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Effect of Solvent, Temperature, and Solvent-to-Solid Ratio on the Total Phenolic Content and Antiradical Activity of Extracts from Different Components of Grape Pomace

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Grape byproducts were subjected to an extraction process under various different experimental conditions (namely, solvent type, temperature, solvent-to-solid ratio, time contact, and raw material) in order to study the effect of these conditions on the yield of phenolic compounds and the corresponding antiradical activity of extracts. Although the order of decreasing capacity to extract soluble materials was ethanol > methanol > water, methanol was the most selective for extracting phenolic compounds. Temperature and solvent-to-solid ratio were found to have a critical role in extraction efficiency; values of 50 °C (between 25 and 50 °C) and 1:1 (between 1:1 and 5:1) maximized the antiradical activity of phenolic extracts. In addition, extracts from grape samples previously subjected to distillation reached higher antiradical values in comparison to those coming directly from pressing; in both cases, seed extracts showed better results than those of stem when ethanol or water was employed, whereas the opposite occurred in the case of methanol. These differences were attributed to the different phenolic compositions of the considered fractions.

KEYWORDS: Grape (*Vitis vinifera*); phenolic compounds; extraction; antiradical activity; solvent; temperature

INTRODUCTION

Because world population is in continuous growth and natural resources are consequently limited, studies dealing with the utilization of renewable sources and the design of processes based on the integral exploitation of natural products have attracted great interest in the past several years. The recovery of byproducts from agricultural industries to be converted into value-added products is a good example. In Europe ∼112 million tons of grape was processed by the wine industry in 1998. An estimated 13 wt % of this amount corresponded to the byproduct after pressing, consisting of skins, seeds, and stems, which can be a rich source of phenols (*1*−*3*). The interest in these compounds is based on their well-known capacity to scavenge free radicals. The generation of these species plays a remarkable role in the progression of a wide range of diseases such as cancer, atherosclerosis, and inflammation processes (*4*, *5*). Furthermore, these compounds were found to be responsible for lipid oxidation, which is a major determinant in the deterioration of foods during processing and storage (*6*). Some phenolic compounds present in natural products showed a higher antiradical activity than the synthetic antioxidants, the utilization of which in the food industry is common and restricted. In Europe, the use of such antioxidants is regulated by Directive 95/2/EC; 3-*tert*-butyl-4-hydroxyanisole (BHA) is permitted in oils and fats up to 200 mg/kg, whereas 3,5-di-*tert*-4-butylhydroxytoluene (BHT) is permitted up to only 100 mg/kg (*7*).

In light of these considerations, the viability of phenolic compounds with antiradical power to be used as food preservatives or dietary supplements for disease prevention is ensured. However, the economical feasibility of an industrial process also requires working in such a way that high values of efficiency are attained. Some factors could contribute to reach this aim: (1) optimizing the values of the variables with a direct influence on the process, (2) correctly choosing the raw materials to extract, and (3) subjecting these materials to appropriate pretreatment.

Extraction efficiency is commonly a function of process conditions. Previous findings have reported the influence of some variables (e.g., temperature, time contact, solvent-to-solid ratio, etc.) on the phenolic yields capable of being extracted from diverse natural products such as almond hulls, pine sawdust, or apple byproducts (*8*, *9*). The positive or negative role of each factor in the mass transfer of the process is not always obvious; the chemical characteristics of the solvent and the diverse structure and composition of the natural products ensure that each material−solvent system shows different behavior, which cannot be predicted.

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It is expected that each component of the grape byproduct possesses a different phenolic composition. Both the nature of the occurring species (structure, polymerization degree, etc.) and their overall quantity could largely vary as a function of the considered fraction (skin, seed, or stem). Therefore, it seems obvious that the properties of related extracts will be a function of each part of the grape.

Antiradical activity of extracts obtained from different fruits can be enhanced by subjecting the raw materials to a controlled pretreatment. For instance, abundant literature can be found on the effect of a thermal treatment on the values of antiradical activity. Jeong et al. (*10*) reached a ~4 times higher antiradical activity of citrus peels aqueous extracts when subjecting them to different heat treatments. During the processing and storage of wine and spirits, grape pomace undergoes some environmental changes (temperature, pressure, aeration, etc.) affecting the composition of the food matrix and so promoting variations in the properties of extracts.

In this work, a study about the effect of temperature, contact time, and solvent-to-solid ratio on the phenolic concentration and antiradical capacity of extracts is undertaken. First, the influence of all these variables on the efficiency of the batch extraction process will be evaluated; conditions maximizing antiradical activity of extracts will be considered as optimal and thus taken as benchmark conditions for the characterization study. Then, because the phenolic content and antiradical activity depends on both the part of the grape pomace analyzed (stem, skin, or seed) and its pretreatment, a comparison of the antiradical activity of the different extracts from the wine- and spirit-making processes (pressing and distillation) will also be carried out.

MATERIALS AND METHODS

Sample Preparation. The grape pomace studied contained skins, stems, and seeds of white and red Garnacha grapes, which had been subjected to two different pretreatments. In the first one, the residues of the harvested grapes were pressed to obtain the grape juice and were named "pressed byproducts". Pressing was carried out at room temperature by using a cylinder press with a flexible polyurethane-coated membrane. The second residues, the "distillated byproducts", were maintained under anaerobic conditions for ~2 months after pressing. Because anaerobic processes are slightly exothermic, a temperature between 25 and 30 °C was kept during this storage time. After this, grape pomace was subjected to an acute increase of temperature (120−130 °C) during a distillation process, to obtain the relevant grape spirit. This last step is probably mostly responsible for the eventual phenol structure and antiradical activity changes. Both types of byproducts assayed were provided by Bodegas Miguel Torres, S.A. (Villafranca del Penedés, Spain), dried by running air at room temperature for 48 h, and stored at room temperature until used. Separation of the samples into their different components was done by hand.

Batch Extraction. The samples (10 g) were subjected to extraction in a rotary shaker G24 New Brunswick Scientific Co. Inc. (New Brunswick, NJ) at a constant stirring rate of 140 rpm. Solvents used were methanol, 96% ethanol (Drogas Vigo, S.L., Porriño, Spain), and distilled water. Mixtures of alcoholic solvents and water are commonly employed by researchers to extract phenolics from natural sources. However, to study the extreme behavior of each solvent, no mixtures were used in this work. The behavior of mixtures may perhaps be inferred from the data for each "pure" solvent. Solids were separated by filtration, and the corresponding extracts were analyzed.

Experimental Design. A full factorial 2^3 experimental design was developed to evaluate the effect of the temperature (**T**), contact time (**t**), and solvent-to-solid ratio (**L**/**S**) (*11*). Temperature values varied between 25 and 50 °C, contact time between 30 and 90 min, and solvent-to-solid ratio between 1:1 and 5:1. Variables were coded in the way that their value ranged between +1 and −1, taking, as central point, the zero value. Therefore

$$t = (\mathbf{t} - 60)/30$$

$$T = (\mathbf{T} - 37.5)/12.5$$

$$L/S = (\mathbf{L}/\mathbf{S} - 7.5)/2.5$$

Table 1. Extraction Conditions of the Experimental Design (Not Coded/Coded Variables)

expt	*t*	*T*	*L/S*	t	T	L/S
1	30	25	5	–1	–1	1
2	30	50	5	–1	1	1
3	30	25	1	–1	–1	–1
4	30	50	1	–1	1	–1
5	90	25	5	1	–1	1
6	90	50	5	1	1	1
7	90	25	1	1	–1	–1
8	90	50	1	1	1	–1
9	60	37.5	3	0	0	0
10	60	37.5	3	0	0	0
11	60	37.5	3	0	0	0
12	60	37.5	3	0	0	0

Table 1 shows the factorial design matrix, with variables in both coded/noncoded form, for better comprehension. Data were adjusted to a response surface *R*

$$R = a_0 + a_1 t + a_2 T + a_3 (L/S) + a_{12} tT + a_{13} t(L/S) + a_{23} T(L/S) + a_{123} tT(L/S)$$

where a_0 is the value of the objective function in the central point conditions, a_1, a_2, and a_3 represent the principal effects associated with each variable, and the others correspond to the crossed effects among variables.

Analytical Methods. *Total Phenolic Compounds.* The total phenolics were assayed colorimetrically by means of the Folin−Ciocalteu method, as modified by Singleton et al. (*12*). Two and a half milliliters of 10-fold diluted Folin−Ciocalteu reagent, 2 mL of 7.5% sodium carbonate, and 0.5 mL of phenolic extract were mixed well. The absorbance was measured at 765 nm after 15 min of heating at 45 °C. A mixture of water and reagents was used as a blank. The content of phenolics was expresed as gallic acid equivalents.

Antiradical Activity. A DPPH radical-scavenging assay was performed using the method described by Brand-Williams et al. (*13*) to determine the hydrogen-donating ability of the crude extract. A volume of 980 μL of 6.1×10^{-5} M DPPH• methanol solution was used. The reaction was started by the addition of 20 μL of sample. The bleaching of DPPH• was followed at 515 nm (Shimadzu UV-160A) at 25 °C for 16 min. The inhibition percentage (IP) of the DPPH• radical was calculated as follows:

$$\mathrm{IP} = \frac{(\mathrm{absorbance}_{t=0\mathrm{min}} - \mathrm{absorbance}_{t=16\mathrm{min}})}{(\mathrm{absorbance}_{t=0\mathrm{min}})} \times 100$$

Statistical Analysis. The results reported in this work are the average of at least three measurements, and the coefficients of variation, expressed as the percentage ratio between standard deviations (SD) and the mean values, were found to be <10 in all cases. Significant variables were calculated, subjecting results to a linear regression, using SPSS statistical program version 10.0 (SPSS Inc., Chicago, IL). Only variables with a confidence level superior to 95% ($p < 0.05$) were considered to be significant.

RESULTS AND DISCUSSION

Influence of Extraction Conditions on the Properties of Extracts: Total Soluble Solids, Total Phenols, and Antiradical Activity. Figure 1 shows the maximum yield of total soluble

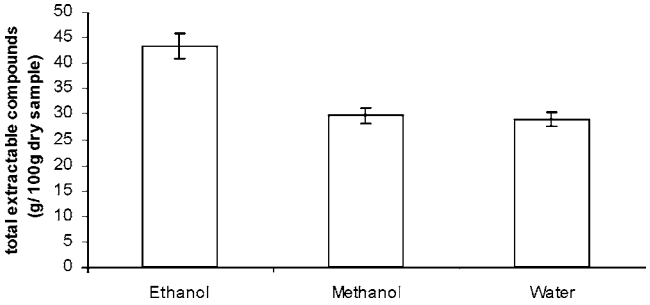

Figure 1. Maximum total soluble solids from red distilled grape byproducts after 8 h in a Soxhlet extractor.

solids extracted with the three solvents in a Soxhlet after 8 h. It is noteworthy that the highest results were attained when ethanol was used (~44%), whereas similar values near 30% were found for methanol and water. The major capacity of ethanol to extract soluble solids can be also deduced from the data shown in **Table 2**, where its results were almost always higher than those detected for the other solvents for any extraction condition of the experimental design. Even so, from a comparison of **Table 2** with **Figure 1**, it can be noted that the yield is far enough from maximum, although this ratio is higher for methanol and water than for ethanol. This suggests that the conditions of the experimental design do not allow one to develop the overall capacity of solvents to extract soluble solids. On the whole, the highest values of extracted solids were reached when extraction was carried out under the conditions of experiment 6 (higher temperature, higher time contact, and higher solvent-to-solid ratio). Statistical analysis of these values showed in the resulting response function models a dependence of these values on the three variables assayed for alcohol extractions, whereas time was not significant for aqueous extraction.

$$\%\ \text{total soluble solids}_{\text{ethanol}} = 10.1 + 1.766t + 2.134T + 1.831(L/S)$$

$$F_{\text{model}} = 31.465;\ p < 0.002;\ R^2 = 0.925$$

$$\%\ \text{total soluble solids}_{\text{methanol}} = 9.176 + 1.895t + 2.203T + 1.833(L/S)$$

$$F_{\text{model}} = 33.921;\ p < 0.002;\ R^2 = 0.968$$

$$\%\ \text{total soluble solids}_{\text{water}} = 9.407 + 1.529T + 1.704(L/S)$$

$$F_{\text{model}} = 6.916;\ p < 0.040;\ R^2 = 0.981$$

Increasing temperature favored extraction by enhancing both the solubility of solute and the diffusion coefficient. As a consequence, an increase of extracted solids was observed at higher values of this variable. Despite the positive effects of higher temperatures on the extraction yields, this cannot be increased indefinitely; the stability of phenolic compounds and the denaturation of membranes can happen at temperatures >50 °C (*14*, *15*). The solvent-to-solid ratio has also a positive effect; in fact, the higher the solvent-to-solid ratio, the higher the total amount of solids obtained, despite the solvent used. This is consistent with mass transfer principles; the driving force during mass transfer is the concentration gradient between the solid and the bulk of the liquid, which is greater when a higher solvent-to-solid ratio is used. Similar results about the effect of temperature and solvent-to-solid ratio on the extraction of

Table 2. Percentage of Total Extractable Compounds from Red Distilled Grape Byproducts Subjected to the Extraction Conditions of the Experimental Design (Highest Values in Bold)

expt	ethanol	methanol	water
1	8.62 ± 0.61	7.06 ± 0.26	8.04 ± 0.42
2	12.07 ± 0.09	11.24 ± 0.05	13.04 ± 0.40
3	4.25 ± 0.24	3.84 ± 0.21	5.12 ± 0.36
4	7.46 ± 0.22	6.07 ± 0.37	5.99 ± 0.03
5	11.03 ± 0.43	9.86 ± 0.57	8.42 ± 0.56
6	**15.07 ± 0.56**	**14.96 ± 0.24**	**13.21 ± 1.02**
7	7.03 ± 0.21	6.22 ± 0.19	8.20 ± 0.17
8	13.40 ± 0.09	12.33 ± 0.12	9.77 ± 0.17
9	10.32	9.29	11.89
10	10.21	10.03	12.02
11	10.88	9.87	12.11
12	10.86	9.34	12.07

Table 3. Percentage of Total Phenolic Compounds from Red Distilled Grape Byproducts Subjected to the Extraction Conditions of the Experimental Design (Highest Values in Bold)

expt	methanol	ethanol	water
1	0.082 ± 0.007	0.041 ± 0.003	0.022 ± 0.001
2	0.134 ± 0.006	0.083 ± 0.004	0.063 ± 0.003
3	0.041 ± 0.003	0.025 ± 0.001	0.016 ± 0.002
4	0.101 ± 0.007	0.045 ± 0.003	0.029 ± 0.001
5	0.114 ± 0.001	0.068 ± 0.004	0.031 ± 0.002
6	**0.163 ± 0.012**	**0.128 ± 0.011**	**0.093 ± 0.006**
7	0.061 ± 0.001	0.044 ± 0.003	0.023 ± 0.001
8	0.143 ± 0.003	0.105 ± 0.006	0.052 ± 0.001
9	0.114	0.059	0.022
10	0.098	0.060	0.043
11	0.100	0.065	0.043
12	0.100	0.053	0.030

phenolic compounds were also reported for milled berries by Cacace et al. (*14*), who also found a linear relationship of temperature and solvent-to-solid ratio with solid yields. Time contact was also detected as a significant variable in ethanol and methanol cases, suggesting a progressive release of solute from solid matrix to solvent during the extraction time interval considered. In contrast, no influence of this parameter was found in aqueous extraction, indicating that there is no benefit in using contact times >30 min for this solvent.

Table 3 shows the yields of phenolic compounds from distilled red grape pomace extracts using ethanol, methanol, and water as solvents. As can be noted, methanol extracts contained a higher quantity of phenolic compounds, followed by ethanol and water. Furthermore, because the phenolic compounds/total soluble solids ratio in methanol was ~30% higher than in the other solvents, a higher selectivity of methanol to extract phenolics can be inferred. In general, values of phenolic compounds yields were between 0.016 and 0.163 g/g of residue, which were similar to those detected for other agricultural materials. As an example, oat hulls and apple byproducts were reported to contain 0.056 g/100 g of solid and 0.11 g/100 g, respectively (*8*, *16*). Likewise, Pastrana-Bonilla et al. (*17*) reported values of 0.169 and 0.195 g of total phenols/100 g of residue for extracts of bronze (Early Fry) and purple (Paulk) Muscadine grapes, respectively. Even so, abundant literature supports the fact that the total phenols capable of being extracted with polar solvents (water, methanol, and ethanol) can vary largely as a function of the employed material, from values of 1.03×10^{-3} g/100 g of solid for *Gevuina avellana* hulls to 3.9 g/100 g of solid found in buckwheat extracts (*18*, *19*). Response surfaces that fit values in **Table 3** are written below, showing

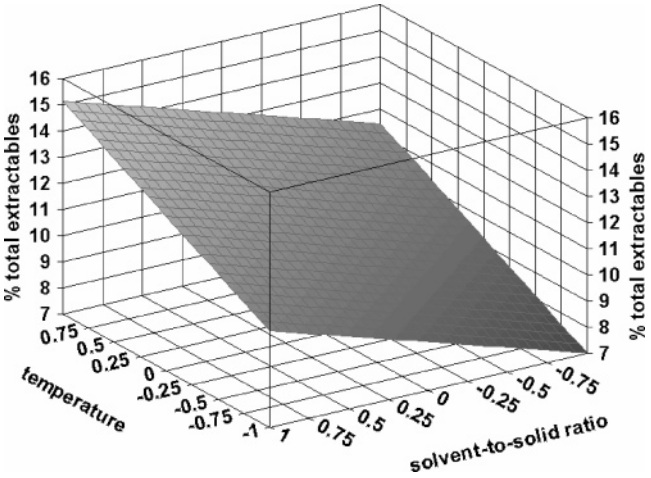

Figure 2. Response surface plot for total soluble solids in methanol extracts of red distilled grape byproducts.

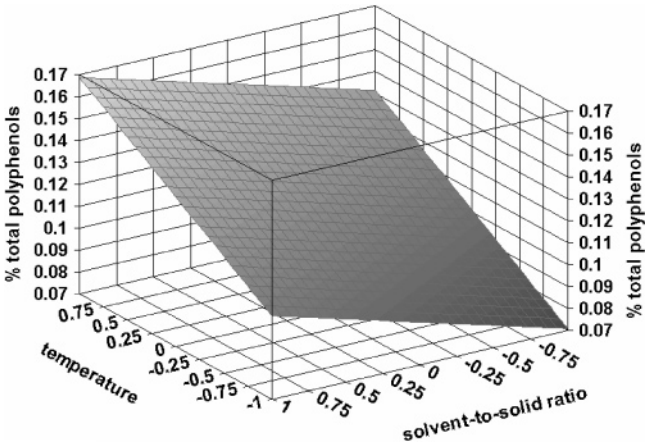

Figure 3. Response surface plot for total phenolic compounds in methanol extracts of red distilled grape byproducts.

tendencies similar to those noted for total solids.

$$\%\ \text{total phenols}_{\text{methanol}} \times 100 = 10.429 + 1.546t + 3.016T + 1.859(L/S)$$

$$F_{\text{model}} = 40.938;\ p < 0.001;\ R^2 = 0.917$$

$$\%\ \text{total phenols}_{\text{ethanol}} \times 100 = 6.471 + 1.865t + 2.290T + 1.263(L/S)$$

$$F_{\text{model}} = 19.265;\ p < 0.006;\ R^2 = 0.930$$

$$\%\ \text{total phenols}_{\text{water}} \times 100 = 3.898 + 1.812T + 1.113(L/S)$$

$$F_{\text{model}} = 6.509;\ p < 0.045;\ R^2 = 0.983$$

Figures 2 and **3** show the response surface plots for the total soluble solids and phenolic content as a function of extraction temperature and solvent-to-solid ratio. The data presented correspond to values found for maximum values of contact time when methanol was used as a solvent. As can be observed, an analogous qualitative trend was detected for both functions, indicating a similar behavior toward variations of temperature and contact time. **Table 4** shows the values of phenol concentration and inhibition percentage of the extracts corresponding to the different conditions of the experimental design. The highest values were reached at conditions of experiment 8 (higher temperature and contact time and lower solvent-to-solid ratio). Different from that observed in previous tables, higher values were obtained when lower solvent-to-solid ratios were employed. It seems obvious, because the lower the amount of solvent used, the higher the concentration of extract attained. Also in this case, the order of decreasing inhibition percentage was found to be methanol > ethanol > water. Several authors showed the higher DPPH inhibition percentage for alcoholic extracts from diverse natural products in comparison to those reached with water. In particular, Oki et al. (*20*) detected a value of this variable 3 times higher when extracts of red-hulled rice were obtained using methanol rather than water. Likewise, the lower power of water versus ethanol for extracting DPPH inhibitors from *Lycium chinese* mill fruits was also reported by Quian et al. (*21*).

Analyzing the results of inhibition percentage, significant models for all solvents were obtained:

$$\%\ \text{Inh}_{\text{methanol}} = 31.1 + 12.36T - 19.13(L/S)$$

$$F_{\text{model}} = 15.13;\ p < 0.006;\ R^2 = 0.950$$

$$\%\ \text{Inh}_{\text{ethanol}} = 18.98 + 7.72t + 7.41T - 12.18(L/S)$$

$$F_{\text{model}} = 16.02;\ p < 0.007;\ R^2 = 0.946$$

$$\%\ \text{Inh}_{\text{water}} = 11.15 + 5.84T - 6.00(L/S)$$

$$F_{\text{model}} = 3.90;\ p < 0.062;\ R^2 = 0.992$$

Contact time has no influence on inhibition percentage of methanol and ethanol extracts, and this was the only difference in comparison to equations reported for phenol yields. In **Figure 4** the behavior of the inhibition percentage of methanol extracts is plotted versus temperature and solvent-to-solid ratio. As can be observed, variations in solvent-to-solid ratio have always (for all three solvents used) a higher effect on the extracts' inhibition percentage than the temperature changes. A correlation between the phenolic concentration and the inhibition percentage was found to occur (**Figure 5**). This is in agreement with other previous findings regarding the relationships of phenolic content and inhibition percentage of extracts from natural products. Mello et al. (*22*) and Ninfali et al. (*23*) also reported a good correlation between both variables when working with tea extracts and vegetable juices, respectively.

Table 4 show the results of phenol concentration and inhibition percentage of distilled red grape pomace extracts for all conditions of the experimental design. Although the extractions were carried out under conditions of the experimental design using all four sample types (pressed red, distilled red, pressed white, and distilled white) and all grape pomace components (seed, skin, and stem), higher values of phenolic concentration and inhibition percentage always corresponded to the same experiment (data not shown by being redundant). Thus, conditions of experiment 8 were considered to be optimal and used for the characterization of the various components of grape byproducts.

Characterization of the Different Components of Grape Pomace. In **Tables 5−10**, phenolic concentration as well as inhibition percentage of the extracts obtained from various parts of grape pomace with all three solvents is presented. The methanol extracts were the most concentrated, followed by ethanol and, finally, by water. The similarity of data corresponding to skin with those of the total byproduct indicates the predominant contribution of this fraction to the total concentra-

Table 4. Phenol Concentration and Inhibition Percentage from Red Distilled Grape Byproducts Subjected to the Extraction Conditions of the Experimental Design (Highest Values in Bold)

	phenol concentration (mg/L)			inhibition percentage		
expt	methanol	ethanol	water	methanol	ethanol	water
1	56.9 ± 4.8	28.4 ± 2.4	15.2 ± 2.8	9.63 ± 0.63	5.23 ± 0.43	2.8 ± 0.17
2	92.5 ± 0.4	57.5 ± 3.0	43.5 ± 2.8	15.62 ± 0.00	10.36 ± 0.47	8.56 ± 0.44
3	141.8 ± 10.7	88.3 ± 3.8	55.2 ± 6.9	26.30 ± 0.14	16.08 ± 0.26	10.23 ± 0.23
4	345.7 ± 9.3	156.6 ± 10.7	100.1 ± 10.4	68.32 ± 1.34	26.39 ± 1.66	20.32 ± 1.21
5	79.0 ± 1.0	46.9 ± 1.0	21.4 ± 0.1	18.24 ± 0.38	8.74 ± 0.39	4.56 ± 0.24
6	112.5 ± 4.8	88.4 ± 2.2	64.2 ± 5.8	23.65 ± 0.00	15.87 ± 0.10	12.39 ± 0.36
7	210.5 ± 8.6	150.8 ± 7.6	79.4 ± 0.3	40.06 ± 2.69	29.27 ± 0.43	11.37 ± 0.18
8	**493.4 ± 9.3**	**361.9 ± 4.5**	**179.4 ± 14.1**	**93.52 ± 4.42**	**65.96 ± 0.42**	**34.41 ± 2.88**
9	131.1	67.4	25.6	25.00	12.20	4.64
10	112.8	68.5	49.6	22.13	12.45	9.63
11	115.0	74.8	49.7	18.63	13.88	8.74
12	115.1	61.1	34.7	20.11	11.36	6.22

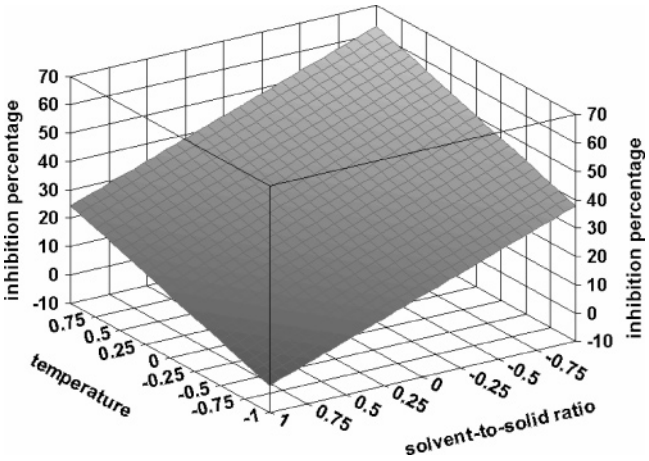

Figure 4. Response surface plot for inhibition percentage in methanol extracts of red distilled grape byproducts.

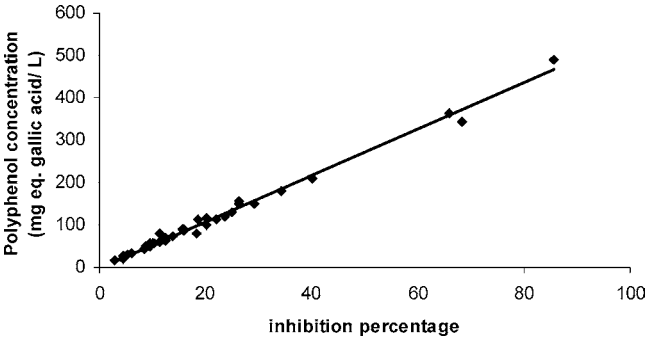

Figure 5. Correlation between phenolic concentration and inhibition percentage ($R^2 = 0.992$).

tion of extract. On the whole, samples subjected to distillation showed both higher phenolic concentration and inhibition percentage than those directly from pressing. During the distillation process, samples can reach temperatures between 120 and 130 °C. Chemical transformations affecting the phenolic composition and, as a consequence, antiradical capacity of extracts will therefore be expected to happen (*24*). Other works showed the positive effects of thermal treatment on DPPH radical scavenging activity of some extracts from various agricultural byproducts. Jeong et al. (*10*), for instance, reported a ∼4 times higher inhibition percentage in aqueous extracts of citrus peels when those were subjected at 150 °C during 60 min. Likewise, remarkable enhancements of inhibition percentage were detected in phenol model systems of quercetin,

Table 5. Phenolic Concentration (Milligrams per Liter) of the Diverse Fractions of Different Grape Pomace Extracts in Methanol[a]

grape sample	stem	seed	skin	total byproduct
white pressed	196.7 ± 39.5	307.0 ± 12.2	284.5 ± 7.2	254.3 ± 6.8
white distilled	182.8 ± 51.6	408.4 ± 15.4	357.1 ± 13.9	340.4 ± 13.5
red pressed	127.6 ± 12.1	432.1 ± 15.6	550.2 ± 33.8	500.7 ± 12.1
red distilled	218.8 ± 14.8	447.5 ± 26.6	492.9 ± 1.6	493.4 ± 9.3

[a] Values obtained by using the optimal extraction conditions (90 min, 50 °C, 1:1).

Table 6. Phenolic Concentration (Milligrams per Liter) of the Diverse Fractions of Different Grape Pomace Extracts in Ethanol[a]

grape sample	stem	seed	skin	total byproduct
white pressed	122.4 ± 9.5	251.3 ± 12.2	182.4 ± 7.2	162.6 ± 6.8
white distilled	118.7 ± 5.6	302.4 ± 9.5	200.2 ± 13.3	199.7 ± 13.5
red pressed	89.6 ± 2.1	326.6 ± 11.5	452.4 ± 3.8	422.2 ± 5.1
red distilled	134.3 ± 11.4	349.2 ± 24.6	368.9 ± 6.1	361.90 ± 4.5

[a] Values obtained by using the optimal extraction conditions (90 min, 50 °C, 1:1).

Table 7. Phenol Concentration (Milligrams per Liter) of the Diverse Fractions of Different Grape Pomace Extracts in Water[a]

grape sample	stem	seed	skin	total byproduct
white pressed	42.1 ± 3.9	113.9 ± 2.2	89.9 ± 7.2	87.2 ± 6.8
white distilled	53.2 ± 4.1	152.4 ± 9.5	90.3 ± 3.3	107.5 ± 3.5
red pressed	32.1 ± 1.2	169.3 ± 11.5	170.4 ± 3.8	157.1 ± 5.1
red distilled	54.9 ± 1.8	177.2 ± 4.6	170.2 ± 6.1	179.4 ± 14.1

[a] Values obtained by using the optimal extraction conditions (90 min, 50 °C, 1:1).

catechin, and resveratrol after storage at 60 °C (*25*). Variations were justified by the well-known tendency of phenols to combine themselves through polymerization reactions; due to the more significant area of charge delocalization, oligomers exerted a higher antiradical activity than the original monomers (*25*, *26*).

When the antiradical activities of extracts obtained from different grape fractions were compared, seed extracts showed the highest values in all three solvents. In white grape samples, the differences in inhibition percentage could be attributed to the higher phenolic concentration of seed extracts with regard to the other fractions. However, a higher phenolic concentration

Table 8. Inhibition Percentage of the Diverse Fractions of Different Grape Pomace Extracts in Methanol[a]

grape sample	stem	seed	skin	total byproduct
white pressed	60.66 ± 2.26	88.74 ± 4.76	64.09 ± 1.98	67.77 ± 4.32
white distilled	97.70 ± 5.56	99.80 ± 5.98	89.64 ± 1.34	95.00 ± 2.26
red pressed	87.15 ± 6.65	92.13 ± 4.46	54.06 ± 3.21	58.08 ± 3.57
red distilled	94.12 ± 5.51	96.67 ± 3.43	82.23 ± 6.31	93.52 ± 4.42

[a] Values obtained by using the optimal extraction conditions (90 min, 50 °C, 1:1).

Table 9. Inhibition Percentage of the Diverse Fractions of Different Grape Pomace Extracts in Ethanol[a]

grape sample	stem	seed	skin	total byproduct
white pressed	79.37 ± 4.87	96.12 ± 1.94	70.21 ± 3.84	74.13 ± 4.92
white distilled	96.76 ± 4.86	99.13 ± 2.85	76.42 ± 8.11	86.76 ± 2.84
red pressed	89.06 ± 3.88	95.87 ± 4.92	60.49 ± 5.83	58.73 ± 1.83
red distilled	89.11 ± 3.68	91.77 ± 7.00	72.76 ± 3.92	65.96 ± 0.42

[a] Values obtained by using the optimal extraction conditions (90 min, 50 °C, 1:1).

Table 10. Inhibition Percentage of the Diverse Fractions of Different Grape Pomace Extracts in Water[a]

grape sample	stem	seed	skin	total byproduct
white pressed	5.86 ± 0.23	13.99 ± 0.83	4.50 ± 0.08	6.67 ± 0.11
white distilled	14.84 ± 1.85	24.87 ± 1.99	19.76 ± 1.54	21.80 ± 1.16
red pressed	10.56 ± 0.72	17.09 ± 1.21	12.86 ± 1.21	15.78 ± 0.73
red distilled	12.85 ± 0.72	26.98 ± 0.73	36.00 ± 2.99	34.41 ± 2.88

[a] Values obtained by using the optimal extraction conditions (90 min, 50 °C, 1:1).

was detected in skin extracts of red grape samples with respect to the seed ones. In this case, the higher inhibition percentage could be explained only on the basis of the different nature of phenols extracted in each case. Previous studies bore out the specific phenolic composition of the diverse grape components. Red skins are known to possess a noteworthy quantity of anthocyanins, which could certainly contribute to increase the phenolic content. Likewise, skin and seeds contain monomers, oligomers, and polymers composed of flavan-3-ols. However, although great amounts of flavan-3-ol dimers and trimers were detected in seed extracts, only monomers and polymers with a considerable polymerization degree were found in skins (*27*−*29*). As already discussed, the more significant area of charge delocalization promotes an increase of the radical inhibition by phenols in the order of progressive polymerization (*25*). Although it has been observed that the scavenging activity of procyanidin fractions from one monomer to four monomers is increased, this trend changes for tannins with a polymerization degree superior to four monomers (*30*). In fact, the antiradical activity could decrease as a consequence of the steric hindrance caused by increasing molecular complexity, which reduces the availability of the hydroxyl groups (*31*). Apart from flavan-3-ols, the presence of other particular phenolic compounds of each fraction could probably have a considerable weight in the different values of the inhibition percentage detected. In fact, gallic acid and resveratrol were reported to occur in both skin and seed, whereas caftaric, coutaric, and glucosides of quercetin, kaempferol, and myricetin were found in stems (*32*, *33*).

To sum up, grape byproducts are a good and cheap source of phenolic compounds, the applications of which as active substances in cosmetic and pharmaceutical compositions steadily increase.

Industrially, the economical feasibility of the extraction process involves the search for the optimal extraction conditions, to maximize the efficiency of the process. In this study, both the higher phenolic concentration and the antiradical activity of extracts were obtained by increasing the temperature and lowering the solvent-to-solid ratio.

These results could mean the first step for the implementation of the process on a large scale, being an adequate starting point for further studies regarding the optimization of the continuous process, of major interest from an industrial point of view.

Finally, the viability of the project might also consider other factors such as the adequate choice of the raw materials and the convenience of subjecting them to a pretreatment. The economic implications of each choice must be evaluated on the basis of the possible value of the final product, which can be presented separately or as a potential additive in food fortification.

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