



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.

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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 et al., 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 pre-mix, is firstly obtained by employing a rotor-stator type device. Then the pre-mix 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.

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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™ 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 and 10^6 CFU/mL.

The tested CBEO concentrations were the same as those previously described, and they were established by considering previous works (Kocevski et al., 2013; Tzortzakakis, 2009). 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.

2.3. Study of O/W emulsions

2.3.1. Emulsions preparation

The CBEO (0.06, 0.08, 0.10, 0.12 mg/g) was used as a lipid phase. To prepare the aqueous phase, 5 mg/g of XG were dispersed in

distilled water and stirred overnight at room temperature. Primary emulsions were obtained following different steps: i) using a rotor-stator homogeniser (Ultraturrax, IKA®, Germany) at 10,000 rpm for 1 min and 20,000 rpm for 3 min; or ii) using a magnetic stirrer for 15 min. In both cases, primary emulsions were subjected to HPH in a Panda Plus 2000 (Gea Niro Soavi S. p. A., Parma, Italy) at 40 or 80 MPa.

2.3.2. Gas chromatography-mass spectrometry analysis

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

After preparing the O/W emulsions, and independently of the process used, the EO was extracted by adding 15 mL of n-hexane to 2 g of the O/W emulsion, followed by 2-min vortex agitations. The mixture was shaken gently and filtered through filter paper. The n-hexane was evaporated at 40 °C in a rota-vapour. The obtained extracts were added to 2 mL of n-hexane and analysed in the 6890/5975 inert GC-MS (Agilent Technologies, USA), equipped with a HP-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature 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 finally to 240 °C at 20 °C/min. Helium gas was used as the carrier gas at a constant flow rate of 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

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