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Optimization of extraction parameters of phenolic compounds from Algerian fresh table grapes, (*Vitis Vinifera*)

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Abstract

The aim of this study is to optimize extraction parameters of phenolic compounds from one variety of Algerian table grapes. The effect of different solvents: distilled water, acetone (20, 40, 60 and 80%), methanol (20, 40, 60 and 80%) and ethanol (20, 40, 60 and 80%), temperature (25, 40, 50, 60 and 70°C), time (30, 60, 90 and 120 min) and four successive extractions were tested. It was found that the solvent nature, time and temperature have a significant effect on total polyphenols (TPP) recovered from the grape. The best extraction conditions were as follows: methanol 80% at 60°C during 30 minutes and three consecutive extractions with content of TPP extracted of 216,81 mg GAE/100 g of fresh weight (FW). This indicates that the rate of polyphenols extracted from grapes depends only on the extraction method used and that Algerian table grape fruits can be considered as a natural source of phenolics compounds with good antioxidant capacity. This study on the optimization of the extraction parameters of phenolic compounds from grapes is very original on scale of Algeria. It is the first made on a variety grown in this country.

Introduction

The extraction of active ingredients with high added value from plant material, especially the case of plant polyphenols, currently attracting a lot of interest in several scientific researches due to their positive effects on health mainly due to their antioxidant and antiradical high power (Bucić-Kojić *et al.*, 2011). According to Menat (2006), phenolic compounds in fruit and vegetables are considered the most important group of natural antioxidants.

The grape is the fruit of the vine cultivated, *Vitis vinifera*). It is the second most cultivated fruit in the world (Chira *et al*., 2008). The fruit is consumed by humans for a long time and is considered an important part of his diet because of its nutritional value such as vitamins, minerals, organic acids and phenolic compounds. The beneficial effects of these constituents are attributed mainly to polyphenols and their antioxidant activity (Zheng *et al*., 2009).

However, the extractability of the phenolic compounds depends on the type of the solvent, nature and preparation of material to be extracted, chemical structure of phenolic compounds, temperature, extraction time, solid-liquid ratio, extraction method employed and possible presence of interfering substances. Solvent extraction, i.e. solid-liquid extraction, is commonly used for the isolation of phenolic compounds from plant material (Bucić-Kojić *et al*., 2011).

The selection of the solvent is one of the most important steps in the extraction process. Methanol, ethanol or acetone and their mixtures in water are so far the most commonly used solvents in the extraction of phenolic compounds from the plant materials (Kim *et al*., 2006; Sanchez-Alonso *et al.*, 2008; Breksa *et al*., 2010).

Despite the complete phenolic profile of the grapes published by some authors (Revilla et Ryan, 2000; Tarara *et al.*, 2008; Rockenbach *et al*., 2011), there are very few studies on the effect of extraction conditions of polyphenols from grapes fruits. Given the above, the objective of this work is to examine the influence of extraction conditions on the extractability of phenolic compounds from fresh table grapes.

Materials and Methods

Plant material

The sample of grapes was purchased at a supermarket in the region of Annaba, NE Algeria) in late October 2012 and is frozen until analysis.

Extraction procedure

The frozen grapes were washed with distilled water, dried with a cloth and mashed in a domestic mill. 10 g of crushed grapes were mixed with 20 ml of the extraction solvent and 0.1 ml/10 ml of solvent

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(v/v) of concentrated HCl to avoid oxidation of the phenolic compounds, the mixture is placed in a water bath with stirring. After agitation, the liquid extract is separated from the solids residue by centrifugation at 3000 rpm/15 min. The supernatant was transferred into vials opaque, the extraction is performed in duplicate and three determination of TPP assays were performed for each extract.

Determination of total polyphenols (TPP)

The content of TPP extracts was determined using the method described by Nickavar *et al.* (2008) modified. Briefly, 50 µl of extract freshly prepared was mixed with 2,5 ml Folin-Ciocalteau reagent (diluted 1/10) and incubated at room temperature. After 10 min, 2 ml of sodium carbonate solution (75 mg/ml) was added. The final solution was mixed thoroughly and allowed to remain for 30 min in a dark place. The absorbance was measured at 765 nm with a spectrophotometer. The TPP for each extract expressed as gallic acid equivalent (g EAG/100 g of fresh material) was determined on the basis of the standard curve using gallic acid as standard (0.1 - 0.9 mg/ml).

Effect of extraction procedures and different parameters

Extraction solvents

Four different solvents were used to determine the most suitable one for the extraction recovery of polyphenols. The solvents used in this experiment were: distilled water, acetone, methanol and ethanol, these three later solvents were tested as a mixture with water at different dilutions of different alcoholic dilutions (20, 40, 60 and 80%) and that at 25°C and for a contact time of 30 minutes. The best extraction solvent was selected according to the value of TPP ,mg GAE/100 g FW).

Extraction time

Samples were extracted using the most effective solvent type and the best solvent dilution concentration, as determined in the first step, for 30, 60, 90, 120 minutes by fixing the extraction temperature constant at 25°C. The best extraction time was selected according to the best value of TPP (mg GAE/100 g FW).

Extraction temperature

Using the most effective solvent and the optimal time as determined during the first and second steps respectively, samples were extracted at various extraction temperature 25, 40, 50, 60 et 70°C. The best extraction temperature was chosen due to the highest value of TPP (mg GAE/100 g FW).

Number of extraction

Setting the solvent, time and temperature as determined in the previous steps, the extraction was repeated three times on the solid residue after centrifugation of the mixture at 3000 rpm/15 min.

Statistical analysis

All statistical analyses were performed using STATISTICA 5.5 (StatSoft Inc., Oklahoma, USA). Values are expressed as mean ± standard deviation of duplicate solvent extraction and triplicate assays. One-way analysis of variance (ANOVA) at $p < 0.05$ was used to determine significant differences between the means.

Results and Discussion

Influence of solvent extraction

In the literature, several solvents are used for extraction of phenolic compounds and often mixed with water at different proportion (Naczk et Shahidi, 2004; Hismath *et al*., 2011). In general, phenolic compounds in plants are polar compounds, which usually are extracted with polar solvents such as aqueous acetone and methanol (Wissam *et al*., 2012). The ability of different solvents to extract phenolic compounds was compared using the method of Folin-Ciocalteu assay. The results were expressed as gallic acid equivalents (mg GAE/100 g FW).

Table 1 shows that the different types of solvent has a significant effect ($p < 0.05$) on TPP content and they were able to extract phenolic compounds, but aqueous methanol 80% was the most effective solvent as acetone and ethanol at the same concentration. Methanol 80% allows to extract the highest quantity TPP which is 94.24 ± 4.93, followed by acetone (80%) 92.09 ± 0.90 and ethanol (80%) 74.80 ± 1.41 mg GAE/100 g FW. The same solvent (methanol) was used by several authors for the extraction of phenolic compounds from grapes (Mélo *et al.*, 2006; Esna-Ashari *et al.*, 2008; Ojeil *et al*., 2010). Combinations of solvents such as methanol, ethanol and acetone with water improve the extraction of phenolic compounds (Bucić-Kojić *et al*., 2011; Chew *et al*., 2011; Caunii *et al*., 2012; Wissam *et al*., 2012). In addition, a 70% methanol minimum is required to inactivate the polyphenol oxidase, enzymes involved in the oxidation of polyphenols, which leads to the phenomenon of browning (Chirinos *et al*., 2007 In Telli *et al*., 2010).

According to the principle of "like dissolve like",

Table 1. effect of solvent extraction on grape phenolic content

Solvent	Total Polyphenols*
Aqueous ethanol	
Ethanol/water (1:4, v/v)	43,99 ± 3,99[b]
Ethanol/water (2:3, v/v)	57,08 ± 1,49[d]
Ethanol/water (3:2, v/v)	65,41 ± 1,60[e]
Ethanol/water (4:1, v/v)	74,80 ± 1,41[g]
Aqueous methanol	
Methanol/water (1:4, v/v)	38,33 ± 0,99[a]
Methanol/water (2:3, v/v)	48,99 ± 3,81[c]
Methanol/water (3:2, v/v)	69,94 ± 1,42[f]
Methanol/water (4:1, v/v)	94,24 ± 4,93[i]
Aqueous acetone	
Acetone/water (1:4, v/v)	57,76 ± 2,58[d]
Acetone/water (2:3, v/v)	66,57 ± 1,55[e,f]
Acetone/water (3:2, v/v)	79,47 ± 3,67[h]
Acetone/water (4:1, v/v)	92,09 ± 0,90[i]
Distilled water	52,46 ± 2,14[c]

Values marked by different letters are significantly different ($p < 0.05$)
*Expressed as mg GAE/100 g fresh weight

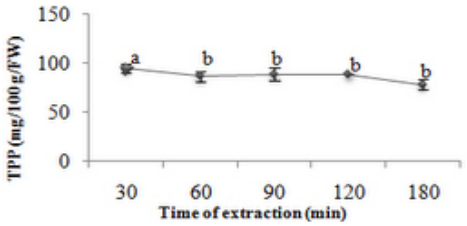

Figure 1. Effect of extraction time on TPP content from grape

Values marked by different letters are significantly different ($p < 0.05$)
Vertical bars represent standard deviation (n = 3)

solvents would only extract those compounds which have similar polarity with the solvents. In other word, the phenolic compounds extracted from grape would have same polarity with the extraction solvent used (Methanol 80%). but due that all other solvents tested could extract phenolics compounds from grapes, hence, we suggested that grape consisted of diverse phenolic compounds with different polarities.

Influence of extraction time

Extraction time is crucial in solvent extraction of phenolic compounds as appropriate extraction time can result in time and cost saving. The effects of extraction time on the phenolic contents of crude extract are showed in Figure 1. As shown in the figure, the highest TPP content is obtained after an extraction time of 30 min with a value of 94.24 ± 4.93 GAE/100 g FW. Several studies have indicated that prolonged extraction lead to a decrease in the phenolic content of crude extracts due to the oxidation of these compounds by prolonging the exposure to environment factors such as light and oxygen (Nazck et Shahidi, 2004; Chan *et al*., 2009; Uma *et al*., 2010; Hismath *et al*., 2011).

In this study, the extraction time has a significant effect on the extraction yield of TPP ($p < 0.05$). However, after 30 min, an increase in the

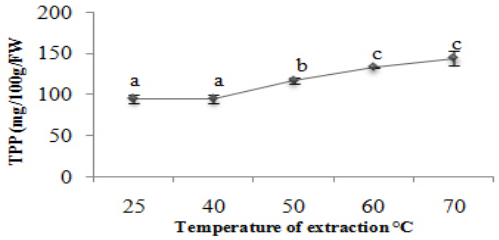

Figure 2. Effect of extraction temperature on TPP content from grape

Values marked by different letters are significantly different ($p < 0.05$).
Vertical bars represent standard deviation (n = 3)

extraction time does not improve significantly the extraction yield (no significant difference ($p > 0.05$) was observed between 60 to 180 min). These circumstances could be well explained by Fick's second law of diffusion, which predicts that after a certain time, there will be a final equilibrium between the solute in the solid matrix (plant sample) and in the bulk solution (extraction solvent) (Chew *et al.*, 2011). Hence, excessive extraction time was no longer useful to extract more phenolic compounds from grapes. From an economic point of view and also taking into account the yield of phenolic compounds from the crude extract, 30 minutes can be considered as optimal extraction time.

Influence of extraction temperature

The extraction yield of TPP increases with increasing the extraction temperature (25°C - 70°C) (Fig.2) indicating that grapes phenolic compounds are relatively stable under high temperature conditions. Significant differences ($p < 0.05$) existed among 25, 40, 50 and 60°C but did not appear between 60 and 70°C. So temperature of 60°C can be used as extraction temperature of grapes TPP. The same observation was made by Ruenroengklin *et al*. (2008) on Litchi fruit (*Litchi chinensis* Sonn.) pericarp from China and Rajaei *et al.* (2010) pistachio (*Pistachia vera*) green hull from Iran.

According to (Wissam *et al*., 2012), an increase in temperature increases the efficiency of the extraction since heat render the cell permeable, increase solubility and diffusion coefficients of the compounds to be extracted and decreases the viscosity of the solvent, thus facilitating its passage through the solid substrate mass, these authors found in their study on pomegranate's peel, that the use of temperatures higher than 50°C decreases the total polyphenols yield which is probably due to their degradation. Kim *et al.* (2006), in their work on rice explained this by the fact that the heat could solubilize the phenolic compounds but without breaking the covalent bonds of these compounds bound to the walls of the rice grains. This indicated that phenolic compounds

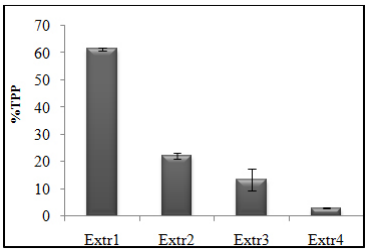

Figure 3. the content of TPP extract in the four extraction successive fractions

Vertical bars represent standard deviation (n = 3)

of plants should be present in different binding status depending on plant species. Thus, effective processing steps to liberate antioxidant compounds from different plants may not be the same (Lee *et al.*, 2006). But according to Sun and Spranger (2005) and Cadot (2012), although high temperatures improve yields, but they can degrade proanthocyanidins which grapes are rich and can cause the appearance of newly formed compounds that absorb at the same wavelength as phenolic compounds, therefore from quantitative and practical point of view, it is preferable that extraction is performed at room temperature.

Influence of multi-stage extraction

A series of successive extractions were performed under operating conditions favoring the best extraction: 80% methanol as solvent, a temperature of 60°C, extraction time of 30 minutes. Three sequential extractions appear sufficient; the first extract contain more than 60% of total extractable polyphenols. This relatively low rate was completed by the second (22%) and third (13%) extraction. Only small amount of these compounds are found in the fourth fraction as shown in figure 3. According to Bonnaillie *et al.* (2012), depletion of the raw material and the concentration of the extraction medium are conducted mainly in the first three stages, the last stage, rather dilutes the volumes retained in the solid. Consequently, the number of extractions can be limited to three successive contacts with fresh solvent, these authors for their part, found in a series of three successive extractions of dandruff peanut following results: 60%, 35% and 5% respectively. A similar result to that found in the present study was obtained by Savova *et al*. (2007) on the red grape seeds (*Vitis Vinifera* L.) with percentages of 60%, 18%, 15% et 7% in the four fractions respectively.

The maximum amount of extractable total phenols of four successive extractions was assessed to 216.81 mg GAE/100 g FW. This result is higher of yields reported by different authors, especially Melo *et al.* (2006) in white grape variety Italia 62.19 ± 3.17 (mg GAE/100 g of FW) and 84.84 ± 6.12 (mg GAE/100 g of FW) in red grape variety Patrícia. This difference may well be explained by the variety and the technique of extraction used.

Conclusion

This study aimed at optimizing conditions for extraction of phenolic compounds from table grape, shows that the nature of the solvent, the solvent / water ratio, time, temperature and number of extraction has a significant effect on the levels of TPP extracted from the fruit. Thus, we can conclude that: the best solvent for the extraction of polyphenols from grapes is methanol, the addition of water to the extraction system improves the yield of extraction, the optimum temperature for the extraction of polyphenols from grapes is 60°C. This parameter is closely related to the extraction time, the optimal time for the extraction of polyphenols from grapes is 30 minutes and three successive extractions seem necessary for the depletion of plant material, and therefore the maximum extraction of polyphenols present in the fruit. The study of other parameters that influence the extraction yield of polyphenols seems interesting and can be used to better optimize extraction. Other criteria may be added in addition to the phenolics compounds, such as the different families of phenolic compounds responsible for the antioxidant properties of grapes and different biological activities. Furthermore, the results obtained in this study indicate that grape fruits can be considered as a natural source of phenolics compounds known for their good antioxidant capacity.

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