Wine lees: from waste to O/W emulsion stabilizer

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Abstract

Wine lees are the major waste from wine production, containing a noticeable amount of biomass (yeast, stalks, and peels), they could be used for the stabilization of dispersed systems such as emulsions. This work is focused on the determination of techno-functional properties of whine less produced from white grapes and industrial-grade blueberries. The interfacial characterisation was carried out by means of dilatational and interfacial shear rheology, whereas the characterisation of emulsion microstructure was assessed by droplet size distribution, rheological characterisation and backscattering measurements. The results obtained indicated that a high amount of polyphenols were present in wine less obtained from grape fermentation with industrial-grade blueberries as an additive, moreover, their presence also caused better interfacial properties (reducing the interfacial tension up to ca. 15 mN/m). However, the comparison of dilatational and interfacial-shear rheology determined that the interfacial response was caused by a densely packed structure. Fairly low droplet sizes (~ 320 nm) were obtained after ultraturrax® homogenization and further passing through the Microfluidizer® device, where the emulsions were stable only in presence of xanthan gum (0.06, 0.12 and 0.25 wt.%). However, the suitable amount of XG gum

was 0.06 and 0.12 wt.% since no phase separation was observed in the emulsions generated over storage time, although flocculation phenomena took place. The results obtained exhibited emulsions with a suitable texture for the preparation of milk-shakes and brewages products, evidencing the potential of wine lees for these products.

Keywords: *Emulsions; O/W interface; Protein adsorption; Interfacial Rheology; berries; Wine lees;*

1. Introduction

Wine has been around for so many thousands of years and is appreciated by many all over the globe. It is a fact, that wine production is one of the most important agricultural activities throughout the world. Grapes are one of the most important fruit crops cultivated worldwide (Gómez-Brandón et al., 2019). The grape production was around 77.8 Mt in 2018 (Ahmad et al., 2020), whereas the reports from the International Organization of Vine and Wine (OIV) show that 292 Mhl wine was produced worldwide in 2018 (Ahmad et al., 2020).

However, agricultural activities and transformation processes have a great environmental impact, because of the inputs required (e.g., energy and water consumption, fertilization and use of pesticides) and the outputs produced (e.g. waste products, polluted water, greenhouse gases), resulting in high disposal costs (De Iseppi et al., 2020). It is therefore essential to take into account that the winery sector produces a large volume of underutilized by-products, which can be a good source if exploited for the extraction of industry-based products (Amulya et al., 2015; Ping et al., 2011; Spigno & De Faveri, 2007). Some studies have demonstrated that winery waste could be a good alternative with immense potential to produce many bioproducts

55 (Ahmad et al., 2020). All this inspires the scientific community to thoroughly address
56 the global problems of grape winery waste with sustainable solutions in an integrated
57 biorefinery approach keeping the environment and the circular economy in the
58 background (Dimou et al., 2015).
59 Typical waste and by-products from wineries include grape pomace (skins and seeds,
60 representing on average about 60% of the total winemaking by-products), grape stalks
61 (14%), grape solids and fermentation (yeast) lees (25%), wastewaters rich in organic
62 compounds (up to about 15 L/L of wine) (Bonamente et al., 2015; De Iseppi et al.,
63 2020), carbon dioxide from the fermentation process, exhausted filtration materials and
64 fining agents. Given a circular economy approach, some of these wastes can be
65 successfully “recycled”, reused or recovered, improving both the economic and
66 environmental sustainability of winemaking activities (Bonamente et al., 2015; Devesa-
67 Rey et al., 2011; Lavelli et al., 2016).
68 Wine lees are the residues that appear at the bottom of the wine production tanks, after
69 the fermentation process, while it is stored or after further treatment. Moreover, wine
70 lees also include the waste generated after filtering and centrifuging the fermented
71 product. The typical composition of the lees includes yeast, tartaric acid, phenolic

72 compounds and other materials of an inorganic nature (Maicas & Mateo, 2020). Due
73 to the high content of bioactive molecules in wine lees, they could be exploited to obtain
74 extracts of high interest for their use in the food, nutraceutical and pharmaceutical
75 industries. In particular, wine lees contain a noticeable amount of proteins that could
76 be used based on their functional properties (Hwang et al., 2009). Some authors have
77 aimed to use plant-protein sources for the next food generation (McClements, 2019).
78 In this sense, there are several examples for the development and stabilization of
79 emulsions based on pea (Liang & Tang, 2014), lupin (Raymundo et al., 2002), faba
80 (Felix et al., 2018), among other proteins.
81 Currently, microbial proteins are postulated as a future sustainable food supply route
82 with a low environmental footprint. Thus, yeasts are a major player in the microbial-
83 derived production of products for food applications. Baker's yeast and alcohols
84 fermentation are the two main processes employing yeasts, with a projected global
85 market value for 2019 of up to 9.2 billion Euro and an annual growth forecast of 7.9%
86 (Matassa et al., 2016).
87 Interestingly, phenolic compounds present in grapes can be adsorbed by wine lees
88 (Mazauric & Salmon, 2005), contributing not only to their organoleptic properties but

89 also their antioxidant ability (Dávalos et al., 2005; Oboh et al., 2009). Nowadays, food
90 with bioactive properties is demanded by society, being antioxidant properties among
91 the most demanded since it prevents cell oxidation which causes serious diseases
92 (Hertog et al., 1993).
93 Wine lees may be considered as an excellent alternative to stabilize dispersed systems
94 since they are wastes rich in proteins which may exhibit interesting functional
95 properties. Moreover, the adsorbed polyphenols may involve antioxidant properties
96 with added value in the product developed.
97 The combined use of other fruits with grapes to obtain fermented beverages involves
98 the development of innovative products that allow the opening of new market niches
99 (Kalli et al., 2018). Thus, the use of red-fruit surplus production supposes the reuse of
100 by-products, contributing to the circular economy and allowing the revaluation of these
101 products (De Sales et al., 2018). Moreover, the use of red fruits in the production of
102 fermented beverages allows the use of its beneficial properties derived mainly from its
103 marked antioxidant character (Bosiljkov et al., 2017).
104 Since wine lees are one of the least studied and exploited winemaking by-products so
105 far, the scientific aim of the manuscript is focused on wine lees revalorization with

applications in the food sector as functional beverage formulations. In this work, wine lees from white wine fermented with and without blueberries were assessed as an emulsifier to obtain, after ultraturrax® homogenization and further passing through the Microfluidizer® device, diluted O/W emulsions as a function of xanthan gum (XG) concentration.

Material and methods

2.1. Materials

Wine lees from white wine were obtained as follows: 100 L of fresh juice from pressing crushed grapes were provided by Agroalimentaria Virgen del Rocío S.A (Almonte, Spain). The yeast used belongs to the *S. cerevisiae* strain (syn. bayanus), it was obtained from commercially prepared active dry yeasts supplied by Lallemand-Agrovin (Ciudad Real, Spain). The fresh juice was divided into four tanks. In the first tank, the fresh juice was fermented over nine months at room temperature. After complete fermentation, wine lees (WL) were collected and stored at -20 ºC. In the other three tanks, the fresh juice was fermented together with 15 % (w/w) industrial grade blueberries provided by Driscoll´s company (Huelva, Spain). Once the primary fermentation was finished, the wine was pumped off into clean tanks. Wine lees of

123 white wine fermented with blueberries (WLBB) precipitated at the bottom of the tank,
124 they were collected and kept at -20 ºC until use. Both wine lees (WL and WLBB) were
125 freeze-dried for their use at -56 ºC using a Telstar LyoQuest Freeze-drier (Barcelona,
126 Spain) during 72 h. Two powders were obtained after WL and WLBB freeze-drying
127 (WLF and WLBBF, respectively), they were kept in hermetic containers at 4ºC until
128 use.
129 The sunflower oil used in the emulsions performed was purchased from a local market.
130 XG, HCl, NaOH and acetic acid were purchased from Sigma-Aldrich Inc. (St. Louis,
131 Missouri, USA). Mili-Q grade water was used for interfacial measurements, whereas
132 distillate-grade water was used for emulsions.

133 ## *2.2. Methods*

134 ### *2.2.1. Proximate composition*

135 The protein content of the WLF and WLBBF extracts were determined in quadruplicate
136 as % N x 6.25. Nitrogen content was determined after combusting the sample at 850
137 ºC in O_2 presence using the LECO CHNS-932 (Leco Corporation, St. Joseph,
138 Michigan, USA). An infrared detector measured the nitrogen content (Dumas method)

139 (Saint-Denis & Goupy, 2004). Lipid, moisture and ash contents were determined
140 according to A.O.A.C. (2000) methods.
141 For phenolic compounds extraction, a rate of 1:5 (w/v, whine lees/mili-Q water) was
142 placed in 2-ml eppendorf reaction tubes using milli-Q water as extracting agent, in
143 compliance with the obligatory conditions required by the U.S. Food and Drug
144 Administration (FDA) and the European Union (Pintać et al., 2018; Sheldon, 2005).
145 After vigorous shaking for 5 seconds using a vortex (Heidolph relax top, JP Selecta,
146 Barcelona), the samples (WLF and WLBBF) were introduced for 10 minutes in an
147 ultrasound bath (Power Sonic 400, Cobos, Barcelona) and then they were centrifuged
148 (Eppendorf MiniSpin Plus microcentrifuges, Eppendorf, Madrid) at 13,400 rpm and 4 °
149 C for 10 min. Finally, the supernatants were stored at 4 °C until use.
150 Total phenolic content was determined using Folin-Ciocalteu´s assay based on the
151 microscale protocol of the assay given by Waterhouse (Prior et al., 2005), modified in
152 order to minimize reactive consumption and time. 20 µl of each extract solution was
153 placed in its corresponding screw cap test tube followed by 1.58 ml of milli-Q water
154 and 100 µl of Folin-Ciocalteu reagent. The tubes were stirred for 2 min and after the
155 addition of 300 µl of Na_2CO_3 (20% w/v) were added and then stirred. After 1 - 1.5 h at

40 ° C in a water bath, all the tubes were cooled and their absorbances were measured at 725 nm (Thermo Electron Corporation, Helios, MA, USA) in a 1 cm quartz cuvette, against a mili-Q water blank. Results were determined as mg gallic acid equivalents (GAE) per g of sample. Calibration was made between 0 and 750 mg/L gallic acid.

2.2.2. Interfacial characterisation

Determination of interfacial tension at equilibrium

The interfacial tension at equilibrium was determined as a function of WLF and WLBBF soluble protein concentration (0.006, 0.03, 0.06, 0.15, 0.3, 0.6, 1.2, 1.8 and 2.4 wt.% soluble protein) after 24 h adsorption at pH 5.0 fixed with acetate buffer at 50 mM. A Wilhelmy plate connected to a sigma 701 tensiometer (KSV, Helsinki, Finland) was placed at the air/water (A/W) interface, whereas oil was added after 10 min equilibrium, forming the oil/water (O/W) interface. The temperature was kept constant at 20 ºC. The interfacial tension was determined along with time until constant value (± 0.1 mN in 5 min).

Pendant droplet tensiometer measurements

The interfacial tension was determined over protein adsorption until the interfacial tension value was nearly constant, reaching a pseudo-equilibrium state after 10,800 s

protein adsorption. The TRACKER pendant droplet tensiometer (IT Concept, France) was used for these measurements. Droplet profile was digitalized and processed according to the Laplace equation (Castellani et al., 2010). Moreover, the transient dilatational moduli were also determined until the pseudo-equilibrium state was reached (10,800 s) at 0.1 Hz. Once the pseudo-steady state was achieved, the dilatational moduli were also obtained as a function of frequency (from 0.0075 to 0.1 Hz). The elastic and viscous dilatational moduli (E'_s and E''_s) were obtained at 10% strain amplitude. All the experiments were carried out in triplicate at the WLF or WLBBF saturation concentration (0.6 wt.% soluble protein), forming an axisymmetric droplet into an optical glass cuvette (7 ml) containing the oil phase whose temperature was thermostatically regulated at 20.0 ± 0.1 °C.

Interfacial shear measurements

The interfacial shear measurements were performed with a DHR-3 rheometer (TA Instruments, Illinois, USA). The transient interfacial viscoelastic moduli (G'_s and G''_s) were determined at the O/W interface during 10,800 s protein adsorption, reaching the above-mentioned pseudo-equilibrium state. The oscillatory tests during protein adsorption were performed at 0.1 Hz. Once the pseudo-steady state was achieved,

190 the interfacial shear moduli were obtained as a function of frequency (from 0.005 to
191 0.1 Hz).

192 *2.2.3. Preparation of emulsions*

193 The WLF and WLBBF solutions were dispersed at the protein saturation concentration
194 (0.6 wt.% soluble protein) and the oil phase (5 wt.%) were dispersed during 2 min with
195 a rotor-stator mixer (Ultraturrax T-25, IKA, Germany) at 20,000 rpm Subsequently, this
196 coarse emulsion obtained was passed through the M-110L Microfluidizer Processor
197 equipped with a F20Y interaction chamber (Microfluidizer®, USA), three times at 80
198 MPa.

199 Supplementary Figure 1 shows the effect of the number of passes of coarse emulsions
200 through the microfluidizer M-110L instrument on the droplet size distribution of the final
201 emulsions. This figure indicates that after three passes the droplet size distribution was
202 optimal. Thus, the coarse emulsions were prepared in batches of 200 g, and they were
203 mixed with a XG solution at 2 wt.% and water until the desired XG concentration (0,
204 0.06, 0.12 and 0.25 wt.%). For this purpose, the T-25 Ultraturrax was used at 500 rpm
205 for 60 s. Emulsions were stored at 5 ºC during 30 days to assess the influence of time
206 on emulsion stability.

2.2.4. Emulsions characterisation

Droplet size distribution measurements

Droplet size distribution (DSD) of the emulsions generated was determined with the particle size analyser Mastersizer 2000 (Malvern, United Kingdom). Sodium dodecyl sulphate (SDS) was added to disrupt the possible floccules formed. The Sauter mean droplet diameter (D [3,2]) was calculated as follows:

From the DSD, the flocculation index (FI) was also determined according to the equation used by Felix et al. (2017):

$$FI = \frac{|D[3,2] - D[3,2]_{SDS}|}{D[3,2]} \quad (1)$$

Where $D[3,2]$ is Sauter mean droplet size in absence of SDS and $D[3,2]_{SDS}$ is Sauter mean droplet size after the addition of 1 % SDS.

Linear viscoelasticity

The mechanical spectra of the emulsions generated were obtained by means of frequency sweep tests, which were performed using the AR-2000 rheometer (TA Instruments, USA). Stress sweep tests were initially carried out at 0.1, 1 and 10 Hz to determine the linear viscoelastic region (LVR). Frequency sweep tests (from 0.05 to 30 rad/s) were performed selecting a stress within the linear viscoelastic range, using

223 a plate-plate geometry (60 mm diameter, 1 mm gap) at 20 ºC controlled by Peltier
224 system.

225 *Backscattering measurements*

226 The physical stability of the emulsions generated was analysed by the application of a
227 light source to a tube where the sample was placed using a Turbiscan Lab Expert
228 device (L'Union, France). The backscattering (BS) was obtained as a function of the
229 tube length (maximum length 70 mm) and storage time (up to 30 days). Relative back
230 scattering (ΔBS) was determined as a function of storage time (Eq. 3):

$$\Delta BS\ (\%) = \frac{(BS_0 - BS_t)}{BS_0} \cdot 100 \qquad (3)$$

231 where BS_0 is the mean value for the BS profile obtained at 35 mm tube length the same
232 day emulsion preparation and BS_t is the mean value for the BS profile obtained at 35
233 mm tube length after 1, 7, 14, 21 and 30 days of storage.

234 *2.3. Statistical analysis*

235 At least three replicates of each measurement were carried out. The measurement
236 uncertainty was determined by means of standard deviation. Significant differences
237 were determined by one-way ANOVA tests (statistical package included in Excel)
238 confidence limits was established at 95% ($p < 0.05$).

3. Result and discussion

3.1. Proximate composition

The proximate composition of the WLF and WLBBF extracts is shown in Table 1. The protein concentration based on the nitrogen content of the studied extracts was 24.3 ± 0.1 and 19.8 ± 0.2% for the WLF and WLBBF extracts. The lower protein content of the WLBBF extact can be attributed to the different wine-growing carried out in presence of blueberries, which can release components such as carbohydrates (i.e., soluble fibres), reducing the protein content of the final product. In any case, the protein content of both wine lees extracts (WLF and WLBBF) is closed to the 15% found in other wine lees from white wine (Nerantzis & Tataridis, 2006). Moreover, moisture content for WLBBF was lower than the moisture content for WLF (6.7 ± 2.8 vs. 2.6 ± 0.2 %) after the same freeze-drying conditions, which together with the presence of carbohydrates can justify the above-mentioned reduction of protein content. Lipids were similar in both samples (9.0 ± 0.4 and 9.3 ± 0.1 % for WLF and WLBBF, respectively), whereas the WLF sample showed a higher amount of ashes (13.1 ±0.1 vs. 20.4 ± 1.4 %). The reduction in ash content can be related to the absorption of soluble salts from blueberries, compensating the salt content of wine and blueberries

256 since the ash amount in wine is higher than in blueberries (Dani et al., 2007;
257 Yemmireddy et al., 2013). Table 1 shows the total polyphenols content for the WLF
258 and WLBBF characterised. The results obtained indicate that the polyphenol content
259 for the WLBBF is almost twice the total polyphenol content of WLF lees (5.4 ± 0.2 and
260 2.0 ± 0.6 mg/g, respectively), which can be attributed to the addition of blueberries in
261 the wine used. This result obtained for WLF is in agreement with the result previously
262 obtained by Giovinazzo and Grieco (2015) for wine lees. The increase of polyphenols
263 noticed for WLBBF not only increase the antioxidant capacity of the WLBBF extract
264 but also can influence the ability of proteins to be adsorbed at the O/W interface
265 (Giovinazzo & Grieco, 2015; von Staszewski et al., 2014).

266 *3.2 Interfacial characterisation*

267 *Interfacial tension*

268 Before any measurement, the saturation concentration of the WLF and WLBBF
269 extracts at the O/W interface was determined. Figure 1A shows that the increase of
270 WLF and WLBBF wine less concentration involves a decrease of interfacial tension
271 since the concentration of surface-active agents at the O/W interface increases
272 (leading to lower surface tension values) (Dickinson, 1998). However, the interfacial

273 tension did not decrease for concentrations higher than 0.6 %, regardless of the wine
274 lees used (Figure 1A). This result was a consequence of the saturation of the interface
275 since an increase of surface-active molecules did not lead to a decrease in surface
276 tension (Tie et al., 2003). This concentration seemed to be similar to the saturation
277 concentration found for other high soluble model proteins such as BSA (bovine serum
278 albumin), lactoglobulin or casein, which is ca. 0.5% (Perez et al., 2010; Tang & Shen,
279 2015). This value indicates that these extracts have an excellent O/W interfacial
280 coverage, being comparable with these model proteins. Regarding the interfacial
281 tension value reached, the minimum was ca. 30 mN/m, which was in line with the
282 results obtained for crayfish, chickpea and fava bean protein concentrates (Felix et al.,
283 2017; Felix, Romero, Carrera-Sanchez, et al., 2019; Felix, Romero, Sanchez, et al.,
284 2019).

285 *Pendant droplet tensiometer measurements*

286 The adsorption of WLF and WLBBF wine-lees soluble proteins (0.6 wt.%) at the O/W
287 interface was monitored by the measurement of interfacial tension over adsorption time
288 (10,800 s) (Figure 1B). The kinetics of the WLF and WLBBF adsorption was
289 characterised by a rapid decrease in the interfacial tension values, followed by a slower

290 evolution and a tendency to reach a constant value. Although both extracts exhibited
291 the same tendency, Figure 1B indicates that the adsorption was faster for the WLBBF
292 extract, where the final interfacial tension was also lower for this extract (14.9 ± 0.3 vs.
293 17.1 ± 0.2 mN/m for WLBBF and WLF wine less, respectively). The additional
294 interfacial tension decrease found for the WLBBF extract can be attributed to protein-
295 phenol interactions or the adsorption of additional molecules (Karefyllakis et al., 2017;
296 von Staszewski et al., 2014). Karefyllakis et al. (2017) suggested that there is a
297 synergistic mechanism as a result of further cross-linking between polyphenols and
298 proteins molecules present at the adsorbed layer. In our study, the reported content of
299 polyphenols in WLBBF and WLF wine lees were polar phenolic due to the extraction
300 method followed. Thus, it can be assumed that additional hydrogen bonds can be
301 developed between the polyphenols bound on adjacent proteins, and thus further
302 unfolding was induced. This additional unfolding could lead to more efficient coverage
303 of the interface, reducing the interfacial tension of the O/W interface stabilized by the
304 WLBBF extract to a larger extend.

305 *Dynamic dilatational measurements*

306 Figure 2A shows the viscoelastic dilatational moduli (E'_s and E''_s) obtained during the
307 WLF and WLBBF adsorption at the O/W interface over 10,800 s. This Figure indicates
308 that both wine lees (WLF and WLBBF) had a similar interfacial response during their
309 adsorption since the elastic modulus (E'_s) experienced an initial increase over its
310 adsorption, which was followed by a tendency to reach a constant value. Otherwise,
311 the viscous modulus (E''_s) experienced a softer initial decrease, also followed by a
312 tendency to reach a constant value. This evolution observed has been previously
313 related to the development of an interfacial film at the O/W interface formed by surface-
314 active agents, exhibiting a predominant gel-like response. This was the case of crayfish
315 protein concentrate (Felix et al., 2017), bovine serum albumin protein isolate
316 (Rangsansarid & Fukada, 2007), pea protein concentrate (Amine et al., 2014) and
317 chickpea protein concentrate (Felix, Romero, Sanchez, et al., 2019), among others. In
318 these cases, the formation of stronger interfacial films (i.e., higher viscoelastic moduli)
319 was also related to a better emulsion capacity and higher emulsion stability, correlating
320 the interfacial dilatational response with the functional properties of the protein system
321 analysed.

322 However, the limitations of dilatational measurements should be considered, since the
323 measurements are affected by changes in droplet area, which may induce some
324 adsorption/desorption phenomena (Kairaliyeva et al., 2017). The results obtained
325 suggested that the WLF wine lees would have better emulsion stability since the
326 dilatational viscoelastic moduli obtained during the protein adsorption exhibited higher
327 values ($E'_s \sim 20$ vs. $E'_s \sim 10$ mN/m). Notwithstanding, this figure indicates that the
328 (rheo)kinetics of the WLBBF extract was faster than the (rheo)kinetics of the WLF
329 extract since the plateau value was reached for the WLBBF extract faster than for the
330 WLF extract.
331 Figure 2B shows the dependence of the viscoelastic dilatational moduli (E'_s and E''_s)
332 on frequency values for WLF and WLBBF extracts. The mechanical spectra obtained
333 confirmed the development of a gel-like structure, where the elastic response was
334 rather than predominant, especially for the interface stabilized by the WLF extract.
335 Moreover, the loss tangent ($\tan\delta = E''_s/E'_s$) can be calculated to analyse the elastic
336 character of the interfacial films formed. The $\tan\delta$ obtained at 0.1 Hz ($\tan\delta_{0.1}$) for the
337 WLF extract was 0.29, whereas the value obtained for the WLBBF extract was 0.37.
338 This parameter also corroborated the higher elastic character for the WLF extract. An

339 increase of almost twice the amount of polyphenols in the WLBBF compared to the
340 WLF resulted in a less elastic behaviour of the interfacial films obtained. These
341 observations could indicate that the complexation of protein lees with the phenolic
342 compounds altered the dilatational rheological behaviour of WLF wine lees. These
343 changes are frequency-dependent where the elasticity of the adsorbed layer can be
344 increased or decreased depending on the nature of the complexing phenols (Pham et
345 al., 2019). However, a comparison of the values obtained with previous studies
346 indicated that the values obtained in both cases were suitable for the stability of
347 emulsions. For instance, the $\tan \delta_{0.1}$ obtained for rice potato isolated adsorbed at O/W
348 interface was ca. 0.6 (Romero, Beaumal, David-Briand, Cordobes, Guerrero, et al.,
349 2011), whereas the $\tan \delta_{0.1}$ for crayfish protein isolate was ca. 0.2 (Romero, Beaumal,
350 David-Briand, Cordobes, Anton, et al., 2011). However, it should be noticed that these
351 values were higher than the values obtained for chickpea protein concentrate (Felix,
352 Romero, Sanchez, et al., 2019), faba bean concentrate (Felix, Romero, Carrera-
353 Sanchez, et al., 2019), or lactoglobilin protein concentrate (Gomes et al., 2018) ($\tan \delta_{0.1}$
354 = 0.19, 0.25 and 0.18 for O/W interfaces stabilized by chickpea fava bean and whey
355 protein, respectively), indicating that the interfacial films developed by WLF and

356 WLBBF extracts are not as strong as these other interfacial films stabilized by
357 lactoglobilin and faba bean proteins. The behaviour observed when WLF and WLBBF
358 extracts are absorbed at the O/W interface can be a consequence of the occurrence
359 of interactions at the liquid-liquid interface or the formation of a densely packed
360 structure (Fischer & Erni, 2007). However, dilatational measurements do not contribute
361 to elucidate the interactions at complex fluid-fluid interfaces (Sagis & Fischer, 2014).
362 Despite the versatility of droplet tensiometers to assess the interfacial responses of
363 complex interfaces, there are some limitations mainly related to the influence of
364 gravitational forces on droplet shape, the absorption/desorption of surface-active
365 agents to the interface during dilatational measurements, and the nature of the
366 interface rigidity (Berry et al., 2015). Interfacial shear measurements were carried out
367 to elucidate the presence of interactions at the O/W interfaces stabilized by WLF and
368 WLBBF.

369 *Dynamic interfacial shear measurements*

370 Figure 3A shows the interfacial viscoelastic moduli (G'_s and G''_s) obtained from small
371 amplitude oscillatory interfacial shear (i-SAOS) measurements for WLF and WLBBF
372 adsorbed at O/W interface over protein adsorption ($t < 180$ min). Contrary to dilatational

measurements, this Figure indicates that the (rheo)kinetics of the WLF and WLBBF adsorption was similar regardless of the extract studied. According to i-SAOS measurements, the adsorption of the two wine lees analysed was characterised by a nearly constant value during the overall adsorption time (note that according to the materials and method section, the first point was obtained after 10 min protein absorption). This evolution was not observed in any of the extracts studied for any of the two wine lees analysed, which indicates that the quasi-equilibrium state was reached faster for i-SAOS measurements than for dilatational measurements. Since i-SAOS measurements are more related to interactions developed at the O/W interface, this result may indicate that the interactions developed in O/W interfaces stabilized by WLF and WLBBF are reached fast in both cases, and they are similar in both cases. However, the viscoelastic moduli values obtained (ca. $3.3 \cdot 10^{-5}$ and $6.7 \cdot 10^{-5}$ Pa·m for G'_s and G''_s, respectively) are lower than the values obtained for other protein systems such as chickpea, fava bean and lactoglobulin adsorbed at O/W interface (at least $1 \cdot 10^{-3}$ Pa·m) (Erni et al., 2011; Felix, Romero, Carrera-Sanchez, et al., 2019; Felix, Romero, Sanchez, et al., 2019). The rheological responses were very different from those previously obtained by the dilatational rheology since a liquid-predominant behaviour

was observed when the interface was analysed by this technique These results can be related to the fact that interfacial shear rheology applies an in-plane deformation, where the response evidence the slippage of molecules along with the interface (Jaensson & Vermant, 2018). However, when the interface was analysed by dilatational rheology, a compression in the surface area took place, leading to a response which was not necessarily related to molecular interactions (Wijmans & Dickinson, 1998).

Figure 3B shows the mechanical spectra obtained by means of a frequency sweep test (from 0.0075 to 0.3 Hz) obtained for the WLF and WLBBF wine less adsorbed at the O/W interface once the quasi-equilibrium state was reached (t > 180 min). This Figure confirms the formation of a protein film with a predominant gel-like behaviour (G'_s > G''_s) at low frequency, whereas at a high frequency (0.03 Hz) a viscous predominant behaviour (G''_s > G'_s) was observed after a crossover point. The mechanical spectra obtained for other interfacial films was different since a gel-like response was obtained in the overall frequency range studied (Felix et al., 2020). Thus, the elastic moduli obtained for both wine less (WLF and WLBBF) showed a marked frequency dependence, indicating that the gel behaviour of the interfacial film formed at high

407 frequency was fairly weak. These results would corroborate the above-mentioned low
408 protein-protein interactions observed during protein adsorption, which is reflected in
409 the mechanical spectra by a crossover point at high-frequency values for interfaces
410 stabilized by WLF and WLBBF extracts. However, this crossover point after protein
411 adsorption was not observed for other proteins such as pea, WPI or chickpea adsorbed
412 at the interface (Manuel Felix, Romero, Carrera-Sanchez, et al., 2019; Manuel Felix,
413 Romero, Sanchez, et al., 2019; Manuel Felix, Yang, et al., 2019), which evidences the
414 formation of a more solid predominant behaviour for these interfacial films.

415 *3.3 Emulsion characterisation*

416 *Droplet size distribution*

417 Figure 4 shows the droplet size distribution (DSD) profiles obtained for emulsions
418 stabilized by WLF and red WLBBF wine lees (A and B, respectively) at four different
419 XG concentration (0, 0.06, 0.12 and 0.25 %), after production and after 30 days.
420 These droplet distribution profiles evidence the reduced droplet sizes obtained for the
421 emulsions generated, regardless of the wine lees used when XG was in the
422 formulation. No influence of XG concentration was observed either.

423 The DSD profile obtained the same day emulsion preparation demonstrated the
424 feasibility of these extracts to form emulsions by the procedure followed, generating
425 emulsions whose DSD maximum value calculated was ca. 500 nm (D[3,2] for
426 emulsions containing XG = 360 - 380 nm). These small droplet sizes of emulsions were
427 obtained after three passes of the emulsions through the microchannel homogenizer,
428 being smaller than the value obtained for other protein-based emulsions such as pea
429 (Peng et al., 2016), chickpea (Felix et al., 2017) and egg albumen (Guerrero et al.,
430 2000), processed by a high-pressure valve homogenizer which ranged around 1-10
431 μm. On the other hand, emulsion storage involved an increase in droplet size up to 500
432 nm in absence of XG. The addition of XG reduced the D[3,2] droplet size from 1.83 to
433 4.05 μm.
434 Table 2 shows Sauter mean diameters (D[3,2]) and Flocculation index (FI) obtained
435 from DSD the profiles during storage time (up to 30 days) as a function of XG
436 concentration (0, 0.06, 0.12 and 0.25 %). This Table indicates that the emulsions
437 stabilized by WLF and WLBB extracts exhibited small droplet sizes, obtaining
438 nanometre sizes in all cases, except for the emulsion in absence of XG, which was not
439 stable and it exhibited a D[3,2] > 1 μm the day after emulsion preparation. Thus, an

440 increase in storage time involved a significant ($p < 0.05$) increase in D[3,2] for the
441 emulsions containing 0 and 0.06 % XG, however, significant changes in Sauter mean
442 diameter were not observed for emulsions containing 0.12 and 0.25 % XG during the
443 storage time evaluated. Moreover, Table 2 also shows that smaller mean diameters
444 were obtained for the WLBBF-based emulsions (i.e., when there was a higher
445 presence of polyphenols), which can be also related to the lower interfacial tension
446 values obtained for this extract (Figure 1B). On the other hand, FI parameter indicated
447 that flocculation increases over storage time, especially for the emulsion containing a
448 higher amount of XG (0.12 and 0.25 %). However, droplet sizes did not show a
449 significant increase over the storage time evaluated, indicating that flocculation did not
450 end up in droplet coalescence and should be regarded as the development of
451 interactions between the wine lees used and the polysaccharide (XG) (Dickinson,
452 2009).

453 *Viscoelastic properties*

454 Frequency sweep tests were performed on all the emulsions generated as a function
455 of XG concentration (0, 0.06, 0.12 and 0.25 %) to determine the dependence of the
456 viscoelastic moduli (G' and G'') on frequency value for emulsions stabilized by WLF

457 (Fig. 5A) and WLBBF (Fig. 5B) wine lees. This figure indicates that the rheological
458 response was dominated by the XG content, where the higher concentration of XG
459 involved higher viscoelastic moduli. Contrary to emulsions with high oil content, these
460 emulsions did not exhibit a gel-like behaviour, showing the mechanical spectra a strong
461 dependence on the frequency value, as well as a liquid-predominant response. This
462 behaviour was previously found for diluted emulsions, where the rheological response
463 was determined by the thickening agent used (XG in this case) (Bayarri et al., 2009;
464 Milas et al., 1990). This thickening agent had a key role in the stability of the emulsions
465 prepared, showing a similar response regardless of the wine lees (WLF or WLBBF)
466 used. A small concentration of this agent can be used to prevent phase separation, as
467 well as droplet coalescence, however big amounts of this agent have been related to
468 flocculation phenomena, as well as an undesirable texture, moving away from the
469 typical texture of food brewages and milkshakes (Bayarri et al., 2009). Thus, strong
470 gel-like responses (G' >> G'') should be avoided for these emulsions because of their
471 texture. Consequently, the emulsions containing 0.25 % XG would not be suitable for
472 the manufacture of brewages with a milkshake-like appearance. For this kind of
473 product, the emulsions generated should exhibit a liquid-predominant behaviour. Table

474 3 shows the viscoelastic moduli at 1 Hz (G'_1 and G''_1) obtained for WLF-, and WLBBF-
475 stabilized emulsions during storage time (up to 30 days) as a function of XG
476 concentration (0, 0.06, 0.12 and 0.25 %). This Table indicates that storage time
477 involves a reduction in G'_1 and G''_1 for the emulsions with the smaller amount of XG
478 (i.e., from $1.96 \cdot 10^{-3}$ to $3.86 \cdot 10^{-5}$ Pa for WLF and $7.56 \cdot 10^{-3}$ to $1.28 \cdot 10^{-4}$ Pa in absence
479 of XG), however, no significant changes were observed for the emulsions containing
480 0.12 and 0.25 % XG, suggesting that no significant structural changes took place
481 during the storage time analysed.

482 *Light scattering measurements*

483 Light scattering diffraction (Backscattering, BS) measurements are performed to
484 anticipate physical destabilization of the emulsions generated (i.e., phase separation,
485 creaming, flocculation and coalescence). Figure 6 shows the relative back scattering
486 (ΔBS) obtained for emulsions stabilized by WLF (Fig. 6A) and WLBBF (Fig. 6B) wine
487 lees during 30 days storage time. This Figure indicates that regardless of the wine lees
488 evaluated (WLF or WLBBF), the emulsion in absence of XG involved noticeable
489 changes in ΔBS over storage time, which can be related to emulsion creaming (which
490 is reflected in the BS profile by a complete reduction of the backscattering signal at the

491 bottom of the tube containing the sample, data not shown). However, the creaming
492 rate decreased with the increment of polyphenols concentration. By contrast, although
493 all the other emulsions (0.06, 0.12 and 0.25 % XG) suffered a certain evolution of ΔBS
494 values until reaching a constant value (where the complete profile of the backscattering
495 does not evidence significant changes either at the top or at the bottom of the tube
496 containing the emulsion sample). In this case, these changes in ΔBS can be attributed
497 to emulsion flocculation. Flocculation phenomena involved a decrease in BS signal
498 (Mengual et al., 1999), which can cause the changes observed in ΔBS. These results
499 are in accordance with the results obtained from DSD profiles, where emulsions
500 increased the FI up to the 15th-day emulsion storage. Moreover, a higher amount of
501 XG involved higher FI values, which was in accordance with the greater dependence
502 of ΔBS on storage time for the emulsions containing a higher amount of XG.

503 ## 4. Concluding remarks

504 WLF and WLBBF are wastes that could be reconverted into by-products with potential
505 applications in the stabilization of food products. Both wine lees extracts (WLF and
506 WLBBF) possessed a similar protein content (~ 20%) and a high number of total
507 polyphenols, where the higher amount was found for WLBBF extract (2.9 ± 0.6 vs. 5.4

508 ± 0.2 mg/g for WLF and WLBBF, respectively), since it resulted from the addition of
509 blueberries during grape fermentation. This additional amount of polyphenols
510 influenced techno-functional properties of WLBBF wine less since it was adsorbed at
511 the O/W interface faster. The adsorption of WLBBF and WLF wine lees involved a
512 reduction in interfacial tension when they were adsorbed at the O/W interface,
513 however, the reduction caused by the WLBBF wine lees was higher than the reduction
514 caused by the WLF (ca. 15 mN/m vs. 17 mN/m). Despite the limitations of droplet
515 tensiometry, the results obtained are quite useful to assess the interfacial responses
516 of proteins adsorbed at complex interfaces. Thus, interfacial dilatational measurements
517 evidenced a more rigid interface when WLF wine lees were adsorbed at the O/W
518 interface, exhibiting higher elastic moduli when it was adsorbed (ca. 20 mN/m vs. 10
519 mN/m). By contrast, i-SAOS measurements did not evidence differences between
520 interfaces stabilized by WLF and WLBBF. The comparison of both techniques
521 indicated that the interfacial film formed was fairly weak regardless of the wine lees
522 used, which suggested that the results obtained by dilatational measurements were
523 caused by a densely packed structure. Results obtained from emulsions indicated that
524 WLF and WLBBF were suitable for the stabilization of diluted O/W emulsions where

525 nanometric-size emulsions (D[3,2] = 360 nm) were obtained. In this sense, XG had a
526 key role in their stability. In absence of XG emulsions were not kinetically stable since
527 droplet coalescence took place over storage time, which ended in phase separation.
528 On the other hand, high amounts of XG (0.25 %) involved a solid-predominant
529 rheological behaviour (which is not desirable for milkshake-like drinks). Intermediate
530 concentrations (0.06 and 0.12 %) led to stable emulsions with a liquid-predominant
531 behaviour. These extracts could be used for the stabilization of dispersed systems,
532 where a suitable amount of XG (0.06 or 0.12 %) is required for the stabilization of food
533 products with the rheological behaviour of shakes and brewages.

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