

CEREAL NON – STARCH POLYSACCHARIDES AS FUNCTIONAL FOOD INGREDIENTS: STRUCTURE – PHYSICAL PROPERTIES RELATIONS

BEZSKROBNI POLISAHARIDI IZ ŽITARICA KAO KOMPONENTA FUNKCIONALNE HRANE: POVEZANOST STRUKTURE I FIZIČKIH OSOBINA

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SUMMARY

The acceptance of cereal non-starch polysaccharides as functional, bioactive ingredients over the last two decades has increased the interest in incorporation of these structural polysaccharides in food formulations. Thus, the use of cereal β -glucan concentrates or isolates as thickening agents, stabilizers and fat mimetics to modify the texture and appearance in calorie-reduced, low-fat foods has been explored. The molecular features of these polysaccharides are important determinants of their physical properties (solubility, water-holding capacity, viscosity enhancement, gelling behavior, etc.), including their physiological responses, when they are considered as ingredients in cereal-based foods and other formulated products. For example, the origin of the viscous behavior of β -glucan and arabinoxylans is attributed to chain physical entanglements, intermolecular associations and network formation via non-covalent and covalent (cross-links) linkages. This review paper will provide updated information on structure, physical properties and functionality (technology aspects, physiological activity-health benefits) of cereal β -glucans and arabinoxylans by focusing attention on structure-function relations of these biopolymers in model systems and real food products.

Key words: β -glucans, arabinoxylans, molecular structure, functional properties, gelation, viscosity.

INTRODUCTION

The recent publicity surrounding the growing incidence of various chronic diseases and obesity and, at the same time, the demonstrated link between the intake of dietary fibre (DF) and various health benefits, have heightened consumer awareness of good nutrition and increased interest in foods and food ingredients enriched in DF, such as cereal-based foods or foods fortified with cell wall-enriched grain fractions and cereal non-starch polysaccharide concentrates and isolates [1, 4, 5, 10, 11]. β -Glucans and arabinoxylans are the chief structural constituents of cell walls (CW) in starch endosperm and aleurone layer of cereal grains representing up to 85% of the total CW polysaccharides. The content of these polysaccharides in the grain depends on the cereal genera. Barley and oat are considered major sources of β -glucans. The levels of β -glucans in barley, oat, rye, wheat, tricale, sorghum, and rice vary in the ranges of 2.5-11.3, 2.2-7.8, 1.2-2.9, 0.4-1.4, 0.4-1.2, 0.1-1.0, and 0.04%, respectively. The amount of arabinoxylans is higher in rye (7.6-12.0%) than wheat (6.1%) and barley (5.8%), and much lower in oat (2.7%), rice (2.6%) and sorghum (1.8%). β -Glucans have been implicated in lowering plasma cholesterol, improving lipid metabolism, and reducing postprandial glycaemic responses. The nutritional value of arabinoxylans has not been investigated to the same extent as those of β -glucans. However, recent studies revealed positive effects of water-soluble maize, wheat and rye arabinoxylans on cecal fermentation, production of short-chain fatty acids, reduction of serum cholesterol and improved absorption of calcium and magnesium. The potential use of β -glucans and arabinoxylans as hydrocolloids in the food industry is based mainly on their rheological characteristics, i.e. their ability to increase the viscosity of aqueous solutions and gelling capacity; thus, they can be utilised as thickening agents to modify the texture and appearance of food formulations or may be used as fat mimetics in the development of calorie-reduced foods. The objective of this review is to address recent research findings on structure – function relationships of cereal non-starch polysac-

charides in the context of their technological and physiological functionality.

STRUCTURAL FEATURES

Mixed linkage (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- β -D-glucans, commonly known as β -glucans, are linear homopolymers of D-glucopyranosyl (Glc_p) residues linked mostly via two or three consecutive β -(1 $\rightarrow$ 4) linkages that are separated by a single β -(1 $\rightarrow$ 3) linkage; less frequent are longer segments of consecutively β -(1 $\rightarrow$ 4) linked Glc_p with degree of polymerization (DP) 5–20 [10, 11]. The molecular features of β -glucans are usually construed from the analysis of the oligomers obtained by digestion of the polymers with a specific (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)- β -D-glucanohydrolase that releases 3-O- β -D-cellobiosyl-D-glucose (trisaccharide unit, DP3) and 3-O- β -D-cellotriosyl-D-glucose (tetrasaccharide unit, DP4) accounting for 90–95% of the total oligosaccharides, and longer oligosaccharides (DP $\geq$ 5) accounting for 5–10% of the total oligosaccharide chain segments. Differences in the proportions of tri- and tetrasaccharides, observed among different β -glucans from various sources and reflected in the molar ratio of cellotriose to cellotetraose units (DP3/DP4), follow the order of wheat (3.0-4.5), barley (1.8-3.5), rye (1.9-3.0) and oats (1.5-2.3); this ratio is considered to be a main fingerprint of the structure of cereal β -glucans.

Arabinoxylans, on the other hand, consist of a linear chain backbone of β -D-xylopyranosyl (Xyl_p) residues linked through (1 $\rightarrow$ 4) glycosidic linkages (Fig. 1). α -L-Arabinofuranosyl (Araf) residues are attached to some of the Xyl_p residues at O-3, O-2, and/or at both O-2,3 positions. A unique feature of arabinoxylans is the presence of ferulic acid, esterified to O-5 of Araf linked to O-3 of the xylose residues. Ferulate esters can dimerize via phenoxy radicals into dehydrodiferulate esters, which are responsible for covalent cross-linking between arabinoxylans chains or between arabinoxylans and other cell wall (CW) constituents (e.g., lignin, proteins) [4, 5]. The ratio of Ara/Xyl residues indicates a degree of branching in the polymer chain, but it

is only a rough indicator of the fine molecular structure which is better described by the substitution pattern along the xylan backbone. In cereals, the Ara/Xyl ratio may vary from 0.3 to 1.1.

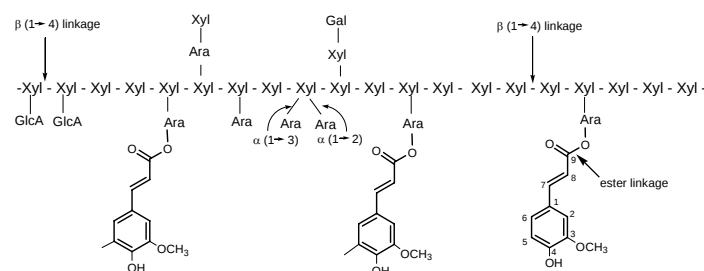


Fig. 1. General structure of arabinoxylans (adapted from ref. 2)
Sl. 1. Osnovna struktura arabinoxilansi
(preuzeto iz ref. 2 uz adaptaciju)

For β -glucans [10] and arabinoxylans [4] molecular weight (Mw) values in the range of $21\text{--}2,700 \times 10^3$ and $220\text{--}2,430 \times 10^3$, respectively, have been reported. Variations in the Mw estimates of cereal non-starch polysaccharides may originate from varietal and environmental (growth) factors, as well as from differences in the methods used for extraction (solvent and temperature affect the solubilization) and purification, aggregation phenomena (dependent on the polysaccharide structural features and solvent quality) and depolymerization events (by endogenous or microbial β -glucanases from contaminating microorganisms) occurring during the extraction-processing steps. Also, the reported molecular weights are dependent on the analytical methodology used for determination of molecular size (including chromatography detector and the standards used).

The molecular and structural features of β -glucans [10,11] and arabinoxylans [4, 5, 8] vary depending on the cereal source of CW polysaccharides and the plant tissue; these are important determinants of their physical properties, such as solubility, extractability and aggregation properties as well as their functionality, when they are considered as ingredients in cereal-based foods and other formulated products, including physiological responses in the human body.

PHYSICAL PROPERTIES

β -Glucans display an extended random coil conformation in solution [10,11]. Arabinoxylans exhibit similar chain conformation in solution characterized as locally stiff, semiflexible coils [4, 5]. As a result of this behaviour, combined with their high molecular weight, β -glucans and arabinoxylans exhibit a very high viscosity in aqueous solutions. The reported intrinsic or limiting viscosity ($[\eta]$) values for cereal β -glucans and arabinoxylans vary in the range of 0.3–9.6 and 2.8–5.9 dl/g, respectively, depending largely on the molecular weights of the polysaccharides [4, 10]. Additionally, isolated arabinoxylans with high $[\eta]$ values generally display high Xyl/Ara ratios, high feruloyl residue and low doubly substituted Xylp contents [8].

Dynamic and steady shear rheological tests of freshly prepared (or heat-treated before measurement) solutions of cereal β -glucans (Lazaridou et al., 2003; Vaikousi et al., 2004; Lazaridou et al., 2004) as well as of arabinoxylans dispersions [8] reveal a behaviour typical of non-interacting disordered polysaccharides with chain entanglements in the concentrated state. Thus, for aqueous dispersions of these polysaccharides a Newtonian region at low shear rates and a shear thinning flow at high shear rates are observed. Moreover, under dynamic rheological measurements, the loss modulus, G'' , is generally larger than the storage (or elastic) modulus, G' at lower frequencies, while the

behaviour approaches that of solid-like materials at higher frequencies, with G' being greater than G'' . As expected, with increasing molecular weight and/or polysaccharide concentration, there is an increase in viscosity as well as in the shear thinning and viscoelastic properties of aqueous dispersions of β -glucans or arabinoxylans.

Although fresh solutions of cereal β -glucans fall rheologically into the category of random coil-type polysaccharides, their rheological properties may change depending on their molecular characteristics (size, structure), storage time (i.e. delay time before analysis) and thermal history [3, 9, 19]. Thus, solutions of β -glucans show unusual shear thinning flow behaviour at low shear rates upon storage, implying a time-dependent formation of transient intermolecular networks. This behaviour becomes more pronounced with increasing polysaccharide concentration and storage time prior to the rheological testing, as well for β -glucans with low molecular weight and high proportions of cellotriosyl units in the polymeric chains. Two possible mechanisms for intermolecular associations among β -glucan chains have been suggested. One involves the side-by-side associations of cellulose-like segments of more than three contiguous β -(1 $\rightarrow$ 4)-linked glucosyl units, leading to formation of chain aggregates, and the other refers to the interactions of chain segments with consecutive cellotriosyl units linked by β -(1 $\rightarrow$ 3) bonds that might form extended intermolecular junction zones^{1,3}. Therefore, higher ratios of tri- to tetrasaccharides and larger amounts of long cellulose-like fragments (DP $\geq$ 5) in β -glucan chains as well as high molecular weight have been associated with decreased solubility or extractability of these polysaccharides from cereal grains [2, 12, 18]. Moreover, it has been found that a higher DP3/DP4 ratio increases the tendency of cereal β -glucans to form aggregates and enhances their gelation potential by increasing the regularity along the polymeric chains [19].

In addition to exhibiting increased solution viscosity on storage, β -glucans have also been shown to form physically cross-linked gels whose three dimensional structure is stabilized mainly by multiple inter- and intra-chain hydrogen bonds in the junction zones of the hydrated polymeric network. Thus, cereal β -glucan thermoreversible hydrogels can be obtained under isothermal (5–45°C, 4–12% w/v polymer concentration) conditions, as well as after repeated freezing and thawing cycles of relatively dilute (1–4% w/v) polysaccharide solutions [3, 4, 9, 10, 11, 19]. Cereal β -glucan hydrogels are also formed at temperatures above 0°C. When the G' , G'' moduli of aqueous dispersions of cereal β -glucans are monitored isothermally by dynamic rheometry at temperatures above 0°C, following an induction period, the G' and G'' increase with time and the aqueous dispersions begin to adopt gel-like properties ($G' > G''$); at the end of the gel curing process, the behaviour becomes typical of an elastic gel network. The time where G' crosses G'' is considered as the gelation time (G_t) and the maximum slope of the G' curve is reported as an index of the gelation rate; the latter, known as “elasticity increment, I_E ”, can be calculated as $I_E = (\text{dlog } G'/\text{dt})_{\text{max}}$. The G_t decreases and the I_E values increase with decreasing molecular weight in cereal β -glucans with similar distributions of cellulose-like fragments (Fig. 2a), possibly due to the higher mobility of the shorter chains that enhances diffusion and lateral interchain associations. Among cereal β -glucans of equivalent molecular weight, the gelation time decreases and the gelation rate increases with increasing molar ratio of DP3/DP4 (Fig. 2b). Moreover, the strength and thermostability of cereal β -glucans hydrogels are enhanced with increasing molecular size and DP3/DP4 ratio in the polysaccharide chains.

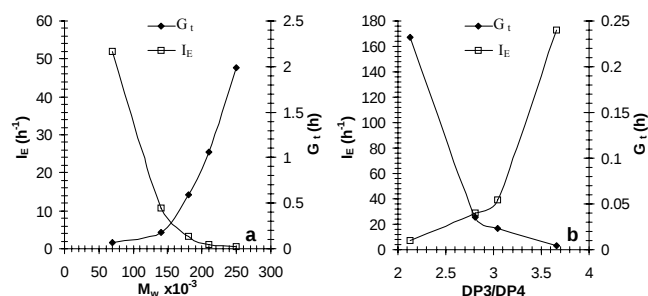


Fig. 2. Molecular weight (a) and structure (b) dependence of elasticity increment (I_E) and gelation time (G_t) for cereal β -glucans cured at 25°C and 8% w/v concentration (frequency 1 Hz, strain 0.1%); the β -glucan preparations of (a) have a common DP3:DP4 ratio, 2.8, and those of (b) have a common molecular weight, 210×10^3 (adapted from ref. 3)

Sl. 2. Zavisnost molekulske težine (a) i strukture (b) od porasta elastičnosti (I_E) i vremena želatinizacije (G) za β -glukane od žitarica konzervisano na 25°C i koncentracije 8% w/v (frekvencija 1 Hz, izduženje 0,1%); β -glukan preparati (a) imaju zajednički odnos DP3:DP4, 2,8, a njihova (b) zajednička molekulska težina je 210×10^3 (preuzeto uz adaptaciju iz ref. 3)

Arabinoxylans have also a tendency to form macrostructures in aqueous solutions via chain aggregation and physical entanglements stabilized by hydrogen bonds. This aggregation tendency may be responsible for the pseudo-gel behaviour of arabinoxylan solutions under certain conditions [4]. The extent of non-covalent self-associations and interactions among β -glucans and/or arabinoxylans chains has been suggested to affect their extractability and solubility [2, 12, 18]. These polymeric associations are governed by certain molecular characteristics of the β -glucan or arabinoxylan chains, e.g. long cellulose-like fragments in β -glucans and unsubstituted chain segments in arabinoxylans.

Thus, the degree of branching and the substitution pattern in arabinoxylans have a role in the solubility and extractability of these polysaccharides; the least soluble fractions of the cereal endosperm CW were found to contain arabinoxylans with the lowest amount of doubly substituted but the highest amount of unsubstituted xylose residues, and long sequences of unsubstituted xylan regions [18]. Covalent cross-linking among arabinoxylans chains, through dimerization of esterified ferulic acid residues, is also believed to contribute to their insolubility [4, 5].

Arabinoxylan solutions possess a unique capacity to form hydrogels in the presence of free-radical generating agents, such as peroxidase/ H_2O_2 , laccase, linoleic acid/lipoxygenase, ammonium persulphate, or ferric chloride [4, 5, 8]. Covalent oxidative cross-linking of arabinoxylan chains through dimerization of ferulic acid substituents is responsible for this unusual property of cereal arabinoxylans. Upon addition of a free-radical generating agent to a solution of arabinoxylans, there is usually a rapid rise in the elastic modulus (G'), attributed to the initial formation of covalent cross-linkages between ferulic acid residues of adjacent arabinoxylan chains, that is followed by a plateau region with very little further increase of G' . The gelation capacity and the strength of gels increase with the concentration of arabinoxylans, their M_w , and the content of ferulic acid [8].

FUNCTIONAL PROPERTIES

Technological aspects

Incorporation of cereal non-starch polysaccharides into various food products (bread, muffins, pasta, noodles, salad dressings, beverages, soups, reduced-fat dairy and meat products) showed that attributes, such as breadmaking performance, water binding and emulsion stabilising capacity, thickening ability,

texture, and appearance appear to be related to the concentration, molecular weight and structure of the polysaccharide.

The addition of enriched in β -glucans and arabinoxylans flour fractions at high levels in yeast-leavened breads leads to high water retention, darker coloured end-products and undesirable effects on crumb texture, as well as to lower loaf volume due to gluten dilution and disruption of the gluten network in dough by the presence of elevated levels of fibers [4, 5, 11]. However, β -glucans and soluble arabinoxylans when used up to a certain concentration in wheat flour dough result in increased loaf volumes and improved crumb structure, probably by increasing the viscosity of the dough aqueous phase, and thereby the dough structure and stability; the increased stability of the films surrounding the gas cells during the early baking processes is useful in prolonging the oven rise and preventing cell coalescence. The optimum concentration of incorporated arabinoxylans [4, 8] and β -glucans [7] (Fig. 3) may depend on their molecular structure (size) and the baking quality of wheat flours. Moreover, with increasing M_w of added arabinoxylans [1] and β -glucans [7] the loaf volume is enhanced. For example, addition of a barley β -glucan isolate in wheat breads with apparent $M_w \sim 200,000$ resulted in higher loaf volumes than those of the respective breads fortified with a lower $M_w \sim 100,000$ preparation (Fig. 3). However, above a certain molecular size, depending on polysaccharide concentration, the effect of polymer incorporation can be detrimental for the quality of baked products [5, 8]. In this context, substantial differences in the reduction of loaf volumes have been observed upon supplementation of wheat bread with 2% β -glucans when the molecular weight of the polysaccharide changed from $\sim 800,000$ to $\sim 200,000$ ⁴. Thus, the addition of optimal doses of endoxylanases during the bread making processes has been found to positively affect the dough handling and bread properties by increasing the solubility of the high amount of insoluble arabinoxylans normally present in the flour; on the other hand, an excessive amount of endoxylanases may lead to an extensive degradation of these polymers and to an average negative effect on bread loaves (stickiness, reduced volume) [4, 5]. Another functional property of arabinoxylans is their antifirming effects on the texture of bread crumb, depending on the amount and M_w of the added polysaccharide into the bread formulation [5, 8].

Cereal β -glucans exhibit the potential to be used as fat replacers due to their high viscosity and water-binding, as well as their foaming and emulsion stabilizing capabilities. Kontogiorgos et al. [18] studied the effects of pure barley and oat β -glucans on the rheological and creaming behaviour of concentrated egg-yolk-stabilized model emulsions (salad dressing model). High molecular weight β -glucans (apparent $M_w \sim 110 \times 10^3$) seem to stabilize the oil-in-water (o/w) emulsions, mainly by increasing the viscosity of the continuous phase, while the low molecular weight counterparts (apparent $M_w \sim 40 \times 10^3$) influence emulsion stability through network formation in the continuous phase.

The molecular features of cereal β -glucans appear to have also an imprint on textural attributes and stability of dairy model systems, such as yogurt [7] and milk drinks [13]. Thus, the impact of barley β -glucan molecular features was investigated on skim milk gels formed at 42°C by acidification with glucono- δ -lactone (GDL). Incorporation of barley β -glucans (0.5-2.0% w/w) into skim milk (10-14% w/w) gels resulted in weakening of the protein network and increasing of spontaneous syneresis¹⁵. However, this weakening effect could be reduced or eliminated by using β -glucans of certain molecular features that either promote the development of a high viscosity medium (high molecular weight, 250 kDa) or the formation of a secondary gel network structure (low molecular weight, 40 kDa and a high DP3/DP4

ratio, 3.5) by the polysaccharide itself. Moreover, using high β -glucan concentrations or polysaccharide preparations with the aforementioned structural characteristics resulted in milk gels with significantly lower serum separation compared to the control formulations. The macroscopic phase separation of oat β -glucans / sodium caseinate mixtures under neutral conditions, and thus the potential stability of these systems on the self, seems to be influenced by the molecular weight of β -glucans and initial composition of the mixtures [13]; the immiscibility between oat β -glucans and milk protein was found to be enhanced with decreasing molecular size of the polysaccharide chains.

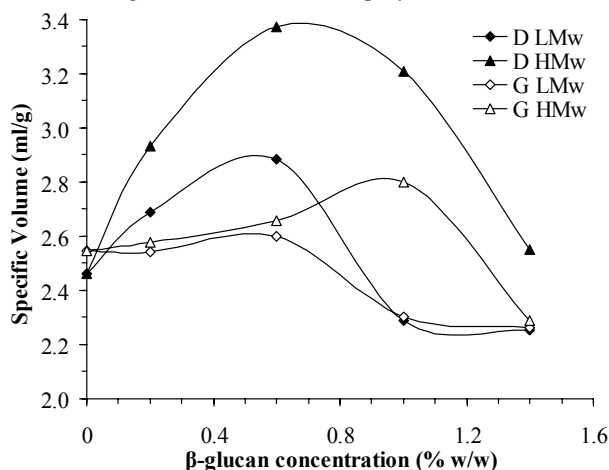


Fig. 3. Effect of concentration of incorporated β -glucans into wheat flours on specific volume of breads. Two barley β -glucan isolates with $M_w \sim 200,000$ (HM) and $M_w \sim 100,000$ (LM) barley at various and two types of wheat flours differing in their bread making quality were used; cultivar G of 'good' and cultivar D of 'poor' breadmaking quality (adapted from ref. 13)

Sl. 3. Efekt koncentracije β -glukana koji se nalazi u pšeničnom brašnu pri specifičnoj zapremini hleba. Dva β -glukana izolovana su sa $M_w \sim 200,000$ (HM) i $M_w \sim 100,000$ (LM) iz dva različita ječma i dva tipa iz različitog pšeničnog brašna, odnosno njihovih hlebova; sorta G „dobra” i sorta D „loša” za proizvodnju hleba (uz adaptacije iz ref. 13)

The molecular weight of β -glucans does not only affect the viscosity of foods but also has a significant impact on the perceived (sensory) thickness of the formulated products, as shown for beverage and soup food prototypes containing 0.25-2% (w/w) β -glucans [14, 15]; significant correlations between textural characteristics and measured viscosity have been found as well as a moderately strong relationship between flavor attributes and the viscosity of soups.

The potential use of β -glucans as biodegradable edible food packaging material has been also suggested [16]. Further to the amount of plasticiser (water, polyol) used in the film formulation, the mechanical properties of cast β -glucan films were affected by the molecular weight of the polysaccharide as well; high molecular weight β -glucans gave stronger and more extensible films than lower molecular weight β -glucan preparations.

Physiological responses

The physiological functions of dietary cereal fibres, such as β -glucans and arabinoxylans, are often attributed to their physico-chemical properties: water holding capacity, swelling, diffusion-suppressing ability through viscosity enhancement and gel formation in the gastrointestinal (GI) tract, binding properties, and susceptibility or resistance to bacterial degradation and fermentation [4, 10, 11]. It has been suggested that these polysaccharides decrease the absorption and reabsorption of cholesterol,

bile acids, and their metabolites by increasing the viscosity of the (GI) tract contents as well as delaying gastric emptying and the intestinal absorption of nutrients, such as digestible carbohydrates (sugars, starch), and thereby reducing postprandial hyperglycemia and insulin secretion. All the above physiological actions are associated with reduced risk of developing cardiovascular diseases, diabetes and insulin insensitivity, and also have health benefits for those individuals with type-2 diabetes.

Wood [6] has suggested that the net result of food processing might be to improve the bioavailability or bioactivity of the β -glucan, and that this is related to the product of ($C \times M_w$); C being the concentration and M_w the molecular weight. Human studies have shown inverse linear relationships between $\log(\text{viscosity})$ and postprandial glucose and insulin responses after consuming glucose solutions containing oat gum (soluble β -glucan). It should also be noted that the food matrix and food processing and storage conditions affect the hypocholesterolemic or hyperglycemic properties of β -glucans [11]. The dose-response for a solid meal might differ from that for a liquid product depending on the amount and state of solubilized β -glucans in the GI tract. Furthermore, reduction of polysaccharide M_w can occur during the isolation stage and/or as a result of food processing conditions. During baking of yeast-leavened oat- and barley-containing breads, the β -glucan is partially degraded (from $1,200\text{--}2,300 \times 10^3$ to $240\text{--}1,920 \times 10^3$), probably due to the action of endogenous β -glucanases in the flour mixtures. Moreover, the typical pasteurization conditions used for an acidic liquid product, such as fruit and tomato juice can result in reduction of molecular weight of added β -glucans as a consequence of acid hydrolysis. On the other hand, food processing of cereal-based foods, such as cooking and extrusion can increase the solubility and thereby, the availability of β -glucans, although the molecular size of the polymer may be partly reduced due to enzymic hydrolysis, milling, stirring, pumping. Food storage can also affect the amount of solubilized β -glucans; frozen storage can result in reduction of β -glucan solubility possibly due to changes in the intermolecular organization (chain aggregation).

For arabinoxylans, *in vitro*, animal and human studies showed that molecular structure is related to the ability of microorganism in the GI tract to ferment the intact polysaccharide and thus, the growth of potential health-promoting bacteria, the so-called probiotics [4]. Un-substituted xylan chains form insoluble aggregates and hinder accessibility of the bacterial enzymes to the substrates. The beneficial role of arabinoxylans in the human diet may also be associated with the presence of ferulic acid covalently bound to these polymers. Cross-linked arabinoxylans by diferulate bridges can limit the fermentation of arabinoxylan chains from the intestinal microbiota. Moreover, recent studies have shown that ferulic acid has strong anti-inflammatory properties. Katapodis et al. [20] evaluated the antioxidant activity of feruloylated oligosaccharides derived by partial hydrolysis of wheat flour arabinoxylans and found that a feruloyl arabinoxylotrisaccharide (FAX₃) had profound antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl reduction assay and inhibited the copper-mediated oxidation of human LDL. This antioxidant activity of FAX₃ may be important in preventing or reducing the progression of arteriosclerosis by inhibiting the peroxidation of lipids.

CONCLUSIONS

The physical and physiological properties of cereal non-starch polysaccharides are of commercial and nutritional importance. The molecular and structural features of these biopolymers are important determinants of their physical properties, such as water solubility, dispersibility, viscosity, and gelation

properties, and thereby affect the functionality of these polysaccharides from a technological viewpoint when added to food systems. The physiological functions of β -glucans also seem to be associated with viscosity enhancement properties, and this may be affected by the amount, molecular weight and state of solubilised β -glucans in the GI tract. Structural characteristics of arabinoxylans that limited their solubility and digestibility by intestinal microorganisms or displayed antioxidant properties were also linked with biological activities of these polysaccharides, such as prebiotic and anti-inflammatory actions.

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