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Study on Grape Seeds Extraction and Optimization: An Approach

Dalia Youssef and Hala El-Adawi

Department of Medical Biotechnology, Genetic Engineering and Biotechnology Research Institute,
Mubarak City for Scientific Research and Technology Applications,
New Borg El-Arab, Alexandria, Egypt

Abstract: The efficient methods for extracting phenolics from grape seeds have shown broad range of pharmacological activities, due to the special health promoting and disease preventing effects of polyphenols. In this research, red grape seeds were subjected to different extraction conditions. The effect of single factor such as the concentration of solvent, the ratio of liquid to solid (L/S), extraction temperature and extraction time were studied. Total phenol was measured by the 4-ammonium antipyrine method to monitor the efficiency of the extraction under different experimental conditions. High Pressure Liquid Chromatograph (HPLC) analysis was applied to identify and quantify some specific dimeric and trimeric phenolics in grape seeds. The extract with highest total phenolics was obtained with ethanol when the ratio of ethanol to water was 1:1 to 3:2 (v/v). A higher extraction yield was obtained at higher temperature. In most cases, when ratio of liquid to solid was 7-8: 1 and extraction time was around 3 h resulted in the best yield.

Key words: Grape seeds, extraction, HPLC, polyphenol

INTRODUCTION

Grape seeds are waste products of the winery and grape juice industry. These seeds contain lipid, protein, carbohydrates and 5000-8000 mg/polyphenol depending on the variety (Amerine and Joslyn, 1967). The average distribution in concentration of polyphenolics about 5% in the juice, 1% in the pulp and about 62% of the remainder in the seeds (Singleton and Esau, 1969; Thorngate and Singleton, 1994). Polyphenols in grape seeds are mainly flavonoids, including gallic acid, monomeric flavan-3-ols catechin, epicatechin, gallic acid, gallocatechin, epigallocatechin and epicatechin 3-O gallate, procyanidin dimers, trimers and more highly polymerized procyanidins (Silva *et al.*, 1991; Prieur *et al.*, 1994). The powerful antioxidant properties of these compounds are now acknowledged. They act as anti-aging, anti-inflammation (Amellal *et al.*, 1985), anti-carcinogenic (Bagchi *et al.*, 1998; Catterall *et al.*, 2000), anti-mutagenic (Liviero and Pulglisi, 1994), anti-ulcer (Saito *et al.*, 1998), antimicrobial effect (Palma *et al.*, 1999) anti-atherogenic and as the inhibitor of human low density lipoprotein oxidation (Mangiapanne *et al.*, 1992; Teissedre *et al.*, 1996; Del Bas *et al.*, 2005).

The objective of this study were to optimize the extraction conditions by varying solvent concentration, ratio of liquid to solid, extraction temperature and time, so as to maximize the recovery yield of polyphenols and

minimize the cost of operation, as well as to avoid/minimize the oxidation, degradation, or polymerization of the desired products.

MATERIALS AND METHODS

Reagents: HPLC grade solvents, including hexane, methanol, ethanol, ethyl-acetate, acetone and acetic acid were obtained from Merck (Darmstadt-Germany). The phenolic compounds, gallic, catechin and epicatechin, were obtained from Sigma-Aldrich (St. Louis, MO) and used as received.

Pre-extraction sample preparation: Red grape seeds (*Vitis vinifera syrah*) were obtained from Ganaclise Veinyard factory (Abu-Elmatameer). Grape seeds were handily separated from grape skin and stem (waste), then washed with tap water and then left to dry in open air away from direct sunlight. They were crushed in a coffee grinder for 2 min, but during this time the grinding was halted for 15 sec at periodic intervals to prevent heating of the sample. The samples were wrapped and stored at -18°C until the extractions were performed.

Extraction process (Egyptian patent filed No. 2006060258): The crushed grape seeds (20 g) were subjected to preliminary treatment (defatting), crushed seeds were soaked in suitable volume of hexane

overnight. Deffated seeds were extracted by temperature controlled percolation with aqueous ethanol, methanol, ethyl-acetate and acetone at different ratios (50 and 70% v/v). Percolation was carried out of different temperature starting at 45°C and ending with the boiling point of each solvent at temperature intervals 5°C. We considered it more adequate to use extraction time that is as low as possible. As a result, an extraction time of two hours was finally established at which no more increase in extraction yield along with extraction time.

Total phenolics determination: Total phenol in the extract was determined by 4-ammonium antipyrine method (Ettinger *et al.*, 1951). The absorbance of the sample solution was measured spectrophotometrically at 500 nm using spectrophotometer. The polyphenol content for each sample was determined using the standard curve previously prepared.

HPLC analysis: The analysis was done using a Beckman-C18 column (100×4.6 mm, 5 µm particle size), equipped with an autosampler, quaternary pump and a UV/Visible multi wavelength detector. The solvents were 2% acetic acid in water (A) and 2% acetic acid in methanol (B). The column was eluted at 1.0 mL min⁻¹.

RESULTS AND DISCUSSION

To optimize the operation parameters, total phenolics yield as final parameters was used to evaluate the effect of each parameter on the extraction yield. An extraction around 3 h resulted in the best extraction yield (data not shown). Longer extraction time decreased the total phenolics extracted, possibly because of some loss of phenolic compounds via oxidation and these products might polymerize into insoluble compounds. When the ratio of liquid to solid was 10-1, total phenolics extracted per gram of dry seed were the same yield at the ratio of 7-8:1 at constant temperature. This may occur due to oxidation, since more liquid might provide more dissolved oxygen, in which, long extraction time and high temperature should be avoided to minimize oxidation.

Effect of heating on the extraction of polyphenols: Hot water extraction of polyphenols works well for tea. In the study of Song (2001), heating at 90°C for 10 min significantly increased the extraction yield of tea polyphenols, compared to extraction in lower temperature. However, for a better quality of tea extract, 80°C was preferable, because fewer undesirable compounds were extracted than at 90°C or higher. In this study, increasing the extraction temperature to 50°C or above resulted in a

Table 1: Effect of extraction temperature on the aqueous extraction of polyphenols from red grape seeds

Methanol 70%	Acetone 70%	Temperature (°C)
48.72	39.40	45
54.44	41.80	50
55.16	43.68	55
57.16		65

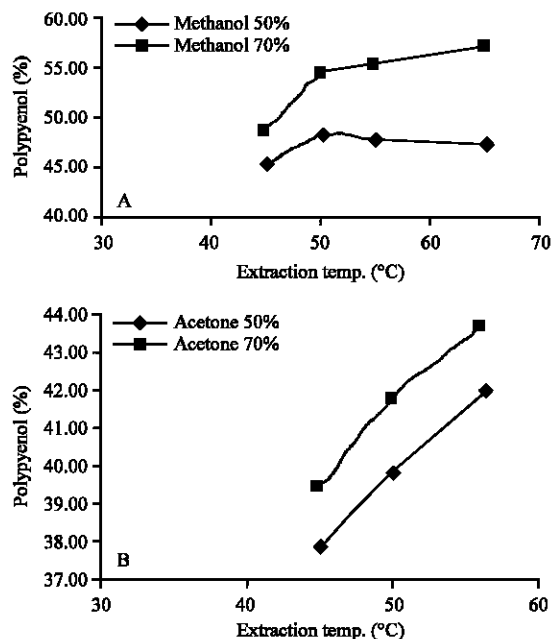


Fig. 1: Effect of solvent/water ratio on the aqueous extraction of Polyphenols from red grape seeds (A: methanol/water and B: acetone/water).

significant increase in concentration of polyphenol (Table 1). Heating might soften the plant tissue and weaken the phenol-protein and phenol-polysaccharide interactions in seed meal, thus more polyphenols would migrate into the solvents. However, more proteins and polysaccharide could be extracted at higher temperature when water was used alone for extraction. These macromolecules caused filtration difficulty when extracts were further purified by membrane, comparing to that of using aqueous solvent. The cost of concentration operation will be very high since water is more difficult to remove than solvent. Therefore, solvent-water mixture could be the better extraction liquid in terms of yield and cost.

Effect of solvent-water ratio on the extraction of polyphenols: The total phenolics in extracts increased with increasing concentration of solvent in water. The phenolic concentration reached a maximum when the ratio of solvent to water was 1:1 to 3:2 (v/v) as can be seen in Fig. 1. At 80 and 95% ethanol concentration, the

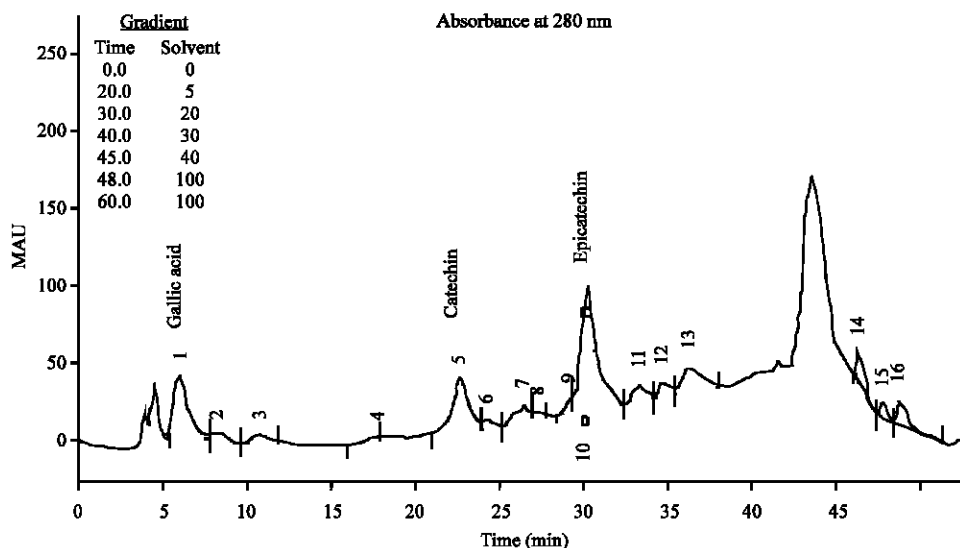


Fig. 2: Resulting chromatogram and gradient elution schedule for HPLC-UV analysis of grape seeds extract

extraction was not completed as reported by Thorngate and Singleton (1994). Consequently we covered the concentration area 50 and 70% for all solvents.

HPLC-UV analysis: Due to the high polarity of compounds presents in the extract, it was analysed by HPLC-UV and the resulting chromatogram is shown in Fig. 2. The two main compounds in this extract were gallic acid and epicatechin. Catechin is also present in a significant amount, as well. These compounds were identified by their retention times against standard samples.

On the basis of the chromatographic conditions employed (i.e., column, elution and detection properties). Other peaks in the chromatogram were not identified due to the lack of standards, they are most probably, phenolic compounds in a sense they contributed significantly to the total phenolics in the extract because total phenolics measured were much higher than the sum of the individual phenolic concentration identified and quantified by HPLC.

CONCLUSIONS

In this study, the yield of extracted phenols was influenced by the concentration of solvent in water, the ratio of solvent-water solution to dry seeds, the extraction temperature and the extraction time. Although polyphenols in grape seeds are water soluble, the addition of solvent improved the extraction efficiency. From an industrial point of view, more work should be done to scale up the optimization conditions for the extraction of polyphenols from red grape seeds.

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