Effect of Drying Temperature on the Stability of Polyphenols and Antioxidant Activity of Red Grape Pomace Peels

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The effect of drying temperature (60, 100, and 140 °C) on the polyphenols' content and antioxidant activity of red grape pomace peels was studied. Freeze-dried samples were used as reference. Differences on the CIE-LAB color, total extractable polyphenols, condensed tannins, UV−vis spectra, and antioxidant activity were evaluated. When drying temperature was 100 and 140 °C, a significant reduction in both total extractable polyphenols (18.6 and 32.6%) and condensed tannins (11.1 and 16.6%) was observed, as well as a decrease of 28 and 50% in the antioxidant activity of the samples, respectively. Hue angle and total color difference in the sample dried at 140 °C were significantly higher than in the freeze-dried reference material. A red color loss at 140 °C was also confirmed by lower absorbance values in the spectra at 525 nm. Drying at 60 °C did not significantly affect the sample characteristics evaluated.

Keywords: *Wine byproducts; grape pomace peels; antioxidant activity; drying temperature*

INTRODUCTION

Over the past few years there has been an increasing interest in natural antioxidants and their role in human health and nutrition. The fact that the oxidation process plays host to several degenerative diseases and can contribute significantly to the risk of human aging and cancer has focused the interest on this subject (Jacob, 1995).

Red wine consumption causes a decrease in platelet aggregation as well as an increase in high-density lipoprotein cholesterol independent of the alcohol content of wine (Seigneur *et al.*, 1990). Phenolic substances found in red wine were reported to inhibit oxidation of low-density lipoprotein cholesterol (Frankel *et al.*, 1993).

The importance of wine making in many countries has led to the availability of large quantities of grape skins that contain a high amount of anthocyanin pigments. These pigments have been commercialized under a number of tradenames, such as Enocianina in the United States, Italy, Germany, and France (Francis, 1992). Moreover, these raw materials have also been reported to have a potent vasorelaxing effect (Fitzpatrick *et al.*, 1993) and antioxidant activity (Kanner *et al.*, 1994), which could be useful to elaborate new products with health promoting capacity.

Drying of wine byproducts may be an essential step in the processing of these materials, and the chemical and biochemical changes that take place during this process should be studied. No information about the polyphenols' stability and antioxidant activity of red grape pomace peels was found. Nevertheless, it has been reported that phenolic antioxidants exhibit significant decomposition at high temperatures, giving rise to a number of breakdown products which, in turn, can be decomposed (Hamama and Nawar, 1991). This effect could also be related to other factors, such as drying conditions, type of bioactive compounds in the sample, etc.

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Nwanguma and Eze (1996) found that an increase of mashing temperature from 65 to 75 °C resulted in a significant reduction in the polyphenolic content of wort. Makkar and Singh (1991) reported a decreased content of total proanthocyanidins in cassava and Leucaena leaves (10.1 and 21.4%, respectively) when heated at 90 °C for 24 h.

The aim of this work was to study the effect of drying temperature on the polyphenolic content and antioxidant activity of red grape pomace peels.

MATERIALS AND METHODS

Sample Preparation. Red grape pomace (*Vitis vinifera* var. Cencidel) obtained from Bodega Los Llanos (Valdepeñas, Spain) was sieved out in order to collect the peels, which were pressed and kept frozen at −20 °C.

Dehydration Rate. Drying curves (kg of water/kg of dry matter) were obtained by periodically weighing red grape pomace peels (RGPP) during dehydration up to a maximum moisture content of 8.0%. The experiments were carried out in an air-circulating oven at a flow rate of 2.3 m^3/min with a tray load of 10 kg/m^2. The air temperature was 60, 100, or 140 °C. Dried samples were milled to less than 1 mm particle size. Freeze-dried RGPP samples were obtained as a reference to evaluate the effect of drying temperature on sample quality.

Color. Samples were placed as a uniform layer (0.5 cm thick) on a 5 cm diameter Petri dish 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. Three CIE-*Lab* values were further incorporated into two functions, which were used to express fiber color, and provided a single measurement of color that simulated visual judgment. The two single-color functions were hue angle: $H^o = (\tan b/a)^{-1}$ and total color difference: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (Shimon, 1992). A freeze-dried sample was used in the last function as reference.

Chemical Analysis. *Total Extractable Polyphenols (TEP).* Powdered samples (500 mg) were extracted sequentially with 40 mL of methanol:water (50:50, v:v) and 40 mL of acetone:water (70:30, v:v) at room temperature for 60 min in each case. After centrifugation at 2500*g* for 15 min, combined supernatants were made up to 100 mL with distilled water. TEP were

S0021-8561(96)00282-8 CCC: $14.00

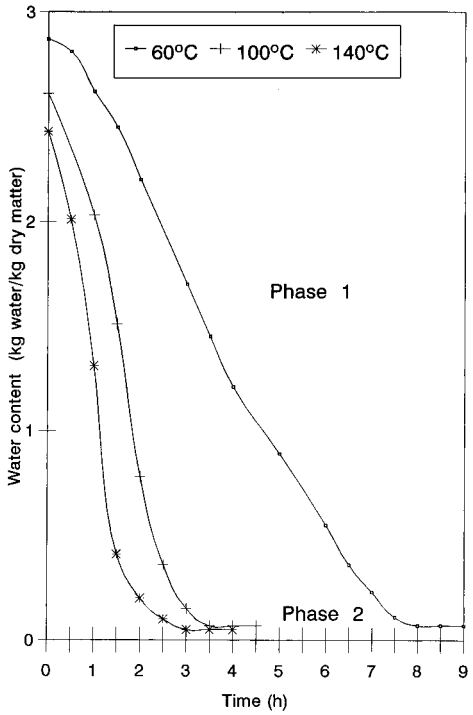

Figure 1. Effect of temperature on moisture content of peels from red grape pomace.

spectrophotometrically assayed by the Folin−Ciocalteu method using tannic acid as standard (Montreau, 1972).

Condensed Tannins (CT). The residues obtained from TEP extraction were treated with 40 mL of hydrochloric acid (50 mL/L) in 1-butanol in a water bath at 100 °C for 3 h. Supernatant obtained by centrifugation (2500*g* for 15 min) was made up to 50 mL with the same solvent, and the absorbance was measured at 555 nm. Carob pod condensed tannins (Nestlé, Ltd.) were selected as standard according to a previous spectral report (Saura-Calixto and Bravo, 1995).

Antioxidant Assay. *Sample Extraction.* The procedure was the same as described above for the extraction of total polyphenols, except that the combined supernatants were concentrated in a vacuum rotatory evaporator at 50 °C, freeze-dried, and dispersed in 10 mL of absolute ethanol.

Antioxidant Activity Determination. The ferric thiocyanate (FTC) method reported by Kikuzaki and Nakatani (1993), slightly modified in our laboratory, was used. A mixture of 0.5 mL of a weighed sample extract (0.25% w/v) in absolute ethanol, 0.5 mL of 2.51% linoleic acid in 99.5% ethanol, 1 mL of 0.05 M phosphate buffer (pH 7), and 0.5 mL of distilled water was placed in a screw-capped tube and then in dark oven at 40 °C. A control without sample extract was used.

Every 24 h, 0.1 mL aliquots of this solution were taken and 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added. Precisely 3 min after the addition of 0.1 mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm every day until the absorbance of the control reached maximum.

The antioxidant activity (AA) was calculated as percentage of inhibition relative to the control using the following equation:

$$AA = 100 - \left[\frac{(\text{sample absorbance}_{96h})/(\text{sample absorbance}_{0h})}{(\text{control absorbance}_{96h})/(\text{control absorbance}_{0h})} \times 100\right]$$

UV−vis Spectra. Ethanolic extract (2 mL), as prepared for the antioxidant assay, was filtered through 0.5 μm filter units (Millex-SR Millipore, France), and the pH was adjusted (pH < 2) with 35% hydrochloric acid (Panreac Química SA, Spain). The spectra over the range 312−550 nm of each sample was recorded (scan speed 240 nm/min) using 96% ethanol as a blank reference in a Perkin-Elmer Lambda 12 spectrophotometer which had been interfaced to a microcomputer through an UV−vis computerized spectroscopy software (PECSS).

Table 1. Polyphenolic Content of Dried Red Grape Pomace Peels (Values Expressed as Dry Basis)[a]

	extractable polyphenols (%)		condensed tannins (%)	
treatment	X^b	s^c	X^b	s^c
freeze-drying	4.3[a]	0.2	27.0[a]	1.0
drying at 60 °C	4.1[a]	0.02	26.2[ab]	1.2
drying at 100 °C	3.5[b]	0.2	24.0[bc]	1.8
drying at 140 °C	2.9[c]	0.2	22.5[c]	1.1

[a] Different superscript letters (a, b, c) in the same column mean significant differences ($p \leq 0.05$). [b] X = mean value. [c] s = standard deviation.

Statistical Analysis. All analytical values represent means of three analytical replications realized at least on two different drying experiments. Data were analyzed by one-way analysis of variance ($p \leq 0.05$). Duncan's multiple range test was used for mean discrimination.

RESULTS AND DISCUSSION

The efficiency of the drying-air temperature increase on accelerating the red grape pomace peels (RGPP) dehydration are illustrated in Figure 1. Similar dehydration curves were obtained by other authors drying onion (Mazza and Lemaguer, 1980) and sugar beet fiber (Bernardo and Dumolin, 1990).

The higher the drying-air temperature, the higher the dehydration rate, as the slopes of the following equations (calculated in the the phase 1 of the curves by a regression analysis) show:

$$Y_{60°C} = 2.94 - 0.391X \qquad r = -0.995$$

$$Y_{100°C} = 2.74 - 0.913X \qquad r = -0.988$$

$$Y_{140°C} = 2.50 - 1.252X \qquad r = -0.989$$

Dehydration rate at 100 °C was 2.3 times faster than at 60 °C but 0.73 times slower than at 140 °C. Similarly, samples limit moisture content (0.05 kg of water/kg of dry matter) was reached after only 3.0 and 3.5 h at 140 and 100 °C, respectively, while it took 8.0 h for the sample dried at 60 °C.

Both extractable polyphenols (TEP) and condensed tannin (CT) in RGPP did not change significantly when the dehydration temperature was 60 °C (Table 1). TEP significantly decreased in the samples dried at 100 and 140 °C. Nevertheless, CT was similar in the samples dried at 100 and 140 °C, but at the last temperature it was significantly lower than 60 °C.

Comparing to freeze-dried samples, a reduction of 18.6 and 32.6% of the TEP content was obtained with drying temperature of 100 and 140 °C, respectively. However, with the same drying temperatures, CT only decreased 11.1 and 16.6%, respectively. These results suggest that extractable polyphenols (TEP) are more sensible to drying temperature than CT.

TEP in grapes and wines are composed by a complex group of substances (phenolic acids, anthocyanins, flavonols, flavan-3-ols, and flavanonols) that are closely associated with the color, taste, and nutritional quality of plant foods, their antioxidant activity being their most important biological property (Macheix *et al.*, 1991; Ho, 1992). Therefore, it should be desirable to avoid the

Table 2. Color Measurements in Dried Red Grape Pomace Peels[a]

	hue angle (deg)		L^*		ΔE^* [b]	
treatment	X[c]	s[d]	X[c]	s[d]	X[c]	s[d]
freeze-drying	23.8[a]	2.7	46.9[a]	1.7		
drying at 60 °C	24.8[a]	2.3	31.0[b]	0.9	13.4[a]	0.8
drying at 100 °C	29.4[a]	1.9	29.8[b]	0.8	14.9[ab]	1.0
drying at 140 °C	45.8[b]	2.3	29.3[b]	0.8	15.4[b]	1.0

[a] Different superscript letters (a, b) in the same column mean significant differences ($p \leq 0.05$). [b] Considering the freeze-dried sample as reference. [c] X = mean value. [d] s = standard deviation.

reduction of the TEP content that takes place in our sample at high drying temperatures. Concerning condensed tannins, these are compounds of higher molecular weight or bound to fiber or protein. This more complex chemical structure of CT may explain their different resistance to thermal degradation.

Three possible mechanisms can be proposed to explain the reduction of the phenolic content of samples dried at high temperature (Maillard and Berset, 1995). Maillard and Berset (1995) proposed three hypotheses to explain the decrease of bound phenolic acids: release of bound phenolic compounds; partial degradation of lignin which could lead to the release of phenolic acid derivatives; and/or the beginning of thermal degradation of the phenolic compounds. In this case, the fact that a significant reduction of the polyphenol content was observed from 100 to 140 °C suggests that thermal degradation is the main mechanism.

Color measurements enabled us to distinguish the influence of the drying-air temperature on some quality characteristics of RGPP. In our samples, which contained anthocyanins, a^* and b^* values were positive, with low hue angle values indicating an intense red color. RGPP samples dried at 140 °C had significantly lower red color than the rest of the samples as shown in Table 2. L^* values of the hot-air samples were significantly lower than for the freeze-dried reference ones, indicating a lower lightness in the last case. The total color difference (ΔE^*) between sample dried at 60 and 140 °C was significantly different. This fact might be due to a loss of TEP during drying at 140 °C (Table 1) and the expected reduction in red color (higher hue angle). Therefore, hue angle and total color difference could be useful to evaluate RGPP quality.

The red color of the grapes and grape byproducts comes mostly from anthocyanins, which have the absorption maxima at 500−550 nm (Tamura and Yamagami, 1994). UV−vis spectra of the samples were more different in the 500−550 nm region than in the 300−350 nm one (Figure 2). Absorbance values at 500−550 nm of the sample dried at 140 °C were lower than the values of other samples, indicating a probable degradation of anthocyanins at this temperature. This could explain the observed reduction of red color samples and potentially its biological activity.

Anthocyanins have begun to be regarded as biologically active substances with, for example, antiinflammatory activity (Vlaskovska *et al.*, 1990), redox potentials (Gabor, 1988), anticonvulsant activity (Drenska *et al.*, 1989), and antioxidative activity (Constantion *et al.*, 1992).

We have not found any information concerning the effects of drying temperature on the antioxidant activity of grape byproducts. Nevertheless, in germinated barley, Maillard and Berset (1995) reported a 20% decrease of antioxidant activity during kilning at 90 °C.

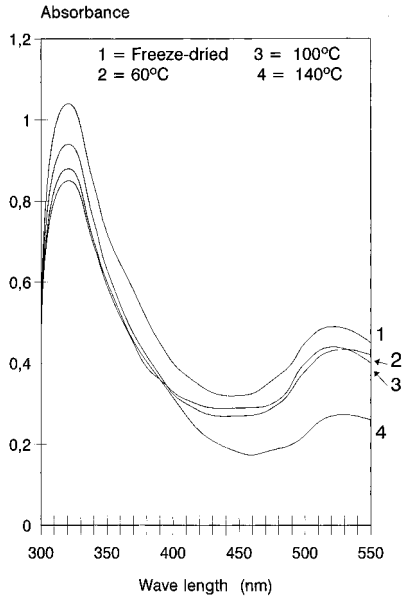

Figure 2. UV−vis spectra of dried peels from red grape pomace.

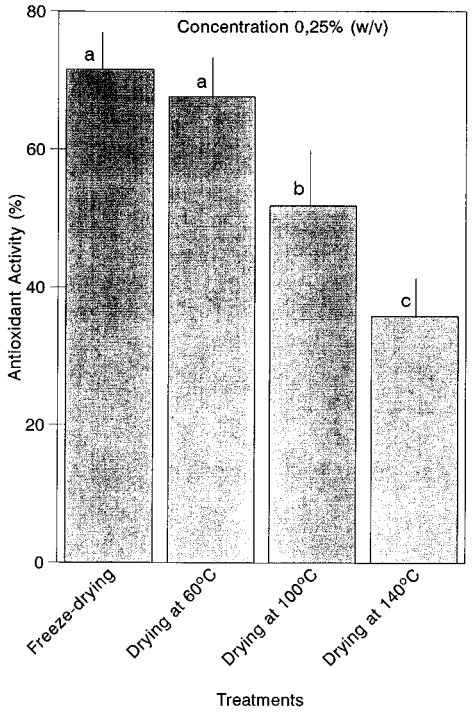

Figure 3. Antioxidant activity of dried peels from red grape pomace. Different letters (a, b, c) mean significant differences ($p \leq 0.05$).

The antioxidant activity of the freeze-dried reference and the sample at 60 °C did not differ significantly, as shown in Figure 3. On increasing the drying temperature to 100 and 140 °C, a reduction of respectively 28 and 50% of the antioxidant activity was obtained. The observed decrease of TEP content at 100 and 140 °C (Table 1) only explains 65−66% of the antioxidant activity reduction found at the same temperatures. Kanner *et al.* (1994) suggest that the high antioxidant activity of grapes and wines could be attributed to the synergistic effects of a mixture of natural phenolic compounds. This could explain the antioxidant activity loss observed in our samples.

These results suggest that RGPP antioxidants can be considered fairly heat stable. Actually, their stability

was comparable to or even higher than that reported by Lee *et al.* (1986) in ginger rhizome, with a remaining 66% of antioxidant activity after prolonged heating at 100 °C.

CONCLUSION

Polyphenolic content, color, and antioxidant activity of red grape pomace peels were not significantly affected when dried at 60 °C. Although dehydration rates were higher when drying at 100 and 140 °C, the loss of antioxidant activity and TEP at these temperatures makes them unsuitable for RGPP drying.

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Received for review April 22, 1996. Revised manuscript received December 18, 1996. Accepted December 19, 1996.[⊗]

JF960282F

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1997.