

# Extraction of Polyphenols from Indian Grape Cultivars: A Review

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**Abstract:** *This paper reviews the polyphenol-compound-extraction systems used to analyse fruit (grape) samples over the last few years. Polyphenol compounds are naturally occurring antioxidants, usually found in some fruits. Sample preparation for analytical studies is necessary to determine the polyphenol composition in these matrices. The most widely used extraction system is liquid-liquid extraction (LLE), which is an inexpensive method since it involves the use of organic solvents, but it requires long extraction times, giving rise to possible extract degradation. Likewise, solid-phase extraction (SPE) can be used in liquid samples. Modern techniques, which have been replacing conventional ones, include: supercritical fluid extraction (SFE), pulsed electrical field (PEP). These alternative techniques reduce considerably the use of solvents and accelerate the extraction process.*

**Keywords:** Grape; polyphenol compounds; liquid-liquid extraction; solid-phase extraction; supercritical fluid extraction; pulsed electrical field treatment.

## 1. Introduction

Polyphenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites found in plants. These phenolic substances or polyphenols include more than 8,000 compounds with great structural diversity (although each has at least one aromatic ring with one or more hydroxyl groups). They can be divided into 10 different classes depending on their basic chemical structure. The most abundant polyphenols in the diet are phenolic acids (benzoic and cinnamic acids), and flavonoids (30 and 60% of the total, respectively). In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids and with one another.

The dietary consumption of grape and its products is associated with a lower incidence of degenerative diseases such as cardiovascular disease and certain types of cancers. Most recent interest has focused on the bioactive phenolic compounds in grape. Anthocyanins, flavanols, flavonols and resveratrol are the most important grape polyphenols because they possess many biological activities, such as antioxidant, cardioprotective, anticancer, anti-inflammation, antiaging and antimicrobial properties. This review summarizes current knowledge on the bioactivities of grape phenolics. The extraction, isolation and identification methods of polyphenols from grape as well as their bioavailability and potential toxicity also are included.

The determination of phenolic compounds in fruits, vegetables, and other foods has been of increasing interest in recent years [10]. Therefore, the objective of the present review is to show the classification of the polyphenolic compounds, taking into account different aspects related to these compounds. Moreover, our aim is to examine the various methods used for preparing and/or treating samples to determine the phenolic content in fruits and vegetables, including the different factors that affect the content in plant bioactive compounds, such as light, temperature, mineral nutrition, pathogens, mechanical damage, plant-growth regulators, and other factors [11].

## 2. Extraction Systems for Phenolic Compounds

Extraction is one of the most important steps in sample pretreatment. Generally, it is a separation process where the distribution of the analyte (in this case, a phenolic compound) between two immiscible phases is made in order to arrive at the appropriate distribution coefficient [6]. The extraction procedure is sequential and systematically carried out using an aqueous organic solvent to extract phenolic compounds in fruit and vegetable samples. This traditional method is called liquid liquid extraction (LLE) and different extraction solvents have been mentioned in the literature such as ethanol, acetone or methanol, or a mixture with water [24]. Soxhlet system is used to extract the lipidic fraction from food and other solid samples, using suitable solvents. Although it is not specific for phenolic compounds extraction, usually the extraction yields are compared to those obtained with another type of polyphenol extraction systems [1]. The ultimate goal of sample preparation is to eliminate or reduce potential matrix interferences [17].

The extraction must be performed with the most adequate solvent and under ideally predetermined analytical conditions of temperature and pH. Moreover, it is essential to take account the polyphenolic structure because these compounds may have multiple hydroxyl groups that can be conjugated to sugars, acids or alkyl groups. Thus, the polarities of phenolic compounds vary significantly and it is difficult to develop a single method for optimum extraction of all phenolic compounds. Hence, the optimisation of the extraction procedure is essential for an accurate assay of phenolic compounds from different food matrices.

## 3. Liquid-Liquid Extraction (LLE)

Solubility of phenolics is governed by their chemical nature in the plant, which may vary from simple to very highly polymerize. Plant materials may contain varying quantities of phenolic acids, phenylpropanoids, anthocyanins, and tannins, among others. There is a possibility of interaction of phenolics with other plant components such as carbohydrates and proteins that may lead to the formation of

complexes that may be quite insoluble. Likewise, the solubility of phenolics is affected by the polarity of solvent(s) used. Therefore, it is very difficult to develop an extraction procedure suitable for the extraction of all plant phenolics. The phenolic extracts from plant materials are always a diversified mixture of plant phenolics soluble in the solvent system used. Additional steps may be required to remove the unwanted phenolics and non-phenolic substances such as waxes, terpenes, fats, and chlorophylls. [21,12].

The extraction methods for simple phenolic compounds (benzoic acids, benzoic aldehydes, cinnamic acids, and catechins) from solid or semi-solid materials have been focused on maceration using organic solvents. The current official analytical method for extracting phenolic compounds is liquid-liquid extraction (LLE) for liquid samples. This method requires expensive and hazardous organic solvents, which are undesirable for health and disposal reasons, and they require a long time per analysis, giving rise to possible degradations. The process of degradation can be triggered both by external and internal factors. Light, together with air and temperature, are the most important factors that facilitate degradation reactions. The extraction temperature usually needs to be high in order to minimise the duration of the process. For these reasons, these traditional extraction sample methods have been replaced by other methodologies which are more sensitive, selective, fast, and environmentally friendly [18].

#### **4. Solid-Phase Extraction (SPE)**

Solid-phase extraction (SPE) is an increasingly useful sample-preparation technique. With SPE, many of the problems associated with liquid-liquid extraction, such as incomplete phase separations, less-than-quantitative recoveries, use and disposal of large and expensive quantities of organic solvents, can be avoided, although the cost of the equipment required for SPE is higher than for LLE. This technique is used most often to prepare liquid samples and extract semivolatile or nonvolatile analytes, but can also be used with solids that are pre-extracted into solvents. They are available in a wide variety of chemistries, adsorbents, and sizes so that it is necessary to select the most suitable product for each application and sample. For phenolic determination in grapes or wines and other beverages, different solid phases have been tested for SPE. Polymers of styrene-divinylbenzene provided good results, while C18-based phases afforded less satisfactory results for polar phenolics [12].

#### **5. Supercritical Fluid Extraction (SFE)**

Usually, phenolic compounds are extracted from plant samples by SPE coupled with other techniques, such as supercritical fluid extraction (SFE). SFE is a relatively recent technique which presents various advantages over traditional methods, such as the use of low temperatures and reduced energy consumption and high product quality due to the absence of solvents in the solute phase. However, this technique is limited to compounds of low or medium polarity. The literature offers descriptions of extraction methods for polyphenols by SFE, the main characteristics of which are the need for high percentages of organic

modifiers; this usually means that the process takes place under subcritical conditions.

Generally, for this extraction procedure, several steps are followed: samples are loaded onto the sorbent of the SPE cartridge, which is inserted into the SPE/SFE extraction cell. The supercritical fluid used can be carbon dioxide, which must go through the SPE cartridge filled with the hydrolysed sample. Thus, analytes (phenolic compounds) are quantitatively trapped by a trapping solvent (for example, methanol) at laboratory temperature (the trapping solvent is cooled naturally during the extraction by the expansion of CO<sub>2</sub>). Finally, the extracts are evaporated to dryness, dissolved in the mobile phase, and injected directly into the HPLC/ESI-MS system [18].

#### **6. Pulsed electric fields (PEF)**

Pulsed electric field (PEF) treatment is an innovative and promising method for non-thermal processing of foodstuff. It is a good alternative to conventional cell membrane permeabilization methods such as thermal treatments and the addition of chemicals as well as of enzymes. Effect of pulsed electric fields (PEF) on polyphenols extraction from grape skin that immersed in diluted water is used for the new utilization of pulse electric energy in food industry. The multi-staged Blumlein-line pulse generator was developed for application of a food processing, and the performance of juice extraction from grape skin was evaluated. The pulse generator is successfully generate several tens kV high-voltage pulse with 140ns pulse width. Subsequently, extracted total polyphenols from the grape skin treated in the needle-to-plate electrode with applying PEF for 30 min from 40 (9 kV charging voltage) to 60 kV (15 kV charging) is measured and compared to that of control. The peak value of the pulse voltage is set to be 60 kV. The purple colored spherical matter shown in left image is disappeared by applying pulse voltage.

#### **7. Total Phenolic Content Analysis**

Total phenolic content was analyzed by a colorimetric assay using Folin–Ciocalteu's phenol reagent. Ferulic acid or gallic acid was used as standard, and the total phenolic content was expressed as mg/L of ferulic acid equivalent, or GAE against the fresh weight of the sample (mg/g). In the literature, much attention has been paid to the determination of anthocynins and flavonoids in grapes. The methods were mainly high-performance liquid chromatography (HPLC) with different detectors, in which HPLC-UV detection was a common tool, followed by HPLC-mass spectrometry (MS) detection. Some complex devices have been employed by more than one MS. Before injection into the HPLC, the crude extract could be purified by solid-phase extraction (SPE) or improved liquid chromatography employed in order to obtain a more perfect profile of phenolic compounds in grape than ever possible before.

#### **8. Conclusions**

In this review, the advantages and disadvantages of different extraction systems for phenolic compounds are discussed.

The most widely used extraction system is liquid-liquid extraction (LLE), which is an inexpensive method, since it involves the use of organic solvents, but it involves long extraction times, which give rise to possible degradations. Consequently, new techniques such as SFE, SPE, PLE, MAE, and UAE have been developed. Normally, extraction efficiency increases at higher extraction temperatures, but the working temperature affects the stability of the phenolic compounds, which also depends on their chemical structure. Thus, factors that influence the extraction processes (temperature, polyphenolic structure, pressure, sample characteristics, and other factors) are discussed using examples.

## Reference

- [1] Arias, M., Penichet I., Ysambert F., Bauzab, R., Zougaghe, M., Rios A. Fast supercritical fluid extraction of low- and high-density polyethylene additives: Comparison with conventional reflux and automatic Soxhlet extraction. *J. Supercrit. Fluid.* 2009, 50, 22-28.
- [2] Balasundram N., Sundram K., Samman S. Phenolic compounds in plants and agri-industrial byproducts: Antioxidant activity, occurrence, and potential uses. *Food Chem.* 2006, 99, 191-203.
- [3] Boudet A.M. Evolution and current status of research in phenolic compounds. *Phytochemistry* 2007, 68, 2722-2735.
- [4] Costa R.M., Magalhaes A.S., Pereira J.A., Andrade P.B., Valentao P., Carvalho M., Silva B.M. Evaluation of free radical-scavenging and antihemolytic activities of quince (*Cydonia oblonga*) leaf: a comparative study with green tea (*Camellia sinensis*). *Food Chem. Toxicol.* 2009, 47, 860-865. *Molecules* 2010, 15 8825
- [5] Dinelli G., Bonetti A., Minelli M., Marotti L., Catizone P., Mazzanti A. Content of flavonols in Italian bean (*Phaseolus vulgaris* L.) ecotypes. *Food Chem.* 2006, 99, 105-114.
- [6] Dobias P., Pavlikova P., Adam M., Eisner A., Benova B., Ventura K. Comparison of pressurized fluid and ultrasonic extraction methods for analysis of plant antioxidants and their antioxidant capacity. *Cent. Eur. J. Chem.* 2010, 8, 87-95.
- [7] En-Qin Xia, Gui-Fang Deng, Ya-Jun Guo and Hua-Bin Li. Biological Activities of Polyphenols from Grapes; *Int. J. Mol. Sci.* 2010, 11, 622-646.
- [8] Escarpa A., Gonzalez M.C. An overview of analytical chemistry of phenolic compounds in foods. *Crit. Rev. Anal. Chem.* 2008, 75, 57-139.
- [9] Fang Z., Zhang, M., Wang L. HPLC-DAD-ESI-MS analysis of phenolic compounds in bayberries (*Myrica rubra* Sieb. et Zucc.). *Food Chem.* 2007, 100, 845-852.
- [10] Ferreres F., Gomes D., Valentao P., Gonçalves R., Pio R., Alves E., Seabra R.M., Andrade P.B. Improved loquat (*Eriobotrya japonica* Lindl.) cultivars: variation of phenolics and antioxidant potential. *Food Chem.* 2009, 114, 1019-1027.
- [11] Gil-Izquierdo A., Gil M.I., Conesa M.A., Ferreres F. The effect of storage temperatures on vitamin C and phenolics content of artichoke (*Cynara scolymus* L.) heads. *Innov. Food Sci. Emerg.* 2001, 2, 199-202.
- [12] Gomez A. M., Carrasco A., Canabate B., Segura A., Fernandez A. Electrophoretic identification and quantitation of compounds in the polyphenolic fraction of extra-virgin olive oil. *Electrophoresis* 2005, 26, 3538-3551.
- [13] Huang Z., Wang B., Eaves D.H., Shikany J.M., Pace R.D. Phenolic compound profile of selected vegetables frequently consumed by African Americans in the southeast US. *Food Chem.* 2007, 103, 1395-1402.
- [14] Karamac M., Amarowicz R., Antioxidant activity of phenolic fractions of white bean (*Phaseolus vulgaris*). *J. Food Lipids.* 2004, 11, 165-177.
- [15] Klejdusa B., Kopecky J., Benesova L., Vacek J. Solid-phase/supercritical-fluid extraction for liquid chromatography of phenolic compounds in freshwater microalgae and selected cyanobacterial species. *J. Chromatogr. A.* 2009, 1216, 763-771.
- [16] Laparra J.M., Glahn R.P., Miller D.D. Bioaccessibility of phenols in common beans (*Phaseolus vulgaris* L.) and iron (Fe) availability to Caco-2 cells. *J. Agric. Food Chem.* 2008, 56, 10999-11005.
- [17] Luthria D.L. Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor. *Food Chem.* 2008, 107, 745-752.
- [18] Mahugo C., Sosa Z., Torres M.E., Santana J.J. Methodologies for the extraction of phenolic compounds from environmental samples: new approaches. *Molecules* 2009, 14, 298-300.
- [19] Mulinacci N., Prucher D., Peruzzi M., Romani A., Pinelli P., Giaccherini C., Vincieri F.F. Commercial and laboratory extracts from artichoke leaves: estimation of caffeoyl esters and flavonoidic compounds content. *J. Pharm. Biomed.* 2004, 34, 349-357.
- [20] Navarro J.M., Flores P., Garrido C., Martinez V. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chem.* 2006, 96, 66-73.
- [21] Naczek M., Shahidi F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J. Pharm. Biomed. Anal.* 2006, 41, 1523-1542.
- [22] Palma M., Piñeiro Z., Barroso C.G. In-line pressurized-fluid extraction-solid-phase extraction for determining phenolic compounds in grapes. *J. Chromatogr. A.* 2002, 968, 1-6.
- [23] Patricia Garcia-Salas, Aranzazu Morales-Soto, Antonio Segura-Carretero, Alberto Fernández-Gutiérrez. Phenolic-Compound-Extraction Systems for Fruit and Vegetable Samples. *Molecules* 2010, 15, 8813-8826
- [24] Ross K.A., Beta T., Arntfield S.D. A comparative study on the phenolic acids identified and quantified in dry beans using HPLC as affected by different extraction and hydrolysis methods. *Food Chem.* 2009, 113, 336-344.
- [25] Serrano M., Zapata P.J., Castillo S., Guillen F., Martinez-Romero D., Valero D. Antioxidant and nutritive constituents during sweet pepper development and ripening are enhanced by nitrophenolate treatments. *Food Chem.* 2010, 118, 497-503.
- [26] Singh A.P., Luthria D., Wilson T., Vorsa N., Singh V., Banuelos G.S., Pasakdee S. Polyphenols content and antioxidant capacity of eggplant pulp. *Food Chem.* 2009, 114, 955-961.

- [27] Wijngaard H.H., Roble C. Brunton N. A survey of Irish fruit and vegetable waste and byproducts as a source of polyphenolic antioxidants. Food Chem. 2009, 116, 202-207.
- [28] Yaginuma S., Shiraishi T., Ohya H., Igarashi K. Polyphenol increases in safflower and cucumber seedling exposed to strong visible light with limited water. Biosci. Biotechnol. Biochem. 2002, 66, 65-72.