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Microencapsulation of maqui (*Aristotelia chilensis* [Molina] Stuntz) leaf extracts to preserve and control antioxidant properties

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Microencapsulation technology is an alternative to stabilize stress factors and protect food ingredients or additives, which include environmentally sensitive bioactive principles in protective matrices to increase their functionality and life span. The objective of this research was to study conditions to obtain microcapsules with antioxidant capacity from a maqui (*Aristotelia chilensis* [Molina] Stuntz, Elaeocarpaceae) leaf extract by emulsification and subsequent retention after microencapsulation. Microcapsules were produced by water-in-oil emulsion (W/O) using a phase of the aqueous maqui leaf extract and gum arabic, and a liquid vaseline phase. Maqui leaf extract antioxidant capacity was 99.66% compared with the aqueous phase of the emulsion at 94.38 and 93.06% for 5% and 15% gum arabic, respectively. The mean yield of maqui leaf extract microencapsulation with 5% gum arabic varied between 38 and 48%, whereas with 15% gum arabic it was 39%. Once the antioxidant microcapsules were formed, mean extract antioxidant capacity ranged between 30 and 35%. Both yields responded similarly to changes in gum arabic concentrations (5% and 15%) in the aqueous phase of the emulsion; 5% concentration produced a microcapsule size from 1.0 to 10 µm. Maqui leaf extracts with high phenolic compound levels, which can be stabilized and protected by the microencapsulation process, produce new natural preservative systems as compared with their synthetic counterparts.

Key words: Maqui, antioxidant capacity, bioactive compounds, gum arabic.

INTRODUCTION

The agro-food sector has expanded Chile's economy through berry production and this could be relevant in world markets not so much because of volume but for quality attributes, opportunity to innovate, and added value. Commercial and scientific interest to produce functional ingredients leads to the development of production, extraction, and purification technologies compatible with the technical and economic application of the product (Onwulata, 2012). However, to transfer the production of these products to the market it is essential to conserve and stabilize their active principles (Parra, 2010; Pasin et al., 2012). Microencapsulation is an alternative technology to stabilize compounds of interest, such as bioactive principles and food ingredients or additives. These compounds are preserved by protective matrices allowing them to be added to foods and increase their life span and functionality (Banjare and Ghillare, 2012).

Current consumer demands are primarily directed to healthy foods with no synthetic preservatives that provide benefits to the physiological functions of the human organism (Manach et al., 2005; Ah-Hen et al., 2012). An alternative to these demands is to include natural active agents such as antioxidants and antimicrobials that not only protect the manufacture of products but also exercise a beneficial effect on human health (Burris et al., 2012). New natural compounds with antioxidant and/or antimicrobial capacity and how to protect them have promoted interest. Phenolic compounds are the most sought by both agro-industry and consumers for their antioxidant and antimicrobial properties that neutralize the action of free radicals, avoid or retard lipid peroxidation processes, and cell damage (Delporte, 2007; Céspedes et al., 2010). It has been estimated that 2% of the oxygen consumed by a normal organism contributes to the formation of reactive oxygen species (ROS) of which various are free radicals. When ROS generation surpasses the organism's antioxidant defenses, independently of the mechanism (UV radiation, environmental pollution, strenuous physical activity, or other), biological structures suffer damage caused by chemical lesions in an oxidative stress process which is involved in developing

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degenerative pathologies and low plasmatic antioxidant concentrations (Seeram, 2008).

Among the usual sources of antioxidants are red fruits and berries rich in phenolic compounds, mainly flavonoids characterized by their anti-carcinogenic, anti-inflammatory, antiatherogenic, antimicrobial (Howell et al., 2005; Nohynek et al., 2006) and antioxidant activity.

Aristotelia chilensis ([Molina] Stuntz, Elaecarpaceae) or maqui is a plant native to the south of Chile well known for its fruit. The native peoples use its leaves as an infusion to treat diarrhea, tonsillitis, pharyngitis, mouth sores, and as an analgesic and febrifuge. This use is supported by phytochemical findings that describe the presence of polyphenols, such as flavonoids in its leaves. There is an interest today for natural and native maqui products exhibiting high levels of phenolic compounds with antioxidant and antimicrobial capacity (Delporte, 2007; Avello et al., 2009; Céspedes et al., 2010). They can conserve these properties over time through the encapsulation process (Cartes et al., 2009) and be included as active ingredients in products produced in the agro-food chain.

The objective of this research was to study conditions to obtain microcapsules with antioxidant capacity from a maqui leaf extract by emulsification and subsequent retention after microencapsulation.

MATERIALS AND METHODS

Phenolic characterization and extraction

Maqui leaves were collected in fields of the Universidad de Concepción, Biobío Region, Chile, in April 2007 and dried at 35 °C. Sieved (EasySieve Retsch, Haan, Germany) leaf powder samples (Ultra Centrifugal Mill ZM 200 Retsch, Haan, Germany) of 50 g were macerated in a mortar and pestle at room temperature by 30 min with an ethanol and water solution (40% v/v) in a 6:1 solvent-solid ratio. The extract was concentrated in a rotary evaporator at 35 °C and lyophilized for 24 h.

Total flavonoid content was determined with the method pointed out in Kumazawa et al. (2004) and results expressed as quercetin equivalent. Total polyphenols were obtained by the Folin-Ciocalteu method (Rubilar et al., 2011) and results expressed as gallic acid equivalents (GAE). Total tannins were determined by the Folin-Ciocalteu method (Velioglu et al., 2006). Total alkaloids of the maqui leaf extract were calculated by alkaline titration (Ordóñez et al., 2006), results were expressed as milligrams of hyoscyamine.

Antioxidant capacity and extract stability

Antioxidant capacity was established by two methods; by inhibiting 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Koksal et al., 2011), and by inhibiting the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical (Kuskoski et al., 2005). Both methods use chromogenic compounds (DPPH and ABTS) to capture free radicals produced and prevent oxidation processes. Results were expressed as gallic acid equivalents.

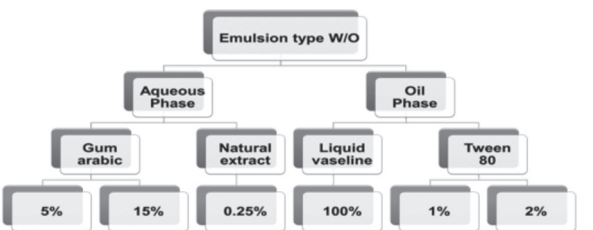
The stability of the maqui leaf extract was determined by quantifying total phenols during 5-mo at 30-d intervals in refrigerated samples.

Identification and quantification of phenolic compounds in the extract

The phenolic components in maqui leaf extract were determined and quantified by high performance liquid chromatography (HPLC) (Series 1050, Hewlett-Packard, Hopkins, Minnesota, USA) with a LiChrospher 100 RP-18 (5 μm, 125-4 mm) column, and elution solvent mixtures A (96% ultrapure water; 3% acetic acid, and 1% acetonitrile) and B (72% ultrapure water; 3% acetic acid, and 25% acetonitrile). The gradient started with 100% A up to 100% B (0-10 min). It was maintained at 100% B for 40 min and then the column was set up at the initial 100% A. The flow was 0.8 mL min^{-1} detected in UV at 280 nm, by absorbance. The standards corresponded to phenolic acids and flavonoids, grade HPLC (Merck, Darmstadt, Germany).

Microencapsulation by extract emulsion

The maqui leaf extract microcapsules were obtained by water-in-oil emulsions (W/O) (Figure 1). The aqueous phase emulsion was prepared by dissolving 5 and 15% gum arabic in distilled water and 0.25% maqui leaf extract in ethanol. The extract was added once the gum arabic was dissolved (10-15 min). The oil phase consisted of liquid vaseline (100%) and 1 and 2% non-ionic surfactant (Tween® 80). Both phases were homogenized in a thermostatic bath at 60 °C and stirred for 5 min. Water-in-oil emulsions were prepared by dissolving 20% aqueous phase with 80% oil phase. The emulsification process was carried out by gradually dispersing aqueous phase into oil phase in a magnetic stirrer agitator at 60 °C for 5 min. They were then mixed in an Ultra-Turrax T18 homogenizer (Fisher Scientific, Waltham, Massachusetts, USA) by increasing the speed from 0 to 10 000 rpm every 20 s for 3 min. Subsequently, emulsions were cooled to room temperature (~ 23 ºC) for later analysis.



W/O: Water-in-oil emulsion.

Figure 1. Aqueous and oil phase concentrations used to obtain microcapsules.

Determination of microcapsule yield, size, and morphology

The overall percentage yield (OY %) was calculated by the ratio of microcapsule mass as a function of the mass of dry solids to encapsulate. The microencapsulation yield (MY %) was calculated from the total microcapsule mass as a function of total prepared emulsion mass.

The microcapsules were directly observed with an optical microscope (Carl Zeiss Standard 25 ICS) with a 100X objective. The diameter was measured on two fields of 20 randomly selected units each to find the mean size.

Internal and external structural characteristics were observed in a scanning electron microscope (SEM, JEOL JSM-6380 LV, Tachikawa, Tokyo, Japan). The microcapsules were dried at room temperature for 1-mo. They were sprinkled onto a 10 × 10 mm sample holder and double-sided adhesive tape was placed on the surface to ensure adhesion. Samples were then covered with gold in a sputter coater (Edwards S150, England) metallizer and observed at 30 kV by SEM at 3700X magnification. Images were taken with a digital camera with the microscope operative system to produce micrographs.

Determination of microcapsule antioxidant capacity

Approximately 1 g of microcapsules was weighed and washed with 10 mL isopropanol-water (1:6) stirred for 5 min in a regulated vortex mixer (ZX3, Velp Scientifica, Usmate, Italy). They were then frozen at -18 °C for 90 min and thawed at room temperature. Samples were stirred in a vortex mixer for 2 min and centrifuged at 6000 rpm for 15 min at room temperature.

The aqueous phase was carefully extracted with syringes after successive washings with isopropanol-water (1:6) to evaluate antioxidant capacity by the ABTS method.

Statistical analysis

ANOVA at a 5% significance level was performed to study the influence of gum arabic concentrations (5 and 15%) in the aqueous phase of the W/O emulsion. Comparison of means was carried out by Duncan's test ($P < 0.05$). Normality was verified according to Modified Shapiro Wilks and homogeneity of variances according to Bartlett. To determine surfactant influence on the antioxidant activity of the extract liberated from the microcapsules, a Kruskal-Wallis non-parametric ANOVA and a Conover (1999) contrast test were performed. The statistical analyses were carried out with the InfoStat Professional version 2008 software (Di Rienzo et al., 2008).

RESULTS AND DISCUSSION

Characterization of maqui leaf extract

The qualitative screening and chemical characterization of maqui leaf ethanolic extract at 1% mostly shows flavonoids at a concentration of 0.061 ± 0.01 mg mL^{-1}, phenolic compounds acting as colorings, antioxidants, and providing flavor to the species that contain them. Tannins and alkaloids were also identified with concentrations of 0.615 ± 0.02 mg mL^{-1} and 1.447 ± 0.01 mg mL^{-1}, respectively. The general phytochemical analysis of *A. chilensis* coincides with Pastene (2009) regarding the presence of flavonoids. These authors point out that flavonoids would be present as heterosides since genins are mostly soluble in non-polar solvents, while tannins have a lower degree of polymerization. Extracts of the Chilean *A. chilensis* species have shown interesting results both for antioxidant action and antibacterial capacity due to the phenolic composition of leaves (Suwalsky et al., 2008; Rubilar et al., 2011).

Flavonols and phenolic components of flavonols found in the analyzed extract coincide with those reported by Céspedes et al. (2010), who determined the presence of ferulic acid, rutin, quercetin, and mirecetin by HPLC in the *A. chilensis* methanolic extract and aqueous extract, as well as isoquercetin and α-catechin in the above fractions.

Quantifying maqui leaf extract phenolic components by HPLC (Table 1) confirmed the preponderance of phenolic acids (54.36%), flavonoids (42.10%), and stilbenes (3.55%). Identifying the components (Figure 2) and their relative quantities (%) in the maqui leaf ethanolic extract showed higher percentages of phenolic

Table 1. Phenolic compounds of maqui (*Aristotelia chilensis*) leaf extract.

Phenolic components	Phenolic compounds	μM	%
Phenolic acids			
	Hydroxybenzoic acid: Gallic acid	399.18	47.55
	Hydroxycinnamic acid: Coumaric acid	57.14	6.81
Flavonoids			
Flavonols	Quercetin	10.92	1.30
	Isoquercetin	2.91	0.35
	Mirecetin	18.95	2.26
	Rutin	15.05	1.79
Anthocyanins	Pelargonidin	121.29	14.45
	Peonidin	1.71	0.20
Flavanols	Catechin	182.59	21.75
Stilbenes	Resveratrol	29.79	3.55

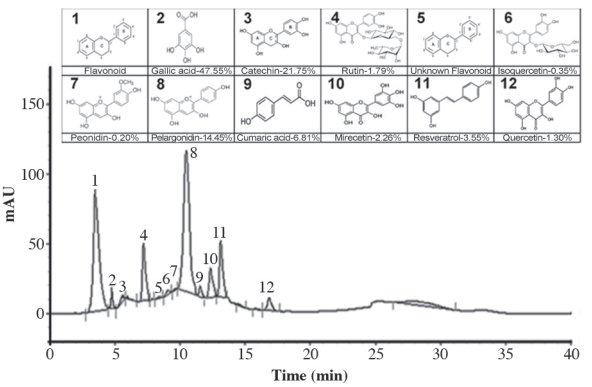


Figure 2. HPLC chromatogram of maqui leaf extract (detection in nm). (1) Flavonoid; (2) gallic acid; (3) catechin; (4) rutin; (5) unknown flavonoid; (6) isoquercetin; (7) peonidin; (8) pelargonidin; (9) coumaric acid; (10) mirecetin; (11) resveratrol; and (12) quercetin.

acids (47.55% gallic acid), flavonols (21.75% catechin), and anthocyanins (14.45% pelargonidin). These have a general structure consisting of two aromatic rings linked by three carbons forming an oxygenated heterocyclic ring substituted with hydroxyl groups whose basic structural formula is made up of anthocyanidins.

Determination of extract antioxidant capacity

Maqui leaf ethanolic extracts exhibited an antioxidant capacity of 158.15 ± 3.36 mM EAG determined by the DPPH method (extract at 0.1%) and 189.50 ± 10.25 mM EAG for extract at 1%; results by the ABTS method were 165.08 ± 7.12 mM EAG (extract at 0.1%) and 165.650 ± 9.94 (extract at 1%). These values are higher than those obtained by Rubilar et al. (2011), who used the DPPH method to obtain values of 47.03 ± 0.1 mM EAG in crude maqui leaf extract with total polyphenols contents of 69.0 ± 0.9 mg EAG g^{-1}. The higher antioxidant capacity found in this research is a result of working with a hydroalcoholic solution (60:40) with a 6:1 solvent-solid ratio, which was previously studied by comparing the quantification of total phenols (40 ± 0.57 mM EAG) in different solvents. Rubilar et al. (2011) also demonstrated the high antioxidant capacity of maqui leaf extract when compared with myrtle leaf extract; polyphenol contents were 32.5 ± 3.1 mg EAG g^{-1} using ethanol-water (50:50) as a solvent and a 5:1 solvent-solid ratio, which confirms the improvement in extraction capacity when the percentage of ethanol is increased. Stability of the extract antioxidant capacity at 1 and 0.1% was 100 and 95% inhibition of the DPPH radical during 5-mo of refrigerated storage. The total polyphenol content of the extract determined by the Folin-Ciocalteu method was 78.5 ± 0.43 mM EAG, which was stable over time and decreased only 10% after 5-mo storage. These results coincide with those reported by Avello et al. (2009), who also obtained high total phenol content (40.00 ± 0.1 mM EAG) in the *A. chilensis* hydroalcoholic extract. Similarly, the study reports 100% inhibition of the DPPH radical and a high antioxidant capacity in the range of 6.0-0.6 μM EAG from the maqui leaf hydroalcoholic extract (60:40) at 1%.

Encapsulation of maqui (*Aristotelia chilensis*) leaf extract

A W/O emulsion preparation was selected since the maqui leaf extract is soluble in water. The aqueous phase was prepared by combining the extract with gum arabic because it exhibits low viscosity, mild flavor, high solubility in water, and good superficial activity.

The oil phase of the emulsion was obtained with liquid vaseline and two concentrations of surfactant (Tween 80) at 1 and 2% with a low hydrophilic-lipophilic balance (HLB 3.3) selected for its ability to form stable W/O emulsions. Tween 80 (HLB 15) was soluble in liquid vaseline (HLB 4). This is consistent with researchers who used hydrocarbons (kerosene, $C_{10}H_{22}$ a $C_{16}H_{34}$) in the oil phase instead of corn oil (Surh et al., 2007). These authors found that edible oils tend to be less hydrophobic and have an active surface with a greater degree of impurities than hydrocarbons.

Emulsion yield

The best microcapsule yields were obtained with emulsions containing 5 and 15% gum arabic. An increase in gum arabic concentration provoked a significant decrease in encapsulation yield since gum arabic viscosity increases when its concentration increases; at 20 °C, a 30% gum arabic solution has a viscosity of 361.5 cP, 16.7 cP at 15%, and only 7.6 cP at 5% (Table 2). Guarda et al. (2011) used gum arabic concentrations of 15 and 30% to obtain microcapsules of active compounds with antimicrobial capacity, for O/W emulsions; this reaffirms that gum arabic viscosity prevented good solubility of maqui leaf extract at concentrations higher than 15%.

Characterization of microcapsules

Table 3 shows the overall and microcapsule yields with the mean droplet size diameter. Yields are within the range reported by Saénz et al. (2009) for phenolic compounds with variations between 20 and 80%.

Size was expressed as the mean diameter value of a total of 30 droplets observed with an optical microscope (Figure 3). Emulsion mean droplet diameter was 2.73 ± 0.37 and 2.91 ± 0.47 μm for microcapsules with 5 and 15% gum arabic, respectively. Size distribution varied in accordance with the percentage of gum arabic. Microcapsules with 5% gum arabic resulted in a bimodal size distribution with two maxima, the first at 2 μm and the second at approximately 5 μm, while microcapsules with 15% gum arabic resulted in a monodisperse size distribution with a maximum of approximately 2 μm. The values are consistent with the range of 0.2-5 µm defined by Mendonça et al. (2009). The droplet size normal distribution is shown in Figure 4. The interval of droplet

Table 2. Gum arabic viscosity as a function of temperature.

	Viscosity concentration				
Temperature	5% GA	10% GA	15% GA	20% GA	30% GA
ºC	cP				
20.0	7.6	12.3	16.7	195.3	361.5
29.5	6.2	10.3	14.6	128.3	238.6
39.2	5.3	8.73	13.7	95.9	159.2
50.0	4.5	7.62	12.2	68.7	108.4
60.0	4.2	6.82	10.3	48.6	76.6

GA = Gum Arabic.

Table 3. Mean size and yield of microcapsules prepared with maqui leaf extracts.

Relationship Gum:Tween 80	Microcapsule yield	Overall yield	Mean size
	%		μm
5:1	37.69 ± 6.71	43.44 ± 8.01	2.93 ± 0.42
5:2	48.05 ± 16.82	55.38 ± 15.52	2.56 ± 0.31
15:1	39.15 ± 3.71	45.13 ± 4.30	2.80 ± 0.73
15:2	39.10 ± 0.64	45.07 ± 0.63	3.36 ± 0.20

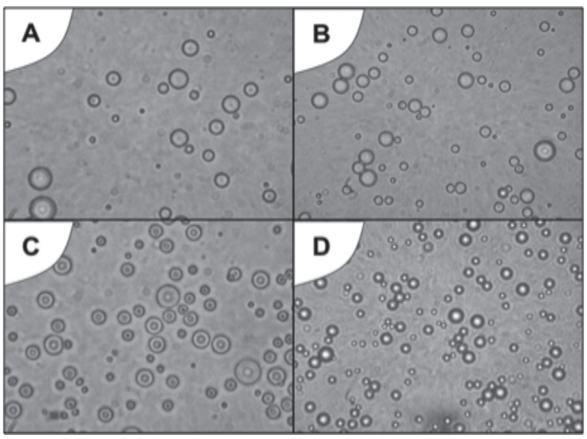


Figure 3. Optical microscopy (100X) of microcapsules prepared in water-in-oil (W/O) emulsion containing 0.25% maqui leaf extract, gum arabic (GA), and Tween 80 (T80). A: 5% GA, 1% T80, B: 5% GA, 2% T80; C: 15% GA, 1% T80; D: 15% GA, 2% T80.

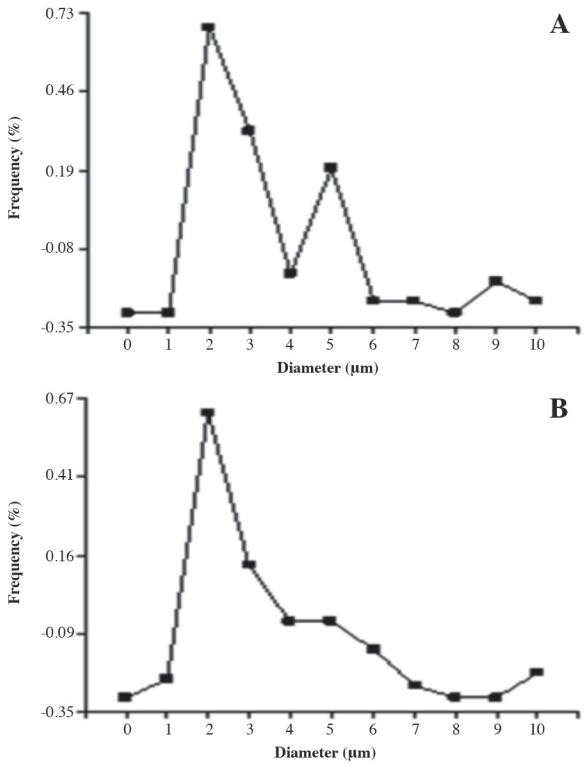


Figure 4. Mean normal distribution of water-in-oil (W/O) emulsion droplet particle size measured by optical microscopy in samples of 5% A and 15% B gum arabic.

size is between 1 and 10 μm with a distribution between 2 and 6 μm. As size decreases, emulsion stability increases against gravitational separation. A creaming process was not observed over time; this suggests that resistant interfacial forces occur, which produce a stable emulsion over time and prevent the coalescence process due to the repulsive forces between the microcapsules for steric stabilization mechanisms (Marcuzzo et al., 2010). The microcapsules diameters were less than 100 μm, which is an advantage to prevent adverse effects on the sensory characteristics when they are incorporated as ingredients.

Morphology of microcapsules with SEM

The morphology of microcapsules was characterized by SEM and indicated that these emulsions contain a microcapsule population forming spherical droplets without any ruptures or pores on the surface (Figure 5), a minimal presence of notches in their surface structure, and a high degree of integrity (McClements, 2012). This is attributable to the method used since results obtained by Saénz et al. (2009) using the spray drying process exhibit a more irregular and notched morphology in its surface because of shrinking that occurs during drying. Figure 5 (1-8) shows the external topography of the microcapsules with 5% and 15% gum arabic. Spherical capsules with different internal compaction can be observed in the oil phase for both surfactant concentrations (1% and 2%). The size distribution is attributable to gum arabic properties, surfactant concentration, and cross-linking of both to form the W/O emulsion. As the surfactant concentration increases, more agglomerates appear making it necessary to dilute them in an aqueous medium to obtain a clearer

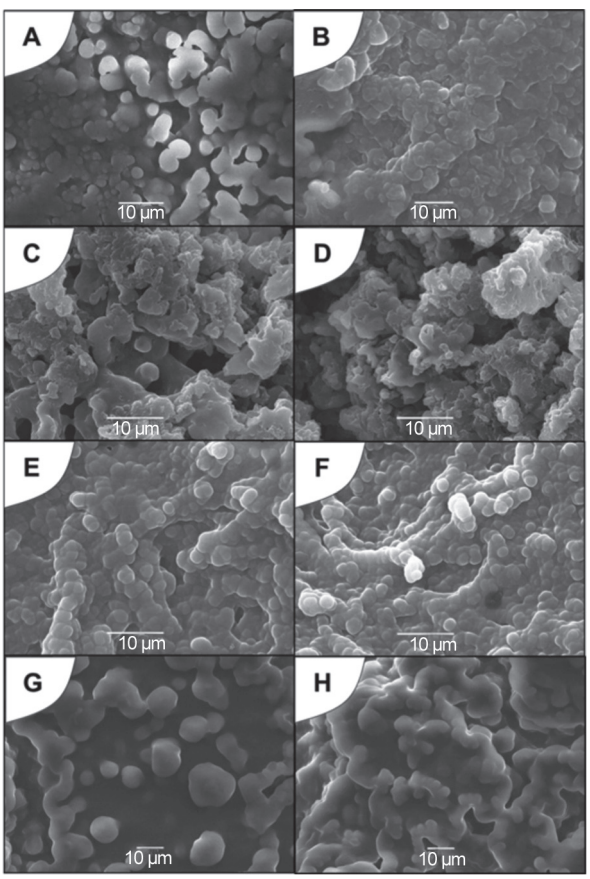


Figure 5. Micrographs (3700X) of microcapsules from water-in-oil (W/O) emulsion with 0.25% maqui leaf extract, gum arabic (GA), and Tween 80 (T80). A-B: 5% GA, 1%T80, C-D: 5% GA, 2%T80; E-F: 15% GA, 1% T80; G-H: 15% GA, 2%T80.

field. This clearly implies a deformation of the droplet. The choice of surfactant concentrations under study is therefore validated.

Study of microencapsulated maqui leaf extract

The maqui leaf ethanolic extract has a protective capacity of 99.66% of the radicals determined by the ABTS method and 94.68% with the DPPH method, which clearly positions the extract as a mixture of compounds with high antioxidant capacity. Incorporating gum arabic also produces a slight decrease in the extract's antioxidant capacity; inhibition in samples with 5% and 15% gum arabic was 94% and 93%, respectively. This would be attributable to the formation of a gum arabic protein complex with the extract, which has been reported by other authors (Su et al., 2008), who point out that the amino acid composition in gum arabic directs the high molecular weight protein fraction strongly adsorbing the oil-water interface. The emulsifying property of gum arabic not only depends on N content but also on the molecular accessibility of the protein component for a rapid adsorption and distribution of the high and low molecular weight fractions of the protein. In this way, the complexation of the maqui leaf extract with the gum arabic microparticles would produce a strong cross-linking effect by forming a three-dimensional network bonded by different polymer chains; this causes reduced mobility of the macromolecular chains and the swelling capacity of the cross-linked microparticles. The interaction of the gum arabic protein fraction after adding the extract would affect the content and type of amino acid, these define the interfacial cross-linking (McClements, 2012).

By comparing treatment means for the percentage antioxidant capacity of the maqui extract with the gum arabic concentration (5% and 15%) and surfactant (1% and 2%) (Table 4), the extract exhibits a high percentage of antioxidant capacity 165.08 mM EAG because it is dissolved in the aqueous phase. The antioxidant capacity of the prepared aqueous phase differs from the liberated microcapsule antioxidant capacity with a decrease from 94% to 30%. This variation involves liberating the active principle from the internal aqueous phase by the external oil phase. Successive washings are carried out in this phase with isopropanol:water, reducing phenolic compound polarity in the initial extract compared with those retained in the microcapsule aqueous phase (Surh et al., 2007).

Current research is oriented to avoiding flavonoid deterioration and finding action mechanisms to prevent its degradation. The proposed microencapsulation technology is an alternative for using, applying, and conserving bioactive agents. The gum arabic concentration under consideration as an encapsulating agent in the aqueous phase produces a protective matrix of the phenolic components of the maqui leaf extract. Surfactant action (Tween 80) in the droplet interface stabilizes the W/O emulsion to obtain microcapsules (Onwulata, 2012).

CONCLUSIONS

Encapsulation technology by water-oil (W/O) emulsion can be applied to maqui leaf extract in a ratio of 20% aqueous phase and 80% oil phase depending on the properties of the component to be encapsulated, microcapsule size, gum arabic composition, and surfactant concentration.

Maqui leaf extract antioxidant activity was 99.66%, whereas results for the aqueous phase of the emulsion were 94.38% and 93.06% for 5% and 15% gum arabic, respectively. This confirms the concentrations of gum arabic under study as encapsulating material to attain the highest antioxidant capacity of the extract in the aqueous phase of the emulsion.

The mean antioxidant activity of the maqui leaf extract microcapsules was 30% compared to 94% in the aqueous phase of the emulsion. This variation is attributable to the loss of polarity of the extract's phenolic components in the aqueous phase after successive washings in an isopropanol-water mixture to remove the external oil phase.

The study of the maqui leaf extract active agent retention in the microcapsules established the influence of the 80% concentration of the external oil phase as a determining factor to measure the antioxidant capacity in the internal aqueous phase of the microcapsule. The results confirm the production of W/O microcapsules as an alternative for new natural preservative systems together with their synthetic counterparts.

ACKNOWLEDGEMENTS

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Table 4. Mean antioxidant capacity of prepared microcapsules compared with maqui leaf extract.

		Viscosity concentration			
Variables	Extract	5% GA/1%T80	5% GA/2%T80	15% GA/1%T80	15% GA/2%T80
ABTS radical inhibition, %	99.66e	30.49a	30.42a	35.62c	31.24b
Antioxidant capacity, mM GAE	165.08d	50.26a	50.07a	59.59c	51.63b

Non-parametric Kruskal Wallis ANOVA. Different letters in the rows indicate differences according to Conover ($P < 0.05$).
GA: Gum arabic; T80: Tween 80; GAE: gallic acid equivalents.

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