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Additional Information

Use of oil-in-water emulsions to control fungal deterioration of strawberry jams

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ABSTRACT

This work aimed to control the fungal deterioration of strawberry jams. The antifungal activity of the clove, cinnamon leaf, lemon and mandarin essential oils and their effectiveness in oil-in-water emulsions were evaluated. According to the results obtained, only clove and cinnamon leaf oils were selected to prepare emulsions. All the tested emulsions were stable, independently the amount of polymer and essential oil used. Essential oil loss was affected by the amount of polymer employed to prepare the emulsions. The oil-in-water emulsions with 5.0 mg/g xanthan gum, and with 0.55 mg/g clove or 0.65 mg/g cinnamon leaf essential oil, were used for the *in vivo* tests. The jams prepared with the oil-in-water emulsions showed a lower fungal decay compared with jams without emulsion. The present work demonstrated that emulsions can be employed to prevent strawberry jam mould spoilage.

Keywords: Essential oils, oil-in-water emulsion, strawberry jam, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium expansum*

1. Introduction

Fungal contamination is a serious problem in the food industry because it has negative impacts on final products. Fungi are the most important microorganisms to contaminate fruit and berry concentrates, like jams with low water activity. Fungal spores present in the raw materials of jams are inactivated while jams are cooked. However, the large jam containers used in the food industry may be re-contaminated by spores of indoor fungi; e.g. during partial container depletion (Nieminen, Neubauer, Sivelä, Vatamo, Silfverberg, & Salkinoja-Salonen, 2008).

There are thousands of known species of moulds, and the commonest genera are *Aspergillus* and *Penicillium*. Moulds are difficult to inhibit because of their complex structure, and using chemical agents is one of the main techniques resorted to for controlling their growth. However, consumer concern about human health has forced the food industry to search for new strategies as alternatives to chemical additives in order to control food spoilage caused by moulds.

Essential oils (EOs) extracted from many plants and fruits are used as antimicrobial agents against bacteria, moulds and yeasts (Perdones, Sánchez-González, Chiralt, & Vargas, 2012; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2014). Their natural character renders them desirable for use in food products. Many EOs have been recognised as safe (GRAS) by the FDA in 21 Code of Federal Regulation part 182.20 (CRF, 2014), and they are widely accepted by consumers (Burt, 2004).

The antimicrobial activity of EOs is attributed mainly to their content in volatile compounds. Eugenol (4-allyl-2-methoxyphenol) is the main compound in cinnamon leaf EO (75-95% (w/w)) (Vangalapati, Satya, Prakash, & Avanigadda, 2012). This EO has demonstrated potent antioxidant and antibacterial activity (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Eugenol is a naturally-occurring phenol extracted from

cloves. Different studies have demonstrated the bactericidal and antifungal activity of clove EO and eugenol (Liang et al., 2015, Hua et al., 2014, Jayashree & Subramanyamm, 1999, and Velluti, Sanchis, Ramos, Egido & Marín, 2003). The antimicrobial activity of EO from citrus fruits has been widely demonstrated against mould, yeast and bacteria (Belletti, Sado-Kamdem, Tabanelli, Lanciotti, & Gardini, 2010, Espina, Somolinos, Lorán, Conchello, García, & Pagán, 2011, and Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008).

The volatile compounds present in EOs are highly insoluble in water because of their lipophilic nature, whose contact with microorganisms in high moisture content foods may be limited (Kalemba & Kunicka, 2003). One way to avoid this problem and to enhance their aqueous solubility and stability is to incorporate essential oils into oil-in water (O/W) emulsions. In this case, their sensory impact on food products could reduce and their water solubility could increase for the contact with microorganisms to suffice and to improve antimicrobial effectiveness (Hill, Gomes, & Taylor, 2013).

The objective of this study was to evaluate the mould decay of strawberry jams by using natural preservatives. For this purpose, the *in vitro* antifungal activity of different EOs and O/W emulsions, and the effectiveness of these emulsions when incorporated into strawberry jams were evaluated.

1. Materials and methods

2. 1 Screening the antifungal activity of EOs

Clove, cinnamon leaf, lemon and mandarin EOs (Sigma-Aldrich, St. Louis, USA) were individually tested against *Aspergillus flavus* (CECT 2685), *Aspergillus niger* (CECT 20156) and *Penicillium expansum* (CECT 20140). The method described by Viuda-Martos et al. (2008) was employed with minor modifications. The stock cultures of

fungi were supplied by the Spanish Type Culture Collection (CECT, Burjassot, Spain). The fungus were inoculated on Potato Dextrose Agar (PDA, Scharlab, Barcelona, Spain) and incubated at 25 ºC for 7 days. Afterwards, spores were counted in a haemocytometer to achieve an inoculum density of 10^6 CFU/mL. Different EO concentrations were tested after taking into account previous studies (Omidbeygi, Barzegar, Hamidi, & Naghdibadi, 2007, Perdones et al., 2012, and Viuda-Martos et al., 2008). The concentrations of tested EOs were: 0.40, 0.45, 0.50 and 0.55 mg/g for clove oil; 0.50, 0.55, 0.60 and 0.65 mg/g for cinnamon leaf oil; 10, 12.50, 15 and 17.50 mg/g for lemon oil; 27.50, 30, 32.50 and 35 mg/g for mandarin oil. Aliquots of 15 g of PDA with the EOs and 0.1% (w/w) Tween 80 (Scharlab, Barcelona, Spain) were poured into Petri dishes. EOs were added to the culture medium at 50°C and Tween 80 was added to the medium to ensure good EO distribution. The petri dishes without EO were used as control samples. The centre of each plate was inoculated with a PDA disc (7 mm diameter) taken from the edge of 0-day-old fungi culture, previously spread with 100 µL of the spore solution (10^6 CFU/mL). Each plate was sealed with Parafilm® and incubated for 7 days at 25°C. Radial mycelial growth was evaluated daily for 7 days by measuring the diameter of each fungus. Values were expressed in mm diameter/day. All tests were run in duplicate.

2.2 Study of O/W emulsions

2.2.1 Emulsion preparation

Xanthan gum (XG, Satiaxane™ CX 911, Cargill, Barcelona, Spain) was dispersed in distilled water at 2.5, 5.0 and 7.5 mg/g, and stirred overnight at room temperature. After biopolymer dissolution, the clove and cinnamon leaf EOs were added to reach the final concentrations of 0.55, 0.65, 0.75 mg/g and 0.65, 0.75, 0.85 mg/g, respectively. The

mixture was emulsified in a rotor-stator homogenizer (Ultraturrax, IKA®, Germany) at 10,000 rpm for 1 min and 20,000 rpm for 3 min. These emulsions were degasified at room temperature with a vacuum pump.

2.2.2 Physico-chemical characterisation of O/W emulsions

The particle size was determined with a laser diffractometer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). Emulsions were diluted in deionised water at 2,000 rpm until an obscuration rate of 10% was obtained. The Mie theory was applied by considering a refractive index of 1.50 and absorption of 0.01.

The ζ-potential was carried out according to Sánchez-González, Cháfer, Chiralt, and González-Martínez (2010), using a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK).

The rheological behaviour of emulsions was analysed by a rotational rheometer (Haake Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a type Z34DIN Ti sensor system of coaxial cylinders, assessed as described by Sánchez-González et al. (2010). Shear stress (τ) was measured as according to shear rate ($\dot{\gamma}$) from 0 to 512 s^{-1}. Apparent viscosity values were calculated at 100 s^{-1}.

2.3.3 GC-MS analysis

The clove and cinnamon leaf EOs and O/W emulsions composition were analysed by GC/MS. Two grams of the EO or O/W emulsions were suspended in a tube that contained 15 mL of n-hexane. The mixture was shaken gently and filtered through filter paper. *n*-hexane was evaporated at 40ºC in a rota-vapour, and the obtained extracts were added to 2 mL of *n*-hexane and analysed by GC-MS.

134 The GC/MS analysis of the EOs was performed in a 6890/5975 inert GC-MS (Agilent
135 Technologies, Santa Clara, CA, US), equipped with a HP-5 fused silica capillary
136 column (30 m x 0.25 mm x 0.25 µm). The oven temperature was held at 60ºC for 3 min,
137 and then raised to 100ºC at 10ºC/min, to 140ºC at 5ºC/min, and finally to 240ºC at
138 20ºC/min. Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min.
139 The injector and MS transfer line temperatures were set at 250°C and 230°C,
140 respectively. The parameters for the MS analysis were EI Ion source, electron energy 70
141 eV, solvent delay 3 min and m/z 40-550 *amu*. EO components were identified by
142 matching mass spectra with the standard mass spectra from the NIST MS Search 2.0
143 library.
144
145 *2.2.4 Antifungal activity of O/W emulsions*
146 The antifungal activity of the clove and cinnamon leaf emulsions against *A. flavus, A.*
147 *niger* and *P. expansum* was determined by the methodology described in Section 2.1. In
148 this case, 0.5 g of each O/W emulsion was added to 49.5 g of PDA at 50°C. Next
149 aliquots of 15 g of PDA with the emulsions were poured into Petri dishes. The PDA
150 with a dispersion prepared with distilled water and XG was used as a control. A disc of
151 mycelial material was placed in the centre of each plate and then incubated. Radial
152 mycelial growth was evaluated daily for 7 days. The results were expressed in mm
153 diameter/day. All tests were run in duplicate.
154
155 *2.3 Study of O/W emulsions in strawberry jams*
156 *2.3.1 Jam preparation*
157 Jam preparation was adapted from Igual, Contreras and Martínez-Navarrete (2010).
158 Strawberry jam was obtained by mixing fruit and sugar in a ratio of 65:35 and cooked at

159 100°C for 30 min to reach a 60°Brix in the product as described in the Spanish quality
160 regulation for fruit jam (BOE, 2003). This process was carried out in an electrical food
161 processor (Thermomix TM 31, Vorwerk M.S.L, Spain). The amount of emulsions
162 added to strawberry jam was established to achieve a concentration of 1 g of the O/W
163 emulsion in 100 g of jam in the final product.
164
165 *2.3.2 Evaluation the antifungal activity of O/W emulsions in strawberry jams*
166 Fifteen grams of strawberry jam that contained the O/W emulsions were inoculated with
167 100 µL of the spore solution (10^6 CFU/ mL). Jams were poured into Petri dishes and
168 incubated for 63 days at two different temperatures: 4°C to simulate product cold
169 storage after opening the jam container, and at 25°C, the optimum growth temperature
170 of fungi. Three Petri dishes were prepared per temperature condition, microorganism
171 and day of analysis (n=54). Mould counts were done in PDA plates after a 72-hour
172 incubation at 25°C (Pascual & Calderón, 2000). All assays were performed in duplicate.
173
174
175 *2.3.3 Sensory analysis*
176 A sensory analysis was carried out by 30 non-expert untrained assessors. The group of
177 assessors was composed of 12 men and 18 women, and panellists' ages ranged from 21
178 to 50 years. Tests were done on a structured 9-point hedonic scale (9=very much like
179 and 1=very much dislike) (UNE-ISO 4123), by which the colour, aroma, taste,
180 consistency and overall acceptance attributes were evaluated. All the samples were
181 presented to the panellists at room temperature in a transparent plastic glass coded with
182 three random numbers.
183

184 *2.4 Statistical analysis*

185 The results obtained in the physico-chemical characterisation of the O/W emulsions,
186 and the antifungal evaluation of the EO and O/W emulsions, were analysed by a
187 multifactor analysis of variance (multifactor ANOVA). The effect of incorporating the
188 O/W emulsion into the sensory attributes of strawberry jam was evaluated by a one-way
189 ANOVA. The least significance procedure (LSD) was used to test for any differences
190 between averages at the 5% level of significance. Data were statistically processed by
191 Statgraphics Centurion XVI.

192

193 **2. Results and discussion**

194 *3.1 Screening the antifungal activity of EOs*

195 The clove, cinnamon leaf, lemon and mandarin EOs all at the tested concentrations had
196 the capacity to reduce or inhibit the growth of *A. flavus, A. niger* and *P. expansum* (Fig.
197 1) since fungi showed slightly retarded growth compared with the control plates, even
198 for the lowest EO concentrations. This behaviour suggests that the active compounds of
199 the EOs could affect initial mould development, and could cause a delay in mould
200 growth, which would confirm their fungistatic effect (Manso, Cacho-Nerín, Becerril &
201 Nerín, 2013).

202 The clove, cinnamon leaf and lemon EOs at all the tested concentrations increased the
203 *Lag phase* of *A. flavus, A. niger* and *P. expansum*, with a diminution of the germination
204 rate. The clove EO provoked a higher delay in mould growth compared to the other EOs
205 tested. The clove EO at the lowest concentration managed to reduce mycelial growth
206 more than 78% for all the studied moulds.

207 The clove, cinnamon leaf and lemon EOs inhibited growth of moulds at the assayed
208 highest concentrations (0.55 and 17.5 mg/g, respectively) during the whole study

period, except for the cinnamon leaf EO tested against *P. expansum*, for which mycelial growth reduced, but was not inhibited. The high lemon EO concentration needed to inhibit fungi development could be due to D-limonene's susceptibility to oxidative degradation, which could cause loss of activity (Sun, 2007).

The mandarin EO caused the lowest percentage of mycelial reduction in all the studied moulds (Fig.1). The highest mandarin EO concentration tested achieved only percentage reductions of 62%, 61% and 73% for *A. flavus*, *A. niger* and *P. expansum*, respectively.

Fig. 1

3.2 Study of O/W emulsions

3.2.1 Physico-chemical characterisation of O/W emulsions

Table 1 shows the $d_{3,2}$ and $d_{4,3}$ values for the particle size analysis, the ζ-potential and the rheological parameters for the various emulsions.

Oil content and XG concentration had a significant impact on $d_{3,2}$ and $d_{4,3}$. Regarding oil content, the clove emulsions at the lowest assayed EO concentration (0.55 mg/g) exhibited a $d_{3,2}$ of 5.19±0.05 μm when the XG concentration was 2.5 mg/g, whereas an increased droplet mean diameter was observed (7.32±0.21 μm) at the highest EO concentration (0.75 mg/g) (Table 1). The same behaviour was observed for $d_{4,3}$ when 2.5 mg/g of XG was used. The mean size values increased from 10.4±2.6 to 17.5±0.7 μm when larger amounts of the clove EO were employed. Generally, the cinnamon leaf O/W emulsions exhibited the same tendency for the $d_{3,2}$ and $d_{4,3}$ values observed in the emulsions formulated with the clove EO.

As observed, the higher the oil content in the emulsions, the bigger particle size became. This could be due to the increase in the dispersed phase concentration, which could facilitate the droplet flocculation rate and therefore the reduction in the ratio between the interfacial stabilising material and the dispersed phase (McClements, 2005). Similar results have been reported by Sánchez-González et al. (2010) in emulsions of bergamot EO and chitosan aqueous systems.

Regarding polymer concentration, the increase in the XG concentration led to a reduction in the droplet mean diameter of emulsions with significant differences ($p<0.05$) (Krstonošić, Dokić, Dokić, & Dapčević, 2009). The cinnamon leaf emulsions prepared with the lowest EO concentration gave $d_{3,2}$ values of around 8.36±0.04 μm when the polymer concentration was 2.5 mg/g. However, a smaller droplet mean diameter was observed when 7.5 mg/g of XG was used at the same EO concentration (6.33±0.29 μm) (Table 1). This could be due to the ability of particles to cover the surface of droplets and to produce a thick interfacial layer around them (Frelichoswska, Bolzinger, & Chevalier, 2009). In contrast, the clove emulsions presented similar $d_{3,2}$ values despite the increase in the XG concentration (Table 1). This suggested that the clove EO amphiphilic components could have greater surfactant activity, and could thus contribute to reduce the droplet particle size under equal homogenisation conditions. This trend has also been observed by Bonilla, Atarés, Vargas, and Chiralt (2012) when they used thyme oil in chitosan-based films.

The effect produced by incorporating larger amounts of polymer was less marked for the $d_{4,3}$ values, for both types of O/W emulsions.

The surface charge of oil droplets in the emulsions prepared with the EOs is shown in Table 1. According to McClements (2005), if the electrical charge of droplets is high enough, the emulsion may be stable against aggregation due to repulsive forces between

droplets. Generally, particles with a ζ-potential that is more positive than + 30 mV, or more negative than - 30 mV, are considered stable. The electrical charge of the lipid droplets of the emulsions were between -67.0±0.6 and -72.0±1.9 for the clove EO emulsions, and between-64.5±1.1 and 70.7±0.9 for the cinnamon leaf EO emulsions. Therefore, it can be stated that both types of O/W emulsions are stable. In the present work, the strong negative ζ-potential observed in the emulsions was due to the presence of XG, which is an anionic hydrocolloid. The polymer was used as an emulsion stabiliser as these stabilisers can absorb into the interfacial layer (Dickinson, 2009). In addition, the stabilisation action of hydrocolloids was due to the viscosity modification in the continuous phase by lowering the rate of creaming and coalescence (Dickinson, 2009 and Garti & Leser, 2001).

Regarding rheological characteristics, all the emulsions showed a shear thickening behaviour with flow behavior index (*n*) values at around 0.47. No thyxotropic effects were observed from the comparison made of the up and down curves. The curves were predicted by the Ostwald de Waele model. Table 1 shows the consistency index (*k*), the flow behaviour index (*n*) and the apparent viscosity values calculated at 100 s^{-1}, which is the typical shear rate of different unit operations, such as mastication (McClements, 2005).

The incorporation of EOs into the XG dispersions did not produce significant changes in the rheological characteristics of the emulsions. Notwithstanding, an increase in the XG concentration in the emulsions resulted in significant increases ($p<0.05$) in the consistency index (*k*), which led to more consistent fluids and is related to the apparent viscosity (η_{ap}) of the emulsions. The clove emulsions formulated with 2.5 mg/g of XG obtained *k* values of 0.127±0.007 Pa·s. The same emulsions prepared with 7.5 mg/g of

polymer gave *k* values of 1.327±0.080 Pa·s (Table 1). The same tendency was observed for the emulsions formulated with cinnamon leaf EOs.

As shown in Table 1, the apparent viscosity values increased significantly ($p<0.05$) when larger amounts of XG were incorporated. These values oscillated between 0.02 and 0.08 Pa·s for the clove emulsions, and from 0.05 to 0.10 Pa·s for the cinnamon leaf emulsions. It is well-known that even at low polymer concentrations, XG dispersions exhibit high viscosities (Laneuville, Turgeon, & Paquin, 2013). Nevertheless, in the samples with the same amount of XG, the n_{ap} values remained constant in spite of using higher EO concentrations. This could be explained by the promotion of the EO-polymer interactions and the complex structure of the network formed by XG, which could cushion the impact of the EOs concentration on η_{ap}. This agrees with previously reported results (Martínez-Padilla, García-Rivera, Romero-Arreola & Casas-Alecáster, 2015) on foams with whey protein concentrate and XG.

Table 1

3.2.2 GC-MS analysis

Screening the antifungal activity of EOs indicated that the clove and cinnamon leaf oils were more interesting for controlling mould growth in food products. Components of these EOs were identified by the GC-MS analysis. In both EOs, eugenol (ca. 86%) was the main compound (Table 2), and similar results have been reported by different authors (Espina et al., 2011 and Singh, Maurya, deLampasona, & Catalan, 2007). The main compound of cinnamon EO from leaves is usually eugenol and, in some cases, there is a small amount of cinnamaldehyde (Tzortzakis et al., 2009 and Vangalapati et al., 2012). The clove and cinnamon leaf extracts and their main compound, eugenol,

303 have been reported as one of the most effective natural antimicrobial agents (Amiri,
304 Dugas, Pichot, & Bompeix, 2008, Hill et al., 2013, Omidbeygi et al., 2007, and Singh
305 et al., 2007).
306 Despite the similar amount of eugenol in both EOs, significant differences in terms of
307 their antifungal activity have been previously observed. The clove EO has marked
308 antifungal activity at the highest tested concentration (0.55 mg/g), whereas the
309 cinnamon leaf EO inhibited the growth of *A. flavus* and *A. niger*, and reduced the
310 growth of *P. expansum* at 0.65 mg/g. These results could suggest synergistic
311 interactions between eugenol and sesquiterpene hydrocarbons (caryophyllene, β-
312 caryophyllene and δ-cadinene) against the evaluated fungi strains. Some studies have
313 reported the antifungal activity of sesquiterpens constituents against several damping-
314 off, root pathogens, etc. (Chang, Cheng, Wu, Chang, Chang, & Su, 2008 and Kumar,
315 Mathela, Tewari, & Bisht, 2014). The sum of the relative areas of eugenol and the
316 sesquiterpene hydrocarbons were 94.67% and 87.76% for the clove and cinnamon leaf
317 EOs, respectively.
318

Table 2

319
320 Emulsions were also analysed and the EOs losses during emulsion preparation were
321 determined. Estimated EOs loss referred to eugenol, the main component of both EOs.
322 Eugenol loss after emulsions preparation came close to 40% in all cases. EOs loss could
323 be attributed to stress applied to samples and to the heating achieved during the
324 homogenisation process. Depending on the type of EO employed for emulsion
325 preparation, non-significant differences were observed. However, EO loss was affected

326 by the amount of XG employed to prepare the O/W emulsions; for instance, 2.5 mg/g of
327 XG led to the greatest eugenol loss compared to 5.0 and 7.5 mg/g XG, while no
328 significant differences were observed between these samples. The lower viscosity of the
329 samples formulated with 2.5 mg/g of XG, compared with the emulsions that contained
330 5.0 and 7.5 mg/g of XG, could cause the diffusion of EOs to the surface of the
331 emulsions to facilitate evaporation and its subsequent loss (Perdones, Escriche, Chiralt,
332 & Vargas, 2016). The greater the viscosity of the samples, the greater the
333 immobilisation of oil droplets.
334
335 *3.2.3 Antifungal activity of O/W emulsions*
336 As previously mentioned, only the clove and cinnamon leaf EOs were selected and their
337 concentrations in emulsions were established by considering the results of the *in vitro*
338 evaluation. As a result, the assayed EO concentrations were 0.55, 0.65 and 0.75 mg/g
339 for clove and 0.65, 0.75 and 0.85 mg/g for cinnamon leaf. No mycelial growth was
340 observed for any tested condition (data not shown), which indicated that the O/W
341 emulsions with 0.55 mg/g of clove and 0.65 mg/g of cinnamon leaf sufficed to inhibit *A.*
342 *flavus, A. niger* and *P. expansum* growth over 7 days.
343 According to the EO loss which occurred during emulsion preparation, the final clove
344 and cinnamon leaf EO content in their O/W emulsions was 0.34 mg/g and 0.39 mg/g,
345 respectively. When comparing the results with those obtained in the *in vitro* tests,
346 increased antifungal activity was noted. This could be attributed to improved water
347 solubility of the encapsulated compounds by enhancing the EOs diffusion rate and,
348 therefore, antifungal activity at the tested concentrations against *A. flavus, A. niger* and
349 *P. expansum*.

According to these results, and to those obtained in the physico-chemical characterisation of the O/W emulsions, the suitable amount of polymer and EO to be employed in strawberry jams was established. The emulsions prepared with 0.55 and 0.65 mg/g of the clove and cinnamon leaf EO, respectively, and with 5.0 mg/g of XG, were added to strawberry jam. Considering this formulation, the final concentration of clove and cinnamon leaf EO in the strawberry jams was 0.34 mg/g and 0.39 mg/g, respectively.

3.3 Study O/W emulsions in strawberry jam

3.3.1 Evaluation of the antifungal activity of O/W emulsions in strawberry jam

In order to evaluate the influence of temperature and the effectiveness of the O/W emulsion on mould growth, samples were stored at 4 and 25°C for 63 days (Fig. 2). In the control samples, the mycelial growth rate was affected by storage temperature for all the tested moulds.

The jams prepared with the O/W emulsions inoculated with *A. flavus* and stored at 4°C showed no fungal growth during the evaluation period. EO type significantly affected ($p<0.05$) the antifungal activity of the O/W emulsions. At refrigeration temperature, the clove and cinnamon O/W emulsions took longer to inhibit A. *niger* and *P. expansum* growth compared with *A. flavus*. The inhibitory effect was stronger when cinnamon leaf O/W emulsion was added to the strawberry jams, compared to clove emulsions. In this regard, no fungal growth was inhibited at days 21 and 28 for *A. niger* and *P. expansum*, respectively when cinnamon leaf was used; however, the clove emulsions inhibited fungal growth at day 49.

Inhibition of *A. flavus* on the samples that contained clove emulsions took place during the first 7 storage days at 25°C, whereas inhibited fungi growth was observed on day 14

375 when the cinnamon leaf EO was used. Unexpectedly, the opposite behaviour was
376 observed for *P. expansum*, whose inhibition at 25ºC was faster than at 4ºC
377 independently of the EO type employed.
378 Despite the oil content in jam samples being the same as that tested in agar media, the
379 time needed to observe the effectiveness of EOs against moulds in jams was longer.
380 This could be related to the different diffusions of the active compounds, which could
381 be easier in agar media than in jam, or could be due to the fact that the antifungals lost
382 through evaporation throughout the storage period were limited since the matrix
383 structure differed and the mass transfer process occurred differently (Perdones et al.,
384 2012). This behaviour could also be explained by the low water content in food
385 compared to agar media, which could hinder the transfer of EO to the active site in the
386 microbial cell (Omidbeygi et al., 2007). Other crucial factors must be taken into
387 account, such as antagonistic interactions with other ingredients (e.g. proteins or
388 carbohydrates) (Pitt & Hocking, 2009).
389

Fig. 2

390
391
392 *3.3.2 Sensory analysis*

393 A sensory analysis was run to check the acceptability of the strawberry jams that
394 contained the O/W emulsions. The samples tested by panellists consisted in jams with
395 the clove and cinnamon leaf O/W emulsions at the established concentrations, and a jam
396 sample with no EO added, which was used as a control. The average scores marked by
397 the assessors for all the evaluated attributes are shown in Fig. 3.

398 The strawberry jams with the added O/W emulsions obtained lower scores for the
399 aroma, taste and overall acceptance attributes compared with the control samples. The
400 consistency and colour attributes did not significantly differ ($p>0.05$) from the control
401 samples. The lowest scores were found in the jams with the cinnamon leaf O/W
402 emulsions because of the higher EO content used and the strong impact of this EO on
403 the typical strawberry jam flavour. However, the incorporation of the O/W emulsions
404 into jams did not affect their texture and colour evaluations.
405 Further studies should be carried out to obtain a good relation between the antimicrobial
406 effectiveness of the active compounds and their sensory impact on the final product.
407

Fig. 3

408

409 **4. Conclusion**

410 The clove and cinnamon leaf EOs showed the highest antifungal properties. The
411 physicochemical characterisation of the different O/W emulsions prepared with these
412 EOs revealed that the EO concentration in the emulsion brings about changes in particle
413 size. XG contributes to the stability of emulsions by adsorption on the oil droplet
414 surface. Indeed the higher the polymer content, the shorter the droplet mean diameter.
415 The main compound of the clove and cinnamon leaf EOs was eugenol, which is
416 responsible, together with sesquiterpene hydrocarbons, for their antifungal activity. The
417 O/W emulsions preparation led to EO loss of about 40%, and such loss was affected by
418 the amount of XG employed, and was not due to the EO type. The antifungal activity of
419 the clove and cinnamon leaf O/W emulsions against several strains, such as *Aspergillus*

flavus, Aspergillus niger and *Penicillium expansum,* was evidenced in the *in vitro* and *in vivo* tests.

The incorporation of the O/W emulsions into strawberry jam did not modify the texture or colour of the product, but negatively affected aroma, taste and the overall acceptance of jam.

The combined results demonstrate the promising advantages of using emulsions as natural additives to preserve and/or extend the shelf life of strawberry jams. Nevertheless, further studies are needed to reduce the sensory impact on final products, such as high pressure homogenisers to reduce the oil droplet's particle size, increasing the interfacial area exposed to microbial cells, or combining different natural agents in order to improve synergistic effects in foodstuff.

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562 **Figure captions**

563 **Fig. 1.** Antifungal activity of the clove, cinnamon leaf, lemon and mandarin EOs against
564 A) *Aspergillus flavus*, B) *Aspergillus niger* and C) *Penicillium expansum* for 7 days.
565 Diameter of mycelial growth (mean value and standard deviation (n=2)).

566 **Fig. 2.** Effect of the oil-in-water emulsions on the growth of A) *Aspergillus flavus*, B)
567 *Aspergillus niger*, C) *Penicillium expansum* on strawberry jams stored at 4ºC and 25°C
568 for 63 days. Mean value and standard deviation (n=2).

569 **Fig. 3.** Sensory profile of strawberry jams. *Indicates 95% significant differences
570 according to the ANOVA test (n=30).

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