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Original Research Article

Phenolic compounds and antioxidant activity of Spanish commercial grape juices

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ABSTRACT

Evaluation of the antioxidant activity of components of the diet is important in order to establish healthy consumption patterns. Data are reported here on the antioxidant activity (FRAP and ABTS), of 20 commercial grape juices and 10 typical Spanish wines and on their content of total phenolic compounds, anthocyanins, flavonoids and 10 individual phenolic compounds (flavanols, benzoic acids and cinnamic acids, measured by HPLC-UV). Red grape juices had a significantly higher ($p < 0.05$) concentration of total phenols (1177 *vs.* 744 mg gallic acid/L), flavonoids (98 *vs.* 63 mg catechin/L) and a higher antioxidant activity (9.16 *vs.* 2.83 meq Trolox/L) in comparison to white grape juices. In comparison to the white wines, white grape juices contained more total phenols (744 *vs.* 286 mg gallic acid/L) and flavonoids (63 *vs.* 29 mg catechin/L) and evidenced higher antioxidant activity ($p < 0.05$). In comparison to the red wines, a lower content of total phenols (286 *vs.* 2358 mg gallic acid/L) and flavonoids (228 *vs.* 29 mg catechin/L) and an absence of anthocyanins were observed in the white wines, which are therefore less antioxidant. Although a two-fold higher concentration of antioxidant compounds was found in red wines than in red grape juices, the latter may be a good option for all age groups because of the absence of alcohol and the potentially beneficial health effects of their phenolic composition and elevated antioxidant activity.

1. Introduction

There has been a steady increase in juice consumption over recent years in Spain (Martín Cerdeño, 2008), which is now the second biggest consumer of grape juice in the world after the USA (L'OIV, 2003). There has also been a five-old increase in juice production in Spain over the past 20 years (MAGRAMA, 2011; L'OIV, 2006).

Red wine has been widely reported to have positive effects (Lachman et al., 2009) and to be more protective of health in comparison to other alcoholic beverages (Alen-Ruiz et al., 2009; Ignat et al., 2011), likely related to the effects on oxidative stress of its high polyphenol content. Positive health effects related to a reduction in oxidative stress have also been reported for non-alcoholic grape juice, whose consumption has been related to reduced ageing and a decrease in atherosclerosis, Parkinson's disease, cancer, and cataracts, among other diseases (Singletary et al., 2003; Park et al., 2009; Dani et al., 2009). The change in oxidative status induced by the juice may also favour a reduction in cardiovascular disease risk by exerting a preventive effect against LDL-c oxidation, endothelial function, atherothrombotic events, inflammatory cascade (Castilla et al., 2006) and hypertension (Mosele et al., 2012). Grape juice has also been reported to improve cognitive and motor function (Joseph et al., 2009; Krikorian et al., 2012). Some of these effects are related to protection against oxidative stress (Yuan et al., 2011), and the antioxidant activity of grape juices may be an indicator of the relative level of health benefit they offer.

Phenolic compounds are secondary plant metabolites that play a key role in the sensory and nutritional quality of fruits, vegetables and other plants (Ignat et al., 2011). These compounds and their antioxidant activity have long been associated with the beneficial effects of grapes and wines (Balasundram et al., 2006). Among phenolic compounds in grapes, catechin, gallic acid and anthocyanin have demonstrated anticancer activities, and flavanols may contribute molecules to anti-inflammation activities (Castilla et al., 2006). Catechin and gallic acid act as free radical scavengers, and epicatechin has also demonstrated antibacterial activity and protective effects against membrane oxidation (Xia et al., 2010). Stalmach et al. (2011) detected a wide array of metabolites and

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derivatives in plasma and urine samples after the absorption of various polyphenolic compounds. The same authors reported that the bioavailability of grape polyphenolic compounds was much greater for phenolic acids and aromatic compounds produced by colonic catabolism than for those produced by absorption in the upper gastrointestinal tract (Stalmach et al., 2013).

The concentrations of polyphenols in grape juices vary according to the species, ripeness, and culture conditions of the grapes and the technology applied to obtain the juice. Their concentrations also differ among grape tissues; thus, the pulp is rich in phenolic acids and the skin is rich in flavonoids (flavonols, flavanols and anthocyanins) (Naczk and Shahidi, 2006). Hence, commercially available grape juices vary widely in the number and type of phenolic compounds, and data on their individual phenolic profiles and associated health benefits are of major interest.

With this background, the objective of this study was to compare the polyphenol profile and antioxidant activity among grape juices commercially available in Spain and a representative sample of Spanish wines. The study followed the guidelines of the International Organisation of Vine and Wine (OIV), which promote the consumption of non-alcoholic grape derivatives, and the nutritional recommendations of the Mediterranean Diet, which promotes the consumption of fruits and vegetables rich in antioxidant substances.

2. Materials and methods

2.1. Samples

The study included 20 grape juices from the main brands available in Spanish supermarkets (9 red and 11 white) and 10 wines widely consumed in Spain (5 red [Rioja and Ribera del Duero] and 5 white [La Mancha]). Packets/bottles of each juice and wine were obtained from three different shops on different dates and each sample was individually analysed by triplicate.

2.2. Reagents

The reagents *p*-coumaric, *o*-coumaric, caffeic, vanillic and syringic acids, diethyl ether, glacial acetic acid, anhydrous sodium sulphate and methanol were from PANREAC Quimica SL (Barcelona, Spain). Reagents *m*-hydroxybenzoic and gallic acids, catechin, epicatechin, Folin-Ciocalteu reagent, sodium carbonate, 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), ferric chloride hexahydrate, sodium acetate, 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), chlorogenic and hydrochloric acids, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), sodium persulphate, sodium phosphate monobasic, sodium nitrite and aluminium chloride were from Sigma–Aldrich (Steinheim, Germany). All reagents were of analytical grade.

2.3. Phenolic compounds

2.3.1. Total phenolic compounds

The amount of total phenols was measured as the absorbance of diluted grape juice (in adimensional units) at 280 nm (Zamora, 2003). The Folin-Ciocalteu reference method(R(CEE) 2676/90), the most widely used approach for measuring total polyphenols (Minussi et al., 2003), was also applied, expressing the results as gallic acid equivalents (mg GAE/L); this method matches the chemistry of the FRAP assay because both are based on electron transfer (Huang et al., 2005).

2.3.2. Total anthocyanin content

The monomeric anthocyanin content of red grape juices was analysed using the pH-differential method of Giusti and Wrolstad (2001). Briefly, two solutions of each sample were prepared by mixing 100 μL of sample and 100 μL of either sodium acetate (0.4 M, pH 4.5) or potassium chloride (0.025 M, pH 1.0) buffers. After allowing the solutions to rest for 15 min, the absorbance was measured with a FLUOStar Omega microplate reader (BMG Labtech, Ortenberg, Germany) both at the maximum absorbance and at 700 nm. Results were expressed as mg cyanidin-3-glucoside/L of sample and obtained by using the following equation: cyanidin-3-glucoside (mg/L) = $(A \times MW \times DF \times 1000)/(\varepsilon \times 0.100)$, where A is $(\text{Absorbance}_{\lambda_{max}} - \text{Absorbance}_{700nm})$ at pH 1.0 minus $(\text{Absorbance}_{\lambda_{max}} - \text{Absorbance}_{700nm})$ at pH 4.5; MW is the molecular weight of cyanidin-3-glucoside; DF is the dilution factor; ε is the molar absorption coefficient of cyanidin-3-glucoside; and 0.100 is the microplate cell width.

2.3.3. Flavonoids content

Total flavonoid content was evaluated using a colorimetric assay with aluminium chloride (Zhishen et al., 1999) and was expressed as catechin equivalents (mg CE/L). A 10 μL aliquot of sample (appropriately diluted) was added to 200 μL of distilled water and 30 μL of a $NaNO_2$ solution (0.5 g/L) on a transparent 96-well polystyrene microplate (Biogen Científica, Madrid, Spain). After 5 min at 37 °C, 30 μL of a 1 g/L solution of $AlCl_3$ was added and, 6 min later, 20 μL of NaOH (1 mol/L) was added to the mixture. The solution was stirred and the absorbance was measured at 510 nm against a water blank on a FLUOStar Omega microplate reader (BMG Labtech, Germany).

2.3.4. Individual phenolic compounds

HPLC was used to identify the different phenolic compounds (Monedero et al., 1998). Samples were prepared by the conventional (discontinuous) extraction method (Diez and Gomez-Cordobes, 1980). One hundred mL of sample was extracted four times with ethyl ether and anhydrous sodium sulphate (used as desiccant when necessary). After vacuum drying, the resulting residue was brought to a constant volume with a methanol/water (1:1) solution and filtered through a 0.45 mm Waters Millipore membrane. Then, 15 μL was injected into a Varian liquid chromatograph equipped with UV/VIS detector (set at 280 and 320 nm), Varian Star 6.41 Chromatography Workstation and Gigabit Integrated Controller. A C-18 reverse-phase Synergy Hydro-RP80A column (25 cm × 0.46 cm, 4 μm internal particle size) was used to separate individual phenolic compounds: mobile phase A was water/glacial acetic acid (98:2) and mobile phase B was methanol/water/glacial acetic acid (60:38:2); compounds were eluted at a flow rate of 0.7 ml/min with the following elution programme: 0% B for 15 min, 100% B at minute 110, 100% B for 10 min and 0% B for 5 min. Chromatographic peaks were identified by comparing the retention time with the reference standards. Given the complexity of the sample, the external standard method was used for the quantification. Each wine and grape juice sample was measured in triplicate, and the results were expressed in mg/L. A full description of the method is given by Monedero et al. (1998).

2.4. Antioxidant activity

2.4.1. FRAP assay

The ferric reducing ability of each sample solution was estimated by using the procedure of Benzie and Strain (1996) adapted to a microplate reader as reported by Pastoriza et al. (2011). Briefly, 280 μL of FRAP reagent, freshly prepared and warmed at 37 °C, was mixed in each well of a transparent 96-well polystyrene microplate (Biogen Científica, Spain) with 20 μL sample or with water as blank reagent. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, plus 2.5 mL 20 mM $FeCl_3 \cdot H_20$ and 25 mL 0.3 M acetate buffer at pH 3.6. Readings at