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Remarks on multidetector HPSEC characterization of native and enzymatically modified cereal arabinoxylans

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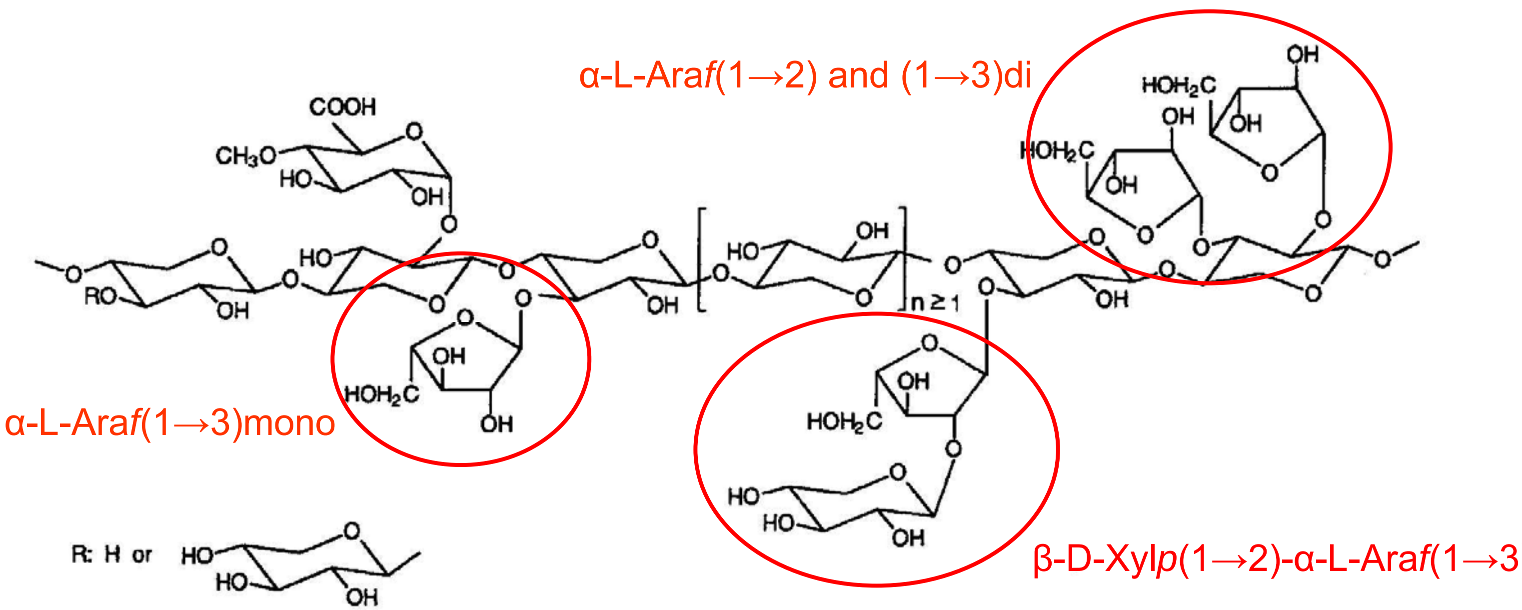
Hemicelluloses

- Large group of non-crystalline heteropolysaccharides found in the primary and the secondary cell walls of plants
- The second most abundant polysaccharide after cellulose produced by plants
- Arabinoxylans are the main hemicellulose component in cereals
  - Due to the abundant nature of arabinoxylans they could be utilized industrially as polymers for biodegradable materials

Structure of arabinoxylans (AX)

- Arabinoxylans consist of a linear (1→4) -linked β-D-xylopyranose backbone with α-L-arabinofuranosidase substituent at the positions of O-2, O-3 or both



Esterified acetyl, feruloyl and p-cumaroyl groups also exist

Ebringerova and Hromadkova 1997
Ebringerova and Heinze 2000

Arabinoxylan content in cereals (%)

Cereal material	Arabinoxylan content (%)
Flour	1 - 5
Whole grain	5 - 10
Bran	15 - 25
Husks (spelts, hulls)	15 - 40
Straw	10 - 25

Henry 1987, Puls 1992

- Cereal husks and straw are rich in arabinoxylans
  - barley husks may contain from one third to 46% of arabinoxylan

Composition of arabinoxylans varies in different cereals and their parts

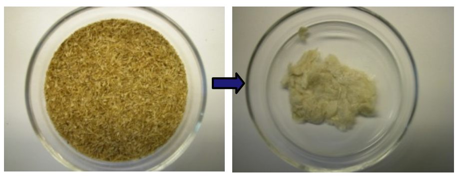
Cereal	Husks Ara : Xyl	Endosperm Ara : Xyl
Wheat	0.55	0.56
Barley	0.36	0.67
Oats	0.11	0.77
Rye	0.48	0.59

Henry, J. Cereal Sci. 1987




Cereal arabinoxylans in our studies

- AX isolated from barley husks (BH) and barley fibre (BF)



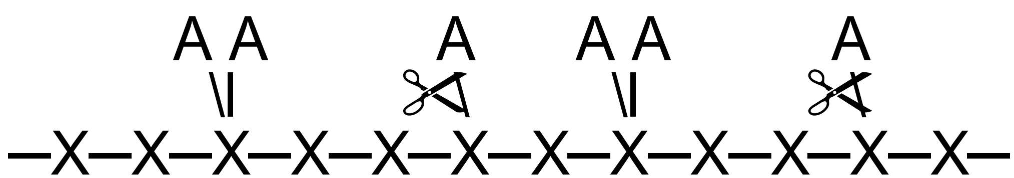
  - laboratory scale alkali-extraction
  - BH and BF side-stream fractions from industrial ethanol process (Koskenkorva, Altia)

- Commercially available arabinoxylans from wheat (WAX) and rye (RAX) endosperm
  - good polysaccharides for structure-function studies due to the high level of purity
  - used for enzymatic modications

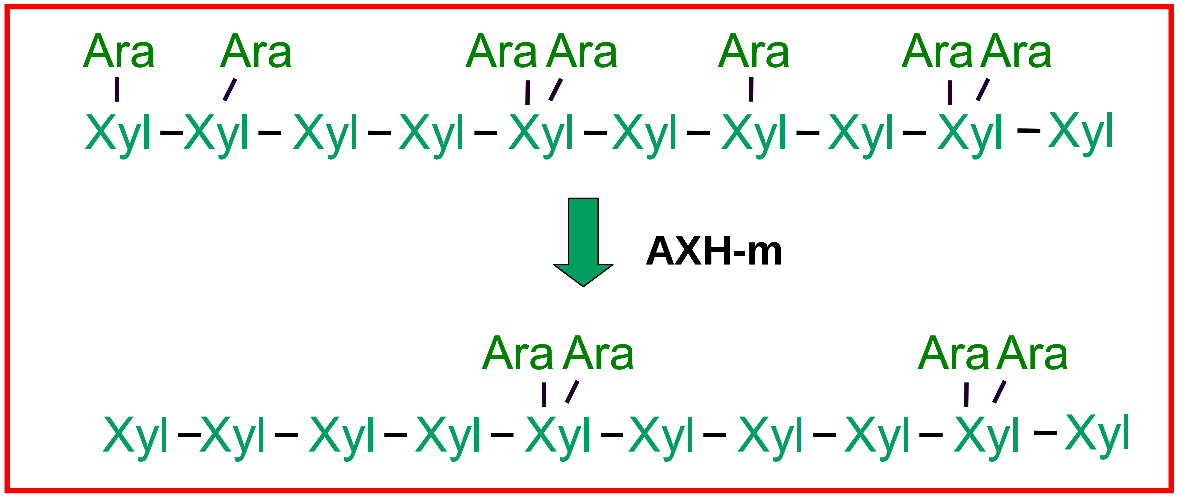
Why to use enzymes for modification of biopolymers?

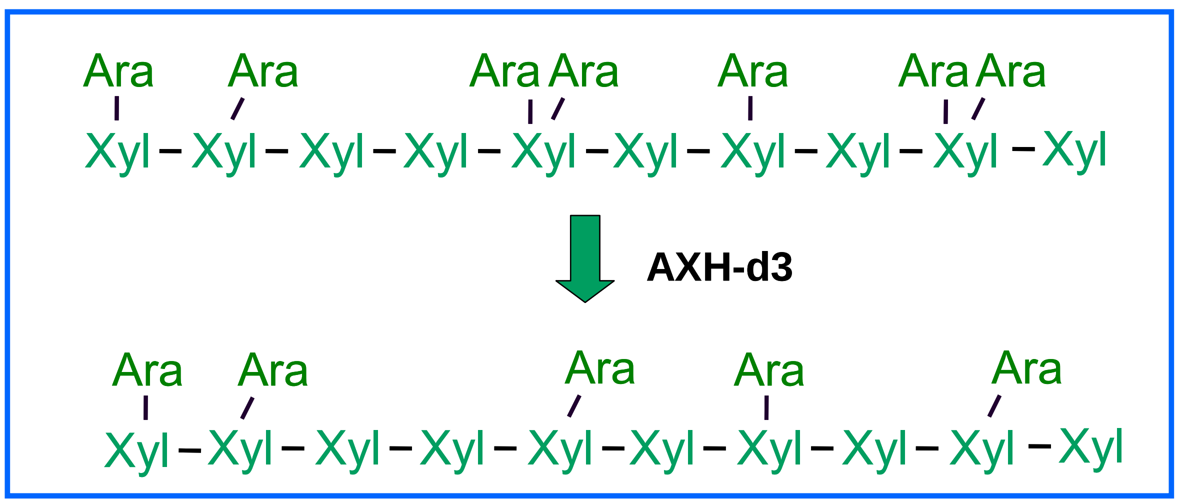
- Production of molecules with varying chemical structure
  - Convenient way to produce chemically different molecules (vs. extraction from plants)

- Controlled modification with spesific enzymes



Action of enzymes (AXH-m and AXH-d3) used for enzymatic modification and for structure elucidation





Van Laere et al., (1999) AMB

Structural differences between wheat and rye arabinoxylans

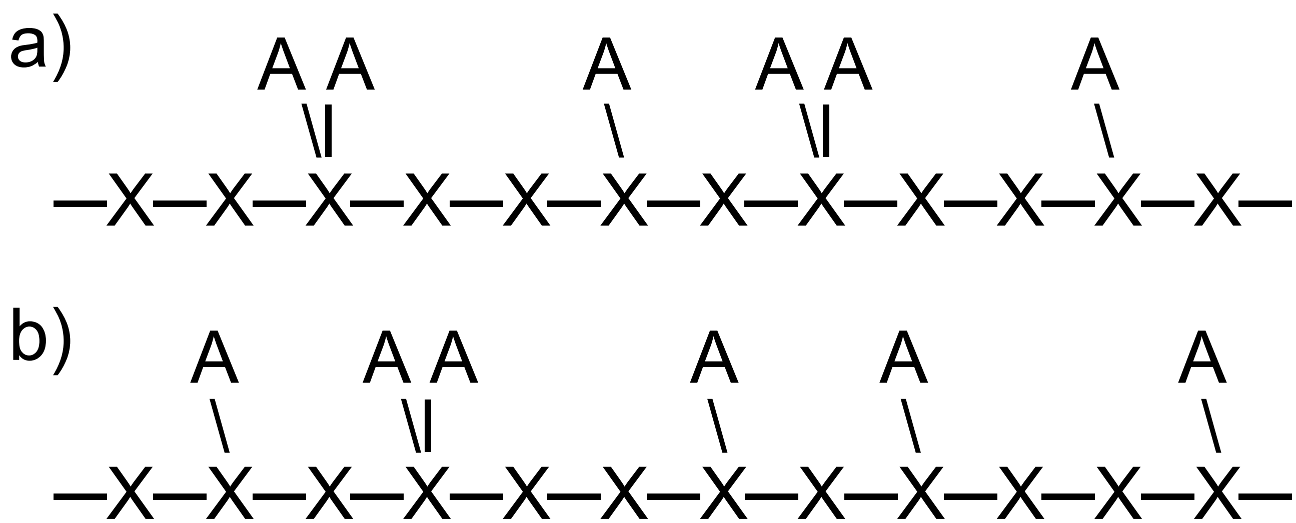
- Relative intensities (%) of α-anomeric protons of α-L-Ara*f* units

Sugar residue	WAX-HV (%)	WAX-MV (%)	WAX-LV (%)	RAX-HV (%)
α-L-Ara*f*(1→3) mono	34.2	34.8	32.8	66.1
α-L-Ara*f*(1→3) di α-L-Ara*f*(1→2) mono	33.3	32.1	40.2	17.3
α-L-Ara*f*(1→2) di	32.5	33.1	27.0	16.6

HV = high viscosity, MV = medium viscosity, LV = low viscosity

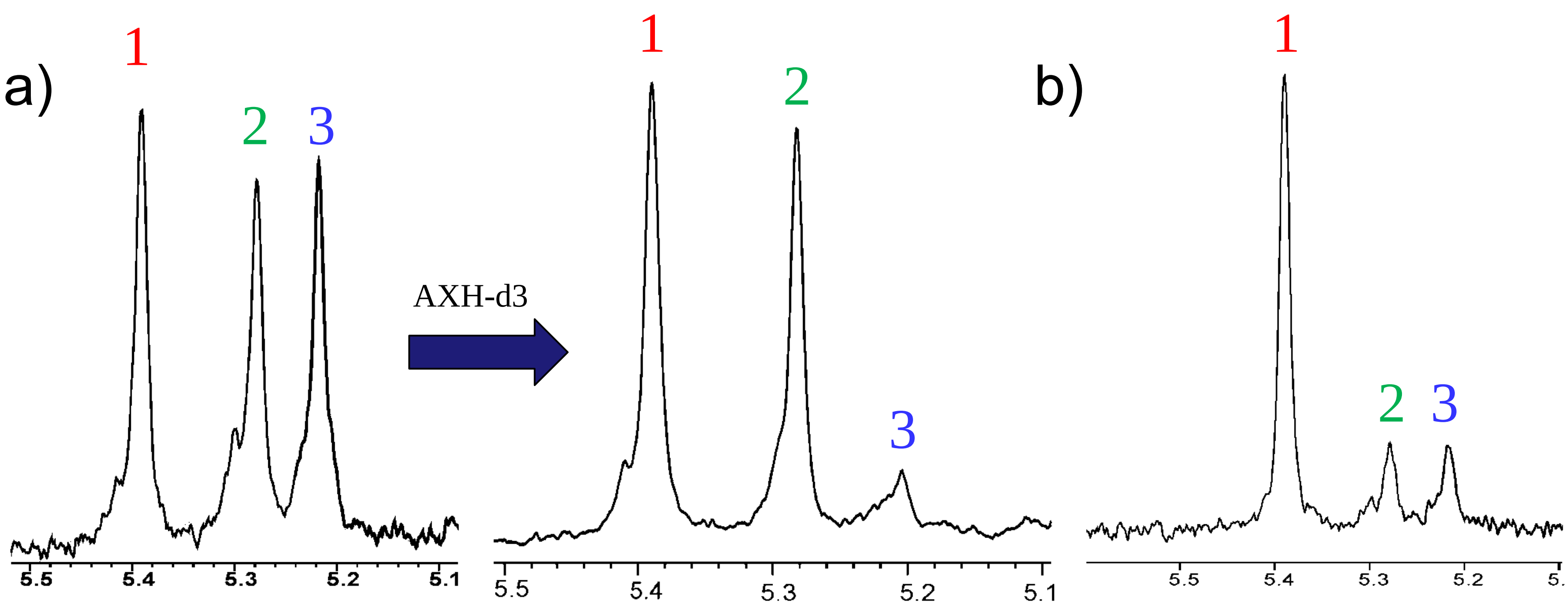
→ WAX 1/3 monosubst., 2/3 disubst.
RAX 2/3 monosubst., 1/3 disubst.

Structures of wheat and rye arabinoxylans



a) Wheat arabinoxylan
b) Rye arabinoxylan

The effect of modification to the structure of arabinoxylans (^{1}H NMR)



a) Wheat AX
b) Rye AX

1) α-L-Ara*f*(1→3)mono

2) α-L-Ara*f*(1→2)mono and α-L-Ara*f*(1→3)di

3) α-L-Ara*f*(1→2)di

HPSEC analyses of native and enzymatically modified arabinoxylans

- Many polysaccharides are known to form molecular assemblies in aqueous environment
  - Arabinoxylans with low ara:xyl ratio tend to precipitate

- Aqueous solution are generally used in HPSEC analyses of arabinoxylans
  - Behaviour in aqueous solution often interesting due to interfaces of arabinoxylans with various biological systems

→ We use both aqueous and DMSO-based solvents for analysis of arabinoxylans

HPSEC with multiple detection

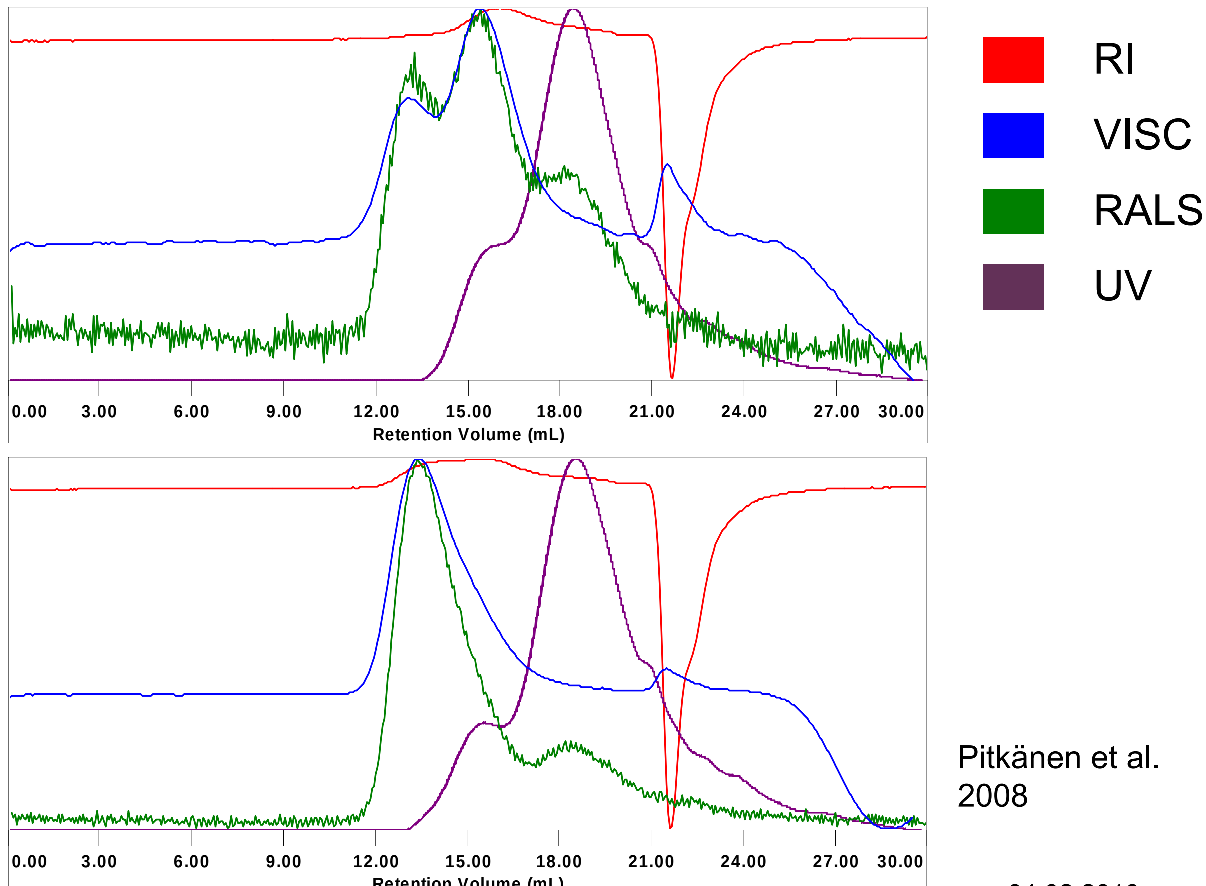
- Molar mass distribution with LS/VISC method
- Simultaneous determination of intrinsic viscosity
  - Valuable information on the molecular density and possible association of molecules
- UV useful for detection of lignin/protein impurities

HPSEC of barley arabinoxylans

- UV detection reveals the presence of protein/lignin impurities

BHAX

BFAX

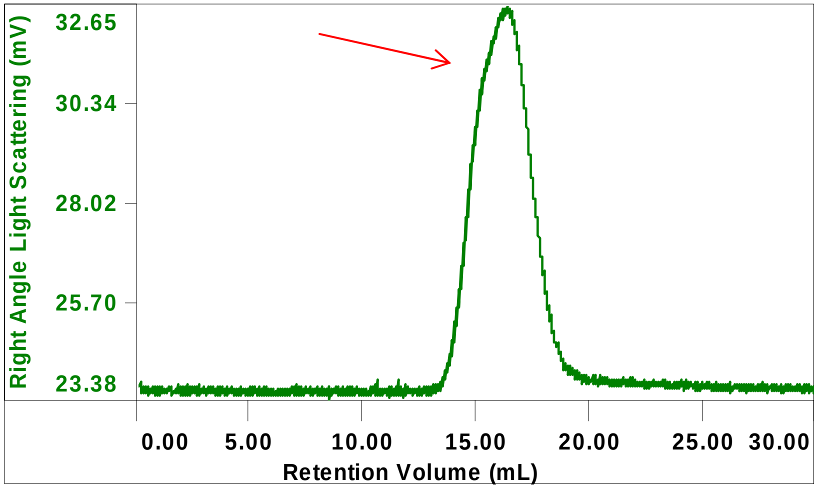


Pitkänen et al. 2008

Comparison of water and DMSO as solvents for arabinoxylan with low degree of substitution

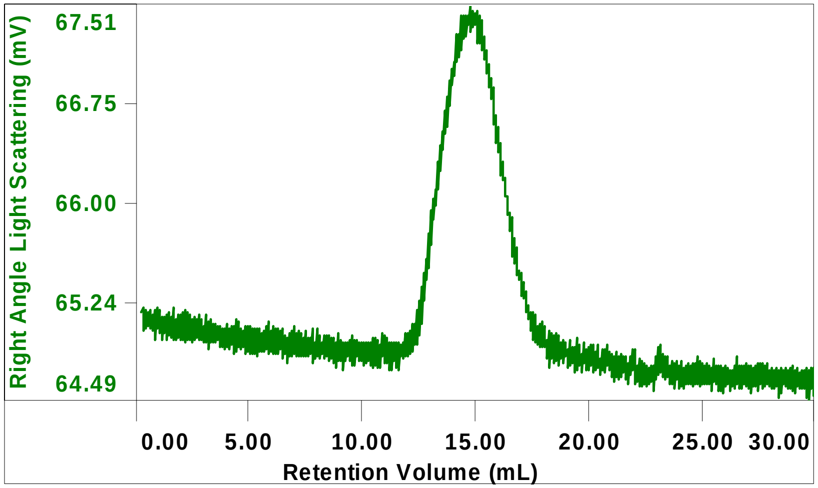
- Wheat arabinoxylan sample (WAX-LV, Megazyme)
  - Ara:xyl ratio 0.32

water + 0.1 M $NaNO_3$



M_w 8.3 · 10^4 g/mol

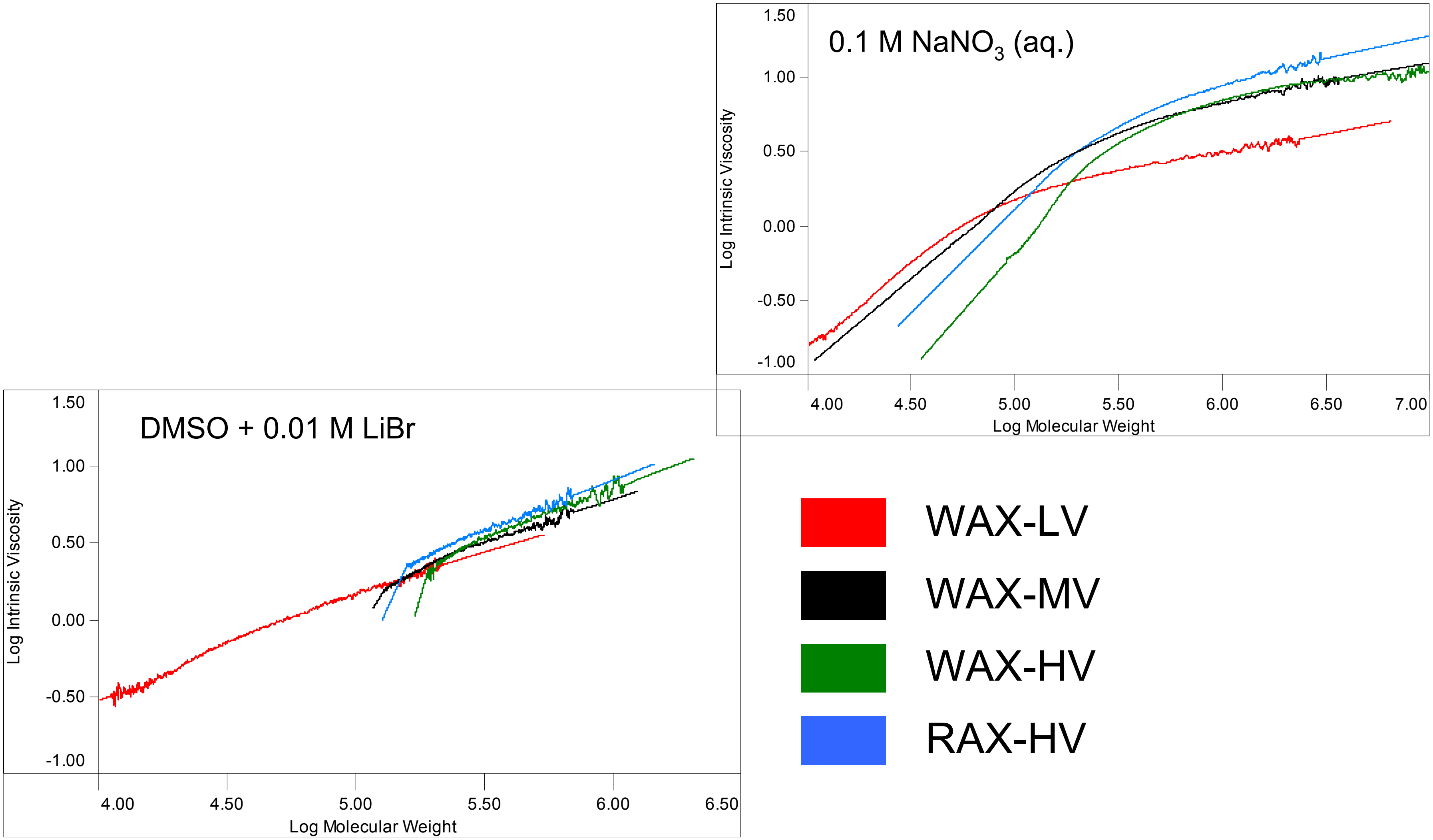
DMSO + 0.01 M LiBr



M_w 4.9 · 10^4 g/mol

Pitkänen et al., 2009

Molar mass-intrinsic viscosity relationship of wheat and rye arabinoxylans in two solvents

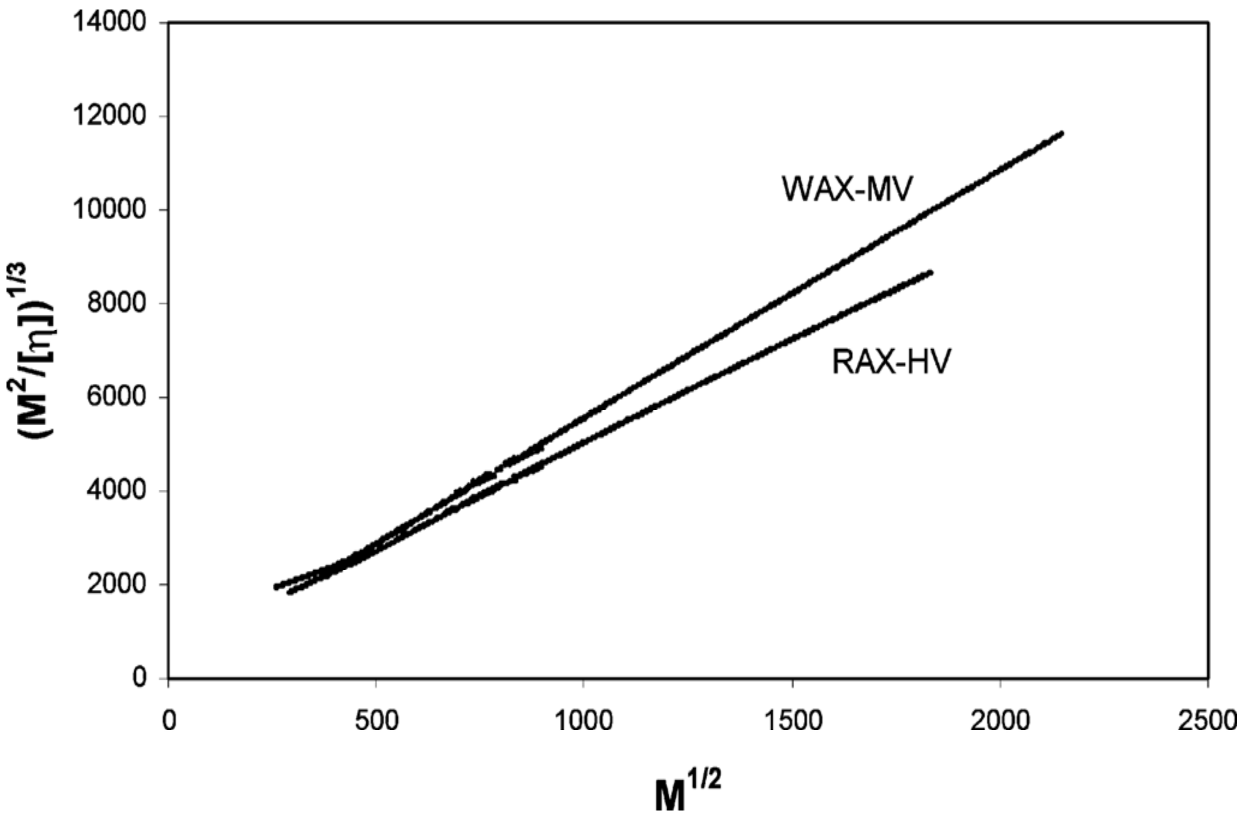


Determination of persistence lenght (L_p) using molar mass and intrinsic viscosity data

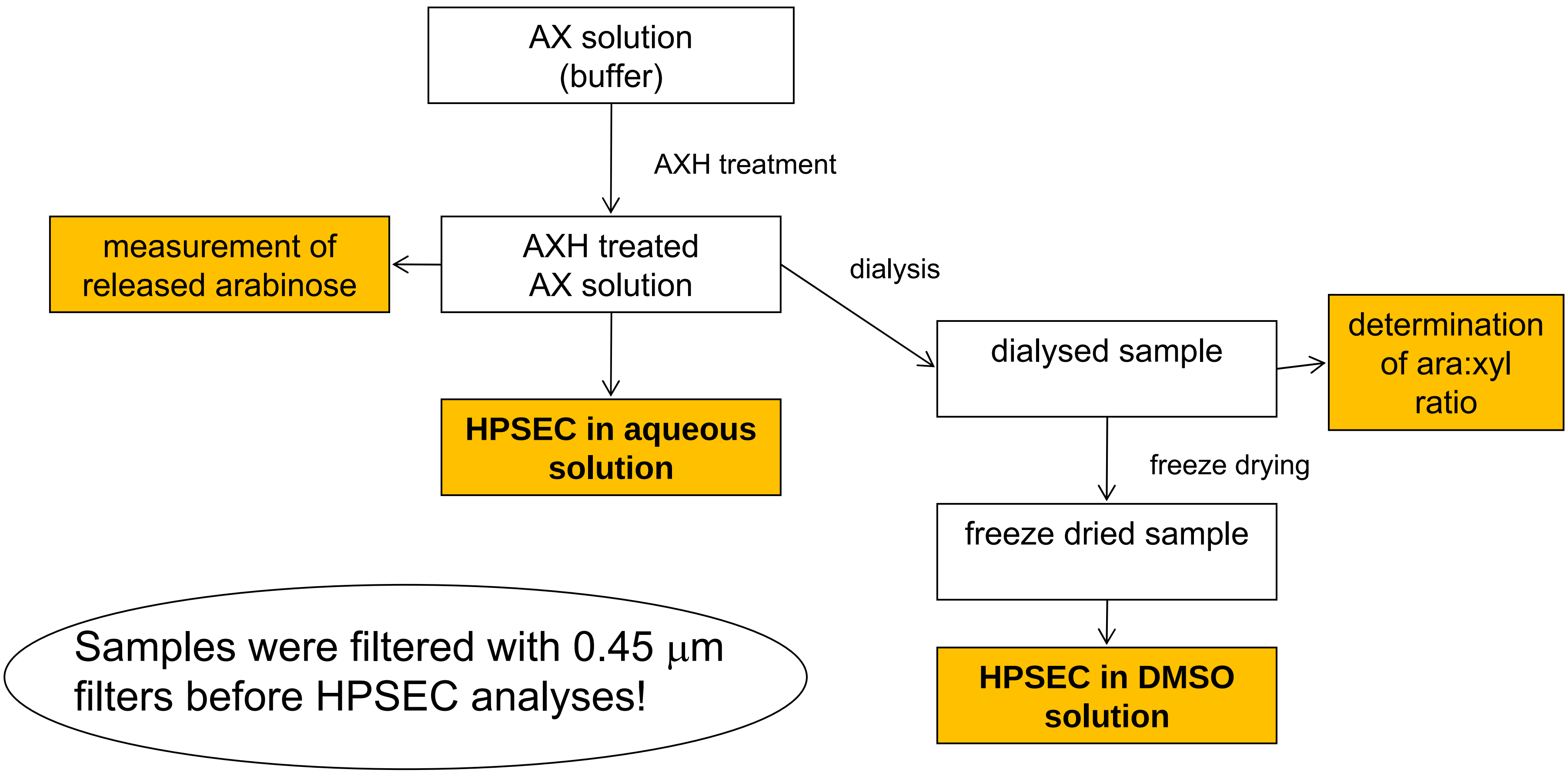
- Bohdanecký model
  - Model dependence → useful for comparison of samples

L_p

WAX-MV = 1.8 nm
RAX-HV = 2.3 nm



Enzymatic modification and HPSEC analysis of wheat and rye arabinoxylans



The effect of enzymatic treatments to molar mass distribution of wheat arabinoxylan sample with initial ara:xyl ratio of 0.51

- Molar mass distributions in DMSO + 0.01 M LiBr

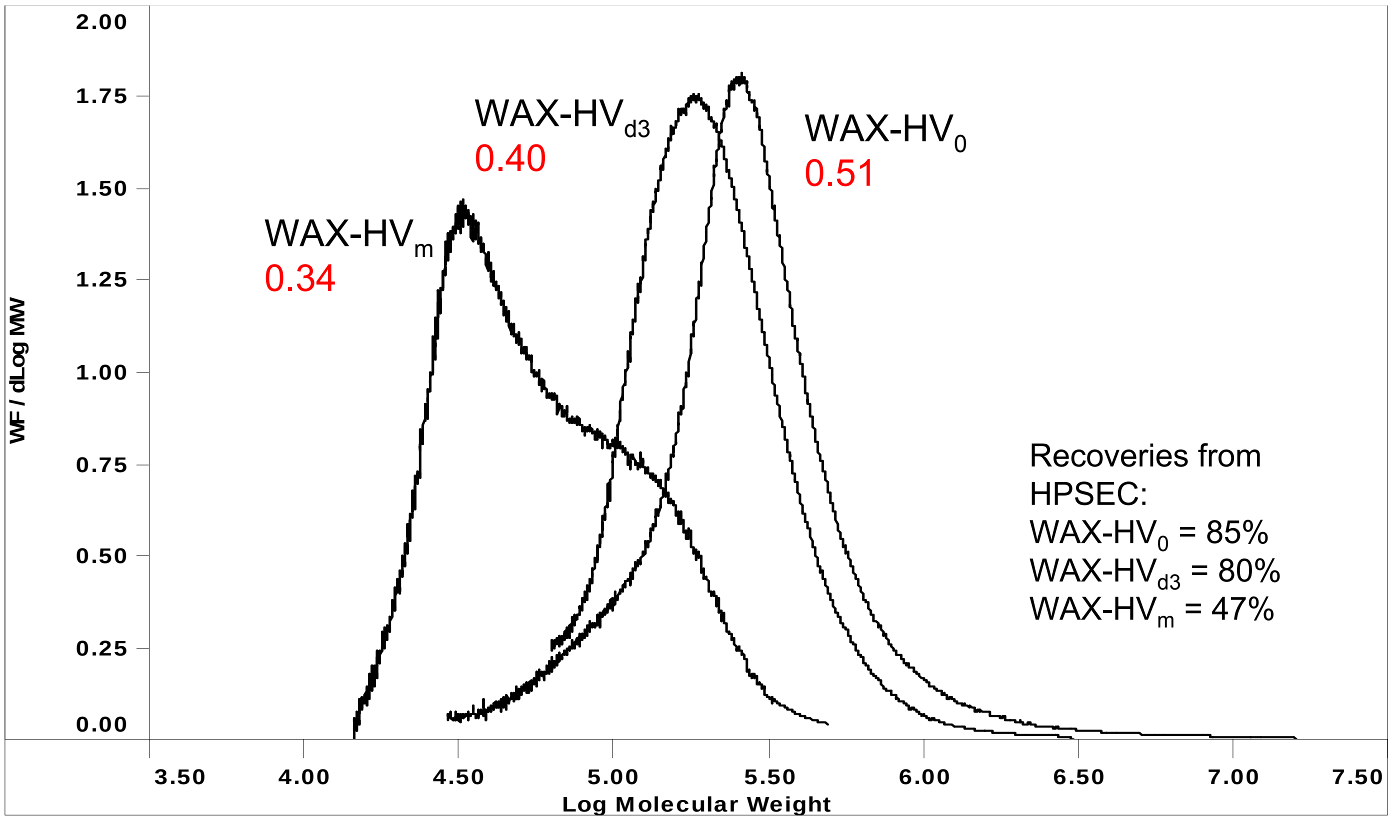
M_w values:

WAX-HV$_0$ = **2.86 ·10^5** g/mol

WAX-HV$_{d3}$ = **2.62 ·10^5** g/mol

WAX-HV$_m$ = **0.80 ·10^5** g/mol

Theoretical reduction of mass caused by enzymes:
AXH-d3 25 000 g/mol
AXH-m 30 000 g/mol



Pitkänen et al., unpublished

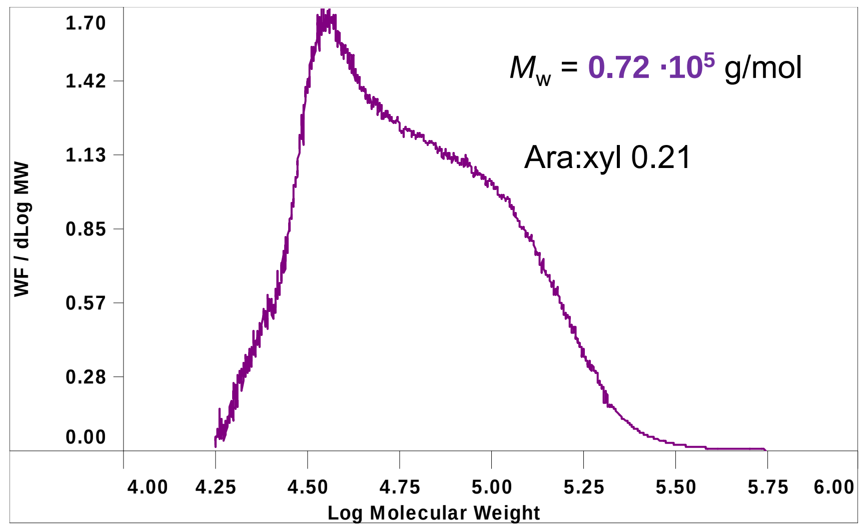
Comparison of water and DMSO as solvents for AXH-m treated rye arabinoxylan (RAX-HV)

- 47% of AXH-m treated sample dissolved in DMSO after modification, freeze drying and redissolution

water + 0.1 M $NaNO_3$

No peaks!

DMSO + 0.01 M LiBr



→ longer arabinoxylan chains could not be dissolved after AXH-m treatment

Conclusions

- UV and viscometric detection coupled to HPSEC very useful in characterization of arabinoxylans extracted from cereal sources (in addition to MALS and RI)
- Analysis of arabinoxylans with both aqueous and DMSO solutions reveals facts about association of xylose chains
- Arabinoxylan assemblies occur in aqueous solution when the amount of arabinose side groups is low
- In addition to low ara:xyl ratio, chain length seems to have an effect on the precipitation of arabinoxylan molecules
- AXH-m enzyme caused partial precipitation of xylan molecules
    - Occurrence of longer regions of unsubstituted xylose residues

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