Food Research International 99 (2017) 571–576

Contents lists available at ScienceDirect

Food Research International

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

Evaluation of natural occurring bioactive compounds and antioxidant activity in Nuragus white wines

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ARTICLE INFO

Keywords:
Polyphenols
Antioxidants
Colour
Wine analysis
Identification
Quantification

ABSTRACT

The aim of the present study is to highlight volatile and targeted non-volatile bioactive compounds in Nuragus wines, as a part of Italian DOC (Controlled Origin Designation) white wines. So far there has not been any systematic study of the chemical compositions and antioxidant activity of this monovarietal wine. Phenolic compounds, volatiles and organic acids were analysed and antioxidant capacity was assessed by spectrophotometric assays. Chromaticity coordinates and technological parameters (alcohol, reducing sugars, pH, total and volatile acidity) were also evaluated. Gallic acid (128 ± 87 mg/L), *trans*-caftaric acid (81 ± 27 mg/L) and tyrosol (25 ± 8 mg/L) were the most abundant phenolic compounds. The major headspace volatiles were isoamyl alcohol (35.8–76.6%) and 2-phenylethanol (5.9–24.9%). In the wine extracts, the most abundant were 2-phenylethanol (12.3–40.0%), 4-hydroxy-2-phenylethanol (12.5–33.3%), diethyl succinate (5.8–30.3%), (*Z*)-octadec-9-en-1-ol (5.9–18.3%) and tryptophol (2.8–15.6%). Nuragus wines exhibited an excellent antioxidant capacity. The data obtained may help Nuragus wine producers to promote this monovarietal wine as a valid complement associated with the Mediterranean diet.

1. Introduction

Moderate consumption of white wine has been associated with several health benefits, taking into account the intake of phenolic substances that are responsible for many of their positive effects (Artero, Artero, Tarín, & Cano, 2015; Basli et al., 2012). Phenols are often less concentrated in white wines than red wines, because of the cultivar and the technological processes involved in their production (Lamuela-Raventós & de la Torre-Boronat, 1999). Nevertheless, they usually show antioxidant capacities similar to red wines, due to the notable activity of several compounds which are also largely present in white wines (Vinson & Hontz, 1995). Among these compounds, hydroxybenzoic and hydroxycinnamic acids, as well as flavonols (in particular quercetin) are widely recognized as the most effective in deactivating free radical species (Chaillou & Nazareno, 2006) which are linked to many diseases (Loperena & Harrison, 2017).

Recent archaeological research carried out in the centre of Sardinia (Italy) showed that white wines were already being produced over 3000 years ago. Indeed, recent excavations have brought to light grape seeds from white cultivars of *Vitis vinifera* which had been cultivated since the Bronze Age (*ca.* 1200 BCE), particularly in the pre-Nuragic and Nuragic periods (Ucchesu, Peña-Chocarro, Sabato, & Tanda, 2015; Ucchesu et al., 2014). One of these cultivars would be the Nuragus, which probably takes its name from the ancient Nuragic buildings. It is one of the popular, traditional white wines of Sardinia, and is produced from grapes of the same variety grown in the South-central part of Sardinia (DOC - Denominazione di Origine Controllata. Disciplinare del Nuragus di Cagliari, 2011). This wine possesses a delicate and pleasant smell, while the flavour is distinctive, going from dry to sweet, depending on the area where the grapes are grown (DOC - Denominazione di Origine Controllata. Disciplinare del Nuragus di Cagliari, 2011; Nieddu, 2011). Although this wine is labelled as DOC (Controlled Origin Designation) at the European level (E-Bacchus, 2007), it is relatively unknown beyond the borders of Italy. Moreover, the chemical characteristics of Nuragus white wines have not yet been explored. In particular, the description of its phenolic profile, which is strongly linked to the antioxidant capacity, could highlight its health benefits. The evaluation of its natural constituents and other properties could be

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http://dx.doi.org/10.1016/j.foodres.2017.06.038
Received 22 March 2017; Received in revised form 9 June 2017; Accepted 17 June 2017
Available online 19 June 2017

useful tool for Nuragus promotion.

Therefore, the goal of the present research was the analysis of the phenolic compounds, volatiles and organic acids profiles of this wine for the first time. In addition, several chemical-physical properties were determined and its total phenols content and antioxidant activity was evaluated by Folin-Ciocalteu's, FRAP (ferric ion reducing antioxidant power) and DPPH$^{\bullet}$ assays, as well as its correlation with the concentration of specific polyphenols.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, Folin-Ciocalteu's phenol reagent, Na_2CO_3, anhydrous $MgSO_4$, NaCl, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), ferric chloride hexahydrate, ferrous sulphate heptahydrate, methanol, orthophosphoric acid 85%, acetic acid, CH_2Cl_2, and acetonitrile were obtained from Sigma-Aldrich (Milan, Italy). Standards of phenolic compounds were purchased from Extrasynthese (Genay, France). All the chemicals used in this study were of analytical grade. Ultrapure water (18 MΩ·cm) was obtained with a Milli-Q Advantage A10 System apparatus (Millipore, Milan, Italy).

2.2. Samples

The Nuragus wine specimens (n = 22) used in this study were commercially available, with certified origins and collected directly from the wineries (Table A.1, supplementary material). Each specimen represented a single batch and was sampled in triplicate in 0.75 L dark glass bottles. Wines were obtained from Nuragus grapes harvested in 2014 and processed according to the traditional oenological processing techniques of Nuragus wines. The five wineries that provided the samples together account for almost 85% of the production of Nuragus wines in Sardinia (Italy). All samples were collected in the second week of February 2015, stored at 8 ± 1 °C and analysed within 3 months. Before analysis, the samples were filtered through an Econofilter RC membrane (0.45 μm, Ø 25 mm, Agilent Technologies, Milan, Italy).

2.3. Headspace solid-phase microextraction (HS-SPME) and solvent extraction

The headspace extraction was performed using a manual solid-phase microextraction (SPME) holder using divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco Co., Bellefonte, PA, USA). The fibre was conditioned according to the instructions by Supelco Co. For HS-SPME, the samples (5 mL) were placed separately in 15 mL glass vials, 2 g of NaCl was added, a magnetic stir bar was inserted, and they were hermetically sealed with PTFE/silicone septa and were maintained in a water bath at 60 °C during the equilibration (15 min) and extraction (45 min). All of the experiments were performed under constant stirring (1000 rpm) with a magnetic stirrer. After sampling, the SPME fibre was withdrawn into the needle, removed from the vial, and inserted into the injector (250 °C) of the GC-FID and GC–MS for 6 min where the extracted volatiles were thermally desorbed directly to the GC column.

For the solvent extraction, each sample (5 mL) was extracted three times with dichloromethane (2 mL) in the test-tube (10 mL) with PTFE/silicone cover and the extracts were combined and dried over anhydrous $MgSO_4$. The combined extracts were concentrated up to 0.3 mL by distillation through Vigreux column and 3 μL were used for GC-FID and GC–MS analyses.

2.4. GC-FID and GC–MS analyses

The GC-FID analyses were carried out with an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7890A equipped with a flame ionization detector (FID) and a HP-5MS capillary column (5% phenyl-methylpolysiloxane, Agilent J and W). The GC conditions were similar to those described previously (Jerković, Tuberoso, Kasum, & Marijanović, 2011). In brief, the oven temp. was programmed isothermally at 70 °C for 2 min, increasing from 70 to 200 °C at 3 °C/min, and held isothermally at 200 °C for 15 min; carrier gas, He (1.0 mL/min). The GC–MS analyses were performed using an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7820A equipped with a mass selective detector (MSD) model 5977E (Agilent Technologies) and a HP-5MS capillary column, under the same conditions as described for the GC-FID analysis. The MSD (EI mode) was operated at 70 eV, and the mass range was 30–300 amu, as previously reported (Jerković et al., 2011).

The identification of the volatile constituents was based on the comparison of their retention indices (RI), determined relative to the retention times of a homologous series of *n*-alkanes (C_9–C_{25}), with those reported in the literature and their mass spectra with authentic compounds available in our laboratories or those listed in Wiley 9 (Wiley, New York, NY, USA) and NIST 14 (National Institute of Standards and Technology, Gaithersburg, USA) mass spectral libraries. The percentage composition of the samples was computed from the GC peak areas using the normalization method (without correction factors).

2.5. HPLC-DAD determination of phenolic compounds

Detection and quantitative analysis of phenolic compounds and xanthine was carried out using an HPLC-DAD method as described by Tuberoso, Serreli, Congiu, Montoro, and Fenu (2017). An HPLC Varian system ProStar was employed, fitted with a pump module 230, an autosampler module 410, and a ThermoSeparation diode array detector SpectroSystem UV 6000lp (ThermoSeparation, San Jose, CA), set at 280, 360 and 520 nm. The separation was obtained with a Kinetex C18 column (150 × 4.60 mm, 3 μm, Phenomenex, Casalecchio di Reno, BO, Italy) using 0.22 M phosphoric acid (solvent A), and acetonitrile (solvent B) at a constant flow rate of 1.0 mL/min. The gradient (v/v) was generated decreasing from 100% solvent A to 80% in 20 min, and then remained stable up to 40 min; to 10% in 50 min; and to 100% in 55 min. The injection volume was 10 μL.

The chromatograms and spectra were elaborated with a ChromQuest V. 2.51 data system (ThermoQuest, Rodano, Milan, Italy). Flavonols were detected and quantified at 360 nm, hydroxycinnamic acids at 313 nm, and all other compounds at 280 nm. Non-commercial hydroxycinnamic acids analysed (*cis*-caftaric acid, 2-S-glutathionyl caftaric acid (GRP), *trans*-coutaric acid and *trans*-fertaric acid) were tentatively identified according to their order of elution and the retention times of pure compounds (caffeic, coumaric and ferulic acids) and comparison with different studies (Makris, Psarra, Kallithraka, & Kefalas, 2003; Mitić, Obradović, Grahovac, & Pavlović, 2010).

Standard solutions were prepared in methanol, and the working standard solutions in ultrapure water. Quantification of non-commercial hydroxycinnamic acids was made using the calibration curves belonging to the most similar compounds: caffeic acid for *cis*-caftaric acid and GRP, *p*-coumaric acid for *trans*-coutaric acid and ferulic acid for *trans*-fertaric acid. The calibration curves were built with the external standard method, correlating the area of the peaks *vs.* the concentration. The correlation values were 0.9989–0.9999 in the range of 0.5–20 mg/L. The samples were diluted with ultrapure water (1:5, v/v) and injected in HPLC without any further purification. The full validation procedure in agreement with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidance note which describes