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Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*)

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ABSTRACT

The purpose of this work was to evaluate chitosan edible films (CH) incorporated with maqui berry extracts (MB) for (i) the inhibition growth of some bacterial strains (ii) their total phenolic (TPC) and total flavonoid content and (iii) their antioxidant activity to define if the chitosan edible films incorporated with maqui berry extract (CH + MB) could be used as natural active films for food use. The antioxidant activity was determined with three different analytical assays: DPPH radical scavenging ability assay, Ferrous chelating capacity (FIC) and ferric reducing activity power (FRAP). The agar disc diffusion method was used to determine the antibacterial activity against *Listeria innocua*, *Serratia marcescens*, *Aeromonas hydrophila*, *Achromobacter denitrificans*, *Alcaligenes faecalis*, *Pseudomonas fluorescens*, *Citrobacter freundii* and *Shewanella putrefaciens*. CH + MB showed higher antioxidant activity, at all concentrations (0.5 and 1%) and with all methods assayed than CH. Furthermore, the antioxidant activity of CH + MB occurs in a concentration-dependent manner. Regarding the antimicrobial activity, CH + MB were effective against seven of the eight tested bacteria. This antibacterial activity takes place in a concentration-dependent manner. CH were not active, against six of the eight bacteria strain tested. CH + MB could improve the quality of foods due to delay the oxidation and the microbial growth.

1. Introduction

At present, there is a significant interest in edible and biodegradable films made from natural polymers due to the severe environmental impact caused by non-biodegradable plastic material residues. The edible or biodegradable films are normally made from compounds present in nature such as proteins, lipids and polysaccharides. However, the major disadvantages of these films are their poor mechanical properties and significantly hydrophilic nature (Shariatinia & Fazli, 2015). On the other hand, the edible films can also be used to carry functional compounds such as preservation agents (antimicrobial or antioxidant compounds), nutrients and nutraceuticals. This practice could tackle challenges faced in food industry improving the shelf life of food and enhances general characteristics of film (Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009).

Chitosan is a linear polysaccharide of randomly distributed β-(1–4)- linked D-glucosamine and N-acetyl-D-glucosamine, is a functional biopolymer obtained from deacetylation of chitin, a biopolymer that is abundant in a variety of crustacean shells, such as crab shells, crawfish shells and shrimp shells (Kim et al., 2011). As a result of its cationic character chitosan has an excellent film-forming ability. This compound is non-toxic, biocompatible, and biodegradable and thus is considered as an environmentally friendly packaging material (Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011). Chitosan films have selective permeability to gases (CO_2 and O_2) and good mechanical properties. In addition, also presenting functional properties such as bacteriostatic and fugistatic (Benhabiles et al., 2012). As mentioned above, besides acting as protective barriers, these films can be used as carriers of bioactive compounds with antioxidant or antibacterial properties. There are several natural ingredients, added to edible films, that present antioxidant or antimicrobial properties such as nisin or

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lysozyme (Pranoto, Rakshit, & Salokhe, 2005), phenolic compounds (Pastor, Sánchez-González, Chiralt, Cháfer, & González-Martínez, 2013), essential oils (Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, & Fernández-López, 2013) or fruit extracts (Rubilar et al., 2013).

Maqui (*Aristotelia chilensis* Mol. Stuntz) is a plant that belongs to Elaeocarpaceae family, which is grown in Chile and Argentina. Its fruits (maqui berry) have been recently reported as one of the healthiest berries in nature, due to its bioactive components (Muñoz et al., 2011; Nakamura, Tanaka, Imada, Shimoda, & Tsubota, 2014). Fruits are usually eaten fresh or used for juice and jams (Escribano-Bailón, Alcalde-Eon, Muñoz, Rivas-Gonzalo, & Santos-Buelga, 2006). In the traditional native herbal medicine, infusions of maqui fruits and leaves have long been used to treat sore throats, kidney pain, digestive ailments (tumours and ulcers), fever, and scarring injuries (Suwalsky, Vargas, Avello, Villena, & Sotomayor, 2008). Many studies, conducted on the biological activities of maqui berry extract have reported, such as antioxidant (Ruiz et al., 2010), antimicrobial (Mølgaard et al., 2011) or antidiabetic effects (Rojo et al., 2012). Another set of data from *in vitro* studies proposes that maqui berry inhibit adipogenesis and inflammation (Schreckinger, Wang, Yousef, Lila, & de Mejia, 2010), and prevent LDL oxidation (Miranda-Rottmann et al., 2002). These biological effects can be attributed to the high content in polyphenolic compounds particularly high concentration of anthocyanins.

Therefore, the purpose of this work was to evaluate chitosan edible films incorporated with maqui berry extracts for (i) the inhibition growth of some bacterial strains (ii) their total phenolic (TPC) and total flavonoid content and (iii) their antioxidant activity by means of three different antioxidant tests to define if the chitosan edible films incorporated with these berry extract could be used as natural active films for coat several foodstuff to delay microbial growth and/or to improve the oxidative stability.

2. Materials and methods

2.1. Plant material

Maqui berry (*A. chilensis*) was collected from the Cañete city in Bio–Bio Region (Chile). The fruit was triturated in the immersion crusher Black and Decker SB400; and then with a mortar, a second fine grinding was performed. The ground sample was lyophilized in 4.5 Labconco Lyophilizer for 72 h. The product obtained was again crushed in a mortar and finally sieved to remove seeds.

2.2. Preparation of edible films

Chitosan-edible films were prepared following the indications of Ojagh, Rezaei, Razavi, and Hosseini (2010) with some modifications. High molecular weight 75–85% deacetylated chitosan (Sigma–Aldrich Chemical Co., Steinheim, Germany) was dissolved in a lactic acid aqueous solution (1% v/v) (Sigma–Aldrich Chemical Co.) at a concentration of 2% (w/v). The chitosan solution was stirred, on a magnetic stirrer/hot plate at room temperature, until it was completely dissolved (24 h). After filtration, the chitosan solution was returned to the magnetic stirrer/hot plate and glycerol (Panreac Química, Barcelona, Spain) was added to a level of 2.5 mL/g chitosan as a plasticizer and mixed for 30 min. After that, lyophilized maqui berry (MB) was added to chitosan solution to reach a final concentration of 0, 0.5 and 1% (v/w) and mixed for 30 min. Twenty grams of the three film forming solutions obtained (chitosan (CH), CH + MB 0.5% and CH + MB 1%) were casted into 9 cm inner diameter sterile Petri dishes (0.31 g/cm^2) covers and then dried for 48 h at 37 °C. Dried films were peeled and stored in a desiccator at 25 °C and 51% relative humidity until evaluation. Saturated magnesium nitrate (Panreac Química) solution was used to meet required relative humidity.

2.3. Antibacterial activity

2.3.1. Microbial strains

The chitosan films incorporated with lyophilized maqui berry were individually tested against several bacteria: *Listeria innocua* CECT 910, *Serratia marcescens* CECT 854, *Aeromonas hydrophila* CECT 5734, *Achromobacter denitrificans* CECT 449, *Alcaligenes faecalis* CECT 145, *Pseudomonas fluorescens*, *Citrobacter freundii* CECT *4626* and *Shewanella putrefaciens* CECT 5346. These microorganisms were chosen as they are commonly associated with refrigerated foods: as indicator of pathogenic microorganism or as spoilage microorganism. All species were supplied by the Spanish Type Culture Collection (CECT) of the University of Valencia (Spain).

2.3.2. Agar disc diffusion method

The agar disc diffusion method described by Tepe, Daferera, Sokmen, Sokmen, and Polissiou (2005) with some modifications was used to determine the antibacterial capacity of chitosan edible films incorporated with lyophilized maqui. Briefly, a suspension (0.1 mL of 10^6 CFU/mL) of each microorganism was spread on the solid medium plates. Nutrient Agar II (Oxoid, Basingstoke, Hampshire, England) in the case of *S. marcescens*, *S. putrefaciens*, *P. fluorescens*, *A. denitrificans* and *A. hydrophila*; Nutrient Agar I (Oxoid, Basingstoke, Hampshire, England) in the case of *A. faecalis* and *C. freundii*; and Brain Heart Infusion agar (Sharlab, Barcelona, Spain) for *L. innocua*. CH, CH + MB 0.5 and CH + MB 1% edible films, 10 mm in diameter were aseptically obtained and placed on the inoculated plates. The plates were left 15 min at room temperature to allow the diffusion of the maqui extracts, and then they were incubated at appropriated temperature (26–37 °C) for each bacteria during 24 h. At the end of the period, the diameter of the clear zone around the films was measured with a caliper (Wiha dialMax® ESD-Uhrmessschieber, CH) and expressed in millimeters (disk diameter included) as its antibacterial activity. All tests were performed in triplicate.

2.4. Total phenol content

The total phenol content (TPC) was determined using the Folin-Ciocalteu's reagent (Singleton & Rossi, 1965). The results were expressed as mg gallic acid equivalents (GAE)/g sample. Each assay was carried out in triplicate.

2.5. Total flavonoid content

For the total flavonoid content (TFC), the method based on Blasa et al. (2005) was used. The results were expressed in mg rutin equivalents (RE)/g of sample as mean of three replicates.

2.6. Antioxidant activity

2.6.1. Sample preparation

To obtain the extracts, 10 g of each film were placed in a capped centrifuge tube and 50 mL of methanol were added, after which the mixture was homogenized in an Ultra-Turrax (IKA, T25D, Staufen, Germany) during 3 min at 18000 rpm. The tube was then centrifuged (Sigma 3-16 PK, Sartorius, Göttingen, Germany) at 2739 g for 20 min at 4 °C and the supernatant was transferred to a round-bottomed flask. The extracts obtained were stored at −20 °C and measured before 24 h.

2.6.2. DPPH radical scavenging assay

The free radical scavenging activity of the samples was measured according to the methodology described by Brand-Williams, Cuvelier, and Berset (1995) using the stable radical DPPH. Results were expressed in mg Trolox equivalent/g film as mean of three replicates.

2.6.3. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the different films samples was determined by using the potassium ferricyanide-ferric chloride method (Oyaizu, 1986). Results were expressed in mg Trolox equivalent/g film as mean of three replicates.

2.6.4. Ferrous ion-chelating ability assay

Ferrous ions (Fe^{2+}) chelating activity (FIC) was measured by inhibiting the formation of Fe^{2+}-ferrozine complex after treatment of test material with Fe^{2+}, following the method of Carter (1971). Results were expressed in mg EDTA equivalent/g sample as mean of three replicates.

2.7. Statistical

Statistical analysis and comparisons among means were carried out using the using the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL). The data collected, for antioxidant and antibacterial properties using disk diffusion assay, were analyzed by one-way analysis of variance (ANOVA) to test the effects of one fixed factor: maqui berry, with three levels: 0, 0.5 and 1%. The Tukey's post hoc test was applied for comparisons of means, differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Antibacterial activity

Inhibition zone diameters yielded by chitosan edible film disks added with different concentrations (0, 0.5 and 1%) of maqui berry extracts against some bacteria are shown in Table 1. Films containing only chitosan (CH) were effective against two of the eight tested bacteria only *S. putrefaciens* and *P. fluorescens* showed inhibition halos. In the scientific literature there are contradictory opinions about the antibacterial properties of chitosan films. Ojagh et al., (2010) or Wang et al., (2011) reported that no significant inhibition zone was obtained for the pure chitosan film against some Gram-positive or Gram-negative bacteria. Nevertheless, Kim et al., (2011) or Du, Zhao, Dai, and Yang (2009) informed that pure chitosan films had antimicrobial effects on several bacteria. There are two possible explanations for this phenomenon. On the one hand, Zivanovic, Chi, and Draughon (2005) reported that a high number of bacteria may exceed chitosan inhibition activity. In this work, the inoculums were approximately 10^6–10^7 CFU per Petri dish. On the other hand, the antimicrobial activity of CH films could be partially affected by the immobilization of chitosan molecules within the film due to the use of high amount of plasticizer substances.

As regards CH + MB films, this edible film showed an inhibitory effect on 7 of the 8 bacteria assayed which occurs in a concentration-dependent manner ($p < 0.05$). Only against *L. innocua* the films added with maqui berry were no active. Fig. 1 showed the inhibition halos obtained with chitosan films added with maqui berry extracts at 1% against *S. marcescens*, *A. faecalis*, *A. hydrophila*, *P. fluorescens*, *C. freundii* and *A. denitrificans*. At all concentrations assayed *A. denitrificans* showed the higher ($p < 0.05$) inhibition halos with values range between 21.43 and 22.94 mm (including film disc) followed by *S. putrefaciens* and *P. fluorescens* with no statistically differences ($p > 0.05$) between them. On the other hand, *S. marcescens* was the bacteria strain more resistant ($p < 0.05$) with inhibition halos comprise between 14.65 and 17.07 mm (including film disc). This is the first study to analyze the antibacterial activity of edible films incorporated with maqui berry extracts. Nevertheless, the antibacterial activity of edible films of chitosan incorporated with bioactive compounds from several sources has been widely studied (Moradi, Tajik, Rohani, & Oromiehie, 2011; Siripatrawan & Noipha, 2012; Ruiz-Navajas et al., 2013).

The antibacterial properties of chitosan edible films incorporated with maqui berry could be related with the presence, in the edible films, of bioactive compounds such as phenolics acids, flavonoids or anthocyanins coming from the maqui extracts. Major components in maqui berry extracts are anthocyanins. Several anthocyanins found in maqui berry are glycosylated forms of delphinidin and cyanidin, such as delphinidin 3,5-*O*-diglucoside, delphinidin 3-*O*-sambubioside-5-*O*-glucoside or cyaniding-3-*O*-glucoside (Escribano-Bailón et al., 2006) which could be the responsible for the antibacterial properties. Therefore, Leitão, Polizello, Ito, and Spadaro (2005) reported a variable sensitivity of *Staphylococcus aureus*, *Enterococcus faecalis*, and *Micrococcus luteus* to proanthocyanidin-rich fractions obtained from berries. Puupponen-Pimiä et al., (2001) found that the extracts, with high content of anthocyanins, obtained from common Finnish berries (blueberry, raspberry, lingonberry, blackcurrant, cloudberry, cranberry, sea buckthorn berry, and strawberry) inhibited the growth of Gram-negative bacteria, while Gram-positive bacteria were quite resistant. Moreover, the antibacterial activity might be due to the action of only one component. However, it is a more widely held point of view that the action is due to a synergistic effect between various components, whether major or minor ones.

The mechanisms behind the antimicrobial activity of phenolic acids, flavonoids or anthocyanins are not completely known, Burdulis et al. (2009) informed that there are several mechanisms of action in the growth inhibition of bacteria by the anthocyanins such as destabilization of cytoplasmic membrane, permeabilization of plasma membrane, inhibition of extracellular microbial enzymes, direct actions on microbial metabolism and deprivation of the substrates required for microbial growth. Antimicrobial activity

Table 1
Antibacterial effect of chitosan edible films incorporated with maqui berry extracts at different concentrations against Gram-positive or Gram-negative bacteria, by disc diffusion method.

Films	Diameter (mean and SD) of inhibition zone (mm) including film disc (10 mm)							
	L. innocua	*A. faecalis*	*S. marcescens*	*A. hydrophila*	*C. freundii*	*S. putrefaciens*	*P. fluorescens*	*A. denitrificans*
CH	N.A.	N.A.	N.A.	N.A.	N.A.	14.32 ± 0.13^{c}	12.86 ± 0.00^{c}	N.A.
CH + MB 0.5%	N.A.	20.05 ± 0.16^{aB}	14.65 ± 0.86^{bD}	16.82 ± 0.24^{bC}	17.10 ± 0.64^{bC}	20.43 ± 0.89^{bAB}	19.13 ± 0.87^{bB}	21.43 ± 0.33^{bA}
CH + MB 1%	N.A.	21.03 ± 0.19^{aC}	17.07 ± 0.36^{aE}	18.59 ± 0.75^{aD}	18.67 ± 0.00^{aD}	21.89 ± 0.13^{aB}	22.03 ± 0.28^{aB}	22.94 ± 0.10^{aA}

N.A.: Non Active.
For a same bacteria, values followed by the same lower case letter are not significantly different ($p > 0.05$) according to Tukey's Multiple Range Test.
For the same film, values followed by the same upper case letter are not significantly different ($p > 0.05$) according to Tukey's Multiple Range Test.

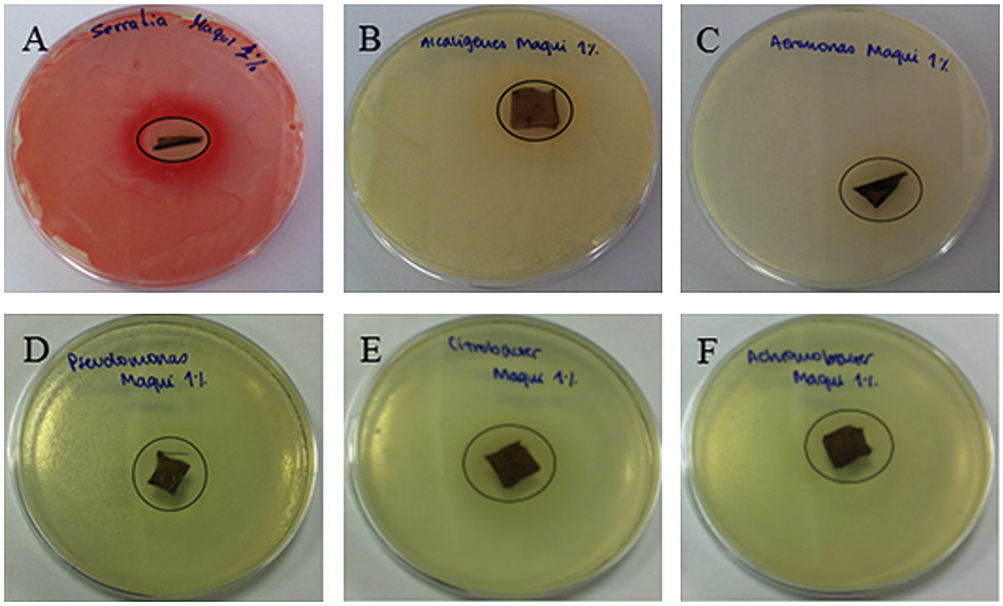

Fig. 1. Inhibition halos obtained with chitosan films added with maqui berry extracts at 1% against A: *Serratia marcescens*, B: *Alcaligenes faecalis*, C: *Aeromonas hydrophila*, D: *Pseudomonas fluorescens*, E: *Citrobacter freundii*, F: *Achromobacter denitrificans*.

of bioactive compounds presents in berries may also be related to anti adherence of bacteria to epithelial cells, which is a prerequisite for colonization and infection of many pathogens. Gene expression studies demonstrated that anthocyanins of cranberry concentrate caused down-regulation of genes encoding outer membrane proteins in *Escherichia coli* O157:H7 (Wu, Qiu, de los Reyes, Lin, & Pan, 2009).

3.2. Total phenol (TPC) and total flavonoid (TFC) content

Phenolic compounds are considered the most antioxidant active metabolites from plants (Bors, Michel, & Stettmaier, 2001). These types of compounds have the ability to donate hydrogen or electrons beyond their capacity to form stable radical intermediates. Therefore, there should be a close correlation between the content of phenolic compounds and antioxidant activity (Pan et al., 2008). Additionally, the phenolic and flavonoid contents can be used as powerful indicators of the antioxidant capacity, which can be used as a preliminary screen for any product when intended as a natural source of antioxidants in functional foods (Viuda-Martos et al., 2011).

Total phenol (TPC) and total flavonoid (TFC) content of chitosan edible films incorporated with maqui berry extracts was shown in Table 2. Chitosan films, without maqui berry extracts added, did not show the presence of phenolic compounds. Nevertheless, there are several scientific works which reported that chitosan films had a low TPC (Moradi et al., 2011; Ruiz-Navajas et al., 2013). This finding might probably be attributed to the formation of chromogens, due to the reaction of Folin-Ciocalteu reagent with non-phenolic reducing substances which can be detected spectrophotometrically (Moradi et al., 2012). After incorporating maqui berry extracts, the TPC of the CH + MB films raised and increased significantly ($p < 0.05$) with the maqui berry extracts concentration. Regarding to TFC (Table 2), again, the Chitosan films did not show the existence of these compounds. However, when the maqui berry extracts were incorporating the TFC of samples (CH + MB) increased ($p < 0.05$) which occurs in a concentration-dependent manner.

Phenolic and flavonoid contents are correlated greatly with antioxidant activity of plant extracts. These constituents are very important from human health point of view mainly due to its free-radical scavenging activity and protection against oxidative stress (Xu & Chang, 2007).

Table 2
Total phenol content (TPC) and Total flavonoid content (TFC) of chitosan edible films incorporated with maqui berry.

	TPC (mg GAE/g)	TFC (mg RE/g)
CH	0.00 ± 0.00	0.00 ± 0.00
CH + MB 0.5%	4.74 ± 0.45[b]	16.39 ± 0.23[b]
CH + MB 1%	8.44 ± 0.14[a]	24.13 ± 0.27[a]

For the same assay, values followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's Multiple Range Test.

3.3. Antioxidant properties

There are huge varieties of bioactive compounds, with antioxidant activity, contained in fruits extracts. Consequently, measuring the antioxidant capacity of each compound alone becomes very difficult. The use of simple "total antioxidant capacity" methods differing in their way of generating free radicals, the strategy to measure the end point of the inhibition reaction, and the sensitivity towards the different reducing molecules in the sample (Roginsky & Lissi, 2005). Therefore, combine more than one method in order to determine *in vitro*, the antioxidant capacity of plant material extracts (Pérez-Jiménez et al., 2008) is necessary. Consequently, the antioxidant properties of chitosan and chitosan added with maqui berry extracts, at different concentrations, edible films were investigated using three diverse methods such as DPPH radical-scavenging assay, FIC assay as well as FRAP assay.

DPPH radical is a stable free radical having an absorption maximum at 517 nm. It has been widely used for measuring the efficiency of several plant extracts as antioxidant agents. The capacity of biological reagent to scavenge the DPPH radical can be expressed as its magnitude of antioxidant capacity. Additionally, compared with other methods, the DPPH assay has many advantages, such as good stability, credible sensitivity, simplicity and feasibility (Deng, Cheng, & Yang, 2011). Chitosan edible films

Table 3
Antioxidant effect of chitosan edible films incorporated with maqui berry at different concentrations by means of three different antioxidant tests such as DPPH, FRAP and FIC assays.

	DPPH (mg TE/g)	FRAP (mg TE/g)	FIC (mg EDTA/g)
CH	0.01 ± 0.00^{c}	0.16 ± 0.02^{c}	107.81 ± 3.71^{a}
CH + MB 0.5%	2.06 ± 0.03^{b}	4.26 ± 0.01^{b}	109.99 ± 4.25^{a}
CH + MB 1%	2.80 ± 0.24^{a}	9.36 ± 0.11^{a}	111.15 ± 3.68^{a}

For a same antioxidant assay, values followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's Multiple Range Test.

(Table 3) had an insignificant antioxidant activity. These results are in accordance with those of Ruiz-Navajas et al., (2013) or Jridi et al., (2014) who reported a slight antioxidant activity of chitosan films, determined with DPPH assay. The scavenging mechanism of chitosan is related to the fact that free radical can react with the residual free amino (NH_2) groups to form stable macromolecule radicals, and the NH_2 groups can form ammonium (NH_3^+) groups by absorbing a hydrogen ion from the solution (Yen, Yang, & Mau, 2008). In the case of chitosan edible films added with maqui berry extracts, the antioxidant activity values ($p < 0.05$) were 2.06 and 2.80 mg TE/g film, for the concentrations of 0.5 and 1%, respectively.

The FRAP assay is usually used to determine the antioxidant potential of plant materials. The antioxidant capacity of the extracts analyzed is determined by the ability of the bioactive compounds presents in these extracts to reduce ferric to ferrous iron (Table 3). Chitosan films showed a little ferric reducing antioxidant activity. The addition of maqui berry extracts onto chitosan films enhanced their antioxidant properties compared to the chitosan films and this enhancement was dependent on the concentration used. Statistical differences were found ($p < 0.05$) between CH + MB 0.5% and CH + MB 1%

Metal ions may catalzse lipid peroxidation that can lead to both free radicals generation and lipid peroxides production (Köksal, Gülçin, Beyza, Sarikaya, & Bursal, 2009). The Fe^{2+} chelating capacity of different chitosan films or chitosan films added with maqui berry extract was shown at Table 3. The results obtained showed that CH + MB films studied were capable of chelating iron (II) and did so in a concentration-dependent manner. However, no statistical differences were found ($p > 0.05$) between chitosan films and chitosan films added with maqui berry. In the same way, there is no differences ($p > 0.05$) between samples added with 0.5 or 1% of maqui berry extracts.

Beyond our knowledge, there are not scientific works where the antioxidant properties of chitosan films added with maqui berry extracts were determined. Nevertheless, the antioxidant properties of maqui berry have been analyzed. Therefore, Cespedes, El-Hafidi, Pavon and Alarcon (2008) analyzed the antioxidant properties of maqui berry extracted with different solvents and determine with diverse methodologies. These authors reported, for inhibition of DPPH radical formation, values of IC_{50} comprised between 1.62 and 20.10 μg/mL or values ranging between 4.81 and 12.97 mmol of Catequin equivalents/gram extract in FRAP assay. In line with this evidence, Ruiz et al., (2010) carried out a study to determined the antioxidant activity of maqui berry collected from several regions of Chile. The authors reported values for antioxidant activity ranging between 69.9 and 100.5 μmol Trolox equivalent/g sample.

As occurs with the antibacterial activity, the antioxidant properties of chitosan films added with maqui berry extracts could be related with the presence, in the edible films, of bioactive compounds such as phenolic acids, flavonoids or anthocyanins coming from the maqui berry extracts. As mentioned above, the main components of maqui berry are the anthocyanins. The antioxidant activity of these compounds is widely demonstrated. However, this activity is greatly dependent on the chemical structure of anthocyanins and not all of them possess similar activities for scavenging diverse radicals (Miguel, 2011). Generally, the antioxidant activity of anthocyanins is associated with the number of free hydroxyls around the pyrone ring. Greater number of hydroxyls greater antioxidant activity. Anthocyanins with their 3′,4′-dihydroxy groups can quickly chelate metal ions to form stable anthocyanin-metal complexes (Sarma, Sreelakshimi, & Sharma, 1997).

In the scientific literature there are several works which reported the relationships between phenolic content and antioxidant activity. In these works, a high correlation between the phenolic content and the antioxidant activity was found (Chirinos, Pedreschi, Rogez, Larondelle, & Campos, 2013; Turumtay et al., 2014). In this work significant correlation between TPC or TFC and antioxidant capacity (FRAP, FIC and DPPH values) of chitosan films added with maqui berry extracts was obtained. Thus, the correlations TPC-DPPH, TPC-FRAP and TPC-FIC were $r = 0.979$; $r = 0.989$ and $r = 0.984$ respectively, whilst the correlations TFC-DPPH, TFC-FRAP and TFC-FIC were $r = 0.998$; $r = 0.964$ and $r = 0.959$ respectively. In the same way, in the present work, the correlations for the DPPH-FRAP, DPPH-FIC and FRAP-FIC methods were $r = 0.946$; $r = 0.944$ and $r = 0.982$, respectively, indicating that chitosan films added with maqui berry extracts has comparable activity in all determinations. However, it should be borne in mind that the antioxidant activity of fruit extracts is not the result of phenolic compounds alone. Other constituents, such as ascorbates, reducing carbohydrates, tocopherols, carotenoids, terpenes, and pigments might contribute to the total antioxidant activity (Babbar, Oberoi, Uppal, & Patil, 2011).

4. Conclusion

The results of this study clearly demonstrated that chitosan edible films added with maqui berry extracts showed a significant antioxidant and antibacterial properties. These properties can be attributed to the bioactive compounds such as phenolic acids, flavonoids or anthocyanins coming from the maqui berry extracts. The incorporation of maqui berry extracts to chitosan edible films may have supplementary applications in food packaging to delay microbial growth and to improve the oxidative stability of foodstuffs.

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