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Characterization of pressurized hot water extracts of grape pomace: Chemical and biological antioxidant activity

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ABSTRACT

Pressurized hot water extracts obtained at different temperatures possess different compositions and antioxidant activities and, consequently, different bioactivities. We characterized two pressurized hot water extracts from grape pomace obtained at 100 °C (GPE100) and 200 °C (GPE200) in terms of antioxidant activity and composition, as well as protective effect on cell growth and mitochondrial membrane potential ($\Delta\psi_m$) in a HL-60 cell culture under oxidative conditions. GPE100 extracts were richer in polyphenols and poorer in Maillard reaction products (MRPs) than were GPE200 extracts. Moreover, hydroxymethylfurfural was detected only in GPE200. Both extracts exhibited similar protective effects on cell growth (comparable to the effect of trolox). In addition, GPE100 strongly decreased the $\Delta\psi_m$ loss, reaching values even lower than those of the control culture. This protective effect may be related to its high polyphenols content. At the highest concentration assessed, both extracts showed strong cytotoxicity, especially GPE200. This cytotoxicity could be related to their MRPs content.

1. Introduction

Pressurized hot water extraction (PHWE) is a highly promising energy-efficient and environmentally benign technique for recovering polyphenols from natural materials. Many of water's solvent properties can be manipulated to optimize phytochemical extraction by raising the temperature, while maintaining water in its liquid state by pressurization (Hawthorne, Miller, Lagadec, Hammond, & Clifford, 2002). Water polarity declines dramatically with increasing temperature, due to hydrogen bond breakdown, and reaches values comparable to organic solvent–water mixtures (Hawthorne et al., 2002). The decrease in viscosity and surface tension with rise of temperature improves the mass transfer rates of compounds from the plant tissue matrix (Hawthorne et al., 2002). Both temperature and pressure play significant roles in disrupting water surface equilibrium, thereby lowering the activation energy required for desorption processes (Ong, Cheong, & Goh, 2006).

However, diverse phenomena, highly dependent on temperature, occur during the PHWE of polyphenols from plant materials: polyphenol degradation and formation of polyphenol-derived antioxidants, selective polyphenol extraction, depolymerization, and polymerization (Ju & Howard, 2005; Vergara-Salinas et al., 2013), as well as the generation of antioxidant-potent Maillard reaction products (MRPs), such as melanoidins (Plaza, Amigo-Benavent, Castillo, Ibáñez, & Herrero, 2010) and hydroxymethylfurfural (He et al., 2012). In PHWE the relationship between polyphenol content and antioxidant activity of the extracts is weak or even inverse (Vergara-Salinas et al., 2013). Therefore, depending on the PHWE temperature used, it is possible to obtain extracts with different compositions and antioxidant activities and, consequently, different activities in biological systems.

The ability of chemical antioxidant activity assays to predict the *in vivo* activity is questionable because it does not take into account several physiological aspects, such as bioavailability and metabolism alterations (Liu & Finley, 2005). However, cell cultures are suitable for assessing the potential action of the antioxidants in a biological system, previous to animal and human studies (Liu & Finley, 2005). The human promyelocytic leukaemia line, HL-60, has been widely used to study oxidative stress-related aspects. This cell line is very sensitive to oxidative stress inducers, such as ultraviolet radiation and hydrogen peroxide (Verhaegen, McGowan, Brophy, Fernandes, & Cotter, 1995) and it is a good option for preliminary evaluation of the potential protective effect of antioxidant compounds.

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Grapes (*Vitis* spp.) are one of the largest fruit crops in the world and grape by-products are produced in massive quantities, especially by the winemaking industry (Djilas, Canadanovic-Brunet, & Cetkovic, 2009). After fermentation in wine production, most of the grapes' original polyphenolic content remains in the pomace (Vergara-Salinas et al., 2013). Polyphenols, such as anthocyanins and condensed tannins (proanthocyanidins), play significant roles in the health benefits of wine (Diebolt, Bucher, & Andriantsitohaina, 2001).

It has been reported that grape polyphenols, in cell line culture ranges, reduce oxidative stress, inhibit DNA damage induced by reactive oxygen species (ROS) (Apostolou et al., 2013) and activate the antioxidant response (Xia, Deng, Guo, & Li, 2010), but also exhibit a dose-dependent toxicity (Gao et al., 2009). This toxicity increases in the case of crude extracts (mixtures of many compounds) and it could be even higher in the pressurized hot water extracts due to the degradation and formation of new compounds (e.g. MRPs) at high temperatures. MRPs can suppress oxidative stress and inflammation in human cells cultures (Kitts, Chen, & Jing, 2012). However, these compounds are considered to be toxic and mutagenic (Husøy et al., 2008).

The objective of this study was to assess grape pomace extracts obtained at different extraction temperatures in terms of polyphenol and MRP contents, chemical antioxidant activity, and bioactivity on HL-60 cell line culture under oxidative conditions, including protective effect on cell growth and mitochondrial membrane potential.

2. Materials and methods

2.1. Chemicals and cell line

Reagents and standards used were of analytical grade. Folin–Ciocalteu reagent, methanol and sodium carbonate were purchased from Merck (Germany). Tripyridyl triazine (TPTZ), $FeCl_3(6H_2O)$, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), 2,5-dihydroxybenzoic acid, ascorbic acid, gallic acid, maleic acid-sodium dodecyl sulphate, triethanolamine, iron(III) chloride, bovine serum albumin, sodium hydroxide, hydrochloric acid (37%), glacial acetic acid, and sodium chloride, 5-hydroxymethylfurfural (5-HMF), RPMI-1640, D-glucose, L-glutamine and Human promyelocytic leukaemia cells HL-60 were obtained from Sigma (USA). Heat inactivated fetal bovine serum was obtained from Gibco (Brazil). 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethyl-benzimidazolyl-carbocyanine iodide (JC-1) was obtained from Life Technologies (USA). Valinomycin was obtained from Santa Cruz Biotechnology (USA).

2.2. Grape pomace

Cabernet Sauvignon pomace was obtained from Carmen Vineyard, Region Metropolitana, Chile. The pre-fermentation process was performed at 18 °C for 10 days and the must was loaded into a 10 m^3 fermentation tank. Fermentation was conducted between 25 °C and 30 °C for 21 days without pectolytic enzymes. The grape pomace sample was taken after the fermentation process had finished. Each sample was reduced to a particle size lower than 1 mm diameter by an Oster® blender (Sunbeam Products, Inc., Boca Raton, FL) and then frozen to −20 °C prior to extraction.

2.3. Pressurized hot water extraction

Grape pomace was subjected to pressurized hot water extraction (PHWE). A 5 g sample (dry weight) of grape pomace was mixed with 100 g of neutral quartz sand to avoid filter clogging in the 100 ml stainless steel extraction cell. The grape pomace was extracted in an Accelerated Solvent Extraction device (ASE® 150, Dionex) with approximately 50 ml of distilled and filtered (0.22 μm) water to obtain a matrix/extractant ratio of 1:10. The extractions were done in triplicate during 5 min at two temperatures: 100 (GPE100) and 200 °C (GPE200). After extraction, the cell contents were rinsed with 100 ml of distilled and filtered (0.22 μm) water and purged for 360 s by applying pressurized nitrogen (10.2 atm). Finally, the collected extracts were freeze-dried and stored in amber vials at −20 °C prior to analysis. Extract solutions of 1 g/l were prepared for analysis.

2.4. Ferric-reducing antioxidant power determination

The ferric reducing ability of plasma (FRAP) test offers a putative index of antioxidant reducing capacity in a sample. A working solution was prepared by mixing 300 mM acetate buffer (pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and a freshly prepared 20 mM $FeCl_3(6H_2O)$ solution in 10:1:1 (v/v/v) proportions. For the assay, 3 ml of working reagent were mixed with 100 μl sample or calibration standard (ascorbic acid), and absorbance was measured at 593 nm after a 30 min reaction time (Pulido, Bravo, & Saura-Calixto, 2000). A calibration curve was constructed, using ascorbic acid (0.1–0.8 mM). The regression coefficient of ascorbic acid was 0.9989. Results were expressed as ascorbic acid equivalents (AAE) per gramme of dry extracts (d.e.).

2.5. Total antioxidant determination by Folin assay

Total antioxidants were determined by Folin assay. Although this method is commonly considered for polyphenol analysis, it indeed determines all compounds in the sample with antioxidant capacity and not only polyphenols (Pérez-Jiménez, Neveu, Vos, & Scalbert, 2010). A mixture of 4.25 ml of phenolic extract (1 mg/ml) and 0.25 ml of Folin–Ciocalteu reagent was diluted 1:1 (v/v) with distilled water, and mixed with 0.5 ml of a 10% sodium carbonate solution (w/v). Absorbance was measured at 765 nm after a 1 h reaction time at room temperature. A calibration curve was constructed, using gallic acid as the calibration standard (20–90 mg/l). The regression coefficient of gallic acid was 0.9987. Results were expressed as gallic acid equivalents (GAE) per g of d.e.

2.6. Anthocyanins and tannin determination by Harbertson-Adams assay

Anthocyanin and condensed tannin contents in grape pomace extracts were determined with the Harbertson-Adams assay

Table 1
Characterization of the pressurized hot water extracts from grape pomace.[a]

Extract		GPE100	GPE200
FRAP (mg AAE/g d.e.)		10.2[b]	15.0[c]
Total antioxidants (mg GAE/g d.e.)		10.6[b]	13.6[c]
Anthocyanins (mg M3GE/g d.e.)		10.5	n.d.
Condensed tannins (mg EC/g d.e.)		52.9[b]	18.3[c]
(+)-Catechin (mg/g d.e.)		0.81[b]	0.65[c]
(−)-Epicatechin (mg/g d.e.)		1.05[b]	1.24[c]
Kaempferol (mg/g d.e.)		0.12[b]	0.03[c]
Myricetin (mg/g d.e.)		0.18[b]	0.16[c]
Resveratrol (mg/g d.e.)		0.02	n.d.
Σ Total polyphenols (mg/g d.e.)		65.58	20.38
MRPs by absorbance (AU)	360 nm	0.318[b]	0.371[c]
	420 nm	0.148[b]	0.281[c]
HMF (mg 5-HMF/g d.e.)		n.d.	0.087

M3GE, malvidin 3-*O*-glucoside equivalents; d.e., dry extract; EC, epicatechin equivalents; FRAP, ferric-reducing antioxidant power; AAE, ascorbic acid equivalents; GAE, gallic acid equivalents; MRPs, Maillard reaction products; AU, absorbance units; HMF, Hydroxymethylfurfural; n.d., not detected.

[a] Values with the same letter (b–c) in each row showed no statistically significant difference at the confidence interval of 95%.