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A new drink rich in healthy bioactives combining lemon and pomegranate juices

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ABSTRACT

Nowadays, the interest in dietary antioxidants, mainly present in fruits and vegetables, has prompted research in the field of commercial polyphenol-rich beverages. The main objective of the present work was to produce new polyphenol-rich beverages using lemon and pomegranate juices in different proportions (at 25%, 50% and 75% for both juices). The bioactive composition (flavonoids and vitamin C) of the mixtures as well as its stability, antioxidant capacity and changes in colour over a 70 days storage period were studied. Our results suggest that the new drink made of 75% of pomegranate juice (PJ) and 25% of lemon juice (v:v), has potential for development of new healthy beverages or food products, emphasised by its high antioxidant capacity determined by its phenolic composition – punicalagin isomers, anthocyanins and vitamin C – and improved colour properties.

1. Introduction

Nowadays, the interest in the role of dietary antioxidants in human health has prompted research in the field of food science. Fruits are good sources of these bioactives, and there are a number of commercial polyphenol-rich beverages, which base their marketing strategies on antioxidant potency.

Pomegranate (*Punica granatum Linn.*) is a very rich source of anthocyanins (cyanidin 3,5-di and 3-*O*-glucoside, delphinidin 3,5-di and 3-*O*-glucoside, pelargonidin 3,5-di and 3-*O*-glucoside), ellagic acid, punicalagin isomers, different flavanols (catechins as catechins and epicatechin, and gallocatechins as gallocatechin and epigallocatechin) (Alighourchi, Barzegar, & Abbasi, 2007; García-Alonso, Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004; Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000; Kulkarni, Mahal, Kapoor, & Aradhya, 2007; Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000; Pérez-Vicente, Serrano, Abellán, & García-Viguera, 2004). In addition, malic and citric acid have been described as the most abundant acids, whilst oxalic, succinic and fumaric are present in lower amounts (Mirdehghan et al., 2006). Moreover, pharmacological activities, anti-inflammatory, hepatoprotective, as well as preventive effects on cancer, cardiovascular and neurodegenerative diseases are associated with these compounds (Faria, Monteiro, Mateus, Azevedo, & Calhau, 2007; Sartippour et al., 2008).

On the other hand, lemon fruit (*Citrus limon* (L.) Burm. f.) is also a rich source of nutrients, including flavonoids, citric acid, vitamin C and minerals (e.g. potassium), which provide numerous health promoting properties (Del Río et al., 2004; González-Molina, Moreno, & García-Viguera, 2008). Among flavonoids, hesperidin and eriocitrin (flavanones), together with small amounts of diosmetin 6,8-di-*C*-glucoside (diosmetin 6,8-diglc), diosmin and vicenin-2 (flavones) are the main compounds present (Gil-Izquierdo, Riquelme, Porras, & Ferreres, 2004; González-Molina et al., 2008; Peterson et al., 2006). Moreover, additional minor flavonoids, such as quercetin and myricetin (Hertog, Hollman, & Van de Putte, 1993), as well as other hydroxycinnamic acids (Gil-Izquierdo et al., 2004) are also known to be present in very low concentrations.

Following our research focused on providing an alternative use for these typical Mediterranean crops, including the second qualities and over-ripe pomegranates (Martí, Pérez-Vicente, & García-Viguera, 2001; Pérez-Vicente et al., 2004) other than fresh consumption, the design of new polyphenol-rich beverages combining lemon juice plus pomegranate juice was carried out in this work.

Lemon juice is widely used as an antioxidant natural substitute for the synthetic ascorbic or citric acids (E300 and E330, respectively) (Martí et al., 2001). Moreover, lemon juice could prevent browning reactions and colour deterioration of pomegranate juice (Özkan, 2002). The aim of the present work was to study the antioxidant activity, organoleptic properties and stability effects that can take place during storage due to the possible synergism/antagonism between the compounds present in these juices in order to design a new beverage with high nutritive value.

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2. Materials and methods

2.1. Reagents and standards

Sweet over-ripe pomegranate juice (PJ) from cv. 'Mollar' was purchased from Cítricos de Murcia, S.A. (Ceutí, Murcia, Spain). The juice was obtained by pressure with a laboratory pilot scale press (Zumonat C-40). This juice was stored frozen (−20 °C) until analysed.

Lemon juice (LJ) was obtained using a domestic squeezer ('Citromatic', Braun Española S.A., Barcelona, Spain) and lemons were collected from the CEBAS-CSIC's Experimental Farm ('La Matanza', Santomera, Murcia, SE Spain) of 'Fino' clones. The juice was stored frozen (−20 °C) until analysed.

Standards. Phenolic compounds were obtained commercially: cyanidin 3-glucoside (Polyphenols, Norway); hesperidin (Merck, Darmstadt, Germany); diosmin (Genay, France); 2,2-diphenyl-1-picryl-hydrazyl (DPPH·) (Sigma, Steinheim, Germany); gallic acid (Doesder. Chem. Co., Barcelona, Spain) and ellagic acid (EA) (Sigma St. Louis, USA). Other reagents were, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Fluka Chemika, Neu-Ulm, Switzerland); Folin–Ciocalteau's reagent (Sigma, Steinheim, Germany); sodium carbonate anhydrous (Panreac Quimica S.A., Barcelona, Spain); potassium dihydrogen phosphate (Panreac Quimica S.A., Barcelona, Spain); citric acid (Sigma, Steinheim, Germany); benzoic acid (Sigma, St. Louis, USA); dimethylsufoxide, 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 used was produced using a Millipore water purification system (Molsheim, France).

2.2. Experimental design

Lemon and pomegranate juices were thawed at room temperature and mixed in different proportions: 75%PJ + 25%LJ, 50%PJ + 50%LJ and 25%PJ + 75%LJ, keeping also two control solutions of 100% pomegranate juice (PJ100) and 100% lemon juice (LJ100). Homogenised mixtures and pure juices were centrifuged (10 min at 2894*g*), and benzoic acid (100 mg l^{-1}) was added in order to prevent spoilage (Martí et al., 2001). Juices and mixtures (8 ml) were placed in screw capped class tubes (10 ml) and, after removal of air under N_2 atmosphere, were stored in the dark at room temperature for 70 days. All analyses were done in triplicate, and the mean values were reported in each case. The analyses were carried out in triplicate every 7 days during the first 30 days, and every 15 days during the last 30 days for each mixture and juice.

2.3. pH, titratable acidity, and total soluble solids

pH, titratable acidity (TA), and total soluble solids (TSS) were evaluated as quality indexes. The pH values were measured using a pH-metre (GLP 21, Crison Ltd., Barcelona, Spain). The TA was determined by titrating 2 ml of the mixture (rising 60 ml final volume with Milli-Q water) with 0.1 N NaOH (pH 8.1). Results were expressed as g citric acid per 100 ml of sample, in accordance with AOAC (1984). The TSS contents were recorded in a refractometre (Abbe WYA-S, Optic Ivymen® System, Barcelona, Spain) at 20 °C with values being expressed as °Brix.

2.4. HPLC analysis, identification and quantification of anthocyanins and noncoloured phenolics

All samples were centrifuged during 5 min at 10,500*g* (model Sigma 1-13, B. Braun Biotech International, Osterode, Germany) at 4 °C. The supernatant (soluble fraction) was filtered through a 0.45 μm PVDF filter (Millex HV13, Millipore, Bedford, Mass, USA) before injection into the HPLC. For identification and quantification of anthocyanins the method previously reported by Pérez-Vicente et al. (2004) was followed. Each sample was analysed on a Merck-Hitachi L6200 liquid chromatograph (Tokyo, Japan), equipped with a Diode Array Detector UV–vis Shimadzu SPD-M6A (Kyoto, Japan) and an autoinjector (Gilson International, model 234, Barcelona, Spain). Chromatograms were recorded and processed on a LC Workstation Class M10A Shimadzu PC-based chromatography data system.

A 20 μl sample was analysed on a Lichrocart RP-18 reversed-phase column (250 × 4 mm, particle size 5 μm) with a precolumn C_{18} (Lichrocart® 4-4, Lichrospher® 100 RP-18 (5 μm)) from Merck (Darmstadt, Germany), using a mobile phase of 5% formic acid (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}. The linear gradient started with 1%B, keeping isocratic conditions during 5 min, reaching 20% B at 20 min, 40%B at 30 min, 95%B at 35 min and 1%B after 41 min. UV chromatograms were recorded at 280, 360 and 520 nm. The analyses were done in triplicate and results expressed as mean value. The different phenolics were characterised by chromatographic comparison with analytical standards, accordingly to previous reports (Gil-Izquierdo et al., 2004; Pérez-Vicente et al., 2004), and quantified by the absorbance of their corresponding peaks in the chromatograms. Anthocyanins were quantified as cyanidin 3-glucoside (detected at 520 nm); punicalagin isomers and ellagic acid as ellagic acid (at 360 and 280 nm, respectively); flavanones, as hesperidin (at 280 nm); and flavones, as diosmin (at 360 nm).

2.5. Extraction and analysis of vitamin C

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined by HPLC-UV as described elsewhere and fully detailed in González-Molina et al. (2008). The vitamin C content was calculated by the addition of ascorbic acid and dehydroascorbic acid contents, and results were expressed as mg per 100 ml.

2.6. Colour measurement

Colour measurement was determined as in Pérez-Vicente et al. (2004). Briefly, solutions were measured in glass cells of 2 mm path length (CT-A22) at 520 nm using a Minolta CM-508i® tristimulus colour spectrophotometer (Osaka, Japan) coupled with a CM-A760 transmittance adaptor, illuminant D65 and 10° observer according to the CIELAB 76 convention. Data (CIEL^*, a^* and b^*), were recorded and processed using the Minolta Software Chromacontrol S, PC-based colorimetric data system. *Hue angle* (H^*) was calculated from $\tan^{-1}$ (b^*/a^*), *Chroma* (C^*) from $(a^{*2} + b^{*2})^{1/2}$ and colour differences (ΔE^*) from $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

2.7. Determination of total phenols by Folin–Ciocalteau's reagent

Total phenol content was determined with the Folin–Ciocalteau method, adapted to a microscale by Arnous, Makris, and Kefalas (2001). In a 1.5-ml Eppendorf microtube, 790 μl of Milli-Q water, 10 μl of sample appropriately diluted with MeOH, and 50 μl of Folin–Ciocalteau reagent were added and vortexed. After exactly 1 min, 150 μl of aqueous 20% sodium carbonate were added, vortexed again and allowed to stand at room temperature in the dark, for 120 min. The absorbance was recorded at 750 nm, and quantified using gallic acid as a standard. Results were expressed as mg per 100 ml of gallic acid equivalents (GAE).