# New Beverages of Lemon Juice Enriched with the Exotic Berries Maqui, Açaí, and Blackthorn: Bioactive Components and in Vitro Biological Properties

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**ABSTRACT:** Following previous research on lemon juice enriched with berries, the aim of this work was to design new blends based on lemon juice mixed with different edible berries of exotic and national origin: maqui (*Aristotelia chilensis* (Molina) Stuntz), açaí (*Euterpe oleracea* Mart.), and blackthorn (*Prunus spinosa* L.). The phytochemical characterization of controls and blends was performed by HPLC-DAD-ESI/MS$^n$. Their antioxidant capacity against DPPH, superoxide, and hydroxyl radicals and hypochlorous acid and their potential to inhibit cholinesterases were also assessed. The profiling of the red fruits and lemon revealed a wide range of bioactive phenolics. The novel beverage based on lemon juice and maqui berry (LM) was the most interesting blend in terms of antioxidant capacity. Berry control samples displayed reduced effects on acetylcholinesterase and butyrylcholinesterase, the lemon juice control being always the most active. This activity was also remarkable for lemon–blackthorn (LB) and lemon–açaí (LA) blends, the last being the most effective inhibitor of cholinesterases among all samples. The results suggested that lemon juice enriched with berries could be of potential interest in the design of new drinks with a nutritive related function on health for chronic diseases.

**KEYWORDS:** *Aristotelia chilensis, Euterpe oleracea, Prunus spinosa, Citrus limon*, antioxidant, cholinesterase inhibition

## INTRODUCTION

In recent years, chronic diseases, including cancer and cardiovascular and neurological disorders, have been taking special relevance in society. For this reason, a continuous flow of information and research results on the positive impact of fruits and vegetables on these diseases has been rising. Previous studies have demonstrated that berries have potential against chronic diseases, and their use in the fresh form or mixed with other juices could act as prevention and improve human health status in these disorders.[1,2]

The loss of basal forebrain cholinergic cells results in an important reduction in acetylcholine, which is believed to play an important role in the cognitive impairment associated with Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis, and Parkinson's disease.[3] Taking into account that cholinesterases, such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are the principal enzymes involved in the hydrolysis of acetylcholine, cholinesterase inhibitors are being developed for the treatment of these diseases. In addition, a wide range of plant compounds with cholinesterase inhibitory activity have been found that may be relevant to the treatment of these neurodegenerative disorders.[4] The available studies are focused on alkaloids,[4,5] xanthones,[6] quercetin,[7] and phlorotannins,[8] among others. Rodent models revealed that the polyphenolic compounds found in berries may decrease the risk of developing age-related neurodegenerative diseases,[9] and cyanidin-3-O-glucoside, commonly present in berries, displayed neuroprotective effects in mice with focal cerebral ischemia.[10]

Current trends and worldwide developments on new foods and products with functionality aim to demonstrate a significant bioactivity of tropical or exotic berries. In this sense, previous research[1,2] designing beverages by combining lemon juice (*Citrus limon* (L.) Burm. f.) with berries resulted in effectively increased antioxidant properties of the lemon juice, as well as improved organoleptic characteristics of the new beverage and offers new possibilities against health problems of chronic diseases.[11] To the best of our knowledge, no previous work about the potential of *C. limon, Aristotelia chilensis* (Molina) Stuntz (maqui), *Euterpe oleracea* Mart. (açaí), and *Prunus spinosa* L. (blackthorn) as AChE and BChE inhibitors has been performed.

Maqui is a common edible berry from central and southern Chile that is a source of natural colorants due to the presence of anthocyanins. This fruit has also been recently reported as one of the healthiest berries, due to its bioactive components.[12,13] Several papers have linked maqui's phenolics with its high antioxidant capacity,[14] in vitro inhibition of adipogenesis and inflammation,[13] protection against oxidative stress,[15] cardioprotection,[12] and in vitro and in vivo antidiabetic effects.[14,16] Açaí is a berry from the palm tree, which is native to the Amazon River area in South America. It is commonly used fresh and in the preparation of beverages and has recently

become popular as a functional food due to its antioxidant potential and phytochemical composition.[17] Potential benefits have been attributed to açaí fruits, extracts, and juices: antioxidant,[18,19] anti-inflammatory,[19] reduction of selected markers of metabolic disease risk[20] or atherosclerosis,[21] pain reduction and improved mobility,[22] and antiproliferative properties.[23] Blackthorn is a fruit of deciduous shrubs native to Europe, mainly Spain, Portugal, and Turkey.[24] It is commonly used in the preparation of jams or macerated with aniseed liqueur to obtain a digestive alcoholic drink called patxarán. Blackthorn is also cited as an astringent, diuretic, and purgative[24] and has recently been proved to be an antioxidant.[25] However, a characterization of its phenolic composition and potential for health-related benefits remains understudied.

Lemon is usually available as fresh produce and is also widely used in the food industry for the elaboration of juices, lemonades, and other processed products.[26] Nonetheless, large quantities of low-quality and over-ripe fruits not optimal for consumers are wasted as byproduct. In this regard, with the aim of minimizing the impact of this bioburden, new alternatives are needed. Lemon juice is rich in nutrients, including vitamin C, minerals, citric acid, and bioactive flavonoids, which can provide health benefits beyond nutrition on cardiovascular disease, cancer, diabetes, and obesity, among other chronic problems of adulthood.[26]

The aims of this work were to perform a phytochemical characterization of lemon juice, maqui, açaí, and blackthorn berries, to design new blends of lemon juice enriched with the berries (5% w/v), to determine their antioxidant capacity, and to evaluate their potential as cholinesterase inhibitors for future applications in nutrition and health.

## ■ MATERIALS AND METHODS

**Chemicals.** Reagents were commercially available: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^\bullet$), $\beta$-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitrotetrazolium blue chloride (NBT), trizina hydrochloride, bovine albumin, sodium chloride, acetylcholinesterase from electric eel, butyrylcholinesterase from equine serum, acetylthiocholine iodide, S-butyrylthiocholine chloride, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), sodium borohydride, sodium hypochlorite solution, ferrium chloride 45% solution, 2-deoxy-D-ribose, and 2-thiobarbituric acid were obtained from Sigma-Aldrich (Steinheim, Germany); potassium dihydrogen phosphate, ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide 30%, and trichloroacetic acid were purchased from Merck (Darmstadt, Germany); magnesium chloride hexahydrate and ascorbic acid were from Fluka Chemika (Neu-Ulm, Switzerland). Ultrapure water was produced using a Millipore water purification system.

**Fruits.** Maqui and açaí lyophilized berries were provided by Ecuadorian Rainforest, LLC (USA). Blackthorn fruit was obtained from Importaciones Samanes S.L. and lyophilized and ground after. Lemon juice was obtained from 'Fino' lemons freshly collected from CEBAS-CSIC's experimental farm (La Matanza, Santomera, Murcia, southeastern 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.

**Experimental Design.** Lyophilized and powdered fruits were added to lemon juice separately to obtain final concentrations of 5% w/v of the fruit in the beverage. In addition, control solutions using 0.18 M citric acid buffer (pH 2.46) were prepared to study the activities of the different fruits without lemon. Lemon juice alone was also assayed (pH 2.15). Homogenized mixtures and control solutions were then centrifuged (7 min at 4000 rpm) and stored frozen (−20 °C) until used.

Samples were labeled as follows: L (lemon juice control), LM 5% (lemon juice plus 5% of maqui berry), M 5% (5% maqui in citric acid buffer), LA 5% (lemon juice plus 5% of açaí berry), A 5% (5% açaí in citric acid buffer), LB 5% (lemon juice plus 5% of blackthorn fruit), B 5% (5% blackthorn in citric acid buffer).

**HPLC-DAD-ESI/MS$^n$.** Chromatographic analyses were carried out on a Luna C18 column (250 × 4.6 mm, 5 mm particle size; Phenomenex, Macclesfield, U.K.). Water/formic acid (99:1, v/v) and acetonitrile were used as mobile phases A and B, respectively, with a flow rate of 1 mL/min. The linear gradient started with 8% of solvent B, reaching 15% solvent B at 25 min, 22% at 55, and 40% at 60 min, which was maintained up to 70 min. The injection volume was 30 $\mu$L. Chromatograms were recorded at 280, 320, 360, and 520 nm. The HPLC-DAD-ESI/MS$^n$ analyses were carried out in an Agilent HPLC 1100 series equipped with a photodiode array detector and a mass detector in series (Agilent Technologies, Waldbronn, Germany). The equipment consisted of a binary pump (model G1312A), an autosampler (model G1313A), a degasser (model G1322A). and a photodiode array detector (model G1315B). The HPLC system was controlled by ChemStation software (Agilent, version 08.03). The mass detector was an ion trap spectrometer (model G2445A) equipped with an electrospray ionization interface and was controlled by LCMSD software (Agilent, version 4.1). The ionization conditions were adjusted at 350 °C and 4 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 65.0 psi and 11 L/min, respectively. The full-scan mass covered the range from $m/z$ 100 to $m/z$ 1200. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Mass spectrometry data were acquired in the positive ionization mode for anthocyanins and in negative ionization mode for other flavonoids. MS$^n$ was carried out in the automatic mode on the more abundant fragment ion in MS$^{(n-1)}$.

Prior to injection, samples were centrifuged (12000 rpm, 5 min) and filtered through a PVDF syringe filter (0.22 $\mu$m). Anthocyanins were quantified as cyanidin 3-O-glucoside at 520 nm, flavonols as quercetin 3-O-rutinoside (rutin) at 360 nm, ellagic acid derivatives as ellagic acid at 360 nm, hydroxycinnamic acids as 5-O-caffeoylquinic acid at 320 nm, flavanones as hesperidin at 280 nm, and flavones as diosmin at 360 nm.

**DPPH Radical Scavenging Activity.** The antiradical capacity was estimated spectrophotometrically in a Multiskan Ascent plate reader (Thermo Electron Corp.) by monitoring the disappearance of DPPH$^\bullet$ at 515 nm, according to Oliveira et al.[27] The reaction mixtures in the sample wells consisted of 25 $\mu$L of samples (five different concentrations to obtain IC$_{50}$) and 200 $\mu$L of DPPH$^\bullet$ dissolved in methanol. Three experiments were performed in triplicate.

**Superoxide Radical (O$_2^{\bullet-}$) Scavenging Activity.** Antiradical activity was determined spectrophotometrically in a 96-well plate reader by monitoring the effect of controls and blends on the O$_2^{\bullet-}$-induced reduction of NBT at 560 nm. Superoxide radicals were generated by the NADH/PMS system according to a described procedure.[28] Samples were tested at five different concentrations to obtain IC$_{50}$. All components were dissolved in phosphate buffer (19 mM, pH 7.4). Experiments were performed in triplicate, expressing results as inhibition percentage of NBT reduction compared to the control.

**Hypochlorous Acid Scavenging Activity.** The inhibition of hypochlorous acid-induced 5-thio-2-nitrobenzoic acid (TNB) oxidation to DTNB was performed according to a described procedure,[29] in a double-beam spectrophotometer (He$\lambda$ios $\alpha$, Unicam, Leeds, U.K.) at 412 nm. Samples are tested at five different concentrations and were diluted in phosphate buffer (50 mM, pH 7.4) to obtain IC$_{50}$. Three experiments were performed in triplicate.

**Hydroxyl Radical Assay.** The deoxyribose method for determining the scavenging effect of samples on hydroxyl radicals was performed as described before[29] in a double-beam spectrophotometer (He$\lambda$ios $\alpha$), programmed in photometric function, with the wavelength fixed at 532 nm. Hydroxyl radical is generated in a Fenton system, in which ascorbic acid accelerates hydroxyl radical formation

by reducing $Fe^{3+}$ ions to $Fe^{2+}$. Therefore, the radical is detected by its ability to degrade deoxyribose into fragments. The heating of the mixture under acid conditions leads to the formation malonaldehyde. This is detected by its reaction with thiobarbituric acid, with the formation of a pink chromogen: Reaction mixtures contained, in a final volume of 1 mL, 50 $\mu$M ascorbic acid, 40 $\mu$M $FeCl_3$, 2 mM EDTA, 2.8 mM $H_2O_2$, 2.8 mM deoxyribose, sample, and 10 mM $KH_2PO_4$−KOH buffer (pH 7.4) as solvent. Samples were tested at five different concentrations to obtain $IC_{50}$. Three experiments were performed in triplicate.

**AChE and BChE Inhibitory Activity.** The inhibition of AChE activity was determined on the basis of Ellman's method, as previously reported.[30] The absorbance was measured at 405 nm, and the rates of reactions were calculated by Ascent Software version 2.6 (Thermo Labsystems Oy). The BChE inhibition assay was performed in a similar way, using 25 $\mu$L of substrate (15 mM butyrylthiocholine) and 25 $\mu$L of enzyme (0.1 U/mL). Three experiments were performed in triplicate. Each sample was evaluated at five different concentrations to obtain $IC_{50}$, and three experiments were performed in triplicate.

**Statistical Analysis.** 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, NY, USA). Pearson correlation analysis was performed to corroborate relationships between selected parameters.

## ■ RESULTS AND DISCUSSION

**Phenolic Compounds.** The analysis of the berry fruits revealed the presence of a wide range of polyphenols. Concerning maqui, different glycosides and diglycosides of delphinidin (**A1**, **A2**, **A5**, **A6**) and cyanidin (**A3**, **A4**) were found (Figure 1; Table 1), in accordance with previous papers.[2,31] Flavonols (quercetin and myricetin derivatives, **F1**−**F5**, **F7**−**F10**), ellagic acid derivatives (**E2** and **E3**, with maximum absorption at 360 nm), 5-O-caffeoylquinic acid (**C6**), and one ellagitannin (granatin B, **E1**, identified and quantified at 320 nm) were also identified (Figure 2; Table 1). Only the maqui sample presented ellagic acid derivatives. With respect to açaí, three derivatives of cyanidin (**A7**, **A8**, **A10**) and one of malvidin (**A14**) were identified (Figure 1; Table 1). Likewise, quercetin (**F5**, **F7**, **F9**, and **F10**) and hydroxycinnamic acid derivatives (**C3**, **C6**, **C8**) were found, too (Figure 2; Table 1), these compounds being also previously reported in açaí.[32,33] Blackthorn presented four anthocyanins (two cyanidin glycosides (**A8**, **A10**) and two peonidin glycosides (**A12**, **A13**)) (Figure 1; Table 1), quercetin derivatives (**F4**, **F6**, **F8**), and hydroxycinnamic acid derivatives (**C1**−**C5**, **C7**) (Figure 2; Table 1), in accordance with previous research.[25] Lemon juice contained flavones, flavanones, flavonols, and hydroxycinnamic acids (Figure 3; Table 2), as described before.[34]

Maqui control (M) and blend (LM) showed considerably high amounts of anthocyanins (Table 3). Delphinidin 3-O-sambubioside-5-O-glucoside (**A1**), delphinidin 3,5-O-diglucoside (**A2**), cyanidin 3,5-O-diglucoside (**A3**), and cyanidin 3-O-sambubioside-5-O-glucoside (**A4**) contributed 49.8% in M and 48.9% in LM of the total anthocyanins amount (Table 3). Delphinidin 3-O-glucoside (**A6**) was the second major anthocyanin in maqui samples (22.1 and 21.8%, respectively). This high content in anthocyanins in maqui has been also previously reported.[2,13,31] Blackthorn (B) and açaí (A) controls and blends (LB and LA, respectively) displayed lower quantities of anthocyanins. With respect to açaí, cyanidin-3-O-rutinoside (**A10**) was the most abundant anthocyanin (60.6% in A and 57.5% in LA), followed by cyanidin 3-O-galactoside (**A7**) (25.6% in A and 27.9% in LA). In blackthorn samples (B and LB), cyanidin-3-O-rutinoside (**A10**) was also
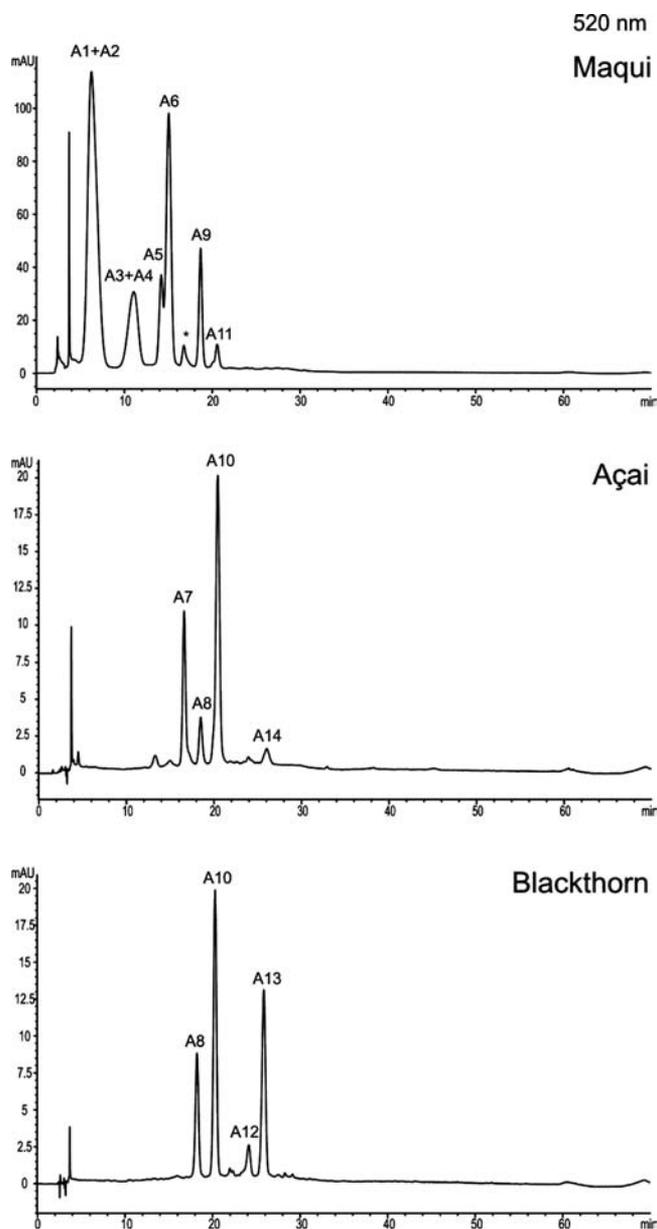


**Figure 1.** Chromatograms of berries registered at 520 nm. The identities of the compounds associated with the peaks shown here are given in Table 1.

the predominant anthocyanin (41.9% in B and 42.2% in LB), followed by peonidin 3-O-rutinoside (**A13**) (32.5% in B and 32.3% in LB).

Maqui samples (M, LM) also showed the highest contents of flavonols (quercetin and myricetin glycosides) (Table 3). Açaí and blackthorn samples displayed similar quantities of flavonols, quercetin 3-O-galactoside (**F5**) being the major flavonol in açaí (67.0% in A and 45% in LA), quercetin 3-O-rutinoside (**F4**) in LB (40.7%), and quercetin 3-O-xyloside (**F8**) in B (39.5%) (Table 3). With respect to hydroxycinnamic acid derivatives, blackthorn samples (B, LB) recorded noticeably higher contents, owing to a large peak of 3-O-caffeoylquinic acid (**C3**) (70.6% in B and 70.5% in LB) (Table 3). Therefore, the new blends analyzed improved the levels of hydroxycinnamic acid derivatives when compared to the controls (Table 3).

Only lemon juice and blends displayed flavones and flavanones (Table 3). Flavones were mainly represented by

**Table 1. Phenolic Compounds Identified in Berries: Maqui, Açaí, and Blackthorn**

| | $t_R$ | $[M + H]^+/[M - H]^-$ | $MS^n$ | nm (max) | maqui | açaí | blackthorn |
|---|---|---|---|---|---|---|---|
| **Anthocyanins** | | | | | | | |
| A1, delphinidin 3-O-sambubioside-5-O-glucoside | 6.2 | 759 | 465, 597, 303 | 524 | + | − | − |
| A2, delphinidin 3,5-O-diglucoside | 6.9 | 627 | 465, 303 | 524 | + | − | − |
| A3, cyanidin 3,5-O-diglucoside | 11.2 | 611 | 449, 287 | 516 | + | − | − |
| A4, cyanidin 3-O-sambubioside-5-O-glucoside | 11.6 | 743 | 449, 581, 287 | 516 | + | − | − |
| A5, delphinidin 3-O-sambubioside | 14.2 | 597 | 303 | 524 | + | − | − |
| A6, delphinidin 3-O-glucoside | 15.1 | 465 | 303 | 524 | + | − | − |
| A7, cyanidin 3-O-galactoside | 16.4 | 449 | 287 | 520 | − | + | − |
| A8, cyanidin 3-O-glucoside | 18.3 | 449 | 287 | 520 | − | + | + |
| A9, cyanidin 3-O-sambubioside | 18.7 | 581 | 287 | 516 | + | − | − |
| A10, cyanidin-3-O-rutinoside | 20.3 | 595 | 287 | 516 | − | + | + |
| A11, cyanidin 3-O-glucoside-5-O-rhamnoside | 20.6 | 595 | 449, 287 | 517 | + | − | − |
| A12, peonidin 3-O-glucoside | 24.2 | 463 | 301 | 520 | − | − | + |
| A13, peonidin 3-O-rutinoside | 25.8 | 609 | 301 | 520 | − | − | + |
| A14, malvidin 3-O-glucoside | 25.9 | 493 | 331 | 520 | − | + | − |
| isomer of cyanidin 3,5-O-diglucoside[a] | 16.7 | 611 | 449, 287 | 524 | + | − | − |
| **Noncolored Flavonoids** | | | | | | | |
| ellagic acid derivatives | | | | | | | |
| E1, granatin B | 23.8 | 951 | 933, 301 | 275 | + | − | − |
| E2, ellagic acid hexoside | 35.8 | 463 | 301 | 255, 360 | + | − | − |
| E3, ellagic acid rhamnoside | 40.1 | 447 | 301 | 265, 360 | + | − | − |
| flavonols | | | | | | | |
| F2, myricetin 3-O-galoylglucoside | 26.9 | 631 | 479, 317 | 260, 355 | + | − | − |
| F3, myricetin 3-O-galactoside | 30.0 | 479 | 317 | 260, 355 | + | − | − |
| F4, myricetin 3-O-glucoside | 30.9 | 479 | 317 | 260, 355 | + | − | − |
| F5, quercetin 3-O-rutinoside | 38.9 | 609 | 301 | 260, 360 | + | − | + |
| F6, quercetin 3-O-galactoside | 39.4 | 463 | 301 | 260, 360 | + | + | − |
| F7, quercetin 3-O-hexoside-5-O-pentoside | 40.2 | 595 | 463, 301 | 260, 360 | − | − | + |
| F8, quercetin 3-O-glucoside | 41.8 | 463 | 301 | 260, 360 | + | + | − |
| F9, quercetin 3-O-xyloside | 47.1 | 433 | 301 | 260, 360 | + | − | + |
| F10, quercetin 3-O-arabinoside | 49.1 | 433 | 301 | 260, 360 | + | + | − |
| F11, quercetin 3-O-rhamnoside | 50.7 | 446 | 301 | 260, 360 | + | + | − |
| hydroxycinnamic acid derivatives | | | | | | | |
| C1 caffeoyldihydrocaffeoylquinic acid (1)[a] | 6.7 | 517 | 335 | 330 | − | − | + |
| C2, caffeoyldihydrocaffeoylquinic acid (2)[a] | 7.0 | 517 | 335 | 330 | − | − | + |
| C3, 3-O-caffeoylquinic acid | 8.9 | 353 | 191, 179 | 330 | − | + | + |
| C4, 3-O-p-coumaroylquinic acid | 13.5 | 337 | 163 | 320 | − | − | + |
| C5, 4-O-caffeoylquinic acid | 16.2 | 353 | 173 | 330 | − | − | + |
| C6, 5-O-caffeoylquinic acid | 16.5 | 353 | 191 | 330 | + | + | − |
| C7, 3-O-feruloylquinic acid | 16.7 | 367 | 193 | 330 | − | − | + |
| C8, 5-O-p-coumaroylquinic acid | 24.9 | 337 | 191 | 330 | − | + | − |

[a]Tentatively identified as two isomers of caffeoyldihydrocaffeoylquinic acid.

diosmetin 6,8-di-C-glucoside (**L2**), followed by diosmetin 7-O-rutinoside (**L4**) and apigenin 6,8-di-C-glucoside (**L1**). With respect to flavanones, eriodictyol 7-O-rutinoside (**L10**) and hesperetin 7-O-rutinoside (**L11**) were found in similar amounts. Ferulic acid (**C9**) was the predominant hydroxycinnamic acid, followed by sinapic acid (**C10**) and 5-O-caffeoylquinic acid (**C6**) in lemon samples (Table 3). This characteristic composition was previously observed.[35] Nevertheless, the concentration of flavanones is lower and the amount of flavones is higher than those in previously found in 'Fino' lemon.[34] It is important to emphasize that flavonoids in lemon are variable according to cultivar, season, growth conditions, or maturity stage.[26,34]

**Antioxidant Capacity.** *DPPH*•. The antioxidant activity was measured by different methods, including DPPH• scavenging. The IC$_{50}$ was used to compare samples (Table

4). Concerning controls, maqui (M) presented the highest activity, which was increased in the LM blend. LA and LB blends were also more effective than the respective controls and lemon juice. This increased antiradical activity in the blends with respect to controls was probably due to the effect of the new matrix enriched in phytochemicals groups in the new mixtures. Nonetheless, we could not find any correlation between anthocyanins and DPPH•. This is a different result from that previously reported with anthocyanin-based fruit extracts[36] or fruits,[37] showing direct correlations between these variables. Then, the higher activity observed with maqui samples (M, LM) explained by their higher anthocyanins content was also supported by the DPPH• scavenging capacity of the maqui fruits[12] and of açaí extracts,[38] as reported for an anthocyanin-rich extract.[23] With respect to blackthorn, a good
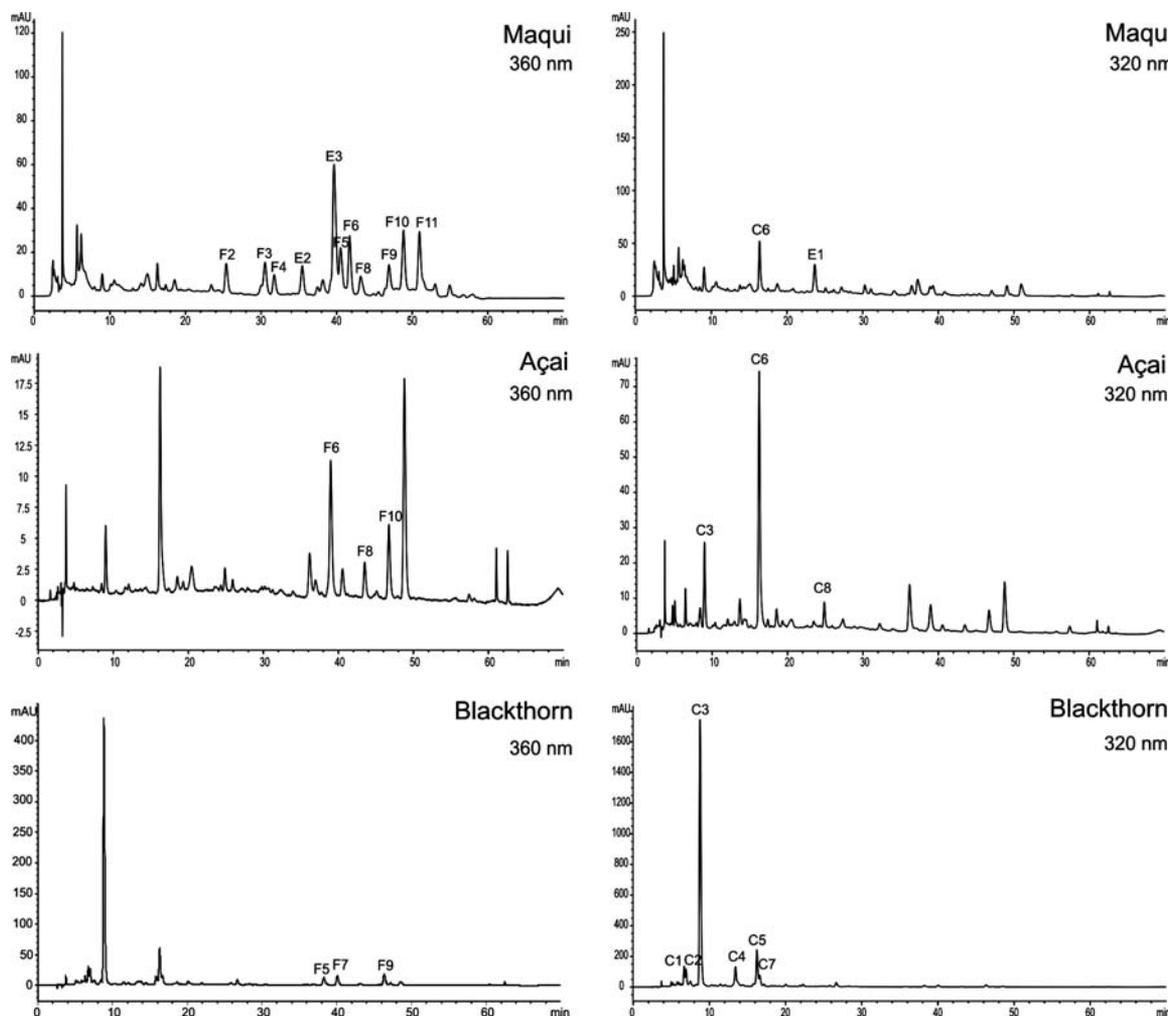
**Figure 2.** Chromatograms of berries recorded at 360 and 320 nm. The identities of the compounds associated with the peaks shown here are given in Table 1.

antioxidant activity against DPPH$^\bullet$ was found in fresh juice and fresh fruits.[24,39]

*Superoxide Radical.* All samples led to low IC$_{50}$ values in the superoxide (O$_2$$^{\bullet-}$) scavenging assay (Table 4), suggesting a high activity against this reactive oxygen species. The values ranged between 2.31 mg/mL (in LM) and 5.49 mg/mL (in B). The addition of blackthorn fruit to lemon juice (LB) resulted in the highest activity when compared to its control (B), whereas the opposite occurred for açaí (LA vs A). In previous studies on the results of O$_2$$^{\bullet-}$ the scavenging activity of flavonoids was associated not only with rutin, apigenin, neohesperidin, neoeriocitrin, and quercetin,[40] but also with anthocyanin-rich extracts,[41] even using freeze-dried açaí.[19] As far as we are aware, no previous studies about O$_2$$^{\bullet-}$ scavenging ability of maqui or blackthorn have been performed, and this result is useful for the biological evaluation of these berries. On the other hand, we found no significant correlation between O$_2$$^{\bullet-}$ scavenging activity or any fruit compounds, but that does not mean that other components of the matrix not detected or analyzed could have some activity.

*Hypochlorous Acid.* The lowest activity was observed against hypochlorous acid (HOCl). The IC$_{50}$ values varied significantly between 15.28 mg/mL (in LM) and 42.80 mg/mL (in A) (Table 4). Lemon juice (L) exhibited higher activity than berry controls (M, A, or B), which was improved in the

blends (Table 4). In addition, a significant correlation between flavones and flavanones and IC$_{50}$ was found ($R = -0.869*$ ($p < 0.05$) and $R = -0.928**$ ($p < 0.005$), respectively). Previous works defined citrus pulp as an effective HOCl scavenger[42] and reported a direct correlation with total phenolics. This is consistent with the slightly higher effect of lemon juice in comparison with berry controls (M, A, or B). In addition, the increased antiradical activity of the new blends may be due by the addition of phytochemicals from the lemon and the compounds from berries, which can give more stability to lemon bioactive compounds, as previously reported.[1,2] These new data on HOCl scavenging ability of maqui, açaí, or blackthorn describe additional modes of action of these fruits in terms of antioxidant activity to support their potential use in food and health.

*Hydroxyl Radical.* Concerning the hydroxyl radical ($^\bullet$OH), samples were characterized by a reactively high antioxidant capacity (Table 4): IC$_{50}$ values varied from 2.79 mg/mL (in LM) to 6.93 mg/mL (in L). Thus, among controls, blackthorn (B) displayed the best activity, and LM and LA were also very effective among the samples. The addition of blackthorn to lemon juice did not seem to affect the antioxidant capacity of the fruit, unlike what happened with the other blends. Anthocyanin-rich extracts from pomegranate (*Punica granatum* L.), and its three major anthocyanidins (delphinidin, cyanidin,
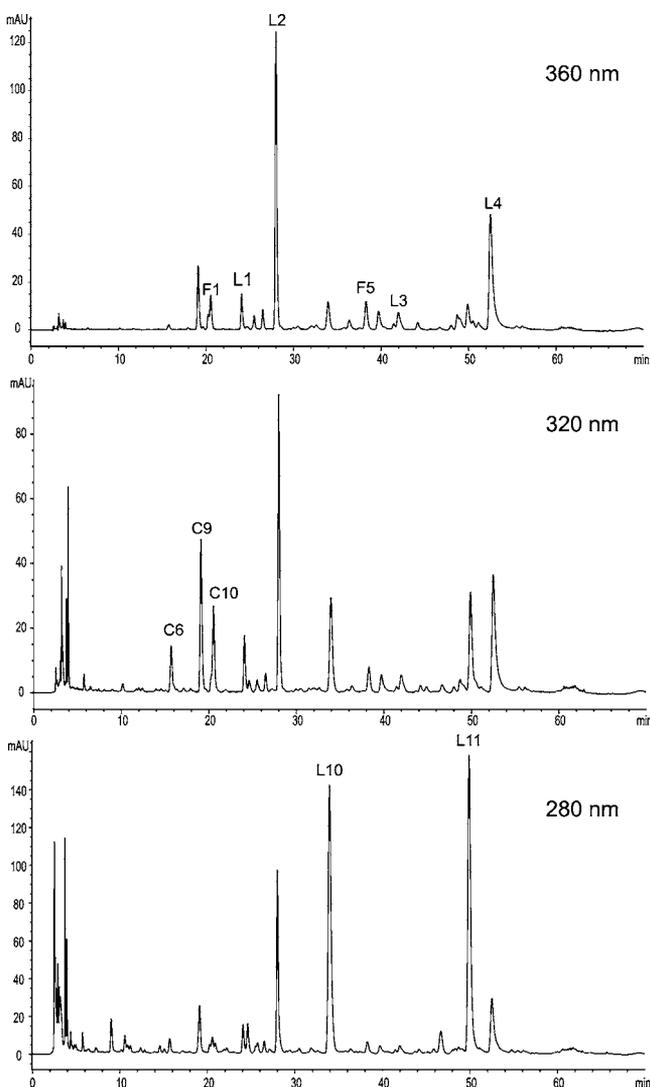
**Figure 3.** Chromatograms of lemon juice recorded at 360, 320 and 280 nm. The identities of the compounds associated with the peaks shown here are given in Table 2.

Fe$^{2+}$ rather than by direct scavenging of the radical. The poor •OH scavenging activity of purple açaí was also demonstrated before,[17] and no previous papers concerned the effect of maqui, blackthorn, or lemon juice. In this assay the effect of ascorbic acid has to be taken into consideration, because of its ability to redox cycle the metal ion required for hydroxyl radical generation, thus increasing the radical production.[29] No ascorbic acid was found in berries (data not shown), although lemon is rich in this nutrient.[26] Furthermore, no pro-oxidant effect was observed with lemon control and blends, probably owing to the phenolics contained in them.

When all of these data are taken into account, different assays can give a broader view to justification of the use of multiple antioxidant tests because most are radicals produced in human cells (superoxide radical, hypochlorous acid, and hydroxyl radical). It is interesting that although samples reacted differently against several reactive species, the novel beverage based on lemon juice and maqui berry (LM) was the most active in all assays (Table 4), as a result of its higher content in phytochemicals belonging to separate bioactive compound groups. The results suggested that phenolic compounds are not the only parameter selective for or indicative of the antioxidant potential; the quality composition and the interactions between compounds in the food matrix, such as the protection of lemon flavonoids by berry polyphenols, may be also involved.

**AChE and BChE Inhibitory Activity.** In recent years, the challenging search for natural inhibitors of cholinesterases involved in the etiology of a broad number of neurological disorders is increasing.[3] The IC$_{50}$ values found for all of the tested controls and blends are presented in Table 4. All samples displayed AChE and BChE inhibitory activities. The results were similar for both enzymes, displaying a strong correlation between them ($r = 0.993$***, $p < 0.001$). Lemon juice (L) exhibited the best activity for both cholinesterases among control samples, showing also a direct correlation with its flavones and flavanones ($R = -0.874$* ($p = 0.01$) and $R = -0.871$* ($p < 0.05$) between total flavones and AChE and BChE, respectively; and $R = -0.909$** ($p = 0.005$) and $R = -0.907$** ($p = 0.005$) between total flavanones and AChE and BChE, respectively). The activity of lemon juice was not enhanced by maqui addition (LM), but significant increases were observed with blackthorn (LB) and açaí (LA), the latter being the most effective. Although this effect is considerably

and pelargonidin),[43] and from rambutan (*Nephelium lappaceum* L.)[41] showed high activity against •OH, possibly by chelating

**Table 2. Phenolic Compounds Identified in Lemon Juice**

| compound | $t_R$ | [M − H]$^-$ | MS$^n$ | nm (max) |
|---|---|---|---|---|
| flavones (360 nm) | | | | |
| **L1**, apigenin 6,8-di-C-glucoside | 24.1 | 593 | 503, 473 | 270, 345 |
| **L2**, diosmetin 6,8-di-C-glucoside | 28.0 | 623 | 503, 413, 383 | 270, 340 |
| **L3**, 8-C-glucosylchrysoeriol | 42.1 | 461 | 300 | 280, 350 |
| **L4**, diosmetin 7-O-rutinoside | 52.5 | 607 | 299 | 280, 345 |
| flavonols (360 nm) | | | | |
| **F1**, quercetin 3-O-rutinoside-7-O-glucoside | 19.7 | 771 | 609, 301 | 265, 365 |
| **F5**, quercetin 3-O-rutinoside | 38.4 | 609 | 301 | 265, 365 |
| hydroxycinnamic acid derivatives (320 nm) | | | | |
| **C6**, 5-O-caffeoylquinic acid | 16.5 | 353 | 191 | 330 |
| **C9**, ferulic acid | 19.1 | 175 | 169 | 330 |
| **C10**, sinapic acid | 20.6 | 205 | 189 | 330 |
| flavanones (280 nm) | | | | |
| **L10**, eriodictyol 7-O-rutinoside | 34 | 595 | 287 | 280 |
| **L11**, hesperetin 7-O-rutinoside | 50 | 609 | 301 | 280 |

**Table 3. Quantification (millgrams per 100 mL) of Different Phenolic Compounds Present in Lemon Juice Control, Berry Controls, and Blends[a]**

| | controls | | | | blends | | |
|---|---|---|---|---|---|---|---|
| | lemon | maqui 5% | açaí 5% | blackthorn 5% | LM 5% | LA 5% | LB 5% |
| **Anthocyanins** | | | | | | | |
| A1, delphinidin 3-O-sambubioside-5-O-glucoside | | 16.66 ± 0.37[b] | | | 16.52 ± 0.30[b] | | |
| A2, delphinidin 3,5-O-diglucoside | | | | | | | |
| A3, cyanidin 3,5-O-diglucoside | | 4.29 ± 0.14[b] | | | 4.53 ± 0.12[b] | | |
| A4, cyanidin 3-O-sambubioside-5-O-glucoside | | | | | | | |
| A5, delphinidin 3-O-sambubioside | | 2.22 ± 0.01 | | | 2.40 ± 0.04 | | |
| A6, delphinidin 3-O-glucoside | | 7.41 ± 0.07 | | | 7.44 ± 0.13 | | |
| A7, cyanidin 3-O-galactoside | | | 0.53 ± 0.01 | | | 0.54 ± 0.03 | |
| A8, cyanidin 3-O-glucoside | | | 0.18 ± 0.00 | 0.70 ± 0.09 | | 0.17 ± 0.00 | 0.64 ± 0.01 |
| A9, cyanidin 3-O-sambubioside | | 2.36 ± 0.03 | | | 2.49 ± 0.04 | | |
| A10, cyanidin-3-O-rutinoside | | | 1.26 ± 0.02 | 1.52 ± 0.16 | - | 1.11 ± 0.06 | 1.42 ± 0.04 |
| A11, cyanidin 3-O-glucoside-5-O-rhamnoside | | 0.52 ± 0.00 | | | 0.58 ± 0.01 | | |
| A12, peonidin 3-O-glucoside | | | | 0.24 ± 0.03 | | | 0.22 ± 0.00 |
| A13, peonidin 3-O-rutinoside | | | | 1.18 ± 0.09 | | | 1.09 ± 0.04 |
| A14, malvidin 3-O-glucoside | | | 0.08 ± 0.00 | | | | |
| total anthocyanins | | 33.45 ± 0.61 | 2.09 ± 0.00 | 3.64 ± 0.37 | 34.00 ± 0.18 | 1.93 ± 0.10 | 3.36 ± 0.09 |
| **Noncolored Flavonoids** | | | | | | | |
| flavones | | | | | | | |
| L1, apigenin 6,8-di-C-glucoside | 0.90 ± 0.10 | | | | 0.38 ± 0.01 | 0.76 ± 0.02 | 0.76 ± 0.02 |
| L2, diosmetin 6,8-di-C-glucoside | 7.02 ± 0.11 | | | | 5.49 ± 0.04 | 5.50 ± 0.18 | 5.71 ± 0.20 |
| L3, 8-C-glucosylchrysoeriol | nq | | | | nq | nq | nq |
| L4, diosmetin 7-O-rutinoside | 4.64 ± 1.64 | | | | 2.82 ± 0.19 | 2.37 ± 0.03 | 2.39 ± 0.25 |
| total flavones | 12.55 ± 1.85 | | | | 8.70 ± 0.22 | 8.63 ± 0.17 | 8.86 ± 0.02 |
| ellagic acid derivatives | | | | | | | |
| E1, granatin B | | 6.35 ± 0.19 | | | 6.57 ± 0.83 | | |
| E2, ellagic acid hexoside | | 0.73 ± 0.07 | | | 0.55 ± 0.02 | | |
| E3, ellagic acid rhamnoside | | 3.73 ± 0.75 | | | 2.27 ± 0.19 | | |
| total ellagic acid derivatives | | 10.81 ± 0.63 | | | 9.40 ± 1.00 | | |
| flavonols | | | | | | | |
| F1, quercetin 3-O-rutinoside-7-O-glucoside | nq | | | | nq | nq | nq |
| F2, myricetin 3-O-galoylglucoside | | 1.22 ± 0.14 | | | 0.71 ± 0.02 | | |
| F3, myricetin 3-O-galactoside | | 1.46 ± 0.19 | | | 1.17 ± 0.04 | | |
| F4, myricetin 3-O-glucoside | | 0.84 ± 0.10 | | | 0.45 ± 0.03 | | |
| F5, quercetin 3-O-rutinoside | 0.97 ± 0.04 | 2.38 ± 0.24 | | 1.11 ± 0.17 | 2.12 ± 0.04 | 0.81 ± 0.02 | 1.98 ± 0.04 |
| F6, quercetin 3-O-galactoside | | 2.36 ± 0.37 | 2.31 ± 0.07 | | 2.09 ± 0.08 | 1.68 ± 0.02 | |
| F7, quercetin 3-O-hexoside-5-O-pentoside | | | | 1.21 ± 0.15 | | | 1.38 ± 0.05 |
| F8, quercetin 3-O-glucoside | | 0.89 ± 0.11 | 0.50 ± 0.04 | | 0.76 ± 0.05 | 0.54 ± 0.07 | |
| F9, quercetin 3-O-xyloside | | 0.45 ± 0.02 | | 1.52 ± 0.21 | 0.60 ± 0.01 | | 1.51 ± 0.01 |
| F10, quercetin 3-O-arabinoside | | 0.95 ± 0.15 | 0.63 ± 0.00 | | 1.50 ± 0.15 | 0.71 ± 0.03 | |
| F11, quercetin 3-O-rhamnoside | | 2.41 ± 0.37 | | | 1.49 ± 0.30 | | |
| total flavonols | 0.97 ± 0.04 | 12.95 ± 1.69 | 3.45 ± 0.11 | 3.84 ± 0.55 | 10.87 ± 0.69 | 3.73 ± 0.13 | 4.88 ± 0.09 |
| hydroxycinnamic acid derivatives (320 nm) | | | | | | | |
| C1, caffeoyldihydrocaffeoylquinic acid (1) | | | | 1.96 ± 0.25 | | | 1.83 ± 0.01 |
| C2, caffeoyldihydrocaffeoylquinic acid (2) | | | | 1.78 ± 0.29 | | | 1.73 ± 0.01 |
| C3, 3-O-caffeoylquinic acid | | | 0.38 ± 0.03 | 31.26 ± 3.47 | | 0.47 ± 0.01 | 33.58 ± 0.05 |
| C4, 3-O-p-coumarylquinic acid | | | | 3.42 ± 0.45 | | | 3.15 ± 0.41 |
| C5, 4-O-caffeoylquinic acid | | | | 4.42 ± 0.60 | | | 4.04 ± 0.14 |
| C6, 5-O-caffeoylquinic acid | 0.28 ± 0.00 | 1.07 ± 0.02 | 1.36 ± 0.19 | | 1.58 ± 0.04 | 2.01 ± 0.02 | 0.45 ± 0.06 |
| C7, 3-O-feruloylquinic acid | | | | 1.44 ± 0.17 | | | 1.65 ± 0.05 |
| C8, 5-O-p-coumaroylquinic acid | | | 0.13 ± 0.01 | | | 0.07 ± 0.01 | |
| C9, ferulic acid | 0.91 ± 0.00 | | | | 1.09 ± 0.03 | 0.94 ± 0.01 | 1.03 ± 0.01 |
| C10, sinapic acid | 0.60 ± 0.01 | | | | 0.87 ± 0.06 | 0.62 ± 0.01 | 0.64 ± 0.01 |

**Table 3. continued**

| | controls | | | | blends | | |
|---|---|---|---|---|---|---|---|
| | lemon | maqui 5% | açaí 5% | blackthorn 5% | LM 5% | LA 5% | LB 5% |
| | | | | **Noncolored Flavonoids** | | | |
| total cinnamic acid derivatives | 1.79 ± 0.01 | 1.07 ± 0.02 | 2.25 ± 0.22 | 44.28 ± 1.77 | 3.54 ± 0.13 | 4.64 ± 0.03 | 47.64 ± 0.64 |
| flavanones (280 nm) | | | | | | | |
| **L10**, eriodictyol 7-O-rutinoside | 3.72 ± 0.09 | | | | 3.28 ± 0.10 | 3.04 ± 0.08 | 3.12 ± 0.09 |
| **L11**, hesperetin 7-O-rutinoside | 3.90 ± 0.38 | | | | 3.35 ± 0.09 | 3.33 ± 0.27 | 3.05 ± 0.11 |
| total flavanones | 7.63 ± 0.47 | | | | 6.63 ± 0.24 | 6.37 ± 0.35 | 6.17 ± 0.20 |

[a]Values are the mean ± standard deviation ($n = 3$). L, lemon juice; M, 5% maqui in citric acid; A, 5% açaí in citric acid; B, 5% blackthorn in citric acid; LM, 5% maqui in lemon juice; LA, 5% açaí in lemon juice; LB, 5% blackthorn in lemon juice; nq, not quantified. [b]Anthocyanins A1 + A2 and A3 + A4 were quantified together.

**Table 4. Antioxidant and Anticholinesterase (AChE and BChE) Activities of Control Fruits and Blends[a]**

| | IC$_{50}$ | | | | | |
|---|---|---|---|---|---|---|
| | DPPH$^\bullet$ | O$_2^{\bullet-}$ | HOCl | $^\bullet$OH | AChE | BChE |
| L | 13.27 ± 0.17 d | 5.24 ± 0.43 e | 25.71 ± 0.43 d | 6.93 ± 0.22 d | 13.18 ± 0.08 c | 12.82 ± 0.22 c |
| M | 9.06 ± 1.07 bc | 3.10 ± 0.13 bc | 40.49 ± 0.03 f | 4.34 ± 0.19 c | 21.76 ± 0.28 e | 19.39 ± 0.36 d |
| A | 18.91 ± 0.68 e | 3.78 ± 0.24 cd | 42.80 ± 0.29 g | 4.72 ± 0.21 c | 21.14 ± 0.38 e | 19.87 ± 0.43 d |
| B | 21.10 ± 0.58 f | 5.49 ± 0.19 e | 39.05 ± 0.63 e | 3.30 ± 0.09 ab | 19.51 ± 0.37 d | 19.13 ± 0.44 d |
| LM | 5.05 ± 0.14 a | 2.31 ± 0.14 a | 15.28 ± 0.06 a | 2.79 ± 0.08 a | 13.70 ± 0.13 c | 13.38 ± 0.19 c |
| LA | 7.39 ± 0.25 b | 4.42 ± 0.18 d | 18.84 ± 0.22 b | 2.85 ± 0.42 a | 8.83 ± 0.11 a | 8.61 ± 0.14 a |
| LB | 9.55 ± 0.83 c | 2.96 ± 0.30 ab | 21.90 ± 0.34 c | 3.49 ± 0.06 b | 10.44 ± 0.29 b | 10.40 ± 0.26 b |
| LSD, $p < 0.05$ | 0.51 | 0.20 | 0.29 | 0.17 | 0.21 | 0.25 |

[a]Results are expressed in IC$_{50}$ ($n = 3$, mg/mL). L, lemon juice; M, 5% maqui in citric acid; A, 5% açaí in citric acid; B, 5% blackthorn in citric acid; LM, 5% maqui in lemon juice; LA, 5% açaí in lemon juice; LB, 5% blackthorn in lemon juice. Means ($n = 3$) in the same columns followed by different letters are significantly different at $p < 0.05$ according to Tukey's test.

lower than that of pharmacological drugs used in neurological diseases, such as physotigmine[5] or galantamine,[44] this is a 100% natural food matrix with potential for daily consumption, without any side effects. Purified plant extracts also showed high activity, as in the case of methanolic extracts of *Lavanda viridis* (IC$_{50}$ = 0.25 and 0.29 mg/mL for AChE and BuChE, respectively),[45] or the hydroxymethanolic extract of *Spergularia rubra* (IC$_{25}$ = 3.68 and 4.29 mg/mL for AChE and BChE, respectively).[46] The cholinesterase inhibitory activity of natural products has been recently tested also in tomato seeds (IC$_{20}$ = 2.4 mg/mL),[27] with slightly lower values than in our samples being reported. These results are of interest for developing natural food products for dietary intervention on cholinesterases using lemon juice, maqui, açaí, and blackthorn.

In this study new blends of lemon juice and different exotic (maqui, açaí) and Iberian (blackthorn) berries with health-promoting activities were developed, and their phytochemical profiles were described, revealing a wealth of bioactive phenolics: anthocyanins, flavonols, hydroxycinnamic acid derivatives, and ellagic acid derivatives in berries and flavones, flavanones, flavonols, and hydroxycinnamic acids in lemon juice. The results of the different radical scavenging methods indicated that the lemon−maqui novel beverage (LM) is the most interesting blend in terms of antioxidant activity. With respect to cholinesterases, all of the samples showed inhibitory activity: the highest potential was found with lemon juice among controls, lemon−açaí (LA) being the most promising blend. These results are of interest for developing natural AChE and BChE inhibitors demanded for health and nutrition, with potential interest in the design of new drinks with a nutritive-related function on cognitive aging-related health conditions as Alzheimer's disease, Parkinson's disease, or senile dementia among others.

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