Effect of Temperature on the Free Radical Scavenging Capacity of Extracts from Red and White Grape Pomace Peels

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Free radical scavenging capacity of the extracts from red and white grape pomace peels (RGPP, WGPP) and as a reference for two commonly used antioxidants (BHA (synthetic) and DL-α-tocopherol (natural) heated at processing temperatures (80, 100, and 120 °C) was evaluated using the 2,2-diphenyl-1-picrylhidrazyl (DPPH•) reagent. Kinetic behavior of the sample extracts did not change when they were heated, following a general multiplicative model in which remaining DPPH• was more affected by temperature than the time needed to reach the steady state. The reduction of this property in the sample extracts heated at 120 °C as compared to those at 20 °C followed an increasing order: BHA (15.3%) < WGPP (22.9%) < RGPP (28.3%) < DL-α-tocopherol (69.8%). Color losses were also measured by a tristimulus colorimeter HunterLab and two parameters were used to express the sample extract color: hue angle (tan $b^*/a^*)^{-1}$ and a^*/b^* ratio. The higher red color losses in RGPP extracts (12.9 times in hue angle) as compared to the yellow color losses in the WGPP extracts (1.5 times in a^*/b^* ratio) during heating may explain their differences in the free radical scavenging capacity.

Keywords: *Grape pomace; extracts; free radical scavenging; temperature*

INTRODUCTION

Marketing costs are directly related to the time foods can remain on the grocery shelf (Buck and Edwards, 1997). Many factors influence keeping the quality of foods, and among them the oxidation process has received more attention. This is probably due to the necessity of replacing synthetic antioxidants [mainly butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)] by others from natural sources in view of the suspected role of the synthetic antioxidants as promoters of carcinogenicity and/or teratogenicity (Krishnakumar and Gordon, 1996).

Oxidation occurs naturally in all foods and may reduce the flavor and nutritive value of fats, oils, and lipid-containing products (Giese, 1996). Nutritional quality losses are related to the losses in essential fatty acids, amino acids, and vitamins. The mechanism of protection by the peroxy radical scavengers (phenolic antioxidants) is more effective during the propagation stage of oxidation because they can interrupt the formation of hydroperoxides; by this, the chain reactions are stopped, and a longer shelf-life of the food is obtained (Basaga et al., 1997).

Commercial natural antioxidants are made from different sources, such as rosemary, tea, spices, etc., but prior to using them, many considerations must be taken into account, such as potency desired, pH of the food, flavor and odor, and type of processing of the food to be added (frying, baking, extrusion, blanching, sterilization) (Buck and Edwards, 1997). In view of this, it is very important to evaluate the thermal stability of the antioxidant to be used so that the final quality of the processed food product can be assured.

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Little information on the effect of temperature on the free radical scavenging capacity of natural antioxidant extracts was found. Reynhout (1991) observed that decreasing the induction time in the stabilized oil containing rosemary extract at 80 °C was higher than when it had added BHA or tocopherol. Other studies were carried out in a model system with caffeic acid at 5, 20, and 35 °C (Cilliers and Singleton, 1989) or in some specific products such as purified lard triacylglycerols with the addition of ferulic acid or tocopherol at 25, 50, 75, and 100 °C (Marinova and Yanishlieva, 1992).

Red grape pomace has been used as a raw material for food colorants production in the form of both powder and liquid (Mazza, 1995). The antioxidant activity of white and red grape pomace peels and seeds has been determined by the ferric thiocyanate method (Larrauri et al., 1996), and the effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels has been studied (Larrauri et al., 1997). Nevertheless, no information on the measurement of its free radical scavenging capacity was found. The objective of this work was to study the effect of processing temperature (80, 100, and 120 °C) on the free radical scavenging capacity of extracts from red and white grape pomace peels.

MATERIALS AND METHODS

White grape pomace peels (*Vitis vinifera* var. Airén) and red grape pomace peels (*Vitis vinifera* var. Cencibel) obtained from Bodegas Los Llanos (Valdepeñas, Spain) were freeze-dried and milled to a particle size less than 0.5 mm.

Preparation of the Sample Extracts. The extracts from red and white grape pomace peels (RGPP; WGPP) were prepared according to the conditions previously reported (Larrauri et al., 1997). In brief, powdered samples (2 g) were extracted sequentially with methanol/water and acetone/water

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Table 1. Kinetic Models and Some Parameters from the Determination of the Free Radical Scavenging Capacity of Sample Extracts Previously Heated at Different Temperatures (Mean ± SD)[a]

samples	equation parameters[b]	temp (°C) 20	80	100	120
BHA (4.5×10^{-3})[3]	*b*	−0.941 ± 0.016	−0.829 ± 0.023	−0.798 ± 0.023	−0.797 ± 0.081
	a	4.31 ± 0.030	3.93 ± 0.059	3.94 ± 0.065	3.89 ± 0.044
	r	−0.954	−0.941	−0.929	−0.913
	remaining DPPH• (%)	5.98 ± 0.10a	7.03 ± 0.55a	6.72 ± 0.32a	6.88 ± 0.61a
	time at the steady state (min)	17.5 ± 1.7a	16.3 ± 1.2a	18.1 ± 0.8a	16.8 ± 0.5a
DL-α-tocopherol (1.25×10^{-3})[c]	*b*	−0.998 ± 0.010	−0.455 ± 0.023	−0.318 ± 0.025	−0.302 ± 0.017
	a	4.14 ± 0.042	3.90 ± 0.041	3.98 ± 0.049	3.96 ± 0.038
	r	−0.966	−0.897	−0.879	−0.907
	remaining DPPH• (%)	4.96 ± 0.10a	16.63 ± 1.94b	18.90 ± 2.24b	18.80 ± 4.22b
	time at the steady state (min)	16.5 ± 1.7a	22.5 ± 2.3b	33.2 ± 6.0c	39.2 ± 7.4c
red grape pomace peel (31.2×10^{-3})[c]	*b*	−0.808 ± 0.030	−0.657 ± 0.022	−0.645 ± 0.043	−0.578 ± 0.015
	a	3.78 ± 0.144	3.45 ± 0.103	3.76 ± 0.042	3.82 ± 0.100
	r	−0.901	−0.936	−0.971	−0.974
	remaining DPPH• (%)	5.84 ± 0.35a	7.88 ± 0.22b	8.95 ± 1.05bc	10.10 ± 0.39c
	time at the steady state (min)	13.0 ± 1.4a	12.6 ± 1.0a	15.3 ± 0.6ab	18.4 ± 3.2b
white grape pomace peel (31.2×10^{-3})[c]	*b*	−0.779 ± 0.015	−0.700 ± 0.040	−0.645 ± 0.023	−0.572 ± 0.039
	a	4.50 ± 0.152	4.64 ± 0.050	4.67 ± 0.021	4.64 ± 0.047
	r	−0.994	−0.994	−0.994	−0.993
	remaining DPPH• (%)	7.30 ± 0.33a	6.90 ± 0.10a	7.55 ± 0.40a	9.36 ± 0.90b
	time at the steady state (min)	39.7 ± 6.6a	38.2 ± 5.0a	52.5 ± 2.3b	59.7 ± 8.1b

[a] Different online letters (a−c) indicate significant differences ($p \leq 0.05$)) in the same characteristics. [b] Multiplicative models: $\ln \text{DPPH}^{\bullet}_{\text{REM}} = b \ln t + \ln a$, where b = slopes; a = intercept; r = correlation coefficient. [c] Expressed as g dry sample/100 mL in the assay mixture.

at room temperature for 60 min, and the supernatants were combined and concentrated up to 5 mL.

Commercial Standards. 3-*tert*-Butyl-4-hydroxyanisole, BHA (Merck Farma y Química, SA, Madrid, Spain), and DL-α-tocopherol (Sigma Chemical Co, St Louis, MO) were directly dissolved in ethanol/water. Other reagents used were 2,2-diphenyl-1-picrylhydrazyl (DPPH•) from Sigma-Aldrich Química, SA (Madrid, Spain), and methanol from Panreac Química, SA (Barcelona, Spain).

The extracts in screw-capped flasks covered with foil were incubated in an oven for 3 h at processing temperatures of 80, 100, and 120 °C. Then, they were immediately cooled and used for the free radical scavenging determination. Duplicate samples were incubated twice. Sample extracts without heating (20 °C) were used as references.

Free Radical Scavenging Method. The antiradical capacity of the sample extracts, previously heated at different temperatures, was estimated according to the procedure reported by Brand-Williams et al. (1995) that was slightly modified in our laboratory (Sánchez-Moreno et al., 1998). In brief, an aliquot (0.1 mL) of the sample extracts was added to 3.9 mL of DPPH• (0.025 g L^{-1}) in methanol. A Perkin-Elmer UV/VIS spectrophotometer model Lambda 12 was used, and the absorbances at 515 nm were measured at different time intervals until the reaction reached a plateau (time at the steady state). Sample concentrations (expressed as g of dry sample/100 mL in the assay mixture) were selected in order to get an adequate time at the steady state: RGPP and WGPP, 31.2×10^{-3}; BHA, 4.5×10^{-3}; and DL-α-tocopherol, 1.25×10^{-3}.

DPPH• concentration in the assay mixture at different time intervals (t) was calculated from the following calibration curve, determined by linear regression:

$$A_{515\text{nm}} = 2935.68[\text{DPPH}^{\bullet}]_t - 2.18 \times 10^{-3}$$

where $[\text{DPPH}^{\bullet}]_t$ was expressed as gram liter^{-1}, correlation coefficient (r) = 0.999. The percentage of remaining DPPH• (% $\text{DPPH}^{\bullet}_{\text{REM}}$) at the steady state was calculated as follows:

$$\%\ \text{DPPH}^{\bullet}_{\text{REM}} = \{[\text{DPPH}^{\bullet}]_t/[\text{DPPH}^{\bullet}]_{t=0}\} \times 100$$

The reduction in the free radical scavenging capacity (RRSC) by heating of the samples was calculated by the following equation:

$$\%\ \text{RRSC} = 100 - [b_T/b_{20\ ^\circ\text{C}} \times 100]$$

where b_T and $b_{20\ ^\circ\text{C}}$ were the slopes from the regression models of the sample extracts heated at different temperatures (T) or at 20 °C, respectively.

Color Measurement. Sample extracts were placed in Petri dishes, and a Tristimulus reflectance colorimeter (HunterLab, model D25) calibrated with a white standard tile (X = 82.45; Y = 84.46; Z = 101.44) was used. Color was recorded using the CIE-L^*,a^*,b^* uniform color space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (−) to red (+) axis, and b^* indicates hue on a blue (−) to yellow (+) axis. Two CIE-Lab values were used to express the sample extracts color: hue angle, $H° = (\tan b^*/a^*)^{-1}$ and a^*/b^* ratio (Shimon et al., 1992).

Statistical Analysis. Data were analyzed by an analysis of variance ($p \leq 0.05$) and means separated by Duncan's multiple range test. Results were processed by the following computer programs: Excel 4.0 and Statgraphics 5.0.

RESULTS AND DISCUSSION

Kinetic behavior of the sample extracts heated at different temperatures (Table 1) followed a general multiplicative model:

$$\ln \text{DPPH}^{\bullet}_{\text{REM}} = b \ln t + \ln a$$

where b is the slope and a is the intercept. High negative correlation coefficient values ($r \geq 0.85$) were obtained for all sample models. The higher the temperature, the lower the model slopes. Similar kinetic models were previously obtained at a standard temperature of 20 °C (Sánchez-Moreno et al., 1998) for different standard polyphenols found in grape peels such as gallic, tannic, caffeic, and ferulic acids; quercetin; rutin; and resveratrol.

An example of the kinetic behavior for RGPP extracts heated at different temperatures is shown in Figure 1. The higher the temperature, the higher the remaining DPPH• and the higher the reaction time. This fact is illustrated in samples and standards in Table 1.

Table 2. HunterLab Color Measurement in the Extracts from Red and White Grape Pomace Peels Previously Heated at Different Temperatures (Mean ± SD)[a]

	*L**		*b**			
temp (°C)	RGPP[b]	WGPP[c]	RGPP	WGPP	hue angle (°) RGPP	*a**/*b** WGPP
20	7.5 ± 0.4a	15.4 ± 1.2a	0.6± 0.04a	1.8 ± 0.09a	4.20 ± 0.07a	0.27 ± 0.04a
80	8.3 ± 0.3a	14.3 ± 1.3a	1.3 ± 0.1b	2.3 ± 0.2b	11.5 ± 0.9b	0.34 ± 0.03ab
100	8.5 ± 0.6a	12.8 ± 0.8ab	5.5 ± 0.5c	5.9 ± 0.4c	47.2 ± 1.3c	0.32 ± 0.04a
120	8.1 ± 0.3a	10.9 ± 0.9b	8.9 ± 0.4d	9.8 ± 0.9d	53.9 ± 1.7d	0.41 ± 0.03b

[a] Different online letters indicate significant differences ($p \leq 0.05$) in the same column. [b] RGPP, red grape pomace peel. [c] WGPP, white grape pomace peel.

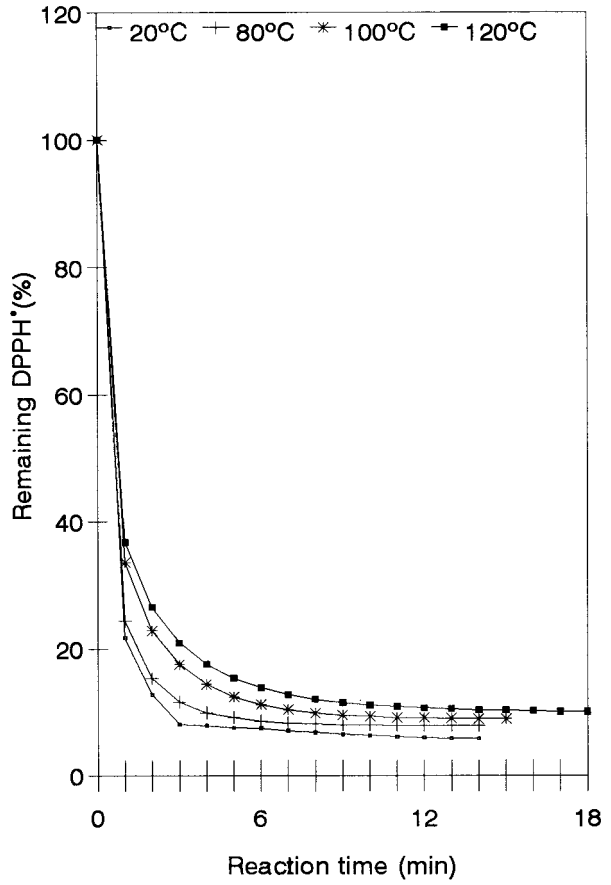

Figure 1. Kinetic behavior in the free radical scavenging determination of extracts from red grape pomace peels heated at different temperatures.

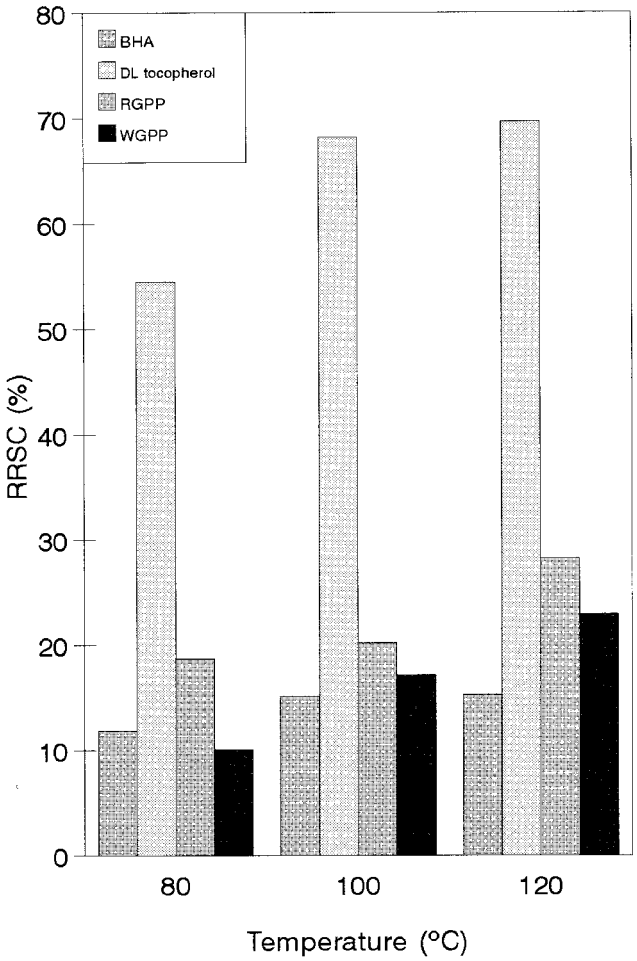

Figure 2. Reduction in the free radical scavenging capacity (RRSC) of the extracts from white and red pomace peels heated at different temperatures.

The remaining DPPH• is negatively correlated to the concentration of the antioxidant compound, and Table 1 shows that this characteristic for WGPP and BHA samples heated at 80 °C was not significantly affected. Nevertheless, DL-α-tocopherol had the greatest increase in remaining DPPH• at 100−120 °C (3.8 times) as compared to that obtained at 20 °C. For other samples this value was 1.7 in RGPP, 1.3 in WGPP, and 1.1 times in BHA. The increase in the remaining DPPH• of these extracts with temperature may suggest some partial losses in the bioactive compounds related with the free radical scavenging capacity.

The time at the steady state for all sample extracts (Table 1) was not significantly different at 80 °C, except for DL-α-tocopherol. BHA was the most stable sample because of the time at the steady state was not significantly affected by heating to 120 °C. An increase in time at the steady state at 120 °C as compared to those at 20 °C were found in the other samples: 2.4 for DL-α-tocopherol, 1.5 for RGPP, and 1.4 times for WGPP.

Taking into account the two main characteristics derived from the free radical scavenging determination in the samples (remaining DPPH• and time at the steady state), it was observed that those values for BHA did not change significantly. Meanwhile, those for DL-α-tocopherol were the most affected. Reynhout (1991) also found higher heat stability in synthetic antioxidants (such as BHA) than in natural ones (such as DL-α-tocopherol).

Comparing the values of the above characteristics from Table 1, it can be postulated that the heating of the sample extracts up to 120 °C influenced the free radical scavenging capacity, being the amount of the remaining DPPH• more affected than the time at the steady state.

The slopes of the kinetic model are useful parameters to characterize the kinetic behavior of the antioxidant compound in the antiradical capacity determination (Sánchez-Moreno et al., 1998). By this, the reduction

in the free radical scavenging capacity (RRSC) was calculated as a ratio between the slopes of the models at different temperatures and at 20 °C (Figure 2). The RRSC at 120 °C of DL-α-tocopherol was the highest (69.8%), and that for BHA was the lowest (15.3%). The RRSC in WGPP (22.9%) was lower than in RGPP (28.5%).

Table 2 shows the color changes in the extracts from RGPP and WGPP when they were heated. A significant decrease in the red color (12.9 times higher hue angle) and an increase in the *b** values (14.8 times) were obtained for RGPP extracts heated at 120 °C as compared to those at 20 °C. Lightness (*L**) was not significantly different. Meanwhile, for WGPP extract, the yellow color (*a**/*b** ratio) and *L** values decreased only 1.4−1.5 times, indicating a smaller color loss than in the RGPP extracts.

Other researches have observed some relation among heating period, color, and polyphenols content in the samples. Skorikova and Lyashenko (1972) obtained a negative correlation between the heating period and the polyphenol content of apple and pear juices, with the leucoanthocyanins being the least stable polyphenol fraction. Similarly, Sato et al. (1996) found a correlation between wine color and the ability of the wine constituents to scavenge superoxide radicals. Specifically in RGPP, losses in the total polyphenols, color, and antioxidant capacity were reported when they were dried at 100 and 140 °C, with the anthocyanins being more affected than other compounds (Larrauri et al., 1997). Bioactive compounds (such as polyphenols, flavonoids, etc.) that are associated to the antioxidant capacity of the samples are usually oxidized at high temperatures (Yoshioka et al., 1990), and this fact may explain the changes in the remaining $DPPH^{\bullet}$ and in the time needed to reach the steady state during the determination of the free radical scavenging capacity of grape pomace peels (Table 1).

In summary, we can denote a lower reduction in the free radical scavenging capacity of WGPP extract with processing temperature as compared to that from RGPP, which could be related to the color losses of these extracts, suggesting that the bioactive constituents of WGPP extract are more heat resistant than those from RGPP.

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