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**Voragen A.G.**

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# The cell wall and its components in cereals and pulse grains, consequences on nutritional value*

A.G. VORAGEN
UNIVERSITY OF AGRICULTURE
WAGENINGEN
HOLLAND

**SUMMARY -** The author describes components present in the cell wall of cereals and pulse grains and explains how polysaccharides are chemically made up. It is essential to find out the composition of cereals, in any case, the analytical methodology used is a very important issue. As regards arabinoxylans, components with a high water absorbing capacity, they are studied regarding the specificity of the industrial enzymes currently available. In conclusion, enzymes according to their specificity in the coupling place on the substract (polysaccharides) produce final results which might be positive or negative. Therefore, the enzyme characteristics, which are or not endo- or exoxylanases, should be considered as a very important issue in enzyme formulation in the feeding industry. Enzymes influence solubility of starch and proteins from polysaccharides, so that there is an enhancement of the kinetics of digestion and production of bioactive polymers. Finally, today there is a new approach of oligosaccharides which may help and stimulate the development of intestinal conditions helping the intestinal probiosis against zoonosis, such as *Salmonella* and *E. coli.*

**Key words:** Cell-wall, cereals, enzymes, NSP, pulse grains.

***RESUME -*** *"La paroi cellulaire et ses composantes dans les graines de céréales et légumineuses, conséquences sur la valeur nutritionnelle". L'auteur décrit les composantes présentes dans la paroi cellulaire des graines de céréales et de légumineuses et explique quelle est la configuration chimique des polysaccharides. Il est essentiel de mettre à jour la composition des céréales, et dans tous les cas la méthodologie analytique utilisée est une question très importante. En ce qui concerne les arabinoxylanes, des composantes ayant une haute capacité d'absorption de l'eau, elles sont étudiées du point de vue de la spécificité des enzymes industrielles actuellement disponibles. Comme conclusion, les enzymes, selon leur spécificité concernant le lieu de connexion sur le substrat (polysaccharides), produisent des résultats finaux qui peuvent être positifs ou négatifs. Par conséquent, les caractéristiques de l'enzyme, selon qu'elle soit ou non une endo- ou exoxylanase, devraient être considérées comme un point très important pour la formulation des enzymes dans l'industrie des aliments pour bétail. Les enzymes influencent la solubilité de l'amidon ainsi que les protéines provenant des polysaccharides, de manière à augmenter la cinétique de la digestion et la production de polymères bioactifs. Finalement, il y a aujourd'hui une nouvelle approche sur les oligosaccharides, qui pourraient aider et stimuler le développement de conditions intestinales favorisant la probiose intestinale contre certains troubles, tels ceux causés par* Salmonella *et* E. coli.

***Mots-clés :*** *Parois cellulaires, céréales, enzymes, polysaccharides non amylacés, légumineuses à grain.*

The subject of my talk is food enzymes used in the feed industry. Enzymes are used on a growing scale in the food and feed industries. More and more applications are being developed, but for many of these we do not have good control of the process, we sometimes have problems with the enzymes, which do not do what we expect them to do. And this is because we do not know exactly what the enzymes are doing to the substrates, we do not know our substrates exactly, and sometimes we do not know our enzymes. A lot of enzyme preparations are mixtures of a number of activities, and it is important to know the composition of the enzyme mixture and to control this. Firstly, here is a model of a plant cell wall. This is part of a growing cell wall, a primary cell. You can see some big bundles, which are cellulose fibrils in the cell wall. On these bundles there is another molecule, which is absorbed to the surface of the bundle and it can grow to another molecule and be absorbed again. This is what we call, these are the cellulose fibrils, these are the xyloglucans. We talk about the xyloglucan complex. You can also see another network going through this network, and this is the pectin or hemicellulose network. In dicotyls we have a lot of pectin, in monocots, cereals and grasses, we often have hemicelluloses. These two networks are interwoven one with other, forming the cell wall. What you do not see here is lignification, which, of course, makes the cell wall structure more

*Transcribed from tape

complicated and also complicates the action of enzymes. Here is a composition of the cell wall. We isolated the polysaccharides out of the cell wall, we call it water-unextractable solids. We did this from maize, a monocot, and soya, a dicot, and we looked at the sugar composition. These are the sugars which make up the polysaccharides, the polymers. Then you see that we always have in the sugar composition quite a large amount of glucose, coming from the cellulose and the xyloglucans. Here you also see the xylose, you see a substantial amount of arabinose here, some galactose. In soya, we see very little xylose, a lot of arabinose, a lot of galactose, a smaller amount of glucose, but a much larger amount of uronic acid. And this tells you that the composition of these cell walls is completely different. Another important point is that these polysaccharides can be acetylated, and when they are acetylated, enzymic degradation is more difficult. The pectins can also be macillated. This gives us the sugar composition then, but does not tell us what the polysaccharides look like. To find this out, we need to know more than the composition: we have to know how these sugars are linked to each other, the glycosidic linkage composition. We can do this using chemical methods, but I will not here go into detail on this subject. We can use periodate oxidation or methylation analysis, or we can carry out analysis, but this is fairly difficult anomer when you have big molecules too complicated to give straight conclusions. You get some information, but not of the detailed kind you want. You can also take the polysaccharide, degrade it with enzymes, preferably pure enzymes, to oligosaccharide structures, and these structures fit into modern equipment like fat amas or pyrolythic amas or into the anamark, and then you can get valuable information enabling you to construct the polysaccharide you are dealing with.

Here is a monocot and another cell wall material where we try to describe the polysaccharide - no, I do not give the sugar composition, but you can see here that in this monocot we have 30% cellulose and in another cell wall material we also have 30% cellulose. And we look at pectin, pectin is a polymer of galacturonic acid, 5%, and 35% Arabinoxylan,(here 30%,and 5%), just the opposite. Glucans, high here, here nothing. Xyloglucan, low here, high here. And here you have structural proteins. So you see the cell walls of these materials are made up of this polysaccharide.

I shall now focus on one cereal, on wheat. We isolated the non-starch polysaccharides, the cell wall polysaccharides, from wheat flour and from wheat bran. Here you see the composition of the flour, and here the composition of the bran. Of course, there is much more cell wall material, the composition has mixed glucans, cellulose, much higher here, arabinogalactans, quite low, and arabinoxylans, 1.9% here and 30.5% here. So you can see that even in one commodity there are large differences in the tissue regarding the composition of the polysaccharides. Well, here is the structure of the mixed-linked glucans, where here you have cellulose, glucose-linked β(1-4) to another glucose and you have very long molecules, thousands of molecules connected to each other. Here in the mixed-linked glucans you have β(1-4), β(1-3), β(1-4), β(1-3). It is not completely alternating, but next to Beta 14, let us say on every three Beta 14 you have one Beta 13. And this is a completely different molecule to this one. This is not soluble, it is a rigid molecule. This is a more flexible molecule and this is very soluble and gives you viscosity, causing problems.

Here you have the structure of a xylan. It is a xylose molecule, β(1-4) linked to each other. But on this backbone are single-unit side chains of arabinose, linked to the two position. It can be linked to the three position, but it can also be linked to both the two and the three positions. This is a single substituted xylose, as is this, and this is a double substituted xylose. I mention this because, as I shall show, this is important for enzyme action.

Then we have arobinoxylam, and in solution they have high water absorbing capacity. They give highly viscous solutions. They can, under certain circumstances, gel. You can use this in bran making. They can also stabilise protein foams upon heating. So they have a number of important functional properties. Also, in a feed mix. I mentioned that you can degrade arabino xylans with enzymes. Here I show the reaction products you get when you degrade such a big molecule, you get small fragments. And these are the fragments we isolated when we degraded arabinoxylans with one enzyme. And I will not go into detail here, but you can see fragments where there is no substitution, fragments where there is single substitution and fragments where there is double substitution. But what is most important is that this enzyme can split next to a substituted xylose residue, it can split next to the sugar molecule. And where you have a double substitution it has always two unsubstituted here. When you have a single substitution, you can have one here. We use another enzyme. When you look now, you see a similar range of products, but when you look here you always have one, or two unsubstituted residues on that side of the fragment, and here, again, when you have

a double substituted you have two sugar here. So this tells us something about the specificity of the enzyme. I can tell you that this is an enzyme which can be used to improve baking quality. The one I showed you before does not. This is very important.

When we now look at the total arabinoxylan molecule, and you have the hypothetical structure where you have some substitution with double and single substituted arabinose, and on the top you see the points where the first enzyme can attack, and on the bottom you can see where the second can attack. And you can see that this one has many more sides of attack than the other one. I will now show you why this is important. When we have an arabinoxylan in solution, and we add the second enzyme, we call it Endo 3, there is a very rapid drop in viscosity. When we use the other enzyme, there is hardly any change in viscosity, and you need very high concentrations to get a drop in the viscosity. So they have a different mechanism of degradation.

When we look at the solubilisation of arabinoxylans from a suspension of wheat flour, you can see that Endo 3 very quickly solubilises arabinoxylans from the insoluble material. Up to 80% within a few minutes. Endo 1 takes much longer to do this.

When we look at a suspension of wheat flour in water, and we add those enzymes, with Endo 1 there is a fall in viscosity, whilst with Endo 3 you get an increase in viscosity, and then this goes down very slowly, and you need high concentrations to bring the viscosity down to the level of Endo 1. This is something which needs to be taken into account when these enzymes are used in feed. Obviously, the first enzyme is better at reducing viscosity than the other, which does not solubilise arabinoxylans. However, I must add that I think that the effect of viscosity on the nutritional value of a feed is overstressed. Viscosity is important, but only when you have extreme values, when you have a very high viscosity, but in the medium range viscosity is not so important for the digestibility of the feed.

Here we have a wheat suspension where we add enzymes numbers 1 and 3. What can we see here? With Endo 3 there is a very quick swelling of the insoluble material. You change the binding properties of the wheat flour. Here, the swelling develops slowly with this enzyme. So you see a distinct difference between the action of these enzymes, and this is determined by the fine structure of the arobinoxylan, and of course, the mechanism, the pattern of action of the enzyme.

Well, here are some structures of arabinoxylan, from wheat, rye, barley and sorgum. You can see that there are a lot of double-substituted silosil residues in rye, more than in wheat. In barley, the frequency of single-substituted silosyl residues in the two position is higher, and the enzymes are also hindered by such a substitution in the two position. Looking now at sorghum, we see a very complicated structure. Almost every silosyl residue is carrying a side-cope next to arabinose, we also have gluroronic acid. They are also acetylated. We also have ferroryl groups on certain arabinose, and when you have this structure, a mix of enzymes, this molecule is hardly affected by the enzymes, it is not degraded. It is a difficult molecule to degrade.

For the complete degradation of arabinoxylans, I have a list of activities you need: you need endoxylanases, which can degrade in the backbone, and you have glucuronic acid in the side-chain, you need a glucuronicxylanase which will split close to a glucuronic acid substitution. You need xylanacetylesterase to remove the acetyl groups. You need an arabinoxylanferruvialesterase to remove the ferruvial groups. You need an arobinoxylanarabinohydrolase to remove the arabinose groups. There is another enzyme which can do that, arabinofuranoxidase when you have longer side chains as in maize or sorghum. You need an endoarabinase and a betacilositase could also be involved. These are the enzymes you need when you want to saccharify your molecule to monosugars. But I think that is not what you want in the feed industry, because we know that when you generate a lot of monomeric xylose and arabinose, the animals have problems with this. So you have to make a choice among these activities to get where you want to be.

What is the consequence of this for feed analysis? Well, up to now, we do an SP or a neutral detergent fibre or acid detergent fibre. These values tell us nothing about the polysaccharides present in a cell wall. Even when we isolate the polysaccharide, what we used to do was to look at the arabinose-xilose ratio and at the molecular weight, but this is not enough. Another thing we did was to look at the amount of unsubstituted, mono and disubstituted silosyl residues. But that is not enough. We have to know how these sidechains are distributed over the xilan molecule, and with this knowledge we can predict what the enzyme will do.

That is wheat, then. I now want to talk about food industry by-products used in the feed industry. Here we can see the difference, what is fibre for us is not always fibre for the cow or the pig or the chicken. Here is the composition of sugarbeet powder, which is used widely in the feed industry. We have a lot of arabinan, a lot of pectin, a lot of cellulose, protein, some non-analysed lichenin and non-analysed material. Now we know the structure of these arabinates. I am not going to show them all, but the polysaccharides in that by-product have been analysed and we have a pretty good picture of what these molecules look like.

Here we have for the sorghum kernel 60% glucuronoarabinoxylan, as I showed you, cellulose, some glucan and some other material.

Here you have palm kernel and meal, where you also have some glucoronoarabinoxylans, a huge amount of manans, cellulose and some arabinoxylan. The mannan is a better one for polymer of mannose, molecules connected to each other. I will return to this subject, because there is something interesting to say about this molecule.

Here you have sunflower meal, where we have xyloglucan, cellulose, glucuronoxylan, etc. You see you have a different set of polysaccharides. I have mentioned pectins. These are very complicated molecules, and when we talk about pectin we usually talk about this part of the molecule. A linear molecule of galacturinan residues connected to each other in an alpha one-four way. This is the pectin we use to make jam and marmalade. But in the cell wall is part of the pectin which is very heavily branched, and we call this the hairy part of the pectin molecule. The set of enzymes to degrade this is known, a whole set of enzymes needed and which are known and available. But in this part we know very little, and in the last five years we have found five new enzymes which were not known before and which are able to degrade this structure.

Here is the pectin molecule, the galacturonic acid, alpha one-four linked. You see that it is a galacturonic acid, they have a carboxyl group which can be macillated to get a high macil pectin, which is what is used to make marmalade. With an enzyme you can remove the methoxyl groups to get a pectic acid, and this pectin you use to make low calory marmalade with a different gelling mechanism. But then you have enzymes which can split into backbone, or flow-esterified pectin, polygalacturonase and pectiliase. Here you have an enzyme, pectiliase, which can split into highly-esterified molecules. When we do this, we obtain degradation products, oligomers. I will come back to this.

Here you have the xyloglucan molecule, which absorbs to a cellulose fibril. On this side of the molecule it is very similar to cellulose, with a cellulose backbone, and it absorbs by hydrogen bonding, it absorbs very strongly. And on this side are some side chains. Very often we talk about cellulases, which are important as an enzyme in application, but among the cellulases, you have enzymes which are in fact endoxyloglucanases, they are specific for this type of substrate, and they can split here, next to a free, unsubstituted glucose residue. And, again, you can get very good oligosaccharides.

Another molecule which is part of the hairy regions of the pectin, this is a galactan, which you have in soya. It is a galactose polymer, beta one-four linked, and sometimes you have a side chain of a galactose or of an arabinose, alpha one-five linked. And here, again, you need a complete enzyme mixture to degrade this. You have enzymes which split in the backbone, enzymes which can take away galactose here, or take away the side chain, and when you take away that side chain this enzyme starts to work much better. And when you take away this side chain with arabinases the enzyme works much better, so you have a very strong synergy between these enzymes. And when you have a complex mix of enzymes, you also get a totally different mixture of oligosaccharides. So the mixture of enzymes determines what reaction products you obtain.

What, then, are the concepts of the mechanism of enzyme action? Why do we add enzymes to feed? Well, the concepts you can find in the literature are solubilisation of the polysaccharides, of starch and proteins; to release starch and protein from the cell wall structure to make them better available for the digestive system; loosening of botanical structures; elimination of gel-forming polymers - this is the viscosity concept, when you have very high viscosities; when you can loosen the structure, enzymes can better penetrate into the system and have a degradation; enhancement of

the kinetics of digestion and production of bioactive polymers. Particularly in Japan and also in Europe some companies are adding oligosaccharides to the feed, as they feel they have a positive effect on animal and human health, it is also used in humans. The idea is that these oligosaccharides stimulate the development of bifido bacteria and lactic acid bacteria in the colon. And there are data which show that the relative amount of these microorganisms increases in the human colon, but also in the colon of piglets. Another theory is that they suppress the pathogenic and putrefying bacteria, instead of a putrefaction in the colon, you get more a saccharolythic fermentation, beneficial for the host. There is a decrease in the pH, activation of the lactoperepside system, production of bacteriocynes and anti-microbial action of certain oligosaccharides. Another theory is that you get the formation of certain oligosaccharides which hinder the binding of certain pathogens to the cell wall of the intestine, particularly *Salmonella* and *E.coli*, and what is important for humans, they can prevent constipation. I have shown you that from cell walls and with our enzymes we can make a whole range of oligosaccharides, and it is interesting to know what these do for animals or for humans. Here is a schematic representation of the cell wall structure, the cellulose, xyloglucan, pectin or the hemicellulase network. We can add enzymes which degrade the pectin particularly in the smooth region, loosening up the cell wall structure, producing degradation products of pectin. This is often done in the fruit juice industry. You can also add hemicellulases, stripping the side chains from the pectin and get only the solubilisation of the hemicellulase fragments. You can also add only cellulases. What happens is that where there is no xyloglucan on the surface, there will be some degradation of the cellulose. But usually when you have cellulases alone, there is not much action, because the pectins prevent the penetration of the enzymes into the system. Now you add cellulases and pectinases, and what you get is the complete liquefaction of the system, with apples and many other things, you can completely saccharify the cell wall, change the cell wall from a solid substrate to a monosugar solution. Here you have a situation where you add a pectinolytic and hemicellulolytic enzyme, where you remove this network, and this network remains. This is also an interesting phenomenon, because here you have certain functional properties which are important. This is what we studied for apples. It is not necessarily true for all tissues - you have to study this, and I must also admit that when you have lichenin in the system, then the enzymes are limited to a large extent in their action on such structures. But on a number of substrates you can induce a number of important and interesting changes in the cell wall. I have mentioned oligosaccharides, and I will show you a number. Here you have xyloglucan degradation, analysed by high-performance chromatography, and here you see the kind of structures we are getting. By the way, in our gut, we have enzymes that can do that. Here you have degradation of arabinans. You get molecules and you go up to molecules with 20 sugar residues, and you have some control over the size of the oligosaccharides you want to make. Here you have fragments of degradation of hairy regions of pectin. You have a molecule, here a tetramer, alternating ramnose galocturonic acid, and you have some large ones, and sometimes you have a galactose here as a side-shape. What is the effect of these oligosaccharides on the gut system? Here I want to go back to kernel meal. When you degrade kernel meal with a gluconase, with a cellulase system, you get some solubilisation of the mannans, 5%. When you do it with a mannanase, you get around 20%. The composition of the digest has 26% mannose and 2.4% glucose. Here you have 4% glucose and 3.4% mannose. You have the combination, you get about 50%, it is 59% mannose, 6% glucose. And here, in this digest, you have 73% dimer, 9% monomer and 22% trimer. And we know, it has been shown in the literature, that these mannose oligosaccharides can prevent the binding of salmonella to the gut of an animal. This is an important effect.

So what are the perspectives for this work? When we know more about structure and function relationships, we can identify relevant enzymes. We also need to know the role of endogenous enzymes, particularly in cereals. There are enzymes in cereals which we do not know and which interfere in the application. We can make better formulated enzyme preparations. We have to make defined cocktails. We can also genetically modify enzymes. First of all, when we know an enzyme, we can find the gene and hyperproduce it. Enzyme companies have large numbers of pure enzymes on their shelves - they have the genes and they can do that. They are even working now on improving the pH optima and temperature optima of these enzymes. We can also go to transgenic crops, we can introduce certain enzymes into the crop itself. And, of course, we can make new applications. Thank you for your attention.