years (i.e., 6 environments).

Further variation in flour composition will result from milling procedures and, in particular, in the proportion of the starchy endosperm that is recovered as white flour. Although

the starchy endosperm accounts for 82 to 83% of the total wheat grain,¹⁴ the actual recovery by milling (called the extraction rate) varies from about 60% for noodle production in some Asian countries⁶⁰ to about 80% for breadmaking flour in Europe. However, because the starchy endosperm is difficult to separate cleanly from the bran the extent of bran contamination increases with higher extractions rates.⁶¹

Contamination of white flour with bran affects the quality of the flour for processing, including whiteness, which is crucial for some types of noodles.

The extraction rate also affects the fiber content of the flour for two reasons. First, bran comprises 45–50% fiber¹⁴ and the presence of even a small proportion can have a significant effect on the fiber content of white flour. Second, cell wall polysaccharides are unevenly distributed within the starchy endosperm, being more highly concentrated in the outer (subaleurone) layers of cells⁶² and this is reflected in the compositions of millstreams.⁶³ Hence, milling procedures will need to be standardized to deliver flours with defined and reproducible fiber contents.

■ HIGH THROUGHPUT ANALYSIS OF FIBER CONTENT

The development of rapid, high throughput, and affordable assays for determination of fiber is essential to underpin the development and exploitation of new types of high fiber wheat.

The complexity of the dietary fiber fraction and the high cost and low throughput of fiber analysis are clearly constraints on screening white flour. A further limiting factor is the low throughput of laboratory scale milling to produce white flour, generally less than 20 samples a day. Furthermore, the flour yields from laboratory scale mills are generally low, 50% or less compared to up to 80% for commercial milling, and variation in the flour yield and in the performance of the mill will result in variability in the analyses.

The development of screening methods has therefore focused on two targets: the determination of AX as the major fiber component in white flour and the prediction of AX in white flour from analyses of whole grain flour.

Rapid Analysis of AX. Arabinoxylans differ from the other major DF components in wheat grain (β -glucan, cellulose, resistant starch, fructans) in consisting of pentose sugars (xylose, arabinose). This allows the use of a specific spectrophotometric assay (based on binding to phloroglucinol) for pentose sugars.^{41,48,64} This assay is sufficiently sensitive and robust (coefficient of variation of around 15% for white flour) for use in screening and has been adapted to a high throughput format⁶⁵ which allows for the analysis of up to 150 samples per day by two trained technicians. Because of the nature of the assay, it is usual to express the total contents of arabinose plus xylose in relation to a xylose standard as “xylose equivalents”. The Theander (AOAC 994.13) method⁴⁵ has also been modified to increase the throughput of analysis of AX in white flour samples.⁶⁶

Soluble arabinoxylan is the major determinant of the viscosity of aqueous extracts of white flour. The relative viscosity (the viscosity of the solution relative to the solvent, RV) of aqueous extracts of white flour has therefore been widely used as a proxy for water-extractable (WE)-AX in genetic and diversity studies which require the analyses of large numbers of samples.^{67–69}

RV can be measured manually on single samples or by using automated viscometers. The specific viscosity (the ratio of the

absolute viscosity to the reference fluid, SV) of aqueous extracts can also be determined online during polymer analysis using viscometers in conjunction with size-exclusion chromatography multiangle-light scattering (SEC-MALS) systems such as the Wyatt Visco-Star system (Wyatt Technology Corporation, Santa Barbara, California, USA).

The solvent retention capacity (SRC) test⁷⁰ determines the ability of flour to retain a set of four solvents that are preferentially absorbed by one or more of the major grain components: 5% lactic acid by glutenin, 5% sodium carbonate and 50% sucrose by starch, and 50% sucrose by pentosans (AX). Hence, the relative retentions of the solvents can be used to predict aspects of the grain composition and quality. Because sucrose is preferentially retained by pentosans, it has been proposed as a predictor of AX content. However, SRC was developed to identify aspects of quality based on the balance of retention by the four solvents, and the use of single solvents to measure individual components (such as sucrose solution for AX) has not been validated.

Near infrared spectroscopy (NIRS) is widely used by researchers as well as in the food and feed industries for determining grain components such as protein and for predicting quality traits.^{71–73} It has also been evaluated for determining AX in flours and grain⁷⁴ but has not so far proved to be sufficiently robust when used to analyze sample sets from different sites and years (authors' unpublished results).

Finally, the availability of specific antibodies may allow enzyme-linked assays (ELISA) to be developed for semi-quantitative analysis, as described for AX,⁷⁵ but this approach has not been developed further as a screening tool.

Determination of AX in White Flour by Analysis of Wholemeal. Wheat bran is rich in AX, accounting for up to about 20% of the dry weight. It is therefore not surprising that comparisons of wholemeal and white flours show no correlations between their total contents of AX (Table 2). However, only a small proportion of the AX in bran is water-soluble, about 2 to 5% of the total AX, compared with 20–50% of the total AX in white flour.¹⁸ Hence, the content of WE-pentosans in wholemeal is highly correlated with that in white flour (0.96), while the relative viscosity (RV) of aqueous extracts of wholemeal flour also correlates with the RV (0.72) and content of WE-pentosans (0.77) in white flour (Table 3).

The ability to predict the content of WE-AX from analyses of wholemeal flours would clearly reduce the cost and increase the sample throughput for screening. However, this may have significant drawbacks.

Table 3. Correlations between Pentosan Contents and Relative Viscosity (RV) of Aqueous Extracts of Wholemeal and White Flours of Wheat Grain Samples Differing in the Content of Arabinoxylan in White Flour (Data from Lovegrove et al., 2020)

| | White flour relative viscosity | White flour WE- pentosans | White flour TOT- pentosans |
|------------------------------------|--------------------------------------|------------------------------|----------------------------------|
| Wholemeal relative viscosity | 0.72 | 0.77 | 0.38 |
| Wholemeal WE- pentosans | 0.50 | 0.96 | 0.52 |
| Wholemeal TOT- pentosans | −0.25 | 0.18 | 0.23 |

First, the content of WE-AX may not correlate with TOT-AX: as noted above, WE-AX varies from about 20%–50% of TOT-AX in white flour.

Second, it is often assumed that the AX content of white flour is correlated with the content of total fiber, and there is no evidence for this. In fact, it is possible that increases in the AX content are accompanied by compensatory decreases in other cell wall polysaccharides, resulting in conservation of the thickness and biomechanical properties of the cell walls.

Finally, the concentrations of other soluble fiber components (β -glucan, fructans, and AGP) also vary and may also affect the RV.

Molecular Breeding. The exploitation of molecular tools has revolutionized crop breeding, particularly of high value and hybrid crops.

The simplest approach is marker-assisted selection (MAS) in which variation in DNA sequences (usually single nucleotide polymorphisms, SNPs) within or closely linked to genes that determine key traits is selected rather than the traits themselves. MAS is particularly effective when applied to “quantitative” traits which are controlled by several genes as opposed to classical Mendelian traits controlled by single genes.⁷⁶ Few studies have been reported on the genetic control of AX in white flour, as opposed to wholemeal.^{68,69,77} These show that multiple loci affect the content and solubility of AX, two of which have been studied in detail: these are a locus on chromosome 1B which controls the contents of total and soluble AX and a locus on 6B which controls the proportion of soluble AX. The gene at the 6B locus has recently been characterized showing that the allele for high WE-AX encodes a defective peroxidase: this reduces the level of cross-linking of AX by diferulate and hence increases the solubility of AX.⁷⁸ The identification of the precise gene allows “perfect” markers for the high soluble AX allele to be designed. By contrast, the responsible gene at the 1B QTL has not been identified and hence the currently available markers are linked but not perfect.^{69,77} In addition, several other “high-AX” loci have also been mapped.⁶⁸ It has been calculated that a 2-fold increase in total AX would have a significant impact on the daily intake of fiber in the UK.⁷⁹ Achieving this may require the use of several markers, adding to the cost and complexity of breeding.

However, classical breeding based on selection for individual traits (either directly or by using MAS) is increasingly being replaced by genomic selection. Genomic selection uses high density genotyping and phenotyping of traits in populations carried out over several years to predict performance, rather than to identify individual loci for traits.⁸⁰ Hence, it allows the simultaneous selection of multiple genes controlling quantitative traits, including multiple fiber components.

Genomic selection is undoubtedly the system of choice for modern plant breeding, but it requires financial and technical resources beyond those available to many traditional wheat breeders.

■ EFFECT OF FIBER ON FLOUR PROCESSING PROPERTIES

Fiber is the third most abundant component in white flour, after starch and protein, and it has a significant effect on processing properties. Its most important characteristics are water binding and viscosity. Notably, water-insoluble arabinoxylans absorb about $\times 10$ their dry weight of water and water-soluble AX about $\times 11$ ^{81,82} while water-extractable AX accounts for about two-thirds of the intrinsic viscosity of aqueous

extracts of white flour.⁸³ We have also shown that AX, β -glucan and AGP may contribute significantly to water absorption of white flours.⁸⁴

A number of studies of the effects of AX on breadmaking have been reported, showing effects on a range of properties which vary depending on the source of the fiber (from wheat flour, wheat bran, or other sources), the bread recipe, and the breadmaking process. Effects of variation in endogenous AX on breadmaking performance have been reported,⁸⁵ while the effects of supplementation with AX and endoxylanase treatment have been reviewed in detail by Courtin and Delcour.⁸³ The latter conclude that whereas soluble AX may increase gas retention, which is the most important aspect of breadmaking, this is reduced by insoluble AX. Consequently, endoxylanases, which are already widely used in the food and feed industries, may be used to optimize the balance between soluble and insoluble forms to improve breadmaking quality.

CONCLUSIONS

To conclude, increasing the intake of dietary fiber is a major priority globally but has been largely targeted by the promotion of high fiber foods, particularly whole grain products, which have low consumer acceptability compared to products made from white flour. The major DF component in white flour is AX which varies in concentration by about 2-fold. This fraction is therefore the focus of breeding efforts to develop genetically enhanced high fiber wheat flour. However, AX only accounts for about half of the total fiber and less is known about variation in the other components. We therefore need greater understanding of the whole fiber fraction in white flour and improved methods of fiber analysis are urgently required to facilitate these studies. Similarly, the exploitation of variation in fiber composition in wheat breeding programs is limited by requirement for simple high throughput methods to measure fiber in white flour.

Ultimately, the greatest challenge is likely to be to reduce the variation in fiber content between grain samples grown in different environments. AX shows high heritability, generally reported as 70–80%, which is high compared to other traits, such as yield, and indicates that it should be amenable to increase by plant breeding. However, this level of heritability also means that the content of AX may vary by 20–30% between samples of the same cultivar. For example, the fiber contents of samples of wheat bred to have 6g fiber per 100g dry weight, which is required for labeling as “high fiber” in the UK, can be expected to range between below 5g and above 7g. Breeding for greater stability is acknowledged as an important target for other wheat grain quality traits in relation to the greater fluctuation in weather conditions associated with climate change and is most likely to be achieved by genomic selection.

Finally, it is important to ensure that the fiber contents of flour samples are standardized by optimizing the performance of mills and that recipes and processes are modified to produce products with high consumer acceptability. This applies not only to hard bread wheat for white bread and noddles, which is the focus of this article, but also to soft wheat for cakes, cookies and pastries, and to durum wheat for pasta.

These challenges will require substantial investments, but the importance of fiber in health and the potential gains in improved quality of life and reduced health care costs mean that this investment is fully justified.

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P.R.S. wrote the original draft, A.P., O.K., and A.L. edited and added to the draft.

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