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Application of thermosonication for *Aloe vera* (*Aloe barbadensis* Miller) juice processing: Impact on the functional properties and the main bioactive polysaccharides

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ABSTRACT

The impact of thermosonication on the functional properties and the main polysaccharides from *Aloe vera* was investigated. Thermal processing was used for comparison purposes. Acemannan was the predominant polysaccharide in *Aloe vera* juice followed by pectins. Interestingly, thermosonication promoted a minor degradation of the acetylated mannose from acemannan than thermal processing. On the other hand, the degree of methylesterification of pectins was slightly reduced as a consequence of thermosonication. Further, swelling and fat adsorption capacities were improved by thermosonication. Thus, the highest values for swelling (> 150 mL/g AIR) and for fat adsorption capacity (~120 g oil/g AIR) were observed when thermosonication was performed at 50 °C for 6 min. Moreover, high inactivation of *L. plantarum* (~75%) was observed when thermosonication was carried out at 50 °C for 9 min. Interestingly, thermosonication promoted a similar color change ($\Delta E = 7.7$) to the modification observed during pasteurization carried out at 75 °C for 15 min ($\Delta E = 8.2 \pm 0.9$). Overall, these results suggested that thermosonication could be a good alternative to thermal procedures of *Aloe vera* juice, since not only caused minor degradation of bioactive polysaccharides but was also able to improve functional properties.

1. Introduction

Aloe vera gel, the mucilaginous aqueous extract of the hydroparenchyma of the succulent leaves of *Aloe vera* plant, has been considered as a raw material of great interest for cosmetic and pharmaceutical industries, representing a new source of compounds with exceptional biological activities [1]. The gel is composed of high amounts of water (> 98%) and a remaining portion mainly composed of polysaccharides, over 60% (w/w), being acemannan the predominant polysaccharide [2]. Acemannan, considered as the main bioactive component of the *Aloe vera* mucilage, is a storage polymer located within the protoplast of the parenchymatous cells [2]. This polysaccharide is mainly composed of acetylated mannose units linked by β-(1 → 4) glycosidic bonds [2–4]. The acetyl groups, the only non-sugar functional groups present in acemannan, seem to play a key role in the physico-chemical properties and biological activity of *Aloe vera* [3,5].

For the last decades, the use of *Aloe vera* gel has been extended to the food industry, mainly as a resource of functional ingredients, especially used for the preparation of healthy food drinks and other beverages, including *Aloe vera* juice. In Aloe industry, thermal procedures are probably the most common methods used for processing *Aloe vera* juice, being pasteurization the most important procedure used for juice production. The high efficiency of pasteurization is due to the elimination of the pathogenic microorganisms and deteriorative enzymes, extending the shelf life of most fluid food products, such as fruit juices [6]. However, thermal procedures tend to promote irreversible modifications to the original structure of the *Aloe vera* polysaccharides, affecting their physiological and pharmacological properties [6–8]. Thus, innovative techniques suitable for food processing such as

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ultrasounds have been explored with the aim of obtaining good results with the application of little or no heat [9].

Ultrasounds (US) have been recognized as a technology with great potential for its application in the food industry. US can be classified as environmentally friendly since the generated sound waves are generally considered as safe and non-toxic [10,11]. Indeed, several studies have been carried out relating the processing of fruit juices using US technology as an alternative for the total or partial substitution of thermal processing procedures [12]. Recently, it has been demonstrated that thermosonication (US combined with mild or low temperature) could not only improve the physico-chemical and functional properties, but also extend the shelf life of different food products [13–16].

Within this context, *Aloe vera* juice could be an interesting material to test the efficiency of US in food processing in order to preserve bioactive components and related functional properties. Therefore, the main aim of this study was to evaluate the effect of thermosonication on the main bioactive polysaccharides from *Aloe vera*, in particular acemannan polymer and cell wall polysaccharides, and also on the functional properties. Further, a conventional thermal procedure was also applied on *Aloe vera* juice for comparison purposes.

2. Materials and methods

2.1. Preparation of the Aloe vera juice

Aloe vera leaves, used as a raw material, were supplied by AMB Wellness Company (Gomez Palacio, Durango, Mexico). Leaves of 3-year-old were selected according to uniform size, shape and color. Prior to the gel extraction, the leaves were washed with a sodium hypochlorite solution (200 ppm) and distilled water. The *Aloe vera* gel was manually extracted as described by Rodríguez-González et al. [6]. The juice was extracted from the gel and filtered throughout a nylon-cloth in order to remove most of the fibrous material. The juice was collected and stored at 4 °C for 8 h prior to processing.

2.2. Processing

2.2.1. Thermosonication processing

Aloe vera juice was subjected to thermosonication as previously described by Saeeduddin et al. [13] with some modifications.

Approximately 150 mL of *Aloe vera* juice was placed into a double jacket vessel (200 mL). A 750 W ultrasonic processor (Sonics VCX750, Materials Inc., Newtown, USA) with a 13 mm diameter probe was used for sonication. The juice was processed at a constant frequency of 20 kHz with an amplitude of 50% and pulse duration of 5 s on and 5 s off. The thermosonication was carried out at two different temperatures, 25 and 50 °C, for three different times, 3, 6 and 9 min. Hence, total processing times were 6, 12 and 18 min. The temperature of the samples was controlled by recirculating water at 20 ± 2 °C (VWR Scientific Model 1166, Niles, IL). The ultrasound probe was introduced into the sample to a depth of 1 cm. All treatments were carried out in triplicate.

In order to determine the power that was acting on the sample, the power density values were determined using a calorimetric method, using the Eq. (1) described by Minjares-Fuentes et al. [17].

$$P = m{\cdot}C\rho{\cdot}\frac{\Delta T}{\Delta t} \tag{1}$$

where P is the ultrasonic power (W), m is the mass of the sample (kg), Cp is the specific heat capacity (J·kg^{-1}·K^{-1}), and $\frac{\Delta T}{\Delta t}$ is the temperature (K) change as a function of time (s). The power density was of 0.213 W·mL^{-1}.

In addition, the ultrasound energy consumption was determined according to Perrier et al. [18]. Thus, the energy consumption W_{US} (kJ·kg^{-1}) could be obtained by using the following Eq. (2):

$$W_{US} = \frac{P * d}{m} \times 10^3 \tag{2}$$

where P is the ultrasonic generator power (P = 750 W), d is the treatment duration (s), m is the mass of the treated samples (kg). Therefore, the energy consumption during processing, taking the longest processing time (18 min) as a reference time, was of 5.4^3 kJ·kg^{-1}.

2.2.2. Thermal processing

Thermal processing was only carried out for comparison purposes. Approximately 150 mL of *Aloe vera* juice were heated at 75 °C for 15 min in a double jacket vessel in order to carry out a conventional pasteurization [6]. Furthermore, two *Aloe vera* juices (150 mL) were heated at 25 °C and 50 °C for 18 min as in the thermosonication procedure. The temperature was measured using a thermocouple and the time was calculated when the inside sample temperature was homogeneous. Finally, the *Aloe vera* juice was cooled up to 10 °C and stored at 4 °C until posterior analysis.

Fresh *Aloe vera* juice without any processing was used as a reference.

2.3. Color

The color was measured using a Konica Minolta colorimeter (CR-400) calibrated with black and white standards. The values of the different color parameter such as lightness (L), greenness/redness (± a*), and blueness/yellowness (± b*) were directly recorded for each juice sample. The chroma parameter (C), indicating color intensity, was calculated using Eq. (3).

$$C = (a^{*2} + b^{*2})^{1/2} \tag{3}$$

Total color difference or change between processed and reference samples was also calculated using Eq. (4):

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \tag{4}$$

where L_0^*, a_0^* and b_0^* are the values of the reference sample, and L^*, a^* and b^* the measured values corresponding to the processed sample. All parameters were measured at least in triplicate for each treatment.

2.4. Alcohol insoluble residues (AIRs)

AIRs from fresh and processed *Aloe vera* juice were obtained by immersing the samples in boiling ethanol (final concentration 85% (v/v) aqueous) as described in Rodríguez-González et al. [6].

2.5. Determination of functional properties

The functional properties determined included hydration properties such as, swelling (Sw) and water retention capacity (WRC), and fat adsorption capacity (FAC). As previously described by Minjares-Fuentes et al. [8], Sw and WRC of AIRs, obtained from the reference and processed samples, were measured in phosphate buffer (1 M; pH 6.3) in order to simulate pH and the buffering conditions of food products.

2.5.1. Swelling

Sw was measured as bed volume after equilibration in an excess of solvent. The samples (0.01–0.10 g) were weighed into a graduated conical tube with an excess of buffer. The suspensions were stirred and after equilibration (16 h) the volumes were recorded and expressed as mL/g AIR.

2.5.2. Water retention capacity

WRC was measured as the water retained by the AIR samples after centrifugation. Samples (0.01–0.10 g) were suspended (24 h) in phosphate buffer (5 mL) and centrifuged (18,000g; 15 min). The residual solids in the supernatant were recovered by filtration (GF/C paper) and

recombined with the pellet. The pellet was weighed (P_1), and dried at 102 °C overnight. After cooling the dry weight was determined (P_2) and hence WRC was calculated using the Eq. (5) proposed by Femenia et al. [19]:

$$WRC = \frac{P_1 - P_2}{P_2 - k} \quad (5)$$

where $k = \alpha(P_1 - P_2)$ with $\alpha = 0.028$ g phosphate/mL.

All WRC results were expressed as g H_2O/g AIR.

2.5.3. Fat adsorption capacity

FAC was measured as the oil retained for the AIR samples after centrifugation. AIRs (0.01–0.10 g) were mixed with sunflower oil (5 mL), left overnight at room temperature and centrifuged at 18,000*g* for 10 min. The excess supernatant was decanted and FAC was expressed as g oil/g AIR.

2.6. Analysis of the main polysaccharides from Aloe vera

2.6.1. Isolation of water-soluble polysaccharides (WSP)

Isolation of WSP was carried out as described in Minjares-Fuentes et al. [20] with slight modifications. AIR preparations from reference, thermally processed and thermosonicated *Aloe vera* juice (100 mg) were suspended in distilled water (250 mL) and stirred for 2 h at room temperature. The suspension was then centrifuged at 18,500*g* during 1 h at 20 °C. The water-soluble and insoluble fractions were collected and lyophilized. The lyophilized extracts were stored in anhydrous conditions until posterior analysis. The mannose-containing-polysaccharide detected in the *Aloe vera* filet and gel water fractions, has been previously identified as the bioactive polysaccharide acemannan whereas the water insoluble material is mainly comprised by cell wall polymers [2,6].

2.6.2. Carbohydrate composition

Carbohydrate analysis was performed as described Minjares-Fuentes et al. [20] for neutral sugars. Sugars were released from residues by acid hydrolysis. About 5 mg of AIR preparations from *Aloe vera* samples were suspended in 12 M H_2SO_4 solution for 3 h followed by dilution to 1 M and hydrolyzed at 100 °C for 2.5 h [21]. A second sample, from AIRs preparations, was hydrolyzed only with 1 M H_2SO_4 (100 °C for 2.5 h). All the hydrolysis procedure was carried out in closed Pyrex culture tubes placed in a heating block; the samples were stirred every 20 min. The cellulose content was estimated by the difference in glucose obtained by Saeman hydrolysis and the hydrolysis carried out with 1 M H_2SO_4 for 2.5 h. Neutral sugars were derivatized as their alditol acetates and isothermally separated at 220 °C by GC with a FID detector and equipped with a 30 m column DB-225 (J&W Scientific, Folsom, CA, USA) with i.d. and film thickness of 0.25 mm and 0.15 μm, respectively. Uronic acids were determined by colorimetry, as total uronic acids [22], using a sample hydrolyzed for 3 h at 20 °C in 12 M H_2SO_4, followed by 1 h at 100 °C in 1 M H_2SO_4.

2.6.3. Fourier transformed infrared (FTIR) spectroscopy analysis

FTIR spectra of the different *Aloe vera* juice samples were obtained using a FTIR spectrometer (Bruker Tensor 27, Massachusetts, USA) as previously described in Minjares-Fuentes et al. [23]. The frequency range used varied from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.7. Microbiology assay

The *Lactobacillus plantarum* C1R1 strain, isolated from plum fruit juice, was supplied by the Chemistry Engineering Department of the School of Chemistry from the Universidad Autónoma de Coahuila. The stocks were prepared by inoculating the starter culture into deMan, Rogosa and Sharpe (MRS) agar (Difco™) and incubated at 37 °C for 48 h. Following to this, *L. plantarum* C1R1 culture was transferred into 21 mL MRS Broth (Difco™) and incubated at 37 °C for 18 h to produce a working stock bacterial culture.

Before innoculation, *Aloe vera* juice was sterilized in an autoclave at 121 °C for 15 min and refrigerated at 8 °C until processed. Inoculum with microbial loads of approximately 10^6 CFU/mL for *L. plantarum* was used to inoculate the sterile juice. This bacteria suspension was used in order to evaluate the effectiveness of thermosonication to eliminate these bacteria from *Aloe vera* juice. Thermal processed samples were also inoculated for comparison purposes.

2.7.1. Enumeration of L. plantarum

Sterile distilled water was used for sample dilution. Each sample was serially diluted (1:10) and 0.1 mL of the appropriate dilutions were plated in duplicate on MRS agar by incubating the surface plated dishes at 37 °C for 48 h. *L. plantarum* populations were manually counted and the results were expressed as the number of logarithmic reductions (−Log [final population/initial population]).

2.8. Statistical analysis

The effect of the different treatments was statistically evaluated by a one-way analysis of variance (ANOVA) with a $p < 0.05$. Further, the Tukey-Kramer test was used as a post-hoc test with a significant level of $\alpha = 0.05$. All calculations and graphics were performed using NCSS software version 2007 and Sigma-plot 10.0 software, respectively.

3. Results and discussion

3.1. Color

Color constitutes an important tool in the evaluation of quality and nutritional losses of liquid foods during processing and/or subsequent storage [12]. Particularly, the color of *Aloe vera* tends to change when is processed as consequence of degradation processes [24]. Thus, the color of thermally and thermosonicated *Aloe vera* juices was measured through the CIEL* a* b* color coordinates [15]. As it can be seen in Table 1, all thermosonicated samples and, also, the sample processed at 75 °C for 15 min (pasteurized sample) were significantly brighter than the reference juice ($p < 0.05$). Likewise, the chroma (C) parameter exhibited higher values for thermosonicated and pasteurized *Aloe vera* juices than the reference sample and, also, than juices processed at mild temperatures (25 and 50 °C) ($p < 0.05$). Interestingly, no significant differences were observed between thermosonicated juices and the pasteurized juice. Overall, the C parameter for thermosonicated samples ranged from 4.3 to 5.7, while the reference sample presented a C value of 3.5.

On the other hand, the visual color differences were evaluated using the ΔE parameter which quantifies the potential color modification of the sample promoted by processing in comparison with the reference sample. As it can be seen in Table 1, the thermally processed samples, excluding pasteurized sample (75 °C for 15 min), exhibited a minor visual color change (ΔE = 1.5) in comparison with the thermosonicated samples (ΔE > 6.0). Interestingly, thermosonicated samples at 25 °C showed a wide ΔE range (between 6.2 and 9.3), whereas samples processed at 50 °C exhibited a closer range comprised between 7.3 and 7.8.

3.2. Effect of thermosonication on the functional properties of Aloe vera juice

Some health benefits associated to *Aloe vera*, such as hypocholesterolemia and hypoglycemia, have been closely related to the functional properties attributed to Aloe polysaccharides, in particular swelling, water retention and fat adsorption capacities [25]. However, it is well known that those properties may be altered during chemical, mechanical and thermal processing [3,8,23,26]. For this reason, the

Table 1
CIEL* a* b* color coordinates and chrome values for *Aloe vera* juices: reference, thermal processing and thermosonication (0.213 W·mL^{-1}).

Sample		L	a*	b*	C	ΔE
Reference		38.7 ± 0.1	0.89 ± 0.1	3.4 ± 0.1	3.5 ± 0.0	–
Thermal processing						
T (°C)	t (min)					
75	15	46.9 ± 0.9	0.91 ± 0.0	4.3 ± 0.1	4.4 ± 0.1	8.2 ± 0.9
50	9	37.9 ± 0.0	1.65 ± 0.1	3.1 ± 0.0	3.5 ± 0.0	1.5 ± 0.1
25	9	39.6 ± 0.2	1.34 ± 0.1	3.1 ± 0.0	3.4 ± 0.1	1.5 ± 0.2
Thermosonication						
T (°C)	t (min)					
50	3	46.3 ± 0.9	−0.13 ± 0.0	4.6 ± 0.2	4.7 ± 0.3	7.8 ± 0.9
50	6	46.1 ± 1.1	−0.04 ± 0.0	4.3 ± 0.0	4.3 ± 0.1	7.5 ± 1.1
50	9	45.8 ± 1.3	1.99 ± 0.1	4.6 ± 0.1	5.0 ± 0.1	7.3 ± 1.2
25	3	47.0 ± 1.0	−0.30 ± 0.0	5.1 ± 0.1	5.1 ± 0.1	8.5 ± 1.0
25	6	47.9 ± 1.2	−0.06 ± 0.0	4.7 ± 0.1	4.7 ± 0.1	9.3 ± 1.2
25	9	44.7 ± 1.9	−0.05 ± 0.0	5.2 ± 0.1	5.2 ± 0.1	6.2 ± 1.9

L (lightness, 0 = black, 100 = white); a* (−a* = greenness, +a* = redness) and b* (−b* = blueness, +b* = yellowness).

functional properties of AIRs from *Aloe vera* fresh juice, thermally processed and those were thermosonication was applied, were determined.

The functional properties of polysaccharides from *Aloe vera* juice are presented in Fig. 1. As it can be seen, for the properties related to hydration, Sw and WRC, the values determined for the reference sample of *Aloe vera* juice were of ~46 mL/g AIR and ~20 g H_2O/g AIR, respectively; while FAC value was of ~49 g oil/g AIR. Interestingly, significant changes in the functional properties were observed in all *Aloe vera* juices processed, either thermally processed or thermosonicated ($p < 0.05$).

Thus, Sw values increased for most of the thermally processed *Aloe vera* juices, excluding those juices processed at 75 °C for 15 min, which exhibited a decrease to ~36 mL/g AIR ($p < 0.05$). On the other hand, thermosonication promoted higher Sw values, reaching up to ~100 and ~156 mL/g AIR in juices thermosonicated at 25 and 50 °C for 6 min, respectively (see Fig. 1a). Interestingly, Sw value of *Aloe vera* juice was increased to around ~200% with the application of US. This increase was higher than the previously reported by Rodríguez-González et al. [6], who observed that the pasteurization process increased Sw values up to 50% in comparison to fresh *Aloe vera* juices. Also, these authors pointed out that the Sw values of those *Aloe vera* samples were exceptionally high. Further, several authors have exposed that the swelling capacity of polysaccharide-rich materials might be linked with their ability to reduce blood cholesterol [27–29].

Regarding to the WRC, processing, either thermal or thermosonication promoted a significant increase in all samples ($p < 0.05$). In general, the samples exhibited a wide range of WRC values, varying from ~20 to ~50 g H_2O/g AIR (Fig. 1b). Interestingly, the WRC values of *Aloe vera* juices treated by thermosonication, both processed at 25 °C for 6 min (49 ± 4 g H_2O/g AIR) and for 9 min at 50 °C (48 ± 2 g H_2O/g AIR), were similar to WRC value determined for the sample processed at 50 °C for 9 min but without ultrasonic assistance (49 ± 4 g H_2O/g AIR). It is important to highlight that the WRC of polymer-rich products has been associated to the reduction of blood glucose, and also to laxative effects [27,29–31]. Noteworthy, thermosonication carried out at 25 °C for 9 min promoted a significant decrease of WRC (50%) ($p < 0.05$) which could be attributed to the generation of water-soluble low molecular weight polymer chains as consequence to the scission of cell wall polysaccharide chains promoted by acoustic energy [32].

Finally, the ability of polysaccharides from *Aloe vera* juice to hold organic molecules, such as lipid molecules, was strongly affected by thermosonication. FAC values of the thermosonicated *Aloe vera* samples varied from ~30 g oil/g AIR, for the juice treated at 25 °C for 9 min, up to 120 g oil/g AIR for *Aloe vera* juice treated at 50 °C for 6 min (Fig. 1c). On the contrary, thermal processing carried out at mild temperatures (25 and 50 °C) for 9 min promoted a FAC value of ~49 g oil/g AIR, whereas for juice processed at 75 °C for 15 min, FAC value decreased to ~27 g oil/g AIR. Previously, Rodríguez-González et al. [26] reported that polysaccharides from *Aloe vera* pasteurized at optimal conditions (70 °C for 15 min) were able to adsorb ~33 g oil/g AIR. Therefore, it should be pointed out that thermosonication seems to promote FAC values higher than values reported, also for *Aloe vera* polysaccharides, by different authors [6–8,26].

The functional properties of cell wall rich materials depend, not only, on the polysaccharides composition, but also, on the manner in which they are interlinked to form the three-dimensional and functional structure of the intact cell wall [33]. Thus, thermosonication could induce changes in the three-dimensional structure of *Aloe vera* polymers, such as acemannan, which might improve their capacity to capture organic molecules, such as lipids. Several authors have observed that the high hydrodynamic shear forces associated with ultrasonic cavitation promoted the disruption of the hydrophobic and electrostatic interactions between polymers, improving their solubility, increasing the surface area and, also, facilitating the rapid oil adsorption [32,34,35]. Further, Chokboribal et al. [3] observed that deacetylation of acemannan polymer promotes changes in its spatial configuration and structure, leading to the generation of hydrophobic regions, which could also explain the high retention of organic molecules observed in this study.

3.3. Effect of thermosonication on the main polysaccharides present in Aloe vera juice

The effect of thermal processing and thermosonication on the main polysaccharides from *Aloe vera* parenchyma tissue was evaluated. Thus, alcohol insoluble residues (AIRs), containing all polysaccharides, and the water-soluble and water-insoluble fractions, obtained from AIRs, were subjected to carbohydrate and FTIR analysis.

Overall, the alcohol insoluble residue (AIR) prepared for processed samples showed a wide range of yields, varying from 115 to 229 mg AIR/100 g juice (Table 2). In most *Aloe vera* juices, either the reference or processed samples, polysaccharides accounted for 50 to 60% of AIR material, except for the juice thermally processed at 75 °C for 15 min where polysaccharides accounted for less than 40%.

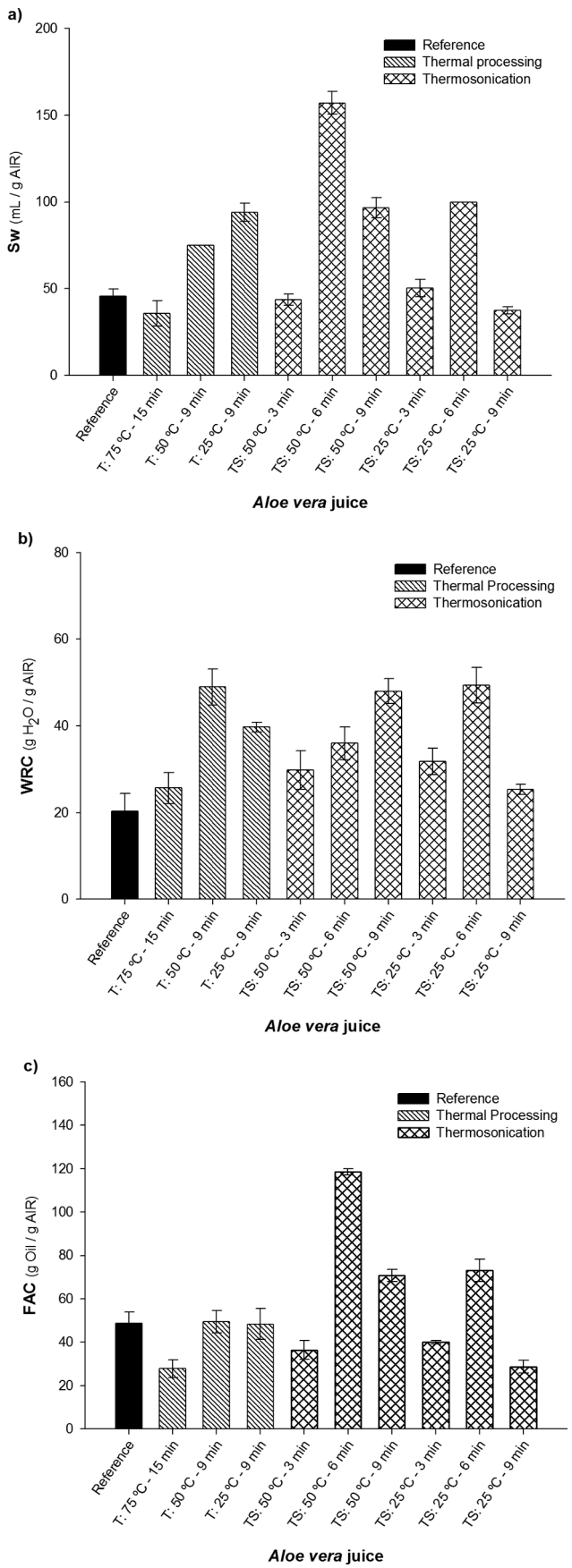

Fig. 1. Functional properties for AIR samples from *Aloe vera* juices: reference, thermally processed (T) and thermosonicated (TS; 0.213 W·mL^{-1}). (a) Swelling (Sw), (b) Water Retention Capacity (WRC), and (c) Fat Adsorption Capacity (FAC).

The carbohydrate analysis revealed that mannose and uronic acids (UA) were the predominant monomers found in AIRs followed by glucose, galactose and xylose. Minor amounts of arabinose, rhamnose and fucose were also found (Table 2).

The occurrence of large amounts of mannose suggested the presence of the bioactive polymer acemannan in all *Aloe vera* juices [2–4,8]. On the other hand, the large amounts of UA together with minor amounts of galactose, arabinose, and rhamnose were a clear indication of the occurrence of pectic substances [2,36]. The presence of cellulose was inferred from the considerable amounts of glucose released after Saeman hydrolysis while hemicelluloses type xyloglucan were associated to the relatively small amounts of xylose and fucose [2].

3.3.1. Water-soluble polysaccharides from Aloe vera juices

The carbohydrate composition from WSP is summarized in Table 3. As it can be observed, WSP accounted for ~74% of the AIR for the fresh sample, whereas in the case of processed Aloe vera samples the WSP content varied from ~57% to ~81% of the AIR ($p < 0.05$), depending on the treatment. Thus, in the thermally processed samples, the WSP yield ranged from ~65%, for the sample treated at 25 °C for 9 min, up to ~75%, when *Aloe vera* juice was processed at 75 °C for 15 min. Whereas, in thermosonicated *Aloe vera* juices, WSP ranged from ~57%, for the juice processed at 25 °C for 6 min, to ~81% when *Aloe vera* juice was treated at 50 °C for 3 min.

The carbohydrate analysis showed that mannose was the predominant monomer present in all WSP fractions of the different *Aloe vera* juices, accounting for more than 60% of the total sugars, followed by UA (~25%), glucose (~10%), and galactose (< 5%). The considerable presence of mannose units together with significant amounts of non-cellulosic glucose (Glc 1 M) in all WSP fractions, confirmed the occurrence of the acemannan polymer. Minor amounts of pectic substances were also present in all WSP fractions. It should be point out that most of these pectic polysaccharides might have arisen from the middle lamellae region and not from the cell walls, as it was previously observed by Femenia et al. [2].

The acemannan polysaccharide was strongly affected for the different procedures, either thermal processing or thermosonication. In the reference *Aloe vera* juice, the mannose and glucose content was of 294 and 41 mg/g WSP, respectively, while the content of these monomers was altered as a consequence of processing. Thus, mannose accounted from 219 up to 284 mg/g WSP in thermally processed samples, at 75 °C for 15 min and 25 °C for 9 min, respectively; whereas in thermosonicated samples for 3 min at 50 °C and, also, at 25 °C, the mannose content accounted for 232 and 306 mg/g WSP, respectively. Moreover, the glucose content varied significantly in all processed samples ($p < 0.05$). Whereas in thermally processed samples, glucose varied from 32 to 46 mg/g WSP, and in thermosonicated samples varied from 28 to 52 mg/g WSP, for juices processed at 50 °C for 6 and 3 min, respectively.

In order to gain more insight about the changes promoted by processing on WSP, and in particular on the bioactive acemannan polysaccharide, FTIR analysis was performed.

In general, the identification of strong bands within the range of 1078–1036 cm^{-1} indicated the presence of mannose and glucose sugars, the main monomers comprising the bioactive acemannan polysaccharide [37] (see Fig. 2). Moreover, the transmittance spectrum at around 1740 cm^{-1} and 1248 cm^{-1} can be attributed to the presence of C═O and C–O–C stretches of acetyl groups, these results being in agreement with the bioactive acetylated polysaccharide acemannan, present in the *Aloe vera* gel [6,38,39]. The acetyl groups of acemannan has been considered as the main responsible not only of the interaction of acemannan with other biomolecules, but also, enabling the transport of other bioactive components across the intestinal epithelium, enhancing their absorption in the intestine [5]. Interestingly, the intensity of the bands at 1740 cm^{-1} and at 1248 cm^{-1} decreased in most of processed samples, except for the sample thermally processed at 50 °C

Table 2
Carbohydrate composition of AIRs from *Aloe vera* juices: reference, thermally processed and thermosonicated (0.213 W·mL^{-1}) samples.

Sample		Yield[a]	Carbohydrates (mg/g AIR)									Total
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc[b]	Glc[c]	UA	
Reference		167	1.6 ± 0.0	0.7 ± 0.0	4.0 ± 0.1	6.7 ± 0.5	244.7 ± 7.2	9.2 ± 0.2	69.3 ± 3.1	68.1 ± 0.6	189.5 ± 14.6	525.7
Thermal processing												
T (°C)	t (min)											
75	15	115	4.4 ± 0.6	0.8 ± 0.0	5.3 ± 0.4	5.8 ± 0.7	142.7 ± 9.1	17.2 ± 2.9	69.9 ± 0.3	56.9 ± 1.3	142.4 ± 19.0	388.5
50	9	189	1.8 ± 0.0	0.9 ± 0.0	3.8 ± 0.0	8.9 ± 0.2	198.5 ± 9.2	9.2 ± 0.1	75.6 ± 0.9	69.6 ± 8.8	183.8 ± 4.1	482.5
25	9	229	1.8 ± 0.0	1.0 ± 0.0	4.2 ± 0.1	9.7 ± 0.2	220.1 ± 16.3	10.3 ± 0.3	78.1 ± 0.7	56.9 ± 3.1	173.2 ± 7.4	498.4
Thermosonication												
T (°C)	t (min)											
50	3	136	1.5 ± 0.1	0.7 ± 0.0	3.8 ± 0.1	7.9 ± 0.5	196.4 ± 3.8	8.6 ± 0.5	79.2 ± 0.1	68.7 ± 1.3	202.5 ± 6.0	500.7
50	6	144	2.5 ± 0.1	1.7 ± 0.3	4.1 ± 0.2	14.4 ± 1.1	119.5 ± 11.2	11.0 ± 0.5	104.0 ± 4.0	73.6 ± 4.5	404.6 ± 17.3	661.8
50	9	130	2.4 ± 0.2	1.4 ± 0.2	4.6 ± 0.2	12.9 ± 1.1	142.1 ± 6.8	10.5 ± 0.2	107.0 ± 9.0	78.5 ± 0.5	370.0 ± 6.7	651.0
25	3	127	2.8 ± 0.2	1.2 ± 0.1	4.7 ± 0.3	11.3 ± 1.4	176.9 ± 9.8	12.3 ± 0.8	93.2 ± 11.2	74.8 ± 4.6	250.0 ± 4.7	552.3
25	6	160	3.8 ± 0.4	1.8 ± 0.2	4.7 ± 0.2	15.2 ± 0.2	152.7 ± 16.6	13.0 ± 1.1	118.7 ± 2.6	82.5 ± 1.3	366.2 ± 26.3	676.1
25	9	133	1.7 ± 0.2	0.7 ± 0.1	5.0 ± 0.1	7.5 ± 0.1	187.3 ± 14.1	11.2 ± 0.4	68.9 ± 3.4	60.4 ± 3.4	133.7 ± 5.5	415.9

[a] mg AIR/100 g *Aloe vera* juice.
[b] Glucose determined using the Saeman hydrolysis conditions [21].
[c] Glucose determined using 1 M sulphuric acid.

Table 3
Carbohydrate composition of water-soluble polysaccharides (WSP) from *Aloe vera* juices: reference, thermally processed and thermosonicated (0.213 W·mL^{-1}) samples.

Sample		Yield[a]	Carbohydrates (mg/g WSP)								Total
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	
Reference		73.5	2.1 ± 0.0	0.0 ± 0.0	4.0 ± 0.1	2.8 ± 0.2	294.2 ± 3.9	8.8 ± 0.1	41.3 ± 0.9	143.6 ± 6.4	496.7
Thermal processing											
T (°C)	t (min)										
75	15	75.5	3.8 ± 0.5	0.0 ± 0.0	5.2 ± 1.5	4.4 ± 0.8	219.2 ± 13.5	15.3 ± 4.0	46.8 ± 12.7	129.5 ± 13.0	424.3
50	9	69.7	2.1 ± 0.4	0.5 ± 0.1	3.3 ± 0.1	3.2 ± 0.2	233.0 ± 19.9	7.1 ± 0.8	34.7 ± 1.8	106.0 ± 6.9	389.8
25	9	65.7	2.1 ± 0.2	0.0 ± 0.0	3.6 ± 0.8	2.7 ± 0.3	284.6 ± 39.7	8.3 ± 1.2	32.4 ± 5.6	74.2 ± 17.7	407.8
Thermosonication											
T (°C)	t (min)										
50	3	81.8	2.0 ± 0.1	0.0 ± 0.0	3.5 ± 0.1	3.3 ± 0.4	232.6 ± 18.0	7.2 ± 0.5	41.8 ± 3.3	87.4 ± 9.9	377.9
50	6	60.0	2.2 ± 0.1	0.0 ± 0.0	3.6 ± 0.4	3.1 ± 0.3	238.2 ± 31.2	9.3 ± 1.1	36.6 ± 4.8	51.7 ± 1.0	344.6
50	9	61.0	2.0 ± 0.0	0.4 ± 0.0	3.9 ± 0.2	2.9 ± 0.0	237.3 ± 10.9	8.6 ± 0.2	35.4 ± 0.4	65.8 ± 3.3	356.3
25	3	71.6	2.2 ± 0.1	0.0 ± 0.0	4.8 ± 0.2	4.0 ± 0.3	305.9 ± 23.4	11.2 ± 0.7	51.6 ± 3.6	88.7 ± 11.8	468.5
25	6	57.4	2.7 ± 0.0	0.0 ± 0.0	3.2 ± 1.5	2.4 ± 1.2	261.2 ± 0.0	8.4 ± 3.9	28.6 ± 12.5	75.8 ± 11.3	382.3
25	9	77.2	2.4 ± 0.1	0.0 ± 0.0	5.1 ± 0.1	3.4 ± 0.1	240.7 ± 2.0	11.5 ± 0.0	46.5 ± 0.2	86.4 ± 8.6	396.1

[a] g WSP/100 g AIR.

for 9 min, and also for those thermosonicated, for 6 and 9 min, at 25 °C. The reduction of these bands has been associated to the deacetylation of the acemannan polymer [3,23,40]. Thus, thermally processed samples, at 75 °C for 15 min, presented the highest deacetylation, ~72%, whereas in the case of thermosonicated samples, deacetylation was lower than 30%.

It is important to highlight that thermosonication seems to promote a lower acemannan deacetylation than different drying procedures [8,23]. Several studies have shown that the acetyl groups of acemannan are involved in the interaction of this polymer with other biomolecules; therefore, this is a key aspect that should be taken into account when assessing the overall quality of *Aloe vera* processed products [3,5,7,8]. In fact, Chokboribal et al. [3], who evaluated the influence of deacetylation of acemannan on its bioactivity, observed that the inductive activity on cell proliferation, vascular endothelial growth factor and collagen I expression was reduced as deacetylation of acemannan increased. On the other hand, rheological studies have demonstrated that acemannan is the main responsible of the pseudoplastic flow behavior of the liquid gel obtained from fresh *Aloe vera* gel, which turn into less viscous, exhibiting Newtonian flow properties, when it is degraded [23,41–43].

On the other hand, pectic substances, accounting for ~25% of WSP, were the second polymer more abundant in WSP fractions from *Aloe vera* juices. Interestingly, pectins were strongly affected for thermosonication. Thus, while pectins accounted for ~150 mg/g WSP in the reference juice and, from ~100 to ~150 mg/g WSP, in thermally

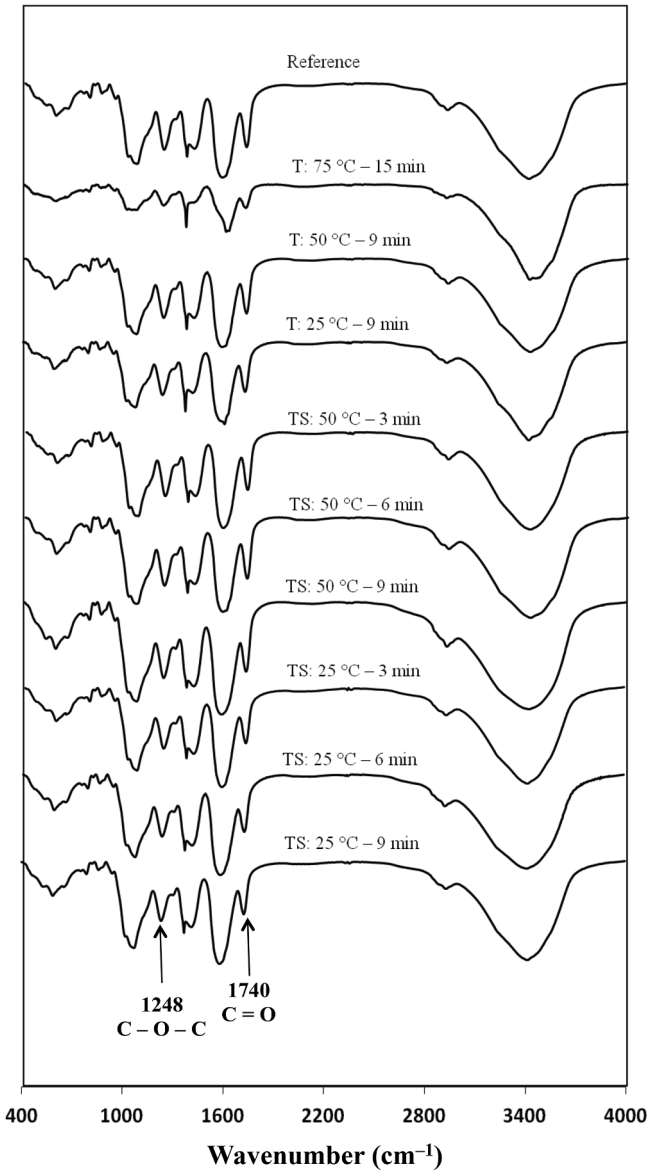

Fig. 2. FTIR spectra of the WSP from *Aloe vera* juices: reference, thermally processed (T) and thermosonicated (TS; 0.213 W·mL^{-1}) samples.

processed samples, in the case of thermosonicated juices, pectin content varied from ~60 to ~95 mg/g WSP.

3.3.2. Water insoluble polysaccharides (WIP) from Aloe vera juices

The results of the analysis of WIP are summarized in Table 4. As it can be seen, WIP represented around 26% of the AIR material in fresh Aloe vera juice, while in processed samples WIP content ranged from 18% to 43%, being the highest value observed for thermosonicated samples ($p < 0.05$). Further, the carbohydrate analysis revealed that WIP were mainly comprised of cell wall polymers, being pectic substances the predominant type of polysaccharides, followed for minor amounts of cellulose and hemicellulosic polysaccharides.

Pectins represented to around 43% of WIP in the fresh *Aloe vera* juice, whereas in processed samples, the content of pectins ranged from ~34% to ~67%. Thermosonicated *Aloe vera* juices exhibited the highest pectin contents whereas samples thermally processed samples exhibited lower pectins content ($p < 0.05$). Pectic substances in all *Aloe vera* juices, fresh and processed, were mainly comprised by homogalacturonans, as inferred from the fact that galacturonic acid represented almost 90% of pectin related monomers. Also, the occurrence of relatively small amounts of galactose (8%), arabinose (1%) and rhamnose (1%) was indicative of the presence of small amounts of rhamnogalacturonans (RG-I).

On the other hand, the degree of esterification (DE) of pectins was estimated as previously described Minjares-Fuentes et al. [17]. Thus, around 31% of the galacturonic acid units from the reference *Aloe vera* juice were esterified. The samples thermally processed at mild temperature (25 and 50 °C) for 9 min exhibited a similar DE (~30%) whereas the DE determined for the sample processed at 75 °C for 15 min was of 41%. On the contrary, thermosonication promoted a slight reduction of DE, varying from 24 to 27% (Fig. 3). These results suggest that thermosonication might promote the degradation of pectic substances by the loss of the methyl groups from homogalacturonan chains, instead of the β-elimination process as occurs in thermal processing.

Xyloglucans were the main type of hemicelluloses present in the WIP fractions for all juices, accounting for 3–4% of total polysaccharides. Interestingly, cellulose represented ~1% or less of WIP in all Aloe vera juice samples. The low amounts of these polysaccharides could be attributed to the filtration process during the preparation of *Aloe vera* juice since most of the CWP are often removed during the filtration step [44].

3.4. Effect of thermosonication on Lactobacillus plantarum

In Aloe industry, the presence of *Lactobacillus* in raw materials, in particular in mucilaginous material, represents a serious problem since they are responsible for the degradation of Aloe polysaccharides in fresh juices. Therefore, the effect of thermosonication on the *L. plantarum* in *Aloe vera* juice was also tested. The results of the logarithmic reduction of the *L. plantarum* are showed in Fig. 4. As it can be observed, the thermally processed sample at 75 °C for 15 min and the thermosonication carried out at 50 °C showed inactivation of *L. plantarum* whereas the samples processed at 25 °C, either thermally processed or thermosonicated, were unable to inactive the *L. plantarum* (see Fig. 4). Interestingly, the inactivation of *L. plantarum* observed in the thermosonicated samples at 50 °C increased as time increased, from 3 to 9 min. It is important to highlight that thermosonication performed at 50 °C for 9 min was able to reduce around 75% the population of *Lactobacillus*. Previous studies have demonstrated that the inactivation efficiency of thermosonication on several microorganisms, *E. coli* and *L. bulgaricus*, increases as temperature and application time increases [45,46].

4. Conclusions

The effect of thermosonication on the functional properties and carbohydrate composition of the main polysaccharides from *Aloe vera* juice was investigated. *Aloe vera* juice was mainly comprised by the bioactive acemannan polymer (> 60%), and pectic substances, in particular homogalacturonans with a low DE. Interestingly, thermosonication promoted minor structural changes of these polysaccharides than thermal processing. In particular, acetylated mannose from acemannan was better preserved during thermosonication than in thermal processing. However, pectins underwent a slight reduction of DE as a consequence of thermosonication.

The changes occurred in these polysaccharides by thermosonication had high influence in the functional properties. In particular, thermosonication improved Sw and FAC whereas WRC remained similar as in thermal processing. Noteworthy, thermosonication carried out at 50 °C for 6 min seems to be a better option for *Aloe vera* processing than thermal procedures since Sw and FAC properties exhibited the highest values. In addition, thermosonication could be an alternative to

Table 4
Carbohydrate composition of water insoluble polysaccharides (WIP) from *Aloe vera* juices: reference, thermally processed and thermosonicated (0.213 W·mL^{-1}) samples.

Sample		Yield[a]	Carbohydrates (mg/g WIP)									Total
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc[b]	Glc[c]	UA	
Reference		26.5	4.0 ± 0.7	2.2 ± 0.6	5.0 ± 0.4	20.7 ± 6.8	131.2 ± 32.5	13.9 ± 1.5	207.3 ± 51.2	203.6 ± 50.3	250.3 ± 27.8	634.5
Thermal processing												
T (°C)	t (min)											
75	15	24.5	6.0 ± 0.0	2.3 ± 0.0	4.6 ± 0.0	18.9 ± 0.0	59.0 ± 1.1	18.1 ± 0.0	191.1 ± 23.5	155.7 ± 19.1	117.2 ± 14.8	417.3
50	9	30.3	4.8 ± 0.5	2.1 ± 0.2	4.5 ± 0.6	20.0 ± 2.3	91.8 ± 9.9	12.6 ± 1.1	164.7 ± 18.4	151.7 ± 16.9	482.2 ± 9.0	782.7
25	9	34.3	5.4 ± 0.5	2.7 ± 0.5	5.1 ± 0.5	23.9 ± 4.1	86.1 ± 4.6	15.8 ± 2.1	202.0 ± 35.7	147.1 ± 26.0	471.4 ± 22.5	812.5
Thermosonication												
T (°C)	t (min)											
50	3	18.2	5.4 ± 0.2	2.7 ± 0.1	5.2 ± 0.3	26.2 ± 1.4	59.0 ± 0.8	15.1 ± 0.3	213.0 ± 9.8	184.9 ± 8.5	607.1 ± 6.9	933.7
50	6	40.0	6.4 ± 0.1	3.2 ± 0.0	5.5 ± 0.1	29.5 ± 0.5	58.2 ± 3.4	17.3 ± 0.1	239.6 ± 6.8	169.6 ± 4.8	621.4 ± 45.9	981.1
50	9	39.0	6.5 ± 0.3	3.7 ± 0.2	5.7 ± 0.4	30.3 ± 1.3	61.0 ± 11.2	16.9 ± 1.5	243.6 ± 10.2	178.7 ± 7.5	621.2 ± 1.8	988.8
25	3	28.4	6.2 ± 0.4	2.4 ± 0.2	5.5 ± 0.4	21.9 ± 1.2	102.9 ± 13.9	17.4 ± 0.9	189.1 ± 9.6	151.8 ± 7.7	576.8 ± 6.2	922.2
25	6	42.6	7.8 ± 0.4	3.3 ± 0.3	5.8 ± 0.4	28.8 ± 2.7	81.5 ± 12.4	19.3 ± 0.4	241.2 ± 19.9	167.7 ± 1.8	570.2 ± 33.1	957.9
25	9	22.8	4.6 ± 0.0	1.8 ± 0.1	4.7 ± 0.2	15.6 ± 1.7	98.9 ± 1.7	14.0 ± 0.5	150.7 ± 15.8	132.2 ± 3.8	366.4 ± 10.9	656.6

[a] mg WIP/100 g AIR.
[b] Glucose determined using the Saeman hydrolysis conditions [21].
[c] Glucose determined using 1 M sulphuric acid.

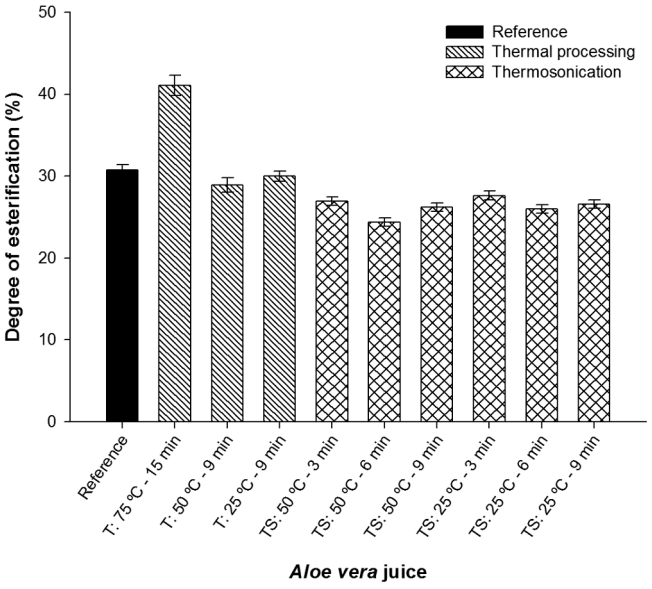

Fig 3. Degree of esterification of pectins from *Aloe vera* juices: reference, thermally processed (T) and thermosonicated (TS; 0.213 W·mL^{-1}) samples.

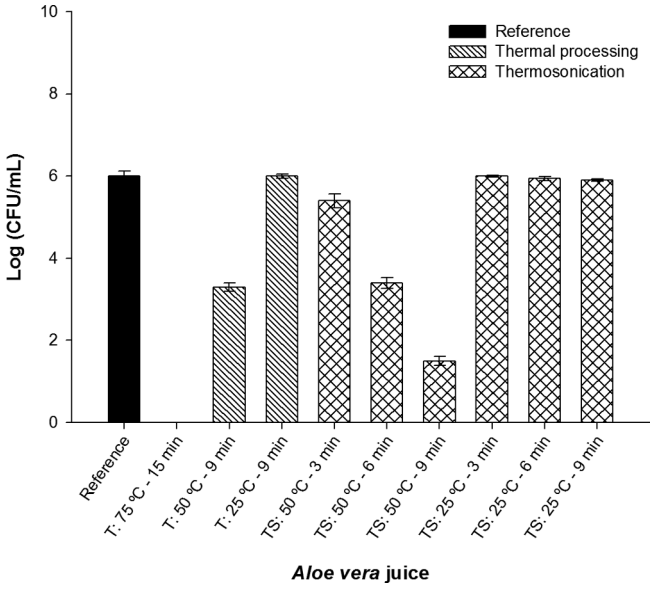

Fig 4. Presence of *L. plantarum* in *Aloe vera* juices: reference, thermally processed (T) and thermosonicated (TS; 0.213 W·mL^{-1}) samples.

pasteurization process since thermosonication might be able to inactive the fermentative bacteria, such *L. plantarum*. Interestingly, the thermosonication, carried out either 50 °C or 25 °C, promoted similar color changes of Aloe vera juice as those observed during the pasteurization procedure (75 °C, 15 min). Further studies about the optimization of the different parameters involved in thermosonication process as well as the effects of this procedure on the beneficial properties of *Aloe vera* are required.

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Artículo

Aplicación de una película de HPMC-parafina sobre melón: efecto sobre aromáticos y actividad de la pectinmetilesterasa

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Resumen

El melón es un fruto apreciado por su aroma y sabor, pero tiene un corto periodo de almacenamiento. Una alternativa para extender este periodo es el uso de películas comestibles. En el presente estudio se evaluó el efecto de la aplicación de una película comestible de hidroxipropilmetilcelulosa-parafina (HPMC-PAR) sobre componentes aromáticos y la actividad de la pectinmetilesterasa de melón almacenado en refrigeración. Frutos de melón Cantaloupe se cubrieron con una película comestible de HPMC-PAR y melones no cubiertos se tomaron como control. Los melones se almacenaron por 20 días a 8 ºC y 80% de humedad relativa. Cada cuatro días, los frutos se sometieron a análisis de concentración de etilbutirato, etilcaproato, butilacetato, benzilalcohol, metilbutanol y actividad de pectilmetilesterasa (PME). Los resultados mostraron que los melones con película tuvieron una mayor concentración de los ésteres analizados, así como una menor actividad de PME en comparación a muestras sin película. Sin embargo, los compuestos alcohólicos no fueron afectados por los tratamientos. Por lo que, la aplicación de la película a base de HPMC-parafina promueve el aumento de compuestos ésteres durante los primeros días de almacenamiento en refrigeración. Además, la actividad de la PME puede influir en la cantidad de estos compuestos aromáticos de la pulpa del melón Cantaloupe.

Palabras claves: aromas, hidroxipropilmentilcelulosa, melón, pectinmetilesterasa.

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Introducción

El melón Cantaloupe es un fruto apreciado por su aroma y sabor los cuales son determinantes para la calidad sensorial y comercial del producto (Kourkoutas *et al.*, 2006). El aroma y sabor del melón son atribuidos a su contenido de compuestos aromáticos volátiles en la pulpa, que incluyen ésteres y alcoholes (Aubert *et al.*, 2005), aunque en algunas variedades de melón se han determinado hasta 240 compuestos por medio de técnicas de cromatografía de gases (Kourkoutas *et al.*, 2006).

Entre los compuestos aromáticos más abundantes en este fruto se encuentran ésteres como el etilbutanoato, etilcaproato, y 3-hexil 2-butanoato, y en menor medida derivados azufrados, aldehídos y alcoholes (Obando-Ulloa *et al.*, 2010). Por otro lado, el melón Cantaloupe tiene una vida de anaquel corta de 10-15 días (Suslow *et al*., 2002), por lo cual es requerida la evaluación de métodos de conservación que contribuyan a mantener la calidad sensorial de este fruto durante periodos de almacenamiento más largos, como lo es la aplicación de cubiertas o películas.

Las cubiertas (soluciones y emulsiones) a base de polímeros comestibles como polisacáridos, proteínas o lípidos han sido aplicadas sobre diversos productos vegetales (Bonilla *et al.*, 2013). La aplicación de películas sobre el melón tiene el potencial de reducir la pérdida de humedad (Reyes *et al.*, 2016), firmeza (Reyes *et al.*, 2017), y tasa de respiración del producto (Reyes *et al.*, 2016). Asimismo, las cubiertas comestibles protegen al producto contra daños mecánicos, oxidativos y microbiológicos, además mejoran la apariencia y la pérdida de aroma del fruto (Genskowsky *et al.*, 2015).

El mayor beneficio del uso de películas y cubiertas es que pueden ser consumidos junto con el producto alimenticio en que están aplicadas, pudiendo dichas películas ser enriquecidas con compuestos que aporten benéficos para la salud o que mejoren las propiedades sensoriales del alimento cubierto (Perdones *et al.*, 2012). Algunos polisacáridos utilizados para preparación de cubiertas comestibles son el alginato de sodio, carragenina y la carboximetilcelulosa (Hamzah *et al.*, 2013; Tavassoli-Kafrani *et al.*, 2016).

La hidroxipropilmetilcelulosa (HPMC) es un polímero derivado de la celulosa usado en la formulación de películas, el cual ha sido probado en diversos vegetales, resultando en menor daño por frío, conservación de la firmeza y menor pérdida de peso en dichos productos (Reyes *et al.*, 2017). Además, las películas comestibles retrasan el proceso de maduración de las frutas (Reyes *et al.*, 2016) por lo que, si las cubiertas pueden disminuir la actividad de las enzimas pécticas, como la pectinmetilesterasa, el fruto mantendrá por más tiempo la firmeza de sus tejidos.

El objetivo de este trabajo fue evaluar el afecto de la aplicación de una película de HPMC-Parafina sobre la concentración de compuestos aromáticos y la actividad de la PME del melón Cantaloupe.

Materiales y métodos

Muestras experimentales

Se utilizaron frutos de melón Cantaloupe (*Cucumis melo* L. var. reticulates) recolectados en Ceballos, Durango (coordenadas geográficas latitud 26.526 y longitud -104.129) en etapa pre climatérica (25 a 27 días después de polinización) de acuerdo con el método publicado por

Nishiyama *et al*. (2007). Los frutos se seleccionaron en estado de madurez tres cuartos desprendido, de tamaño y dimensiones similares (frutos de 1.2 a 1.5 kg libres de daños físicos). El proceso de producción fue riego por goteo.

Materiales y reactivos

La HPMC ($C_{56}H_{108}O_{30}$) fue donada por Colorcon (México). Los siguientes reactivos fueron adquiridos de Sigma-Aldrich (St Louis MI, EUA) Metabisulfito de sodio, sorbato de potasio, monoestearato de propilenglicol, D-metilo de ácido poligalacturónico, Tris, sulfato de amonio, dodecil sulfato de sodio, Triton X-100, azul de bromotimol y estándares de etilbutirato, etilcaproato, butilacetato, benzilalcohol y metilbutanol. Se empleó parafina grado reactivo Analítika (Monterrey, Nuevo León, México).

Tratamientos

Los melones recolectados se seleccionaron libres de daños físicos y contaminación microbiana aparente. Se lavaron con agua clorada a 200 ppm de hipoclorito de sodio, y distribuidos aleatoriamente en dos lotes: tratamiento control (sin aplicación de película), y película (frutos cubiertos con la película HPMC-parafina). La preparación y aplicación de la película de HPMC-parafina se describió previamente en Meza *et al*. (2013). Todos los frutos se almacenaron luego en un frigorífico a 8 ±2 ºC con humedad relativa de 80 ±4% durante 20 días. Posteriormente, los melones se analizaron cada cuatro días a lo largo del periodo de estudio (0, 4, 8, 12, 16 y 20 días). Los análisis practicados fueron concentración de etilbutirato, etilcaproato, butilacetato, benzilalcohol, metilbutanol y la actividad de la pectinmetilesterasa (PME) llevados a cabo por triplicado. Los tratamientos (control y película) se repitieron ocho veces y cada repetición contenía 24 melones.

Análisis de compuestos volátiles

El análisis se desarrolló con el método propuesto por Aubert *et al.* (2005) con algunas modificaciones. Una muestra compuesta de 125 g de melón de cada una de las repeticiones de los tratamientos se mezcló con 125 ml de galato de n-propilo (10 mM) y triturada en una mezcladora por 2 min, posteriormente, homogeneizada por un min con el ultraturrax. La mezcla se centrifugó (4 500 rpm, 20 min, 4 ºC) y se recolectó el sobrenadante.

Para llevar a cabo las pruebas de porcentaje de recuperación, se prepararon soluciones de 5 estándares de compuestos volátiles presentes en el melón; estas soluciones se prepararon a 40 ppm en diclorometano. Posteriormente, 150 ml de la solución de estándares (conteniendo etilbutirato, etilcaproato, butilacetato, benzilalcohol y metilbutanol) o el extracto obtenido de la pulpa de melón, fueron extraídos tres veces con 50 ml de diclorometano (3 x 15 min) bajo agitación constante a una temperatura de 4 ºC. Posteriormente, la mezcla se concentró hasta 8 ml usando un equipo kuderna-danish a 70 ºC. Los 8 mL se colocaron en un microkuderna y concentrados hasta 1 mL a 45 ºC. El concentrado se inyectó en un cromatógrafo de gases HP 6820 con detector de ionización en flama y una columna capilar DB-5 de 30 x 0.25 x 0.25 (Supelco, PA, EUA).

Las condiciones del cromatógrafo fueron de 250 ºC en el inyector, 250 ºC en el detector; la columna se sometió a un programa de 35 ºC de temperatura inicial con rampa de 5 ºC por min hasta alcanzar 150 ºC, la temperatura final se mantuvo por 10 min. Las lecturas del cromatógrafo

se registraron y analizaron con el software Agilente Cerity NDS y comparadas con una curva de calibración de estándar de los compuestos mencionados. Los resultados se reportaron como mg kg^{-1} de fruto fresco.

Preparación de la muestra para la actividad de pectinmetilesterasa

El análisis se llevó a cabo con el método descrito por Lamikanra y Watson (2004) con algunas modificaciones. Se utilizaron rebanadas del centro de la fruta (ecuador) de medidas aproximadas de 80 x 30 x 2 mm de cada tratamiento. A 40 g de rebanadas de melón se les agregó 80 mL de buffer de Tris (pH 7.8, 0.05 M) y se homogenizó en una mezcladora por 2 min para después ser centrifugada a 4 ºC y 4 800 G durante 30 min. El sobrenadante se mezcló con sulfato de amonio de manera de obtener una concentración del mismo de 60% y se colocó en un congelador a -18 ºC por 1 h. La mezcla fue entonces centrifugada a 4 ºC y 4 800 G por 1 h. El sobrenadante fue descartado y los residuos se homogenizaron en 4 ml de Tris por 1 min. La mezcla se centrifugó a 4 ºC y 4 800 G por 1.5 h. El sobrenadante fue la muestra para ensayo de la actividad enzimática.

Actividad de la pectinmetilesterasa

La prueba se realizó con una modificación del método propuesto por Lamikanra and Watson (2003). Una solución de éster D-metilo de ácido poligalacturónico (0.1%) se preparó en una solución de NaCl 0.4 M. Se usó como indicador azul de bromotimol 0.01% en un buffer de fosfato de potasio. Antes de cada reacción, la solución péctica (2.5 mL) era ajustada a un pH de 7.5 con NaOH 2 M. A la solución péctica se le adicionó el azul de bromotimol (0.2 mL), 0.1 mL del extracto enzimático y se agitó en vórtex. La muestra se leyó a una absorbancia a 620 nm en 20 y 80 s para determinar la velocidad de la reacción. Los resultados se reportaron en actividad enzimática relativa donde 100% de actividad era de muestras de melón antes de tratamientos.

Diseño experimental y análisis estadístico

Se usó un diseño factorial con dos factores: tiempo de almacenamiento (0, 4, 8, 12, 16 y 20 días) y aplicación de película (sin y con película), y se llevaron a cabo ocho repeticiones por tratamiento. Los resultados de las variables evaluadas se analizaron mediante análisis de varianza. La diferencia entre medias de tratamientos se realizó por la prueba de comparación múltiple de diferencia mínima significativa de Fisher (DMS de Fisher) con un nivel de significancia de 0.05, usando el programa estadístico SAS versión 8 (SAS Institute Inc., 2005).

Resultados y discusión

Compuestos volátiles

Se presentaron diferencias significativas en las concentraciones de etilbutirato, etilcaproato y butilacetato entre los melones sin y con película ($p \leq 0.05$). Se observó que, en los melones con película, las concentraciones de etilbutirato y etilcaproato presentaron un aumento súbito a los 4 días de almacenamiento (Figura 1 y 2), mientras tanto la cantidad de butilacetato tuvo aumento repentino hasta los 8 días (Figura 3).

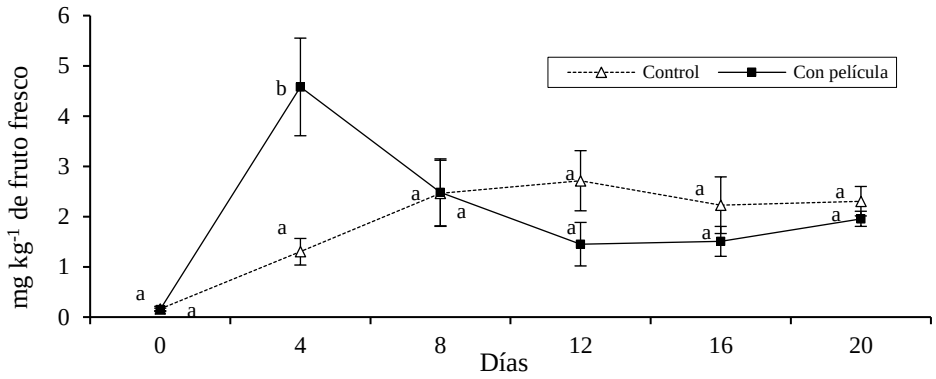

Figura 1. Concentración de etilbutirato (mg kg^{-1} de solido fresco) en melones con y sin película almacenados durante 20 días en refrigeración. Barras sobre la media de los resultados representa ±desviación estándar (n= 4). Diferentes literales indican diferencia significativa, por LSD de Fisher ($p \leq 0.05$), entre melones con y sin película durante el tiempo de almacenamiento.

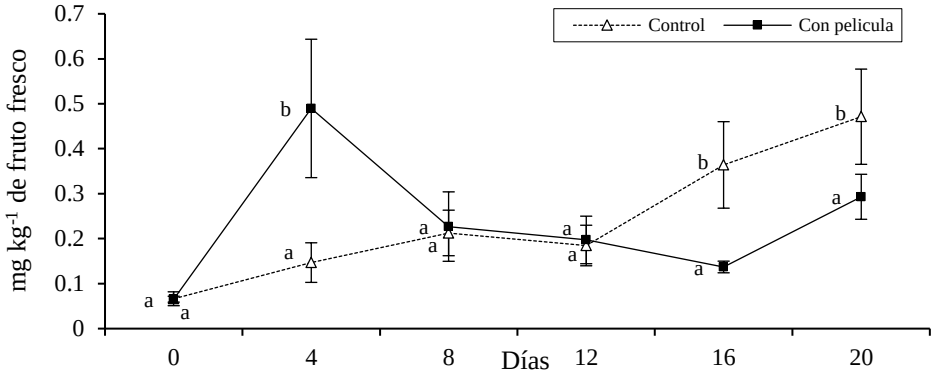

Figura 2. Concentración de etilcaproato (mg kg^{-1} de solido fresco) en melones con y sin película almacenados durante 20 días en refrigeración. Barras sobre la media de los resultados representa ±desviación estándar (n= 8). Diferentes literales indican diferencia significativa, por LSD de Fisher ($p \leq 0.05$), entre melones con y sin película durante el tiempo de almacenamiento.

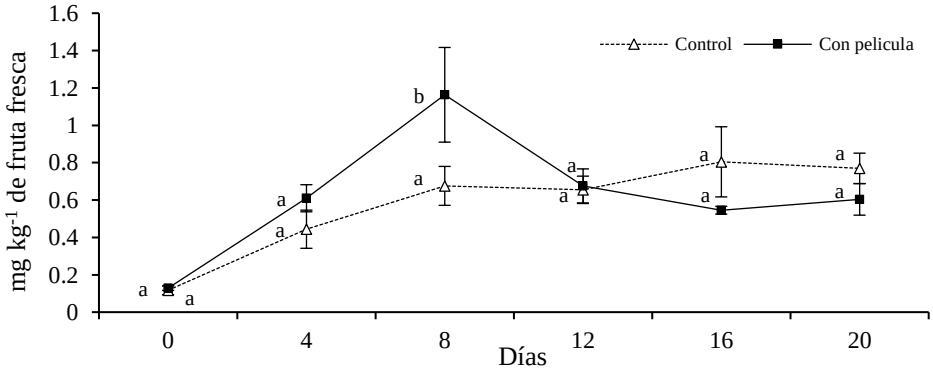

Figura 3. Concentración de butilacetato (mg kg^{-1} de solido fresco) en melones con y sin película almacenados durante 20 días en refrigeración. Barras sobre la media de los resultados representa ±desviación estándar (n= 8). Diferentes literales indican diferencia significativa, por LSD de Fisher ($p \leq 0.05$), entre melones con y sin película durante el tiempo de almacenamiento.

La concentración de estos compuestos fue significativamente menor ($p \leq 0.05$) en los frutos control durante los periodos de tiempo mencionados. Asimismo, los melones sin cubierta presentaron un aumento gradual en la concentración de los ésteres analizados a lo largo del periodo de estudio. Además, los resultados obtenidos en el estudio mostraron que la concentración de los compuestos alcohólicos monitoreados no tuvo cambios ($p > 0.05$) entre los diferentes tratamientos durante el almacenamiento. Resultados similares se han encontrado al cubrir fresas con una película de quitosán (Almenar *et al.*, 2009) y mango con carnauba (Dang *et al.*, 2008), los autores citados consiguieron aumentar la concentración de aromas con el uso de estas cubiertas.

Diversos estudios han demostrado que la producción o síntesis de compuestos aromáticos en frutas y vegetales (especialmente de tipo éster) están directamente relacionados con la madurez y la presencia y concentración de etileno (Günther *et al.*, 2015; Li *et al.*, 2016), por lo que el rápido aumento observado en la cantidad de los compuestos ésteres puede estar relacionado con la concentración relativamente alta de etileno encontrada en el interior de los melones cubiertos con la película de HPMC-parafina, reportada con anterioridad (Meza *et al.*, 2013). La presencia de esta hormona activa enzimas como lipasas, alcohol aciltransferasa y alcohol acetiltransferasa, las cuales promueven una mayor síntesis de compuestos ésteres responsables de aromas y sabores en frutas y vegetales (Hui *et al.*, 2010. En contraste, se observó que las concentraciones de alcoholes, presentes en manzana, no fueron afectados por la inhibición de síntesis de etileno (Dandekar *et al.*, 2004).

Actividad de la pectinmetilesterasa (PME)

La actividad de PME fue menor en los melones con cubierta durante el periodo de 8 a 16 días de almacenamiento ($p \leq 0.05$) (Figura 4). Los frutos con película presentaron una actividad relativa de 25% (donde 100% es la actividad relativa de los frutos sin tratamiento al tiempo inicial) a los 8 días de almacenamiento; mientras que las muestras sin película tuvieron actividad relativa mayor a la inicial de hasta ~122% en el periodo de 12-16 días. Ha sido reportado que el uso de cubiertas comestibles puede disminuir la actividad enzimática de los vegetales al promover la formación de atmosferas modificadas internas (Meza *et al.*, 2013), lo cual puede retardar su proceso de maduración (Reyes *et al.*, 2016).

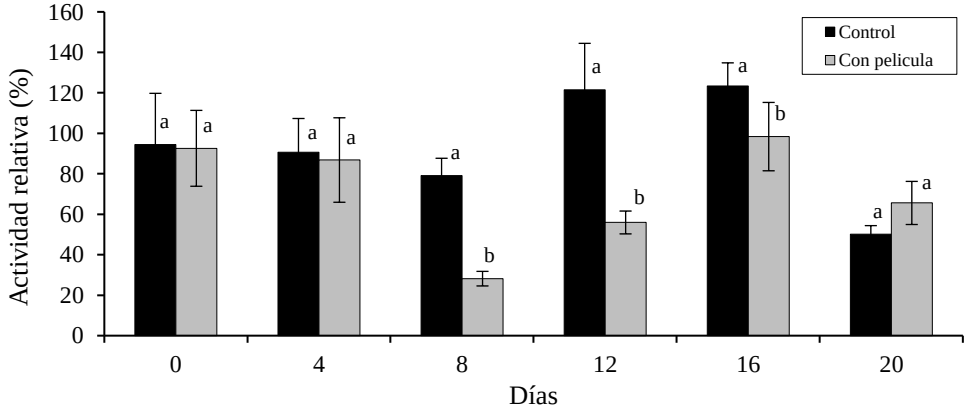

Figura 4. Actividad relativa de la pectinmetilesterasa en pulpa de melón tratado con una cubierta polimérica y almacenado por 20 días en refrigeración. Barras sobre la media de los resultados representa ±desviación estándar (n= 8). Diferentes literales indican diferencia significativa, por LSD de Fisher ($p \leq 0.05$), entre melones con y sin película durante el tiempo de almacenamiento.

La función de la PME es iniciar el cambio y degradación estructural de las pectinas, lo que afectará directamente en la disminución de la firmeza del vegetal (Giovane *et al*., 2004). La síntesis de aromas y los cambios de textura durante la maduración y senescencia de las frutas tiene una cierta correlación debido a los cambios en la estructura celular (Dos-Santos *et al.*, 2013).

Algunas investigaciones concuerdan que los aromas pueden estar atrapados o ligados en la estructura celular formado por polisacáridos, principalmente pectinas (Bezman *et al.*, 2003; Savary *et al.*, 2006; Harker y Johnston, 2008), y probablemente cambios estructurales puedan afectar la liberación o acumulación de aromas en el tejido del fruto, por lo que frutos con menores cambios estructurales, cambios promovidos por enzimas como la PME, podrían contener mayor cantidad de compuestos aromáticos.

Conclusiones

La aplicación de la película a base de HPMC-parafina aceleró la síntesis de compuestos ésteres en melón durante etapas tempranas de almacenamiento en refrigeración. Asimismo, esta película disminuyó la actividad de la PME, lo cual puede disminuir cambios estructurales en los tejidos del fruto y consecuentemente mejorar la retención de compuestos volátiles.

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Effect of High Hydrostatic Pressures on Antioxidant Properties of Mexican Fig (*Ficus Carica L.*) Paste

Abstract

The fig is a fruit with important nutritional and functional properties for human health. The aim of this study was to evaluate the impact of processing with high hydrostatic pressures (HPP) at different times and temperatures of pressurization on anthocyanin content and antioxidant capacity of fig paste. This work showed that HPP at 350 MPa for 5, 10 and 20 min at 20 and 40°C did not change total acidity, total soluble solids and color. However, pressurized samples at 350 MPa for 5, 10 and 20 min showed a significant increase in anthocyanin content compared to the control. The antioxidant capacity, measured by 2,2-azino-bis-3-ethylbenzatholine-6-sulfonic acid assay was not altered by HPP at different times, but when it was treated at 350MPa by 20 and 40°C, this result was modified and had a significant increase when method of 2,2-diphenyl-1-picrilhydrazil was used. These data demonstrate that the HPP can be used in the fig products generation with higher nutritional quality.

Keywords: Fig paste; HHP; Antioxidant capacity; Anthocyanin; PPO

Research Article

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Introduction

Common fig (*Ficus carica L.*) belongs to the family Moracea. It is commonly known as fig, which is a medium-sized deciduous tree widely distributed in sub-tropical and tropical countries. The fig was introduced in Mexico by Spanish Franciscan missionaries in the 16th century and it is assumed that Mexican figs are the Spanish cultivar Black Mission [1]. This fruit has been used as medicine for several centuries [2], hence being an important harvest worldwide for its dry and fresh consumption. Moreover, fruits can be eaten canned, or in other preserved forms [3]. However, these procedures may alter the phytochemical composition of the product.

Nowadays, there is a general trend to increase fresh fruit consumption mainly due to their health properties. Different studies have demonstrated that figs are an important source of minerals and vitamins such as iron, calcium, potassium, thiamin and riboflavin [4]. Figs are free of sodium, fat and cholesterol; contain at least 17 amino acids and high concentrations of aspartic and glutamic acid [5,6]. They also possess relatively high fiber content (5.8%, w/w), which more than 28% is soluble, and can help to lose weight and to control blood sugar and cholesterol [7]. Dehydrated figs have the highest concentration of polyphenols when compared to regular fruits and generally consumed beverages [5]. For all these characteristics, figs are really considered as functional foods [8].

Thermal processing is traditionally used in food industries causing unwanted changes in food quality such as loss of aroma, color, flavor, texture and nutritional value. The use of high hydrostatic pressures (HHP) for food processing has increased its application by food industry. HHP is a non-thermal technology capable to produce high quality food, preserving the characteristics of fresh food and extending its shelf life. In addition, HHP have the ability to inactive microorganisms as well as enzymes responsible for shortening the life product while maintaining food sensorial and nutritional properties [9,10]. However, in some cases, HHP may activate undesirable enzymes such as polyphenoloxidase (PPO) resulting in short shelf life due to flavor and color changes [11].

Phenolic compounds are closely associated with the sensory and nutritional quality of fresh and processed foods. Among the chemical reactions that occur during fruits processing, phenolic compounds oxidation is a phenomena responsible for profound modifications of the initial plant polyphenols concentration. Since it is generally agreed that PPO is mainly responsible for browning, and its increased activity after peeling and cutting would be expected [12].

Due fig is a climacteric fruit, very perishable, with high metabolic activity, fast ripening and reduced store time at room temperature, make difficult its commercialization. Furthermore, the industry of fresh-cut fruits and vegetables is constantly growing due to the current consumption needs for minimally

processed foods. Therefore, new techniques for maintaining quality and inhibiting undesired microbial growth are demanded in all steps of the production and distribution chain [13]. Thus, the application of HPP seems to be a good processing alternative for fig. The main aim of this work was to evaluate the effect of HPP on physicochemical properties, anthocyanin content and antioxidant capacity in fig paste (Black mission).

Materials and Methods

Reagents

ABTS reagent (2,2-azino-bis-3-ethylbenzthia-6-sulfonic acid), potassium persulfate and Trolox was purchased from Sigma-Aldrich. Anhydrous ethanol, anhydrous methanol, HCl and all other reagents were of analytical grade.

Sample preparation

Figs (cursives L.), Black mission variety, were freshly cut and received from Huerto La Linda, S.P.R de R.L. of C.V. located in the Ejido Álvaro Obregón from Lerdo Durango, Mexico. Figs were washed with fresh water and stored in a cooler with crushed ice, in a proportion less than 20% of the volume of fig to slow maturation without causing cold damage, until its use (less than 6 hours). Peduncle from fruit was removed with a knife and figs were liquefied without adding water in an industrial blender of 4 Kg (International model LI-5 with serial number 037-ago-98). Liquefied samples were placed into a 60 L aluminum container where the whole fig batch was mixed and stored under refrigeration until processing (less than 12 hours). The homogenized fig paste was weighed (100 g) inside vacuum packing bags, impervious to oxygen and moisture, and vacuum sealed with a sealer (Food Saver® model U3835 Bag Sealer). Bags were labeled and subjected to pressurization. Paste was always in contact with ice to prevent the growth of microorganisms. The samples already packed in vacuum were placed in warm water to reach the pressurization chamber temperature.

Fig paste processing with high pressures

Treatments were carried out in an isostatic press (Laboratory Cold Isostatic Press model LCIP402260NCEP1MLN) located in the Integral Food Laboratory of the Technological Institute of Tepic (Nayarit, Mexico) with a maximum pressure of 700 MPa. The pressure transmitted to the fluid was distilled water containing 5% (v/v) of ethylene glycol, according to the manufacturer's instructions. The time required to reach the desired pressure was independent of the pressure level (350 MPa/ 4.18-5.29 min) and the time of pressure relief was 15-43 seconds. The pressurization times reported in this study do not include an increase in pressure. Black mission samples were pressurized at 350 MPa for 5, 10 and 20 min at 20 and 40°C. The pressurized samples at 0 min, means to pressurize enough time to reach the pressure of 350 MPa and at that time the pressurization is suspended. Control samples reached room temperature (20 or 40°C) and were cooled again. After processing, samples were stored at -20°C until its analysis. The experiments were developed using homogenized samples of each of two fig varieties and analyzed by triplicate.

Extraction and quantification of anthocyanins

To determine the total anthocyanin content, approximately 1 g of fig paste sample was weighed in a 25 mL Erlenmeyer flask and a solution to extract anthocyanins (10 mL) was added (HCl: methanol: water: 0.02:8:1.8; v/v/v). The flask was placed in a sonicator at 50°C for 1 h. The extract was centrifuged at 3000 rpm for 10 min at 4°C and supernatant was collected and stored in an amber vial at 4°C until its use. Finally, absorbance was measured at a wavelength of 525 nm and total anthocyanins concentration in fig paste was reported as µg equivalent of cyanidin 3-glucoside/g of fig paste.

Total antioxidant capacity

Antioxidant capacity was evaluated using the ABTS method according to Nenadis et al. [14]. A 7mM aqueous solution of ABTS and a 140 mM solution of potassium persulfate were prepared. Then, 88 µL of potassium persulfate were mixed with 5 mL of ABTS and left to rest in the dark for 12 h. Then, 500 µL of the activated radical was taken and mixed with 25 mL of ethanol. The absorbance was determined at 734 nm and adjusted from 0.7 to 1 if necessary. The calibration curve of Trolox was used in a range of 0.5-8 µM from a methanolic solution of Trolox sonicated for 5 minutes.

The inhibition percentage of DPPH was calculated as follows:

DPPH inhibition (%) = [(A ctrl – A) / A ctrl]* 100

Results are expressed as antioxidant activity equivalent to Trolox (µM Trolox/100 g of paste).

For DDPH determinations of fig paste samples, a plate containing the calibration curve and samples was read at 520 nm in an Elisa reader by following kinetic times of 0, 4, 10, 30, 60 and 90 min until the percentage of inhibition of the antioxidant (Trolox) was observed. Different Trolox alcoholic solutions were used for calibration curves and the analyses were performed in triplicate and results were expressed as DPPH inhibition percentage.

Statistical analysis

Data were analyzed by Statistica for Windows version 7.0 applying two-way analysis of variance and differences between means were determined ($P< 0.05$) using Duncan´s multiple range test.

Results and Discussion

Diverse studies on change in the state of food products have shown the impact of the quality of processing methods on the organoleptic properties (e.g. taste, texture, smell and appearance). The color is an intrinsic property of foods, and therefore, a change in color and often caused by change in quality. While other important quality attribute of processing figs is titratable acidity because the presence of citric, acetic and a small quantity of malic acids has been reported in figs and the level of acid contributes markedly to the flavor of the products [15]. This study showed that HPP at 350 MPa for 0, 5, 10 and 20 min at 20 and 40°C did

not change total acidity, total soluble solids and color (data not shown).

Figs are an important source of anthocyanins. In this study, the total anthocyanins content of fig paste at different pressurized-times are summarized in Table 1. Their values range from 237.3 to 271 µg/g of paste. Thus, the total anthocyanins content was significantly increased with increasing pressurized time ($P<0.05$). The fig paste at 10 min of pressurized time, showed the highest total anthocyanins when compared to other treatments. In this study, the content of anthocyanins did not change significantly in all the samples treated at different temperatures (20 and 40°C) at 350 MPa. However, pressurized samples at 350 MPa at different times (0, 5, 10 and 20 min) showed a significant increase in anthocyanin content compared to the control. In these samples, the contents were significantly higher (5-14%) and may be the result of cell disruption caused by pressure and leading to a higher extractability of these compounds [16].

Table 1: Anthocyanins content in pressurized and non-pressurized fig paste.

Time (min)	Anthocyanins Content (µg of Cyanidin 3-glucoside/g of Fig Paste)
0	233.59^{b}
5	251.68^{ab}
10	271.08^{a}
20	266.20^{a}
Control	237.31^{b}

Mean with different letter are significative different, p= 0.029.

The effect of HHP on anthocyanins content has been well studied. When products rich in polyphenols and anthocyanins are pressurized, anthocyanins content is affected and increased with processing conditions (temperature and time) [17]. These results are agreed with Corrales et al. [18] who have demonstrated the increase in anthocyanins recovery when HPP are applied. However, lower anthocyanin content was observed in samples treated for 20 min and it may be due to a remaining activity of enzymes such as PPO and β-glucosidase, due PPO is a stable enzyme to pressure, which oxidizes polyphenols (anthocyanins) generating dark compounds. In addition, García et al. [19] observed the activation of this enzyme in red raspberries treated at 600 MPa for 10 min. However, PPO and other peroxidases are inactivated by applying a pressure equal or greater than 400 MPa in combination with temperatures between 20 to 90°C. Under these conditions, PPO activity can be reduced by up to 50%, although the percentages may vary depending on intrinsic properties of processed foods [20].

Generally, temperature is one of the most important factors affecting antioxidant activity. The antioxidant capacity of a substance is reflected by its ability to scavenge reactive oxygen species and reactive electrophiles. The antioxidant capacity of the fig paste was 376.7 µmol Trolox/100 g of paste by ABTS. The antioxidant capacity of our treatments decreased when temperature is increased (Figure 1). HPP had a light negative effect on the antioxidant capacity measured by ABTS, reducing the antioxidant capacity by 21.5% when the pressurization temperature was raised from 20 to 40°C. As shown in Figure 1a, from 0 to 5 min of pressurization at 40°C, antioxidant capacity was increased, but from 10 to 20 min a slight decrease in antioxidant activity was observed. The decrease in antioxidant capacity can be the result of eliminating part of enzymatic oxidation, responsible for polyphenols and anthocyanins loss.

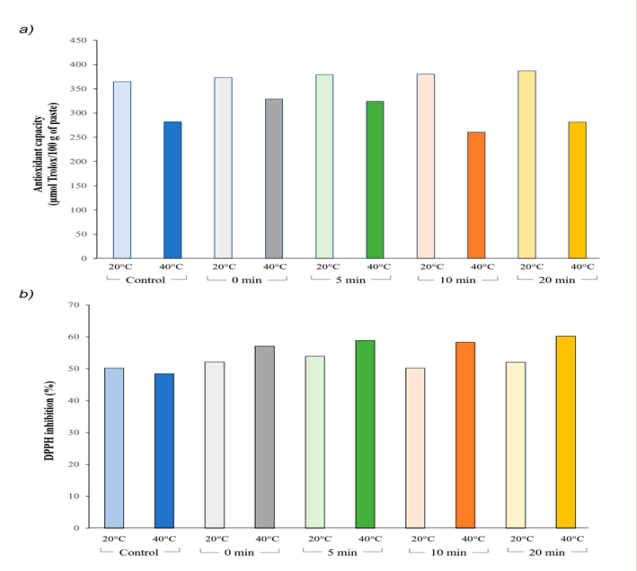

Figure 1: Effect of different temperature and time of pressurization on antioxidant properties of fig paste.

a) Antioxidant capacity with ABTS

b) DPPH radical scavenging activity of pressurized and non-pressurized fig paste. Data were expressed as mean. Values are significative different at $P<0.05$.

A number of methods are used to determine the radical scavenging effects of natural compounds with antioxidant properties. DPPH method is a preferred procedure due it is fast, easy and reliable and does not require a special reaction or device. When the stable DPPH radical accepts an electron from the antioxidant, a violet color of the DPPH is reduced to yellow which is measured spectrophotometrically. All treatments showed same level of DDPH radical scavenging activity, about 49.3% of antiradical activity by inhibition of DPPH. HHP treatment increased antioxidant activity in fig paste, which ranged from 50 to 60%. Thus, the DPPH method showed an increase of 14.4% of the antioxidant capacity after 5 min at 350 MPa (Figure 1b) and a 9.4% raise when the pressurization temperature ranges from 20 to 40°C.

It has been shown that the effect of HHP on antioxidant capacity depends on the treated product. For instance, Patras et al. [21] reported higher antioxidant capacity of blackberry puree after pressurization, but found no effect of the process on strawberry puree. Tomato and carrot purees subjected to HHP

presented higher antioxidant capacity than unprocessed samples [22]. Cao et al. [23] studied the total phenolic levels of strawberry pulps submitted to HHP, at 400 MPa there was a decrease in total phenolic content regardless the time of HHP treatment.

Conclusion

Time and temperature of HHP treatment showed the strongest influence on anthocyanin content and antioxidant activity. Thus, our results suggest that 10 min of HPP at 20°C would be appropriate operating conditions to produce favored anthocyanin levels, antioxidant capacity and scavenging activity in fig paste.

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Conflict of Interest

The authors declare that there is not a conflict of interests regarding the publication of this paper.

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ORIGINAL PAPER

Improvement of the Quality and the Shelf Life of Figs (*Ficus carica*) Using an Alginate–Chitosan Edible Film

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Abstract The consumption of fresh figs (*Ficus carica*) has long been associated with longevity and health benefits. However, fresh figs are highly perishable. An alternative to extend the shelf life of this fruit might be the application of an edible film. Thus, in this study, fig fruits collected at two maturity stages (referred as stages III and IV) were coated with an alginate–chitosan bilayer edible (A–Ch BE) film and the main physiological and quality attributes were evaluated during storage at low temperature (6 °C) at 0, 3, 6, 9, 12, and 15 days of storage. Ethylene and CO_2 productions were considered as physiological parameters, while firmness, color changes, weight loss, and visual infection were the quality attributes evaluated. The application of the film reduced CO_2 production and increased ethylene emission, regardless of the maturity stage of the figs. Firmness for uncoated figs underwent a significant decrease during storage, exhibiting values lower than 1.0 N. On the contrary, for coated figs, firmness was not only maintained but also even increased up to 3 N, for figs of stage III, and up to 2.5 N for the more mature figs (stage IV). Furthermore, coated figs exhibited better external color retention ($\Delta E < 2$), lower weight loss (~8 %), and also lower visual fungal contamination (<5 %) than uncoated fruits during the 15 days of storage at 6 °C, regardless the maturity stage. Therefore, the results indicate that application of an A–Ch BE film not only can improve the overall quality of figs (*F. carica* var. Mission) stored at 6 °C but is also able to extend their shelf life.

Keywords Alginate · Chitosan · Edible film · Figs · Shelf life

Introduction

Fig (*Ficus carica* L.) is a fruit with a high commercial value. The production of this fruit is mainly located around the Mediterranean Sea, the USA (CA), Brazil, India, and Japan (FAOSTAT 2013). Fig is a nutritious fruit containing considerable amounts of vitamins, amino acids, antioxidants, and dietary fiber (Elleuch et al. 2011; Martínez-García et al. 2013; Solomon et al. 2006). In addition, figs contain higher levels of potassium, calcium, and iron than those usually found in other common fruits such as bananas, grapes, oranges, strawberries, and apples. Also, figs are free of sodium, fat-free, and, like other fruits, cholesterol free (Chessa 1997; Crisosto et al. 2010; Michailides 2003).

The fig is considered as a climacteric fruit with a moderate ethylene production and respiration rate (Marei and Crane 1971; Paul et al. 2012; Villalobos et al. 2016) and its commercial quality depends, mainly, on the maturity stage at which the fruit is harvested (Crisosto et al. 2010). Commercially, a very large percentage of figs is consumed dried in cookies or bars, and to a lesser extent, figs are eaten as fresh fruits. However, the fig is a very sensitive product to microbial growth even when stored at a low temperature; thus, it is important to consider alternative processes to extend its shelf life (Martínez-García et al. 2013). A feasible alternative could

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be the application of an edible coating or film, which is a preservation method used to improve food appearance and to maintain the quality of different vegetable products. In addition, the edible films used are environmentally friendly (Khwaldia et al. 2004).

Edible coatings minimize vegetable and fruit respiration rate; act as a water vapor barrier; reduce microbial proliferation; and delay dehydration during processing, handling, and storage (Benítez et al. 2015; Cha and Chinnan 2004; Park 1999; Xu et al. 2007). These coatings are regularly made of proteins or polysaccharides; however, there are several new approaches to improve the functional and mechanical properties of such coatings, like the addition of such hydrophobic compounds as lipids, to improve film water impermeability (Vu et al. 2011) and the design of protein–protein and protein–polysaccharides films (Di Pierro et al. 2006), which include the use of polymers like chitosan and alginate.

Alginate is a polysaccharide widely used for encapsulation due to its ability to form gels in the presence of several divalent cations such as calcium, barium, and strontium by ionotropic gelation (Braccini and Pérez 2001; Ramadas et al. 2000). Besides, the effectiveness of alginate coatings to improve the quality and shelf life of different minimally processed fruits, such as apples (Olivas et al. 2007), papayas (Tapia et al. 2008), and pineapples (Montero-Calderón et al. 2008), has been evaluated. On the other hand, chitosan is a cationic polysaccharide obtained from partial deacetylation of chitin, the main constituent of the crustacean exoskeleton (Elsabee and Abdou 2013). Compared with other polysaccharides, chitosan has several important advantages, including biocompatibility and biodegradability. It is non-toxic and demonstrates antimicrobial properties (Hosseinnejad and Jafari 2016; Shapiro and Cohen 1997; Suyatma et al. 2005); moreover, it can be easily modified by physical or chemical methods (Le Tien et al. 2003).

Based on these premises, the main aim of this study was to evaluate the effects of the application of an alginate–chitosan bilayer edible film on the physiological and quality attributes of figs (*F. carica* var. Mission), harvested at two maturity stages, during storage at low temperature. In particular, external color, firmness, weight loss, and visual fungal contamination were analyzed.

Material and Methods

Chemical Reagents

Sodium alginate was acquired from CYTECSA, S.A from C.V. (Mexico, D.F.), soy lecithin from Golden Bell (Mexico, D.F.), and olive oil was purchased at a local store. Chitosan (reagent grade), lactic acid, calcium chloride, and CO_2, as well as ethylene standards were supplied for Sigma-Aldrich Company (St. Louis, MI, USA).

Plant Material

Figs used in the current study were collected at two maturity stages: stage III (when the receptacle has three fourth reddish-purple coloration) and stage IV (receptacle has a deep purple coloration) (Crisosto et al. 2010; Owino et al. 2004). Fruits were harvested in August–September of 2014 in Cd. Lerdo (Durango, Mexico; 25° 32′ 10″ N/103° 31′ 28″ W). Fruits, free of physical damage and without microbiological contamination, were selected for this study. All fruits were washed by immersion in 0.02 % sodium hypochlorite solution during 1 min, and then, according to their maturity stage, divided into two batches before treatments were applied.

Preparation of Sodium Alginate and Chitosan Layers

Different formulations were tested in preliminary trials and, finally, a bilayer film formulation was selected in order to obtain a transparent, silky, elastic, and flexible coating which had to be resistant to handling. Besides, the bilayer film was easy to apply and adhered well to both smooth and corrugated surfaces, as well as being easy to remove (Andreuccetti et al. 2011).

Sodium alginate layer was developed according to Raybaudi-Massilia et al. (2008). Two grams of sodium alginate was solubilized in 100 mL of distilled water, and this solution was heated to 70 °C for 5 min. Then, the alginate solution was cooled to 10 °C, and the following reagents were added: 0.5 g of soy lecithin, 4 g of glycerol, and 15 mL of olive oil. The mixture was homogenized at 15,000 rpm during 10 min using an Ultraturrax T18 (IKA® Works, Inc., Wilmington, USA). Finally, the emulsion was cooled at 25 °C for its application. Chitosan layer was prepared by the methodology proposed by Romanazzi et al. (2013). One gram of chitosan was diluted in 100 mL of a 1 % (*v*/*v*) lactic acid solution, and afterward, 0.5 g of soy lecithin, 2 g of glycerol, and 5 mL of olive oil were added. The mixture was homogenized at 15,000 rpm during 10 min using an Ultraturrax T18 (IKA® Works, Inc., Wilmington, USA). Finally, the emulsion formed was cooled to 25 °C before its application.

The sodium alginate emulsion was extended on a smooth glass plate to form a film, which was submerged in a 2 % (*w*/*v*) calcium chloride solution for 2 min until a gel was formed. The sodium alginate film was then dried at room temperature for 8 h. Finally, the chitosan film was applied over the dried sodium alginate film and allowed to dry at room temperature (25 °C) overnight. Thickness of the bilayer (0.065 ± 0.001 mm) edible film was measured using an optical micrometer Labomed VF10X (Labomed Inc., CA, USA).

Characterization of the Film Forming Emulsion

The viscosity and mechanical flow properties of the alginate and chitosan emulsions were analyzed prior the formation of the A–Ch BE film. The viscosity of alginate and chitosan emulsions (100 mL) was measured using a RVDV-I PRIME Brookfield viscometer (Brookfield Engineering Laboratory Inc., Middleboro, MA, USA) equipped with an RV5 spindle. All measurements were performed at 100 rpm and 25 °C. According to this, the sodium alginate emulsion showed a viscosity and a consistency of 2944 ± 67 cP and 456.33 ± 16.1 N s m^{-2}, respectively, whereas in chitosan solution, these parameters were of 404 ± 35 cP and 330 ± 25.1 N s m^{-2}.

Bilayer Alginate–Chitosan Film Characterization

Water Vapor Permeability

The water vapor permeability (WVP) of the sodium alginate and chitosan bilayer film was measured according to the ASTM 96-92 (1990) method as suggested by Bourbon et al. (2011). The films were placed inside glass flasks (50 mL) with 20 mL of distilled water. The flasks were stored in an atmosphere with a relative humidity (RH) of 21 %. The weight change was recorded at lapses of 30 min during 12 h. The WVP was calculated by the following equation (Eq. 1):

$$\mathrm{WVP} = \frac{\mathrm{WVRT}}{|S(R_1 - R_2)|} D \tag{1}$$

where WVRT is the water vapor transport rate (g h^{-1} m^{-2}); S is the water vapor pressure (Pa) at 25 °C; R_1 and R_2 are the external (21 %) and internal (100 %) relative humidities (RH) into the flask, respectively; and D is the thickness of the film (m). The WVRT was determined as follows:

$$\mathrm{WVRT} = \frac{\left(\frac{dm}{dt}\right)}{A} \tag{2}$$

where $\frac{dm}{dt}$ is the transmission rate of water vapor (g h^{-1}), and A is the area of film (m^2).

All measurements were carried out in triplicate.

CO_2 and Ethylene Permeability

Carbon dioxide (PCO_2) and ethylene (PE) permeabilities were measured at 25 °C in a controlled-temperature room using a quasi-isostatic method (García et al. 2000), based on the measurement of the amount of gas diffusing through a film. A desiccator of 4.1 L was used to determine the gas permeability of the films, which was exposed to a carbon dioxide (9.14 mL/L)–ethylene (0.304 mL/L) mixture at the bottom of the desiccators at atmospheric pressure and 95 % of RH. An aliquot of 1 mL of gas from the top of the desiccator was taken every 10 min during 4 h. The gas sample was analyzed by gas chromatography. The gas chromatograph was equipped with two columns connected in parallel. Thermal conductivity (TCD) and flame ionization (FID) detectors were used to determine the CO_2 and ethylene concentration, respectively.

The PCO_2 and PE were calculated using Fick's law equation (Eq. 3) (Gontard et al. 1996):

$$P = \frac{\left(\frac{dm}{dt}\right)(\delta)}{(A)(\Delta p)} \tag{3}$$

where P is the permeability of CO_2 or ethylene (mol m^{-1} s^{-1} Pa^{-1}); $\frac{dm}{dt}$ is the transmission rate of oxygen or carbon dioxide through the film (in mol s^{-1}); δ is the thickness of the film (in m); A is the surface area of the film (in m^2); and Δp is the differential partial pressure across the film (in Pa). Three replicates of each film type were tested.

Mechanical Properties

The films were cut to obtain 50 × 20 mm rectangular samples (0.065 mm wide) which were then conditioned at 95 % RH and 25 °C for 48 h prior to the analytical determinations (De Moura et al. 2009). A texture analyzer TA-XT plus (Stable Micro Systems, London, England) was used to determine the maximum tensile strength (TS), maximum percentage of elongation at break (%), and elastic modulus (or Young's modulus). Films were stretched using a speed of 0.5 mm s^{-1}. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of the original length). Data were analyzed with software Exponent version 5.1.1.0.

Bilayer Edible Film Application and Storage

Fig fruits of both maturity stages were divided into two batches. The first batch was selected as a control group (without bilayer edible film application), while the second batch was coated with the alginate–chitosan bilayer edible (A–Ch BE) film.

The application of the edible film was performed in two phases to form the bilayer edible film. First, figs were submerged in the sodium alginate emulsion during 30 s and the emulsion excess was removed. Then, the sodium alginate emulsion was gelled by immersion in a 2 % (*w*/*v*) calcium chloride solution and dried at room temperature. Following that, the coated fruits were dipped in the chitosan emulsion for 30 s. Finally, they were dried using air at room temperature at 5 m s^{-1} during 15 min. All figs, coated and uncoated, were stored at 6 °C and 95 % RH for 15 days. The CO_2 and ethylene

productions, firmness, color, weight loss, and visual infection were evaluated at 0, 3, 6, 9, 12, and 15 days of storage.

CO_2 and Ethylene Productions

Ten fruits were placed in a 1.5 L container during 1.5 h at 6 ± 1 °C, and then a gas sample (1.5 mL) was taken from the headspace gas of the container and injected in an Agilent 6820 gas chromatograph (AgilenteTechnology, CA, USA) equipped with TCD and FID detectors, two 6 ft. × 1/4 in Alltech CTR I columns (Alltech Associates, Inc., Deerfield, IL, USA), and a 30 m × 0.53 mm × 0.25 μm Carboxen column (Supelco, PA, USA). The determination of temperatures in the test were 95 °C in the oven, 300 °C in the FID, 170 °C in the TCD, and 70 °C in the injector; and the determination was an isothermical process. The CO_2 and ethylene productions were expressed in mL kg^{-1} h^{-1} of CO_2 and in μL kg^{-1} h^{-1} of C_2H_4.

Firmness Measurement

The figs' firmness was measured according to an adaptation of the method published by Meza-Velázquez et al. (2013). The firmness measurement was performed using a Texture Analyzer TA-XT plus (Stable Micro Systems, England) with a 30 kg load cell. A compression assay with a spherical probe of 1/2 in. was used to evaluate the firmness in whole figs. The assays were carried out at a speed of 1.5 mm s^{-1} and 1.5 mm of compression distance. Firmness was measured on five figs per treatment at each storage time. All fruit samples were tempered at 25 °C before measurements. The texture parameter measured on the resulting force–distance curves was firmness (in Newtons), reporting the mean value of five replicates.

Color Evaluation

A Minolta CR-300 Colorimeter, equipped with CIE illuminant C and 0° viewing angle geometry, was used to obtain L^* (lightness), a^* (redness), and b^* (yellowness) values. The color was measured on the surface of 15 fruits of each treatment, obtaining the chroma (C^*) value using the following equation (Eq. 4):

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (4)$$

while the total color difference (ΔE^*) was calculated using the next equation (Eq. 5):

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad (5)$$

Weight Loss

Samples made up of 20 figs were weighed at the beginning of the experiment and after 3, 6, 9, 12, and 15 days of storage, and the weight loss was reported as the percentage change from the initial weight, running three treatment replicates.

Fruit Visual Infection

The fruit visual infection was evaluated as described Velickova et al. (2013). Eighteen figs per treatment were examined to detect visible mold growth during storage, reporting as infected figs those fruit samples with mycelia development, brown spots, and softening of the infected area. Three treatment replicates were run simultaneously, and the results were expressed as a percentage of the infected fruits.

Data Analysis

The effects of the A–Ch BE film, the maturity stage of figs and the storage period on CO_2 and ethylene emission, firmness, color, weight loss, and visual infection of figs were evaluated by analysis of variance (ANOVA) using the SAS statistical software version 8 (SAS Institute Inc. 2005). Furthermore, the post hoc analysis was carried out using Fisher's least significant difference (LSD) test with a significance level of 0.05.

Results and Discussion

Bilayer Edible Film Characterization

Water Vapor, CO_2, and Ethylene Permeability

The determination of the water vapor permeability (WVP) and the permeability of gases, such as CO_2 and ethylene, of a film, provides important information for the film formulation, especially if the objective is to obtain modified atmosphere conditions (McMillin 2008). The alginate–chitosan bilayer edible (A–Ch BE) film developed to coat fig fruits had a WVP of $6.31 \pm 0.7 \times 10^{-12}$ g m^{-1} h^{-1} Pa^{-1}. This WVP value is lower than those reported by Bourbon et al. (2011) in a chitosan-based edible film and by Olivas and Barbosa-Cánovas (2008) in alginate-based coatings. The lower WVP of the A–Ch BE film could be attributed to the presence of hydrophobic components, such as lipids, in the bilayer film formulation, since it is known that the addition of lipids to polysaccharide-based films produced effective barriers to water vapor due to its hydrophobicity (García et al. 2000). On the other hand, the A–Ch BE film had a CO_2 permeability of $2.28 \pm 0.98 \times 10^{-13}$ mol m^{-1} s^{-1} Pa^{-1} and an ethylene permeability of $1.4 \pm 0.9 \times 10^{-14}$ mol m^{-1} s^{-1} Pa^{-1}. Interestingly, the A–Ch BE film exhibited a higher CO_2 and ethylene

permeability than films composed of galactomannans and collagen blends (Lima et al. 2010) and of wheat gluten (Mujica Paz et al. 2005). The application of an edible film with adequate permeability properties promotes the formation of a modified atmosphere which can extend the shelf life of the coated products (Villalobos-Acuña et al. 2011a, b).

Bilayer Edible Film Mechanical Properties

Packaging films must maintain their integrity and withstand external stress during their application and use; thus, it is important to determine the film's mechanical properties in order to predict its performance when applied to a food product (Yang and Paulson 2000). In the current study, the tensile strength (maximum tensile stress that a film can hold), percentage elongation (maximum length change of a sample before breaking), and Young's modulus were evaluated as the main mechanical property indicators of the A–Ch BE film (Pereda et al. 2012) (Table 1).

The tensile strength of the bilayer edible film was of 0.96 ± 0.22 MPa, while the elongation percentage and Young's modulus were of 16.62 ± 4.18 % and 6.55 ± 1.4 MPa, respectively. These results are different from those published by Pereda et al. (2012), who reported a higher tensile strength (8.4 MPa), elongation percentage at break (19 %), and Young's modulus (76 MPa) in films composed of chitosan and olive oil. It is possible that such differences could be due to the olive oil concentration added to the coating. Yang and Paulson (2000) also suggested that film extensibility can be improved by increasing the plasticizer concentration used in the film formulation. However, the A–Ch BE film mechanical properties (elasticity, flexibility and breakage resistance) were better than those reported in other studies (Benavides et al. 2012; Galus and Lenart 2013; Olivas and Barbosa-Cánovas 2008; Palma et al. 2016; Sun et al. 2014). These film mechanical properties are important in anticipating whether the coating will adequately resist its application and manipulation, contributing to the maintenance of the overall food product quality.

Table 1 Physical properties of the A–Ch BE film

Physical properties		
Permeability properties		
Water vapor permeability	$6.31 \pm 0.70 \times 10^{-12}$	g m^{-1} h^{-1} Pa^{-1}
CO_2 permeability	$2.28 \pm 0.98 \times 10^{-13}$	mol m^{-1} s^{-1} Pa^{-1}
Ethylene permeability	$1.4 \pm 0.91 \times 10^{-14}$	mol m^{-1} s^{-1} Pa^{-1}
Mechanical properties		
Tensile strength	0.96 ± 0.22	MPa
Elongation percentage	16.62 ± 4.18	%
Young's modulus	6.55 ± 1.4	MPa

Effect of the Alginate–Chitosan Bilayer Edible Film on Figs

CO_2 and Ethylene Productions

CO_2 and ethylene productions are good indicators of metabolic activity and can be used to predict the shelf life of vegetable products (Khaliq et al. 2015). In this study, the application of the A–Ch BE film affected the CO_2 emissions of figs at both maturity stages, and in general, the application of the film promoted a significant reduction of CO_2 emissions for figs at both stages of maturity during storage (Table 2). Besides, a decrease in the CO_2 production in fig fruits (coated and uncoated) from ~3.0 to ~1.0 mL kg^{-1} h^{-1} in the first 3 storage days was observed, regardless of the maturity stage. Moreover, in the subsequent days of storage, the CO_2 emission remained lower than ~1.0 mL kg^{-1} h^{-1} until day 15.

Several authors have also reported a reduction in the CO_2 production in coated fruits, including Chinese pears (Ju et al. 2000), nectarine (Ahmed et al. 2009), and mangoes (Khaliq et al. 2015), which resulted from the reduction of the oxygen availability promoted by the impermeability of the film (Khaliq et al. 2015).

With regard to ethylene production, the application of the A–Ch BE film caused a significant decrease in coated figs after the first 3 storage days from 37 to 25 μL kg^{-1} h^{-1} for fruits in stage III and from 34 to 25 μL kg^{-1} h^{-1} for figs in stage IV (see Fig. 1). Then, there was a significant increase in figs of both maturity stages throughout the storage period at 6 °C (up to 55 μL kg^{-1} h^{-1} for figs in stage III and to 77 μL kg^{-1} h^{-1} for figs in stage IV), while in uncoated fruits,

Table 2 CO_2 emission values (mL kg^{-1} h^{-1}) of coated figs with an A–Ch BE film and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C

Maturity stage	Storage days	Uncoated fruits	Coated fruits
III	0	2.20 ± 0.2^{aX}	3.01 ± 0.18^{aY}
	3	1.02 ± 0.1^{bX}	0.99 ± 0.09^{bX}
	6	0.59 ± 0.01^{cX}	0.63 ± 0.01^{cdX}
	9	0.78 ± 0.01^{dX}	0.59 ± 0.00^{dY}
	12	0.87 ± 0.04^{bdX}	0.67 ± 0.02^{cdY}
	15	0.95 ± 0.02^{bX}	0.78 ± 0.01^{cY}
IV	0	2.16 ± 0.00^{aX}	2.95 ± 0.18^{aY}
	3	0.95 ± 0.03^{bX}	1.00 ± 0.01^{bX}
	6	0.80 ± 0.08^{bdX}	0.62 ± 0.01^{cdY}
	9	0.77 ± 0.03^{dX}	0.57 ± 0.03^{dY}
	12	0.83 ± 0.01^{bdX}	0.74 ± 0.01^{cdX}
	15	0.92 ± 0.01^{bX}	0.77 ± 0.00^{cY}

Values in a column followed by different low case letter are statistically different. Values in a row followed by different capital letter are statistically different ($p < 0.05$)

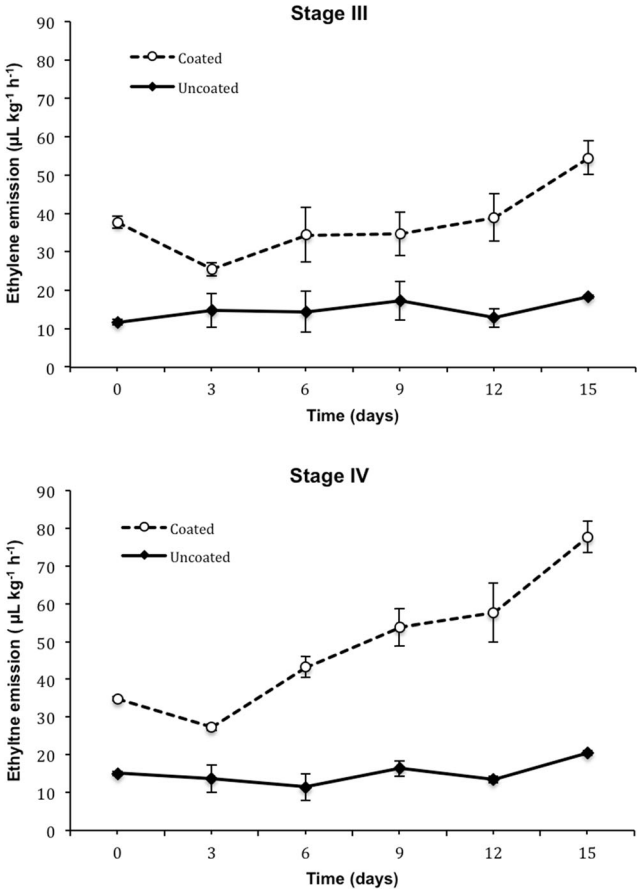

Fig. 1 Ethylene emission of coated (with an A–Ch BE film) and uncoated (control) figs at two maturity stages stored up to 15 days at 6 °C. *Vertical bars* indicate standard deviation

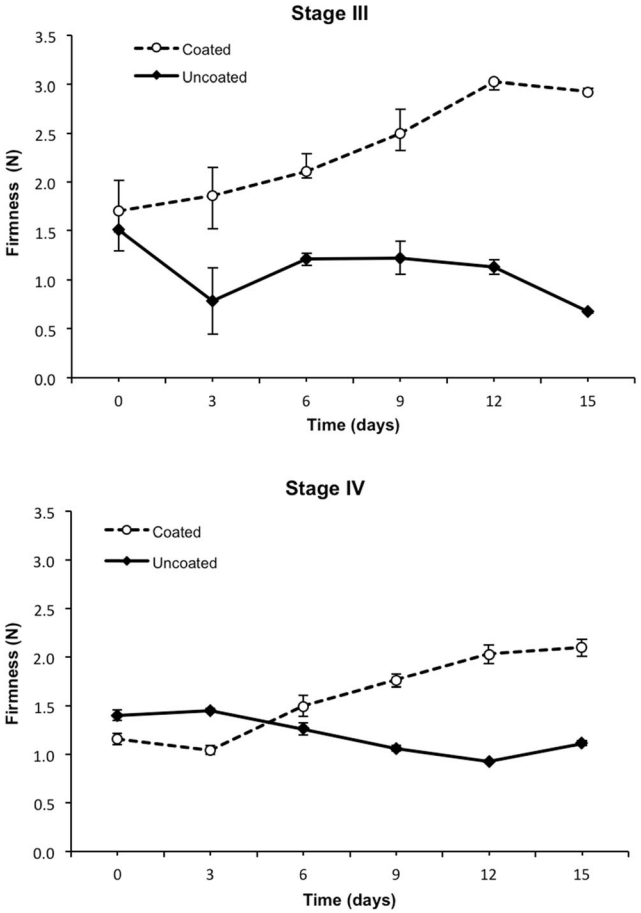

Fig. 2 Texture (compression force) changes of figs, collected at two maturity stages, coated with an A–Ch BE film and uncoated (control) stored up to 15 days at 6 °C. *Vertical bars* indicate standard deviation

the ethylene production remained almost constant, with values around 15 µL kg^{-1} h^{-1}, at both maturity stages. Ahmed et al. (2009) reported that *Aloe vera* gel coatings reduced the ethylene production in "Arctic Snow" nectarines during 6 weeks of cold storage (0 ± 0.5 °C), and Ju et al. (2000) published reports showing that emulsions containing 9 % of corn oil used as coating delayed the ethylene production in two varieties of Chinese pears stored for 2 months at 0 °C. However, in the current study, the fact that the ethylene production increased more in coated fruits could be attributed to the inclusion of olive oil in the film formulation. Thus, Owino et al. (2006) reported that ethylene synthesis can be stimulated by the direct application of several types of vegetable oils; although, the mechanism of such an increase of the ethylene production in mature figs has not yet been elucidated.

Firmness

The application of the A–Ch BE film affected the figs firmness at both maturity stages throughout the storage period. In general, coated figs exhibited higher firmness values than uncoated fruits ($p < 0.05$) (Fig. 2). The coated figs could maintain their firmness due to the retention of their water content, which results in the preservation of the cell integrity (Toivonen and Brummell 2008). Furthermore, Hernández-Muñoz et al. (2008) reported that the combined use of chitosan and calcium gluconate increased the firmness of strawberry under cold storage (10 °C) for up to 6 days. It is known that calcium plays an important role in cell wall integrity, thereby promoting the formation of calcium pectate in figs (Chardonnet et al. 2003; Ortiz et al. 2011; Serrano et al. 2004; Verdini et al. 2008).

Changes in Fruit Color

Color is one of the most important attributes of fruit quality. The application of A–Ch BE film affected L^* (lightness) and C^* (chroma) parameters in fig fruits throughout the storage period (at 6 °C). Thus, L^* values for uncoated figs, at both maturity stages, decreased from ~32 to ~29 after 15 days of storage (Table 3), resulting in a darker fruit peel, whereas the A–Ch BE film helped to retain the figs peel lightness during the storage period. On the other hand, the C^* parameter observed in uncoated fruits exhibited a significant decrease at

Table 3 L^*, C^*, and ΔE^* parameter values of coated (with an A–Ch BE film) and uncoated (control) figs at two maturity stages stored up to 15 days at 6 °C

Maturity stage	Storage days	L^*		C^*		ΔE^*	
		Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
III	0	31.93 ± 0.44^{aX}	29.82 ± 0.15^{abcY}	7.24 ± 1.04^{aX}	6.54 ± 0.59^{aX}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	3	30.34 ± 0.54^{bcX}	30.38 ± 0.46^{aX}	7.55 ± 0.85^{aX}	7.11 ± 0.35^{aX}	1.74 ± 0.77^{abX}	1.57 ± 0.48^{aX}
	6	30.58 ± 0.27^{bX}	29.41 ± 0.56^{abcY}	6.54 ± 0.51^{acX}	6.76 ± 0.51^{abX}	1.84 ± 0.71^{abX}	1.72 ± 0.49^{aX}
	9	29.81 ± 0.38^{bdX}	30.07 ± 0.39^{abX}	6.21 ± 0.33^{acX}	6.91 ± 0.50^{abX}	3.32 ± 0.98^{bcX}	1.34 ± 0.69^{aX}
	12	29.17 ± 0.23^{dX}	29.18 ± 0.17^{bcdX}	4.70 ± 0.53^{bX}	6.01 ± 0.41^{acX}	4.19 ± 0.72^{cX}	1.43 ± 0.50^{aY}
	15	29.07 ± 0.58^{dX}	29.63 ± 0.09^{abcX}	3.84 ± 0.61^{bX}	6.25 ± 0.14^{acY}	4.99 ± 1.56^{cX}	0.96 ± 0.27^{aY}
IV	0	31.03 ± 0.77^{acX}	28.94 ± 0.27^{cdY}	7.10 ± 0.90^{aX}	6.12 ± 0.21^{abcX}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	3	29.89 ± 0.45^{bdX}	29.33 ± 0.21^{acdX}	5.17 ± 0.12^{bcX}	5.94 ± 0.53^{abcX}	2.89 ± 1.07^{bcX}	0.93 ± 0.26^{aX}
	6	30.62 ± 0.22^{bcX}	29.18 ± 0.48^{acdY}	5.15 ± 0.31^{bcX}	5.69 ± 0.48^{bcX}	2.73 ± 1.09^{bX}	1.04 ± 0.49^{aX}
	9	29.74 ± 0.02^{bdX}	28.95 ± 0.19^{cdX}	4.48 ± 0.19^{bX}	5.07 ± 0.22^{cX}	3.46 ± 0.93^{bcX}	1.17 ± 0.14^{aY}
	12	29.61 ± 0.24^{bdX}	29.21 ± 0.13^{acdX}	4.57 ± 0.06^{bX}	5.13 ± 0.19^{cX}	3.18 ± 1.17^{bcX}	1.13 ± 0.11^{aY}
	15	29.26 ± 0.23^{dX}	28.43 ± 0.12^{dX}	4.04 ± 0.06^{bX}	5.06 ± 0.32^{cX}	4.19 ± 1.23^{cX}	1.33 ± 0.01^{aY}

Lowercase letters (a, b, c, d) indicate significant differences in maturity stages and storage days ($p < 0.05$). Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits ($p < 0.05$)

both maturity stages, from ~7.4 up to ~4, while in coated fruits, this parameter ranged from 7.1 to 6.0 and from 6.1 to 5.0 in stages III and IV, respectively (Table 3). Interestingly, the uncoated fruits also showed high ΔE values at both maturity stages, whereas in coated fruits, this parameter was lower than 2 units during all storage periods (Table 3). These results suggest that the use of the A–Ch BE film retains the external color of the figs better during storage at low temperature. This is in agreement with the results reported by Hernández-Muñoz et al. (2008) using a chitosan coating that contributed to reducing color changes in strawberries during storage.

Several authors have suggested that a decrease in L^* and C^* parameters may be associated with the fruit ripening process (Díaz-Mula et al. 2009; Durmaz et al. 2010; Mikulic-Petkovsek et al. 2015). Moreover, Usenik et al. (2009) proposed that the C^* parameter is also related to the presence of fruit pigments, such as anthocyanins. Interestingly, the L^* and C^* values observed in coated figs, after 15 days of storage, corresponded to a commercially attractive product (see Fig. 3).

Weight Loss

The weight loss of fruit and vegetables is closely related to transpiration and respiration processes. Also, the moisture loss of fresh fruits can be considered as one of the most critical factors in the loss of quality (Guilbert et al. 1997).

The application of the A–Ch BE film had a significant effect on the weight loss of figs, since weight loss for uncoated figs reached up to 18 % of their initial weight, at the day 15 of storage, while coated fruits had only around 5–7 % of weight loss at the end of the storage period (Fig. 4). Recently, Villalobos et al. (2016) reported that figs from "Cuello Dama Blanco," "Cuello Dama Negro," and "San Antonio" varieties, packaged with a microperforated biaxially oriented polypropylene film

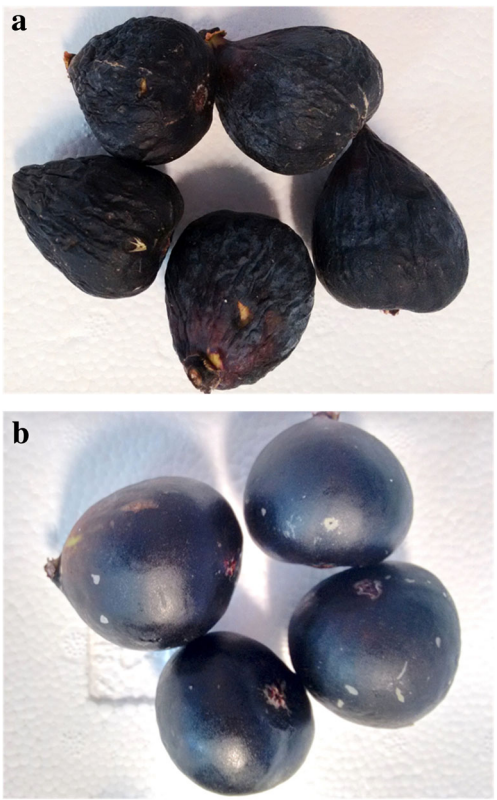

Fig. 3 Figs (*Ficus carica* L.) var. Mission after 15 days of storage at 6 °C: **a** uncoated and **b** coated figs

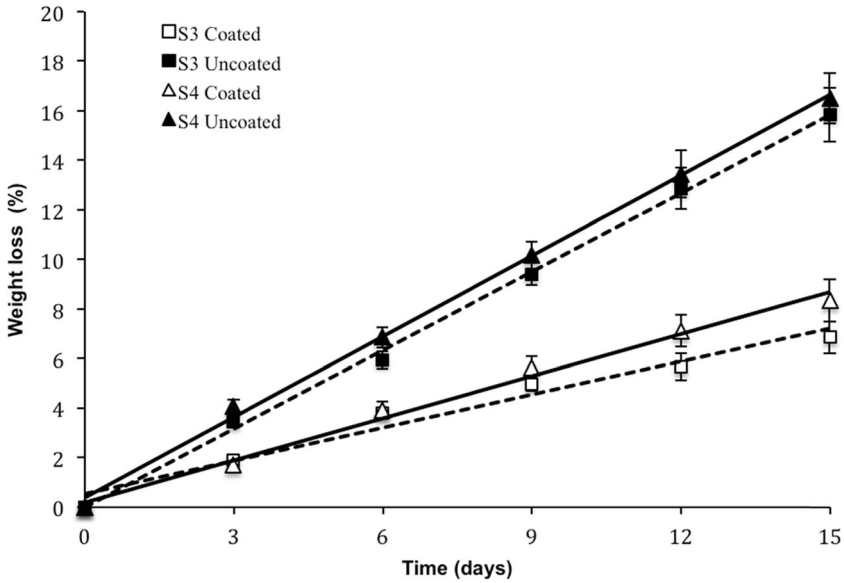

Fig. 4 Weight changes of coated (with an A–Ch BE film) and uncoated (control) figs at two maturity stages (S3 and S4 stand for stages III and IV respectively) stored up to 15 days at 6 °C. *Vertical bars* indicate standard deviation

(thick 40 μm; ∅ 100 μm), exhibited lower weight losses than fruits packaged with a macroperforated film (thick 40 μm; ∅ 9 mm). Similar results have also been reported in *Capsicum annum* L. var. King Arthur coated with different hydrocolloids based in locus bean gum, maltodextrin, or xanthan gum (Conforti and Zinck 2002). Several authors have proposed that the addition of lipid compounds to the film formulation improves its hydrophobic capacity (Fabra et al. 2012, 2013; Guillén et al. 2013; Pereda et al. 2012). Lipids act as a seal over the fruit's surface, and the water is retained since it is not miscible with lipids (Conforti and Zinck 2002).

On the other hand, the maturity stage did not show any significant effect on the weight loss which is in agreement with the results reported by Crisosto et al. (2010), who evaluated the fresh weight of four fig varieties harvested at two different maturity stages, particularly commercial and tree ripe. These authors reported that fruit's weight depended more on the fig variety than on the maturity stage.

Fruits' Visual Contamination

The application of the A–Ch BE film also retarded the fruits' visual contamination (Fig. 5). Thus, uncoated fig fruits presented visual fungal infection after just 3 days of storage. On the contrary, fungal infection was only observable in coated figs after 15 days of storage. In fact, at the end of storage, coated figs presented a visual fungal contamination on, approximately, 5 % of the fruits, whereas about 80–87 % of the uncoated figs were contaminated. Romanazzi et al. (2013) reported that chitosan coatings were capable of reducing microbial and fungal contamination in strawberries stored at 20 °C and 95–98 % RH during 4 days. Also, Gol et al. (2013) demonstrated that the coatings of carboxymethyl cellulose, hydroxypropylmethyl cellulose, and their combination with the antifungal agent chitosan reduced fungal contamination in strawberry fruit during storage.

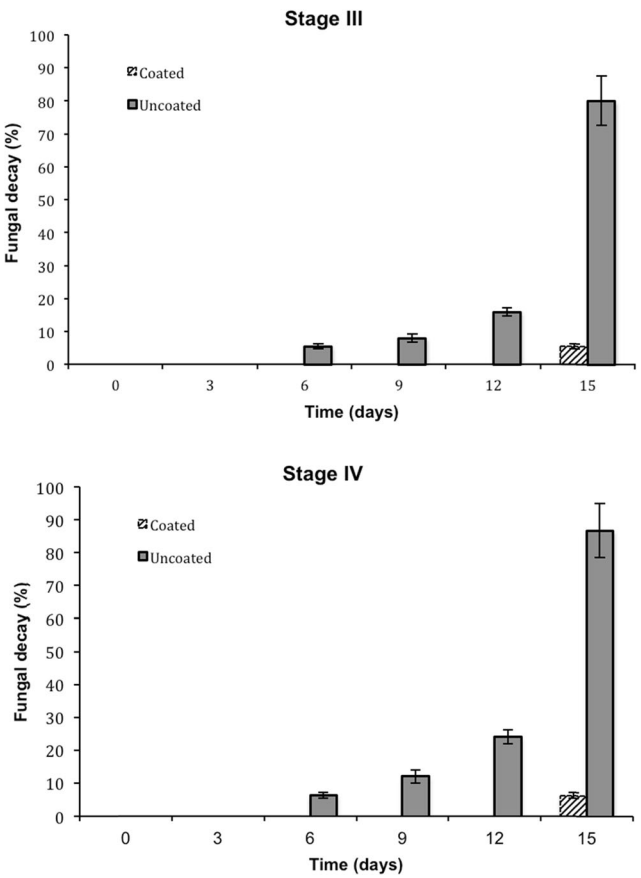

Fig. 5 Visual fungal contamination of coated (with an A–Ch BE film) and uncoated (control) figs at two maturity stages stored up to 15 days at 6 °C. *Vertical bars* indicate standard deviation

Conclusion

The present study was focused on the development and application of an alginate–chitosan bilayer edible (A–Ch BE) film in order to improve the quality and shelf life of figs (*F. carica* var. Mission). The A–Ch BE film developed in this study exhibited adequate mechanical properties of elasticity and elongation and also appropriate WVP and PCO_2 values, which promoted a significant increase in the ethylene emission of figs throughout the storage period, regardless their maturity stage. Furthermore, the application of the A–Ch BE film contributed to retain the color of figs during storage at 6 °C. Interestingly, the firmness of figs was not only maintained but also even increased when the film was applied. Moreover, the excellent moisture barrier properties of the developed film helped to reduce the transpiration rate of fruits during storage, which could be associated to the significant reduction of the weight loss of fruits. Additionally, the low permeability to moisture of the A–Ch BE film retarded the visual fungi contamination for 12 days of storage. Therefore, the application of the A–Ch BE film might be considered as a potential and effective alternative to extend the shelf life of fresh figs during storage at low temperature (6 °C) and high RH (95 %).

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Calidad de melón cantaloupe (*Cucumis melo*) cubierto con una película comestible de alginato-hpmc-parafina

Quality of Cantaloupe melon (*Cucumis melo*) covered by an alginate-hpmc-paraffin edible coati

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Resumen

Introducción: El melón es un fruto que tiene un corto periodo de almacenamiento. Una alternativa para extender este periodo es el uso de películas comestibles. En el presente estudio se evaluó el efecto de la aplicación de una película comestible de alginato de sodio-hidroxipropilmetilcelulosa-parafina (ALG-HPMC-PAR), sobre la calidad de melón Cantaloupe durante dos tipos de almacenamiento.

Método: Frutos de melón Cantaloupe se cubrieron con una película comestible de alginato-hidroxipropilmetil celulosa-parafina (ALG-HPMC-PAR), y no cubiertos (CONTROL). Los melones se almacenaron por 21 días a 5ºC y 95% de humedad relativa (Hr). Cada siete días, los frutos se sometieron a análisis de índice de daños por frío, pérdida de peso, textura, y concentración de CO_2 y etileno de la atmósfera interna del fruto. Además, para simular manejo comercial, al término de cada periodo de siete días en refrigeración, una muestra de melones era extraída del frigorífico y expuesta a condiciones ambientales de temperatura y humedad relativa (25ºC y 21-25% respectivamente) durante tres días (almacenamiento combinado), y medidos los parámetros anteriormente mencionados.

Resultados: La aplicación de la película comestible provocó que los frutos cubiertos tuvieran una mayor concentración de CO_2 y menor concentración de etileno en la atmósfera interna del fruto, contribuyendo a que los melones cubiertos mantuvieron su calidad por un mayor tiempo de almacenamiento, manteniéndose más firmes, con menor pérdida de peso y sufrir menor índice de daños por frío con respecto los frutos control.

Discusión: Los resultados demuestran la factibilidad de la aplicación de películas comestibles para mantener la calidad del melón Cantaloupe almacenado en refrigeración y en almacenamiento combinado.

Palabras Clave: calidad comercial; melón; película; alginato de sodio

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Abstract

Introduction: Melon is a fruit with a short storage period. An alternative to extend the shelf-life of this fruit is the application of an edible film. In present study was evaluated the effect of the application of an edible coating based on alginate-hydroxypropilmethyl-cellulose-parafine over the quality of Cantaloupe melon under two storage types.

Method: Cantaloupe melon fruits were covered by an alginate-hydroxypropilmethyl-cellulose-parafine edible coating (ALG-HPMC-PAR), and uncovered (CONTROL). Experimental melons were stored for 21 days at 5 ºC and 95 % relative humidity. Every seven days, the fruits were subjected to analysis of chilling injury index, loss of weight, texture, and CO_2 and ethylene concentration of internal atmosphere of the fruit. In addition, to simulate commercial management, at the end of each period of seven days in refrigeration, a sample of melons was extracted from the refrigerator and exposed to environmental conditions of temperature and relative humidity (25ºC and 21-25% respectively) during three days (combined storage), and measured the parameters mentioned above.

Results: The application of the edible coating induced that melons had a higher CO_2 and lower ethylene concentration in the fruit inner atmosphere, contributing to maintain their quality for a longer storage time than the control fruits, since the covered melons were firmer, had a lower weight loss, and suffered a lower chilling injury index.

Discussion or **Conclusion**: The obtained results prove the feasibility of the application of edible coatings to maintain the Cantaloupe melon quality in cooling and combined storage.

Keywords: Commercial quality, melon, coating, sodium alginate

Introducción

El melón (*Cucumis melo* L.) es un fruto apreciado por su propiedades sensoriales, principalmente por su aroma y sabor, además de ser un producto de gran importancia comercial, ya que su valor de producción bruta mundial supera los 19,700 millones de dólares (FAOSTAT, 2013). El mayor productor de melón en el mundo durante el 2013 fue China, con 14,400 millones de toneladas, seguido por Turquía e Irán con 1,699 y 1,501 millones de toneladas respectivamente. Por su parte, México ocupó el doceavo lugar con una producción de 561,953 ton, y es el sexto país exportador de melón a nivel mundial (FAOSTAT, 2013). Sin embargo, el melón es un fruto climatérico sensible al etileno que tiene una maduración rápida, lo cual reduce su vida de anaquel, resultando en una cantidad grande de producto con calidad comercial baja, por lo que se requiere establecer métodos de conservación para este fruto.

El almacenamiento a temperaturas de refrigeración es uno de los métodos más utilizados para conservar y aumentar la vida de anaquel del melón, ya que desacelera procesos bioquímicos relacionados con la maduración del fruto. No obstante, el almacenamiento en refrigeración de algunos productos vegetales incluyendo el melón Cantaloupe, regularmente induce a la aparición de diferentes patologías post-cosecha, tales como el daño por frio, lo cual afecta negativamente la calidad del producto ya que causa maduración anormal y aparición temprana de senescencia de vegetales y frutos (Millán-Trujillo *et al*., 2001, 173; García-Sahagún *et al*., 2005, 161; Meza-Velázquez *et al*., 2013, 259). Un método novedoso para mantener la calidad y extender la vida de anaquel de vegetales mínimamente procesados es la aplicación de cubiertas (soluciones y/o emulsiones) a base de polímeros comestibles como polisacáridos, proteínas y/o lípidos (Gómez-Estaca *et al*., 2009, 480; Monedero *et al*., 2009, 509; Fabra *et al*., 2012, 109; Bonilla *et al*., 2013, 303; Arnon *et* al., 2015, 465). Las cubiertas aplicadas forman una barrera entre el producto y el medio ambiente, retardando el intercambio de humedad, oxígeno, dióxido de carbono y etileno (Lima *et al*., 2010, 101; Meza-Velázquez *et al*., 2013, 259). Otras propiedades de estas cubiertas comestibles incluyen la protección del producto contra daños mecánicos, oxidativos y microbiológicos, además que mejoran la apariencia y previenen la pérdida de aromas de frutos y vegetales (Fabra *et al*., 2012, 109; Han *et al*., 2014, 1; Genskowsky *et al*., 2015, 1057). Algunos materiales que han sido utilizados para preparación de cubiertas comestibles son el alginato y derivados de la celulosa, como la hidroxipropilmetil celulosa. El alginato es un polisacárido usado en procesos de encapsulación debido a su capacidad para formar geles en presencia de

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cationes divalentes como calcio, bario y estroncio (Ramdas *et al.*, 2000, 405; Braccini y Pérez, 2001, 1089). La eficacia del alginato en el desarrollo de cubiertas comestibles es abordada por diversos autores en diferentes productos incluyendo manzanas (Olivas *et al.*, 2007, 89), piñas (Montero-Calderón *et al.*, 2008, 182) y papayas (Tapia *et al.*, 2008, 1493). Por otra parte, la hidroxipropilmetilcelulosa es un polímero derivado de la celulosa usado en la formulación de películas, el cual ha sido probado en diversos vegetales, resultando en la extensión de la vida de anaquel de dichos productos (Pérez-Gago *et al.*, 2002, 2903; Meza Velázquez *et al.*, 2013, 259).

El objetivo del presente estudio consistió en evaluar el efecto de la aplicación de una cubierta comestible a base de alginato de sodio-hidroxipropilmetilcelulosa-parafina sobre la textura, daño por frio, pérdida de peso, y concentración de dióxido de carbono y etileno interno de frutos de melón cantaloupe (*Cucumis melo* L.) almacenados en refrigeración y en melones sometidos, posterior a la refrigeración, a condiciones ambientes de temperatura y humedad relativa.

Método

Muestras experimentales. Los frutos de melón Cantaloupe (*Cucumis melo var. reticularis*) utilizados en el estudio se recolectaron de un predio del Ejido Andalucía de Matamoros, Coahuila (México; 103°13´42" longitud oeste/ 25° 31´41" latitud norte) en etapa pre-climatérica (25-27 días después de polinización), siguiendo la metodología descrita por Nishiyama *et al*. (2007, 1281). Los melones seleccionados fueron frutos de 1.2-1.5 kg recolectados en estado de madurez 3/4 desprendido (Beaulieu *et al.*, 2004, 250), y que no presentaran daños físicos y contaminación microbiana visible.

Materiales y Reactivos. La hidroxipropilmetilcelulosa la proporcionó Colorcon de México (México). El alginato de sodio y la parafina se adquirieron en CyTCSA, S.A de C.V. (México). La lecitina de soya y los reactivos mono estearato de propilenglicol, meta bisulfito de sodio, sorbato de potasio y mono estearato de propilenglicol se adquirieron de Golden Bell, S.A. de C.V. (México) Los estándares de dióxido de carbono y etileno se obtuvieron de Sigma-Aldrich Company (St. Louis, MI, EUA).

Preparación de la cubierta. La cubierta de alginato de sodio-hidroxipropilmetilcelulosa-parafina (ALG-HPMC-PAR) se preparó siguiendo una adaptación del método publicado por Raybaudi-Massilia *et al*. (2008, 1150), la cual consiste primeramente en la preparación por separado soluciones de alginato de sodio al 3 % y de HPMC al 2 % p/v en agua destilada a 90 ºC

en agitación constante por 5 min. Posteriormente, las soluciones preparadas se enfriaron rápidamente a 4-5 ºC para lograr una hidratación adecuada de los polímeros. Las dos soluciones frías se mezclaron y calentaron a 80 ºC, añadiendo lecitina de soya y mono estearato de propilenglicol en 0.6 % (p/v) y parafina en 25 % p/v respecto a la mezcla, la cual fue emulsificada inicialmente a 12000 rpm por 2 min usando un Ultraturrax T18 (IKA® Works, Inc, Wilmington, EUA), y después a 25000 rpm por 5 min. La emulsión finalmente se dejó enfriar a temperatura ambiente y se desgasificó por 10 min mediante una bomba de vacío Felisa (Fabricantes Feligneo, Jalisco, México) para su posterior aplicación.

Tratamientos. Los melones recolectados se lavaron con agua clorada a 200 ppm de cloro, y distribuidos aleatoriamente en dos lotes: tratamiento control (sin aplicación de película), y película (frutos cubiertos con la película ALG-HPMC-PAR. Las muestras experimentales (melón entero y pulpa) se analizaron antes y después de aplicar la cubierta de ALG-HPMC-PAR. Posteriormente, los frutos (cubiertos y control) se sometieron a un almacenamiento a 5 ºC y 95% de humedad relativa (Hr) durante 21 días. Los frutos eran analizados cada siete días a lo largo del periodo de estudio (0, 7, 14 y 21 días). Las pruebas analíticas practicadas a los melones fueron concentración de CO_2 y etileno (en los gases internos del fruto), medición instrumental de firmeza, índice de daño por frío y pérdida de peso. Además, y para simular condiciones de manejo comercial (Valero *et al*., 2013, 1), al finalizar cada periodo de siete días en refrigeración, una muestra de melones fue extraída del frigorífico y expuesta a condiciones ambientales de temperatura (25ºC) y humedad relativa (21-25%) por 3 días, y se les determinó los mismos parámetros descritos con anterioridad. Los tratamientos (control y película) se repitieron cuatro veces y cada repetición contenía 24 melones.

Aplicación de la película. La emulsión formadora de película se aplicó manualmente a melones enteros lavados y secos por medio de una brocha hasta quedar completamente cubierto el fruto, y luego el exceso de emulsión fue retirado del producto (Conforti y Zinck, 2002, 1360). Los melones cubiertos se sumergieron en una solución de cloruro de calcio al 1 % por un minuto para la gelificación de la película. Los frutos cubiertos se expusieron a una corriente de aire de 5 m/s para secar la película, y luego almacenados en frio. El grosor de la película aplicada se midió con un micrómetro, obteniendose un espesor final de 14 µm.

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Pruebas analíticas

Determinación de CO_2 y etileno en espacio interno del fruto. La concentración de CO_2 y etileno en el espacio interno del fruto se llevó a cabo por el método descrito por Pérez-Gago *et al.* (2002, 2903), tomándose una muestra de 5 mL del gas contenido en el espacio interno del fruto, posteriormente se inyecto 1 mL de la muestra de gas en un cromatógrafo de gases HP 6820 (Agilent Technology, CA, EUA**)** equipado para la determinación de CO_2 con una columna empacada Alltech CTR I de 6 pies × ¼ de pulgada (Alltech Associates, Inc., Deerfield, Illinois, EU), y un detector de conductividad térmica. La temperatura del inyector, del detector y de la columna fue de 20, 170 y 35 ºC, respectivamente. Por otra parte, la concentración de etileno se determinó usando una columna Carboxen de 30 m × 0.5 mm × 0.25 µm (Supelco, PA, EUA), con un detector de ionización de flama. La temperatura del inyector fue de 120 ºC, y la del detector de 250 ºC. La separación se llevo a cabo usando una rampa de temperatura iniciando a 35 ºC con un incremento de 20 ºC/min hasta alcanzar 120 ºC. Las lecturas del cromatógrafo se analizaron con el software Agilent Cerity NDS (Agilent Technologies, EUA), y comparadas con curvas de calibración de estándares de CO_2 y etileno. La medición se llevó a cabo en tres frutos de melón, de cada repetición de los tratamientos, en cada tiempo de almacenamiento. Cada fruto fue considerado como réplica analítica.

Textura. La firmeza del fruto entero se realizó usando el método de compresión con un texturómetro TAXT2i equipado con el software Exponent 6.1.4.0 (Microsystem, Londres, Inglaterra), utilizando un punzón de punta redondeada de media pulgada de diámetro, con una velocidad de desplazamiento de 1.5 $mm \cdot s^{-1}$ y una distancia de recorrido de 2 mm. Tres melones, de cada repetición de los tratamientos, se usaron para el análisis. Luego, a cada fruto se les practicaron diez mediciones a lo largo de su zona ecuatorial (30 réplicas). La firmeza se especificó como la fuerza máxima de compresión (N) (Meza Velázquez *et al*., 2013, 259), y se reportaron los cambios respecto al valor inicial de firmeza del fruto entero de melón no cubierto al inicio del estudio.

Índice de daños por frío (IDF). La determinación del IDF se realizó utilizando la escala de valores de 0 a 4 propuesta por García-Sahagún *et al.* (2005, 161), donde 0 = fruto sin daño; 1= daño ligero (10 % o menos de la superficie del fruto dañada); 2 = daño moderado (10 a 15 % de la superficie); 3 = daño regular (15 a 25 % de la superficie dañada, producto no apto para comercialización); y 4 = daño severo (más del 25 % de la superficie del fruto dañado). La

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evaluación se realizó en cuatro frutos de melón de cada repetición de los tratamientos. El cálculo del IDF fue obtenido usando la fórmula 1:

$$IDF = [(n)0 + (n)1 + (n)2 + (n)3 + (n)4] / N \quad \text{.....(Fórmula 1)}$$

Donde:
n = número de frutos dañados;
N = número de frutos por tratamiento.

Pérdida de peso. Una muestra de 5 melones, de cada repetición de los tratamientos, se pesó al inicio del experimento y después a los 7, 14 y 21 días de almacenamiento en refrigeración. La pérdida de peso se reportó como el cambio porcentual, sufrido por el fruto, desde su peso inicial.
Diseño experimental y análisis estadístico. Se usó un diseño factorial con tres factores: tipo de almacenamiento (refrigeración y combinado), tiempo de almacenamiento (0, 7, 14 y 21 días) y aplicación de película comestible (con y sin película), y se llevaron a cabo cuatro repeticiones por tratamiento. Los resultados de las variables evaluadas se analizaron mediante Análisis de Varianza. La diferencia entre medias de tratamientos se realizó por la prueba de comparación múltiple de Diferencia Mínima Significativa de Fisher (DMS de Fisher) con un nivel de significancia de 0.05, usando el programa estadístico SAS versión 8 (SAS Institute Inc., 2005).

Resultados y Discusión

Determinación de CO_2 y etileno en espacio interno del melón

En la tabla 1 se presentan los resultados de efecto de la aplicación de la película de ALG-HPMC-PAR sobre la concentración de CO_2 y etileno de melones expuestos a dos tipos de almacenamiento (refrigeración y combinado) durante 21 días. Los resultados muestran que los melones refrigerados (cubiertos y control) tuvieron una concentración de CO_2 y etileno menor que los frutos sometidos al almacenamiento combinado en frio y después en condiciones ambientales ($P < 0.05$). Además, en general, durante el almacenamiento la aplicación de la película ALG-HPMC-PAR afectó la concentración de los gases evaluados solo en los frutos expuestos a almacenamiento combinado ($P < 0.05$), observándose una concentración de CO_2 mayor y concentración de etileno menor ($P < 0.05$) que en los melones no cubiertos. Diversos

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estudios reportan que aplicar películas comestibles en frutas y hortalizas modifica su atmósfera interna y provoca retraso en los procesos metabólicos asociados con la maduración y senescencia (Vigneault *et al.*, 2000, 1314; Falguera *et al*., 2011, 292; Bonilla *et al*., 2012, 303), repercutiendo a la posibilidad de que la aplicación de la película de alginato-HPMC-parafina haya modificado el ritmo respiratorio del fruto debido a la permeabilidad selectiva de la cubierta a los gases y humedad. Estos resultados concuerdan con los publicados por Meza Velázquez *et al*. (2013, 259), que reportaron que la aplicación de cubiertas comestibles contribuye a que aumente la concentración de CO_2 interno en frutos, lo cual disminuye el ritmo metabólico de frutos y vegetales mínimamente procesados, lo que resulta en una extensión de la vida de anaquel de estos productos (Paul y Pandey, 2014, 1223).

Tabla 1. Concentración de CO_2 y etileno en melones cubiertos con una película comestible, y no cubiertos sometidos a dos tipos de almacenamiento

Tipo de Almacenamiento	Días	CO_2 (mL/L)		Etileno (µL/L)	
		Control[1]	Película[2]	Control[1]	Película[2]
Refrigeración*	0	6.96 ± 0.44 ^{aX}	7.41 ± 0.96 ^{aX}	113.7 ± 16.3 ^{aX}	97.97 ± 8.76 ^{aX}
	7	3.92 ± 0.26 ^{bX}	5.02 ± 0.85 ^{bX}	18.56 ± 0.67 ^{bX}	48.55 ± 5.90 ^{bX}
	14	3.76 ± 0.08 ^{bX}	4.29 ± 0.27 ^{bX}	17.01 ± 0.68 ^{bX}	20.71 ± 0.90 ^{bX}
	21	4.24 ± 0.29 ^{bX}	5.61 ± 0.57 ^{bX}	26.02 ± 0.45 ^{bX}	37.53 ± 7.51 ^{bX}
Combinado**	0+3	9.37 ± 0.25 ^{cX}	12.77 ± 0.56 ^{cY}	148.3 ± 6.10 ^{aX}	173.35 ± 13.6 ^{cX}
	7+3	12.46 ± 0.67 ^{dX}	14.00 ± 0.46 ^{cX}	236.6 ± 22.3 ^{cX}	243.82 ± 7.59 ^{dX}
	14+3	14.29 ± 0.58 ^{eX}	15.97 ± 0.51 ^{dX}	358.4 ± 46.7 ^{dX}	265.38 ± 32.3 ^{dY}
	21+3	17.23 ± 1.08 ^{fX}	20.29 ± 0.84 ^{eY}	297.4 ± 41.5 ^{eX}	179.08 ± 15.4 ^{cY}

Valores promedio ± desviación estándar (n=4 repeticiones por tratamientos).
[1] Frutos de melón no cubiertos (control). [2] Frutos de melón cubiertos con la película comestible de alginato-hidroxipropilmetilcelulosa-parafina.
* Almacenamiento en refrigeración a 5ºC y 95% Hr. **Almacenamiento combinado: Almacenamiento en refrigeración a 5ºC y 95% Hr seguido de almacenamiento a temperatura ambiente a 25ºC y 21-25% Hr.
Valores seguidos de diferente letras a, b, c, d, e, f, indican diferencia significativa en el tipo de almacenamiento y días de almacenamiento en una misma columna ($p<0.05$, DMS de Fisher).
Valores seguidos de diferente letra X, Y indican diferencia significativa entre frutos cubiertos con película y no cubiertos (control) en un mismo periodo de tiempo ($p<0.05$, DMS de Fisher).

Textura

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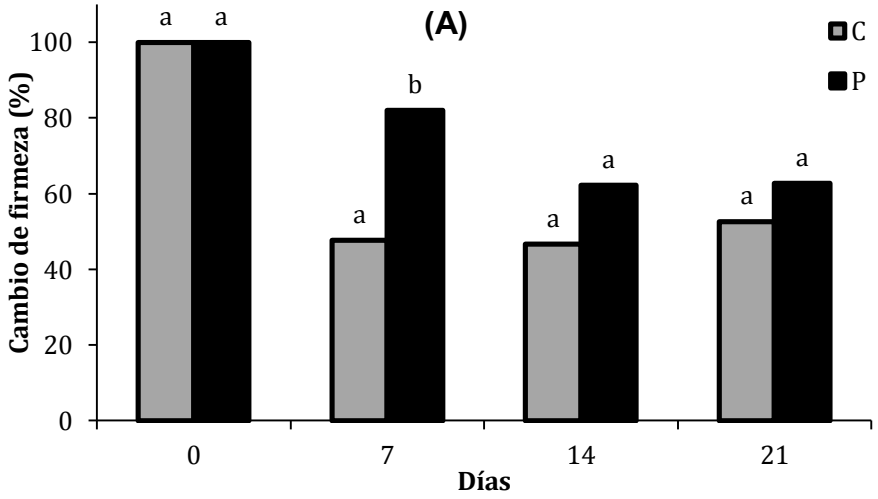
En las figuras 1a y 1b se ilustran los cambios en la firmeza de melones expuestos a dos tipos de almacenamiento (refrigeración y combinado) durante 21 días, tratados con una película de ALG-HPMC-PAR. Se observa que los melones cubiertos con la película comestible fueron más firmes que los frutos sin aplicación de la cubierta en los dos tipos de almacenamiento ($P < 0.05$), con una pérdida de textura de 51% en frutos almacenados en frio por 21 días, y de 61% en melones después del periodo de almacenamiento combinado (21 días de almacenamiento en refrigeración y 3 días a temperatura ambiente). La textura de frutas y vegetales está directamente relacionada con la composición y características de la pared celular, la cual está estrechamente relacionada con el grado de madurez del fruto (Oms-Oliu *et al*., 2007, 301; Giongo *et al*., 2013, 480), indicativo para que la firmeza mayor de los melones cubiertos con la película comestible sea atribuida a que dichos frutos mantuvieron su integridad celular en el tejido durante el almacenamiento (Toivonen y Brummell, 2008, 1). Estos resultados coinciden con los publicados por Pérez-Gago *et al*. (2002, 2903), Fan *et al*. (2009, 84), Valero *et al*. (2013, 1), y Meza-Velázquez *et al*. (2013, 259) quienes reportan una conservación de la textura de diversas frutas y hortalizas mediante la aplicación de cubiertas a base de alginato, así como de HPMC.



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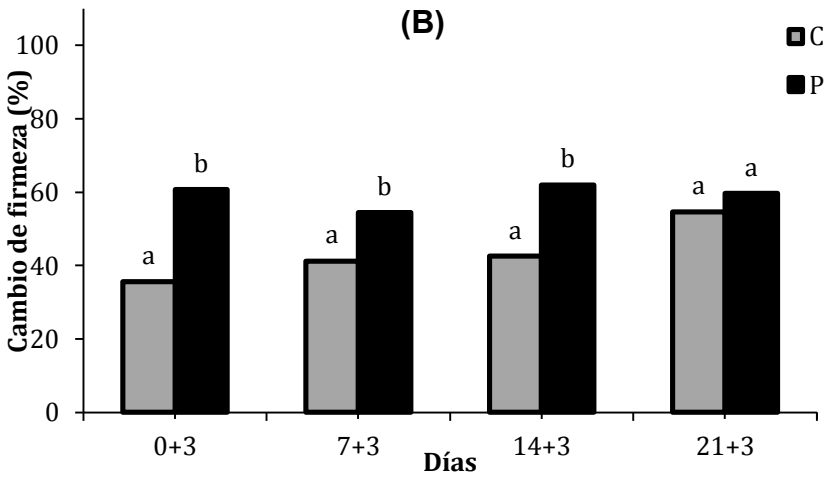

Figuras 1a y 1b. Cambios de textura (%) de frutos de melón cubiertos con la película comestible de alginato-hidroxipropilmetilcelulosa parafina (P), y no cubiertos o control (C), almacenados en refrigeración (a 5ºC y 95% Hr) (A), o sujetos a almacenamiento combinado (almacenamiento en refrigeración a 5ºC y 95% Hr seguido de almacenamiento a temperatura ambiente a 25ºC y 21-25% Hr)(B). Diferentes letras sobre las barras de valores de tratamientos (control y película) indican diferencia significativa en cada periodo de tiempo de almacenamiento ($P<0.05$, DMS de Fischer).

Índice de daños por frío (IDF)

En las figuras 2a y 2b se muestran los valores promedio de daños por frío y deterioro de melones expuestos a dos tipos de almacenamiento (refrigeración y combinado) durante 21 días, tratados con una película de ALG-HPMC-PAR. Los resultados muestran que los melones no cubiertos presentaron un índice de daños por frío (IDF) mayor (~3.8) en comparación con los frutos cubiertos (~2.3) al ser almacenados a 5 ºC ($P < 0.05$). Asimismo, los frutos no cubiertos estuvieron más deteriorados después del almacenamiento combinado que los melones cubiertos con la película ALG-HPMC-PAR ($P < 0.05$), los cuales mantuvieron una apariencia considerada comercialmente aceptable después de los 21 días de refrigeración seguidos de 3 días de almacenamiento a temperatura ambiente. El almacenamiento a bajas temperaturas es uno de los métodos más comunes para conservar frutas y hortalizas, pero muchos frutos, como el melón Cantaloupe, son susceptibles a sufrir daños por frío (Artes y Artes-Hernández, 2003, 299). Los resultados del presente estudio concuerdan con lo reportado por Pérez-Gago *et al*. (2002, 2903) y Meza-Velázquez *et al*. (2013, 259), quienes encontraron que el uso de una película de comestible de HPMC disminuyó el daño por frío en mandarina y melón Cantaloupe, respectivamente. Lo anterior comprueba las ventajas de la aplicación de la película comestible de alginato-hidroxipropilmetilcelulosa-parafina para mantener la calidad de este fruto en condiciones de

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almacenamiento de refrigeración y a temperatura ambiente.

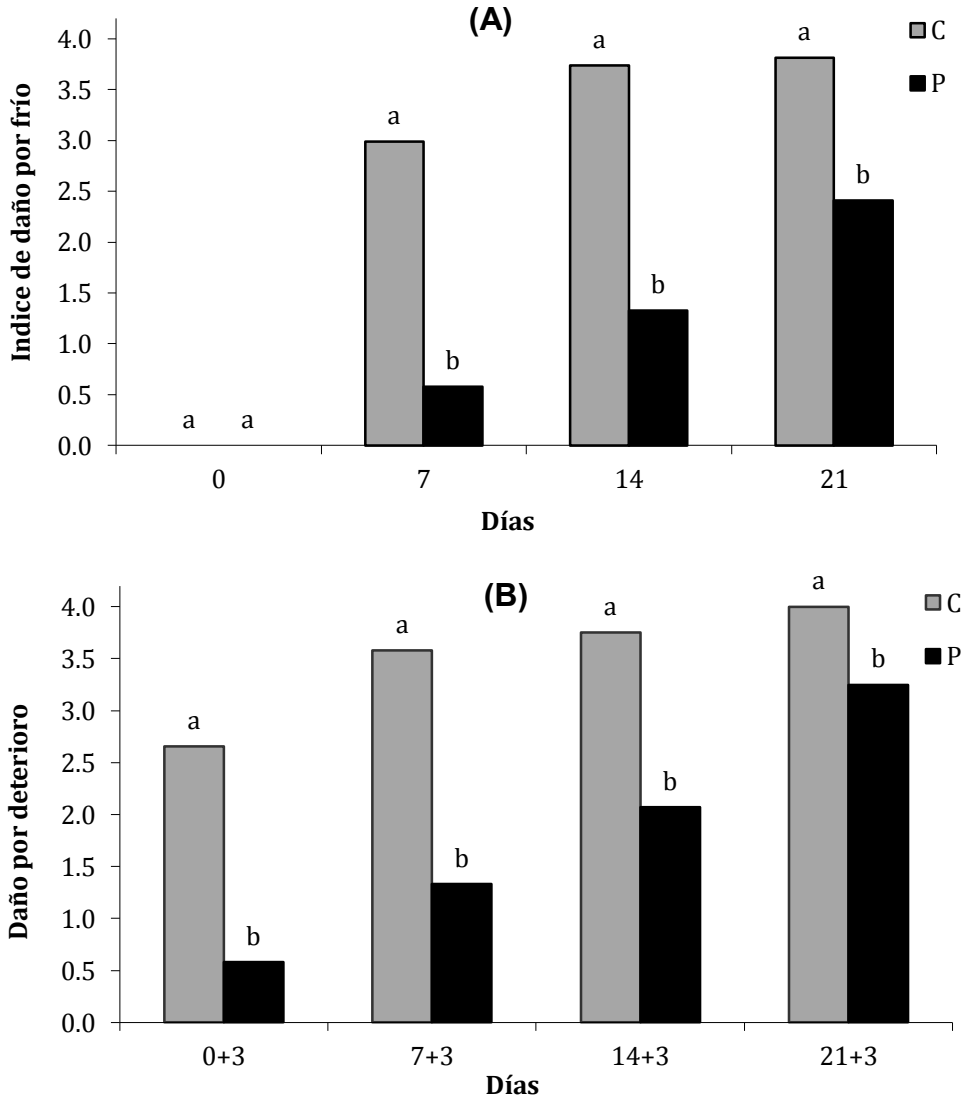

Figura 2a y 2b. Valores promedio del índice de daños por frío y deterioro de frutos de melón cubiertos con la película comestible de alginato-hidroxipropilmetilcelulosa-parafina (P), y no cubiertos o control (C), almacenados en refrigeración (a 5ºC y 95% Hr) (A), o en almacenamiento combinado (almacenamiento en refrigeración a 5ºC y 95% Hr seguido de almacenamiento a temperatura ambiente a 25ºC y 21-25% Hr) (B). Diferentes letras sobre las barras de valores de tratamientos (control y película) indican diferencia significativa en cada periodo de tiempo de almacenamiento ($P<0.05$, DMS de Fischer).

Pérdida de peso

En la figura 3 se ilustra el porcentaje de pérdida de peso de melón Cantaloupe, con y sin película de ALG-HPMC-PAR, almacenado por 21 días en refrigeración. Los resultados indican que la aplicación de la cubierta a base de alginato-HPMC-parafina redujo la pérdida de peso de los

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melones en comparación con los frutos control ($P < 0.05$), los cuales perdieron hasta el 51 % de su peso después de 21 días de almacenamiento. Los productos hortofrutícolas, incluyendo el melón, poseen un alto contenido de agua, y están expuestos a la pérdida de dicha humedad en sus tejidos por transpiración y/o respiración (Eitenmiller *et al*., 1985, 136; Fan *et al*., 2009, 84), resultando en la pérdida de peso del producto. Los resultados del presente estudio concuerdan con los publicados por Navarro-Tarazaga *et al*. (2008, 9502) y Velickova *et al*. (2013, 80). La pérdida de peso menor de los melones cubiertos con la película comestible de ALG-HPMC-PAR contribuye a que se reduzcan los cambios en otros parámetros sensoriales indicadores de la calidad de este producto, como la textura y apariencia del fruto (Saladie *et al*., 2007, 1012; Fan et al., 2009, 84).

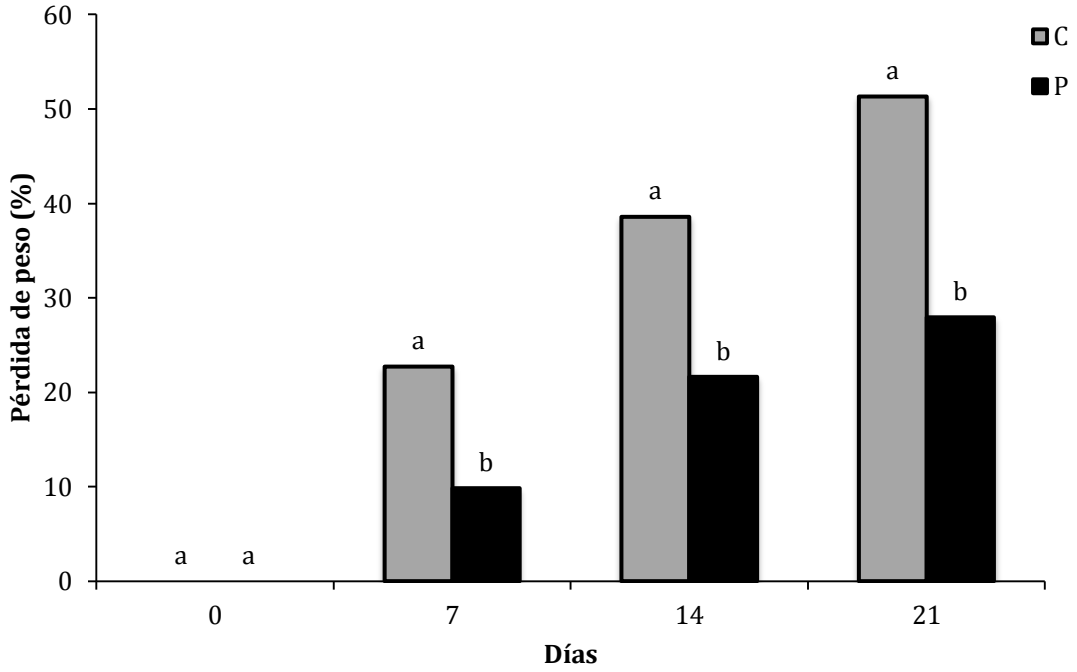

Figura 3. Valores promedio del porcentaje de pérdida de peso de frutos de melón cubiertos con la película comestible de alginato-hidroxipropilmetilcelulosa parafina (P), y no cubiertos o control (C), almacenados en refrigeración (a 5 ºC y 95 % Hr) durante 21 días. Diferentes letras sobre las barras de valores de tratamientos (control y película), indican diferencia significativa en cada periodo de tiempo de almacenamiento ($P < 0.05$, DMS de Fisher).

Conclusiones

La aplicación de una cubierta comestible de alginato-HPMC-parafina sobre frutos de melón Cantaloupe entero, promovió un mantenimiento de la calidad de este fruto tanto almacenado en

frio como en condiciones de temperatura ambiental después de su periodo de refrigeración. Los frutos de melón cubiertos con la película comestible resultaron ser más firmes y perdieron menos peso, lo cual es atribuido a la modificación del ritmo respiratorio del fruto por la permeabilidad selectiva de la cubierta a los gases y humedad. Además, la cubierta comestible contribuyó a que el melón almacenado en refrigeración presentara daños menores por frío, lo cual es una ventaja adicional de la aplicación de esta película comestible. Se concluye que la aplicación de la cubierta comestible a base de alginato-HPMC-parafina es una alternativa recomendable para preservar la calidad de frutos de melón durante su almacenamiento a baja temperatura y en condiciones de almacenamiento comercial a temperatura ambiente. Estudios relacionados con otras frutas y hortalizas deben ser considerados para tener un entendimiento mayor de los efectos de películas comestibles sobre la calidad y cambios fisiológicos de los mismos.

Agradecimientos

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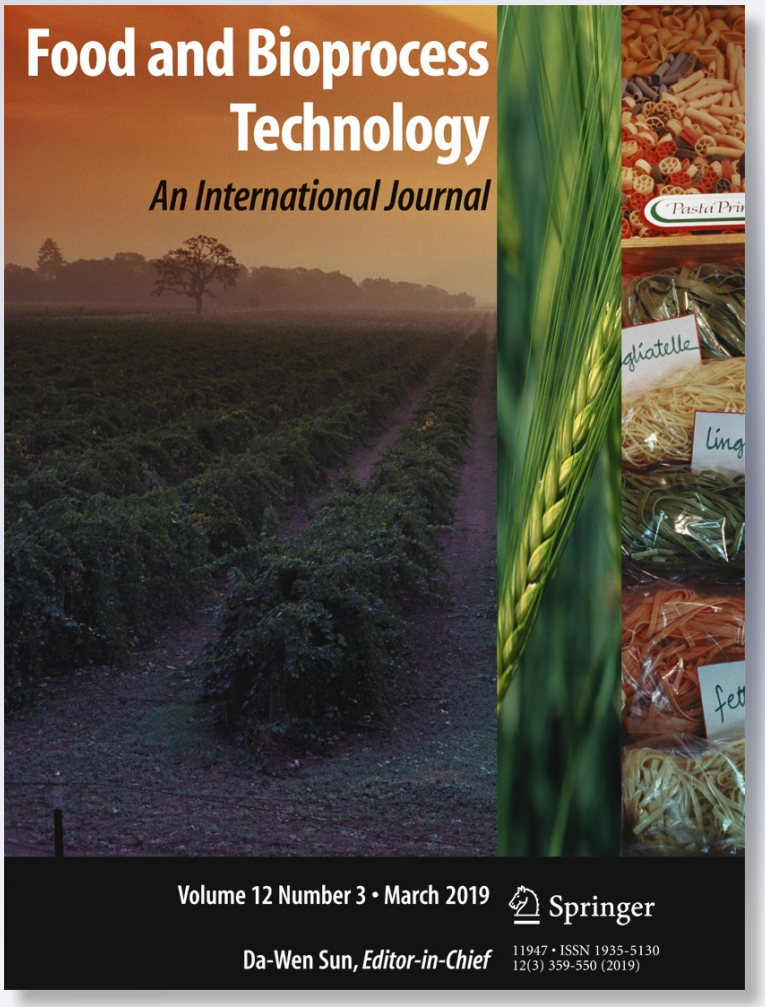

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ORIGINAL PAPER

Application of an Alginate–Chitosan Edible Film on Figs (*Ficus carica*): Effect on Bioactive Compounds and Antioxidant Capacity

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Abstract

The main aim of this study was to evaluate the effect of the application of an alginate–chitosan (A–Ch) coating on the bioactive compounds and the antioxidant capacity of fresh figs (*Ficus carica*), collected at two maturity stages (referred to as stages III and IV), and stored for 15 days at 6 °C. The composition of the internal atmosphere of the figs, as well as the polyphenol content and antioxidant capacity, was analyzed at 0, 3, 6, 9, 12, and 15 days, respectively. The sensory quality of coated and uncoated figs, stored for 15 days, was also assessed. Fresh figs were used as a reference in the sensory quality evaluation. The A–Ch coating caused considerable modifications in the internal atmosphere of the figs at the two maturity stages evaluated. The ripening process was delayed as O_2 was reduced and CO_2 concentrations were increased. Further, the total polyphenol content of the figs and, also, identified individual polyphenols, were preserved by the application of the A–Ch coating. Anthocyanins, in particular cyanidin-3-O-rutinoside, were the most abundant bioactive compound. Uncoated figs also exhibited higher antioxidant capacity than coated figs at maturity stage III, whereas in coated figs antioxidant capacity was kept constant along storage period regardless of their maturity stage. Interestingly, the coated figs stored for 15 days at 6 °C showed a high acceptability in the sensory evaluation, being similar to fresh figs. Therefore, the A–Ch coating could be an excellent post-harvest technology useful in preserving not only the organoleptic and sensory attributes but also bioactive components of figs during storage at low temperature.

Keywords Figs · Alginate–chitosan coating · Bioactive compounds · Antioxidant capacity · Sensory quality · Low-temperature storage

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Introduction

Figs (*Ficus carica*) have long been associated with longevity and health benefits due to their high content of vitamins, amino acids, antioxidants, and dietary fibers (Martínez-García et al. 2013; Solomon et al. 2006; Turan and Celik 2016). This fruit, in particular the dark or purple varieties, represents an important source of bioactive compounds, in particular high amounts of anthocyanins (Dueñas et al. 2008; Çalişkan and Aytekin Polat 2011; Kamiloglu and Capanoglu 2015); the content being similar to that of black plums (Usenik et al. 2009), blueberries (Bunea et al. 2013), sweet cherries, and strawberries (Wu et al. 2006). This high content of anthocyanins has made figs an attractive fruit to consumers, since it has been

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observed that their intake can inhibit inflammatory processes and heart failure, in addition to promoting anticarcinogenic and hypoglycemic activities (Szymanowska et al. 2015; Castro-Acosta et al. 2016). Besides, figs contain large amounts of the flavonoid quercetin-3-O-rutinoside (Del Caro and Piga 2008; Kamiloglu and Capanoglu 2015) which has been associated with different health benefits, such as the reduction of oxidative stress (Nishimura et al. 2016) and anti-inflammatory activity (Mascaraque et al. 2014).

Generally, figs are harvested at two different maturity stages, either physiological maturity (stage III) or commercial maturity (stage IIV) (Crisosto et al. 2010; Owino et al. 2004). Figs in maturity stage IV are completely mature, being more susceptible to overripe, microbial infection, and loss of the organoleptic properties (Villalobos et al. 2014) whereas figs at physiological maturity tend to show long shelf life since the biochemical and physiological changes continue after harvesting (Marei and Crane 1971).

Commercially, a very large percentage of figs are consumed dried in cookies or bars, and to a lesser extent, figs are also eaten in fresh form (Reyes-Avalos et al. 2016). However, dehydration affects the sensorial, nutritional, and functional fruit quality (Kamiloglu and Capanoglu 2015; Martínez-García et al. 2013), while fresh figs are highly sensitive to microbial growth even when stored at low temperatures; therefore, it may be useful to evaluate alternative processes to extend the shelf life of this fruit (Martínez-García et al. 2013). A feasible alternative could be the application of edible coatings, which is a preservation method used not only to improve food appearance, but also to maintain the quality of different fruits and vegetables (Meighani et al. 2015; Arnon et al. 2015).

Edible films can modify the internal atmosphere (O_2 and CO_2) of coated products, delaying the metabolic changes associated with the ripening process of fruits and, therefore, extending the shelf life (Meza-Velázquez et al. 2013; Tzoumaki et al. 2009). Previously, we characterize an alginate–chitosan bilayer edible film and evaluated the effectiveness of this film on the quality and shelf life of figs stored at low temperature (Reyes-Avalos et al. 2016). Interestingly, this edible film demonstrated excellent water vapor and gas barrier properties which contributed to decreasing the transpiration and respiration rates of stored figs. Further, figs coated with the film exhibited a better resistance to fungal contamination than uncoated figs, improving the overall quality and also extending shelf life during low-temperature storage. However, the information about possible effects of this coating on bioactive compounds, such as antochyanins, is very scarce. Thus, the aim of this study was to evaluate the effect of the application of this coating on the bioactive compounds and the antioxidant capacity of figs (*Ficus carica*) var. Mission, harvested at two maturity stages, during storage at low temperature. In particular, internal atmosphere gas, polyphenol content, antioxidant capacity, and the sensorial quality of figs were analyzed in this study.

Material and Methods

Chemical Reagents

Sodium alginate was acquired from CYTECSA, S.A. de C.V. (México, D.F.), soy lecithin from Golden Bell (México, D.F.), and olive oil was purchased at a local store. J.T. Baker (Phillipsburg, NJ, USA) supplied water, phosphoric acid, ammonium diacid phosphate, and acetonitrile HPLC grade. Chitosan, sodium carbonate, lactic acid and calcium chloride, Folin–Ciocalteu and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ABTS diammonium salt (2, 2-azinobis-3-ethylben zothiazoline-6-sulphonic acid) reagents, potassium persulfate, 2,4,6-Tri-(2-pyridyl)-s-triazine (TPTZ), hydrochloric acid, ethyl alcohol, iron (III) chloride hexahydrate, sodium acetate 3-hydrate, glacial acetic acid; CO_2, oxygen standard grade, gallic acid, chlorogenic acid, quercetin-3-O-rutinoside, cyanidin-3-O-glucoside, and cyanidin-3-*O*-rutinoside were all purchased from Sigma-Aldrich Company (St. Louis MI, USA).

Plant Material

The figs used in this study were collected in August–September in Cd. Lerdo (Durango, Mexico; 25° 32′ 10″ N/103° 31′ 28″ W) as previously described in Reyes-Avalos et al. (2016). Fruits free of any physical damage were selected and washed using 0.02% sodium hypochlorite solution. Finally, according to their maturity stage, either stage III or stage IV, the fruits were divided into two batches before applying any treatment. Each treatment was repeated four times ($n = 4$).

The first batch was selected as a control group (without coating application), while the second batch was coated with the alginate–chitosan (A–Ch) film. The preparation and application of the A–Ch coating has previously been described in Reyes-Avalos et al. (2016). All figs, coated and uncoated, were stored at 6 °C and 95% RH for 15 days.

The internal CO_2 and O_2 concentrations, total phenolic, polyphenol profile, and antioxidant capacity were evaluated at 0, 3, 6, 9, 12 and 15 days of storage. Sensory quality of coated and uncoated figs was determined at 15 days of storage; fresh figs were used for comparison purposes. Approximately, 15 g of coated and uncoated figs from each storage period was homogenized and lyophilized at − 20 °C for 72 h in a Labconco FreeZone Triad Cascade Benchtop Freeze drier (Kansas City, MO, USA) for subsequent analysis.

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Internal CO_2 and O_2

Two milliliters of gas from the internal atmosphere of the fruit was extracted with a syringe inserted through the fruit blossom ends. The CO_2 and O_2 concentrations in the gas sample were determined using an Agilent 6820 gas chromatograph (Agilent Technology, Palo Alto, CA, USA) equipped with a TCD detector, two 6 ft × 1/4 in Alltech CTR I columns (Alltech Associates, Inc., Deerfield, IL, USA) as previously described in Reyes-Avalos et al. (2016). The internal gas sample was isothermally separated at 95 °C while the injector and detector temperatures were 70 °C and 170 °C, respectively. All determinations were measured in triplicate. CO_2 and O_2 concentrations were expressed as μM (micromoles of gas/L air).

Polyphenol Compound Analysis

Extraction

Polyphenol compounds were extracted according to the method proposed by Eim et al. (2013) with slight modifications. Approximately, 0.5 g of lyophilized figs was homogenized with 10 mL of methanol (MeOH) using an Ultra-Turrax IKA T25D (IKA® Works, Inc., Wilmington, USA) at 13,500 rpm for 1 min. The samples were stored in darkness and mechanically stirred for 16 h at 4 °C. The samples were centrifuged (ALC 4218, Milano, Italy) at 1750*g* for 15 min and filtered through a Whatman no. 4 filter paper. The supernatants were stored at 4 °C for later analysis. All extractions were performed in duplicate.

Total Phenolic Content

Total soluble polyphenols were spectrophotometrically measured in accordance with the Folin–Ciocalteu method, using 96-well microplates, as previously described by González-Centeno et al. (2014) with some modifications. Briefly, 95 μL of distilled water was placed in each well, and 10 μL of the extract was added, followed by 5 μL of the Folin–Ciocalteu reagent. Then, the mixture was incubated for 5 min and 80 μL of 7.5% (*w*/*v*) Na_2CO_3 solution was added. A Multiskan Spectrum spectrophotometer (Thermo Fisher Scientific, Vanda, Finland) was used to incubate the mixture for 30 min, in the absence of light, at 25 °C before measuring the absorbance at 765 nm. Gallic acid (0–200 ppm) was used as standard for calibration and the phenolic content results were expressed as milligram of gallic acid per 100 g of dry matter (dm). Each of the given values is the mean of six experimental determinations.

Identification and Quantification of Polyphenol Compounds by HPLC–DAD

The polyphenolic compounds were extracted and analyzed by HPLC–DAD according to the method described by Del Caro and Piga (2008) with slight modifications. Approximately, 2 g of lyophilized figs was homogenized with 10 mL of MeOH (HPLC grade) and stirred for 16 h at 4 °C. Then, the samples were centrifuged at 4000*g* for 5 min at 4 °C and concentrated to 5 mL at 30 °C in vacuum conditions. The concentrated samples were then diluted to 10 mL with Milli-Q water and filtered through a ∅0.45-μm PTFE filter prior to HPLC analysis. The chromatographic analysis was carried out using an HPLC Agilent 1200 (Agilent Technology, Palo Alto, CA, USA) equipped with a diode array detector (DAD), a quaternary pump, and two LiChrospher C_{18} 5-μm (4 mm × 150 mm) columns (Phenomenex) connected in series. The temperature, flow rate, and injection loop were of 25 °C, 0.5 mL/min, and 20 μL, respectively. The mobile phase was comprised of (A) 50 mM ammonium diacid phosphate solution brought to 2.6 pH with phosphoric acid, (B) 80% acetonitrile and 20% phase A, and (C) 200 mM phosphoric acid. The mobile phase gradient was of 100% A at 5 min, 92% A and 8% B at 8 min, 14% B and 86% C at 20 min, 16.5% B and 83.5% C at 25 min, 21.5% B and 78.5% C at 35 min, 50% B and 50% C at 70 min, 100% A at 75 min, and 100% A at 80 min. The polyphenol compounds were analyzed at four different wavelengths: 280 nm for catechins and benzoic acids, 316 nm for hydroxycinnamic acids, 365 nm for flavonols, and 520 nm for anthocyanins. High purity standards of gallic acid, chlorogenic acid, quercetin-3-O-rutinoside, cyanidin-3-*O*-rutinoside, and cyanidin-3-O-glucoside were used not only to identify but also quantify these polyphenolic compounds.

Evaluation of the Antioxidant Capacity

The effect of the A–Ch bilayer edible film and, also, of the maturity stage on the antioxidant capacity of figs was also tested. The 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays were used as described by González-Centeno et al. (2012). For both antioxidant assays, an automated microplate reader, in particular a Multiskan Spectrum (Thermo Scientific, Waltham, MA USA) was used.

Sensory Quality

The sensory analysis of coated and uncoated (control) fig fruits was carried out following the methodology previously described by Gol et al. (2013) with some modifications. Specifically, the sensory quality was evaluated from the following parameters: appearance, color, odor, firmness, flavor, and overall acceptability for a total of 3 samples; one sample

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corresponding at 0 day (fresh) and 2 samples (coated and uncoated) stored for 15 days. All figs were labeled with 4-digit code numbers and randomly provided to the panelists. The samples were evaluated by a panel of 50 volunteers aged between 19 and 53 years old, who were habitual consumers of figs. Drinking water was provided to panelists for eliminating the residual taste between samples. Each attribute was scored on a structured hedonic scale labeled from "extremely unpleasant" (0) to "extremely pleasant" (9).

Data Analysis

All data were statistically analyzed using analysis of variance (ANOVA). Further, the post hoc analysis was carried out using Fisher's least significant difference (LSD) test with a significance level of 0.05. All calculations were carried out using Minitab 18 statistical software (Minitab Inc. 2018).

Results and Discussion

Internal Concentrations of CO_2 and O_2 of Figs

Regardless of the maturity stage of the evaluated figs, the application of the alginate–chitosan (A–Ch) coating caused important changes in the composition of the internal atmosphere of figs during storage at low temperature ($p < 0.05$). Thus, coated figs exhibited a higher CO_2 concentration than uncoated figs (Fig. 1), whereas O_2 concentration was higher in uncoated figs (Fig. 2).

As can be observed in Fig. 1, the CO_2 concentration of coated figs decreased during storage, from ~1600 to ~710 μM, for figs at stage III, and from ~1850 to ~740 μM, for figs at stage IV; whereas in uncoated figs, the CO_2 concentration decreased from ~600 to less than 200 μM, regardless of the maturity stage.

However, the internal O_2 of uncoated figs exhibited a slight but significant increase during storage, from ~5890 to 7650 μM for figs of both stages of maturity (III and IV), whereas in coated figs internal O_2 increased from ~3100 to ~3990 μM for figs stage III, and from ~2900 to ~3250 μM for figs at stage IV (Fig. 2). As can be observed, CO_2 concentration decreased during the first days of storage, which could be attributed to the low respiration rate as consequence of low temperature. In the subsequent days of storage, the A–Ch coating promoted the CO_2 accumulation inside of fruit as consequence to the low gas permeability of coating (Reyes-Avalos et al. 2016).

The composition of the internal atmosphere of coated figs showed a similar behavior to other coated fruits since it has been observed that coated fruits and vegetables tend to exhibit a CO_2-rich internal atmosphere (Cisneros-Zevallos and Krochta 2002; Meza-Velázquez et al. 2013). Several authors have also observed that edible coatings are able to decrease the respiratory rate of figs, which in turn may lead to the modification of the internal atmosphere (Reyes-Avalos et al. 2016; Bourbon et al. 2011; Fabra et al. 2012). It is well known that the modification of the internal atmosphere, by increasing CO_2 and decreasing O_2 concentration, is one of the key factors in delaying the ripening process of fruits (Díaz-Mula et al. 2011; Majidi et al. 2014; Chaudhary et al. 2015).

Total Polyphenols

The influence of the A–Ch coating on the total polyphenol content (TPC) of figs at the two maturity stages (III and IV) stored at 6 °C can be observed in Fig. 3. Here, TPC was influenced by both the application of the coating and also the maturity stage of the figs. Thus, in uncoated figs, TPC decreased as the maturity stage increased ($p < 0.05$), whereas in coated figs TPC remained almost unchanged, accounting for about 206 mg GAE/100 g dm, regardless of the maturity stage and storage time ($p > 0.05$). In fact, only uncoated figs at stage III exhibited a slight but significant increase in the TPC as storage time increased, achieving a maximum of 246.15 ± 8.84 mg GAE/100 g dm at 12 days of storage ($p < 0.05$) (Fig. 3). Interestingly, these results are concomitant with those from other studies performed in sweet cherry fruits coated with the sodium alginate film (Díaz-Mula et al. 2012), and in blue berries coated with the A–Ch film (Chiabrando and Giacalone 2015), showing that the TPC of coated fruits was lower than in uncoated fruits. Several authors have suggested that lower TPC in coated samples could be the result of the hypoxia and the inhibition of the phenylalanine ammonia-lyase promoted by the lower O_2 and higher CO_2 concentration which result from the coating (Imahori et al. 2008; Wang et al. 2015).

Identification and Quantification of Anthocyanins, Flavonols, and Polyphenol Acids of Figs by HPLC

In order to gain more insight into the different polyphenolic compounds present in figs, methanolic extracts were subjected to HPLC–DAD analysis. The chromatograms from the HPLC–DAD analysis of the methanolic extracts from figs are shown in Fig. 4.

As can be seen, five different polyphenolic compounds were identified at different wavelengths (280–520 nm). In particular, gallic acid (GA) and chlorogenic acid (CA) were identified at 280 and 316 nm, respectively. The flavonol quercetin-3-O-rutinoside (Q3R) was also found at 365 nm whereas at 520 nm two anthocyanins were identified: cyanidin-3-O-glucoside (C3G) and cyanidin-3-O-rutinoside (C3R). Interestingly, C3R, with around 90%, was the predominant compound followed by Q3R,

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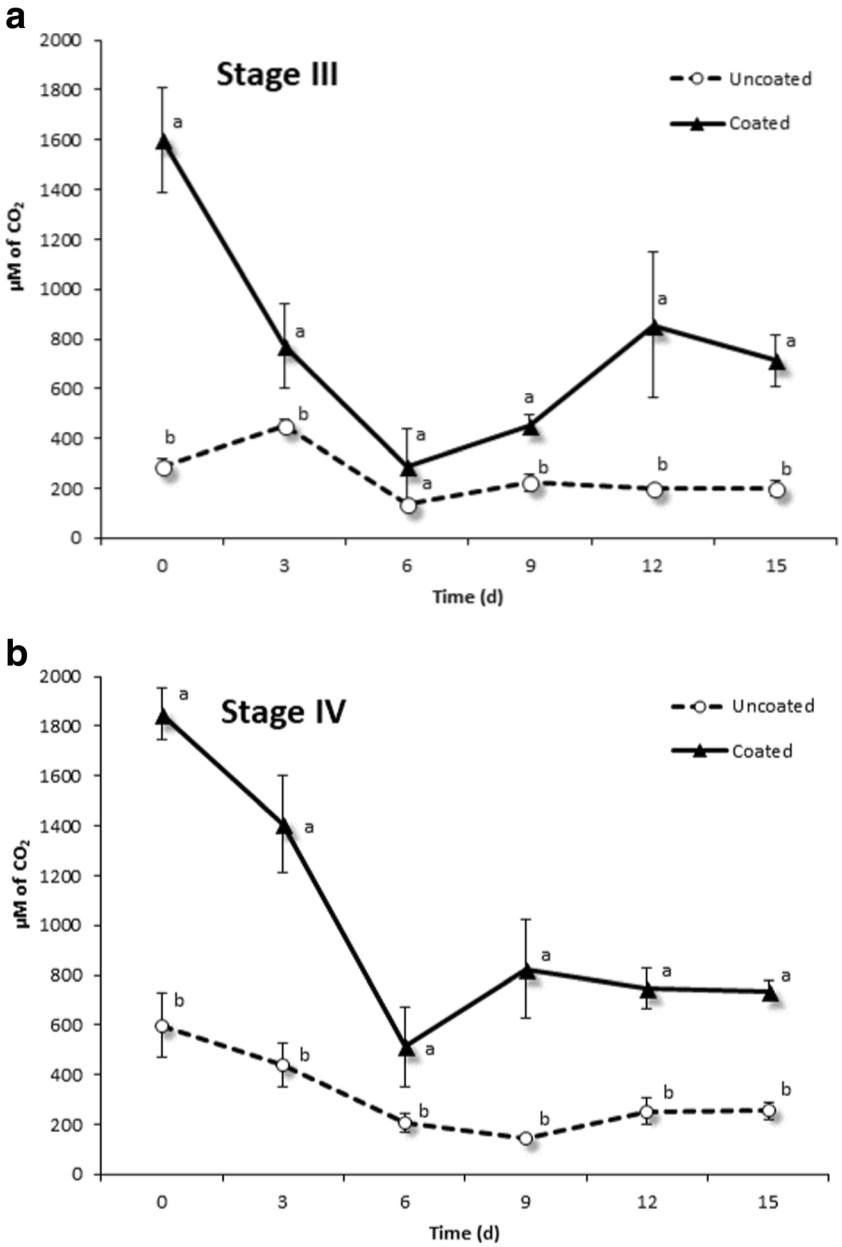

Fig. 1 Internal CO_2 concentration (μM) of coated figs with an A–Ch coating and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C. Bars over the mean results stand for ± standard deviation ($n = 4$). Different lowercase letters indicate significant differences in coated and uncoated figs according to the LSD test ($p < 0.05$) ($n = 4$)

whereas C3G, CA, and GA were found in minor amounts (listed in order of abundance).

The influence of the application of the A–Ch film on each of these polyphenolic compounds is described below.

Effects of the A–Ch Coating on Anthocyanins

The results from the quantification of two anthocyanins, cyanidin-3-O-glucoside (C3G) and cyanidin-3-O-rutinoside (C3R), found in the methanolic extracts obtained from the different samples are shown in Table 1.

As can be observed, the application of the film significantly affected the concentration of C3G and C3R at maturity stage III ($p < 0.05$) whereas at maturity stage IV it was not significant ($p > 0.05$). Interestingly, uncoated figs at maturity stage III showed a significant increase from 4.8 to 13 mg/100 g dm for C3G and from 53 to 117 mg/100 g dm for C3R during the first 9 days of storage ($p < 0.05$). After this period, these anthocyanins, C3G and C3R, exhibited a significant decrease ($p < 0.05$), up to 5.84 mg/100 g dm for C3G and to 63.81 mg/100 g dm for C3R.

The coated figs at maturity stage III did not exhibit a clear trend, and in this case C3G and C3R anthocyanin concentrations during the storage period varied from ~ 2.5 to ~ 5 mg/100 g dm and from ~ 35 to ~ 65 mg/100 g dm, respectively ($p > 0.05$).

Similar results have been reported by other authors for plums coated with alginate (Valero et al. 2013) and blackberries coated with chitosan (Oliveira et al. 2014). The lower

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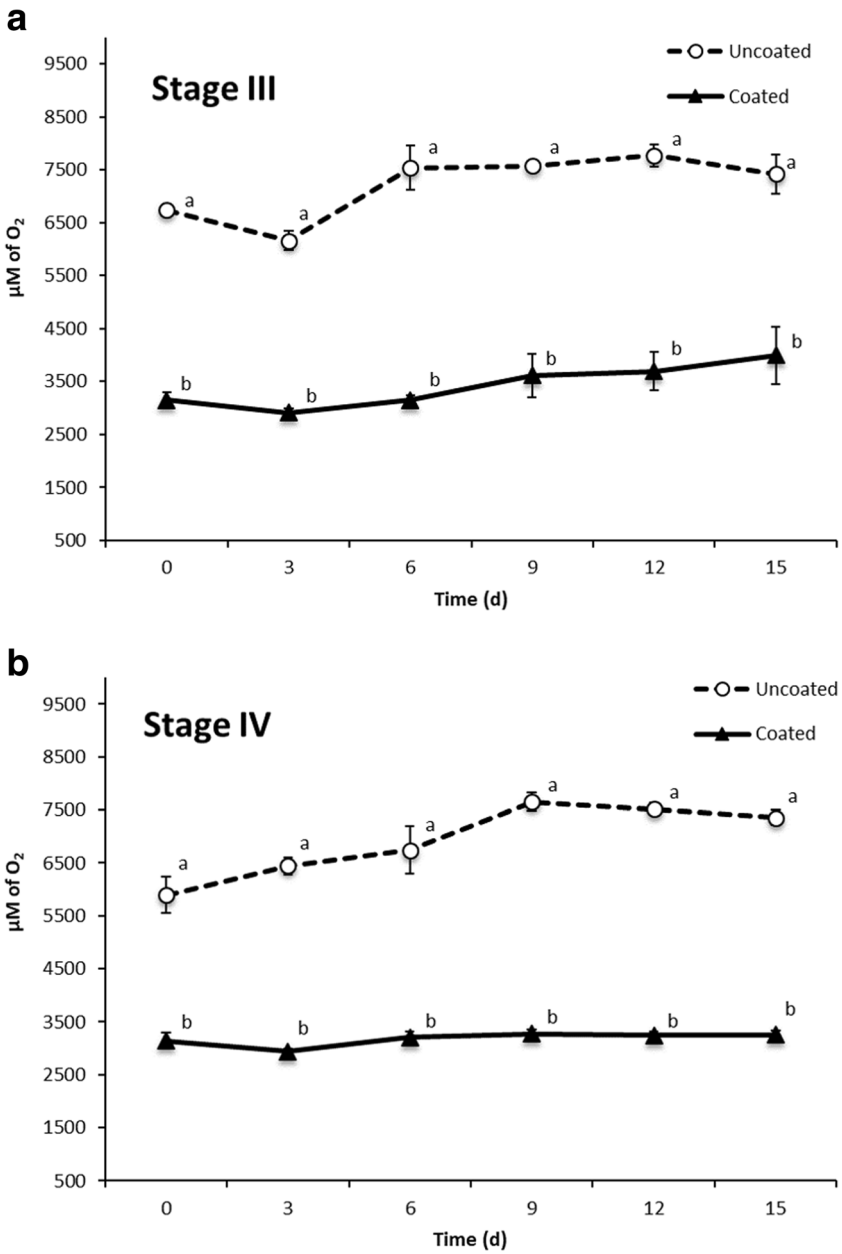

Fig. 2 Internal O_2 concentration (μM) of coated figs with an A–Ch coating and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C. Bars over the mean results stand for ± standard deviation ($n = 4$). Different lowercase letters indicate significant differences in differences in coated and uncoated figs according to the LSD test ($p < 0.05$) ($n = 4$)

anthocyanin content in the coated fruits could be a consequence of the internal atmosphere modification (high CO_2–low O_2), since it has been observed that the biochemical reactions involved in the anthocyanin synthesis can be regulated by modified atmosphere (Díaz-Mula et al. 2011; Selcuk and Erkan 2014).

Effect of the A–Ch Coating on Quercetin-3-O-Rutinoside

The quercetin-3-O-rutinoside (Q3R) was the most abundant flavonoid found in the methanolic extracts of figs. Table 2 shows the concentration of this compound in coated and uncoated figs, at the two maturity stages, during low-temperature storage.

Interestingly, the application of the A–Ch coating on figs at stage III did not show any significant effect on the Q3R concentration. However, when the A–Ch coating was applied on figs at stage IV, the concentration of Q3R was slightly higher than in uncoated figs, in particular at the end of the storage period evaluated. Overall, the Q3R content ranged from ~20 to ~30 mg/100 g dm in figs at stage III; whereas for figs at stage IV, it varied from ~15 to ~25 mg/100 g dm. These results are in agreement with Rößle et al. (2011), who observed that the application of an alginate film did not affect the content of Q3R in apple samples. Nevertheless, it should be emphasized that information relating to the effect of edible coatings on the content of Q3R is very scarce.

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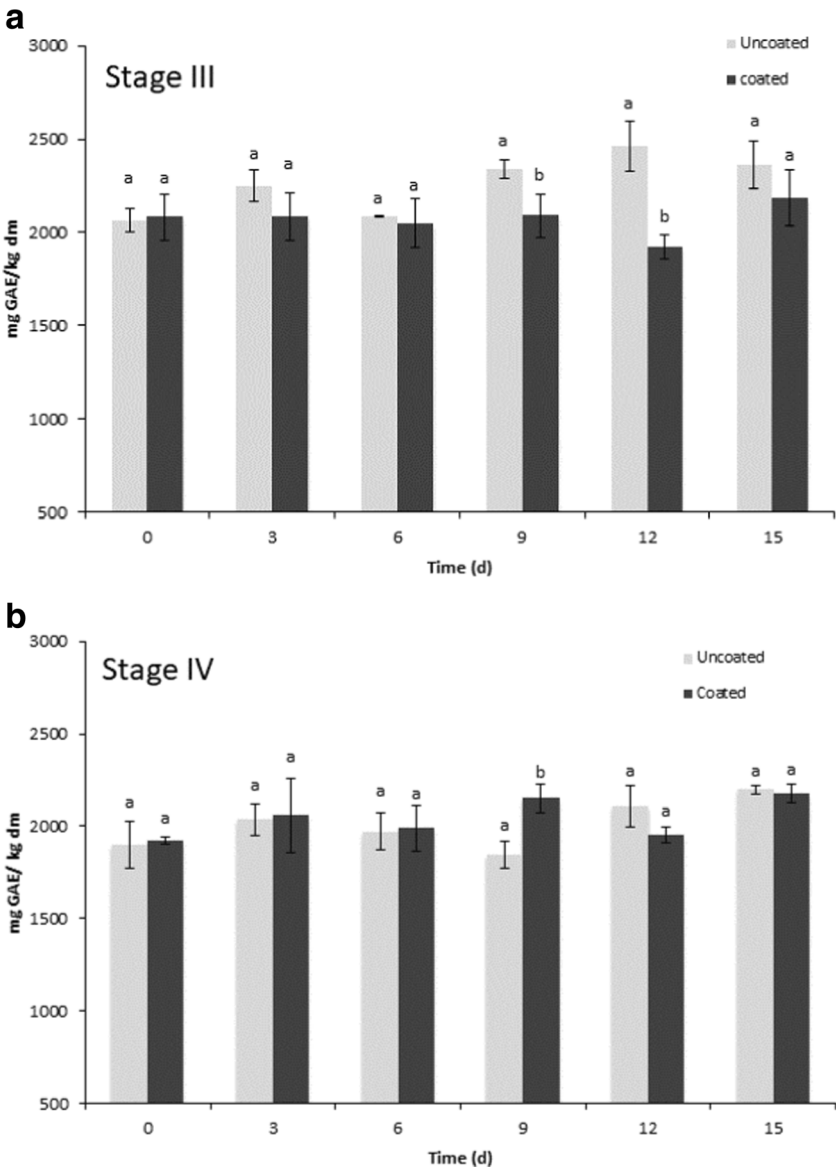

Fig. 3 Total polyphenol concentration (mg of GAE/ 100 g dm) of coated figs with an A–Ch coating and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C. Bars over the mean results stand for ± standard deviation ($n = 4$). Different lowercase letters (a, b, c, d) indicate significant differences in differences in coated and uncoated figs according to the LSD test ($p < 0.05$) ($n = 4$)

Effect of the A–Ch Coating on Phenolic Acids

The influence of the A–Ch coating on the content of chlorogenic acid (CA) and gallic acid (GA) of figs, at two maturity stages (III and IV), stored at low temperature (6 °C) is presented in Table 3.

As can be seen, significant changes were observed in the CA content when figs, at stage III, were coated with A–Ch coating ($p < 0.05$), whereas the application of A–Ch coating in figs at stage IV did not cause such changes ($p > 0.05$). Interestingly, the CA content increased, both for uncoated and coated figs, as storage time increased ($p < 0.05$). In particular, for uncoated figs, CA increased from 1.21 ± 0.16 to 3.14 ± 0.45 mg/100 g dm while a similar increase from 1.31 ± 0.2 to 3.62 ± 0.14 mg/100 g dm was observed for coated figs.

The application of the A–Ch coating changed GA content significantly at the end of the storage period ($p < 0.05$). In particular, coated figs exhibited higher GA levels than those found in uncoated samples. Thus, an increment in GA content in figs at stage III, from ~ 1.7 to ~ 2.8 mg/100 g dm, was caused by the coating application, whereas for uncoated samples the GA content remained almost constant. However, no significant changes in the GA content were observed for coated figs at stage IV, while for uncoated figs, this phenolic acid decreased during storage period. These results suggested that CA could be more affected by the internal atmosphere modification as consequence of the application of the A–Ch coating than GA. It is important to note that most of polyphenol compounds showed high variability along storage period, in particular those in maturity stage III, which could be attributed to different biochemical changes involved in ripening process. Again it is important to emphasize that there is very limited information in the scientific literature relating to the influence of coatings on this type of phenolic compounds. Further

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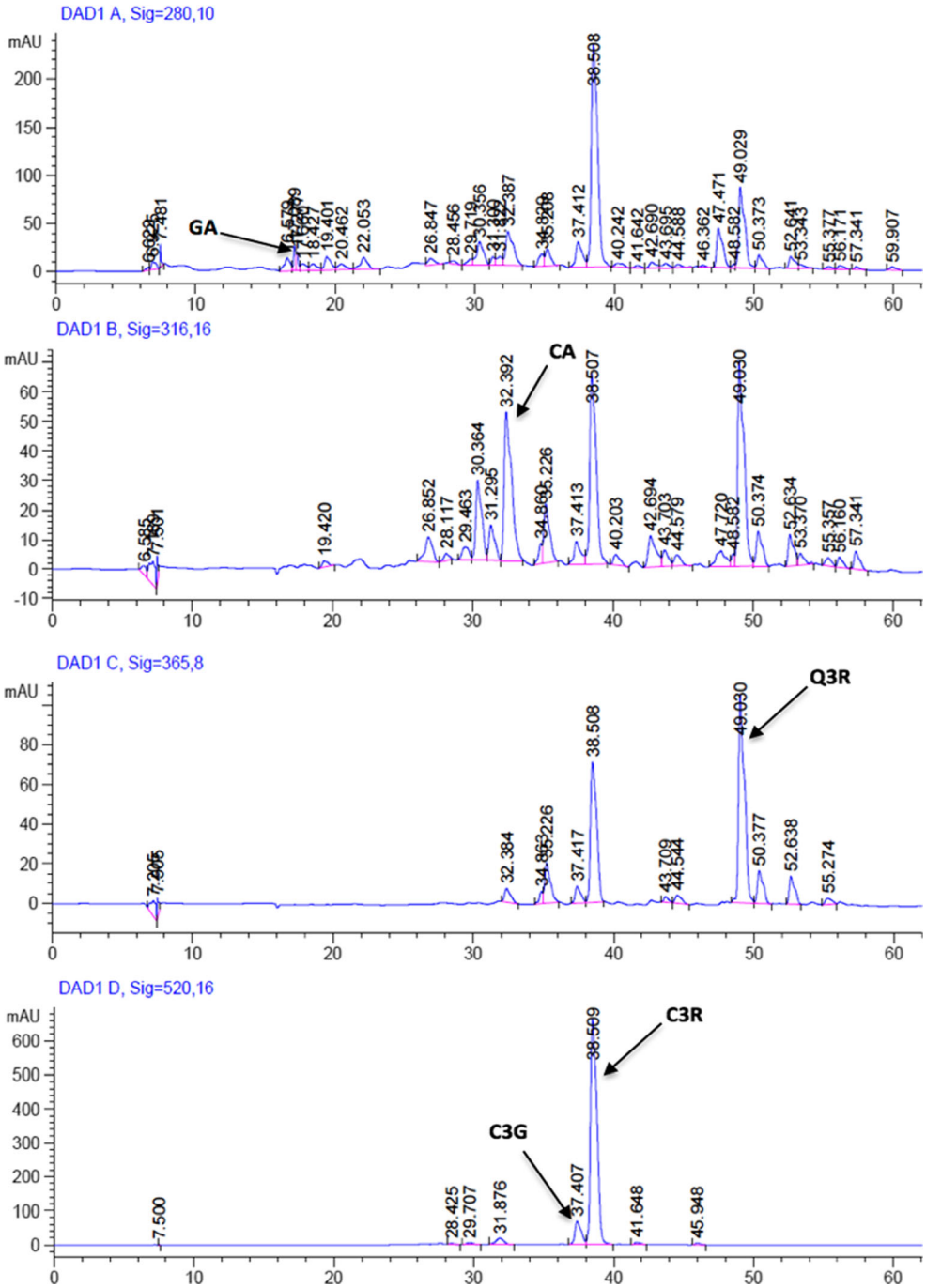

Fig. 4 HPLC chromatogram of the individual polyphenols present in the methanolic extracts of fig samples. GA gallic acid (280 nm; 17.08 min), CA chlorogenic acid (316 nm; 32.39 min), Q3R quercetin-3-rutinoside (365 nm; 49.03 min), C3G cyanidin-3-glucoside (520 nm; 37.40 min), C3R cyanidin-3-rutinoside (520 nm; 38.50 min)

studies focused in the biochemical mechanisms involved in the synthesis of this type of bioactive compounds during storage at low temperatures should be carried out in order to explain this phenomenon.

Effect of the Application A–Ch Coating on the Antioxidant Capacity of Fig Fruits

The results corresponding to the antioxidant capacity, determined by the ABTS and FRAP assays, of uncoated figs and, also, of figs coated with an A–Ch film stored at 6 °C during 15 days are presented in Table 4.

Interestingly, significant differences were observed in the antioxidant capacity, measured by ABTS and FRAP, as a consequence of the application of the A–Ch coating ($p < 0.05$). Thus, in general, the antioxidant capacity of uncoated figs was higher than in coated figs. Further, the maturity stage of figs was also an important parameter since figs at stage IV exhibited lower antioxidant capacity than fruits collected at stage III (see Table 4).

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Table 1 Concentration of two different anthocyanins, cyanidin-3-O-glucoside (C3G) and cyanidin-3-O-rutinoside (C3R), identified in the methanolic extracts of coated figs with an A–Ch coating and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C (mg/100 g dm)

Maturity stage	Storage days	Cyanidin-3-O-glucoside		Cyanidin-3-O-rutinoside	
		Uncoated	Coated	Uncoated	Coated
III	0	4.80 ± 0.07^{aX}	3.73 ± 0.04^{acX}	53.10 ± 9.97^{aX}	46.79 ± 3.37^{abX}
	3	7.19 ± 0.03^{acX}	5.35 ± 0.12^{abX}	80.92 ± 4.03^{aX}	35.56 ± 5.04^{abY}
	6	5.73 ± 0.12^{acX}	3.46 ± 0.05^{acX}	60.54 ± 12.74^{aX}	39.65 ± 8.75^{abX}
	9	13.00 ± 0.03^{bX}	4.68 ± 0.08^{abY}	116.93 ± 4.45^{bX}	49.97 ± 10.84^{abY}
	12	8.10 ± 0.17^{cX}	2.79 ± 0.04^{aY}	83.68 ± 18.08^{aX}	19.43 ± 1.37^{aY}
	15	5.84 ± 0.13^{acX}	5.36 ± 0.05^{abY}	63.81 ± 21.49^{aX}	65.35 ± 3.15^{bX}
IV	0	6.73 ± 0.08^{acX}	5.21 ± 0.05^{abX}	77.02 ± 3.12^{aX}	63.96 ± 2.98^{bX}
	3	7.21 ± 0.07^{acX}	3.53 ± 0.10^{acY}	78.88 ± 3.57^{aX}	52.12 ± 8.52^{abX}
	6	5.13 ± 0.11^{aX}	6.73 ± 0.09^{bX}	64.14 ± 3.70^{aX}	61.70 ± 12.91^{bX}
	9	5.45 ± 0.10^{acX}	6.04 ± 0.08^{bcX}	64.37 ± 12.34^{aX}	58.65 ± 7.95^{bX}
	12	6.64 ± 0.22^{acX}	5.06 ± 0.06^{abX}	74.86 ± 26.48^{aX}	60.38 ± 5.86^{bX}
	15	6.33 ± 0.21^{acX}	5.39 ± 0.09^{abX}	68.51 ± 25.85^{aX}	67.38 ± 9.04^{bX}

Lowercase letters (a, b, c, d) indicate significant differences in maturity stages and storage days according to the LSD test ($p < 0.05$) ($n = 4$)

Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits according to the LSD test ($p < 0.05$) ($n = 4$)

For instance, the antioxidant capacity of uncoated figs at stage III increased during storage, either for ABTS (from 1930.7 ± 39.0 to 2140.1 ± 18.2 μM TE/100 g dm) or FRAP assay (1928.8 ± 41.4 and 2322.5 ± 82.4 μM TE/100 g dm). On the contrary, no significant differences ($p > 0.05$) were observed in coated figs at this maturity stage, accounting for a mean value of 1672.2 ± 34.9 μM TE/100 g dm for ABTS, and 1726.0 ± 55.4 μM TE/100 g dm for the FRAP assay. It is important to point out that the maximum antioxidant capacity of uncoated figs at maturity stage III, for both ABTS and FRAP assays, was observed after 9 days of storage, decreasing afterwards.

Table 2 Concentration of quercetin-3-O-rutinoside (Q3R) identified in the methanolic extracts of coated figs with an A–Ch coating and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C (mg/100 g dm)

Maturity stage	Storage days	Quercetin-3-O-rutinoside	
		Uncoated	Coated
III	0	25.24 ± 0.88^{acX}	28.09 ± 3.46^{abdX}
	3	30.14 ± 0.55^{aX}	31.78 ± 0.43^{aX}
	6	29.69 ± 0.58^{acX}	21.94 ± 2.31^{bX}
	9	25.59 ± 1.23^{acX}	20.15 ± 2.34^{bX}
	12	34.53 ± 7.73^{aX}	18.78 ± 2.17^{bY}
	15	32.18 ± 11.20^{aX}	33.21 ± 11.85^{aX}
IV	0	25.08 ± 3.55^{abX}	24.72 ± 1.67^{abX}
	3	25.46 ± 4.99^{abX}	23.96 ± 3.93^{abX}
	6	28.13 ± 2.12^{acX}	22.76 ± 3.11^{abX}
	9	16.23 ± 2.73^{bdX}	25.24 ± 4.08^{abX}
	12	19.77 ± 2.58^{bcdX}	17.71 ± 1.99^{cbX}
	15	13.50 ± 4.07^{dX}	20.11 ± 3.90^{dbX}

Lowercase letters (a, b, c, d, e, f) indicate significant differences in maturity stages and storage days according to the LSD test ($p < 0.05$) ($n = 4$)

Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits according to the LSD test ($p < 0.05$) ($n = 4$)

On the other hand, no significant changes ($p > 0.05$) in the antioxidant capacity of coated figs at stage IV were observed, accounting for a mean value of 1505.9 ± 26.3 and 1432.3 ± 41.4 μM TE/100 g dm for ABTS and FRAP assays, respectively. However, uncoated figs at the same maturity stage exhibited a significant decrease in the antioxidant capacity as storage time increased. In the case of the antioxidant capacity measured by ABTS, a decrease from 1860.8 ± 65.3 to 1500.0 ± 98.7 μM TE/100 g dm was observed, while a decrease from 1861.9 ± 61.1 to 1427.9 ± 76.8 μM TE/100 g dm was determined by the FRAP method.

Interestingly, the antioxidant capacity of figs, measured for both ABTS and FRAP assays, showed a good correlation with the C3R content ($R^2 = 0.80$).

Several authors have also observed that the lower antioxidant capacity exhibited by the coated fruits could be associated with the delay of the ripening process (Díaz-Mula et al. 2012; Wang and Gao 2013). Thus, these results suggested that the ripening delay caused by the A–Ch coating promotes a high preservation of the antioxidant capacity of figs during storage at low temperature (6 °C), regardless of the maturity stages (III and IV) evaluated.

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Table 3 Concentration of the phenolic acids (mg/100 g dm) identified in methanolic extracts of coated figs with an A–Ch coating and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C

Maturity stage	Storage days	Chlorogenic acid		Gallic acid	
		Uncoated	Coated	Uncoated	Coated
III	0	1.21 ± 0.16^{adX}	1.31 ± 0.20^{acfX}	1.96 ± 0.07^{aX}	1.70 ± 0.17^{afX}
	3	1.65 ± 0.07^{acdX}	1.18 ± 0.11^{aX}	2.07 ± 0.06^{aX}	2.12 ± 0.12^{bcX}
	6	1.99 ± 0.20^{acX}	1.81 ± 0.07^{abX}	2.09 ± 0.13^{aX}	1.86 ± 0.06^{acX}
	9	3.22 ± 0.17^{bX}	2.33 ± 0.14^{bY}	2.20 ± 0.11^{aX}	2.36 ± 0.07^{bdX}
	12	3.20 ± 0.66^{bX}	2.06 ± 0.06^{bcY}	2.23 ± 0.05^{aX}	2.58 ± 0.21^{deX}
	15	3.14 ± 0.45^{bX}	3.61 ± 0.14^{dX}	2.13 ± 0.29^{aX}	2.78 ± 0.25^{eY}
IV	0	1.40 ± 0.08^{acdX}	1.44 ± 0.10^{acfX}	1.78 ± 0.11^{abcX}	1.85 ± 0.05^{acX}
	3	1.14 ± 0.18^{dX}	1.46 ± 0.13^{acfX}	1.85 ± 0.08^{abcX}	1.42 ± 0.08^{fY}
	6	2.09 ± 0.04^{cX}	1.31 ± 0.29^{acfX}	1.89 ± 0.08^{abX}	1.67 ± 0.19^{afX}
	9	1.44 ± 0.12^{adX}	1.92 ± 0.41^{bfX}	1.46 ± 0.10^{cdX}	1.67 ± 0.10^{afX}
	12	2.05 ± 0.63^{cX}	1.48 ± 0.16^{acfX}	1.58 ± 0.17^{bcdX}	1.42 ± 0.06^{fX}
	15	2.02 ± 0.53^{cX}	1.67 ± 0.19^{abX}	1.31 ± 0.12^{dX}	1.50 ± 0.14^{afX}

Lowercase letters (a, b, c, d, e, f) indicate significant differences in maturity stages and storage days according to the LSD test ($p < 0.05$) ($n = 4$)

Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits according to the LSD test ($p < 0.05$) ($n = 4$)

Sensory Quality Evaluation

The results obtained after the sensory quality evaluation of fresh figs as well as coated and uncoated figs stored for 15 days at 6 °C are shown in Fig. 5.

Color, texture, appearance, odor, taste, and general acceptability were the main sensory attributes assessed. Overall, the results clearly show that coated figs did not present significant differences, for the different sensory attributes evaluated, in comparison with fresh figs ($p > 0.05$), the score being higher than 7 for all attributes.

On the contrary, uncoated figs exhibited a significant reduction of the score related to the sensory attributes evaluated, taking the fresh figs as a reference ($p < 0.05$), and obtaining a score lower than 6 for all attributes.

Generally, the sensory attributes of fruits undergo a significant reduction as the time of storage increases, reducing the overall quality of fruits. Similar results have been observed for

Table 4 Antioxidant capacity (μmol Trolox equivalent/ 100 g dm) of coated figs with an A–Ch coating and uncoated (control) figs, collected at two maturity stages and stored for 15 days at 6 °C

Maturity stage	Storage days	ABTS		FRAP	
		Uncoated	Coated	Uncoated	Coated
III	0	1930.7 ± 39.0^{bdX}	1661.7 ± 51.1^{abY}	1928.8 ± 41.4^{bcX}	1735.4 ± 68.1^{abX}
	3	1963.9 ± 23.8^{abX}	1795.9 ± 93.4^{aX}	2226.9 ± 84.8^{aX}	1894.0 ± 41.7^{aY}
	6	1916.9 ± 42.9^{bdX}	1504.5 ± 73.5^{bcY}	2104.0 ± 78.5^{abX}	1542.5 ± 137.7^{bcY}
	9	2140.1 ± 18.2^{aX}	1669.7 ± 58.7^{abY}	2322.5 ± 82.4^{aX}	1710.7 ± 95.4^{abY}
	12	2008.5 ± 101.6^{abX}	1561.1 ± 95.3^{bcY}	2163.6 ± 94.2^{abX}	1619.8 ± 170.0^{abcY}
	15	1862.7 ± 156.2^{bdX}	1840.2 ± 85.4^{aX}	1924.4 ± 241.3^{bcX}	1853.7 ± 220.2^{aX}
IV	0	1474.1 ± 44.1^{cX}	1590.8 ± 65.2^{bcX}	1485.2 ± 44.0^{deX}	1636.7 ± 41.7^{abcX}
	3	1860.8 ± 65.3^{bdX}	1525.5 ± 22.1^{bcdY}	1861.9 ± 61.1^{bcX}	1521.8 ± 46.6^{bcdY}
	6	1480.6 ± 65.8^{cX}	1550.6 ± 53.6^{bcdX}	1408.4 ± 71.0^{dX}	1451.2 ± 126.5^{bcdX}
	9	1734.2 ± 49.4^{dX}	1469.5 ± 67.4^{cdX}	1713.4 ± 72.4^{ceX}	1360.1 ± 65.5^{cdY}
	12	1771.0 ± 33.5^{bdX}	1384.8 ± 46.6^{dY}	1495.7 ± 124.9^{deX}	1234.4 ± 14.4^{dX}
	15	1500.0 ± 98.7^{cX}	1514.2 ± 90.2^{bcdX}	1427.9 ± 76.8^{deX}	1390.0 ± 122.2^{cdX}

Lowercase letters (a, b, c, d, e) indicate significant differences in maturity stages and storage days according to the LSD test ($p < 0.05$) ($n = 4$)

Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits according to the LSD test ($p < 0.05$) ($n = 4$)

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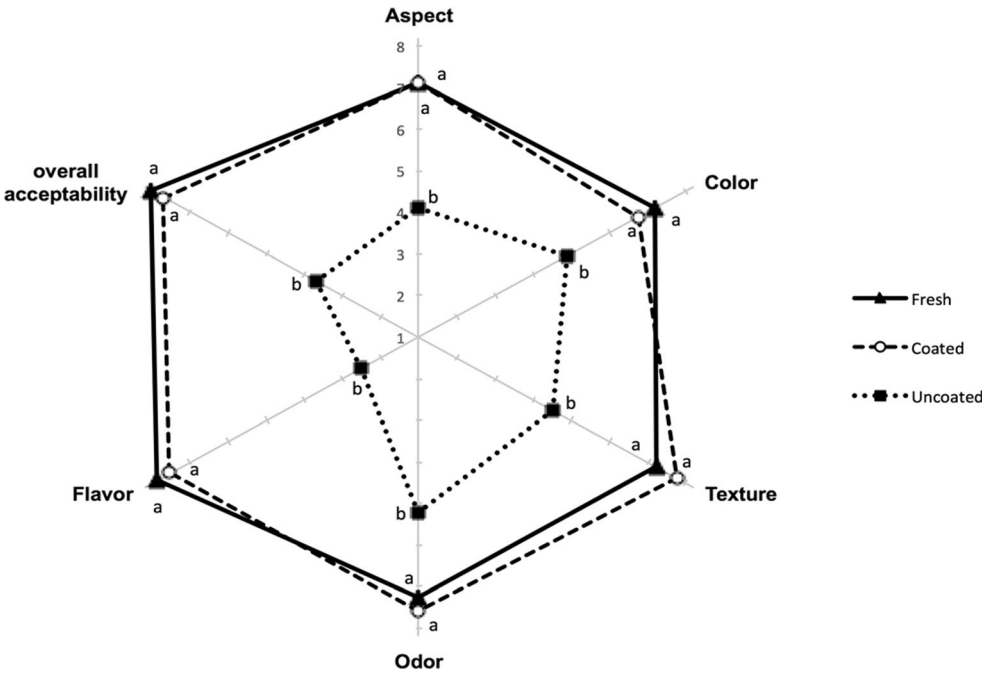

Fig. 5 Comparison of the sensory quality of coated figs with an A–Ch coating and uncoated (control) figs, stored for 15 days at 6 °C with fresh figs. Means with the same letters are not significantly different according to the LSD test ($p > 0.05$)

strawberries coated with a chitosan-based film (Valenzuela et al. 2015) and for pineapple coated with the alginate film (Mantilla et al. 2013) during storage at low temperature (< 6 °C). Thus, these results showed that the application of the A–Ch coating could reduce the loss of the sensory characteristics of figs, retaining its main sensory attributes at least during 15 days of storage at 6 °C.

Conclusions

The effect of the application of an alginate–chitosan (A–Ch) coating on the internal atmosphere, the bioactive compounds, antioxidant capacity, and sensorial quality of figs, collected at two stages of maturity (III and IV), has been evaluated. Thus, the A–Ch coating modified the internal atmosphere (increasing CO_2 and decreasing O_2 content) of figs, delaying the ripening process. In particular, the application of the A–Ch coating promoted, on the one hand, a controlled synthesis of polyphenol compounds ensuring a better retention of these compounds, in particular of C3R and Q3R, whereas for GA was not only retained but also increased. Further, during the storage period evaluated, the antioxidant capacity and CA of coated figs were preserved. Finally, it should be highlighted that the application A–Ch coating caused a much more efficient preservation of the overall sensory quality of the fruit during storage, especially more noticeable when figs at maturity stage IV were coated.

Therefore, the application of A–Ch coating can be a potential and effective alternative to preserve not only the bioactive compounds, but also, the organoleptic characteristics assuring the shelf life of the fruit during storage at low temperature.

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Effects of UV-C irradiation and traditional thermal processing on acemannan contained in *Aloe vera* gel blends

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ABSTRACT

The effects of pH (3.5, 4.5, and 5.5) and UV-C irradiation dose (12.8, 24.2, 35.8, and 54.6 mJ/cm^2) on the physicochemical properties changes in 10% *Aloe vera* gel blends; in addition, the acemannan concentration and structural changes in the precipitated polysaccharides were evaluated. A thermal treatment (TT; 45 s at 90 °C) was used for comparison. In contrast to TT, a dose of 24.2 mJ/cm^2 did not induce significant changes of free sugar content. Moreover, TT and UV-C irradiation did not significantly affect the content of mannose but increased those of galactose, fructose, and glucose. ^{1}H NMR analysis revealed minimal changes in the isolated fractions of acemannan, indicating that compared to the unprocessed control sample, the acemannan deacetylation was more pronounced by TT (27%) than by UV-C irradiation (11% at 54.6 mJ/cm^2), without any significant difference between the two. UV-C irradiation of *Aloe vera* gel blends at pH 3.5 and 24.2 mJ/cm^2 was an alternative to TT and efficiently preserve the characteristics of acemannan.

1. Introduction

The leaves of *Aloe barbadensis* Miller, commonly called *Aloe vera,* have an outer green rind and a transparent mucilaginous inner pulp (or gel), which accounts for 70–80% of leaf weight (Femenia, Sánchez, Simal, & Rosselló, 1999) and comprises 98.5–99.5 wt% water. The remaining solids (0.5–1.5 wt%) are known to contain more than 200 individual substances with diverse biological activities (Choi & Chung, 2003; Rodríguez-González et al., 2011), *e.g.*, wall polysaccharides (pectin, hemicellulose, and cellulose) and acetylated polysaccharides such as acemannan (Femenia et al., 1999; Ni, Yates, & Tizard, 2004). Acemannan mainly contains mannose units (> 60%, often partially acetylated), glucose units (~20%), and small amounts of galactose residues (< 10%) (Chokboribal et al., 2015; Femenia et al., 1999; Rodríguez-González et al., 2011). The mannose-bound acetyl groups of acemannan strongly affecting the physicochemical properties and biological activity of *A. vera* (Campestrini, Silveira, Duarte, Koop, & Noseda, 2013; Chokboribal et al., 2015; Ni et al., 2004). The molecular weight (MW) of acemannan varies between 30 and 45 kDa, although MWs exceeding 200 kDa have also been reported (Chokboribal et al., 2015; Femenia, García-Pascual, Simal, & Rosselló, 2003). High-temperature processing and pH changes are common strategies of *A. vera* gel conservation. However, acemannan is unstable and is easily degraded upon change of pH or exposure to high temperatures, enzymes (mannases), or bacteria (Javed & Atta-Ur, 2014; Kim, 2006). For example, at temperatures exceeding 70 °C, acemannan undergoes degradation (Femenia et al., 2003), experiencing small changes of mannose content, notable changes of galactose content, and deacetylation of mannose units (Minjares-Fuentes & Femenia, 2017). The deacetylation of acemannan can also be induced by exposure to alkaline media (Chokboribal et al., 2015; Ni et al., 2004) and is known to result in physical property and biological activity changes (Chokboribal et al., 2015; Femenia et al., 2003; Minjares-Fuentes et al., 2016). Thus, the preservation of the natural properties of *A. vera* gel is challenging and requires the application of emerging non-thermal technologies such as shortwave ultraviolet (UV−C) irradiation (Worobo, 1999), which is

Abbreviations: UV-C, shortwave ultraviolet; P, processed treatment; UP, unprocessed treatment; TT, Thermal treatment; TC, total carbohydrate; MW, molecular weight; SD, standard deviation; ANOVA, analysis of variance; HPAEC-PAD, high performance anionic chromatography coupled with amperometric pulse detection; HPSEC-PAD, size exclusion chromatography coupled with amperometric pulse detection; ^{1}H NMR, proton nuclear magnetic resonance

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known to effectively kill pathogens in fruit juices (Hanes et al., 2002; Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004). However, the effectiveness of UV−C irradiation treatment depends on a multitude of factors (*e.g.*, irradiation dose, medium pH, and medium optical properties (color, turbidity, viscosity, and absorbance coefficient) that must be identified prior to process optimization (Gopisetty et al., 2018; Koutchma, 2009). As *A. vera* gel is an important component of different products, it is important to preserve it without changing the properties of acemannan, since such changes may alter product quality and functional characteristics. The UV−C irradiation of *A. vera* gel in continuous flow mode with a short residence time avoids the increase of liquid temperature and is therefore a safe alternative to thermal treatment. However, the impact of UV light on sugars has not been extensively explored. Simple sugars are known to absorb UV radiation at 240–360 nm (Fan & Geveke, 2007), while long-chain complex carbohydrates such as acemannan mainly absorb at wavelengths outside the UV−C range and are therefore not sensitive to this radiation (Koutchma, 2009). However, UV radiation may induce some changes in simple sugars linked to the acemannan chain, with the extent and position of these changes depending on the irradiation dose, residence time, optical properties of the evaluated matrix, and the operation mode (batch or continuous) of the UV−C system. Consequently, the use of UV−C irradiation as an alternative technology for the stabilization of *A. vera* gel and the impact of this irradiation on acemannan have not been explored. To bridge this gap, we herein evaluate the effect of UV−C irradiation dose and pH on the content and characteristics of acemannan in a 10% *A. vera* gel blend, hypothesizing that UV−C irradiation can be used as a viable alternative to thermal treatment for stabilizing *A. vera* gel.

2. Materials and methods

2.1. Materials

Four-year-old *A. vera* plants used as the raw material were obtained from the greenhouse of the Chemical Sciences Department, University of Chihuahua. Homogenous leaves were washed and disinfected, and the yellow liquid seeping from the leaves, known as acibar, was removed by 1-h vertical draining. The pulp was separated from the skin, washed with distilled water, triturated in a blender (Phillips Electric Blender, Mexico), and filtered to obtain a gel that was stored at 4 °C for 1 h or less prior to treatment.

2.2. Chemicals

Standards of D-Glucose, mannose, galactose (assay ≥ 99.5%), formic acid and a pullulan set (342–720,000 Da), as well as D_2O (99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Another analytical- and HPLC-grade reagents were purchased from Sigma-Aldrich and J. T. Baker (Mexico City, Mexico).

2.3. A. vera gel treatment

A. vera gel diluted with water to 10% was separated into three batches, the pH of which was adjusted to 3.5, 4.5, and 5.5. Each batch was subjected to irradiation doses of 12.8 ± 0.18, 24.2 ± 0.12, 35.8 ± 0.36, and 54.6 ± 0.39 mJ/cm^2 corresponding to residence times of 2.6, 4.9, 7.4, and 11.2 s, respectively. All experiments were performed in duplicate at room temperature (20 °C) in a continuous-flow CiderSure 3500 commercial UV unit (FPE Inc., Macedon, NY, USA) comprising a stainless-steel outer housing and three chambered inner quartz tubes connected in series. Eight low-pressure mercury lamps (λ = 254 nm) used to irradiate the passing fluid were concentrically placed within the interior of the quartz/stainless steel cylinder with a gap of 0.08 cm. The UV unit was equipped with two UVX-25 sensors (UVP, Inc., Upland, CA, USA). The UV dose was changed by adjusting the flow rate (10–40 gallon per hour). Additionally, a thermal treatment (TT) control experiment was performed by heating gel samples with different pH at 90 °C for 45 s in a continuous tubular pasteurizer (UHT/HTST unit, Micro Thermics, Raleigh, NC, USA) (He, Changhong, Kojo, & Tiah, 2005). Total carbohydrate (TC), free sugars, and acemannan content changes of processed (P; TT and UV-C) and unprocessed (UP) samples were analyzed immediately.

2.4. UV dose measurement

The intensity of incident UV radiation was measured every 50 ms using two UVX-25 sensors (UVP, Inc.) placed at the top and bottom of the cylinder. The absorption coefficient was calculated at sample depth according to Beer's law as 0.0403 cm^{-1}. This value was then multiplied by the sensor placement factor (supplied by the manufacturer) to obtain the actual intensity. Exposure times were determined from the flow rate for each test. UV dose (mJ/cm^2) was calculated as irradiance × exposure time (Quintero-Ramos et al., 2004).

2.5. Optical properties

The optical properties of the gel blend were measured according to methods described by Koutchma, Keller, Chirtel, & Parisi (2004). The absorption coefficient was obtained at λ = 254 nm using a spectrophotometer (PerkinElmer model Lambda 25 UV/VIS, Waltham, MA, USA) and matched demountable fused quartz cuvettes (FireflySci, Inc., NY, USA) with path lengths of 0.1, 0.2, 0.5, and 1.0 mm. Turbidity was measured using a micro Turbidimeter 100 instrument (Scientific, Inc., Fort Myers, FL, USA). The content of soluble solids was measured using an Abbe refractometer (American Optical Corporation, NY, USA). All measurements were performed in triplicate, and the results were reported as mean ± standard deviation (SD).

2.6. Total carbohydrates (TC) content

TC content was determined according to the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as a standard, and the results were expressed as mg/g dry mass (d.m.) of the gel blend.

2.7. Measurement of free sugar content

Free sugar content was determined by high-performance anion-exchange chromatography with pulsed amperometric detection according to a modification of the method of Bozzi, Perrin, Austin, & Arce Vera (2007). A suspension of precipitated polysaccharides (2 mg) in distilled water (2 mL) was stirred for 24 h at 4 °C, filtered (0.45 μm), and analyzed by a Thermo Scientific Dionex ICS-5000+ system (Sunnyvale, CA, USA) equipped with an autosampler and an electrochemical detector using a Au working electrode and a AgCl reference electrode. Sugars were separated on an anion-exchange resin (CarboPac PA1 analytical column, 250 × 4 mm; CarboPac PA1 pre-column, 50 × 4 mm) using gradient elution with water (mobile phase A) –300 mM NaOH (mobile phase B) mixtures. The following gradient was employed: 0–10 min with 100% A, 10–85 min 0–45% B, and 85–90 min 45 to 0% B. Glucose, mannose, and galactose were used as standards. All measurements were done in triplicate, and the content of free sugars was expressed as mg/g dry mass (d.m.) of the gel (mean value ± SD).

2.8. Size exclusion chromatography

The effects of different treatments on the MW distribution of polysaccharides in *A. vera* gel were evaluated by size exclusion chromatography coupled with amperometric pulse detection (HPSEC-PAD) using a Zorbax BioPlus™ SE-450 size exclusion column. The mobile phase (10 mM acetate buffer) was pumped with an auxiliary pump and mixed

on a post-column with 300 mM NaOH using a flow rate of 1 mL min^{-1} for both components. Precipitated polysaccharides (2 mg) were suspended in distilled water (20 mL), and the suspension was stirred overnight at 4 °C. Samples of this suspension were filtered (0.22 μm), injected by the autosampler, and analyzed by a Thermo Scientific Dionex ICS-5000+ system (Sunnyvale, CA, USA) equipped with an electrochemical detector using a Au working electrode and a AgCl reference electrode. Fractionated pullulans (342–720,000 Da) were used as standards. Each sample was analyzed in triplicate, and the results (kDa) were expressed as mean ± SD.

2.9. Treatment effect on acemannan structure

The effects of processing on the content and deacetylation degree of acemannan were verified by ^{1}H NMR analysis. Four treatments were chosen from the original experimental design and performed once again for ^{1}H NMR analysis. The selected treatments were at pH 3.5, analyzing (a) unprocessed (UP), (b) UV-irradiated (12.8 mJ/cm^2; D-UV1), (c) UV-irradiated (54.6 mJ/cm^2; D-UV4), and (d) TT. The four treatments were subjected to fractionation using an HPLC system, under the same conditions as those used for size exclusion chromatography (Section 2.8). Enough sample was collected at the retention time corresponding to the acemannan fraction. Afterward, the acemannan fractions were isolated and ^{1}H NMR structural analysis was performed.

2.9.1. Isolation of polysaccharides

Fraction of polysaccharides from the four treatments were isolated by precipitation using a modification of the method of Campestrini et al. (2013). UP and P samples were freeze-dried, subjected to precipitation by adding 4 times volumes of anhydrous ethanol and maintained for 48 h at 4 °C. The precipitates were separated by centrifugation, dried in a vacuum oven at 40 °C for 6 h, and milled to a 200-mesh particle size. Same amount (2 mg) of sample (isolated acemannan) from each treatment was utilized for ^{1}H NMR analysis.

2.9.2. ^{1}H NMR analysis

An indirect quantification and monitoring of the structural changes of acemannan were performed by ^{1}H NMR analysis, based on the integrals of the signals corresponding to malic acid and acemannan (Minjares-Fuentes et al., 2017). ^{1}H NMR analysis of polysaccharides was carried out using a modification of the method proposed by Bozzi, Perrin, Austin, and Arce Vera (2007). Spectra were recorded at 400 MHz on a Bruker Avance Spectrometer (Massachusetts, USA) equipped with a 5-mm broadband multi-nuclear *z*-gradient probe head. A precipitated polysaccharide sample (2 mg) was weighed into an Eppendorf tube, solubilized with 1 mL of D_2O, and 0.6 mL of the solution was transferred to a Wildman NMR tube. All measurements were carried out at pH 3.5 using a pre-saturated ZGPR pulse sequence program and 128 scans. The content and acetylation degree of acemannan were determined using a slight modification of the method proposed by Jiao et al. (2010). Formic acid (10 mM) was added as the internal standard, and its singlet at 8.22 ppm was well separated from peaks of the *A. vera* gel. The relative acetylation degree of processed samples was calculated as 100% × A_P/A_{UP}, where A_P and A_{UP} are areas under the curves of acetyl group signals corresponding to processed and unprocessed gel samples, respectively. The content of acemannan (W_{ti}) was calculated as

$$W_{ti} = \frac{W_{is} \times IN_s \times NA_{is} \times MW_s}{IN_{is} \times NA_s \times MW_{is}} \times 100\%$$

Where, s = sample, is = internal standard, *IN* = integral, *NA* = number of atoms, and *MW* = molecular weight. The acemannan content was reported as mg/g d.m. of isolated polysaccharide.

2.10. Experimental design and statistical analysis

A completely randomized 4 × 3 factorial experiment performed in duplicate was employed to determine the influence of UV-C dose (12.8, 24.2, 35.8, and 54.6 mJ/cm^2) and pH (3.5, 4.5, and 5.5) on the carbohydrates of the 10% *A. vera* gel blend. UP and TT samples were used as controls. The obtained data were subjected to an analysis of variance (ANOVA), and differences of means were characterized by Tukey's test. In addition, a contrast analysis of mean differences between treatments was performed. For ^{1}H NMR analysis, a Tukey's test was done. Significance was defined as $p < 0.05$ using Minitab version 16 software (Minitab, 2010, State College, PA) and Microsoft Excel software version 16 (Microsoft Excel software version 16, 2016, Redmond, Washington, USA).

3. Results and discussion

3.1. Optical properties of A. vera gel blend

The 10% *A. vera* gel blend used for all the treatments featured had pH 6.15, 0.13°Bx, absorption coefficient of 0.493 cm^{-1}, turbidity of 21.50 ± 0.91 NTU, total solids content of 0.269 ± 0.08%, and a TC content of 54.60 ± 1.83 mg/g d.m. These low optical properties were advantageous for UV irradiation-based processing. The solids content is related to the TC content represented by complex sugars or high-molecular-weight polysaccharides, which are the main solid components of the gel (Minjares-Fuentes & Femenia, 2017).

3.2. TC content

Table 1 shows that both the processed and unprocessed gel blends featured TC contents between 47.68 and 53.80 mg/g d.m. Considering the 10-fold dilution of *Aloe vera* gel, these TC results are in agreement with those reported previously (Bozzi et al., 2007; Femenia et al., 1999). Table 2 shows that UV-C dose and pH did not significantly affect TC content, whereas TT at different pH had a significant influence, which was ascribed to the thermal degradation of complex sugars at the employed temperature (Rodríguez-González et al., 2011).

3.3. Influence of UV-C and pH on free sugar content

The effects of diverse treatments on free sugar content are shown in Table 2. The content of glucose, one of the main monosaccharides in *A. vera* gel and the second most abundant component of acemannan, increased after processing (Fig. 1a), with the greatest increase observed for TT (Fig. 1b). This finding agreed with the results obtained for thermally processed *A. vera* gel samples by Bozzi et al. (2007), Femenia et al. (2003) and Rodríguez-González et al. (2011) and was ascribed to the release of simple sugars due to the thermally induced hydrolysis of polysaccharides. UV-C doses of greater than 24.2 mJ/cm^2 caused a significant increase of glucose content (Fig. 1c), which was ascribed to the irradiation-induced hydrolysis of oligo- and polysaccharides (acemannan) present in the *A. vera* gel. Previous reports showed that processing-induced acemannan degradation results in an increase of glucose content and explored the influence of factors such as temperature, pH, and enzymes (Minjares-Fuentes & Femenia, 2017), whereas the impact of UV light on sugars has been underexplored. Fan and Geveke (2007) irradiated apple cider and sugar solutions, demonstrating that glucose absorbs little UV light in the range of 240–360 nm. The effects of UV irradiation on simple sugars were explored by Günter, Borisenkov, & Ovodov (2009), who found that irradiation of plant tissues decreased the contents of arabinose and galactose therein. However, despite the proven impact of UV light on monosaccharides, conjugated long-chain carbohydrates are not particularly sensitive to UV radiation, since their maximum absorption wavelengths are outside the UV-C range (Koutchma, 2009). Similar results were reported by Islam

Table 1
Effect of different treatments on the free sugar, TC contents, and MW distributions of 10% Aloe vera gel blends.

Treatments	Glucose (mg/g d.m.)	Fructose (mg/g d.m.)	Sucrose (mg/g d.m.)	Mannose (mg/g d.m.)	Galactose (mg/g d.m.)	TC (mg/g d.m.)	MW (kDa)
UP/3.5	14.084 ± 0.88^{de}	3.289 ± 0.41^{g}	0.476 ± 0.05^{ef}	0.339 ± 0.003^{a}	0.046 ± 0.006^{bcde}	52.70 ± 2.52^{ab}	372.90 ± 20.5^{ab}
UP/4.5	14.255 ± 0.85^{de}	4.453 ± 0.53^{efg}	0.650 ± 0.04^{cd}	0.343 ± 0.005^{a}	0.048 ± 0.003^{bcde}	53.18 ± 2.31^{ab}	415.47 ± 0.432^{a}
UP/5.5	14.605 ± 0.45^{bcde}	4.452 ± 0.77^{efg}	0.885 ± 0.03^{ab}	0.354 ± 0.006^{a}	0.043 ± 0.003^{de}	53.80 ± 3.04^{a}	399.37 ± 14.9^{a}
D-UV1/3.5	13.530 ± 1.17^{e}	3.981 ± 0.53^{fg}	0.506 ± 0.02^{ef}	0.342 ± 0.034^{a}	0.046 ± 0.004^{cde}	52.54 ± 2.40^{ab}	387.33 ± 7.82^{a}
D-UV1/4.5	14.372 ± 0.59^{bcde}	4.486 ± 0.43^{efg}	0.459 ± 0.06^{efg}	0.325 ± 0.011^{a}	0.048 ± 0.002^{bcde}	51.82 ± 1.77^{ab}	396.33 ± 9.73^{a}
D-UV1/5.5	14.763 ± 0.26^{bcde}	4.865 ± 0.41c^{def}	0.911 ± 0.09^{a}	0.352 ± 0.011^{a}	0.042 ± 0.005^{e}	48.94 ± 1.81^{ab}	382.72 ± 14.3^{a}
D-UV2/3.5	13.900 ± 0.29^{de}	4.765 ± 0.78^{def}	0.546 ± 0.01^{de}	0.351 ± 0.015^{a}	0.050 ± 0.003^{bcde}	53.08 ± 1.47^{ab}	386.92 ± 16.4^{a}
D-UV2/4.5	14.333 ± 1.13^{cde}	5.806 ± 0.31^{abcde}	0.400 ± 0.07^{fg}	0.360 ± 0.032^{a}	0.052 ± 0.004^{abcde}	51.64 ± 1.89^{ab}	397.16 ± 1.07^{a}
D-UV2/5.5	14.142 ± 0.27^{de}	5.017 ± 0.62^{cdef}	0.756 ± 0.08^{bc}	0.357 ± 0.020^{a}	0.041 ± 0.003^{e}	51.10 ± 1.59^{ab}	383.74 ± 2.74^{a}
D-UV3/3.5	15.611 ± 0.51^{abcde}	5.190 ± 0.63^{bcdef}	0.493 ± 0.03^{ef}	0.366 ± 0.063^{a}	0.050 ± 0.001^{bcde}	53.80 ± 3.18^{a}	386.90 ± 21.0^{a}
D-UV3/4.5	16.158 ± 0.73^{abcd}	4.647 ± 0.16^{defg}	0.405 ± 0.05^{fg}	0.368 ± 0.015^{a}	0.054 ± 0.002^{abcde}	50.92 ± 2.01^{ab}	397.16 ± 1.07^{a}
D-UV3/5.5	16.237 ± 0.30^{abcd}	5.137 ± 0.70^{bcdef}	0.331 ± 0.03^{gh}	0.355 ± 0.017^{a}	0.051 ± 0.004^{bcde}	50.38 ± 2.93^{ab}	383.74 ± 2.74^{a}
D-UV4/3.5	15.386 ± 1.09^{abcde}	5.769 ± 0.47^{abcde}	0.474 ± 0.04^{ef}	0.384 ± 0.028^{a}	0.052 ± 0.003^{abcde}	53.26 ± 1.06^{a}	389.80 ± 23.3^{a}
D-UV4/4.5	17.526 ± 1.78^{a}	6.531 ± 0.70^{ab}	0.382 ± 0.04^{fgh}	0.355 ± 0.015^{a}	0.062 ± 0.004^{ab}	51.46 ± 1.59^{ab}	408.10 ± 46.4^{a}
D-UV4/5.5	17.807 ± 1.14^{a}	5.697 ± 0.44^{abcde}	0.439 ± 0.09^{efg}	0.390 ± 0.068^{a}	0.048 ± 0.006^{bcde}	51.28 ± 4.68^{ab}	394.38 ± 2.87^{a}
TT/3.5	17.650 ± 1.68^{a}	6.281 ± 0.54^{abc}	0.256 ± 0.02^{h}	0.340 ± 0.003^{a}	0.057 ± 0.004^{abcd}	48.94 ± 1.26^{ab}	337.21 ± 0.679^{b}
TT/4.5	16.824 ± 0.88^{ab}	5.988 ± 0.47^{abcd}	0.384 ± 0.02^{fgh}	0.339 ± 0.006^{a}	0.067 ± 0.003^{a}	48.40 ± 3.03^{ab}	376.52 ± 4.27^{ab}
TT/5.5	16.772 ± 1.19^{abc}	6.864 ± 0.56^{a}	0.555 ± 0.07^{de}	0.333 ± 0.003^{a}	0.061 ± 0.004^{abc}	47.68 ± 3.53^{b}	376.94 ± 3.43^{ab}

*Means ± standard deviation (n = 3). Different letters by column are significantly different according to Tukey test ($p < 0.05$). UP, unprocessed; TT, thermal treatment (90 °C, 45 s); D-UV1, 12.8 mJ/cm^2; D-UV2, 24.2 mJ/cm^2, D-UV3, 35.8 mJ/cm^2, D-UV4, 54.6 mJ/cm^2; TC, total carbohydrates; MW, molecular weight.

et al. (2016), who observed that the glucose and fructose contents of apple juice increased upon high-dose irradiation (> 40 mJ/cm^2).

Processing of *aloe vera* gel blends (TT and UV-C) caused a significant change in the fructose content (Table 2), compared with UP treatments. Increases in UV-C doses resulted in high fructose content (Table 1), while TT released higher fructose amount than UV-C treatment. This finding had been associated the hydrolysis of complex carbohydrates like fructans and oligofructans present in *aloe vera* gel (Salinas, Handford, Pauly, Dupree, & Cardemil, 2016) due to processing. The release of large amounts of fructose from *aloe vera* gel was related to thermal pasteurization at temperatures above 80 °C (Femenia et al., 2003; Rodríguez-González et al., 2011).

Sucrose content was affected by both TT and UV-C treatments, as well as by the pH (Table 2). Table 1 shows that TT and UV-C doses for the *aloe vera* gel blend cause low sucrose contents at pH < 4.5, compared to UP treatment. In addition, TT presented the lowest sucrose content at pH 3.5 (Table 1) due to its hydrolysis at low pH and high temperatures (L'homme, Arbelot, Puigserver, & Biagini, 2003). The same effect was observed at irradiation dose > 24.2 mJ/cm^2 (Table 1). These led to the release of glucose and fructose, consistent with the behavior shown by both monosaccharides, as discussed previously.

The content of galactose (contained in acemannan as small fractions linked to the polysaccharide backbone) in the *A. vera* gel blend was low (Fig. 2) and depended on pH (Table 2), slightly increasing at pH > 3.5 (Fig. 2a), whereas irradiation dose had no significant influence. Processing of *A. vera* gel blends increased their galactose content (Fig. 2b), and the greatest increase was observed for TT (Fig. 2c), in agreement with the results of Rodríguez-González et al. (2011). These changes were caused by the hydrolysis of fractions bound to the structural chain of acemannan, since this polysaccharide is known to release galactose at temperatures above 70 °C (Femenia et al., 1999; Rodríguez-González et al., 2011).

Mannose is the monomeric structural unit of acemannan, and changes of its content may therefore be an indicator of acemannan degradation (Femenia et al., 1999). Table 1 shows that mannose content was not significantly affected by treatment type or pH, *i.e.*, the polysaccharide backbone comprising partially acetylated mannose and glucose units (Femenia et al., 1999) linked by β-(1,4) glycosidic bonds was not altered by any of evaluated treatments.

3.4. MW distribution

Fig. 3 shows the elution profiles of *A. vera* gel blend fractions for different treatments, revealing that these heterogeneous and polydisperse profiles featured two or three peaks of different intensity. The first peak (8 min) corresponded to a compound with a high molecular weight (372–400 kDa) and was assigned to a partially acetylated glucomannan suggested as acemannan according to Campestrini et al.

Table 2
Contrast analysis of the effects of treatments on free sugar, TC contents and MW distributions of 10% Aloe vera gel blends at different pH.

Source	DF	Sum of Squares						
		Glucose	Fructose	Sucrose	Mannose	Galactose	TC	MW
Model	17	68.37*	29.33*	1.0916*	9.70×10^{-3}	1.71×10^{-3}*	121.146	10308.13
UP *vs* P	1	9.150*	8.934*	0.1689*	3.87×10^{-4}	2.02×10^{-4}*	24.440*	347.66
TT *vs* UV-C	1	15.01*	7.146*	0.0585*	2.28×10^{-3}	7.20×10^{-4}*	53.766*	4326.68*
pH	1	2.893	1.268*	0.2111*	6.01×10^{-5}	3.75×10^{-5}	20.681*	1023.99
pH^2	1	0.333	0.350	0.0888*	3.92×10^{-4}	3.04×10^{-4}*	0.391	2246.07*
pH *UP *vs* P	1	0.036	0.593	0.0585*	1.68×10^{-4}	1.20×10^{-6}	10.541	215.45
pH^2* UP *vs* P	1	0.138	0.221	0.0091	1.00×10^{-5}	9.34×10^{-6}	0.038	253.16
pH *TT *vs* UV-C	1	3.226	0.087	0.0302*	7.60×10^{-5}	3.51×10^{-5}	1.766	1076.66
pH^2* TT *vs* UV-C	1	0.703	0.862	0.0161*	1.67×10^{-4}	3.04×10^{-6}	0.195	45.09
D	3	33.70*	7.478*	0.1972*	4.23×10^{-3}	2.96×10^{-4}	3.043	502.74
pH*D	6	3.177	2.394	0.2528*	1.92×10^{-3}	1.02×10^{-4}	6.281	270.58
Error	18	20.02	5.131	0.0567	0.01267	6.11×10^{-4}	84.375	5091.56

*Significance level at $p < 0.05$. UP, unprocessed treatment; P, processed treatment; TT, thermal treatment; UV-C, UV treatment; D, UV dose; TC, total carbohydrates; MW, molecular weight.

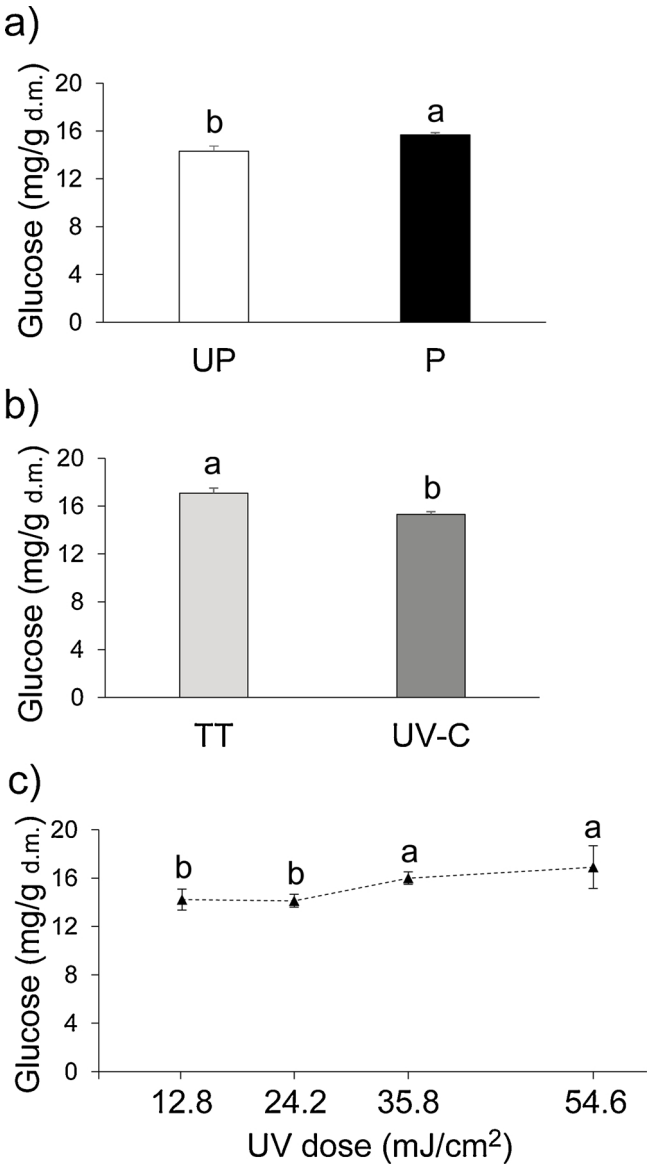

Fig. 1. Effects of treatments on the glucose content of 10% *Aloe vera* gel blends. Comparison between (a) processed (P) and unprocessed (UP) samples and (b) thermally and UV-C–treated samples. (c) Effects of UV-C dose on glucose content. For (a) and (b), different letters denote significant differences in the contrast test. For (c), the differences of means were analyzed using Tukey's test. $p < 0.05$ was employed as a significance level.

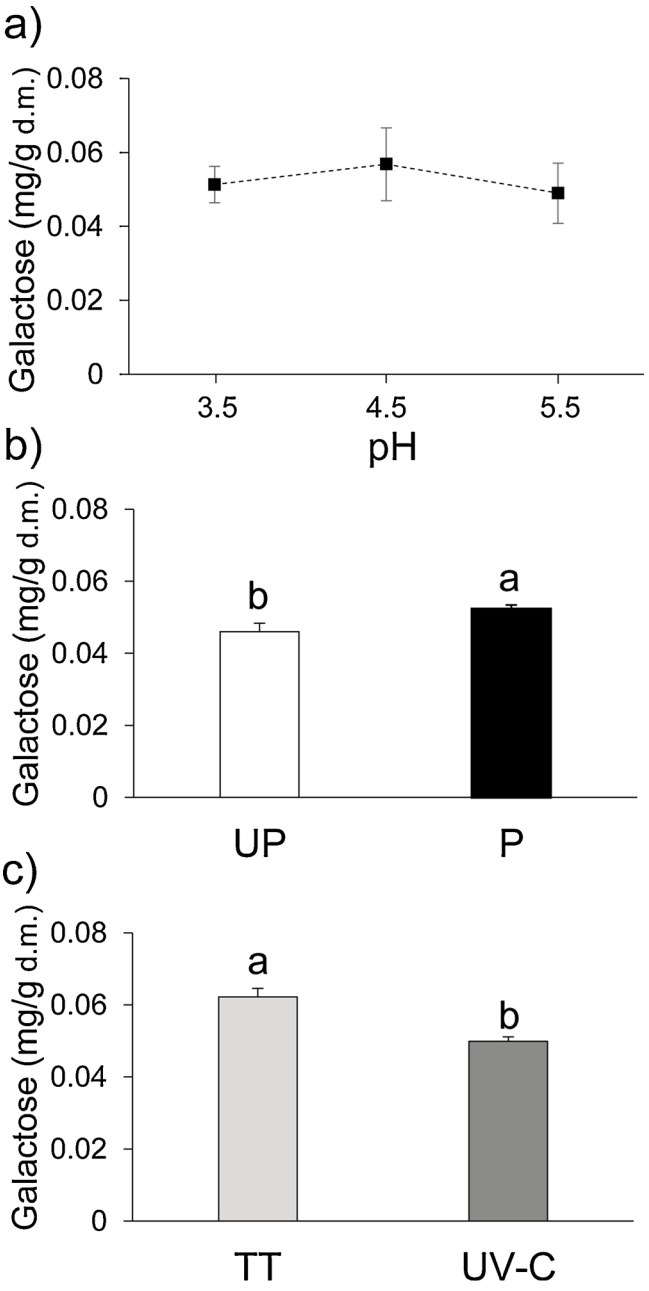

Fig. 2. Effects of treatments on the galactose content of 10% *Aloe vera* gel blends. (a) Effect of pH. Comparison between (b) processed (P) and unprocessed (UP) samples and (c) thermally (TT) and UV-C–treated samples. Differences between treatments ($p < 0.05$) were determined using the contrast test.

(2013) and Femenia et al. (1999), who identified and characterized these mannose-rich polymers. The two remaining peaks eluted at 10 and 13.8 min were assigned to a monosaccharide (MW < 0.342 kDa) highly abundant in the gel. Table 1 shows the MW distributions of the compound corresponding to the first peak in the chromatogram for *A. vera* gel blends subjected to different treatments. Although the MW of acemannan typically ranges between 30 and 45 kDa (Femenia et al., 2003; Minjares-Fuentes & Femenia, 2017), Campestrini et al. (2013) found that it can reach 1.2 MDa. This variability was attributed to factors such as plant age, plant growth temperature, harvesting time, gel extraction conditions, *etc.* (Femenia et al., 1999; Lee et al., 2012; Ray, Ghosh, Ray, & Aswatha, 2015). Contrast analysis (Table 2) showed that the MW of the first-peak compound was significant affected by the pH of treated samples. This dependence is illustrated in Fig. 4a, which shows that maximum MW was observed at pH 4.5, *i.e.*, at the initial pH of the gel. In general, UV—C treatment afforded higher MWs (382–408 kDa) than TT (337–376 kDa) (Fig. 4b). This finding was corroborated by data in Fig. 3c, where a decrease of first-peak (8 min) intensity and the generation of another peak (10 min) were observed in TT. Similar results were reported by Minjares-Fuentes et al. (2017) and Rodríguez-González et al. (2011), who observed a decrease in the MW of acemannan in gel samples spray-dried and pasteurized at different temperatures, respectively. This decrease was attributed to the breakdown of glycosidic bonds between the monomeric bases of the polysaccharide, since an increase of bound mannosyl residue (1,4) content was detected in treated samples (Rodríguez-González et al., 2011).

3.5. ^{1}H NMR analyses of *A. vera* gel blend fractions

^{1}H NMR analysis provides vital information for determining the composition and quality of *A. vera* gel preparations (Bozzi et al., 2007;

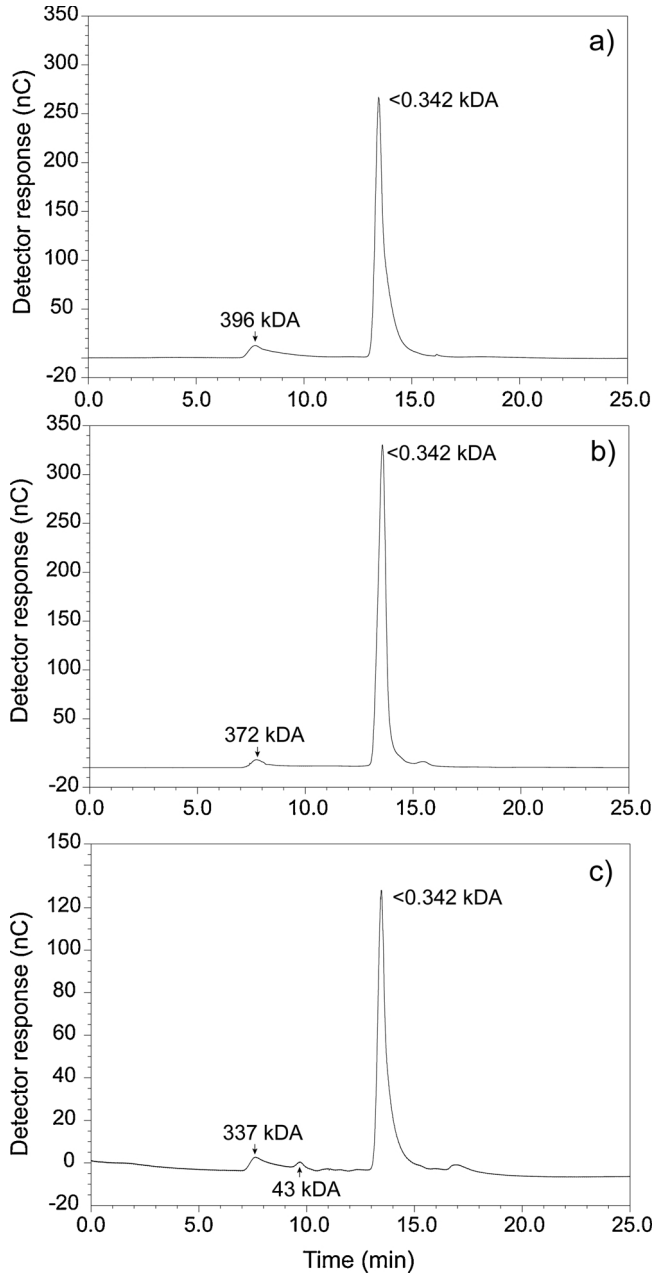

Fig. 3. HPSEC-PAD elution profiles of (a) unprocessed (UP), (b) UV-C–irradiated at 54.6 mJ/cm^2, and (c) thermally treated 10% *Aloe vera* gel blends fractions (pH 3.5).

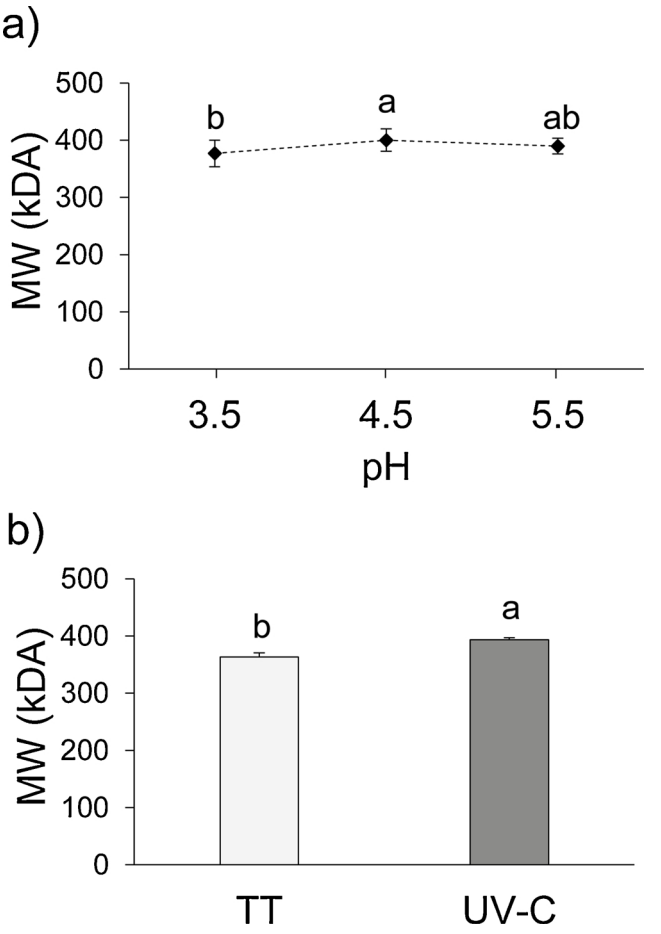

Fig. 4. (a) Effect of pH (mean differences determined by Tukey's test) and (b) TT and UV-C treatment (mean differences determined by contrast test) on the MW of polysaccharides in the 10% *Aloe vera* gel. Significance level: $p < .05$.

Chokboribal et al., 2015). Fig. 5 shows ^{1}H NMR spectra of acemannan isolated from different *A. vera* gel treatments at pH 3.5. The acetyl groups of acemannan afford a characteristic signal at δ 1.99–2.26 ppm that can be considered as the fingerprint of *A. vera* acemannan (Bozzi et al., 2007; Campestrini et al., 2013; Jiao et al., 2010; Minjares-Fuentes et al., 2017). The two doublets with coupling constants of 3.8 and 8 Hz at 4.4 and 5.1 ppm, respectively, in the region of anomeric hydrogens, were compared with the NMR library Human Metabolome Data Base (Wishart et al., 2018). Therefore, it can be inferred these doublets correspond to the presence of a glucose residue in the polymeric chain of the acemannan. The region between 3 and 4 ppm corresponds to hydrogens inside the sugar core, and not the anomeric hydrogens. A clear *O*-acetyl signal was observed for all samples, and its intensity did not strongly depend on UV radiation dose, decreasing upon thermal treatment. Similar effects of *A. vera* gel processing were reported by Bozzi et al. (2007) and Minjares-Fuentes et al. (2017). Quantitative analysis of the relative acemannan acetylation degree showed that TT decreased the extent of acetylation by ˜27% (relative to that of the unprocessed sample), while UV irradiation at doses of 12.8 and 54.6 mJ/cm^2 resulted in decreases of only 13 and 11%, respectively compared to UP sample. No significant change is observed in the region of anomeric and non-anomeric hydrogens of the polysaccharide chain of acemannan, which could indicate that the acemannan polysaccharide did not undergo any side reactions upon thermal and UV−C treatments. Although small changes in the relative degree of acemannan acetylation were found, they are not apparent between the TT and UV−C treatments (Fig. 5). Fig. 6 shows the amounts of acemannan in the isolated fractions obtained from different treatments. Although UV−C treatment did not present any significant changes in acemannan concentration compared to UP treatment, its indirect quantification gave acemannan yields of 84 and 87%. This is because long-chain carbohydrates are not particularly sensitive to UV radiation (Koutchma, 2009) and the low UV−C doses used in this research. TT significantly reduced acemannan yield to 78% compared to UP treatment but showed no significant differences from UV−C treatment (Fig. 6). Similar results for thermally treated acemannan gel were reported by Rodríguez-González et al. (2011), who observed a reduction in acemannan content for all the evaluated pasteurization treatments. The small effect of UV−C and TT treatments on the structural changes of acemannan was favored by the mild conditions used in this study.

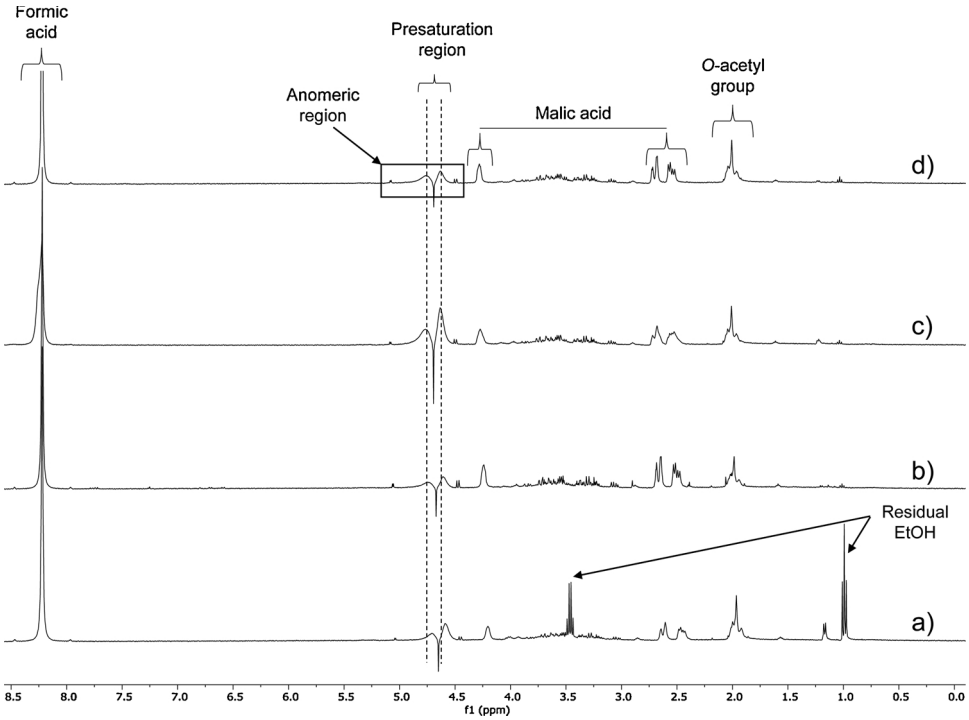

Fig. 5. Pre-saturated ^{1}H NMR spectra of isolated acemannan fractions (isolated from 10% *A. vera* gel blends at pH 3.5) recorded in D_2O using 10 mM HCOOH as an internal standard for (a) unprocessed (UP), b) UV-C–irradiated (12.8 mJ/cm^2; D-UV1), (c) UV-C–irradiated (54.6 mJ/cm^2; D-UV4), and (d) thermally treated samples.

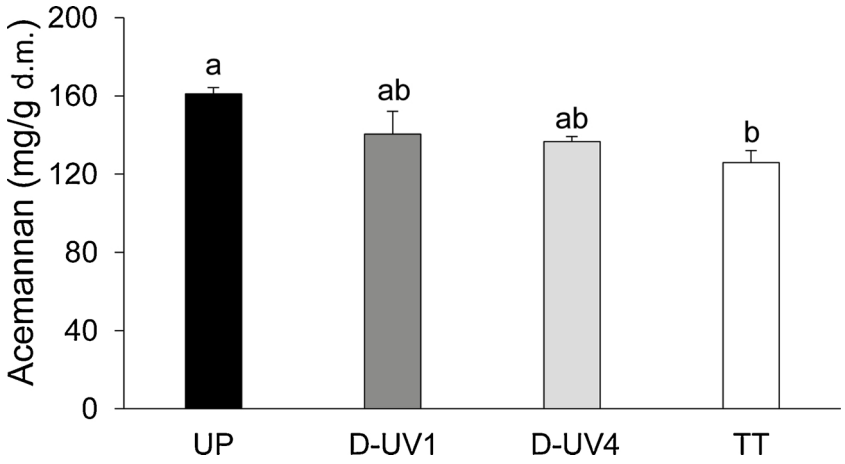

Fig. 6. ^{1}H NMR-determined acemannan contents in isolated acemannan fractions obtained from 10% *Aloe vera* gel (pH 3.5) subjected to (a) unprocessed, (b) UV-C–irradiation (12.8 mJ/cm^2; D-UV1), (c) UV-C–irradiation (54.6 mJ/cm^2; D-UV4), and (d) TT.

4. Conclusion

Continuous-flow UV−C irradiation of *A. vera* gel blends preserved the chemical and structural characteristics of acemannan better than TT. The free sugar contents of *A. vera* gel blends were not affected by an irradiation dose of 24.2 mJ/cm^2, whereas an increase was observed for TT. Neither UV−C nor thermal treatments changed the content of mannose, which indicates that these treatments did not modify the acemannan structure. Compared to UV−C irradiation treatments, TT resulted in a higher extent of acemannan degradation (up to 27%), as determined by analysis of MW distribution, deacetylation percentage, and acemannan content. ^{1}H NMR was found to be a useful tool for indirect estimation of the deacetylation degree of acemannan in isolated acemannan fractions, because the intensity of the acetyl group signal could be correlated to the amount of remaining acetylated groups in the polysaccharide. It was concluded that UV−C irradiation of 10% *Aloe vera* gel blends at a dose of 24.2 mJ/cm^2 and pH 3.5 can be effectively used to preserve the characteristics of acemannan and can therefore be utilized for the pasteurization of beverages or diverse products containing *A. vera*. Thus, the hypothesis was confirmed.

Acknowledgments

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Utility of Milk Coagulant Enzyme of *Moringa oleifera* Seed in Cheese Production from Soy and Skim Milks

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Abstract: In this study, the potential use of *Moringa oleifera* as a clotting agent of different types of milk (whole, skim, and soy milk) was investigated. *M. oleifera* seed extract showed high milk-clotting activity followed by flower extract. Specific clotting activity of seed extract was 200 times higher than that of flower extract. Seed extract is composed by four main protein bands (43.6, 32.2, 19.4, and 16.3 kDa). Caseinolytic activity assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and tyrosine quantification, showed a high extent of casein degradation using *M. oleifera* seed extract. Milk soy cheese was soft and creamy, while skim milk cheese was hard and crumbly. According to these results, it is concluded that seed extract of *M. oleifera* generates suitable milk clotting activity for cheesemaking. To our knowledge, this study is the first to report comparative data of *M. oleifera* milk clotting activity between different types of soy milk.

Keywords: soy milk; milk-coagulant activity; *Moringa oleifera*; seeds

1. Introduction

Nowadays, foods are intended not only to satisfy hunger and provide necessary nutrients for humans, but to prevent nutrition-related diseases and improve consumers' physical and mental well-being [1]. Moreover, there is plenty of scientific literature that demonstrates the close connection between diet and health, particularly related to chronic diseases, which have encouraged the development of growing spectrum products such as nutraceuticals, medifoods, and vitafoods [2,3].

In México, data from three national surveys conducted in 1988, 1999, and 2006, using the International Obesity Task Force classification system, described the upward trends on overweight and obesity in school-age children and teenagers at a national level [4]. Besides, increased world population, and the continuous rise of morbid obesity and other nutritional diseases have led to the employment of protein from vegetal sources, along with the preference of low-fat dairy products in consumers from our country. Therefore, people are constantly pursuing better life quality by eating low-fat dairy products which may reduce the risk of stroke or coronary heart disease [5]. Also, the remarkable sales in soy-based products can be attributed to the beneficial health properties of soy-derived foods.

Cheese is a widely consumed product by the general population, it is highly-concentrated, rich in proteins and lipids, essential amino acids, and minerals such as calcium and phosphorus. The first

step in cheesemaking is the milk clotting process where κ-caseinolytic enzymes contribute to micelle aggregation, usually performed by animal rennet, which has been the traditional coagulant in cheese manufacture for centuries [6]. However, dairy products have recently come under fire from researchers showing the detrimental effects of high saturated fat and cholesterol concentrations to the body [7]. Since full-fat dairy products contain more calories, many experts assumed that avoiding them would lower the risk of diabetes. However, some studies have found that yogurt intake, but not milk, is consistently associated with lower incidence of diabetes mellitus. On the other hand, cheese intake, despite the higher calories, fat, and saturated fat content, is also associated with lower diabetes risk in several but not all studies [7–9]. These findings suggest that health effects of dairy products may depend on multiple complex characteristics and represent promising areas for further research [10]. Although the data are controversial, Mexican gastronomy is innovating products (lactose-free, high calcium, and weight-control foods) using vegetables harvested in large amounts in Mexican soil.

Nutritionists have mentioned that the incorporation of bioactive components in dairy products might confer several advantages [11]. For example, phenolic compounds have been extracted from a variety of plant sources and used as matrix food ingredients improving functional properties of dairy products such as storage and heat stability, as well as foaming properties [12,13]. Dairy product enrichment with phenolic compounds has been proposed for beverages [13], yogurt [14], milk powder [15], and processed cheese [16]. Given that polyphenols interact with proteins [17], their addition to milk may result in a high yield recovery in cheese, mainly attributed to hydrophobic and hydrophilic interactions [18] that depend on pH, molar ratio, and molecular properties of the polyphenols [15]. However, recent studies have shown that rennet-induced coagulation is altered by the addition of tea polyphenols to milk [19,20] but in spite of this limitation, cheddar type cheese has been produced from milk enriched with green tea extract [21]. In addition, plant extracts could increase the shelf life of dairy products by inhibiting oxidation of polyunsaturated fatty acids and the development of off-flavor aromas [22].

Yadav et al. [23] stated that soy milk is a well-known protein enriched bio-functional food, but its acceptability is reduced by the presence of complex sugars which give a bean-like flavor. However, it was shown that fermentation produces a reduction of such off-flavor compounds. Hence, soy by-products such as yogurt and cheese can be nutraceutical products with antioxidant potential.

It has been reported that commercial plant proteases, such as bromelain and papain, can clot the protein in soy milk forming a curd [24]. Unfortunately, unlike casein in bovine milk, enzymatic curdling of soy milk produces poor flavor and texture since proteolysis is more pronounced in cheese processed with vegetable coagulants which leads to a soft and buttery cheese texture and, partly, to liquefaction and loss of shape [25,26]. In addition, short peptides produced by its high proteolytic activity affect the flavor, which results in an excessively acidic and bitter cheese [27]. For this reason, the commercial use of bromelain, ficin, and papain to clot soy milk has not been successful [28–30]. However, a more recent study showed that *Saccharomyces bayanus* SCY003 protease produced a soy curd with elasticity that resembles milk-casein cheese [31]. Therefore, it is important to continue the search of proteases with the capability to coagulate soy milk and improve the acceptability of soy cheese.

Moringa oleifera is grown in rural regions of Mexico and its different parts, such as leaves, flowers, and seeds, are edible. It is a source of protein, calcium, iron, carotenoids, and phytochemicals, and it is employed for several applications in developing countries [32]. Previously, it has been reported that *M. oleifera* is an interesting source of milk clotting enzyme. Pontual et al. [33] reported the caseinolytic and milk-clotting activities of *M. oleifera* flowers using azocasein and skim milk as substrates, respectively. *M. oleifera* seed extract was also used as a milk-clotting agent, and the resulting curd was white and firm [34]. Despite the aforementioned studies on milk-clotting enzyme from *M. oleifera*, a deep evaluation of this potential source of a rennet substitute is still absent. In addition, there are no studies that evaluate the efficiency of *M. oleifera* proteases to clot soy milk proteins. Thus, the aim of this research was to determine the potential ability of different parts from *M. oleifera* to

coagulate whole, skim, and soy milks and to investigate the use of *M. oleifera* in the production of soft cheese.

2. Materials and Methods

2.1. Vegetal and Dairy Material

Moringa oleifera seeds, flowers, and leaves were obtained from Lombardia, Michoacán, located at 19°01′30″ N and 102°05′39″ W. All the samples were dried and crushed at room temperature and stored in closed containers at −20 °C. Commercial cow whole and skimmed milk (Lala® and Carnation®, Nestle®, respectively) and soy milk powders (Soyapac®, Colpac; AdeS®, and Soyalac®) were used to evaluate different parameters related to the milk coagulant enzyme (MCE).

2.2. Enzyme Extraction

Briefly, duplicate samples of 2 g of powder of the different parts of the plant were immersed in 10 mL of 50 mM phosphate buffer, pH 7.0 (1:10; w/v), the mixtures were macerated by two methods: traditional stirring for 4 h and ultrasonic bath (42 KHtz) for 15 min at room temperature. The extracts were centrifuged at 3500 rpm for 10 min. Finally, the supernatants were stored at 4 °C until used.

2.3. Milk-Clotting Activity (MCA)

The milk-clotting activity was determined following the procedure described by Arima et al. [35] with some modifications. Briefly, a suspension of skim milk powder was used as a substrate with 10 mM $CaCl_2$, pH 6.5. The milk was previously incubated at 35 °C for 5 min, then MCE was added at a 10:1 ratio (v/v, skim milk to enzyme extract). One unit of milk-clotting activity (MCA) is defined as the amount of enzyme to clot 1 mL of a solution containing 0.1 g skim milk powder in 40 min at 35 °C.

The time between the addition and the appearance of clots was registered and the total MCA was calculated as follows:

$$MCA\left(\frac{SU}{mL}\right) = \frac{2400}{Coagulation\ time\ (s)} \times dilution\ factor \quad (1)$$

In order to compare the efficiency of the milk clotting enzyme, rennet from calf stomach (mixture of chymosin and pepsin) from Sigma-Aldrich (Toluca, Mexico) and 5% NaCl were used as the positive and negative controls, respectively. The specific activity was determined by dividing the MCA between protein concentrations of extracts [Soxhlet units (SU)/mg protein].

2.4. Caseinolytic Activity

Caseinolytic activity was measured by Sarath's method [36]. Suitably diluted seed extract solution was added to 2.5 mL of 1% casein dissolved in 50 mM NaH_2PO_4 (pH 6.5). The mixture was incubated at 37 °C for 30 min and then 2.5 mL of 5% trichloroacetic acid solution (TCA) was added. After precipitation, all mixtures were centrifuged at 10,000 rpm for 20 min and the absorbance of the supernatant at 280 nm was registered. The blank sample was prepared in the same way by adding TCA prior to the addition of the substrate. One unit of caseinolytic activity of enzyme was defined as the amount of enzyme that delivers 1 µg of tyrosine (Tyr) and causes a 0.01 increase in absorbance at 280 nm through 1 cm of cuvette path length. Rennet from calf stomach (mixture of chymosin and pepsin) from Sigma-Aldrich and 5% NaCl were used as positive and negative controls, respectively.

2.5. Protein Determination

The protein concentration was determined according to Lowry et al. [37]. A standard curve was generated using bovine serum albumin (10–500 µg/mL) as the standard. Alkaline copper sulphate reagent was added to the different dilutions and sample solutions, which were incubated at room temperature for 10 min. Then, Folin & Ciocalteu's reagent (commercially available, F9252 from

Sigma-Aldrich) was added to each tube and incubated for 30 min. The mixture absorbance was measured using a spectrophotometer (600 nm, Hach DR 5000, USA Hach, Loveland, CO, United States.

2.6. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The crude extract from *M. oleifera* seed was characterized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [38]. The crude extract was first boiled for 5 min in the presence of SDS and β-mercaptoethanol and 50 µg/mL of protein concentration was loaded into the gel (5% of stacking gel and 12% of separating gel). SDS-PAGE was run at 120 volts (Miniproteam II cell, Bio-Rad, Hercules, CA, USA) until the bromophenol blue dye marker disappeared from the separating gel. Proteins were stained with Coomassie blue R250 and washed with methanol/acetic acid/water (40:50:10) solution to remove the dye excess. A low molecular weight marker was used in a range of 20 to 110 KDa as the standard (Bio-Rad).

2.7. Cheese Elaboration

Cheesemaking was carried out by preheating portions of 1000 mL of skim and soy milks at 60 °C. Then, 5 mL of $CaCl_2$ (2 M, pH 6.5) was added only to skim milk, and 50 mL of seed crude extract (10 g/100 mL of milk) or renin (0.14 mg/mL) were incorporated into the mixture. After, both milks were incubated for 60 min at 50 °C, the curd was cut and stirred at 150 rpm in an orbital shaker, and then the whey were drained. Finally, the curds were placed in round-bottomed containers and cheeses were stored in polyethylene bags at 4 °C.

2.8. Statistical Analysis

The data of three independent experiments were collected and statistically analyzed using one-way analysis of variance (one-way ANOVA), followed by Tukey's honestly significant difference (HSD). Probability $p < 0.05$ indicated statistically significant differences.

3. Results and Discussion

Plant enzymes are widely studied as potential coagulants in cheese production, for example, extracts of *Cynara scolymus* L. [39], *Albizia lebbeck* [40], *Centaurea calcitrapa* [41], the latex of *Sideroxylon obtusifolium* [42], the flowers of *Silybum marianum* [43], *Cynara scolymus* [44], and *Jacaratia corumbensis* O. Kuntze [45]. Ginger rhizome has been used as a source of milk coagulating clotting cysteine protease [46]. However, *Cynara cardunculus* L. extract is particularly popular due to its traditional use in elaborating artisanal sheep milk cheese [25]. In contrast, Mexican plants with milk-clotting activity have been scarcely studied. It has been reported that *Solanum elaeagnifolium* berry extract can be suitable for soft cheese manufacturing, for example for cream cheeses [47]. Therefore, generating knowledge and expanding the field of Mexican natural coagulants is of great importance.

3.1. Initial Analysis of Coagulant Activity in Different Parts of the M. oleifera

Previous studies have described milk clotting activity in different *M. oleifera* tissues [33,34]. To gain a more complete picture of the tissue-level localization of milk clotting activity in *M. oleifera*, we prepared extracts from seeds, flowers, and leaves. As summarized in Figure 1, the coagulant activity of each extract was studied using whole cow's milk as the substrate. Commercial rennet (tube 1) and 5% of NaCl (tube 2) were used as positive and negative controls, respectively. Seed extract showed high clotting activity (tube 3), while milk clotting activity was only slightly detected for flower extract (tube 4) and was absent for leaf extract (tube 5). The total milk clotting activity of the seed extract was 3419 SU/mL, which corresponds to 50% of the total activity obtained with calf rennet, however, in terms of specific activity only represents a 30% decrease (Table 1). These data are consistent with those reported by Talajsir et al. [34] for seeds only, but were not similar for other tissue extracts (leaves and flowers). The activity with flower extract was 13.66 SU/mL, which agrees with the activity reported by

Pontual et al. [33]. It is noteworthy that milk clotting activity of seed extract does not seem to depend on the milk type (whole or skim milks) and along with a previous background where similar activity values were reported on skim milk, our *M. oleifera* seed extract has approximately 8 times more activity. Therefore, we chose seed extract for the following experiments.

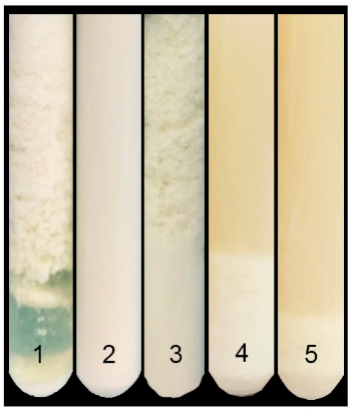

Figure 1. Test tubes of curd formation using *M. oleifera* crude extracts on whole milk. 1: Calf rennet (positive control); 2: NaCl 5% (negative control); 3: Seed extract; 4: Flower extract; 5: Leaf extract.

Table 1. Values of whole milk-clotting activity from the different parts of *M. oleifera*.

Crude Extract	Protein Concentration (mg/mL)	Total MCA (SU/mL)	Specific Activity (SU/mg Protein)
Seeds	10.30 ± 0.45	3419.26 ± 186.80	331.96
Flowers	10.62 ± 0.77	13.66 ± 1.12 **	0.77
Leaves	28.43 ± 3.04 *	ND	ND
Calf rennet	14.10 ± 0.95	6060.6 ± 0.71 *	429.82

The values are expressed as mean ± standard deviation. ND: None detected. MCA: milk-clotting activity. SU: Soxhlet unit. Asterisks represent statistical significance (three separate experiments) based on variance (one-way ANOVA), followed by Tukey's honestly significant difference (HSD) (* $p < 0.05$ vs. Seeds and Flowers; ** $p < 0.05$ vs. Seeds).

3.2. Electrophoretic Pattern of Moringa oleifera Extracts

Electrophoretic patterns of each extract were evaluated to determine the molecular weight of proteins with greater abundance that possibly participate in milk coagulation. The results of analysis by SDS-PAGE of *M. oleifera* extracts are shown in Figure 2. A comparison of protein content of different crude extracts shows the variable protein levels in leaves, flowers, and seeds. Both leaves' and flowers' protein patterns showed some discrete bands. On the other hand, predominant bands were found in seed crude extract. From molecular marker and sample protein, an electrophoretical mobility plot was made in order to determine the molecular weight of seed extract proteins. There were four main bands (a to d) and their apparent molecular mass were 43.6, 32.2, 19.4, and 16.3 kDa. Some bands observed in the extract had similar molecular weights as reported for calf rennet (mixture of chymosin and pepsin) and exhibited one prominent band of 48 kDa, suggesting that *M. oleifera* seeds may possess one or more enzymes with rennet-like activity. However, more studies on the structural characterization of these proteins are needed since lectin, an acid protein with 30 kDa, from *M. oleifera* seeds has coagulant properties which are mainly used to reduce water turbidity [48,49].

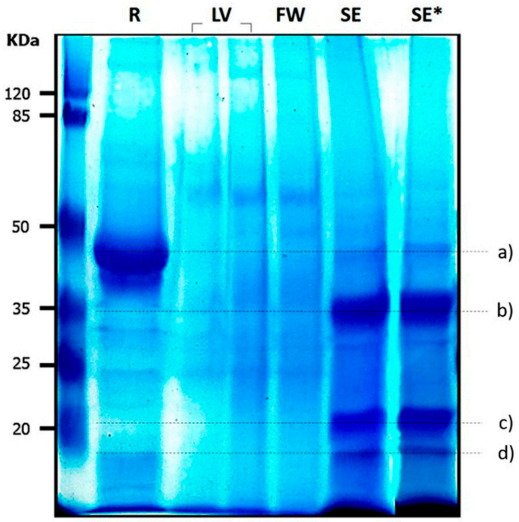

Figure 2. Electrophoretic pattern of *Moringa oleifera* crude extracts. R: Rennet, LV: Leaves, FW: flowers, SE: Seed extract, SE*: Seed extract macerated with ultrasonic bath. (**a**–**d**): Proteins of interest. Data are representative of three experiments.

3.3. Effect of Substrate and Enzyme Concentration on Skim Milk Clotting Activity

Measuring enzyme and substrate concentration, in terms of observed activity, is a key task to determine the quality of the milk coagulation process. It is well known that milk sources and enzyme types significantly affect the cheese yield and curd formation time [50]. Therefore, it is necessary to explore the effect of these parameters to control the hydrolysis of casein by *M. oleifera* seed extract.

In order to evaluate the effect of substrate concentration on milk-clotting activity, skim milk varied from 10 to 90 g/L. The use of this range of substrate concentrations allowed an accurate determination of time at the onset of lump formation. However, it is important to mention that it was almost impossible to measure the time required for coagulation when a low concentration of substrate was used (<10 g/L) because the concentration of 20 mg/mL of enzyme (seed extract) caused an instant coagulation; on the other hand, when a higher concentration of substrate was used, there was a marked increase in milk coagulation time. This is probably due to the increase in the viscosity of the reaction mixture. As shown in Figure 3, the milk-clotting activity decreased as substrate concentration was increased in a directly proportional manner until the concentration of substrate reached about 90 g/L. In addition, according to the clotting time present in these experiments, it was observed that 10 g/L of substrate, coagulated at 35 °C and pH 6.5 with $CaCl_2$, presented a clotting time of 5.47 ± 0.33 s. The reaction mixture with 90 g/L of substrate had a clotting time of 35.74 ± 10.04 s, which is 6.5-fold higher. Thus, our results revealed that the optimum substrate concentration was 10 g/L, suggesting that a high concentration of skim milk has an adverse effect over the catalytic efficiency. These results do not agree with those reported by Ahmed and Helmy [51] for *Aloe variegate* and *Bacillus licheniformis* 5A5 milk-clotting enzymes, who report that increasing skim milk concentration caused a significant increase in MCA up to 60 g/L for both enzymes. However, Wahba et al. [52] reported that substrate concentration over 60 to 210 g/L increased the clotting time. In addition, a study with *Mucor pusillus* pepsin showed a gradual decrease in milk clotting activity to 36.5% with skim milk concentration reaching the 200 g/L [53]. In any case, such retardation of milk-clotting by seed extract may be attributed to several factors. There is an insufficient amount of substrate (casein molecules) due to dilution which restricts the ability of the enzyme to act at its full capacity [54]. The increased viscosity of the solution at higher concentrations of milk diminishes the enzyme activity or it may be due to the smaller amount of hydrolyzed κ-casein when coagulation is initiated; both facts have been stated by Dalgleish [55] and Low et al. [56].

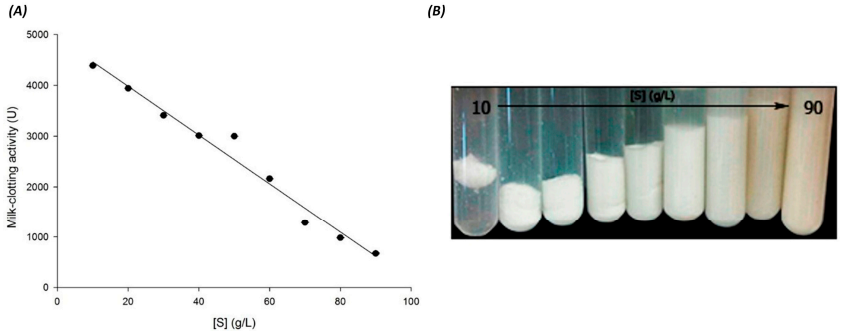

Figure 3. Substrate concentration effect on skimmed milk-clotting activity of seed extract. (**A**): Left plot was drawn using the data obtained from the milk-clotting activity assay. (**B**): Right image shows the curd formation in test tubes. One unit of milk-clotting activity (MCA) is defined as the amount of enzyme to clot 1 mL of a solution containing 0.1 g skim milk powder in 40 min at 35 °C. Substrate concentration is expressed as [S]. All experiments were performed in triplicate, and each data point represents the means of at least three determinations. The regression line equation is ($y = -48.03x + 4941.39$) with a correlation coefficient of $R = 0.99$.

The influence of enzyme concentration is an important parameter that affects cheese quality and yield. The effect of enzyme concentration on the milk coagulant activity of seed extract is shown in Figure 4. At a lower concentration of enzyme (<20 mg/mL) in 10 mL of the skim milk, the seed extract did not show any sign of coagulation for two or more hours at 35 °C. The relative activity increased linearly from 20 to 90 g/mL, suggesting that the maximum milk-clotting activity is reached when the enzyme concentration is up to 90 mg/mL. From the results, it can be deduced that a proportional relationship between the enzyme concentration and milk clotting activity exists. However, greatly diluted enzyme did not have enough activity to coagulate the skim milk. These results agree with those reported by other authors, who have mentioned that the clotting time decreased as the concentration of enzyme increased [51,57,58]. Lopez et al. [59] and Najera et al. [58] attributed this phenomena to a higher level of κ-casein proteolysis. On the other hand, at lower concentrations of enzyme, the activity decreased due to the insufficient amount of enzyme to clot the milk. It is noteworthy that milk coagulation by rennet combines an initial enzymatic hydrolysis reaction and a subsequent enzyme-independent protein aggregation reaction [60].

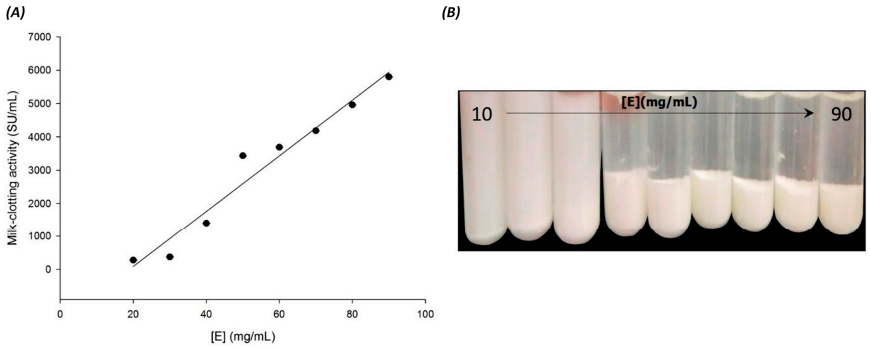

Figure 4. Enzyme concentration effect on skimmed milk-clotting activity of seed extract. (**A**): Left plot was drawn using the data obtained from the milk-clotting activity assay. (**B**): Right image shows the curd formation in test tubes. One unit of milk-clotting activity (MCA) is defined as the amount of enzyme to clot 1 mL of a solution containing 0.1 g skim milk powder in 40 min at 35 °C. Enzyme concentration is expressed as [E]. All experiments were performed in triplicate, and each data point represents the means of at least three determinations. The regression line equation is ($y = 83.55x + (-1582.85)$) with a correlation coefficient of $R = 0.98$.

3.4. Effect of Enzyme Concentration on Caseinolytic Activity

The dairy industry characterizes rennet enzyme using two parameters. The first is the milk-clotting activity (MCA) expressed in International Milk-clotting units, determined by a standard method [48] that describes the ability to aggregate micelles by cleaving the Phe105-Met106 bond or a nearby bond of κ-casein. The second property is the general proteolytic activity (PA), which is the ability to cleave any bond of casein [61]. The MCA/PA ratio captures the essential quality of a milk-clotting enzyme to cheese's elaboration.

Figure 5 shows the effect of seed extract concentration (enzyme) in the assays from 10 to 50 (mg/mL) on the rate of caseinolytic activity. The proteolytic activity was measured by the casein digestion method. It can be observed that the PA is dependent of the enzyme concentration. This result suggests that seed extract has an excellent catalytic property. In addition, the MCA/PA ratio (2995:1) for seed extract was observed, and it was much higher than that reported in most previous studies [33,34]. The high quality of the enzyme in *M. oleifera* seed extract seems to be a promising asset for industrial purposes.

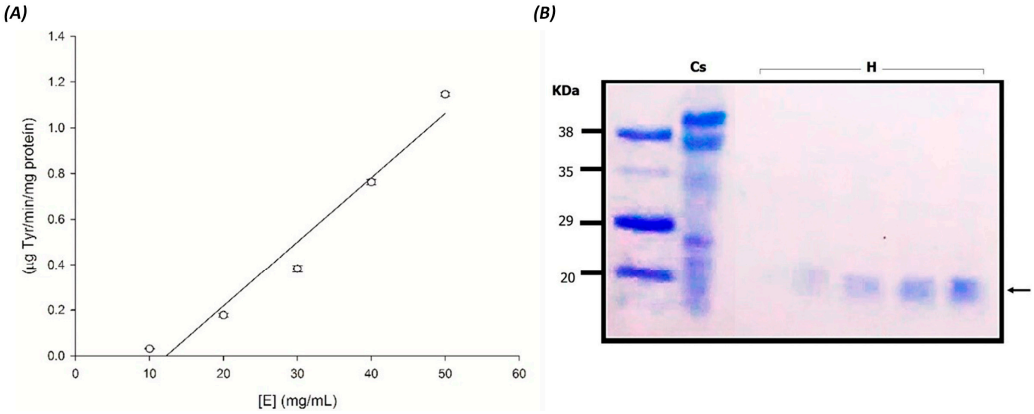

Figure 5. Effect of enzyme (seed extract) concentration on caseinolytic activity. (**A**): Left plot was drawn using the data obtained from the casein hydrolysates with different enzyme concentrations ([E]: 10, 20, 30, 40, and 50 mg/mL). (**B**): Right image shows SDS-PAGE of Cs: 1% Casein; and H: casein hydrolysates. All experiments were performed in triplicate.

3.5. Composition of Soy Milk

Many methods have been developed to measure the activity of milk clotting enzymes. Most are based on the time necessary to coagulate a casein-based substrate. These methods measure the enzymatic and non-enzymatic reactions in milk coagulation. The work of Zhao et al. [62], based on the Arima et al. [35] study, determined MCA, and the results were expressed in Soxhlet units. However, it is not clear if these methods are also regularly used to measure the coagulant activity of plant extracts with other types of milk such as soymilk. Therefore, we decided to assess if the constituents of different soy milks, such as protein and fat content, fiber, carbohydrates, and some minerals, may influence the milk clotting process and, consequently, the finished cheese.

Soy milk is a highly diverse fluid consisting of a vast number of substances, the main ones being soluble carbohydrates (sucrose, raffinose, stachyose, others), protein, fiber, minerals, and fat [63]. Soy milk has approximately 20% lipids but this concentration varies among regions. Soybeans harvested in the United States have more lipids than those from China. Thus, the actual composition of soy milk depends on many factors, including varieties, growing season, geographic location, environmental conditions, and methods of making soy milk (nama-shidori and kanetsu-shibori) [64].

Soy milk is an emulsion composed of soy protein, mainly glycinin and β-conglycinin which represent 60% of total soy milk protein, and lipids, mainly triacylglyceride composed of linoleic acid,

oleic acid, and phospholipids. In addition, phytic acid, minerals, and oligosaccharides are present in the soluble component of the emulsion and when some bivalent ions such as calcium and magnesium are added to soy milk in order to elaborate tofu, the ions combine with phytic acid [65]. As a result, the pH decreases and the protein immobilizes, forming a large body constructed with proteins, lipids, and minerals [66].

The chemical compositions of the different types of soy milk used in this study are presented in Table 2. From the results, all types of soy milk were mainly composed of protein and fat. However, a lower percentage of nutritional composition was found for Soyalac. To elaborate firm tofu, the average ratio of fat-protein is 0.55 to 1. The ratios for the different soy milks AdeS, Soyalac, and Soyapac were 0.72, 0.5, and 0.9, respectively due the protein content variations in commercial soy milk ranging from 2.0 to 6.6 g, while total fat content also varied from 1.0 to 4.5 g. These data suggest that Soyalac maintains the correct ratio to produce a cheese like firm tofu. However, the process of soy milk manufacturing might cause chemical changes, which leads to soy protein degradation. Therefore, it is known that the physicochemical properties of soy milk play an important role in tofu making. In addition, if the MCA of *M. oleifera* seed extract accepts different substrates, the question arises as to which soy milk should be used for the MCA assay. Thus, we decided to assess the effect of substrate concentration on the coagulant activity with different soy milks.

Table 2. Nutritional composition of different soymilk (per 1 cup).

Commercial Milk Type	Fat (g)	Fiber (g)	Protein (g)	CHO * (g)	Ratio $^{+}$	Ca	Fe	P	Zn	Calories (Kcal)
[50]	4.67	3.18	6.73	4.43	0.69	9.8 mg	1.4 mg	120.05 mg	NR	79
AdeS	4.5	3.0	6.2	10.8	0.72	27%	9%	NR	22%	109
Soyalac	1.0	2.0	2.0	10.0	0.50	19%	21%	NR	NR	60
Soyapac	6.0	0	6.6	9.1	0.90	25%	13%	28%	25%	117

* Carbohydrates; $^{+}$ the fat to protein ratio; NR, not reported. Nutritional facts were taken from the package labels of each type of milk.

3.6. Effect of Substrate (Soy Milk Types) on Milk Clotting Activity

Several factors affect the milk clotting activity, among them, different types of substrates and enzyme concentrations which modify the rate of an enzyme-catalyzed reaction. When the enzyme concentration is small, the enzyme can completely combine with the substrate, and the degree of hydrolysis is increased. When more enzyme is added, its amount is higher than that of the substrate, resulting in a relatively small substrate concentration. Then, some amount of enzyme cannot combine with its substrate, leading to a modification in the enzymatic activity.

Soy milk composition is variable, depending mainly of soybean varieties and processing methods [64]. Substrate specificity was determined using three different commercial soy milks (Figure 6). A high level of MCA was shown by substrate 1 (23,500 SU/mL) at 20 g/L and a moderated and poor MCA was revealed by substrates 2 and 3, respectively (Figure 6). Interestingly, seed extract activity is approximately 5 times higher using substrate 1 than using substrate 2. It is worth mentioning that when substrate 3 was used at low concentration (20 g/L), seed enzyme was not suitable to perform its activity. However, only at higher concentration (100 g/L of substrate 3), a good milk clotting activity of 6100 SU/mL was obtained, and it was even better than that obtained with skim milk at the same concentration (Figure 3). The differences between activities from all types of soy milk might be due to the difference in its physicochemical composition, mainly fats, proteins, and total solids in each kind of milk. Thus, seed extract possesses an exceptionally abundant and diverse specificity. Therefore, these results indicate that the milk clotting enzyme from *M. oleifera* seeds has a broad catalytic spectrum and suggests its usefulness for different applications in the dairy industry. Interestingly, our results indicated that Soyalac was the best substrate and hence it was selected for cheese production.

Numerous factors influence the primary and secondary coagulation phases as well as rheological properties to form gels. The most essential factors are protein (substrate) concentration, milk pH value,

milk clotting enzyme type and concentration, calcium concentration, and temperature [59,67]. Earlier reports indicated that bovine milk clotting time is affected by type and protein content of the coagulant. Mehaia [68], for instance, indicated that clotting time of bovine milk can be longer when the concentration ratio of the protein content of the coagulant enzyme is increased because of the increased effectiveness of collisions due to a decreasing aqueous phase. Bruno et al. [69] also reported that a higher dilution of hieroymain fruit extract prolonged bovine milk clotting time. In contrast, a significant decrease in bovine milk clotting time was observed with an increase in the amount of *Solanum macrocarpon* extract [70]. Moreover, it was reported that MCA is increased when crude extract concentration of ginger rhizome is diluted [71].

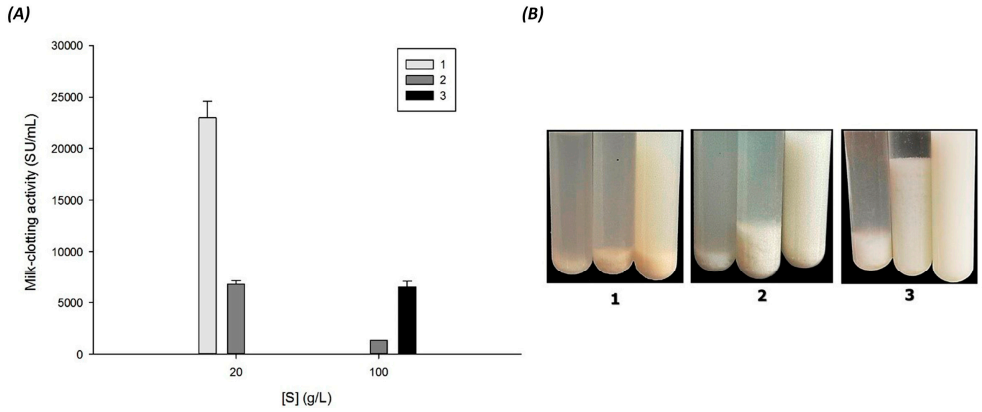

Figure 6. Milk-clotting activity from seed crude extract of *M. oleifera* on different types of commercial soy milk. (**A**): Left plot was drawn using the data obtained from the milk-clotting activity assay with different types of soy milk. (**B**): Right image shows the curd formation in test tubes. Substrate 1: AdeS; Substrate 2: Soyapac; Substrate 3: Soyalac. Asterisks represent statistical significance (three separate experiments) based on variance (one-way ANOVA), followed by HSD ($p < 0.05$ vs. Substrate 2: Soyapac).

3.7. Cheese Processing

Soy milk has been used as a cow milk alternative due to its high amounts of protein, iron, unsaturated fatty acids, and niacin but low amounts of fat, carbohydrates, and calcium compared with those of cow's milk [72]. Various soy cheeses are made in some countries and have attracted increasing attention and have been used as a soft cheese-like product [73–75].

To further confirm the suitability of the *M. oleifera* seed extract as a rennet substitute in cheesemaking, the potential of the milk-clotting enzyme from seed extract was proved. A soft-white cheese was obtained with soymilk (Soyalac) while the cheese made with skim milk was hard and crumbly (Figure 7). The results demonstrated no significant differences in the coagulation time using both types of milk, and cheeses of 3.6 to 6.8 g were obtained.

Liu and Chang [76] reported that tofu texture made from soy milk is affected by several factors including soybean composition, soy milk processing, type and amount of coagulant, and processing methods. Glycinin and β-conglycinin are the major storage proteins (globulins) in soy foods. Glycinin corresponds to the 11S protein, and β-conglycinin is the principal component of the 7S protein. Tezuka et al. [77] reported that higher amounts of coagulant are required for the 7S globulin-rich soy milk than 11S globulin-rich or normal soy milks. The gel hardness depended mainly on glycinin content, hence, if the 11S/7S ratio increases, the hardness of gel is enhanced [78]. In accordance with this background, we assumed that commercial soy milks used in this study presented a low glycinin fraction and an enriched 7S-globulin content. However, additional studies are needed to know the quality and composition of the main protein present in commercial soy milks.

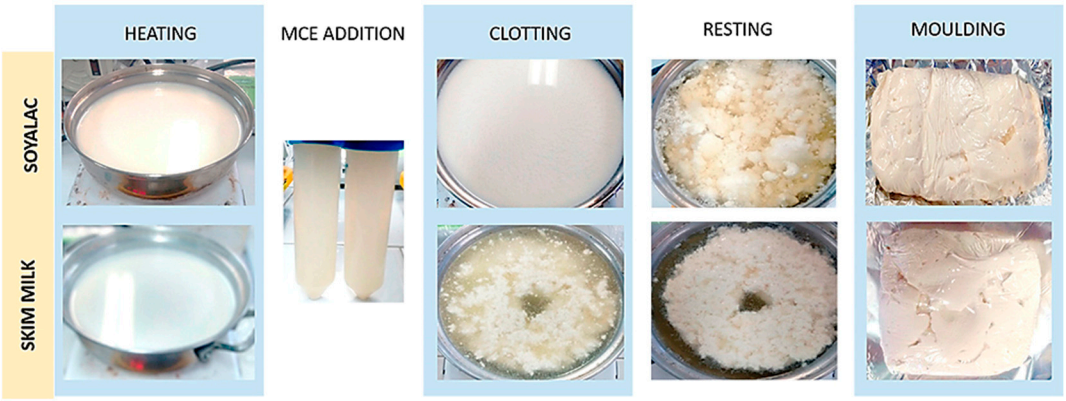

Figure 7. Workflow employed in cheese making using seed crude extract. The upper photos show soy milk cheese's elaboration while the lower photos show skim milk cheese's elaboration, both processed with Moringa seed extract. All experiments were performed in triplicate.

4. Conclusions

Leaf and flower enzymes displayed a negligible value of milk-clotting activity, whereas the seed enzyme demonstrated a high milk-clotting activity on whole, skim, and soy milks. In addition, it would be interesting to consider if other components, such as phytochemicals found in *M. oleifera* seed extract, could have antioxidant effects, which may help to reduce the risk of cardiovascular diseases. With these results it can be inferred that Mexican *M. oleifera* seed extract can be successfully used for different types of cheese manufacture with nutritional benefits, as well as several industrial applications.

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Conflicts of Interest: The authors hereby declare that there are no competing interests in this work.

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