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REVIEW PAPER

Cell wall polysaccharides in cereals: chemical structures and functional properties

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Abstract β-Glucans and arabinoxylans are the two primary cell wall structural components in cereals, such as wheat, oat, barley and rye. The relative amounts of the two polysaccharides vary with species and growing environments. The cell walls of barley and oats are generally rich in β-glucan, whereas rye and wheat cell walls contain higher levels of arabinoxylans. Cereal β-glucan is a mix linked $(1 \rightarrow 3)$ $(1 \rightarrow 4)$-β-D-glucan composed of two major building blocks: a trisaccharide and a tetrasaccharide unit: the combination of the two units is over 90%. The ratio of the two building blocks is used as a fingerprint for each β-glucans: it is 4.5, 3.3, 2.2 for wheat, barley and oat β-glucan, respectively. Of the two types of cell wall polysaccharides in cereals, β-glucan received greater attention due to its proved beneficial physiological effect as an excellent source of soluble dietary fibre for significantly attenuating blood glucose and insulin levels and its demonstrated ability to reduce low-density lipoprotein cholesterols (LDL) in serum. The ability of cereal β-glucan to attenuating blood glucose, insulin levels is linked to the viscosity produced by cereal β-glucans in a linear relationship. It is also demonstrated that the functional properties of cereal β-glucans are determined by their structural features and molecular weight: a higher trisaccharide to tetrasaccharide ratio favours gel formation and faster gelation process, and ultimately, gives stronger gels. Cereal β-glucan also demonstrated an unusual behaviour by forming gel faster and yielding stronger gels at lower molecular weight (above minimum gelation molecular weight). Such a structure–function relationship was established based on rheological, light scattering and computer modelling studies which cover both dilute and concentrated concentration regimes: high tri/tetra ratio gives high proportion of consecutive trisaccharide unit which favours the intermolecular association of β-glucan chains; on the other hand, low molecular weight chains have higher mobility that promotes intermolecular chain–chain interactions, hence, lead to faster gelation process and formation of stronger gels. Research also revealed that processing and storage conditions, such as temperature, pH, extrusion, baking and frozen before eating, have significant effects on the bioavailability of cereal β-glucans, hence, its ability to reduce blood glucose and cholesterol levels.

Keywords Cereal β-glucan · Polysaccharide · Structure · Functional properties · Physiological effect

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Introduction

Cell walls are the major source of dietary fibre which are multi-component composites involving polysaccharides, structural proteins and in some cases, lignin. $(1 \rightarrow 3)$, $(1 \rightarrow 4)$-β-glucans and arabinoxylans (AX) are the major structural components of cereal endosperm cell walls, which differentiates themselves from the other plant cell wall structural components: i.e. cellulose, xyloglucan and pectin. Although the chemical structures of individual component of cereal cell walls have been determined, how these macromolecules are oriented and interact with each other in the native state of cell walls are still not fully understood. In cereal grains, plant cell wall materials have a major impact on the grain processing (milling, baking, malting etc.) and the quality of the end product, including textures, organoleptic properties, shelf life and nutritional

values. Much research interests have been focused on mixed (1 → 3), (1 → 4)-linked β-D-glucans due to their significant physiological benefits and bioactivities towards human health. Animal studies and human clinical trials have led to the health claim of oats- and barley-based products specifically attributed to the levels of (1 → 3) (1 → 4)-linked β-D-glucans [1–4]. The current article reviews the most recent advancement in cereal β-glucans, covering extraction process, structural characteristics, structure–function relationships and potential applications.

Sources and structures

Cereal β-glucan is a polysaccharide occurs in the sub-aleurone and endosperm cell walls of the seeds of cereals, including oats, barley, rye and wheat. A typical distribution of the β-glucans in grain seeds is illustrated in Fig. 1. The fluorescence micrographs indicate the distribution of β-glucans along the endosperm cell walls in oats and barley kernels, respectively. β-glucan in oats is more concentrated in the sub-aleurone layers than in the endosperms; in contrast, a more even distribution of β-glucan is observed for barley and rye grains. Although there is a lack of information where exactly the β-glucan resides in the seed of wheat, it is likely concentrated in the walls of the sub-aleurone cells as evidenced by the enrichment of β-glucans in the bran fractions using a debranning or conditional milling processes [5, 6]. The level of β-glucans in cereal kernels varied from as low as 0.5–1% in wheat to as high as 3–9% in barley, as shown in Table 1.

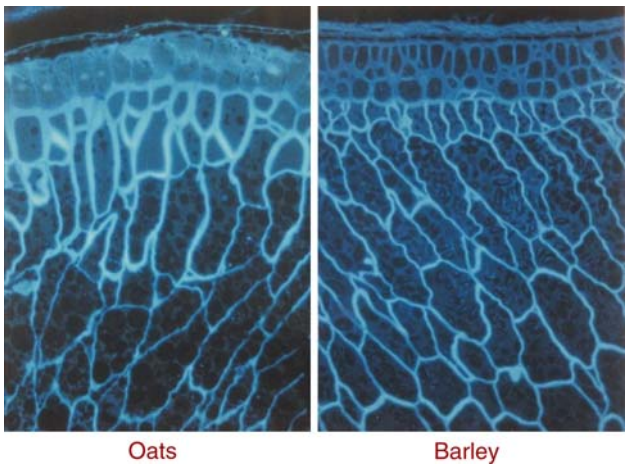

Fig. 1 Fluorescence micrographs of sections of oat (*left*) and barley (*right*), showing the fluorescence (*white*) of calcofluor-stained β-glucan in the endosperm cell walls. Oat, which has thicker cell walls, is enriched in β-glucan. Adapted from Wood [4]

Table 1 Chemical composition of cereals kernels

	Starch (%)	Protein (%)	β-Glucan (%)	Pentosans (%)
Wheat	64	11–16	0.5–1	2–6
Barley	46	11	3–9	Smaller amount
Oat	59	16	2–6	Smaller amount

The structure of cereal β-glucans is typical of a linear homopolysaccharide as it contains only a single type of sugar unit, i.e. β-D-glucopyranose (β-D-Glc*p*). Over 90% of the β-D-Glc*p* residues in cereal β-glucans are arranged as blocks of two or three consecutive (1 → 4)-linked units separated by a single (1 → 3)-linked unit, which forms the two types of building blocks of cereal β-glucans: a cellotriosyl unit and a cellotetraosyl unit, as shown in Fig. 2. The remaining 10% of the polymer chain is mainly composed of longer cellulosic sequences ranged from 5 up to 14 β-D-Glc*p* residues. The structural features of cereal β-glucans are represented by the oligosaccharide profiles released by a specific enzymatic hydrolysis (lichenase: EC 3.2.1.73, *endo*-1,3(4)-β-D-Glucanase). Of the oligomers released, the trisaccharide and tetrasaccharide units are particularly important and their ratio constitutes the finger print of a particular grain. For example, the tri/tetrasaccharide ratio for β-glucan from wheat, barley and oats are 4.5, 3.0 and 2.3, respectively (Table 2) [7].

The C-13 NMR spectra of the three β-glucans appeared same, which is not surprising, because the three β-glucans shared the same structural features: they all contained 1 → 3 and 1 → 4 mix linked β-glucopyrinosyl units. As shown in Fig. 3, there are four clearly identified resonances: three anomeric signals (101.9–103 ppm); a single 3-linked glucopyrinosyl signal (86.4 ppm), three 4-linked glucopyrinosyl signal (79.1, 79.2 and 79.3 ppm, respectively) and non-linkage sugar ring resonances (60.1–74.6 ppm). The single peak at 86.4 ppm indicates that there is only one type of 1,3 linkage. In other words, the NMR spectra confirm there is no consecutive β-1,3-linkage in cereal β-glucans. On the contrary, split signals from 79.1 to 79.3 ppm indicates there are a number of types of 1,4 linkages in the β-glucan chain. Two dimensional NMR spectroscopic techniques such as H^1–C^{13}-heteronuclear correlation, COSY, TOCSY were used to derive all the signals on the same sugar ring, while the long range Heteronuclear Multi-Bond Correlation spectroscopy was used to establish the connectivity between two neighbouring sugar units through space coupling. By summarizing all the information obtained from these one- and two-dimensional NMR spectroscopy, an unambiguous assignment of all NMR resonant signals for each proton and carbon of every glucose residue was achieved, as shown in Table 3 [6]. Based on the structural information gathered, a model structure of cereal β-glucan is proposed as shown in Fig. 4. It is believed that the distribution of the trisaccharide and tetrasaccharide building blocks is random unless future evidence indicates otherwise.

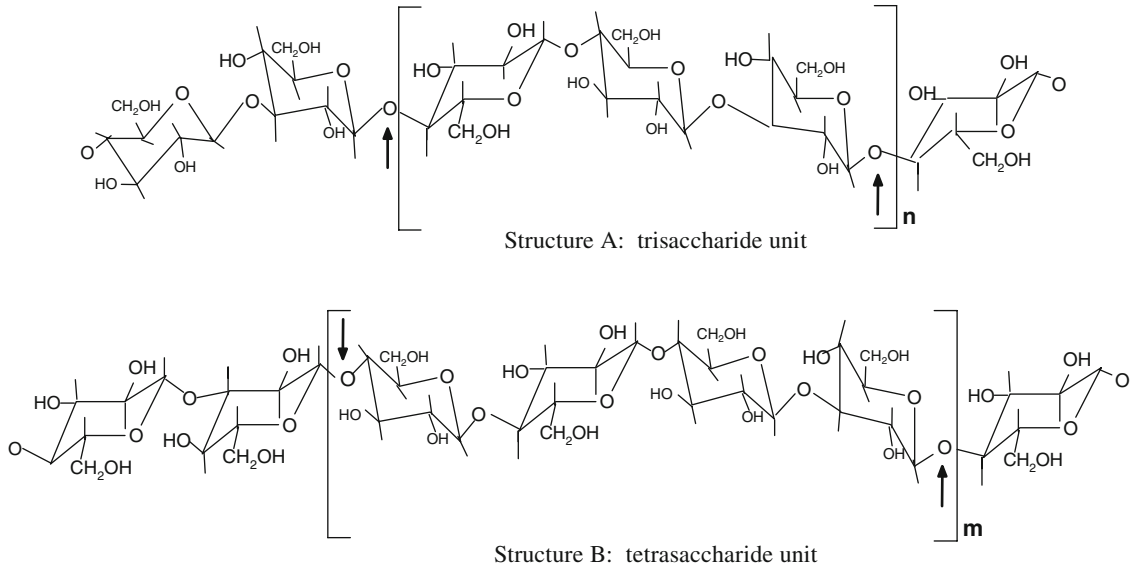

Fig. 2 Structural features of cereal β-glucans

Table 2 Structural features of β-D-glucans from cereals after lichenase hydrolysis

β-D-Glucan source	Peak area (%)		Total (%)		Ratio
	Tri	Tetra	Tri + tetra	Penta–nona	Tri/tetra
Wheat bran (pre-processed)	72.3	21.0	93.3	6.7	4.5
Barley	63.7	28.5	92.2	7.8	3.3
Oat	58.3	33.5	91.9	8.1	2.2

Functional properties

Molecular weight, molecular weight distribution and conformational properties

Molecular weight of β-glucans in the cell wall matrix has so far not been measured. The apparent molecular weight obtained for isolated β-glucans is scattered in the range of 10^4–10^6 g/mol, depending on sources, methods of isolation, degree of aggregation and determination techniques.

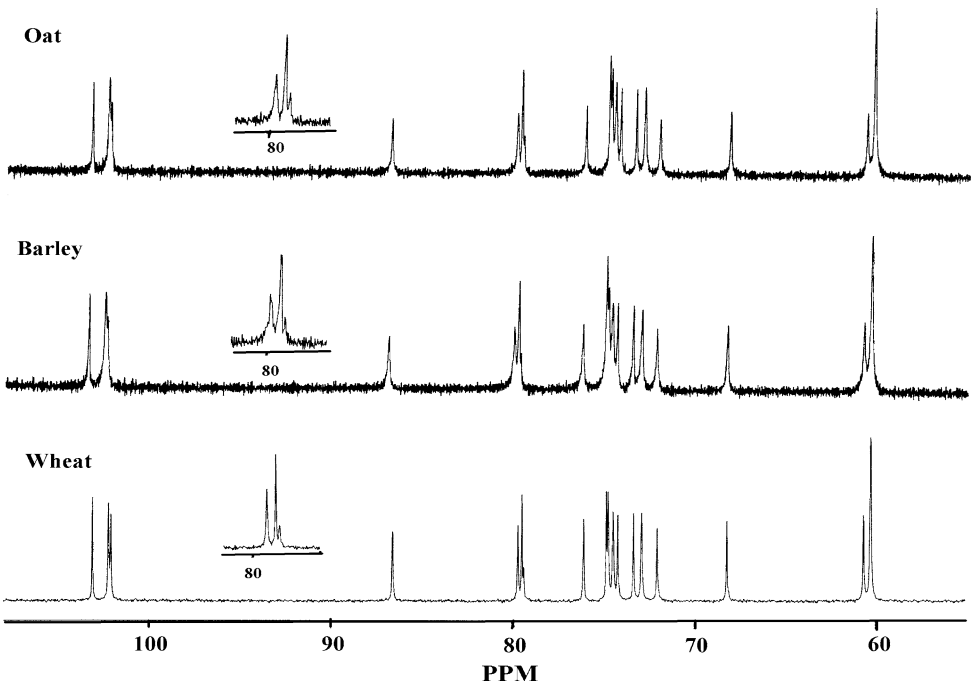

Fig. 3 Carbon-13 NMR spectra of cereal β-glucans

Table 3 Inter-residue connectivity of cereal β-glucans

Resonance (ppm)	Correlated resonance (ppm)
4.47 (H-1 of A)	86.4 (C-3 of B)
4.42 (H-1 of B)	79.5 (C-4 of C)
4.38 (H-1 of C)	79.3 and 79.2 (C-4 of A and D, respectively)
103.3 (C-1 of A)	3.46 (H-3 of B)
101.9, 102.1 (C-1 of B and C)	3.42 (H-4 of A and C)
3.19 (H-2 of A)	74.0 (C-3 of A) and 103.0 (C-1 of A)
3.14 (H-2 of C)	74.3 (C-3 of C) and 102.1 (C-1 of C)
3.28 (H-2 of B)	86.4 (C-3 of B) and 101.9 (C-1 of B)
68.0 (C-4 of B)	3.46 (H-3 of B)
75.9 (C-5 of B)	3.30 (H-4 of B)

A, B, C, D corresponds to the model structure assignments illustrated in Fig. 4, Cui et al. [6]

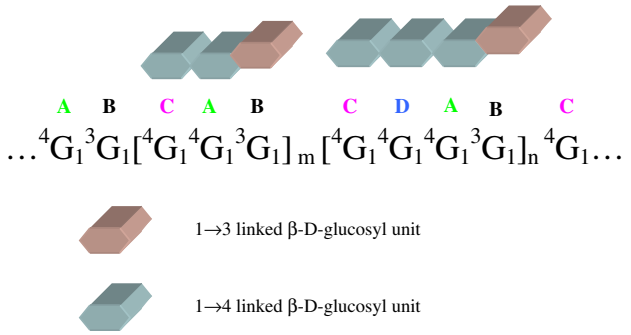

Fig. 4 Model structure of cereal β-glucan building blocks

Generally, the molecular weights of carefully isolated β-glucans follow the trend of oat > barley > rye > wheat, coinciding with their ease of extractability. One of the popular methods for determination of molecular weight and its distribution is size exclusion chromatography (SEC). In the absence of molecular weight detector, molecular weight standards are required to calibrate the columns. Isolated β-glucans are highly polydispersed in molecular weight, giving a broad molecular weight distribution. Wang et al. developed a method which allowed separations of β-glucans into many fractions with low polydispersity. These fractions are found to be identical in chemical structure, thus, are particularly suitable for the use as molecular weight standards (Fig. 5) [8].

Cereal β-glucans in aqueous solution adopt a disordered random coil conformation. Recent studies suggest that there are no significant differences in molecular conformation between cereal β-glucans when measured in extreme dilute solution in a good solvent. The Mark-Houwink-Sakurada exponents obtained for oat, barley and wheat fall in the range of 0.70 ± 0.05, which implies an expanded, semi-flexible chain conformation. With such a

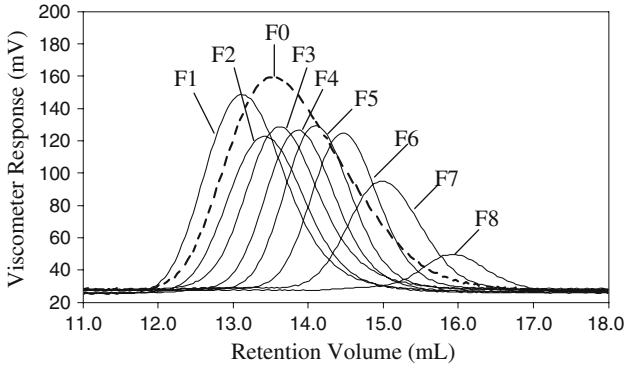

Fig. 5 Molecular weight distribution of six fractions and unfractionated β-glucans isolated from barley

conformation, the estimated persistence length, which is a measure of the chain stiffness, should not be much bigger than ~4 nm [9]. However, the measured values of persistence length were sometimes 10–20 times higher than this value [10]. This is the result of inter-molecular association among β-glucan molecules through hydrogen bonding leading to formation of aggregates, which imparts greater stiffness to the polymer chain, thus higher solution viscosity. The existence of molecular aggregates in water solution was clearly demonstrated by dynamic light scattering measurements (Fig. 6) [11]. These aggregates could be effectively eliminated in 0.5 M or higher concentration of sodium hydroxide or, but not by other treatments, including the use of 6 M urea as a solvent and a repeated filtration process or ultrasonic treatment. The success of obtaining an aggregate-free solution allowed the measurement of true molecular weight and conformational properties of single molecules. It confirmed an extended random coil conformation for all cereal β-glucans, and the persistence length of wheat β-glucan was determined to be in the range of ~5 nm [11].

Flow behaviour and gelation properties

Semi-dilute and concentrated solutions of cereal β-glucans are appreciably different from one another as a

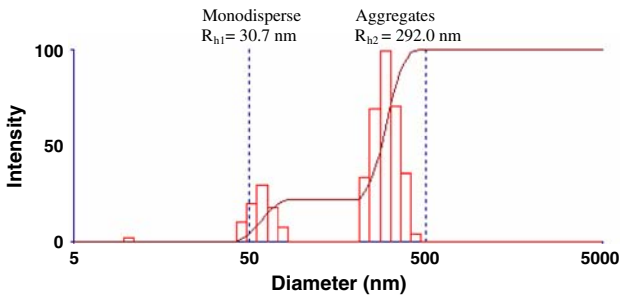

Fig. 6 Particle size distribution of wheat β-glucan demonstrating the presence of aggregates in aqueous solutions

consequence of varied chemical structures and molecular weight. For example, high molecular weight oat β-glucan solutions exhibit typical viscoelastic flow behaviour, and do not form a gel within a reasonable time period. Freshly prepared barley and wheat β-glucan solutions are also viscoelastic fluids as demonstrated in Fig. 7; however, a thermo-reversible gel could be formed if the β-glucan solutions are allowed to stand for a period of time under lower temperature (Fig. 8) [12, 13]. Partially hydrolysed oat β-glucans can also form a gel, but the gel development time is much longer [14, 15]. Figure 8 shows the mechanical spectrum of a gel formed by a 5% wheat β-glucan fraction (M_w = 340 KD). It is interesting to note that the gel strength of cereal β-glucans increased with the decrease in molecular weight (unpublished data).

Accumulating evidence suggests that the arrangement of cellotriosyl and cellotetraosyl units and their ratio in the polymer chain are important factors controlling the solution properties of these polysaccharides [12–16], although earlier studies ascribed this mainly to the long runs of cellulosic segments [17, 18]. Recent evidence from our laboratories and others suggested that sections of consecutive cellotriose units were mainly responsible for forming stable junction zones that lead to aggregation and gelation. According to this mechanism, the gelation ability of cereal β-glucans is in the order of wheat > barley(rye) > oat, which has been repeatedly demonstrated experimentally [13–15]. Molecular weight is another important factor that exerts significant influence on the solution properties of β-glucans. The gelation rate generally increases with decrease in molecular weight as shown in Fig. 9 (must be above the critical gelation chain length); this phenomenon is in disagreement with regular gelling polysaccharides in which longer polymer chains favour the formation of gels.

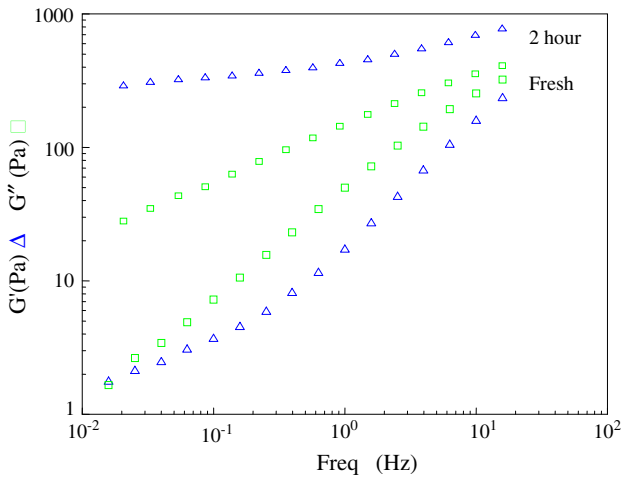

Fig. 7 Frequency sweep of 5.0% wheat β-glucan (WF1, M_w = 3.4 x 10^4) measured at 25°C: freshly prepared versus standing for 2 h at room temperature (25°C)

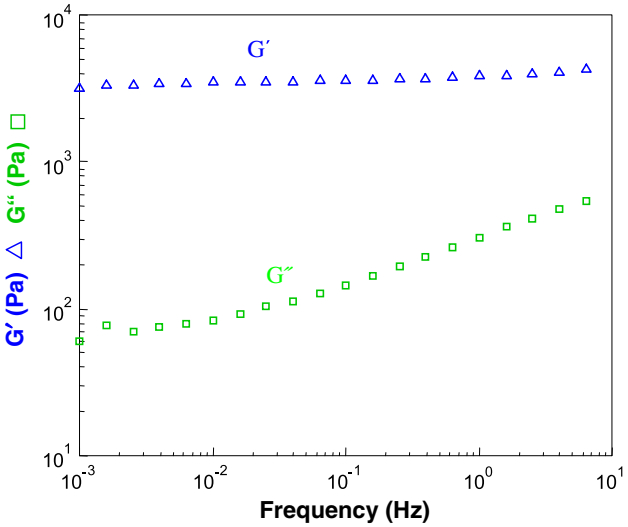

Fig. 8 Frequency sweep of 5.0% wheat β-glucans fraction 1 (WF1, $M_w = 3.4 \times 10^4$) at 5°C

The hypothesis for this phenomenon has been that small β-glucan molecules have higher mobility (less restriction of diffusion) than their large counterparts, thus are more readily to interact with each other to form a stable junction zone. The further association of the aggregates forms the three-dimensional gel networks at concentrated solution regime. It may also be that large molecules favour intra-molecular hydrogen bonding, whereas small molecules favour inter-molecular hydrogen bonding, which promotes network formation.

Physiological effect and health benefits

Oat and barley β-D-glucans have demonstrated health benefits, including lowering cholesterol levels and attenuating postprandial glycemic response. However, the postprandial blood glucose and insulin responses differ with different foods, and can be significantly affected by the present (amount) and status (bioavailability) of β-glucan in the foods. The Food and Drug Administration of the USA allowed a health claim for both oat and barley β-glucans for lowering the risk of coronary heart disease [1, 2]. These claims were based on substantial scientific evidence from both animal models and human clinical trials. For example, Kalra et al. [19] showed that barley β-glucan lowered the levels of total cholesterol, LDL-cholesterol and triglycerides in rats while Kahlon et al. demonstrated that barley as well as oat β-glucan significantly lowered the cholesterol levels of hamsters [20]. Bourdon et al. reported that barley β-glucan lowered the cholesterol concentration and attenuated the insulin response in humans [21]. Cavallero et al. studied the effect

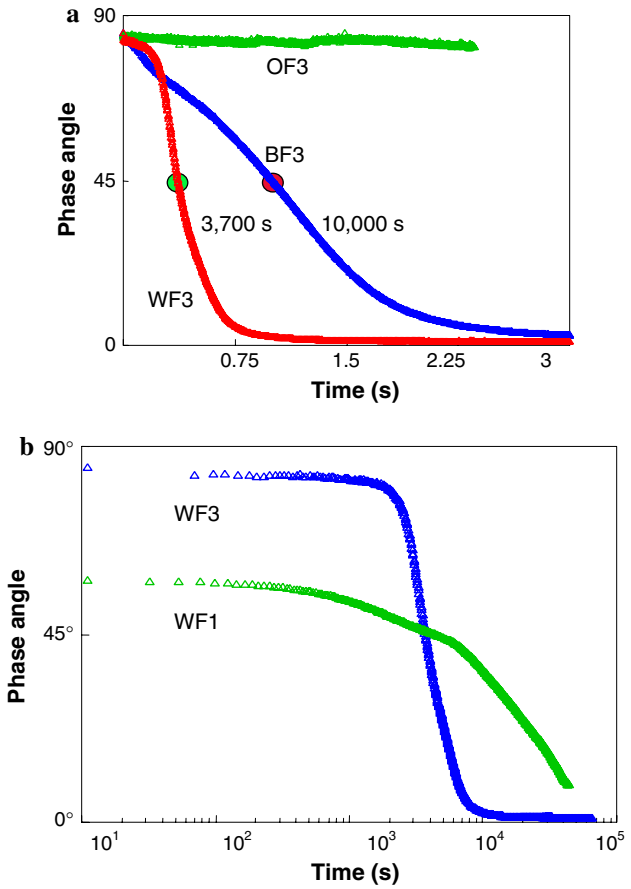

Fig. 9 Effect of structure and molecular weight on the gelation rate of cereal β-glucans. The phase angle less than 45° indicates the formation of gel. *OF3*, Oat β-glucan, $M_w = 97$KD; *BF*-3, Barley β-glucan, $M_w = 96$KD; *WF3*, Wheat, $M_w = 97$KD; *WF1*, $M_w = 340$KD

of barley β-glucan on human glycemic response and found a linear decrease in glycemic index with the increase of β-glucan content [22]. The presence of 5 g of oat β-glucan in extruded breakfast cereals caused a 50% decrease in glycemic response [23]. In a drinking model, both oat gum and guar gum significantly decreased the postprandial glucose rise and reduced the total- and LDL-cholesterol levels in human subjects [24, 25]. Wood later demonstrated that the observed postprandial glucose-lowering effect of oat β-glucan was positively correlated to the viscosity of the drink, which in turn, is determined by molecular weight and concentration of β-glucan used. A linear relationship between blood glucose levels and the product of concentration and molecular weight of β-glucan was established based on a number of studies, as shown in Fig. 10 [3].

Cereal β-glucans also demonstrated other health benefits. For example, oat fibre prolongs satiety after meals and alleviates constipation [26]. It has been shown that the solubility of the β-glucans is critical for exerting many reported positive physiological effect. By the same principle, the structural features, molecular weight or any

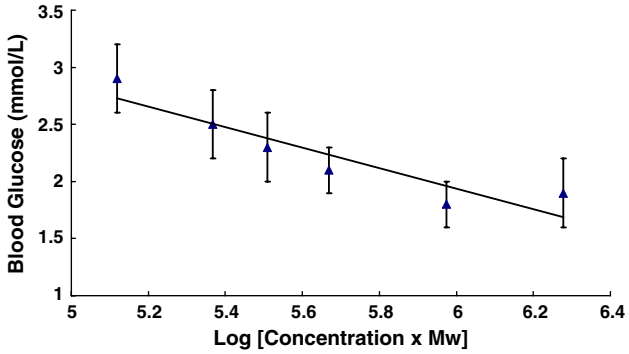

Fig. 10 A linear relationship between blood glucose levels and the product of concentration and molecular weight of β-glucan (viscosity). Adapted from Wood [3]

characteristics that could affect the viscosity-producing properties of β-glucans would have impact on their physiological effect. However, there is lack of information on whether β-glucan gels have any significant influence on physiological effects.

Applications

Oat and barley flours and minimum processed bran products are readily available as food ingredients in North America, Europe and other parts of the world. Advanced processing and fermented oat products have emerged during the last decade, including Oatrim from oats, Glucagel™ from barley and more recently *Viscofiber*® from both oats and barley. Oatrim is a product prepared by treating oat bran or oat flour with a thermostable α-amylase at high temperatures. The application of Oatrim in food includes bakery products, frozen desserts, processed meats, sauces and beverages. Oatrim is also used as a fat replacer [27]. Glucagel™ is based on barley β-D-glucans prepared by extraction and partial hydrolysis. The low molecular weight β-D-glucans (15,000–150,000 D) form gels at 2% polymer concentration and above. The major functionality of Glucagel™ is its gelling and fat mimetic properties, which is used as fat substitutes in bakeries, dairy products, dressings and edible films [28]. β-D-glucan isolated from oats can also be used in the personal care industry, as a moisturizer in lotions and hand creams. *Viscofiber*® is a product recently developed by a Canadian company [29]. It is a highly viscous product produced by a new technology using a combination of water–alcohol treatment of barley and oat flours with or without enzymes. The product is basically the intact cell walls of the endosperms. The current primary market for this product is as dietary supplements for the functional food and nutraceutical industry. In the Scandinavian countries, oat-based dairy substitute products can be found from the market, such as beverages

(Oatly), a fermented oat product (e.g. oat yoghurt) and oat ice creams. With more evidence on the health benefits of cereal β-glucans and the development of novel processing technologies, it is predictable that the world-wide applications of β-glucans in the food, nutraceutical and cosmetical industries will surge in the near future.

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