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Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on *Penicillium italicum*

Laura Sánchez-González*, Maite Cháfer, Amparo Chiralt, Chelo González-Martínez[1]

Departamento de Tecnología de Alimentos – Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

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ABSTRACT

Chitosan-based films containing bergamot essential oil (BO) at 0.5%, 1%, 2% and 3% w/w were prepared to evaluate their physical and antifungal properties. Film-forming dispersions (FFD) were also characterized in terms of rheological properties, particle size distribution and ζ-potential. In order to study the impact of the incorporation of BO into the chitosan (CH) matrix, water vapour permeability (WVP), mechanical and optical properties of the dry films were evaluated. Furthermore, the antifungal effectiveness of CH–BO composite films against *Penicillium italicum* was studied. Results showed that the incorporation of BO provoked a decrease in the water vapour permeability, this reduction being around 50% when using a BO–CH ratio of 3:1. Concerning mechanical and optical properties, CH–BO composite films were less resistant to break, less deformable and less glossy. The load parameters (TS and EM) decreased more than 50% and the percentage of elongation at break was also dramatically reduced from 22% to 5%, as compared with the pure chitosan films. CH–BO composite films showed a significant inhibitory effect on the growth of *P. italicum*, which depended on the BO concentration. Chitosan films with the maximum bergamot oil content (3:1 BO–CH ratio) led to a total inhibition of the fungus growth during the first 5 days at 20 °C. Although the antifungal effectiveness of the films decreased throughout the storage time, a significant reduction of 2 logarithm units as compared with the control remained possible, after 12 days at 20 °C, using the highest BO content.

1. Introduction

To design proper edible antimicrobial films to be used in food preservation can be considered as one of the major challenges for food technologists in the next few years. In the future, these films will be tailor made to solve some specific problems for a given product. It is important to know the influence of different factors on their properties in order to design the most suitable film for a determined use and functionality.

Among the active biomolecules, chitosan has a great potential for a wide range of food applications due to its biodegradability, biocompatibility, antimicrobial activity, non-toxicity and film-forming capacity (Arvanitoyannis, 1999; Li, Dunn, Grandmaison, & Goosen, 1992; Tharanathan & Kittur, 2003). Chitosan-based films have been proven to present moderate oxygen barrier properties and good carbon dioxide barrier properties but high water vapour permeability, due to their hydrophilic nature (Butler, Vergano, Testin, Bunn, & Wiles, 1996). Usually, hydrophobic compounds, such as lipids, are incorporated into this type of hydrophilic hydrocolloid films to improve their water barrier properties. One possibility is the use of essential oils (EOs), as hydrophobic constituents, which have also been demonstrated to present potential antimicrobial activity against a wide variety of bacteria, moulds and yeast (Fischer & Phillips, 2006). Generally, phenolic and terpene compounds are major contributors to these antimicrobial properties. The specific advantage of EO appears to be the synergistic effects of their compounds as evidenced in the greater activity when applied as natural EO, as compared with the sum of the effects of the individual substances (Duke & Beckstrom-Sternberg, 1992).

Recently, the application of citrus essential oils to food preservation has received increased attention because not only do they lend themselves to use in food products but are also generally recognized as safe at flavouring concentrations (GRAS). These factors made them into very promising compounds to be used as a natural alternative to chemical-based preservatives, in line with the changes in legislation and consumer trends (Brul & Coote, 1999).

Bergamot oil is a citrus oil (from *Citrus bergamia*), whose major chemical compounds are volatile, such as limonene (32–45%) and linalool (around 10.23%) (Moufida & Marzouk, 2003; Svoboda & Greenaway, 2003). The antimicrobial efficiency of BO, and its com-

* Corresponding author. Tel.: +34 96 387 7000x83609; fax: +34 96 387 73 69.
E-mail addresses: lausango@upvnet.upv.es (L. Sánchez-González), cgonza@tal.upv.es (C. González-Martínez).
[1] Tel.: +34 96 387 7000x73656; fax: +34 96 387 73 69.

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ponents, linaool and citral, has been found to be effective against *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Arcobacter butzleri* and *Penicillium digitatum* (Fisher & Phillips, 2008), among others, both when oil is applied directly and when in contact with the oil vapour. The mechanisms by which essential oils bring about their antimicrobial effect are not clear but there are a number of proposed mechanisms (Holley & Patel, 2005): terpenes have the ability to disrupt and penetrate not only the lipid structure of the cell membrane, but also the mitochondrial membrane, leading to the denaturing of proteins and the destruction of cell membrane, cytoplasmatic leakage, cell lysis and eventually, cell death.

In composite chitosan–essential oil based films, the possible presence of interactions between the active antimicrobial agent and the chitosan should be taking into account, as they could affect the efficiency of the antimicrobial response. Chitosan might interact with terpens which are the major components of essential oils, mainly by weak interactions such as hydrogen bonding (Mayachiew, Devahastin, Mackey, & Niranjan, 2010). Hosseini, Razavi, and Moussavi (2009) found that the intensity of these interactions depended on the nature of the essential oil. These authors showed that the incorporation of essential oils into chitosan films led to a loose in the compactness of the film structure, this effect being more accused with cinnamon essential oil in comparison with thyme and clove oils. These interactions could affect the release of the added antimicrobial agent (Hosseini et al., 2009) and so, the antimicrobial response of the composite films.

On the other hand, films and coatings should be designed to fulfill a number of requirements, such as to have proper mechanical properties, good appearance (adequate gloss and transparency) and water and gas barrier properties. Thus, knowing how different factors influence these physical film properties is relevant to be able to improve and optimize the film functionality. Among these factors, the stability related properties of the film-forming dispersions (FFD), such as rheological behaviour, particle size and distribution and ζ-potential of the dispersed lipid particles, play an important role in the properties of lipid–hydrocolloid composite films. The control of the FFD properties could allow us to design films with determined functional properties (McClements, 2007)

The aim of this work was to evaluate how the functionality of chitosan-based films was affected by the incorporation of different ratios of bergamot essential oil, through the analysis of different physical and structural properties of the FFD and films. The antifungal properties of the films against *Penicillium italicum* were also evaluated. This fungus is one of the major causes of citrus fruit decay (blue mould) and films containing citrus essential oils could be used, without any impact on the fruit flavour, to prevent the microbial growth.

2. Materials and methods

2.1. Materials

High molecular weight chitosan (CH) with a deacetylation degree of 82.7% (Batch 10305DD, Sigma–Aldrich Química, Madrid, Spain), 98% glacial acetic acid (Panreac, Barcelona, Spain) and bergamot essential oil (BO) supplied by Herbes del Molí (Alicante, Spain) were used to prepare the film-forming dispersions.

2.2. Preparation of the film-forming dispersions

Chitosan (1% w/w) was dispersed in an aqueous solution of glacial acetic acid (0.5% w/w) at 25 °C. After stirring overnight, bergamot essential oil (BO) was added to chitosan (CH) solution to reach a final concentration of 0%, 0.5%, 1%, 2% and 3% (w/w). CH–BO mixtures were emulsified at room temperature (25 °C) using a rotor–stator homogenizer (Ultraturrax DI 25 basic-Yellowline, Janke & Kunkel, Staufen, Germany) at 13,500 rpm for 4 min. These emulsions were vacuum degasified at room temperature (25 °C) with a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany). Sample nomenclature was CH–*n*BO, the *n* value being the ratio BO:CH in the film or FFD.

2.3. Characterization of the film-forming dispersions

The density of the FFD was measured by means of a digital densimeter DA-110M (Mettler Toledo, Barcelona, Spain). A pH-meter C831 (Consort, Tumhout, Belgium) was used to determine the pH of the FFD at 20 °C.

2.3.1. ζ-Potential measurements

In order to perform ζ-potential measurements, FFD were diluted to a droplet concentration of 0.02% BO using an aqueous solution of glacial acetic acid (0.5%w/w). ζ-Potential was determined using a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK). The Smoluchowsky mathematical model was used to convert the electrophoretic mobility measurements into ζ-potential values.

2.3.2. Particle size measurements

Particle size analysis of the FFD was carried out using a laser diffractometer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The samples were diluted in deionised water at 2000 rpm until an obscuration rate of 10% was obtained. The Mie theory was applied by considering a refractive index of 1.52 and absorption of 0.1 for BO. Three samples of each FFD were measured in quintuplicate.

2.3.3. Rheological behaviour

The rheological behaviour of FFD was analysed in triplicate at 25 °C by means of a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti. Rheological curves were obtained after a stabilization time of 5 min at 25 °C. The shear stress (σ) was measured as a function of shear rate ($\dot{\gamma}$) from 0 to 512 s^{-1}, taking 5 min to reach the maximum shear rate and another 5 min to attain zero shear rate. The power law model (Eq. (1)) was applied to determine the consistency index (K) and the flow behaviour index (n). Apparent viscosities were calculated at 100 s^{-1}.

$$\sigma = K \cdot \dot{\gamma}^n \quad (1)$$

2.4. Preparation of films

A casting method was used to obtain films. FFD were poured onto a framed and levelled polytetrafluorethylene (PTFE) plate (ϕ = 15 cm) and were dried in atmospheric conditions (25 °C, 60% relative humidity) for 48 h. Film thickness was controlled by pouring the amount of FFD that will provide a surface density of solids in the dry films of 56 g/m^2 in all formulations. Dry films were peeled off the casting surface and preconditioned in desiccators at 20 °C and 54.4% relative humidity (RH) prior to testing. A hand-held digital micrometer (Palmer-Comecta, Spain, ±0.001 mm) was used to measure film thickness at three different points of the same sample at least.

2.5. Water vapour permeability

Water vapour permeability (WVP) was measured in dry film discs (ϕ = 7 cm), which were equilibrated at 54.4% RH and 20 °C, according to the "water method" of the ASTM E-96-95 (ASTM,