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Adsorption study on the formation of interfacial layers based on birch glucuronoxylans

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ABSTRACT

Glucuronoxylans (GX), particularly crude fractions obtained by pressurized hot water extraction of birch wood, act as potent emulsifiers and stabilizers against physical separation and lipid oxidation. Herein, we studied the adsorption of GX on hydrophobic interfaces to correlate their multicomponent character towards the formation of interfacial layers in emulsions. Dynamic interfacial tension (DIFT) and quartz crystal microgravimetry with dissipation monitoring (QCM-D) were applied to various GX fractions and the results compared with those from cellulose-based emulsifiers. The roles of residual lignin and polysaccharides are discussed considering the formation of interfacial layers during emulsification. The DIFT of the different GXs reached quasi-equilibrium faster as the lignin concentration increased, implying a correlation between the rate of adsorption and the residual lignin content. The effect of NaCl addition was more pronounced in polysaccharide-rich fractions, indicating that the polysaccharide fraction modulated the effect of ionic strength. QCM-D showed that despite the fast adsorption exhibited by the lignin-rich GX extract in the DIFT curves, the adsorbed materials were lightweight, suggesting that the polysaccharide fraction built the bulk of the interfacial layer. These results provide a foundation towards understanding the role of GX in interfacial stabilization beyond traditional plant-based counterparts.

1. Introduction

Given its abundance and renewability, lignocellulosic biomass is a prominent source of sustainable materials. Compared to cellulose, the hemicellulose fraction has been less utilized (Mikkonen, 2020). A class of hemicelluloses, xylans, can be isolated from wood, straw, and sugarcane bagasse. The detailed molecular makeup and functionality of xylans depend on the source and method used for its extraction (Abik et al., 2023); of particular interest is the emulsifying property of glucuronoxylans (GX) recovered from birchwood by pressurized hot water extraction (PHWE). Wood-derived GX are composed primarily of linear poly-(1→4)-β-o-xylopyranosyl chains, with side groups including partial acetylation at the C-2 and C-3 hydroxyl groups and 4-O-methyl-α-glucuronic acid residues connected by a (1→2) glycosidic linkage (Sjöström, 1993); the latter imparts a negative charge to the polymer while the former imparts solubility in water by moderating the hydrogen bonds between the backbone chains. The PHWE method is unique as it yields GX of smaller molar mass (Ho et al., 2023; Mikkonen et al., 2019) than its counterparts extracted by alkali and other extraction methods (Saake et al., 2001), as well as a higher proportion of phenolic residues originated from lignin (Ho et al., 2023; Mikkonen et al., 2019) which exists either in the free form or as lignin-carbohydrate complexes (LCCs, covalently-bound complexes between lignin and polysaccharides) (Carvalho et al., 2021; Mikkonen, 2020). Besides its excellent emulsifying properties, comparable to those of commercially-established cellulose-based emulsifiers, GX presents an enhanced protection against...
lipid oxidation (Lahtinen et al., 2019) and displays prebiotic properties (Kynkääniemi et al., 2022). Hence, GX is a potential candidate as a sustainable and environmentally friendly plant-based emulsifier. Moreover, contrary to other polysaccharide-based emulsion stabilizers, GX emulsions have low viscosity (Ho et al., 2023; Mikkonen et al., 2019), and therefore can be used to fortify liquid foodstuff with oil-soluble nutrients with no major changes in sensory properties.

Despite its advantages, several knowledge gaps need to be addressed to fully realize GX's potential. Previous studies have mostly highlighted the important role of lignin in the emulsion stabilization. Fractionating the adsorbed GX at the oil-water interface showed that of the different types of LCC contained in the GX extracts, the benzyl ether LCC, which is present in the crude extract and lignin-rich fraction, are exclusively found at the interface instead of the continuous phase (Carvalho et al., 2021). Additionally, insoluble particles may also participate in the stabilization of emulsions, as indicated in our systematic study of the rheological properties of different GX-stabilized emulsions (Ho et al., 2023). It is therefore clear that lignin plays an important role in the emulsifying property of GX. However, lignin-rich and hemicellulose-rich extracts obtained from ethanol precipitation of crude extract both showed inferior performance compared to the spray-dried crude extracts (Carvalho et al., 2021; Ho et al., 2023; Lahtinen et al., 2019). This implies a contribution by both the hemicellulose and the lignin fraction towards stabilization, whereby an optimal lignin-hemicellulose proportion was expected to exist. Such a topic, namely, the extent of each fraction's contribution remains an unclear subject that awaits elucidation. Understanding the interplay between the hemicellulose and lignin fractions in the mechanism of emulsion stabilization is critical to furthering their applications. It will also provide a rationale to adjust the extraction and post-extraction treatments within a biorefinery to obtain the desirable properties.

An important feature of an emulsifier is the rate at which it adsorbs at the oil-water interface to subsequently form an interfacial layer. The adsorption of an emulsifier to the oil-water interface is a time-dependent process which can be broadly divided into two steps: 1) the translational diffusion of the emulsifier from the bulk phase to the sub-interface (i.e., the molecular-scale layer before the oil-water interface), and 2) the adsorption/attachment of the emulsifier from the sub-interface to the interface. These processes can be described from the evolution of the interfacial tension over time, and can be divided into the 1) induction, 2) rapid fall, 3) meso-equilibrium, and 4) equilibrium phases (Hua & Rosen, 1988). The adsorption is considered to be diffusion-controlled if the rate of diffusion is slower than the rate of adsorption, and vice-versa (Bazhlekov & Bazhleкова, 2018; Ravera et al., 2000). The process was first described quantitatively using a mathematical model by Ward and Tordai in 1946 (Ward & Tordai, 1946), and subsequent studies have aimed to expand and refine the model. During emulsification, the adsorbed emulsifier reduces the interfacial tension between the aqueous and oil phases, effectively lowering the energy barrier to increase the interfacial area and thus facilitate the formation of smaller droplets (McClements, 2015). However, the formation of new droplets leads to emulsifier-free fresh interfaces that are energetically prone to coalesce, effectively increasing the droplet size. Such coalescence can be prevented if the emulsifier adsors at the interface at a faster rate than coalescence, regardless of whether the emulsification is carried out under high or low energy conditions (i.e., turbulent flow of fluids breaking into the droplets or instantaneous formation of droplets through phase boundary (McClements & McClements, 2015). Once adsorbed, emulsifiers prevent coalescence through the Gibbs-Marangoni effect (which is associated to the lateral diffusion of the emulsifier molecules at the oil-water interface following successful adsorption) or, in the case of macromolecular emulsifiers, by forming a protective viscoelastic layer at the interface (Clark et al., 1991; McClements, 2015; McClements et al., 2017; Ravera et al., 2021). However, it must be noted that the size of a macromolecular emulsifier (i.e., degree of polymerization, hydrodynamic radius, etc.) may reduce the translational mobility (Viel et al., 2003), which hinders translational diffusion from the bulk phase to the oil-water interface.

This study aimed to understand the interplay between the different components of birch GX during their interfacial adsorption. We approached the analysis by comparing GX to established cellulose-based emulsifiers. We assessed the adsorption behavior primarily by dynamic interfacial tension (DIFT) at the water-hexadecane interface, combined with quartz crystal microgravimetry with dissipation monitoring (QCM-D), the latter of which would shed light on the viscoelasticity of the adsorbed layer. At the end, we compared the DIFT and QCM-D results to the emulsification properties of the emulsifiers. We also attempted to correlate the trend in adsorption by looking at the attractive forces between GX and paraffin wax by performing colloidal probe force spectroscopy; the experimental details and results of which are included in the supplementary information. We hypothesized that the amount of residual lignin in GXS influences the rate of adsorption and the properties of the formed interfacial layers. The results of this study brought forth an understanding over the formation of interfacial layer in GX-stabilized emulsions, subsequently providing a rationale for a tailored refining of GX and other biomass-derived materials as well as contributing to the development of a characterization approach for surface-active complex biomaterials.

2. Experimental section

2.1. Materials

Methylcellulose [MC, Methocel™ A15LV; 12–18 mPa.s at 20 °C as 2 % solution in water; degree of methylation = 2.19 by nuclear magnetic resonance (Kono et al., 2017); Mw (weight-averaged molar mass) = 87.5 kDa, Mw/Mn (dispersity) = 8.97 (pullulan equivalent, by size-exclusion chromatography in aqueous 0.1 M NaNO3)] and sodium carboxymethylcel lulose [CMC, Walocel™ CRT 30; 30 mPa.s at 20 °C as 2 % solution in water; degree of carboxymethylation = 1.24 by nuclear magnetic resonance (Kono et al., 2016); Mw = 227.1 kDa, Mw/Mn = 4.81 (pullulan equivalent, by size-exclusion chromatography in aqueous 0.1 M NaNO3)] were obtained as a gift from Dupont (Meyrin, Switzerland). Sulfated cellulose nanocrystals (CNC, from sulfuric acid hydrolysis of wood pulp. Zeta potential = ~45 mV; dimensions: 5–20 nm wide, 140–200 nm long) were purchased as a never-dried dispersion from cellulose lab (Fredericton, Canada). PHWE GX isolated by spray-drying (sGX) and ethanol precipitation (epGX) were sourced from the Natural Resources Institute Finland (LUKE). Ethanol-soluble GX (esGX) was isolated from sGX by antisolvent precipitation followed by lyophilization, as previously reported (Carvalho et al., 2021). Carboxymethylated GX (CMGX) were synthesized from epGX according to published procedures (Ho et al., 2023). Table 1 contains a summary of the different samples while Table S1 includes the full chemical characterization of sGX, epGX, CMGX (Ho et al., 2023), and esGX (Carvalho et al., 2021).

Chloroform, hexadecane, sodium chloride, and paraffin wax were obtained from Sigma-Aldrich (ST Louis, United States). Citric acid monohydrate was purchased from Merck (Darmstadt, Germany). Sodium hydroxide was obtained from Alfa Aesar (Massachusetts, United States). Beeswax was obtained as a sample from BIONORD Biokemi (Stenungsund, Sweden). All chemicals used in this study were of analytical or synthesis grade.

2.2. Preparation of emulsifier dispersions

All dispersions used in this study were prepared by dispersing the given emulsifier in 25 mM citric acid buffer pH 4.5 and adjusted to the required concentrations (1 %, 0.3 %, 0.03 %, or 0.003 % w/v) using a volumetric flask. This pH value was chosen considering the tradeoff between dissolution of the lignin fraction (high pH) and possible hydrolysis of hemicelluloses (low pH). Each emulsifier was dispersed differently in order to minimize undispersed aggregates. The sGX, epGX,
esGX, and CMGX were dispersed directly in the buffer and kept under stirring overnight. The esGX was ultrasonicated (Branson 450 Digital Sonifier, Marshall Scientific, Hampton, USA) three times at 3000 rpm. The wax was melted at 70 °C in an oven for at least 3 h. Changes in oscillation frequency and dissipation were measured at 23 °C at the fundamental resonance frequency of 5 MHz. Citric acid buffer 25 mM pH 4.5 was applied for about 30 min to equilibrate the film, followed by 3.5 h exposure to 0.03 % emulsifier solution in the same buffer, and ended with at least 1 h rinsing using emulsifier-free buffer. This concentration was chosen as it gave a measurable adsorption signal without clogging the fluidic system in the instrument. The measurements were performed in duplicate for each sample, and the data was presented as a representative curve from one of the replicates (Figs. 1–4, S5) as each replicate measurement was performed with freshly-dispersed drop and fresh buffer, and therefore the interfacial tension value of each time point cannot be averaged.

2.5. Measurement of emulsifier adsorption at the solid-liquid interface

QCM-D (E4 instrument, Q-Sense AB, Sweden) was used to follow the adsorption of the emulsifiers on hydrophobic solid-liquid interface. Gold-coated quartz sensors were cleaned by UV-ozone cleaner (ProCleaner™, BioForce Nanosciences, Virginia, USA) and 1 % (w/v) paraffin wax in chloroform was spin-coated (WS-650SZ-6NPP, Laurell Technologies, PA, USA) three times at 3000 rpm. The wax was melted at 70 °C in an oven for at least 3 h. Changes in oscillation frequency and dissipation were measured at 23 °C at the fundamental resonance frequency of 5 MHz. Citric acid buffer 25 mM pH 4.5 was applied for about 30 min to equilibrate the film, followed by 3.5 h exposure to 0.03 % emulsifier solution in the same buffer, and ended with at least 1 h rinsing using emulsifier-free buffer. This concentration was chosen as it gave a measurable adsorption signal without clogging the fluidic system in the instrument. The measurements were performed in duplicate for each sample, and the data was presented as a representative curve from one of the replicates (Fig. S5) as the time points were recorded in real-time and thus the frequency change and dissipation for each one cannot be averaged.

An important feature of QCM-D is that it is possible to obtain the mass of adsorbed layer, which is normally obtained by converting the frequency change into mass using the Sauerbrey equation. However, QCM-D does not distinguish between adsorbed dry mass and the mass of hydrated layers, which must be taken into account during data interpretation. The full frequency- and dissipation-over-time plot (Figs. S3 and S4, respectively) for each GX showed that the dissipation values were spread over the different overtones, indicating a soft, viscoelastic film, and thus Sauerbrey equation cannot be applied. Moreover, while it was possible to fit the data from sGX, epGX, and CMGX using the Kelvin- Voigt model, it was not possible for esGX, and therefore we opted to directly compare the change of frequency as a measure of adsorbed mass.

### Table 1

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
<th>Mass (g/100 g)</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>sGX</td>
<td>Spray-dried GX</td>
<td>24.63 ± 3.82</td>
<td>Spray-dried (Kilpelainen et al., 2013) powder of the crude extract</td>
</tr>
<tr>
<td>epGX</td>
<td>Ethanol-precipitated GX</td>
<td>15.26 ± 0.07</td>
<td>Hemimicelle-rich fraction obtained from the crude extract by precipitation in 8:1 v/v ethanol/water (Carvalho et al., 2021).</td>
</tr>
<tr>
<td>esGX</td>
<td>Ethanol-soluble GX</td>
<td>30.40 ± 1.30</td>
<td>Lignin-rich fraction obtained from the ethanol-soluble fraction during the production of epGX (Carvalho et al., 2021).</td>
</tr>
<tr>
<td>CMGX</td>
<td>Carboxymethyl GX</td>
<td>18.67 ± 0.01</td>
<td>Synthetic derivative of epGX where the hydroxyl groups are partially carboxymethylated (Ilo et al., 2023).</td>
</tr>
<tr>
<td>MC</td>
<td>Methylcellulose</td>
<td>–</td>
<td>Methyl-ether derivative of cellulose, representing uncharged polysaccharide-based emulsifier.</td>
</tr>
<tr>
<td>CMC</td>
<td>Carboxymethylcellulose</td>
<td>–</td>
<td>Carboxymethyl-ether derivative of cellulose, representing charged polysaccharide-based emulsifier.</td>
</tr>
</tbody>
</table>

Unfortunately, unlike regular small molecule or protein emulsifiers, it is difficult to define the molecular properties of GXs relevant to their interfacial activities due to their high molar mass dispersity, their multicomponent nature (i.e., being a mixture of polysaccharide and lignin), and the possibility of having multiple LCCs in one hemicellulose chain (i.e., the structure may not follow the classical surfactant model). Therefore, we were not able to fit our DIFT data to the currently available empirical models, such as the Ward-Tordai model or the Gibbs’ adsorption isotherm (Miller et al., 2017). Instead, we describe the findings primarily qualitatively relative to the different types of GX.
2.6. Emulsion formation capability

To correlate the adsorption profile with the emulsification properties, we analyzed the emulsions' droplet size soon after emulsification, both before and after high-pressure homogenization. The emulsions were formulated with 1% (w/w) emulsifier in 25 mM citric acid buffer (pH = 4.5) and 5% hexadecane (w/w); the concentration was chosen to be in line with our previous emulsion stability studies (Ho et al., 2023; Lahitten et al., 2019). The mixture was first mixed with a high-speed homogenizer (Ultra-Turrax T18 basic equipped with 252N-25G dispersing head, IKA, Staufen, Germany) at 12000 rpm for 3 min to obtain a coarse emulsion, which was then further processed using a high-pressure homogenizer (Microfluidizer 110Y with a series of 75 mm Y-type P20Y and 200 mm Z-type H30Z interaction chambers, Microfluidics, Westwood, MA, USA) at 800 bar for 3 cycles. The droplet size of the emulsions both before (UT - Ultra-Turrax) and after (MF - microfluidizer) high-pressure homogenization were analyzed by laser light scattering (Malvern Hydro 3000 SM, Malvern Instruments Ltd., Malvern, UK), assuming 1.33 and 1.434 as the refractive indices for water and hexadecane, respectively. The results are presented as droplet size distribution (Fig. 6A) and surface-weighted average diameter (D_{[3,2]}). The average and standard deviation values of D_{[3,2]} were taken from two replicate measurements of three readings (n = 6).

3. Results and discussion

3.1. Effect of GX type towards adsorption

The interfacial adsorption of the GXs at the oil-water interface was compared to that of a cellulose-based emulsifiers. The three reference emulsifiers MC, CMC, and CNC were chosen to represent various emulsion stabilization mechanisms based on polysaccharides: MC is known to adsorb readily at the oil-water interface (Sarkar, 1984); CMC is known as a viscosity modifier (Arancibia et al., 2013; Radi & Amir, 2013); and CNC is a Pickering emulsifier which requires a high-energy emulsification (Bai et al., 2019; Dai et al., 2020). While the emulsification in the current and previous experiments used 1% (w/w) emulsifiers, our efforts to compare the emulsifiers primarily used low concentrations (0.03%, w/v) to ensure light transmission to allow appropriate imaging of the suspended drop (sGX and esGX, in particular, formed opaque dispersions at high concentrations). As such, we include measurements at high concentrations only when experimentally feasible.

We first compared the DIFT profiles of the GXs (Fig. 1A) and the reference cellulose-based emulsifiers (Fig. 1B). All GXs showed a reduction of interfacial tension over time. The DIFT curves also showed the common characteristic of macromolecular emulsifiers, where the interfacial tension value continued to decrease until reaching a quasi-equilibrium state (Beverung et al., 1999; Castellani et al., 2010; Reinhard Miller et al., 1994). This phenomenon occurs as the macromolecular emulsifier may undergo conformational rearrangements as the hydrophobic domains undergo conformational changes in the aqueous environment. The rearrangement does not stop as the emulsifier is adsorbed at the interface and then it interacts with other molecules that co-adsorb, leading to a maturation of the interfacial layer (Beverung et al., 1999). MC and esGX were adsorbed relatively fast (no initial rapid fall phase were apparent). Such behavior was in direct contrast with the results from CMC and CNC, which showed a similar DIFT profile when compared to water and emulsifier-free buffer (Fig. S5). Meanwhile, there was a clear difference when we compared the DIFT profiles of the GXs. CMGX was adsorbed at the slowest rate and to the least extent. Over the other GXs, adsorption occurred increasingly faster following the order epGX > sGX > esGX.

From the results above, it is clear that lignin is a driving force that enables GXs adsorption to the surface of the oil. This is especially apparent when we compared the results for CMGX and CMC. Both polysaccharides are water-soluble and negatively charged; however, CMGX contains residual lignin, while CMC does not. Based on Fig. 2A, 0.03% CMGX exhibited adsorption to the interface within the measured timeframe (despite being the slowest among the GXs), while CMC did not show any significant drop of interfacial tension. Given that the main difference between CMC and CMGX was the presence of lignin, it can be stated that lignin played a key role in the interfacial properties of GXs. However, MC was still adsorbed despite the absence of lignin, and even at a faster rate compared to all GXs. This suggested that lignin modified the hydrophobicity of GX as such that its interfacial activity increased.

The DIFT profile differences observed between epGX, sGX, and esGX appeared to be in line with their respective lignin content. From Fig. 2A, esGX, which had the highest lignin content, was adsorbed the fastest and the most, whereas epGX was adsorbed the slowest and the least between the three. This result indicated that the amount of lignin also plays a role in addition to its presence, where a higher amount of lignin corresponded to enhanced adsorption. Meanwhile, CMGX was adsorbed even slower than epGX. This slower dynamic may be due to a lower lignin content compared to epGX, as the synthesis process involved precipitation in methanol further removed the lignin content (Ho et al., 2023), but could also be caused by the added presence of carboxyl groups. Being ionizable and hydrophilic, the carboxyl groups rendered CMGX less hydrophobic and hence were adsorbed at a slower rate. Such a
model supports the conclusion that the adsorption of GX at the oil-water interface is driven by the presence of lignin that imparts a hydrophobic character into the system.

3.2. Concentration dependence of GX adsorption

As the first step in interfacial adsorption involves the diffusion of emulsifiers from the bulk onto the interfacial layer, it is useful to evaluate the concentration dependence of the DIFT profile as the diffusion process of polymers could be concentration-dependent (Bouchaud & Daoud, 1987; Han & Ziya Akcasu, 1981; Van De Ven, 1994). From Fig. 2, it was shown that as the concentration of GX increased, the onset of rapid fall became earlier; with the exception of CMGX, the rapid fall even occurred before the measurable time frame limit of the reverse pendant drop technique at concentrations starting from 0.1 to 0.3 %. In fact, at 1 % w/v the drop of interfacial tension was rapid enough that the oil pendant detached from the needle. Additionally, the quasi-equilibrium interfacial tension values also decreased with increasing concentration, marking an increase in the surface excess concentration. The concentration dependence indicated that the adsorption of GXs may be diffusion-controlled. Consequently, this showed that it would be possible to achieve optimal emulsification by these GXs by optimizing their concentrations.

Note that the highest concentrations that were measured for sGX and esGX were 0.15 % and 0.1 % respectively; above these concentrations, the solutions were too turbid for the drop shape measurements, unlike epGX and CMGX which were still clear enough for the measurements to be conducted even at 1 %. The high turbidity highlighted the presence of insoluble particles in GX extracts. In fact, at 0.03 %, while the solutions were clear enough for the measurement to be conducted, there was a visible build-up of precipitations on top of the drop at the end of the measurements, which may have contributed to the lower quasi-equilibrium interfacial tension. The presence of insoluble particles in the crude extract of PHWE hemicelluloses has been previously indicated to contribute to emulsion stabilization in the form of a Pickering system (Ho et al., 2023; Valoppi et al., 2019). However, it is currently unclear whether or not these particles need a high energy input in order to adsorb at the oil-water interface.

We also probed the multi-component aspect of GX towards adsorption by utilizing its concentration dependence. As mentioned above, benzylether-type LCC, which is only present in the crude and ethanol-soluble extracts, is preferentially adsorbed at the interface, indicating that it may be integral to the stabilization mechanism (Carvalho et al., 2021). However, it is currently not possible to isolate the individual LCCs and analyze their adsorption separately. By analyzing the DIFT profiles at a very dilute concentration, we assume that the adsorption rates of all the different components are slowed down enough to the point where the overall adsorption profile of the mixture would primarily be dominated by the fastest-adsorbing species. This observation is shown in Fig. 3, where we compared the DIFT profile of the GXs at 0.003 % (w/v). It was shown that sGX and esGX showed almost identical DIFT profiles, confirming that the fastest-adsorbing species in sGX is contained in the ethanol-soluble fraction. Given the more nonpolar nature of ethanol relative to water, the more hydrophobic fraction of GX would be solubilized and separated from the more hydrophilic fraction. The more-hydrophobic nature would explain why this fraction was adsorbed faster. With the current level of knowledge, however, we cannot yet fully decipher the origin of this enhanced hydrophobicity aside from the higher lignin content; we currently do not know the size and conformation of the lignin and polysaccharide moieties to suggest whether the hydrophobicity could have arisen from the increased accessibility of the hydrophobic domains to orientate towards the oil phase.

3.3. Sensitivity of GX adsorption against ionic strength

Electrostatic interactions are known to alter the interfacial activity of surfactants (Diamant & Andelman, 1996) and polyelectrolytes (Roberton & Richmond, 2014). In GX, the presence of 4-O-methylglucuronic acid residues in the polysaccharide chain imparts some negative charge to the hemicelluloses. Additionally, given the polyphenolic nature of lignin, there might be some deprotonation of the phenolic hydroxyl groups, which would contribute to the overall negative charge (Osterberg et al., 2020). It is therefore relevant to also investigate how the electrostatic interactions may influence the adsorption behavior of GX. We probed the ionic interactions in two ways. The first way was by the inclusion of salt (in this case NaCl) into the buffer, which acts by screening the electrostatic interactions (Diamant & Andelman, 1996), effectively neutralizing the charge by providing counterions against the charge-carrying groups. This effect was evident in our dispersions, where the presence of 100 mM NaCl lowered the zeta potential of the dispersions (Table 2). Secondly, we also introduced negative-charge-carrying groups by carboxymethylation to produce CMGX. This way, we would be able to evaluate the effect of electrostatics arising from the chain modifications.

The effect of introducing salt towards the DIFT profile can be seen in Fig. 4. The DIFT drop seemed to progress in the same profile regardless of the salt content. However, there was an overall reduction in the nominal value of the interfacial tension except for esGX, suggesting a higher surface excess concentration for the other GXs in the presence of salt. Interestingly, the extent of reduction appeared to be inversely correlated with lignin content; lignin-rich esGX was not affected by the

<table>
<thead>
<tr>
<th>Name</th>
<th>Zeta potential (mV)</th>
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<tbody>
<tr>
<td></td>
<td>0 mM</td>
</tr>
<tr>
<td>sGX</td>
<td>-8.12 ± 0.67</td>
</tr>
<tr>
<td>epGX</td>
<td>-7.45 ± 0.86</td>
</tr>
<tr>
<td>esGX</td>
<td>-15.42 ± 1.37</td>
</tr>
<tr>
<td>CMGX</td>
<td>-22.67 ± 1.63</td>
</tr>
</tbody>
</table>
3.4. Adsorption at the solid-liquid interface

In the presence of salt, the adsorption of GXs at the solid-liquid interface was found to be negligible, since this adsorption occurred at a much later time point compared to the DIFT profile. This discrepancy could mean that the adsorbed layer was little amount of adsorbed material. Meanwhile, the results for the GXs (Fig. S4A) were largely consistent to the DIFT profiles, with the exception of esGX. There was a similar extent of frequency change between esGX and CMGX, indicating a lightweight adsorbed layer. While this result is in agreement with the small molar mass of esGX (Carvalho et al., 2021), and CMGX, indicating a lightweight adsorbed layer. However, after the rinsing step, both emulsifiers equilibrated to approximately the same frequency value. This was likely a result of the desorption of loosely-bound materials. Meanwhile, the difference in DIFT profile between CMC and CMGX was found as well in the QCM-D data, which could suggest that their higher quasi-equilibrium interfacial tension value was due to a general lack of material adsorption that resulted in a lower surface excess concentration.

3.5. Implication of interfacial adsorption on emulsification

The adsorption of emulsifiers at the oil-water interface has been demonstrated to be correlated to their emulsifying capability, with faster adsorption and lower equilibrium interfacial tension correlated to smaller initial droplet sizes (Amine et al., 2014; Goloub & Pugh, 2005; Kalogiannis et al., 2010). However, based on Fig. 6, it is reasonable to conclude that there was no clear correlation between the adsorption
dynamics of the tested emulsifiers and the emulsifying performance, both before and after microfluidization. Both CMC and CNC, which barely showed any adsorption, exhibited the smallest droplets prior to microfluidization; the other emulsifiers showed similar droplet sizes. Microfluidization reduced the D[3,2] significantly; however, larger droplet size variations were seen among the different emulsifiers. CNC emulsions had the largest D[3,2] after microfluidization; the size of the CNCs may have limited the size of the smallest achievable droplets. There were differences between the GXs after microfluidization; sGX and epGX were smaller than CMGX, which was expected; however, esGX, which was the fastest to adsorb among the four GXs, produced the largest droplets. Meanwhile, CMGX yielded the larger droplets, given the lack of adsorption. The result for esGX was unexpected since the DIFT results indicated that it should cover the surface of the fresh droplets quicker than sGX and epGX, and hence the droplets were supposed to be smaller. However, we observed extensive flocculation of esGX emulsions immediately after microfluidization, which was also reflected in the droplet size distribution (Fig. 6A). The submicron droplet population appeared to be in the same diameter range to the other GX; hence, the larger D[3,2] value was due to the contribution of the larger flocs. CNC-stabilized emulsion presented the largest droplets after microfluidization, as the size of the CNC particles may have limited the smallest achievable droplet size (Binks & Lumsdon, 2001; Chevalier & Bolzinger, 2013). The emulsification results showed that the adsorption of the GXs was not enough to produce a clear effect on emulsification performance - the low viscosity of the aqueous phase and the intermolecular attraction between GXs may destabilize the emulsions even soon after emulsification. Moreover, the physical state of the GXs in their aqueous dispersion is not yet fully understood. It is known that the aggregation of wood hemicelluloses is time-dependent in addition to concentration dependent (Bhattarai, Sulaeva, et al., 2020; Bhattarai, Valoppi, et al., 2020), and as such their aggregation is more complicated than less-complex emulsifiers. Considering how the conformation of a polysaccharide influences its emulsifying capability (Tang & Huang, 2022), an understanding of the aggregation behavior of GXs may be required to connect their interfacial behavior to their emulsifying properties.

3.6. Interfacial behavior of multicomponent emulsifiers via DIFT and QCM-D

The complexity of GXs, given their multicomponent and highly disperse nature, made it challenging to characterize their physical behavior at the oil-water interface. In this study, we focused on the transfer from the bulk phase to the interface, an important aspect in the emulsification process. We employed DIFT and QCM-D to follow the adsorption process (i.e., without any external energy input). Despite the fact that DIFT and QCM-D were used to probe different interfaces (liquid-liquid and solid-liquid), they were used to gain complementary information on the formation of interfacial layer by the GXs. The interfacial tension relates to the surface excess and describes the lateral density of the anchoring moiety at the oil-water interface. Unfortunately, the high dispersity of GXs prevented conversion of DIFT results to mass- or molar-based areal density (i.e., the mass or number of molecules per unit area). QCM-D, on the other hand, filled this need given that it senses the apparent mass of the adsorbed layer. Combining the respective results, we concluded that the lignin acts as an anchor for the GXs, facilitating adsorption at the oil-water interface. Meanwhile, the bulk of the interfacial layer was likely composed of the polysaccharide fraction. These results complemented our previous study, where we discussed the effect of its chemical compositions to its emulsion stabilization (Ho et al., 2023).

When it comes to the emulsification process, no direct relation between DIFT and QCM-D results was expected, as elaborated in Section 3.5; these techniques rely on adsorption in the absence of external energy input. Particularly, the role of insoluble particles formed by sGX and esGX cannot be easily assessed. The paraffin wax was used as a model hydrophobic surface to simulate the hydrophobic interactions between GXs and the nonpolar surfaces (oil). However, being a solid, the lateral dynamics of the adsorbed layer on wax would be different to that at the liquid-liquid interface. It is accepted that oil molecules participate in the interfacial activity of surfactants (Fainerman et al., 2019), which may not be possible at the solid-liquid interface. As such, despite the fact that the QCM-D dissipation data showed interfacial layers with a viscoelastic behavior, the latter cannot be directly applied to liquid-liquid interfaces. The viscoelasticity of the liquid-liquid interface may impart a dissipative property that could alter the adsorption dynamics (Yeung et al., 1997), which unfortunately could not be assessed due to instrument limitation. While a thin layer of liquid hexadecane coated on the QCM-D sensor may have represented the liquid-liquid interface better, there was a risk of detergency action by the emulsifiers (Olesen et al., 2016) that might strip the hexadecane layer instead of having them adsorb at the interface. Lastly, neither method probed droplet-droplet interactions that may be detrimental to emulsion stabilization; for example, the rapid destabilization of esGX emulsions were not apparent from either DIFT or QCM-D data and was only visible after actual emulsification. Nevertheless, the characterization of the interfacial structure may be useful in understanding other functionalities of the emulsion, such as its digestibility and performance as delivery systems.

4. Conclusion and outlook

By studying the adsorption behavior of different grades of GX at the liquid-liquid and solid-liquid hydrophobic interfaces (using a combination of DIFT and QCM-D experiments), and by comparing them to those of established polysaccharide-based emulsifiers, we have obtained additional information about the formation of interfacial layers by GX. Additionally, we have attempted to correlate the adsorption behavior to their emulsifying properties. Firstly, the lignin fraction was the main driving force for the adsorption of GX to the oil-water interface by introducing hydrophobicity to the system. The extent of this hydrophobicity was proportional to the amount of lignin in the system, which was confirmed by the high interfacial activity of esGX even at low concentrations. This was the first time the adsorption dynamics of GX had been described. Secondly, based on the salt screening experiment and the results from the zeta potential measurements, the ionic environment of the aqueous phase may influence the chain packing of the polysaccharide fraction, which would affect the surface excess concentration. Thirdly, we found indications of a more central role that the polysaccharide fraction of GX plays in its emulsion stabilization mechanism, which pointed to its role in forming the bulk of the interfacial layer. Finally, the emulsification tests indicated that controlling the droplet-droplet interactions may be the key to optimizing the emulsifying properties of GX beyond the formation of an interfacial layer. These results emphasized the need to further understand the functionalities of GX as emulsifier, as well as confirmed our hypothesis that the lignin content affects the rate of interfacial adsorption and the properties of the interfacial layer.

We also evaluated the combined use of DIFT and QCM-D to probe the interfacial structure of multicomponent emulsifiers, though a thorough understanding of GX interfacial behavior requires further studies. The latter should include dynamic measurements of interfacial viscoelasticity at the liquid-liquid interface and consideration of lignin solubility (pH and salt screening in the aqueous phase). The finer structure of the interfacial layer, such as its thickness and roughness, remains unknown along with the hierarchical arrangements that may exist within the adsorbed layer (i.e., localization of lignin and polysaccharide in the layer). The influence of their aggregation behavior on the emulsifying capability, including the time- and concentration-dependence of it, should also be understood further. Moreover, the uniformity of the layer, whether patchy or continuous, is a subject that deserves attention. Additionally, the interactions between GX and industrially-relevant
systems, such as vegetable oil and plant wax, should be explored. These aspects will provide a more comprehensive knowledge on the role of lignin and polysaccharide fractions of GX, relevant to their tailorable functionality.

Abbreviations

CMC sodium carboxymethylcellulose
CMGX carboxymethylated PHWE GX
CNC cellulose nanocrystals
DIFT dynamic interfacial tension
epGX ethanol-precipitated PHWE GX
esGX ethanol-soluble PHWE GX
GXs glucuronoxylans
LCCs lignin-carbohydrate complexes
MC methylcellulose
MF emulsions after microfluidization
Mw weight-averaged molar mass
Mw/Mn dispersity
PHWE pressurized hot water extraction
QCM-D quartz crystal microgravimetry with dissipation monitoring
sGX spray-dried PHWE GX
UT emulsions before microfluidization, after homogenization using Ultra-Turrax

CRedit authorship contribution statement

Felix Abik: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. Katarina Solin: Writing – review & editing, Methodology, Investigation. Sami Hietala: Writing – review & editing, Resources, Methodology. Orlando J. Rojas: Writing – review & editing, Resources, Methodology, Funding acquisition. Thao Minh Ho: Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. Kirsi S. Mikkonen: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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