Food Chemistry 196 (2016) 833–841

Contents lists available at ScienceDirect

Food Chemistry

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

Evaluation of the anthocyanin release and health-promoting properties of Pinot Noir grape juices after pulsed electric fields

Sze Ying Leong [a,b], David John Burritt [b], Indrawati Oey [a,*]

[a] Department of Food Science, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[b] Department of Botany, University of Otago, PO Box 56, Dunedin 9054, New Zealand

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 4 October 2015
Accepted 6 October 2015
Available online 13 November 2015

Keywords:
Pulsed electric fields
Pinot Noir
Maceration
Anthocyanins
Caco-2 cells
Oxidative stress
Antioxidant activity

ABSTRACT

This study evaluated the health-promoting properties of Pinot Noir juices (*Vitis vinifera* L.) obtained at different maceration times after pulsed electric fields (PEF) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and human intestinal Caco-2 cells assays. Juice quality, anthocyanins, total phenolics and vitamin C were also determined. The evaluation of bioprotective capacity of the juice against H_2O_2-induced oxidative stress in Caco-2 cells was determined using biomarkers for cellular health and integrity: cell viability and lactate dehydrogenase (LDH) leakage. Compared to untreated grape juice, PEF pre-treatment on grapes enhanced the release of the major anthocyanin found in Pinot Noir, i.e. malvidin-3-*O*-glucoside (+224%). Increase in the content of total phenolic (+61%) and vitamin C (+19%) as well as improvement in the DPPH scavenging activity (+31%) and bioprotective capacity (+25% for cell viability and +30% for LDH leakage) were observed in grape juices following PEF treatment. Bioprotective capacity determined by the cellular biomarkers had significant linear correlations with malvidin-3-*O*-glucoside content ($0.71 \leqslant r \leqslant 0.73$) whereas DPPH scavenging activity was not well correlated with malvidin-3-*O*-glucoside ($r = 0.30$) and total phenolics ($r = 0.30$). Therefore, evaluation of the bioprotective capacities using Caco-2 cell assay performed in this study makes a novel contribution to the current knowledge that demonstrates the capability of PEF technology to produce plant-based foods with better phytochemical composition and exhibiting the capacity to protect cells from oxidative stress.

1. Introduction

Oxidative stress in biological systems is a condition when there is an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidants (endogenous or exogenous) to neutralise the ROS produced (Apel & Hirt, 2004). Consumption of sufficient amounts of dietary antioxidants, with bioprotective capacity, from fruits and vegetables has been shown to reduce oxidative stress and thus reduce the risk of developing some diseases such as cardiovascular disease, cancer and problems associated with ageing (Lampe, 1999).

Grapes are rich source of polyphenols, such as anthocyanins, phenolic acids, flavonols, flavan-3-ols and stilbenes, that possess the capability to protect biological systems from oxidative stress (Day, Kemp, Bolton, Hartog, & Stansbie, 1997; Freedman et al., 2001; Stein, Keevil, Wiebe, Aeschlimann, & Folts, 1999). Most of these compounds have been demonstrated individually to be capable, albeit at different extents, of positively correlating to their *in vitro* total antioxidant activity (Cho, Howard, Prior, & Clark, 2004; Iacopini, Baldi, Storchi, & Sebastiani, 2008). Anthocyanins and phenolics are most abundant in the thick walled cell that makes up the skin of the grape berries (Pinelo, Arnous, & Meyer, 2006). To increase the release of these compounds, prolonging maceration of the berries is widely used in the production of grape juice and winemaking (Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas, & Fernández-Fernández, 2001). In addition, pulsed electric field (PEF) technology can accelerate the release of anthocyanins and other phenolics from grape skins (Puértolas, López, Condón, Álvarez, & Raso, 2010) due to its effect on cell membrane permeability (Angersbach, Heinz, & Knorr, 2000). However, little is known whether the bioactive compounds released due to PEF processing do enhance the bioprotective/antioxidant capacity of the resultant juice and more importantly, whether PEF processing does in fact result in a food product with enhanced bioprotective capacity at the cellular level.

In vitro chemical assays are frequently used to assess the antioxidant activity of plant foods such as ABTS (2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate)), DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant potential) and ORAC (oxygen radical absorption capacity) assays (Carocho & Ferreira, 2013). Considering the fact that antioxidants may act

* Corresponding author.
E-mail address: indrawati.oey@otago.ac.nz (I. Oey).

http://dx.doi.org/10.1016/j.foodchem.2015.10.025
0308-8146/

synergistically rather than individually, these chemical assays have been shown useful to assess the total antioxidant capacity of various plant-based liquid foods (Barba, Esteve, Tedeschi, Brandolini, & Frígola, 2013). These assays measure the ability of natural antioxidants to scavenge free radicals using different chemical reaction mechanisms, either via electron transfer or hydrogen atom transfer. However, very often, the free radicals scavenging capacity of plant-based foods does not necessary reflect their health benefits *in vivo* since different bioactive compounds may act *in vivo* through different mechanisms under biological conditions and these cannot easily be mimicked using *in vitro* chemical assays (López-Alarcón & Denicola, 2013). For this reason, there is a need to employ more relevant biological experimental approach that takes into consideration the interaction of bioactive metabolites to associate, interact and permeate the membranes of biological systems in order to assess the resulting physiological response (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014).

Animal models and human studies are the best approach to determine the bioprotective capacity of plant-based foods, however they are often very costly and are not suited to the optimisation of food processing conditions, which may require testing many combinations of processing parameters. In this regard, model cell culture systems have been considered as a relevant biological assay (López-Alarcón & Denicola, 2013; Wan et al., 2014).

Human colon adenocarcinoma (Caco-2) cells are commonly used as a cell culture system for bioprotective/antioxidant related research because of their similar morphology with human small intestinal epithelial cells (Glahn et al., 1998) to simulate the uptake and absorption of plant bioprotective molecules/antioxidants. Wan, Liu, Yu, Sun, and Li (2015) developed a Caco-2 cell-based quantitative antioxidant assay and demonstrated a good correlation between the cell-based assay and animal model studies. Several studies have clearly shown the potential of pure antioxidant compounds (Rodríguez-Ramiro, Martín, Ramos, Bravo, & Goya, 2011) or extracts isolated from plants with fixed concentrations of bioactive compounds (Aherne, Kerry, & O'Brien, 2007; García-Nebot, Recio, & Hernández-Ledesma, 2014) in protecting Caco-2 cells from induced oxidative damage. However, limited studies in the current literature have investigated the bioprotective capacity of digested plant extracts in their natural state and composition especially in processed plant-based foods that have different chemical compositions compared to their fresh or untreated counterparts.

The objective of the present work was to evaluate the anthocyanin release and the health-promoting properties of Pinot Noir grape juices obtained during maceration of up to 14 days after PEF processing at different intensities (1.5 kV/cm, either at 15 or 70 kJ/kg). The amount and leaching efficiency of selected bioactive compounds, namely malvidin-3-*O*-glucoside anthocyanin, total phenolics and vitamin C into the juice during maceration was analysed. The health-promoting properties of grape juice was assessed using DPPH radical scavenging capacity assay to determine the total antioxidant capacity and using a Caco-2 cell assay system to determine the bioprotective capacity against hydrogen peroxide (H_2O_2) induced oxidative stress. The correlations between the results of the DPPH assay and the Caco-2 cell assays and the contents of malvidin-3-*O*-glucoside, total phenolics and vitamin C were then determined and are discussed with respect to their significance for minimally processed plant-based foods.

2. Materials and methods

2.1. Grape mash preparation

Pinot Noir grapes (*Vitis vinifera* L.) grown in Marlborough region (41.88°S 173.67°E; South Island, New Zealand) were supplied by Villa Maria Estates winery. The grapes were harvested in late March 2013 at their desirable ripeness stage; determined on the basis of sugar (20–22 °Brix), acidity (60–80 g/L) and pH (3.3–3.5) levels standardised by the growers. After harvest, the grapes were immediately dispatched and transported overnight to the Food Science Department at University of Otago (Dunedin, New Zealand) using a refrigerated (4 °C) truck. On arrival (within 24 h after harvest), the grapes were visually screened for any damaged and mouldy areas and manually destemmed. The destemmed grapes are referred as "grape mash".

2.2. Pulsed electric field treatment

The grape mash (200 g) was treated using batch configuration of PEF equipment (ELCRACK-HVP 5, German Institute of Food Technologies, Quakenbrück, Germany). The time delay between destemming and PEF treatment for each sample was standardised to 5 min. The batch treatment chamber (100 mm length × 80 mm width × 50 mm height, 400 mL capacity) consisted of two parallel stainless steel electrodes of 5 mm thickness separated by a distance of 80 mm. In this study, square wave bipolar pulses were applied and monitored on-line with oscilloscope (Model UT2025C, Uni-Trend Group, China). The PEF operating variables were used as follows: constant pulse width of 20 μs, pulse frequency of 50 Hz, electric field strength of 1.5 kV/cm, pulse numbers of 243 (referred as "PEF Low") and 1033 (referred as "PEF High"). The specific energy input was calculated according to Zhang, Barbosa-Cánovas, and Swanson (1995) using Eq. (1).

$$\text{Specific energy input (kJ/kg)} = \frac{V^2 \times n \times \tau}{R \times w} \quad (1)$$

V is the measured output pulse peak voltage (in kV), n is the number of pulses applied (dimensionless), τ is the pulse width of square width (in μs), R is the effective load resistance (in Ω) and w is the total weight of grape mash in the PEF treatment chamber (in g). V is the result of the measured output pulse current value and the effective resistance of the grape mash in the treatment chamber on the basis of Ohm's Law. The effective load resistance is also directly related to the conductivity value of grape mash and the cross-sectional area of treatment chamber.

The estimated specific energy inputs were 14.48 ± 0.11 kJ/kg and 69.99 ± 0.52 kJ/kg for "PEF Low" and "PEF High", respectively. The change in conductivity and temperature of grape mash prior to and after PEF treatment was measured using an electrical conductivity meter (CyberScan CON 11, Eutech Instruments, Singapore). Initial electrical conductivity of grape mash prior to PEF treatment averaged at 3.91 ± 0.10 mS/cm. Changes in the electrical conductivity (in percentage %) after "PEF Low" and "PEF High" were 9.42 ± 2.64% and 14.63 ± 3.61% respectively. The initial temperature of grape mash before PEF treatment averaged at 15 ± 2 °C and the temperature of grape mash after PEF treatment did not exceed 25 ± 2 °C. Changes in treatment temperature were 2.61 ± 0.42 °C and 8.10 ± 0.44 °C respectively due to "PEF Low" and "PEF High". Each PEF condition was independently conducted in triplicates for each maceration time point (0, 2, 4, 8 and 14 days).

2.3. Cold soak maceration

The grape mash (untreated and PEF-treated) was immediately sealed inside a 500 mL autoclaved polycarbonate container with polypropylene screw cap (LabServ, Thermo Fisher Scientific, Victoria, Australia) to avoid any evaporation and degradation of grape polyphenols due to exposure to atmospheric air and light during maceration and stored at 10 ± 2 °C under dark condition. In this study, cold soak maceration was conducted to decrease the