WHEAT FLOUR ARABINOXYLANS STRUCTURE AND FUNCTIONAL PROPERTIES

-review-

A. Stoica
Valahia University of Tîrgovişte, Environmental Engineering and Biotechnologies Faculty
tel. 0245/206108, e-mail: stoicaandi@yahoo.com

Abstract

In recent years, wheat flour arabinoxylan has attracted attention since it has proven to have a significant influence on the water balance and rheological properties of dough, retrogradation of starch and bread quality. Therefore, it is essential to know the structure and the properties of this substances in order to understand the functionality of wheat flour arabinoxylans in bread-making.

Keywords*: arabinoxylans, ferulic acid, oxidative gelation.*

Introduction

Arabinoxylan is a minor component of entire cereal grain, but constitutes an important part of plant cell walls. Together with other polysaccharides it builds up the cell walls of grain tissue and thus becomes a part of the skeletal framework. It is a part of the so-called hemicellulose that is generally believed to act as cement between cellulose fibres (Rimsten, 2003).

The term hemicellulose refers to a group of homo- and heteropolymers consisting largely of anhydro β-(1,4)-D-xylopyranose, mannopyranose, glucopyranose and galactopyranose main chains with a number of substituents (Jeffries, 1994) and they are generally classified according to the main sugar residue in the backbone in xylans, mannans, galactans and glucans (Palonen, 2004).

Hemicelluloses are more heterogeneous polysaccharides and are the second most abundant organic structure in the plant cell wall. The major hemicellulose polymer in cereals is xylan (de Vries and Visser, 2001). Hemicelluloses are generally found in association with cellulose in the walls of plants (Jeffries, 1994).

The non-starch polysaccharides in the endosperm cell walls of wheat constitute up to 75% of cell wall dry matter weight. Of these the AX are the most proeminent group (85%) (Courtin and Delcour, 2002). Non-starch polysaccharides (NSP), originating from the cell wall of the aleurone and endosperm of wheat kernel, represent different polysaccharides, which are built up of pentose sugars [sometimes named pentosans, mainly arabinoxylans (AX)] and hexose sugars. AX together with cellulose, β-glucan, arabinogalactan-peptide and other minor constituents like glucomannan and xyloglucan are referred to as NSP (Wang, 2003).

Arabinoxylans can be divided into soluble water-extractable (WE-AX) and insoluble water-unextractable arabinoxylans (WU-AX) (Vardakou et al., 2004). 25-30% of the 1,5-2,5% AX found in wheat flour endosperm is water-extractable. Possible reasons for their water-extractable nature are incomplete cross-linking with other components, small structural differences or initial enzymic degradation in the kernel (Courtin and Delcour, 2002). The water-unextractable nature of WU-AX is due to a combination of non-covalent interactions and covalent bonds with neighbouring AX molecules and other cell wall components such as proteins, cellulose and lignin (Vardakou et al., 2004).

It is necessary to make a distinction between AX and arabinogalactan-peptides (AGP), another class of non-starch polysaccharides found in the endosperm of wheat. In spite of large differences between AX and AGP in both structure and properties, they have often been and sometimes still are regarded or studied as one group called pentosans. Recent investigations by Loosveld and co-workers on the functionality of AGP in breadmaking have clearly shown that AGP behave very differently from AX in bread-making. Therefore, it seems wise to abandon use of the term pentosans (Courtin and Delcour, 2002).

The structure of wheat flour arabinoxylans

The structure of AX is a linear backbone of β-1,4 linked xyloses carrying single arabinose residues on C(O)-3 and/or on C(O)-2 position (Wang, 2003; Bordei, 2000; Åman et al., 1999) (fig.1.). Some of the arabinose residues are ester linked on C(O)-5 to ferulic acid (FA) (3-methoxy, 4 hydroxy cinnamic acid) (Carvajal-Milan, 2006) (fig.2.). FA is concentrated in the cell wall of the outer coverings of wheat kernel (Wang, 2003).

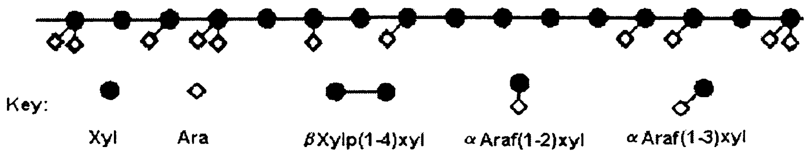


Fig. 1: The structure of wheat arabinoxylans (Egi et al., 2004)

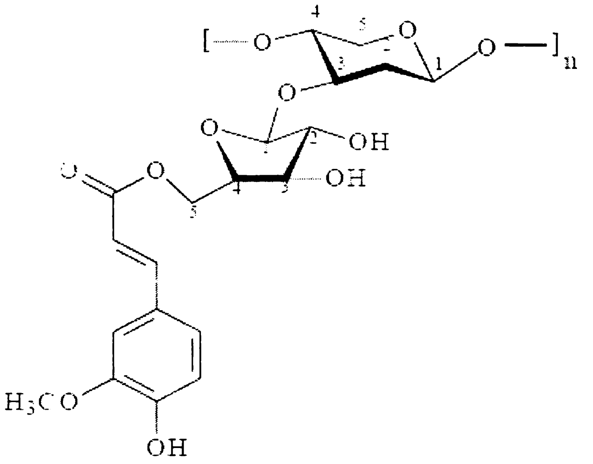
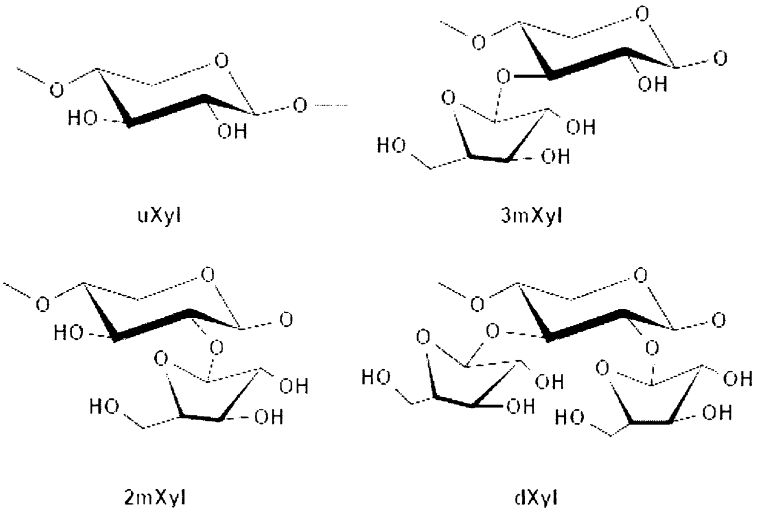


Fig.2: Structure of FA bound to arabinoxylan D-xylopyranosyl residue substituted on C (O)-3 with L-arabinofuranosy residues. The link of FA to C(O)-5 of a L-arabinofuranosyl residue (after Wang, 2003)

This gives four differently linked xylose residues, one unsubstituted (uXyl), two monosubstituted at either C2 (2mXyl) or C3 (3mXyl) and one disubstituted (dXyl) (fig.3.). Other minor, but common substituents bound to the xylan backbone are 4-O-methyl-glucuronic acid and glucuronic acid. These uronic acids are bound to the C2 atom of the xylose residue (Rimsten, 2003).

Arabinoxylans generally consist of between 1500 - 5000 residues (Chaplin, 2004). The chemical structures of wheat WE-AX and WU-AX are basically the same, the WU-AX having a slightly higher molecular weight. The differences in extractability are probably due to differences in chemical and physical interactions. Substituents such as phenolic acids, acetyl groups and proteins may be found in cereal arabinoxylans. A highly substituted arabinoxylan fraction linked to wheat gluten has recently been found (Autio, 2006).

An important parameter for AX behaviour is the arabinose to xylose ratio (A/X), with a typical average value of 0,5 to 0,6 for the general wheat WE-AX population but extreme values of 0,31 to 1,06 for WE-AX subfractions. In the strongly branched regions, that constitute about three quarter of the AX molecules, 4 out of 7 xylose residues would be substituted with a high level of disubstitution. The branched regions are alternated with open regions which can contain up to five or more consecutive unsubstituted xylose residues.


uXyl

3mXyl

2mXyl

dXyl

Fig.3: Main structural elements of arabinoxylan (after Rimsten, L., 2003)

Arabinoxylan conformation. It is generally considered that four parameters on the primary structure level define the tridimensional conformation and, thus, the behaviour of the AX:

1) the length of the xylan backbone,
2) the degree of substitution (A/X ratio),
3) the substitution pattern or the way the arabinose residues are implanted in the backbone and
4) the coupling of ferulic acid to other AX molecules or cell wall components.

Fiber studies indicate that when the xylan chain is unsubstituted, its conformation is a flexible left turning helix with 3 xylose residues per turn (Courtin and Delcour, 2002). The free molecules in solution may, however, take up a wide variety of conformations with only moderately extended structures. Although the backbone xylan structure is similar to that occurring in cellulose there is little driving force to produce crystalline type structures as the intra- and inter-molecular hydrogen bonds associated with the 6-hydroxyl groups are necessarily absent. The presence of arabinose side chains reduce interaction between chains due to their inherently more flexible water-hungry furanose conformations. However, where there are sections of disubstituted xylan the chain is relatively inflexible (Chaplin, 2004).

While the degree of substitution of WE-AX would not influence the conformation or the rigidity of the WE-AX, the presence of diferulic acid bridges between WE-AX would lead to molecules with an increased molecular weight and changed conformational properties (Courtin and Delcour, 2002).

Functional properties of wheat flour arabinoxylans

Solubility

The wheat flour arabinoxylans are divided on the basis of solubility into water-soluble (WS) and water-insoluble (WIS). The WS arabinoxylans are extractable with cold water, whereas alkali is needed to extract the WIS arabinoxylans. The solubility of the pentosans varies widely with climatic conditions. The percentage of soluble pentosans is higher in the endosperm than in the bran and shorts fractions (Autio, 2006). The AX solubility depend primarily on the substitution degree of AX. Removal of arabinose residues from AX lowered their solubility in water. Insolubility of the low A/X fraction was attributed to an increased aggregation of unsubstituted regions of the AX, stabilised by hydrogen bonds. In such regions, the AX chains are more flexible and able to align whith each other (Courtin and Delcour, 2002).

Water-Holding Capacity

It is generally accepted that the arabinoxylans have an excellent water-holding capacity (Banu ş.a., 2000), although few studies have been published on this property. Bushuk (1966) suggests that the water uptake of pentosans is about 15 g water per g dry pentosans; thus, pentosans have an impact on the water absorption of dough (Autio, 2006).

Surface Activity

Wheat arabinoxylans and arabinogalactans decrease the surface tension of water (Izydorczyk et al., 1991). Whether this is the property of pentosans or proteins present in the preparations has been discussed by Eliasson and Larsson (Autio, 2006). They concluded that the obtained curves are typical of uniform and amphiphilic molecules, indicating that not only the arabinogalactans but also the arabinoxylans are truly linked to proteins.

Molecular Weight

In practice it has proved difficult to determine the molecular weight of polysaccharides because it is difficult to define their conformation and especially at high solute concentrations some polysaccharides self-associate (Autio, 2006). Molecular weight of AX was reported to be in the range between 22,000-5,000,000 Da (Wang, 2003).

Viscosity

AX form highly viscous solutions when dissolved in water (Courtin and Delcour, 2002). The intrinsic viscosity of arabinoxylans varies from one wheat flour to another (Autio, 2006). Values from 1.9 to 6.9 dl/g have been reported for wheat arabinoxylans and 0.045 to 0.062 dl/g for wheat arabinogalactans, indicating that arabinoxylan is the main contributor to the high viscosity of pentosans in aqueous solution (Izydorczyk et al., 1991; Autio, 2006). The arabinoxylans show a value similar to that of guar gum (2.3 to 6.8 dl/g) but are more viscous than dextran (0.214 dl/g) and gum arabic (0.12 to 0.25 dl/g).

Oxidative Gelation

This unique property of oxidative gelation of flour water-solubles was first described by Durham (1925) and later shown to be caused by the pentosans (Miller and Hoseney, 1999; Moore et al., 1990). Arabinoxylans containing ferulic acid have the property to cross-link and form gels at room temperatures upon the addition of oxidizing agents. Only a few oxidizing agents have the ability to induce gelation. These include ammonium persulfate, formamidine disulfide, and hydrogen peroxide in the presence of peroxidase, which causes the formation of free radicals. Potassium bromate and ascorbic acid, two oxidizing agents commonly used in bread, do not cause gelation. Hydrogen peroxide is effective, working together with the peroxidase that flour contains naturally. No heat treatment is required (Autio, 2006). Gelation was affected by the pentosan concentration, oxidant oncentration, pH, ash, and presence of organic compounds. Fincher et al (1974) determined that the water-soluble pentosan fraction consists of three components: an arabinoxylan, a free protein, and a protein covalently linked to an arabinogalactan (Moore et al., 1990).

Neukom and Markwalder (1978), Hoseney and Faubion (1981) suggested several hypotheses for the oxidative gelation mechanism, but no consensus could be found:

(1) Arabinoxylan ferulic acid is linked to tyrosine residues or to ferulic acid complexed with protein;

(2) Arabinoxylan ferulic acid is linked to N-terminal protein groups;

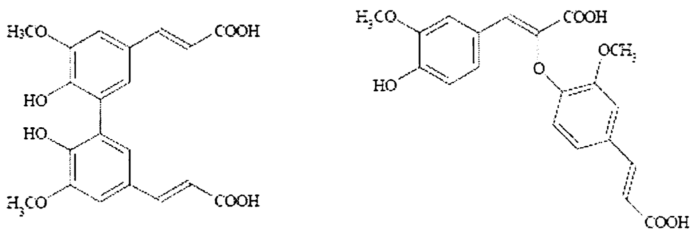
(3) Part of ferulic acid is bound to the glycoprotein and serves as a bridge between the protein and pentosan; or

(4) A covalent binding of protein with the arabinoxylan chain via a ferulic acid group is involved.

All the data make it clear that ferulic acid plays an important role (Autio, 2006; Miller and Hoseney, 1999). Figueroa-Espinoza şi Rouau (1998) considered that the AX cross-linking is caused by formation of diferulic acid. Five main diferulic acids (di-FA) (5-5', 8-5' benzo, 8-O-4', 8-5' and 8-8' di-FA) are identified in gelled WE-AX, the 8-5' and 8-O-4' forms being always preponderant (Figueroa-Espinoza and Rouau, 1998; Carvajal-Millan et al., 2006) (fig.4.). The involvement of a trimer of ferulic acid (4-O-8', 5'-5''-dehydrotriferulic acid) in laccase crosslinked wheat WEAX has been recently reported. In addition to covalent cross-links (di-FA, tri-FA), the involvement of physical interactions between AX chains was suggested to contribute to the arabinoxylan gelation and gel properties (Carvajal-Millan, 2006). The main result of oxidative cross-linking is a strong increase in the viscosity of AX in solution. When the AX concentration is sufficiently high, a gel is formed (Courtin and Delcour, 2002).

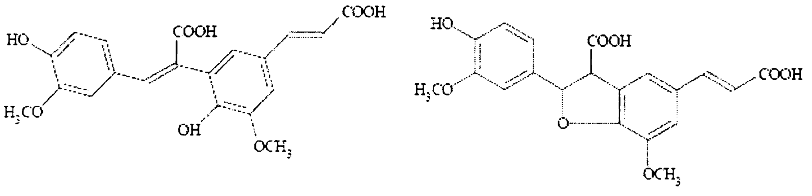
WE-AX gels present interesting properties like neutral taste and odour, high water absorption capacity (up to 100 g of water/g of dry polymer) and absence of pH or electrolyte susceptibility (Izydorczyck and Biliaderis, 1995).

It has not been confirmed that protein has a role in the gelation, but experiments involving inhibitors of the oxidative gelation process suggest that the aromatic ring and not the propenoic moiety is involved in the reaction of ferulic acid.



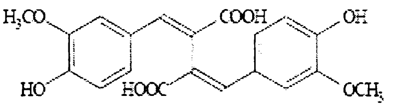
5,5'-diferulic acid

8-O-4'-diferulic acid



8,5'-Diferulic acid

8,5'-Diferulic acid benzofuran form



8,8'-Diferulic acid

Fig. 4: Diferulic acid (di-FA) structures identified in WE-AX gels

The most important gel properties are water-binding capacity and gel strength. The term "gel strength" often is used to describe both the waterbinding and texture, even though the two properties are not always correlated. Although the gels are very soft, they nevertheless have a large capacity to bind water. Water-insoluble arabinoxylans reportedly form a gel under the same conditions as water-soluble arabinoxylans (Autio, 2006).

References

Åman, P, Andersson, R., Fredriksson, H., (1999). Grain polysaccharides, versatile substrates for enzymes. *2nd European Symposium on Enzymes in Grain Processing ESEPG-2*, p.9-21.

Mortimer, R.K. (2000). Evolution and variation of the yeast (Saccharomyces) genome. Genome Res., 10, 40[illegible]-409;

Naumov GI, James SA, Naumov ES, Louis EJ, Roberts IN (2000) Three new species in the Saccharomyces sensu stricto complex: Saccharomyces cariocanus, Saccharomyces kudriavzevii and Saccharomyces mikatae. Int J Syst Evol Microbiol 50:1931-1942;

Nguyen, H-V., Lepingle, A., and Gaillardin, C. (2000). Molecular typing demonstrates homogeneity of Saccharomyces uvarum strains and reveals the existence of hybrids between S. uvarum and S. cerevisiae, including the the S. bayanus type strain CBS380. Syst. Appl. Microbiol., 23, 71-85;

Rainieri, S., Zambonelli, C., Hallsworth, J.E., Pulvirenti and giudici,P.(1999). Saccharomyces uvarum: a distinct group within Saccharomyces sensu stricto.FEMNS Microbiol.Lett., 177, 177-185;

Seoighe, C. and Wolfe, K.H. 1998. Extent of genomic rearrangement after genome duplication in yeast. *Proc. Natl. Acad. Sci.* 95: 4447–4452;

Sherman, F., Fink, G.R., and Hicks, J. B. 1986. *Methods in yeast genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Sicheritz-Ponten, T. and Andersson, S.G. 2001. A phylogenomic approach to microbial evolution. *Nucleic Acids Res.* 29: 545–552;

Yamagishi H, Ogata T (1999) Chromosomal structures of bottom fermenting yeasts. Syst Appl Microbiol 22:341-353;

WEB SITE REFERENCES

http://www.cogeme.man.ac.uk/Facilities/projects.html; COGEME.

http://evolution.genetics.washington.edu/phylip.html; Phylip.

http://www.yeastgenome.org; SGD.