Mannans as film formers and emulsion stabilizers

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ACADEMIC DISSERTATION

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Abstract

Mannans are abundant plant polysaccharides found in the endosperm of certain leguminous seeds (guar gum galactomannan, GG; locust bean gum galactomannan, LBG), in the tuber of the konjac plant (konjac glucomannan, KGM), and in softwoods (galactoglucomannan, GGM). This study focused on the effects of the chemical structure of mannans on their film-forming and emulsion-stabilizing properties. Special focus was on spruce GGM, which is an interesting new product from forest biorefineries.

A plasticizer was needed for the formation of films from mannans other than KGM and the optimal proportion was 40% (w/w of polymers) glycerol or sorbitol. Galactomannans with lower galactose content (LBG, modified GG) produced films with higher elongation at break and tensile strength. The mechanical properties of GG-based films were improved by decreasing the degree of polymerization of the polysaccharide with moderate mannanase treatments.

The improvement of mechanical properties of GGM-based films was sought by blending GGM with each of poly(vinyl alcohol) (PVOH), corn arabinoxylan (cAX), and KGM. Adding other polymers increased the elongation at break of GGM blend films. The tensile strength of films increased with increasing amounts of PVOH and KGM, but the effect of cAX was the opposite. Dynamic mechanical analysis showed two separate loss modulus peaks for blends of GGM and PVOH, but a single peak for all other films. Optical and scanning electron microscopy confirmed good miscibility of GGM with cAX and KGM. In contrast, films blended from GGM and PVOH showed phase separation.

GGM and KGM were mixed with cellulose nanowhiskers (CNW) to form composite films. Addition of CNW to KGM-based films induced the formation of fiberlike structures with lengths of several millimeters. In GGM-based films, rodlike structures with lengths of tens of micrometers were formed. Interestingly, the notable differences in the film structure did not appear to be related to the mechanical and thermal properties of the films.

Permeability properties of GGM-based films were compared to those of films from commercial mannans KGM, GG, and LBG. GGM-based films had the lowest water vapor permeability when compared to films from other mannans. The oxygen permeability of GGM films was of the same magnitude as that of commercial polyethylene / ethylene vinyl alcohol / polyethylene laminate film. The aroma permeability of GGM films was low. All films were transparent in the visible region, but GGM films blocked the light transmission in the ultraviolet region of the spectra.

The stabilizing effect of GGM on a model beverage emulsion system was studied and compared to that of GG, LBG, KGM, and cAX. In addition, GG was enzymatically modified in order to examine the effect of the degree of polymerization and the degree of substitution of galactomannans on emulsion stability. Use of GGM increased the turbidity of emulsions both immediately after preparation and after storage of up to 14 days at room temperature. GGM emulsions had higher turbidity than the emulsions containing other mannans. Increasing the storage temperature to +45 °C led to rapid emulsion breakdown, but a decrease in storage temperature increased emulsion stability after 14 days. A low degree of polymerization and a high degree of substitution of the modified galactomannans were associated with a decrease in emulsion turbidity.

Acknowledgements

Contents

List of original publications

This thesis is based on the following original publications, which are referred to by their Roman numbers I–V:

I   Mikkonen, K. S., Rita, H., Helén, H., Talja, R. A., Hyvönen, L., and Tenkanen, M. 2007. Effect of polysaccharide structure on mechanical and thermal properties of galactomannan-based films. Biomacromolecules, 8(10), 3198–3205.

II   Mikkonen, K. S., Yadav, M. P., Cooke, P., Willför, S., Hicks, K. B., and Tenkanen, M. 2008. Films from spruce galactoglucomannan blended with poly(vinyl alcohol), corn arabinoxylan, and konjac glucomannan. BioResources, 3(1), 178–191.

III   Mikkonen, K. S., Mathew, A. P., Pirkkalainen, K., Serimaa, R., Xu, C., Willför, S., Oksman, K., and Tenkanen, M. Glucomannan composite films with cellulose nanowhiskers, submitted.

IV   Mikkonen, K. S., Heikkilä, M. I., Helén, H., Hyvönen, L., and Tenkanen, M. Spruce galactoglucomannan films show promising barrier properties, submitted.

V   Mikkonen, K. S., Tenkanen, M., Cooke, P., Xu, C., Rita, H., Willför, S., Holmbom, B., Hicks, K. B., and Yadav, M. P. 2009. Mannans as stabilizers of oil-in-water beverage emulsions. LWT – Food Science and Technology, 42(4), 849–855.

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Abbreviations

cAX	corn arabinoxylan
CNW	cellulose nanowhiskers
DMA	dynamic mechanical analysis
DP	degree of polymerization
DS	degree of substitution
DSC	differential scanning calorimetry
E'	storage modulus
E"	loss modulus
EVOH	ethylene vinyl alcohol
GA	gum arabic
GC	gas chromatography
GG	guar gum galactomannan
GGM	galactoglucomannan
KGM	konjac glucomannan
LBG	locust bean gum galactomannan
OM	optical microscopy
OP	oxygen permeability
PE	polyethylene
PEG	polyethylene glycol
PVOH	poly(vinyl alcohol)
RH	relative humidity
RT	room temperature
SEM	scanning electron microscopy
T_g	glass transition temperature
TGA	thermal gravimetric analysis
WVP	water vapor permeability

1 Introduction

Hemicelluloses are the most abundant plant polysaccharides other than cellulose and thus comprise a remarkable and unexploited natural resource. Galactoglucomannans (GGM) are the main hemicelluloses in softwoods and can be recovered as a by-product from mechanical pulping of spruce in high yield and purity. Use of GGM has been suggested as a film former, emulsion stabilizer, and immunostimulatory agent (Hartman *et al.*, 2006a, 2006b, Ebringerová *et al.*, 2007, Willför *et al.*, 2008).

The development of biodegradable films is one of the most important aims of the packaging industry today. Biodegradable packaging prepared from renewable materials could contribute to managing the carbon cycle in a sustainable manner (Narayan, 2007). Polysaccharides isolated from natural materials are more environmentally friendly than synthetic polymers not only because of their biodegradability, but also by taking account of the amount of energy and environmental impact required for the production of synthetic polymers (Shen and Patel, 2008). Many polysaccharides have the advantage of being soluble in water, which is the most important "green" solvent and the most suitable for polysaccharides (Šimkovic, 2008). Polysaccharide-based films can also be edible. Edible films have potential to reduce the amount of packaging required to protect the product. Polysaccharide-based edible films are attractive, non-greasy, and low-calorie and can be applied, e.g., to extend the shelf-life of fruit, vegetables, seafood, or meat products by preventing dehydration, oxidative rancidity, and surface browning. The ability of water-soluble polysaccharides to reduce oxygen and increase carbon dioxide levels in internal atmospheres reduces respiration rates, thereby prolonging the shelf-life of fruits and vegetables in a manner analogous to controlled atmospheres. The beneficial effects of edible films include improved retention of flavor, acids, sugars, texture, and color, increased stability during shipping and storage, improved appearance, and reduced spoilage (Nisperos-Carriedo, 1994).

In order to maintain their protective properties, the films must be relatively strong and flexible. This can be achieved with high cohesion between film-forming polymers, such as hydrogen bonding. Plasticizers are used to modify the physical and mechanical properties

of packaging films. Use of plasticizer generally decreases the tensile strength and increases the elongation at break of films (Banker, 1966).

Polysaccharides are also utilized as stabilizers of oil-in-water emulsions. Gum arabic is widely considered as the best emulsifier in a dilute oil-in-water emulsion system, such as beverages. However, the supply of gum arabic is variable, uncertain and subject to climatic, economic, and political conditions in the Sahelian region of Africa, where it is produced. Thus the availability of an effective substitute for gum arabic would be desirable for the producers of beverage emulsions (Yadav *et al*., 2007a). Other polysaccharides used or studied for emulsion stabilization include modified starches, galactomannans, pectins, and corn arabinoxylan (corn fiber gum).

The present study concentrated on the properties of mannan-based films. In addition, the emulsifying properties of mannans were investigated. Special focus was on spruce galactoglucomannan. The literature review of this thesis focuses on mannans and their film formation as well as on the use of polysaccharides as emulsion stabilizers. The experimental part of this thesis is a summary of the attached papers **I**-**V**, in which galactomannans were modified enzymatically and used for the preparation of films and emulsions; blend films were prepared from GGM with poly(vinyl alcohol), konjac glucomannan (KGM), and corn arabinoxylan; composite films were made from GGM and KGM with cellulose nanowhiskers; and the emulsion stabilizing properties of mannans were compared.

2 Literature review

2.1 Mannans

Mannans are heteropolysaccharides found in plant cell walls as supporting material (hemicellulose) and as a storage polysaccharide in the endosperm of certain legume seeds and in the tuber of *Amorphophallus konjac* (plant gums) (Maier *et al.*, 1993; Sjöström, 1993, Takigami, 2000). Hemicelluloses are polysaccharides soluble in alkali that are associated with cellulose of the plant cell wall (Dekker, 1985). Hemicelluloses are the most abundant plant polysaccharides other than cellulose, and wood contains 20–30% hemicellulose on a dry weight basis. The most abundant hemicelluloses are xylans and mannans, and galactoglucomannans (GGM) are the principal hemicelluloses in softwoods (about 20%), while hardwoods contain 2–5% glucomannan (Sjöström, 1993). In the mechanical pulping of Norway spruce (*Picea abies)*, part of the GGM is dissolved and removed with the waste water, and this is considered a disadvantage, as it reduces pulp and paper yield and increases the impact load on either the environment or on the waste water management (Willför *et al.*, 2008). However, GGM can be recovered as a by-product from process waters of mechanical pulping of spruce by purification, ultrafiltration, and ethanol precipitation or spray-drying at a yield of 5 kg/ton pulp (Willför *et al.*, 2003). The cost of GGM was estimated to be 670 €/t (Persson *et al*., 2007). Despite the availability, abundance, and low cost of GGM, there are currently no industrial processes utilizing it.

Guar gum (GG) and locust bean gum (LBG) galactomannans are widely used as thickeners and stabilizers in the food, cosmetics, and textile industries, and in the paper industry to improve paper strength (Maier *et al*., 1993). GG is obtained from the seed of the legume *Cyamopsis tetragonolobus*, which grows mainly in the arid and semiarid regions of the Indian subcontinent, and LBG is from *Ceratonia siliqua*, grown in Mediterranean countries. To extract and purify galactomannan gums on a commercial scale, the tough seed-coat is removed by milling or grinding. For research purposes, the whole seed is normally ground to a fine powder, and the gum is extracted by prolonged

stirring with hot or cold water, dilute aqueous sodium hydroxide, or 1% acetic acid. The soluble portion is separated by centrifugation or filtration, and the polysaccharide is recovered by precipitation with ethanol, methanol, or isopropyl alcohol (Dea and Morrison, 1975).

Amorphophallus konjac is grown in Japan, China, and Indonesia. Konjac glucomannan (KGM) is produced from two-year-old konjac tubers. The tubers are washed, sliced into thin chips, and dried. The dried chips are pulverized into flour that is then separated by wind sifting and is often washed with ethanol to remove impurities. KGM is suitable for thickening, gelling, texturing, and water binding (Takigami, 2000).

Mannans are a structurally heterogeneous group of polysaccharides varying in their monosaccharide composition, substitution pattern, and degree of polymerization. Galactomannans (GG, LBG) consist of a β-1,4-D-mannopyranosyl backbone with single D-galactopyranosyl residues α-linked to mannose C-6 sites (Figure 1A) (Maier *et al.*,
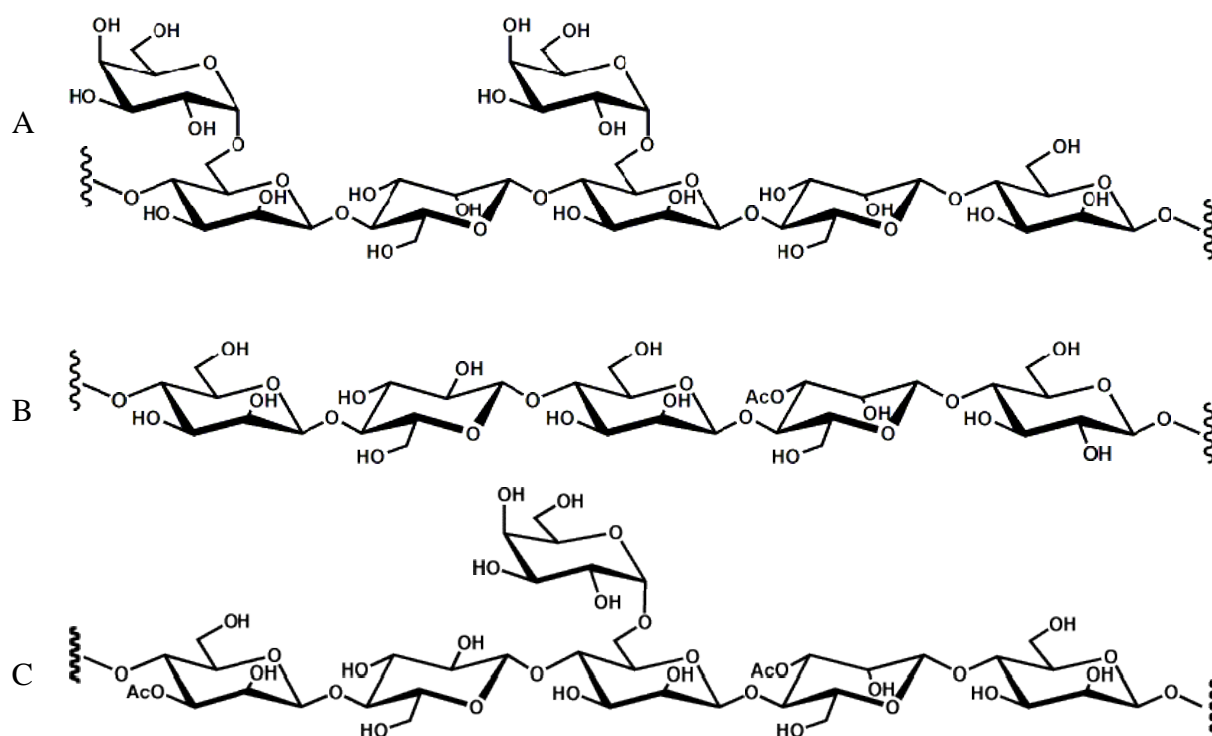
Figure 1. Chemical structure of A) galactomannan, B) glucomannan, and C) galactoglucomannan.

1993). Mannose/galactose ratios of LBG and GG are approximately 3.5:1 and 1.5:1, respectively. KGM has a chain of β-1,4-D-glucopyranosyl and β-1,4-D-mannopyranosyl units, about 5% of which are acetylated, and a mannose/glucose ratio of 1.6:1 (Figure 1B) (Takigami, 2000). Spruce GGM contains all these structural elements with a mannose/glucose/galactose ratio of approximately 4:1:0.5 and a degree of acetylation of about 30% (Figure 1C) (Willför *et al*. 2003). The molar masses of GG, LBG, and KGM are approximately 1000 kDa (Dea and Morrison, 1975; Li *et al.*, 2006a) and that of water-extracted GGM ranges from approximately 30 to 60 kDa (Willför *et al*., 2008).

2.2 Biodegradable films and coatings

A variety of polysaccharides and their derivatives, along with proteins and lipids, have been tested for potential use as edible films or coatings. These include starch, starch hydrolysates, cellulose derivatives, alginate, pectin, carrageenan, gelatin, casein, gluten, acetoglycerides, waxes and surfactants (Kester and Fennema, 1986). Edible films are thin layers of edible material placed on or between food components (Krochta and de Mulder-Johnston, 1997). They can act as an adjunct for improving overall food quality, extending shelf life, and possibly improving economic efficiency of packaging materials (Kester and Fennema, 1986). Edible films can be used to inhibit migration of moisture, oxygen, carbon dioxide, aromas, lipids, etc.; to carry food ingredients; and / or to improve the mechanical properties of the food. Edible films can reduce the amount of packaging, but not eliminate it (Krochta and de Mulder-Johnston, 1997). Films can be formed directly on food surfaces by dipping, spraying or brushing, or as separate, self-supporting films by casting. Film forming techniques include: 1) Coacervation, which involves separation of a polymeric coating material from a solution by heating, altering pH, adding solvents, or altering the charge of the polymer involved; 2) Solvent removal, which is a necessity for solid film formation from film-forming materials dispersed in aqueous solutions; and 3) Solidification of melt by cooling, which is a common technique for preparing lipid films (Greener Donhove and Fennema, 1994).

Biodegradable, but not necessarily edible, materials are developed to replace synthetic packaging materials in some applications. Biodegradable polymers include polylactide (PLA), the polymerization product of lactic acid; polycaprolactone (PCL); polyhydroxyalkanoates (PHA), which are polyesters produced by microorganisms; and poly(vinyl alcohol) (PVOH). However, natural polymers, such as polysaccharides and proteins, have an advantage over synthetic biodegradable plastics, because their biodegradability and environmental compatibility are assured (Swift, 1993). Replacing conventional synthetic packaging materials with biodegradable polymers can reduce use of non-renewable resources and decrease waste through biological recycling to the biosystem. The potential uses for such biopolymeric packaging materials include: 1) Single use, disposable packaging materials; 2) Daily consumer goods, such as plates, cups, containers, and egg boxes; 3) Disposable personal care napkins, sanitary pads, diapers, etc.; 4) Lamination coating; and 5) Bags for agricultural mulching (Tharanathan, 2003). To compete with non-biodegradable synthetic polymers, the critical mechanical, optical, and barrier properties for the intended application should be matched (Krochta and de Mulder-Johnston, 1997). The main functions of the package are to keep, present, and dispense the product. Protection of the product may be regarded as the rate of change of quality and this includes mechanical damage, loss of consistency or crispness, loss of appearance, and sensory changes (Ashley, 1985). The films must withstand mechanical stress during wrapping, shipping, and handling to maintain their integrity and barrier properties (Gontard *et al*., 1992).

Formulations of packaging films and coatings include at least one component able to form an adequately cohesive and continuous matrix (Gontard and Guilbert, 1994). The degree of cohesion depends on polymer structure, solvent used, temperature, and the presence of other molecules, such as plasticizers (Tharanathan, 2003). Packaging films from naturally occurring biopolymers are generally prepared in the laboratory scale by wet casting of the aqueous solution on a suitable base material and later drying. Extruded sheets have been made from a blend of pectin and starch and later compression molded to obtain thin films (Fishman *et al*. 2004). Addition of 24% PVOH to pectin-starch blend enabled film blowing (Fishman *et al*. 2006).

2.2.1 Film properties

The tensile properties of films are measures of their behaviour under mechanical stress. In the tensile testing of films, a stress-strain experiment is carried out so that the film specimen is stretched at a constant speed until it breaks and the force needed to deform the specimen is recorded. Tensile strength, elongation at break and Young's modulus are obtained from the stress-strain curve. Tensile strength (MPa), σ, is defined as $\sigma = F/A$, where F is the force applied and A is the cross-section area of the specimen. Elongation at break (%), ε, is the distance of deformation before breaking defined as $\varepsilon = \Delta L/L$, where L is the initial length of the specimen and ΔL is the increment of length caused by the applied force. Young's modulus (MPa) is calculated as the slope of the linear part of the stress-strain curve. High tensile strength indicates that the material is strong, high elongation at break indicates flexibility, and Young's modulus indicates stiffness. The desired properties depend on the application of the film. For films to be used as edible coatings, the tensile properties are not as important as for stand-alone films.

The properties of a material depend on its physical state, either glassy or rubbery. In the glassy state, the film is brittle and breaks easily, whereas in the rubbery state, it is elastic and flexible. In the glassy state, the film is less permeable to gases than in the rubbery state (Biliaderis *et al*., 1999). The glass transition temperature (T_g), is defined as the point at which the material passes from glas to rubbery during heating. The T_g is determined using differential scanning calorimetry (DSC), dynamic mechanical analysis (DMA), or dielectric analysis (DEA). In DSC, the difference in the amount of heat required to increase the temperature of a sample and a reference are measured as a function of temperature. In DEA, an oscillating electrical field, provided by an alternating voltage via sensor electrodes, is used to measure changes in dipole orientation and ion mobility in polymers and cross-linked systems. DMA is the measurement of the mechanical properties of materials as they are deformed under periodic stress. The sample is deformed by a sinusoidal strain, $\gamma(t) = \gamma_0(t)\sin(\omega t)$, where γ_0 is the amplitude, ω the angular frequency and t the time, resulting in a stress, $\sigma(t) = \sigma_0(t)\sin(\omega t + \delta)$, with the amplitude σ_0 and phase difference δ. These data are used to calculate the storage modulus (E'), loss modulus (E"), and tangent delta of the material. DMA is commonly done as a

function of temperature, but it can be done also as a function of RH (Hartman *et al.*, 2006a, Sternemalm *et al.*, 2008, Mikkonen *et al.*, 2009).

Thermal gravimetric analysis (TGA) monitors the change in a mass of a substance as a function of temperature or time as the sample is subjected to a controlled temperature program. TGA can be used to study the thermal degradation temperature of a material as well as the water content.

The crystallinity of films is measured using x-ray diffraction. When a beam of monochromatic x-radiation is directed at a crystalline material, the x-rays are reflected or diffracted at various angles with respect to the primary beam (Patnaik, 2004). The relationship between the wavelength of the x-radiation (λ), the angle of diffraction (θ), and the distance between each set of atomic planes of the crystal lattice (d) is given by Bragg's law: $m\lambda = 2d\sin\theta$, where m is an integer that defines the order of diffraction. The intensity is plotted as a function of twice the angle, 2θ. From Bragg's law, the interplanar distances of the crystalline material can be calculated. The ratio of crystalline material to amorphous material can be measured by integrating the intensity of the crystal diffraction peaks and dividing it by the intensity of the amorphous diffraction. In addition, the crystallinity of films can be studied using DSC, in which an endothermic peak indicates the melting of crystalline structures during heating.

The structure and miscibility of films consisting of polymer blends can be studied using Fourier transform infrared spectroscopy (FTIR). In FTIR, the infrared light is guided through an interferometer. After passing through the sample, the measured signal is the interferogram, on which a mathematical Fourier transform is performed. FTIR can give information about the intermolecular hydrogen bonds between blended polymers.

The visual appearance of the films can be studied using microscopy, which shows the presence of one or several phases in the films. Optical microscopy (OM) uses visible light and a system of lenses to magnify images of small samples. OM is used to study the film surface. Scanning electron microscopy (SEM) images the sample surface by scanning it with a high-energy beam of electrons. The atomic force microscope (AFM) utilizes a very

sharp tip fixed on the end of a cantilever. The sample is fixed on a piezoelectric scanner which can move the sample under the tip in x, y, and z-directions. SEM and AFM can be used to study the surface or cross-section of films.

The hydrophilicity or hydrophobicity of films is studied by measuring the contact angle of water on the film surface. Low contact angle of water indicates that the film is hydrophilic, while high contact angle indicates the opposite. Water sorption isotherms are determined by conditioning a film specimen at different RH conditions and weighing the film. Water solubility can be tested by placing a film specimen in a beaker containing water and then drying and weighing the insoluble part.

Permeability properties of films are important when the films are used as protective barriers against water vapor or gases. The requirements for barrier materials depend on the application. If the food is high in polyunsaturated fat, a film extremely resistant to oxygen transport would be desired. However, if the edible film is applied to fresh fruits or vegetables to retard desiccation, a certain degree of oxygen and carbon dioxide permeability is necessary to avoid anaerobic respiration (Kester and Fennema, 1986). According to McHugh and Krochta (1994), film permeability data can be used in product shelf life predictions and in tailoring the permeability properties of films for specific food applications. Water vapor permeability (WVP) and oxygen permeability (OP) are considered crucial with respect to many food destabilization processes. Carbon dioxide and ethene permeability can also be of interest. The light transmittance of a package affects the rate of oxidation of lipids, and thereby food quality. To preserve the sensory properties of food, such as taste and odour, aroma barrier properties are needed. Grease barrier properties are important in applications involving fatty products. Polysaccharide-based films are generally good barriers against grease and oxygen, but poor against water vapor.

2.2.2 Plasticizers

Plasticizers function by weakening the intermolecular forces between adjacent polymer chains, resulting in decreased tensile strength and increased flexibility. Plasticizers are necessary adjuncts to most polymeric films in order to reduce brittleness, improve flow, and impart flexibility (Banker, 1966). In addition, plasticizers increase the free volume of a system, which permits increased motion of polymer molecules (Sears and Darby, 1982). Plasticizers generally increase gas, water vapor, and solute permeability and decrease the glass transition temperature (T_g) of the film. The lowering of the T_g below room temperature changes a material that is hard, brittle, and glass-like at room temperature to one that is soft, flexible, and tough (Banker, 1966).

The basic requirements of any plasticizer in a polymer system are compatibility and permanence. To be compatible, the plasticizer should be miscible with the polymer, indicating similar intermolecular forces in the two components. The most effective plasticizers will generally resemble most closely in chemical structure the polymers they plasticize. The plasticizer should also approximately match the solubility properties of the polymer in the solvent system used (Banker, 1966).

Non-volatile, high boiling, non-separating substances, such as polyols, mono-, di-, or oligosaccharides, and lipids, are used as plasticizers to change the physical and mechanical properties of biopolymer films (Gontard *et al*., 1993). Volatile plasticizers may also be used as processing aids. In that case, the major portion of the plasticizer is flashed off during processing, but traces remain to give some plasticization. The most abundant plasticizer is water. Sometimes the plasticizer may not be added as a separate material but may be produced *in situ* by cleaving occasional polymeric molecules to produce smaller ones (Sears and Darby, 1982). Migration and crystallization of plasticizers can take place over time and reduce the elongation at break of films.

2.3 Films from mannans

A relatively small number of studies on film formation from mannans has been published. KGM films are the most widely studied (Table 1), and there are a few publications on films from LBG and GGM. Glycerol and sorbitol have been the most commonly used plasticizers for mannan films. KGM forms films even without additional plasticizer, so in many studies on it plasticizers were not used. To improve the mechanical properties of films, KGM was often blended with other polymers, such as pullulan, alginate, poly(vinyl alcohol), carboxymethyl cellulose, chitosan, gellan, and gelatin. The films were commonly characterized using tensile testing, DSC, X-ray diffraction, FTIR, and SEM. Some research groups reported also results from TGA, DMA, light transmittance, OP, WVP, and moisture uptake of the films.

Aydinli and Tutas (2000) studied the water vapor permeability (WVP) of LBG films plasticized with polyethylene glycol (PEG) 200, PEG 400, PEG 600, and PEG 1000. The WVP was lowest for films plasticized with PEG 200. The authors concluded that the WVP values of LBG films were at the same level as those of cellulose-derived films. The effect of the amount and molar mass of PEG on the light transmittance and mechanical properties of LBG films was also examined (Aydinli *et al*., 2004). The tensile strength varied from 5 to 40 MPa and the elongation at break was between 1 and 10% at 50% RH depending on the type and amount of PEG. PEG 200 was found to be the most suitable plasticizer in terms of both mechanical and light transmittance properties.

KGM films were reported to be strong and flexible, with tensile strength of 80 MPa and elongation at break of 33% (Ye *et al*., 2006). However, the authors did not report the RH in which the measurement was done, and the plasticizing effect of water on polysaccharide-based films should be taken into account (Talja *et al.*, 2007). In the studies on KGM-blend films, there was often an optimum blend ratio which resulted in improved film properties when compared to films from the pure polymers. For example, when blended with chitosan, the maximum tensile strength, 88 MPa, was achieved at a KGM:chitosan ratio of 80:20 (Ye *et al*., 2006). The tensile strength of KGM/gelatin

Table 1. Studies on mannan-based films.

Film former	Plasticizer	Measurements															Reference
		TT	TGA	DMA	DSC	x-ray	FTIR	SEM	LT	OP	WVP	SO	MU	WS	CA	HS	
LBG	Polyethylene glycol 200, 400, 600, and 1000, Glycerol Propylene glycol Sorbitol	X							X		X	X					Aydinli and Tutas, 2000; Bozdemir and Tutas, 2003; Aydinli *et al.* 2004
KGM Acid-hydrolyzed KGM KGM + CMC + palm olein lipid	Glycerol Sorbitol Not used	X			X	X		X			X	X					Cheng *et al.* 2006, 2007, 2008
KGM KGM + pullulan pullulan	Glycerol	X										X					Yue *et al.* 1995
KGM KGM + alginate Alginate KGM + PVOH PVOH KGM + CMC CMC	Not used	X	X		X	X	X	X	X			X	X				Xiao *et al.* 2000a, 2000b, 2001
KGM KGM + chitosan chitosan	Not used	X			X	X	X	X									Ye *et al.* 2006

Table 1. Continued.

Film former	Plasticizer	Measurements															Reference
		TT	TGA	DMA	DSC	x-ray	FTIR	SEM	LT	OP	WVP	SO	MU	WS	CA	HS	
KGM + PVOH KGM + gelatin	Not used	X			X	X	X		X		X		X	X		X	Li and Xie, 2004; Li *et al*. 2006b
KGM KGM + gellan gellan	Not used	X			X	X	X	X	X				X				Xu *et al*. 2007
KGM + collagen + alginate	Not used	X				X	X	X			X		X				Wang *et al*. 2007
KGM KGM + starch Starch	Glycerol	X	X		X	X	X	X					X				Chen *et al*. 2008
GGM GGM + alginate GGM + CMC Benzylated GGM Styrene grafted GGM	Glycerol Sorbitol Xylitol Not used			X	X					X					X		Hartman *et al*., 2006a, 2006b

TT = tensile testing, TGA = thermal gravimetric analysis, DMA = dynamic mechanical analysis, DSC = differential scanning calorimetry, x-ray = x-ray diffraction, FTIR = Fourier transform infrared spectroscopy, SEM = scanning electron microscopy, LT = light transmittance, OP = oxygen permeability, WVP = water vapor permeability, SO = water sorption, MU = moisture uptake, WS = water solubility, CA = contact angle, HS = heat-sealability

blends reached its maximum at 40% KGM content (Li *et al.*, 2006b). Partial acid hydrolysis of KGM resulted in decreased molecular weight and increased polydispersity, which initially increased the Young's modulus and tensile strength of films (Cheng *et al.*, 2007). However, water sorption and WVP of films also increased with increasing degree of hydrolysis. Pure KGM films were not well heat-sealable, but the addition of gelatin improved this characteristic, with the highest heat-sealability at 40% KGM content (Li *et al.*, 2006b). KGM films dissolved in cold water in 30 s, which is a desired property for packages used for such products as instant noodles, instant coffee, instant tea, and instant milk powder.

Hartman *et al.* (2006a) considered GGM films containing either alginate or carboxymethylcellulose to have interesting oxygen barrier and mechanical strength properties. Polyol plasticizers were needed to decrease the brittleness of films and to make them easier to handle. The oxygen permeability of GGM/alginate blend was 0.55 $(cm^3 \cdot \mu m)/(m^2 \cdot d \cdot kPa)$, and that of sorbitol-plasticized GGM film was 2.00 $(cm^3 \cdot \mu m)/(m^2 \cdot d \cdot kPa)$. Characterization of the mechanical properties of films using humidity scan dynamic mechanical analysis showed that the sorbitol-plasticized GGM films softened at higher RH than the glycerol-plasticized films. Hartman *et al.* (2006b) produced benzyl GGM and used plasma treatment followed by styrene addition, vapor-phase grafting of styrene, and lamination with the benzylated GGM to increase the humidity tolerance of GGM films.

2.4 Cellulose nanowhiskers as reinforcements

The use of cellulose nanowhiskers (CNW) as reinforcements of various synthetic and some natural polymer matrices has been studied (Samir *et al.*, 2005). CNW are prepared by acid hydrolysis of cellulose from different sources, such as wood, cotton, or animal origin. The hydrolysis yields a suspension of highly crystalline, nanoscale, whisker-shaped rods in water, the dimensions of which depend on the cellulose origin and the hydrolysis conditions (de Sourza Lima and Borsali, 2004, Elazzouzi-Hafraoui *et al.*, 2008). The

aqueous suspensions are stable because of the introduction of negative charges on the surface of the crystallites during the hydrolysis process (de Sourza Lima and Borsali, 2004).

Crystalline structures are extremely tightly packed and tend to be impermeable to gases (McHugh and Krochta, 1994). Due to the extensively ordered structure, the tensile strength of CNW is high (Samir *et al*., 2005). Uniform dispersion of the reinforcement leads to large interfacial area between the reinforcement and matrix, which alters the molecular mobility, the relaxation behavior, and the thermal, mechanical, and gas barrier properties of the nanocomposite (Sorrentino *et al*., 2007). When reinforcement-reinforcement interactions are favored, nanocomposites containing CNW can have greatly improved mechanical properties in comparison to the matrix polymer alone. However, high matrix-reinforcement interactions, such as transcrystallization, can result in low mechanical performance (Samir *et al*., 2005). The tensile properties of a starch-CNW system were also found to be dependent on CNW content, moisture conditions, and the type of plasticizer (Angles and Dufresne, 2001; Lu *et al*., 2006; Mathew *et al*., 2008). Angles and Dufresne (2000) found that glycerol accumulated in the cellulose/amylopectin interfacial zones. The soft interphase most probably prevented any effective stress transfer at the reinforcement/matrix interface and the tensile properties of films were little affected by the addition of CNW (Angles and Dufresne, 2001). In contrast, the tensile strength of a starch-CNW-sorbitol system increased gradually with increased reinforcement load, showing that the CNW were the load-bearing entity in the composites (Mathew *et al*., 2008). Kvien *et al*. (2007) also reported enhanced tensile properties in a starch-sorbitol system with the addition of CNW.

2.5 Beverage emulsions

Flavor, cloudiness, and color of beverages are often generated by emulsification of hydrophobic components in a continuous water phase. Citrus flavors are among the most popular of all beverage flavors. The citrus flavor products are based primarily on the

water-insoluble essential oils from the peel of the fruits, e.g. orange oil or lemon oil. Although citrus oils are important for the beverage emulsions, they also present a major problem for the emulsions because of their low density. It is therefore difficult to stably disperse the oils into the sugar solution (Tan, 2004). Weighting agents are a group of materials that are added to the essential oils to increase their specific gravity and to help maintain a stable dispersion of the oil (Chanamai and McClements, 2000, Tan, 2004).

To prepare beverage emulsions, the measured amounts of preservative, citric acid, coloring, and gum are first dissolved in water and the weighting agent is dissolved in the oil. Secondly, the oil phase is mixed with the water phase to make a crude emulsion. Usually this step can be achieved by the use of a high-speed mixer, colloid mill, hydroshear, or similar type of equipment. Thirdly, the crude emulsion is homogenized by pumping it through the homogenization valve of the homogenizer at high pressure (Tan, 2004).

Beverage emulsions are consumed in a highly diluted form. They are first prepared as an emulsion concentrate that is later diluted in sugar solution in order to produce the finished beverage. The emulsions in both the concentrated and diluted forms must have a high degree of stability (Tan, 2004). However, emulsions are thermodynamically unstable systems that tend to break down during storage. Generally, emulsion breakdown can be due to flocculation, creaming, sedimentation, coalescence, Ostwald ripening, and/or phase inversion (McClements, 2005). The long-term stability of emulsions is normally extended by adding a variety of stabilizers. The stabilization can be sought through control of either the rheology of the continuous phase, i.e. by enhancing its viscosity, or of its interfacial properties (Doublier and Cuvelier, 2006). A stabilizer is any ingredient that can be used to enhance the stability of an emulsion and may be classified as either an emulsifier or a texture modifier depending on its mode of action (McClements, 2005). Furthermore, an emulsifier is a compound that promotes emulsion formation and short-term stabilization by interfacial action (Dickinson, 2003).

2.6 Polysaccharides as emulsion stabilizers

Beverage emulsions are commonly stabilized using polysaccharides, which have advantages over proteins and small-molecule surfactants because the former are more sensitive to environmental conditions (pH, ionic strength, temperature) than polysaccharides, and the latter may have problems associated with flavors (McClements, 2005). The most well-known hydrocolloid for use in acidic beverage emulsions is gum arabic (Dickinson, 2003; Tan, 2004). Gum arabic or gum acacia is a tree gum exudate obtained from *Acacia senegal* and *seyal* species. The trees grow widely across the Sahelian belt of Africa situated north of the equator up to the Sahara desert and from Senegal in the west to Somalia in the east. Gum arabic is a complex arabinogalactan and contains a small amount of nitrogenous material that cannot be removed by purification. *Acacia senegal* gum contains 44% galactose, 27% arabinose, 13% rhamnose, 14.5% glucuronic acid, and 1.5% 4-*O*-methyl glucuronic acid. The average molar mass is 380 kDa (Williams and Phillips, 2000). Gum arabic is completely soluble in twice its weight of water and has very low viscosity in water with regard to concentration. Gum arabic is an efficient emulsifier for oil-in-water emulsions. It reduces the interfacial tension between oil and water and facilitates the formation of fine oil droplets in the emulsion. It forms interfacial films on the oil droplets and stabilizes the emulsion (Tan, 2004). The E number (European Union number code for food additives) for gum arabic is E 414.

There is significant natural variability among gum arabic batches from different growing regions with respect to their performance as emulsifiers (Buffo *et al*., 2001). The level of surface activity of gum arabic is rather low and to compensate for this, it is necessary to use a rather high gum-to-oil weight ratio, approximately 1:1. Because of this and the high cost of gum arabic, other hydrocolloids have been suggested to replace gum arabic (Dickinson, 2003). The most widely accepted alternatives to gum arabic for use as the beverage emulsion stabilizer are modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, and E 1451). They are a group of specially designed starch derivatives with balanced lipophilic and hydrophilic groups on the starch molecules. Modified starch is a cleaner material than gum arabic, as it contains fewer foreign substances (Tan, 2004).

Galactomannans GG (E 412) and LBG (E 410) are in wide commercial use as beverage emulsion stabilizers. Fenugreek galactomannan (from *Trigonella foenum-graecum*) has also been studied as emulsion stabilizer. Huang *et al*. (2001) compared the emulsification properties of several hydrocolloid gums and found that fenugreek galactomannan produced a very stable emulsion and was more efficient than other gums in lowering the interfacial free energy. This is in agreement with an earlier study by Garti *et al*. (1997), who wrote that fenugreek galactomannan adsorbed on the oil interface, forming a relatively thick interfacial film. Fenugreek galactomannan has higher galactose substitution and water solubility than the other galactomannans. Emulsions containing GG were more stable than those containing LBG (Huang *et al*., 2001). According to Dickinson (2003), GG and LBG stabilize emulsions mainly by modifying the rheological properties of the aqueous phase between the dispersed particles. Garti and Reichman (1994), however, suggested galactomannans to be considered as emulsifiers as well as stabilizers, because they showed strong interfacial activity. However, Brummer *et al*. (2003) showed that enzymatic removal of protein diminished the ability of fenugreek galactomannan to reduce interfacial tension.

Pectins (polygalacturonic acids) (E 440) extracted from plant cell walls are commonly used as gelling and thickening agents in foods (Dickinson, 2003). Citrus pectin and beet pectin are able to reduce the interfacial tension between an oil phase and a water phase and can be effective for the preparation of emulsions (Leroux *et al*., 2003, Mirhosseini *et al*., 2008a). The surface activity and emulsion stabilization characteristics of pectin are improved by a high degree of acetylation and a reduction in its molar mass (Dickinson, 2003). Leroux *et al*. (2003) concluded that the emulsifying properties of pectin are most probably due to protein residues present within it. According to Dickinson (2003), the hydrophobic protein component is responsible for the surface activity of pectin, acting as the strong anchor point at the oil-water interface, with the hydrophilic polysaccharide chains providing the protective layer that confers effective steric stabilization during extended storage.

Corn fiber gum (arabinoxylan, cAX) is the main, low value by-product of the corn milling process. Its emulsion-stabilizing capacity has recently been studied and compared to that of gum arabic (Yadav *et al*., 2007a, 2007b, 2007c, 2008, 2009). Corn fiber gum was considered to be a potential replacement for gum arabic in beverage flavor emulsification. The emulsification capacity was suggested to be due to its high protein content and/or the high level of branching in the polysaccharide backbone (Yadav *et al*., 2007a). High molar mass was associated with higher emulsion stability (Yadav *et al*., 2007b). Increasing the corn fiber gum content beyond 0.25% (w/w of emulsion) did not further enhance the stability of an emulsion containing 5% (w/w) orange oil, and this polysaccharide content was considered to be enough to achieve a stable emulsion (Yadav *et al*., 2007c).

3 Aims of the study

The overall aim of this thesis was to study the properties of mannan-based films and the emulsion-stabilizing properties of mannans, with an emphasis on films and emulsions from GGM. The main objectives of the individual studies were:

1. To prepare enzymatically modified galactomannans and films from them, and study the effect of the chemical structure of mannans on the mechanical and thermal properties of the films.

2. To study the morphology and mechanical properties of films from glucomannans GGM and KGM.

3. To improve the mechanical properties of GGM-based films by blending GGM with PVOH, KGM, and cAX, and by mixing GGM and KGM with CNW.

4. To compare the permeability properties of GGM-based films to those of films from other mannans.

5. To study the emulsion-stabilizing properties of GGM and other mannans and to examine the effect of the DP and DS of mannans on emulsion stability.

4 Materials and methods

This section summarizes the materials and methods presented in more detail in the original papers (I-V).

4.1 Materials

The polymers used and their suppliers are listed in Table 2. Three different batches of GGM were used. In Paper II, GGM was prepared on a laboratory scale from thermomechanical pulp of spruce according to the method of Willför *et al*. (2003), with the exception that the pulp was taken after the second refiner in a two-stage refining system. In Paper III, GGM was obtained from an industrial-scale isolation trial (Xu *et al.*, 2007). The concentrated solution was spray-dried (GGMSpDr) and then dissolved in water at 10 g/l, filtered through a glass fiber filter to remove a small amount of agglomerated particles, concentrated using a rotary evaporator, and vacuum-dried. In Paper IV, GGM was from the industrial-scale trial, but ethanol precipitated (GGMEtOH) instead of spray-dried and filtered as described above, after which it was lyophilized. In Paper V, unfiltered GGMSpDr and GGMEtOH were used. Two different batches of KGM were also used (Table 2).

Table 2. Polymers used.

Material	Supplier	Paper
GG	Sigma, USA	I, IV,V
LBG	Fluka, Switzerland	I, IV,V
KGM	Baoji, China	II, V
KGM	Arthur Branwell, UK	III, IV
PVOH	Sigma, USA	II, IV
GGMLab (laboratory scale isolation)	Åbo Akademi, Finland (Willför *et al.,* 2003)	II
GGMSpDr (spray-dried)	Åbo Akademi, Finland (Willför *et al.,* 2003; Xu *et al*., 2007)	III, V
GGMEtOH (ethanol precipitated)	Åbo Akademi, Finland (Willför *et al.,* 2003; Xu *et al*., 2007)	IV, V
CNW	Technical University of Luleå, Sweden (Bondenson *et al*., 2006)	III, IV
cAX	ERRC, USDA, USA (Yadav *et al.*, 2007a)	II, V
Gum arabic	Tic Gums, USA	V

The enzymes used were the purified β-mannanase of *Trichoderma reesei* (from VTT, Finland) and commercial α-galactosidase (Biogalactosidase, Quest, The Netherlands). The enzyme mixture for complete hydrolysis was from VTT (Buchert *et al*., 1993).

4.2 Enzymatic modification of galactomannans (I)

GG was enzymatically modified to different molar masses and degrees of galactose substitution. Stepwise enzymatic modifications were carried out at VTT (Finland) as described by Hannuksela *et al*. (2002). The degree of polymerization of GG was decreased by treating GG (3 g/l) with different dosages of β-mannanase (0, 2, 10 or 50 nkat/g) for 4 h. The degree of substitution of the resultant samples was modified by selectively removing galactose side groups by α-galactosidase (0, 200 or 500 nkat/g, 5 h). After the treatments, the GGs were lyophilized, washed with ethanol to remove monosaccharides and short oligosaccharides, and re-lyophilized.

Carbohydrate composition of modified GGs was analyzed by gas chromatography (GC) after complete enzymatic hydrolysis (Buchert *et al*., 1993). After hydrolysis, all samples were clear solutions. The GC equipment and method of analysis of monosaccharides was described by Virkki *et al*. (2005), except that the GC oven temperature was maintained at 210ºC for 10 min, then increased to 220ºC at 1ºC/min and held at 220ºC for 6 min. Molar mass of modified GGs could not be determined due to incomplete solubilization of samples in any eluents tested. Sample codes, enzyme dosages applied and galactose : mannose ratios are shown in Table 3.

Table 3. Sample codes, enzyme dosages used in modifications and galactose : mannose ratios of modified galactomannans.

Code	Galactosidase dosage (nkat/g)	Mannanase dosage (nkat/g)	Gal / 10 mannose units	Molar mass code
6Gal-H	0	0	4.9	High
6Gal-MH	0	2	5.2	Medium high
6Gal-ML	0	10	5.7	Medium low
6Gal-L	0	50	5.4	Low
4Gal-H	200	0	3.6	High
4Gal-MH	200	2	3.7	Medium high
4Gal-ML	200	10	3.9	Medium low
4Gal-L	200	50	3.6	Low
2Gal-H	500	0	2.3	High
2Gal-MH	500	2	2.3	Medium high
2Gal-ML	500	10	2.0	Medium low
2Gal-L	500	50	1.9	Low

4.3 Preparation of films (I-IV)

The film-forming polymers were dissolved in hot, deionized water (I, III: 80ºC, II, IV: 95ºC). In Papers I and II, mannans were added to water that already contained plasticizer. In Papers III and IV, mannans were first dissolved, then CNW were added when used and the suspension was stirred for 5 minutes before the addition of plasticizer. The solutions or suspensions were degassed by ultrasonication under vacuum for 5 minutes, cast on polystyrene petri dishes or Teflon plates, and dried at 60ºC.

The effect of the amount of plasticizer was tested on GG and LBG-based films (I). Later, a plasticizer level of 40% (w/w of mannans) was used. In Paper I, glycerol and sorbitol were used as plasticizers. In Papers II and III, glycerol was used and in Paper IV, sorbitol was used.

4.4 Characterization of films (I-IV)

4.4.1 Thickness

The thickness of films was measured using a micrometer. A Lorentzen & Wettre micrometer (precision 1 µm) was used in Paper I for films analyzed with a tensile tester, in Paper III for films tested with DMA, and in Paper IV for all films. A Mitutoyo micrometer (precision 10 µm) was used in Paper I for films tested with DMA. In Paper II, a micrometer from Ames, Waltham, USA, (precision 1 µm) was used. In Paper III, a micrometer from NSK, Japan (precision 10 µm), was used for films analyzed with a tensile tester.

4.4.2 Tensile properties

A tensile tester was used to obtain the stress-strain data of the films (I-III). In Paper I, Instron 4465 universal testing machine was used. In Paper II, an updated Instron 1122 mechanical property tester was used and in Paper III, an Instron 4411 mechanical property tester was used. The tensile tests were done either in a climate room, with controlled relative humidity (I: RH 50%, II: RH 65%) or in a normal room with conditioned specimens (III: RH 54%). Ten to thirteen replicate specimens were analyzed.

4.4.3 Thermal analyses

In Papers I and III, thermal gravimetric analysis (TGA) was used to determine the water content of the films by heating the specimens to 120ºC using a TGA 850 with STAR[e] software (Mettler Toledo). In Paper III, the thermal degradation of films was also studied with TGA by heating the specimens to 600ºC using a TGA Q500 (TA Instruments). Three replicate specimens were analyzed.

Dynamic mechanical analysis (DMA) was done as a function of temperature to determine the glass transition temperature (T_g) of the films. At the T_g, the loss modulus

shows a peak and the storage modulus decreases. DMA was used instead of DSC, because it is a more sensitive method. A DMA 242 (Netzsch-Gerätebau GmbH) was used in Paper I, a Rheometrics RSA II solids analyzer in Paper II, and a DMA Q800 (TA Instruments) in Paper III. T_g was taken as the peak temperature of the loss modulus at 1 Hz, which was determined by fitting a parabolic curve to loss modulus data with Origin 7.0 (I) or Origin 7.2 (II) (OriginLab Corporation) software. Three replicate specimens were analyzed.

4.4.4 Microscopy

Transmitted light (II, III), fluorescence (II), and polarizing (III) imaging was done with an optical microscope (OM). Light microscopy was used for examining relatively large structures and phase separation in the blend and composite films. Autofluorescence was used for visualizing the impurities in the films and polarizing microscopy for visualizing the crystalline structures.

Cross-sections of films were viewed with scanning electron microscopy (SEM) (II, III). Rectangular film strips were plunged into liquid nitrogen and fractured manually using tweezers. The fractured fragments were mounted onto aluminium stubs and coated with a thin layer of gold by direct current sputtering. Images of oriented fracture faces were collected using an SEM operated in the high vacuum-secondary electron imaging mode.

4.4.5 X-ray diffraction

The degree of crystallinity of mannan-CNW composites was studied with wide angle X-ray scattering (WAXS) (III). A setup with a Rigaku rotating anode (fine focus) X-ray tube and an MAR345 image plate detector (Rayonix) was used. The measurements were done in the Department of Physics, University of Helsinki.

4.4.6 Water vapor permeability (WVP)

Water vapor permeability (WVP) was measured by a gravimetric method (IV) (ASTM, 2005a). Films were sealed on aluminum cups containing desiccant or saturated salt solution, and placed in a cabinet in which the relative humidity was controlled with saturated salt solution and the air velocity was controlled with a fan. The salt solutions were selected so that there was low RH on one side of the film and high RH on the other. The change in the weight of the cup was monitored with repeated weighing. The water vapor transmission rate (WVTR) was calculated from a linear regression of the slope of weight gain against time by dividing the slope by the mouth area of the test cell. The water vapor partial pressure at the underside of the film was calculated using the correction method described by Gennadios *et al.* (1994). The WVP was obtained from WVP = (WVTR × L) / Δp, where L is the thickness of the film and Δp is the water vapor partial pressure difference between the two sides of the film. Three replicate specimens of each film type were measured.

4.4.7 Oxygen permeability (OP)

The oxygen gas transmission rate (OTR) of the films was measured using an oxygen permeability (OP) tester with a coulometric sensor (Ox-Tran Twin, Modern Controls Inc.) (ASTM, 2005b). The film was placed in a diffusion cell and exposed to 100% oxygen on one side and to a mixture of 98% nitrogen and 2% hydrogen on the other side. Oxygen molecules diffusing through the film reached the coulometric sensor, generating a signal proportional to the amount of oxygen in the carrier gas. The OP was calculated by multiplying the OTR by the thickness of the film and dividing it by the difference in oxygen gas partial pressure between the two sides of the film. Four replicate specimens of each film type were measured.

4.4.8 Aroma permeability

The aroma permeability of the films was studied with a system containing a test cell and oven, a control system and a gas chromatograph (GC) at KCL (Finland). The test cell was divided into aroma source and receiving cells. The test specimen was placed between the two cells, and these were pressed together. Aroma concentrations were measured as a function of time, while the compounds diffused from the source cell containing the model aroma solution through the tested material to the receiving cell. The test solution contained D-limonene, cis-3-hexenol, isoamyl acetate, and R-carvone in mineral oil. Gas was automatically sampled from the test cells to the GC. The test cell was placed in a test cell oven with temperature control from ambient to 60ºC. The sample transfer oven could be heated up to 150ºC. A heated transfer line connected the sample loop to the GC (Vähä-Nissi *et al.*, 2008). Two replicate films were measured.

4.4.9 Light transmission

To determine the light transmittance, the intensity of the light source was measured by a photoelectric cell, both with and without the interposition of the film sample. The light transmittance was the percentage of the light intensity measured with the film to that obtained without it. The light transmittance of the films was scanned from 190 to 800 nm wavelength using a Shimadzu UV-2501 PC spectrophotometer equipped with an integrating sphere detector. The haze of films was determined in triplicate at 550 nm wavelength as described in the ASTM Standard Test Method for Haze and Light Transmittance of Transparent Plastics (2007).

4.5 Preparation of emulsions (V)

Mannans, cAX, and gum arabic were dissolved by stirring overnight in water containing sodium benzoate and citric acid. Coarse emulsion concentrates were prepared from the mannan solution, orange oil, and water, by pre-homogenization using a bench top

homogenizer. A fine emulsion was then prepared by passing the coarse emulsion through a high pressure homogenizer. The resulting homogenized concentrates were diluted 31.25 × to 78.125 ml in 10% (w/w) sucrose solution containing 0.1% sodium benzoate and 0.3% citric acid.

4.6 Characterization of emulsions (V)

4.6.1 Turbidity

The emulsion stability was measured as turbidity $T = 2.303 AD/l$, where T was the turbidity in reciprocal centimeters, A was the observed absorbance at 650 nm, D was the dilution factor, and l was the path length of the cuvette in centimeters. The turbidity was determined immediately after preparing the dilute emulsion to obtain emulsion activity (EA) by measuring absorbance at the wavelength of 650 nm using a Shimadzu UV-1700 spectrophotometer. The bottles containing the emulsion were set on the bench at room temperature (23ºC), in a refrigerator at 4ºC, and in an oven at 45ºC without agitation for gravity separation. The emulsion stability (ES) was monitored daily by absorbance (loss of turbidity) measurement for 14 days.

4.6.2 Confocal microscopy

Emulsions were dyed with Nile Red (0.1% in acetone) and studied by confocal microscopy (TCS SP, Leica Microsystems) after 3, 7, and 14 days aging. A 10 µl drop of the dyed emulsion was placed in a glass-bottomed microwell dish and spread into a thin film by placing a coverslip on top of it. The oil droplets labeled with Nile Red were viewed by excitation of the dye in the sample with the 488 nm laser line of an argon laser, and images of the visible fluorescence were collected at 590–620 nm within single focal planes. The number and size of particles in the microscopic images were determined with Fovea Pro 3.0 (Reindeer Graphics) image manipulation software.

4.7 Statistical analyses (I, V)

Analysis of variance (ANOVA) was used to study the effects of the type and amount of plasticizer and the effect of the type of polymer (for films from native GG and LBG) and the effects of the type of plasticizer, DS and DP (for films from modified GGs) on the results of elongation at break and tensile strength of the films (I). The effect of factors with more than two levels was analyzed using linear and quadratic trend contrasts. ANOVA was also used to study the effects of the DS and DP of the modified GGs on the turbidity of emulsions (V). To obtain detailed interpretation of these comparisons, linear, quadratic, and cubic trend contrasts were used. The stabilizing performance of GGMSpDr and GGMEtOH at different conditions was compared by two-way analysis of variance.

5 RESULTS

5.1 Thickness of films

Galactomannan-based films had a thickness of approximately 80 µm when prepared for tensile testing, and approximately 100 µm when prepared for DMA. Blend films from GGM with cAX, KGM, and PVOH were on average 70 µm thick. GGM/CNW composite films had a thickness of 80 µm and KGM/CNW composite films had a thickness of 40 µm. Films prepared for permeability measurements were approximetely 40 µm thick, except the films from GG and LBG that had a thickness of approximately 80 µm.

5.2 Tensile properties of mannan-based films

5.2.1 Need of plasticizer (I, III)

Film formation from KGM and GGM was studied without plasticizer and with glycerol, sorbitol, xylitol, and mannitol. As expected, KGM formed films without added plasticizer, but other mannans required a plasticizer for cohesive film formation. Migration and crystallization of xylitol and mannitol from KGM-based films was visible within a week from film preparation. Sorbitol also crystallized, but more slowly, within six weeks from film preparation. GGM did not form cohesive films with xylitol or mannitol. The effect of plasticizer dosage on tensile properties of galactomannan films was studied using plasticizer contents of 20, 40 and 60% (w/w of polymers). The elongation at break of GG and LBG films increased and the tensile strength generally decreased with increasing content of glycerol or sorbitol, as expected (Figure 2). GGM formed cohesive films only with 30% and 40% plasticizer contents, and 40% plasticizer content was selected for further experiments. Figure 3 presents photographs of mannan films plasticized with 40% glycerol. GGM films were brownish, KGM films were transparent, and GG and LBG films were opaque with a rough surface.

The tensile strength of the films was generally slightly higher when glycerol rather than sorbitol was used as the plasticizer (Figures 2A, 2B, and 5A). As exceptions, the tensile strengths of films from native GG containing 60% sorbitol, LBG-based films prepared with 40% sorbitol and films from low molecular weight GGs plasticized with sorbitol were equal to or greater than those of the corresponding glycerol-plasticized films. The elongation at break of films was notably higher for films plasticized with glycerol than for those plasticized with sorbitol (Figures 2C, 2D, and 5B). Therefore, glycerol was used as plasticizer in further studies on mechanical properties of films (II, III). Sorbitol
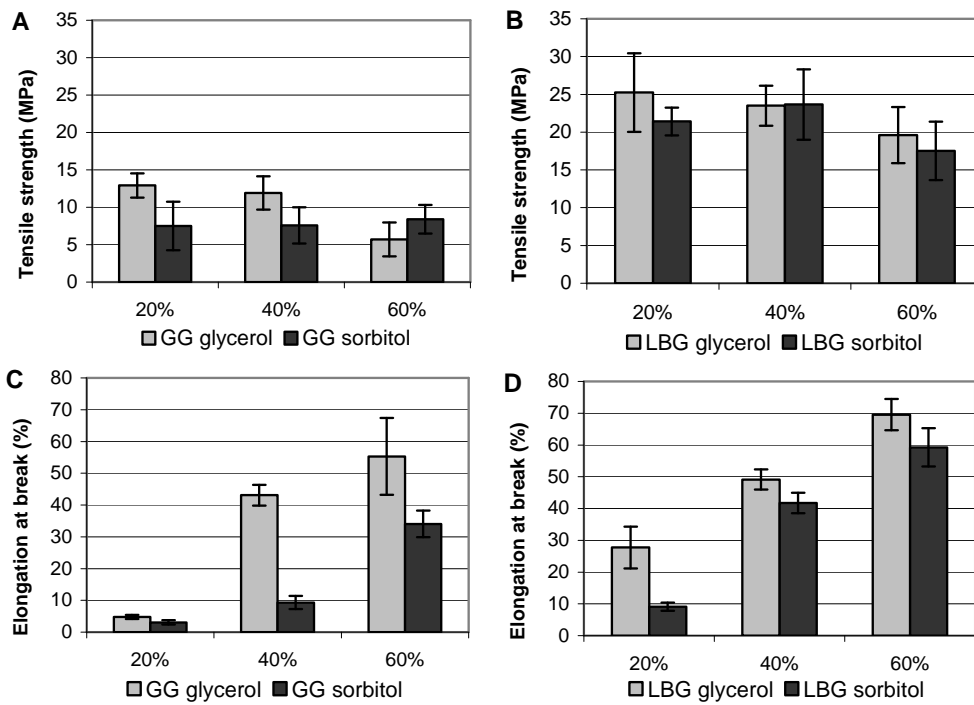

Figure 2. Effect of the amount of plasticizer on (A, B) tensile strength and (C, D) elongation at break of films from (A, C) native guar gum (GG) and (B, D) locust bean gum (LBG) plasticized with different amounts of glycerol or sorbitol. Each average is based on n = 12 measurements and the error bars indicate standard deviation. The measurements were done at 50% RH.
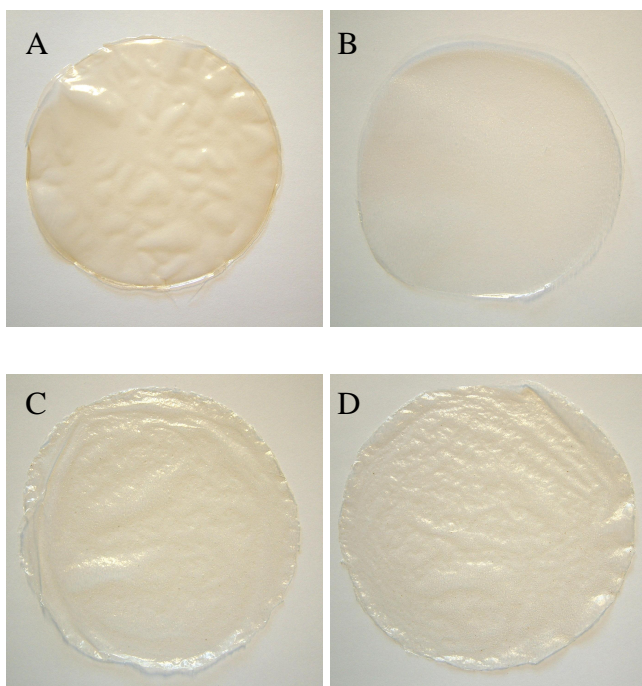
Figure 3. Photographs of films from (A) GGMEtOH, (B) KGM, (C) GG, and (D) LBG plasticized with 40% glycerol.

was selected as plasticizer for the study on permeability properties of the films (IV), because experiments on oat spelt xylan films showed that sorbitol gives better oxygen and water vapor barrier properties than glycerol (Mikkonen *et al.*, 2009). In addition, Hartman *et al.* (2006a) obtained a low value for oxygen permeability of GGM films plasticized with sorbitol.

5.2.2 Effect of mannan structure (I-III)

The chemical structure of mannans significantly affected the mechanical properties of films. Films from LBG (galactose : mannose ratio approximately 3:10) were clearly stronger and more flexible (24 MPa and 49%) than films from native GG (galactose : mannose ratio approximately 6:10) (12 MPa and 43%) (Figure 4). The strongest films, 43 ± 15 MPa, were formed from KGM. GGMSpDr formed films with relatively high tensile strength, approximately 12 MPa at 54% RH, which was close to that of GG films. GGMEtOH and GGMLab formed weaker films with a tensile strength of 5 and 6 MPa,

respectively, at 50% RH. The elongation at break of GGM films was clearly lower than that of films from other mannans.
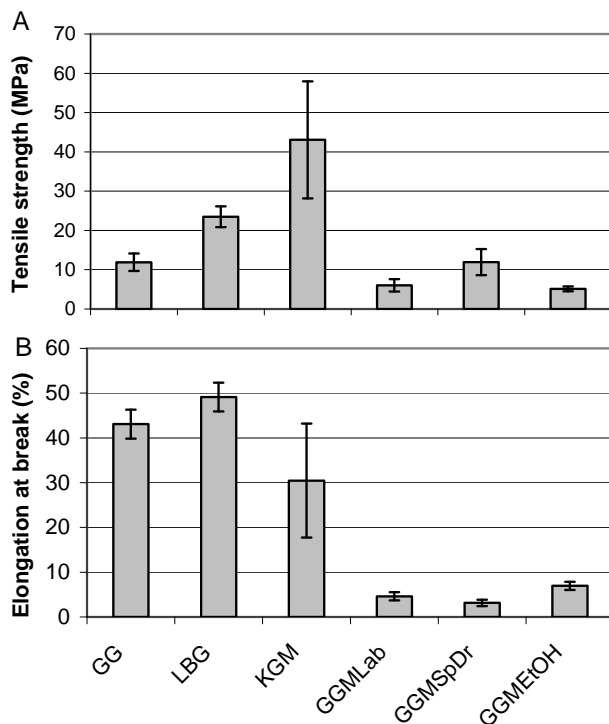

Figure 4. (A) Tensile strength and (B) elongation at break of films from different mannans plasticized with 40% (w/w) glycerol. Each average is based on n = 12–13 measurements and the error bars indicate standard deviation. The measurements were done at about 50% RH.

The effect of chemical structure on film properties was further studied with enzymatically modified GGs. Removal of galactose substituents increased both the tensile strength (Figure 5A) and elongation at break (Figure 5B) of both glycerol- and sorbitol-plasticized films from enzymatically modified GGs. The mechanical properties of films were improved also by moderately reducing DP. Controlled decrease in DP interestingly resulted in an increase in the elongation at break of films until medium-low DP level, but decreased again at the lowest DP (Figure 5B). Controlled decrease of DP also improved the tensile strength of some samples (Figure 5A). Among the modified GGs, the highest tensile strength values were measured for glycerol-plasticized films from 2Gal-MH. The tensile strengths of films from 2Gal-MH containing sorbitol and from 2Gal-ML plasticized with either glycerol or sorbitol were also high. However, films made from the most degraded GGs with the lowest DP were clearly weaker than the other films (Table 5 in I).
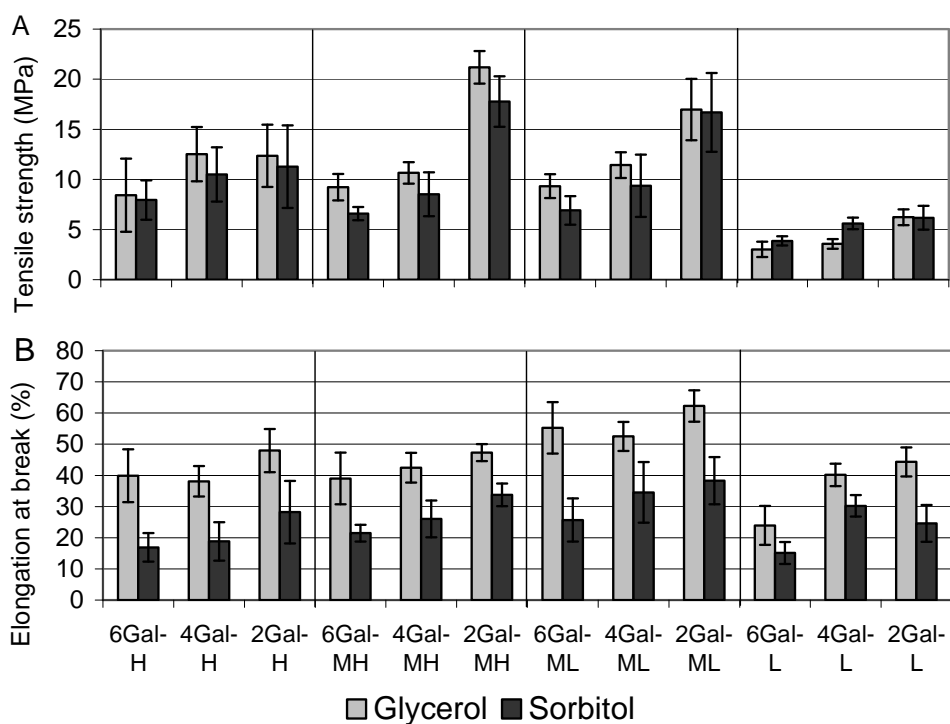
Figure 5. Effect of the degree of substitution (2Gal, 4Gal or 6Gal), degree of polymerization (H, MH, ML or L) and type of plasticizer on (A) tensile strength and (B) elongation at break of films from modified GGs plasticized with 40% glycerol or sorbitol. Each average is based on $n = 12$ measurements and the error bars indicate standard deviation. The measurements were done at 50% RH.

5.2.3 Blend films containing spruce galactoglucomannans (II)

Further experiments concentrated on GGM, because they are a potential new product from forest biorefineries, and compared them to KGM because of the similarities in their sugar composition. The improvement of the tensile properties of GGM films was sought by blending GGMLab with PVOH, KGM, and cAX. PVOH was selected because it was previously successfully blended with pectin (Coffin *et al*., 1996, Fishman *et al*., 2006) and PVOH-KGM blends were previously studied (Xiao *et al*., 2000b, Li and Xie, 2004). KGM was selected because of its excellent film forming properties and chemical similarity to GGM, and cAX because it is an interesting new gum and a by-product of corn milling.

Increasing content of KGM increased the tensile strength of the films in a curvilinear manner (Figure 6A). A small amount of KGM notably enhanced the elongation at break of

films, which was high for all films containing KGM, although not as high as that of films with a high content of PVOH (Figure 6B). The measurements were done at 65% RH and due to the plasticizing effect of water, the tensile strengths of pure GGMLab and KGM films were lower and the values of elongation at break were higher than the the results obtained at 50% RH (Figure 4).

The tensile strength of films clearly increased when PVOH was used in GGMLab / PVOH at the ratio of 1:3 (Figure 6A). At lower PVOH levels, the differences in the tensile strength of GGMLab / PVOH blend films were small. The elongation at break of GGMLab / PVOH films increased with increasing PVOH content from GGMLab : PVOH ratio of 1:1 to pure PVOH, but a small addition of PVOH did not have an effect as the elongation at break of GGMLab : PVOH 3:1 films was similar to that of films from pure GGM (Figure 6B).

Adding cAX to GGMLab at a GGMLab : cAX ratio of 3:1 did not significantly affect the tensile strength of the films, but at the ratio of 1:1, the tensile strength decreased to almost zero (Figure 1A in II). In contrast, the elongation at break of films increased slightly with increasing cAX content (Figure 1B in II). The mechanical properties of GGMLab : cAX 1:3 and 0:1 films could not be measured, because cAX was very sensitive to changes in ambient RH, and these films were difficult to handle at 65% RH, which was used for tensile testing.
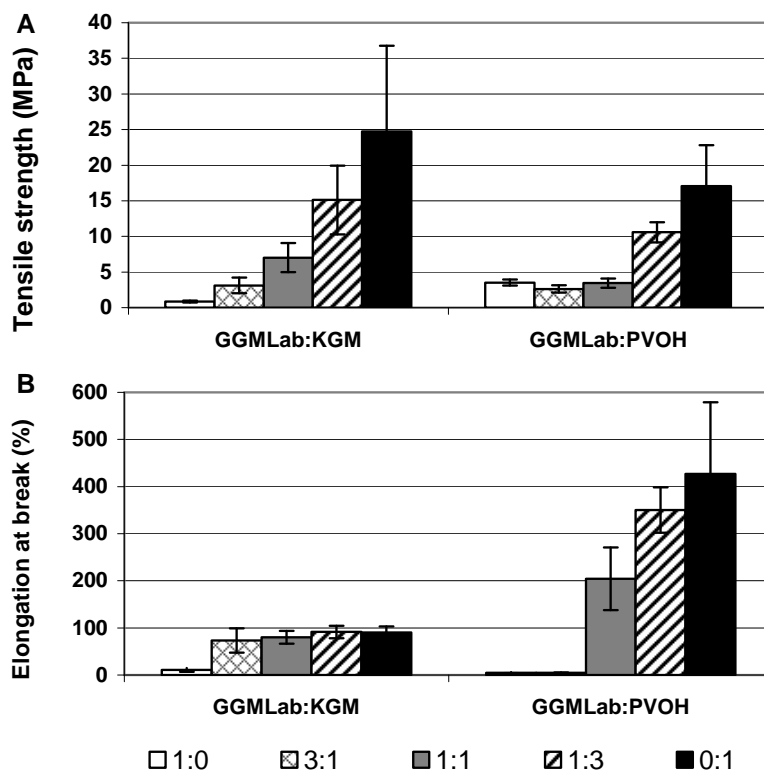
Figure 6. (A) Tensile strength and (B) elongation at break of films from GGMLab blended with PVOH and KGM at different ratios and plasticized with 40% (w/w of polymers) glycerol. Each average is based on n = 10 measurements, and the error bars indicate standard deviations. The measurements were done at 65% RH.

5.2.4 Glucomannan composites with cellulose nanowhiskers (III)

The improvement of the tensile properties of GGM and KGM-based films was also studied by mixing GGMSpDr and KGM with cellulose nanowhiskers (CNW). CNW are highly crystalline and have high tensile strength. Thus they are potential reinforcements and have been studied as reinforcements of polymer films.

The tensile strength of the KGM-based films not containing glycerol increased with increasing CNW content (Figure 7 in III). However, CNW did not significantly affect the other tensile properties of films. The tensile strength and Young's modulus of KGM-based films decreased and the elongation at break increased when glycerol was added, as expected. The tensile strength and elongation at break of GGMSpDr-based composite

films, plasticized with glycerol, were significantly lower than those of KGM-based composite films. However, the Young's modulus of GGMSpDr films was similar to that of the glycerol-plasticized KGM-based films. Surprisingly, the addition of CNW did not show any clear effect on the tensile properties of glycerol-plasticized KGM and GGMSpDr films.

5.3 Thermomechanical characterization

Thermal behavior was studied on films from the least and the most intensively degraded GGs, on the blend films from GGMLab with cAX, KGM, and PVOH, as well as on the GGMSpDr / CNW and KGM / CNW composite films.

5.3.1 Galactomannan films (I)

A decrease in E' and one or two peaks in E'' of galactomannan films were observed with increasing temperature, which indicates α-relaxation (glass transition, T_g) (Table 4). The DS clearly affected the T_g. Films from GGs with low DS (2Gal) showed a single peak in the E'' spectra (T_{g2}). Interestingly, for films from highly branched GGs (6Gal), two incorporating peaks were observed (T_{g1} and T_{g2}) (Figure 7). The DP also had an effect on the T_g. With the exception of films from high DS GGs (6Gal) plasticized with glycerol, a decrease in T_{g2} with decreasing DP was observed. The T_gs of films plasticized with sorbitol were at higher temperature than those of corresponding glycerol-plasticized films.

Table 4. Glass transition temperatures (T_{g1} and T_{g2}) of selected galactomannan films plasticized with 40% glycerol or sorbitol and conditioned at 50% RH (mean value $\pm$ s.d. from at least three measurements at 1 Hz frequency).

	Glycerol		Sorbitol	
	T_{g1} (°C)	T_{g2} (°C)	T_{g1} (°C)	T_{g2} (°C)
6Gal-H	−64 ± 1	−54 ± 2	−33 ± 4	−4 ± 1
2Gal-H	nd	−55 ± 3	nd	−7 ± 4*
6Gal-L	−62 ± 4	−24 ± 12	−36 ± 2	−11 ± 3
2Gal-L	nd	−61 ± 3	nd	−13 ± 1

nd = not detected

* only two measurements
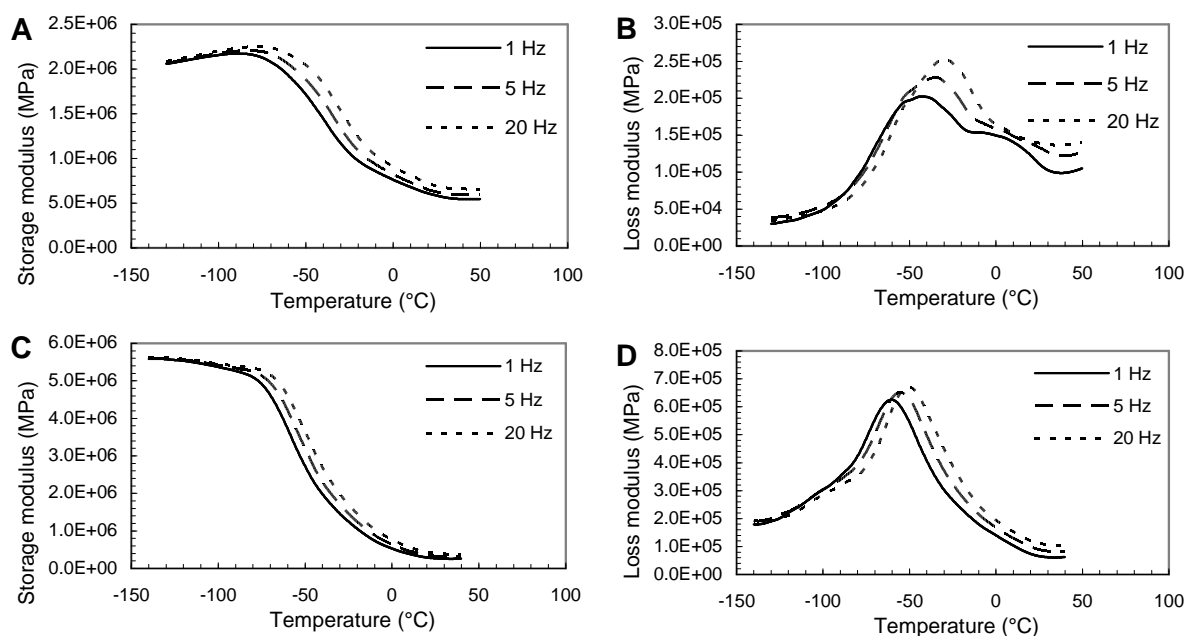
Figure 7. Representative storage modulus (E') and loss modulus (E") spectra for glycerol-plasticized 6Gal-L (A, B) and 2Gal-L (C, D) films at frequencies of 1, 5, and 20 Hz. The specimens were conditioned at 50% RH before analysis.

5.3.2 Glucomannan films (II, III)

The films from GGMLab and its blends with other polymers were dried in a vacuum oven before DMA measurements. All GGMLab / KGM and GGMLab / cAX films had a peak in E", which indicates a single glass transition (Figure 2 in II). The T_g of GGMLab / KGM

films varied within only a narrow range. The T_g of GGMLab / cAX blends increased with increasing cAX content, but the T_g of pure cAX-based films was lower and close to that of films based on pure GGMLab. There were two peaks in the E" spectra of films containing both GGMLab and PVOH (Figure 2 in II). The second peak at approximately –20 to –30ºC was more pronounced for GGMLab : PVOH 1:1 and 1:3 films than for films with the GGMLab : PVOH ratio of 3:1. The E" curves of pure PVOH-based films had a single peak with a shoulder.

The GGMSpDr- and KGM-based composite films were conditioned at 54% RH before measurement. Addition of plasticizer clearly affected the E' of KGM films (Figure 8). The films containing glycerol softened more than the films prepared without added plasticizer. The E' of GGMSpDr films, plasticized with 40% glycerol, decreased with increasing temperature even more than that of plasticized KGM films. The E" of plasticized KGM- and GGM-based films showed a peak at approximately –50°C indicating T_g. The addition of CNW did not have an effect on these properties, as the dynamic mechanical behavior of the mannan-CNW composites at both ratios studied was similar to that of the corresponding films from KGM and GGMSpDr not containing CNW (Figure 6 in III).

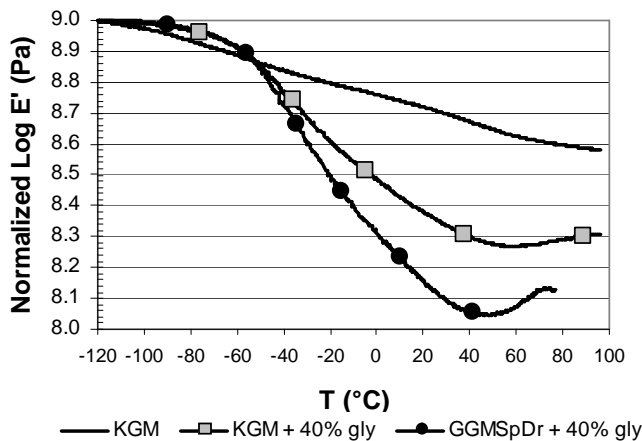Figure 8. Logarithm of the storage modulus (E') of KGM films with and without glycerol (gly) and GGMSpDr film with 40% glycerol. The E' at –120 ℃ was normalized at 1 GPa for all the samples. The specimens were conditioned at 54% RH before analysis.

5.4 Thermal degradation of glucomannan films (III)

To study the thermal degradation of the films, the specimens were heated to 600ºC using a thermal gravimetric analyzer (Figure 5 in III). The loss of water was seen close to 100ºC. KGM-based films not containing glycerol maintained their weight until higher temperatures than the glycerol-plasticized films. Their thermal degradation began steeply at approximately 250ºC, whereas that of the glycerol-plasticized KGM- and GGMSpDr-based films began between 100 and 150ºC and took place more gradually. The TGA curves of the KGM-based films not containing glycerol were almost identical up to approximately 280ºC, regardless of the use of CNW, after which the films with CNW degraded somewhat faster up to about 400ºC (Figure 5B in III). When glycerol was used, the addition of CNW at the KGM:CNW ratio of 95:5 slightly increased the degradation stability of the KGM-based films (Figure 5C in III). The glycerol-plasticized KGM:CNW 85:15 composite film had a lower degradation stability and a TGA curve of somewhat different shape than the corresponding composite film at the ratio of 95:5 and the pure KGM-based film. The degradation stability of the pure GGMSpDr-based film and of the composite film at the GGMSpDr:CNW ratio of 95:5 were also slightly higher than that of the GGMSpDr:CNW 85:15 composite film (Figure 5D in III).

5.5 Water content of films (I, III)

Water content was determined on films from galactomannans with the widest structural differences plasticized with glycerol or sorbitol and conditioned at 50% RH and on GGMSpDr and KGM films and GGMSpDr / CNW and KGM / CNW composite films conditioned at 54% and 76% RH. The water content of galactomannan films was about 10% (Table 5). Films from galactomannans with low DS (2Gal) had approximately 1% greater water content than the films from galactomannans with high DS (6Gal) (Table 6 in I). In addition, the water content of the films plasticized with glycerol was approximately 1% higher than that of the films plasticized with sorbitol. DP did not affect water content (Table 6 in I).

When conditioned at 54% RH, the water content of unplasticized KGM films was 8.2%, that of KGM films plasticized with 40% glycerol was 10.8% and that of GGMSpDr films plasticized with 40% glycerol was 11.8%. The water content of GGMSpDr / CNW and KGM / CNW composite films varied from 7.6% to 11.8%. KGM-based films not containing glycerol had the lowest water content, and GGMSpDr-based films that were plasticized with 40% glycerol, had the highest. The water content at 54% RH did not show a clear dependence on the amount of CNW (Table 5). In contrast, at 76% RH, the water content generally decreased with increasing amount of CNW, especially in the GGMSpDr-based films. The water content of the GGMSpDr film was 22%, whereas that of the GGMSpDr:CNW 85:15 film was only 15%. Similarly as at 54% RH, the KGM films not containing glycerol absorbed least water, 10.7–13.9% (Table 2 in III).

Table 5. Water content of films at 54% RH (mean value ± s.d. from three measurements).

Film	Water content (± s.d.) (wt.-%)
6Gal-H + 40% glycerol	10.4 ± 0.4*
2Gal-H + 40% glycerol	11.7 ± 0.5*
6Gal-L + 40% glycerol	10.3 ± 0.3*
2Gal-L + 40% glycerol	11.5 ± 0.5*
KGM	8.2 ± 0.6
KGM:CNW 95:5	9.9 ± 0.8
KGM:CNW 85:15	7.6 ± 0.4
KGM + 40% glycerol	10.8 ± 1.2
KGM:CNW 95:5 + 40% glycerol	10.2 ± 0.3
KGM:CNW 85:15 + 40% glycerol	10.7 ± 1.0
GGMSpDr + 40% glycerol	11.8 ± 0.6
GGMSpDr:CNW 95:5 + 40% glycerol	10.7 ± 0.4
GGMSpDr:CNW 85:15 + 40% glycerol	11.8 ± 0.6

* 50% RH

5.6 Morphology of glucomannan films

5.6.1 Optical microscopy of films (II, III)

Blend films from GGMLab with KGM, PVOH, and cAX, as well as GGMSpDr / CNW and KGM / CNW composite films were viewed with OM and SEM. OM imaging with visible light showed that pure GGMLab-based films had a slightly grainy surface (Figure 9A). The films contained elevated spots that autofluoresced. When viewed with the unaided eye, the KGM-based films were clear and transparent, but OM showed the presence of some irregular-shaped agglomerates that could have originated from less soluble fractions of KGM (Figures 9C and 11A). The surfaces of GGMLab / cAX and GGMLab / KGM blend films looked much alike. Figure 9B presents an image of GGMLab : KGM 1:1 film as an example. All films containing GGMLab had a somewhat grainy surface, whereas those from pure cAX were smooth. Films not containing GGMLab did not show significant fluorescence.

Figure 9. Optical microscopic images of surfaces of glycerol-plasticized (gly) films from (A) GGMLab; (B) GGMLab : KGM 1:1; and (C) KGM obtained using visible light. Scale bar = 1 mm. All images are at the same magnification.

Addition of PVOH resulted in increased unevenness of the film surfaces (Figure 10). Films with GGMLab : PVOH ratios of 3:1 and 1:1 had a much rougher surface than the films from pure GGMLab. The films with GGMLab : PVOH ratio of 1:3 had a pattern of irregular minor shapes immersed in a continuous phase. In contrast, the surfaces of pure PVOH-based films were very smooth.
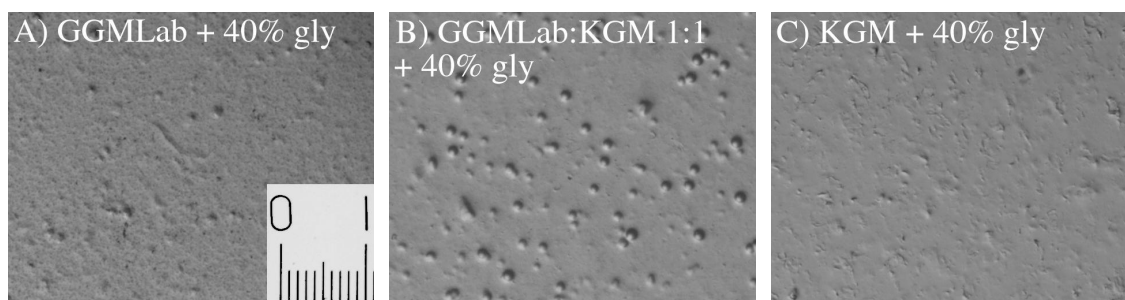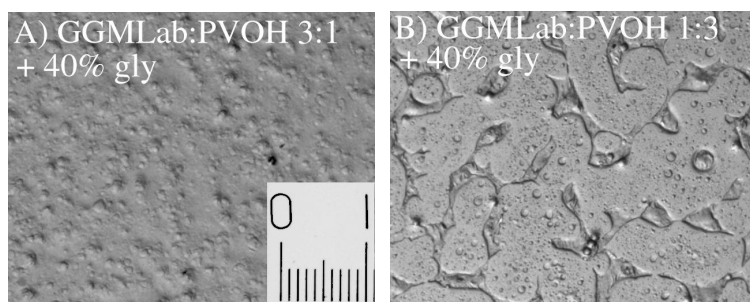
Figure 10. Optical microscopic images of surfaces of glycerol-plasticized (gly) films from (A) GGMLab : PVOH 3:1 and (B) GGMLab : PVOH 1:3 obtained using visible light. Scale bar = 1 mm. Both images are at the same magnification.

Addition of CNW caused a remarkable change in the appearance of the films immediately after drying. In KGM films, CNW induced the formation of fibrous structures visible with naked eye and shown also by OM (Figure 11B). The appearance of the KGM-CNW composite films was similar regardless of the use of glycerol or of the KGM:CNW ratio. The fibrous structures were not aligned, but ran randomly in all directions along the film plane. Polarizing OM confirmed the extraordinary effect of CNW on the structure of KGM-based films. Bright components seen with polarizing OM are visible due to their characteristic of orientating polarized light, which indicates crystalline structures. In the films from pure KGM, the crystalline structures appeared as single, relatively small, bright spots scattered all over the films (Figure 11C). KGM films containing glycerol had more structures visible with polarizing OM than the unplasticized films. In the KGM-CNW composites, the bright components were organized as long and fibrous structures resembling those seen with regular OM (Figure 11D).

CNW also clearly changed the structure of GGMSpDr-based films. OM study of the GGMSpDr / CNW composites showed the presence of rods with a length of tens of micrometers, which were absent in the film from pure GGMSpDr (Figure 2A in III). When viewed with polarizing OM, the film from pure GGMSpDr had some poorly visible bright spots a few micrometers in size, but the GGMSpDr-CNW composites contained bright rods corresponding to the size of structures seen with regular OM (Figure 2B in III). The size of polarizing structures increased with increasing content of CNW.

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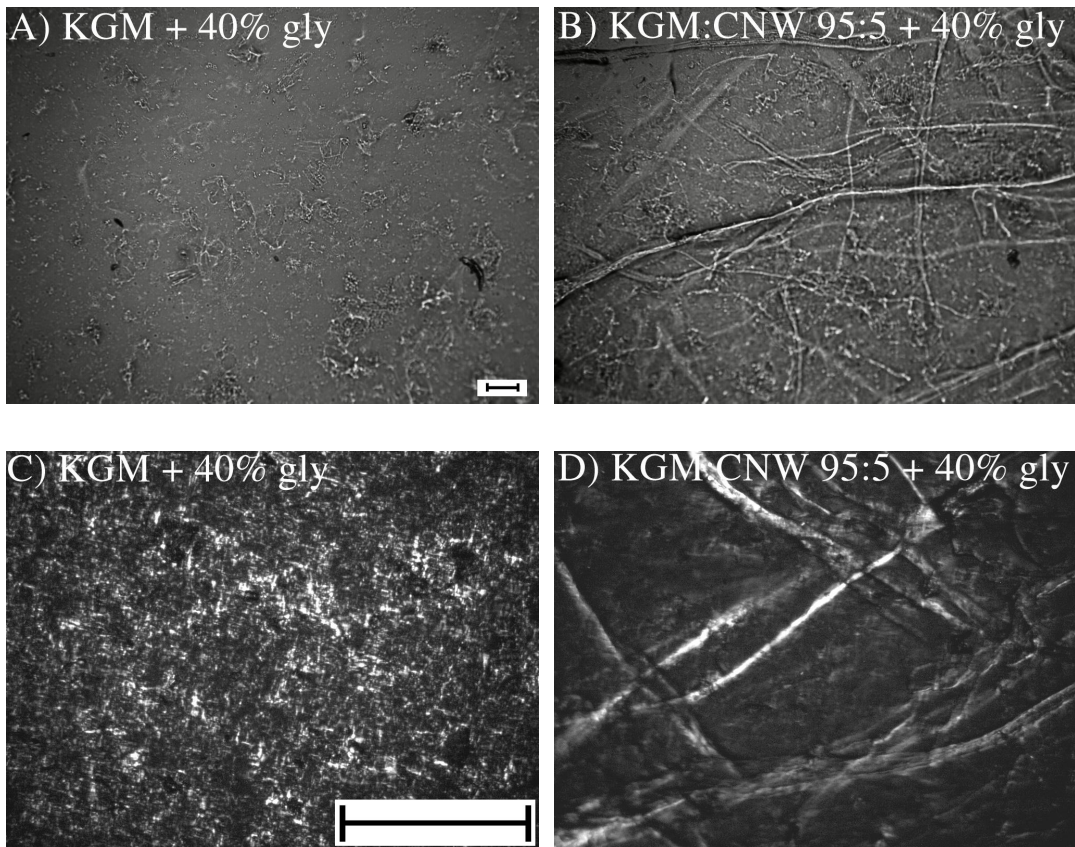
Figure 11. Optical microscopic images of films from (A) KGM and (B) KGM:CNW 95:5 (weight ratio) plasticized with 40% glycerol (gly) (wt.-% of KGM and CNW) and polarizing optical microscopic images of films from (C) KGM and (D) KGM:CNW 95:5 plasticized with 40% glycerol. The scale bar in A is 0.1 mm and images A and B are at the same magnification. The scale bar in C is 1 mm and images C and D are at the same magnification.

5.6.2 Scanning electron microscopy of film cross-sections (II, III)

The cross-section of pure GGMLab-based film, viewed with SEM, was somewhat grainy with some dark and dense areas (Figure 12A). The cross-sections of KGM film and GGMLab : KGM 1:1 blend film were even and homogeneous (Figure 12B and 12C). The cross-sections of GGMLab / cAX blend films showed a homogeneous structure consisting of a single phase (Figure 6 in II).

A) GGMLab + 40% gly

B) GGMLab:KGM 1:1 + 40% gly

20.0μm

C) KGM + 40% gly

Figure 12. Scanning electron micrographs of cross-sections of glycerol-plasticized (gly) films from (A) GGMLab; (B) GGMLab : KGM 1:1; and (C) KGM. Scale bar = 20 µm. All images are at the same magnification.

The blend films from GGMLab with PVOH clearly showed separation into two distinct phases. The film with GGMLab : PVOH ratio of 3:1 (Figure 13A) had spherical particles of different sizes in a continuous phase that resembled the cross-section of the film from pure GGMLab. When the ratio of GGMLab to PVOH was 1:1, the two phases were formed as layers on top of each other (Figure 5C in II) and in some areas they had separated (Figure 5D in II). At the GGMLab : PVOH ratio of 1:3 (Figure 13B), the continuous phase looked similar to the cross-section of pure PVOH-based film (Figure 5F in II), and there were small round and oval particles inside.

A) GGMLab:PVOH 3:1 + 40% gly

B) GGMLab:PVOH 1:3 + 40% gly

20.0μm

Figure 13. Scanning electron micrographs of cross-sections of glycerol-plasticized (gly) films from (A) GGMLab : PVOH 3:1 and (B) GGMLab : PVOH 1:3. Scale bar = 20 µm. Both images are at the same magnification.

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The effect of CNW was seen as increased heterogeneity of the film cross-section (Figure 3 in III). The layered structure of KGM-CNW composite films viewed with SEM (Figure 14 A) could correspond to the fibrous shapes visible with naked eye and with OM. In GGMSpDr-based films, the increase of CNW content caused increasing porosity (Figure 14B).
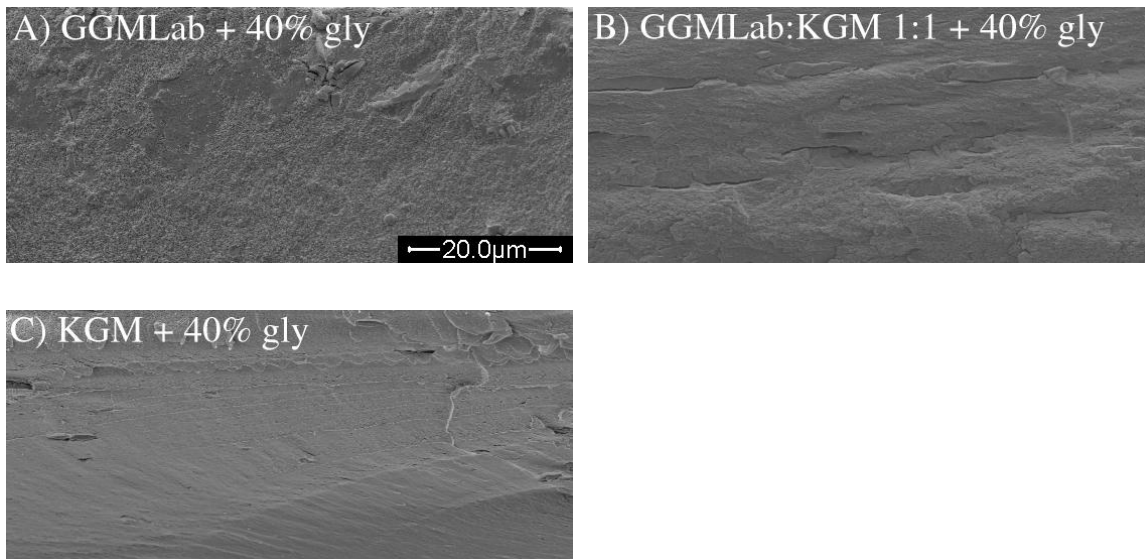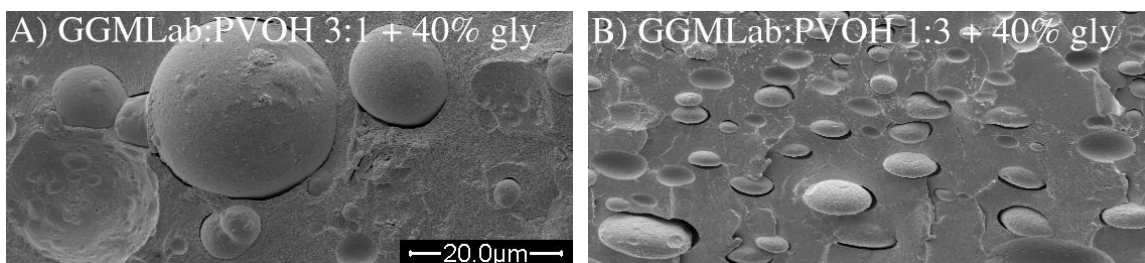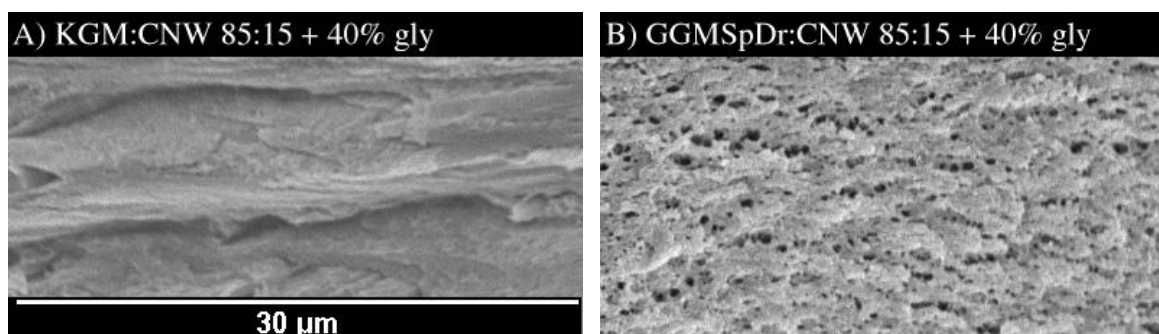

Figure 14. Scanning electron micrographs of cross-sections of glycerol-plasticized (gly) films from (A) KGM : CNW 85:15 and (B) GGMSpDr : CNW 85:15. Scale bar = 30 µm. Both images are at the same magnification.

5.6.3 Crystallinity of mannan (III)

The degrees of crystallinity of mannan in the films calculated from X-ray diffraction patterns are shown in Table 1 in III. Figure 4 in III shows the X-ray diffraction patterns of unplasticized and plasticized KGM:CNW 85:15 composite films, from which the diffraction of cellulose has been subtracted. The unplasticized KGM films had the lowest degree of mannan crystallinity, about 12–13%. Addition of glycerol increased the crystallinity of mannan in the KGM films to 25–31% and the mannan crystallinity in the glycerol-plasticized GGMSpDr films was 24–27%. The addition of CNW somewhat increased the crystallinity of mannan in the plasticized KGM films.

5.7 Permeability properties of mannan-based films (IV)

The most interesting film types were selected from studies I-III for the comparison of the different permeability properties. The films were plasticized with sorbitol because it was recently shown to result in lower WVP and OP than glycerol in xylan films (Mikkonen *et al.*, 2009).

5.7.1 Water vapor permeability

The WVP test conditions were selected so that the RH gradient was approximately 50%. Use of desiccant inside the test cups is recommended by the ASTM Standard Test Methods for Water Vapor Transmittance of Materials (2005a) and it gives reliable and comparable results with small standard deviation. However, the water activity of food is rarely even close to zero, so also the gradient of 32/86% RH was used to have conditions closer to those of a real food system. At both gradients used, the WVP of GGMEtOH films was lower than that of any other mannan film, 1.6 and 19 [g·mm/(m^2·d·kPa)], respectively (Table 6). When GGMEtOH was blended with PVOH, particularly at the ratio of GGMEtOH:PVOH 1:3, the WVP was even lower. Use of GGMEtOH significantly decreased the WVP of films containing KGM at the gradient of 0/54% RH, but not at 32/86% RH. Addition of CNW did not affect the WVP of GGMEtOH films, but somewhat decreased the WVP of KGM films at 0/54% RH.

5.7.2 Oxygen permeability

The OP of KGM films was slightly higher than that of GGMEtOH films (Table 6). GGM and KGM seemed to have some synergism, as the OP of their blends was lower than that of films from either of the raw materials alone. Unexpectedly, the use of CNW did not affect the OP of films, even though CNW remarkably changed the visual appearance of the films. The GGMEtOH:PVOH 3:1 blend and the film from GG leaked and thus only one and two replicate OP measurements were successful, respectively. The OP of those

films as well as of the film from LBG was much higher than that of all others. Interestingly, at the ratio of GGMEtOH:PVOH 1:3, PVOH was able to decrease the OP of GGM films.

The OP of a commercial PE/EVOH/PE film was tested as comparison, because EVOH is widely used as an oxygen barrier material. Due to its high cost and sensitivity to water, it is usually laminated with some cheaper and more hydrophobic material. The OP of the PE/EVOH/PE laminate used was 4.4 $[cm^3 \cdot \mu m/(m^2 \cdot d \cdot kPa)]$, which is of the same magnitude than that of GGMEtOH films whether pure, with CNW, or blended with KGM,

Table 6. Water vapor permeability (WVP) of films at different RH gradients and 22 ºC (mean value ± standard deviation from three measurements) and oxygen permeability (OP) of films at 50–75% RH and 22 ºC (mean value ± standard deviation from four measurements). The films were plasticized with 40% (w/w of polymers) sorbitol.

Film	WVP (RH 0/54%) ± sd [g·mm/(m²·d·kPa)]	WVP (RH 32/86%) ± sd [g·mm/(m²·d·kPa)]	OP (RH 50–75%) ± sd [cm³·µm/(m²·d·kPa)]
GGMEtOH	1.6 ± 0.05	19 ± 1	6.8 ± 3***
KGM	3.6 ± 0.3	24 ± 1	8.1 ± 3
GG	3.4 ± 0.5	38 ± 1	39.7 ± 15**
LBG	2.6 ± 0.1	37 ± 1	17.7 ± 7
GGMEtOH:KGM 3:1	1.7 ± 0.2	26 ± 3	4.2 ± 1
GGMEtOH:KGM 1:3	1.9 ± 0.3	29 ± 1	5.1 ± 3
GGMEtOH:PVOH 3:1	1.0 ± 0.1	22 ± 3	21.9*
GGMEtOH:PVOH 1:3	0.4 ± 0.06	15 ± 2	3.6 ± 2
PVOH	0.5 ± 0.03	15 ± 2	9.2 ± 2
GGMEtOH:CNW 95:5	1.9 ± 0.3	22 ± 3	6.5 ± 2
KGM:CNW 95:5	2.6 ± 0.4	24 ± 2	6.6 ± 2***

* Only one successful measurement
** Two replicate measurements
*** Three replicate measurements

or PVOH at the ratio of GGMEtOH:PVOH 1:3. The result can also be calculated by taking into account only the thickness of the EVOH layer in the laminate, because that layer is responsible for the oxygen barrier properties of the laminate film. That way the OP of EVOH was 0.3 [$cm^3 \cdot \mu m/(m^2 \cdot d \cdot kPa)$], which is clearly lower than the OP measured for GGMEtOH-based films.

5.7.3 Aroma permeability

Due to the long testing time, only two film types, GGMEtOH and KGM plasticized with 40% glycerol, were selected for aroma permeability measurement. The films tested had very low aroma permeability. The diffusion coefficients were lower for GGMEtOH films than for KGM films (Table 2 in IV). GGMEtOH and KGM films were especially good barriers against R-carvone, which did not pass the films during the measurement time (10 days).

5.7.4 Light transmission and haze

All films were transparent to visible light (Figure 15). KGM films had the highest light transmittance throughout the scanned wavelength region. All films showed a small drop in the light transmittance at approximately 290 nm, and the films from galactomannans GG and LBG had another drop at about 350 nm wavelength. GGMEtOH-based films blocked the transmission of ultraviolet light. Use of PVOH at the GGMEtOH:PVOH ratio of 3:1 somewhat decreased the light transmittance. Addition of CNW did not affect the light transmittance.

The haze values were low for KGM films and for films with a high content of KGM (KGM:CNW 95:5, and GGMEtOH:KGM 1:3) (Table 3 in IV). The haze of GGMEtOH films was 23.5%, which was clearly higher than the haze of KGM films, 4.6%, but of the same order of magnitude as the haze of GG and LBG films. Addition of CNW slightly increased the haze of both KGM and GGMEtOH films. Blending of PVOH clearly

increased the haze of GGM-containing films. The highest haze value was measured for pure PVOH films.
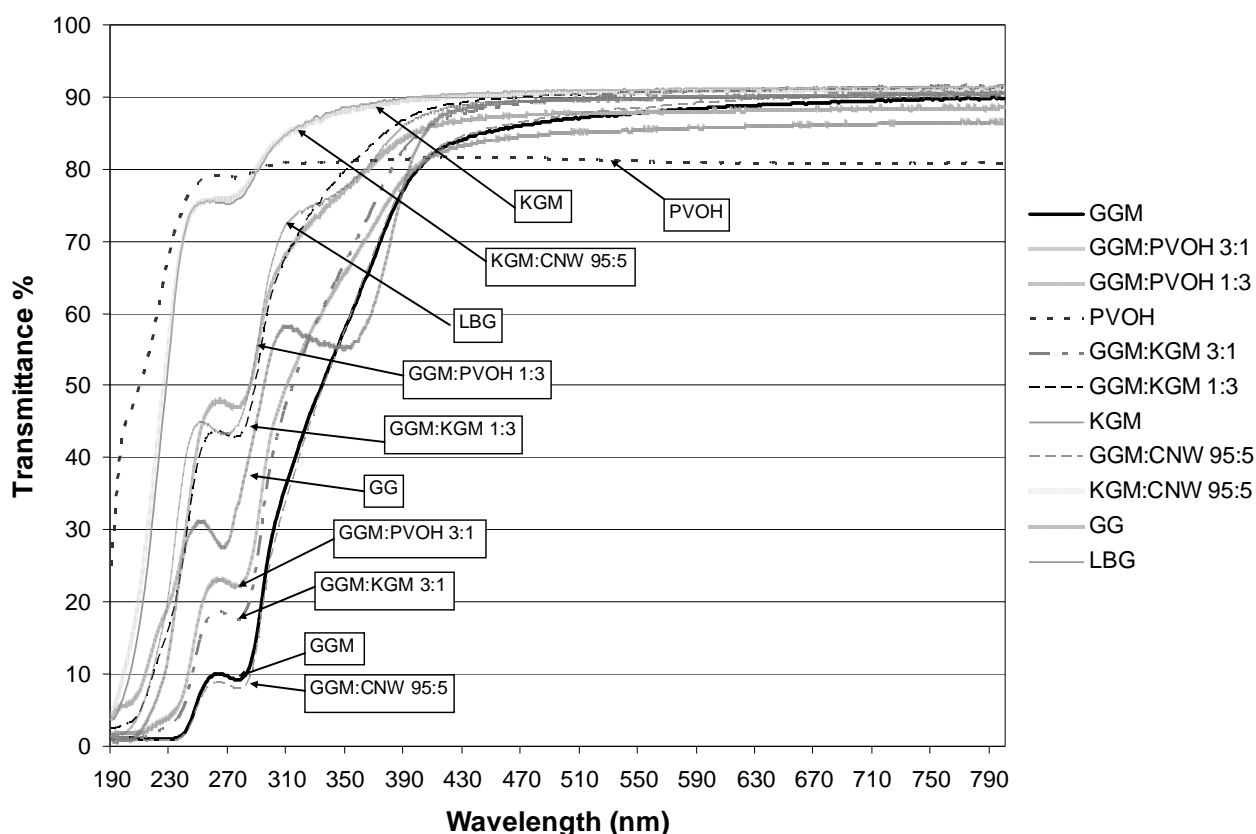
Figure 15. Light transmittance of sorbitol-plasticized films.

5.8 Mannans as stabilizers of oil-in-water beverage emulsions (V)

Emulsions were stabilized with GGMSpDr, GGMEtOH, GG, LBG, KGM, and cAX. Immediately after preparation, the emulsions containing GG or LBG had a lower turbidity than the control emulsions (Figure 16A). The turbidity of KGM emulsions was similar to that of the control samples. Addition of either of the GGMs (GGMEtOH or GGMSpDr) or cAX increased the turbidity of the emulsions. During 14 days at RT, the control emulsions separated almost completely and their turbidity decreased to close to zero (Figure 16B). The turbidities of emulsions containing GG, LBG, and KGM were low after 14 days. The higher turbidity of GGM emulsions when compared to the other studied mannans was

seen at each measuring point. The average turbidity of GGMEtOH emulsions after 14 days was 9.8 cm^{-1} and that of cAX emulsions was 13.6 cm^{-1}. Decrease in the DP of galactomannans clearly decreased emulsion stability (Figure 3 in V). Decreasing DS resulted in increased turbidity.

For comparison, emulsions were prepared also using GA, which is the most well-known hydrocolloid for emulsion stabilization. At a polysaccharide/oil ratio of 0.05, the turbidity of GA-stabilized emulsions was at the same level or slightly lower than that of
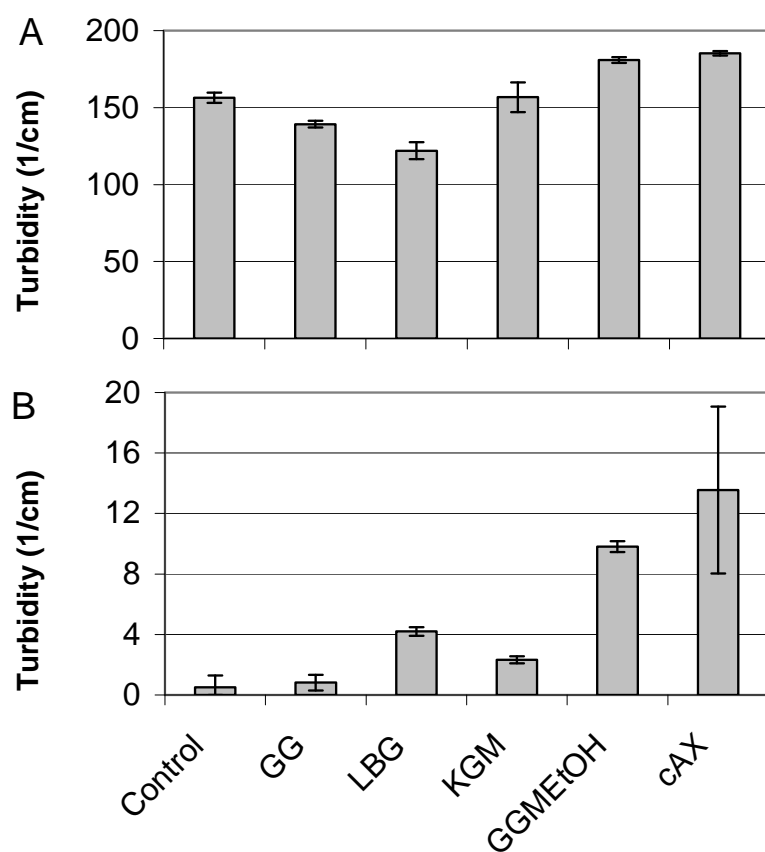
Figure 16. Turbidities of emulsions containing different mannans and cAX at polysaccharide/oil ratio of 0.05 (A) immediately after preparation and (B) after 14 days at RT. The averages are based on three absorbance measurements at 650 nm wavelength and the error bars indicate standard deviations.

the GGMEtOH emulsions throughout the observation time. However, this polysaccharide/oil ratio is not optimal for GA-stabilized emulsions (Dickinson, 2003; Mirhosseini *et al.*, 2008b; Tse and Reineccius, 1995) and therefore further studies are required to extensively compare the emulsion-stabilizing properties of GGM to those of GA.

Using either of the GGMs increased the emulsion turbidity (Figure 17), with an increasing amount of GGMs generally increasing the turbidity. After 14 days, GGMSpDr showed the highest turbidity at the highest studied polysaccharide/oil ratio, 0.20:1. In contrast, the emulsions containing GGMEtOH reached the maximum turbidity at the ratio of 0.10:1. Differences in turbidity between the polysaccharide/oil ratios of 0.05:1 and 0.10:1 were relatively small for the both GGMs.

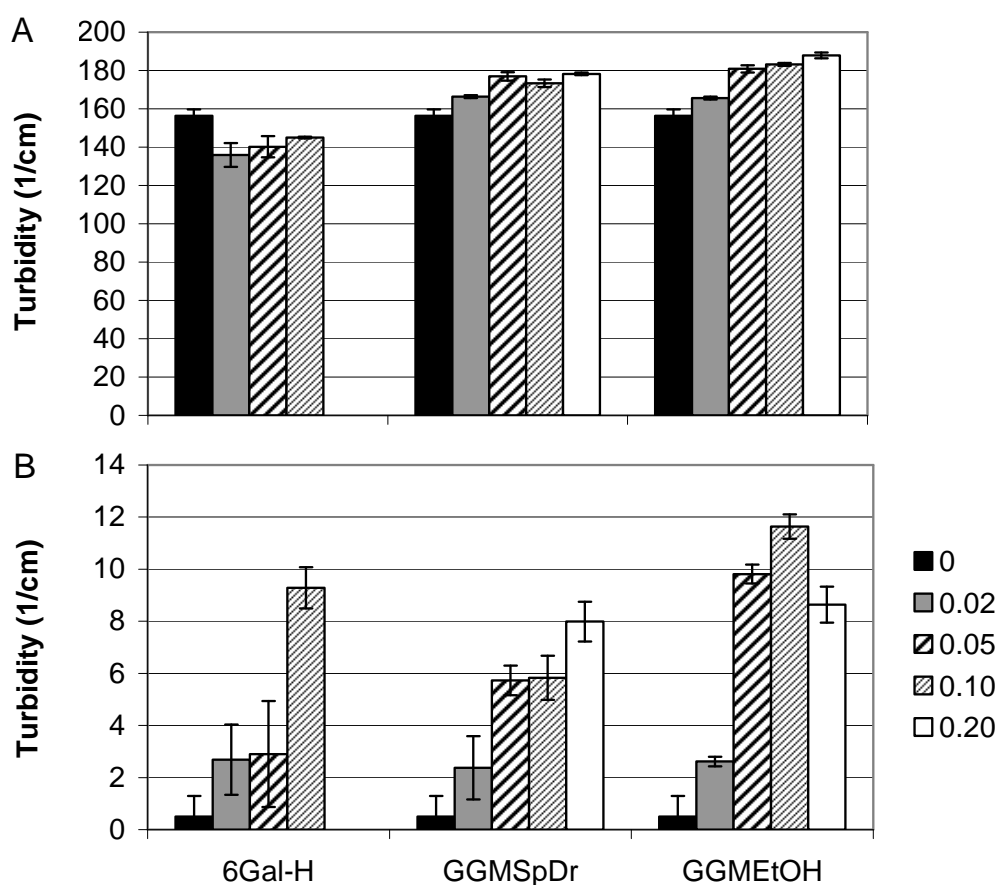Figure 17. Turbidity of 6Gal-H, GGMSpDr, and GGMEtOH-stabilized emulsions at different polysaccharide/oil ratios (A) immediately after preparation and (B) after 14 days storage at RT. The averages are based on three absorbance measurements at 650 nm wavelength and the error bars indicate standard deviations.

Storage tests at elevated temperatures are considered as the most relevant means to shorten the analysis time to determine emulsion stability. The emulsion breakdown promoting effect of increased temperature is due to the loss of viscosity and to the greater mobility of the system (Tse and Reineccius, 1995). Such an effect was clearly seen in the present study, as the turbidity of emulsions stored at 45ºC for three days was generally lower than that of the emulsions stored at lower temperatures (Figure 5A in V). During seven days storage at 45ºC, the emulsion turbidity decreased to close to zero, except for GGMEtOH emulsions, which had a slightly higher turbidity (Figure 5B in V). Interestingly, the emulsions stored at 23ºC for three days had higher turbidity than the corresponding emulsions stored at 4ºC (Figure 5A in V).

After seven days, the emulsions containing 6Gal-H and cAX showed the highest turbidity when stored at 4ºC (Figure 5B in V). For GGMSpDr emulsions, the storage temperature of 23ºC resulted in a higher turbidity than 4ºC and the turbidity of GGMEtOH emulsions did not differ significantly between those two storage temperatures. However, after 14 days, the decreasing trend in turbidity with increasing temperature was clearly seen for all samples studied (Figure 18).
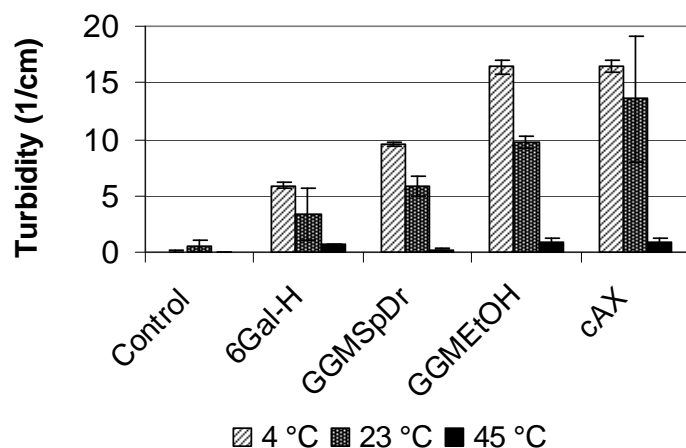
Figure 18. The effect of storage temperature on the turbidity of emulsions stabilized by 6Gal-H, GGMSpDr, GGMEtOH, and cAX after 14 days. The averages are based on three absorbance measurements at 650 nm wavelength and the error bars indicate standard deviations.

The GGM emulsions stored at RT were viewed by confocal microscopy after 3, 7, and 14 days (Figure 6 in V). The size of the detected particles did not significantly change

from approximately 1 µm during the observation time, but the particle number decreased with time.

6 DISCUSSION

6.1 Films

6.1.1 Tensile properties (I-III)

Apart from KGM, mannans did not form cohesive and continuous films without the addition of plasticizer. Hartman *et al.* (2006a) also discovered that a plasticizer was needed to avoid brittleness of GGM films, even though they reported the preparation of a fragile film from pure GGM. This is the first study on the tensile properties of GGM films, as Hartman *et al.* (2006a, 2006b) did not analyze the tensile strength or elongation at break of films.

The tensile testing results with modified GG-based films suggest that removal of side groups might facilitate the sliding of polymers across each other, thereby increasing the elongation at break of films. The increase in tensile strength could be due to more dense packing of mannan chains resulting in increased hydrogen bonding. The results are in good agreement with a previous study on interactions of galactomannans (Dea *et al.*, 1986). The distribution pattern of galactose side groups of the galactomannan samples was not examined. It is not known whether α-galactosidase is selective for galactose residues attached to mannose units next to other substituted mannose units in the galactomannan backbone, or those next to unsubstituted mannose units. It is therefore difficult to speculate on the possible distribution pattern of the enzymatically modified samples in the present study.

Höije *et al.* (2008) found that stronger and more flexible films were formed when some of the arabinose side groups of rye arabinoxylan were enzymatically removed. However, further debranching decreased the tensile properties of films, whereas in the present study, the GGs with the lowest DS formed films with the highest tensile strength and elongation at break. KGM, which does not contain galactose branches, formed the strongest mannan films. Instead of galactose, KGM has acetyl groups, which are responsible for its water-

solubility. Acetyl groups are smaller molecules than galactose and present in KGM only in a small number, so they may not hinder the dense packing of KGM chains in the films.

The results showed an optimum DP for galactomannans at which they formed the strongest and most flexible films. The improvement of tensile properties by a controlled decrease in the DP could be due to enhanced solubility and decreased viscosity, and thus better orientation of the polymers during film formation. Decreasing DP further by mannanase dosage of 50 nkat/g resulted in polymeric chains that were too short, with poor film-forming properties. Thus reduction of DP seemed to have two contrasting effects: the improvement of solubility and the decrease of cohesive forces between film-forming polymers. The results are in agreement with a study by Cheng *et al.* (2007), who hydrolyzed KGM with hydrochloric acid and found that the tensile strength of films increased with initial increase of hydrolysis level. They suggested that the increase of tensile strength could be explained by chain flexibility and mobility, degree of entanglement, and available segmental motion.

Although the solubility of GGs was not good enough for a proper M_w determination that would have allowed more detailed information to be obtained about the optimum DP of GGs, M_w values for some similarly modified GGs have been reported as 100–250 kDa following 10 nkat/g mannanase treatment (medium low DP) and 85–150 kDa following 50 nkat/g treatment (low DP) (Hannuksela *et al.*, 2002). Thus a relatively small decrease in M_w might have resulted in significant changes in the mechanical properties of films prepared from GGs, when comparing GGs with medium low DP to those with low DP. The M_w of GGM is clearly lower than that of other mannans and of low DP GGs, which most probably explains the lower tensile properties of GGM films when compared to films from other mannans. Interestingly, films from filtered GGMSpDr and GGMEtOH had higher tensile strength than films from some of the modified GGs, including those with low DP. On the other hand, the elongation at break of GGM films was lower than that of films from the modified GGs.

The effect of the enzymatic treatment can be evaluated also by comparing the properties of films from 6Gal-H and native GG. The 6Gal-H sample went through the

dissolving, heating, ethanol washing, and lyophilization treatments required for the modification, but without the addition of enzymes. In comparison, native GG was not treated in any way. The mechanical properties of films from native GG and 6Gal-H containing 40% plasticizer were similar. The elongation at break and tensile strength of films from LBG containing 40% of glycerol or sorbitol were higher than those of corresponding 2Gal-H and 4Gal-H-based films, which were expected to have DP and DS close to those of LBG. The differences could be due to a decrease in the water solubility of GGs after lyophilization, especially those with high DP and low DS. The distribution pattern of galactose substituents along the mannan backbone may also be different in LBG and modified GGs. The mechanical properties of films made from 2Gal-MH were close to those of films from LBG when 40% plasticizer was used. Hence, it is possible to get films resembling LGB-based films from GG by applying targeted enzymatic modifications. The elongation at break and tensile strength of the best modified GG-based films were high also in comparison with those of potato starch-based films plasticized with comparable glycerol dosage and analyzed in similar conditions (Talja *et al*., 2007).

Several studies on KGM-based blend films have shown that there is an optimum polymer ratio that gives higher film tensile strength than either of the two polymers alone (Yue *et al*., 1995, Xiao *et al*., 2000a, Xiao *et al*., 2000b, Xiao *et al*., 2001, Li and Xie, 2004, Li *et al*., 2006b, Ye *et al*., 2006). For example, blends of KGM and PVOH reached a maximum tensile strength at 20% PVOH content (Xiao *et al*. 2000a), and also in the presence of glutaraldehyde, the tensile strength of KGM / PVOH blends was higher than that of films purely from either KGM or PVOH (Li and Xie 2004). Relatively similar or high molar masses of the blended polymers could promote synergism. The molar mass of GGM is significantly lower than that of PVOH and KGM, and in the present study there was not an optimum blend ratio, as the films from pure PVOH and KGM had clearly higher tensile strength than any of the GGMLab / PVOH or GGMLab / KGM blends. Based on SEM imaging, it can be reasoned that the tensile properties of the GGM blend films were determined mainly by the properties of the continuous phase, which was formed of a homogeneous mixture of two polysaccharides in GGMLab / cAX and GGMLab / KGM blends, but of either GGM-rich or PVOH-rich phase in the films made from those two polymers, which seem to be incompatible.

At least three explanations for the lack of a larger effect of CNW on the mechanical strength and stiffness of its composite films can be considered: 1) CNW were heterogeneously dispersed in the films, 2) The fibrous and rod-like structures induced by CNW had low mechanical strength, or 3) The interaction between these structures and the film matrix was weak. In all cases, the major continuous and mainly amorphous film matrix would be responsible for the mechanical strength of the films. Glycerol and/or water could attach on the interface of the crystallites and the amorphous film matrix, lubricating the interfacial area and reducing the load transfer between these components. The increase in tensile strength with increasing CNW content in KGM-based films not containing glycerol supports this reasoning. Unfortunately, GGM does not form cohesive films without external plasticizer and thus the difference noticed with KGM could not be verified with GGM.

6.1.2 Dynamic mechanical properties (I-III)

The two separate T_gs for the modified GGs with high DS and GGMLab / PVOH blends indicated the release of the motion of the molecules in two stages, which could result from the presence of weaker and stronger interactions between film components. The separate glass transitions observed in the waxy maize starch-glycerol system have been explained by at least partial phase separation of polymer and plasticizer (Mathew and Dufresne, 2002). Gaudin *et al.* (1999) associated clusters of sorbitol in starch-based films with greater system mobility. In the present study, the high DS of GGs might have led to uneven distribution of plasticizers and to the formation of polyol-rich and polymer-rich phases in the film matrix. In the polyol-rich phase, there could have been greater than average amounts of polyol-polyol and galactomannan-polyol-polyol interactions. The motion of the bonds associated with plasticizer could be released at a lower temperature, resulting in the first detected T_g (T_{g1}). The second T_g (T_{g2}), at a higher temperature, could be due to the release of the motion of molecules in the polymer-rich phase. Removing galactose side groups possibly enabled more homogeneous distribution of polyols and GGs and thus resulted in only a single T_g. This reasoning is supported by the fact that the T_{g2} of films from highly substituted GGs was on average somewhat higher than that of

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films from GGs with low DS, which showed only one glass transition. In a system consisting of two phases, the polymer-rich phase would contain less plasticizer and therefore have higher T_g than a system with an even distribution of polyols. The dependence of thermal behavior of films on DS of GGs was seen in both glycerol- and sorbitol-plasticized films.

Distinct glass transitions clearly indicate that GGMLab and PVOH were incompatible and that phase separation took place. Interestingly, the T_g values of the films from pure GGMLab and PVOH were close to each other and to the first detected T_g of the blends (T_{g1}), but the second T_g of the blends (T_{g2}) was clearly higher. It is possible that glycerol was unevenly distributed in the two separate phases, the GGM-rich and PVOH-rich phase. Plasticization decreases T_g, so the T_{g1} could have resulted from the phase containing more glycerol and T_{g2} from the phase with less glycerol.

The similar temperature of relaxation of the amorphous phases of the KGM:CNW and GGM:CNW composite films, shown by DMA, indicates that the degree of plasticization by water and glycerol in that phase did not change when CNW were added, which also supports the idea that some water and glycerol was located either inside or on the surface of the fibrous and rod-like structures as well as in the main film matrix. This would also be in agreement with a study of starch-CNW composites by Anglès and Dufresne (2000), who concluded that glycerol accumulated in the amylopectin-cellulose interface. To reduce the interference of glycerol during film preparation, mannans and CNW were first mixed in water and after that glycerol was added. The formation of the fibrous and rod-like structures could occur during the mixing of the film solution, and glycerol, when added afterwards, could attach on the surface of these structures. An experiment was made by mixing KGM and CNW in water containing glycerol, and the formation of fibrous structures similar to that described earlier in this thesis was noted.

Hartman et $al.$ (2006a, 2006b) used moisture scanning DMA to characterize GGM-based films. A drop in E' of the plasticized film samples was observed between approximately 35% and 50% RH in the DMA graph. The glycerol-containing film clearly softened more rapidly than the sorbitol- or xylitol-plasticized films. The E' measured by

Hartman *et al*. (2006a, 2006b) was lower than that measured in the present study (Figure 2 in II), indicating that the films prepared in the present study were stronger than those prepared by Hartman *et al*.

6.1.3 Morphology (II, III)

GGMLab isolated in laboratory scale contained a small amount of insoluble particles, some but not all of which were removed by filtration through a steel sieve of 45 µm used in the preparation of GGMLab / cAX sample set. Those particles most probably formed the elevated spots observed by optical microscopy using visible light. The same spots autofluoresced, indicating that they could have contained residual aromatic structures originating from spruce wood. Because the aromatic substances are present in GGM only in trace amounts, reliable analytical data of their composition has not been obtained up to date. However, it is most likely that these structures arise from fragments of lignin-like moieties, possibly from so-called lignin-carbohydrate complexes.

OM and SEM showed clearly that GGMLab and PVOH were immiscible. Microscopical studies by Xiao *et al*. (2000b) showed phase separation of PVOH and KGM, the latter of which is similar in sugar composition to GGM. IR spectroscopy showed that there was some hydrogen bonding between the two polymers, but not as much as between KGM and other polysaccharides. This could also explain the results of the present study. GGMLab might form strong hydrogen bonding and homogeneous mixtures with the other studied polysaccharides cAX and KGM, but not with PVOH, which has only one hydroxyl group attached to every second carbon atom of the polymeric chain.

The addition of CNW greatly changed the appearance of KGM-based films. This could be seen even with naked eye, and was confirmed with microscopy. The visible structures in the composites were several magnitudes larger than the size of CNW. Chanzy *et al*. (1982) showed that glucomannan can crystallize on cellulose microfibrils, which, however, are much longer than CNW. Structures of similar size have not been reported

earlier in films from other polymers with CNW. Study with OM and SEM showed that CNW induced morphological changes also in the GGMSpDr-based films. The structures formed in the GGMSpDr / CNW composite films were considerably smaller than those in the KGM / CNW films. This was most likely affected by the lower molar mass of GGMSpDr than that of KGM. The pores seen in GGMSpDr / CNW films with SEM could derive from air bubbles, pulled-out undissolved structures and/or moisture evaporation during the film drying and the sputtering operation (Nordqvist *et al.*, 2007).

Polarizing OM imaging suggested that the formation of the fibrous and rod-like structures was due to the reorganization of nanosized crystalline components in the films. X-ray diffraction measurements showed that the pure KGM- and GGMSpDr-based films were semicrystalline. The degree of crystallinity of KGM films was higher when glycerol was used, and this could be seen also with polarizing OM. Surprisingly, the crystalline structures in GGMSpDr films not containing CNW, detected with X-ray diffraction, were poorly visible with polarizing OM even though the degree of crystallinity of those films was of the same magnitude as that of KGM films containing glycerol. This could be due to the small size of crystalline structures in GGMSpDr films. The degree of crystallinity of mannan in KGM-based films slightly increased with increasing CNW content, but in GGMSpDr-based films, it did not show a significant difference. The differences in the degree of crystallinity of mannans due to the addition of CNW were small and did not correspond to the remarkable differences in the visual appearance of the films. Therefore, increased mannan crystallization is unlikely to be responsible for the formation of the fibrous structures. In contrast, mannans could gather CNW into larger crystalline structures visible with polarizing OM.

6.1.4 Permeability properties (IV)

The WVP of films was clearly higher at the gradient of 32/86% RH than at the gradient of 0/54% RH, even though the RH difference was 54% in both cases. Theoretically, the calculated permeability of a film should be the same at different RH conditions, if the gradient is similar. However, this is not the case with hydrophilic films, in which water

molecules interact with polar groups in the film structure causing plasticization and swelling (Gennadios *et al*., 1994).

The WVP of GGMEtOH films was lower than that of films from other mannans. GGM contains acetyl groups, which have a more hydrophobic character than the free hydroxyl groups in sugar units. Therefore, GGM has fewer sorption sites for water than the other studied mannans. KGM carries a small number of acetyl groups, and GG and LBG are not acetylated. The higher WVP of films from GG and LBG in comparison to GGMEtOH films could also be due to the differences in the molecular mobility of mannans in the film matrix. GG and LBG are much more highly substituted with galactose than GGM. As discussed previously, a smaller number of galactose side groups may allow denser packing of mannan chains in the films. This could further lead to decreased molecular mobility and lower WVP than in films from GG and LBG. The films from GG, which is more highly substituted with galactose than LBG, had higher WVP at the gradient of 0/54% RH than the films from LBG. At the gradient of 32/86% RH, however, the uncorrected WVP of those films was similar and the corrected WVP was higher for the LBG film. Thus, the RH seems to affect the mechanism of WVP in galactomannan films. At high RH, the swelling of films can affect the solubility and diffusivity of water in the films.

The WVP of PVOH and GGMEtOH:PVOH 1:3 films was lower than that of GGMEtOH films. As shown by microscopy, at the ratio of GGMLab:PVOH 1:3 the continuous phase was formed from PVOH, and GGMLab was located as small spheres inside the film, while at the ratio of 3:1, the continuous phase was mainly GGMLab with large PVOH particles. Thus the WVP was low when PVOH formed the continuous phase.

The WVP values obtained in the present study are higher than reported earlier for polyethylene glycol-plasticized LBG films by Aydinli and Tutas (2000), where the WVP was 6.3–11.6×10^{-8} [g/(m·h·Pa)], corresponding to 1.5–2.8 [g·mm/(m^2·d·kPa)], at a gradient of 0/84% RH for LBG films plasticized with varying amounts of different polyethylene glycols. The WVP of sorbitol-plasticized potato starch films at a gradient of 0/54% RH (Talja *et al*., 2007) was also slightly lower than the WVP of mannan films at that gradient in the present study. However, Aydinli and Tutas (2000) and Talja *et al*.

(2007) did not report the use of a fan inside the desiccator, which increases the air circulation and WVP results. The WVP of sorbitol-plasticized oat spelt arabinoxylan films at the gradient of 0/54% RH, studied using the same desiccator cabinet equipped with the fan as in the present study, was similar to that of GGMEtOH films (Mikkonen *et al*., 2009).

In the OP measurements, the GGMEtOH:PVOH 3:1 film leaked, but at the GGMEtOH:PVOH ratio of 1:3, PVOH was able to decrease the OP of GGMEtOH films. The visual appearance and phase separation of GGMLab:PVOH blends provided reasons for these results. At the ratio of GGM:PVOH 3:1, when large PVOH particles were located in the continuous GGM matrix, holes, or zones with a thin layer of continuous phase may have been formed and allowed the high oxygen permeability. The OP of GGMEtOH films, 6.8 $[cm^3 \cdot \mu m/(m^2 \cdot d \cdot kPa)]$, was higher than that reported for GGM-sorbitol films by Hartman *et al*. (2006a), 2.0 $[cm^3 \cdot \mu m/(m^2 \cdot d \cdot kPa)]$. The OP of the GGM films in the present study was lower than that measured previously for glycerol-plasticized amylose and amylopectin films by Rindlav-Westling *et al*. (1998), but not as low as that of sorbitol-plasticized aspen glucuronoxylan films studied by Gröndahl *et al*. (2004). The OP of oat spelt arabinoxylan films plasticized with 40% sorbitol was 4.7 $[cm^3 \cdot \mu m/(m^2 \cdot d \cdot kPa)]$, which is slightly lower than that of GGM films (Mikkonen *et al.*, 2009).

GGMEtOH and KGM films had very low aroma permeability. The diffusion coefficients for both GGMEtOH and KGM films were lower than those measured previously for low density polyethylene coated paper (Vähä-Nissi *et al*., 2008). The results indicate that GGM and KGM films have potential as aroma barrier materials.

GGMEtOH films were transparent to visible light, but blocked the light transmission at the ultraviolet range of the spectra, which can be beneficial for product preservation in some food applications as the most pronounced deleterious effects of light on food are caused by ultraviolet light (Bekbölet, 1990). The haze of LBG films, plasticized with sorbitol in the present study, was 22.5%, which is of the same magnitude as measured by Aydinli *et al*. (2004) for LBG films plasticized with a low amount of polyethylene glycol.

The haze results indicate that KGM films were very clear, but the other studied mannan films were slightly translucent.

6.2 Emulsions (V)

In general, the galactomannan-containing emulsions were unstable in the present study, and a decrease in the DP of galactomannans further decreased emulsion stability. The greatest decrease took place between the DP levels of MH and ML. The recent study of Yadav *et al*. (2007b) showed that cAXs with molar masses of 360-490 kDa (CFG-2) were more effective emulsion stabilizers than those with molar masses of 250-290 kDa (CFG-1). However, the molar mass of a polysaccharide is not alone responsible for its emulsion stabilization capacity, as M_w of GGM is significantly lower than that of GG and LBG, and in the experimental conditions used in the present study, GGM were more effective emulsion stabilizers than GG and LBG. The effectiveness of GGM as emulsion stabilizer could be at least partly due to acetyl groups.

Decreasing DS increased the turbidity of emulsions. This is in contrast with the comparison of the stability of emulsions containing GG and LBG, which are galactomannans with naturally different galactose substitution. GG, with a higher DS, produced emulsions with higher turbidity than LBG. In previous studies, fenugreek galactomannan, which has a mannose/galactose ratio of 1:1, was found to be a more efficient emulsion stabilizer than the less substituted galactomannans, in spite of its production of a lower viscosity than LBG (Garti *et al*., 1997; Huang *et al*., 2001).

The water-solubility of low-DS galactomannans was lower than that of high-DS galactomannans, and the solubility of the enzymatically modified GGs in water was further reduced after ethanol precipitation and lyophilization. This low solubility probably affected their emulsion-stabilizing performance. GG and LBG are in commercial use as emulsion stabilizers and therefore their relatively poor performance in the present study was unexpected. Garti and Reichman (1994) concluded that relatively high galactomannan concentrations are required for full coverage of oil droplets and emulsion stabilization, and

water dilution of those emulsions will cause fast desorption of the galactomannan and emulsion destabilization. In the present study, a model beverage system was used to study the emulsion stabilization and therefore all emulsions were diluted after homogenization. In the diluted form, the polysaccharide concentration (0.032–0.32 mg/ml) as well as the visually observed viscosity of the emulsions was low. It is possible that similar concentrations and emulsion preparation conditions are not optimal for mannans from different sources due to differences in both their molar mass and viscosity, and that more stable emulsions could be obtained using GG and LBG in different settings.

It has been suggested that the origin of the emulsifying ability of hydrocolloids is generally the presence of proteinaceous material covalently bound or physically associated with the carbohydrate polymer (Dickinson, 2003). However, contradictory conclusions have been made about the role of protein in the interfacial properties of galactomannans (Brummer *et al.*, 2003, Garti and Reichman, 1994). In the present study, the protein content of the studied samples did not correlate with the emulsion stability.

The results on the effect of the polysaccharide/oil ratio with 6Gal-H are in agreement with a study by Vélez *et al.* (2003), who demonstrated that GG concentrations of 0.1% (w/w) or higher are necessary for oil-in-water emulsion stabilization. However, above a critical polysaccharide concentration, depletion flocculation of oil droplets is induced (Chanamai and McClements, 2001) so the maximum emulsion stability is achieved at intermediate polysaccharide concentrations. The results with GGM suggest that the formation of emulsion was enhanced by an increasing amount of GGMEtOH, but their stability during storage decreased due to excess of GGMEtOH. The emulsion breakdown mechanism at different concentrations could be studied further, e.g., by light scattering methods.

7 SUMMARY AND CONCLUSIONS

Plasticizer was needed for film formation from mannans other than KGM. Glycerol and sorbitol were found suitable for GGM and the optimal amount of plasticizer was 40% (w/w of polymers). The chemical structure of mannans clearly affected film formation. KGM formed the strongest films with high elongation at break. To further study the effect of chemical structure of mannans on film properties, GG was modified enzymatically to different molar masses and degrees of galactose substitution. The strongest and the most flexible films that behaved as a single phase in DMA were formed from galactomannans with the minimum substitution with galactose. Interestingly, the elongation at break of GG-based films reached a maximum at medium-low polymerization, and their tensile strength in the medium-high – medium-low range. By controlled enzymatic hydrolysis, the mechanical properties of guar gum galactomannan-based films can be made comparable to those of LBG-based films.

GGM / KGM blend films were homogeneous and had higher tensile strength than the films from pure GGM. Even a small addition of KGM notably increased the elongation at break of GGM-based films. Blending GGM with KGM was found to be an effective way to improve the mechanical properties of GGM-based films. Although the use of PVOH increased the mechanical properties of GGM / PVOH blend films, DMA and microscopic study showed that the two polymers were immiscible. GGM and cAX formed homogeneous blend films, but they were very sensitive to high relative humidity. The addition of CNW greatly changed the appearance of the KGM-based films. Microscopic study with OM and SEM showed that CNW also induced morphological changes in GGM-based films. Polarizing microscopy suggested that the formation of the fibrous and rodlike structures was due to reorganization of crystalline components in the films. The differences in the degree of crystallinity of mannans due to the addition of CNW were small and did not correspond to the remarkable differences in the visual appearance of the films. Therefore, increased mannan crystallization could not have been responsible for the formation of the fibrous structures. In contrast, mannans could gather CNW into larger crystalline structures visible with polarizing OM. Unexpectedly, the remarkable

differences in the film structure did not indicate great changes in the other film properties studied.

Sorbitol-plasticized GGM films showed promising barrier properties. The WVP and OP of GGM films were lower than those of films from other mannans and the OP of GGM films was comparable to that of a commercial PE/EVOH/PE laminate film. The WVP was even lower with PVOH as the continuous phase of the film. The OP of GGM-PVOH blends depended on the blend ratio and at the ratio of GGM:PVOH 1:3, it was lower than that of films from either of the polymers alone. The WVP of GGM-KGM blends was lower than that of films from pure KGM at the RH gradient of 0/54%. GGM and KGM showed synergism as the OP of the blends was lower than that of the films from pure GGM and KGM. Unexpectedly, the presence of 5% of CNW did not significantly affect the WVP or OP of the films despite the structural changes. Aroma permeability of both GGM and KGM films showed low diffusion coefficients of the aroma compounds tested. All films were transparent to visible light, but films containing GGM blocked the light transmission in the ultraviolet region. The haze of GGM films was higher than that of KGM films and the use of CNW further increased the haze.

GGM enhanced the formation of emulsions and increased the emulsion stability during 14 days at RT and 4 °C. Particularly ethanol-precipitated GGM was found to be a more effective emulsifier and emulsion stabilizer than the other studied mannans, but not as effective as cAX, in the present experimental conditions. The optimal GGM concentration was dependent on emulsion age and GGM type. Storage at elevated temperature caused a rapid emulsion breakdown of all samples studied. Decrease in the DP of galactomannans clearly decreased emulsion stability. A beverage emulsion is a complex system in which many factors, such as molar mass, branching, concentration, viscosity, and dilution of the stabilizing hydrocolloid have a profound effect on emulsion stability. Thus, despite the similarities in the sugar composition of GGM and other mannans, they differed significantly in emulsifying performance.

GGM films could be used as oxygen or aroma barriers at relatively low RH conditions or if coated with a more hydrophobic material. The drawback with GGM is that it needs a

high amount of plasticizer, which can be a problem in food applications because of migration. Thus the stability of plasticizer can be a subject of further studies. The usefulness of mannan-based films in different applications also requires further studies on preparation of films by extrusion. Decreasing the hydrophilicity and sensitivity of mannan-based films to water is a future challenge. This study is the first indication of the potential of GGM as a stabilizer of food emulsions and shows that GGM is an interesting option for an emulsion stabilizer to be studied further in future.

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