1 **Influence of the welan gum biopolymer concentration on the rheological properties,**

2 **droplet size distribution and physical stability of thyme oil/W emulsions**

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11 **Abstract.**

12 The objective of this work is to obtain a stable and natural antimicrobial delivery system.

13 Thus, the effect of the addition of a natural polysaccharide such as welan gum on the

14 linear viscoelastic properties, flow behaviour, droplet size distribution and physical

15 stability of thyme oil/W emulsions formulated with a wheat-derived surfactant was

16 studied. All emulsions obtained show submicron diameters regardless of the

17 concentration of welan. Emulsion without gum shows Newtonian behaviour under steady

18 shear. Meanwhile, emulsions containing welan gum show a weak gel-like behaviour with

19 higher G' and G" values on increasing the content of gum in the emulsion. Their flow

20 curves illustrate a shear thinning behaviour with much greater viscosity than that

21 exhibited by emulsions without gum. This behaviour fits well to the Cross model. The

22 main destabilization process of thyme oil/W emulsion without gum is creaming versus

flocculation and coalescence in emulsions containing welan. Rheology, diffraction laser and multiple light scattering techniques have proved that welan gum is an important rheological modifier for thyme oil/W ecological emulsions, it being possible to control the rheological properties of these emulsions by adjusting the concentration of gum. However, welan gum does not improve the physical stability of these emulsions.

Keywords: Welan gum, Thyme oil, Emulsions, Rheology, Physical stability.

1.- Introduction.

Nowadays, many industries are looking for new ecofriendly and sustainable products, which can be used in different applications. Ecological emulsions are one of these products. Finding the right formulation which fulfils environmental and non-toxicity requirements is essential for researchers within both the research and industrial fields.

As far as formulations are concerned, one of the most important ingredients in emulsions is the surfactant. Surfactants are amphiphilic molecules formed by a polar head and a lipophilic tail which can be grouped by the charge of their polar head groups: cationic, anionic, amphoteric (zwitterionic) or non-ionic. In addition, surfactants can also be classified by their hydrophilic lipophilic balance (HLB) value. The range of HLB values is from 0 to 20. HLB <9 refers to lipophilic surfactants and HLB> 11 to hydrophilic. Therefore, generally for emulsions of type O / W the emulsifiers have an HLB between 8 and18 and for W / O emulsions of 3-8 [1]. It should be noted that the use of emulsifiers whose hydrophilic lipophilic balance (HLB) value is close to the required HLB for oil could enable the formulation of stable emulsions. In this work, a non-ionic surfactant, specifically the alkyl poly pentoside commercially known as Appyclean 6548 (D-xylofuranose, oligomeric tetradecyl and octodecyl glycoside, C14, C18 alcohol) is used. This surfactant possesses an HLB value of 9.25 which has been shown to be the optimum

47   HLB value necessary to obtain stable thyme essential oil emulsions [2]. Appyclean 6548

48   is a newly-developed surfactant derived from renewable raw materials such as wheat

49   biomass. The alkyl poly pentoside surfactants are made of pentosides derived from

50   hemicellulose [3], which is not digestible by human intestinal digestive enzymes.

51   However, this kind of food fiber could be utilized by members of the intestinal microbial

52   microbiota like a prebiotic product.[4]. In addition, this surfactant fulfils the requirements

53   to be used in ecolabel formulations.

54   In this work, thyme essential oil is used as oil phase. This essential oil is classified as a

55   GRAS product by the United States Food and Drug Administration [5]. Thyme Vulgaris,

56   has very high antioxidant activity due to its main components: thymol, carvacrol, γ-

57   terpinene, myrcene, linalool, p- cymene, limonene, 1,8-cineole, and α-pinene. Among

58   other essential oils, thyme oil has relatively strong antimicrobial activity which it makes

59   it suitable for use in the food field [6,7], Thyme oil is found to be effective against

60   *Salmonella typhimurium* L. monocytogenes, A. hydrophila and autochthonous flora

61   spoilage in meat products [8]. Nevertheless, thyme essential oil emulsions might undergo

62   one or several destabilization mechanisms, such as flocculation, coalescence, Ostwald

63   ripening or creaming. The incorporation of biopolymers into the continuous phase of

64   emulsions decreases or prevents the movement of droplets and increases the viscosity of

65   the continuous phase, reducing the creaming destabilization process. The use of natural

66   polysaccharides in food or cosmetic applications as thickening, stabilizing and

67   emulsifying agents is due to their biocompatibility, biodegradability and non-toxicity. In

68   this study, welan gum is used as a polysaccharide in order to enhance the rheological

69   properties of emulsions.

Welan is an anionic extracellular polysaccharide, secreted by the micro-organism Sphingomonas *sp.*, namely from *Alcaligenes* sp., ATCC 31555, a gram negative microorganism. It is used in a wide variety of applications in several fields such as food, coating materials, medicine and the petroleum and cement industries [9]. Specifically, this gum can be used as an ingredient in food products like jellies, beverages or dairy products in which it can act as a thickening, suspending, binding, emulsifying, stabilizing and viscosifying agent [10]. Structurally, it is composed of l-mannose, l-rhamnose, d-glucose, and d-glucuronic acid.[11]. The chemical structure is essential in order to explain the behaviour in aqueous phase. For example, although gellan, welan or rhamsan, all of which are derived from Sphingomonas sp, are structurally close, they have different behaviours in different mediums. Thus, while gellan can make stable gels in the presence of salts, rhamsan and welan give only weak gels or very viscous solutions with a high degree of thermal, pH and salinity stability [12].

Despite the advantages of this environmentally friendly polysaccharide, there are only a few studies dealing with the use of welan gum in oil-in-water emulsions. In addition, there have been no previous studies concerning the physical stability and rheological behaviour of thyme essential oil emulsions prepared with welan gum.

In this study, we investigate the influence of welan gum concentration to improve the rheological properties of emulsions using thyme essential oil as dispersed phase. Additionally, the effect on the physical stability of emulsions is studied. All the components selected to formulate the emulsions are environmentally friendly materials (thyme essential oil, welan and appyclean 6548). The combination of rheology, laser diffraction and multiple light scattering techniques demonstrated that these results have

important implications for the design and utilisation of emulsions as antimicrobial delivery systems in the food industry.

2.-Materials and Methods.

2.1. Materials.

Thyme essential oil (938 g/l), supplied by Bordas Chinchurreta S.A, and a wheat-derived surfactant called Appyclean 6548 obtained from Wheatoleo are used as oil phase and emulsifier respectively. Sodium azide (0.1wt%) is used to avoid microbiological contamination. Welan gum (K1A96) is used as supplied by CP Kelco Company (San Diego, USA).

2.2. Emulsification Procedure.

The continuous phases are prepared by dissolving 0.1 wt% sodium azide in ultrapure water cleansed using a Milli-Q water purification system. The emulsifier, Appyclean 6548 (6wt%) is melted and dissolved in the dispersed phase (thyme oil – 40wt%) due to the fact that it is solid and immiscible in water. The mixture of oil and surfactant is heated to 70ºC for 30 minutes and then cooled to 25ºC. Once at this temperature, a total mass of 200 g of the coarse emulsions is prepared in two steps using a rotor-stator device equipped with a mesh screen (Silverson L5M) at 2000 rpm. Firstly, the mixture of solvent and surfactant is gradually added for 180s to the aqueous phase. Subsequently, a batchwise procedure is carried out for 30s. High pressure homogenization is carried out with a microfluidization system (M110P with an F12Y interaction chamber, Microfluidics, USA) at 2500psi. Coarse emulsions are passed once through the microfluidization system.

115 Finally, the gum is added very slowly to the final emulsions. The emulsions are

116 mechanically stirred using an Ika-Visc MR-D1 (Ika, Germany), for two hours at 500rpm.

117 Five emulsions are prepared using different gum concentrations (0, 0.05, 0.1, 0.15, 0.2

118 wt%). Two replicates of each emulsion with a final weight of 200g are prepared.

119 *2.3. Rheological properties.*

120 The rheological characterization involved stress and frequency sweeps in small amplitude

121 oscillatory shear experiments (SAOS) and stepwise steady shear flow tests. Haake-

122 MARS controlled-stress rheometer (Thermo-Scientific, Germany) is used to carry out the

123 rheological experiments. Flow curves are performed using a 60/1° double cone geometry

124 for emulsions without gums. Emulsions with gums are measured using a serrated plate-

125 plate sensor (60 mm). Flow curves are carried out in a range of 0.05 to 19 Pa. Stress

126 sweeps are performed in a range of 0.05 to 19 Pa at 1 Hz. Equilibration time prior to

127 rheological tests is 300s. All measurements are performed at 25 °C ± 0.1 °C using a C5P

128 Phoenix circulator (Thermo) and in duplicate, the values shown being the average of the

129 two replicates.

130 *Microscopy*

131 An optical microscope Axio Scope A1 (Carl Zeiss) with a 40x objective is used to observe

132 the microstructure of the emulsions at room temperature.

133 *2.4. Droplets size distribution measurements.*

134 Size distribution of oil droplets is determined by the laser diffraction technique

135 (Mastersizer 2000, Malvern, Worcestershire, United Kingdom). Sauter diameter (D3,2),

136 volume-weighted mean diameter (D4, 3) and span are the parameters selected to discuss

137 the results. D3,2 and D4,3 values are obtained from the following equation:

138 $$D_{3,2} = \frac{\sum_{i=1}^{N} n_i d_i^3}{\sum_{i=1}^{N} n_i d_i^2} \qquad (Eq.\,1) \qquad\qquad D_{4,3} = \frac{\sum_{i=1}^{N} n_i d_i^4}{\sum_{i=1}^{N} n_i d_i^3} \qquad (Eq.\,2)$$

139 where n_i is the number of droplets with diameter d_i. On the other hand, span values are

140 given by:

141 $$Span = \frac{D(v,0.9) - D(v,0.1)}{D(v,0.5)} \qquad (Eq.\,3)$$

142 where D(v, 0.9), D(v, 0.5) and D(v, 0.1) are the highest droplet size contained in 90%,

143 50% and 10% of volume of dispersed phase, respectively.

144 *2.5. Physical Stability.*

145 The physical stability of thyme essential oil emulsions is evaluated for 15 days at 30ºC

146 by means of a Turbiscan Lab Expert device (Formulaction, France) using the multiple

147 light scattering technique. Emulsion stability is studied by analysing the variation of the

148 backscattering (BS) profiles as a function of length of tube containing the sample, and

149 time. This technique makes it possible to determine the major mechanisms of

150 destabilization in each sample as well as the kinetics of the destabilization process.

151 *2.6. Statistical Analysis*

152 A one-way analysis of variance (ANOVA) was used to determine the occurrence of

153 significant differences among the obtained results. For this purpose, OriginPro 8 software

154 is used.

155 **3.- Results and Discussion.**

156 *3.1. Rheological properties*

157 3.1.1. Small amplitude oscillatory shear tests

158   Figure 1 shows stress sweeps at 1Hz as a function of welan gum concentration. Thanks

159   to this test, the linear viscoelastic range (LVR) can be measured, which indicates stress

160   region within which the response of the material does not depend on the applied stress.

161   G´ (storage modulus) and G´´ (loss modulus) remain practically constant until a critical

162   stress (τ_C) is reached. From this point, generally, both moduli sharply decrease. However,

163   in these emulsions loss modulus values increase before the decrease. This behaviour is

164   observed in other systems and it can be explained by considering that a reorganization of

165   the structure occurs before the nonlinear region is reached [13]. The critical stress value

166   is important because from this point the deformation in the structure is irreversible with

167   increasing stress [12]. This parameter is often known as yield stress although it should be

168   noted that its value depends on the evaluation technique.

169   As can be observed in Figure 1, the linear viscoelastic range, and thus the critical stress

170   values, are clearly dependent on gum concentration. The critical stress values for

171   emulsions are 0.06, 0.14, 0.36 and 0.59 Pa at 0.05 wt%, 0.1 wt%, 0.15 wt% and 0.2 wt%

172   of welan, respectively. This result indicates that increasing gum concentration in the

173   emulsions increases the resistance to shear, which implies a higher stability of the

174   emulsion under the stress amplitude. In addition, the storage modulus and the loss

175   modulus values at the LVR slightly increase with increasing welan concentration, which

176   means an increase in the structural strength of the emulsions. Emulsions without gum do

177   not present a linear viscoelastic response in the range of shear stress amplitude studied.

178   Figure 1

179   In order to obtain more information concerning the linear viscoelastic properties, figure

180   2 illustrates the changes in G' and G"as a function of the frequency and the concentration

181   of welan gum for thyme oil/W emulsions aged for 24h. The results of these experiments

182   provide insights into the emulsion structure because these tests are non-destructive and

183   do not interfere with it. According to Steffe (1996) [14,15] and other authors, in weak

184   gels, storage and loss moduli exhibit a slight dependence on frequency, and G' values

185   exceed G" values in the whole frequency range studied. Emulsions formulated with

186   thyme essential oil containing different concentrations of welan gum present clear

187   viscoelastic properties with a predominance of the elastic over the viscous component at

188   all frequencies and with both moduli practically independent of frequency. Therefore,

189   these emulsions behave as weak gel-like materials.

190   Figure 2

191   The dependence of the storage modulus on the frequency can be quantitatively described

192   by a power law type equation:

193    $G'(\omega) = K'.\omega^{n'} \quad Eq.(4)$

194   where K' is the intercept and n' is the slope in the double logarithmic plot of G' against

195   frequency.

196   The curves of G'as a function of angular frequency fit fairly well to the power law

197   (R^2>0.99). The fitting parameters are plotted in figure 3. The values for the slopes of G'

198   (n') are similar for all welan gum concentrations, except for 0.1wt% which exhibits a

199   slight maximum value. This result indicates that no change in the structure occurs but

200   only a change of consistency of the system connected with the addition of welan gum

201   (thickening effect). The value of this slope also makes it possible to classify the behaviour

202   of system. It is known that n'> 0 denotes weak gels whereas n'=0 corresponds to strong

203   gels [16]. Our emulsions present n' values between 0.1 and 0.08 so it can be concluded

204   that these emulsions exhibit a weak gel-like behaviour. It is worth noting that a plateau

205   zone is obtained for the emulsion containing 0.05 wt% welan gum. The plateau zone is

206 characterised by a trend of G´´ to reach constant values at low frequencies. In this case a

207 minimum value of G" was achieved at 0.3 Hz.

208   Figure 3

209 An increase in gum concentration causes a significant increase in G´and G". This rise can

210 be related to the formation of a stronger and more elastic structure in the continuous phase

211 [17]. The natural structure of welan gum is a perfect double helix [18]. As the gum

212 concentration in the continuous phase of emulsions increases, the interaction between

213 adjacent double helixes promotes the formation of a three-dimensional network that

214 increases by increasing the gum concentration [19].

215 The dependence of the storage modulus, which is related to the solid-like response, on

216 the welan concentration present in the emulsion is investigated. With this aim, the double-

217 logarithmic plot of G' at 1 rad/s against welan gum concentration is shown in Figure 4.

218 The experimental data are well fitted ($R^2 = 0.998$) to the following equation:

219 $G'_{1rad/s} = a \cdot C^b$ $(Eq. 5)$

220 As can be observed G' increases linearly with the concentration, the slope being 2.2. This

221 value is close to that commonly found for normal polysaccharide gels at concentrations

222 above the minimum critical gelling concentration (b = 2) [20]. The result obtained reveal

223 that the rheology of these emulsions is dominated by the continuous phase.

224   Figure 4

225 3.1.2. Steady state flow test

226 The emulsion formulated with no gum shows Newtonian behaviour and the flow curve

227 fits fairly well to the Newtonian law. The viscosity value of 24 h aged Newtonian

228 emulsion is 7.4±0.3 mPa.s. When the different concentrations of gum are added, not only

229 is the rheological behaviour changed, but also an increase in viscosity occurs (Figure 5).

230 It is noteworthy that an increase in viscosity inhibits some destabilization processes such

231 as creaming and/or phase separation by slowing down the droplet motion. Figure 5 shows

232 the influence of welan gum on the flow properties of thyme oil/W emulsions after an

233 aging time of 24 h. All emulsions containing gum show a pseudoplastic behaviour, with

234 viscosity versus shear rate exhibiting a trend to a viscosity plateau region at low shear

235 rates and, subsequently, a shear-thinning behaviour. Furthermore, it can be observed that

236 the apparent viscosity (η) increases as the welan gum concentration increases, this being

237 due to the well-known thickening effect of polysaccharides. However, the increase in

238 viscosity exhibited for these emulsions may be also promoted by droplet flocculation

239 which would be induced by the presence of welan in the continuous phase (depletion

240 flocculation). The greater the welan concentration, the greater the depletion flocculation

241 and the higher the viscosity. The fact that these emulsions are flocculated is consistent

242 with the flow behaviour observed. The flocs act as a solid at low shear rate. Once the

243 critical shear rate is exceeded, the applied forces are sufficient to disrupt the bonds

244 between droplets and the emulsion flows, showing a shear thinning behaviour.

245 The behaviour found fits the Cross model fairly well ($R^2 > 0.99$):

246 $$\eta = \frac{\eta_0}{1+\left(\frac{\gamma}{\gamma_c}\right)^{1-n}} \qquad Eq.\,(6)$$

247 Where γ_c is related to the critical shear rate for the onset of the shear-thinning response,

248 η_0 stands for the zero-shear viscosity and (1-n) is a parameter related to the slope of the

249 power-law region; n being the so-called "flow index". For shear thinning materials, n

250 values are between 0 and 1. A solid material would show n = 0, while a Newtonian liquid

251 would show n = 1.

252 For emulsions aged for 24 h, an increase in the gum concentration leads to an increase in

253 the zero shear viscosity (Table 1). It is worth noting that a small content of welan gum

254 causes a significant increment in emulsion viscosity (7.4 mPa·s in emulsion without gum

255 versus 556 Pa·s in emulsion containing only 0.05 wt% welan gum) due to, as mentioned

256 above, the thickening effect of welan. The η_0 evolution is gradual from 0.05 wt% to 0.15

257 wt% of welan gum added to the emulsion, but a sudden increase in this parameter from

258 0.15 wt% to 0.2 wt% gum concentration was observed. This rheological change may be

259 due to an improved viscosity of the continuous phase and/or a more marked effect of

260 depletion flocculation. In Table 1, we can also observe that there is a trend to decreasing

261 critical shear rate with increasing the gum concentration. In other words, the higher the

262 welan concentration in the emulsion, the narrower the Newtonian region. Regarding the

263 flow index, significant differences with concentration were not observed. The values are

264 close to 0, indicating that emulsions containing welan gum exhibit a strong pseudoplastic

265 character.

266 Figure 5

267 Table 1

268 The flow curve results obtained are very interesting because they make it possible to

269 control the viscosity of the thyme essential oil/W emulsions by adjusting the

270 concentration of welan gum.

271 *3.2. Droplet size distribution*

272 Figure 6 shows the droplet size distributions for all emulsions studied after an ageing time

273 of 24 h prepared with Silverson L5M at 2000rpm and one pass through the Microfluidizer

274 M110P at 2500psi. For these emulsification conditions, all emulsions formed present a

275 bimodal distribution as a consequence of the recoalescence phenomenon induced by an

276   excess of mechanical energy-input [21]. This result is usual in emulsions obtained with

277   Microfluidizers [22]. Droplet size distribution shifts towards higher values with the

278   addition of gum, and this effect is more marked from 0.15 wt% to 0.2 wt% welan gum

279   concentration. Additionally, the second peak in droplet size distribution increases with

280   increasing welan gum concentration.

281   Figure 6

282   Except for the emulsion containing 0.2 wt% welan, all emulsions show submicron mean

283   diameters. Figure 7 illustrates D3,2, D4,3 and span parameters as a function of gum

284   concentration. The incorporation of welan gum to thyme essential oil in water emulsions

285   causes an increase in both diameters (D3,2 and D4,3) and polydispersity. Subsequently,

286   these parameters slightly increase with concentration until 0.2 wt% welan is reached. At

287   this gum concentration a significant increase in Sauter diameter, volume mean diameter

288   and polydispersity occur. These results are consistent with those obtained from the flow

289   curves, where zero-shear viscosity also gradually increases up to 0.2 wt% welan,

290   concentration, at which point an important rise occurs. Again, this may be explained by

291   considering the depletion flocculation effect. This aggregation of droplets provokes an

292   increase in mean droplet size, which is more important at higher concentrations of welan

293   in the continuous phase.

294   Figure 7

295   Microphotographs taken after one day for emulsions with different gum concentrations

296   (Figure 8) also are in concordance with the increase in mean diameters and polydispersity

297   as a function of welan concentration found by laser diffraction.

298   Figure 8

3.3. Multiple Light Scattering

Figure 9 illustrates, by way of example, the results of the physical stability study performed at room temperature by the multiple light scattering technique for emulsions containing 0 wt% (Figure 9a), 0.1 wt% (Figure 9b) and 0.2 wt% (Figure 9c). In this figure a plot of BS versus measuring cell height as a function of time is shown. Additionally, an inset is included where backscattering is plotted against tube length in reference mode ($\Delta BS\% = BS\% - BS_0\%$) to better show the backscattering changes.

Creaming is observed for the emulsion without gum (Figure 9a), which is the migration of dispersed phase droplets from the lower area to the upper part of the sample due to the difference in densities between the aqueous and dispersed phases. This destabilization is also related to the low viscosity exhibited by this emulsion. Furthermore, it should be noted that $\Delta BS\%$ decreases at the top of the tube length, which may be interpreted as a coalescence process of droplets into the creamed phase [23]. No increase in droplet size implying flocculation, coalescence or Ostwald ripening is found in this emulsion, as can be deduced from the lack of variation in the backscattering profiles from the middle of the tube.

The addition of welan gum to the emulsion, in spite of increasing the viscosity of the emulsion, fails to inhibit the creaming, although the clarification height decreases. It also causes a slight increase in droplet size with ageing time due to a destabilization mechanism such as flocculation, coalescence or Ostwald ripening. Additionally we can observe in Figure 9b a clarification at the top of the measuring cell. This result can be attributed to the occurrence of some coalescence which led to an oiling-off process (phase separation). The increase in droplet sizes with ageing time is very slight up to 0.2 wt% welan concentration. At this concentration, this phenomenon is quite significant, as well

323   as a phase separation in the upper part of the sample. In order to quantify this

324   phenomenon, the kinetics of the destabilization process in the middle zone of the

325   measuring cell (between 20 and 25 mm of sample height) was studied. The ΔBS (%) of

326   each concentration has been plotted as a function of aging time in figure 10 and the results

327   are fitted to an exponential equation (R2 > 0.99), typically used for a first- order kinetic

328   model:

329     $\Delta BS = \Delta BS_E + (\Delta BS_0 - \Delta BS_E).exp(-kt) \quad Eq.(7)$

330   where ΔBS stands for the variation in the BS as a function of aging time, ΔBS_E is the

331   corresponding decrease in BS when equilibrium is reached, BS_0 is the initial value of

332   backscattering, and k is the first-order kinetic coefficient. This kinetic equation has

333   proved to be useful to monitor the physical stability of oil-in-water emulsions [24]. Table

334   2 lists the values of the parameters of equation 7 as a function of welan gum concentration.

335   The first-order kinetic coefficient (k), the most relevant parameter, exhibits a tendency to

336   increase with the rise in the relative concentration of the welan gum, indicating a higher

337   rate of increase in droplet size. Nevertheless, the low values of this parameter should be

338   noted for emulsions with smaller gum concentrations. In contrast, the emulsion with a

339   greater gum content (0.2wt%) exhibits higher values of k, indicating a significant increase

340   in droplet size with aging time. These results are in concordance with those previously

341   obtained from flow curves and droplet size distribution. The incorporation of gum into

342   the aqueous phase of emulsions causes an increase in both droplet size and viscosity, the

343   thickening effect and depletion flocculation being the responsible factors. This

344   aggregation of droplets continues with time, leading to coalescence and, finally,

345   provoking phase separation. The greater the welan content in the emulsion, the greater

346   the phase separation.

347   Figure 9

348   Figure 10

349   Table 2

350

351   **4.-Conclusions.**

352   Thyme essential oil-in-water emulsions of submicron range were obtained with

353   microfluidizer M100P at 2500psi-one pass using a bio-based surfactant called Appyclean

354   6548. In order to improve the rheological properties of emulsions, welan gum

355   polysaccharide was included in the emulsion formulation at different concentrations.

356   Emulsion without gum does not exhibit linear viscoelastic region. All the emulsions with

357   gum show a weak-gel like behaviour with stronger interactions as the welan concentration

358   in the emulsion increases. Flow curves of emulsions containing gum exhibit a shear

359   thinning behaviour and they are fitted to the Cross-model equation. The viscosity of these

360   emulsions also increases with concentration of welan. Conversely, the emulsion without

361   gum shows Newtonian behaviour. Multiple light scattering indicates that creaming is the

362   main destabilization mechanism for emulsion without gum. Creaming is decreased by the

363   better rheological properties of emulsions with polysaccharide. However, an increase in

364   droplet size, probably due to flocculation, occurs, this being significant at 0.2wt% welan

365   gum. This emulsion shows the highest kinetics of destabilization. From the results

366   obtained, it can concluded that welan gum is an excellent rheological modifier of thyme

367   essential oil/W emulsions, making it possible to control the viscosity of the emulsion by

368   adjusting its concentration. However, welan gum is not effective in increasing the

369   physical stability of these emulsions. The main destabilization process is flocculation,

370   which finally provokes phase separation.

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455 **Tables**

456 Table 1. Fitting parameters to the Cross model for emulsions studied. Standard deviation
457 of the mean (2 replicates) for η_∞ <2%, η_0 < 10%, $\dot{\gamma}$ < 1% and n < 5% .

Welan gum concentration (wt%)	n	η_∞ (Pa.s)	η_0 (Pa.s)	$\dot{\gamma}$ (s^{-1})
0.05	0.14	0.015	556	$1.5,10^{-4}$
0.1	0.18	0.017	2103	$1.1,10^{-4}$
0.15	0.13	0.03	5736	4.8,10-5
0.2	0.16	0.033	17514	$5.6,10^{-5}$

458

459

460 Table 2. Fitting parameters of the first-order kinetic equation for the BS in the 20−25 mm
461 zone of the measuring cell versus aging time as a function of welan gum concentration.

Welan gum concentration (wt%)	ΔBS_E (%)	$BS_0 - \Delta BS_E$ (%)	K (s^{-1})	R^2
0	-1.05	1.17	4.38	0.95
0.05	-1.36	1.35	1.69	0.98
0.1	-2.65	2.54	2.74	0.99
0.15	-2.10	2.04	3.77	0.97
0.2	-38.38	40.20	4.03	0.97

462

463 **Table captions**

464 Table 1. Fitting parameters to the Cross model for emulsions studied. Standard deviation
465 of the mean (2 replicates) for η_∞ <2%, η_0 < 10%, $\dot\gamma$ < 1% and n < 5% .

466 Table 2. Fitting parameters of the first-order kinetic equation for the BS in the 20−25 mm
467 zone of the measuring cell versus aging time as a function of welan gum concentration.

468 **Figure captions**

469 Figure 1. Oscillatory shear stress sweeps as a function of welan concentration.
470 Temperature = 25 °C

471 Figure 2. Mechanical spectra of emulsions as a function of welan gum concentration.
472 Standard deviation for the mean is also plotted. Temperature = 25 °C

473 Figure 3. Fitting parameters to the power law equation for all the emulsions studied. K´
474 is the intercept and n´ is the slope in the double logarithmic plot of G´ against frequency
475 as a function of welan gum concentration.

476 Figure 4. Storage modulus versus welan gum concentration. Continuous lines illustrate
477 the data fitting to the power law. Standard deviation for the mean is also plotted.
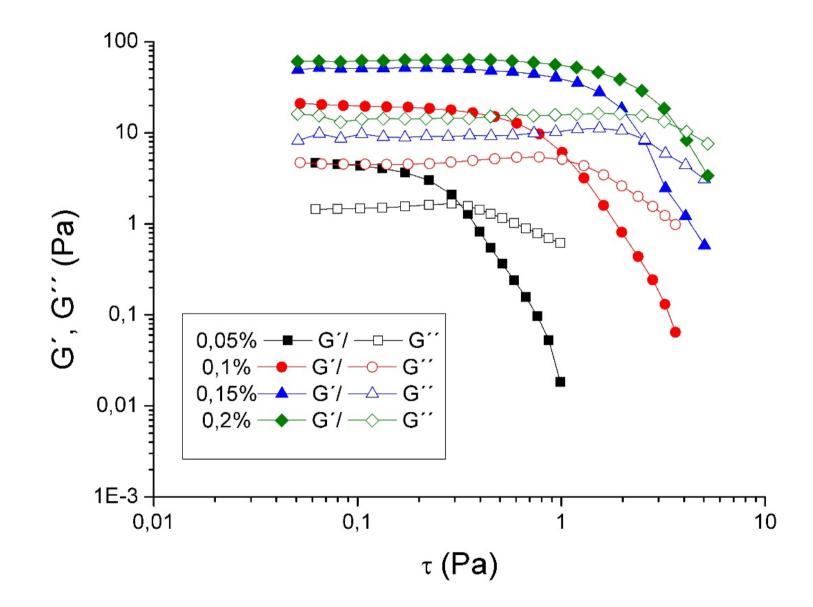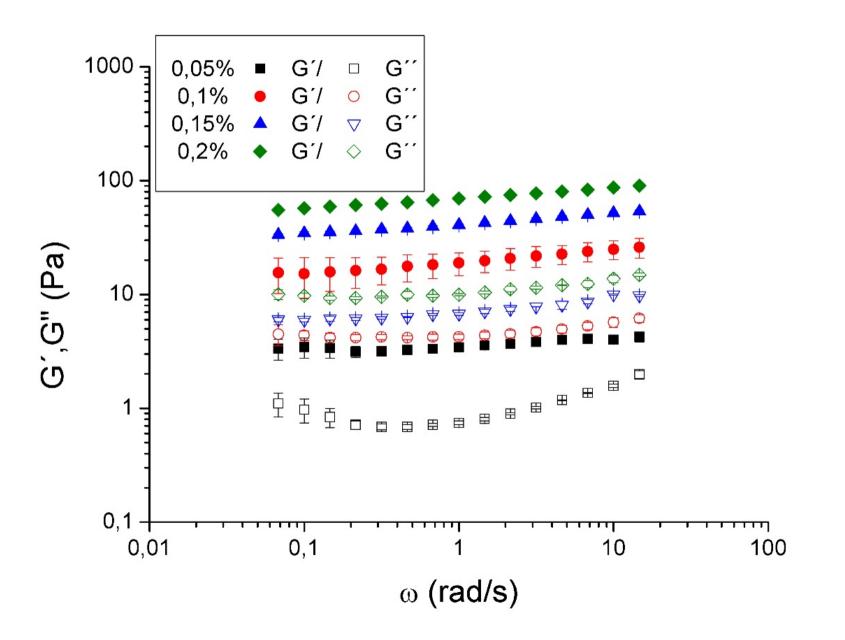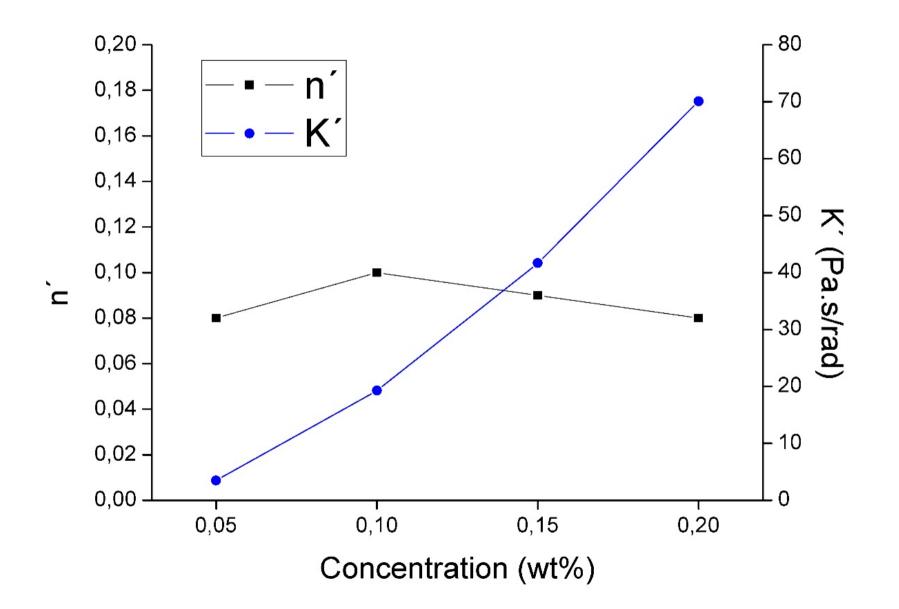478 Temperature = 25 °C.

479   Figure 5. Flow curves of emulsions aged for 24 as a function of welan gum concentration.
480   Continuous lines illustrate the data fitting to the Cross equation. Temperature = 25 °C.
481   Standard deviation of the mean (two replicates) for η < 8%.

482   Figure 6. Droplet size distributions for emulsions aged for 24 h as a function of welan
483   gum concentration.

484   Figure 7. Sauter mean diameters, volumetric mean diameters and span for all emulsions
485   aged for 24 h as a function of welan gum concentration.

486   Figure 8. Photomicrographs for all emulsions prepared as a function of welan gum
487   concentration a) without gum, b) 0.05wt%, c) 0.1wt%, d) 0.15wt% and e) 0.2wt% at 24
488   h of aging time.

489   Figure 9. Backscattering versus measuring cell height as a function of time and reference
490   mode (insets) for emulsions made a)without gum, b)0.1wt% welan gum and c) 0.2wt%
491   welan gum, at 25°C.

492   Figure 10. Destabilization kinetics in the 20−25 mm zone monitored over 15 days for all
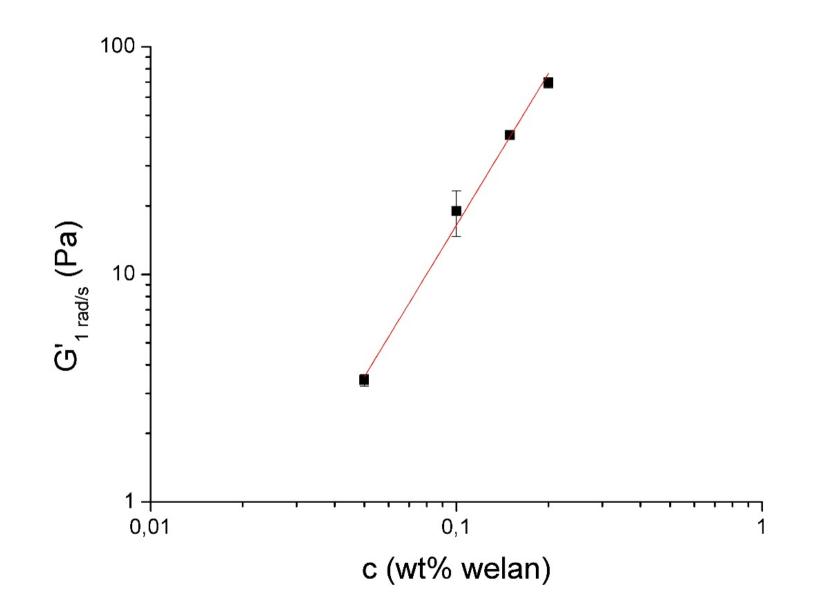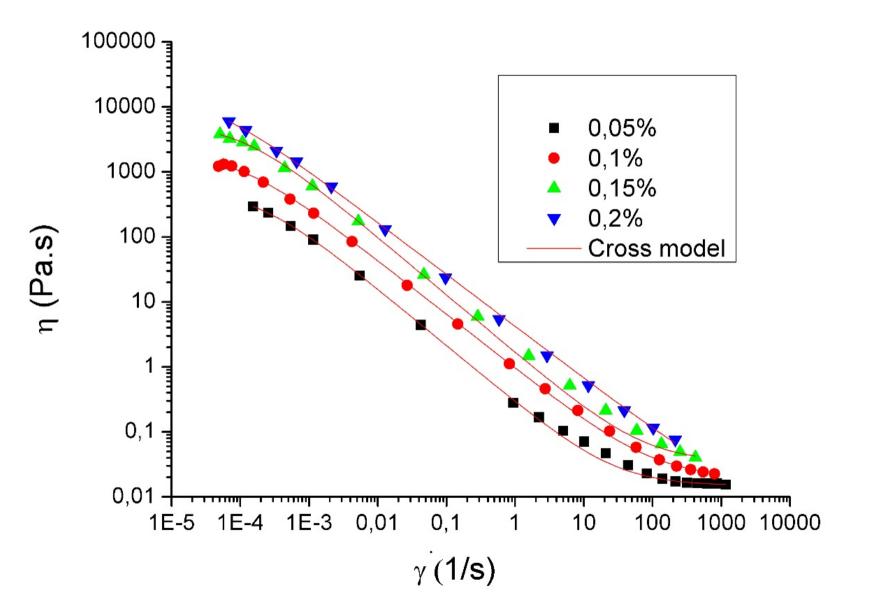493   studied emulsions as a function of welan gum concentration.
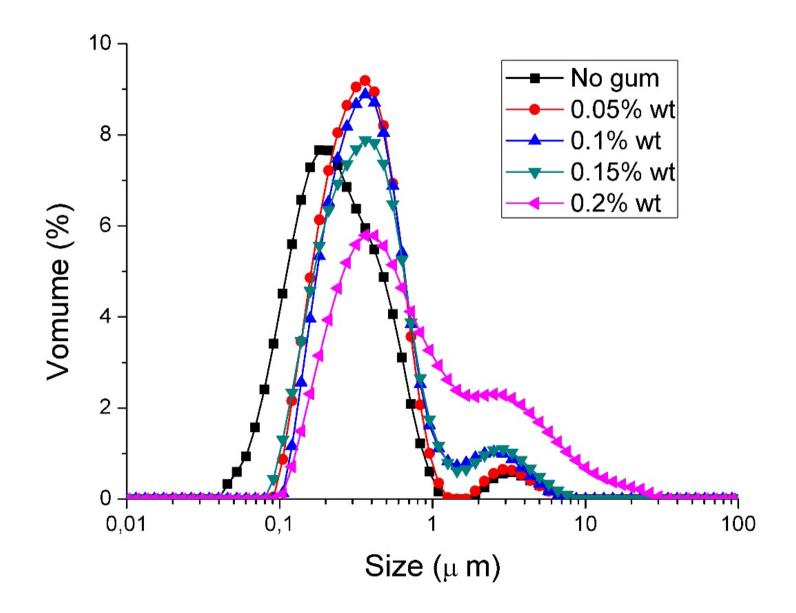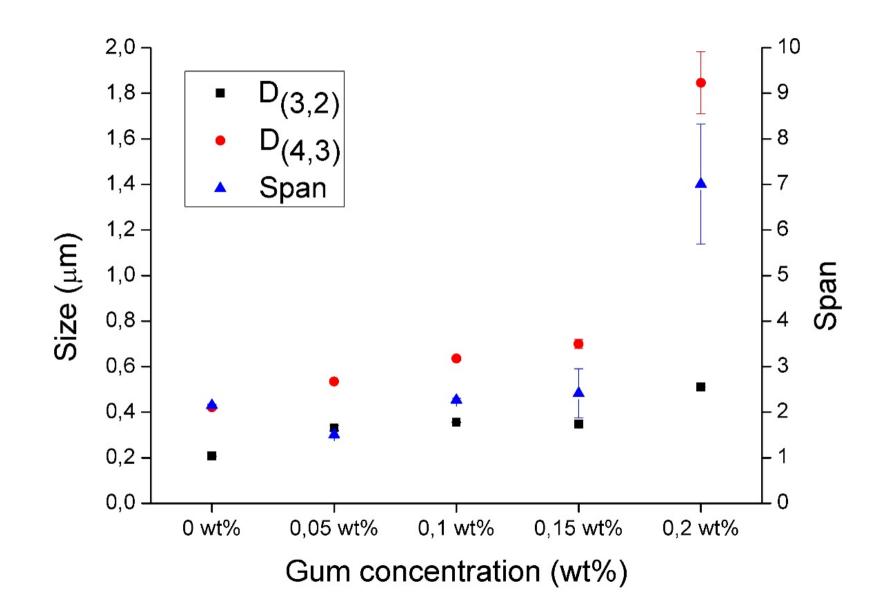
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