

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF BIOACTIVE CHITOSAN FILMS

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Abstract— In this work the preparation of chitosan films containing different bioactive compounds (gallic acid or salicylic acid) and plasticizers (sorbitol or Tween 80) was assessed. In addition, the component composition influence on the performance of chitosan-based films over antimicrobial and antioxidant activity were researched using the Response Surface Methodology from a Doehlert two-factor model. Both films (A: chitosan/gallic acid/Tween 80 and B: chitosan/salicylic acid/sorbitol) showed a good antioxidant capacity where a higher bioactive compound content and a low plasticizer concentration led to the best performance. Furthermore, the chitosan/gallic acid/Tween 80 film evidenced antimicrobial activity against *Escherichia coli* ATCC 25922. Chitosan-based films developed from formulation A and B showed a promising performance as bacteriostatic agent, which is sufficient evidence to assess these films as successful materials for food packaging.

Keywords— Chitosan, gallic acid, salicylic acid, antioxidant capacity

I. INTRODUCTION

The development of biodegradable food packaging has been a research topic of great interest in recent times, where the use of the chitosan biopolymer has been favored due to its capability to form films with an excellent biodegradability, biocompatibility, antimicrobial and anticancer properties and non-pulling features (Ruiz-Navajas *et al.*, 2013). This polysaccharide is obtained by deacetylation of chitin, a biopolymer that is abundant in a variety of crustacean shells, such as crab, crawfish and shrimp (Cho *et al.*, 2011). Chitosan is a promising compound intensively used as an alternative additive in agriculture, food and pharmaceutical industry (Abdelmalek *et al.*, 2017; Yuan *et al.*, 2019; Qu and Luo, 2020). These films can be modified with specific ingredients such as antioxidants, antimicrobial agents, flavors, spices or colorants, which improve the packaging performance by adding novel functions (Salmieri and Lacroix, 2006). In addition, in recent times the incorporation of bioactive hydroxybenzoic acid compounds such as gallic acid (GA) or salicylic acid (SA) in chitosan films has reached

a particular interest due to their antioxidant activity (Soleimani Aghdam *et al.*, 2016; Souza *et al.*, 2017). Gallic acid (3,4,5-trihydroxybenzoic acid) and its derivatives are widely present in the plant kingdom (blueberries, apples, flax and tea). These molecules represent a large family of plant secondary polyphenolic metabolites which are true natural antioxidants (Aruoma *et al.*, 1993; Chanwitheesuk *et al.*, 2007). Salicylic acid is widely used in various applications such as topical pharmaceutical products or food protectants (Singh *et al.*, 2010; Zhang, Zhang and Yang, 2015). In the present work, different bioactive chitosan films containing a bioactive compound (gallic acid or salicylic acid) and a plasticizer agent such as sorbitol or Tween 80 were prepared, and the chemical structure influence of the former over their antioxidant and antimicrobial activity was assessed.

II. METHODS

A. Materials

Low molecular weight chitosan (~100 kDa, 86.64 % of deacetylation degree, Parafarm, USA); glacial acetic acid (99.5 wt.-%, Cicarelli, Argentina); anhydrous gallic acid (98 wt.-%, Biopack, Argentina); salicylic acid (>99 wt.-%, Cicarelli, Argentina) sorbitol (SB) solution (70 wt.-%, Ingredion, USA); Tween (TW) 80 (p.a., Anedra, Argentina), Folin-Ciocalteu phenol reagent (p.a., Biopack, Argentina), sodium carbonate (99.5 wt.-%, Ciccarelli, Argentina), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (p.a., Sigma-Aldrich, USA) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (97 wt.-%, Sigma-Aldrich, USA) were the reagents used. Antimicrobial activity of the films was assessed against *Escherichia coli*-ATCC 25922, *Leuconostoc mesenteroides* MS1 isolated from industrial sausage (Serra *et al.*, 2018) and *Lactobacillus plantarum* ES147 isolated from raw cereal (Salvucci, LeBlanc and Pérez, 2016) and ATCC 8014.

B. Films preparation

Two kinds of films based on chitosan such as chitosan/salicylic acid/sorbitol and chitosan/gallic acid/Tween 80 were prepared. According to previous studies, the reaction mixture (100 g) was prepared dissolving 1.0 g of chitosan in acetic acid buffer at pH 4 (Raspo, Gomez and Andreatta, 2018). Then, different

concentrations of bioactive compound and the plasticizers were solubilized in the mixture under stirring at 25 °C. A bioactive compound concentration of 0.25, 0.50, 0.75 or 1 wt% with a sorbitol content of 1, 2.5, 5, 7.5 or 10 wt% or a Tween 80 concentration of 0.5, 1 or 3 wt% were used. In all cases, the reaction mixture was spread on Petri dish with a ratio of 0,16 mL of mixture per cm² of plate area. The film formation took place through self-assembly chitosan backbones during casting process after water evaporation at 25°C.

C. Antioxidant activity

Aqueous extracts were previously obtained to carry out antioxidant assays, where 3 mL of distilled water were left in contact with near 25 mg of film for 2h. Total phenolic compounds (TPC) was determined with the Folin-Ciocalteu reagent at pH 9 resulting in a blue coloration determined spectrophotometrically according Ivanova et al. (2011) with slight modifications and quantified as mg equivalents of gallic acid per mg of film. DPPH inhibition assay was determined as the equivalent antioxidant capacity of Trolox (TEAC) expressed as μmol of Trolox (Tr) per g of film, according to and Siripatrawan and Harte (2010).

D. Antimicrobial activity

The antibacterial activity by agar diffusion method (Clinical and Laboratory Standards Institute, 2013) of chitosan-based films and their compound mixture solutions were tested against a pathogenic bacterium such as *Escherichia coli* ATCC 25922 (Gram-negative), a food-borne bacterium as *Leuconostoc mesenteroides* MS1 (Gram-positive) and two beneficial bacteria *Lactobacillus plantarum* ES147 and ATCC 8014 (Gram-positive). *Escherichia coli* ATCC 25922, *Leuconostoc mesenteroides* MS1 and *Lactobacillus plantarum* ES147 and ATCC 8014 were grown on tryptic soy broth for 24 h at 37°C, de Man Rogosa and Sharpe broth (MRS) for 48 h at 30°C and MRS broth for 24 h at 37°C. Then, tryptic soy agar plates (*Escherichia coli*) or MRS agar plates (*Leuconostoc mesenteroides* MS1 and *Lactobacillus plantarum* ES147 and ATCC 8014) were inoculated with the respective bacterial inoculum.

The inoculation was prepared following the direct colony suspension method in a saline solution to obtain a 0.5 optical density in the Mc Farland scale, which is approximately equivalent to a 1.5×10^8 CFU/mL concentration. Chitosan films (sterilized with UV light) were cut into a disc shape of 15 mm diameter and then placed on the surface of MRS agar for *Lactobacillus plantarum* strains and *Leuconostoc mesenteroides* and on tryptic soy agar for *Escherichia coli*. After incubation for 24 h at 37° C for *Escherichia coli* and at 30° to *Lactobacillus plantarum* strains and *Leuconostoc mesenteroides*, the inhibition halo diameter of films was determined. In the case of film forming solutions, 10 μL of solution were previously placed on a 5mm diameter sterile paper disc (125 μm, Munktell), and their antimicrobial activity assessed using the same procedure described above.

E. Fourier transform infrared spectroscopy (FTIR)

Different chitosan films generated by self-assemble after incorporation of tween 80/gallic acid or sorbitol/salicylic acid were analyzed by Fourier transform infrared spectroscopy. FTIR spectra were performed using a Nicolet 5-SXC spectrophotometer (USA), recorded within a (4000 to 400) cm⁻¹ range with a 4 cm⁻¹ resolution, using air as background.

F. Response Surface Methodology (RSM)

The experimental design for the development of this work was carried out using the Response Surface Methodology from a Doehlert two-factor model (Doehlert, 1970). This mathematical model was selected to identify the interaction between the response variables studied (TPC and DPPH) and the independent variables. The two factors, i.e., the independent variables used in the formulation of films were sorbitol and salicylic acid concentration or Tween 80 and gallic acid concentration. The range of these variables is detailed in Material and Methods Section which were based on previous studies of antioxidant properties and solubilities (Raspo, Gomez and Andreatta, 2018). The response surface computation was performed using Statgraphics Centurion XVI software (v16.1, USA) and was estimated from experimental data with a confidence interval of 95%.

G. Statistical analysis

Results were expressed as the mean of three determinations. The data were analyzed by ANOVA and the results were compared by DGC test at a significance level of 0.05. All analyses were performed using the INFostat statistical software (Di Rienzo *et al.*, 2018).

III. RESULTS AND DISCUSSION

A. Film characterization

The liquid reaction mixture presented a quite homogeneous appearance due to the presence of a certain turbidity given by the bioactive compounds. However, once the casting process was finished the final films presented a very good appearance in terms of homogeneity, transparency and gloss, and being soft to the touch. The obtained films were characterized by FTIR, and their antioxidant and antimicrobial properties were analyzed.

B. Antioxidant activity

TPC and DPPH values were analyzed to obtain the antioxidant activity. Table 1 and 2 show the data of antioxidant capacity quantified for chitosan/salicylic acid/sorbitol and chitosan/ gallic acid/ Tween 80 film. The first two columns of Table 1 and 2 show the bioactive compound content employed in the reaction mixture while the third and four columns exhibit the final compound concentration in film after the solvent is evaporated.

Figure 1 shows the response surface of TPC for chitosan / salicylic acid / sorbitol and chitosan / gallic acid / Tween 80 formulations. It was found that both kind of films presented a similar performance where an increasing in the amount of the plasticizer and the bioactive compound determines a higher TPC value. This trend is consistent with the fact that film formation is based on the hydrogen bond interactions generated between small molecules of

plasticizer and bioactive compound with chitosan backbones, yielding a tridimensional crosslinked network. As a higher content of small molecules containing hydroxyl groups is used during film formation, only a fraction of these groups participates in the crosslinking of chitosan chains while a growing number of free molecules remain occluded within the mesh of the network formed.

Table 1. Antioxidant experimental values of chitosan/salicylic acid/sorbitol films at different compositions

Mixture		Film		TPC <i>mg GA</i> <i>g film</i>	DPPH <i>μmol Tr</i> <i>g film</i>
AS wt%	SB wt%	AS wt%	SB wt%		
0.00	0.00	0.00	0.00	1.31	a
0.00	1.00	0.00	41.35	1.70	a
0.00	2.50	0.00	64.04	0.67	b
0.00	5.00	0.00	78.39	0.75	b
0.00	7.50	0.00	84.71	0.6	b
0.00	10.00	0.00	88.27	0.19	a
0.25	0.00	20.04	0.00	1.55	a
0.25	1.00	12.88	36.07	1.11	b
0.25	2.50	8.39	58.72	0.62	b
0.25	5.00	5.31	74.27	0.87	b
0.25	7.50	3.88	81.46	0.37	a
0.25	10.00	3.06	85.60	0.50	a
0.50	0.00	33.44	0.00	8.03	b
0.50	1.00	22.85	31.99	3.17	b
0.50	2.50	15.49	54.22	2.15	b
0.50	5.00	10.08	70.56	1.01	c
0.50	7.50	7.47	78.45	0.27	c
0.50	10.00	5.93	83.09	0.26	d
0.75	0.00	43.04	0.00	8.36	d
0.75	1.00	30.79	28.74	5.16	d
0.75	2.50	21.58	50.36	4.75	c
0.75	5.00	14.40	67.21	1.84	c
0.75	7.50	10.81	75.65	0.94	d
0.75	10.00	8.65	80.71	0.50	d
1.00	0.00	50.25	0.00	7.17	d
1.00	1.00	37.27	26.09	7.25	c
1.00	2.50	26.86	47.01	7.01	c
1.00	5.00	18.33	64.16	2.20	d
1.00	7.50	13.91	73.04	0.95	d
1.00	10.00	11.21	78.48	0.43	d

Standard deviations determined for AS or SB = 0.01 wt%, TPC = 0.44 mg GA/g film and for DPPH = 34.1 μmol Tr/g film.
n.a.: not available.

For all the tables, values followed by the same letter within a column show no significant differences (P < 0.05).

Table 2. Antioxidant experimental values of chitosan-gallic acid-Tween 80 films at different compositions

Mixture		Film		TPC <i>mg GA</i> <i>g film</i>	DPPH	
GA wt%	TW wt%	GA wt%	TW wt%		<i>μmol Tr</i> <i>g film</i>	
0.00	0.50	0.00	33.44	5.49	a	n.a.
0.00	1.00	0.00	50.25	1.00	b	25.0
0.25	0.50	14.35	28.69	10.8	c	159
0.25	1.00	11.17	44.69	9.51	d	426
0.50	0.50	25.13	25.13	32.4	c	264
0.50	1.00	20.12	40.24	7.42	d	229
0.50	3.00	11.20	67.19	12.0	e	445
0.75	1.00	27.45	36.60	33.4	f	247
0.75	3.00	15.92	63.66	39.0	g	450
1.00	1.00	33.56	33.56	37.0	f	275
1.00	3.00	20.16	60.48	36.2	g	380

Standard deviations calculated were for AS or SB = 0.01 wt%, TPC = 1.21 mg GA/g film while for DPPH = 26.6 μmol Tr/g film. n.a.: not available.

The letters (a-g) are the results of the statistical analysis: values followed by the same letter within a column show no significant differences (P < 0.05).

Figure 2 shows the response surface of DPPH expressed in TEAC for each film type. For chitosan/salicylic acid/sorbitol film this parameter has different trends depending on the SB content evaluated. When the SB concentration is null, the DPPH value rises as the bioactive compound amount increases while the DPPH parameter reaches a maximum value within 0.1 - 35 wt% SB range. DPPH value decreases with higher AS concentration in the formulation for SB contents higher than 35 wt%. This behavior suggests that the interaction between plasticizing agent and the bioactive molecule takes place from the hydrogen bonds formation. The structuring of the bioactive compound by self-arrangement with chitosan backbones produces in these conditions an antioxidant activity decrease due to the lesser diffusion of the former. A similar phenomenon is observed for chitosan/gallic acid/Tween 80 films where the DPPH parameter rises with the increase of GA concentration for a 25 wt% of TW content. However, within the analyzed TW range between 25 and near 75 wt% the DPPH parameter reaches a maximum value centered between 10 and 30 wt% of GA content. Finally, DPPH decreases for a higher GA concentration at 75 wt% of TW due to crosslinked structure formation between chitosan, GA, and TW.

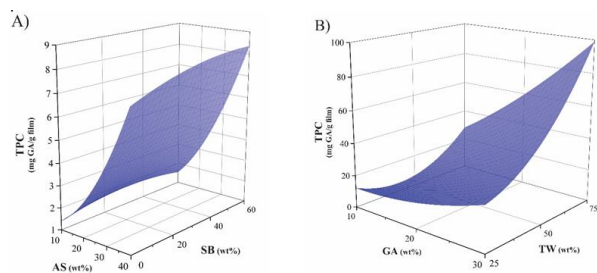


Figure 1. Response surface of TPC for chitosan/salicylic acid/sorbitol (A) and chitosan/gallic acid/Tween 80 (B) films. The values wt% corresponds to the final compound concentrations in the film attained.

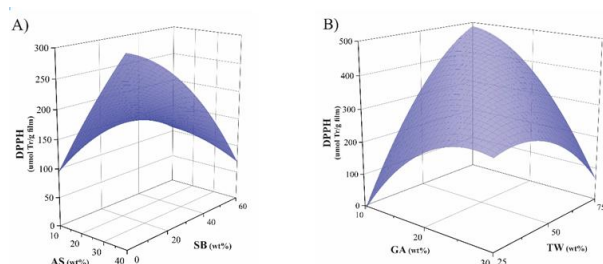


Figure 2. Response surface of DPPH expressed by TEAC for chitosan/salicylic acid/sorbitol (A) and chitosan/gallic acid/Tween 80 (B) films.

C. Fourier transform infrared spectroscopy (FTIR)

FTIR spectrometry was used to evaluate the modification of chitosan backbones interaction after the incorporation of salicylic acid, gallic acid, Tween 80, or sorbitol. Figure 3 shows the FTIR spectra of four films based on chitosan with different contents of plasticizer and bioactive molecule. The profile of the spectral curves shows characteristic signals of functional groups belonging to the components of each film. The characteristic bands of chitosan such as stretching vibrations of O-H and N-H overlap within range of $3550\text{--}3200\text{ cm}^{-1}$, and $\text{C}_{\text{sp}^3}\text{-H}$ at 2940 cm^{-1} of alkyl groups are shown in Fig. 4. In addition, the signal at 1640 cm^{-1} corresponding to $\text{C}=\text{O}$ stretching band of amide and the flexion N-H at 1550 cm^{-1} of amino group were observed. However, the bands of O-H stretching and N-H flexion vibration of chitosan, were strongly affected by the interaction with the active compound and the plasticizer in the mixture. In all case, the band of N-H flexion vibration exhibits a shift from near 1550 cm^{-1} , which is mostly associated to the formation of hydrogen bond with hydroxyl and carbonyl groups of the incorporated compounds. Moreover, the FTIR spectrum of film containing TW and GA shows a decrease in the O-H stretching band which supports the formation of hydrogen bond interaction between chitosan's amino and hydroxyl groups and the active compound and plasticizer. The small size of these compounds compared to the size of chitosan favors their diffusion and distribution in liquid bulk and the hydrogen bond

formation with chitosan backbones. Therefore, it is expected that the active compound and the plasticizer play a relevant role in the final properties of the chitosan film obtained from these non-covalent interactions.

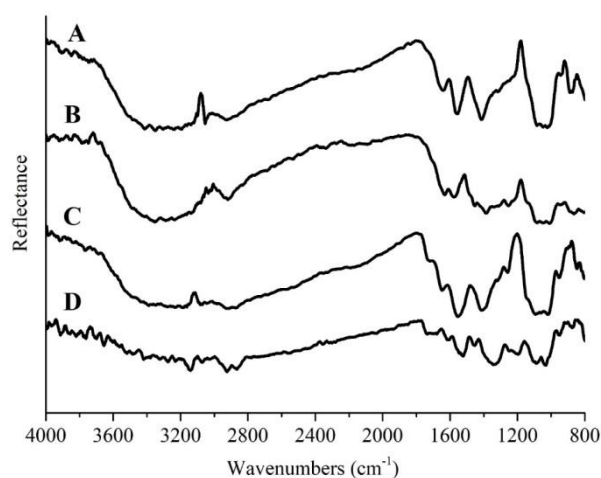


Figure 3. FTIR spectra of chitosan-based films containing 5 wt% SB (A), 5 wt% SB / 1wt% SA (B), 1 wt% TW (C) and 1 wt% TW / 1 wt% GA (D)

D. Antimicrobial activity

Considering the trends found for antioxidant properties, two formulations at the highest concentrations of bioactive compound and an intermediate concentration of plasticizers were assessed against the film antimicrobial activity. One of them, the formulation A constituted by chitosan / salicylic acid 1 wt% / sorbitol 5 wt% while the formulation B was composed of chitosan / gallic acid 1wt% / Tween 80 1wt%. Furthermore, liquid controls such as gallic acid and salicylic acid solutions were tested. Antimicrobial activity of films based on chitosan was assessed against *Escherichia coli* ATCC 25922 (Gram-negative), foodborne bacteria as *Leuconostoc mesenteroides* MS1 (Gram-positive) and two beneficial bacteria *Lactobacillus plantarum* ES147 and ATCC 8014 (Gram-positive). Serra et al. (2018) reported that no antimicrobial activity was found for gallic acid solutions while salicylic acid solution showed bactericide action against *Leuconostoc mesenteroides* MS1. In this work, it was found that neither gallic acid or salicylic acid solution were able to inhibit the growth of *Lactobacillus plantarum* ES147 and ATCC 8014, and *Escherichia coli* ATCC 25922.

On the other hand, when evaluating the antimicrobial activity of film forming solutions embedded over a circular support (disk) results in no zone of inhibition on agar solid medium for any bacteria strain, irrespective of bioactive compound and plasticizers used and neither the liquid controls. In addition, the films of pure chitosan, chitosan + sorbitol and chitosan + Tween 80 were evaluated as control and a no inhibition zone was found on all bacteria strain assessed. However, it has to be highlighted that there was no bacterial growth under the film indicating that chitosan-based films can exhibit bacteriostatic

effect against *Leuconostoc mesenteroides* MS1, *Escherichia coli* ATCC 25922 and *Lactobacillus plantarum* ES147 and ATCC 8014.

The same results were observed on formulation B with exception for *Escherichia coli* where a notable bacteria number diminution was observed although no clear inhibition halo was registered (Figure 4). This behavior can be explained in terms of the diffusion of the free bioactive compound occluded within crosslinked chitosan backbones, and then, on the agar plate.

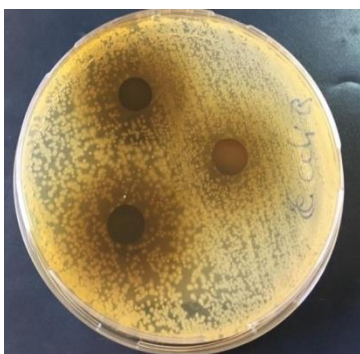


Figure 4. Bacteria number diminution for Formulation B: chitosan/gallic acid 1wt%/Tween 80 1wt% against *Escherichia coli* ATCC 25922.

IV. CONCLUSIONS

The chitosan-based films exhibited a good appearance in terms of homogeneity, color, and brightness. The trends obtained allowed to establish what characteristics are necessary for the development of bioactive chitosan films for a specific application. Considering the antioxidant capacity evidenced by these chitosan-based films where a higher bioactive compound content and a low plasticizer concentration led to the best performance. The films obtained from chitosan/gallic acid 1 wt%/Tween 80 1 wt% showed a decreasing number of *Escherichia coli* ATCC 25922 bacteria although no clear inhibition halo was observed. Chitosan-based films developed from formulation A and B shown a promising performance as a bacteriostatic agent, which is sufficient evidence to assess these films as successful materials for food packaging.

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REFERENCES

Abdelmalek, B. E. *et al.* (2017) β -chitin and chitosan from squid gladius: Biological activities of chitosan and its application as clarifying agent for apple juice. *International Journal of Biological Macromolecules*. 104, 953–962.

Aruoma, O. I. *et al.* (1993) Evaluation of the Antioxidant

- and Prooxidant Actions of Gallic Acid and Its Derivatives. *Journal of Agricultural and Food Chemistry*. 41, 1880–1885.
- Chanwitheesuk, A. *et al.* (2007) Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk.. *Food Chemistry*. 100(3), 1044–1048.
- Cho, Y. S. *et al.* (2011) Preparation, characterization, and antioxidant properties of gallic acid-grafted-chitosans. *Carbohydrate Polymers*. 83(4), 1617–1622.
- Clinical and Laboratory Standards Institute (2013) M100-S23 Performance standards for antimicrobial susceptibility testing. *CLSI document M100-S23*, 1–35.
- Doehlert, D. H. (1970) Uniform Shell Designs. *Journal of the Royal Statistical Society*, 19(3), 231–239.
- Ivanova, V. *et al.* (2011) Polyphenolic content of Vranec wines produced by different vinification conditions. *Food Chemistry*, 124(1), 316–325.
- Qu, B. and Luo, Y. (2020) Chitosan-based hydrogel beads: Preparations, modifications and applications in food and agriculture sectors – A review. *International Journal of Biological Macromolecules*. 437–448.
- Raspo, M. A., Gomez, C. G. and Andreatta, A. E. (2018) Optimization of antioxidant, mechanical and chemical physical properties of chitosan-sorbitol-gallic acid films by response surface methodology. *Polymer Testing*. 70.
- Di Rienzo, J. A. *et al.* (2018) InfoStat versión 2018. Córdoba, Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Available at: <http://www.infostat.com.ar>.
- Ruiz-Navajas, Y. *et al.* (2013) In vitro antibacterial and antioxidant properties of chitosan edible films incorporated with *Thymus moroderi* or *Thymus piperella* essential oils. *Food Control*. 30(2), 386–392.
- Salmieri, S. and Lacroix, M. (2006) Physicochemical Properties of Alginate / Polycaprolactone-Based Films Containing Essential Oils AND. *Journal of Agricultural and Food Chemistry*. 54(10205), 10214.
- Salvucci, E., LeBlanc, J. G. and Pérez, G. (2016) Technological properties of Lactic acid bacteria isolated from raw cereal material. *LWT - Food Science and Technology*. 70, 185–191.
- Serra, M. *et al.* (2018) Assessment of natural vegetal extracts in the inhibition of *Leuconostoc mesenteroides* MS1. *Agriscientia*. 35, 51–62.
- Singh, N. *et al.* (2010) Physical properties of zein films containing salicylic acid and acetyl salicylic acid. *Journal of Cereal Science*. 52(2), 282–287.
- Siripatrawan, U. and Harte, B. R. (2010) Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids*. 24(8), 770–775.
- Soleimani Aghdam, M. *et al.* (2016) Impact of salicylic acid on postharvest physiology of fruits and

- vegetables. *Eco-Friendly Technology for Postharvest Produce Quality*. 243–268.
- Souza, V. G. L. *et al.* (2017) Physical properties of chitosan films incorporated with natural antioxidants. *Industrial Crops and Products*. 107(May), 565–572.
- Yuan, X. *et al.* (2019) A review on the preparation of chitosan oligosaccharides and application to human health, animal husbandry and agricultural production. *Carbohydrate Polymers*. 60–70.
- Zhang, Y., Zhang, M. and Yang, H. (2015) Postharvest chitosan-g-salicylic acid application alleviates chilling injury and preserves cucumber fruit quality during cold storage. *Food Chemistry*. 174(September), 558–563.