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Elaboration and characterization of O/W cinnamon (*Cinnamomum zeylanicum*) and black pepper (*Piper nigrum*) emulsions

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ABSTRACT

Essential oils of black pepper (*Piper nigrum*) and cinnamon (*Cinnamomum zeylanicum*) have shown an antimicrobial effect against disease-related pathogens and food spoilage bacteria. However, the hydrophobic nature of oils limits their use as natural preservatives within a food matrix. Therefore, the main objective of the present work was to prepare and characterise oil/water (O/W) micro- and nanoemulsions of black pepper and cinnamon essential oils elaborated by ultrasound and high-pressure homogenisation (HPH). For both methods, optically transparent systems with a particle size <100 nm and ζ-potential < −30 mV were obtained. During storage, cinnamon essential oil and blended cinnamon-pepper essential oil nanoemulsions exhibited greater physical stability compared to micro-emulsions. Furthermore, the ultrasound processed cinnamon essential oil nanoemulsion presented greater physicochemical stability and antimicrobial properties against *Listeria monocytogenes* and *Escherichia coli* than the pepper essential oil nanoemulsion. The results of this study could be useful for designing antimicrobial delivery systems for food safety and preservation.

1. Introduction

Essential oils are compounds derived from plant material with a potential to be used as natural food preservatives (Holley & Patel, 2005; Nielsen et al., 2017). The growing consumer demand to use antimicrobial agents from natural sources justifies the incorporation of essential oils into food (Burt, 2004). Essential oils of black pepper (*Piper nigrum* L.) and cinnamon (*Cinnamomum zeylanicum*) have been shown to have an antimicrobial effect against pathogenic and spoilage bacteria (Hamidpour, Hamidpour, Hamidpour, & Shahlari, 2015). Chemical studies have demonstrated that besides essential oil, cinnamon contains mucilage, tannins, sugar and resin (Gilles, Zhao, An, & Agboola, 2010). Non-volatile compounds (mainly condensed tannins), particularly, cinnamaldehyde (64.1%), which is the essential aroma and taste compound but also possesses antibacterial properties, is the main constituent of a crude cinnamon extract, alongside proanthocyanidins (23.2%) and catechins (3.6%) (Hamidpour et al., 2015). Whereas, black pepper essential oil is composed primarily of monoterpenes and sesquiterpenes (Ravindran & Kallupurackal, 2012). This essential oil has also been used to inhibit the growth of microorganisms, such as *Vibrio cholerae*, *Staphylococcus albus*, *Clostridium diphthereae*, *Shigella dysenteriae*, *Streptomyces faecalis*, *Bacillus* spp. and *Pseudomonas* spp., in addition to suspending the growth and production of aflatoxins produced by *Aspergillus parasiticus*. These effects are due to chemical constituents, such as piperazine, piperanine, piperidine A and piperolein B (Ravindran & Kallupurackal, 2012). Due to their complex composition, the mechanisms by which essential oils inhibit bacteria are likely to involve several targets in the bacterial cells (Burt, 2004). The hydrophobicity of essential oils allows them to interact with cell membranes that primarily cause an alteration in the fatty acid composition and leakage of the cellular components (Burt, 2004; Donsì, Annunziata, Vincensi, & Ferrari, 2012). Essential oils have been classified by the US Food and Drug Administration as generally recognised as safe. However, a major drawback of many essential oils is their hydrophobic nature, which makes them insoluble in water based media and matrices (Liang, Yuan, Vriesekoop, & Lv, 2012). So that, their direct incorporation into aqueous-based foods and beverages is limited by

Abbreviations: HLB, hydrophilic-lipophilic balance; HPH, high-pressure homogenisation; PdI, polydispersity index; WI, whiteness index.

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their low water solubility and interactive binding with food components, such as protein and lipids (Niu, Pan, Su, & Yang, 2016). One approach to extending the application of essential oils in foods is liposomal encapsulation or nanoencapsulation (Jo et al., 2015), which allows the solubilisation of the essential oil in the water phase through molecular dispersion, and improves the stability and bioavailability of the active substance. The great challenge of incorporating essential oil into food matrices could be overcome if they are incorporated into nanoemulsions. Recently, the use of essential oil nanoemulsions has been shown to control the growth of pathogens present in certain foods. Spice oil nanoemulsions were used as potential natural inhibitors against pathogenic *E. coli* and *Salmonella* spp. from fresh fruits and vegetables (Amrutha, Sundar, & Shetty, 2017). At the same time, essential oil oregano nanoemulsion was shown to inhibit pathogens microorganisms present in chicken paté (Moraes-Lovison et al., 2017). On the other hand, essential oil nanoemulsions incorporated into chitosan coatings inhibited the growth of pathogenic microorganisms on green beans (Severino et al., 2015). The optimal preparation of nanoemulsions is necessary for the maximisation of antimicrobial activity, using sufficiently low concentrations to minimise alteration of the food quality (Yeon-Ji et al., 2015). The physical stability of the active substance within the nanoemulsion is improved by protecting them from interacting with the food ingredients, while the subcellular size of the emulsion droplets increases their bioactivity (Donsì, Annunziata, Sessa, & Ferrari, 2011).

Contrariwise, antimicrobial compounds may undergo rapid depletion within foods, due to physical diffusion within the food system or degradation by proteases (Bhatti, Veeramachaneni, & Shelef, 2004). Therefore, to protect antimicrobials from depletion in foods, the use of protective delivery agents could be beneficial (Niu et al., 2016), improving the dispersibility and efficacy of essential oils against certain microorganisms. Nanoemulsions can be formed by low-energy and high-energy emulsification methods. High-energy approaches require the input of a substantial amount of mechanical energy and typically involve the methods of microfluidisation, high-pressure homogenisation (HPH) and ultrasound-assisted emulsification (Hashemiravan, Mazloom, & Farhadyar, 2013). A comparison of essential oil-loaded conventional emulsions and nanoemulsions prepared by microfluidization and ultrasonication has been useful to evaluate the antimicrobial activity (Sugumar, Ghosh, Mukherjee, & Chandrasekaran, 2016; Yildirim, Oztop, & Soyer, 2017). However, the protective effect also depends, to a great extent, on the type and concentration of essential oil used. The novelty of this research lies in knowing the best conditions of preparation of micro and nanoemulsions of essential oil of cinnamon, essential oil of pepper and its mixture obtained by ultrasound and homogenization by high pressure. Also, this help to understand the mechanisms on the inactivation of the emulsified compounds on gram positive and gram negative bacteria, which is of interest for further investigations and to functional foods industry. Therefore, the objective of this work was to study the effect of emulsification methods (ultrasound and HPH) on the physicochemical, stability and antimicrobial properties (against Gram (+) and Gram (−) bacteria) of micro- and nanoemulsions prepared using cinnamon and pepper essential oils as active substances.

2. Material and methods

2.1. Raw material

The essential oils of black pepper (*P. nigrum* L.) and cinnamon (*C. zeylanicum*) were donated by the company "Aromatic Victoria S.A de C.V". Sodium alginate was purchased from the "Cosmopolitan S.A de C.V" drugstore. The surfactants used included polyoxyethylene sorbitan monolaurate (Tween 20), sorbitan monohexadecanoate (Tween 40), polyoxyethylene sorbitan monooleate (Tween 80) and sorbitan monolaurate (Span 20), which were all trademarks of Sigma−Aldrich, USA. The bacterial strains used in the study were *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 43895) and *Salmonella enterica* serovar Typhimurium (ATCC 14028).

2.2. Emulsion preparation

The preparation of the microemulsion or primary emulsion was carried out according to the modified technique of Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, and Martín-Belloso (2014). The aqueous phase was a solution of sodium alginate in distilled water (1% w/v). For the oily phase (1% v/v), three formulations were made: the first consisted only of black pepper essential oil, the second contained only cinnamon essential oil and the third was a mixture of both oils at 1:1 (v/v) ratio. Span 20 (hydrophilic-lipophilic balance (HLB 8.6), Tween 80 (HLB 15), Tween 40 (HLB 15.6) and Tween 20 (HLB 16.7) were added to adjust the HLB of all the solutions to 12, 14 and 16, respectively. Once the HLB number was adjusted, a test was performed using a fixed amount of the dispersed phase (1% v/v) and varying the amount of surfactant to obtain an oil:surfactant ratio of 1:1, 1:3 and 1: 5 (v/v), respectively. Data analysis was performed using response surface methodology.

The nanoemulsions were prepared using two high-energy emulsification methods: ultrasound and HPH. In the first instance, the primary emulsion was ultrasonicated using a 20 kHz ultrasonic processor with a maximum power of 750 W (Cole-Parmer instruments, model CPX750, USA), operating at 30% amplitude with pulses of 5 × 5 s for 12 min and immersed in an ice bath to mitigate the ultrasound thermal effect. For HPH, the solution was homogenised (Nano Bee homogeniser, Bee International Inc., South Easton, Massachusetts, USA) at 150 MPa, with five cycles.

2.3. Emulsion characterisation

2.3.1. Physical properties

The pH of the emulsion was measured using a potentiometer (Orion 5 Star, Thermo Fisher Scientific Inc., Beverly, USA) according to the Association of Official Analytical Chemists (AOAC., 2000). The colour was evaluated using a Hunter Lab colourimeter (Color-Flex, model CX115 45/0, USA) to record the CIE *L, a* and *b* parameters and whiteness index (*WI*) were calculated using Eq. (1).

$$WI = \left((100 - L)^2 + \left(a^2 + b^2\right)\right)^{0.05} \quad (1)$$

2.3.2. Particle size and polydispersity index (PdI)

Particle size and PdI of the emulsion was evaluated by using a dynamic light-scattering particle size analyser (Zetasizer Nano ZS, model ZEN 3600, Malvern Instruments, UK). The experiments were performed on suspensions diluted with deionised water (1:10 v/v), which were stirred continuously to ensure they were well dispersed. Using this method, a laser beam is directed through the samples and scattered by the droplets in a characteristic pattern, depending on their size. The average droplet size is determined by an array of photodiodes located behind the cuvette (Anarjan, Mirhosseini, Baharin, & Tan, 2010).

2.3.3. Zeta (ζ)-potential measurement

The ζ-potential values of the emulsions were measured at room