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ABIOTIC STRESS BASED BIOPROCESSES FOR THE PRODUCTION OF HIGH VALUE ANTIOXIDANT PHENOLIC COMPOUND IN PLANTS: AN OVERVIEW

BIOPROCESOS BASADOS EN LA APLICACIÓN DE ESTRESES ABIÓTICOS EN PLANTAS PARA LA PRODUCCIÓN DE COMPUESTOS FENÓLICOS ANTIOXIDANTES DE ALTO VALOR: UNA REVISIÓN

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Abstract

Phenolic compounds (PC) are secondary metabolites produced by plants that have diverse applications in the pharmaceutical, cosmetics, nutraceutical and food industries. Therefore, the design of bioprocesses for their production, extraction and purification is of major relevance. The application of postharvest abiotic stresses (*i.e.*, wounding, modified atmospheres, UV radiation) can be used as an approach to increase the concentration of PC during postharvest of diverse plant tissues. Herein, we propose an abiotic stress based bioprocess for the production of high commercial value antioxidant PC. The strategy proposed was exemplified with experimental data showing how abiotic stresses can be applied to produce resveratrol and quercetin-3-*O*-glucoside in grapes, and chlorogenic acid in carrots. Finally, different procedures to extract and purify PC produced in the stressed plant tissue are discussed.

Keywords: abiotic stresses, phenolic compound, wounding, UV-C light, plant tissue, by-products, downstream processing.

Resumen

Los compuesto fenólicos (PC) son metabolitos secundarios producidos por plantas que tiene diversas aplicaciones en la industria farmacéutica, cosmética, nutracéutica y alimentaria. Por lo tanto, el diseño de un bioproceso para su producción, extracción y purificación es de gran importancia. La aplicación de estrés abiótico poscosecha (*i.e.*, daño por corte, atmósferas modificadas, radiación UV) puede ser usada como una estrategia para incrementar la concentración de PC durante la poscosecha de diversos tejidos vegetales. En este artículo proponemos un bioproceso basado en la aplicación de estrés abiótico para la producción de PC antioxidantes de alto valor comercial. La estrategia propuesta es ejemplificada con datos experimentales que muestran cómo el estrés abiótico puede ser aplicado para producir resveratrol y quercetina-3-*O*-glucósido en uvas y ácidos clorogénicos en zanahorias. Finalmente, se discuten procedimientos para extraer y purificar PC producidos en tejidos estresados de plantas.

Palabras clave: estreses abióticos, compuesto fenólico, daño por corte, luz UV-C, tejido vegetal, subproductos, procesos de separación.

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1 Introduction

Phenolic compounds (PC) are secondary metabolites produced by plants, algae, and fungi, which have a wide range of applications in the pharmaceutical, cosmetics, dietary supplements, and food industries. The scientific interest for the PC increased in the 1990s when the *French Paradox* (low occurrence of cardiovascular diseases despite diets rich in cholesterol and saturated fats) was attributed to resveratrol present in red wine (Renaud and de Lorgeril, 1992). In addition to the health-promoting properties reported for PC, they also possess *in vitro* antioxidant activity (Xia *et al*., 2010) and thus, they have many applications as additives in food and cosmetic formulations (Llorac *et al*., 2002; Peschel *et al*., 2006).

The health benefits and diverse industrial applications of PC have prompted the interest of the scientific community to increase their content in plant tissues by using different tools. The most widely used tool to achieve this purpose is genetic engineering. In this strategy, the phenylpropanoid pathway is modified to produce specific PC at high levels. For instance, Niggeweg *et al*., (2004) reported that the overexpression of hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase (*Hqt*) gene in tomato resulted in a 2-fold increase of 5-*O*-caffeoylquinic acid (5-CQA) content, compared with the non-genetically modified tomato. Rommens *et al*., (2008) increased the content of kaempferol by 100-fold in potato by silencing the flavonoid-3', 5'-hydroxylase (*F3'5'h*) gene and increased by 4-fold the content of 5-CQA by overexpressing the *Hqt* and prephenate dehydratase (*Pdh*) genes. In addition to tomato and potato, tobacco was also genetically engineered to increase its PC content. Howless *et al*., (1996) overexpressed the L-phenylalanine ammonia lyase (PAL) gene in tobacco, which code for the key enzyme on the biosynthesis of PC. The overexpression of PAL gene resulted on the accumulation of hydroxycinnamic acids, particularly chlorogenic acid.

Although several crop lines have been genetically engineered to obtain high levels of PC, genetic modification is technically complicated. Likewise, the extensive use of genetically modified plants is still under debate because they are considered biological hazards due to their potential risk of overcrossing between the genetically modified plant and their wild relatives as well as the danger of toxicity or allergy for consuming genetically modified foods (Schouten *et al*., 2006; Jacobo-Velázquez and Cisneros-Zevallos, 2012).

Recently, Cisneros-Zevallos (2003) proposed the application of controlled postharvest abiotic stresses as an approach to increase the concentration of PC in fruits and vegetables. Cisneros-Zevallos (2003) mentioned that selected abiotic stress treatments (*i.e*., wounding, exogenous applications of phytohormones, modified atmospheres, UV radiation, etc.) can affect the production of PC, resulting in an increase of the nutraceutical value of plant foods. Furthermore, individual PC produced under stress conditions in plants exhibit a higher antioxidant activity as compared with PC present in non-stressed plant tissues (Reyes and Cisneros-Zevallos, 2007; Heredia and Cisneros-Zevallos, 2009a; Jacobo-Velázquez *et al*., 2011).

The aim of this paper is to provide an overview of how the application of abiotic stresses could be included in a bioprocess to induce the production of PC in different sources (plants, fungi and algae), with particular attention to plant tissue. In addition, different standard procedures for the extraction and purification of PC produced in the stressed tissue are described. To exemplify the abiotic-stress based bioprocess proposed herein, abiotic stress conditions that can be applied in carrots and grapes to induce the production of individual PC (*i.e*., chlorogenic acid, resveratrol and quercetin-3-*O*-glucoside) are described using experimental data obtained in our research group.

2 Sources of phenolic compounds (PC)

Fresh fruits and vegetables are the most abundant natural source of PC in the human diet (King *et al*., 1999; Shahidi, 2000; Boskou, 2006; Gharras, 2009; Isabelle *et al*., 2010a; Isabelle *et al*., 2010b). In addition, PC can also be found in cereals, teas, juices, coffee, and wines (de Beer *et al*., 2002; Balasundram *et al*., 2006). However, the presence of PC is not limited to plant tissue since fungi and algae also represent a source of phenolics. For instance, Onofrejová *et al*., (2010) extracted phenolic acids (chlorogenic, vanillic, caffeic, *p*-cumaric, cinnamic, and protocatechuic acids) from *in vivo* culture of both freshwater algae and marine macroalgae using pressurized liquid and solid phase extraction. It is believed that these PC play a role in the response of algae to different biotic and abiotic stresses. López *et al*., (2011) also identified

different PC (gallic acid, cathechin, rutin, myricetin, quercetin, protocatechuic acid, vanillic acid, caffeic acid, ferulic acid, chlorogenic acid and syringic acid) in aqueous extracts of the brown algae *Stypocaulon scoparium*. Ribeiro *et al*., (2007) identified five PC (hyperoside, quercetin, caffeic acid, *p*-cumaric acid, and ellagic acid) in *Fistulina hepatica*, which is a mushroom used for human consumption. Huang *et al*., (2008) reported 37 phenolics in 31 endophytic fungal taxa. Some of the PC identified in the endophytic fungi were galloyl-3-*O*-*β*-glucuronide, quercetin-3-*O*-*β*-glucuronide, kaempferol-3-*O*-rutinoside, and resveratrol-3-*O*-glucoside.

There are some synthetic antioxidant PC such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butyl hydroquinone (TBHQ), however the use of these compounds has decreased due to concerns about their carcinogenic effect (Shahidi, 2000). In recent years, some authors have proposed that fruit and vegetables processing by-products like rice hulls, peels and seeds of citrus fruits, grapes and tomatoes may be a natural and abundant source of antioxidant PC (Mource *et al*., 2001; Schieber *et al*., 2001; Balasundram *et al*., 2006; Goñi and Hervert-Hernández, 2011). Interestingly, some fruit and vegetables processing by-products have shown a higher content of PC as compared to the edible part of plant foods (Balasundram *et al*., 2006). These by-products considered as waste can be used as an important source of PC. Furthermore, since by-products contain plant cells that respond to abiotic stress, they can be further stressed to increase the PC content prior to downstream processing. Some by-products that can potentially be used as raw material for the production of PC by means of applying abiotic stress are shown in Table 1. Likewise the total PC content of some by-products is shown in Table 2.

3 Application of abiotic stresses in plant tissues as a strategy to increase high value phenolic compounds (PC)

The application of postharvest abiotic stresses induce the accumulation of PC in different plant tissues including grape, carrot, lettuce, apple, cranberry, jicama, celery, sweetpotato, and red cabbage (Cantos *et al*., 2001; Cisneros-Zevallos, 2003; Reyes *et al*., 2007; Heredia and Cisneros-Zevallos, 2009a; González-Aguilar *et al*., 2010; Eichholz *et al*., 2011). Reyes and Cisneros-Zavallos (2007) reported that the increase of total PC content depends on wounding intensity and the type of plant tissue stressed. The stress-induced synthesis of PC in plants is mediated by signaling molecules such as the reactive oxygen species (ROS) and some phytohormones including ethylene (ET) and methyl jasmonate (MJ) (Jacobo-Velázquez, 2010).

Table 1. By-products generated in the industrial processing of fruits and vegetables that can be used as starting material for the stress-induced production and extraction of phenolic compounds (Goñi and Hervert-Hernández, 2011).

Tissue	Edible part (%)	By-product (%)	Worldwide production in 2010[a] (millions of tons)	Annual estimate of by-products (millions of tons)
Tomato	93-97	Peel and seeds (3-7)	145.75	4.37-10.20
Banana	70	Peel (30)	102.11	30.63
Apple	60	Pulp and seeds (40)	69.56	27.82
Orange	44	Peel (66)	69.41	45.81
Grape	80-85	Peel (15-20)	68.31[b]	10.24-13.66
Carrot	60-70	Bagasse (30-40)	33.65[b]	10.09-13.34
Pineapple	48	Peel, pulp and core (62)	19.41	12.03
Artichoke	40	Outer bracts, receptacles and stems (60)	1.44	0.86

[a] Food and Agricultural Organization of the United Nations, 2012 (<< http://faostat.fao.org/#>>).

[b] Estimated production of carrots and grapes in Mexico was 346,466 and 307,147 tons, respectively (http://faostat.fao.org/#).

Table 2. Total phenolic content (TPC) in by-products generated in fruits and vegetables processing (Peschel *et al*., 2006).

By-product	Tissue	TPC[a]
Residues from juice production		
	Apple	52.18
	Strawberry	59.77
	Pear	18.41
Waste from canning factory		
	Artichoke	95.65
	Asparagus	69.43
	Tomato	37.29
Harvest remains		
	Broccoli	25.58
	Cucumber	27.26

[a]mg gallic acid equivalents per g DW.

These signaling molecules induce the production of PC by activating the phenylpropanoid metabolism. PC produced under stress conditions help to mitigate the cellular damage provoked by the stress (Reyes *et al*., 2007; Heredia and Cisneros-Zevallos, 2009a; Moglia *et al*., 2008; Jacobo-Velázquez *et al*., 2011).

From the different abiotic stresses already investigated to induce the accumulation of PC in plants, wounding is regarded by many as the most effective (Reyes *et al*., 2007; Jacobo-Velázquez and Cisneros-Zevallos, 2012). The effectiveness of wounding to induce the biosynthesis of PC may be further enhanced if an additional abiotic stress is applied in the wounded tissue. For instance, the exogenous application of phytohormones as well as the storage of wounded carrot tissue under hyperoxia conditions (80% O_2 oxygen in the atmosphere) enhanced the accumulation of PC in carrots (Heredia and Cisneros-Zevallos, 2009a; Jacobo-Velázquez *et al*., 2011).

In the present study, we evaluated the application of wounding and UV-C light stress to determine abiotic stress conditions that can induce the production of PC in carrots and grapes. In the case of carrots, the accumulation of PC was evaluated in shredded-tissue as well as in bagasse to show how by-products of the fruits and vegetables industry can be used for this purpose.

3.1 Experimental approach

Carrots (*Daucus carota* L.) and grapes (*Vitis vinifera*) were obtained from a local market (HEB, N.L. Mexico). Carrot shreds were generated using a commercial vegetable shredder (diameter 0.7 cm). Carrot bagasse was obtained by a juice processor (Philips juice extractor HR1854/00). The stressed carrot tissue (shreds and bagasse) was stored in an incubator (BOD incubator, VWR International, USA) at 25°C for 48 h. Grapes were exposed to UV-C light (30 W, G30T8, GE, Fairfield, CT) at different times intervals (30, 60 and 120 min) and stored at 30°C for 5 days. The PC from carrot shreds and bagasse were obtained using the procedure reported by Jacobo-Velázquez *et al*., (2011). PC were extracted by homogenization using methanol, centrifuged (29,000 xg, 20 min, 4°C), and the supernatant (methanol extract) was used to determine the total PC content. Likewise, the methanol extract was passed through nylon membranes (0.45 μm) (VWR, Wester Chester, PA) and used to determine the HPLC phenolic profile. The identification of PC in methanol extracts of stressed carrots was performed by high performance liquid chromatography-photodiode array detection (HPLC-DAD) following the procedure reported by Becerra-Moreno *et al*., (2012).

To extract the PC from grapes, the skins from the stressed tissue were first manually removed. Thereafter, PC from skins were extracted following the procedure reported by Cantos *et al*., (2001) with some modifications. 10 ml of methanol:formic acid (97:3 v/v) were added with grape skins (2 g). The fraction was homogenized (AHS250, VWR, West Chester, PA) and centrifuged (29,000 xg, 5 min, 4°C). The supernatant was filtered using nylon membranes (0.45 μm) (VWR, Wester Chester, PA) and used to determine the phenolic profiles of grapes. The identification of PC from grapes was performed as described by Cantos *et al*., (2001).

For the quantification of individual PC, standard curves of 5-*O*-caffeoylquinic acid, resveratrol and quercetin-3-*O*-glucoside were prepared in the range of 0.5-100 μM. The concentration of the phytochemicals was expressed as milligrams of each individual compound per kilogram of plant tissue dry weight (DW).

Statistical analyses were performed using triplicates. Analyses of variance (ANOVA) were conducted using JMP software version 5.0 (SAS Institute Inc., Cary, NC, USA) and mean separations performed using the LSD test ($p < 0.05$).

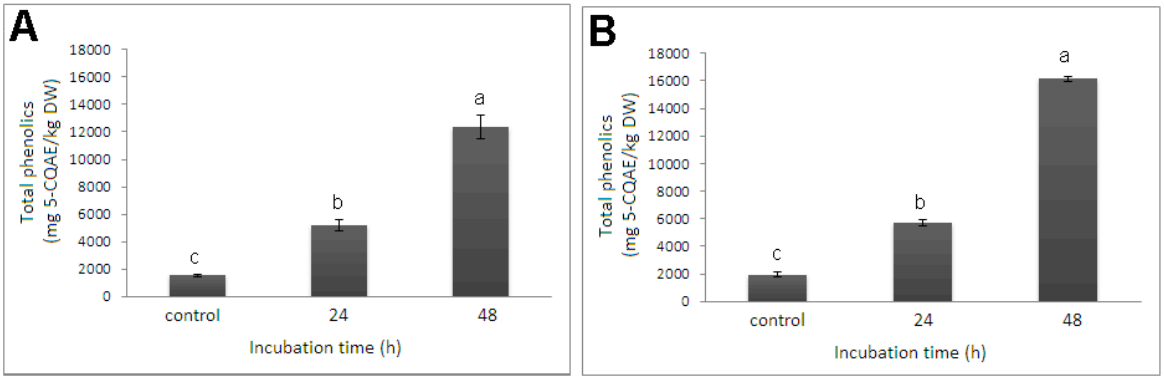

Fig. 1. Production of phenolic compounds during storage (25°C) of carrot bagasse (A) and shreds (B). Total phenolic content is expressed as mg 5-caffeoylquinic acid equivalents per kg dry weight (mg 5-*O*-CQAE/kg DW). Values represent the mean of three replications with their standard error bars. Different letters indicate statistical difference by the LSD test ($p < 0.05$). Data shown from bagasse and shreds was obtained from independent studies.

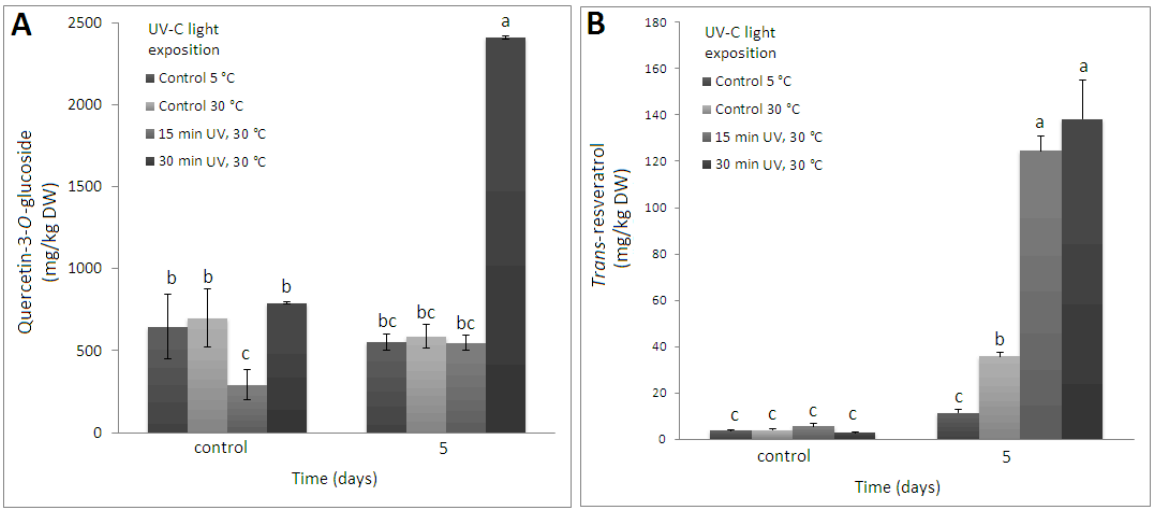

Fig. 2. Quercetin-3-*O*-glucoside (A) and *Trans*-resveratrol (B) content in grapes exposed to UV-C light and incubated for 5 days at 30°C. Values represent the mean of three replications with their standard error bars. Different letters indicate statistical difference by the LSD test ($p < 0.05$).

Table 3. Concentration of CQA in carrots shreds incubated for 48 h at 25°C

Incubation time (h)	Chlorogenic acids (mg chlorogenic acid equivalents/kg DW)		
	5-CQA	3,4-diCQA	3,5-diCQA
0	30	14	20
24	300	18	49
48	1192	52	195

Abbreviations: 5-*O*-caffeoylquinic acid (5-CQA); 3,4-diCQA (3,4-dicaffeoylquinic acid); 3,5-diCQA (3,5-dicaffeqoylquinic acid).

3.2 Production of chlorogenic acids in carrots bagasse and carrots shreds incubated for 48 h

With the aim to increase the total PC content, carrots were wounded to produce shreds. Likewise, carrot bagasse, which is produced under severe wounding stress, was obtained from a carrot juice extractor equipment. Both wounded tissues (bagasse and shredded carrots) showed an increase in the total PC content as the time of incubation increased (Fig. 1). The analyses by HPLC-DAD showed that under this abiotic stress conditions the main compounds produced in carrots are the chlorogenic acids (CQA). Specifically, shredded carrots showed an increase in 5-CQA, 3,4-diCQA, and 3,5-diCQA, while the 5-CQA was the compound exhibiting the highest accumulation when compared with the control sample (Table 3).

The application of other abiotic stresses in carrots has also been shown to increase the content of CQA. For instance, Heredia and Cisneros-Zevallos (2009b) reported an increase of 627% of 5-CQA per kg in carrots shreds exposed to ET, indicating that applying phytohormones and wounding stress have a synergistic effect on increasing the total PC content in this tissue. Otherwise, Jacobo-Velázquez *et al*., (2011) reported an increase of 349% in the TPC in carrots shreds exposed to hyperoxia (80% O_2). Under these conditions, the 5-CQA had the highest increase (~ 2966%) compared to control group. Likewise, Du *et al*. (2012) reported an increase of 270% in the TPC in carrots slices exposed to UV-B light. On the other hand, Becerra-Moreno *et al*. (2012) reported that the application of glyphosate (482 g/L) in wounded carrots induced an increase in shikimic and 5-CQA content by ~1735% and ~5700%, respectively. The differences in the content of PC in stressed carrots are due to the type of abiotic stress-applied, the incubation conditions, and the varieties of the crop used in the analysis. The PC produced in wounded-carrots are of high pharmaceutical and nutraceutical value since they have shown anti-cancer, anti-genotoxic, anti-viral, anti-microbial, and antioxidant activity (Clifford, 2000).

3.3 Production of resveratrol and quercetin-3-O-glucoside in grape exposed to UV-C light

Grapes were exposed to UV-C to evaluate the effect of this abiotic stress on the accumulation of individual PC. The analysis by HPLC-DAD revealed that at 5 days of incubation, resveratrol and quercertin-3-*O*-glucoside are accumulated in the irradiated samples. Specifically, the treatment that showed the highest accumulation of both PC was the grapes irradiated with UV-C light for 30 min. Quercertin-3-*O*-glucoside concentration increased by 4-fold, whereas the concentration of resveratrol increased by 30-fold after 5 days of incubating the irradiated sample (Fig. 2). Resveratrol and quercetin-3-*O*-glucoside have shown diverse health-promoting properties, especially on the prevention of cardiovascular diseases (Careri *et al*., 2003; Xia *et al*., 2010).

As mentioned earlier herein, there are different natural sources for the extraction of PC including fresh fruits and vegetables, food industries by-products, vegetable tissues that do not meet quality standards for human consumption, or even fungi and algae. These sources can be stressed to obtain higher concentration of PC. The general abiotic stress based strategy proposed herein to induce the production of PC is summarized in Fig. 3. To increase the content of PC in plant tissue, wounding can be applied as a first stress in carrots. Furthermore, the application of an additional stress in wounded tissue can induce higher accumulation of individual PC. On the other hand, the direct application of UV-C radiation in plant tissues such as grapes induces higher accumulation of certain PC such as resveratrol and quercetin-3-*O*-glucoside. The stressed tissue can be subjected to downstream processing in order to recover and purify PC produced and accumulated. Methods for the recovery and purification of PC are described in the following section.

4 Recovery and purification of phenolic compound (PC) produced in stressed plant tissues

Once PC are produced (*i.e*., stressed plant tissue), they need to be extracted and purified accordingly to their final application. Fig. 4 shows separation, purification and polishing technologies typically utilized for the downstream processing of PC. PC comprise an assorted group of organic compounds with very diverse physicochemical properties. Therefore, an adequate strategy to recover a particular compounds should be based on their specific physicochemical properties as well as those of the contaminants presents.

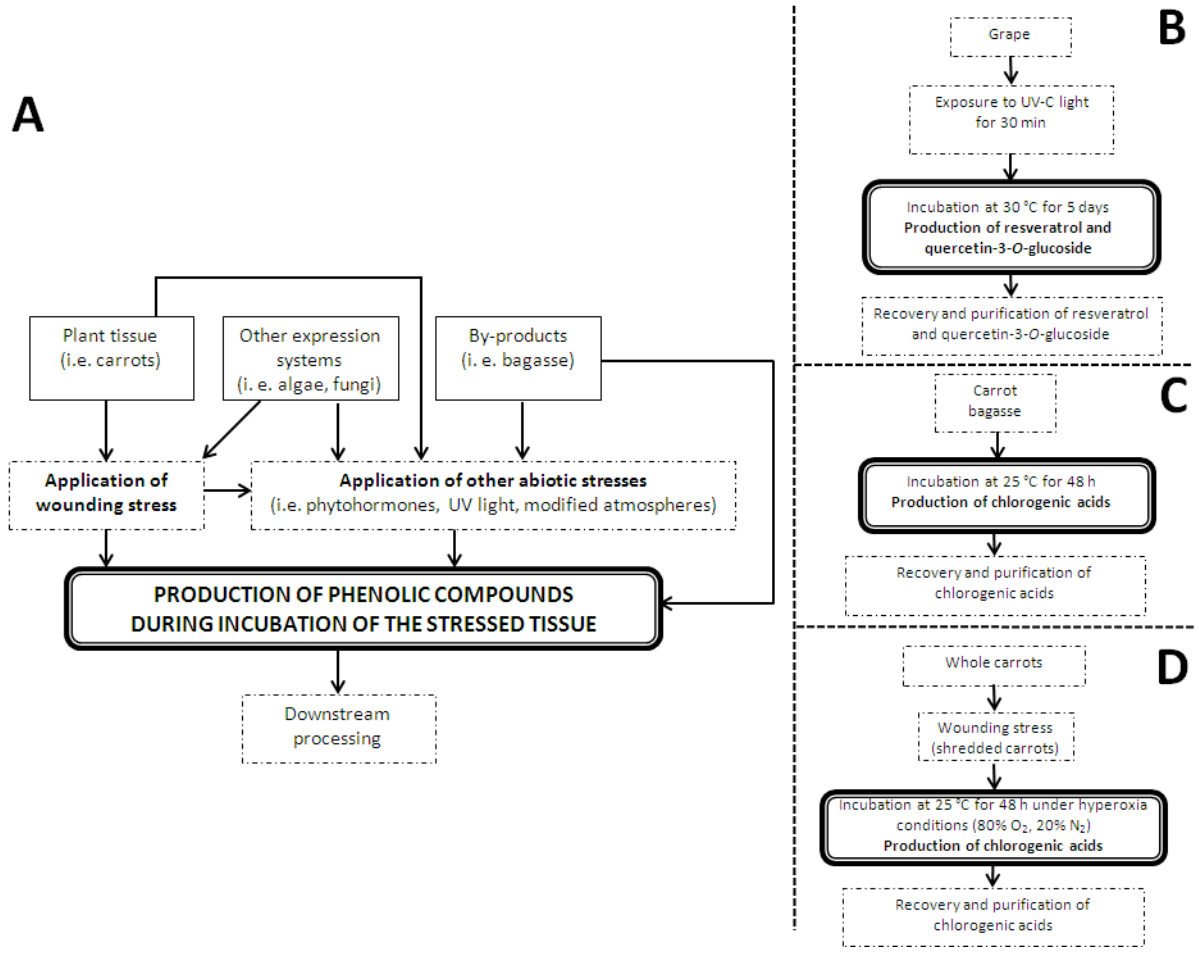

Fig. 3. Abiotic stress based bioprocesses for the production of high value phenolic compounds from different sources (A). Production of resveratrol and quercetin-3-*O*-glucoside in grape (B). Production of chlorogenic acids in carrot baggase (C) and whole carrots (D).

After the production has taken place, PC are usually extracted from the tissue (vegetal, fungal, algal, etc.) using solid-liquid extraction (Perez-Magarino *et al*., 2008; García-Márquez *et al*., 2012). In this method, an adequate solvent, selected based on the polarity of the solutes of interest, is put in contact with the solid matrix (*i.e*., plant tissue) (Tsao and Deng, 2004). As the solvent interacts with the solutes, these are extracted from the matrix. In order to favor this process, different pre-treatments can be used on the solid matrix, being the most common the homogenization/grinding of the tissue. This increases the surface area between the sample and the solvent, thereby enhancing the mass transfer (Kim and Lee, 2002). Drying the sample may also favor the extraction process, particularly when the water content in the matrix is high and the solvent being used is hydrophobic in nature (dichloromethane, hexane, etc.). However, since most PC range from hydrophilic to amphipathic usually polar solvents/solvent mixtures are used.

Some strategies have been reported to enhance the extractions of PC from plant tissues. Sonication/ultrasonication has been used to increase extraction yields of low molecular weight compounds. Shock waves generated as part of the cavitation process increase mixing and weaken solid barriers (cell wall and membrane), favoring mass transfer towards the liquid fraction (Kim and Lee, 2002). Additionally, microwave-assisted extraction (MAE) is a novel method for obtaining PC. This strategy consists in mixing the plant tissue with a specific

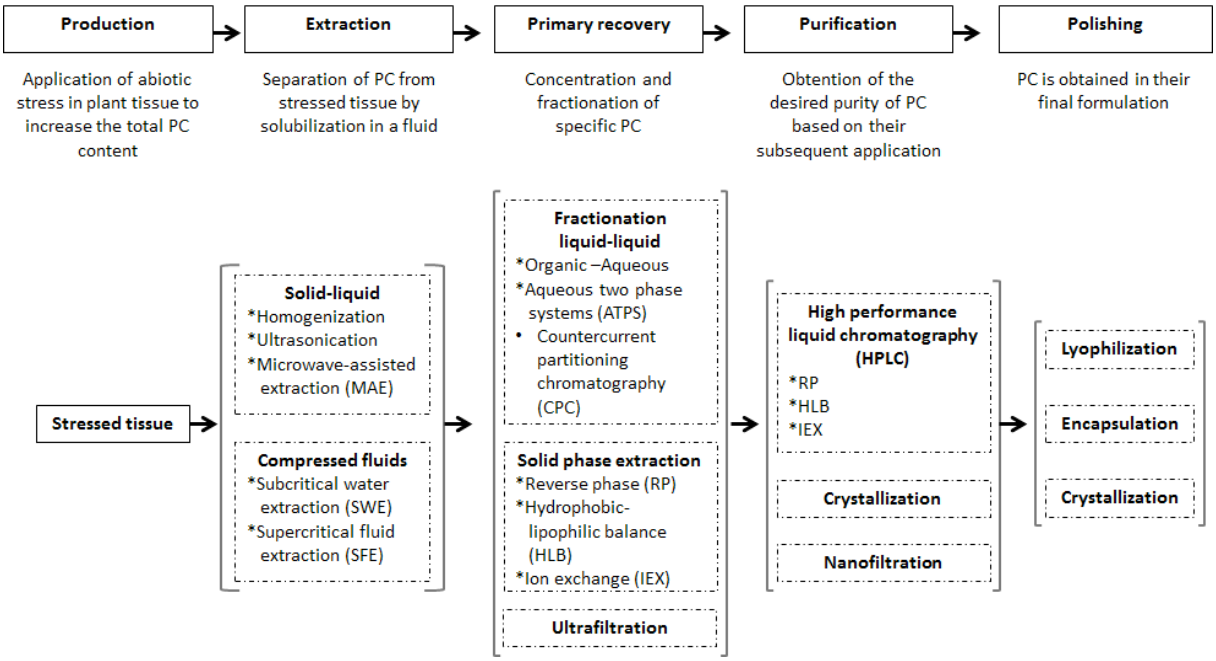

Fig. 4. Separation, purification and polishing technologies typically utilized for the downstream processing of phenolic compounds.

solvent, and then the solid suspension is exposed to microwaves for short periods. This method has efficiently assisted in the extraction of PC from plant tissues such as tealeaves and grape seeds (Escribano-Bailón and Santos-Buelga, 2003). Regarding solvents selection, low molecular weight alcohols (mainly methanol and ethanol, pure or in solution 20-80 %v/v) are used to extract low molecular weight polyphenols, hydroxycinnamic acids, glycosylated PC and some flavonoids (Robbins, 2003), while slightly less polar solvents, such as acetone (usually in solution), may be used for extracting insoluble-bounded phenolic acids, anthocyanins, and procyanidins (Escribano-Bailón and Santos-Buelga, 2003).

The use of compressed fluids such as subcritical water extraction (SWE) and supercritical fluid extraction (SFE) has been proposed as an alternative to solid-liquid extraction to recover low molecular weight compounds (Escribano-Bailón and Santos-Buelga, 2003). These techniques combine high temperatures (40-200°C) and pressures (1-20 MPa) to extract PC from vegetal tissue reducing extraction time and solvent consumption. This results on a more environmental friendly strategy (Dai and Mumper, 2010). However, these techniques are not yet fully standardized, particularly for the extraction of PC (Tsao and Deng, 2004), and since they have not been yet widely adopted at industrial level their application at large scale is still expensive (Dai and Mumper, 2010).

The fractions obtained from the extraction process, containing the PC of interest, require subsequent recovery steps in order to remove contaminants. In this context, PC are usually fractionated and/or concentrated according to their physicochemical properties using strategies based on liquid-liquid fractionation (LLF) or solid phase extraction (SPE). Furthermore, ultrafiltration and precipitation-based strategies may be also used in order to remove high molecular weight contaminants such as polysaccharides, proteins, and genetic material (Doran, 1995; Verrall, 1996). These high molecular weight contaminants change their structural conformation based on the system conditions. Therefore, the increase of ionic strength (adding salt, "salting out" effect), the elimination of electrostatic repulsion between molecules (adjusting pH to their specific isoelectric point), and the modification of the dielectric constant (by adding a secondary solvent or reagent) can promote their aggregation and further precipitation (Verrall, 1996).

Concerning liquid-liquid fractionation strategies, one of the most common approaches are the organic-aqueous biphasic systems. In this case, the solutes are fractioned between two phases, one hydrophobic (*i.e.*, dichloromethane, hexane, acetonitrile, etc., usually

the top phase due to its lower density) and one hydrophilic (water, low molecular weight alcohols, acetone or solutions). Based on their characteristics most PC are fractionated towards the hydrophilic phase, being separated from less polar contaminants that migrated to the opposite phase. A major drawback of this approach is that hazardous organic solvents are utilized, representing a risk to human health and the environment (Escribano-Bailón and Santos-Buelga, 2003). A more environmentally friendly approach for the liquid-liquid fractionation of PC are the aqueous two phase systems (ATPS). In ATPS both phases are primarily hydrophilic, avoiding the use of hazardous volatile organic solvents. Although is a technique that has been mainly use for fractionating high molecular weight compounds (mostly proteins), it may be also used for low molecular weight compounds (Benavides and Rito-Palomares, 2008). Particularly, alcohol-salt ATPS are suitable for obtaining PC from crude extracts. Another option for the liquid-liquid fractionation of PC is the countercurrent partitioning chromatography (CPC) (Dai and Mumper, 2010). In this technique, immiscible or partially miscible liquid effluents are confronted on opposite directions generating a high number of thermodynamic equilibriums as solutes migrate through the interphase between effluents. This is a high-resolution technique from which a high number of fractions can be obtained. However, it is mainly used at preparative lab scale due to technical difficulties related to the scaling-up of the operation.

Solid phase extraction (SPE) is another strategy of fractionation that involves the adsorption of solutes on a solid matrix according to their physicochemical properties. There is a wide diversity of stationary phases (solid matrixes) that can be used for SPE (ionic exchange, reverse phase, normal phase, hydrophobic interaction, affinity, etc.) based on their adsorption principle. The most commonly used stationary phases to fractionate PC are reverse phase matrixes (C_{18}) although ion exchange (IEX, mainly anion exchange) and hydrophobic-lipophilic balance (HLB) phases are also utilized (Zwir-Ferenc and Biziuk, 2006). Strategies based on SPE are usually straight-forward and versatile, being even able to obtain multiple fractions if the elution is conducted on gradient or steps. However, stationary phases can be used just a limited number of times before losing resolution and adsorption capacity (due to unspecific irreversible interactions between the functional groups in the matrix and the solutes in the sample) and therefore need to be replaced constantly, adding costs to the process.

After the major contaminants have been removed on the primary recovery stage, purity of the PC can be further increased using strategies based on high performance liquid chromatography (HPLC), crystallization and membrane fractionation (nanofiltration). As in the case of SPE, most of the stationary phases used for fractionating PC in HPLC are based on polarity, being the C18 stationary (reverse) phase the most common (Escribano-Bailón and Santos-Buelga, 2003; Tsao and Deng, 2004). As previously mentioned, also IEX and HLB can be used for this purpose. Depending on the complexity of the fraction, more than one HPLC stage may be required in order to obtain a pure fraction. HPLC is a well-characterized technique and it is the most commonly used purification technique in the biotechnology industry, despite the drawbacks that its scaling-up represents. Crystallization is another method to purify chemicals compounds, while concentrating the product of interest at the same time. In this strategy, the fraction that contains both the compound of interest and the few remaining contaminants is mixed with an appropriate solvent in which the contaminants are more soluble that the product. Then, the fraction is evaporated and cooled, generating a supersaturated solution, promoting the crystallization and sedimentation of the product of interest. This process is repeated until the desired purity and recovery yield are achieved (Zhang *et al*., 2008). Nanofiltration is another technique than may be used for the purification and concentration of PC, although not widely utilized for this purpose. This technique fractionates based on molecular weight (molecular mass cut-off, MMCO, between 150 to 1000 Da), eliminating saline ions at the same time. Although it is a versatile strategy it has some drawbacks, mainly related with the obstructions of the membrane due to unspecific deposition, as well as the extremely high pressure needed to achieved significant flux values (Tylkowski *et al*., 2010; Paun *et al*., 2011).

Once the purity required is achieved, the product (a fraction highly enriched in PC or even a completely isolated compound) may undergo a polishing process in which its final form is reached. This usually involves the elimination of water (using a suitable drying process), crystallization, mixing of final formulation, and/or encapsulation. Lyophilization (freezing drying) and crystallization may be used to go from solution to solid state. Spray drying is not commonly used for this purpose since it favors the oxidation of PC when air is used as drying effluent. However, it may be used if

proper conditions are considered to avoid an excessive loss of product. This includes the microencapsulation of compounds of interest as well as the use of a non-oxidative gas (*i.e.*, nitrogen) as drying effluent (Munin and Edwards-Lévy, 2011).

Conclusions

Herein a general bioprocess based on the application of abiotic stresses to produce PC in diverse sources (with special emphasis in plant tissue) was proposed. Different factors to consider when designing the bioprocess were discussed such as the type of abiotic stress conditions to apply and the different types of plant tissues that can be utilized for PC production. The production of chlorogenic acids, resveratrol and quercetin-3-*O*-glucoside were exemplified using carrots and grapes as model systems. Ones the PC of interest have been accumulated in the stressed tissue they can be extracted, recovered and purified using conventional or emerging downstream technology. The design of the downstream process is directly related to the biochemistry properties of the products of interest and those of the contaminants present, the grade of purity needed and the final application of the product. The application of abiotic stresses has proven to have great potential for the production of compounds of commercial interest.

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