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Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison

M. Corrales [a,*], S. Toepfl [b], P. Butz [a], D. Knorr [c], B. Tauscher [a]

[a] Institute of Chemistry and Biology, Federal Research Centre for Nutrition and Food, D-76131 Karlsruhe, Germany
[b] German Institute of Food Technology, D-49610 Quakenbrück, Germany
[c] Department of Food Biotechnology and Food Process Engineering, Berlin University of Technology, D-14195 Berlin, Germany

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Abstract

Extracts from grape by-products contain bioactive substances such as anthocyanins which could be used as natural antioxidants or colourants. The effect of heat treatment at 70 °C combined with the effect of different emerging novel technologies such as ultrasonics (35 KHz), high hydrostatic pressure (600 MPa) (HHP) and pulsed electric fields (3 kV cm^{-1}) (PEF) showed a great feasibility and selectivity for extraction purposes. After 1 h extraction, the total phenolic content of samples subjected to novel technologies was 50% higher than in the control samples. Therefore, the application of novel technologies increased the antioxidant activity of the extracts being the extractions carried out with PEF four-fold, with HHP three-fold and with ultrasonics two-fold higher than the control extraction. In addition, the extraction of individual anthocyanins was studied showing a selective extraction based on the glucose moieties linked to the anthocyanidins; anthocyanin monoglucosides were better extracted by PEF, whereas the acylated ones were extracted by HHP.

Keywords: Anthocyanins; Total phenolic content; Antioxidant capacity; Ultrasonic; High hydrostatic pressure; Pulsed electric fields

Industrial relevance: This study examines the feasibility of different emerging technologies such as high hydrostatic pressure, pulsed electric fields and ultrasonics as potential extraction methods for bioactive substances from grape by-products. Grape by-products represent a low-cost source of valuable bioactive compounds such as anthocyanins, with great industrial applications as colourants or nutraceuticals. The higher yields obtained in extractions carried out by high hydrostatic pressure and pulsed electric fields are of major interest from an industrial point of view, since solvent amounts were reduced and extraction times shortened. Thus, the combination of emerging technologies for extraction purposes and low-cost raw materials is an economical alternative to traditional extraction methods according to industry demands and a sustainable development.

1. Introduction

The search for environmentally friendly and low-cost row materials and technologies is forcing the food industry to develop new methods to guarantee the sustainability of the food chain. As industrialisation continues, food production will become more concentrated, creating greater quantities of waste at a given location. While this can create greater environmental problems, the concentrated waste can often be more easily reassimilated into the food cycle. Grape by-products, for instance, constituted mainly by peels, contain a high amount of secondary metabolites including phenolic acids, flavanols and anthocyanins (Macheix, Sapies & Fleuriet, 1991; Singleton & Trousdale, 1983) which are reported to possess antibacterial, antiviral, antioxidant, anti-inflammatory, anti-cancerogenic properties and can prevent cardiovascular diseases (Bravo, 1998; Dugan, 1980; Frankel, Waterhouse, & Teissedre, 1995). These compounds exist in plants enclosed in insoluble structures such as the vacuoles of plant cells and lipoproteins bilayers which complicate their extraction. Thus, different novel extraction methods including subcritical water, polymeric adsorber resins and pressurised liquid extraction have been reported to enhance secondary metabolites extraction from grape by-products (Ju & Howard, 2003, 2005; Kammerer,

* Corresponding author. Tel.: +49 721 6625536; fax: +49 721 6625503.
E-mail address: margarita.corrales@bfel.de (M. Corrales).

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Kljusuric, Carle & Schieber, 2005; Metivier, Francis, & Clydesdale, 1980; Revilla, Ryan, & Martin-Ortega, 1998; Zhi & Howard, 2005). However, the effect of other technologies such as high hydrostatic pressure and pulsed electric fields still remain to be investigated. Nevertheless, these studies highlighted the benefits of moderate temperatures (60 °C–70 °C) for an optimal extraction since high temperature enhances mass transfer phenomena increasing the internal liquid phase which raises the pressure, causing centrifugal circulation of the solutes through plant membranes. Furthermore, a heat treatment can also break the phenolic–matrix bonds and influence the membrane structure of plant cells making them less selective by coagulation of lipoproteins.

Thus, the use of novel technologies that are able to enhance cell disruption combined with a temperature of 70 °C may represent not only an economical but also an environmental alternative, since grape by-products could be recycled in the food industry in form of value-added ingredients or additives. High hydrostatic pressure (HHP), pulsed electric fields (PEF) and ultrasonics belong to the environmentally friendly and energy efficient technologies being able to enhance mass transfer processes within plant or animal cellular tissues, as the permeability of cytoplasmatic membranes can be affected (Dörnenburg & Knorr, 1993; Toepfl, Mathys, Heinz, & Knorr, 2006).

The use of HHP enhances mass transfer rates increasing cell permeability as well as increasing secondary metabolite diffusion according to changes in phase transitions (Richard, 1992). In fact, the potential use of HHP for extraction of flavanoids from propolis has recently been demonstrated (Shouqin, Jun, & Changzheng, 2005).

PEF enhances mass transfer rates by electroporation of plant cell membranes improving tissue softness and influencing textural properties. For instance, carrots, potatoes and apples treated with PEF lose their water content more rapidly during osmotic drying when they were subjected to PEF (Angersbach, Heinz, & Knorr, 2000; Lebovka, Praporscic, & Vorobiev, 2004). Moreover, PEF has been reported to be an ideal method to enhance juice production, increasing the content of valuable components and even replacing the enzymatic maceration (Eshtiaghi & Knorr, 2000).

Ultrasonics is one of the most industrially used methods to enhance mass transfer phenomena. Its feasibility for the extraction of secondary metabolites such as tea, mint, chamomile, ginseng etc. has been highlighted in many research works (Li, Ohdaira, & Ide, 1995; Mason & Zhao, 1994). Ultrasonics enhance mass transfer rates by cavitation forces, where bubbles in the liquid/solid extraction can explosively collapse and generate localized pressure causing plant tissue rupture and improving the release of intracellular substances into the solvent (Knorr, Ade-Omowaye, & Heinz, 2002).

In spite of the literature dealing with the efficiency of PEF, HHP and ultrasonics for extraction purposes, a comparison of these three novel technologies for the extraction of anthocyanins from grape by-products has not been studied before. For this reason, its feasibility for anthocyanin recovery has been here investigated. This study is of great relevance, since an optimal recovery of anthocyanins and other antioxidants from grape by-products provides an enormous potential for product development according to current industry and consumer demands.

2. Materials and methods

All reagents and solvents were of analytical grade and purchased from Merck (Darmstadt, Germany). Compounds used for identification and quantification purposes with LC-DAD/ESI-MS were cyanidin-3-*O*-monoglucoside (Cy-3-glu) and malvidin-3-*O*-monoglucoside (Mv-3-glu) (Extrasynthese, Lyon, France). Grape by-products from Dornfelder *Vitis vinifera* ssp. were kindly supplied by Timrott Bio-Produkte GmbH (Ilbesheim, Germany). Grape by-products were separated into the following different fractions: skins, stems and seeds. Skins were stored in sealed plastic bags (3 kg), kept at −80 °C and defrosted before utilisation.

2.1. General extraction procedure

All extractions were conducted by a solid/liquid ratio of 1:4.5 where the solvent was a mixture of ethanol and water (50:50, v/v). After each of the treatments extracts were filtered and supernatants were collected for further analysis.

2.2. Control extraction

Control extraction was carried out in a water bath incubated at a temperature of 70 °C held during 1 h.

2.3. Pulsed electric fields extraction

A pulsed electric field treatment was applied using a PurePulse (PurePulse Technologies, San Diego, USA) exponential decay pulse generator with a maximum voltage of 10 kV and a maximum average power of 8 kW. The peak pulse voltage used was 9 kV, resulting in an electric field strength of 3 kV cm^{-1}. A serie of 30 pulses was applied at ambient temperature to obtain a specific energy input of 10 kJ kg^{-1}. The temperature increase after the treatment was less than 3 °C. A parallel plate treatment chamber consisting of stainless steel electrodes with an electrode area of 140 cm^2 and a gap of 3 cm was used. The pulse repetition rate was 2 Hz, the total treatment time was 15 s, for filling and unfilling of the sample the time required was 1 min. The subsequent extraction was performed at 70 °C and held during 1 h in a shaken Erlenmeyer flask.

2.4. High hydrostatic pressurised extraction

Experiments were conducted in a high hydrostatic pressure device consisting of a series of thermostated microautoclaves (i.d. 16 mm, ca. 25 ml) connected by valves (aad GmBH, Frankfurt, Germany). Pressure was generated by a hydraulic pump in combination with a pressure intensifier. The pressure-transmitting medium was water and glycol (80:20, v/v). Due to adiabatic heating there was an initial temperature rise of not

more than 4 °C on pressure build-up: the initial temperature was usually restored after 1–2 min. The temperature in the vessels was controlled by a thermostat Polystat from Huber (Offenburg, Germany). Samples were pressurised at 600 MPa, 70 °C and held during 1 h in Teflon tubes (inner/outer diameter 6–8 mm; 2–5 ml) with silicon stoppers.

2.5. Ultrasonics extraction

Ultrasonics extraction was carried out in an ultrasonics bath Sonorex Bandelin RK 100H with heating frequency of 35 KHz (Schalltec, Mörfelden-Walldorf, Germany), at 70 °C held during 1 h.

2.6. Total extraction

A total extraction from grape skins was carried out in a water bath incubated at a temperature of 70 °C held during 3 h. In this case, the solid/liquid ratio was increased to 1:20.

2.7. Determination of moisture

The moisture content of fresh and pressurised samples was determined following the 934.06 AOAC (1998) method. All results were expressed on dry matter (DM) basis.

2.8. Determination of soluble solids

Soluble solids in the extracts were determined using a digital refractometer (Digital ABBE Refractometer AR 2008, A. Krüss, Germany).

2.9. Analysis of anthocyanins by LC-DAD/ESI-MS

An aliquot of 1 ml of the extracts was evaporated to dryness in a centrifugal vacuum dryer (Speed Vac SC110 Savant, Germany) coupled to a refrigerated condensor trap (RT 100 Savant, Germany), and the residue was dissolved in 500 µl of acidified water (pH 3) and membrane filtered (0.45 µm) before injection. Anthocyanins were analysed by direct injection of the solutions. They were analysed according to the method reported by Kammerer, Claus, Carle, and Schieber (2004) slightly modified using LC-DAD/ESI-MS technologies (Agilent, Waldbronn, Germany). The mass spectrometer was fitted with an ESI source in positive mode. The column eluate was recorded in the range m/z 50–1000. Nitrogen was used both as drying gas at flow rates of 11.0–12.0 ml min^{-1}, and as nebulising gas at a pressure of 65 psi. The nebuliser temperature was 350 °C. The separation was performed with a Phenomenex (Torrance, CA) Aqua C_{18} column, (250×4.6 mm i.d; 5 µm particle size), operated at ambient temperature. The diode array detector was set to an acquisition range of 520 nm. The mobile phase consisted of water/formic acid/acetonitrile (87:10:3, v/v/v; eluent A) and water/formic acid/acetonitrile (40:10:50, v/v/v; eluent B) using a gradient program as follows: from 10 to 25% B (10 min), 15% B isocratic (3 min), from 15 to 25% B (7 min), from 25 to 55% B (30 min), from 55 to 100% B (1 min), 100% B isocratic (5 min), from 100 to 10% B (0.1 min). Total run was 50 min. The injection volume for all samples was 20 µl and the flow rate was of 1.0 ml min^{-1}.

Table 1
Variation of moisture and soluble solids of control extractions and those assisted by Ultrasonics, HHP and PEF

Extraction	Moisture (g 100^{-1} g fresh grape by-products)	Soluble solids °Brix
Raw matter	96.19±0.03[a]	–
Control	80.12±1.81[b]	15.2±1.03[b]
Ultrasonics	78.47±0.23[b]	16.7±2.19[b]
HHP	77.44±1.63[b]	18.3±0.30[b]
PEF	73.65±2.65[b]	17.2±0.03[b]

Values represent as a mean±standard deviation, $n=3$. Different letters in columns indicate significant differences among mean values of treatments ($p<0.05$).

2.10. Quantification of individual compounds

Individual compounds were quantified using the calibration curve of Cy-3-glu. Calibration of structurally related substances was determined including a molecular weight correction factor (Chandra, Rana, & Li, 2001). All determinations were performed in triplicate and expressed as mg Cy-3-glu eq. g^{-1} dried matter (DM).

2.11. Total phenolics

Total phenolics were determined using the Folin–Ciocalteau method described by Singleton and Rossi (1965). A 100-fold diluted extract aliquot of 125 µl was mixed with 625 µl of Folin–Ciocalteau reagent (previously diluted 10-fold with distilled water; and incubated at 45 °C) and set 3 min at room temperature. 500 µl of sodium carbonate (105.99 g mol^{-1}; and previously incubated at 45 °C) were added to the mixture and incubated at 45 °C during 15 min after which the absorbance was measured at 750 nm.

Gallic acid hydrate (Roth, Karlsruhe, Germany) was used as standard for the calibration curve and results were expressed as µmol of gallic acid eq. (GAE) g^{-1} dry matter (DM).

2.12. Antioxidative capacity

The $ABTS^{+}$ method described by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993) was slightly modified for the determination of the polar antioxidative capacity. A stock solution of 5 mM $ABTS^{+}$ (2.2′-azinobis [3-ethylbenzothiazoline-6-sulphonic acid]) was diluted in water and preincubated for at least 12 h with 140 mM $K_2S_2O_8$ to produce the radical cation $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ solution was then diluted in 5 mM saline phosphate buffer pH 7.4 (0.695 g $Na_2HPO_4\times2\ H_2O$+0.159 g $NaH_2PO_4\times2\ H_2O$+4.5 g NaCl per l) until absorbance readings reached a value of 1.5 at 735 nm. An aliquot extract of 100 µl was diluted 200-fold in buffer mixed with 2.9 ml $ABTS^{\bullet+}$ and set 15 min at 30 °C then absorbance was measured at 735 nm. A calibrated curve using Trolox as standard was used to calculate the antioxidant activity of the samples, expressed as µmol trolox eq. (TE) g^{-1} dry matter (DM).

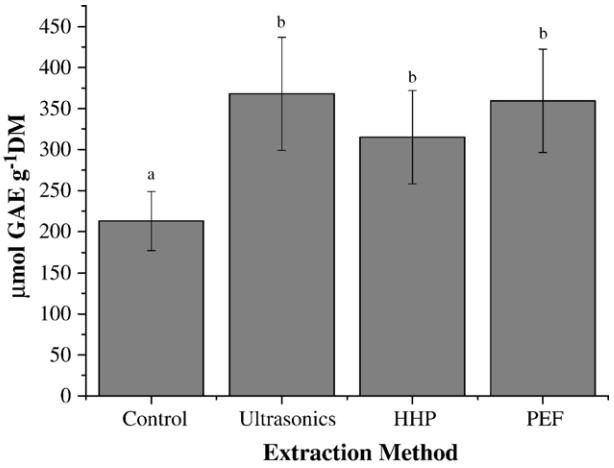


Fig. 1. Total phenolic content (µmol GAE g^{-1} DM) from grape by-products extracted by Ultrasonics, HHP and PEF. Different letters above bars indicate significant differences between mean values ($p<0.05$).

2.13. Statistical analysis

The results reported in this work are the average of at least three measurements. The data presented in tables and figures represents mean values ± standard deviation ($n=3$). Significance levels ($p<0.05$) were evaluated using statistical package of Microsoft Office software, Student's *t* test.

3. Results and discussion

3.1. Total phenolic content and antioxidant capacity of grape by-products extracts as a function of the methodology

Dornfelder *V. vinifera* ssp. by-products were selected for the recovery of anthocyanins and other antioxidants, because of their higher content in anthocyanins compared to other grape varieties grown in the zone of the Palatine in Germany. Indeed, it is well popular extended the mixture of Dornfelder grapes with other varieties of grapes with the aim of improving red colour and flavour of low quality table wines. The extraction of Dornfelder anthocyanins and other antioxidant compounds can be described as a mass transport phenomenon where solids contained in plant structures migrate into the solvent up to equilibrium. Mass transport phenomena can be increased by heating, changes in concentration gradients and with the influence of technologies such as ultrasonics, HHP and PEF (Weltin-Chanes, Vélez-Ruiz, & Barbosa Cánovas, 2003). The treatment intensities and an ethanol concentration of 50% were chosen as a result of previous studies carried out in our group to establish the optimal extraction conditions (Corrales, Butz, & Tauscher, 2006; Knorr, 1994; Lebovka et al., 2004). Moreover, Pinelo, Rubilar, Sineiro, and Nunez (2003) and more recently of Spigno, Tramelli, and Marco de Faveri (2006) also highlighted the advantages of a mixture of ethanol/water for anthocyanin recovery from grape skins. Results represented in Table 1 shows the higher content in soluble solids and moisture loss when extractions were assisted by ultrasonics, HHP and PEF compared to control extractions. In connection with the effectiveness of the extraction, the determination of the content in secondary metabolites in the extracts was estimated by the total phenolic content and antioxidant activity. The total phenolic content of the samples extracted with different methods ultrasonics, HHP and PEF was not significantly different ($p<0.05$) but in comparison to the control samples (Fig. 1). Ultrasonics, HHP and PEF increased phenolic compounds recovery approximately two-fold higher than the control extraction at 70 °C. On the contrary, the results obtained from the antioxidant activity expressed as µmol TE g^{-1} DM were significantly different among the different treatments used ($p<0.05$). The highest antioxidant content (784.34± 150.41 µmol TE g^{-1} DM) was obtained in the PEF treated samples followed by HHP (548.49±47.97 µmol TE g^{-1} DM), ultrasonics (308.13±46.54 µmol TE g^{-1} DM) and the control (187.13±28.45 µmol TE g^{-1} DM) (Fig. 2). The maximal extraction aimed by PEF was a 75% of the possible total extraction to be obtained. Folin–Ciocalteau determined mainly the number of aromatic rings in molecules present in the sample, certainly indicating the content of polyphenols. However, as a result of the extraction treatments, it is also to be acknowledged that some other substances with antioxidant activity were extracted and better determined by $ABTS^{+}$ radical assay. The radical activity test $ABTS^{+}$ determines the scavenging properties, not only of polyphenolic compounds but also from other substances with antioxidant capacity present in the extracts such as anthocyanin condensate products, vitamins, aminoacids, minerals and synergistic effects among them. The use of electrical fields of 3 kV cm^{-1} seems to cause irreversible pores in plant membranes increasing the extractability of polyphenols by the release of solutes into the solvent. Moreover, PEF provides the possibility of inactivating degrading enzymes which may explain the higher yields in antioxidant activity compared to the other methods (Fig. 2). In support to these results, Estiaghi and Knorr (2000) reported that the nutritional content of fruit juices as well as the number of intracellular compounds from grapes during wine production was enhanced when applying an external electrical field in a range of 1–3 kV

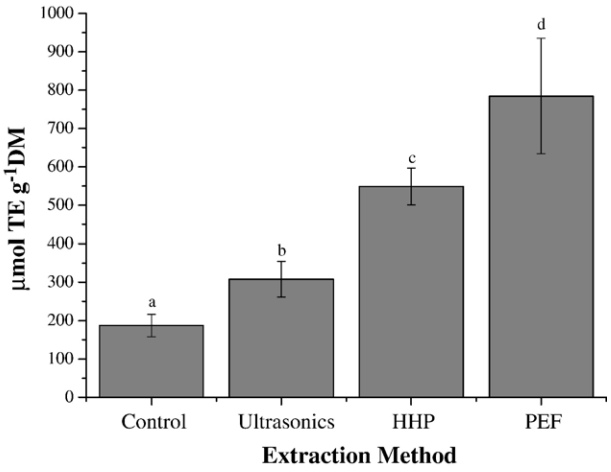


Fig. 2. Antioxidant capacity (µmol TE g^{-1} DM) from Dornfelder grape by-products extracted by Ultrasonics, HHP and PEF. Different letters above bars indicate significant differences between mean values ($p<0.05$).

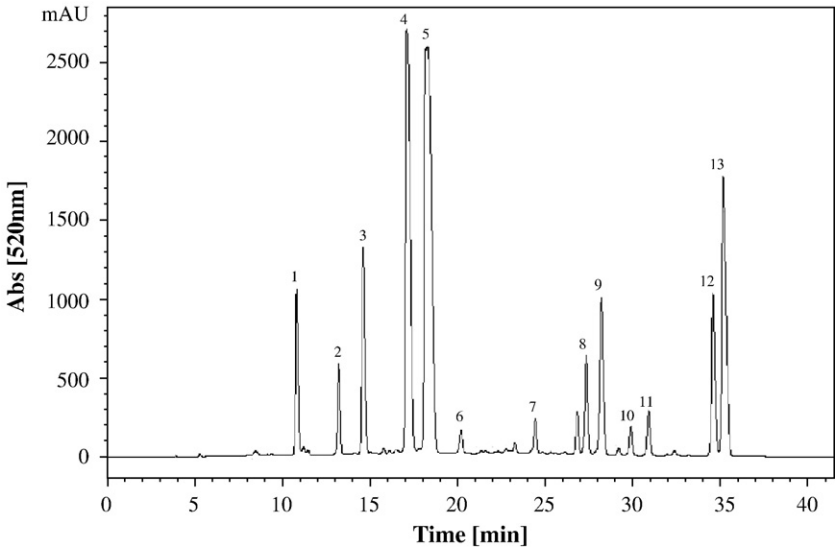
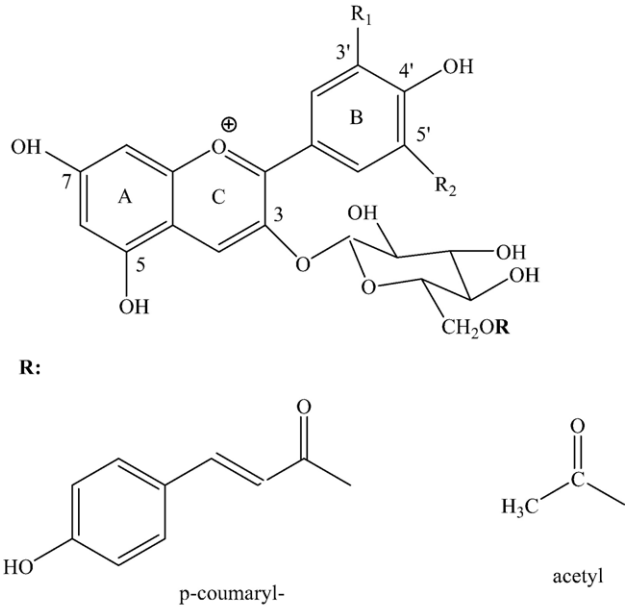


Fig. 3. HPLC anthocyanin profile from Dornfelder (*Vitis vinifera* ssp.) extract obtained at 600 MPa, 70 °C during 1 h. Compounds: **1**. Dl-3-glu, M^+=465; **2**. Cy-3-glu, M^+=449; **3**. Pt-3-glu, M^+=479; **4**. Pn-3-glu, M^+=463; **5**. Mv-3-glu, M^+=493; **6**. Dl-3-acglu, M^+=507; **7**. Pt-3-acglu, M^+=521; **8**. Pn-3-acglc, M^+=505; **9**. Mv-3-acglu, M^+=535; **10**. Cy-3-pcmglu, M^+=595; **11**. Pt-3-pcmglu, M^+=625; **12**. Pn-3-pcmglu, M^+=609; **13**. Mv-3-pcmglu, M^+=639.

cm^{-1}. Similarly, HHP increased the extraction yields due to its aptitude to deprotonate charged groups and disrupt salt bridges and hydrophobic bonds in cell membranes which may lead to a higher permeability (Barbosa-Canovas, Pothakamury, Palou & Swanson, 1998). In addition, the decrease in the dielectric constant of water caused under HHP combined with temperature leads to a decrease in the polarity of the media which may contribute to the higher levels of total phenolics and other antioxidants yielded. (Fernandez, Goodwin, Lemmon, Levelt-Sengers & Williams, 1997). Flavonols and anthocyanins are well-known to be less soluble in water than in an organic media (Revilla, Ryan & Martin-Ortega, 1998). Indeed, the medium plays an important role not only in the extraction of polyphenolic compounds but also in the antioxidant activity of the extracts. Pinelo, Rubilar, Sineiro, and Nunez (2005), demonstrated that phenolic compounds presented a higher antioxidant activity when they were dissolved in ethanol>methanol>water; ordered from less to high polarity. Similar results were obtained by Van der Berg, Haenen, Van der Berg, and Bast (1999) who reported that the antioxidant activity of quercetin in ethanol solution was nearly two-fold higher than that observed in the organic solvent Triton X-100. Therefore, the fact that high pressure treatments do not cause significant changes on antioxidant activity in food matrices (Butz et al., 2003), explains that the higher antioxidant activity here achieved, was according to a mechanism other than diffusion. Ultrasonics also improved the extraction yields compared to the control samples; however, the effect of ultrasonics on antioxidant recovery was less pronounced than for PEF and HHP.

3.2. Anthocyanin extraction yields as a function of the methodology

The HPLC separation of anthocyanins from a Dornfelder skins extract is represented in Fig. 3. The anthocyanins present in extracts from Dornfelder were identified in the monoglucoside forms (3-glu) of delphinidin (Dl), cyanidin (Cy), petunidin (Pt), peonidin (Pd), and Malvidin (Mv); the acetylglucoside forms (3-acglu) and the *p*-coumaroylglucoside forms (3-pcmglu) of Dl, Cy, Pt, Pd and Mv. Table 2 shows the extraction yields in total anthocyanins, total anthocyanin monoglucosides and acylatedglucosides obtained from the differently treated samples. PEF increased the anthocyanin extraction yields up to 10% in



Anthocyanidin	R_1	R_2
Cyanidin (Cy)	OH	H
Delphinidin (Dl)	OH	OH
Malvidin (Mv)	OCH_3	OCH_3
Peonidin (Pn)	OCH_3	H
Petunidin (Pt)	OH	OCH_3

Fig. 4. Anthocyanins' general structure.

Table 2
Individual anthocyanin content (mg Cy-3-glu eq. g^{-1} DM) from Dornfelder grape by-products extracted by Ultrasonics, HHP and PEF

Compound	Control	Ultrasonics	HHP	PEF
Anthocyanins monoglucosides				
Dl-3-glu	0.40±0.016	0.33±0.008	0.47±0.229	0.43±0.017
Cy-3-glu	0.30±0.002	0.27±0.003	0.33±0.073	0.37±0.061
Pt-3-glu	0.48±0.002	0.39±0.017	0.66±0.034	0.95±0.456
Pn-3-glu	4.22±0.017	1.06±0.125	2.90±0.333	6.08±0.204
Mv-3-glu	2.06±0.061	2.79±0.577	1.68±0.260	2.42±0.447
Subtotal	7.46±0.098[a]	4.85±0.73[a]	6.05±0.929[a]	10.25±1.185[b]
Acylated anthocyanin monoglucosides				
Dl-3-acglu	0.28±0.001	0.28±0.002	0.74±0.597	0.30±0.001
Pt-3-acglu	0.30±0.008	0.29±0.003	0.32±0.032	0.31±0.005
Pn-3-acglu	0.35±0.002	0.32±0.007	0.39±0.097	0.36±0.017
Mv-3-acglu	0.50±0.005	0.41±0.018	0.62±0.326	0.54±0.054
Cy-3-pcmglu	0.30±0.006	0.30±0.009	0.32±0.023	0.31±0.003
Pt-3-pcmglu	0.38±0.004	0.35±0.004	0.43±0.053	0.38±0.008
Pn-3-pcmglu	0.53±0.012	0.39±0.044	0.59±0.168	0.51±0. 038
Mv-3-pcmglu	1.17±0.053	0.58±0.115	1.70±0.222	1.08±0.217
Subtotal	0.48±0.091[a]	2.91±0.202[b]	5.15±1.518[c]	3.79±0.343[b]
Total content	7.93±0.189[a]	7.76±0.932[a]	11.21±2.447[b]	14.05±1.528[c]

Values represent as a mean±standard deviation, $n=3$. Different letters in rows indicate significant differences among mean values of treatments ($p<0.05$).

comparison with HHP and up to 17% compared to the conventional extraction whereas the differences between the control samples and those extracted with ultrasonics were not significantly different ($p>0.05$). These results are in agreement with the studies reported by Toepfl (2006) in which a higher extraction of anthocyanins from purple fleshed potatoes treated with PEF was described. Studies of Gimenez, Kajda, Margomenou, Piggott, and Zabetakis, (2001) reported also a better colour retention of jams and grape juices when treated by HHP. It can be observed that the improvement of extractability of individual anthocyanins was dependent on the type of treatment and the substitution pattern of B ring in the flavylium structure, as well as the different glycosilation of A and C rings (Fig. 4). When a glucose was linked covalently to the OH- at C_3 (anthocyanin monoglucoside), the extraction was of 10.25±1.17 mg g^{-1} in extractions assisted by PEF, whereas the control, HHP and ultrasonicated treated samples were of 7.46±0.45 mg g^{-1}, 6.04± 0.62 mg g^{-1} and 4.84±0.70 mg g^{-1} respectively.

PEF remarkably enhanced the extraction of anthocyanin monoglucosides compared to the amount of acylatedglucosides extracted. Acylatedglucoside anthocyanins seemed to be physically entrapped within the matrix, or form hydrogen bonds with cell wall polysaccharides and were consequently extracted in less proportion. The highest anthocyanin recovery was obtained by PEF and corresponded to a 60% of the maximal recovery which was obtained when a solid/liquid ratio of 1:20 and longer holding times were used.

The proportion of anthocyanins in solution was related to their chemical structure and stability to process conditions. The stability of anthocyanins was shown to be dependent on the OH- and OCH_3- groups at position R_1 in $C_{3'}$ and R_2 in $C_{5'}$ of the B ring, as well as the sugar moieties, and phenolic acyl groups of the C ring (Fig. 4). The higher content in OH- and OCH_3- and acyl groups, the higher the stability, Mv being the most stable one followed by Pd, Pt, Cy and Dl. These significant chemical differences explain the higher yields of Pt, Pd and Mv, obtained by PEF and HHP extractions (Table 2). Contrary to PEF extractions, the content of acetylglucoside and coumaroylglucoside anthocyanins (acylated ones) in pressurised extracts was remarkably higher. The decrease in solvent polarity enhances their release since they are less polar and the most stable anthocyanins. Therefore, HHP has the ability to reduce the pH of the solvent during the extraction not only because of the higher release of phenolics into the solvent but also because of the deprotonation of molecules present in the extracts. This decrease in the pH might also enhance the extraction of acylated anthocyanins since they are more stable at pH<4 where the flavyliums cations are predominant. At this pH, aromatic acyl groups of acylated anthocyanins are known to stack on the flavylium nucleus and thereby protect the pyrylium ring from the nucleophilic addition of water, which lead to their colourless forms (chalcones) (Brouillard, 1983).

4. Conclusions

The application of advanced technologies such as ultrasonics, HHP and PEF has demonstrated to offer an extraordinary potential and selectivity for extraction purposes. They enhanced the antioxidant capacity of the samples up to four-fold higher than control extractions at 70 °C and extracted anthocyanins selectively. However, the multitude of process dimensions which present themselves when they are combined with conventional process variables such as temperature, time, change in pH, solid/liquid ratios should be further studied in order to optimize the whole process for a future industrial implementation. The combination of effective extraction technologies and low-cost raw materials represent an environmental and economical alternative to conventional extraction methods where large amounts of organic solvents and long extraction times are required. The use of these novel processing technologies will reduce food processing wastes and facilitate the production of natural valuable products which will guarantee food sustainability and meet consumer demands.

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