Food Research International 65 (2014) 462–468

Contents lists available at ScienceDirect

Food Research International

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

A comparative study of physical pretreatments for the extraction of polyphenols and proteins from vine shoots

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ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form 2 April 2014
Accepted 13 April 2014
Available online 24 April 2014

Keywords:
Polyphenols
Proteins
Ultrasounds
High-voltage electrical discharges
Pulsed electric fields

ABSTRACT

This work examined the potential of valorization of vine shoots through their polyphenol and protein contents. However the choice of the experimental conditions targeted polyphenol extraction at the expense of proteins for further simplification of the purification process. The intensification of polyphenol and protein extraction by physical treatments (pulsed electric fields (PEF), high-voltage electrical discharges (HVED) and ultrasound (US)) was studied. A significant enhancement of polyphenol extraction was noticed with HVED, PEF and US. However, and for each treatment, the improvement of the extraction process started beyond a specific energetic threshold (HVED (10 kJ/kg), PEF (50 kJ/kg) and US (1010 kJ/kg)). HVED had the highest polyphenol and protein extraction yields with the lowest energetic prerequisite. Extracts of high polyphenol yield (34.5 mg of gallic acid equivalent (GAE) per g of dry matter (DM)) and high purity (89%) were obtained with HVED. Polyphenol and protein diffusion coefficients (m^2/s) demonstrated HVED to better enhance the extraction process of those biomolecules. Similarly, the calculation of the electrical conductivity disintegration index, Z, showed the highest tissue damage for HVED and a rising cellular damage with the increased energetic requirement of each treatment.

1. Introduction

Vine shoots are agricultural byproducts conventionally used as a heating source or left on the ground to rot (Luque-Rodríguez, Pérez-Juan, & Luque de Castro, 2006). The valorization of vine shoots has been focused over the production of ethanol and paper pulp (Delgado-Torre, Ferreiro-Vera, Priego-Capote, Pérez-Juan, & Luque de Castro, 2012), however using this raw material as a source of polyphenols would increase its economic value (Luque-Rodríguez et al., 2006). Vine shoots were shown to be an important source of polyphenols and proteins, which contents varied depending on the cultivars and experimental conditions. Solid–liquid extraction gave polyphenol yield varying from 25.36 ± 1.62 (Atasarısı variety) to 36.56 ± 2.67 mg GAE/g (Trakya İlkeren variety), while protein content changed from 12.09 g/100 g to 28.13 g/100 g (Çetin, Altinöz, Tarçan, & Göktürk Baydar, 2011). Superheated ethanol–water extraction of polyphenols from vine shoots gave yields from 17 to 41 mg of GAE/g depending on the experimental parameters (Luque-Rodríguez et al., 2006). Whether forming part of lignin or found as extractives (non-structural components) (Romero & Sánchez, 2005), polyphenols are extracted from vine shoots and contribute in obtaining high-added value products in nutraceutical, pharmacological and oenological industries (Delgado-Torre et al., 2012). Moreover, grape canes can be considered as dietary supplements, since they are protein rich plant materials (Çetin et al., 2011). During the last decencies non-conventional environmentally friendly methods have been developed to enhance the extraction processes, giving higher yields and better extract quality than classic extraction methods (Soxhlet, etc.). Non-conventional physical methods can decrease chemical use, and reduce operational time (Azmir et al., 2013). Recently, pulsed electric fields (PEF) and high-voltage electrical discharges (HVED) have been tested for polyphenol extraction from various byproducts (Boussetta, Grimi, Lebovka, & Vorobiev, 2013; Boussetta, Lanoisellé, Bedel-Cloutour, & Vorobiev, 2009; Boussetta, Lebovka, et al., 2009; Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008; Grimi, Lebovka, Vorobiev, & Vaxelaire, 2009; Grimi, Mamouni, Lebovka, Vorobiev, & Vaxelaire, 2011; Puértolas, López, Condón, Álvarez, & Raso, 2010). PEF induce the membrane electroporation phenomenon. When subjected to an external electric field, the electrical potential difference across the cell membrane increases. If the induced electrical potential exceeds some threshold value (1–2 V for most plant tissues), the cell membrane loses its semipermeability leading to pore creation named electroporation (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999). HVED induce the electrical breakdown in water (Boussetta, Lesaint, & Vorobiev, 2013). Formed as a result of local heating, or already present in water, air bubbles are implicated in this

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http://dx.doi.org/10.1016/j.foodres.2014.04.024
0963-9969/

phenomenon. If the electrical field intensity is sufficient, it provokes an electron avalanche, representing a starting point for the streamer propagation from the positive to the negative electrodes. Numerous secondary phenomena might occur, such as bubble cavitation, high-amplitude pressure shock waves, and liquid turbulence, causing particle fragmentation and cell structure damage. Subsequently, extraction of biomolecules from cell cytoplasm can be enhanced. The ultrasonic method has been extensively studied for the extraction of many molecules, amongst which polyphenols (Chemat, Lagha, AitAmar, Bartels, & Chemat, 2004; Ghafoor, Choi, Jeon, & Jo, 2009; Khan, Abert-Vian, Fabiano-Tixier, Dangles, & Chemat, 2010). Ultrasound (US) waves generate mechanical vibrations in a solid, liquid or gas, having the ability to deform. US of high intensity (10–1000 W/cm^2) can disrupt the cell tissue. Waves propagate longitudinally or perpendicularly through the solid–liquid medium near the product's surface, creating cycles of expansion and compression. The expansion is likely to produce gas bubbles in the liquid, stimulating local pressure and temperature elevations, up to 50 MPa and 5000 °C, respectively (Mason, 1997). The potential collision of the bubbles is responsible for the cavitation phenomenon. Cell membranes in the vicinity undergo extensive and repetitive shearing; they are altered and thus release their intracellular content more easily (Chemat et al., 2004). Few studies have been conducted on the use of grape vine shoots as a source of polyphenols (Çetin et al., 2011; Delgado-Torre et al., 2012; Karacabey & Mazza, 2008; Karacabey, Mazza, Bayındırlı, & Artık, 2012; Luque-Rodríguez et al., 2006; Max, Salgado, Cortés, & Domínguez, 2010), and as far as we know, the comparison of the three aforementioned treatments on the extraction of polyphenols and proteins from vine shoots has not been done. The effects of the physical treatments and their energetic thresholds are not yet well understood. The objective of this study was to examine vine shoot potential valorization through their polyphenol and protein contents. However, the main biomolecules of interest in this study were polyphenols, and the experimental conditions were chosen to maximize their extraction yields and diminish that of proteins. This strategy of guiding the extraction towards polyphenol extraction and simultaneously minimizing the protein yield is likely to simplify a subsequent purification process. The intensification of polyphenol extraction from vine shoots by PEF, HVED and US was conducted. The resulting yields were compared taking into consideration the required energy input and the induced cellular damage. The effect of the treatment on the polyphenol purity in the extracts was also determined.

2. Materials and methods

2.1. Raw material

Industrial vine shoots of *Vitis vinifera* var. Grenache Blanc (France) were used. Vine shoots were cut into cylinders of 1 cm in height and a mean diameter of 5 mm. The dry matter content (DM) of vine shoots was 91%.

2.2. Extraction experiments

2.2.1. Pulsed electric field pretreatment

The PEF apparatus consisted of a pulsed high voltage power supply (Tomsk Polytechnic University, Tomsk, Russia) and a batch one-liter treatment chamber with stainless electrodes (Fig. 1a). The electrodes of the treatment chamber were two parallel disks. The electrode area was 95 cm^2. The circuit configuration and the electrode shape generated exponential decay pulses. Vine shoot cylinders (15 g) were introduced between the electrodes. Extraction solvent (water) at 50 °C was added to the solid. The liquid-to-solid ratio (w/w) was 20. The high voltage pulse generator provided 40 kV–10 kA pulses. The distance between electrodes was fixed to 3 cm. Consequently, the corresponding electric field strength E was 13.3 kV/cm. The total treatment duration T_t was changed by increasing the number of pulses n from 0 to 1500 (Eq. (1)). The temperature of the suspension was controlled each 100 pulses and a pause was made after a series of 100 pulses for 1 min to maintain the temperature at 50 °C. The temperature elevation during the whole treatment was limited (<5 °C). The pulse duration t_{PEF} was approximately 10 μs. The pulses were applied with a repetition rate of 0.5 Hz, which was imposed by the generator. The total treatment duration T_t was calculated as

$$T_t = n \times t_{PEF}. \quad (1)$$

The specific energy input W (kJ/kg) was obtained from Eq. (2):

$$W = \frac{\sum_{i=1}^{n} W_{PEF}}{m} \quad (2)$$

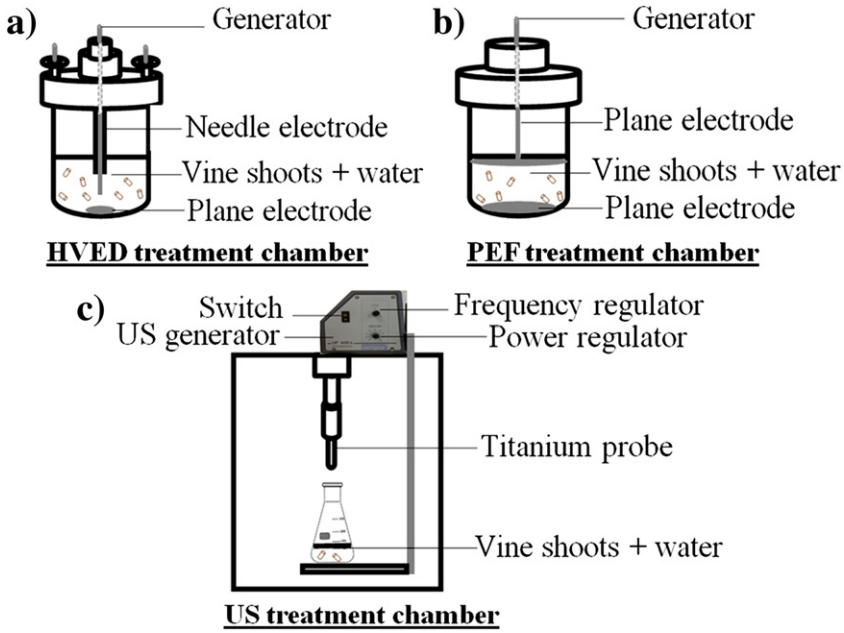

Fig. 1. Instrumental set-up for (a) pulsed electric fields, (b) high voltage electrical discharges, and (c) ultrasounds.