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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).

Table 1
pH, Titratable Acidity (TA) and Total Soluble Solids (TSS) during 70 days of storage.

Mixtures	pH	TA	TSS
L	2.12 ± 0.02a	5.53 ± 0.03b	7.20 ± 0.07c
M 2.5%	2.49 ± 0.05b	2.89 ± 0.08a	4.00 ± 0.08a
M 5%	2.52 ± 0.06b	2.95 ± 0.04a	5.00 ± 0.07b
LM 2.5%	2.14 ± 0.04a	5.84 ± 0.04c	8.40 ± 0.07d
LM 5%	2.17 ± 0.04a	6.03 ± 0.06d	9.20 ± 0.13e
LSD, $p < 0.05$	0.02	0.02	0.03

Values are mean ± standard deviation ($n = 18$).
TA (Titratable Acidity) is expressed as g citric acid per 100 ml juice.
TSS (Total Soluble Solids) is expressed as °Brix (25 °C).
Means (n=3) in the same columns followed by different letters are significantly different at $p < 0.05$ according to Tukey's test.

2.9. $ABTS^+$ assays of antioxidant capacity

All samples were centrifuged at 10,500 rpm (model EBA 21, Hettich Zentrifugen, Tuttlingen, Germany) during 5 min at room temperature. The free radical scavenging activity was determined using the $ABTS^+$ method in aqueous media according to Mena et al., (Mena et al., 2011). The antioxidant activity was evaluated by measuring the variation in absorbance at 414 nm after 50 min. Assays were measured by using 96-well micro plates (Nunc, Roskilde, Denmark) and Infinite® M200 micro plate reader (Tecan, Grödig, Austria). All reactions started by adding 2 μl of the corresponding diluted sample to the well containing the stock solution (250 μl). Final volume of the assay was 252 μl. Results were expressed as mM Trolox.

2.10. Statistical analyses

Data shown are mean values ($n = 3$). All data were subjected to analyses of variance (ANOVA) and a Multiple Range Test (Tukey's test), using PASW Statistics 18 software (Somers, New York, USA). Pearson correlation analysis was performed to corroborate relationships between selected parameters.

3. Results and discussion

3.1. Quality parameters

Concerning both pH and TA (Titratable Acidity) values, non-significant or relative differences were observed in the mixtures or the control juices for 70 days of storage, and also non-significant differences were found at the two studied temperatures; because of this, results were presented as average values for the whole experiment (Table 1). The pH of beverages with L juice (L, LM 2.5% and LM 5%) was lower than 2.2, whereas maqui berry controls (2.5% and 5%), were 2.49 and 2.52, respectively. In relation to TA, citric acid content in maqui control solutions was lower than in mixtures containing L juice, which presented normal values (around 6 g citric acid · 100 ml^{-1}) (González-Molina, Moreno, & García-Viguera, 2008b).

On the other hand, TSS content of both lemon and maqui control solutions were lower than those registered in the mixtures, as expected (Table 1). TSS values of all samples did not change considerably throughout the storage period at two temperatures considered.

3.2. Flavones and flavanones stability

Flavonoids concentration in lemon juice depends on the cultivar, and maturity stage, among other factors (González-Molina et al., 2008a). Flavanones and flavones were provided by lemon juice to the new mixtures, since these kind of phenolic compounds were not found in maqui controls. Main flavones quantified in lemon mixtures were vicenin-2 and diosmetin 6,8-diglucoside, whereas main flavanones were eriocitrin and hesperidin. Flavones initial values were similar in all beverages containing lemon juice:

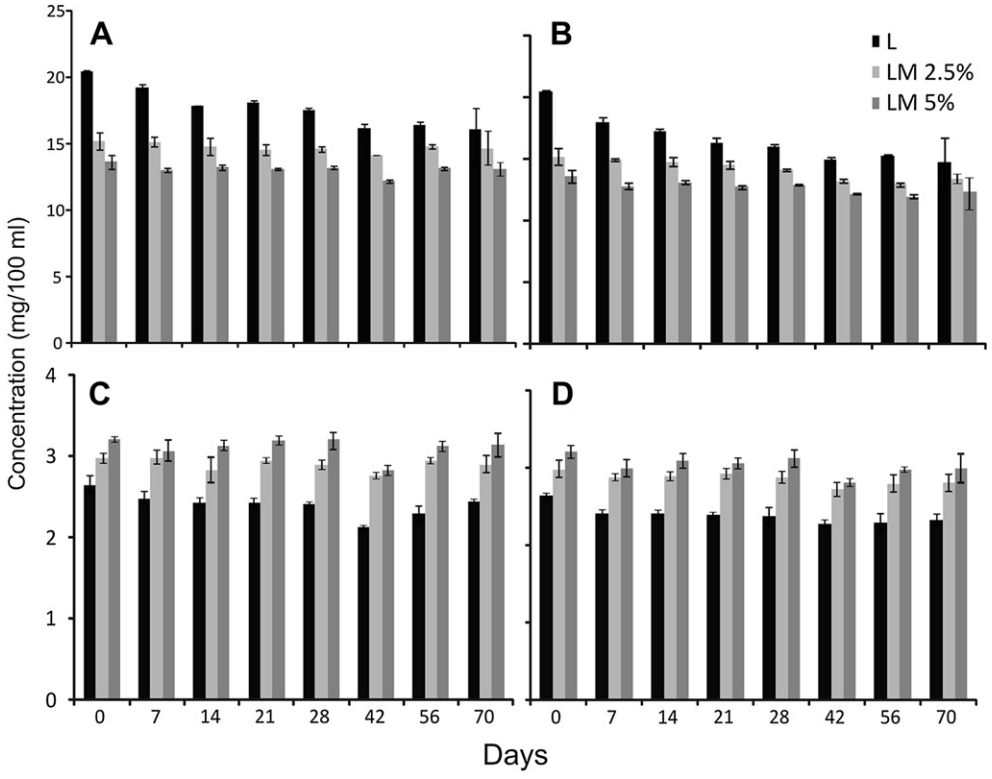

Fig. 1. Total lemon flavonoids at 4 °C (A), 25 °C (B) and diosmetin 6,8-diglucoside at 4 °C (C) and 25 °C (D) in 70 days of storage.

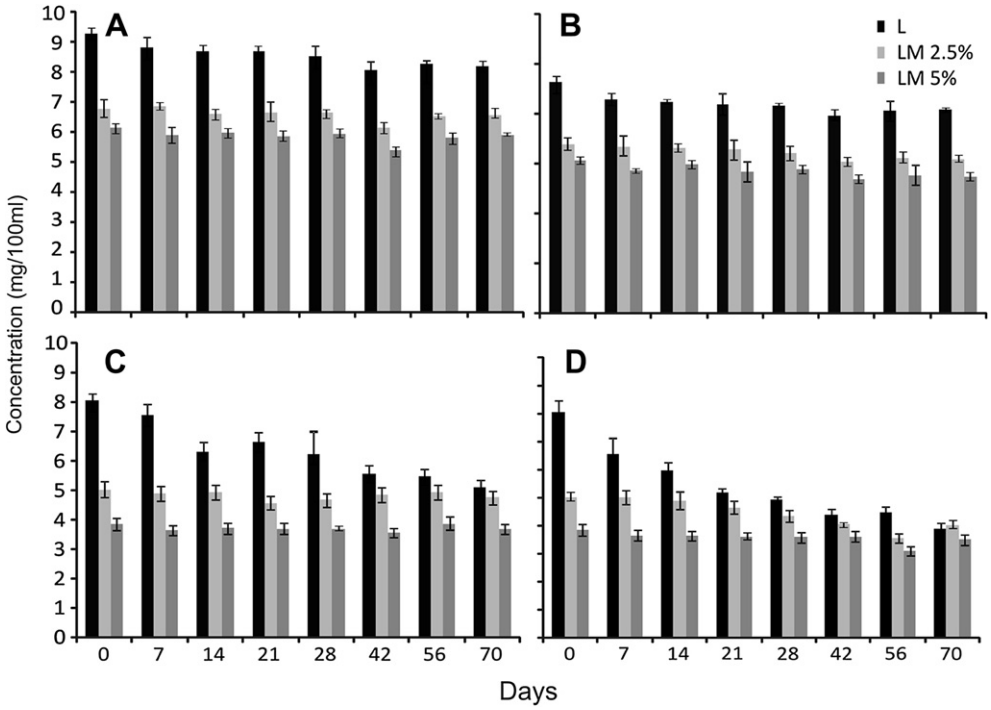

Fig. 2. Flavanones eriocitrin at 4 °C (A), 25 °C (B) and hesperidin at 4 °C (C) and 25 °C (D) in 70 days of storage.

0.44 and 2.95 mg 100 ml^{-1} for vicenin and diosmetin 6,8-diglucoside, respectively. In relation to flavanones, lemon juice showed the highest initial quantity for both eriocitrin and hesperidin (9.27 mg 100 ml^{-1} and 8.09 mg 100 ml^{-1}, respectively), whereas initial values of mixtures with maqui were lower in LM 2.5%: 6.78 mg 100 ml^{-1} and 5.05 mg 100 ml^{-1} for eriocitrin and hesperidin, respectively and in LM 5%: 6.14 mg 100 ml^{-1} and 3.86 mg 100 ml^{-1}.

Regarding the changes in concentration during the storage period, total lemon flavonoids (flavones plus flavanones) showed a decrease in concentration, stressed in the mixtures stored at 25 °C (Fig. 1). Lemon juice had the highest loss among all samples, 21.1% at 4 °C and 27.7% at 25 °C. On the contrary, maqui mixtures displayed a slight protection, as the fall in total lemon flavonoids was lower (Fig. 1). With respect to flavones, only small losses were observed in lemon juice control at 25 °C and they have been related to variations in diosmetin 6,8-diglucoside (12.1%), since vicenin-2 did not suffer almost any change (Fig. 2). Concerning flavanones, eriocitrin did not display significant losses, even though hesperidin was the most affected lemon flavonoid during storage (Fig. 2). Main decreases of hesperidin were registered in lemon juice control (L), reaching almost a 50% of the initial content by the end of the experiment. This fact could be attributed to the tendency to precipitate of flavanones as a consequence of their low solubility (Gil-Izquierdo et al., 2004). However, only 20.4 and 9.1% of the initial hesperidin was degradated in lemon-maqui mixtures (LM 2.5% and LM 5%, respectively) stored at 25 °C (Fig. 2). Thus, a possible stabilization effect of maqui berries on the hesperidin was observed, that had protected hesperidin of degradation, keeping lemon flavonoids content, in a dose-dependent manner, because with higher proportion of maqui, the hesperidin decreased. Similar observations, although in a lesser degree (5.6% and 4.3%, in LM 2.5% and 5%, respectively), have also been seen in samples stored at 4 °C.

3.3. Vitamin C content and changes during storage

Lemon juice is a natural source of vitamin C, as it was aforementioned, in contrast to maqui freeze-dried berries, where vitamin C was not detected. Consequently, vitamin C (calculated as the sum of AA and DHAA) was analysed only in those mixtures containing lemon juice (Fig. 3). Initially, the vitamin C content of

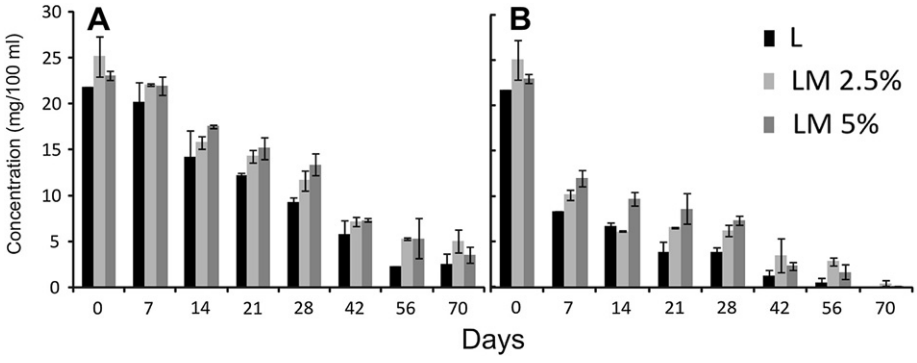

Fig. 3. Total vitamin C (AA + DHAA) for 70 days of storage at 4 °C (A) and 25 °C (B).

Table 2
Anthocyanins in maqui berries (*Aristotelia chilensis*) recorded at 520 nm.

Anthocyanins	Tr	nm (max)	M^+ (*m*/*z*)	MS^2 (*m*/*z*)
Delphinidin 3-sambubioside 5-glucoside	23.4	524	759	465, 597, 303
Delphinidin 3,5-diglucoside	24.7	524	627	465, 303
Cyanidin 3-sambubioside 5-glucoside	26.7	516	743	449, 581, 287
Cyanidin 3,5-diglucoside	26.7	516	611	449, 287
Delphinidin 3-sambubioside	28.7	524	597	303
Delphinidin 3-glucoside + unidentified acylated anthocyanin	29.5	524	465	303
Tentatively identified as isomer of 3b	30.4	516	611	449, 287
Cyanidin 3-sambubioside	31.1	516	581	287

lemon control (L) and mixtures (LM 2.5% and 5%) was similar, ~25 mg 100 ml^{-1}.

During the storage period, a significant fall in vitamin C content over first days was seen, being more striking in those samples stored at 25 °C. Concretely, vitamin C of lemon juice control (L) decreased 68% during the first 14 days of storage at 25 °C, while at 4 °C, the losses were only of a 35% (Fig. 3). However, the rate of decrease in vitamin C content was lower in the mixtures with maqui, with retention of this nutrient until the end of the experiment. Like this, LM 5% was the best mixture protecting vitamin C during the first 14 days, showing a degradation rate of 24% and 57% at 4 °C and 25 °C, respectively (Fig. 3). The LM 2.5% mixtures did not offer such protection with similar levels to the lemon juice control (L) (Fig. 3). This protective effect of berries on vitamin C has already been reported in models of beverages of lemon juice enriched with berry concentrates by González-Molina et al. (González-Molina et al., 2008b).

3.4. Anthocyanins

Anthocyanins of *A. chilensis* (Table 2) have been identified previously by Escribano-Bailón et al., and Schreckinger et al., (Escribano-Bailón et al., 2006; Schreckinger, Lotton, et al., 2010b). Seven of them plus one isomer were confirmed by HPLC-DAD-ESI-MS^n analysis. Initial values of each anthocyanin were similar in all samples with the same percentage of maqui berries powder, as well as in the respective total content (Table 3).

The total content of anthocyanins tended to decrease in all samples tested at 25 °C (Fig. 4), whereas the losses were of less intensity at 4 °C, condition that preserved anthocyanins during storage. It is remarkable that the anthocyanin degradation rate was clearly influenced by the presence of lemon juice in both studied temperatures. In fact, any loss of anthocyanins in maqui controls (M 2.5% and M 5%) was recorded at 4 °C; nevertheless, anthocyanins content of mixtures with lemon juice decreased by 19% and 11% for LM 2.5% and LM 5%, respectively, by the end of the experiment. The same results were achievable in a more emphasized way at 25 °C, as the fall in anthocyanins for both M 2.5% and M 5% was about 37%, while it was 74% and 56% for LM 2.5% and LM 5%, respectively. This effect can be attributed to: 1) the mutual degradation of anthocyanins and ascorbic acid (AA) in the presence of oxygen (Sondheimer & Kertesz, 1953), probably by a free radical mechanism (Iacobucci & Sweeny, 1983); and 2) the degradation products of AA (DHAA, H_2O_2, and furfurals, among others) that can also led to the breakdown of anthocyanins (Özkan, 2002).

Likewise, it is worth mentioning that the mixture with lower maqui concentration (LM 2.5%) was more affected with regard to the total anthocyanins than that with higher maqui dose (LM 5%) at the two studied temperatures.

3.5. Anthocyanins vs. vitamin C

Mutual degradation of both anthocyanins and ascorbic acid has been broadly reported (García-Viguera & Bridle, 1999; Özkan, 2002). Nonetheless, as it was aforementioned, whereas degradation rates of vitamin C were lower in lemon mixtures than in lemon controls, the anthocyanins from mixtures showed higher degradation than in the maqui controls. Therefore, a protective effect of anthocyanins on vitamin C has been exhibited. In this way, other authors have recorded the same protective effect in different models or beverages: Poei-Langston and Wrolstad (Poei-Langston & Wrolstad, 1981), and Bordignon-Luiz et al. (Bordignon-Luiz, Gauche, Gris, & Falcão, 2007), suggesting the protective effect of flavonols on ascorbic acid in an "ascorbic acid-anthocyanin-flavonol" model system. Iversen (Iversen, 1999), found that the degradation rate of anthocyanins was 3–4 times faster than the ascorbic acid in blackcurrant nectar, and Kaack and Austed (Kaack & Austed, 1998), remarked a protective effect on ascorbic acid when flavonols and anthocyanins were both present simultaneously. Hence, regardless implicated processes remain still unknown, it is a point worth mentioning the reduction of vitamin C degradation rate as a likely consequence of the combination of lemon juice phytochemicals with other bioactive compounds from the maqui berries.

3.6. Colour changes during storage

In general, colour parameters were similar among samples with the same concentration of maqui berry, even over time. Lightness (CIEL^* value) tended to increase among all the samples and for both temperatures, being more stressed in those samples stored at 25 °C. Likewise, despite statistical significant differences recorded for samples stored at 4 °C, changes were not too relevant, except for L, probably due to flavanones precipitation (Gil-Izquierdo et al., 2004) (Tables 4 and 5).

Table 3
Initial values of anthocyanins content from maqui berry of the different mixtures studied.

Anthocyanins from maqui	M 2.5%	M 5%	LM 2.5%	LM 5%
Dp 3-smb-5-glc	38.84 ± 2.49d	75.55 ± 0.06f	38.15 ± 1.15c	72.94 ± 4.04c
Dp 3,5-diglc	37.73 ± 1.91cd	71.45 ± 0.54e	38.20 ± 1.63c	73.52 ± 3.70c
Cy 3,5-digly	15.53 ± 1.11ab	30.02 ± 0.04b	15.80 ± 0.82ab	29.82 ± 2.23ab
Dp 3-smb	18.61 ± 0.99b	35.75 ± 0.73c	19.17 ± 0.71b	36.77 ± 1.78b
Dp 3-glc	34.41 ± 1.75c	61.51 ± 1.48d	39.51 ± 1.88c	75.36 ± 3.15c
Cy 3-smb	12.78 ± 1.15a	23.96 ± 0.66a	13.82 ± 0.65a	26.90 ± 1.76a
LSD, $p < 0.05$	1.35	0.62	1.01	2.38
TAnt	**157.87 ± 8.60**	**300.34 ± 3.36**	**165.86 ± 8.60**	**317.45 ± 16.83**

Dp 3-smb-5-glc, (Delphinidin 3-sambubioside-5-glucoside); Dp 3,5-diglc (Delphinidin 3,5-diglucoside); Cy 3,5-digly, (Cyanidin 3,5-diglucoside + Cyanidin 3-sambubioside-5-glucoside); Dp 3-smb (Delphinidin 3-sambubioside); Dp 3-glc (Delphinidn 3-glucoside); Cy 3-smb, (Cyanidin 3-sambubioside).
Values are mean ± standard deviation ($n = 3$) expressed as mg·100 ml^{-1} juice.
Means (n=3) in the same columns followed by different letters are significantly different at $p < 0.05$ according to Tukey's test.

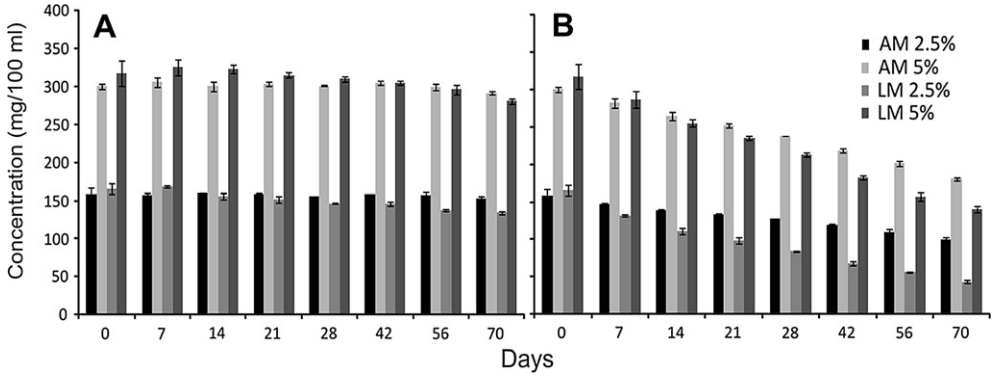

Fig. 4. Total anthocyanins content from maqui-samples during storage at 4 °C (A) and 25 °C (B).

Taking into account both redness (CIELa^*) and yellowness (CIELb^*), a similar trend was found among them for all the samples (a strong correlation between these parameters was recorded: $R^2 \sim 0.99$; $p < 0.001$). While a decrease in L was detected, an augment for all the other samples, both mixtures and controls, occurred. Likewise, variations were higher in those samples stored at 25 °C and, in spite of the statistically significant differences that there were not so considerable.

Table 4
Stability of CIE$L^*a^*b^*$ values in samples during storage at 4 °C.

4 °C	Days	L	M 2.5%	M 5%	LM 2.5%	LM 5%
CIEL^*	0	75.26a	29.06a	15.47a	24.82a	15.02a
	7	82.98b	31.27b	16.62b	25.32ab	15.04a
	14	83.57b	31.68b	17.10bc	26.04bc	15.76ab
	21	88.74c	32.37b	17.78d	26.96d	16.14b
	28	89.27c	31.72b	17.33cd	26.55cd	15.62ab
	42	89.46c	31.76b	17.18bcd	29.08e	16.21b
	56	89.39c	30.99b	16.79bc	31.22f	16.28b
	70	89.90c	32.16b	17.75d	32.41g	17.27c
	LSD	0.55***	0.44***	0.18***	0.25***	0.25***
CIEa^*	0	2.35e	60.42a	47.92a	57.29a	47.83a
	7	1.20d	62.32b	49.46b	57.71ab	47.96ab
	14	0.90c	62.77bc	50.08bc	58.31bc	48.86c
	21	0.28b	63.23c	50.89c	59.20d	49.35c
	28	0.02ab	62.74bc	50.37bc	58.94cd	48.74bc
	42	−0.02a	62.61bc	50.14bc	61.16e	49.36c
	56	0.03ab	62.24b	49.75b	63.41f	49.50c
	70	−0.01a	62.99bc	50.84c	63.99f	50.55d
	LSD	0.08***	0.26***	0.28***	0.23***	0.23***
CIEb^*	0	12.47d	49.95a	26.52a	42.63a	25.75a
	7	8.17c	53.77b	28.50ab	43.50ab	25.78a
	14	7.37c	54.46b	29.32bc	44.73bc	27.01ab
	21	4.01ab	55.64b	30.49c	46.32d	27.68b
	28	4.36b	54.55b	29.72bc	45.62cd	26.77ab
	42	3.36a	54.61b	29.47bc	49.99e	27.79b
	56	4.58b	53.29b	28.79abc	53.67f	27.91b
	70	4.32ab	55.30b	30.45c	55.72g	29.63c
	LSD	0.28***	0.77***	0.53***	0.44***	0.43***
Chroma	0	12.69d	78.38a	54.77a	71.41a	54.16a
	7	8.26c	82.31b	57.09b	72.25ab	54.45ab
	14	7.42c	83.10b	58.04bc	73.50bc	55.83bc
	21	4.02ab	84.23b	59.32c	75.17d	56.58c
	28	4.36ab	83.13b	58.48bc	74.56cd	55.61abc
	42	3.36a	83.08b	58.16bc	78.81e	56.65c
	56	4.58b	81.93b	57.48b	83.00f	56.83c
	70	4.32ab	83.84b	59.26c	84.85g	58.59d
	LSD	0.29***	0.70***	0.49***	0.41***	0.43***
Hue angle	0	79.34a	39.57a	28.96a	36.65a	28.28a
	7	81.68b	40.79b	29.95ab	37.00ab	28.26a
	14	83.08b	40.95b	30.34ab	37.50bc	28.94ab
	21	85.97c	41.35b	30.93b	38.04d	29.29b
	28	89.73d	41.00b	30.55ab	37.74cd	28.78ab
	42	90.37d	41.09b	30.44ab	39.26e	29.38b
	56	89.69d	40.57ab	30.05ab	40.29f	29.41bc
	70	90.14d	41.26b	30.92b	41.06g	30.37c
	LSD	0.54***	0.30**	0.34**	0.15***	0.28***

Means (n=3) in the same columns followed by different letters are significantly different at $p < 0.05$ according to Tukey's test. LSD, $p < 0.01$ (**), $p < 0.001$ (***).

Table 5
Stability of CIE$L^*a^*b^*$ values in samples during storage at 25 °C.

25 °C	Days	L	M 2.5%	M 5%	LM 2.5%	LM 5%
CIEL^*	0	75.26a	29.06a	15.48a	24.82a	15.02a
	7	77.46ab	32.39b	17.87b	28.29b	17.00b
	14	79.39b	33.29bc	19.47c	30.83c	18.79c
	21	87.88c	34.65cde	20.32d	33.59d	20.07d
	28	88.37cd	34.18cd	20.64d	34.02d	19.92d
	42	90.23cd	35.76ef	22.72e	36.42e	21.98e
	56	90.62d	35.21de	22.67e	36.98e	22.57ef
	70	90.86d	36.94f	24.01f	39.30f	23.69f
	LSD	0.77***	0.44***	0.24***	0.46***	0.42***
CIEa^*	0	2.35d	60.42a	47.92a	57.29a	47.83a
	7	2.05d	63.02b	50.91b	59.53b	50.08b
	14	1.41c	63.70bc	52.86c	61.54c	52.28c
	21	0.27b	64.42cd	53.75cd	63.92d	53.71c
	28	−0.04ab	63.81bcd	54.02d	63.91d	53.55c
	42	−0.17ab	64.49cd	56.00e	64.39d	55.55d
	56	−0.21a	64.47cd	56.04e	64.59d	56.26d
	70	−0.27a	64.91d	57.06f	64.34d	57.02d
	LSD	0.15***	0.32***	0.30***	0.25***	0.47***
CIEb^*	0	12.47d	49.95a	26.52a	42.63a	25.75a
	7	11.83cd	55.69b	30.66b	48.72b	29.16b
	14	10.88c	57.24bc	33.41c	53.00c	32.24c
	21	5.92b	59.59cde	34.88d	57.76d	34.44c
	28	5.88b	58.78cd	35.43d	58.49d	34.20c
	42	3.62a	61.50ef	39.02e	62.62e	37.91d
	56	4.75ab	60.54de	38.94e	63.61e	38.75d
	70	4.40a	63.42f	41.24f	67.60f	40.68e
	LSD	0.36***	0.76***	0.42**	0.76***	0.75***
Chroma	0	12.69d	78.38a	54.77a	71.41a	54.16a
	7	12.01cd	84.10b	59.43b	76.86b	57.95b
	14	10.97c	85.64bc	62.53c	81.22c	61.42c
	21	5.93b	87.71cd	64.08d	86.13d	63.81c
	28	5.88b	86.76cd	64.60d	86.63d	63.54c
	42	3.60a	89.11de	68.25e	89.82e	67.16d
	56	4.75ab	88.44de	68.24e	90.65e	68.32de
	70	4.42a	90.75e	70.41f	93.34f	70.05e
	LSD	0.41***	0.73***	0.47**	0.65***	0.79***
Hue angle	0	79.34a	39.57a	28.96a	36.65a	28.28a
	7	80.18ab	41.47b	31.04b	39.23b	30.11b
	14	82.62b	41.95bc	32.30c	40.73c	31.66c
	21	87.49c	42.78cde	32.98d	42.12d	32.67c
	28	90.36d	42.65cd	33.26d	42.47d	32.55c
	42	92.66de	43.64ef	34.86e	44.20e	34.19d
	56	92.51de	43.20de	34.79e	44.56e	34.56d
	70	93.59e	44.34f	35.85f	46.41f	35.50d
	LSD	0.86***	0.27***	0.19***	0.33***	0.41***

Means (n=3) in the same columns followed by different letters are significantly different at $p < 0.05$ according to Tukey's test. LSD, $p < 0.01$ (**), $p < 0.001$ (***).

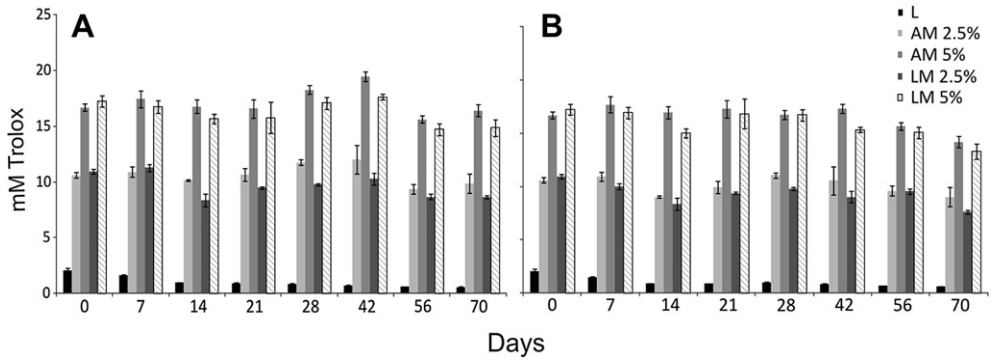

Fig. 5. Antioxidant capacity (mM Trolox) reported by ABTS$^+$ method for 70 days of storage at 4 °C (A) and 25 °C (B).

With respect to *Chroma* and *Hue* angle, an increase was noted for all the samples at all temperatures, apart from a fall in *Chroma* values of L, as it was expected considering the strong correlation existing between CIE*La** and CIE*Lb**.

On the other hand, regardless that losses in total anthocyanin contents over time were found, the red colouration remained quite stable, until the end of the study, as a result of the formation of other coloured-polymers (Boulton, 2001), or copigmentation between anthocyanins and flavonols that can also modify the colour expression by increasing *Chroma* along with a tone shift towards orange tonalities over time (González-Manzano, Dueñas, Rivas-Gonzalo, Escribano-Bailón, & Santos-Buelga, 2009). The same effect has recently been reported by González-Molina for elderberry concentrate mixed with lemon juice (Unpublished data), unlike in other red fruits (pomegranate, aronia, and grapes) this effect has been pointed out in opposite way (González-Molina et al., 2008b, 2009).

3.7. *In vitro antioxidant activity (ABTS$^+$ assay)*

Concerning the total *in vitro* antioxidant capacity measured by the ABTS$^+$ method, Lemon juice controls were lower (2.03 ± 0.21 mM Trolox) than the maqui controls (10.61 ± 0.25 and 16.69 ± 0.33 mM Trolox for M 2.5% and M 5%, respectively). Likewise, the new beverages reported similar levels than red-controls ones (10.94 ± 0.24 and 17.25 ± 0.46 for LM 2.5% and 5%, respectively), supporting the strong *in vitro* antioxidant capacity of maqui, attributed to their polyphenolic content (Céspedes, Alarcon, Avila, & Nieto, 2010; Céspedes et al., 2008; Miranda-Rottmann et al., 2002).

Regarding changes over the storage period, there were not remarkable differences between the two studied temperatures (Fig. 5). The L controls displayed a decrease of antioxidant capacity by about 70% at the end of the study, correlated to vitamin C degradation ($R^2 = 0.933$, $p < 0.01$ in L 4 °C; and $R^2 = 0.956$, $p < 0.001$ in L 25 °C). Nevertheless, when new mixtures (LM 2.5 and 5%) were analysed, the antioxidant capacity was rather constant and did not exceed 20% losses at 4 °C and 30% at 25 °C. This protective effect was probably due to the protection of vitamin C already discussed.

3.8. *Total phenolics*

The total phenolics content (TPC) analysed by the Folin–Ciocalteu's Reagent method was 62.97 ± 1.83, 143.07 ± 5.53, and 243.64 ± 8.08 mg GAE·100 ml^{-1} for L, M 2.5%, and M 5% controls, respectively. Moreover, when TPC of the new mixtures was measured, LM 2.5% and LM 5% showed the additive TPC of their components (187.80 ± 5.41 and 279.84 ± 5.15 mg gallic acid·100 ml^{-1}, respectively). Likewise, phenolic content determined by FCR method reported considerably higher concentrations than those determined by HPLC methods (Fig. 6). The results obtained by this method are not suitable to the total phenolics determination because the reagent react not only with phenolics but also with a variety of non-phenolic reducing compounds including tertiary aliphatic amines, tertiary amine-containing biological buffers, amino acids (tryptophan), hydroxylamine, hydrazine, certain purines, and other organic and inorganic reducing agents leading to overvaluation of the total phenolics content (Ikawa, Schaper, Dollard, & Sasner, 2003). Furthermore, different phenolics can present different answers with the Folin–Ciocalteu's Reagent, presenting lower absorption which it leads to a underestimation of various compounds (Vinson, Su, Zubik, & Bose, 2002).

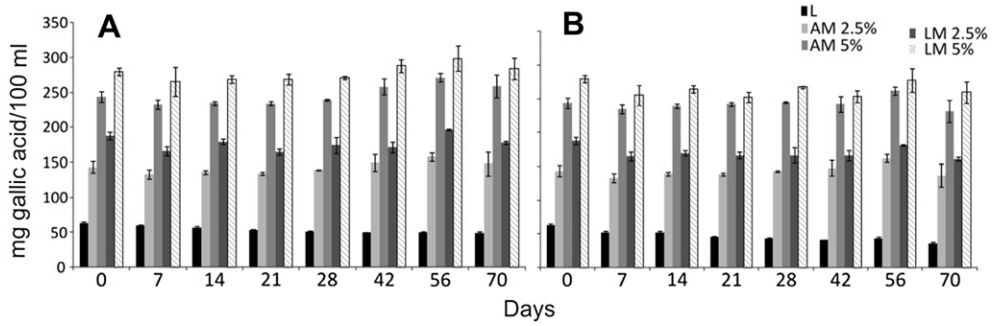

Fig. 6. Total phenolic content (TPC) measured for 70 days at 4 °C (A) and 25 °C (B) by Folin–Ciocalteu's method.

Concerning the evolution of juices over time, the TPC remained quite stable for all the beverages, recording only significant losses for L controls (22% at 4 °C and 44% at 25 °C), due in some extent to the possible precipitation of flavanones (Gil-Izquierdo et al., 2004; González-Molina et al., 2008b) (Fig. 6). Curiously, in maqui controls increased their TPC over time, which could be associated with the formation of secondary metabolites able to react with Folin–Ciocalteu's Reagent.

4. Conclusions

The novel new designed beverages of maqui berries and lemon juice have shown protective interactions among bioactive phytochemicals and a good stability over time with respect to the analytical parameters studied. The vitamin C of lemon juice was preserved in those mixtures containing maqui, thanks mainly to the anthocyanins from these berries. Likewise, hesperidin and, hence, lemon flavonoids, were protected by maqui as well. On the contrary, anthocyanins from new mixed-drinks suffered a severe decrease by the presence of lemon juice. CIE*Lab* parameters were generally stable, showing the new beverages a powerful and attractive red colour throughout the study. Finally, initial high levels of antioxidant capacity and total phenolics content from the mixtures remained quite stable over time, except for the lemon juice. In summary, new drinks rich in bioactive phytochemicals, had a high *in vitro* antioxidant activity as well as an attractive colour well preserved throughout the study period, especially at 4 °C. Further approaches in the evaluation of their bioavailability and biological activity are necessary to verify their potential *in vivo* beneficial effects for nutrition and health.

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