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

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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.

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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

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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 ± 0.2 μm) and "small", SFFE (0.43 ± 0.02 μm). 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},

in a spectral range from 450 at 6000 cm^{-1}. Experiments were performed in triplicate, at 3 random points of film.

2.3.2. *Optical properties: color and opacity*

Color variation and opacity of the active films were determined using a computer vision system, which consists of a black box with four natural lights D65 (Philips, 18 W, Netherlands) and a digital camera (Canon, EOS Rebel XS, Japan) at 22.5 cm of distance from the sample. The angle between the camera lens and the light corresponds to 45° (Matiacevich et al., 2012). Calibrations of the camera and image parameters used were obtained following the protocol stated by Pedreschi et al. (2006). The photographs were taken using black and white background, then intensity of RGB digital characteristics were extracted using Adobe Photoshop 7.0 software (Adobe Systems Incorporated, USA), and finally, converted to CIEL*a*b* space, where L^* indicates lightness, a^* red–green axis and b^* yellow–blue axis.

Color variation of active films versus controls (alginate and alginate-antimicrobial) was determined using $CIE\Delta E_{2000}$ equation described by Luo et al. (2001). Opacity was determined using Eq. (1) (Matiacevich et al., 2015b).

$$\text{Opacity}\,(\%) = \frac{L^*\text{black background}}{L^*\text{white background}} \times 100 \quad (1)$$

where L^* black background and L^* white background represent lightness of active films in black and white background, respectively.

2.3.3. *Solubility*

The solubility of active films was determined at two temperatures, 4 °C, to simulate films behavior onto a food, and 37 °C, to evaluate behavior during microbiological analysis. The solubility was determined following the methodology described by Matiacevich et al. (2013). The amount of solubilized sample was calculated according to Eq. (2).

$$\%S = \left(\frac{W_0 - W_f}{W_0}\right) \times 100\% \quad (2)$$

where %S is the solubility percentage of film, W_0, the initial weight of sample, on dry basis, and W_f, the final weight of dried undissolved film.

2.3.4. *Molecular mobility*

The study of relaxation times by H^1 NMR as a function of temperature provides information about mobility of protons in water and/or solid matrix according to the pulse sequence used (Blümich and Pretsch, 2016). A time-resolved proton nuclear magnetic resonance (^1H NMR) (Bruker Minispec, mq20, Germany) was used to obtain time of transverse or spin-spin relaxation (T_2), consisting in a magnetic field of 0.47 T, operated at frequency of 20 mHz and 40 °C.

Free induction decay (FID) of protons from water and solid matrix was performed following the method described by Navarro et al. (2016). The test time was about 15 s, whereby samples do not suffer significant changes during NMR measurements.

The FID curves obtained were fitted to a bi-exponential behavior, using Origin Pro 8 software (OriginLab Corporation, USA), following Eq. (3).

$$I = A1^{-t/T_{2FID1}} + A2^{-t/T_{2FID2}} \quad (3)$$

where I is the signal intensity of protons, $A1$ and $A2$ are constants, T_{2FID1} and T_{2FID2} correspond to relaxation times of polymer chains protons and tightly-bonded water protons, respectively.

2.3.5. *Microstructure of film morphology*

The surface and edges of active films was observed using scanning electron microscopy (SEM) (Zeiss, Supra 40, Germany). Active films were fractured with liquid nitrogen and conditioned before being photographed. Film pieces (~2 mm^2) were placed in a platform and coated with platinum in order to confer conductive properties. Photographs were taken at different magnifications, from 500× to 50,000×, of surface and edges of active films.

2.4. *Antimicrobial properties of active films during storage time*

2.4.1. *Storage conditions of active films*

Active films were stored for 15 days, at different humidity and temperature conditions, 75%RH/4 °C, 75%RH/20 °C, 11%RH/4 °C and 11%RH/20 °C, in order to evaluate effects of the storage conditions in the antimicrobial properties of active films.

Relative humidity was conditioned by placing active films into desiccators with saturated salt solutions (sodium chloride for 75%RH and lithium chloride for 11%RH), at temperatures studied (Greenspan, 1977). Measurements were made at 0, 3, 7, 10 and 15 days of storage.

2.4.2. *Inhibition of Escherichia coli growth*

The antimicrobial activity of active films against *E. coli* was determined for studying prolonged release of the antimicrobial agent under different storage conditions. For this, *E. coli* was previously grown at 37 °C overnight in an incubator with constant stirring (N-Biotek, NB-205, Korea). The concentration of bacteria (colony forming units (CFU)/mL) was obtained by optical density, measuring absorbance at 625 nm in a spectrophotometer (Shimadzu, UVmini-1240, Japan). The concentration of the bacterial suspension obtained was 10^8 CFU/mL by turbidity McFarland scale (CDCP and WHO, 2003). The bacterial suspension were serially diluted until obtain a final concentration of 10^6 CFU/mL. Then, 1 mL of bacterial suspension was spread in Mueller-Hinton agar. Moreover, pieces of 1 cm^2 of active film were placed on the agar surface previously inoculated with *E. coli*, and, subsequently, incubated at 37 °C for 24 h, in an incubator (Velp Scientifica, FOC 225E, Italy), and the numbers of developed colonies were counted. Finally, the percentage of colony reduction was calculated using Eq. (4) (Kavoosi et al., 2013).

$$\%\text{colony reduction} = \frac{N^\circ\text{ of colony in test samples} - N^\circ\text{ of colony in control}}{N^\circ\text{ of colony in test samples}} \times 100\% \quad (4)$$

2.4.3. *Inhibition of Botrytis cinerea growth*

Antifungal properties of volatile compounds of essential oil encapsulated in edible films were observed using vapour contact (Velázquez-Nuñez et al., 2013). *B. cinerea* was spread into a PDA agar acidified with tartaric acid 10% w/w and stored for 2 days at 22 °C. After obtaining fresh *B. cinerea*, films pieces

Table 1 – Characterization of physical properties of films: Moisture content (%dry basis), thickness, color parameters of CIELab space, color variation (ΔE_{2000}) comparing with control film, opacity and solubility at two temperatures.

	Moisture content (%)	Thickness (mm)	Color (CIELab)			ΔE_{2000}	Opacity (%)	Solubility (%)	
			L^*	a^*	b^*			4 °C	37 °C
Control	9.96 ± 0.97^a	$0.09 \pm 0{,}02^a$	69.31 ± 0.54^a	6.14 ± 0.19^a	-13.67 ± 0.13^a	0.00 ± 0.00^a	1.73 ± 0.25^a	100.0 ± 0.0^a	100.0 ± 0.0^a
Control LFFE	8.60 ± 1.22^{ab}	0.13 ± 0.03^b	69.00 ± 0.13^a	5.89 ± 0.07^a	-12.94 ± 0.45^a	0.48 ± 0.42^a	1.43 ± 0.19^a	100.0 ± 0.0^a	100.0 ± 0.0^a
LFFE	8.31 ± 0.54^{ab}	0.19 ± 0.03^c	65.15 ± 0.38^b	-0.89 ± 0.72^b	4.50 ± 2.33^b	17.27 ± 2.40^b	5.31 ± 3.24^a	100.0 ± 0.0^a	64.2 ± 18.6^{bc}
Control SFFE	8.07 ± 0.99^{ab}	0.10 ± 0.03^{ab}	68.07 ± 0.36^a	5.42 ± 0.06^a	-12.99 ± 0.30^a	1.24 ± 0.21^a	2.18 ± 0.14^a	100.0 ± 0.0^a	98.5 ± 1.4^{ac}
SFFE	7.61 ± 0.72^b	0.19 ± 0.03^c	54.53 ± 0.79^c	-3.10 ± 0.92^c	39.05 ± 0.38^c	37.67 ± 0.13^c	6.40 ± 0.59^b	81.9 ± 15.5^a	65.0 ± 3.5^c

Note: Different letters indicate significant differences ($p < 0.05$) in the parameters (same column) for different active films.

of 1.5 cm^2 were placed in the cover of each Petri dish (previously spread with *B. cinerea*), so that it does not directly touch the surface of agar, in order to evaluate the antifungal activity of volatiles in the active compound, and stored at 22 °C for 2 days. In addition, positive controls, without the active agent, and negative controls, without fungi, were performed. Inhibition percentage was calculated comparing growth diameter of the positive control with growth diameter in presence of active film.

2.5. *Statistical analysis*

Statistical analysis of results was performed by ANOVA (analysis of variance) and multiple comparison by the Tukey test, with a confidence level of 95%, in order to assess significant differences between mean values, using the GraphPad Prism 5.01 software (GraphPad Prism Ink., USA).

3. Results and discussion

3.1. *Characterization of active films*

3.1.1. *Moisture content and thickness*

Table 1 shows that there are not significant differences ($p > 0.05$) between moisture contents of the LFFE films and moisture contents of controls. On the other hand, the SFFE films showed different moisture contents regarding to control films moisture contents. These differences were attributed to the fact that the SFFE active films have higher concentration of encapsulating agent (sodium caseinate) than control films, which could explain the lower moisture content of these films regarding to second ones. As sodium caseinate is an amphiphilic molecule (Casanova and Cardona, 2004), it can expose hydrophobic groups (not associated with the molecules of the active compound) repelling water which could be absorbed by the matrix.

Thickness values are showed in Table 1, where this one increased with the addition of encapsulating agent, being observed significant differences ($p < 0.05$) regarding to the control films, for both active films (the LFFE and the SFFE).

3.1.2. *FT-IR spectra*

The FT-IR spectra of active films are shown in Fig. 1. For the alginate control, peaks at 3271, 2932, 1300, 1077 and 1025 cm^{-1} were observed, corresponding to bonds: O—H, C—H, C—C—H and/or O—C—H, C—O—C of sodium alginate, respectively. Similar results were reported by Lawrie et al. (2007) and Aguirre Calvo and Santagapita (2016). Although active films also showed these signals, by adding different compounds (antimicrobial agent, encapsulating agent and the mixture of both), new signals, corresponding to new interactions, were found in the spectra (Fig. 1). For the LFFE films, new bonds were observed (in relation to the alginate control, Fig. 1a), when it added antimicrobial agent (1737 and 815 cm^{-1}) (Fig. 1b), encapsulating agent (1535 cm^{-1}) (Fig. 1c) and the mixture of all components (2971 and 1619 cm^{-1}) (Fig. 1d). For the SFFE films, the observed peaks were 1737 cm^{-1} for antimicrobial agent (Fig. 1e), 1535 cm^{-1} for encapsulating agent (Fig. 1f), and 1622, 1537 and 1240 cm^{-1} for the mixture of all components (Fig. 1g). Therefore, interactions between the antimicrobial-alginate, alginate-encapsulating agent and encapsulating agent-antimicrobial-alginate were detected since these new peaks (bonds) were observed, as was reported by Anbinder et al. (2011), in alginate and yerba mate extract matrixes, with formation of a shoulder between 1540 and 1570 cm^{-1}, assigned to interaction of polar groups of the active compound with hydroxyl or carboxyl groups of alginate. The interactions were characterized by adding antimicrobial and encapsulating agents separately, and, for both films (the LFFE and the SFFE), similar results were found. However, when all components were added, their interactions into the LFFE and the SFFE films were different, indicating that interactions were related to the droplet size of film forming emulsion.

3.1.3. *Optical properties*

The optical properties of active films are of great importance since these directly influence the acceptability of foods on which these are applied. Table 1 shows the optical properties of active films. Color parameters of the CIEL$^*a^*b^*$ space of active films such as the L^* value, showed significant differences ($p < 0.05$) between active films and their controls. Also significant differences ($p < 0.05$) between the LFFE and the SFFE films were observed. These results can be explained by an increase in the concentration of the encapsulating agent in the SFFE films (higher amount of solids particles of sodium caseinate after drying by casting). Values of the parameter a^* decreased in the LFFE and SFFE films regarding to controls. Secondly, the b^* values, which are associated to yellow color, of the LFFE and the SFFE films increased significantly ($p < 0.05$) regarding to controls, indicating a high tendency to yellowness (Table 1). Thus, the CIEL$^*a^*b^*$ space parameters showed that active films tended more to yellowness as the sodium caseinate concentration increased.

The color differences of active films in relation to alginate control were evaluated using ΔE_{2000}; values are reported in Table 1. Significant color differences ($p < 0.05$) between active films and the control were observed, where the SFFE films showed a greater color difference than the LFFE films, which would probably limit the SFFE films application.

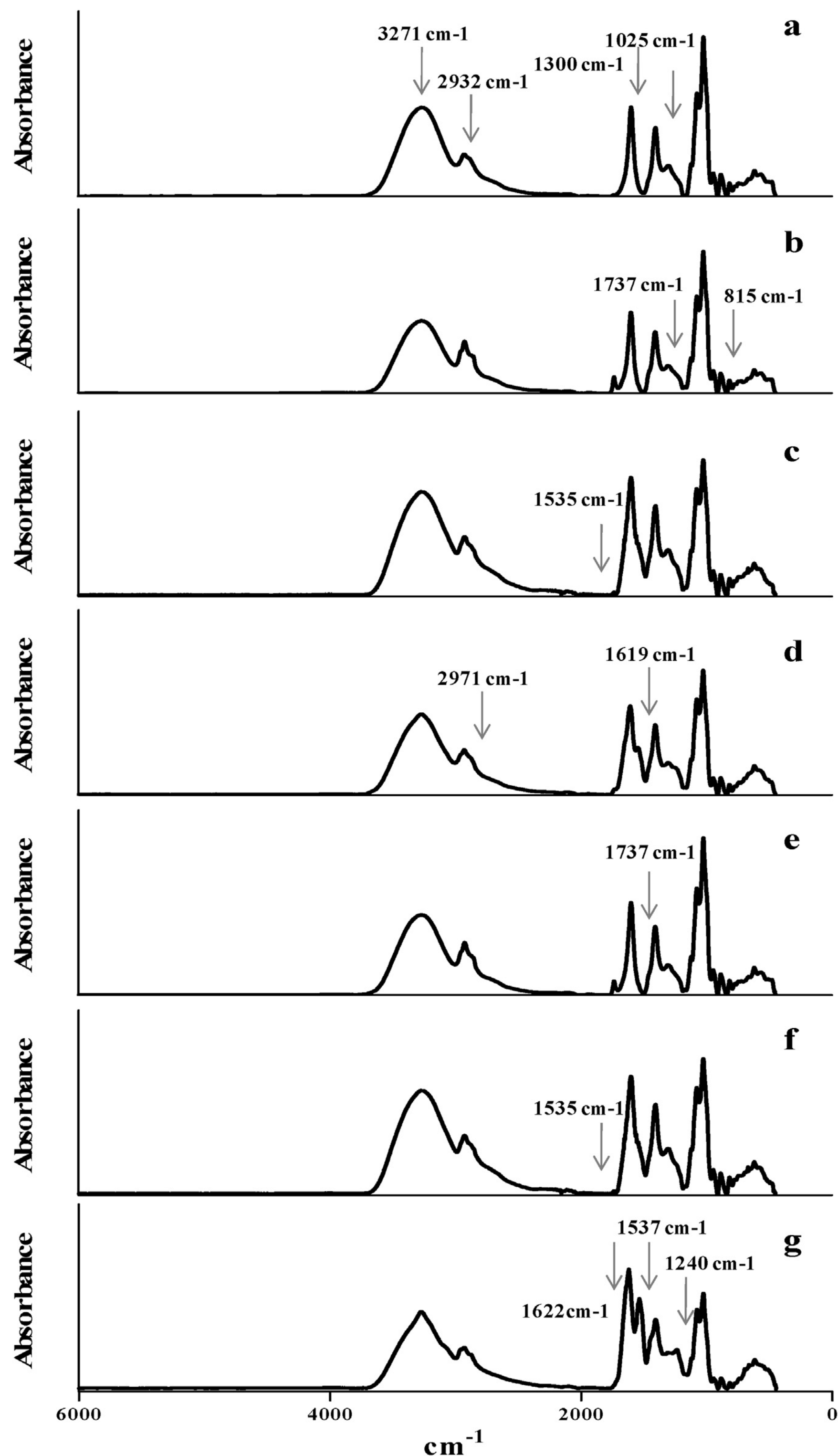

Fig. 1 – FT-IR (a) control, (b) control LFFE (antimicrobial), (c) control LFFE (encapsulating agent), (d) LFFE, (e) control LFFE (antimicrobial), (f) control SFFE (encapsulating agent), and (g) SFFE.

Opacity of active films is shown in Table 1. Controls are considered transparent, because their opacity values are less than 5% (Banerjee and Bhattachrya, 2011). Addition of the encapsulating agent caused a significant increase in opacity values (~10%) of active films, which is consistent with the results obtained for the CIEL*a*b* space parameters and the color differences (ΔE_{2000}), where the encapsulating agent generates yellow films, increasing color at higher concentrations.

3.1.4. *Solubility*

The solubility percentages of all active films, at two temperatures, are reported in Table 1. Controls films were 100% soluble in water, independently of temperature, mainly due to the hydrophilic nature of the matrix (sodium alginate). This behavior can be attributed to its binding capacity with water, with the plasticizing agent and the functional groups (Rubilar et al., 2013); a similar result was observed by Santana and Kieckbusch (2013), in sodium alginate films plasticized with glycerol, xylitol and mannitol, which were completely

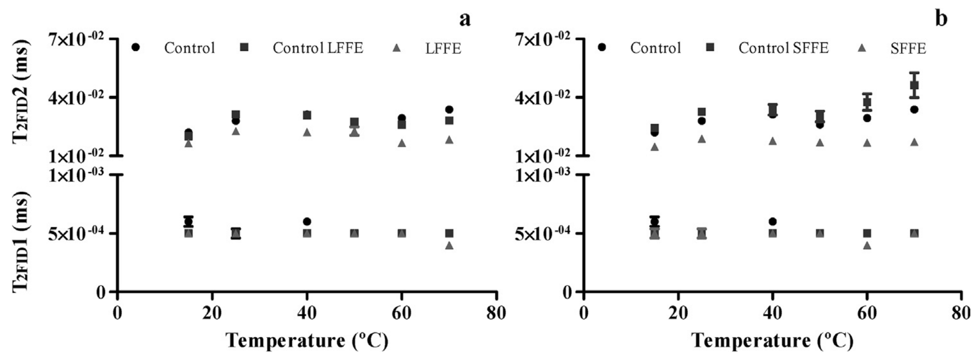

Fig. 2 – Molecular mobility (T_{2FID}) by H^1 NMR for active films made from (a) LFFE and (b) SFFE at different temperatures.

solubilized in distilled water. Furthermore, addition of the antimicrobial agent did not modify the solubility (Table 1), although its hydrophobic nature, since the concentration used was relatively low, as was reported by Salmieri and Lacroix (2006), for alginate/polycaprolactone-based films with 1% of essential oils (oregano, savory and cinnamon), where the films were entirely soluble in water. Furthermore, the active films based on LFFE were 100% soluble at 37 °C, while its solubility decreased at 4 °C (~60%). A same behavior was found for the films based on SFFE; solubility at 37 °C was about 80%, but decreased at 4 °C, being around 60% at 4 °C (Table 1). Solubility values of the films based on LFFE and SFFE were lower than their respective controls. This behavior can be attributed to the addition of the encapsulating agent, which stabilizes the physical structure of films, reducing its solubility, due to an increase of bond energy. Similar results were observed by Deepa et al. (2016), in alginate-based films with cellulose nanofibrils (CNF) as encapsulating agent, where was found a water solubility reduction from 8,6 to 1,7%, with incorporation of 10% of CNF, indicating a strong hydrogen bonds formation between the CNF and film matrix, where water molecules cannot break them.

3.1.5. *Molecular mobility*

Relaxation times for active films are reported in Fig. 2. All films showed two protons populations with different FID behaviors, where the first population was associated to solids found in the matrix ($T_{2FID}1$) and, the second population, to water absorbed by the matrix (water monolayer) ($T_{2FID}2$). Furthermore, $T_{2FID}1$ values were not affected by increase of temperature in all active films (Fig. 2), while $T_{2FID}2$ values increased at higher temperature. This increase is associated with greater mobility of protons of water absorbed into the matrix (Farroni et al., 2008; Ritota et al., 2008), since temperature rising increases the vibrational energy of protons. The increase of $T_{2FID}2$ in control films was higher than others films, which can be explained by the higher moisture content observed in these films in relation to the LFFE and SFFE films (Table 1). A system that contains greater amount of water in multilayer structure could show greater molecular mobility. On the other hand, in Fig. 2, no change of slope in curves $T_{2FID}1$ and $T_{2FID}2$ versus temperature were observed in all active films, which made no possible to observe the glass transition temperature (Tg) of the films (Farroni et al., 2008). This behavior could occur because the films are in rubbery state, since they were made by casting (40 °C), at a temperature where the molecules have no time to arranged and form a crystal structure.

3.1.6. *Microstructure*

In Fig. 3, the microstructure of active films was observed, both its surface (Fig. 3a–e) and their edges (Fig. 3f–j). Control film showed a smooth and homogenous surface, which could indicate that their components are miscible with each other, while certain roughness on its edge (Fig. 3f) was observed.

When antimicrobial agent was added (Fig. 3b, c, g and h), oil droplets were observed on the surface and at the edges. In the LFFE films, these droplets are larger compared to the droplets observed in the SFFE film, which is consistent with the droplet sizes of film forming emulsions. Furthermore, in the surface of the LFFE control film, cracks were observed, which is consistent with that reported by Hosseini et al. (2009); these authors reported that the incorporation of active compounds of hydrophobic nature (thymol and essential oil clove) caused cracks and structure loss of chitosan films, which could also explain the formation of cracks in active films. In the cases of the LFFE and SFFE films, differences in the droplet sizes were observed respect to their control films (Fig. 3d, e, g and h). The antimicrobial agent droplets are not observed in the surface of the SFFE films, because smaller droplets increase interaction between polymer-plasticizer and antimicrobial agent causing the droplets not to be recognizable in the structure (Atarés and Chiralt, 2016). Also, it was observed that the droplets of the antimicrobial agent were not uniformly distributed in the SFFE and LFFE films, but were distributed forming a line through the film. Sánchez-González et al. (2009) reported similar results in hydroxymethylcellulose (HPMC) films with tea tree essential oil, where patterns of the droplets were observed in the films, attributed to the occurrence of creaming in a high viscosity continuous phase. Although changes were observed in the morphology of surface and edges of active films at microscopic level, these differences are not noticeable at macroscopic level.

3.2. *Characterization of active films under different storage conditions*

Storage could affect antimicrobial and antifungal properties of active films, therefore, it is very important to study activity of these ones under different storage conditions, in order to know the conditions that maximizes their activity and determine foods types where these would have better functionality.

3.2.1. *Antimicrobial properties*

Fig. 4 reports antimicrobial activity against *E. coli* for all active films under different storage conditions (75%RH/4 °C, 75%RH/20 °C, 11%RH/4 °C and 11%RH/20 °C) by a period of 15 days. No high reduction in the number of *E. coli* colonies was observed for all active films, independent of storage

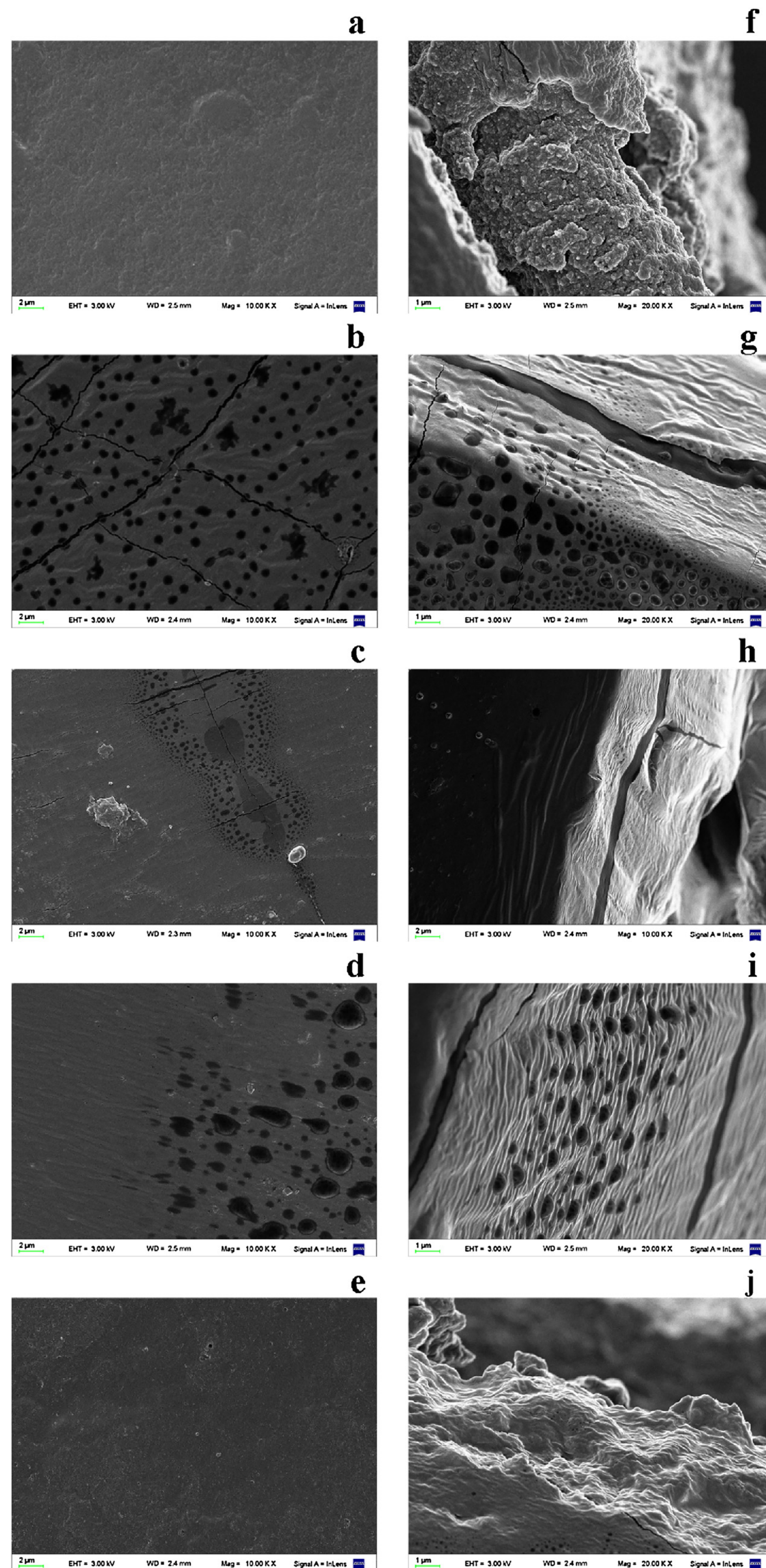

Fig. 3 – Morphology of surface of active films by SEM. Magnification 10,000×: (a) control, (b) control LFFE, (c) control SFFE, (d) LFFE, (e) SFFE and morphology of edges of active films magnification 20,000×: (f) control, (g) control LFFE, (h) control SFFE, (i) LFFE and (j) SFFE.

conditions, whereby the active agent could be encapsulated. Particularly, the LFFE and SFFE films showed greater inhibition, under different storage conditions, compared to control films. The highest values of inhibition were observed in the SFFE films (60% of inhibition of colony growth), which was attributed to the antimicrobial agent protected by the encapsulating agent, increasing antimicrobial activity of these films. In Fig. 4, an increase in colony reduction percentage of *E. coli*

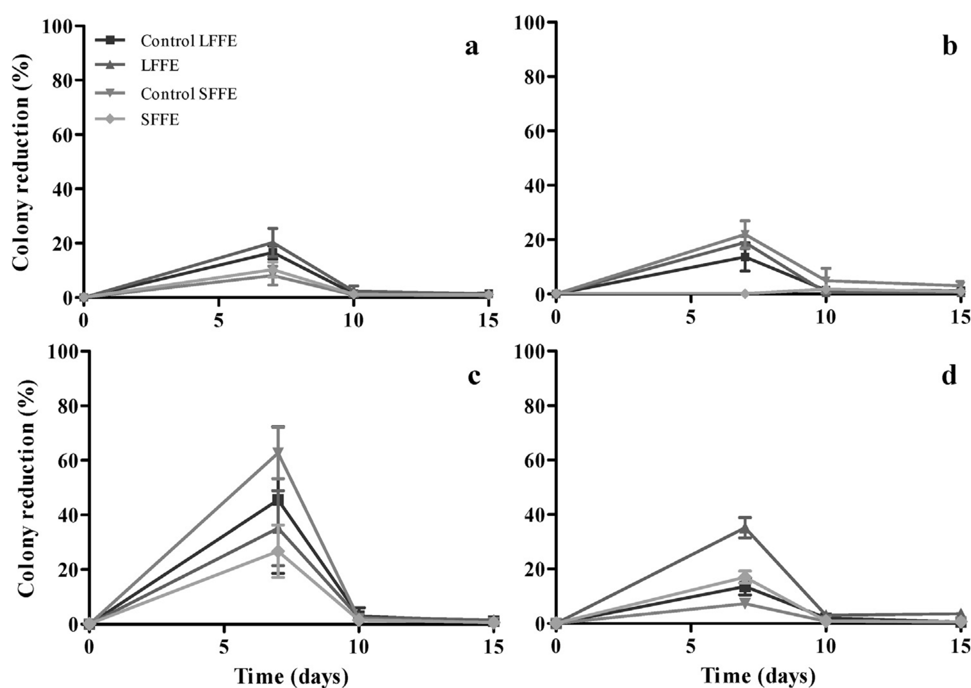

Fig. 4 – Antimicrobial activity of active films against *Escherichia coli* as percentage of colony reduction, at different storage conditions: (a) 75%RH–20 °C, (b) 75%RH–4 °C, (c) 11%RH–20 °C and (d) 11%RH–4 °C.

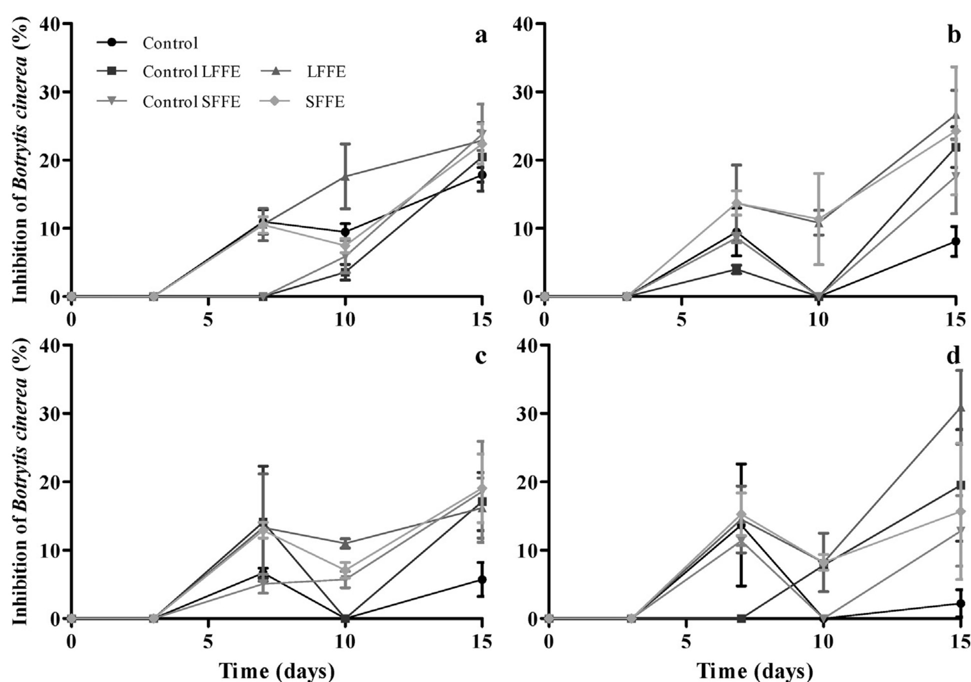

Fig. 5 – Antifungal activity of active films against *Botrytis cinerea* as percentage of inhibition of growth, at different storage conditions: (a) 75%RH–20 °C, (b) 75%RH–4 °C, (c) 11%RH–20 °C and (d) 11%RH–4 °C.

was observed in some active films (SFFE at 11%RH–20 °C and LFFE in other storage conditions), after 7 days storage, reaching approximately over 20% inhibition. The behavior could be because the active compound is released from active film as a function of storage time. Comparing the different storage conditions of films, the condition of 11% RH at 20 °C was the one in which the greatest colony reduction percentage was achieved, after 7 days of storage. This condition showed more release of the antimicrobial agent because, under this condition and storage time, there is greater diffusion of the antimicrobial agent from the active film into the culture medium and thus greater growth inhibition of *E. coli*. At a subsequent storage time, colony reduction percentage was less than 5%, independent of film type and storage conditions, not achieving a total release of the active agent in the time interval studied.

3.2.2. *Antifungal properties*

The antifungal activities of active films against *B. cinerea*, under different storage conditions, were observed in Fig. 5. First of all, antifungal activity was not observed at zero time in all active films, which could be due to the fact that the antimicrobial agent is encapsulated. At 3 days of storage, in all conditions, films showed inhibition of the sclerotia (structure developed by the mold to survive in unfavorable conditions) (data not

shown), demonstrating that the antimicrobial agent inhibits survival mechanisms of *B. cinerea*. At later storage times (starting from 7 days), active films inhibited growth of *B. cinerea*, for all storage conditions studied. Comparing the antifungal activity of films stored at 75% RH at 4 °C, it can be observed that the LFFE films exerted the highest activity, followed by the SFFE films, and then, by their respective controls (Fig. 5a). Furthermore, activity was maintained during storage, reaching a relatively high growth inhibition percentage of *B. cinerea*, at 15 days of storage. A same behavior was observed in the other storage conditions studied, for example, in the conditions with 75 and 11% RH, at 4 °C, showing about 30% of inhibition (Fig. 5b, d), demonstrating the release of the active agent in a sustained storage. Matiacevich et al. (2015) previously reported the efficacy of essential oils incorporated to alginate films, who studied the antifungal activity of carvacrol against *B. cinerea* during storage. Results showed that *B. cinerea* growth was inhibited due to the prolonged release of carvacrol during storage. Finally, active films can serve as a carrier for antimicrobial agents, due to their ability to maintain high concentrations of these last on the food surfaces during storage; these could also be an alternative to extend shelf life of foods (Supardan et al., 2016).

4. Conclusions

The droplet sizes of film forming emulsions influence characteristics and physical properties of active films made from such emulsions. Different interactions, at molecular level, between encapsulating agent-alginate and encapsulating agent-alginate-antimicrobial agent were detected in the microstructure of films. The interactions generated differences mainly in the optical properties of films, limiting application of high concentrations of encapsulating agent due to a detectable yellowing emerging.

The antimicrobial and antifungal properties of active films depended primarily on storage conditions, being higher in the films made from emulsions with large droplet size (LFFE). The storage conditions that allowed prolonging the release of the antimicrobial agent and, consequently, improved antimicrobial activity of active films, differed according to the type of microorganism; for *E. coli*, this condition was 11% RH at 20 °C while, for *B. cinerea*, were two conditions, 75% and 11% HR at 4 °C.

Finally, differences in the prolonged release of the antimicrobial agent depended both on the processing and storage conditions of films. It is noteworthy that the highest activity, during storage, was obtained in films with the larger droplets.

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References

Anbinder, P.S., Deladino, L., Navarro, A.S., Amalvy, J.I., Martino, M.N., 2011. Yerba mate extract encapsulation with alginate and chitosan systems: interactions between active compound encapsulation polymers. J. Encapsul. Adsorpt. Sci. 1, 80–87.

Aguirre Calvo, T., Santagapita, P., 2016. Physicochemical characterization of alginate beads containing sugars and biopolymers. J. Qual. Reliab. Eng. 2016, http://dx.doi.org/10.1155/2016/9184039, Article ID 9184039, 7 pages, 2016.

Aloui, H., Khwaldia, K., Sánchez-González, L., Muneret, L., Jeandel, C., Hamdi, M., Desobry, S., 2014. Alginate coatings containing grapefruit oil or grapefruit seed extract for grapes preservation. Int. J. Food Sci. Technol. 49, 952–959.

Anal, A., Singh, H., 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery: a review. Trends Food Sci. Technol. 18, 240–251.

Arancibia, M., Giménez, B., López-Caballero, M.E., Gómez-Guillén, M.C., Montero, P., 2014. Release of cinnamon essential oil from polysaccharide bilayer films and its use for microbial growth inhibition in chilled shrimps. LWT: Food Sci. Technol. 59, 989–995.

Arzate-Vázquez, I., Chanona-Pérez, J., Calderón-Domínguez, G., Terres-Rojas, E., Garibay-Febles, V., Martínez-Rivas, A., Gutiérrez-López, G., 2012. Microstructural characterization of chitosan and alginate films by microscopy techniques and texture image analysis. Carbohydr. Polym. 87, 289–299.

Atarés, L., Chiralt, A., 2016. Essential oil as additives in biodegradable films and coatings for active food packaging. Trends Food Sci. Technol. 48, 51–62.

Azarakhsh, N., Osman, A., Ghazali, H.M., Tan, C.P., Adzahan, N.M., 2014. Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. Postharvest Biol. Technol. 88, 1–7.

Banerjee, S., Bhattachrya, J.H., 2011. Compressive textural attributes, opacity and syneresis of gels prepared from gellan, agar and their mixtures. J. Food Eng. 102, 287–292.

Barba, C., Eguinoa, A., Maté, J.I., 2015. Preparation and characterization of β-cyclodextrin inclusion complexes as a tool of a controlled antimicrobial release in whey protein edible films. LWT: Food Sci. Technol. 64, 1362–1369.

Bierhalz, A., da Silva, M., de Sousa, H., Braga, M., Kieckbush, T., 2013. Influence of natamycin loading methods on the physical characteristics of alginate active films. J. Supercrit. Fluids 76, 74–82.

Blümich, B., Pretsch, E., 2016. Introduction to compact NMR: a review of methods. Trends Anal. Chem. 83, Part A.

Casanova, H., Cardona, S., 2004. Emulsiones O/W estabilizadas con caseinato de sodio: Efecto de los iones calcio, concentración de proteína y temperatura. Vitae 11, 13–19.

CDCP (Center for Disease Control and Prevention), WHO (World Health Organization), 2003. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world., pp. 209–214.

Chiabrando, V., Giacalone, G., 2015. Effect of essential oil incorporated into an alginate-based edible coating on fresh-cut apple quality during storage. Qual. Assur. Saf. Crop 7, 251–259.

Deepa, B., Abraham, E., Pothan, L.A., Cordeiro, N., Faria, M., Thomas, S., 2016. Biodegradable nanocomposite films based on sodium alginate and cellulose nanofibrils. Materials 9, 50.

De'Nobili, M., Soria, M., Martinefski, M., Tripodi, V., Fissori, E., Rojas, A., 2016. Stability of L-(+)-ascorbic acid in alginate edible films loaded with citric acid for antioxidant food preservation. J. Food Eng. 175, 1–7.

Farroni, A., Matiacevich, S., Guerrero, S., Alzamora, S., Buera, M.P., 2008. Multi-level approach for the analysis of water effects in corn flakes. J. Agric. Food Chem. 56, 6447–6453.

Gammariello, D., Incoronato, A.L., Conte, A., Del Nobile, M.A., 2016. Effect of sodium alginate coating with ascorbic acid on shelf life of raw pork meat. J. Food Technol. Res. 3, 1–11.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R., 2007. Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res. Int. 40, 1107–1121.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. J. Res. Natl. Bur. Stand. Sect. A 81, 89–96.

Hanaa, A.R., Sallam, Y.I., El-Leitht, A.S., Aly, S.E., 2012. Lemongrass (*Cymbopogon citratus*) essential oil as affected by drying methods. Ann. Agric. Sci. 57, 113–116.

Hosseini, M.H., Razavi, S.H., Mousavi, M.A., 2009. Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. J. Food Process. Preserv. 33, 727–743.

Kavoosi, G., Dadfar, S.M.M., Purfard, A.M., 2013. Mechanical, physical, antioxidant, and antimicrobial properties of gelatin films incorporated with thymol for potential use as nano wound dressing. J. Food Sci. 78, 244–250.

Keawchaoon, L., Rangrong, Y., 2011. Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids Surf. B 84, 163–171.

Lawrie, G., Keen, I., Drew, B., Chandler-Temple, A., Rintoul, L., Fredericks, P., Grondal, H., 2007. Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS. Biomacromolecules 8, 2533–2541.

Luo, M.R., Cui, G., Rigg, B., 2001. The development of the CIE 2000 colour difference formula: CIEDE2000. Color Res. Appl. 26, 340–350.

Matiacevich, S., Silva, P., Osorio, F., Enrione, J., 2012. Evaluation of blueberry color during storage using image analysis. In: Caivano, J.L., Buera, M.P. (Eds.), Color in Food: Technological and Psychophysical Aspects. CRC Press, Boca Raton, pp. 211–218.

Matiacevich, S., Celis, D., Schebor, C., Enrione, J., 2013. Physicochemical and antimicrobial properties of bovine and salmon gelatin-chitosan films. CyTA-J. Food 11, 366–378.

Matiacevich, S., Acevedo, N., López, D., 2015a. Characterization of edible active coating based on alginate-thyme oil-propionic acid for the preservation of fresh chicken breast fillets. J. Food Process. Preserv. 39, 2792–2801.

Matiacevich, S., Riquelme, N., Herrera, M.L., 2015. Conditions to prolonged release of microencapsulated carvacrol on alginate films as affected by emulsifier type and pH. J. Appl. Polym. Sci. 2015, 1–12.

Naik, M.L., Fomda, B.A., Jaykumar, E., Bhat, J.A., 2010. Antibacterial activity of lemongrass (*Cymbopogon citrates*) oil against some selected pathogenic bacterias. Asian Pac. J. Trop. Med. 3, 535–538.

Navarro, R., Arancibia, C., Herrera, M.L., Matiacevich, S., 2016. Effect of type of encapsulating agent on physical properties of edible films based on alginate and thyme oil. Food Bioprod. Process. 97, 63–75.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., Bugarski, B., 2011. An overview of encapsulation technologies for food applications. Procedia Food Sci. 1, 1806–1815.

Pan, K., Chen, H., Davidson, M., Zhong, Q., 2014. Thymol nanoencapsulated by sodium caseinate: physical and antilisterial properties. J. Agric. Food Chem. 62, 1649–1657.

Pascall, M., Lin, S.J., 2013. The application of edible polymeric films and coatings in the food industry. J. Food Process. Technol. 4, 1–2.

Pedreschi, F., León, J., Mery, D., Moyano, P., 2006. Development of a computer vision system to measure the color of potato chips. Food Res. Int. 39, 1092–1098.

Ritota, M., Gianferri, R., Bucci, R., Brosio, E., 2008. Proton NMR relaxation study of swelling and gelatinization process in rice starch–water samples. Food Chem. 110, 14–22.

Rojas-Graü, M.A., Soliva-Fortuny, R., Martín-Belloso, O., 2009. Edible coatings to incorporate active ingredients to fresh-cut fruits: a review. Trends Food Sci. Technol. 20, 438–447.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G., Martín-Belloso, O., 2013. Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. LWT: Food Sci. Technol. 50, 240–246.

Rubilar, J.F., Cruz, R.M.S., Silva, H.D., Vicente, A.A., Khmelinskii, I., 2013. Physico-mechanical properties of chitosan films with carvacrol and grape seed extract. J. Food Eng. 115, 466–474.

Salmieri, S., Lacroix, M., 2006. Physicochemical properties of alginate/polycaprolactone-based films containing essential oils. J. Agric. Food Chem. 54, 10205–10214.

Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., Martín-Belloso, O., 2015. Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. Food Hydrocoll. 43, 547–556.

Sánchez-González, L., Vargas, M., González-Martínez, C., Chiralt, A., Cháfer, M., 2009. Characterization of edible films based on hydroxypropylmethylcellulose and tea tree essential oil. Food Hydrocoll. 23, 2102–2109.

Santana, A.A., Kieckbusch, T.G., 2013. Physical evaluation of biodegradable films of calcium alginate plasticized with polyols. Braz. J. Chem. Eng. 30, 835–845.

Sipahi, R.E., Castell-Pérez, M.E., Moreira, R.G., Gomes, C., Castillo, A., 2013. Improved multilayered antimicrobial alginate-based edible coating extends the shelf-life of fresh-cut watermelon (*Citrullus lanatus*). LWT: Food Sci. Technol. 51, 9–15.

Supardan, M.D., Annisa, Y., Arpi, N., Satriana, S., Mustapha, W.A.A., 2016. Cassava starch edible film incorporated with lemongrass oil: characteristics and application. Int. J. Adv. Sci. Eng. Inf. Technol. 6, 216–220.

Velázquez-Nuñez, M.J., Avila-Sosa, R., Palou, E., López-Malo, A., 2013. Antifungal activity of orange (*Citrus sinensis* var. Valencia) peel essential oil applied by direct addition or vapor contact. Food Control 31, 1–4.

Zhang, I., Cui, L., Che, X., Zhang, H., Shi, N., Li, C., Chen, Y., Kong, W., 2015. Zein-based films and their usage for controlled delivery: origin, classes and current landscape. J. Control Release 206, 206–219.