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Antimicrobial activity of lavandin essential oil formulations against three pathogenic food-borne bacteria

Salima Varona[a,*], Soraya Rodríguez Rojo[a], Ángel Martín[a], María José Cocero[a], Ana Teresa Serra[b], Teresa Crespo[b,c], Catarina M.M. Duarte[b,c]

[a] High Pressure Processes Group, Department Chemical Engineering and Environmental Technology, University of Valladolid, Spain
[b] Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
[c] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal

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ABSTRACT

Lavandin (*Lavandula hybrida*) essential oil contains components with biocide and antiviral properties that can be used as substitutes of antibiotics. This application requires an appropriate formulation of the essential oil. In the present work, the antimicrobial activity of free and encapsulated lavandin essential oil against three pathogenic bacteria (Gram-negative: *Escherichia coli*; Gram-positive: *Staphylococcus aureus* and *Bacillus cereus*) was determined. The formulations were prepared using innovative high-pressure techniques (PGSS and PGSS-drying) as well as spray-drying. Carrier materials used for the encapsulation were soybean lecithin, n-octenyl succinic anhydride (OSA) modified starch and poly-caprolactone. Results demonstrated that lavandin oil antibacterial activity could be enhanced by encapsulation, due to the protection and control release of the oil. As well, encapsulation might present an interesting opportunity to facilitate the action of antimicrobials, improving essential oil penetration inside of the outer membrane.

1. Introduction

Public concern about the use of antibiotics in livestock feed has increased, because the emergence of antibiotic resistant bacteria and their possible transmission from livestock to humans. In fact, in the European Union the use of synthetic antibiotics, health and growth promoters as additives in livestock feed is prohibited since 2006 (European Parliament and Council Regulation (EC) No. 1831/2006). In this context, one possible solution is the used of essential oils. Essential oils are recognized as safe substances (ESO, GRAS – 182.20) by the Food and Drug Administration (2005) and some contain compounds which can be used as antibacterial additives (Ait-Ouazzou et al., 2011; Cox et al., 2001; Nerio et al., 2010; Mišić et al., 2008; Muyima et al., 2002). However, the required concentration of the essential oils for an effective biocide action can be about 100 times higher than that of a standard antibiotic (e.g. streptomycin and nystatin) (Hanamanthagouda et al., 2010). Thus, essential oils must be adequately formulated to protect them from degradation, evaporation and to provide a controlled release. The encapsulation has demonstrated to improve the antibacterial activity of several antibiotics (Drulis-Kawa and Dorotkiewicz-Jach, 2010).

The formulations tested in this work are based on lavandin essential oil and were prepared using three types of carriers, namely: lecithin, OSA modified starch and polycaprolactone, using three different encapsulation processes: PGSS (particles from gas saturated solutions), PGSS-drying (PGSS-D) and spray-drying (SD). The preparation of these formulations is reported in Varona et al. (2009, 2010, 2011).

Spray-drying is one of the best-known conventional technologies for the precipitation and co-precipitation of particles for food and pharmaceutical application, but its main drawback is the high temperature needed. PGSS and PGSS-drying are new technologies which use compressed carbon dioxide as solvent. This solvent is non toxic, environmentally friendly and can be eliminated completely from the final product by depressurization. PGSS and PGSS-drying processes allow to work mild conditions and therefore to reduce lavandin oil degradation. The PGSS process takes advantage to the fact that polymers can be saturated with carbon dioxide decreasing their melting temperature (De Paz et al., 2010).

The present study takes into consideration the possible use of lavandin essential oil (*Lavandula hybrida*) as a natural biocide by means of a suitable formulation. With this aim, several lavandin oil formulations has been produced and tested against

* Corresponding author.
E-mail address: salimavarona@gmail.com (S. Varona).

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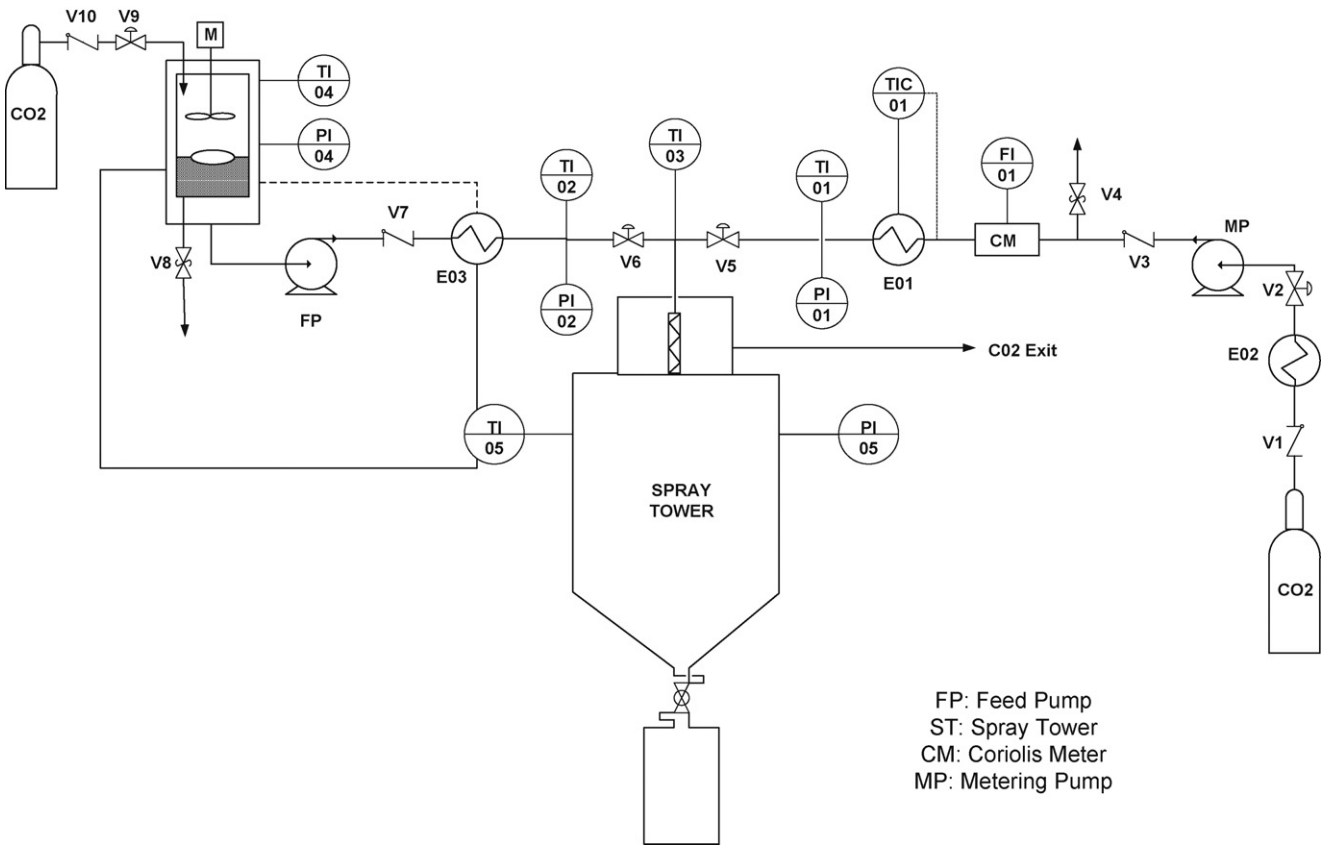

Fig. 1. Flow diagram of the PGSS-drying plant.

three foodborne pathogenic bacteria. *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram-positive) and *Bacillus cereus* (Gram-positive).

2. Materials and methods

2.1. Materials

Lavandin essential oil "*Lavandula hybrida super*" used in this project was purchased from COCOPE (Valladolid, Spain). This oil was produced by steam distillation. Modified OSA starch derived from waxy maize was provided from National Starch Group (New Jersey, USA). Poly-(ε-caprolactone) 2403D (mean molecular weight: 4000 g/mol; melting temperature: 55–60 °C) was supplied by Solvay Caprolactones (Solvay Interox Ltd., United Kingdom). Soybean lecithin (97% phospholipids) was obtained from Glama-Sot (SOTYA, Madrid, Spain). Sodium chloride was provided by Sigma–Aldrich (Madrid, Spain). Trans-2-hexen-1-al 98% was provided by Sigma–Aldrich (Madrid, Spain).

2.2. Experimental design

The bacteria strains chosen for were *E. coli* (Gram negative), *S. aureus* (Gram-positive) and *B. cereus* (Gram-positive). These bacteria are already reported to be sensitive to Reydovan Lavandin (*L. hybrida reydovan*) (Oussalah et al., 2007). *E. coli* has been chosen as a model of a bacterial infection in livestock that could be treated by ingestion of the essential oil formulations. *S. aureus* is responsible for skin infections, being a model infection that could be treated by a topical treatment (application to body surfaces such as the skin or mucous membranes). *B. cereus* can contaminate rice and vegetables (as well as dairy products and meat) and therefore is a possible target for essential oil formulations added as biocides to the irrigation of such cultivations.

2.3. Preparation of formulations

2.3.1. Spray-drying

Oil-in-water emulsions were dried by spray drying. The spray drier used was Mobile Minor model MM-Basic PSR from GEA Niro. This equipment has a maximum treatment capacity of 4 l/h, inlet air temperature of 330 °C and rotary spray capacity of 15 N m^3/h.

2.3.2. Particles from gas saturated solutions (PGSS)-drying

Oil-in-water emulsions were dried by PGSS-drying. In this processes the emulsion is saturated with CO_2 causing a decrease of the emulsion viscosity. The emulsion saturated with CO_2 is contacted with the supercritical CO_2 in a static mixer and then expanded through a nozzle. This expansion facilitates the formation of extremely fine droplets which dry very fast, leading to the production of small particles. By the combination of co-extraction in the static mixer and evaporation of residual water in the spray tower the consumption of CO_2 is reduced (Weidner, 2009). A flow diagram of the PGSS-drying plant is presented in Fig. 1. Different conditions of pre-expansion pressure (6–10 MPa), pre-expansion temperature (104–130 °C) and gas to product ratio (GPR) (5–35) were assayed in order to study their influence in powder characteristics. Further details can be obtained in previous works (Varona et al., 2010).

2.3.3. Particles from gas saturated solutions (PGSS)

Poly-caprolactone particles loaded with lavandin essential oil were produced by the PGSS process. Poly-caprolactone and lavandin oil were filled together in a pressure cell where they were