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1 Phenolic residues in spruce galactoglucomannans

2 improve stabilization of oil-in-water emulsions

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10

11 ABSTRACT

12 **Hypothesis.** Amphiphilic character of surfactants drives them at the interface of dispersed systems,
13 such as emulsions. Hemicellulose-rich wood extracts contain assemblies (lignin-carbohydrate
14 complexes, LCC) with natural amphiphilicity, which is expected to depend on their chemical
15 composition resulting from the isolation method. Lignin-derived phenolic residues associated with
16 hemicelluloses are hypothesized to contribute to emulsions' interfacial properties and stability.

17 **Experiments.** We investigated the role of phenolic residues in spruce hemicellulose extracts in the
18 stabilization of oil-in-water emulsions by physical and chemical approach. Distribution and changes

19 occurring in the phenolic residues at the droplet interface and in the continuous phase were studied during
20 an accelerated storage test. Meanwhile, the physical stability and lipid oxidation in emulsions were
21 monitored.

22 **Findings.** Naturally associated lignin residues in GGM act as vehicles for anchoring these
23 hemicelluloses into the oil droplet interface and further enable superior stabilization of emulsions. By
24 adjusting the isolation method of GGM regarding their phenolic profile, their functionalities, especially
25 interfacial behavior, can be altered. Retaining the native interactions of GGM and phenolic residues is
26 suggested for efficient physical stabilization and extended protection against lipid oxidation. The results
27 can be widely applied as guidelines in tailoring natural or synthetic amphiphilic compounds for
28 interfacial stabilization.

29 **Keywords**

30 Spruce galactoglucomannans, phenolic residues, emulsion stability, lipid oxidation

31 **Abbreviations**

32 eTMP ethanol precipitated galactoglucomannan (GGM) from thermomechanical pulping, LCC lignin-
33 carbohydrate complexes, OHB hydroxybenzoates, OHC hydroxycinnamates, PE spruce phenol extract,
34 PHWE GGM from pressurized hot-water extraction, PR phenolic residue, PV peroxide value, sTMP
35 spray-dried GGM from thermomechanical pulping.

36 **Introduction**

37 To deliver and protect lipophilic compounds in both technical applications and foods, lipids are
38 preferably dispersed into polar media, most often water. This formulation — emulsion — enhances the
39 physical properties of the product and enables efficient delivery of bioactive compounds, for example.
40 To mix two immiscible liquids and stabilize emulsions, additional components are needed, namely,
41 emulsifiers and stabilizers.¹ Small-molecular amphiphilic compounds, such as phospholipids and
42 organosulphates, adsorb readily at the emulsion interface and decrease the energy needed for
43 emulsification. However, the interface is sensitive to environmental changes, such as the presence of
44 other amphiphilic compounds or mechanical forces. In addition to classical surfactants, macromolecules,
45 such as proteins and polysaccharides, are used as emulsifiers and/or stabilizers. The advantage of using
46 polysaccharides as stabilizers is that their properties are stable under a broad pH and ion strength range,
47 while the function of proteins is highly pH and ion strength dependent.^{2–3} Moreover, polysaccharide-
48 stabilized emulsions tolerate thermal processing.

49 Pure polysaccharides are polar and lack a clear amphiphilic nature. Their emulsification and
50 stabilization ability is considered to occur either via residual protein fractions that would introduce
51 amphiphilicity, or via increment of steric repulsion and/or continuous phase viscosity.^{4–5} Examples
52 include proteinous gum Arabic and corn fiber gum, and very large polysaccharides, such as pectin and
53 xanthan.^{6–9} Pickering-type stabilization has also been proposed for polysaccharides.^{10–11} That is, nano-
54 sized particles adsorb to the interface, creating a rigid barrier against coalescence and transfer of
55 compounds between the dispersed and continuous phases.

56 The high surface area of emulsions predisposes lipids into contact with oxygen and oxidation initiators,
57 such as transition metals. Lipid oxidation decreases the functionality and nutritional value of lipids and
58 produces off-flavors. Moreover, oxidation causes physical changes to the emulsion structures, e.g.,
59 polymerization of lipids and eruption of emulsions.¹² Thus, lipids should be protected against oxidation,
60 and at the same time, the interfacial structure may be protected. The rate of oxidation is dependent on

61 the amount and type of oil, emulsifiers, and stabilizers, and on the partitioning of the emulsifier and
62 stabilizer between the interface and continuous phase.^{2,13–14}

63 Approaches to stabilizing interfaces include thickening the interface or altering its charge with
64 multilayer coatings^{14–16} or incorporating antioxidants, such as polyphenols, to the interface.¹⁷ These
65 multi-step techniques principally use electrostatic deposition of polymer layers. That is, oppositely
66 charged polymers are layered one by one on the droplet surface. This technique is laborious and requires
67 great control of the process parameters, such as pH and ion strength, thus leading to costly production.

68 Biorefineries are currently being developed for profitable isolation of various lignocellulosic fractions
69 into precursors of bio-based chemicals and materials. Availability of potential new renewable raw
70 materials has extended the possibilities to explore their suitability for industrial applications; however,
71 in many cases, their applicability or sustainability may be limited due to the need for hazardous solvents
72 or reagents in biomass derivatization. Conversion of non-food biomass into a source of energy or raw
73 materials for technical products often involves a sequence of chemical derivatization reactions aided by
74 solvents and reagents. Within the biorefinery approach, biomass isolation techniques are being
75 developed to control the composition and functions of isolated fractions. In this paper, we present a green
76 chemistry approach towards dispersing agents by exploring and exploiting the natural characteristics of
77 softwood extracts, namely, spruce *O*-acetyl-galactoglucomannans (GGM), wood-derived hemicelluloses
78 that are still widely untapped, as multifunctional stabilizers.

79 We have previously shown that spruce GGM, abundant future forest biorefinery products that are
80 relatively small, linear chain polysaccharides, adsorb at the lipid droplet interface and stabilize it against
81 physical breakdown and lipid oxidation.^{18–20} However, the action mechanisms are yet to be discovered.
82 GGM are envisioned as multifunctional, sustainable, natural stabilizers that could replace several less
83 effective additives and be applied in various types of industrial dispersions, including food, cosmetics
84 and pharmaceuticals after safety evaluations (Pitkänen et al., submitted) and in technical applications,
85 e.g. coatings and chemicals.

86 GGM are hemicelluloses that consist of chains of (1→4)-linked β-D-mannopyranosyl and β-D-
87 glucopyranosyl units with single (1→6)-linked α-D-galactopyranosyl units attached to mannose.²² Part
88 of the secondary hydroxyl groups of mannose units are acetylated, which introduces hydrophobic sites
89 to the structure. Yet, the backbone of GGM lacks clear non-polar parts that would give GGM an
90 amphiphilic nature. In softwood, hemicelluloses may be associated or partially covalently bound to
91 lignin, forming lignin-carbohydrate complexes (LCC).²³ Lignin-originating phenolic residues (PR) are
92 likely to accompany hemicelluloses during the isolation process and provide additional characteristics
93 to GGM.

94 The remarkable physical emulsion stabilization capacity of GGM is considered to arise from steric
95 repulsion and/or Pickering-type stabilization by GGM assemblies. We have previously shown that GGM
96 adsorbs at the oil droplet interface, but the driving force enabling adsorption is not known.¹⁸ At high
97 GGM concentrations, stabilization also occurs via increment of viscosity.¹⁹ GGM also inhibits lipid
98 oxidation both in oil-in-water emulsions and in microcapsules.^{20,24}

99 The isolation method of hemicelluloses affects both the content and composition of hemicelluloses
100 and accompanying PR. For example, by altering the temperature and extraction time in flow-through
101 pressurized hot water extraction, the composition and size of recovered hemicelluloses and lignin can be
102 controlled.^{25–27} However, the role of PR for the functionality of GGM is largely unknown; their presence
103 may be either beneficial or detrimental, depending on the application.

104 The wide availability of raw materials, the economical isolation processes, and the multifunctionality
105 of GGM make them a highly attractive stabilizer alternative for industrial use. To understand the features
106 of GGM and enable development of applications, knowledge of their function mechanisms is needed.
107 The aim of this study was to demonstrate the role of naturally occurring lignin-originating PR in GGM
108 in the stabilization of oil-in-water emulsions. Thereby we illustrate the interaction and interfacial
109 functionality of lipophilic structures bound with hydrophilic polysaccharide tails. GGM isolated with
110 different techniques and having either hemicellulose-phenolic compound complexes or added phenolic

111 extract were compared for their stabilization capacity. Partitioning of PR between the interface and
112 continuous phase of emulsions and the changes occurring in them were determined during an accelerated
113 storage test. Meanwhile, physical stability and lipid oxidation in emulsions was monitored. This study
114 reveals that GGM-phenolic complexes play a key role in emulsion stabilization.

115 **Materials and methods**

116 Materials

117 GGM, a natural mixture containing variety of galactoglucomannans and wood extractives, were
118 obtained in powder form from process water of a Finnish pulp mill in an industrial-scale isolation trial
119 after spray-drying (sTMP) or after ethanol precipitation (eTMP)^{28–29} and as aqueous concentrate from
120 pressurized hot water flow-through extraction (PHWE)²⁵. The average molar mass of sTMP and eTMP
121 were 20 000–30 000 g/mol¹⁸ and that of PHWE GGM below 10 000 g/mol¹⁹. Lignan-rich spruce phenol
122 extract (PE) was isolated from spruce knotwood according to Willför et al.³⁰ Rapeseed oil, consisting of
123 60% of monounsaturated, 31% of polyunsaturated, and 4% of saturated fatty acids (Bunge Finland Oy,
124 Raisio, Finland), was purchased from a supermarket and purified from natural anti- and pro-oxidants by
125 adsorption chromatography according to a previously described method.^{20,31}

126 Emulsion preparation

127 Emulsions containing 5 wt.-% of stripped rapeseed oil and 1 wt.-% of GGM were prepared by high-
128 pressure homogenization according to a previously described method.²⁰ For the addition of spruce
129 phenol extract (PE) to eTMP emulsion, PE was dissolved into acetone (30 mg/mL) and then dispersed
130 into 1 wt.-% GGM solution before emulsification to yield 5.5 mg added phenolic compounds in 100 g
131 emulsion. The total phenol content of spruce phenol extract was estimated using the Folin-Ciocalteu

132 method ³² and the amount of added extract was adjusted in order to double the amount of phenolic
133 compounds in the emulsions compared to samples with plain eTMP GGM.

134 Accelerated storage test

135 For the accelerated storage test, 100 g of emulsion was stored in a 250-mL glass bottle at 40 °C in the
136 dark up to 2.5 months. Stability of the emulsion was monitored several times during the first two weeks
137 of storage and then in intervals of two weeks for a total of 11 weeks. At each sampling, a few drops of
138 emulsion were withdrawn for determining droplet size distribution and for visual investigation with
139 optical microscopy. At the same sampling, 0.5 g of emulsion was withdrawn for the analysis of peroxide
140 value and polymerized lipids. For monitoring the changes occurring in PR, 30 g of emulsion was
141 withdrawn after 0, 7 and 14 days of storage. Before each sampling, the emulsion was mixed by turning
142 the containers upside down ten times. For the analysis of volatile oxidation products, 1.5 g of fresh
143 emulsion was placed in 20-mL glass vials (75.5 × 22.5 mm), sealed with caps, and the vials were stored
144 at 40 °C in the dark. At each sampling, three replicate samples were withdrawn (n = 3).

145 Droplet size distribution

146 The droplet size distribution was characterized by static light scattering using a Mastersizer Hydro
147 3000 SM (Malvern Instruments Ltd, Worcestershire, UK). Before each sampling, the emulsion was
148 mixed gently by turning the container upside down ten times. Interfacial area was calculated from the
149 average droplet size and volume fraction of the added oil.

150 Microscopy

151 The emulsion morphology was characterized using optical microscopy (AxioScope A1, Carl Zeiss
152 Inc., Oberkochen, Germany). Before each sampling, the emulsion was mixed gently by turning the
153 container upside down ten times.

154 Partitioning of emulsions

155 Emulsions were partitioned into aqueous and creamed phases by centrifugation.¹⁸ 30 g of emulsion
156 was centrifuged at 24 000 g at RT for 15 min. The creamed phase was collected and the residue was
157 centrifuged for additional 5 min. The second creamed phase was combined with the first one for the
158 analysis of adsorbed GGM fraction. 1 mL of continuous phase was collected for the analysis of the non-
159 adsorbed GGM fraction. Both the creamed phase and continuous phase were subjected to further analysis
160 (Fig. 1).

161 Determination of phenolic residues by UHPLC-DAD-FLD

162 Distribution and changes occurring in interfacial and dispersed free and bound phenolic residues of
163 GGM were determined in order to evaluate their possible contribution to emulsion stability.

164 The creamed phase was divided into two equal portions. The adsorbed GGM were precipitated with
165 80 mL of 80% ethanol, which is also compatible for the extraction of free phenolic compounds (Fig. 1).
166 Released lipids were removed from the aqueous phase by extraction with 80 mL of iso-octane. The
167 organic phase was removed and the remaining aqueous phase was centrifuged (10 min at 24 000 g).
168 Supernatant and precipitated GGM were collected separately. The supernatant was evaporated to
169 dryness.

170 Non-adsorbed GGM in the continuous phase (1 mL) were precipitated with 4 mL of 80% ethanol.
171 Remaining lipids were removed by extraction with 5 mL of iso-octane. The aqueous phase and
172 precipitate were separated by centrifugation (10 min at 3000 g). Supernatant and precipitated GGM were
173 collected separately. The supernatant was evaporated to dryness.

174 The hydrolyses and analysis of PR were performed according to a previously described method using
175 the same equipment and reagents.²⁰ The analysis included fractions of ethanol-soluble free PR, ester-
176 bound PR to ethanol-soluble GGM, ester-bound PR to ethanol-precipitated GGM and glycosidically

177 bound or otherwise entrapped PR in GGM (Fig. 1). All the analyses were performed in triplicates (n =
 178 3). The results were expressed as $\mu\text{g/kg}$ of emulsion. The average and standard deviation were calculated
 179 across three analytical replicates (n = 3).

180
 181 Figure 1. Sample preparation for the analysis of adsorbed and non-adsorbed phenolic residues. Each
 182 creamed phase and aqueous phase provided results of six different compound groups in four different
 183 fractions. The analysis was performed in triplicates.

184 Determination of peroxide value

185 The primary oxidation of the emulsions was evaluated by the total content of hydroperoxides in the
186 lipid phase determined by the peroxide value (PV). The analysis was performed according to a previously
187 described method²⁰ where lipids were first released and extracted and then PV was determined according
188 to a ferric thiocyanate method. The average and standard deviation were calculated across three
189 analytical replicates (n = 3).

190 Determination of hexanal by SHS-GC-FID

191 The secondary stage of lipid oxidation in emulsions was followed in terms of hexanal formation
192 according to a previously described static headspace gas chromatography (SHS-GC-FID) method.³³ At
193 each sampling time, three replicate vials were withdrawn for the analysis. Hexanal contents were
194 reported as peak area per gram of emulsion (peak area/g emulsion). The average and standard deviation
195 were calculated across three analytical replicates (n = 3).

196 Determination of polymerized lipids by SEC-RI

197 Lipid oxidation in emulsions was also followed in terms of polymerization. Formed oligomeric lipids
198 and remaining monomers were determined utilizing size-exclusion chromatography (SEC) in
199 combination with refractive index (RI) detection, as described previously.²⁰ The results were reported as
200 relative peak area proportions (%). The average and standard deviation were calculated across three
201 analytical replicates (n = 3).

202 **Results**

203 Droplet size distribution and morphology

204 Physical stabilization of emulsions by studied GGM extracts were evaluated by droplet size
205 distributions. The structures of emulsions were also visualized by optical microscopy to reveal possible
206 breakdown.

207 Emulsions stabilized by PHWE GGM had a unimodal droplet size distribution with an average droplet
208 size $D[3,2]$ of $0.4\ \mu\text{m}$ (Fig. 2). The droplet size distribution remained similar during six weeks of
209 accelerated storage test at $40\ ^\circ\text{C}$. In sTMP GGM-stabilized emulsions, the $D[3,2]$ was larger ($2.1\ \mu\text{m}$)
210 and increased to $3.4\ \mu\text{m}$ during storage. The eTMP GGM-stabilized emulsions showed bimodal droplet
211 size distribution and an increase in droplet size during storage. The addition of PE to eTMP GGM did
212 not significantly influence the droplet size distribution, as the eTMP+PE emulsions formed and aged in
213 a similar manner to those containing plain eTMP GGM, in terms of droplet size distribution. Optical
214 microscopy images confirmed the presence of mainly very small droplets of less than $1\ \mu\text{m}$ in diameter
215 in PHWE emulsions, whereas large droplets and flocculation were seen in sTMP emulsions. In eTMP
216 and eTMP+PE emulsions, droplets with a large size range were observed, which could be due to
217 coalescence or Ostwald ripening (Fig. 3).

218 The total oil-water interfacial area of PHWE emulsion was $800\ \text{m}^2/\text{kg}$ while that of sTMP emulsion
219 was $150\ \text{m}^2/\text{kg}$ (Fig. 4). No significant changes were observed in PHWE emulsion while the interfacial
220 area of sTMP emulsion decreased to $100\ \text{m}^2/\text{kg}$ during the first week of storage at $40\ ^\circ\text{C}$. The interfacial
221 area of eTMP and eTMP+PE emulsions decreased continuously from $1300\ \text{m}^2/\text{kg}$ to $400\ \text{m}^2/\text{kg}$ during
222 two weeks of storage.

Figure 2. Droplet size distributions of emulsions with 5 wt.-% rapeseed oil stabilized with 1 wt.-% GGM obtained from (a) pressurized hot water extraction (PHWE), (b) thermomechanical pulping after spray-drying (sTMP), (c) thermomechanical pulping after ethanol precipitation and addition of spruce phenol extract (eTMP + PE), and (d) eTMP as fresh and after 1–6 weeks storage at 40 °C. The graphs are the averages of three replicate measurements.

Figure 3. Optical microscopy images of emulsions with 5 wt.-% rapeseed oil stabilized with 1 wt.-% GGM, stored at 40 °C for 2 weeks. GGM obtained from (a) pressurized hot water extraction (PHWE), (b) thermomechanical pulping after spray-drying (sTMP), (c) thermomechanical pulping after ethanol precipitation with addition of spruce phenol extract (eTMP + PE), and (d) eTMP without additives.

Figure 4. Total interfacial area of lipid droplets (5 wt.-%) in GGM-stabilized (1 wt.-%) emulsions during accelerated storage test at 40 °C. GGM obtained from pressurized hot water extraction (PHWE), thermomechanical pulping after spray-drying (sTMP) and thermomechanical pulping after ethanol precipitation (eTMP) and addition of spruce phenol extract (eTMP + PE).

Distribution of phenolic residues in emulsions

To understand how phenolic residues (PR) in GGM affect emulsion stability, their distribution between the interface and continuous phase was determined.

In all the studied emulsions, less than 0.1% of PR were located at the interface while the majority remained in the continuous phase (Fig. 5). The content of interfacial PR in PHWE GGM-stabilized emulsion was 2.6 µg/kg emulsion, while the content in the continuous phase was 111 mg/kg emulsion. The majority of the adsorbed PR (77%) were ester bound to ethanol-soluble GGM fraction, but some of the free or weakly associated PR (21%) were located at the interface. The continuous phase of PHWE emulsion had similar distribution of ester bound and free PR as at the interface though the contents were greater. The chemical structures of the adsorbed PR and those of remaining in the continuous phase were different. While ester-bound hydroxycinnamyls (OHC) (57%) and hydroxybenzoyls (OHB) (32%) were the main PR units at the interface, ester-bound OHB (62%) and flavanols (35%) were dominant in the

251 continuous phase. In the free PR, the proportion of flavanols (39%) and OHC (29%) were greater at the
252 interface than in the continuous phase (19% and 10%, respectively) while the proportion of OHB was
253 far greater (71%) in the continuous phase than at the interface (32%).

254 In sTMP GGM-stabilized emulsion, the content of interfacial PR was 0.4 µg/kg emulsion and the
255 content of continuous phase PR was 59 mg/kg emulsion (Fig. 5). PR ester bound to ethanol-soluble
256 GGM and free PR comprised equal fractions (47%) at the interface. Free PR were the most abundant
257 fraction (53%) in the continuous phase. Flavanols constituted the majority (65–70%) of the adsorbed
258 and continuous phase PR followed by OHB (24–26%).

259 In eTMP+PE GGM-stabilized emulsion, PR of 1.7 µg/kg emulsion were adsorbed at the interface
260 while 41 mg/kg emulsion remained in the continuous phase (Fig. 5). PR ester bound to ethanol-soluble
261 GGM was the main fraction (54%) at the interface, but also a fraction of free (21%) and glycosidically
262 bound (22%) PR were adsorbed. Free PR (57%) and glycosidically bound PR (39%) constituted the main
263 fractions in the continuous phase. Flavanols constituted the majority (53–65%) of the adsorbed and
264 continuous phase PR followed by OHB (35–47%).

265 As the content of PR in eTMP GGM was considered relatively low,²⁰ that is, much lower than in sTMP
266 GGM, the distribution of PR in eTMP emulsions were not studied in more detail.

Figure 5. Composition of adsorbed (A) and non-adsorbed (B) phenolic residues in stripped rapeseed oil (5 wt.-%) emulsions stabilized by GGM (1 wt.%). GGM obtained from pressurized hot water extraction (PHWE), thermomechanical pulping after spray-drying (sTMP) and thermomechanical pulping after ethanol precipitation and addition of spruce phenol extract (eTMP + PE). The graphs presenting hydroxybenzoates (OHB), hydroxycinnamates (OHC), flavonols and flavanols are the averages of three replicate analyses.

274 Changes occurring in the phenolic residues during storage

275 In order to understand the role of phenolic residues (PR) in the physical and oxidative stabilization of
276 emulsions, changes occurring in the distribution and contents of PR were monitored during the first two
277 weeks of accelerated storage test.

278 Changes in the contents of PR were observed both in the interfacial and continuous phases throughout
279 the accelerated storage test (Fig. 6). In PHWE emulsion, the changes were more substantial in the
280 continuous phase than at the interface: a decrease of 55–62% in the total PR content was observed in the
281 continuous phase while the decrease was only 24% at the interface. Further changes were not observed
282 after one week of storage. The changes in the continuous phase occurred mainly in PR ester bound to
283 ethanol-soluble GGM (72%) and at the interface in free PR (55%). In the continuous phase, the decrease
284 was more pronounced in OHB (89%) compared to flavanols (51%). At the interface, the reduction was
285 similar for both OHB and flavanols (60–68%).

286 In sTMP emulsions, changes also occurred mainly in the continuous phase PR: a continuous decrease
287 of 35% in their content was observed during two weeks. The changes occurred primarily in PR ester
288 bound to ethanol-precipitated GGM (84%) and in PR ester bound to ethanol-soluble GGM (60%). The
289 contents of all of the PR, OHB, OHC and flavanols decreased (51–87%).

290 Unlike in the other emulsions, in eTMP+PE emulsion, changes occurred only in the interfacial
291 phenolic residues: a continuous decrease of 74% occurred over two weeks. The changes could be
292 observed in all of the studied fractions, being most abundant in glycosidically bound PR (87%). The
293 greatest decrease occurred in OHB and OHC (87–94%).

294 As the content of PR in eTMP GGM was relatively low,²⁰ the changes occurring in their contents
295 during emulsion storage were not studied in more detail.

296

Figure 6. Changes occurring in the adsorbed (A) and non-adsorbed (B) phenolic residues in stripped rapeseed oil (5 wt.-%) emulsions stabilized by GGM (1 wt.%) during accelerated storage test at 40 °C. GGM obtained from pressurized hot water extraction (PHWE), thermomechanical pulping after spray-drying (sTMP) and thermomechanical pulping after ethanol precipitation and addition of spruce phenol extract (eTMP + PE). The graphs are the averages of three replicate analyses.

Lipid oxidation in GGM-stabilized emulsions

304 In PHWE GGM-stabilized emulsion, formation of hydroperoxides was detected (PV of 61 mEq/kg)
 305 only after 9 weeks of storage at 40 °C (Fig. 7). In sTMP emulsion, hydroperoxides were detected after 2
 306 weeks, and PV of 20 mEq/kg oil was measured after 6 weeks. eTMP GGM inhibited oxidation, but not
 307 as efficiently: hydroperoxides were detected after 4 days of storage and PV of 264 mEq/kg was measured
 308 after two weeks. Addition of spruce phenol extract to eTMP GGM enhanced the stability of lipids:
 309 hydroperoxides were detected after 9 days (10 mEq/kg oil) and PV of 228 mEq/kg oil was measured
 310 after 8 weeks of storage.

311
 312 Figure 7. Formation of hydroperoxides in the stripped rapeseed oil phase (5 wt.%) of GGM (1 wt.%)
 313 stabilized emulsions during accelerated storage test at 40 °C. GGM obtained from pressurized hot water
 314 extraction (PHWE), thermomechanical pulping after spray-drying (sTMP) and thermomechanical
 315 pulping after ethanol precipitation (eTMP) and addition of spruce phenol extract (eTMP + PE). The
 316 graphs are the averages of three replicate analyses.

317 In PHWE and sTMP emulsions, hexanal was not detected during the first four weeks of storage (Fig.
 318 8). As was seen in PV, spruce phenol extract added to eTMP GGM delayed the oxidation but not as
 319 efficiently as PHWE GGM and sTMP GGM. In eTMP GGM-stabilized emulsion, hexanal was detected
 320 after 2 weeks and was detected in eTMP+PE emulsions after 3 weeks. After 1.5 months of storage, the
 321 samples in headspace vials were physically separated and thus volatile oxidation products were no longer
 322 determined.

323 No polymerization or interaction products of the lipids were detected during the 2.5-month accelerated
 324 storage test in any of the studied emulsions.

325
 326 Figure 8. Formation of hexanal in the stripped rapeseed oil phase (5 wt.%) of GGM (1 wt.%) stabilized
 327 emulsions during accelerated storage test at 40 °C. GGM obtained from pressurized hot water extraction
 328 (PHWE), thermomechanical pulping after spray-drying (sTMP) and thermomechanical pulping after
 329 ethanol precipitation (eTMP) and addition of spruce phenol extract (eTMP + PE). The graphs are the
 330 averages of three replicate analyses.

331 Discussion

332 This study clearly demonstrates that GGM containing bound phenolic residues are more efficient
333 emulsion stabilizers than purified (ethanol precipitated) GGM alone or with incorporated phenolic
334 compounds. This applies both for the physical stability and for the protection of dispersed lipids against
335 oxidation. The structure and composition of residual phenolic compounds (PR) in GGM define their
336 ability to adsorb to the oil-water interface and stabilize it. In addition, PR are responsible for the
337 remarkable oxidative stability of the dispersed lipids. These observations will be discussed further.

338 Although only small portion of PR adsorbed at the interface, they had a remarkable effect on emulsion
339 morphology and stability. According to our earlier findings, the surface load of eTMP GGM in
340 corresponding 5% oil-in-water emulsion was 0.8 mg/m².¹⁸ This surface load corresponds to
341 approximately 10% of GGM present in the emulsion. However, in the present study, less than 1% of the
342 PR present in the emulsions were located at the interface. As the molar mass of the adsorbed GGM tend
343 to be greater compared to those remaining in the continuous phase,¹⁸ greater surface load is achieved
344 with fewer molecules. This knowledge leads us to hypothesize either that the size of GGM is of
345 importance or that these larger molecules contain other functionalities, most likely PR, which aid their
346 anchoring at the interface. Acetylation of hydroxyl groups alters the overall polarity of GGM, making
347 them less polar. Acetyl groups may play a role in the functionality of GGM; however, they are not
348 expected to carry the main responsibility for GGM's stabilization capacity. In addition, GGM contain
349 only negligible portions of residual proteins;³⁴ thus, their role in stabilization is considered insignificant.

350 PHWE GGM-stabilized emulsions had the greatest content of PR at the interface (2.6 µg/kg emulsion),
351 especially of those that were ester bound to ethanol-soluble GGM, and at the same time had a unimodal
352 droplet size distribution with smallest average droplet size and largest droplet surface area compared to
353 emulsions prepared with the other types of GGM. In addition, PHWE emulsion remained extremely
354 stable throughout the 6 weeks of the accelerated storage test. With the other types of GGM, a smaller

amount of PR (0.4–1.7 $\mu\text{g/kg}$ emulsion), most of them being free or only weakly associated with GGM (i.e., extractable with ethanol), were adsorbed at the droplet interface. These emulsions had larger average droplet sizes and were less stable. More importantly, when phenolic compounds were introduced as a separate extract (in eTMP+PE), emulsification was efficient, but the long-term physical stability of emulsion was not comparable to that of PHWE emulsion. Free PR may act as emulsifiers (i.e. facilitate emulsion formation), but may destabilize the interface during storage.³⁵ Moreover, their partitioning between the continuous and interfacial phases is highly pH dependent due to the dissociation of the acid and hydroxyl groups. Most of the ester-bound PR, on the other hand, lack the acid group and thus remain neutral at a wide pH range.

PR may be bound to the monosaccharide units of GGM either via ester or ether bonds or via glycosidic bonds.^{23,36} Part of these bonds may occur naturally, but they are also formed during the isolation processes. Each monosaccharide unit has several hydroxyl groups available for ester bonding, but GGM have only one site for glycosidic bonding, that is, at the reducing end of the molecule. Non-polar units attached to the polysaccharide backbone are considered to increase the amphiphilic nature of GGM and enable their anchoring to the oil droplet interface. Larger GGM chains have more possible sites available for ester linking and therefore a greater number of PR could be bound. Further increase in the amphiphilic nature would make these larger GGM molecules more susceptible to adsorb at the interface. Ester-bound PR would also enable even packaging of GGM parallel to the interface (Fig. 9) and therefore could lead to greater physical stability and protection against lipid oxidation, as shown for PHWE emulsions in this study. Glycosidically bound PR, on the other hand, may be located only at the reducing end of the GGM molecules and could therefore lead to perpendicular anchoring of GGM to the surface. Our hypotheses of orientation of GGM-PR assemblies on droplet interface are presented in Fig. 9. This orientation is hypothesized to form a thick, but porous interfacial layer due to steric hindrance caused by the GGM chains with attached galactopyranosyl side groups. It is likely that this perpendicular orientation and steric hindrance would lead to larger droplet size than if GGM chains were aligned with the interface, as

380 demonstrated with sTMP and eTMP emulsions containing a considerable fraction of glycosidically
 381 bound PR at the interface. In addition, a thick and porous layer could be responsible for the observed
 382 flocculation of these emulsions. Flocculation may eventually lead to coalescence and increased average
 383 droplet size. However, thick interfacial layer, if not too porous, could inhibit lipid oxidation by acting as
 384 a steric barrier between the oxidation initiators and dispersed lipids,^{13–14} which could explain the
 385 oxidative stability of sTMP emulsion. The presently hypothesized nature of GGM-PR assemblies at
 386 droplet interface explains the anchoring of intermediate-sized GGM tails on the droplet surface by
 387 lipophilic PR structures. This is analogic to protein anchors in gum Arabic^{4,5}, with the difference that the
 388 present GGM-PR assemblies were more efficient stabilizers than gum Arabic studied earlier¹⁸. Such
 389 structural assemblies are suggested as guidelines in targeted isolation of bio-based hydrocolloid
 390 structures with high functionality or in derivatization or synthesis of structural elements designed for
 391 efficient interfacial stabilization.

392
 393 Figure 9. Simplified illustration of the hypothetical orientation of GGM containing ester-bound (A) and
 394 glycosidically bound (B) phenolic residues at the interface of oil-in-water emulsion.

395 GGM may also stabilize emulsions via Pickering-type stabilization, that is, forming an interfacial layer
 396 by assemblies.¹⁹ Assemblies comprise parallel-orientated non-branched carbohydrate chains, which are
 397 associated via hydrogen bonding of the hydroxyl groups.³⁷ Alternatively, PR may promote GGM-GGM

398 associations due to their polarity difference with the aqueous medium or due to the hydrogen bonding of
399 PR. In Pickering-type stabilization, the size and orientation of the assemblies determine the droplet size
400 and thickness of the interfacial layer.^{10–11,19}

401 The chemical structures of PR in the studied GGM samples varied from simple free aromatic phenolic
402 acids to polymers of flavonoids. Lignin is a polymer of ester, ether, glycosidically and carbon-bound
403 phenyl propanoid units.³⁸ Lignin may exist in free form or it may be bound or associated to surrounding
404 hemicelluloses and cellulose forming LCC.^{23,36} In these complexes, lignin is connected to sugar units
405 mainly with hydroxycinnamyl or hydroxybenzyl units via glycosidic, ester or ether bonds. In base and
406 acid hydrolyses of GGM, as used in the present work, various bond types existing between the
407 carbohydrate chain and phenolic residues and between different phenyl units in the PR are cleaved. Thus,
408 this technique provided information about the various groups of phenyl units present in GGM, even
409 though it did not reveal their complex native structures. As lignin fragments in LCC seem to be mainly
410 monomers and dimers,³⁹ we hypothesize that the PR in the studied GGM are rather small units. The bond
411 type and composition of released PR clearly correlated with the stabilization capacity of GGM.
412 Hydroxycinnamyls ester bound to ethanol-soluble GGM were most efficiently adsorbed to the oil droplet
413 interface. Emulsions containing higher amounts of hydroxycinnamyls appeared to be most stable.

414 Previous studies have proposed that PR are more likely to interact with hemicelluloses with higher
415 numbers of galactose side groups.²³ If the residues are bound to galactose units, this could enable PR to
416 get in contact with the lipid surface despite the possible steric hindrance caused by rigid GGM. While
417 attaching to the non-polar medium, the PR would simultaneously anchor GGM to the interface.

418 The large interfacial area in emulsions predisposes lipids into enhanced contact with oxidation
419 initiators. In addition, flocculation may bring dispersed lipid droplets into close contact, enhancing the
420 transfer of lipid radicals and hydroperoxides from one droplet to another. This interaction may lead to
421 an increased oxidation rate. If the interfacial layer is sufficiently thick and/or dense, it acts as physical
422 barrier between active initiators and lipid species, while at the same time preventing further oxidation.^{13–}

423 ¹⁴ In addition to acting as physical barrier, GGM containing PR may protect lipids against oxidation via
424 scavenging radicals and binding iron.⁴⁰

425 Lipids were most stable against oxidation in PHWE emulsion that had the smallest average droplet
426 size, the largest surface area and the greatest content of PR, especially those that were bound to GGM,
427 among the studied emulsions. In contrast, lipid oxidation was most intensive in eTMP emulsion which
428 had larger average droplet size, smaller surface area and contained less PR compared to the other
429 emulsions.²⁰ The addition of phenolic compounds as a separate extract (eTMP+PE) improved oxidative
430 stability, but not to the level of PHWE emulsion. Thus, PR, especially those associated with GGM,
431 seemed to have a significant role in the stabilization of lipids against oxidation. Lipid oxidation and
432 physical stability are also expected to be interlinked, for example, oxidized lipid droplets may be more
433 prone to Ostwald ripening due to their increased miscibility with the continuous phase after increased
434 polarity.

435 In all of the studied emulsions, decrease in the contents of PR occurred during the storage. The changes
436 were most evident in the ethanol-soluble fractions, both in free PR and in ester-bound PR. In PHWE and
437 sTMP emulsions, changes in the PR occurred mainly in the continuous phase. At the same time, these
438 emulsions were most stable against oxidation. In eTMP+PE emulsion, containing only minor amount of
439 GGM-bound PR, decrease in phenolic compounds was most apparent at the interface. In addition, lipid
440 oxidation was greater in this emulsion compared to PHWE and sTMP emulsions. Adsorbed free phenolic
441 compounds may have deattached from the interface during storage, leading to a decrease in their contents
442 and possible destabilization of the interface. Reactions of PR with radicals may also promote
443 polymerization of GGM and formation of interaction products with lipids. Even if the polymerized or
444 interacted GGM would remain at the droplet interface, the number of antioxidant active PR would be
445 reduced. Decrease in the coverage of the interface and reduced content of antioxidants at the site of
446 radical formation could have led to increased lipid oxidation.

447 In addition to the physical stabilization of emulsions and its protective effect against lipid oxidation,
448 GGM-bound hydroxycinnamyls located at the interface could act as antioxidants. Phenolic compounds
449 may scavenge primary carbon-centered lipid radicals and stop the chain reaction before it escalates and
450 leads to elevated oxidation.⁴¹ PR in the continuous phase, on the other hand, could have functioned via
451 binding or reducing transition metals and thus inhibiting oxidation. Polysaccharides themselves may also
452 retard lipid oxidation in emulsions.^{2,6} The action may be due to increment of viscosity or due to transition
453 metal binding. Polysaccharides have also been proposed to scavenge carbon and hydroxyl radicals.^{42–43}
454 Other wood extractives, such as triterpenes and sterols, may also possess antioxidant activity.
455 However, their contents in wood are relatively low, usually less than 1–2% in heartwood and sapwood.³⁰
456 Half of this content (i.e., 0.5–1%) may have been present in the studied GGM fractions.²⁵ If this was the
457 case, their content could have been significant in eTMP but negligible in PHWE. Yet, PHWE emulsions
458 were more stable against oxidation than eTMP emulsions. Knotwood extract of Norway spruce has been
459 reported to contain considerable amounts of lignans, 7-hydroxymatairesinol being the predominant one
460 (70–85%).⁴⁴ These compounds may have been responsible for the increased oxidative stability of
461 eTMP+PE emulsion.

462 Current results support the use of natural non-purified GGM fractions in emulsion stabilization.
463 Extraction of a sufficient amount of GGM containing bound PR will give GGM superior functionality.
464 Regarding applications, it is noteworthy that the color of GGM extracts is correlated to the content and
465 composition of lignin residues. Obtained results provide crucial information for industry, which is
466 currently developing non-hazardous and economical processes for the isolation of GGM. For example,
467 with flow-through pressurized hot water extraction, the size of GGM and content of PR can be
468 controlled.^{25,45} Moreover, the composition of PR and thus also the color of the extract may be adjusted
469 by extraction temperature and time.

470 **Conclusions**

471 We showed that the phenolic profile in GGM contribute to their interfacial functionality. PR act as
472 vehicles that enable the anchoring of GGM into the oil droplet interface. GGM-PR assemblies provide
473 steric stabilization against physical breakdown and antioxidants against lipid oxidation. The composition
474 of PR in natural GGM extracts can be tailored by sustainable and economical isolation processes. GGM
475 are multifunctional plant-based emulsifiers and stabilizers exhibiting both physical and chemical
476 stabilization capacity over extended storage time. According to the hypothesis, we demonstrated that by
477 retaining interactions between isolated GGM and PR, stabilization is achieved without adding other
478 surfactants or antioxidants. Previously known emulsifiers are typically small-molecular amphiphilic
479 surfactants, such as Tween20,⁴⁶ whereas macromolecular hydrocolloids are mainly used as viscosity-
480 modifying stabilizers.⁶⁻⁹ The present work suggests that lipophilic structures bound with intermediate-
481 sized polysaccharide tails result in highly efficient interfacial stability. Superior physical stabilization
482 and protection against lipid oxidation compared to e.g. gum Arabic^{18,20} or Tween 20⁴⁶ was obtained by
483 one-ingredient formulation and one-step emulsification process. The shelf life of PHWE GGM-
484 stabilized emulsions, in terms of physical and oxidative stability, at room temperature would be up to 8
485 months. Correspondingly, the shelf life would be prolonged up to 1.5 years at cooled conditions, such as
486 in a refrigerator. Even with less efficient GGM fractions, the emulsions would remain stable for 1–4
487 months. This high and long-term emulsion stability and abundant availability of raw materials makes
488 “spruce gum” an attractive future alternative for conventionally used emulsifiers and stabilizers in
489 various technical, pharmaceutical and food applications. Future work includes investigation of
490 continuous phase composition and dispersed phase volume fraction on the stabilization capacity of
491 GGM. Furthermore, understanding the functionality of LCC at the emulsion interface provides
492 perspectives for future tailoring of both natural and synthetic compounds for efficient stabilization of
493 dispersed systems.

494 **Conflicts of interest**

495 There are no conflicts to declare.

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