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Active films based on alginate containing lemongrass essential oil encapsulated: Effect of process and storage conditions

Natalia Riquelme[a], Maria Lidia Herrera[b], Silvia Matiacevich[a,*]

[a] Departamento de Ciencia y Tecnología de los Alimentos, Facultad Tecnológica, Universidad de Santiago de Chile, Obispo Umaña 050, Estación Central, Santiago, Chile

[b] Instituto de Tecnología en Polímeros y Nanotecnología ITPN (UBA-CONICET), Facultad de Ingeniería, Universidad de Buenos Aires, Av. Las Heras 2214, Ciudad Autónoma de Buenos Aires, Argentina

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ABSTRACT

Antimicrobial active films are studied to increase fresh food shelf life. The aim of this study was to evaluate the effect of process and storage conditions on physical properties and antimicrobial activity of alginate-based films with encapsulated lemongrass essential oil.

Films were obtained from film forming emulsions with different droplet sizes ($2.9 \pm 0.2\,\mu m$ and $0.43 \pm 0.02\,\mu m$), containing 1%w/w alginate, 1%w/w sorbitol, 0.75%w/w–1.35%w/w sodium caseinate, 0.5%w/v lemongrass essential oil and 0.02%w/w calcium carbonate. Each film was characterized by physical properties and antimicrobial activity against *Escherichia coli* and *Botrytis cinerea*. Films were evaluated in different storage conditions (75%RH, 4 °C; 75%RH, 20 °C; 11%RH, 4 °C; 11%RH, 20 °C).

Droplet sizes of film forming emulsions affected significantly the physical properties and antimicrobial activity of films, being more effective large droplet size. Particularly, high concentration of sodium caseinate affected optical properties of films. Moreover, storage conditions affected antimicrobial activity of films. The greatest inhibition of microbial growth was observed at 4 °C, reaching the highest percentages, after 15 days of storage, demonstrating the release of the active agent in a prolonged manner.

In conclusion, differences in sustained release of the antimicrobial depended mainly on the processing and storage conditions of active film.

1. Introduction

In view of the increasing demand and consumption of healthier products, one of the biggest challenges of the food industry is to increase the shelf life of fresh and minimally processed foods. To achieve this, several technologies have been studied and applied; however, some of them alter the properties and quality of foods. Meanwhile, emerging technologies have been developed which does not alter significantly the organoleptic properties of foods, such as edible films and coatings (Pascall and Lin, 2013). Edible films are developing in order to permit incorporation and prolonged release of active compounds, such as antimicrobial agents, into a polymer matrix, improving safety and/or nutritional and sensory attributes of foods, extending the shelf life and reducing the risk of pathogens growth on the surface (Rojas-Graü et al., 2009).

Alginate gels are used in the food industry as edible films for application on fruits and vegetables (Arzate-Vázquez et al., 2012). There are several studies about properties of alginate edible films that contained natamycin (antifungal compound produced by the bacterium *Streptomyces natalensis*), i.e., distinguishing that these have good properties and can be processed by different methods without loss its properties (Bierhalz et al., 2013). Films based on agar and sodium alginate

* *Corresponding author.*
E-mail address: silvia.matiacevich@usach.cl (S. Matiacevich).
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were developed by incorporation of cinnamon essential oil, where these shown high antioxidant and antimicrobial activities, being able to reduce growth of *Listeria monocytogenes* (Arancibia et al., 2014). Aloui et al. (2014) studied biodegradable coatings based on sodium alginate with grapefruit seed extract or grapefruit essential oil, with the objective of preserve the quality of grapes, founding preservation of antioxidant activity, antifungal effect, weight loss reduction and firmness maintenance. Navarro et al. (2016) studied active edible films, incorporating thyme oil as antimicrobial agent by emulsions, using different encapsulating agents (trehalose, β-cyclodextrin and Tween 20), founding the highest antimicrobial activity with β-cyclodextrin. Besides, alginate films with citric and/or ascorbic acid were developed by De'Nobili et al. (2016), who reported that films can be potentially useful to avoid oxidation in nuts.

In addition, different applications in foods have been reported such as multilayered antimicrobial alginate-based edible coating to increasing the shelf life of fresh-cut watermelon (*Citrullus lanatus*), without affecting its quality attributes and maintaining sensory acceptance (Sipahi et al., 2013). Robles-Sánchez et al. (2013) indicated that the combination of alginate with an antibrowning agent (ascorbic and citric acid) preserved the color of mango (*Mangifera indica* L.) and increased the antioxidant potential of fruits until 12 days at 4 °C. Also, alginate-based coating incorporated with different concentration of lemongrass were used for fresh-cut pineapple (*Anana scomosus* cv. Josapine) during low temperature storage, concluding that coatings has potential to extend the shelf-life and maintain the quality of this one (Azarakhsh et al., 2014). Chiabrando and Giacalone (2015) applied biodegradable alginate-based coatings with essential oils (cinnamon and rosemary) to fresh-cut apple (cv. Golden delicious), finding that addition of essential oils was more effective than alginate alone on weight loss, preserving the original color and lightness of fruits. Additionally, Matiacevich et al. (2015a) studied active edible coatings based on alginate with propionic acid and thyme essential oil, applied by spray system on fresh chicken breast fillet, where the selected coating increased the shelf life by about 33%, with the lowest dehydration of fillets. Moreover, a sodium alginate coating with ascorbic acid was added to raw pork meat slices, where the coating allowed a shelf life prolongation of 11 days (Gammariello et al., 2016).

Essential oils contain a complex mixture of volatile and non-volatile compounds produced in aromatic plants due to its secondary metabolism, whose antimicrobial properties are attributed to their interaction with the cell membrane of bacteria (Salvia-Trujillo et al., 2015). *Cymbopogon citratus*, known as lemongrass, is an herb that has been cultivated for medicinal purposes in various countries (Naik et al., 2010). It is characterized by a strong odor of lemon due to its high content of citral (75% of its composition) (Hanaa et al., 2012). Lemongrass essential oil has shown antimicrobial activity against various microorganisms such as fungi, yeasts and bacteria Gram (+) and Gram (−) (Naik et al., 2010). An important issue is that application of essential oils directly into edible films may have limited benefits, because these can be easily neutralized or diffused into food causing sensory problems, which has a major impact on the product acceptability. In addition, these active compounds are generally volatile and susceptible to oxidation due to direct exposure to high temperature, pressure changes, presence of light and oxygen (Keawchaoon and Rangrong, 2011). For these reasons, the micro and nano-encapsulation by emulsification are an alternative to maintain the functional properties of active compounds during storage and protecting them from deterioration. In addition, encapsulation allows better handling of the final product, since it facilitates incorporation of compounds at different matrices, prevents interaction of flavors and aromas and improves absorption of active compounds in the body (Nedovic et al., 2011). Besides, other important benefit of encapsulation is the gradually release of active compounds under influence of specific conditions (Anal and Singh, 2007). However, selection of the encapsulating agent is critical since it may influence the emulsion stability (Gharsallaoui et al., 2007). Particularly, sodium caseinate presents emulsifying properties, due to their high content of hydrophobic amino acids and its amphiphilic character, provides good encapsulation properties for hydrophobic active compounds (Pan et al., 2014).

The prolonged release of active compounds added to edible films demands a high-level of coordination to be produced and applied at a specific site, time, and rate onto a food (Barba et al., 2015). In this sense, to optimize the functional properties of encapsulated active compounds, it is necessary to know process conditions (such as pH, type/concentration of the encapsulating agent and type/time of homogenization) and storage conditions (such as relative humidity and temperature), in order to allow gradually release and prolonged of the active compound (Matiacevich et al., 2015b; Zhang et al., 2015). The aim of this study was the physical characterization of alginate-based films with encapsulated lemongrass oil, and evaluation of the effects of processing and storage conditions on antimicrobial properties of the films.

2. Material and methods

2.1. Materials

Sodium alginate, sorbitol and sodium caseinate were purchased from Blumos (Chile), lemongrass essential oil was obtained from Sigma–Aldrich (USA), and calcium carbonate was purchased from Winkler (USA). The salts (sodium chloride and lithium chloride) used to condition relative humidities were supplied by Merck (Germany).

For antimicrobial activity measurements, the microorganisms used were *Escherichia coli* ATCC 25922, obtained by Instituto de Salud Pública (Chile), and *Botrytis cinerea* (previously isolated and identified in the laboratory). The culture media used for microbial growth were Mueller-Hinton and Potato Dextrose (PDA) Agar, supplied by Biokar Diagnostics (France).

2.2. Preparation of active films

Active films were prepared by casting, at 40 °C for 15 h, from film forming emulsions containing 1%w/w sodium alginate as matrix, 1%w/w sorbitol as plasticizer, and 0.5% w/v lemongrass essential oil as antimicrobial agent. Film forming emulsions were prepared with different droplet sizes, "large", denominated LFFE ($2.9 \pm 0.2\,\mu m$) and "small", SFFE ($0.43 \pm 0.02\,\mu m$). Sodium caseinate as encapsulating agent at optimum concentration to obtain stable emulsions (preliminary studies), 0.75%w/w and 1.35%w/w for the LFFE and the SFFE, respectively.

Films from the LFFE were homogenized in a high-speed homogenizer (Thristor Regler, TR50, Germany), at 10000 rpm for 2.5 min. On the other hand, the SFFE were pre-homogenized, at 7500 rpm for 2.5 min, and then by ultrasound homogenization (Sonics, VCX500, USA), for 20 min, using a pulse on 15 s and pulse off 10 s, and 90% amplitude. Finally, cross linker agent (0.02% w/w calcium carbonate) was added at emulsions, adjusting pH at 4.0 using a pHmeter (Jenway, 3505, UK), to obtain relatively stable emulsions (determined by preliminary studies). Furthermore, control films were prepared: alginate and alginate-antimicrobial. The moisture content was determined by gravimetric analysis (at 105 °C for 24 h) and thickness of films was measured using a micrometer (Mitutoyo, Japan).

2.3. Characterization of active films

2.3.1. Fourier transform infrared spectroscopy (FT-IR)

The spectra were recorded on a FT-IR spectrometer with attenuated total reflectance unit (UATR) (Perkin Elmer, Spectrum Two System, USA), using 16 scans with a resolution of $1\,cm^{-1}$,