Research Article

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Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stuntz] a Chilean blackberry

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Abstract

BACKGROUND: The aim of the present study was to determine (1) the polyphenolic profile (phenolic acids, flavonoids and anthocyanins), (2) the antioxidant using four different methodologies (DPPH, ABTS, FRAP and FIC) and (3) the antibacterial properties of maqui berry [*Aristotelia chilensis* (Molina) Stuntz] (MB) grown in Chile.

RESULTS: The HPLC analysis of MB showed a total of 19 polyphenolic compounds identified as anthocyanins (eight compounds), flavonols (10 compounds) and ellagic acid. Delphinidin derivatives were the predominant anthocyanins while quercetin derivatives were the predominant flavonols. MB showed an antioxidant activity measured with DPPH, ABTS, FRAP and FIC methods of 28.18, 18.66, 25.22 g Trolox equivalent kg^{-1} and 0.12 g ethylenediaminetetraacetic acid equivalent kg^{-1}, respectively. With regard to the antibacterial activity, all strains tested were affected by MB. *Aeromonas hydrophila* and *Listeria innocua* showed the highest sensitivity to maqui berry extracts with MIC values of 40 and a 50 mg mL^{-1}, respectively.

CONCLUSIONS: The results suggest that maqui berry has a great potential to be employed in the food industry as potential food ingredient to functional food development or as bio-preservative.

Keywords: maqui berry; antioxidant activity; antibacterial; polyphenolic profile

INTRODUCTION

The world population, mainly in USA and Europe, typically consumes diets high in meat and saturated fat but low in fruits, vegetables and whole grains. This dietary pattern increases the risk of developing a great number of diseases. There are campaigns encouraging people to take regular physical exercise, to have diets rich in fruits and vegetables and, in general, to take on healthy habits.

It has been known that fruits exert a beneficial impact on human health. Within the fruits, small berries represent a diverse group, including a variety of red, blue or purple, small-sized and highly perishable fruits which are greatly appreciated for their intense colour, delicate texture and unique flavour.[1] These fruits are widely consumed in fresh or in processed forms such as juices, juice concentrates, beverages, jams and jellies.[2] Moreover, several studies recognise the protective effect that the consumption of berry fruits may have against chronic diseases like cancer or cardiovascular disorders, among others.[3,4] The biological effects of berry fruits can be attributed to their rich source of bioactive compounds like phenolic acids, tannins, stilbenes, flavonoids and mainly anthocyanins. Anthocyanins are natural water-soluble pigments which have a deep implication in human health through different pathways.[5] Thereby, population-based investigations revealed an association between anthocyanins and low incidence of cardiovascular disease, diabetes mellitus and cancer.[6–8] Likewise, several studies showed clinical and biomedical indexes enhancement after intakes of fruits, rich in anthocyanins, by volunteers with various health conditions.[9]

The fruit from *Aristotelia chilensis* (Molina) Stuntz (Elaeocarpaceae), commonly known as maqui berry, Chilean blackberry or 'maqui, is a common wild edible berry that grows in central and southern Chile as well as south-western Argentina.[10] In the traditional native herbal medicine, infusions of maqui berries and leaves have long been used to treat sore throats, kidney pain,

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digestive ailments, ulcers, fever, and scarring injuries.[11] The berries, which are about 6 mm in diameter, are extremely rich in anthocyanins. These compounds are responsible of their intense blackish colour and the major contributor to their antioxidant potential, which is one of the highest among known berry fruits of the entire world.[12] Many studies have linked anthocyanins content in maqui berry with its wide biological activities such as anti-diabetic effects,[13] prevention of Alzheimer's disease,[14] inhibition of adipogenesis and inflammation,[10] prevention against oxidative stress[15] and prevention of low-density lipoprotein oxidation.[12]

Whilst there are several works, in the scientific literature, reporting the biological effects and the anthocyanins contents of berry fruits in general and maqui berry in particular, there is lack of information regarding the phenolic acids and flavonoids presents in this fruit. Likewise, there are limited works where the antibacterial properties of maqui berry are determined. Therefore, the aim of the present study was to determine (1) the polyphenolic profile (phenolic acids, flavonoids and anthocyanins), (2) the antioxidant properties and (3) the antibacterial properties of maqui berry grown in Chile.

MATERIALS AND METHODS

Plant material

Two kilograms of maqui berries [*Aristotelia chilensis* (Molina) Stuntz] were collected from the Cañete city (37° 48′ 00″ S, 73° 23′ 00″ W) in the Bio-Bio Region (Chile). The berries were triturated in an immersion crusher (Black and Decker SB400) and then they were sieved to remove seeds. After that, with a mortar, a second fine grinding was performed. The ground sample was lyophilised in a Christ Alpha 2–4 (Christ, Osterode am Harz, Germany) lyophiliser for 72 h and finally the product obtained was, again, crushed in a mortar.

Sample preparation

To obtain the extract, 3 g of ground maqui berry were added to 30 mL of acidified (0.1% HCl) methanol/water (80:20) and then homogenised with an Ultra-Turrax (IKA T-25 homogeniser; IKA. Staufen, Germany) at 18 000 rpm for 2 min. The extract was centrifuged at 3000 × *g* for 7 min at 4 °C and the supernatant was collected. The pellet was mixed with 30 mL of acetone/water (70:30) and homogenised with an Ultra-Turrax at 18 000 rpm for 2 min. Finally, the sample was centrifuged at 3000 × *g* for 7 min at 4 °C. The supernatants of the two phases were mixed in a round bottom flask and evaporated until dryness using a rotary evaporator R-205 (Büchi, Flawil, Switzerland) under reduced pressure (<100 mbar) at 40 °C. For the antioxidant activity and polyphenolic profile, 10 mL of methanol were added to the dried extract, and the mixture was well shaken in a vortex for 2 min, finally it was passed through a 0.45 µm Millipore filter and stored at −20 °C. With regard to antibacterial activity, dried extract of maqui was dissolved in Muller Hinton broth at 100 g L^{-1}.

Total phenol content

The total phenol content (TPC) was determined using Folin–Ciocalteu reagent.[16] The results were expressed as g gallic acid equivalents (GAE) kg^{-1} sample dry weight (DW).

Total flavonoid content

For the total flavonoid content (TFC), the method based on Blasa *et al.*[17] was used. The results were expressed in g rutin equivalents (RE) kg^{-1} of sample DW.

Total anthocyanin content

The assessment of total anthocyanin content (TAC) was carried out by the pH differential method according to AOAC as described by Simirgiotis and Schmeda-Hirschmann.[18] Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5. Pigment concentration is expressed as g cyanidin-3-glucoside equivalents kg^{-1} DW and calculated using the formula:

$$\text{TAC}\ \left(\text{mg}\ \text{g}^{-1}\right) = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times 1}$$

where $A = (A_{510\text{nm}} - A_{700\ \text{nm}})_{\text{pH1}} - (A_{510\text{nm}} - A_{700\ \text{nm}})_{\text{pH4.5}}$; MW (molecular weight) = 449.2 g mol^{-1}; DF is the dilution factor; 1 = cuvette path length, in cm; ε = 26 900 L mol^{-1} cm, the molar extinction coefficient for cyanidin-3-glucoside; and 1000 is the factor to convert grams to milligrams.

Determination of polyphenolic compounds

Maqui berry extracts (20 µL) were injected into a Hewlett-Packard HPLC series 1200 instrument (Woldbronn, Germany) equipped with UV–visible diode array detector. Separations were achieved on a C_{18} Teknokroma column (Mediterranean sea_{18}, 25 × 0.4 cm, 5 µm particle size; Teknokroma, Barcelona, Spain) and the chromatograms were recorded at 280, 360 and 520 nm. Phenolic compounds were analysed, in standard and sample solutions, using a gradient elution at 1 mL min^{-1} with the following gradient program, started with 95% A, 75% A at 20 min, 50% A at 40 min, 20% A at 50 min and 20% A at 60 min. The mobile phases were composed of formic acid in water (4.5:95.5, v/v) as solvent A and acetonitrile as solvent B. The identification of non-anthocyanin compounds was carried out by comparing UV absorption spectra and retention times of each compound with those of pure standards injected under the same conditions. The compounds were quantified through calibration curves of standard compounds (phenolic acid standards: caffeic, ellagic, gallic, and chlorogenic acids; flavonoids standards: rutin, quercetin, myricetin, apigenin, luteolin, quercetin-3-*O*-glusoside and quercetin-3-*O*-galactoside) from Extrasynthese (Genay, France). Phenolic acid standards were dissolved in methanol at different concentrations between 10 and 200 µg mL^{-1}; flavonoids standards were dissolved in methanol at different concentrations between 1 and 250 µg mL^{-1}. High linearity ($r^2 > 0.995$) was achieved for each standard curve for each compound. When reference compounds were unavailable, compounds were tentatively identified by comparing their UV–visible spectra with previously published data.[19–21] Quantification of anthocyanins was carried out based on linear curves of authentic standards. A delphinidin-3-glucoside calibration (concentration between 1 and 250 µg mL^{-1} ; linearity 0.999) was used for the quantisation of delphinidin derivatives, while the cyanidin 3-glucoside calibration (concentration between 1 and 250 µg mL^{-1} ; linearity 0.998) was used for cyanidin derivatives. The estimated concentrations were subsequently multiplied by a respective molecular weight correction factor according to Chandra *et al.*[22]

Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of the samples was measured according to the methodology described by Brand-Williams *et al.*[23] using the stable radical DPPH. Results were expressed as g Trolox equivalent kg^{-1} sample DW.

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the maqui berry extracts was determined by using the potassium ferricyanide–ferric chloride method following the suggestion by Oyaizu.[24] Results were expressed in g Trolox equivalent kg^{-1} sample DW.

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.[25] Results were expressed in g EDTA equivalent kg^{-1} sample DW.

ABTS radical cation scavenging activity assay

The ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity assay was determined as described by Leite *et al.*[26] Absorbance values were measured on a spectrophotometer at 734 nm. The results were calculated based on a calibration curve of Trolox, and results were expressed as g Trolox equivalent kg^{-1} of sample DW.

Antibacterial activity

Microbial strains

The lyophilised maqui berry extract was 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, *Enterobacter gergoviae* CECT 857, *Enterobacter amnigenus* CECT 4078 and *Shewanella putrefaciens* CECT 5346. These microorganisms were chosen because they are commonly associated with refrigerated foods, as indicators of pathogenic microorganisms or as spoilage microorganisms. All species were supplied by the Spanish Type Culture Collection (CECT) of the University of Valencia (Spain).

Minimum inhibitory concentration and minimum bactericidal concentration assays

Antibacterial activity was determined based on a colorimetric broth microdilution method proposed by Abate *et al.*[27] Dried extract of maqui berries were prepared in the concentration range from 10 to 100 g L^{-1} in Muller Hinton broth (MHB) and sterilised by filtration through a 0.22 µm Millipore filter. Briefly, MIC was assayed using sterile 96-well microplates. Each well was filled with a total volume of 250 µL containing ca. 10^6 colony forming units (CFU) mL^{-1} of test bacteria and variable concentrations of the extract prepared in MHB. Negative controls contained non-inoculated medium with extract samples and positive control wells were prepared with inoculated medium without extract samples. Contents of each well were mixed on a plate shaker at 150 rpm for 2 min prior to incubation for 24 h at the optimal temperature depending on the microbial inoculums. After the incubation, 25 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dissolved in DMSO (0.8 g L^{-1}) was added to each well and incubated for 1 h in order to allow the viable microorganism to metabolise the yellow MTT dye into formazan. The minimum inhibitory concentration (MIC) value was considered as the concentration of the first well that did not undergo colour change and was confirmed by plating 10 µL samples from clear wells on Mueller Hinton agar medium. Minimum bactericidal concentrations (MBC) were determined as the lowest concentrations of extract at which microbial growth did not occur, and the initial viability was, in addition, reduced by at least 99.9% within 24 h; it was obtained by inoculation of 50 µL aliquots of negative tubes (absence of colour changes in the MIC determination) on Muller Hinton agar, using the spread plate technique and enumeration after incubation for 24 h at 26 °C for *A. hydrophila*, *A. denitrificans*, *S. marcescens* and *S. putrefaciens* or at 37 °C for *L. innocua*, *A. faecalis*, *E. amnigenus* and *E. gergoviae*.

Statistical assay

Statistical analysis and comparisons among means were carried out using the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL, USA). All experiments were carried out in triplicates and data were reported as mean ± standard deviation. The differences of mean values among polyphenolic profile was analysed by one-way analysis of variance (ANOVA). The Tukey's post hoc test was applied for comparisons of means, differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Total phenol, total flavonoid and total anthocyanin content

Polyphenolic compounds (phenolic acids, flavonoids and anthocyanins) are considered the most important bioactive constituents of plants and fruits. The presence of polyphenolic compounds in food, especially in fruit can be particularly important for consumers, because of their beneficial health properties. In addition, they can be used as an important indicator of several functional properties like antioxidant or antibacterial properties.[28]

The three main groups of polyphenolic compounds in maqui berries are phenolic acids, flavonoids and anthocyanins. Table 1 shows the total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) of maqui berry. The TPC of maqui berries analysed in this work was significantly higher than reported by Fredes *et al.*[20] in different maqui genotypes belonging to four geographical areas in Chile which values ranged between 11.1 and 14.5 g GAE kg^{-1} fresh weight and Brauch *et al.*[21] who reported that the TPC on fresh and dry maqui berries, collected in Aysén region (Patagonia), were 19.7 and 32.0 g GAE kg^{-1}, respectively.

As regards the TFC of maqui berries (Table 1), to our our knowledge, there are no studies where this parameter was determined. However, TFC of several berries, native of Chile, were determined by Ramirez *et al.*[29] This authors reported a TFC of six Chilean berries: highbush blueberries, murta, calafate, arrayán, chequén and meli comprised between 2.57 and 45.72 g quercetin equivalent kg^{-1} DW.

Anthocyanins are the main polyphenolic compound present in maqui berry. The TAC reported in this study (Table 1) was higher than those reported by Brauch *et al.*[21] who found a TAC on fresh and dry maqui berries of 12.6 and 21.1 g CGE kg^{-1}, respectively and Fredes *et al.*[30] who reported that TAC of maqui berries cultivated in Chile was 9.3 g CGE kg^{-1}. In a previous work, Fredes *et al.*[31] determined the TAC of two wild populations of maqui fruits harvested at different maturity stages in central Chile. This author reported that the late maturity stage resulted in the highest anthocyanin content ranging from 7.9 to 8.8 g CGE kg^{-1}.

The results obtained in this work showed a huge variation with scientific literature studies where the maqui polyphenolic content (total phenolic, flavonoids and anthocyanins) were determined. This variation may be due to the extraction methodology used or the solvents employed. Additionally, it should be borne in mind that the bioactive compounds content and especially the

Table 1. Total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) of maqui berry extracts

Parameter	Amount
TPC (g GAE kg^{-1})	49.74 ± 0.57
TFC (g RE kg^{-1})	12.19 ± 0.02
TAC (g C-3-GE kg^{-1})	22.58 ± 0.24

Each assay was carried out in triplicate.
GAE, gallic acid equivalents; RE, rutin equivalents; C-3-GE, cyanidin 3-glucoside equivalents.

polyphenolic compounds (phenolic acids, flavonoids or anthocyanins) depend of several factors, such as genotype, environmental factors, geographical location and altitude (due to the lower temperatures of the higher elevation sites, as well as, the higher UV radiation, the mechanism for producing higher concentration of polyphenolic compounds is triggered), season, soil, maturity stage at harvest, post-harvest conditions, etc.

Polyphenolic profile

The HPLC analysis of the maqui berry extracts (Table 2), showed a total of nineteen polyphenolic compounds identified as anthocyanins (eight compounds), flavonols (10 compounds) and ellagic acid. As regards anthocyanins content (Fig. 1A), delphinidin derivatives and cyanidin derivatives were the compounds detected. These results were in agreement with several authors who reported the presence of eight anthocyanins in maqui berries identified as delphinidin or cyanidin-derivatives.[10,21,32,33] Delphinidin derivatives were the predominant anthocyanins detected which represented 66.42% of the total anthocyanins with delphinidin-3-glucoside as the main component ($P < 0.05$) followed by delphinidin-3,5-diglucoside and delphinidin-3-sambuboside with no statistical differences ($P > 0.05$) between them. In reference to the main anthocyanin present in maqui berries there are contradictory results. Escribano-Bailon *et al.*[32] identified delphinidin-3-5-sambubioside as the main anthocyanin (34% of total anthocyanins). However, Tanaka *et al.*[33] and Céspedes *et al.*[15] reported that the major anthocyanin compound present in maqui berry was delphinidin-3,5-diglucoside, whereas Rojo *et al.*[13] informed that delphinidin-3-glucoside as the main component. This great variability, as mentioned above, could be caused by two main factors, such as genotype and maturity stage. On the other hand, it is important to note that the total anthocyanin content estimated by the pH differential method was lower than those obtained by HPLC quantification (1.8-fold). Several works with maqui berries have indicated that higher estimation is obtained using the HPLC method as compared to the pH differential method.[20,21]

With reference to non-anthocyanin compounds (Fig. 1B), 11 compounds were identified, mainly quercetin and its derivatives (seven compounds), myricetin and its derivatives (three compounds) and ellagic acid which was found in the highest ($P < 0.05$) concentration. As regards to quercetin and its derivatives, the compounds dimethoxy-quercetin showed the highest concentration ($P < 0.05$) followed by rutin (quercetin-3-rutinoside) and quercetin-3-galactoside. With reference to myricetin and its

Table 2. Polyphenolic profile (anthocyanins and non-anthocyanins compounds) of maqui berry

Compound	R_t (min)	Peak no.	λ_{max} (nm)	Concentration (g kg^{-1} DM)
Anthocyanins				
Delphinidin-3-sambubioside-5-glucoside	9.92	1	276, 344, 524	4.36 ± 0.01^{e}
Delphinidin-3,5-diglucoside	9.95	2	276, 342, 524	7.23 ± 0.04^{b}
Cyanidin-3-sambubioside-5-glucoside	10.51	3	278, 330, 514	6.89 ± 0.06^{c}
Cyanidin-3,5-diglucoside	11.86	4	278, 330, 514	5.36 ± 0.05^{d}
Delphinidin-3-sambubioside	12.66	5	276, 344, 526	7.06 ± 0.15^{bc}
Delphinidin-3-glucoside	13.25	6	276, 344, 526	9.48 ± 0.25^{a}
Cyanidin-3-sambubioside	14.49	7	278, 334, 512	0.73 ± 0.11^{g}
Cyanidin-3-glucoside	14.86	8	278, 332, 512	1.24 ± 0.02^{f}
TOTAL	–	–	–	42.35 ± 0.08
Non-anthocyanins				
Myricetin-3-galactoside	17.29	9	258, 356	0.32 ± 0.01^{c}
Myricetin-3-glucoside	18.79	10	258, 356	0.62 ± 0.01^{b}
Quercetin-galloyl-hexoside	19.99	11	260, 356	0.12 ± 0.00^{h}
Quercetin-galloyl-hexoside	20.52	12	252, 356	0.07 ± 0.00^{i}
Rutin	21.18	13	256, 354	0.20 ± 0.01^{f}
Ellagic acid	21.60	14	254, 366	0.94 ± 0.01^{a}
Quercetin-3-galactoside	22.18	15	256, 352	0.17 ± 0.00^{g}
Quercetin-3-glucoside	23.86	16	256, 352	0.07 ± 0.00^{i}
Dimethoxy-quercetin	26.04	17	252, 346	0.28 ± 0.00^{d}
Myricetin	26.59	16	254, 372	0.25 ± 0.01^{e}
Quercetin	31.94	19	254, 370	0.06 ± 0.00^{j}
TOTAL	–	–	–	3.11 ± 0.02

Each assay was carried out in triplicate.
For each group (anthocyanins or non-anthocyanins) values followed by the same letter within the same column are not significantly different ($P > 0.05$) according to Tukey's multiple range test.
R_t, retention time; λ_{max}, absorbance.

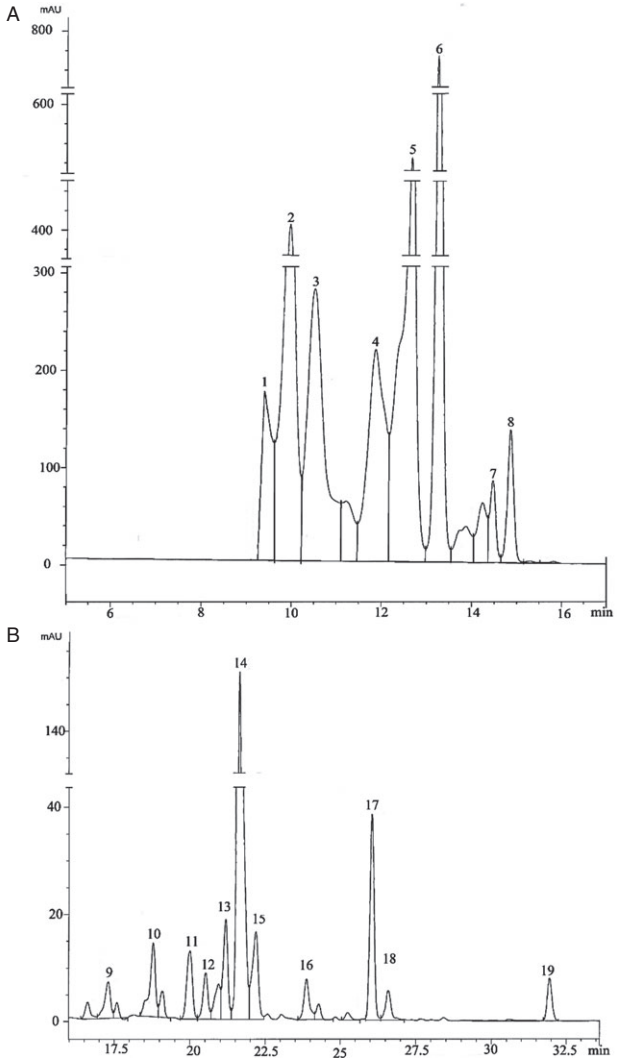

Figure 1. (A) Anthocyanin profile of maqui berry. 1. Delphinidin-3-sambubioside-5-glucoside; 2. delphinidin-3,5-diglucoside; 3. cyanidin-3-sambubioside-5-glucoside; 4. cyanidin-3,5-diglucoside; 5. delphinidin-3-sambubioside; 6. delphinidin-3-glucoside; 7. cyanidin-3-sambubioside; 8. cyanidin-3-glucoside. (B) Non-anthocyanin profile of maqui berry. 9. Myricetin-3-galactoside; 10. myricetin-3-glucoside; 11. quercetin-galloyl-hexoside; 12. quercetin-galloyl-hexoside; 13. rutin; 14. ellagic acid; 15. quercetin-3-galactoside; 16. quercetin-3-glucoside; 17. dimethoxy-quercetin; 18. myricetin; 19. quercetin.

derivatives, myricetin-3-glucoside showed the highest ($P < 0.05$) concentration. The results obtained were very similar to those reported by Brauch *et al.*[21] who mentioned that quercetin derivatives and myricetin derivatives were the main non-anthocyanin constituents of maqui berries. In the same way, Gironés-Vilaplana *et al.*[34] found that the main non-anthocyanins compounds of several lyophilised maqui samples from Chile were quercetin and myricetin derivatives and ellagic acid. Nonetheless, Schreckinger *et al.*[10] and Céspedes *et al.*[15] found the occurrence of proanthocyanidins or hidroxycinnamic acids like ferulic, sinapic or *p*-coumaric acids in maqui berry fruits.

In this case, the total amount of flavonoids found in maqui berries, measured with HPLC, was much lower than those of total flavonoids measured by colorimetry. This could be explained, at least partly, by the performance of colorimetry which tended to be less accurate than that of HPLC.[35]

Table 3. Antioxidant activity of maqui berry extracts measured with four different methodologies: DPPH, ABTS, FRAP and FIC

Method	Result
DPPH (g TE kg^{-1})	28.18 ± 0.37
ABTS (g TE kg^{-1})	18.66 ± 0.26
FRAP (g TE kg^{-1})	25.22 ± 0.38
FIC (g EDTAE kg^{-1})	0.12 ± 0.02

Values are expressed as mean ± SD.
Each assay was carried out in triplicate.
TE, Trolox equivalent; EDTAE, ethylenediaminetetraacetic acid equivalent.

Antioxidant properties

Owing to the complexity of the antioxidant compounds and their mechanism of action, to assess the antioxidant properties *in vitro* of foodstuff it is generally accepted that diverse methods should be employed. So, all aspects of antioxidant efficacy are covered. A single method will provide basic information about antioxidant properties, but a combination of methods describes the antioxidant properties of the sample in more detail.[36] To determine the antioxidant properties of maqui berries, four different methodologies were used in this work. Table 3 shows the values obtained for the antioxidant activity of maqui berry fruits using the DPPH, ABTS, FRAP and FIC assays.

DPPH and ABTS assays are the antioxidant methods most extensively used. The ABTS method is generally indicated for evaluating the antioxidant activity of hydrophilic compounds, the reactions with $ABTS^{\bullet+}$ involve an electron transfer process. The DPPH method is commonly used for aqueous/organic extracts with hydrophilic and lipophilic compounds, the reactions with $DPPH^{\bullet}$ involves H atom transfer.[37] In DPPH assay, the results obtained in this study (Table 3) confirm the results found by Céspedes *et al.*[38] and Fredes *et al.*,[20] who reported an IC_{50} values for DPPH assay of 0.0016 g L^{-1} and 0.0012 g, respectively. The maqui berry extract had an antioxidant activity measured with ABTS assay (Table 3) of 18.66 g TE kg^{-1} DW. Gironés-Vilaplana *et al.*[34] reported values comprised between 0.18 and 0.25 mol L^{-1} TE kg^{-1} in several lyophilised maqui samples from Chile.

Ferrous ion, usually found in food products, is well known as an effective pro-oxidant agent. Polyphenolic compounds showed the ability to chelate pro-oxidant metal ions, such as iron and copper and consequently avoiding free radical formation from this pro-oxidants.[39] Maqui berries showed FIC value of 0.12 g EDTA kg^{-1} sample DW. In FRAP assay the antioxidant capacity of the extracts analysed is determined by the ability of the bioactive compounds, presents in these extracts, to reduce Fe^{3+} to Fe^{2+}. Maqui berries analysed in this work (Table 3) showed a FRAP value of 25.22 g TE kg^{-1} DW. These results were consistent with those reported by Céspedes *et al.*[38] who analysed the antioxidant properties of maqui berry extracted with different solvents. These authors reported values ranged between 4.81 and 12.97 mol L^{-1} catequin equivalents kg^{-1} extract in FRAP assay. In line with this evidence, Ruiz *et al.*[40] carried out a study to determined the antioxidant activity of maqui berries collected from several regions of Chile. The authors reported values for antioxidant activity ranging between 69.9 and 100.5 mmol L^{-1} TE kg^{-1} sample.

In scientific literature there are several works which reported that correlation between polyphenolic compounds and

antioxidant activity occurs. These studies indicated that these compounds largely contributed to the antioxidant properties.[36,39] Nevertheless, the action mechanism set in motion by the antioxidant activity of these compounds is still not clearly understood. Miguel[41] reported that these compounds are known for their properties to inhibit lipid oxidation by acting as chain-breaking peroxyl-radical scavengers and to scavenge free radicals or reactive oxygen species like hydroxyl radicals, peroxy nitrite and hypochlorous acid. In addition, for Liyana-Pathirana and Shahidi[42] polyphenolic compounds exerted the antioxidant activity, mainly due to their redox properties, by various possible mechanisms such as hydrogen donation, transition metal chelating activity, and/or singlet oxygen quenching capacity.

Obviously, the high content of anthocyanins are linked with antioxidant properties. 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.[43] Generally, the antioxidant activity of anthocyanins is associated with the number of free hydroxyls around the pyrone ring: the greater the number of hydroxyls, the greater is the antioxidant activity. Anthocyanins with their 3′,4′-dihydroxy groups can quickly chelate metal ions to form stable anthocyanin–metal complexes.[44] Nevertheless, it should be borne in mind that the antioxidant properties of polyphenolic compounds present in fruits, in general, and in maqui berry, in particular, are difficult to link to a specific compound or group compounds due to their complexity and variability. Therefore, the antioxidant activity could be caused by the major compounds present in maqui berry or due to a synergistic effect between the major compounds and the minor ones. Another aspect to consider with respect to the antioxidant activity of polyphenolic compounds is the extraction process and the solvents used. Thus, Sindi *et al.*[45] evaluated the influence of the extraction methodology and solvent in the contents of total polyphenolic and anthocyanins, as well as antioxidant capacity of *Hibiscus sabdariffa*. The results showed that there were significant differences in the phytochemical content and antioxidant activity.

Antibacterial properties

The the best of our knowledge there are no studies where the antibacterial activity of maqui berries was determined. Therefore, the antibacterial properties of maqui berry extracts, expressed as minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC), were evaluated against six bacterial strains (Table 4). These microorganisms were chosen because they are commonly associated with refrigerated foods as indicators of pathogenic microorganisms or as spoilage microorganisms and considering a future use of maqui berry extracts as a possible food ingredient.

As regards antibacterial activity of each microorganism, *A. hydrophila* and *L. innocua* showed the highest sensitivity to maqui berry extracts with MIC values of 40 and 50 g L^{-1}, respectively. Silva *et al.*[46] reported an MIC value of 50 g L^{-1} for extracts of blueberries against *L. innocua*. On the other hand, *E. amnigenus*, *E. gergoviae* and *A. denitrificans* were the bacterial strains more resistant, with MIC values of 90, 80 and 80 g L^{-1}, respectively. With reference to MBC (Table 4), in this work all the bacterial strains tested had MBC with values between 50 and 100 g L^{-1}.

The antibacterial properties of maqui berries could be related to the high content of bioactive compounds, predominantly anthocyanins. Thus, the antibacterial activity of berry fruits with high content of anthocyanins has previously been proposed. Wu *et al.*[47] found that American cranberry (*Vaccinium macrocarpon*), at 100 μL mL^{-1}, reduced *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* by 3–8 log CFU mL^{-1}. Puupponen-Pimiä *et al.*[48] found that the extracts, with high contents of anthocyanins, obtained from common Finnish berries inhibited the growth of Gram-negative bacteria. Shen *et al.*[49] reported that blueberry (*Vaccinium corymbosum* L.) extracts, at 112.5–900 mg mL^{-1}, exhibited a dose-dependent growth-inhibitory effect against *L. monocytogenes* and *Salmonella enteritidis*.

Table 4. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) (g L^{-1}) of Maqui berry extracts

Bacterial strain	MIC	MBC
Listeria innocua	50	60
Alcaligenes faecalis	60	70
Enterobacter amnigenus	90	100
Enterobacter gergoviae	80	90
Serratia marcescens	60	70
Aeromonas hydrophila	40	50
Shewanella putrefaciens	70	80
Achromobacter denitrificans	80	90

Values are expressed as g L^{-1}.
Each assay was carried out in triplicate.

There are different mechanisms through berry polyphenolic compounds and mainly the anthocyanins can lead to microorganism toxicity. Anthocyanins and phenolic compounds have demonstrated permeative action by destabilising the lipopolysaccharide membrane and increasing the efflux of ATP from the cytoplasm.[50] Additionally, Kleerebezem *et al.*[51] reported that blueberry phenolics could have deep effects on membrane fluidity, changes in the fatty acid profile, and disrupt metabolism disruption. Nevertheless, as occurs with antioxidant activity, it is difficult to attribute the antibacterial activity of a complex mixture of bioactive compounds, as with the maqui berry extracts, to a single or particular constituent, especially taking into account the great variability in the composition of bioactive compounds of maqui berries, which is greatly influenced by several factors such as genotype, altitude, environmental conditions, etc. In this way, Bassole *et al.*[52] reported that the presence of several bioactive compounds with a wide antibacterial properties combined with other minor constitutes might be involved in improving overall antibacterial activity of fruit extracts.

CONCLUSIONS

This study demonstrates that maqui berry has a great potential to be employed, in the food industry, as potential food ingredient to development functional food or as bio-preservative due to (1) the high content in polyphenolic compounds mainly anthocyanins and (2) the promising antioxidant and antibacterial properties. Nevertheless, in-depth studies are necessary to analyse the changes produced by human digestion which could have dramatic effects on bioaccessibility and bioavailability of the bioactive compounds present in this berry fruit.

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