Food Chemistry 156 (2014) 258–263

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Health benefits of wine: Don't expect resveratrol too much

Limin Xiang [a,b], Lingyun Xiao [b], Yihai Wang [a,b], Haifeng Li [b], Zebo Huang [b,c], Xiangjiu He [a,*]

[a] College of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China
[b] School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, China
[c] College of Life Science and Bio-pharmaceutical, Guangdong Pharmaceutical University, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 27 October 2013
Received in revised form 31 December 2013
Accepted 3 January 2014
Available online 18 January 2014

Keywords:
Red wine
Polyphenols
Resveratrol
Antioxidant activity
SH-SY5Y cell

ABSTRACT

Moderate consumption of red wine reduces the risk of heart disease and extends lifespan, which these healthy benefits are often attributed to its high antioxidant content. The relative contributions of wine polyphenols in healthy benefits were studied in this study. Among all wine polyphenols, caffeic acid was the richest one, while gallic acid showed the highest free radical scavenging activity. There was no significant difference between the prime red wine and the red wine adding 10-fold resveratrol on neuroprotective effects on SH-SY5Y cell line. The contribution percentage of resveratrol to the antioxidant activity of red wine was less than other tested polyphenols. It suggested that resveratrol may be negligible with respect to healthy benefits of red wine.

1. Introduction

Long-term, moderate consumption of red wine has reduced the incidence of many diseases, such as risk of coronary heart disease (Leikert, Rathel, Wohlfart, Cheynier, Vollmar, & Dirsch, 2002), atherosclerosis (Vinson, Teufel, & Wu, 2001), cancers (Middleton, Kandaswami, & Theoharides, 2000), etc. Recent evidence suggests that wine consumption is correlated with a reduction in the incidence of those neurodegenerative diseases associated to oxidative stress such as Alzheimer's disease, Parkinson's disease (Sun, Wang, Simonyi, & Sun, 2008).

Red wines are rich in polyphenols, such as phenolic acids (gallic acid, caffeic acid, *p*-coumaric acid, etc.), stilbenes (trans-resveratrol), flavonoids (catechin, epcatechin, quercetin, rutin, myricetin, etc.) (Kammerer, Claus, Carle, & Schieber, 2004). Resveratrol has been considered as the major functional constituent in red wine, which could prevent or slow the progression of a wide variety of illnesses, including cancer (Jang, Cai, Udeani, Slowing, Thomas, Beecher, et al., 1997), cardiovascular disease (Bradamante, Barenghi, & Villa, 2004) and ischemic injures (Sinha, Chaudhary, & Gupta, 2002), as well as enhance stress resistance and extend the lifespans of various organism from yeast (Howitz, Bitterman, Cohen, Lamming, Lavu, Wood, et al., 2003) to vertebrates (Baur & Sinclair, 2006; Valenzano, Terzibasi, Genade, Cattaneo, Domenici, & Cellerino, 2006). However, compared with other polyphenols, the concentrations of resveratrol is low in red wine (Gerogiannaki-Christopoulou, Athanasopoulos, Kyriakidis, Gerogiannaki, & Spanos, 2006). How many real benefits can we get from resveratrol when we drink a cup of red wine (Corder, Mullen, Khan, Marks, Wood, Carrier, et al., 2006)? Here we demonstrate that resveratrol is negligible in the health benefits of red wine.

2. Materials and methods

2.1. Chemicals

HPLC grade methanol was purchased from Hanbon Sci. & Tech. (Jiangsu, China). Standard chemicals of gallic acid, caffeic acid, syringic acid, *p*-coumaric acid were purchased from Aladdin Chemical Co., Ltd. (Shanghai, China). Polydatin was purchased from Shanghai Tauto Biotech. Co., Ltd. Shanghai, China. Resveratrol, quercetin, protocatechuic acid, and β-D-glucosidase were purchased from Sigma–Aldrich Chemical Co., (St. Louis, MO, USA). DPPH (1,1-diphenyl-2-picrylhydrazyl) in free radical form was a product of Aladdin Chemical Co., Ltd. (Shanghai, China). PMS (Phenazine methosulfate) was obtained from J&K Chemical Ltd. (Shanghai, China). NADH (β-nicotinamide adenine dinucleotide), NBT (nitroblue tetrazolium chloride), ethyl acetate and all other reagents ($Na_2WO_4{\cdot}2H_2O$, $Na_2MoO_4{\cdot}2H_2O$, Na_2CO_3, HCl, $LiSO_4{\cdot}H_2O$, H_3PO_4, and Br_2) were purchased from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). 3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide (MTT) was purchased from Amersco (Solon, OH, USA). Water was double distilled water by automatical double pure water distillatory (Shanghai, China).

* Corresponding author. Tel./fax: +86 20 3935 2132.
E-mail address: hexiangjiu@hotmail.com (X. He).

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http://dx.doi.org/10.1016/j.foodchem.2014.01.006

2.2. Wine sample

Five wine samples, CHANGYU (CH), DYNASTY (DY), FRENCH PARADOX (FR), GREAT WALL (GR), and STONE CELLARS (ST) were purchased from Carrefour supermarket (Wuhan, China). The samples were opened, stored in darkness at 4 °C. Analyses were carried out within 2 weeks.

2.3. Preparation of wine sample

2.3.1. Wine sample for HPLC

After added 20 mg β-D-glucosidase, 5 mL wine sample in 20 mL tube was shaken at 45 °C for 20 h. Under this condition, the polydatin in red wine was converted to resveratrol completely. Then, hydrolyzed wine was extracted with 5 mL ethyl acetate for 3 times. The organic phase was evaporated to dryness by rotary evaporation at 35 °C. The dried extract was dissolved in 1 mL 30% methanol aqueous solution. 20 μL of the aliquot was injected into the HPLC system.

2.3.2. Wine sample for cell viability assay

100 mL wine sample was freeze-dried and weighed. These extracts were stored at 4 °C for assay.

2.4. Preparation of standard solutions

The standard mixtures were dissolved in methanol (HPLC grade) to a concentration of 1 mg mL^{-1} and were stored in darkness at −20 °C until analyzed. Prior to analysis, methanolic solution was diluted with water to at least five concentration levels. 20 μL of the aliquot was injected into the HPLC system (Porgali & Buyuktuncel, 2012).

2.5. Quantitation of individual polyphenols

The individual polyphenols was analyzed and quantified by HPLC using a waters 600 instrument equipped with a photodiode array detector and empower software (Milford, MA, USA). Separations were performed at 40 °C with a Sepax Amethyst C18 column (5 μm, 4.6 × 250 mm). Gradient elution with two solvents was used: solvent **A** consisted of methanol: water: acetic acid (5:94.5:0.1, v/v) and solvent **B** was methanol: acetic acid (99.9:0.1, v/v). The following linear gradient of eluents was used: 10% **B** initially, 10–35% **B**, 0–35 min; 35–50% **B**, 35–50 min; 50–10% **B**, 50–55 min. The system was equilibrated using the starting conditions for 10 min prior to injection of the next sample. The flow rate was 0.8 mL/min. The detection wavelength was 254 nm for caffeic acid, syringic acid and quercetin, 306 nm for gallic acid, protocatechuic acid, *p*-coumaric acid, and resveratrol. Polyphenols were identified by comparing retention times and UV spectral data with those of pure standards. Quantification was carried out with the external standard method.

2.6. Determination of total phenolic content

The total phenolic contents of wine were measured using a modified colorimetric Folin–Ciocalteu method (Wolfe, Wu, & Liu, 2003), using resveratrol as the standard. 0.25 mL of a suitable diluted wine sample and 1.0 mL water were added to a test tube. Then 0.25 mL Folin–Ciocalteu reagent (diluted with water 1:1, v/v) was added to the solution and mixed well and allowed to react for 6 min. Then, 2.5 mL of 7% sodium carbonate (Na_2CO_3) solution and 2.0 mL water was added to the mixture to a final volume of 6 mL and mixed well. After incubation for 90 min at room temperature in the dark, the absorbance was measured at 760 nm on a UV–vis spectrophotometer (UV-1700, Shimadzu, Japan). Folin–Ciocalteu reagent was substituted by the same volume of distilled water in the blank. Total phenolic content of red wine was expressed as milligrams of resveratrol equivalent per 1 L wine (mg of RE/L) through the calibration curve with resveratrol. Data were reported as mean ± SD for triplicate measure ments.

2.7. Determination of antioxidant activity

2.7.1. DPPH scavenging activity

The capacity of sample to scavenge DPPH· radical was assessed as previously reported with some modification (Cheel, Van Antwerpen, Tumova, Onofre, Vokurkova, Zouaoui-Boudjeltia, et al., 2010). Briefly, 200 μM solution of DPPH in methanol was prepared fresh before used. Then 100 μL of the solution was mixed with 100 μL of tested sample in a 96-well flat-bottom plate. After 30 min of incubation at 37 °C in the dark, the absorbance was measured at 546 nm using a Microplate reader (Kehua Technologies, Inc., Shanghai, China). The DPPH· radical-scavenging activity was calculated as follows:

$$\text{Inhibition } \% = [(E - S)/(E)] \times 100,$$

where $E = A - B$ and $S = C - D$; A, absorbance of the control; B, absorbance of the control blank; C, absorbance of the sample; D, absorbance of the sample blank.

Gallic acid was used as a reference compound. The IC_{50} (the inhibitory concentration at which there is 50% reduction of free radical) of each sample (red wine, red wine adding 10-fold resveratrol extra, all polyphenols tested) was determined. The values were reported as mean ± SD for triplicate sample.

2.7.2. Superoxide anion radical-scavenging activity

Superoxide radical were generated by the NADH/PMS system according to a reported protocol (Valentao, Fernandes, Carvalho, Andrade, Seabra, & Bastos, 2001). The reaction mixtures in the sample wells consisted of NADH (166 μM), NBT (43 μM), PMS (2.7 μM) and test sample in a final volume of 0.8 mL. NADH and NBT and PMS were dissolved in Tris HCl buffer (50 mM, pH 8.1). Samples were dissolved in methanol and diluted with water to at least five concentrations. The reaction was started by the addition of PMS and conducted for 5 min at room temperature. The absorbance was read at 546 nm using a Microplate reader (Kehua Technologies, Inc., Shanghai, China). The scavenging effect was calculated as follows:

$$\text{Inhibition } \% = [(E - S)/(E)] \times 100,$$

where $E = A - B$ and $S = C - D$; A, absorbance of the control; B, absorbance of the control blank; C, absorbance of the sample; D, absorbance of the sample blank.

Gallic acid was used as a reference compound. The IC_{50} (the inhibitory concentration at which there is 50% reduction of free radical) of each sample (red wine, red wine adding 10-fold resveratrol extra, all polyphenols tested) was determined. The values were reported as mean ± SD for triplicate sample.

2.8. Cell viability assay

Human neuroblastoma cell SH-SY5Y was obtained from American Type Culture Collection (ATCC) and maintained in an atmosphere of 5% CO_2 and 95% air at 37 °C in DMEM/F12 (1:1) medium supplemented with 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT, USA). Cell viability assay was determined by MTT assay as described (Zhang, Yu, Sun, Lin, Chen, Tan, et al., 2007). Briefly, SH-SY5Y was seeded at a density of 2×10^4 cells per well in 96-well plate, grown for 24 h, and then treated with different concentration of wine extracts and 100 μM H_2O_2 for 24 h.