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Assisted extraction of bioactive compounds from plum and grape peels by ultrasonics and pulsed electric fields

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ABSTRACT

The industrial use of fruits for the production of juices results in the accumulation of large amounts of by-products such as peels, with are still a good source of phytochemicals such as phenols and anthocyanins. In this work, the impact of two different processing configuration of pulsed electric fields (PEF-I and PEF-II in continuous, with 25 mm and 7 mm of treatment chamber diameter, respectively) and ultrasonication (US25 and US50 in batch, at 25 and 50 °C, respectively) were evaluated in order to assess these technologies as environmental friendly alternatives to water extraction at 70 °C (WE70) in plum and grape peels. US was able to increase the extraction of anthocyanins and flavonoids in plum peels, being less effective than PEF with total phenols. In grape peels, when US was performed at higher temperature (US50), the yields were significantly higher. PEF was more successful when the diameter of the chamber was larger (PEF-I), and consequently the residence time and number of pulses greater. Particularly, PEF allowed to augment several folds the extraction of anthocyanins and flavonoids from grape peels, but was deleterious for ascorbic acid. Principal component analysis (PCA) showed that US50 and PEF-I were clustered and positively correlated with bioactive compounds recovery and antioxidant capacity.

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1. Introduction

The industrial use of fruits, especially for the production of juices, results in the accumulation of large amounts of by-products such as peels and seeds. Peels have a high added value, since they are still a good source of phytochemicals such as phenols and anthocyanins (Gil et al., 2002a). Plum and grape are among the fruits distinguished for their high content in bioactive compounds in peel and seeds. Plums belong to *Rosaceae* family, and are emerging as one of the most important crops in USA, being the states of California, Oregon and Washington the mayor producers (NASS, 2012). Plum contains high amounts of natural phenolic phytochemicals, such as flavonoids and phenolic acids (Gil et al., 2002a; Kim et al., 2003a,b). In plums, the proportion of skin varies from 10% to 25% of the total weight (Renard and Ginies, 2009). Plums extracts could contribute to the inhibition of proliferation of cancer cells; flavonoid and procyanidins fractions and in a less extent phenolic and anthocyanins fractions inhibited the proliferation of breast cancer cell line up to 50% (Olsson et al., 2004). Among the mechanisms proposed, this class of phytochemicals seemed to have pro-apoptotic effects against colon cancer cell lines (Seeram et al., 2006). On the other side, grape peels represent one of the most important food by-product, since grapes are the second world's largest fruit crop (almost 70 millions of tons produced in 2012), of which 70% is used for wine production (FAO, 2012). Flavonoids contained in grape peel have shown protective effects against cardiovascular events: incubation of platelets with peel extract led to a decrease in platelet aggregation from 70% to 30%, and in a dramatic inhibition of the release of superoxide, a potent intrinsic pro-oxidant (Vitseva et al., 2005). More recently, it has been demonstrated that grape peel extracts also exert anti-hyperglycemic activity in diet-induced obese rats (Hogan et al., 2011).

Conventional solvent extractions of bioactive compounds from peels are time and solvent consuming, representing a serious energetic and environmental issue. For example, the industrial batch extraction of polyphenols from grape peels is generally performed for about 20 h at 50–60 °C. At the same time, in plants these

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compounds exist enclosed in insoluble structures such as the vacuoles of plant cells and membrane bilayers, which are not accessible to solvents (Corrales et al., 2008). The use of heat to enhance mass transfer and reduce time can have deleterious effects, since temperature >70 °C has been shown to cause rapid degradation of some class of compounds, such as anthocyanins (Ju and Howard, 2003). Industrial interest in increasing the rate of the mass transfer and as a consequence in reducing the operation time is based on increasing productivity, preserving the nutritional or physiological value of the food components and reducing the economic cost of the process (Puértolas et al., 2012). Ultrasound (US) and pulsed-electric fields (PEF) are promising nonthermal technologies and potential alternatives to traditional solvent extraction. Both technologies have been tested in several fruit by-products, including orange peel (Luengo et al., 2013), citrus peel (Khan et al., 2010; Ma et al., 2008; Sampedro et al., 2013) and grape peel (Corrales et al., 2008; Ghafoor et al., 2009), among others. PEF is generally conducted in batch (Boussetta et al., 2014; Lopez et al., 2008; Luengo et al., 2013), and only a few examples of continuous PEF processing are reported in literature (Plaza et al., 2011; Sampedro et al., 2013), which are limited to juices. Thus, there is a lack of knowledge in the application of continuous PEF treatment for solid/liquid extraction, as in peels processing. At the same time, despite the increasing interest in peels and seeds as a source of antioxidants, the use of PEF and US in the fruits object of this study is scarce, incomplete or absent, as in plum peels.

In this study we performed a continuous PEF and US treatments with different process configurations on plum and grape peels, evaluating the effects on several bioactive compounds (flavonoids, anthocyanins, phenols, ascorbic acid) and antioxidant capacity (DPPH• radical scavenging). We compared nonthermal processing with a water-assisted extraction at 70 °C, using principal component analysis to statistically discriminate between the different treatments.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), concentrated sulfuric acid, sodium thiosulphate, pyrogallol, Folin–Ciocalteu reagent (2 N), iodine, aluminum chloride, sodium carbonate, sodium acetate, gallic acid, quercetin and *l*-ascorbic acid were obtained from Sigma Aldrich (St. Luis, MO, USA). Acetone, methanol and concentrated hydrochloric acid were purchased from JT Baker (Capitol Scientific, Austin, TX, USA). All the buffers were prepared fresh, and stored at 4 °C before use.

2.2. Sample preparation

Fresh grapes (*Vitis vinifera* L.) and plums (*Prunus domestica* var. Casselman) were bought from a local store (Pullman, WA, USA). The fruits were washed in cold water, and then the grapes were de-stemmed by hand, while the plums were cut into 6 wedges and the core removed. The skins and pulp were separated by juice extractor (Black & Decker JB2200B, Towson, MD, USA). The yield of the separation process was determined by weighing (Balance Sartorius, MC1 LC6200D, WA, USA) the contents of two batches. Peels were mixed with water in a 1:4 (w/v) ratio.

2.3. PEF assisted extraction (PEF)

Plum and grape peels were treated with a continuous PEF equipment DIL ElCrack® (Quakenbrück, Germany), consisting of an exponential decay pulse generator with a maximum voltage of 25 kV, rated power of 5 kW pulse shape rectangular and alternating polarity. A digital oscilloscope, Tektronix TDS 3064B (Beaverton, OH, USA) (600 MHz, 50 S/s), was connected to the power tower in the PEF system to monitor pulse shape, pulse width, and frequency. The pump used (Dayton electric MPG Co. 2M168A, Chicago, IL, USA) is able to generate a flow of between 290 L/h up to a maximum of 2000 L/h. For PEF extractions two different chamber conditions were used:

- PEF-I: flow 290 L/h, diameter of chamber 25 mm, gap 26 mm, 25 kV voltage, 10 Hz frequency, 6 μs pulse width (τ).
- PEF-II: flow 290 L/h, diameter of chamber 7 mm, gap 10 mm, 25 kV voltage, 10 Hz frequency, 6 μs pulse width (τ).

The increase of temperature after both treatments was less than 3 °C. Effective resistance of food in the treatment chamber and the specific energy per pulse were obtained according to Zhang et al. (1995), as following. Resistance (R) is obtained through the equation:

$$R = \frac{\rho L}{A}$$

where ρ is the resistivity of the food (Ω m), L is the gap between the two parallel electrodes and A is the electrode area. Resistivity (measured at 25 °C) was determined by direct immersion of a conductivity meter (Orion Research Inc., Boston, Massachusetts, USA). The total dissipated energy per pulse (W') was calculated by the following equation:

$$W' = \int_0^T V(t)I(t)dt$$

where $V(t)$ and $I(t)$ are the measured voltage and current, respectively. The total dissipated energy (W) was calculated by multiplying the energy per pulse (W') by the number of pulses. The number of pulses (n) was calculated considering the volume flow ($\bar{u}$, m s^{-1}), the diameter of the cell (ϕ, cm) and the pulse repetition rate (f, Hz), as following:

$$f = \frac{P}{\tau V I} \times 1000 \quad \text{and} \quad n = f \times \frac{\phi}{\bar{u}} \times 1000$$

Finally, the treatment time is defined as $t = n \times \tau$. Results are summarized in Table 1. After each treatment samples were collected and kept at 4 °C for further analyses.

Table 1
Volume flow ($\bar{u}$, m s^{-1}), number of pulses (n), treatment time (t, μs), resistivity (ρ, Ω cm), resistance (R, Ω), specific energy per pulse (W', W/pulse) and total specific energy applied (W, W) for continuous PEF-I and PEF-II processing in grape and plum peels.

	$\bar{u}$ (m s^{-1})	n	t (μs)	ρ (Ω cm)	R (Ω)	W' (W/pulse)	W (W)
Grape peels							
PEF-I	0.16	25.2	151.2	6.13	325.3	11.5	289.8
PEF-II	2.09	9.7	58.2	6.13	1593.0	3.91	37.8
Plum peels							
PEF-I	0.16	25.2	151.2	7.81	414.6	9.05	228.0
PEF-II	2.09	9.7	58.2	7.81	2030.0	1.84	17.8