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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