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Antimicrobial efficiency of carvacrol vapour related to mass partition coefficient when incorporated in chitosan based films aimed for active packaging

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ABSTRACT

Chitosan-based packaging films were investigated for antimicrobial efficiency against food spoilage microorganisms through headspace technique. Five chitosan based films containing carvacrol with known mass partition coefficient (K_{mass}) in the range from 10^{-4} to 10^{-8} were produced and their antimicrobial activity was evaluated. *Bacillus subtilis*, *Escherichia coli*, *Listeria innocua* and *Salmonella enteritidis* were exposed to active films either just after inoculation or just after an incubation phase. When tests were made at inoculation time for carvacrol concentration of 3.13×10^{-8} g mL^{-1} and low K_{mass}, films revealed antimicrobial efficiency against all tested microorganisms. When already grown colonies were exposed to films, those with lower K_{mass} displayed moderate inhibitory action against tested Gram-negative and strong efficiency against tested Gram-positive bacteria. From all results, the minimal vapour inhibitory concentration was considered as 4.62×10^{-8} g mL^{-1} ($K_{mass} = 1.13 \times 10^{-6}$) for *B. subtilis*, *E. coli* and *L. innocua*, 1.08×10^{-7} g mL^{-1} ($K_{mass} = 1.01 \times 10^{-4}$) for *S. enteritidis*. Films with the carvacrol vapour concentrations of 4.62×10^{-8} g mL^{-1} and 6.41×10^{-8} g mL^{-1} had strong antimicrobial effect at t_0 and t_1 except for *S. enteritidis* that was the most resistant bacterium. Concentration of 3.13×10^{-8} g mL^{-1} was efficient at t_0 but not at t_1. Concentration of 6.28×10^{-9} g mL^{-1} did not have any antimicrobial effect against all tested microorganisms, both at t_0 and t_1. Lag phase, doubling time of bacteria and concentration of released volatile compounds are key factors of antimicrobial efficacy. Antimicrobial tests and observations permitted a comparison of bioactivity potential of the activated chitosan films and the examination of the relationship between the antimicrobial properties and the mass partition coefficient.

1. Introduction

Microorganisms, temperature, sunlight, oxygen, humidity etc. are often main causes of quality loss of packed food products. These factors might induce microbial proliferation in the food, degradation of food components and alterations in the organoleptic properties with consequent consumers rejection. Despite increase in awareness of importance of high level hygiene in food supply chain, foodborne illnesses caused by microorganisms are still large public health problem. Therefore, research and development in the past decade have been focused on the antimicrobial packaging. Besides, consumer's demands for both minimally processed and preservative-free products and concerns related to the use of chemical additives, use of natural additives with antimicrobial properties, such as essential oils, seems to be a promising alternative for food industry. Therefore extensive research focused on the delivery of natural antimicrobial substances to food products has gained extreme attention of worldwide strong food packaging industry. In the selection of an antimicrobial compound, it must be considered the effectiveness against the targeted microorganisms and also the possible interactions among the antimicrobial compound, the film-forming biopolymer and food components. These interactions can modify both antimicrobial activity and film characteristics. Mentioned factors are keys for development of successful antimicrobial films and coatings (Avila-Sosa et al., 2012; Campos, Gerschenson, & Flores, 2011).

Most reporting cases of foodborne illness have documented significant increases over past few decades in the incidence caused by microorganisms in food, including pathogens such as *Salmonella*, *Listeria*, and *Escherichia coli* (Amalaradjou & Bhunia, 2012; Olaimat & Holley, 2012; Pires, Vieira, Perez, Wong, & Hald, 2012; Viazis & Diez-Gonzalez, 2011).

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To date, it has been demonstrated that essential oils (EO) that are generally recognized as safe for food application, effectively inhibit microbial growth and thus could be used to control foodborne pathogenic bacteria (Burt, 2004). Different results are observed depending on the test conditions, microorganisms, and source of the antimicrobial compound (Roller, 2003). According to literature, high degree of inhibition by volatile components of EOs in the vapour phase has been demonstrated (Goñi et al., 2009; Inouye, Takizawa, & Yamaguchi, 2001; Kloucek et al., 2011; Nedorostova, Kloucek, Kokoska, Stolcova, & Pulkrabek, 2009). Furthermore, it was reported that incorporation of active components of essential oils into edible films for special application by vapour phase is very efficient. Thus smaller concentrations compared with those required for direct application of EO are needed to inhibit different microorganisms. Because of ability to suppress growth of microorganisms in foods and application technology, use of essential oils and their constituents has to be carefully checked from organoleptic point of view. Similar inhibition for *in vitro* tests and the assays conducted with food were reported (Rodriguez, Nerin, & Batlle, 2008).

Carvacrol is a phenolic compound extracted from oregano and thyme oil. Its inhibitory effect on the growth of various microorganisms is well documented and described extensively. Different studies report its activity using vapour phase method (Ben Arfa, Combes, Preziosi-Belloy, Gontard, & Chalier, 2006; Kloucek et al., 2011; Nostro et al., 2009), diffusion methods (Liolios, Gortzi, Lalas, Tsaknis, & Chinou, 2009) and contact methods (Friedman, Henika, & Mandrell, 2002; Lambert, Skandamis, Coote, & Nychas, 2001). Besides studies were performed on different food products such as fish (Kim, Marshall, Cornell, Preston, & Wei, 1995), fruit juices (Kisko & Roller, 2005), meat (Skandamis & Nychas, 2002), and against different microorganisms such as *E. coli* (Friedman et al., 2002; Burt et al., 2007), *Bacillus* (Ultee, Kets, Alberda, Hoekstra, & Smith, 2000; Burt et al., 2007), *Listeria* (Friedman et al., 2002), *Salmonella* (Friedman et al., 2002) etc. Furthermore, it can be used to improve functional and antimicrobial properties of biopolymer films to extend product shelf-life (Ben Arfa et al., 2006; Mastromatteo, Barbuzzi, Conte, & Del Nobile, 2009).

This study aimed to evaluate the inhibition of *Bacillus subtilis*, *E. coli*, *Listeria innocua* and *Salmonella enteritidis* by the only vapour phase using volatile carvacrol incorporated into chitosan-based films. The concentrations of carvacrol in the chitosan-based films after drying were determined. In addition, the mass partition coefficients were also detected. They were used to control the concentration of carvacrol in the vapour phase. From the quantification of the aroma compounds in the air, minimum inhibitory concentration and the correlation between the carvacrol concentration in the vapour-phase and the film/headspace partition coefficient were studied.

2. Materials and methods

2.1. Materials and reagents

Commercial grade chitosan (CS) (France Chitine, Marseille, France, powder 652, having a molecular mass of 165 kDa, low viscosity, food grade, degree of deacetylation of 85%) and arabic gum (AG) (Spraygum, CNI, France) were used to constitute the film matrix. Anhydrous glycerol (Fluka, 98% purity, Fluka Chemical, Germany), lecithin (Sigma–Aldrich, France), nanoclays (montmorillonite, which synthesis and characterization has been detailed by Reinholdt, Miehé-Brendlé, Delmotte, Le Dred, & Tuilier, 2005) were used in order to improve aroma compound retention and mechanical properties. Acetic acid (glacial 100%, Merck, Darmstadt, Germany) was used as solvent in the preparation of the film forming solutions. All the chemicals were used without further purification. Freshly prepared solutions were always used in all experiments. Carvacrol with a purity >97% (Fluka) was used as a model antimicrobial volatile compound. *n*-hexane (Chromasolv, purity of >97%, Sigma–Aldrich) was used as the extraction solvent. Culture media Luria Broth (LB) Agar, Brain Heart Infusion Broth (BHI Broth), Brain Heart Infusion Agar (BHI Agar) and Potato Dextrose Agar (PDA) were obtained from Sigma–Aldrich. Columbia Agar was obtained from Biokar Diagnostic.

2.2. Chitosan based films preparation

A chitosan solution was prepared by dissolving the chitosan powder in a 1% (v/v) aqueous acetic acid, to obtain 1 and 2% (w/v) film forming solutions (FFS). To achieve a complete dispersion of the chitosan, the solution was stirred for 2 h at room temperature. Composite films were achieved according the same procedure as previously described by Kurek, Descours, Galić, Voilley, and Debeaufort (2012). Carvacrol (from 0.01 to 3%, w/v) was dispersed in the FFS and the mixture was homogenized for 10 min with an Ultra Turrax (T25 IKA). FFS was then poured into a glass Petri dish. In order to obtain films, solvents were removed by drying (from 18 h to 24 h) in a ventilated climatic chamber (KBF 240 Binder, ODIL, France) at 20 °C and 30% RH. After drying, the films were peeled off and stored in the same ventilated climatic chamber at 25 °C and 30% RH before measurements.

2.3. Film characterization

2.3.1. Carvacrol retention in biopolymer matrix

In order to follow the aroma compound release/retention, an extraction technique and a gas chromatography analysis were performed. *n*-hexane was used as the extraction solvent (having better affinity for carvacrol). Slices of dry film were put in the glass vial and a known amount of *n*-hexane was added at ratio 0.2:1.5, followed by stirring until all the aroma compound was extracted. The one step extraction yield was taken into account for the final calculation. The extraction yield was previously determined by successive extractions until the sample was exhausted.

The aroma compound was quantified by injecting the extract into a gas chromatograph and calculated from an external calibration curve. For each sample three replicates were performed.

2.3.2. Measuring of the aroma compound in gas phase

In order to measure the carvacrol release from the film (solid phase) into a headspace (gas phase), a static headspace method was used. The film was put into a headspace vial (40 mL Supelco) and then sealed immediately with Mininert Valves (Supelco). Each measurement was carried out at 25 °C after the equilibrium was reached. After equilibration, a volume of 1 mL of headspace air was taken out using a gastight syringe (Hamilton, Switzerland) and injected in the gas chromatograph. Two headspace injections were made per vial. The amount of carvacrol released in the gas phase was calculated from an external calibration curve.

2.3.3. Gas chromatography analysis

Carvacrol in the film extracts and in the headspace above the film were analysed by a Shimadzu GC 14B gas chromatograph, equipped with a flame ionization detector (GC-FID) and 30 m length DB-Wax column (J&W) with 0.53 mm i.d. and 1.0 μm film thickness and nitrogen as carrier gas (60 kPa). Hydrogen and air were used as ignition gases. The oven temperature programme was set at 210 °C, isothermal. The injector and detector temperature were at 240 °C.