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Biodegradable films from cereal arabinoxylans

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ACADEMIC DISSERTATION

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Abstract

Cereal arabinoxylans (AXs) belong to the heterogeneous group of hemicelluloses. They are branched polysaccharides located in the plant cell wall, where they strengthen the cell wall together with cellulose and lignin. AXs vary in their molecular structure depending on the plant source from which they are extracted. Cereal AX can be extracted, for example, from the side streams of cereal processing such as bran, husks/hulls, and straw. AXs are sustainable raw materials with a film-forming ability; they can therefore be utilized more efficiently in biodegradable packaging and coatings.

In this thesis, structurally different cereal-based AXs were used in the film studies. The effect of the type and amount of polyol plasticizer on the film properties as well as their storage stability during four and five months' time was investigated with oat spelt arabinoxylan (OsAX). The structure–function relationship of AXs in films was studied by comparing differently substituted rye and wheat flour arabinoxylan (RAX and WAX, respectively) and was further investigated by using specific arabinofuranosidases to tailor arabinose to xylose ratios and arabinose substitution patterns of RAX and WAX. The usability of crude biomass extracts in larger-scale film/sheet preparation was studied via the sheet extrusion of two wheat bran extracts (WBEs), which contained similar amounts of AX and lignin, whereas their starch and protein contents differed depending on the purification process. The film and sheet properties, such as mechanical and barrier properties as well as crystallinity and the physical state of the films, were investigated in order to evaluate the suitability of these materials for packaging.

This study revealed that AX, having relatively low arabinose substitution and molar mass, such as OsAX, in general needs plasticization for cohesive film formation. It was found that the amount and type of plasticizer clearly affects the tensile and permeability properties of the films as well as their water sensitivity. In general, the sorbitol-plasticized films were stronger and had lower water vapor permeability (WVP) and oxygen gas permeability (OP) than the films plasticized with glycerol. Structure correlation studies carried out with RAX and WAX showed that the AXs' fine structure affects film formation and film properties. With specific enzymatic tailoring, it was observed that the Ara/Xyl ratios of RAX and WAX did not contribute alone to the behavior of AXs in the films; instead, both the amount and distribution of arabinose side units in the xylan chain had a high impact on the results. When the number of un-substituted xylose units was high, the water solubility of AX decreased and the formed films had a semi-crystalline structure. Mild de-branching improved some film properties, for example, the tensile strength of the RAX films increased. Additionally, it was observed that the OP of the RAX and WAX films decreased along with the de-branching. A storage study showed that the polyol-plasticized OsAX film properties changed during storage; possible reasons for this were lowered plasticization caused by glycerol migration out from the film matrix and/or formation of heterogeneous structures in the film with plasticizer-rich and polymer-rich areas. The WBEs turned out to be potential raw materials in the up-scaled sheet preparation method, which was carried out with a single-screw extruder. Both of the extracts that were studied formed cohesive sheets with polyol addition.

In this study, all of the AX films showed low OP properties, which were further decreased by lowering the Ara/Xyl ratio or with plasticized films by decreasing the polyol content. Thus, AX films can be thought of as a potential choice for those applications where high oxygen barrier capacity is needed to prevent the oxidative deterioration of the packaged food product. Present study on AXs' fine structure gave information that can be used when various structurally different agricultural side streams are considered as raw materials for film applications. In the current study, the main challenge of the AX films and sheets was their water sensitivity, and with the plasticized films, their decreased storage stability. The sheet extrusion results proved that highly purified fractions are not essential in material production; instead, in this study, moderately purified fractions with a high starch and low protein content formed strong sheets with lowered water sensitivity.

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Espoo, January 2016

Susanna Heikkinen

List of original publications

This thesis is based on the following publications, which are referred to in the text by the Roman numerals **I–V**. In addition, some unpublished data is included.

I Mikkonen, K.S., Heikkinen, S., Soovre, A., Peura, M., Serimaa, R., Talja, R.A., Helén, H., Hyvönen, L., and Tenkanen, M. 2009. Films from oat spelt arabinoxylan plasticized with glycerol and sorbitol. J. App. Polym. Sci. 114:457-466.

II Heikkinen, S.L., Mikkonen, K.S., Koivisto, P., Heikkilä, M.I., Pirkkalainen, K., Liljeström, V., Serimaa, R., and Tenkanen, M. 2014. Long-term physical stability of plasticized hemicellulose films. BioResources 9:906-921.

III Höije, A., Sternemalm, E., Heikkinen, S., Tenkanen, M., and Gatenholm, P. 2008. Material properties of films from enzymatically tailored arabinoxylans. Biomacromolecules 9:2042-2047.

IV Heikkinen, S.L., Mikkonen, K.S., Pirkkalainen, K., Serimaa, R., Joly, C., and Tenkanen, M. 2013. Specific enzymatic tailoring of wheat arabinoxylan reveals the role of substitution on xylan film properties. Carbohydr. Polym. 92:733-740.

V Heikkinen, S.L., Jacquemin, L., Rouilly, A., Sablayrolles, C., Tenkanen, M., and Pontalier, P.-Y. Comparison of two wheat bran extracts in sheet extrusion process. Submitted.

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Contribution of the author to papers I–V:

I The author planned the study with the other authors and performed most of the experimental work. She participated in interpreting the results and preparing the manuscript.

II The author planned the study on xylans with the other authors and participated in the experimental work. She had the main responsibility for interpreting the results and served as the corresponding author of the paper.

III The author planned the study with the other authors and performed a portion of the experiments with E.S. She participated in interpreting the results and preparing the manuscript.

IV The author planned the study with the other authors and performed most of the experimental work. She had the main responsibility for interpreting the results and served as the corresponding author of the paper.

V The author planned the study with the other authors, participated in sheet production, and performed the water vapor sorption experiment. She interpreted the results together with the other authors and served as the corresponding author of the paper.

Abbreviations

Ac	*O*-acetyl group
Ara	arabinose
Ara/Xyl	arabinose-to-xylose ratio
Ara*f*, α-L-Ara*f*	α-L-arabinofuranosyl
AX	arabinoxylan
AGX	arabinoglucuronoxylan
AXH	arabinoxylan arabinofuranohydrolase
AXH-d3	α-L-arabinofuranosidase (release of (1→3)-linked α-L-Ara*f* unit from disubstituted β-D-Xyl*p* residues)
AXH-m	α-L-arabinofuranosidase (release of (1→ 2)- and (1→3)-linked α-L-Ara*f* unit from monosubstituted β-D-Xyl*p* residues)
DEA	dynamic electrical analyzer
DMA	dynamic mechanical analyzer
DP	degree of polymerization
DS	degree of substitution
GC	gas chromatography
Glc*p*A	α-D-glucopyranosyl uronic acid
GX	glucuronoxylan
MeGlc*p*A	4-*O*-methyl-α-D-glucopyranosyl uronic acid
M_w	weight average molar mass
NMR	nuclear magnetic resonance
OP	oxygen permeability
OsAX	oat spelt arabinoxylan
OTR	oxygen transmission rate
RAX	rye flour arabinoxylan
RAX-d3	RAX de-branched with AXH-d3
RAX-m	RAX de-branched with AXH-m
RAX-ref	RAX, no enzymatic modification
RH	relative humidity
RT	room temperature
T_β	β-relaxation temperature
T_g, T_α	glass transition temperature, α-relaxation temperature
TGA	thermal gravimetric analysis
WAX	wheat flour arabinoxylan
WAX-d3	WAX de-branched with AXH-d3
WAX-m	WAX de-branched with AXH-m
WAX-ref	WAX, no enzymatic modification
WBE-P	wheat bran extract with high protein content
WBE-S	wheat bran extract with high starch content
WVP	water vapor permeability
WVS	water vapor sorption
WVTR	water vapor transmission rate
XRD	X-ray diffraction
Xyl	xylose
Xyl*p*, β-D-Xyl*p*	β-D-Xylopranosyl

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1 Introduction

The agriculture and forestry industries provide, as their side streams, a large quantity of unutilized biomass containing a significant amount of plant polysaccharides. Hemicelluloses are the second most abundant plant polysaccharides after cellulose, constituting approximately one-third of the plant biomass. Since the reservoir of this polymeric material is substantial, and hemicelluloses have a film-forming ability, they are potential raw material for value-added products such as those in packaging and coatings. Petroleum-based plastic materials are conventional and widely used in the packaging industry, and their properties have been well studied. However, the growing packaging markets need increasing amounts of raw materials, and from the environmental point of view, there is a need for sustainable alternatives whose usage would decrease petroleum dependency and thus lower non-biodegradable plastic waste production. It is also important that the valorization of hemicelluloses from side streams and residues to packaging materials does not compete with food production. In addition to packaging applications, the feasibility of polymeric hemicelluloses has been evaluated for other uses such as emulsion stabilizers, food thickeners, binders in wood adhesives, and in the pharmaceutical industry (Mikkonen et al. 2009; Norström et al. 2015).

Hemicelluloses are a heterogeneous group of polysaccharides that participate in plant cell wall strengthening together with cellulose and lignin (Scheller and Ulvskov 2010). Hemicelluloses are composed of various monosaccharide units, and their composition varies between plant species. The most abundant hemicelluloses are xylans and mannans. In angiosperms (cereals, grasses, and hardwood), they are mainly xylans, whereas in gymnosperms (softwood), they are glucomannans. Structural differences both in the degree of substitution and length of the polymer affect its film-forming properties. In general, water-soluble hemicelluloses form films, but in some cases, external plasticizers are needed to enhance cohesive film formation (Mikkonen et al. 2007). Packaging and coating materials need to fulfill many requirements, like storage stability and material properties that will protect packaged food from, for example, spoilage. The standards for new film materials are high, so the materials must be thoroughly tested to discover which have the properties needed for food packaging.

Packaging applications represent the largest sector in the European plastic industry, representing a share of almost 40% (www.plasticseurope.org). A large part of these synthetic plastics still end up in landfills. In order to minimize the environmental impact of this packaging, new raw materials need to be studied. The development of bio-based plastics, for example, from starch and cellulose, has decreased the plastic industry's dependency on petroleum. Bio-based plastics are not necessarily biodegradable. For example, bio-based polyethylene does not biodegrade, although it is manufactured from sugarcane-derived bio-ethanol. The use of sugarcane- and starch-derived materials in the plastic industry competes with food production, unlike biomass-derived hemicelluloses, which are non-digestible.

Polysaccharides, such as cereal arabinoxylans (AXs), can be extracted from biomass and are one alternative raw material for bio-based biodegradable films. Agricultural side

streams can contain AXs that compose up to 40% of their dry weight (Sun et al. 1996; Hettrich et al. 2006). Due to their abundance in plants and their original function as elastic cross-linkers in the cell wall matrix, they are interesting raw materials for film studies.

Before AX films can replace current plastic packaging materials in food packages, their film formation and film properties need to be studied thoroughly. Systematic study is needed to understand how, for example, plasticizers and AX structure affects films and what might cause the main challenges in implementing AXs as packaging materials.

In the present study, AXs from cereal sources were utilized as raw materials for biodegradable films in order to increase the knowledge of their film-forming ability and film properties. Commercially available AXs with varying Ara/Xyl ratios and substitution patterns were used as they were or after enzymatically tailoring. External plasticizers were used when needed for cohesive film formation. This work focused on how the amount and type of external plasticizer affected film properties, how stable these films remained during storage, and what the effect of AX structure on film properties was. In addition, the effects of wheat bran extract composition and external plasticizer on material properties of extruded sheets were tested. The literature review of this thesis focuses on xylans and their use in biodegradable films. The experimental part of this thesis summarizes the data published in the attached papers I–V.

2 Review of the literature

2.1 Xylans

Xylans are a heterogeneous group of polysaccharides that belong to the hemicelluloses. Xylans are a major hemicellulose in angiosperms (hardwoods, cereals, and grasses), composing up to 25–40% of the dry weight and minor hemicellulose in gymnosperms (softwoods), in which the xylan content is between 5–10% (Sjöström 1993; Sun *et al*. 1996; Ebringerová 2006; Hettrich *et al*. 2006). The backbone of xylans is composed of (1→4)-linked β-D-xylopyranosyl (Xyl*p*) units. Some of the Xyl*p* units can be substituted at position *O*-2, *O*-3, or both with other monosaccharide units or *O*-acetyl groups (Ac) (Figure 1) (Ebringerová and Heinze 2000). Xylans are usually classified based on their side groups. In arabinoxylans (AXs), α-L-arabinofuranosyl (Ara*f*) substituents are linked to mono-substituted Xyl*p* units mainly at position *O*-3; or, two Ara*f* units can be linked to di-substituted Xyl*p* units at positions *O*-2 and *O*-3. Ara*f* substitution in the mono-substituted Xyl*p* unit at position *O*-2 is less common, but it has been reported to exist to a small extent, for example, in rye arabinoxylan (Vinkx *et al*. 1995). In glucuronoxylans (GXs), α-D-glucopyranosyl uronic acid (Glc*p*A) or its 4-*O*-methyl ether (MeGlc*p*A) is linked to Xyl*p* at position *O*-2. In arabinoglucuronoxylans (AGXs), both Ara*f* and MeGlc*p*A (or Glc*p*A) groups are attached to the xylan backbone. Ara*f* groups may be further substituted with feruloyl or *p*-cumaroyl groups, or in some cases, with Xyl*p* units, in which case, Xyl*p*-Ara*f* disaccharide is formed and linked to *O*-3 of the Xyl*p* in the xylan chain (Höije *et al*. 2006) The degree of polymerization (DP) and the degree of substitution (DS) varies between plant species and between parts of the plant.

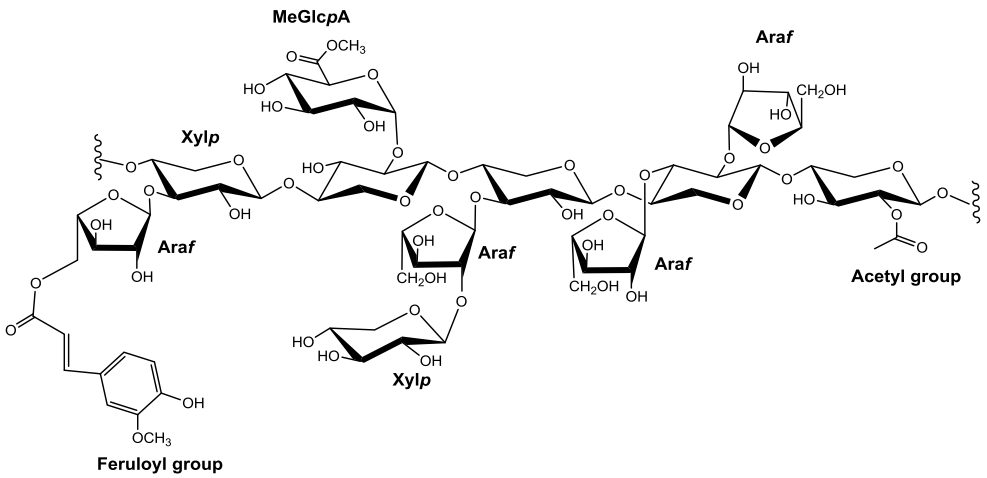

Figure 1. Schematic xylan structure with different substituents.

Part of the cereal-based xylans is water-extractable, but in most cases, crosslinks between the plant cell wall components hinder the extraction. An aqueous alkaline solution can be used to open up the cell wall structure (e.g., by hydrolyzing ferulate-ester crosslinks between xylans or xylan and lignin, water-un-extractable xylans can be liberated). In wheat flour xylan, 23% is water-extractable (WE-AX) and 77% water un-extractable (WU-AX) (Saulnier *et al*. 2012). Alkali that are often used in extraction include sodium hydroxide (NaOH), potassium hydroxide (KOH), and barium hydroxide ($Ba(OH)_2$), of which $Ba(OH)_2$ has been shown to be the most selective for cereal arabinoxylans (Gruppen *et al*. 1991). In addition, hydrolytic enzymes can be utilized in extraction, for example, starch- and protein-degrading enzymes were used in an ultrasound-assisted extraction of AX from wheat bran (Wang *et al*. 2014). The extraction yield from lignocellulosic materials, such as from straw, can be further increased by de-lignification of the material (e.g., with hydrogen peroxide prior to extraction) (Sun *et al*. 2000). The use of pressurized hot water to extract xylan from birch chips has been studied (Kilpeläinen *et al*. 2012; Grigoray *et al*. 2014). For the large-scale extraction of arabinoxylan from wheat bran and straw, the use of a twin-screw extruder has been suggested (Maréchal *et al*. 2004; Zeitoun *et al*. 2010; Jacquemin *et al*. 2015). This method achieves lowered chemical consumption and waste water volume.

Xylan structure has been shown to have an effect on water solubility. A low substitution degree generally indicates low water solubility. In AXs, decreased arabinose substitution results in an increased number of un-substituted Xyl*p* units, which lowers the water solubility (Andrewartha *et al*. 1979; Sternemalm *et al*. 2008; Pitkänen *et al*. 2011; Zhang *et al*. 2011). Both the number of Ara*f* substituents and their distribution along the xylan chain has been reported to affect the solution and hydrodynamic properties of wheat flour AXs (Pitkänen *et al*. 2011). The presence of Ara*f* residues, as well as their distribution over the xylan backbone, has been suggested to affect the interaction of AXs with each other (Dervilly-Pinel *et al*. 2001a). Low Ara*f* substitution enhances the stronger hydrogen bonding between the Xyl*p* groups, resulting in shorter average distances between the chains and increased intermolecular interaction (Rondeau-Mouro *et al*. 2011). As a result, large, un-substituted segments in xylan chains can cause aggregation. In acetylated xylans, the hydrolysis of acetyl groups due to alkaline treatment decreases the water solubility, remarkably resulting in water-insoluble xylans. On the other hand, fully acetylated xylans are water-insoluble, as shown by Gröndahl *et al*. (2003). In their study, the solubility properties of aspen glucuronoxylan were affected by the degree of acetylation: non-acetylated GX was partially soluble in hot water, whereas fully acetylated GX was soluble in non-polar solvents.

2.1.1 Xylans in angiosperms

Monocots (cereals and grasses)

Arabinoxylans (AXs) in monocots consist of a (1→4)-linked Xyl*p* backbone, to which Ara*f* substituents are connected by (1→2)- and/or (1→3)-glycosidic linkages. In arabinoglucuronoxylan (AGX), in addition to Ara*f* groups, Glc*p*A or MeGlc*p*A groups are attached to backbone Xyl*p*. The weight average molar mass (M_w) and arabinose-to-xylose ratio (Ara/Xyl) vary among plant species and between parts of the plant (Table 1). In cereals, generally, AXs located in the primary cell walls in the endosperm and bran are more substituted and have higher M_w than AXs from the secondary cell walls of lignified tissues, such as husks/hulls and straw. For example, the M_w of rye bran and flour (endosperm) is ten times higher than that of rye straw, and the xylan backbone of rye bran and flour AX contains on average three times more Ara*f* substituents than the AX of rye straw (Table 1).

The Ara/Xyl ratio indicates the proportion of Ara*f* groups to Xyl*p* units and is an average number. It does not illustrate how Ara*f* groups are distributed over the xylan chain, nor does it show whether the Xyl*p* units are mono- or di-substituted with Ara*f* groups. The chemical structures of biopolymers such as AXs are difficult to determine exactly because they have natural variation in their fine structure. Distribution of the Ara*f* substituents varies over the xylan chain, and it has been suggested that there is alteration of highly and less branched parts in the chain (Gruppen *et al*. 1993). Dervilly-Pinel *et al*. (2004) conclude that in wheat flour, AX Ara*f* residues are distributed irregularly along the chain. AX populations varying in molecular weight and type and degree of Ara*f* substitution can be fractionated with precipitation according to their water solubility (Izydorczyk and Biliaderis 1993).

In addition to the Ara/Xyl ratio, substitution patterns vary between the cereal species. For example, in highly branched rye arabinoxylan (RAX), such as from endosperm, approximately two-thirds of the Ara*f* groups are attached in mono-substituted Xyl*p* units and one-third in the di-substituted Xyl*p* units, whereas in wheat endosperm arabinoxylan (WAX) with a similar Ara/Xyl ratio, the situation is reversed (Pitkänen *et al*. 2009). The average content of un-substituted Xyl*p* residues is 66% for WAX and 58% for RAX (Pitkänen *et al*. 2011; Saulnier *et al*. 2012). The extractability of AX has been associated with structural differences. In water-extractable arabinoxylans (WE-AX) from rye grain, the Ara/Xyl ratios were slightly higher and the M_w was lower compared to water-unextractable AX (WU-AX) (Buksa *et al*. 2014). On the other hand, in wheat flour, both the Ara/Xyl ratio and the M_w were somewhat higher in WU-AX than in WE-AX (Izydorczyk and Biliaderis 1995).

Table 1. Examples of weight average molar masses (M_w) and the Ara/Xyl ratios of some AXs and AGXs from monocots.

Source	M_w (g/mol)	Ara/Xyl	Reference
Bamboo	13400	0.06–0.08	Guan *et al*. 2014, Zhong *et al*. 2013
Barley fiber	81000	0.51	Pitkänen *et al*. 2008
Barley flour	177000	0.62	Dervilly-Pinel *et al*. 2001b
Barley husk	20200–49300	0.22–0.28	Gröndahl *et al*. 2006, Höije *et al*. 2005, Köhnke *et al*. 2008, Pitkänen *et al*. 2008
Barley straw	28000	0.34	Sun and Sun 2002
Corn cob	19300–54000	0.13–0.23	Bahcegul *et al*. 2013, Egüés *et al*. 2013, Egüés *et al*. 2014, Gordobil *et al*. 2014
Corn hull	506000	0.64	Zhang *et al*. 2003
Corn stem	20200	0.14	Xiao *et al*. 2001
Oat spelt	23000	0.13	Saake *et al*. 2001
Rice straw	21800	0.26	Xiao *et al*. 2001
Rye bran	232000	0.57	Sárossy *et al*. 2013
Rye flour	255000	0.50	Pitkänen *et al*. 2011
Rye straw	22700	0.16	Xiao *et al*. 2001
Wheat bran	152000–218000*	0.2–1.3*	Zhang *et al*. 2011
Wheat flour	286000	0.51	Pitkänen *et al*. 2011
Wheat straw	28000–40900*	0.13–0.23*	Sun *et al*. 1996

*Variation results from the fractionation study

Dicots (hardwood)

Glucuronoxylan (GX) is the main hemicellulose in hardwoods, such as aspen, beech, birch, and eucalyptus. GX is composed of (1→4)-linked Xyl*p* backbone, where 4-*O*-methyl-α-D-glucopyranosyl uronic acid groups (MeGlc*p*A) are connected with (1→2)-linkages in approximately one MeGlc*p*A group per ten Xyl*p* units (Sjöström 1993). In addition, hardwood xylans contain *O*-acetyl-groups (Ac) in approximately seven Ac-group per ten Xyl*p* units. The reported weight average molar masses (M_w) of hardwood GXs vary between 8000 g/mol and 17000 g/mol (Jacobs and Dahlman 2001; Teleman *et al*. 2002; Gröndahl *et al*. 2004; Šimkovic *et al*. 2011a).

2.1.2 Xylans in gymnosperms

Softwood xylan is mainly arabinoglucuronoxylan (AGX), composed of Ara*f*, MeGlc*p*A, and Xyl*p* (1:2:11) (Sjöström 1993; Escalante *et al*. 2012). The M_w of AGX from larch, spruce, and pine were 17300; 19200; and 20800 g/mol, respectively (Jacobs and Dahlman 2001).

2.1.3 De-branching of arabinoxylan

The natural structural variation among AXs is wide. Both the length of the backbone and the number of Ara*f* side groups vary depending on the source. In addition to naturally occurring variation, AXs can be modified systematically to study their structure functions. Ara*f* substituents can be removed via hydrolytic enzymes or mild acids.

Enzymatic de-branching

AXs can be enzymatically tailored by specific α-L-arabinofuranosidases (EC 3.2.1.55), which hydrolyze terminal Ara*f* units from polymeric AXs. These arabinoxylan arabinofuranohydrolases (AXH) are divided into two groups depending on their substrate specificities. The enzyme AXH-m acts on α-(1→2)- and (1→3)-linked Ara*f* units on mono-substituted Xyl*p* residues, whereas AXH-d3 releases only α-(1→3)-linked Ara*f* units from the di-substituted Xyl*p* residues (Figure 2) (Van Laere *et al*. 1997). The efficient action and specificity of AXH-m- and AXH-d3-enzymes have been verified by ^{1}H NMR spectroscopy (Sørensen *et al*. 2006; Pitkänen *et al*. 2011). AXH-m and AXH-d3 have been utilized when the effect of Ara*f* substitution on solution and the hydrodynamic properties of WAX was studied (Pitkänen *et al*. 2011). Additionally, WAX was tailored with AXH-m and AXH-d3 in order to study how the degree of substitution and substitution pattern affected WAX adsorption into cellulosic surfaces (Köhnke *et al*. 2011). AXH-m has been used to specifically tailor RAX in composite film studies (Stevanic *et al*. 2011; Mikkonen *et al*. 2012).

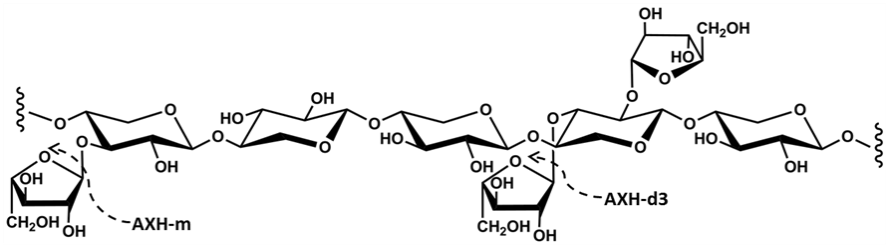

Figure 2. De-branching of arabinoxylan with arabinofuranohydrolases AXH-m and AXH-d3.

Chemical de-branching

Mild acid can be used in the chemical de-branching of AXs, since it primarily hydrolyzes Ara*f* side units, as reported by Whistler and Corbett (1955). In more recent studies, oxalic acid has been used in the acid-catalyzed de-branching of RAX (Sternemalm *et al.* 2008; Stepan *et al*. 2012; Bosmans *et al*. 2014). However, as a disadvantage, the chain length was reduced along with the de-branching during the treatment, resulting in AX with both low DS and DP. Higher DP but lower de-branching were obtained by keeping the treatment time short.

2.1.4 Other modifications of xylans

In recent studies, xylans, especially from wood and pulp, have been chemically derivatized in order to enhance their application potential as, for example, raw film material. The extraction conditions may cause reactions, such as the loss of acetyl groups, due to high pH decreasing the water solubility of xylans, which might therefore limit further use. Water-soluble derivatives from water-insoluble xylans have been successfully produced. Water-insoluble birch GX and beech GX have been carboxymethylated to increase water solubility and thus to improve film formation (Alekhina *et al*. 2014; Simkovic *et al*. 2014). The carboxymethylation of beech GX and oat spelt AX was shown to increase the anionic nature of the film surface (Velkova *et al*. 2015). Beech GX has been derivatized with hydroxypropylsulfonate and sulphate groups (Simkovic *et al*. 2011a, 2011b, 2014). Xylan from birch pulp has been hydroxypropylated to retrieve water-soluble derivatives for film preparation (Laine *et al*. 2013; Mikkonen *et al*. 2015). Intensive chemical acetylation of aspen GX resulted in decreased water solubility but improved thermal stability of the xylan (Gröndahl *et al*. 2003). In addition, chemical acetylation of agro-based AX, such as from rye flour, and corn cobs, increased thermal stability and hydrophobicity (Stepan *et al*. 2012; Egüés *et al*. 2014). Although most of the derivatizations have been made for xylan polymers, some trials on film surface modifications have been reported. Barley husk AX film surface was fluorinated, resulting in less hydrophilic film compared to the unmodified one (Gröndahl *et al*. 2006).

2.2 Xylan films

2.2.1 Film preparation

On the laboratory scale, films are usually produced by casting the aqueous film solution into a plastic Petri dish or Teflon dish. After water evaporation, self-standing film can be removed from the dish. Films are often dried at room temperature (RT) to avoid too fast evaporation resulting in unwanted aggregation (Zhong *et al*. 2013). However, in cases where the water solubility of the xylan was low, elevated temperatures of up to 80°C were used to avoid sedimentation of the polymer during evaporation (Zhong *et al*. 2013). Although water is an environmentally friendly, non-toxic option as a solvent, its use is not always possible. Water-insoluble xylans, such as highly acetylated xylans, have been dissolved in chloroform or dimethyl formamide prior to casting (Stepan *et al*. 2012, 2014). In a newly presented laboratory-scale production method, xylan film solution is casted on a moving web (Vartiainen *et al*. 2015).

Extrusion is a larger-scale method for film production compared to casting, and even on the laboratory scale, it is closer to industrial production. Extrusion is considered to be a continuous process where an extruder equipped with one or two screws is used for mixing the material, often under a temperature gradient. The moisture content of the material and the extrusion temperature have been shown to have an impact on the extrudability of the bio-based materials (Bachegul *et al*. 2013). In the extrusion process, raw material is fed to the system as it is or after pre-pelleting, after which the screw (or screws) mixes and transfers the materials toward the die. The thickness and width of the slit in the die define the thickness and width of the films or sheets. Film extrusion of biopolymers, like polysaccharides and proteins, has been reported (e.g., corn cob AGX, starch, pectin, and sugar beet pulp have been successfully utilized in sheet extrusion) (Fishman *et al*. 2000, 2004; Rouilly *et al*. 2009; Bahcegul *et al*. 2013; Akkus *et al*. 2014). In addition, sunflower protein isolate and soy protein films have been produced by extrusion (Zhang *et al*. 2001; Rouilly *et al*. 2006). Extrusion is also used in cellophane production, in which a cellulose-based viscose solution is extruded through the narrow slit into an acidic bath, where it regenerates back into a cellulose film.

In industrial production, requirements for polymer properties differ from laboratory-scale tests. Synthetic thermoplastics are industrially shaped by various methods, such as extrusion blow molding. Hemicelluloses are not thermoplastics; therefore, their material properties may create a challenge in industrial production, and conventional processing equipment is not necessarily suitable for them. For example, hemicelluloses, like all polysaccharides, are sensitive to the relative humidity of the environment; this may create a challenge in the material production conditions when increased water sorption softens and plasticizes the material. Another challenge is caused by the thermal stability of the polysaccharides, which is low compared to moldable thermoplastics (Mensitieri *et al*. 2011).

2.2.2 Film properties

To be able to function as a packaging or coating material, hemicellulose films or sheets need to meet the requirements appointed to these materials. Food packaging needs to maintain the quality and safety of the food. One of the main purposes of packaging material is to prevent or retard unwanted changes in packaged food that would otherwise lead to spoilage. Material properties define how applicable the film is for packaging or coating purposes and what kind of protection it gives to the packaged food product (Table 2). Mechanical properties indicate how films and sheets behave under mechanical stress—how strong, stiff, and flexible they are and how well they can give physical protection, for example, against mechanical stress. Permeability properties tell how much and how fast gases, like O_2, CO_2, or N_2; water vapor; or grease, permeate through material. Permeability measures a permeant's solubility in the polymer and its diffusivity through the film. It is affected by the film's thickness and gas pressure difference between the different sides of the film.

Oxygen gas takes part in many reactions, leading to the decreased shelf life of foods and even turning foods inedible. For example, lipid oxidation induces off flavors and the rancidity of fat-containing foods. Oxidation decreases food quality and nutritional value, and it may cause undesirable health implications. Water also takes part in many unwanted reactions. In dry and low-moisture foods, increased water activity may increase harmful microbial growth, which in the worst case, if consumed, leads to food poisoning. Packaging material has to prevent the drying of high-moisture foods. In addition to WVP properties, the water sensitivity of packaging material can be followed by water vapor sorption (WVS), a method that measures the water adsorption capacity of packaging materials. WVS can determine the amount of water intake in different relative humidity conditions in certain temperatures. Water plays an important role when studying hydrophilic materials. Water softens and plasticizes the amorphous part of these materials by increasing its molecular mobility, which in turn decreases mechanical strength and usually increases gas permeability. Most film-measuring methods were developed for synthetic plastics, which generally are stable, for example, in varying humidity conditions.

The requirements of the packaging applications direct the choice of the material, for example, in flexible packaging, low-density polyethylene (LDPE) with up to 1000% elongation at break is used (Robertson 2008). On the other hand, it has high oxygen permeability 1870 [(cm^3 μm)/(m^2 d kPa)] but low water vapor permeability 0.08 [(g mm)/(m^2 d kPa)] (McHugh and Krochta 1994). Ethylene vinyl alcohol (EVOH), with an OP of 0.01–0.1 [(cm^3 μm)/(m^2 d kPa)], and polyvinylidene chloride (PVDC), with an OP of 0.1–3.0 [(cm^3 μm)/(m^2 d kPa)], are examples of good oxygen barriers (Lange and Wyser 2003). In the case of water vapor and oxygen barrier properties, WVP values under 0.1 [(g mm)/(m^2 d kPa)] and OP values under 10 [(cm^3 μm)/(m^2 d kPa)] are considered to be good values in food packaging (Krochta and De Mulder-Johnston 1997).

Film material may be completely amorphous, or it may contain both amorphous and crystalline parts. The material properties of the amorphous part of the film are dependent on its physical state. In the amorphous phase of the film, two separate thermal relaxations, α- and β-relaxation, are possible. At sub-ambient temperatures and when small-scale

molecular motions are released, β-relaxation occurs (Butler and Cameron 2000). At higher temperatures, α-relaxation occurs; this is often referred to as glass transition temperature (T_g). Below T_g, amorphous material is in a glassy state, molecular movements are slow, and film is brittle and might fracture easily under mechanical stress. However, in a glassy state, film has low gas permeability. Also, the crystalline regions in the films have been shown to lower the permeability properties (McHugh and Krochta 1994). The crystallinity of the films is often associated with low substitution of the xylan chain, where un-substituted segments form crystalline structures. However, very slow removal of water may also induce crystalline formation in highly substituted AX (Nieduszynski and Marchessault 1972).

Table 2. Selected film properties and their relevance to the package and packaged food product.

Measured property	**Relevance to the film/package performance**	**Relevance to the packaged food product**
Tensile strength (TS)	Strength, durability	Physical protection
Elongation at break (EB)	Flexibility	Physical protection
Young's modulus (YM)	Stiffness	Physical protection
Oxygen transmission rate (OTR) / Oxygen permeability (OP)	Function as an oxygen gas barrier	Physical and chemical protection against oxidation
Water vapor transmission rate (WVTR) / Water vapor permeability (WVP)	Function as a water vapor barrier	Physical and chemical protection against moisture loss or gain / Protection against microbial growth
Water vapor sorption (WVS)	Water sensitivity, softening, swelling, shrinkage	Effect of relative humidity of the storage conditions
Contact angle	Hydrophilicity/hydrophobicity	Physical and chemical protection
Aroma permeability	Function as an aroma barrier	Physical and chemical protection against aroma loss or gain, or off flavors
Grease permeability	Function as a grease barrier	Physical and chemical protection
Light transmittance	Light exposure, visibility through the package	Protection against light-induced reactions (e.g., off flavors and rancidity).
Glass transition temperature (T_g)	Physical state of the amorphous part of the film, Effect on film properties	Chemical and physical protection
Thermal stability	Tolerance of thermal processes	Physical protection
Crystallinity (ϕ)	Effect on film properties	Chemical and physical protection
Density/porosity	Effect on permeability properties	Chemical and physical protection

Effect of plasticizer on polysaccharide film properties

Some polysaccharides need external plasticization to enhance their cohesive film formation. Plasticizer increases the free volume in the polymer network by reducing the number of hydrogen bonds between the polymers, therefore increasing the flexibility and reducing the brittleness of the film (Banker 1966; Gontrad *et al*. 1993). As a result, the physical and mechanical properties of the films change. For instance, glass transition temperature (T_g) and tensile strength decrease, whereas elongation at break increases along with increased plasticization. Polyols, such as glycerol, sorbitol, and xylitol, are natural and biodegradable plasticizers (Vieira *et al*. 2011). Other plasticizers, for example, poly(ethylene glycol) methyl ether (mPEG) in the plasticization of rye AX films and propylene glycol (PG) in the plasticization of corn hull AX films, have been used (Zhang and Whistler 2004; Sárossy *et al*. 2012). The molecular structures of some external plasticizers used in the film studies are illustrated in Figure 3. The amount of plasticizer used in the film studies varies greatly, and up to 100% (w/w of hemicellulose) amounts have been reported (Saxena *et al*. 2009).

The plasticizer needs to be compatible with the polymer to function properly, but this is not always the case. For example, xylitol has been shown to migrate and crystallize on the surface of aspen glucuronoxylan film, therefore not plasticizing the film (Gröndahl *et al*. 2004). Instead of plasticization, a low amount of plasticizer may result in an anti-plasticization effect, a commonly known phenomenon in synthetic polymers. In anti-plasticization, film properties change in the opposite way that they do in plasticization: tensile strength and the Young's modulus increase and elongation decreases, making the material stiffer than it was originally. This phenomenon is associated with lowered molecular motions due to links between the plasticizer and the polymer (Gaudin *et al*. 2000). Anti-plasticization of the starch films containing 12% glycerol has been observed (Lourdin *et al*. 1997). Also, it was suggested that low sorbitol content induced an anti-plasticizing effect in starch films (Gaudin *et al*. 1999).

Water also acts as an external plasticizer, especially in hydrophilic biopolymer films, by interfering with the hydrogen bonding between the polymer chains and thus enhancing the mobility of the molecules. Due to the hydrophilicity of the polysaccharides, the water content of the films is affected by the relative humidity of the environment. Hydroxyl groups of polysaccharides form hydrogen bonds with the water molecules; the number of free hydroxyl groups in pentosyl residues is two in Xyl*p* and three in Ara*f*. In addition, the hydrophilic nature of the plasticizer has an effect on the water content of the film. For example, glycerol is more hydrophilic than sorbitol; thus, the water content of glycerol-plasticized mannan films was higher compared to the sorbitol-plasticized films (Cheng *et al*. 2006). The diffusivity of water in hydroxypropylated beech xylan film was shown to increase by increasing the sorbitol content of the film (Bayati *et al*. 2014).

Film properties are affected by the addition of polyol plasticizer. Examples of the mechanical properties of un-plasticized and plasticized AX films with varying amounts or types of plasticizer are presented in Table 3. In general, un-plasticized films are stronger and stiffer than plasticized ones. The barrier properties of AX films have been studied less than those of mechanical properties, although they are important parameters when

considering their applications. The water vapor and oxygen barrier properties reported for xylan films in the literature are shown in Table 4. Almost all of the reported OP values are under 1 [(cm^3 μm)/(m^2 d kPa)], revealing that AX films can retard oxygen permeation.

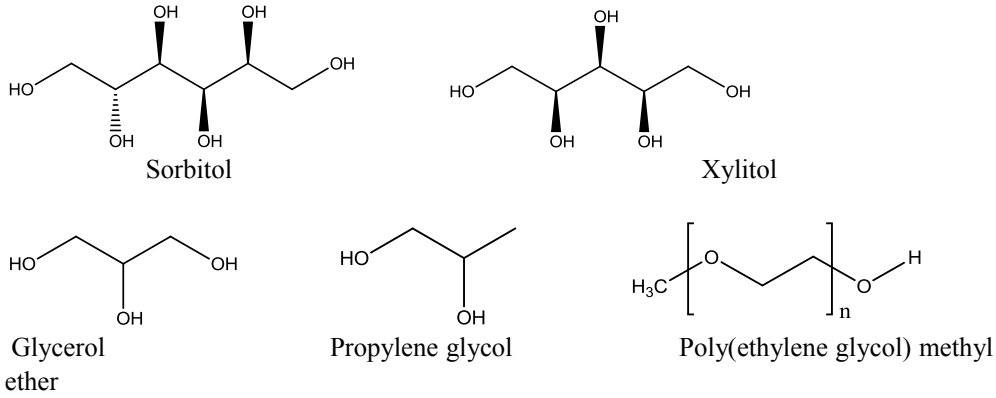

Figure 3. Examples of external plasticizers used in xylan film studies.

Internal plasticizers in xylan films

In internal plasticization, side chains or groups are chemically attached to the main chain of a polymer. As a result, these groups act as a plasticizer by decreasing the intermolecular forces and therefore increasing the mobility of the polymers and flexibility of the material. Naturally occurring high Ara*f* substitution in AX may also be considered as an internal plasticizer, since it increases the flexibility of the film compared to low substitution. Chemical acetylation of rye AX was shown to increase the flexibility of the film, similar to an external plasticizer (Stepan *et al*. 2012). Chemical acetylation of the corn cob AX increased the tensile properties of the film (Egüés *et al*. 2014). Long-chain anhydride modification of bamboo xylan resulted in film with an amorphous structure, decreased moisture sensitivity, and increased tensile properties (Zhong *et al*. 2013). Hydroxypropylation of birch xylan enhanced cohesive film formation and decreased the crystallinity of the film (Mikkonen *et al*. 2015). Carboxymethylation of birch xylan increased the plasticization of the film, since elongation at break increased and the tensile strength and Young's modulus decreased (Alekhina *et al*. 2014).

Table 3. Mechanical properties of plasticized and un-plasticized xylan films.

Film material	External plasticizer	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)	RH(%)	T (°C)	Reference
Aspen GX	20% sorbitol	~ 40	~ 2	nr	50	23	Gröndahl *et al*. 2004
Aspen GX	35% sorbitol	~13	~ 7	nr	50	23	Gröndahl *et al*. 2004
Aspen GX	50% sorbitol	~ 3	~13	nr	50	23	Gröndahl *et al*. 2004
Aspen GX	20% xylitol	~ 40	~ 2	nr	50	23	Gröndahl *et al*. 2004
Aspen GX	35% xylitol	~12	~ 5	nr	50	23	Gröndahl *et al*. 2004
Aspen GX	50% xylitol	~ 3	~ 8	nr	50	23	Gröndahl *et al*. 2004
Bamboo AGX	25% sorbitol	12 ± 1	3.4 ± 0.2	735 ± 87	50	nr	Peng *et al*. 2011
Barley husk AX	-	50	2.5	2930	50	nr	Höije *et al*. 2005
Corn cob AX	-	54 ± 13	7.1 ± 1.3	1662 ± 268	50	25	Egüés *et al*. 2014
Chemically acetyl. Corn cob AX	-	67 ± 12	13.4 ± 1.6	2241 ± 353	50	25	Egüés *et al*. 2014
Corn cob AX	40% glycerol	3.3 ± 0.4	5.3 ± 1.7	3 ± 1	50	25	Gordobil *et al*. 2014
Corn hull AX	-	54 ± 0.4	6.2 ± 1.6	1316 ± 90	54	nr	Zhang and Whistler 2004
Cotton stalk xylan	-	1.3 ± 0.1	48 ± 5	0.4 ± 0.03	nr	nr	Goksu *et al*. 2007
Cotton stalk xylan	2% glycerol	0.8 ± 0.05	89 ± 10	0.08 ± 0.01	nr	nr	Goksu *et al*. 2007
Oat spelt AX	100% sorbitol	~ 2.2	~ 22	nr	nr	nr	Saxena *et al*. 2009
Rye bran AX	-	~16	~ 6	~ 550	50	23	Sárossy *et al*. 2013
Rye flour AX	-	58 ± 11	8.1 ± 3.3	2500 ± 400	50	30	Stevanic *et al*. 2011
Rye flour AX	-	~ 64	~12	~1800	50	23	Mikkonen *et al*. 2012
Rye flour AX	-	~ 60	~ 22	~ 2100	nr	nr	Stepan *et al*. 2012
Rye flour AX	30% mPEG	33 ± 7	42 ± 10	700 ± 100	nr	nr	Sárossy *et al*. 2012
Chemically acetylated Rye AX	-	65 ± 4	21 ± 3	2160 ± 80	50	23	Stepan *et al*. 2014

Table 3. Continued.

Film material	External plasticizer	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)	RH (%)	T (°C)	Reference
Spruce AGX	-	55 ± 7	2.7 ± 0.7	2735 ± 388	50	25	Escalante *et al*. 2012
Spruce AGX	5% sorbitol	57 ± 1	3.1 ± 0.3	2612 ± 160	50	25	Escalante *et al*. 2012
Spruce AGX	15% sorbitol	44 ± 2	3.3 ± 0.8	2063 ± 109	50	25	Escalante *et al*. 2012
Spruce AGX	25% sorbitol	26 ± 8	4.3 ± 1.8	1163 ± 140	50	25	Escalante *et al*. 2012
Wheat flour AX	-	98 ± 16	39 ± 9	2475 ± 255	59	25	Ying *et al*. 2013
Wheat flour AX	-	59 ± 8.8	37 ± 8	1175 ± 323	75	25	Ying *et al*. 2013
Wheat flour AX	-	8.1 ± 4.5	7.2 ± 3.3	325 ± 133	91	25	Ying *et al*. 2013

- = no external plasticizer used

nr = not reported

Table 4. Barrier properties of plasticized and un-plasticized xylan films.

Film material	External plasticizer	RH (%)	OTR [(cm^3 /(m^2 d)]	OP [(cm^3 µm)/(m^2 d kPa)]	RH (%)	WVTR [(g /(m^2 d)]	WVP [(g mm)/(m^2 d kPa)]	Reference
Aspen GX	35% sorbitol	50	nr	0.21	nr	nr	nr	Gröndahl *et al*. 2004
Barley husk AX	-	50	nr	0.16	nr	nr	nr	Höije 2008
Corn bran AX	15% glycerol	nr	nr	nr	84/22	nr	15.3	Péroval *et al*. 2002
Corn hull AX	-		nr	nr	0/54	nr	4.1	Zhang and Whistler 2004
Corn hull AX	13% sorbitol	nr	nr	nr	0/54	nr	2.0	Zhang and Whistler 2004
Oat spelt AX	100% sorbitol	nr	355	nr	nr	nr	nr	Saxena *et al*. 2010
Oat spelt AX	100% sorbitol	nr	nr	nr	nr	550	nr	Saxena *et al*. 2011
Rye bran AX	-	50	2.32	0.87	0/52	nr	7.7	Sárossy *et al*. 2013
Rye flour AX	-	50	nr	0.15–0.22	0/50	nr	2.6	Sárossy *et al*. 2012
Spruce AGX	-	50	nr	0.12	nr	nr	nr	Escalante *et al*. 2012
Wheat bran AX	-	nr	nr	0.84	nr	nr	nr	Zhang 2012

OTR = oxygen transmission rate, OP = oxygen permeability, WVTR = water vapor transmission rate, WVP = water vapor permeability

- = no external plasticizer used

nr = not reported

Effect of arabinoxylan fine structure on film properties

Water is often used as a solvent in arabinoxylan film studies, and the degree of Ara*f* substitution has been shown to have an impact on water solubility. Structure correlations of AX films have been studied both by fractionating extracted AX according to their water solubility (i.e., according to their DS) and by de-branching AX using enzymes or mild acid. When the fine structure of rye AX (RAX) was modified by enzymatic de-branching, the content of the Ara*f* substitution was found to affect the material properties of the composite films (Stevanic *et al*. 2011; Mikkonen *et al*. 2012) (Table 5). The water solubility of RAX decreased, and the crystallinity of the films increased along with the Ara*f* de-branching. In chemically de-branched and further acetylated RAX films, elongation at break was lowest when the Ara/Xyl ratio was the lowest, whereas tensile strength and Young's modulus were not dependent on the Ara/Xyl ratio (Stepan *et al*. 2012). In wheat flour AX films, elongation at the break and tensile strength were highest when the Ara/Xyl ratio was the lowest (Ying *et al*. 2015) (Table 5). The DS of wheat bran AX fractions has been reported to affect the thermal properties and density of the films (Zhang *et al*. 2011; Zhang 2012). Films with a low Ara*f* content had higher crystallinity and increased local macromolecular mobility than films prepared from highly substituted AX (Table 5). Ara*f* content was also shown to affect film density: measurements conducted in a dry environment showed increased density with increased substitution. On the contrary, low Ara*f* substitution of the xylan chain is suggested to enhance the formation of a more compact film structure with smaller nanopores than AX with a higher substitution (Rondeau-Mouro *et al*. 2011). The latter result was associated with lowered molecular motions of AX chains or shorter average distances of these chains.

Table 5. Effect of Ara/Xyl ratio on tensile strength (TS), elongation at break (EB), Young's modulus (YM), glass transition temperature (T_g), and crystallinity (ϕ) of the AX films.

Film material	Ara/Xyl	M_w (g/mol)	RH (%)	TS (MPa)	EB (%)	YM (MPa)	T_g (°C)	ϕ (%)	Reference
Rye flour AX	0.45	184000	50	~64	~12	~1800	nr	0	Mikkonen *et al*. 2012
Rye flour AX	0.27	152000	50	~60	~11	~1700	nr	11 ± 2	Mikkonen *et al*. 2012
Rye flour AX	0.50	289000	50	58 ± 11	8.1 ± 3.3	2500 ± 400	135	0	Stevanic *et al*. 2011
Rye flour AX	0.16	289000	50	32 ± 3	2.1 ± 0.6	2200 ± 200	141	20 ± 3	Stevanic *et al*. 2011
Wheat bran AX	0.65	152000	nr	nr	nr	nr	nr	10 ± 2	Zhang 2012
Wheat bran AX	0.15	166000	nr	nr	nr	nr	nr	24 ± 4	Zhang 2012
Wheat flour AX	0.73	233000	40	61 ± 8	22 ± 9	nr	82	nr	Ying *et al*. 2015
Wheat flour AX	0.53	197000	40	98 ± 16	39 ± 9	nr	85	nr	Ying *et al*. 2015
Wheat flour AX	0.33	177000	40	131 ± 25	46 ± 8	nr	83	nr	Ying *et al*. 2015

nr = not reported

2.2.3 Storage stability of polyol-plasticized polysaccharide films

Packaging must protect products, such as foods, from the environment and retain the products' quality during their shelf life. Synthetic polymers stay relatively stable, but with increasing interest in biopolymers in packaging applications, their storage stability must be evaluated. So far, stability studies of polysaccharide films have been conducted mostly with polyol-plasticized starch films. Polysaccharide film does not necessarily stay stable when aged; instead, it may undergo some structural changes. In potato starch films, long-term storage was reported to induce recrystallization of the starch (Van Soest *et al*. 1996). On the other hand, plasticization with binary polyols was shown to retard the crystallization of the potato starch in the films (Talja *et al*. 2008). Due to the migration of the plasticizer, externally plasticized films might undergo some structural changes during long-term storage. Glycerol migration from starch and chitosan films has been observed during storage (Kuutti *et al*. 1998; Suyatma *et al*. 2005). In chitosan films plasticized with glycerol, 90 days of aging resulted in increased tensile strength and decreased elongation at break, whereas in un-plasticized films, both tensile strength and elongation at break increased. There were also color and gloss changes, and the researchers concluded that structural changes, such as the rearrangement of the polymer chains, were related to the Maillard reaction (Leceta *et al*. 2015).

3 Aims of the study

The aim of this thesis was to study the applicability of cereal-derived AXs as biodegradable film materials for future packaging or coating applications. Due to its relatively low degree of substitution and M_w commercially available OsAX was chosen to represent AXs typically found in agricultural side streams such as husks/hulls and straw. Commercial RAX and WAX were selected due to their purity, high substitution levels, and M_w. The effect of external polyol plasticization on OsAX film properties and the physical stability of the plasticized films were studied. The effect of AXs' fine structure on film formation and film properties was studied by comparing RAX and WAX. The fine structure of RAX and WAX were further tailored with specific enzymes in order to systematically cleave Ara*f* side groups from the main chain, mimicking, in a controlled manner, the structural variations of different native AXs. On a laboratory scale, films are often prepared by casting, a method that cannot be up-scaled. To be able to move toward larger film/sheet preparation methods and to test the applicability of crude biomass extracts in film/sheet formation, sheet extrusion was tested with two wheat bran extracts (WBEs).

The individual objectives in studies **I–V** were as follows:

- To study the role of external polyol plasticization on OsAX film properties (**I**, **II**).
- To study the role of arabinose substitution on AX film properties (**III**, **IV**).
- To investigate the water sensitivity of the AX films **(I–V)**.
- To follow the storage stability of polyol-plasticized OsAX films (**I**, **II**).
- To evaluate the applicability of wheat bran extracts as raw materials for extruded sheets (**V**).

4 Materials and methods

This section briefly summarizes the materials and methods used in the present study. More detailed descriptions can be found in the original publications (**I–IV**). The earlier unpublished OsAX results presented in the results section were obtained by using the same materials and methods used in study **II**.

4.1 Materials

4.1.1 Cereal arabinoxylans and wheat bran extracts

In order to study the potential use of AXs from side streams of cereal processing in packaging film applications, suitable commercial products were selected to serve as raw materials for the films. AXs from three different sources were chosen for film studies (Table 6). Oat spelt AX represents a relatively short xylan chain and a low Ara/Xyl ratio, making it similar to AX extracted from husks/hulls and straw. As for rye and wheat flour AX, they resemble the AX found in cereal bran, having a relatively long xylan chain and high Ara/Xyl ratio. Due to their purity, high arabinose content, and different substitution pattern, both RAX and WAX were used in more detailed studies on how Ara*f* substitution affects film formation and film properties. Structural modification of RAX and WAX was carried out with specific Ara*f* cleaving enzymes. Two wheat bran extracts (WBEs) that were prepared in a separate study (Jacquemin *et al*. 2015) were used as raw materials in sheet extrusion in order to study larger-scale material production with crude biomass extract. Production of the two extracts was achieved by a twin screw extrusion fractionation process. Prior to the extraction, the bran was de-starched by either washing the bran with 40°C water (WBE-S) or enzymatically using an α-amylase (BAN 480L, Novozymes, Denmark) treatment (WBE-P).

Table 6. Arabinoxylans used in studies **I–V**.

Arabinoxylan source	Abbreviation	Supplier	Study
Oat spelt	OsAX	Sigma-Aldrich	**I**
Oat spelt	OsAX	TCI Europe	**II**
Rye flour (high viscosity)	RAX-hv	Megazyme	**III**
Wheat flour (medium viscosity)	WAX-mv	Megazyme	**IV**
Wheat bran extract (high starch content)	WBE-S		**V**
Wheat bran extract (high protein content)	WBE-P		**V**

All of the AXs and WBEs were characterized prior to the film or sheet preparation. The monosaccharide compositions of AXs used in studies **I–III** were determined using GC after acid methanolysis (Sundberg *et al*. 1996). The data for WAX and molecular weights of RAX and Sigma OsAX was published separately (Virkki *et al*. 2008; Pitkänen *et al*. 2009; Eronen *et al*. 2011). The molecular weight of OsAX (study **II**) was determined in this study by high-performance size exclusion chromatography (HPSEC) according to a method described previously (Pitkänen *et al*. 2009). The compositions of the wheat bran extracts were analyzed in Jacquemin *et al*. (2015) (Table 7).

Table 7. Main components of the wheat bran extracts (WBE-S and WBE-P) shown as a percentage of the dry matter (Jacquemin *et al*. 2015).

Main components	WBE-S	WBE-P
Protein	17.9	38.2
Lignin	11.0	11.9
Carbohydrates	61.8	29.8

4.1.2 Enzymatic hydrolysis of rye and wheat flour arabinoxylans

The Ara/Xyl ratios are similar in the rye and wheat flour AXs, but the distribution of the substituents differs. In the rye flour AX, two-thirds of the Ara*f* substituents are located in the mono-substituted Xyl*p* units and one-third in the di-substituted Xyl*p* units, whereas in the wheat flour AX, the situation is reversed. Because of the high number of Ara*f* units in the mono-substituted Xyl*p* in the rye flour AX, systematic enzyme hydrolysis was used to target them. Modification of RAX was done using α-L-arabinofuranosidase AXH-m (from *Meripilus giganteus*, GH 51, kind gift from Novozymes, Bagsvaerd, Denmark), which removed the (1→2)- and (1→3)-linked α-L-Ara*f* units from mono-substituted β-D-Xyl*p* residues. Hydrolysis was performed by varying the enzyme dosage in order to achieve three different modified RAXs in addition to one unmodified one (RAX-ref) (i.e., in total, four RAX-m samples with different Ara/Xyl ratios). Preliminary hydrolysis was performed in an Eppendorf scale, after which the enzyme dosages for five-hour incubation were chosen for film study (Table 8). The WAX, having a high number of di-substituted Xyl*p* units, was hydrolyzed; this was in addition to AXH-m with AXH-d3 (E-AFAM2, from *Bifidobacterium adolescentis*, GH 43, Megazyme, Wicklow, Ireland), which removed the (1→3)-linked α-L-Ara*f* units from the di-substituted β-D-Xyl*p* residues. In order to remove all of the targeted Ara*f* units, small-scale pre-hydrolysis was performed, after which the enzyme dosages and incubation time were chosen to prepare the de-branched WAX for film studies. Three different WAX samples were obtained for film

studies: unmodified WAX-ref and de-branched WAX-m and WAX-d3. The amount of released arabinose was analyzed with a commercial kit (K-LACGAR, Megazyme, Wicklow, Ireland). To verify the AXH-m action in RAX on only Ara*f* residues linked to mono-substituted Xyl*p* units, ^{1}H-NMR spectroscopy was conducted with a Varian Unity 500 spectrometer (Palo Alto, USA) operating at 500 MHz for ^{1}H. Measurements were performed at 50°C, and ^{1}H chemical shifts (ppm) were referenced to an internal acetone signal at 2.225 ppm.

Table 8. De-branching of RAX and WAX. Enzymes and hydrolysis conditions used.

Arabinoxylan	Enzyme	Enzyme dosage (nkat/g of AX)	Incubation time (h)	T (°C)	pH
RAX-hv	no	0	5	40	5.0
	AXH-m	200	5	40	5.0
	AXH-m	1000	5	40	5.0
	AXH-m	10000	5	40	5.0
WAX-mv	no	0	48	40	5.0
	AXH-m	5000	48	40	5.0
	AXH-d3	250	48	40	6.0

RAX-hv = high-viscosity RAX, WAX-mv = medium-viscosity WAX

4.1.3 Plasticizers

In OsAX studies, polyol plasticizers were used to enhance film formation. Polyol plasticizers glycerol (BDH Laboratory Supplies, Lutterworth, England), sorbitol (Cerestar, Krefeld, Germany), and xylitol (Danisco, Kotka, Finland) were chosen and used as obtained.

4.2 Film preparation

Prior to casting, AXs were suspended in milli-Q water and mixed by magnetic stirring at 90–95°C for 15 min. OsAX was not totally water-soluble, and in pre-tests, OsAX suspension containing an insoluble part did not result in cohesive films. Therefore, the insoluble part of the suspension was removed via centrifugation (22500 g for 15 min), and the supernatant containing 10 g/l soluble OsAX was taken for the film studies. Glycerol, sorbitol, their blends, and xylitol were added in varying amounts (0–40% of the weight of the OsAX) to the OsAX supernatant by magnetic stirring at 80–85°C. RAX and WAX

were both water-soluble, and a clear 1% (w/v) solution was obtained. In RAX and WAX, enzymatic hydrolysis (described in paragraph 4.1.2) was conducted, after which the film solutions were dialyzed against water for 48 h using the membrane with the molecular weight cut-off 12000–14000 g/mol in order to remove the buffer salts. For the unmodified reference films, RAX and WAX were treated similarly, except no enzyme was added. All of the prepared film solutions were degassed by ultrasonication *in vacuo* to remove air from the solutions, after which they were cast into polystyrene Petri dishes or Teflon dishes. The OsAX films in storage study **I** were dried at 60°C for 4 h, while all of the other films were dried at RH 50% at 23°C. The films were stabilized at RH 50% at 23°C for at least seven days before the measurements, excluding the films for the water vapor sorption study, which were stabilized in a desiccator containing P_2O_5 to RH 0% prior to measurement, and the films for dielectric analysis, which were conditioned at RH 54% in a desiccator containing saturated $Mg(NO_3)_2$. The films in storage study **I** were stored between the printing papers, and in study **II**, they were stored on the printing paper at RH 50% at 23°C.

4.3 Sheet production

Dried wheat bran extracts (WBE-S and WBE-P) were mixed with water (30 g of water for 100 g of dry powder) and plasticizer (glycerol, sorbitol, or their blends) in the amount of 30% (g/g of the extract powder) in a Perrier 32.00 mixer (Montrouge, France). The mixture was stored in sealed plastic bags at 4°C for 12 h before sheet extrusion. The extruder used was a Polylab Haake system (Karlsruhe, Germany) with a single single-screw mechanism (18 mm diameter screw, compression rate of 1.8) fitted with a 100 mm-wide sheet die. The operating conditions were chosen after pre-tests and are given in Table 9.

Table 9. Operating conditions used in the film extrusion.

Parameters	Value
Barrel temperature (°C)	50/80/100/110 (die)
Screw speed (rpm)	75, 100, 125
Torque (Nm)	1.2–6.1
Die pressure (Bar)	24.3–46.4
Die thickness (μm)	100, 250, 400

4.4 Film properties

4.4.1 Thickness (I–V)

The thickness of the films in the tensile and barrier property studies was measured with a micrometer (Lorentzen & Wettre, Kista, Sweden; precision 1 μm) at five points; then, the average was calculated. In the DMA analysis (study **I**) and the sheet extrusion study (**V**), a Mitutoyo micrometer (Mitutoyo Corp., Kanagawa, Japan; precision 10 μm) was used.

4.4.2 Tensile properties (I–V)

Tensile testing in studies **I–IV** was performed at RH 50% at 23°C using an Instron 4465 universal testing machine (Instron Corp., High Wycombe, England) with a load cell of 100 N to determine the tensile strength, elongation at break, and Young's modulus of the films. The tensile properties of the extruded sheets in study **V** were determined at RT with an H5KT universal testing machine (JFC, France) with a 100 N load cell.

4.4.3 Permeabilities (I–V)

Water vapor permeability (I–V)

The water vapor permeability (WVP) of the films was determined by the cup method according to the ASTM E 96/E 96M-05 standard (ASTM, 2005) with RH gradients of 0/54% ($Mg(NO_3)_2$) (**I, III, IV**), 0/66% ($NaNO_2$) (**I**), and 0/86% (KCl) (**I**) at 22°C. In study **V**, the RH gradient was 0/60% at 25°C; the cups were placed in a climate room (RH 60%). In all of the experiments, $CaCl_2$ was used as a desiccant (RH 0%) inside the cups. The cups were weighed four to seven times at intervals of 1–24 h. The water vapor transmission rate (WVTR) was calculated from the linear regression of the slope of weight gain versus time by dividing the slope by the test cell mouth area. The water vapor partial pressure on the underside of the film was calculated with the correction method described by Gennadios *et al*. (1994). The WVP was obtained by multiplying the WVTR by the thickness of the film and dividing it by the water vapor partial pressure difference between the two sides of the film.

Oxygen gas permeability (I–IV)

The oxygen gas transmission rate (OTR) of the films in studies **I**, **II**, and **IV** was measured using an Ox-Tran Twin equipped with a coulometric sensor (Modern Controls, Inc., Minneapolis, USA). The films were measured at RH 50–75% at 22°C. In study **III**, a

Systech Instrument 8001 analyzer (Johnsburg, USA) was used at RH 50% and 23°C. Oxygen permeability (OP) was calculated by multiplying OTR by film thickness and then dividing that by the oxygen gas partial pressure difference between the two sides of the film.

4.4.4 Water vapor sorption (IV, V)

A DVS Intrinsic sorption microbalance (Surface Measurement Systems, Alperton, UK) was used to analyze the water sorption isotherms of the films. The measurements were carried out at a humidity range of 0–90% and at 25°C. The humidity was raised by increments of RH 10%. The sample weight was equilibrated at each step. The moisture uptake (%) was calculated according to Equation (1):

$$Moisture\ uptake = 100[(W_{moist} - W_{dry})/W_{dry}] \quad (1),$$

where W_{moist} is the weight of the sample equilibrated at the chosen relative humidity and W_{dry} is the weight of the dry sample.

4.4.5 Thermal analysis (I, IV, V)

A dynamic mechanical analyzer (DMA 242, Netzsch-Gerätebau GmbH, Selb, Germany) was used to study the α-relaxation (glass transition, T_g) of the OsAX films in study **I**. The storage modulus and loss modulus data were collected at frequencies of 1, 2.5, 5, 10, and 20 Hz in a temperature range of –120 to 50°C and a heating rate of 2°C/min. Glass transition temperature was taken as a peak temperature of loss modulus at 1 Hz. Humidity scans in study **I** were performed on a PerkinElmer DMA 7e (Waltham, USA). Two amplitudes (5 µm for the films plasticized with 10% polyol and 10 µm for the films plasticized with 40% polyol) were used at a frequency of 1 Hz. Storage modulus data was collected in an RH range of 0–90% at the rate of 0.1%/min at 30°C.

For dielectric testing in study **IV**, a TA Instruments Dynamic Electrical Analyzer (DEA 2970, New Castle, USA) was used in multi-frequency mode. Conditioned films (RH 54%) were positioned between two electrodes. A Viton® gas barrier rubber O-ring seal was added between the sensor plates to prevent water evaporation during the measurement. Samples were measured as duplicates from –120°C to 150°C at a heating rate of 3°C/min. Constant force (340 N) was applied during the experiment. The frequencies used were 1; 10; 100; 1,000; 10,000; and 100,000 Hz. The peak temperatures of tangent delta at 1 Hz were taken as α- and β-relaxations (T_α and T_β), of which T_α was considered as a glass transition temperature (T_g).

The glass transition temperature (T_g) of the extruded sheets in study **V** was analyzed with Tritec 2000 DMA (Triton Technology Ltd., Grantham, UK) with a temperature range of –100°C to 100°C. The applied frequencies were 1 and 10 Hz; the amplitude was 50 µm.

The water content of the films conditioned at RH 50% (climate room) in study **I** and at RHs 50%, 75.5% (desiccator containing saturated NaCl), and 98.0% (desiccator containing water) in study **III** was determined via a thermal gravimetric analyzer equipped with STAR^e software (Mettler Toledo TGA 850, Giessen, Germany). Film specimens were heated from 25°C to 120°C at a heating rate of 50°C/min, at which point the temperature was maintained at 120°C for 10 min. The weight loss of the sample was considered to be water content.

4.4.6 X-ray diffraction (I–IV)

Wide-angle X-ray scattering measurements (XRD) were carried out to determine the degree of crystallinity of the films and the size of the xylan crystallites. XRD setups and interpretation of the diffraction patterns are described in more detail in studies **I**, **II**, and **IV**. The films were measured at room temperature (25 ± 1°C) and at a relative humidity of 21 ± 2%.

5 Results

5.1 Characteristics of the raw materials (I–V)

In this thesis, commercially available oat spelt AX and rye and wheat flour AXs were used in film studies. In addition, two previously extracted wheat bran extracts were utilized for sheet extrusion. The monosaccharide compositions as well as the weight average molar masses of the commercial AXs and separately produced extracts were characterized; the results are summarized in Table 10.

Table 10. Monosaccharide compositions, Ara/Xyl ratios, and weight average molar masses of arabinoxylans and wheat bran extracts used in this study.

AX	Xyl (%)	Ara (%)	Me-Glucuronic acid (%)	Glc (%)	Other (%)	Ara/Xyl	M_w (g/mol)	Study
OsAX	83	12	-	5.0	-	0.14	58000[a]	**I**
OsAX	78	12	2.0	6.7	2.1	0.15	58500	**II**
RAX-hv	66	33	-	1.0	-	0.50	246400[b]	**III**
WAX-mv	64[c]	36[c]	-	0[c]	-	0.56	213900[b]	**IV**
WBE-S	11[d]	5.1[d]	-	45[d]	1.2[d]	0.46	nd	**V**
WBE-P	11[d]	5.7[d]	-	11[d]	1.2[d]	0.50	nd	**V**

- = not detected, nd = not determined

[a]Eronen *et al*. (2011), [b]Pitkänen *et al*. (2009), [c]Virkki *et al*. (2008), [d]Jacquemin *et al*. (2015)

5.2 Effect of de-branching on rye and wheat flour arabinoxylans' fine structure (III–IV)

Rye and wheat flour AXs were both highly substituted with similar Ara/Xyl ratios of 0.50 and 0.56, respectively (Table 10). Additionally, their molar masses were close to each other (Table 10). The different substitution patterns of RAX and WAX enzymatic tailoring that were carried out with two specific enzymes (AXH-m and AXH-d3) resulted in unmodified chain length but varying Ara/Xyl ratios and Ara*f* distribution (Figure 4, Table 11).

In RAX, having a high amount of mono-substituted Ara*f* units, three different dosages of AXH-m gradually decreased the number of Ara*f* units linked to mono-substituted Xyl*p* residues, whereas the small number of doubly substituted Xyl*p* remained intact. As a result, RAXs with four different Ara/Xyl ratios were obtained for film studies: one

unmodified (RAX-ref) and three modified RAX-m samples (Table 11). Cleavage of the Ara*f* units from the RAXs was confirmed to originate from mono-substituted Xyl*p* units by ^{1}H NMR (Figure 1 in study **III**). In the WAXs, AXH-m and AXH-d3, having different substrate specificities, sufficiently removed the targeted Ara*f* substituents, since their amount decreased approximately to two-thirds of the original (as expected). As a result, WAXs with a different number of un-substituted Xyl*p* units but similar Ara/Xyl ratios were obtained (Table 11).

Table 11. Ara/Xyl ratios and estimated number of un-substituted Xyl*p* units after enzymatic de-branching of RAX and WAX.

Arabinoxylan	Enzyme used	Ara/Xyl	Un-substituted Xyl*p* units (%)
RAX-hv	-	0.50	58[a]
	AXH-m	0.37	71
	AXH-m	0.30	78
	AXH-m	0.20	88
WAX-mv	-	0.56	66
	AXH-m	0.29	86
	AXH-d3	0.34	66

[a]Pitkänen *et al*. (2011)

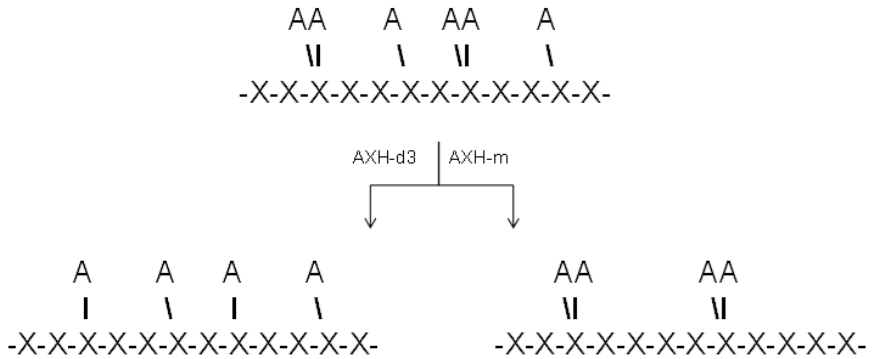

Figure 4. Hypothetical structures of enzymatically tailored wheat AXs.

5.3 Film formation and characteristics of the films

The water solubility of the AXs turned out to be a critical factor in cohesive film formation. OsAX, with a naturally low Ara*f* substitution, was not totally water-soluble, and cohesive films were not formed from the suspension containing insoluble material. Removal of the insoluble part by centrifugation was not enough, and external plasticization was needed for cohesive film formation. As an exception, one experiment with OsAX from TCI was carried out successfully without polyol plasticization. All of the OsAX films were transparent, but they had a brownish shade. The unmodified RAX and WAX were both water-soluble. Highly de-branched RAX and WAX, in which the number of un-substituted xylose units increased the most, had clearly lowered water solubility, and visible agglomeration was observed in the film suspensions; the formed films were translucent. Low de-branching did not alter water solubility, and both the formed films and the unmodified films were transparent. Due to the casting method used in the film preparation, the films were somewhat heterogeneous in their thicknesses, which varied in all of the studies, the average being 20–50 μm.

Film morphology affects film properties; therefore, the crystallinity of the films was determined. Unmodified RAX-ref and WAX-ref films were fully amorphous, but lowered solubility due to de-branching was observed to increase film crystallinity. All of the OsAX films in studies **I** and **II** were semi-crystalline (i.e., there were regions of xylan crystallites surrounded by an amorphous region). The crystallinity of plasticized OsAX films varied between 20–38% (Table 12). The average size of the single xylan crystallites was 5–10 nm. Also, the highly de-branched WAX-m in study **IV** was semi-crystalline (12%, Table 12). Instead, WAX-d3 was still water-soluble, and the films formed were fully amorphous (crystallinity 0%). The crystallinity values of the RAX-ref and RAX-m films were not determined in study **III**, but X-ray diffractograms with increasing peak intensity indicated increased crystallinity along with de-branching.

All of the studied AX films absorbed water and were hydrophilic. The unmodified RAX-ref and WAX-ref films had similar water contents, at RH 50% and room temperature; 11% and 12%, respectively. Water sorption of the RAX and WAX films was measured by different methods, and in Table 12, the water contents at RH 50% are reported. In the RAX films, the water content was analyzed in three different relative humidities. At RH 50%, and RH 76% water content increased slightly along with the de-branching, from 11% to 13% and from 15% to 17%, respectively (except RAX-m Ara/Xyl 0.20, which had a lower water content than the films with more moderate de-branching). At RH 98%, the water content decreased along with the de-branching from 55% to 41%. In the case of WAX, de-branching did not affect the water content of the films at RH 50% (Table 12). In study **IV**, the water sorption of the WAX films was studied in more detail in an RH range of 0–90% (Figure 5). At RHs below 50%, the isotherms were almost identical, but above 50%, there were small differences, indicating that the amount and distribution of the Ara*f*

groups affects the amount of bound water molecules in the film matrix. In the OsAX films, glycerol seemed to somewhat increase the water content of the films compared to sorbitol when the plasticizer content was 40% (Table 12). When 10% of the plasticizer was added, the values for the glycerol- and sorbitol-plasticized films were the same. The results from the DMA humidity scan demonstrated the high impact of polyol type and amount on softening of the films along with the increasing RH, which was observed as a decrease of storage modulus (Figure 6). Softening of the OsAX film plasticized with 40% glycerol began at RH 10%, and at RH 30%, the relative storage modulus was decreased to half (compared to the value at RH 0%). When the films were plasticized with 40% sorbitol, softening of the films started at a higher RH than in the case of the 40% glycerol-plasticized films; a steep drop of the storage modulus was observed in the RH range of 40–60%. The films plasticized with 10% glycerol and sorbitol behaved similarly to each other, and the decrease of storage modulus occurred in a broader RH range than when 40% polyol was used.

Glass transition temperature was studied and observed in the OsAX and WAX films. In OsAX, plasticization affected T_g of the amorphous part the films. As expected, T_g decreased along with the increased polyol amount (i.e., increased plasticization) (Table 12). The lowest value was obtained with 40% glycerol, indicating that at a high content, glycerol was a more effective plasticizer than sorbitol. In the WAX films, both α- and β-transitions (T_g and T_β) were determined with DEA to find how de-branching affected the molecular mobility of AX in the amorphous part of the film. In WAX and WAX-d3, T_β values, which give information about local AX chain mobility, were similar, but in WAX-m, it was significantly lower than in the other two films. In contrast to the T_β results, the T_g values of WAX and WAX-m were similar, but the T_g of WAX-d3 was higher.

Table 12. Crystallinity, thermal transitions (T_β and T_g), and water content of the films in studies **I–IV**. The T_g of OsAX films was determined with DMA, whereas the T_β and T_g of WAX were determined with DEA.

Study	AX	Plasticizer	Crystallinity ϕ (%)	T_β (°C)	T_g (°C)	Water content at RH 50% (% w/w)
I	OsAX	10% Gly	23 ± 5	nd	17 ± 6	11.2 ± 0.5
	OsAX	10% Sor	20 ± 5	nd	–10 ± 1	11.1 ± 0.2
	OsAX	40% Gly	26 ± 5	nd	–59 ± 6	12.5 ± 0.9
	OsAX	40% Sor	22 ± 5	nd	–24 ± 11	10.4 ± 0.9
II	OsAX	40% Gly	38 ± 3	nd	nd	nd
	OsAX	40% Gly–Sor (3:1)	38 ± 3	nd	nd	nd
	OsAX	40% Gly–Sor (1:1)	37 ± 3	nd	nd	nd
	OsAX	40% Gly–Sor (1:3)	37 ± 3	nd	nd	nd
	OsAX	40% Sor	35 ± 3	nd	nd	nd
III	RAX-ref	-	nd	nd	nd	11.0 ± 0.2
	RAX-m (0.37)	-	nd	nd	nd	12.5 ± 0.2
	RAX-m (0.30)	-	nd	nd	nd	13.4 ± 0.2
	RAX-m (0.20)	-	nd	nd	nd	13.3 ± 0.3
IV	WAX-ref	-	0	–100.3 ± 2.9	40.4 ± 0.5	12.0 ± 0.1
	WAX-m (0.29)	-	12 ± 3	–105.6 ± 0.4	40.6 ± 0.3	12.3 ± 0.1
	WAX-d3 (0.34)	-	0	–100.9 ± 2.0	52.2 ± 2.6	11.7 ± 0.4

- = no external polyol plasticizer

nd = not determined

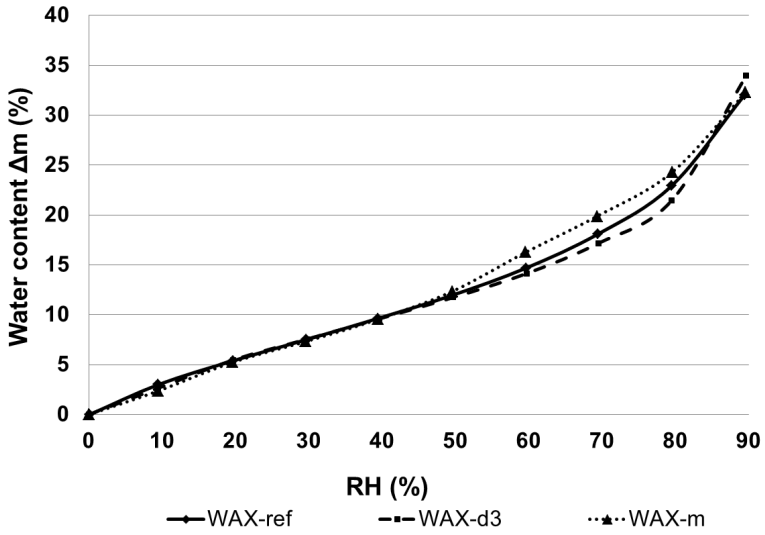

Figure 5. Water sorption isotherms of WAX films in RH range of 0–90% (Figure 4 in study **IV**).

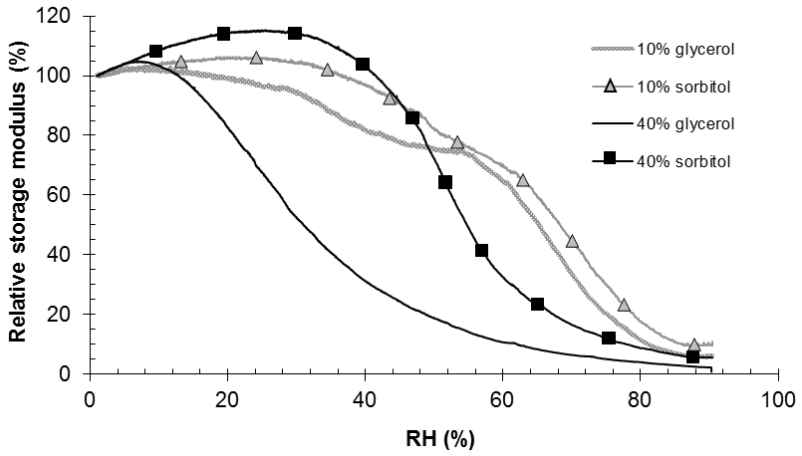

Figure 6. Effect of relative humidity on storage modulus of plasticized OsAX films (Figure 2 in study **I**). The sorbitol curves are marked with squares and triangles to separate them from the glycerol curves.

5.4 Tensile properties

5.4.1 Effect of external plasticization on oat spelt arabinoxylan films (I–II)

Preliminary tests demonstrated that in general, OsAX needed external plasticization for cohesive film formation. Glycerol and sorbitol were compatible with OsAX, but xylitol crystallized on the film surface, which was observed visually, and it was not chosen for further experiments. Instead, glycerol, sorbitol, and their blends were used, added in amounts varying between 0–40% (w/w of OsAX). As can be seen from the tensile property values, the type and amount of plasticizer had a clear impact on the OsAX films (Table 13). The highest tensile strength and Young's modulus were obtained when no plasticizer or 10% (w/w of OsAX) glycerol or sorbitol were used. By increasing the plasticizer amount, the tensile strength and Young's modulus decreased in both the glycerol- and sorbitol-plasticized films; in addition, elongation at break increased in most of the studies. With a plasticizer content of 10%, the tensile properties of the glycerol- and sorbitol-plasticized films were similar to each other and to un-plasticized film. When 20% or more plasticizer was used, the sorbitol-plasticized films were generally stronger, stiffer, and more elongated than the glycerol-plasticized films.

Table 13. Tensile strength (TS), elongation at break (EB), and Young's modulus (YM) of OsAX films plasticized with 0–40% (w/w of OsAX) of glycerol, sorbitol, or their blends (gly–sor). The measurements were taken at RH 50%.

Plasticizer	TS (MPa)	EB (%)	YM (MPa)	Study
no	25.0 (± 4.1)	6.0 (± 0.8)	626 (± 126)	unpubl.
10% gly	19.6 (± 4.7)	4.7 (± 1.2)	507 (± 187)	**I** and unpubl.
	–27.5 (± 5.0)	–9.5 (± 1.1)	–1093 (± 422)	
20% gly	7.9 (± 1.0)	10.0 (± 3.0)	274 (± 60)	unpubl.
30% gly	5.4 (± 1.6)	12.3 (± 2.2)	130 (± 31)	unpubl.
40% gly	3.0 (± 0.6)	10.5 (± 3.7)	81 (± 13)	**I**, **II**, and unpubl.
	–4.2 (± 0.9)[a]	–13.1 (± 3.3)[a]	–113 (± 16)[a]	
10% sor	16.8 (± 6.6)	4.2 (± 1.1)	537 (± 167)	**I** and unpubl.
	–23.8 (± 4.8)	–9.0 (± 2.5)	–699 (± 170)	
20% sor	14.1 (± 1.4)	13.9 (± 4.3)	318 (± 65)	unpubl.
30% sor	11.1 (± 2.1)	19.7 (± 4.7)	224 (± 46)	unpubl.
40% sor	8.0 (± 0.8)	9.7 (± 4.0)	155 (± 29)	**I**, **II**, and unpubl.
	–10.8 (± 3.2)[a]	–24.4 (± 2.7)[a]	–304 (± 64)[a]	
40% gly–sor (3:1)	5.6 (± 0.6)	15.8 (± 1.9)	104 (± 17)	**II**
40% gly–sor (1:1)	7.6 (± 1.2)	17.9 (± 2.4)	156 (± 28)	**II**
40% gly–sor (1:3)	8.7 (± 1.0)	18.3 (± 5.1)	175 (± 38)	**II**

[a]Variation of average value in three separate studies

5.4.2 Role of arabinose substitution on film properties (III–IV)

The effect of Ara*f* side groups on the mechanical properties of AX films was studied by comparing highly branched RAX (Ara/Xyl 0.50) and WAX (Ara/Xyl 0.56) as reference films (RAX-ref and WAX-ref) with enzymatically tailored ones. Because a high number of Ara*f* groups are located in the mono-substituted Xyl*p*, RAX was systematically de-branched with arabinofuranohydrolase AXH-m acting specifically on those arabinoses (study **III**). WAX was extensively de-branched with both AXH-m and AXH-d3 (study **IV**) in order to study the effect of these two types of substitutions on film properties. OsAX has a naturally low Ara*f* content (Ara/Xyl 0.15), and in addition, it has a lower M_w than RAX and WAX (approx. 60000 g/mol vs. 250000 g/mol). In the RAX films, the highest tensile strength and elongation at break were found in the film with moderate de-branching (Ara/Xyl 0.37) (Figure 7A and 7B). This film was the strongest when all of the studied films (OsAX, RAX, and WAX) were compared with each other; it had a tensile strength of 58 MPa, whereas the OsAX film had the lowest tensile strength (25 MPa). The RAX-ref film had the highest Young's modulus (Figure 7C). Of the WAX films, WAX-ref had the highest tensile strength, whereas the Young's modulus was similar in the WAX-ref and WAX-m films and the lowest in the WAX-d3 film (Figures 7A and 7C). Elongation at break was similar in the WAX-ref and WAX-d3 films and the lowest in the WAX-m film (Figure 7B). The Young's modulus of the OsAX film was similar to the highly de-branched RAX-m (Figure 7C). Elongation at break of OsAX was similar to all of the other films (except RAX-m at 0.37) (Figure 7B).

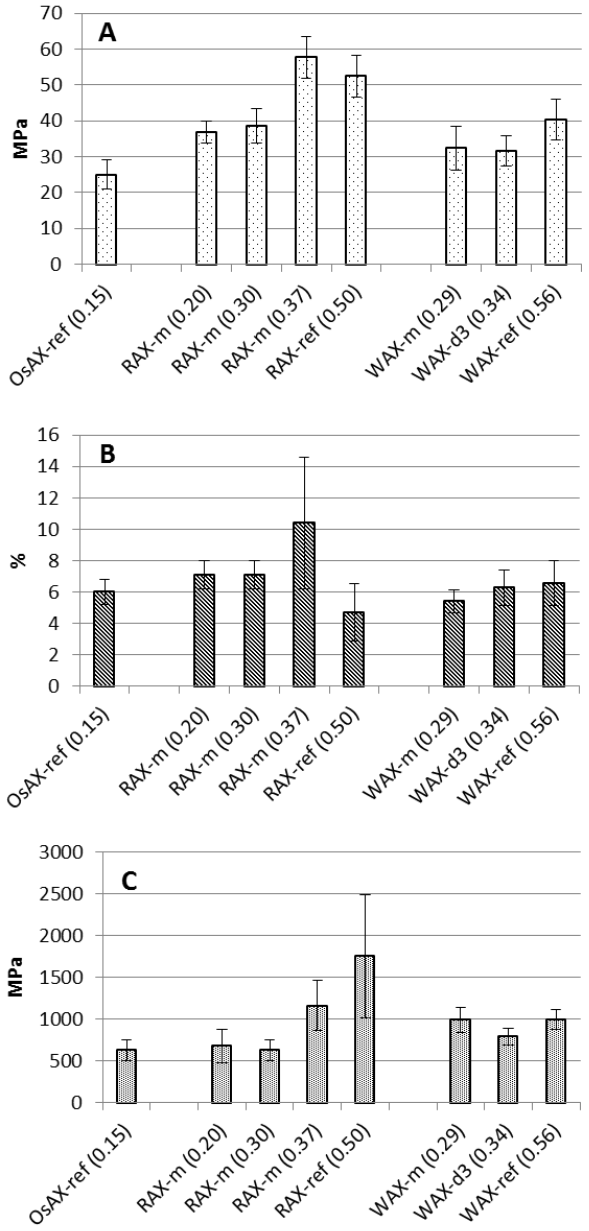

Figure 7. Effect of Ara*f* branching on the tensile properties of cereal AX films without external plasticization. A: Tensile strength, B: Elongation at break, and C: Young's modulus. Ara/Xyl ratios are shown in parenthesis after the sample name. The measurements were done at RH 50% at RT.

5.5 Permeability properties

5.5.1 Effect of external plasticization on permeability properties

Oxygen permeability of plasticized OsAX films

The plasticizer effect on the oxygen permeability properties of the OsAX films was determined in studies **I** and **II.** Glycerol, sorbitol, and their blends were used in the amounts of 10% and 40% (Table 14). In addition, previously unpublished OP results with 0%, 20%, and 40% glycerol or sorbitol are presented in Table 14. In all of the measurements, the RH was 50–75% and the temperature was 23°C. The results show that the average OP values of un-plasticized film, films with 10% glycerol or sorbitol, and films with 40% sorbitol or a glycerol–sorbitol blend were similar to each other. The OP of the films containing 40% glycerol was the highest; there was, however, higher variation in those results than in the OP results of the other measured films.

Table 14. Oxygen transmission rate (OTR) and oxygen permeability (OP) of OsAX films.

Plasticizer	OTR [$cm^3/(m^2\ d)$]	OP [$cm^3\ \mu m/(m^2\ d\ kPa)$]	Study
no plasticizer	13.8 (± 3.1)	4.3 (± 0.6)	unpubl.
10% glycerol	10.7 (± 0.9)	3.0 (± 0.9)	**I**
20% glycerol	17.3 (± 0.88)	8.4 (± 3.7)	unpubl.
40% glycerol	11.8 (± 0.2)	5.4 (± 0.3)	**I**, **II** and unpubl.
	–32.9 (± 6.3)[a]	–19.2 (± 10.4)[a]	
10% sorbitol	12.5 (± 2.7)	3.2 (± 0.6)	**I**
20% sorbitol	14.7 (± 1.3)	5.9 (± 1.3)	unpubl.
40% sorbitol	9.6 (± 1.8)	3.7 (± 2.0)	**I**, **II**, and unpubl.
	–11.8 (± 5.0)[a]	–4.7 (± 1.6)[a]	
40% glycerol–sorbitol (1:1)	10.2[b]	4.1[b]	**II**

[a]Variation of average value in three separate studies

[b]One successful measurement

Water vapor permeability of plasticized OsAX films

The WVP of OsAX films plasticized with glycerol, sorbitol, and their blends was analyzed in studies **I** and **II**; the results are collected in Table 15. In addition, previously unpublished WVP results are shown in Table 15. In all measurements, the RH gradient was 0/54% and the temperature was 23°C. The results clearly indicate that sorbitol

plasticization lowers the WVP of the films compared to glycerol plasticization. In the films plasticized with glycerol–sorbitol blends, the WVP decreased when the amount of sorbitol in the blend increased. Sorbitol-plasticized films (10%, 20%, and 40%) had WVP values of 1–2%, which is lower than that of the un-plasticized OsAX film (4%) (Table 15). Clearly, the highest values were obtained when glycerol was used alone as a plasticizer or when most of the plasticizer blend was composed of glycerol (glycerol–sorbitol 3:1).

Table 15. Water vapor transmission rates (WVTRs) and water vapor permeabilities (WVPs) of OsAX films plasticized with 0–40% (w/w of the polysaccharide) glycerol, sorbitol, and their blends. RH gradient 0/54% at 23°C.

Plasticizer	WVTR [g/(m^{2}d)]	WVP [(g·mm)/(kPa·m^2·d)]	Study
no plasticizer	186.6 (± 13.9)	4.3 (± 0.6)	unpubl.
10% glycerol	180.0 (± 5.0)	3.3 (± 0.2)	**I**
20% glycerol	288.6 (± 21.3)	8.9 (± 0.8)	unpubl.
40% glycerol	580.0 (± 34.0)	13.0 (± 3.0)	**I**, **II** and
	–667.1 (± 50.6)[a]	–27.0 (± 2.9)[a]	unpubl.
10% sorbitol	70.0 (± 16.0)	1.1 (± 0.1)	**I**
20% sorbitol	40.0 (± 1.1)	1.2 (± 0.2)	unpubl.
40% sorbitol	62.0 (± 2.1)	1.9 (± 0.2)	**I**, **II**, and
	–90.0 (± 10.0)[a]	–2.1(± 0.1)[a]	unpubl.
40% glycerol–sorbitol (3:1)	412.8 (± 9.7)	14.6 (± 1.0)	**II**
40% glycerol–sorbitol (1:1)	216.8 (± 1.4)	7.5 (± 0.9)	**II**
40% glycerol–sorbitol (1:3)	97.2 (± 4.7)	3.1 (± 0.2)	**II**

[a]Variation of average values in three separate studies

The effect of the RH gradient on WVP was further studied by comparing the RH gradient of 0/54% to higher gradients of 0/66% and 0/86%. Films plasticized with 10% or 40% glycerol and sorbitol, were studied at a temperature of 23°C. With both studied polyol types and amounts, the WVP increased when the RH gradient increased and when the polyol content of the films increased (Figure 8). The glycerol-containing films had a clearly higher WVP than the sorbitol-containing films in all of the studied RH gradients.

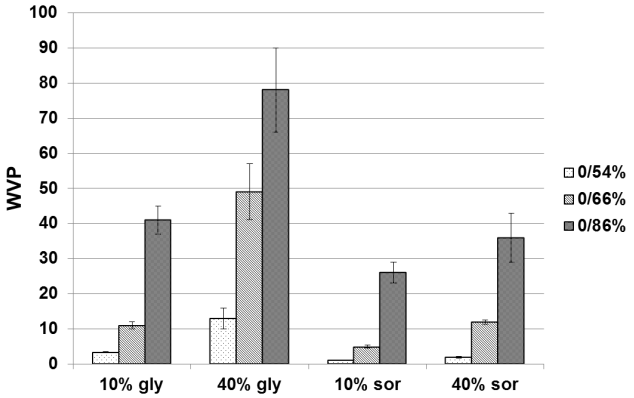

Figure 8. Effect of RH gradient on the water vapor permeability (WVP) of OsAX films plasticized with 10% and 40% of glycerol and sorbitol at 23°C.

5.5.2 Role of arabinose substitution on permeability properties

Oxygen permeability measurements were done for unmodified and modified RAX and WAX films (studies **III** and **IV**). RAX-ref and WAX-ref films acted as moderate oxygen gas barriers. In both the RAX and WAX films, de-branching decreased oxygen permeability (Figure 6 in study **III** and Table 16, respectively). In the RAX films, the OP was somewhat lower than in the WAX films. All of the RAX films had an OP of under 2 [cm^3 µm/(m^2 d kPa)], whereas in the WAX films, the values were higher: 7.6 and 4.6 [cm^3 µm/(m^2 d kPa)]. WVP was determined for the WAX films, and the results for WAX-ref and WAX-d3 were similar. WAX-m could not be measured due to leaking of the film.

Table 16. OP and WVP of WAX films.

	OP [cm^3 µm/(m^2 d kPa)]	**WVP** [(g mm)/(kPa m^2 d)]	**Study**
WAX-ref (0.56)	7.6 (± 1.4)	7.9 (± 0.9)	**IV**
WAX-d3 (0.34)	4.6 (± 0.6)	7.2 (± 1.2)	**IV**
WAX-m (0.29)	nd	nd	**IV**

5.6 Storage stability of polyol-plasticized OsAX films (I–II)

The long-term physical stability of polyol-plasticized OsAX films was studied in order to get information on how aging affects the properties of the films containing arabinoxylan, polyol, and water. Since water acts as a plasticizer, films were stored in a climate room in controlled RH and temperature to keep the water content similar. Two separate studies were carried out: first, a five-month study with 40% (w/w of polysaccharide) glycerol and sorbitol (**I**), second, a four-month study where the effect of glycerol–sorbitol blends was investigated (**II**). In the stability study (**I**), the tensile strength of the glycerol-plasticized films increased significantly, from 3.2 (± 0.8) MPa to 19.1 (± 3.4) MPa during four months of storage. After five months, the level was similar to the four-month value: 16.8 (± 5.9) MPa. Elongation at break decreased from 10.4 (± 3.4)% to 2.8 (± 1.0)% during the five months of storage. Similarly to study **I**, in study **II**, the tensile strength of the glycerol-plasticized films increased, and elongation at break decreased during the four months of storage; however, the shift was more moderate (Figure 9). Against the hypothesis, sorbitol did not stabilize glycerol in the blends; instead, the more glycerol there was in the blend, the more the tensile strength increased over the four months of storage. In the sorbitol-plasticized films, the change in the tensile properties was opposite to the glycerol plasticization: the tensile strength and Young's modulus decreased and elongation at break increased during storage (Figure 9).

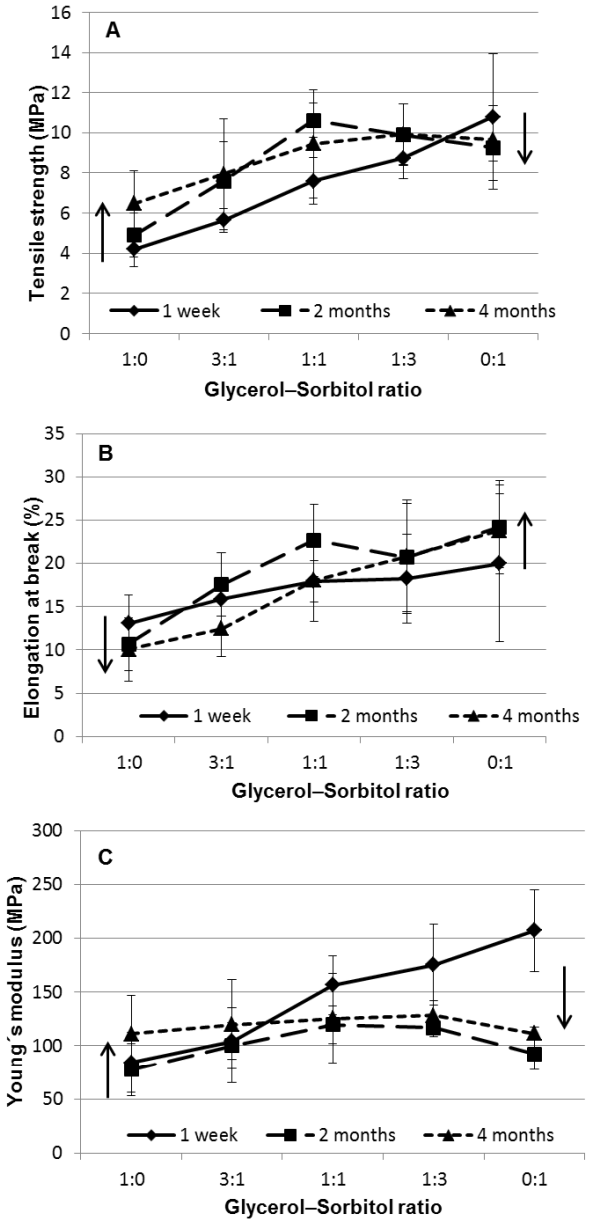

Figure 9. Effect of two and four months of storage on mechanical properties of OsAX films plasticized with 40% glycerol, sorbitol, and their blends (Figure 2 in study **II**).

Four months of storage decreased the WVP of all of the films (Figure 10). The decrease was not identical in all of the films; instead, the decrease was the highest (63%) when the glycerol–sorbitol ratio was 3:1 and the lowest (21%) when sorbitol was used alone. On the contrary to the WVP results, the OP of the films, increased during storage (Table 17). The OP was measured successfully from the OsAX films plasticized with 40% glycerol and 40% sorbitol; the films plasticized with a glycerol–sorbitol blend (1:1) leaked, and no value was obtained.

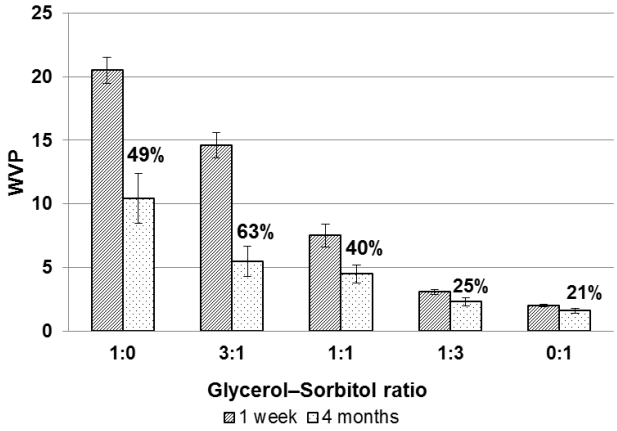

Figure 10. Effect of four months of storage time on the water vapor permeability of OsAX films plasticized with 40% glycerol, sorbitol, and their blends. The films were stored at RH 50% at 23°C. The percentages above the bars represent the decrease of the WVP after storage compared with fresh films (Figure 4 in study **II**).

Table 17. Oxygen transmission rates (OTR) and oxygen permeabilities (OP) of OsAX films plasticized with 40% sorbitol, glycerol, and their blends (1:1) after one week or four months of storage.

Plasticizer (40%)	OTR (1 week) [$cm^3/(m^2\ d)$]	OP (1 week) [$cm^3\ \mu m/(m^2\ d\ kPa)$]	OTR (4 months) [$cm^3/(m^2\ d)$]	OP (4 months) [$cm^3\ \mu m/(m^2\ d\ kPa)$]
Gly	11.8 ± 0.2	5.4 ± 0.3	16.7[a]	8.9
Gly–sor 1:1	10.2[a]	4.1	-[b]	-
Sor	11.8 ± 5.0	3.7 ± 2.0	13.4 ± 3.3	6.4 ± 1.4

[a]Only one successful measurement was made

[b]No result was obtained due to leaking of the film

5.7 Sheet extrusion of wheat bran extracts (V)

Two wheat bran extracts, WBE-S and WBE-P, were utilized in sheet extrusion in order to study the suitability of crude biomass extract in larger-scale film/sheet preparation. With both studied plasticized extracts, a 100 mm-wide continuous sheet was successfully produced via single screw extrusion. The thickness of the sheets varied from 280 µm to 655 µm. In the pre-tests, slit thicknesses of 100, 250, and 400 µm were tested, and since the most regular and continuous sheets were obtained with a slit thickness of 250 µm, this was chosen for this study. The effect of the cutting direction was tested with glycerol- and sorbitol-plasticized sheets prior to the actual measurements. Somewhat higher mechanical properties were obtained when the specimens were cut in the direction of the extrusion flow than when the specimens were cut in the opposite direction. Therefore, extrusion flow direction was chosen for all of the samples. Due to the limited amount of raw material WBE-S, only three separate tests were conducted; this is compared to five for WBE-P.

The results indicated that the type of the polyol plasticizer had an effect on both the mechanical properties and WVP of the extruded sheets. In both WBE-S and WBE-P, sorbitol increased the tensile strength and Young's modulus and decreased elongation at break compared to glycerol (Figure 11). The difference between glycerol and sorbitol plasticization was higher in WBE-S than in WBE-P. In the polyol blends, the increased amount of sorbitol had only a minor effect on the tensile values of the WBE-P sheets. WBE-S contained a high amount of starch, which was discovered to have a high impact on the tensile properties and WVP. Starch in the films clearly increased the strength and the stiffness of the sorbitol-plasticized sheets and decreased the WVP. The effect was similar in the glycerol-plasticized sheets; furthermore, elongation at break increased. The WBE-S sheet plasticized with sorbitol had the highest tensile strength (20.7 MPa). Elongation at break of the sorbitol-plasticized WBE-S sheet was similar to the plasticized WBE-P sheets (2–3%) (Figure 11B).

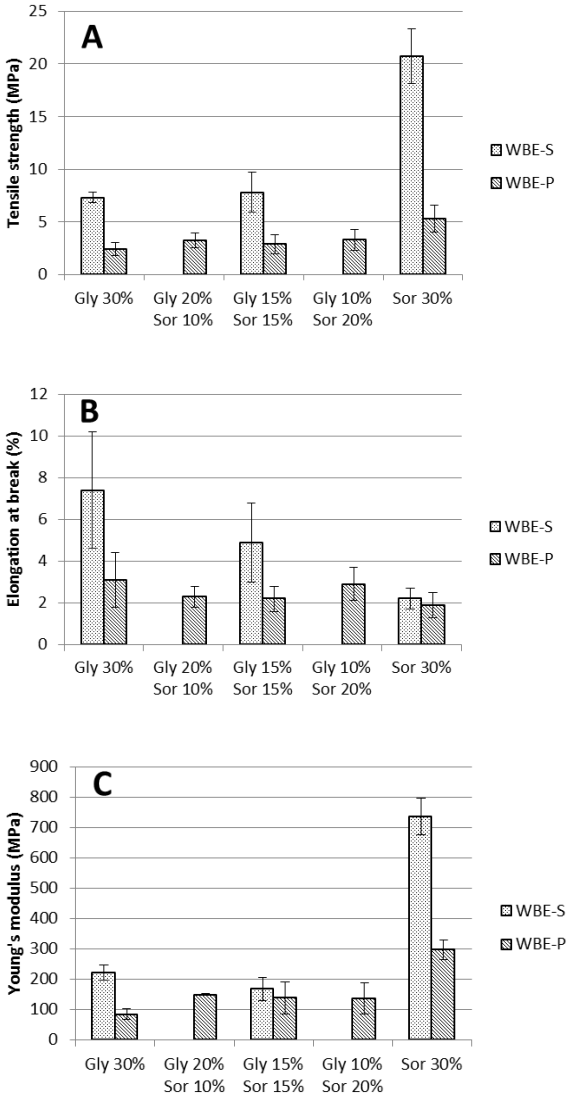

Figure 11. Mechanical properties of extruded sheets. WBE-S is a wheat bran extract with a high starch content; WBE-P has a high protein content.

The glass transition (T_g) of the glycerol- and sorbitol-plasticized sheets was analyzed using DMA (Table 18). In all of the measurements, two separate tan δ peaks were obtained, indicating two T_g values. Sheets contained arabinoxylan, lignin, protein, starch, and polyol plasticizer, and it is possible that polyol was not evenly distributed in the sheet, causing two relaxation peaks. The protein/starch content did not have an effect on the T_g's of the sheets, since the values seemed to be more dependent on the polyol content than on the extract composition.

The water sensitivity of the sheets was studied both by WVS and WVP measurements. In both WBE-S and WBE-P, the WVS was higher when glycerol was used as a plasticizer instead of sorbitol (Figure 12). In the glycerol-plasticized WBE-P sheet, the WVS was higher than in the WBE-S when RH was above 50%, indicating that starch removal made the sheets somewhat more hydrophilic. In the sorbitol-plasticized sheets, a similar effect was not observed. The starch-containing WBE-S sheets had a lower WVP than the sheets containing protein (Table 18).

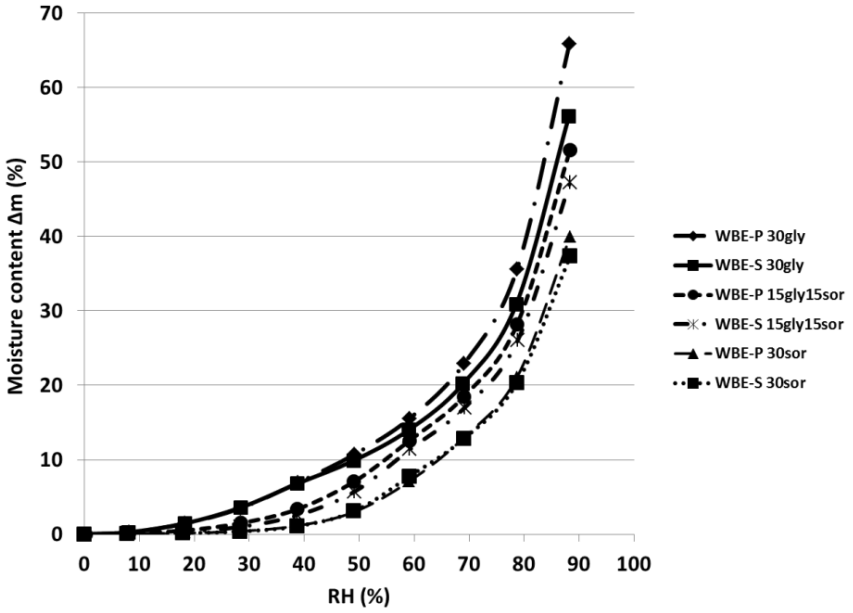

Figure 12. Water vapor sorption (WVS) of plasticized WBE-S and WBE-P sheets in the RH range of 0–90% (Figure 1 in study **V**).

Table 18. Glass transition temperatures (T_g) and water vapor permeability (WVP) of extruded WBE sheets.

	T_{g1} (°C)	T_{g2} (°C)	WVP [g mm/m^2 d kPa]
WBE-S			
30% glycerol	–59.9	9.1	28.8 ± 0.4
15% glycerol + 15% sorbitol	nd	nd	10.5 ± 0.1
30% sorbitol	–21.2	33.5	3.2 ± 0.1
WBE-P			
30% glycerol	–56.9	3.6	34.8 ± 0.4
20% glycerol + 10% sorbitol	nd	nd	19.9 ± 0.4
15% glycerol + 15% sorbitol	nd	nd	21.7 ± 0.6
10% glycerol + 20% sorbitol	nd	nd	15.3 ± 0.3
30% sorbitol	–20.7	33.3	8.7 ± 0.1

nd= not determined

6 Discussion

6.1 Role of external polyol plasticizer

Both of the commercial OsAXs used in this study had low Ara*f* substitution (Ara/Xyl ratios of 0.14 and 0.15, respectively) and low M_w (approximately 58,000 g/mol), which affected their water solubility and film-forming capacity. Low Ara*f* substitution has been shown to lower the solubility of AX (Pitkänen *et al*. 2011). Additionally, the presence of some glucose-rich impurities in OsAX that were not water-soluble was proven by GC analysis in study **I**. The water-insoluble part was removed by centrifugation to enhance film formation, since precipitates clearly lowered this. Although the OsAX film solution mainly contained soluble polymers, in most of the trials, no cohesive films were formed without the addition of a polyol plasticizer. The low M_w of the OsAX lowered its film-forming ability, an effect that has also been observed in galactomannan-based films (Mikkonen *et al*. 2007). As an exception, and unexpectedly, one experiment was successfully carried out without polyol plasticization. The findings concerning the need for external plasticization in OsAX films are in agreement with Saxena *et al*. (2009), who enhanced the film formation of OsAX by having equal amounts of sorbitol and OsAX in the film solution. Similarly, films made from aspen GX (M_w 15,000 g/mol, ratio of methyl glucuronic acid and xylose 0.17) and bamboo AGX (M_w 13,400 g/mol, Ara/Xyl 0.07) needed external plasticization for film formation (Gröndahl *et al*. 2004; Peng *et al*. 2011). In this study, the adequate amount of polyol plasticizer in the OsAX films was between 10–40% (w/w of the polymer). Higher amounts, especially in the case of glycerol, resulted in sticky films that could not be removed from the dish.

The role of plasticizer type and amount on OsAX film properties was investigated in study **I**. The films were plasticized with 10% and 40% (w/w of the polymer) glycerol or sorbitol. The results showed that the OsAX films plasticized with 10% of glycerol or sorbitol, were stronger and stiffer than the films with 40% polyol plasticizer. On the other hand, the films with 40% polyol plasticizer elongated more than the films plasticized with 10% polyol. In general, 40% glycerol proved to soften OsAX films more efficiently than 40% sorbitol; the films had a lower tensile strength and Young's modulus than the films plasticized with sorbitol. Interestingly, the situation was the opposite when the films were plasticized with 10% polyol, since the plasticizing effect of glycerol was lower than that of sorbitol; further, the glycerol-plasticized films were stronger and stiffer compared to the sorbitol-plasticized films. The results concerning elongation at break were somewhat conflicting in study **I**, where two separate experiments were conducted. In the first experiment, the films were stored for one week, and in the second experiment, the films were stored from one week to five months. In the first experiment, the films plasticized with 10% glycerol or sorbitol had similar elongation at break with each other, as did the films plasticized with 40% glycerol or sorbitol. However, the films stored for one week in the second experiment

had a different result; the films with 40% sorbitol had a clearly greater elongation at break than films with 40% glycerol. The films for these two separate experiments, in the study **I**, were prepared similarly to each other, except that the drying of the films differed. The films for the second experiment were dried at 60°C; therefore, they dried in a much shorter amount of time than when dried at RT (first experiment). This may have affected the formation of the film matrix (e.g., how the xylan chains orientate in the presence of sorbitol when water evaporates faster than when dried at RT). In study **II**, where the films were dried at RT, OsAX films plasticized with 40% sorbitol were strongest, stiffest, and elongated the most compared to films plasticized with 40% glycerol or glycerol–sorbitol blends.

The OsAXs used in studies **I** and **II** were from different suppliers, and although most of the results are consistent in both studies, there were some differences in the tensile properties as well as in the crystallinity of the films. The crystallinities of the OsAX films varied between 20–26% in study **I** and between 35–38% in study **II**. In both studies, the glycerol-plasticized films had higher crystallinity than the sorbitol-plasticized films. The higher plasticizing effect of glycerol compared to sorbitol increased the molecular mobility of the OsAX chains, which in turn enhanced the formation of xylan crystallites. The same phenomenon has been seen in other plasticized polysaccharide films, such as starch-based films (Rindlav-Westling *et al*. 1998; Talja *et al*. 2008) and galactoglucomannan films (study **II**). Similarly, the plasticized OsAX film (100% sorbitol) had a crystallinity of 26% (Saxena *et al*. 2011).

Plasticizer type and amount clearly affected the T_g of the films. In study **I**, films plasticized with 10% glycerol had the highest T_g (17°C), while the lowest T_g was found in the 40% glycerol film (–59°C). Plasticizer type and amount affected the mobility of the OsAX molecules in the film. Glycerol and sorbitol differ in size: glycerol is a smaller molecule than sorbitol, and the larger molecular dimensions of sorbitol were found to decrease the density of the film by increasing the free volume of the polymer network (Cheng *et al*. 2006). In addition to the plasticizing effect in the films, plasticizer added in low amounts (< 15%) may induce an anti-plasticization effect, as discussed when glycerol-plasticized starch films were studied (Lourdin *et al*. 1997). The anti-plasticization phenomenon is known to take place in synthetic polymers where elongation at break decreases, tensile strength increases, and T_g value increases with the addition of a low amount of plasticizer. In contrast, in the plasticizing-effect where the molecular mobility of the polymers increases, the softness and flexibility of the material increases and the T_g decreases. Lourdin *et al*. (1997) discovered that in glycerol-plasticized starch films, elongation at break was the lowest when the glycerol amount was approximately 12%, but on the other hand, the tensile strength was not the highest, leaving the question of the anti-plasticization effect partly unresolved. In study **I**, a clear anti-plasticization effect was not observed in the films plasticized with 10% glycerol at RH 50%, although tensile strength and T_g were clearly higher than in the sorbitol-plasticized films (10% plasticization).

When the plasticizer amount was 40%, the situation was the opposite. The data from the unpublished experiment did not indicate anti-plasticization of the films when low plasticizer amounts were used. In that study, the tensile strength of un-plasticized OsAX film and film with 10% sorbitol had similar tensile strengths (approximately 25 MPa) and elongation at break (6–9%), whereas films with 10% glycerol had lower tensile strength (20 MPa) and similar elongation at break (9.5%) than un-plasticized film. Glass transition measurements were not carried out in that series. Signs of the anti-plasticization effect of water were observed in a DMA humidity scan, in which the relative storage modulus of the OsAX films increased at low RH levels.

The results from the permeability measurements indicated the high impact of plasticizer type and amount on OP and WVP. Both OP and WVP decreased by lowering the amount of polyol from 40% to 10%. Additionally, with glycerol, films had a higher WVP and OP than with sorbitol when the polyol amount was 40%. When 10% was used, both the glycerol- and sorbitol-plasticized films resulted in similar permeability properties at approximately RH 50%. In the OsAX films, the lowest OP value, approximately 3 [cm^3 µm/(m^2 d kPa)], was obtained with 10% of glycerol or sorbitol. By increasing the polyol amount from 10% to 40%, glycerol-containing films had a somewhat higher OP than sorbitol-containing films, but the differences were small, and all of the values were under 10 [cm^3 µm/(m^2 d kPa)], which is, according to Krochta and De Mulder-Johnston (1997), the limit of a good oxygen barrier. In this study, the OTR values were much lower (9.6–32.9 [cm^3/ m^2 d]) than those of Saxena *et al*. (2010), where the OTR of OsAX films with 100% sorbitol was 355 [cm^3/ m^2 d]. Saxena *et al*. (2010) found that by adding cellulose nanocrystals to the film matrix, film density and porosity as well as the OTR of the films decreased dramatically. Polyols may decrease the density of the AX films by increasing the free volume; OsAX film with 100% sorbitol had a density of 0.6 g/ml (Saxena *et al*. 2010), whereas un-plasticized WAX film had a density of 1.5 g/ml (Ying *et al*. 2013). The density and porosity of the films were not determined in this study; however, from the permeability results, it can be seen that films with the 10% polyol content at RH 50% have lower permeability values than when the polyol content is 40%. This may be an indication of a denser film structure. Especially, the difference was seen in the WVP results of the glycerol-plasticized films. With 10% glycerol, the OsAX film had a WVP of 3.3 [(g mm)/(kPa m2 d)] in the RH gradient of 0/54% at RT, whereas when the glycerol content was increased to 40%, the WVP values ranged from 13.0–27.0 [(g mm)/(kPa m^2 d)]. The WVPs of all of the sorbitol-plasticized films (10%, 20%, and 40% plasticization) were similar, varying from 1.1–2.1 [(g mm)/(kPa m^2 d)]. Similar results have been obtained with the corn hull AX with 13% sorbitol (2.0 [(g mm)/(kPa m^2 d)], whereas without plasticization, the WVP of the films was 4.1 [(g mm)/(kPa m^2 d)] (Zhang and Whistler 2004). Crystalline structures are reported to lower permeability. In this study, however, the OsAX film with the highest crystallinity also had the highest OP and WVP, indicating that softening of the film resulting from the polyol type and amount used has a higher impact on permeability properties than do xylan crystallites.

6.2 Role of arabinose substitution

One of the main interests in this study was to evaluate how Ara*f* substitution affects film formation and film properties. The role of Ara*f* substitution was elucidated in studies **III** and **IV** with a highly substituted RAX and WAX featuring similar Ara/Xyl ratios but different substitution patterns. Fine structures were further modified by systemically de-branching RAX and WAX by specific enzymes. The target of this substitution study was to get information on how AXs from different sources, having different substitution degrees and patterns, would function as raw materials in film applications.

Both enzymes, AXH-m and AXH-d3, worked as expected: RAX films with Ara/Xyl ratios of 0.50, 0.37, 0.30, and 0.20, and WAX films with Ara/Xyl ratios of 0.56, 0.34, and 0.29, were obtained. All cleaved Ara*f* substituents in RAX-m were from mono-substituted Xyl*p* units, as in WAX-m (0.29), whereas in WAX-d3 (0.34), all of the removed Ara*f* residues originated from di-substituted Xyl*p* units. As reported earlier, the amount and distribution of Ara*f* substituents play an important role in the water solubility of AXs, and the de-branching of RAX and WAX resulted in partial precipitation of the solutions (Köhnke *et al*. 2011; Pitkänen *et al*. 2011). The same was discovered in this study: the de-branching of Ara*f* groups from mono-substituted Xyl*p* units increased the amount of un-substituted Xyl*p* units. Further, it is probable that the amount of longer un-substituted segments increased the aggregation of the chains, which in turn notably lowered solubility. This was visually observed in the film solution; in addition, the formed films were translucent. Lowered solubility resulted in brittle films that were difficult to handle.

A low Ara/Xyl ratio, as in barley husk (0.22), has been previously associated with a semi-crystalline film structure (Höije *et al*. 2005). Similarly, OsAX films in this study, having Ara/Xyl ratios of 0.14 and 0.15, were semi-crystalline. As lowered solubility was associated with the semi-crystalline nature of the OsAX films in studies **I** and **II**, the crystallinity of the de-branched RAX and WAX films was studied. The crystallinity index of the RAX films was not determined in study **III**, but X-ray diffractograms clearly indicated increased crystallinity along with de-branching. The reference RAX film (Ara/Xyl 0.50) had an amorphous structure (0% crystallinity). This is in agreement with earlier studies in which the crystallinity of the RAX in composite films increased with increased de-branching: the crystallinities of the RAXs with an Ara/Xyl ratio of 0.27 were reported to be 11 ± 2%, and with an Ara/Xyl ratio of 0.16, 20 ± 3% (Mikkonen *et al*. 2012; Stevanic *et al*. 2011). In WAX, the distribution of Ara*f* substituents after de-branching determined water solubility and affected crystallinity. It was observed that similarly to RAX, in WAX, the de-branching with AXH-m increased the number of un-substituted Xyl*p* units, therefore decreasing water solubility. As a result, the WAX-m film had a semi-crystalline structure and a crystallinity of 12 ± 3%. The WAX-d3 film structure remained amorphous, since removal of the Ara*f* branches from the di-substituted Xyl*p* units did not increase the number of un-substituted Xyl*p* units, although the Ara/Xyl ratio

decreased to the same level with the WAX-m. Thus, it can be concluded that a low Ara/Xyl ratio does not affect the crystallinity of the film alone, but the distribution of Ara*f* units along with the xylan chain has a high impact. The number of un-substituted Xyl*p* residues correlates with the degree of crystallinity; a high number of un-substituted Xylp units in the WAX-m (86%) and in the RAX-m (80%, Pitkänen *et al*. 2011), resulted in the films with crystallinities of 12 ± 3% and 11 ± 2% (Mikkonen *et al*. 2012), respectively.

The effect of Ara*f* substitution on thermal relaxations of AX chains in the amorphous part of the WAX films was studied via DEA. Two relaxations (α and β) were observed in all of the films. The mobility of the AX chains decreased when the number of di-substituted Xyl*p* units decreased, and T_α (T_g) clearly took place at a higher temperature in the WAX-d3 film (52.2°C) than in the WAX-ref and WAX-m films (40.4°C and 40.6°C, respectively). The T_β of the WAX-ref and WAX-d3, having the same amount of un-substituted and substituted Xylp units, were similar (–100.3°C and –100.9°C), whereas the T_β of the WAX-m was lower (–105.6°C). These results indicate that the number of substituted/un-substituted Xyl*p* units affects the local mobility of WAX (T_β) in the amorphous part of the WAX film, whereas the type of substitution (mono- or di-) affects the mobility of the whole molecule (T_α, T_g). Similarly, the T_g results of WAX-ref and WAX-m are in accordance with the study by Stevanic *et al*. (2011), in which the removal of all Ara*f* groups from mono-substituted Xyl*p* units did not significantly alter the T_g of the RAX film.

Mild de-branching of RAX (from Ara/Xyl of 0.50 to 0.37) proved to strengthen the films and make them more flexible, since both tensile strength and elongation at break increased, but with further de-branching, tensile strength clearly decreased and elongation at break increased slightly. The latter effect was also seen with highly de-branched WAX films, where in both the WAX-m and WAX-d3 films tensile strength was lower than in the unmodified WAX-ref film. Interestingly, the tensile strength of WAX-m and WAX-d3 was similar, although the WAX-m was semi-crystalline and the WAX-d3 amorphous. The unmodified RAX film was stronger and stiffer than the unmodified WAX film, whereas their elongation was similar. Although the Ara/Xyl ratios were similar in RAX and WAX, their Ara*f* distributions differed: RAX had less un-substituted Xyl*p* units than WAX. Based on the tensile properties, it can be concluded that Ara*f* substitution plays an important role in the chain interactions, and a high number of substituted Xyl*p* units seems to have a beneficial influence on the tensile strength of the films. Additionally, the molecular mobility of the xylan chains (α-relaxation) was dependent on the type of Ara*f* substituents attached to the xylan backbone. Differences in molecular mobility may influence the xylan chain orientation during drying when there is still water in the system and the films are in a rubbery state. For instance, WAX-d3 had a higher T_g than WAX-ref and a lower tensile strength and Young's modulus. In semi-crystalline films, such as WAX-m, the decrease in tensile strength could be due to inadequate interactions between the crystalline and amorphous parts of the film.

The OP of the RAX and WAX films varied depending on the Ara*f* substitution. The water contents of the films were similar at RH 50%, and all of the films were in a glassy state, indicating that the ratio of un-substituted and substituted Xyl*p* units mostly influenced the OP values. In the RAX films, the OP decreased systematically along with the de-branching and increased crystallinity from approximately 2 to 1 [cm^3 µm/(m^2 d kPa)]. The length of the diffusion path of the oxygen gas increased due to crystalline areas in the films, therefore decreasing the OP. In the WAX films, the OP values were higher than in the RAX films, but their values decreased when Ara*f* was de-branched from di-substituted Xyl*p*, keeping the number of substituted Xyl*p* units the same and the film amorphous. WAX-ref and WAX-d3 had similar water contents, and they were both in a glassy state at the measurement conditions, yet the OP was lower in WAX-d3 (4.6 [cm^3 µm/(m^2 d kPa)]) than in WAX-ref (7.6 [cm^3 µm/(m^2 d kPa)]). Decreased OP results indicate that by modifying WAX chains so that they only contain mono-substituted Xyl*p* units, the packing of the chains in the films is denser compared to unmodified WAX, lowering the oxygen gas diffusion through the film. In cereal-based AX films, OP values of under 1 [cm^3 µm/(m^2 d kPa)] have been reported for barley husk AX (0.16 [cm^3 µm/(m^2 d kPa)]) (Höije *et al*. 2005; Höije 2008), for rye bran AX (0.87 [cm^3 µm/(m^2 d kPa)]) (Sárossy *et al*. 2013), for rye endosperm AX (0.15–0.22 [cm^3 µm/(m^2 d kPa)]) (Sárossy *et al*. 2012), and for wheat bran AX (0.84 [cm^3 µm/(m^2 d kPa)]) (Zhang 2012). These values show the potential of AX films as oxygen barriers in food packaging.

Although de-branching significantly decreased the OP values of the WAX films, there were no differences in the WVPs of WAX-ref and WAX-d3 (7.9 and 7.2 [(g mm)/(m^2 d kPa)], respectively). The results are in accordance with the WVP of un-plasticized rye bran AX film (7.7 [(g mm)/(m^2 d kPa)]) (Sárossy *et al*. 2012). However, lower values were measured in the rye endosperm AX films (2.6 [(g mm)/(m^2 d kPa)]) (Sárossy *et al*. 2013) and in study **I** with the sorbitol-plasticized OsAX films (1.1 and 1.9 [(g mm)/(m^2 d kPa)], respectively).

This study showed that AXs' fine structure has a clear impact on film properties. The type and amount of Ara*f* substitution affects water solubility and film formation, which in turn has an effect on film properties. The information concerning AXs' fine structure is useful when various structurally different agricultural side streams are considered as raw materials for film applications. An optimal AX structure with the desired film properties can be accomplished, for example, by selective fractionation techniques or by specific enzymatic modifications.

6.3 Water sensitivity of the AX films

According to water sorption studies, all of the studied films were hydrophilic, and their water content was dependent on the relative humidity conditions. Water acts as an external plasticizer, since AXs have a high amount of free hydroxyl groups that can interact with water, resulting in increased plasticization and swelling (Gennadios *et al*. 1994). Due to the plasticizing effect of water, it is important to know how much film absorbs water in the measurement conditions. The effect of film composition on water content was relatively low. All of the measured water contents of the AX films at RH 50% were between 10.4% (OsAX with 40% sorbitol) and 13.4% (de-branched RAX with Ara/Xyl 0.30). Glycerol increased the water content of the OsAX films at RH 50% slightly, when the plasticizer amount increased from 10% to 40%, whereas in sorbitol, the situation was reversed; by increasing the sorbitol amount from 10% to 40%, the water content of the films decreased. The differences in the water content of glycerol- and sorbitol-plasticized OsAX films can be explained by the more hydrophilic nature of glycerol. Glycerol can draw more water into the OsAX film than sorbitol can, as stated by Cheng *et al*. (2006). Although the differences in the water contents of the OsAX films at RH 50% are not high, it is important to notice the effect of RH on film properties above 50%. The WVP of plasticized OsAX films increased notably along with the RH gradient and with the increasing polyol content both in sorbitol- and glycerol-plasticized films. The OsAX film plasticized with 10% sorbitol had the lowest WVP, and the film plasticized with 40% glycerol had the highest WVP in all of the RH gradients studied (0/54%, 0/66%, and 0/96%). A DMA humidity scan confirmed that softening of the OsAX film plasticized with 40% glycerol started at a considerably lower RH than softening of the films plasticized with 10% glycerol, 10% sorbitol, or 40% sorbitol.

Water acts as the only external plasticizer in the AX films when no polyol plasticization is needed for film formation. The RH of the measurements and the stabilization conditions affect the water content of the AX films, which in turn affects the softening of the film structure. Water has shown to act as an efficient plasticizer in amorphous WAX films by lowering the strength and stiffness of the films with various Ara/Xyl ratios (Ying *et al*. 2013). These researchers' results indicate reduced molecular interactions of AX chains and increased distances between chains. The water contents of the WAX and RAX reference films as well as the de-branched films were analyzed in order to evaluate the possible changes in hydrophilicity due to de-branching. Water sorption of unmodified and de-branched WAX films was almost the same in the RH range of 0–50% (study **IV**) at RT. In these conditions, all of the films were in a glassy state, where water sorption is typically surface adsorption. When RH increased from 50% to 80%, the semi-crystalline WAX-m absorbed most of the water, but at RH 90%, the highest water uptake was found in the WAX-d3 film. However, the differences between WAX films were small. Similar water sorption isotherms were obtained by Ying *et al.* (2011), with WAX films having three different Ara/Xyl ratios. On the contrary to study **IV** and Ying *et al*. (2011), Zhang *et al*.

(2011) found that water uptake of wheat bran AX films with varying Ara/Xyl ratios increased notably above RH 80%; the results correlated with the DS. Under RH 80%, water sorption was similar between the films. In study **III**, the water content of the RAX films was measured with TGA instead of sorption microbalance, and the results were somewhat different than what was obtained with the WAX films. Additionally, the Ara*f* distribution, and therefore the number of substituted, and un-substituted Xyl*p* units, vary between RAX and WAX, possibly affecting the hydrogen bonding between the water and hydroxyl groups of AX. In RAX films at RH 50% and RH 76%, de-branching increased the water content of the films, whereas at 98%, the highest water content had the most substituted RAX-ref. When RH increases, at some point, the mobility of the AX chain will increase, and the films turn from a glassy state into a rubbery state. Water enhances the chain separation of the films, which allows more water to enter into the film structure. Ying *et al*. (2013) studied WAX films with different Ara/Xyl ratios in three different RHs. They concluded that when the water content of the films is under 15%, the water bonds strongly to hydroxyl groups of polysaccharides, but above 15%, the mobility of the water increases, enhancing the desorption and exchange of bound water molecules. One explanation of the behavior of RAX films at a high RH (98%) is that xylan crystallites prevent swelling; therefore, the water content of semi-crystalline films is lower than in amorphous RAX-ref. This phenomenon was not seen in the WAX films, where the highest RH was 90%, which is clearly lower than that of the RAX study.

6.4 Stability of the polyol-plasticized OsAX films

Both studies **I** and **II** showed that storage affected the tensile and barrier properties of the polyol-plasticized films. The films were stored at constant conditions at RH 50% at 23°C. The films in study **I** were stored between printing papers, and the films in study **II** were stored on the printing paper (i.e., the paper was attached to the film only on one side). In study **I**, the glycerol-plasticized films had increased strength and decreased elongation at break after storage. Expectedly, glycerol migration out from the film matrix decreased the plasticization of the film, therefore increasing the tensile strength and decreasing the elongation at break. This phenomenon has been observed in starch and chitosan films (Kuutti *et al*. 1998; Suyatma *et al*. 2005). In the sorbitol-plasticized films, both tensile strength and elongation at break showed a decreasing tendency, but the effect was not as significant as in the glycerol-plasticized films. Plasticized OsAX films were in a rubbery state at RT, and the molecules were mobile. In addition to glycerol migration, the mobility of the OsAX may create heterogeneous film structures in the sorbitol-containing film, resulting in polyol-rich and polymer-rich regions. Formation of sorbitol clusters has been observed in sorbitol-plasticized starch films (Gaudin *et al*. 1999). In study **II**, the plasticization of glycerol–sorbitol blends in addition to glycerol and sorbitol alone were investigated. A previous study of maize starch films indicated that sorbitol in blends may

prevent glycerol migration (Krogars *et al*. 2003). In study **II**, similarly to study **I**, the tensile strength and Young's modulus of the glycerol-plasticized film increased and elongation at break decreased. In the sorbitol-plasticized films, the result was the opposite, and both the tensile strength and Young's modulus decreased and elongation at break increased. Unexpectedly, the most significant change was observed in the Young's modulus of the sorbitol-plasticized films. After two months of storage, the Young's modulus dropped from 200 MPa to 100 MPa. Although the tensile properties of sorbitol-plasticized films changed during storage, indicating an altered film structure, no sorbitol crystallization was observed either visually or with XRD. Against the hypothesis of stabilizing effect of sorbitol in glycerol-sorbitol blends (Krogars *et al.* 2003), in this study the presence of sorbitol did not retard glycerol migration; instead, the more glycerol there was in the blend, the more the tensile strength increased during storage, indicating glycerol migration and lowered plasticization. One explanation of the different result obtained in this study is that the plasticizer amount was much higher (100% of the weight of the polymer) (Krogars *et al.* 2003).

In addition to tensile properties, film aging and storage time affected permeability properties. The WVP of the stored OsAX films was lower than the WVP of the fresh films (study **II**) in all of the studied polyol plasticizers or blends; the decrease was highest when the glycerol–sorbitol blend (3:1) was used. Altogether, when glycerol was used as a plasticizer alone or as part of the polyol blend, a clear indication of lowered plasticization, resulting from glycerol migration during storage, was observed. The WVP of the sorbitol-plasticized films decreased only slightly. Although the WVP of both the glycerol- and sorbitol-containing films decreased to some extent during storage, the effect on the OP was the opposite. The OP of both the glycerol- and sorbitol-containing OsAX films increased. Structural changes, such as decrease of free volume in the film due to glycerol migration or possible phase separation of OsAX and sorbitol, seemed to enhance oxygen diffusion and/or solubility in the film instead of decreasing it, therefore increasing OP. In addition, oxygen is non-polar, which probably affects the way of permeation through hydrophilic film. The crystallinity of the fresh and stored films was determined in study **II** to be similar, so this does not explain the changes in the permeability properties of the films. The formation of OsAX crystallites from the OsAX-glycerol film solution was studied by Peura *et al*. (2008). They discovered that small xylan di-hydrate crystallites formed during drying, and the number of crystallites increased along with water evaporation; however, the average size of the crystallites did not increase. According to the findings of Peura *et al*. (2008) and this study, it can be concluded that OsAX crystallites were formed during drying and that no further crystallization of xylan occurred after film drying when stored at RH 50% at RT, although in these conditions, the OsAX films are in a rubbery state, which enables the molecules to move.

This storage study revealed the challenges that polyol-plasticized films may encounter in packaging applications. The choice of a plasticizer type and amount plays a key role in

film stability. In this study, 40% polyol was added, and in addition, there was a plasticizing effect of water. Although glycerol and sorbitol were compatible with OsAX, on the contrary to xylitol, their high percentages caused instability of the film matrix during storage. The storage stability of polyol-containing films may differ with varying polyol contents. Lower plasticizer amounts may diminish glycerol migration and possible formation of separate polyol–polymer phases in film.

6.5 Applicability of wheat bran extracts for sheet extrusion

Film preparation using a solution casting method is not feasible in larger-scale film production; therefore, other methods that are easier to upscale need to be studied. Although the properties of AXs may differ remarkably from those of synthetic polymers, the suitability of the production methods of plastics should be investigated in the case of these less-exploited biomass fractions. One potential method is sheet extrusion, which has been carried out for starch- and protein-containing materials (Fishman *et al*. 2000, 2004; Rouilly *et al*. 2006, 2009). Only recently, sheet extrusion of hemicellulose fraction was studied, resulting in successful production of corn cob AX strips (Bachegul *et al*. 2013; Akkus *et al*. 2014).

Similarly, to cast AX films, the extruded sheets in this study were hydrophilic. Both the WVS and WVP results were in agreement with the cast polysaccharide films: the water content of the sheets increased notably when RH increased. Additionally, the use of a hydrophilic plasticizer, such as glycerol, further increased this phenomenon. Water sensitivity of the extruded sheets was affected both by the chosen polyol and the starch content of the material. Starch-containing sheets plasticized with 30% sorbitol clearly had the highest tensile strength (20.7 MPa) and the lowest WVP (3.2 [(g mm)/(m^2 d kPa)]). Somewhat lower TS values have been reported for glycerol-plasticized soy protein sheets (15.6 MPa) and sunflower protein isolate sheets (6.7 MPa), but these had clearly higher elongation at break (8.8% and 46%, respectively) (Zhang *et al*. 2001; Rouilly *et al*. 2006). Corn cob AX sheet strips produced without external plasticization were reported to have a clearly higher tensile strength (76 MPa) and elongation at break (35%) than the sheets in this study (Bachegul *et al*. 2013). When the results of the WBE-S and WBE-P sheets are compared, it can be concluded that further purification of WBE i.e. starch removal did not necessarily improve the sheet properties; on the contrary, this made the sheets weaker and more susceptible to water vapor. This is in accordance with Saadatmand *et al*.'s (2013) findings, where the use of crude wood hydrolysate showed promising results by working as well as the further upgraded fraction in film studies.

7 Conclusions

OsAX needed external plasticization to form cohesive films, and it was observed that the amount and type of polyol had a clear effect on the film properties. Glycerol and sorbitol were proven to be effective plasticizers in OsAX films. In general, the sorbitol-plasticized films were stronger and stiffer and had a lower WVP and OP than those plasticized with glycerol. Similarly, 10% polyol content resulted in stronger films with a lower OP and WVP than films with 40% polyol, which in turn elongated the most. This study showed that polyol performance varied according to the amount and structure; this has to be taken into account when choosing a plasticizer for the application. With suitable polyol selection, film properties can, to some extent, be optimized.

This study revealed that AXs' fine structure greatly affected film properties. Specific enzymes were proven to be effective tools, and systematic de-branching was successfully carried out. The type and amount of Ara*f* substitution was shown to affect water solubility and hence film formation. As a result, film properties changed. In general, the OP decreased along with the de-branching. With mild de-branching, stronger films were obtained. The increased amount of un-substituted Xyl*p* units increased crystallinity. The results of this structure correlation study are useful when various structurally different agricultural side streams are considered as raw materials for film applications. Additionally, an optimal AX structure with the desired film properties can be obtained, for example, by selective fractionation techniques or by specific enzymatic modifications.

All of the films in this study were hydrophilic, and they clearly reacted to the changes in the RH of the environment. Water vapor sorption studies indicated that the higher the RH, the higher the water content of the film. The addition of a hydrophilic plasticizer, glycerol, to the OsAX film further increased the water content of the film measured at RH 50%, and the highest value was obtained when the film was plasticized with 40% glycerol. A DMA humidity scan confirmed the water sensitivity of OsAX film plasticized with 40% glycerol, since the softening of this OsAX film clearly began at a lower RH than the softening of other plasticized OsAX films. When the water contents of OsAX, RAX, and WAX were compared at RH 50%, the highest water content had the de-branched RAX film, yet all of the values were between 10.4–13.4%.

The stability studies highlighted the challenges encountered in the use of external plasticization. There was a clear indication of glycerol migration when used in high amounts (40% w/w of the AX). The problem in the instability of the glycerol-plasticized film matrix concerns not only the changes of film properties due to decreased plasticization, but also the question of where the glycerol goes (e.g., into the packaged food product). In sorbitol-plasticized films, sorbitol crystallization was not observed during storage; however, storage notably decreased the stiffness of the films, and it is

possible that the heterogeneity of the film was increased by formation of plasticizer-rich and polymer-rich areas.

The extrusion study demonstrated that crude biomass extracts, such as WBEs, have potential as raw materials for sheet extrusion. The WBEs formed continuous sheets when polyol-plasticizer was added. The relatively high starch content in the extract made the sheets stronger and less water sensitive compared to sheets in which the starch was enzymatically removed. This result supports the use of milder purification steps in extract preparation.

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