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Application of cinnamon bark emulsions to protect strawberry jam from fungi

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Abstract

The objective of the work was to evaluate the use of cinnamon bark-xanthan gum emulsions to preserve strawberry jam. The optimisation of the methodology used to prepare the emulsions and, the evaluation of their antimicrobial activity in culture media and in the strawberry jam were investigated. Emulsions were prepared in either a rotor-stator homogeniser or a magnetic stirrer combined with a high pressure homogeniser. Microorganism suspensions (10^3 and 10^6 CFU/mL), essential oil concentration and microbial sensitivity were decisive in the emulsions' antimicrobial activity. The high stress applied to samples and their heating during homogenisation caused essential oil content losses. The jams prepared with the oil-in-water emulsions inoculated with *Aspergillus flavus*, *Penicillium expansum*, *Zygosaccharomyces rouxii* and *Zygosaccharomyces bailii* exhibited no growth during the 28 days of analysis The obtained results indicated the suitability of cinnamon bark oil-xanthan gum emulsions for preserving strawberry jam.

Keywords: Natural agents; cinnamaldehyde; oil-in-water emulsions; preservation; strawberry jam

1. Introduction

Jams are defined as mixtures, with a suitable gelled consistency, of sugars, pulp and/or purée of one or more fruits and water. Despite jam is a stable product due to its high sugar level (69%, USDA, 2016), there are particular microorganisms, such as moulds and yeasts, which are able to grow in products with an elevated amount of sugar.

The use of chemical additives is very effective to prevent food spoilage owing to moulds and yeasts proliferation. Nevertheless, consumers have become more concerned about the adverse impact of synthetic additives on human health (Stević, Berić, Šavikin, Soković, Godevac, Dimkić & Stanković, 2014). In this sense, natural preservatives such as essential oils (EOs) had been extensively used during the last years due to its antioxidant and antimicrobial properties (Perdones, Sánchez-González, Chiralt, & Vargas, 2012).

EOs are categorised as flavourings in Europe (Official Journal of the European Communities, Commission Decision 2002/113/EC, notified under document number C (2002) 88) and their constituents are categorised as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration. Cinnamon EO has demonstrated a strong antimicrobial activity but few reports show the behaviour versus moulds and yeasts (Manso, Becerril, Nerín, & Gómez-Lus, 2015). EOs contain volatile compounds and they are highly insoluble in water because of their lipophilic nature, and may have limited contact with microorganisms in high moisture content foods (Kalemba & Kunicka, 2003). This problem can be successfully overcome by using oil-in-water (O/W) emulsions, improving the water solubility of EOs, ensuring sufficient contact with microorganisms and enhancing their antimicrobial effectiveness (Hill, Gomes, & Taylor, 2013). O/W emulsions can be obtained by a two-step process (McClements, 2005). A coarse emulsion, or premix, is firstly obtained by employing a rotor-stator type device. Then the premix is processed in a high pressure homogeniser. High

pressure homogenisation (HPH) reduces particle droplet size and is used to produce emulsions with uniform composition and greater stability (Lee, Lefèvre, Subirade, & Paquin, 2009).

The main objective of this work was to study the use of cinnamon bark oil-in-water emulsions to preserve strawberry jams from fungi contamination. The optimisation of the methodology employed to prepare the emulsions by reducing active compounds losses, and their antimicrobial potential against moulds and spoilage yeasts in strawberry jam were investigated.

2. Materials and methods

2.1 Microorganism, culture media and reagents

Strains of *Aspergillus flavus* (CECT 20156), *Aspergillus niger* (CECT 20156), *Penicillium expansum* (CECT 20140), *Zygosaccharomyces rouxii* (CECT 1229) and *Zygosaccharomyces bailii* (CECT 12001) were supplied by the Spanish Type Culture Collection (CECT, Burjassot, Spain). For culture media, Potato Dextrose Agar (PDA), Yeast Peptone Dextrose broth (YPDB) and agar were used, all provided by Scharlab (Barcelona, Spain).

In the emulsions formulation, the cinnamon bark EO (CBEO) was supplied by Ernesto Ventós S.A. (Barcelona, Spain) and the xanthan gum (XG, Satiaxane^{TM} CX 911) by Cargill (Barcelona, Spain). *Trans-cinnamaldehyde* 99% was supplied by Sigma-Aldrich (St. Louis, USA) and n-Hexane by Scharlau (Barcelona, Spain).

2.2 Screening the antimicrobial activity of the CBEO

The CBEO was individually tested against *A. flavus*, *A. niger* and *P. expansum* following the methodology proposed by Ribes, Fuentes, Talens, and Barat (2016). Moulds were inoculated on PDA and incubated at 25 °C for 7 days. The spore solutions (10^3 and 10^6 CFU/mL)

harvested from a 7-day-old PDA were prepared in NaCl 0.7% with a haemocytometer. Next 100 µL of each fungal suspension were spread on the surface of a PDA Petri dish and an agar plug of this dish (7 mm diameter) was transferred to the centre of 15 g PDA's Petri dishes with different EO concentrations, which were established by considering previous studies (Kocevski, Du, Kan, Jing, & Pavlović., 2013; Manso et al., 2015). The tested EO concentrations were: 0.03, 0.04, and 0.05 mg/g. To secure EO distribution, 0.1% of Tween 80 was added to the medium. The controls with the same amount of Tween 80 were added to the test. Each dish was sealed with Parafilm® and incubated for 7 days at 25 °C.

Radial mycelial growth was determined after 1, 3, 5 and 7 days of incubation by measuring the diameter of the fungal colony. Values were expressed as mm diameter/day.

The Minimal Inhibitory Concentration (MIC) and the Minimal Fungicidal Concentration (MFC) of the CBEO were evaluated by observing the revival or growth of the inhibited mycelial disc transferred to the untreated PDA for 7 days. The dishes that showed no growth were taken as the MFC value, whereas those with mycelial growth indicated the MIC value.

The antimicrobial activity of the CBEO against *Z. rouxii* and *Z. bailii* was also evaluated by the methodology adapted from Tyagi, Gottardi, Malik, and Guerzoni (2014). Yeast strains were grown in YPD broth medium at 25 °C for 48 h in an orbital shaking incubator at 120 rpm. Cells were counted in a haemocytometer to obtain an inoculum density of 10^3 and10^6 CFU/mL.

The tested CBEO concentrations were the same as those previously described, and they were established by considering previous works (Tzortzakis, 2009; Kocevski et al., 2013). Aliquots of 15 g of YPD agar with the EO and 0.1% Tween 80 were poured into Petri dishes. Next 100 µL of the cell solution were spread on the surface of the YPD agar media dishes. As controls, the YPD agar dishes were supplemented with the same amount of Tween 80. The inoculated plates were incubated at 25 °C for 48 h. The MIC values were determined at the lowest EO concentration with non-visible growth. All the tests were run in triplicate.

116 *2.3 Study of O/W emulsions*

117 *2.3.1 Emulsions preparation*

118 The CBEO (0.06, 0.08, 0.10, 0.12 mg/g) was used as a lipid phase. To prepare the aqueous
119 phase, 5 mg/g of XG were dispersed in distilled water and stirred overnight at room
120 temperature. Primary emulsions were obtained following different steps: i) using a rotor-stator
121 homogeniser (Ultraturrax, IKA®, Germany) at 10,000 rpm for 1 min and 20,000 rpm for 3
122 min; or ii) using a magnetic stirrer for 15 min. In both cases, primary emulsions were subjected
123 to HPH in a Panda Plus 2000 (Gea Niro Soavi S. p. A., Parma, Italy) at 40 or 80 MPa.

124

125 *2.3.2 Gas chromatography-mass spectrometry analysis*

126 The final EO content in the CBEO emulsions was quantified according to the methodology
127 employed for emulsion preparation: rotor-stator device and/or a high pressure homogenisation
128 at 40 and 80 MPa. For this purpose, 5 mg/g of the XG were dispersed in distilled water and
129 stirred overnight at room temperature. After biopolymer dissolution, the CBEO was added to
130 reach a final concentration of 0.50 mg/g.

131 After preparing the O/W emulsions, and independently of the process used, the EO was
132 extracted by adding 15 mL of n-hexane to 2 g of the O/W emulsion, followed by 2-minute
133 vortex agitations. The mixture was shaken gently and filtered through filter paper. The n-
134 hexane was evaporated at 40 ºC in a rota-vapour. The obtained extracts were added to 2 mL of
135 n-hexane and analysed in the 6890/5975 inert GC-MS (Agilent Technologies, USA), equipped
136 with a HP-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm). The oven temperature
137 was held at 60 °C for 3 min, and then raised to 100 °C at 10 °C/min, to 140 °C at 5 °C/min, and
138 finally to 240 °C at 20 °C/min. Helium gas was used as the carrier gas at a constant flow rate of
139 1 mL/min. The injector and MS transfer line temperatures were set at 250 °C and 230 °C,

respectively. The parameters for the MS analysis were EI Ion source, electron energy 70 eV, solvent delay 3 min and *m/z* 40–550 amu. EO components were identified by matching mass spectra with the standard mass spectra from the NIST MS Search 2.0 library (Ribes et al., 2016). The analysis was repeated three times for each sample.

According to the results obtained in this part of the study, and those obtained while evaluating the antimicrobial activity of the CBEO, the concentration of the EOs in the emulsions (0.06, 0.08, 0.10, 0.12 mg/g) and the methodology for preparing emulsions (use of magnetic stirrer for 15 min and HPH process) were established.

2.3.3 Physico-chemical characterisation of the O/W emulsions

The pH of the emulsions was measured by a Crison Basic 20+ pH meter (Crison S.A. Barcelona, Spain), and density was determined in a pycnometer.

Particle size was determined in a laser diffractometer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK) following the methodology described by Ribes et al. (2016).

The ζ-potential was determined according to Ribes et al. (2016) with a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK). All the analyses were run in triplicate.

2.3.4 Antimicrobial activity of the O/W emulsions

The antifungal activity of the CBEO emulsions against *A. flavus*, *A. niger* and *P. expansum* was determined by the methodology described in Section 2.2. In this case, 0.50 g of each emulsion (0.06, 0.08, 0.10, 0.12 mg/g of the CBEO and 5 mg/g of XG) was added to 49.50 g of PDA at 50 °C. The controls with a dispersion prepared with distilled water and XG were added to the test. Each Petri dish was sealed with Parafilm® and incubated for 7 days at 25 °C. Radial

mycelial growth was determined after 1, 3, 5 and 7 days. Values were expressed as mm diameter/day. The MIC or MFC values of the O/W emulsions were studied.

The antimicrobial action of the CBEO emulsions against *Z. rouxii* and *Z. bailii* was also assessed by the previously described methodology. 100 µL of the cell solution (10^3 or 10^6 CFU/mL) was spread on the surface of each dish that contained YPD agar with emulsion. The YPD agar with the dispersion prepared with distilled water and XG was used as a control. The inoculated plates were stored at 25 °C for 48 h. The MIC values were determined. All the tests were run in triplicate.

2.4 Study of the O/W emulsions in strawberry jam

2.4.1 Jam preparation

Strawberry jam was prepared according to Ribes et al. (2016). The O/W emulsions were added to jam after cooling the product at ambient temperature and then homogenising. The amount of emulsions added to strawberry jam was established in order to achieve a concentration of 1 g of the O/W emulsion in 100 g of jam in the final product.

2.4.2 Sensory analysis

A sensory analysis was carried out by a semi-trained panel. The group of assessors was formed by 11 men and 19 women, whose ages ranged from 21 to 50 years. They were recruited due to their interest and availability, following the general guidelines UNE-ISO 8586:2012. Training sessions were carried out in order to introduce the panellists to the sensory analysis and to identify and score the quality attributes which describes the samples. Tests were run on a structured 9-point hedonic scale (9=like very much and 1=dislike very much) (UNE-ISO 4121),

by which colour, aroma, taste, consistency and overall acceptance attributes were evaluated. All the samples were presented to panellists at room temperature under normal lighting conditions in a transparent plastic cup coded with random, three-digit numbers. Bread pieces and spoons were provided to the panellists; drinking water was also provided for oral rinsing.

2.4.3 Shelf-life of inoculated strawberry jam

Fifteen grams of strawberry jam that contained the O/W emulsions (0.08 and 0.10 mg/g of EO and 5 mg/g of XG, homogenised at 40 MPa) were inoculated with 100 µL of the spore and cell solution (10^3 CFU/ mL). Plates were incubated at 25 °C for 28 days. Three Petri dishes were prepared per EO concentration, microorganism and analysis day (n=150). Moulds and yeast counts were taken in PDA plates after 72 h of incubation at 25 °C (Pascual & Calderón, 2000). All the assays were performed in triplicate.

2.5 Statistical analysis

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

3. Results and discussion

3.1 Antimicrobial activity of the CBEO

The results obtained while screening the antifungal activity of the CBEO are found in Fig. 1. The CBEO increased the *Lag phase* of all the moulds evaluated, with a diminution on the

germination rate for both fungal suspensions (10^3 CFU/mL and 10^6 CFU/mL). At the highest EO concentration (0.05 mg/g), mycelial growth was totally inhibited in all the studied moulds, irrespectively of the fungal concentration employed. The use of 0.03 and 0.04 mg/g of the CBEO reduced the growth of *A. flavus, A. niger* and *P. expansum*, regardless of the evaluated fungal suspension.

The inoculum concentration affects the degree of inhibition. In the most diluted suspension (10^3 CFU/mL), the CBEO caused the total inhibition of *P. expansum*, independently of the EO concentration employed. Furthermore, *A. flavus* and *A. niger* were totally inhibited when 0.04 and 0.05 mg/g of the CBEO was used, respectively. This behaviour reflects the greater resistance of *A. niger* and the highest sensitivity of *P. expansum* to CBEO exposure. The highest assessed fungal concentration showed 100% mycelial growth inhibition when 0.05 mg/g of the CBEO was incorporated into the media.

Antifungal activity could be the result of different activity sites on microbial cells, such as damage of the enzymatic cell systems that correlate with the energy production or structural compounds of EOs, or even the denaturation of the enzymes involved in spore germination (Gutiérrez, Batlle, Sánchez, & Nerín, 2010). The efficacy of cinnamaldehyde, the main CBEO compound, to inhibit growth of the fungi of genera *Penicillium* and *Aspergillus* has been demonstrated by Lopez, Sanchez, Batlle, and Nerín (2007). They found that *P. islandicum* and *A. flavus* were completely inhibited by 4.36 μL/L and 34.9 μL/L, respectively, of a cinnamaldehyde-fortified cinnamon EO in the vapour phase, and reported the MIC of cinnamaldehyde against *A. flavus* to be 21.8 μL/L.

The MFC values for *P. expansum, A. flavus* and *A. niger* were 0.03, 0.04 and 0.05 mg/g, respectively, at the most diluted spore suspension. However, 0.05 mg/g of the CBEO was the MIC at 10^6 CFU/mL for the three strains. These results indicate the relation between the EO

concentration and spore solution, and confirm that the concentration of fungal suspensions plays an important role in fungal development (Manso, Cacho-Nerin, Becerril, & Nerín, 2013).

The MIC of the CBEO was determined against different yeast strains (*Z. rouxii* and *Z. bailii*) at the 10^3 and 10^6 CFU/mL cell suspensions. The EO exhibited concentration-dependent inhibition of growth, and the MIC of the CBEO varied from 0.04 to 0.05 mg/g. The results indicated greater antimicrobial activity of the CBEO against *Z. rouxii* than against *Z. bailii*, with a MIC value of 0.04 mg/g. The highest MIC value (0.05 mg/g) at 10^3 cells/mL was shown against *Z. bailii* (data not shown). The same trend was observed for the MIC value when the highest cell suspension was used (10^6 CFU/mL). The obtained data indicated that the yeast suspension concentration plays a key role in reducing yeast spoilage. Similar results were obtained by Monu, Techathuvanan, Wallis, Critzer, and Davidson (2016) when determining the MIC of cinnamon bark and *trans-cinnamaldehyde* against *Z. bailii*.

3.2 Study of the O/W emulsions

3.2.1 Gas chromatography-mass spectrometry analysis

The CBEO components were identified by a GC-MS analysis (Table 1). The main EO compounds were *trans*-cinnamaldehyde (74.56%), caryophyllene (6.5%), eugenol (5.14%), cinnamylacetate (2.83%) and β-linalool (2.62%). Similar results have been reported by different authors (Fei, Yi-cheng, Xing-qian, & Yu-ting, 2011; Mazzarino et al., 2015). The antifungal properties of the CBEO and their main component, *trans-cinnamaldehyde*, have been demonstrated by several authors (Manso et al., 2013). Some research works have attributed the antifungal properties of cinnamaldehyde to the high electrophilic properties of the carbonyl group adjacent to the double bound, which render it reactive with the nucleophiles present in microorganisms (Gill & Holley, 2004).

Given the volatility of EOs, it is important to quantify the EO retained by O/W emulsions, and to, therefore, adjust the EO content to be used in emulsion formulations. These results are useful for optimising the methodology to prepare O/W emulsions. Emulsions were analysed by a GC-MS analysis, and losses of EOs while being prepared using different treatments (rotor-stator homogenisation and/or HPH process) were determined. EO losses were referred to *trans-cinnamaldehyde*.

Trans-cinnamaldehyde losses in the O/W emulsions prepared with the rotor-stator device were around 40%, and became higher in combination with HPH (Fig. 2). In contrast, the % of *trans-cinnamaldehyde* losses in the emulsions obtained by magnetic stirring, and subjected to 40 and 80 MPa of pressure, were 6.80±1.29 and 15.27±2.21, respectively. The emulsion subjected to high pressure showed a significant ($p<0.05$) reduction in the *% of trans-cinnamaldehyde* losses compared with the emulsion obtained in the rotor-stator type device. This could be caused by the high stress applied to samples and their heating during the homogenisation process, which would promote the degradation of constituents. Indeed, the higher the pressure applied during the homogenisation process, the greater the degradation of the EO compounds. These results agree with those reported by Donsì, Annunziata, Sessa, and Ferrari (2011) for a terpenes mixture, who observed the degradation of different active compounds, due to the stress that samples had to withstand during high shear homogenisation and HPH.

3.2.2 Physico-chemical characterisation of stable O/W emulsions

Different formulations and pressures were used to obtain stable emulsions. The pH, density, $d_{3,2}$, $d_{4,3}$ and ζ-potential values for the different emulsions are summarised in Table 2.

282 The pH values of the emulsions prepared at 40 MPa varied between 6.56±0.02 and
283 7.30±0.05 at ambient temperature, and the values obtained from the emulsions prepared at 80
284 MPa varied between 6.80±0.02 and 7.37±0.02. The pH decrease may be related with the acid
285 nature and dissociation in the aqueous solution of some CBEO compounds. Similar results
286 were reported by Sánchez-González, Vargas, González-Martínez, Chiralt, and Cháfer (2009)
287 and Sánchez-González, Chiralt, González-Martínez, and Cháfer (2011) when incorporating
288 different EOs into hydroxypropylmethylcellulose film-forming dispersions.
289 No changes were observed for density when EO content increased.
290 As can be observed in Table 2, the higher the oil content in emulsions, the bigger particle
291 size becomes. This could be due to an increase in the dispersed phase concentration, which
292 facilitates the droplet flocculation rate, as well as the reduction in the ratio between the
293 interfacial stabilising material and the dispersed phase (McClements, 2005). Similar results
294 have been reported by Sánchez-González, Cháfer, Chiralt, and González-Martínez (2010) in
295 emulsions of bergamot EO and chitosan aqueous systems. Only for the emulsions prepared
296 with 0.10 mg/g and 0.12 mg/g of the CBEO a significant ($p<0.05$) impact on $d_{3,2}$ was observed.
297 The mean size values lowered from 3.397±0.127 to 3.112±0.228 μm in the emulsions prepared
298 using 0.10 mg/g of the CBEO at 40 and 80 MPa, respectively. The reduction in the mean size
299 values for the emulsions formulated with 0.12 mg/g of the EO was more marked, and the mean
300 size values lowered from 3.397±0.127 to 2.949±0.073 μm in the emulsions subjected to 40 and
301 80 MPa, respectively. In contrast, the primary emulsions formulated with 0.06, 0.10 and 0.12
302 mg/g of the EO and subjected to high pressure had a significant ($p<0.05$) impact on $d_{4,3}$, and
303 showed a reduction around 1.5 μm. Only in case of the emulsion with 0.08 mg/g of the CBEO
304 the impact of HPH on $d_{4,3}$ did not affect significantly (Table 2).
305 According to McClements (2005), if the electrical charge of droplets was sufficiently high, the
306 emulsion could become stable against aggregation due to repulsive forces between droplets.

307 Generally, particles with a more positive ζ-potential than + 30 mV, or a more negative one than
308 - 30 mV, are considered stable (Heurtault, Saulnier, Pech, Proust, & Benoit, 2003). The
309 electrical charge of oil droplets in emulsion is shown in Table 2. A strong negative ζ-potential
310 was observed in emulsions. The increase in pressure applied during the homogenisation
311 procedure of emulsions diminished the surface charge of particles with significant differences
312 ($p<0.05$). The decrease in their ζ-potential values was more negative than -45.0 mV. The
313 mechanical stress during HPH can break up the XG, and thus increase the number of molecules
314 to be potentially adsorbed on the O/W interface. This would explain the observed ζ-potential
315 strengthening (Salvia-Trujillo, Rojas Graü, Soliva Fortuny, & Martín Belloso, 2015).
316 The obtained O/W emulsions were stable regardless of the effect caused in the electrical charge
317 of droplets by HPH.
318

319 *3.2.3 Antimicrobial activity of the O/W emulsions*

320 According to the results obtained in the Section 3.1 and the % of *trans-cinnamaldehyde*
321 losses, the concentrations of the tested EO were 0.06, 0.08, 0.10, 0.012 mg/g.
322 The antifungal activity of the CBEO emulsions obtained at 40 and 80 MPa against *A. flavus, A.*
323 *niger* and *P. expansum* for 7 days by using 10^3 and 10^6 CFU/mL is shown in Fig. 3.
324 The O/W emulsions under the tested conditions increased the *Lag phase* of all the tested
325 moulds, and the germination rate lowered. The O/W emulsions prepared with 0.08 mg/g of the
326 CBEO at 40 MPa for the lowest assayed spore solution (10^3 CFU/mL) had a significant
327 antifungal effect ($p<0.05$) on all the studied moulds. These emulsions inhibited the growth of
328 *A. flavus, A. niger* and *P. expansum* for 7 days.
329 The assays in which the fungal suspension was 10^6 CFU/mL obtained an increased mycelia
330 growth rate for the three evaluated fungi. These results once again confirmed the relevance of

the initial fungal concentrations on fungal development (Manso et al., 2013). Under these conditions, the O/W emulsions prepared with 0.08 mg/g of the CBEO at 40 MPa were able to inhibit the total growth of *A. flavus, A. niger* and *P. expansum.* On the contrary, when the EO concentration rose above 0.08 mg/g and the pressure applied to the primary emulsions was 80 MPa, *A. niger* development on culture media occurred.

A similar trend in the growth rate was observed for both fungal suspensions when the pressures applied to the primary emulsions increased. The O/W emulsions formulated with 0.06 mg/g of the CBEO at 80 MPa showed higher mycelial growth than the same emulsions prepared at 40 MPa. This finding could be related with the % of *trans-cinnamaldehyde* losses, which could diminish the antifungal effectiveness of the O/W emulsions.

The MIC and MFC values of the O/W emulsions formulated with 0.08 mg/g of the CBEO at 40MPa and 0.10 mg/g of CBEO homogenised at 40 and 80 MPa were also evaluated. The MFC of the O/W emulsions process at 40 MPa was 0.08 mg/g.

The MIC of the O/W emulsions was also determined against *Z. rouxii* and *Z. bailii* at different cell suspensions (10^3 and 10^6 CFU/mL). The lowest MIC (0.06 mg/g) value was obtained at 10^3 CFU/mL for both strains by subjecting the primary emulsions to 40 MPa. In contrast at 10^6 CFU/mL, a remarkable antimicrobial effect was observed for *Z. rouxii*. At this cell suspension, the emulsion's MIC values for *Z. rouxii* and for *Z.bailii* were 0.06 and 0.08 mg/g of the CBEO, respectively, when applying 40 MPa of pressure (data not shown). As previously mentioned, the higher the pressures applied in homogenisation, the bigger the % *trans-cinnamaldehyde* losses. This fact affected yeast growth inhibition, which became less effective due to loss of active compounds.

356 *3.3 Study of the O/W emulsions on strawberry jam*

357 *3.3.1 Sensory analysis*

358 A sensory analysis was carried out to check the acceptability of the strawberry jam that
359 contained the O/W emulsions. The samples tested by panellists consisted of the jam with the
360 CBEO emulsions at the established concentrations. One jam sample with no EO was used as a
361 control. The strawberry jam with the O/W emulsions scored lower for the aroma, taste and
362 overall acceptance attributes compared with the control samples. No significant differences
363 were observed between EO concentrations. Consistency and colour attributes did not
364 significantly differ ($p>0.05$) from the control samples (Fig. 4).

365

366 *3.3.2 Study of the O/W emulsions on strawberry jam*

367 The emulsions prepared with 0.08 and 0.10 mg/g of the CBEO and homogenised at 40
368 MPa, were added to strawberry jam. Jams were inoculated to simulate a possible product
369 contamination and samples with no inoculation were used as controls.

370 Microbial development on the strawberry samples that contained the O/W emulsions for 28
371 days at 25 °C was studied (Fig. 5). The jams prepared with the O/W emulsions inoculated with
372 *A. flavus*, *P. expansum*, *Z. rouxii* and *Z. bailii* showed no growth throughout the study. For *A.*
373 *niger*, a reduction of around 1 log was observed between the control plates and the samples.
374 These results agree with those reported above. *A. niger* showed the greatest resistance against
375 the CBEO treatment and the O/W emulsions added to strawberry jam.

376 Strawberry is sensitive to pathogens, and fungal contamination is common in this product.
377 Major threatening fungi that reduce the post-harvest storage life of strawberries include
378 *Botrytis*, *Aspergillus*, *Rhizopus* and *Penicillium* (Lazar, Jobling, & Benkeblia, 2010; Sharma,
379 2014). Various reports have demonstrated that *A. niger* species members are responsible for the

post-harvest decay of fresh fruits like apples, peaches, citrus, grapes, strawberries and tomatoes, among others (Perrone et al., 2007). This opportunistic effect could suggest the greater resistance of *A. niger* to the O/W emulsions incorporated into strawberry jam.

4. CONCLUSIONS

The optimisation of the methodology to prepare cinnamon bark-xanthan gum emulsions achieves a *trans-cinnamaldehyde* losses around 40%, which are higher in combination with high pressure homogenisation. Nevertheless, the losses of the emulsions obtained by magnetic stirring, and subjected to 40 and 80 MPa of pressure, are below 16%. Moreover, the antimicrobial activity of the emulsions was determined by fungal suspension, essential oils concentration and microbial sensitivity to essential oils.

The incorporation of emulsions containing 0.08 mg/g of cinnamon bark oil into strawberry jam allows their preservation against *Aspergillus flavus*, *Penicillium expansum, Zygosaccharomyces rouxii* and *Zygosaccharomyces bailii* during the whole evaluation period. Furthermore, this incorporation does no modify product texture or colour, but negatively affects the aroma, taste and overall acceptance of jam.

Although, the obtained results suggest some advantages in the use of the cinnamon bark emulsions as natural preservatives in strawberry jam, more studies are needed to reduce the sensory impact of essential oils. The combination of different natural antifungal agents such as phenolic compounds or zinc salts, could be a promising alternative to reduce or suppress the changes produced in foods due to the strong flavour of essential oils.

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Figure captions

Fig. 1. Antimicrobial activity of the CBEO against (A) *Aspergillus flavus*, (B) *Aspergillus niger* and (C) *Penicillium expansum* after 7 days of incubation at 25 °C (fungal suspensions: 10^3 and 10^6 CFU/mL). Media values (n=3) ± SD.

Fig. 2. Percentage (%) of *trans-cinnamaldehyde* loss from the CBEO emulsions with different treatments (rotor-stator and/or HPH at 40 or 80 MPa). Mean values (n=3) ± SD.

Fig. 3. Antimicrobial activity of the O/W emulsions obtained at 40 and 80 MPa against (A) *Aspergillus flavus*, (B) *Aspergillus niger* and (C) *Penicillium expansum*, after 7 days of incubation at 25 °C. Fungal suspensions: 10^3 and 10^6 CFU/mL. Media values (n=3) ± SD.

Fig. 4. Sensory profile of strawberry jam. *Indicates 95% significant differences according to the ANOVA test (n=30).

Fig. 5. Effect of the O/W emulsions on growth of A) *Aspergillus flavus*, B) *Aspergillus niger*, C) *Penicillium expansum*, D) *Zygosaccharomyces rouxii* and E) *Zygosaccharomyces bailii* on the strawberry jam stored at 25 °C. Inoculum density: 10^3 CFU/mL. Mean values (n=3) ± SD.