

Long-Term Physical Stability of Plasticized Hemicellulose Films

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Oat spelt arabinoxylan (OsAX) and spruce galactoglucomannan (GGM) are hemicelluloses that can be extracted in large quantities from side-streams of the agriculture and forest industries. They both form self-standing films, making them potential future packaging materials. This systematic study focuses on the effect of long-term storage on the physical stability of hemicellulose-based films. OsAX and GGM films were plasticized with 40% (w/w of the polysaccharide) of glycerol, sorbitol, or their blends, and their stability was followed for four months. Ageing especially affected the glycerol-containing films, in which the tensile strength and Young's modulus increased and elongation at break decreased. Although the mechanical properties were altered, storage did not affect crystallinity of the films. Oxygen gas permeability (OP) and water vapor permeability (WVP) properties were monitored in OsAX films. Interestingly WVP decreased during storage; more than a 40% decrease was seen when plasticizer blends contained 50% or more glycerol. In contrast, there were no drastic changes in the OP during storage; all the OPs obtained were between 3.7 and 8.9 [cm³ μm/ (m² d kPa)].

Keywords: Arabinoxylan; Galactoglucomannan; Films; Plasticizer blends; Storage; Mechanical properties; Barrier materials

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INTRODUCTION

Food packaging has many purposes, such as protecting food products from the environment, retaining food quality, and increasing product shelf life. Oil-based plastic materials are widely used in food packaging, and their properties have been well-studied. In spite of their suitability for food packaging, after use they are non-biodegradable solid waste in the environment. In addition, growing packaging markets will need increasing amounts of raw materials in the future, and the need for sustainable and biodegradable alternatives is evident. One option is the use of plant polysaccharides such as hemicelluloses, which form biodegradable films and are, therefore, a green alternative to oil-based products. In addition, valorization of hemicelluloses to packaging materials does not compete with food production, unlike the use of starch. Hemicelluloses are a group of heteropolysaccharides closely associated with the cellulose and lignin in plant cell walls. The most abundant hemicelluloses are xylans and mannans. In angiosperms they are mainly xylans, whereas in gymnosperms they are mainly glucomannans. Large quantities of xylans and glucomannans are extractable, for example, from side-streams of the agriculture and forest industries (Mikkonen and Tenkanen 2012).

Oat spelt arabinoxylan (OsAX) and spruce galactoglucomannan (GGM) are potential packaging-film materials. These two hemicelluloses have similar molecular weights (30,000 to 60,000 g/mol), and both have a low substitution degree, but their monosaccharide compositions are different (Willför *et al.* 2003; Saake *et al.* 2004; Xu *et al.* 2007). OsAX can be extracted from oat spelt, the major byproduct in oat milling, which contains approximately 35 to 40% arabinoxylan (Saake *et al.* 2004). OsAX has a (1→4)-linked β -D-xylopyranosyl (Xylp) backbone to which a few α -L-arabinofuranosyl (Araf) groups are connected by (1→3)-glycosidic linkages at irregular intervals (Fig. 1). OsAX also contains low amounts of α -4-O-methylglucopyranosyluronic acid (MeGlcA). OsAX has low arabinose-to-xylose ratio (Ara:Xyl), which varies, according to the literature, between 1:10 and 1:6 (Saake *et al.* 2004; Mikkonen *et al.* 2009). GGM consists of a backbone in which (1→4)-linked β -D-glucopyranosyl (Glc p) and β -D-mannopyranosyl (Manp) residues alternate. GGM has α -D-galactopyranosyl (Galp) units, which are (1→6)-linked to the Manp residues (Fig. 1). Manp residues are also partially substituted with O-acetyl groups at positions C-2 and C-3 (Sjöström 1993; Willför *et al.* 2008). GGM contains mannose, glucose, and galactose in an average ratio of 4:1:0.5 (Willför *et al.* 2003). Acetylated GGM is water-soluble and can be extracted from for example, processing waters of pulp mills, wastewaters of fiberboard mills, and by water extraction from wood chips (Willför *et al.* 2003; Albertsson *et al.* 2010; Edlund *et al.* 2010; Song *et al.* 2011).

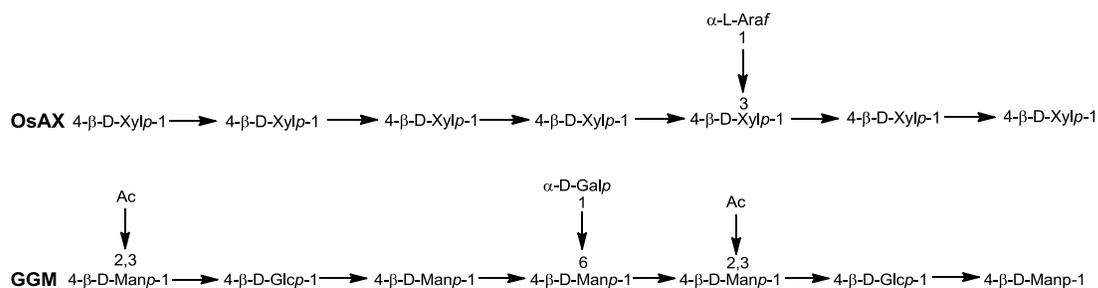


Fig. 1. Schematic partial structures of oat spelt arabinoxylan (OsAX) and spruce galactoglucomannan (GGM)

An increasing number of studies have investigated the use of hemicelluloses as sustainable packaging material, as pointed out in reviews by Hansen and Plackett (2008) and Mikkonen and Tenkanen (2012). Film formation from agro-based xylans, such as those from cotton stalks, barley husks, oat spelt, corn hulls, and wheat bran, has been studied lately (Zhang and Whistler 2004; Höije *et al.* 2005; Mikkonen *et al.* 2009; Zhang *et al.* 2011; Bahcegul *et al.* 2012). GGM film formation has been studied with plasticizers, with reinforcement using nano-sized cellulose, and with cross-linking agents (Hartman *et al.* 2006, Mikkonen *et al.* 2010; 2011; 2012). However, stability studies of hemicellulose films have only been briefly addressed in our previous study on OsAX films (Mikkonen *et al.* 2009), which opened questions on, *e.g.*, the function of plasticizer blends relative to the behavior of barrier properties and the crystallinity of films during storage. The long-term stability of films is relevant, because packaging materials need to be usable and functional for long periods of time. Synthetic polymers stay relatively stable for the shelf life of the packed product, but this may not necessarily be true for biopolymers (Kuutti *et al.* 1998; Forssell *et al.* 1999).

Some biopolymers need external plasticization for film formation. Plasticizers change the physical and mechanical properties of the biopolymer films, *e.g.* reducing brittleness and increasing flexibility (Banker 1966; Gontrad *et al.* 1993). Polyols are commonly used plasticizers in biopolymer films since they are natural and biodegradable (Vieira *et al.* 2011). However, the plasticizing effects of polyols, such as glycerol, sorbitol, or xylitol, may not be stable during long-term storage. Migration of the plasticizer inside or outside the film matrix may cause changes in, for example, mechanical and permeability properties.

If the plasticizer migrates outside the film matrix, then it can potentially move into the food product. Crystallization of plasticizers may also occur, as reported with potato starch films (Talja *et al.* 2007), when crystallization of sorbitol and xylitol was observed at relative humidity of $\leq 54\%$ during seven days of storage. Sorbitol has also been found to crystallize from corn starch films when used alone as a plasticizer (Krogars *et al.* 2003). Xylitol crystallization from aspen glucuronoxylan films (Gröndahl *et al.* 2004) and from OsAX films (Mikkonen *et al.* 2009) has been observed. Our previous study indicated that when glycerol is used as a plasticizer in OsAX film, it may migrate from the film matrix (Mikkonen *et al.* 2009). A similar effect has been reported with oat-starch films and chitosan films plasticized with glycerol (Kuutti *et al.* 1998; Suyatma *et al.* 2005). Krogars *et al.* (2003) concluded that a glycerol–sorbitol blend (1:1) added at amounts equal to the polymer weight is more stable than glycerol or sorbitol alone in maize-starch films.

Films made from hemicelluloses with low substitution degrees, such as OsAX, aspen glucuronoxylan, and GGM, are semi crystalline (Gröndahl *et al.* 2004; Mikkonen *et al.* 2009; Mikkonen *et al.* 2010). Morphological changes in films, like changes in crystallinity, may occur during storage. Crystallinity of films is associated with altered tensile and barrier properties of arabinoxylan films (Höije *et al.* 2008; Heikkinen *et al.* 2013). In starch films, long-term storage has been shown to induce recrystallization of the starch (Van Soest *et al.* 1996). However, the use of binary polyol plasticizers was shown to retard the crystallization of potato starch in films (Talja *et al.* 2008).

In this study, the physical stability of films prepared from two main hemicellulose types, xylan and glucomannan were monitored. OsAX and GGM were chosen as film materials because both are potential raw materials for packaging films and can be extracted in large amounts from non-food sources. Both OsAX and GGM need external plasticization for cohesive film formation, and the films have similar tensile properties (Mikkonen *et al.* 2009; 2011).

Mechanical properties and crystallinity of the OsAX and GGM films were monitored over a four-month period. The effect of ageing on the crystallinity of hemicellulose films has not been addressed before. Water vapor permeability (WVP) and oxygen gas permeability (OP) properties were studied in OsAX films. Our earlier work (Mikkonen *et al.* 2009) showed that OsAX film properties differ depending on whether they are plasticized with glycerol or sorbitol. In the present study, in addition to pure glycerol and sorbitol, as well as their blends, was used in both OsAX and GGM films, therefore deepening further the knowledge of plasticization of hemicellulose films and their long-term stability.

EXPERIMENTAL

Materials

Oat spelt arabinoxylan (OsAX) (CAS 9014-63-5, TCI Europe NV, Antwerp, Belgium) was determined to contain 77.7% xylose, 11.5% arabinose, and 2% MeGlcA, in addition to 6.7% glucose and 2.1% galactose impurities. For monosaccharide analysis, OsAX was degraded to monomers by acid methanolysis, after which the monosaccharides were analyzed for their trimethylsilyl derivatives by GC-FID (Sundberg *et al.* 1996). The molecular weight of OsAX was determined to be 58,500 g/mol by high-performance size exclusion chromatography (HPSEC) in DMSO eluent containing 0.01 M LiBr, according to a method described previously (Pitkänen *et al.* 2009). Spruce galactoglucomannan (GGM) was obtained from the processing waters of a Finnish pulp mill in an industrial-scale isolation trial after ethanol precipitation (Willför *et al.* 2003; Xu *et al.* 2007). Dried GGM was dissolved in water, filtrated to remove a small amount of insoluble material, and lyophilized prior to use. The monosaccharide composition of GGM has been determined to be 58% mannose, 18% glucose, 13% galactose, and 11% other sugars, with the degree of acetylation being 14% (Xu *et al.* 2007). The molecular weight of GGM has been determined to be approximately 30,000 g/mol (Mikkonen *et al.* 2012). The glycerol and sorbitol used were from BDH Laboratory Supplies (Lutterworth, England) and Cerestar (Krefeld, Germany), respectively. Anhydrous CaCl₂ with a granule size of 1 to 2 mm was obtained from Merck (Whitehouse Station, NJ).

Preparation of the Films

OsAX was suspended in water (16 g/L) and mixed by magnetic stirring at 90 to 95 °C for 1 hr. The suspension was centrifuged at 17,000 rpm for 15 min, after which the insoluble part (6 g/L) was removed. Plasticizer (40% of the weight of the soluble OsAX) was added at 60 to 65 °C, with magnetic stirring. GGM was suspended in water (10 g/L) and mixed by magnetic stirring at 85 °C for 10 min. Plasticizer (40% of the weight of the GGM) was added at 85 °C, with magnetic stirring. Film solutions were degassed by ultrasonication *in vacuum* for 5 min and cast onto Teflon plates or Teflon-coated petri dishes. Films were dried on plates at 23 °C and relative humidity (RH) 50%, and then removed from the plates and stored on printing paper at 23 °C and RH 50% for from 1 week to 4 months before analysis. Glycerol, sorbitol, and their blends (gly:sor 3:1, 1:1, 1:3) were used as plasticizers.

Mechanical Properties

Tensile testing was performed at 23 °C and 50% RH with an Instron 4465 universal testing machine (Instron Corp., High Wycombe, England) with a load cell of 100 N. The initial grip distance was 50 mm, and the rate of grip separation was 5 mm/min. The specimen width was 10 mm. The thickness of the films was measured with a micrometer (Lorentzen & Wettre, Kista, Sweden; precision 1 µm) at five points, the average of which was calculated. Mechanical properties of the films were measured after 1 week, 2 months, and 4 months of storage. Six to twelve replicate specimens of each film type were measured.

X-Ray Diffraction (XRD)

XRD measurements were used to determine the crystallinity of OsAX and GGM films 1 week and 4 months old. Wide-angle X-ray scattering measurements were carried

out in the perpendicular transmission geometry using Cu $K\alpha_1$ radiation. The beam (point focus) was monochromated and focused with a bent Si(111) crystal and a totally reflecting mirror. The diffraction patterns were collected with a MAR345 image plate detector (Rayonix, Evanston, IL).

The diffraction intensities were plotted as a function of scattering angle. The scattering angles were given in 2θ , which is twice the value of the Bragg angle in Bragg's law $\lambda = 2d_{hkl} \sin(\theta[^\circ])$. In the formula, λ is the wavelength of the incident radiation and d_{hkl} is the distance of the planes given by Miller indices hkl . Films were measured at room temperature (25 ± 1 °C) and RH ($21 \pm 1\%$). In order to acquire more accurate statistics, 4 to 8 films were stacked parallel to each other during diffraction measurements to get an average sample thickness of about 250 μm . For OsAX films, the 100% crystalline xylan diffraction pattern was calculated using the known crystal structure and atomic coordinates of xylan dihydrate (Nieduszynski and Marchessault 1972), and the 0% crystalline pattern (*i.e.* amorphous, or non-crystalline, background) was measured from the pure rye arabinoxylan film (Stevanic *et al.* 2011). The diffraction pattern of crystalline GGM was computed on the basis of the known crystal structure of mannan I (Atkins *et al.* 1988). The non-crystalline background was measured from the filtrated and lyophilized GGM powder.

Water Vapor Permeability (WVP)

The WVP properties of OsAX films after 1 week and after 4 months of storage were determined using the RH gradient of 0/54%. Films were sealed on aluminum cups containing 43 g CaCl_2 to give 0% RH. There was an air gap of 6 mm between CaCl_2 and the underside of the film. The cups were placed in a desiccator cabinet equipped with a fan. The RH in the cabinet was maintained at 54% with saturated $\text{Mg}(\text{NO}_3)_2$ solution. The temperature inside the cabinet was 22 °C, and the air velocity above the samples was 0.15 m/s. The cups were weighed three times a day. The temperature and RH of the cabinet were measured before each weighing with use of a Rotronic Hygro-Palm RH meter (Bassersdorf, Switzerland).

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 at 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. The thickness of the films was measured at 10 points at 1- μm precision prior to testing. Three replicates of each film type were tested.

Oxygen Permeability (OP)

The oxygen gas transmission rate (OTR) of the OsAX films was measured after 1 week and after 4 months of storage using an Ox-Tran Twin equipped with a coulometric sensor (Modern Controls, Minneapolis, MN). One to three replicates of the glycerol, sorbitol, or gly:sor (1:1) plasticized films were measured at 22 °C and RH 50 to 75%. The film area was 5 cm^2 , and the thickness of the film was measured at 5 points at 1- μm precision. 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.

Statistical Analysis

Variance analysis was used to test the differences in tensile properties of the films. Differences were considered statistically significant when p was ≤ 0.05 . PASW Statistics 18.0.2 (IBM SPSS Software, Armonk, NY) was used for the analysis.

RESULTS AND DISCUSSION

Tensile Properties

OsAX and GGM both need an external plasticizer to form self-supporting films, and 40% plasticizer (w/w of the polysaccharide) was chosen, since it has been found to be the optimal amount for GGM (Mikkonen 2009) and suitable for OsAX (Mikkonen *et al.* 2009). This relatively high amount of plasticizer also made it easier to observe possible changes in plasticization during storage. Since absorbed water also acts as a plasticizer of polysaccharide-based films (Mikkonen *et al.* 2009), tensile properties were measured in controlled RH and temperature.

OsAX and GGM films (1 week)

Tensile properties of fresh OsAX and GGM films varied depending on the hemicelluloses, plasticizers, and their blends. In fresh OsAX films, mechanical properties (tensile strength, elongation at break, and Young's modulus) increased when the amount of sorbitol increased in the plasticizer blend, and the values were highest when sorbitol was used alone (Fig. 2A–C). Tensile strength of OsAX film plasticized with glycerol was 4 MPa and elongation at break 13%, whereas the values with sorbitol were 11 MPa and 20%, respectively. Similar results for fresh OsAX films plasticized with pure glycerol and sorbitol were obtained in our preceding study (Mikkonen *et al.* 2009). Saxena *et al.* (2009) prepared films from a suspension in which OsAX was plasticized with 100% of sorbitol (of the weight of the OsAX), resulting in lower tensile strength (2 MPa) but similar elongation at break (22%) than in the current study.

In fresh GGM films, tensile strength and Young's modulus increased when the amount of sorbitol was increased in the plasticizer blend, but, in contrast to OsAX films, elongation at break decreased with increasing sorbitol content (Fig. 2D–F). Tensile strength and Young's modulus were highest when sorbitol was used alone and lowest when glycerol was used alone (Fig. 2D,F). Elongation at break had the lowest value when sorbitol was used alone and the highest when glycerol was used alone (Fig. 2E). Again, the tensile property values for the films plasticized with glycerol–sorbitol blends lay between the highest and lowest values, and the increase of tensile strength and Young's modulus and the decrease of elongation at break were observed to depend on composition of the blend. Although differences depending on plasticizer type were observed, the values were rather low, which is typical for polymers with relatively low degree of polymerization. In previous studies, where GGM was plasticized with glycerol or sorbitol alone, films had similar tensile strengths (12 MPa), but sorbitol-plasticized film had higher elongation at break (7%) and lower Young's modulus (250 MPa) than glycerol-plasticized film (3 to 4% and 500 to 750 MPa, respectively) (Mikkonen *et al.* 2010; 2011; 2012). Differences in tensile properties of glycerol- and sorbitol-plasticized GGM films between the current and previous studies may be due to a different GGM batch being used by Mikkonen *et al.* (2010) and to film solution preparation that used a high-pressure homogenizer (Mikkonen *et al.* 2011).

Glycerol and sorbitol are commonly used external plasticizers that differ in size. Sorbitol, having six carbon atoms and hydroxyl groups, is a bigger molecule than glycerol, which has three carbon atoms and hydroxyl groups. The larger molecular dimensions of sorbitol increase the free-volume of the polymer network, making it less dense (Cheng *et al.* 2006). However, when added in the same mass quantities (40% of the weight of the hemicellulose) to the film solution, the molecular content of the glycerol is higher in the films compared with sorbitol.

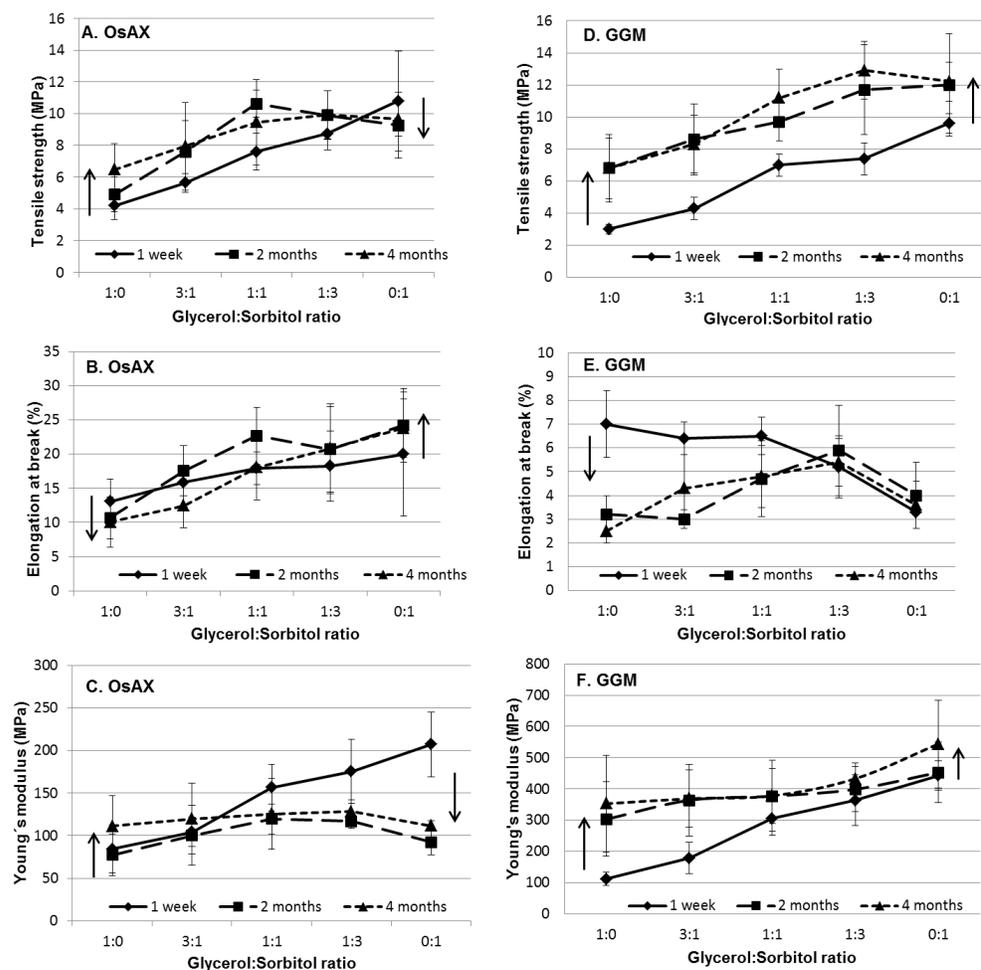


Fig. 2. Tensile strength (A), elongation at break (B), and Young's modulus (C) of the OsAX films, and tensile strength (E), elongation at break (F), and Young's modulus (G) of the GGM films plasticized with glycerol–sorbitol blends after 1 week, after 2 months, and after 4 months of storage. The total amount of plasticizer was 40% of the weight of the OsAX and GGM. Arrows represent the direction of change in tensile properties of stored films plasticized with 40% glycerol and 40% sorbitol.

When the fresh OsAX and GGM films were compared with each other, it was seen that tensile strength increased similarly from ~4 MPa to ~10 MPa, when the amount of sorbitol was increased in the plasticizer blend (Fig. 2A,D). Also, Young's modulus increased in both OsAX and GGM films when the amount of sorbitol increased in the plasticizer blend (Fig. 2C,F). Interestingly, in sorbitol-plasticized GGM films, Young's modulus was twice as high as in sorbitol-plasticized OsAX films, but in glycerol-plasticized films, Young's modulus values were similar in both hemicellulose types.

External plasticizers affected elongation at break differently in fresh OsAX and GGM films. Both film types were prepared similarly, and due to the film preparation procedure (casting), there was no preferred orientation of the polymers in the film matrix, which would affect the tensile properties. Sorbitol appeared to strengthen the GGM film network, preventing the polymers from sliding across each other and, therefore, lowering the elongation values. However, in OsAX films, sorbitol made the films stronger and more flexible compared with glycerol. OsAX and GGM have different monosaccharide compositions, and, in addition, GGM contains acetyl groups, which might have an impact on GGM–sorbitol interactions, making the films stiffer and less flexible. The number of free hydroxyl groups is higher in GGM than in OsAX since GGM is composed of hexose sugars and OsAX pentose sugars. It is possible that these free hydroxyl groups of GGM form more hydrogen bonds with sorbitol than OsAX does, resulting in more compact and stiffer film structure. In sorbitol plasticized pea starch films, compact packing has been concluded to be due to the compatibility of glucose units and sorbitol (Zhang and Han 2006).

OsAX and GGM films (2 and 4 months)

In both film types, storage affected the mechanical properties. The change in tensile properties was seen at two months, after which the properties, in most cases, remained the same over the next two months. In the OsAX films plasticized with glycerol alone, tensile strength and Young's modulus increased and elongation at break decreased during storage, whereas in the sorbitol-plasticized films, the result was the opposite: Tensile strength and Young's modulus decreased and elongation at break increased (Fig. 2A–C). The most significant change was in Young's modulus of sorbitol-plasticized film, which decreased after two months of storage from 200 MPa to 100 MPa (Fig. 2C). Young's modulus was similar in all OsAX films after two and four months of storage.

In GGM films plasticized with glycerol alone, as in OsAX films, tensile strength and Young's modulus increased and elongation at break decreased significantly during storage (Fig. 2D–F). This indicates lowered plasticization in the films, caused, most probably, by glycerol migration out of the film matrix. This is a phenomenon that we have observed also earlier (Mikkonen *et al.* 2009). In the sorbitol-plasticized GGM films, tensile strength and Young's modulus increased during storage (Fig. 2D,F), but elongation at break was similar after one week, two months, and four months of storage (Fig. 2E).

Storage affected the mechanical properties of both OsAX and GGM films plasticized with glycerol–sorbitol blends. In OsAX films, the glycerol amount clearly affected tensile strength: The more glycerol there was in the plasticizer blend, the more the tensile strength increased over four months (Fig. 2A). Elongation at break decreased in OsAX films plasticized with polyol blend containing more than 50% glycerol, whereas when the polyol blend contained more than 50% sorbitol, the effect was the opposite (Fig. 2B). Young's modulus of OsAX films clearly decreased more when more sorbitol was added to the plasticizer blend. According to our previous study of glycerol and sorbitol plasticization (Mikkonen *et al.* 2009), the studied OsAX films are in a rubbery state in which the molecules are mobile. By the end of two months, this may create a more heterogeneous film with sorbitol-rich and polymer-rich regions. The heterogeneity of the film could then affect its strength and stiffness. Gaudin *et al.* (1999) studied sorbitol-plasticized wheat starch films with NMR, and their results indicated that with high sorbitol content, clusters of sorbitol were formed. In the present study, there was no

sorbitol migration out of the film matrix, since sorbitol crystals were not observed on the film surface. In starch films crystallization of sorbitol has been observed (Talja *et al.* 2007). Similarly in our other experiments sorbitol crystallized out from the konjac glucomannan (KGM) film matrix. OsAX and GGM both have rather low molecular weight, whereas KGM and starch both have high molecular weight. The differences in molecular weight might affect the intra- and intermolecular interactions as well as polymer-plasticizer interactions, further affecting the sorbitol crystallization in the starch and KGM films. A significant decrease in stiffness was not seen in GGM films; therefore, it is likely that the above-mentioned molecular structure of the polysaccharide, especially the number of free hydroxyl groups, has an effect on sorbitol–polysaccharide interactions. There was also a difference in elongation at break between sorbitol-plasticized films. In GGM films, when the plasticizer blend contained more glycerol than sorbitol, storage decreased elongation at break and increased Young's modulus, possibly due to glycerol migration (Fig. 2E,F). Interestingly, the increase in tensile strength of GGM films was on the same order with all plasticizer blends (Fig. 2D). When studied statistically with two-way variance, the interaction effect of age and plasticizer in GGM films was significant in elongation at break and Young's modulus, but not in tensile strength. However, when studied alone, both plasticizer and age had significant effects on the results. In OsAX films, plasticizer and age significantly affected all mechanical properties, and there was also an interaction effect between age and plasticizer.

Unlike in the earlier study with maize-starch films (Krogars *et al.* 2003), the presence of sorbitol did not prevent glycerol migration. However, the amount of plasticizer was clearly higher in Krogars *et al.* (2003) (100% of the weight of maize starch) than in this study (40% of the weight of hemicellulose). So, it seems that plasticizer type, plasticizer amount, and polymer network have impacts on the stability of the plasticized film, and therefore the properties of polysaccharide-based films with varying compositions need to be studied separately.

Crystallinity

Crystalline structures in films are often associated with lowered permeability properties (McHugh and Krochta 1994). They may also have an effect on mechanical properties. OsAX and GGM films have been analyzed as semi crystalline in previous studies (Mikkonen *et al.* 2009; 2011). Current crystallinity analysis of the films also indicated that the xylan and mannan were semicrystalline, *i.e.* there were regions of xylan and mannan crystallites surrounded by amorphous regions. OsAX had crystallinity of 32 to 38% (Table 1), which was higher than in our previous study, in which crystallinity varied from 22 to 26% (Mikkonen *et al.* 2009). However, in that study, the OsAX used was from a different supplier. The crystallinity of 26% was also obtained by Saxena *et al.* (2011) who studied OsAX films plasticized with 100% sorbitol (of the weight of the OsAX). In the present study the average size of single xylan crystallites in OsAX films was determined to be 5 to 10 nm.

Crystallinity values for all GGM-films were in the range 16 to 23% (Table 1), which is similar as published earlier (20 to 25%) (Mikkonen *et al.* 2011). The GGM crystallites in the films had a mean width the size of a few unit cells, indicating that the size of GGM crystallites was less than a few nanometers, which results in (due to XRD line broadening) no clear diffraction peaks, which would give unambiguous information about the crystalline structure. The most probable crystalline structure for native GGM is mannan I.

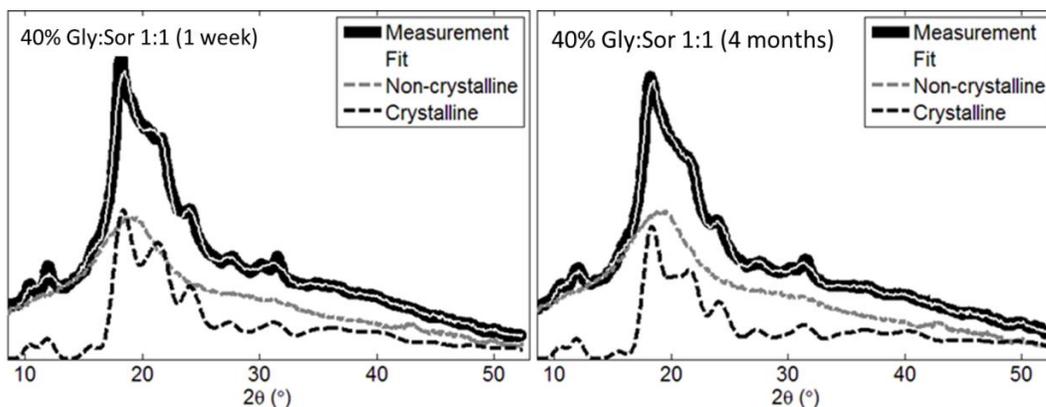
Table 1. Crystallinities (Φ [%]) of OsAX and GGM Films After 1 Week and After 4 Months of Storage

Gly: Sor	OsAX, Φ [%]		GGM, Φ [%]	
	1 Week	4 Months	1 Week	4 Months
1:0	38	37	23	23
3:1	38	35	22	24
1:1	37	34	20	22
1:3	37	34	17	17
0:1	35	32	16	18

The uncertainty (error) in crystallinities was $\pm 3\%$.

Both fresh (one week) and stored (four months) OsAX and GGM films plasticized with glycerol had somewhat higher crystallinity than sorbitol-plasticized films. The same effect of plasticizer type on crystallinity was observed in our earlier work (Mikkonen *et al.* 2009). The higher plasticizing effect of glycerol compared with sorbitol has been associated with increased molecular mobility of the polymer chains, which increases the crystallinity. This also has been seen in starch-based films (Rindlav-Westling *et al.* 1998; Talja *et al.* 2008).

For both OsAX and GGM, crystallinity of the fresh and stored films was similar (Table 1, Fig. 3). Possible changes in molecular mobility of OsAX and GGM in the glycerol containing films due to plasticizer migration did not affect the crystallinity. Interestingly, the small changes in the crystallinity observed after four months were in the opposite directions: The crystallinity of OsAX films slightly decreased, whereas the crystallinity of GGM films slightly increased during storage. However, the changes were inside the uncertainty value of the measurement ($\pm 3\%$). XRD measurement confirmed the visual observation that sorbitol did not crystallize during storage, since no sorbitol peaks were observed in diffractograms (Fig. 3). Crystallite formation occurs during film drying as stated by Peura *et al.* (2008). They studied OsAX-glycerol film solution and discovered that small xylan dihydrate crystallites were formed and the number of crystallites increased as water was evaporated, but the average size of the crystallites did not increase. Similarly to OsAX films, this study indicates, that the crystallites in GGM films are formed during drying.

**Fig. 3.** Example of the average radial diffraction pattern and crystallinity fit of OsAX films after 1 week and after 4 months of storage. Films were plasticized with glycerol–sorbitol blend (1:1).

External plasticizer and remaining water in the dried films did not enhance the rearrangement of polymer chains in the amorphous part of the film to an extent sufficient to increase the crystallinity during storage. Therefore it seems that the semi-crystalline OsAX and GGM network, once formed, is relatively stable when stored in constant conditions, and the migration of glycerol does not affect the crystallinity of the polymer network.

Water Vapor and Oxygen Gas Permeability

Barrier properties of the packaging films affect the stability of the packed product. Deterioration of the quality of foods, such as oxidation in lipid-containing products, drying in water-containing products, or wetting of dry products, may occur if migration of oxygen or moisture through the packaging film or coating is high. In this work, the WVP and OP of plasticized OsAX films were measured in an RH gradient of 0/54% and an RH of 50 to 75%, respectively. Earlier studies have shown that at an RH of 50%, the water content of OsAX films with 40% glycerol is 12.5%, and with 40% sorbitol, 10.4% (Mikkonen *et al.* 2009). The higher water content of glycerol-plasticized films compared with sorbitol-plasticized ones can be explained by the higher hydrophilicity of glycerol, enabling it to draw more water into the OsAX film than sorbitol does (Cheng *et al.* 2006).

The type of plasticizer significantly affected the WVP of fresh OsAX films, since films plasticized with 40% sorbitol had a WVP of 2.0 [(g mm)/(kPa m² d)] whereas when films were plasticized with 40% glycerol the WVP was ten times higher, 21 [(g mm)/(kPa m² d)], and the WVP increased systematically when the amount of glycerol in the glycerol–sorbitol blend was increased (Fig. 4). OsAX formed a more compact film structure with decreased water permeation when plasticized with sorbitol instead of glycerol. Better compatibility of sorbitol with OsAX might be related to a higher number of hydrogen bonds formed between OsAX and sorbitol than between OsAX and glycerol resulting from the differing number of hydroxyl groups in sorbitol (six) than in glycerol (three). The WVP value for sorbitol-plasticized OsAX film in our earlier study was similar, 1.9 [(g mm)/(kPa m² d)], but for glycerol-plasticized film, somewhat lower, 13 [(g mm)/(kPa m² d)] (Mikkonen *et al.* 2009). Decreased WVP values for glycerol-plasticized OsAX films have been obtained with 10% glycerol (3.3 [(g mm)/(kPa m² d)]), whereas lowering the sorbitol content to 10% did not alter the WVP as significantly (1.1 [(g mm)/(kPa m² d)]) (Mikkonen *et al.* 2009). The WVP increase caused by the hydrophilic plasticizer has been seen earlier and is associated with increased polymer chain mobility in the film network, which increases the water diffusion rate (Bertuzzi *et al.* 2007).

Storage decreased the WVP of all films (Fig. 4). The decrease was highest (63%) when the glycerol–sorbitol ratio was 3:1 and lowest when sorbitol was used alone (21%). Crystalline structures are known to lower permeability (McHugh and Krochta 1994). However, in this study, fresh and stored OsAX films had similar crystallinity. The significant decrease of the WVP in glycerol-containing films during storage indicates decreased plasticization, decreasing the free-volume after glycerol migration out of the film matrix. The WVP of sorbitol-plasticized films also decreased, but to a lesser extent. Sorbitol did not crystallize during storage, but the stiffness of the sorbitol-plasticized films decreased significantly. However, the possible phase separation of sorbitol and OsAX in the film matrix during storage did not have a drastic effect on the WVP.

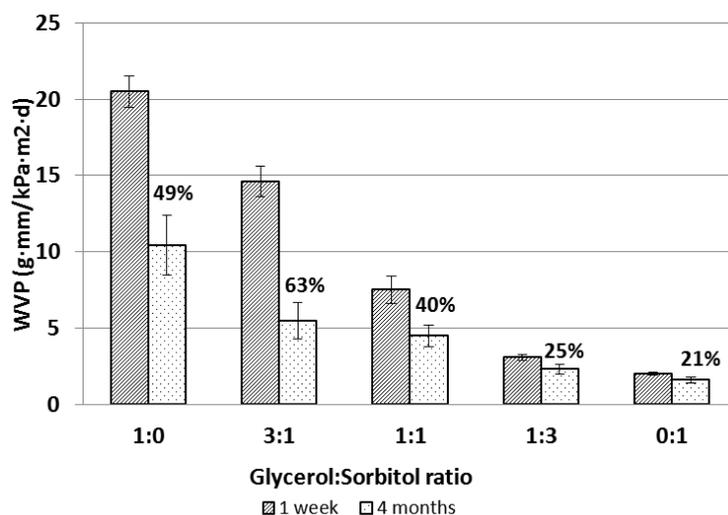


Fig. 4. The water vapor permeability of OsAX films plasticized with glycerol–sorbitol blends after 1 week and after 4 months of storage. Three parallel measurements were done for each film type. The percentages above the bars represent the decrease of the WVP after storage compared with fresh films.

The OP of OsAX films plasticized with glycerol, sorbitol, and a glycerol–sorbitol blend (1:1) were measured after one week and after four months of storage. After one week, all the studied films had similar OPs, varying from 3.7 to 5.4 [$\text{cm}^3 \mu\text{m}/(\text{m}^2 \text{ d kPa})$] (Table 2). This result is in agreement with our previous study (4.7 to 7.4, Mikkonen *et al.* 2009). Clearly lower WVP values (0.18 [$\text{cm}^3 \mu\text{m}/(\text{m}^2 \text{ d kPa})$]) have been obtained by adding cellulose nanocrystals to OsAX films plasticized with 100% sorbitol (of the weight of the OsAX) (Saxena *et al.* 2010). After four months the OP values were somewhat higher (6.4 to 8.9). So, although the migration of glycerol out of the film matrix decreased the WVP of glycerol-plasticized OsAX film, the same migration slightly increased the diffusion and/or solubility of oxygen in the glycerol-plasticized OsAX film, increasing the OP. Changes in Young's modulus and tensile strength of sorbitol-plasticized films after storage indicate structural changes other than altered crystallinity in the film. These changes may also affect the OP. However, the differences in the OP of the studied films were small, and all OP values were under 10 [$\text{cm}^3 \mu\text{m}/(\text{m}^2 \text{ d kPa})$], which is regarded as the limit for a good oxygen barrier (Krochta and De Mulder-Johnston 1997).

Table 2. Oxygen Transmission Rates and Oxygen Permeabilities of OsAX Films Plasticized with 40% Sorbitol, Glycerol, or Sorbitol–Glycerol Blend (1:1)

Plasticizer (40%)	OTR (1 Week) [$\text{cm}^3/(\text{m}^2 \text{ d})$]	OP (1 Week) [$\text{cm}^3 \mu\text{m}/(\text{m}^2 \text{ d kPa})$]	OTR (4 Months) [$\text{cm}^3/(\text{m}^2 \text{ d})$]	OP (4 Months) [$\text{cm}^3 \mu\text{m}/(\text{m}^2 \text{ d kPa})$]
Gly	11.8 ± 0.2	5.4 ± 0.3	16.7 ^a	8.9
Sor–Gly 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.

CONCLUSIONS

1. Long-term physical stability of polyol-plasticized hemicellulose films was studied systematically. Sorbitol-plasticized films were more stable during storage than glycerol-plasticized films. However, some properties were improved after glycerol migration: Films became stronger, stiffness increased, and water vapor permeability decreased.
2. The tensile properties of sorbitol-plasticized OsAX and GGM films changed in opposite directions during storage, indicating differing interactions between sorbitol and OsAX and GGM.
3. The degree of crystallinity of OsAX and GGM films stayed relatively stable during four months of storage so the number of crystallites was not changing notably after film formation. Despite the glycerol migration the crystalline part of the polymer network in the films did not change when stored in constant conditions.
4. The presence of sorbitol in plasticizer blends did not prevent glycerol migration.

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