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Enhanced antimicrobial activity of essential oil components immobilized on silica particles

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ABSTRACT

The antimicrobial activity of essential oils components (EOCs) is well-known. However, their high volatility and powerful aroma limit their application in the formulation of a wide range of food products. In this context, the antimicrobial activity of carvacrol, eugenol, thymol and vanillin grafted onto the surface of three silica supports with different morphologies, textural properties and chemical reactivities (fumed silica, amorphous silica and MCM-41) was evaluated herein. Materials characterization revealed a good immobilization yield and all the devices showed a micro-scale particle size. Sensory evaluation revealed that sensory perception of EOCs decreases after covalent immobilization. Moreover, immobilization greatly enhanced the antimicrobial activity of the essential oil components against *Listeria innocua* and *Escherichia coli* compared to free components. The incorporation of EOCs immobilized on silica particles into pasteurized milk inoculated with *L. innocua* demonstrated their effectiveness not only for *in vitro* conditions, but also in a real food system.

1. Introduction

New techniques to prevent food spoilage and to guarantee food safety have rapidly and innovatively developed in recent years as a result of the current inadequacy of traditional antimicrobial methods and the growing spread of antibiotic resistant strains of bacteria and fungi (Capeletti et al., 2014). Some new tendencies in this field include the use of naturally-occurring antimicrobial compounds, e.g., plant metabolites. Essential oils (EOs), lipophilic extracts of bioactive compounds with antimicrobial activity against several food-borne microorganisms have grown the most in research publications and industrial applications (Hyldgaard, Mygind, & Meyer, 2012).

The antimicrobial activity of EOs has been attributed to their phenolic compounds and their interaction with microbial cell membranes, which cause the leakage of ions and cytoplasmic content, and can thus lead to cellular breakdown (Burt, 2004; Suntres, Coccimiglio, & Alipour, 2015). Despite the described antimicrobial behaviour, the direct application of EOs to food products has several limitations: strong sensory properties (Nostro & Papalia, 2012), high volatility (Majeed et al., 2015), poor water solubility (Burt, 2004), degradability (Turek & Stintzing, 2013) and potential toxicity (Smith et al., 2005). Moreover, the concentration of an essential oil component (EOC) needed to inhibit microbial growth in a food system is higher than in *in vitro* studies. This is not only due to interactions with food matrix components (Hyldgaard et al., 2012), but also to difficulties in their dispersion in the food water phase (Weiss, Gaysinsky, Davidson, & McClements, 2009).

Hence, research has focused on the development of technologies, such as encapsulation or immobilization, to improve the functionality of natural antimicrobials (Weiss et al., 2009).

Regarding encapsulation, different organic delivery systems have been used to encapsulate EOs for their later application directly or after incorporation to films or coatings for food preservation purposes (Guarda, Rubilar, Miltz, & Galotto, 2011; Ravichandran, Hettiarachchy, Ganesh, Ricke, & Singh, 2011; Ribes, Fuentes, Talens, & Barat, 2016).

Besides traditional organic matrices, new inorganic materials (i.e. porous siliceous materials) have been used as supports to

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prepare antimicrobial devices through the encapsulation of a payload molecule in the voids of porous silica particles. Of all of them, MCM-41-like supports are the most widely used porous silica in applications in the food sector, where they can be used as catalysts in the synthesis of nutrients and bioactive molecules, in sensor technology and also as carriers to design smart delivery systems (Pérez-Esteve, Ruiz-Rico, Martínez-Máñez, & Barat, 2015). Besides porous materials, other silica particles are widely used in animal feed and in the food industry (Uboldi et al., 2012). This is the case of amorphous silica, particles which are considered GRAS, an authorized additive in Europe and E-551-classified (Contado, Ravani, & Passarella, 2013).

Entrapment of antimicrobial compounds in these materials can also protect bioactive substances from environmental stress, mask undesirable sensory properties, prevent interactions with food components, and achieve the controlled release of the antimicrobial compound at the site of action. Bearing in mind these features, different naturally-occurring antimicrobial compounds, such as allyl isothiocyanate (Park, Barton, & Pendleton, 2012), caprylic acid (Ruiz-Rico et al., 2015), lysozyme (Yu et al., 2015), and EOCs (Bernardos et al., 2015), have been encapsulated in mesoporous silica supports. It is noteworthy that all these studies have managed to preserve or enhance the inhibitory effect of bioactive compounds.

Apart from voids capable of entrapping active compounds, siliceous materials present a large surface capable of reacting with organic molecules, and of creating hybrid organic-inorganic systems where silica materials act as a support and organic molecules create a functional layer on the support's surface. Based on this approach, Li and Wang (2013) reported lysozyme-coated mesoporous silica nanoparticles that exhibited efficient enhanced antibacterial activity against *Escherichia coli*. Qi, Li, Yu, and Wang (2013) used vancomycin-modified mesoporous silica nanoparticles to kill pathogenic gram-positive bacteria. Pędziwiatr-Werbicka et al. (2014) synthesized fatty acids functionalized mesoporous silica particles with relative antimicrobial activity. Despite these promising results, as far as we know, the preparation of antimicrobial devices from EOs by this innovative approach has not yet been explored.

Accordingly, this study aimed to design a collection of antimicrobial devices based on anchoring several volatile EOCs (carvacrol, eugenol, thymol and vanillin) to the surface of three types of silica particles with different surface areas, textural properties and chemical reactivities (silica-fumed, MCM-41 and amorphous silica), and to evaluate their antimicrobial activity against some food-borne pathogens, e.g., *Listeria innocua* and *Escherichia coli*, compared with that of free bioactive compounds.

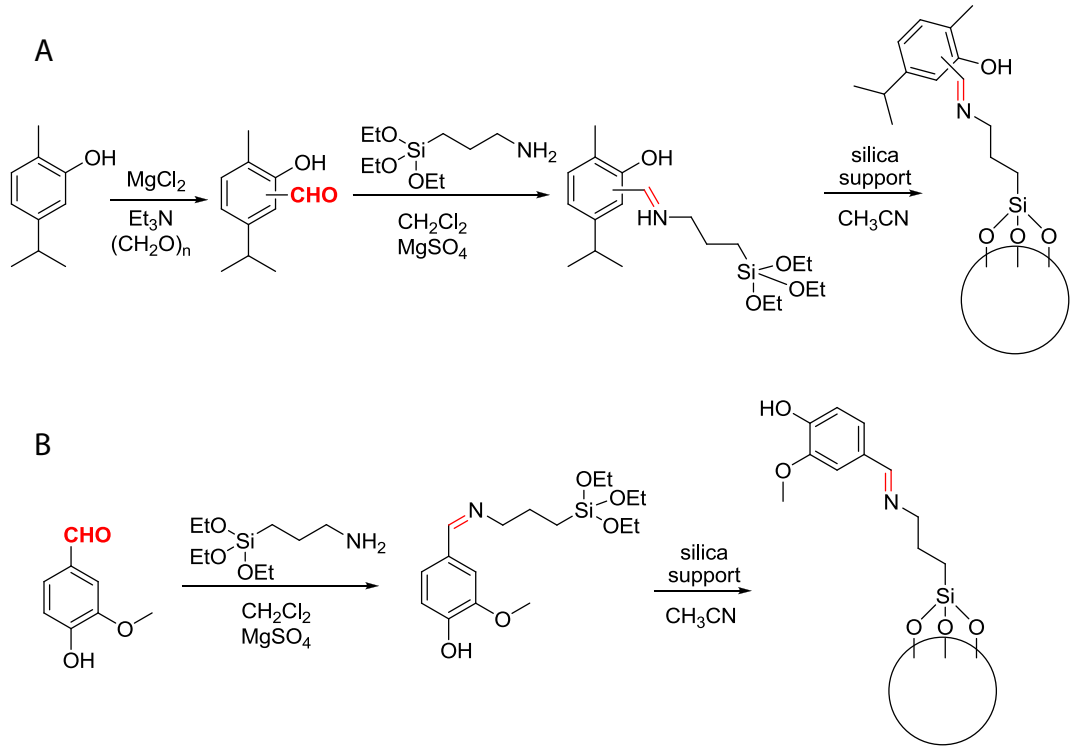
2. Materials and methods

2.1. Chemicals

N-cetyltrimethylammonium bromide (CTABr), sodium hydroxide, triethanolamine ($TEAH_3$), tetraethylorthosilicate (TEOS), (3-Aminopropyl)triethoxysilane (APTES), trimethylamine, paraformaldehyde, diethyl ether, chloroform, *n*-butanone, dimethyl sulfoxide, carvacrol, eugenol and thymol were provided by Sigma-Aldrich (Madrid, Spain). Vanillin was purchased from Ventós (Barcelona, Spain). Acetonitrile, hydrochloric acid, magnesium sulfate, potassium hydroxide, sulfuric acid and microbiological media grade were provided by Scharlab (Barcelona, Spain). Fumed silica (FS) nanoparticles (AEROSIL® 200) were purchased from Evonik Industries (Essen, Germany) and amorphous silica (AS) microparticles (SYLYSIA® SY350/FCP) were provided by Silysiamont (Milano, Italy).

2.2. Mesoporous silica particles synthesis

MCM-41 microparticles were synthesized following the so-called "atrane route", where CTABr was used as the



Scheme 1. Representation of the synthesis procedure of the antimicrobial devices. A. The three-step synthesis of the carvacrol, eugenol and thymol immobilized systems (carvacrol provided as an example). Step 1. Aldehyde derivatization. Step 2. Alkoxysilane derivatization. Step 3. Anchoring to the silica support (FS, MCM-41 or AS). B. The two-step synthesis of the vanillin immobilized systems. Step 1. Alkoxysilane derivatization. Step 2. Anchoring to the silica support (FS, MCM-41 or AS).