Industrial Crops & Products 111 (2018) 379–390

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Research paper

Solvent selection for efficient extraction of bioactive compounds from grape pomace

Diandra Pintać[a,*], Tatjana Majkić[a], Ljilja Torović[b,c], Dejan Orčić[a], Ivana Beara[a], Nataša Simin[a], Neda Mimica–Dukić[a], Marija Lesjak[a]

[a] Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[b] Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[c] Institute of Public Health of Vojvodina, Futoška 121, 21000 Novi Sad, Serbia

ARTICLE INFO

Keywords:
Grape pomace
Polyphenols extraction
Serbian grape varieties
Ursolic acid
Winery by-products

ABSTRACT

Pomace is a winemaking by-product, rich in bioactive compounds which exert various health benefits and are used as dietary supplements, cosmetic ingredients, food colorants and preservatives. Isolation and further utilization of these compounds is an important issue in pomace waste management. Different industries have different requirements for specific classes of compounds and thus, selective extraction methods for isolation of these classes should be developed. In this work, the efficacy of six solvents (80% MeOH, 80% EtOH, EtOAc, acetone, acidified 50% and 80% MeOH) for the extraction of polyphenolic and triterpenoid compounds was examined. Pomaces of different grape varieties from Fruška Gora winery region in Serbia were used for extraction. 47 phenolics and 3 triterpenoids were analyzed by LC–MS/MS and 5 anthocyanin glucosides by LC-UV/VIS, while contents of total phenols and flavonoids were determined spectrophotometrically in obtained extracts. The most efficient solvent for each class and each compound was defined: EtOAc was the best for obtaining an extract rich in polyphenols, acidified 50% MeOH for isolation of anthocyanins, and acetone for ursolic acid. For industries that work on a large scale and aim at the highest utilization of raw material, 80% MeOH was found to be a solvent of choice, providing the highest yield of all polyphenols. This study confirmed that pomaces from Fruška Gora vineyards are a promising source of bioactive compounds, suggesting that their utilization could be very attractive for pharmaceutical, food and cosmetic industries.

1. Introduction

Grape is one of the world's most important fruit crops, mainly due to a wide range of dietary products deriving from it, such are fresh fruit and juice, wine, raisins, jam, jelly, etc. and its high content of health-giving phytonutrients (Schieber et al., 2001). Grape is a rich source of numerous classes of natural products − carbohydrates, organic acids, vitamins and minerals, but most importantly, polyphenols (Vauzour et al., 2010). Polyphenols, such as flavonols (quercetin, kaempferol, myricetin), flavanols (catechins, epicatechins), anthocyanins (malvidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, petunidin 3-*O*-glucoside) and stilbenes (resveratrol), are predominantly distributed in the skin, seeds, stems and leaves of grapes (Xia et al., 2010). These compounds are known for their various beneficial effects on human health, among which antioxidant (Lachman et al., 2013; Rockenbach et al., 2011), cardioprotective (Otero-Pareja et al., 2015), anti-inflammatory (Manca et al., 2016; Rodríguez-Morgado et al., 2015), antimicrobial (Oliveira et al., 2013), antiaging (Xia et al., 2010) and anticancer (Jara-Palacios et al., 2015; Tounsi et al., 2009) activities are the most acknowledged. Besides polyphenols, triterpenoid compounds (e.g. ursolic acid) can also be present. These compounds have high potential for medical application since they possess antidiabetic, anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective and gastroprotective properties, with ursolic acid being one of the promising substances of natural origin in cancer prevention and therapy (Grigoras et al., 2013; Strzemski et al., 2016; Woźniak et al., 2015). Thus, efficient isolation and further utilization of bioactive constituents of grape and its products has been attracting attention in the past decade (Dai and Mumper, 2010; Oliveira et al., 2013).

During winemaking, only a small part of phytochemicals is

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; EtOAc, ethyl acetate; GAE/g fw, mg of gallic acid equivalents per gram of fresh weight; TFC, total flavonoid content; TPC, total phenolic content; QE/g fw, mg of quercetin equivalents per gram of fresh weight; de, dry extract; fw, fresh weight; 80% MeOH, 80% methanol; 80% EtOH, 80% ethanol; 50% MeOH + acid, methanol:distilled water:formic acid = 50:48.5:1.5; 80% MeOH + acid, methanol:distilled water:formic acid = 80:19:1

* Corresponding author.
E-mail addresses: diandra.pintac@dh.uns.ac.rs, diandra90@live.com (D. Pintać).

http://dx.doi.org/10.1016/j.indcrop.2017.10.038
Received 20 March 2017; Received in revised form 5 October 2017; Accepted 22 October 2017

transferred from grape to wine, while large quantities remain in pomace, the by-product consisting of pressed grape leftovers (e.g. seeds, skin, stems; Barcia et al., 2014; Jara-Palacios et al., 2014). The chemical composition of pomace depends on the grape variety, soil type, agroclimatic factors and applied winemaking techniques. Winemaking process is different for red and white wines, because white grape must is usually not fermented with the solid grape particles which is opposite to red must (Rodríguez Montealegre et al., 2006), but it also differs among wineries which often apply their own unique manufacturing practice. Although it can be utilized as animal feed, organic fertilizer and raw material for obtaining ethanol in distilleries, the annual disposal of 5–9 million tons of grape pomace worldwide is becoming a serious environmental and economic problem (Đilas et al., 2009; Jara-Palacios et al., 2015). Thus, it would be of a great interest to efficiently exploit disposed pomace as a rich and inexpensive source of beneficial phytochemicals, which could be successfully used in food, cosmetic and pharmaceutical industries.

The main goal of this study was to define the most suitable solvent for extraction of polyphenols and ursolic acid from grape pomaces. Pomaces of two red grape varieties (Merlot and Cabernet Sauvignon) and one white variety (Italian Riesling) grown in Fruška Gora mountain in Serbia were chosen to be studied. Six solvents were selected for the extraction of pomaces: 80% methanol, 80% ethanol, acetone, ethyl acetate, acidified 50% and 80% methanol. Chemical composition of the obtained extracts was monitored by spectrophotometric, LC-UV/VIS and LC–MS/MS methods. Beside investigating the capacity of different solvents to extract specific constituents, the aim of this study was also to evaluate red and white pomaces from Serbia as potential sources of bioactive natural compounds, and promote their utilization.

2. Materials and methods

2.1. Chemicals and reagents

Standards of phenolic and triterpenoid compounds were purchased from ChromaDex (Santa Ana, USA), Fluka Chemie GmbH (Buchs, Switzerland), Extrasynthese (Lion, France) and Sigma–Aldrich Chem (Steinheim, Germany). Standards of anthocyanins were purchased from AppliChem (Darmstadt, Germany; malvidin 3-*O*-glucoside chloride, cyanidin 3-*O*-glucoside chloride, delphinidin 3-*O*-glucoside chloride) and Phytolab (Vestenbergsgreuth, Germany; petunidin 3-*O*-glucoside chloride, peonidin 3-*O*-glucoside chloride). HPLC gradient grade methanol, ethanol, dimethyl sulfoxide (DMSO) and petroleum ether were purchased from J. T. Baker (Deventer, The Netherlands), acetonitrile HPLC grade from Promochem LGC (Wesel, Germany), hydrochloric acid 35% from Roth (Karlsruhe, Germany), p.a. formic acid and sodium acetate from Merck (Darmstadt, Germany), methanol and ethyl acetate were obtained from Zorka Pharma Hemija (Šabac, Serbia), formic acid from AppliChem PanReac GmbH (Darmstadt, Germany), Folin–Ciocalteu (FC) reagent from Fluka Chemie GmbH (Buchs, Switzerland), sodium carbonate was provided from Centrohem (Stara Pazova, Serbia) and aluminium chloride from Kemika (Zagreb, Croatia).

2.2. Plant material and extract preparation

Grape pomace, consisting of grape skin, seeds, pulp and stems, obtained by industrial pressing for complete separation of juice, was generously provided by wineries Bajilo (pomace of Cabernet Sauvignon and Italian Riesling varieties), Šukac (pomace of Merlot variety) and Agner (pomace of Italian Riesling variety) in September and October of 2014. The vineyards and wineries are located on Fruška Gora mountain in northern Serbia and are greatly renowned for wine quality.

Six extracting solutions were prepared: 80% methanol (80% MeOH), 80% ethanol (80% EtOH), acetone, ethyl acetate (EtOAc) and methanol:distilled water:formic acid in two different ratios (MeOH:H_2O:HCOOH = 50:48.5:1.5 (50% MeOH + acid) and MeOH:H_2O:HCOOH = 80:19:1 (80% MeOH + acid)). One gram of each pomace was macerated with 10 mL of each solvent, for 6 h at room temperature with moderate shaking (Šibul et al., 2016). After filtration, the six hour maceration followed by filtration was repeated two more times with the same amount of solvent added to the pomace leftovers. The collected extract fractions were merged and the solvent was evaporated to dryness under vacuum at 45 °C and dissolved in warm distilled water (40–50 °C, 10 mL per g of dry extract). To remove the nonpolar compounds, the extracts were washed repeatedly with petroleum ether (fraction 40–60 °C) till full decolorization of the petroleum ether fraction occurred. The extracts were dried under vacuum, and dissolved in DMSO (w/v) to obtain stock solutions (20 mg/mL). The extract preparations were done in triplicate.

2.3. Determination of total phenolic content

The total phenolic content (TPC) of pomace extracts was determined according to the spectrophotometric method adapted for 96-well microplates, described in Lesjak et al. (2011). For the calibration curve, 11 concentrations of gallic acid dissolved in water were used, ranging from 0.625 μg/mL to 80 μg/mL. The concentrations of all 24 extracts were: 0.625 mg/mL, 0.313 mg/mL and 0.156 mg/mL. Thirty μL of each extract or gallic acid solution were added to 150 μL of 0.1 mol/L FC reagent and after 10 min mixed with 120 μL of Na_2CO_3 (75 mg/mL). In the sample correction probes, 150 μL of distilled water was added instead of the FC reagent. Absorbance was read after 2 h at 760 nm. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of dried extract (de) and per g of fresh weight (fw). The results were presented as a mean value of triplicate tests.

2.4. Determination of total flavonoid content

Total flavonoid content (TFC) of pomace extracts was measured by the colorimetric assay based on the formation of a flavonoid-aluminium complex. The method was adapted for 96-well microplates as described in Lesjak et al. (2011). Quercetin was used for the construction of the calibration curve in eleven concentrations ranging from 0.625 μg/mL to 80 μg/mL. All 24 extracts were tested in three concentrations in a range of 1.25 mg/mL − 5.00 mg/mL. Briefly, 30 μL of extract or standard solution were diluted with 90 μL of methanol, and 6 μL of 10% $AlCl_3$ (substituted with distilled water in the correction probe), 6 μL of 1 mol/L CH_3COONa and 170 μL of distilled water were added. Absorbance was read at 415 nm after 30 min. Total flavonoid content was expressed as mg of quercetin equivalents (QE) per g of de and per g of fw.

2.5. Quantitative analysis of pomace extracts

2.5.1. Quantitative LC–MS/MS analysis of selected phenolic and triterpenoid compounds

All extracts were dissolved in 50% methanol to the final concentration of 2 mg/mL. Determination of the selected compounds was done using Agilent Technologies 1200 Series high-performance liquid chromatograph coupled with Agilent Technologies 6410A Triple Quad tandem mass spectrometer with electrospray ion source, by the previously published procedure (Orčić et al., 2014), with the addition of six compounds with the following optimized compound-specific parameters (retention time, precursor ion, product ion, fragmentor voltage, collision voltage): ellagic acid (2.23 min, *m/z* 301, *m/z* 301, 152 V, 0 V), morin (2.92 min, *m/z* 301, *m/z* 149, 120 V, 29 V), resveratrol (2.26 min, *m/z* 227, *m/z* 185, 130 V, 15 V), ursolic acid (9.44 min, *m/z* 455, *m/z* 455, 300 V, 12 V), glycyrrhetinic acid (8.89 min, *m/z* 469, *m/z* 425, 280 V, 40 V) and glycyrrhizin (6.98 min, *m/z* 821, *m/z* 351, 220 V, 45 V). For all compounds, peak areas were determined using Agilent MassHunter Workstation Software − Qualitative Analysis (ver. B.03.01). Calibration curves were plotted by OriginLabs Origin Pro