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A novel beverage rich in antioxidant phenolics: Maqui berry (*Aristotelia chilensis*) and lemon juice

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ABSTRACT

In recent years, the interest in dietary antioxidants and bioactive compounds, mainly found in vegetables, has prompted research in the field of new polyphenol-rich drinks. The aim of the present work was to design new beverages using lemon juice and maqui (*Aristotelia chilensis*), rich in flavonoids and vitamin C. The composition of the new beverages as well as their compounds stability, antioxidant capacity and phenolic content over 70 days of storage period were studied. Results showed how anthocyanins and other phytochemicals from maqui preserved vitamin C and other flavonoids in the new mixtures owing to a higher rate of anthocyanin degradation. However, for the colour characteristics, the CIE*Lab* parameters displayed only slight variations, and the samples presented attractive colour during storage. The new beverages also had high values of *in vitro* antioxidant capacity, mainly owed to the maqui polyphenols, with a strong stability throughout study. Therefore, a new designed drink for the growing market of high nutritional and health-promoting food products has been developed.

1. Introduction

Nowadays, there is a growing dietary interest on health-promoting berries, which are fruits rich in anthocyanins among other phenolic phytochemicals, and other bioactive compounds (Seeram et al., 2008). Among berries, maqui (*Arisotelia chilensis* (Mol.) *Stuntz*) is a Chilean native evergreen shrub of the *Elaeocarpaceae* family that grows in Central and Southern areas of the country and produces red/purple colour berries about 6 mm in diameter. 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). Recently, scientific research has demonstrated that these fruits have a strong *in vitro* antioxidant, anti-atherogenic and cardioprotective activities, and *in vitro* both adipogenesis and inflammation inhibitory effects, among others (Céspedes, El-Hafidi, Pavon, & Alarcon, 2008; Schreckinger, Wang, Yousef, Lila, & De Mejia, 2010a).

Therapeutical properties of maqui have been related to their high polyphenols content, concretely anthocyanins: delphinidin 3-sambubioside-5-glucoside, delphinidin 3,5-diglucoside, delphinidin 3-sambubioside, delphinidin 3-glucoside, cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, and cyanidin 3-glucoside (Escribano-Bailón et al., 2006; Schreckinger, Wang, et al., 2010a). Consequently, owing to the presence of these anthocyanins, maqui berries can also be used as natural colourants (Escribano-Bailón et al., 2006), giving an attractive red colour to new mixed-juices.

On the other hand, *Citrus* genus is the most important fruit crop in the world. Lemon (*Citrus limon* (L.) Burm. f.) is the third most important citrus crop (González-Molina, Domínguez-Perles, Moreno, & García-Viguera, 2010). Furthermore, lemon fruit is also a rich source of nutrients, including vitamin C (ascorbic acid + dehidroascorbic acid), minerals, citric acid, and flavonoids, which provide numerous health benefits (González-Molina et al., 2010). Vitamin C is probably the most important water-soluble antioxidant as well as an efficient scavenger of reactive oxygen species, and lemon is a rich source of this nutrient (González-Molina et al., 2010). With respect to flavonoids, hesperidin and eriocitrin (flavanones) and diosmetin glycosides (flavones) are the main compounds (Gil-Izquierdo, Riquelme, Porras, & Ferreres, 2004). Other notable flavonoids have been identified in lemon: vicenin-2 (flavone), diosmin (flavone), quercetin and myricetin (flavonols) as well as other hydroxycinnamic acids (Gil-Izquierdo et al., 2004;

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Hertog, Hollman, & Van de Putte, 1993). Taking into account the bioactive composition of lemon, a wide range of beneficial effects on the prevention of different kinds of cancer, cardiovascular diseases, glucose, lipid metabolism and obesity have been reported (Adibelli, Dilek, & Akpolat, 2009; Hertog et al., 1993).

Therefore, to supply antioxidants through diet it is of great interest polyphenol-rich beverages. The aim of this work was to produce new drinks using lemon juice and maqui berries at different concentrations (2.5% and 5% w/v), following the scope of previous reports directed towards the research of new beverages based on rich-in-antioxidants berries. Likewise, the phytochemical composition, antioxidant capacity, colour, and stability during storage at two different temperatures (4 °C and 25 °C) were studied to characterize these newly designed mixed-juices as novel, safe and acceptable drinks.

2. Materials and methods

2.1. Chemicals

Phenolic compounds were obtained commercially: cyanidin 3-glucoside (Polyphenols, Norway; >97% purity); hesperidin (Merck, Darmstadt, Germany; >90% purity); diosmin (Genay, France; >95% purity); gallic acid (Doesder. Chem. Co., Barcelona, Spain; >99% purity). Other reagents were, 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt ($ABTS^{+}$) (Sigma, Steinheim, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Fluka Chemika, Neu-Ulm, Switzerland); Folin-Ciocalteu's Reagent (Sigma, Steinheim, Germany); sodium carbonate anhydre (Panreac Química S.A., Barcelona, Spain); potassium di-hydrogen phosphate (Panreac Química S.A., Barcelona, Spain); hexadecyltrimethylammonium bromide (Sigma, Steinheim, Germany); citric acid (Sigma, Steinheim, Germany); sodium benzoate (Panreac Quimica S.A., Barcelona, Spain); dimethylsulphoxide, formic acid, and methanol were all of analytical grade (Merck, Darmstadt, Germany); ascorbic acid (AA) and dehydroascorbic acid (DHAA), both from Sigma–Aldrich (Steinheim, Germany); 1,2-phenylenediamine dihydrochloride (OPDA) (Fluka Chemika, Neu-Ulm, Switzerland). Ultrapure water was produced using a Millipore water purification system (Molsheim, France).

2.2. Fruits

Commercial maqui berries were provided by 'Altalena' (Chile), freeze-dried and thawed at −20 °C until tests. Lemon juice was obtained from 'Fino' lemons freshly collected from CEBAS-CSIC's Experimental Farm ('La Matanza', Santomera, Murcia, SE Spain; 38°6′14″ N, 1°1′59″ W), using a domestic squeezer ('Citromatic', Braun Española S.A., Barcelona, Spain). Juice was stored frozen (−20 °C) until used.

2.3. Experimental design

Freeze-dried maqui berries were grinded and added to a volume of lemon juice to obtain final concentrations of 2.5% (w/v) and 5% (w/v) of the grind fruit in the beverage. In addition, control solutions in a 0.18 M citric acid buffer (pH 2.46) using the same proportions were prepared to study the behaviour of maqui phytochemicals without the lemon juice. Lemon juice was also assayed as control.

Homogenized mixtures and control solutions were centrifuged (5 min at 3500 rpm). After that, sodium benzoate (200 mg l^{-1}) was added in order to prevent spoilage. Mixtures and controls were stored in transparent glass vials (56 mm × 18 mm Ø; vol. 10 ml) with plastic screw-caps, and stored at both 4 °C and 25 °C in the dark for 70 days. Triplicate solutions were prepared for each experiment and all analytical measurements were done in triplicate. Analyses were carried out every 7 days for the first 28 days, and every 14 days during the rest of the experiment.

Samples were labelled as follows: L (lemon juice control), LM 2.5% (lemon juice plus 2.5% of maqui berry), LM 5% (lemon juice plus 5% of maqui berry), M 2.5% (2.5% maqui control solution in citric acid buffer), M 5% (5% maqui control solution in citric acid buffer).

2.4. pH, Titratable Acidity (TA), and Total Soluble Solids (TSS)

pH, Titratable Acidity (TA), and Total Soluble Solids (TSS) were evaluated as quality indexes following the method reported by Mena et al. (Mena et al., 2011). Results were expressed as g citric acid per 100 ml of sample in TA, and °Brix in TSS.

2.5. Analysis of phenolic compounds by RP-HPLC-DAD

All samples were centrifuged for 5 min at 10,500 rpm (model Sigma 1–13, B. Braun Biotech International, Osterode, Germany). Supernatant was filtered through a 0.45 μm PVDF filter (Millex HV13, Millipore, Bedford, Mass., USA) before injection into the HPLC system. For the identification and quantification of anthocyanins the method previously reported by González-Molina et al. (González-Molina, Moreno, & García-Viguera, 2009) was followed. The HPLC system was equipped with a Luna C_{18} column (25 cm × 0.46 cm i.d., 5 μm particle size; Phenomenex, Macclesfield, UK) with a C_{18} security guard (4.0 × 3.0 mm) cartridge system (Phenomenex, Macclesfield, UK), using as mobile phases 5% formic acid in water (v/v) (solvent A) and HPLC-grade methanol (solvent B) (Merck, Darmstadt, Germany). Elution was performed at a flow rate of 1 ml min^{-1} using a gradient starting with 1% B, reaching 20% B at 20 min, 40% B at 30, and 95% B at 35 and 39 min. Finally gradient came back at 1% B at 41 min until the end at 50 min. Chromatograms were recorded at 280, 360 and 520 nm. Different phenolics were characterised by chromatographic comparison with analytical standards and accordingly to previous reports (Schreckinger, Lotton, Lila, & de Mejia, 2010b; González-Molina et al., 2010) as well as quantified by the absorbance of their corresponding peaks. Flavanones were quantified as hesperidin at 280 nm; flavones as diosmin at 360 nm, and anthocyanins as cyanidin 3-glucoside at 520 nm.

2.6. Extraction and analysis of vitamin C

Vitamin C content were determined by HPLC as described by González-Molina et al. (González-Molina et al., 2010). AA and DHAA were identified and quantified by comparison with pattern areas from AA and DHAA. The vitamin C content was calculated by adding AA and DHAA content, and results were expressed as mg l^{-1}.

2.7. Colour measurements

Colour measurement was determined following the method reported by González-Molina et al. (González-Molina, Moreno, & García-Viguera, 2008a). Data (CIEL^*, a^* and b^*), were recorded and processed using the Minolta Software Chromacontrol S, PC-based colourimetric data system. *Hue angle* (H) was calculated from $\tan^{-1}(b^*/a^*)$ and Chroma (C^*) from $(a^{*2} + b^{*2})^{1/2}$.

2.8. Total phenolic content by the Folin–Ciocalteu's Reagent

Total phenolic content (TPC) was determined by the Folin–Ciocalteu's Reagent method adapted to a microscale according to a described procedure (Mena et al., 2011). Results were expressed as mg per 100 ml of gallic acid equivalents (GAE).