Food Chemistry 145 (2014) 327–334

Contents lists available at ScienceDirect

Food Chemistry

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

Assessment of pomegranate wine lees as a valuable source for the recovery of (poly)phenolic compounds

Pedro Mena [a,1], Juan A. Ascacio-Valdés [b,1], Amadeo Gironés-Vilaplana [a], Daniele Del Rio [c], Diego A. Moreno [a], Cristina García-Viguera [a,*]

[a] Department of Food Science and Technology, CEBAS-CSIC, P.O. Box 164, E-30100, Espinardo, Murcia, Spain
[b] Department of Food Science and Technology, Universidad Autónoma de Coahuila, 25280 Saltillo, Coahuila, Mexico
[c] The φ^2 Laboratory of Phytochemicals in Physiology, Department of Food Science, University of Parma, 43125 Parma, Italy

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form 19 July 2013
Accepted 1 August 2013
Available online 17 August 2013

Keywords:
Punica granatum L.
Ellagitannin
Anthocyanin
Mineral
Yeast

ABSTRACT

Wine lees are the residue formed at the bottom of recipients containing wine after fermentation and are mainly composed of yeast. They can be applied for the recovery of value-added phytochemicals owing to the ability of yeast to form molecular interactions with (poly)phenolic compounds. This study aimed to evaluate the potential use of lees obtained as by-products after winemaking of pomegranate (*Punica granatum* L.) juice. Pomegranate wine lees showed high both phenolic content (about 30 mg GAE/g dry matter) and antioxidant capacity by DPPH. and $ABTS^{\cdot+}$ assays. The phytochemical screening of this by-product by UHPLC–ESI-MS^n allowed the identification of up to 39 chemicals, being hydrolysable tannins and anthocyanins the predominant structures. Ellagic acid and gallic acid were present in high amounts. In addition, mineral composition was also assessed. Overall, pomegranate wine lees resulted in a promising source for the recovery of bioactive polyphenols with potential applications in different industrial fields.

1. Introduction

Food grade yeast is used as an excellent source of protein, B vitamins, and some essential minerals, with applications in the agri-food and health industries as nutritional supplements, food additives, and livestock feeds. *Saccharomyces cerevisiae* yeast biomass is a major by-product from breweries and wineries that can be used to produce yeast protein concentrates while still retaining their functional properties and nutritive values (Bekatorou, Psarianos, & Koutinas, 2006). Actually, commercial, nutritional brewer's yeast is recommended as a dietary supplement generally recognised as safe (GRAS) for healthy hair and nails.

Wine lees are the residue formed at the bottom of recipients containing wine after fermentation or during storage. Lees are mainly composed of yeast used for winemaking and, in a minor proportion, of organic molecules (such as tartaric acid in grape wine lees) and inorganic matter (Martínez-Rodríguez & Polo, 2000). Lees play a major role in wine processing as they interact with (poly)phenolic compounds directly related to the colour and other organoleptic properties (Mazauric & Salmon, 2005). Actually, lees yeast can form molecular interactions with (poly)phenolic compounds and adsorb them (Mazauric & Salmon, 2006). Moreover, lees liberate enzymes favouring the hydrolysis and transformation of (poly)phenolic substrates in phenolics with high added-value and interest like gallic acid or ellagic acid (Vattem & Shetty, 2003). Attending to these properties related to wine lees, they could be applied for the adsorbance and recovery of (poly)phenolic compounds with potential use for the food, nutraceutical, cosmetic, and pharmaceutical industries presented and/or formed during winemaking.

A dietary source with high content in diverse bioactive phytochemicals is pomegranate (*Punica granatum* L.). Pomegranate fruit contains anthocyanins, ellagitannins, gallotannins, non-coloured flavonoids, and lignans, among others (poly)phenolic compounds (Bonzanini, Bruni, Palla, Serlataite, & Caligiani, 2009; Fischer, Carle, & Kammerer, 2011; Mena et al., 2012a). This particular phytochemical profile has been associated with the broad array of biological properties of pomegranate products (Mena, Gironés-Vilaplana, Moreno, & García-Viguera, 2011b), turning this fruit into a product of growing interest for consumers and industry. Pomegranates fruits are usually earmarked for arils fresh consumption, juices, jams, and dried arils. Moreover, the elaboration of pomegranate wine has recently been pointed out as a novel use for pomegranate. Significant compositional changes take part during

* Corresponding author. Address: Phytochemistry Lab, Department of Food Science and Technology, P.O. Box 164, E-30100, Espinardo, Murcia, Spain. Tel.: +34 968 396304; fax: +34 968 396213.
E-mail addresses: pmena@cebas.csic.es (P. Mena), jaav2_07@hotmail.com (J.A. Ascacio-Valdés), agvilaplana@cebas.csic.es (A. Gironés-Vilaplana), daniele.delrio@unipr.it (D. Del Rio), dmoreno@cebas.csic.es (D.A. Moreno), cgviguera@cebas.csic.es (C. García-Viguera).
[1] These two authors have contributed equally to the present work.

0308-8146/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.foodchem.2013.08.039

pomegranate winemaking processes, resulting in wines with a promising phytochemical profile (Mena, Gil-Izquierdo, Moreno, Martí, & García-Viguera, 2012b; Mena, Gironés-Vilaplana, Martí, & García-Viguera, 2012c). Likewise, pomegranate wine lees (PWL) are generated as by-products and their exploitation may allow more profits along with a sustainable use of wastes.

The aim of the present study was to evaluate the phytochemical characteristics of PWL as potential source for the recovery of high added-value compounds and as nutraceutical supplement. For that, the (poly)phenolic profile by UHPLC–ESI-linear ion trap mass spectrometer (UHPLC–ESI-MS) and antioxidant activities of this novel dietary product together with its mineral composition were assessed and compared with common yeast.

2. Materials and methods

2.1. Chemicals and reagents

2,2-diphenyl-1-picryl-hydrazyl (DPPH**.**), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), ellagic acid, and Folin–Ciocalteau's reagent were purchased from Sigma–Aldrich (Steinheim, Germany); formic acid, methanol, and sodium carbonate anhydrous, all of analytical grade, from Panreac Química SA (Barcelona, Spain). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and gallic acid from Fluka Chemika (Neu-Ulm, Switzerland) were used as standards for spectrophotometric assays. Milli-Q water used was produced using an Elix®3 Millipore water purification system coupled to a Milli-Q module (model Adventage10) (Molsheim, France). Yeast, *S. cerevisiae* var.*bayanus*, (Awri R2; Mauri Yeast Australia, Toowoomba, Queensland, Australia), potassium metabisulfite, and a fermentation activator containing ammonium phosphate, Actimax Plus (Agrovin, Alcázar de San Juan, Ciudad Real, Spain), were used for winemaking.

2.2. Pomegranate winemaking and lees recovery

Second quality pomegranate fruits from cv. *Wonderful*, harvested in Alicante region (SE Spain), were provided by "Cambayas Coop. V." (Elche, Alicante, Spain). Pomegranates were cut, deseeded, and juice was obtained by pressure with a screw press.

Pomegranate wine was produced in accordance with (Mena et al., 2012c). Juice was placed in a 50 L steel vessel where 60 mg/L of potassium metabisulfite and 200 mg/L of Actimax Plus were added as recommended by the producer to protect the juices and to favour yeasts activity. Next, fermentation was started after yeasting (300 mg/L) and the temperature was kept at 19 °C ± 1 during the fermentation process (6 days). Once fermentation was finished, the wine was racked for one day at 4 °C. Then, the wine was transferred to new vessels and the lees along with residual wine were collected. Wine elaboration was done in triplicate.

The PWL where separated from residual wine by centrifugation. For that, lees with residual wine were placed in Falcon tubes (50 mL of capacity) and centrifuged at 3100 rpm by 10 min. Then, the supernatants where decanted and the pellets recovered. This recovered material was freeze-dried in order to eliminate undesirable liquid material and grounded to obtain the samples as a powder.

2.3. Extraction of phenolic compounds from PWL

For the extraction of bioactive phytochemicals, powdered lyophilized lees (0.2 g) were placed in Eppendorf tubes (2 mL of capacity) and extracted with 2 mL of MeOH 70% (v/v). Yeast used for winemaking, *S. cerevisiae* var. *bayanus*, was used as control and was treated in the same way as the samples. Then, all tubes were vortex and placed in a sonicator bath for 10 min to improve the methanol extraction. All the tubes were centrifuged at 10,480 g (model EBA 21, Hettich Zentrifugen, Tuttlingen, Germany) during 5 min at room temperature. Supernatants were recovered and filtered through 0.22 μm PVDF filters (Membrane Solutions, Spring View Lane Plano, TX, USA).

2.4. Total phenolic content by Folin–Ciocalteau's reagent

Total phenolic content (TPC) was determined by the Folin–Ciocalteu's reagent method adapted to microscale (Mena, Martí, Saura, Valero, & García-Viguera, 2012d). TPC was evaluated by measuring the variation in absorbance at 765 nm after 1 h of reaction. Assays were measured by using 96-well micro plates (Nunc, Roskilde, Denmark) and Infinite® M200 micro plate reader (Tecan, Grödig, Austria). Samples were diluted 1:16, except control yeast, and were quantified using gallic acid as standard. Results were expressed as milli gram of gallic acid equivalents (GAE) per gram of dry material.

2.5. Identification of (poly)phenolic compounds by UPLC-MS^n

Methanolic extracts of PWL were analysed using an Accela UHPLC 1250 equipped with a linear ion trap-mass spectrometer (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionisation probe (H-ESI-II; Thermo Fisher Scientific Inc., San Jose, CA, USA). Separations were performed using a BlueOrchid C18 column (50 × 2 mm), 1.8 μm particle size (Knauer, Berlin, Germany). Volume injected was 5 μL and column oven was set to 30 °C.

Three MS experiments were performed for each analytical column, of which two in negative mode and one using positive ionisation (anthocyanins) (Mena et al., 2012a). In negative mode, an experiment optimised for epicatechin analysis was carried out using the following conditions. The MS worked with a capillary temperature equal to 275 °C, while the source heater temperature was set to 200 °C. The sheath gas flow was 40 units, while both auxiliary and sweep gas were set to 5 units. The source voltage was 4 kV. The capillary voltage and tube lens were −42 and −118 V, respectively. The mobile phase, pumped at a flow rate of 0.2 mL/min, was kept at 0.1% aqueous formic acid up to 2 min, and then 3-min linear gradient of 0–20% acetonitrile in 0.1% formic acid. From 5 to 13 min the acidified acetonitrile turned up to 40%, followed to 3 min of 80% acetonitrile and then 4 min at the start conditions to reequilibrate the column. Analyses were carried out using full scan, data-dependent MS^3 scanning from *m/z* 100–2000, with collision induced dissociation (CID) equal to 30 (arbitrary units).

Then, the MS worked with conditions optimised to hydrolysable tannin analysis. The capillary temperature was set to 275 °C, while the source heater temperature was 250 °C. The sheath gas flow was 60 units, while auxiliary and sweep gas were set to 15 and 4 units, respectively. The source voltage was 4 kV. The capillary voltage and tube lens were −49 and −153 V, respectively. Analyses were carried out using full scan, data-dependent MS^3 scanning from *m/z* 100–2000, with CID equal to 13.8 in MS^2 and 30 in MS^3. The function "Stepped Collision Energy" using a width of 20% and 3 collision energy steps was activated. The chromatographic conditions were the same used for preliminary phenolic analyses.

In positive mode, the MS worked with a capillary temperature equal to 275 °C, while the source heater temperature was set to 300 °C. The sheath gas flow was 40 units, while auxiliary gas was set to 5 units, without sweep gas. The source voltage was 4.5 kV. The capillary voltage and tube lens were 20 and 95 V, respectively. The mobile phase, pumped at a flow rate of 0.2 mL/min, was a