Effect of Different Winemaking Technologies on Phenolic Composition in Tinta Miúda Red Wines

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The influence of different types of winemaking technology on the contents of catechins, proanthocyanidins, and anthocyanins in Tinta Miúda red wines was studied. The Tinta Miúda red wines were made by fermentation with carbonic maceration, fermentation with stem contact, and fermentation without stem contact, respectively. The analysis of individual catechins, procyanidins, and anthocyanins in these wines was performed by HPLC, and quantification of total catechins, total oligomeric proanthocyanidins, total polymeric proanthocyanidins, and total anthocyanins was carried out by spectrophotometric methods. The wine made by carbonic maceration contained the highest amounts of both catechins and oligomeric and polymeric proanthocyanidins, followed by the wine made by fermentation with stem contact, whereas the wine made by fermentation without stem contact contained the lowest of these compounds. On the other hand, the concentrations of total anthocyanins and nearly all individual anthocyanins in the carbonic maceration wine were lower than those in the wines made by fermentation with stem contact and fermentation without stem contact. These results indicated that, although the carbonic maceration technique could retain higher amounts of catechins and proanthocyanidins in wine, it did not favor retaining or stabilizing anthocyanins in wine.

Keywords: *Catechin; proanthocyanidin; anthocyanin; winemaking technology*

INTRODUCTION

Phenolic compounds play a very important role in enology owing to their contribution to the wine sensory properties of color, flavor, astringency, and bitterness (*1*−*4*), enzymatic or nonenzymatic browning (*5*−*9*), haze formation due to their reactions with proteins (*3*, *10*−*13*), and aging behavior (*14*−*16*). Several studies have suggested that some phenolic compounds, in particular catechins and proanthocyanidins, may play a positive role in human health, in particular their protective action with regard to heart disease and their radical scavenger ability (*17*−*21*). For this reason, enologists have been interested, for several years, in producing wines that are rich in bioactive phenolic compounds. The most important factors affecting the content of these compounds in wine are their concentrations in grape, the winemaking technology, and their transformation during the wine aging process.

Various works have been realized on the quantification of phenolic compounds in grapes (*4*, *22*−*25*). Bourzeix et al. (*22*) quantified catechins and procyanidins in various French grapevine varieties, demonstrating that Pinot Noir was richest in these compounds. In our previous work (Baoshan Sun, unpublished data, 1994), we found that Tinta Miúda grape was one of the richest in catechins and proanthocyanidins among the studied red grapevine varieties from Portugal.

For given grape varieties, the type of winemaking technology can significantly affect the levels of phenolic compounds of wine. Wines made by skin fermentation with stem-contact contained much higher polymeric phenols than those wines made by skin fermentation without stem-contact (*26*). Extending pomace-contact time increased both total and polymeric phenol levels (*26*). Timberlake and Bridle (*27*) studied the effect of processing on the color characteristics of some red wines. It was found that wine made by thermovinification (60 °C for 30 min) was much more colored than the traditional one, but it contained less anthocyanins and more polymeric compounds; the wine made by carbonic maceration was the least colored. Auw et al. (*28*) determined the effect of several processing treatments including immediate press, hot press, and skin fermentation on the phenol composition and color of some red wines and juices. Immediate press wines and juices had the lowest of all measured phenols (i.e., phenolic acids, catechins, and dimeric procyanidins), whereas skin fermentation wines had higher levels of nearly all these compounds than hot press wines or juices (*28*). Kovac et al. (*29*−*30*) reported that the addition of supplementary quantities of seeds during fermentation could significantly increase catechins and dimeric procyanidins of wines, but this manipulation is generally not used in the winemaking process.

Bourzeix et al. (*22*) studied the influence of carbonic maceration, fermentation with stem contact, and fermentation without stem contact after heat treatment, on the composition of catechins and low molecular mass procyanidins in several red wines. The maceration time was four to 6 days (at 25−30 °C) for stem-contact wine, 8 days (at 30 °C) for carbonic maceration wine, and 30 min after heat treatment at 75 °C for non-stem-contact wine. It was found that for all grapevine varieties studied, the stem-contact wines had the highest catechin

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and procyanidin levels, followed by the carbonic maceration wines, and the non stem-contact wine with heat treatment contained the lowest amounts of these compounds. Similar results were also obtained by Ricardo-da-Silva et al. (*24*). These authors studied the effect of carbonic maceration, stem-contact, and non stem-contact winemaking technologies on the dimeric and trimeric procyanidin contents of Carignan and Mourvèdre red wines. The maceration time for all three types of winemaking technologies was 9 days, but the maceration temperature was 22−28 °C for stem-contact and non stem-contact wines, and 32 °C for carbonic maceration wine. These authors found that the stem-contact wine produced the highest levels of both non-galloylated and galloylated procyanidins, followed by carbonic maceration, whereas the non-stem-contact wine produced the lowest levels of these compounds.

For white wine, on the other hand, Ricardo-da-Silva et al. (*31*) also studied the effect of pomace contact, carbonic maceration, and hyperoxidation on the composition of dimeric and trimeric procyanidins in the wines made with Grenache Blanc grapes. The maceration times for the pomace-contact wine and the carbonic maceration wine were identical (i.e., 20 h), but the maceration temperatures for the pomace-contact wine and the carbonic maceration wine were 14 °C and 30 °C, respectively. These authors found that carbonic maceration wines contained the higher amounts of these compounds than the pomace-contact wines. Hyperoxidation induced important losses of all phenolic compounds analyzed.

However, all these works were concerned only with several phenolic compounds. Furthermore, the effects of different winemaking technologies on the contents and structural composition of higher oligomeric and polymeric proanthocyanidins in wine is still unknown. The main goal of this work was, therefore, to study the effect of winemaking technologies on the composition of catechins, oligomeric and polymeric proanthocyanidins, and anthocyanins in Tinta Miúda red wines.

MATERIALS AND METHODS

Standards. (+)-Catechin and (−)-epicatechin were purchased from Fluka A. G. (Buchs, Switzerland). Malvidin-3-glucoside was obtained from Extrasynthèse (Genay, France). Procyanidins B_1, B_2, B_3, B_4, B_1-3-*O*-gallate, B_2-3-*O*-gallate, B_2-3′-*O*-gallate, trimer C_1, and trimer T_2 were isolated from the methanol extract of grape seeds, in our laboratory, by Toyopearl TSK HW-40 (F) and semipreparative HPLC, as described earlier (*32*).

Grapes. Tinta Miúda (*Vitis vinifera* L.) grapes were sampled at the end of September 1998 from vineyards of the INIA-Estação Vitivinícola Nacional (Dois Portos, Portugal).

Preparation of Skin Fermentation Wines. Two 50-kg lots of Tinta Miúda grape clusters were crushed and destemmed using a destemmer-crusher (Gandra, Vila Nova de Famalicão, Portugal) and collected respectively in 60-L stainless steel tanks. The stems of one lot isolated from the destemmer-crusher were added back to the tank containing the crushed grapes from the same lot. Both lots were treated with sulfur dioxide (80 mg/L) prior to undergoing skin fermentation at 25 °C (with or without stem contact, respectively). The cap was punched down three times daily until it remained submerged. After six-days of maceration, when alcoholic fermentation was finished, the mash was pressed. Free-run and press wines were combined and stored in 20-L vessels at room temperature. After one month of conservation, the wines were racked, treated with sulfur dioxide (30 mg/L), and stored at room temperature. After three months, the wines were racked, treated with sulfur dioxide (30 mg/L), bottled, and stored at room temperature for another one month prior to analysis.

Preparation of Carbonic Maceration Wine. A 50-kg lot of Tinta Miúda grape clusters was used for preparation of carbonic maceration wine, from which a 3-kg portion of Tinta Miúda grape clusters was crushed using a destemmer-crusher (Gandra, Vila Nova de Famalicão, Portugal), collected together with stems in 90-L stainless steel tanks, and treated with sulfur dioxide (80 mg/L). Then the remaining 47 kg of the Tinta Miúda grape clusters was carefully added in the same tank and stored at 25 °C under CO_2 atmosphere. After seventeen days of intracellular fermentation/maceration (density = 1013), the mash was pressed. Free-run and press wines were combined, collected in the tank, and stored at 25 °C to undergo extracellular fermentation. After 3 days (density = 1003), when alcoholic fermentation was finished, the wine was treated with sulfur dioxide (60 mg/L) and stored in 20-L vessels at room temperature. After one month of conservation, the wines were racked, treated with sulfur dioxide (30 mg/L) and stored at room temperature. After three months, the wines were racked, treated with sulfur dioxide (30 mg/L), bottled, and stored at room temperature for another one month prior to analysis.

Fractionation of Proanthocyanidins on the Basis of Their Polymerization Degree. The wines were separated into three fractions (F_I, F_{II}, and F_{III}), containing respectively, catechins, oligomeric proanthocyanidins (degree of polymerization ranging from 2 to 12−15), and polymeric proanthocyanidins (degree of polymerization > 12−15), using C_{18} Sep-Pak cartridges as already described (33). Each fraction was evaporated to dryness at < 30 °C and dissolved in methanol with desired concentration, prior to vanillin assay or thioacidolysis.

Isolation of Total Proanthocyanidin Fraction. The procedure of isolation of the total (oligomeric plus polymeric) proanthocyanidin fraction is similar to that of fractionation of proanthocyanidins as already described (*33*). Furthermore, 3−6 mL aliquots of the wines were dealcoholized by rotary evaporation at less than 30 °C and adjusted to pH 7.0 with 0.1 N NaOH solution and/or with phosphate buffer (pH 7.0). With the aid of vacuum, this sample was then passed through the two preconditioned neutral Sep-Pak cartridges with series connection: the superior one was a tC_{18} Sep-Pak and the inferior one was a C_{18} Sep-Pak. Elution (flow rate ≤ 2 mL/min) was carried out with 10 mL of H_2O adjusted to pH 7.0 to eliminate phenolic acids. After drying the cartridges with N_2, elution was carried out with 15 mL of diethyl ether to eliminate catechins and some other unwanted phenolic compounds, and then with 15 mL of methanol to elute global proanthocyanidins (oligomers and polymers). The latter fraction was evaporated to dryness, and dissolved in methanol (0.5−1 mL), to give a desired concentration prior to thioacidolysis.

Vanillin Assay for Catechins and Proanthocyanidins. Quantification of total flavan-3-ols in catechin, and oligomeric proanthocyanidin and polymeric proanthocyanidin fractions obtained from C_{18} Sep-Pak cartridges was performed by the modified vanillin assay using, respectively, (+)-catechin, purified grape seed oligomeric procyanidins, and purified grape seed polymeric procyanidins as reference standards (*34*).

HPLC Analysis of Individual Catechins and Procyanidins. Analyses of individual catechins and procyanidins were performed by HPLC as described previously (*33*).

Analysis of Individual and Total Anthocyanins. Individual anthocyanins were analyzed by HPLC using malvidin-3-glucoside as reference as described previously (*35*). Total anthocyanins were determined by spectrophotometric method based on SO_2 bleaching (*36*), using malvidin-3-glucoside as reference standard.

Analysis of Total Phenolics. Total phenolics (index) was analyzed according to Ribéreau-Gayon (*37*).

Color Measurements. Color measurements of the wines were performed according to the CIELAB 76 convention (*38*), by determining the transmission data at multiwavelengths ranging from 380 to 770 nm with 10-nm intervals. The

Table 1. Individual Catechin and Procyanidin Contents (mg/L) in Tinta Miúda Wines Made by Three Different Winemaking Technologies[a]

	type of winemaking technology					
	carbonic maceration		stem-contact		non-stem-contact	
individual flavanols	mean	SD	mean	SD	mean	SD
(+)-catechin	67.2***c***	6.2	42.7***b***	2.2	18.8***a***	1.9
(−)-epicatechin	26.8***b***	2.7	10.3***a***	1.5	14.3***a***	1.3
procyanidin B_1	76.2***c***	7.6	47.1***b***	2.1	18.3***a***	2.5
procyanidin B_2	21.2***b***	0.3	8.6***a***	0.3	7.4***a***	1.3
procyanidin B_3	13.9***c***	0.8	8.5***b***	0.0	2.8***a***	0.8
procyanidin B_4	11.5***b***	0.8	4.7***a***	0.9	3.9***a***	0.6
procyanidin C_1	15.0***c***	0.4	6.5***b***	0.6	3.6***a***	0.5
procyanidin T_2	32.6***c***	2.4	16.5***b***	0.8	5.3***a***	1.9
procyanidin B_1-3-*O*-gallate	1.3***a***	0.0	2.3***b***	0.4	0.6***a***	0.0
procyanidin B_2-3-*O*-gallate	5.9***a***	0.4	5.1***a***	0.7	5.0***a***	1.3
procyanidin B_2-3′-*O*-gallate	3.2***ab***	0.4	3.8***b***	0.6	1.5***a***	0.6

[a] Means ($n = 2$) followed by the same letter in a row are not significantly different (LSD, 5%).

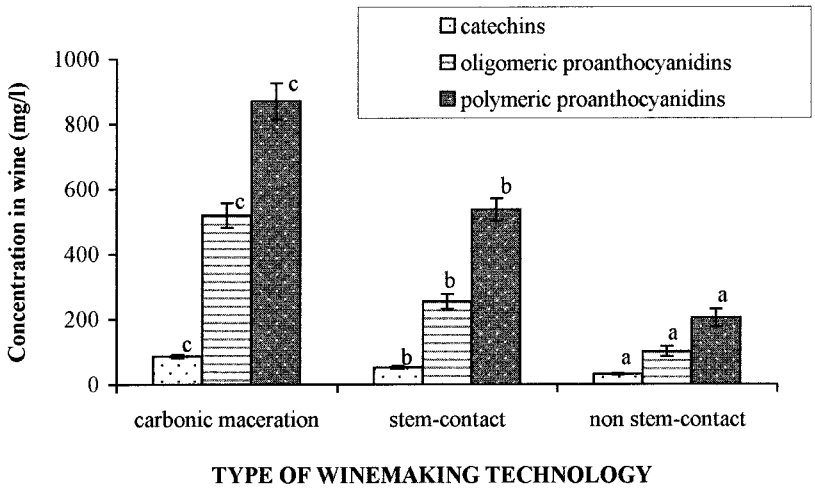

Figure 1. Effect of winemaking technologies on total catechins, total oligomeric proanthocyanidins, and total polymeric proanthocyanidins in red wines. Vertical bars represent the standard deviation ($n = 3$). For the same fraction, means followed by the same letter are not significantly different (LSD, 5%). The concentrations of total catechins, total oligomeric proanthocyanidins, and total polymeric proanthocyanidins were expressed as (+)-catechin, purified grape seed oligomeric procyanidin, and purified grape seed polymeric procyanidin equivalents, respectively (*34*).

cylindrical coordinates *L** (psychometric lightness), *C** (psychometric chroma), and *h* (hue-angle) values were obtained by using the Triest 1.0 program (*39*). The axes of a three-dimensional color space *a** (measure of redness) and *b** (measure of yellowness) were calculated as described (*38*).

Degradation of Proanthocyanidins with Toluene-α-thiol. Acid-catalyzed degradation of proanthocyanidins in the presence of toluene-α-thiol, followed by HPLC analysis to determine their structural composition, was performed as described earlier (*40*).

Analysis of Other Enological Parameters. Ethanol concentration, pH values, density, total and volatile acidity, free and total sulfur dioxide concentrations, and the concentrations of several minerals (i.e., Ca, Cu, Fe, Mg, Na, and K) were determined according to the official methods of OIV (*41*).

Statistical Analysis. Sampling and analyses were performed in duplicate or triplicate, and the data are presented as mean ± SD. Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Statgraphic 5.0 v. (STSC Inc., Rockville, MD).

RESULTS

Individual Catechins and Procyanidins. The results of HPLC analysis of individual catechins, and dimeric and trimeric procyanidins, in different types of wines are presented in Table 1.

It can be seen that for all three types of wines, (+)-catechin was always present in a higher concentration than (−)-epicatechin. Among the HPLC-detectable procyanidins, procyanidin B_1 was presented in highest concentration; the galloylated procyanidins were generally present in lower amount than the nongalloylated ones. These results agree with those of other authors (*22*, *24*, *31*).

It is important to note that catechin and nongalloylated procyanidin contents in carbonic maceration wine were much higher than those in skin fermentation wines. As expected, the wine made by skin fermentation with stem contained higher amounts of nongalloylated procyanidins than than that without stem, except (−)-epicatechin, procyanidin B_2, and procyanidin B_4, each of which was presented in similar concentrations in the two types of skin fermentation wines. On the other hand, the amounts of all galloylated procyanidins were much lower than the nongalloylated ones, and the concentration of each galloylated procyanidin is independent of the winemaking technologies.

Total Catechins, Total Oligomeric, and Polymeric Proanthocyanidins. The total catechins, and total oligomeric proanthocyanidins and total polymeric proanthocyanidins, in the three types of wines obtained by vanillin assay are presented in Figure 1.

It has been shown that the carbonic maceration wine contained the highest amount of total catechins, total

Table 2. Structural Composition (Percent in Moles) of Oligomeric Proanthocyanidins[a]

type of winemaking technology		terminal units				extension units			
		(+)-cat	(−)-epicat	(−)-epiG	(−)-epig	(+)-cat	(−)-epicat	(−)-epiG	(−)-epig
carbonic maceration	mean	14.3***a***	5.5***a***	0.2***b***	0.0	10.3***b***	62.7***b***	3.1***c***	3.8***a***
	SD	0.3	0.2	0.0		0.4	0.1	0.0	0.5
stem-contact	mean	15.4***a***	6.9***b***	0.2***b***	0.0	9.7***ab***	59.8***b***	2.0***b***	6.0***b***
	SD	0.4	0.2	0.0		0.3	0.2	0.0	0.4
non-stem-contact	mean	17.6***b***	9.5***c***	0.1***a***	0.0	8.7***a***	53.2***a***	1.7***a***	9.2***c***
	SD	0.9	2.0	0.0		0.6	1.7	0.1	0.3

[a] Abbreviations: (+)-cat, (+)-catechin; (−)-epicat, (−)-epicatechin; (−)-epiG, (−)-epicatechin 3-*O*-gallate; (−)-epig, (−)-epigallocatechin. Means ($n = 2$) followed by the same letter in a column are not significantly different (LSD, 5%).

Table 3. Structural Composition (Percent in Moles) of Polymeric Proanthocyanidins[a]

type of winemaking technology		terminal units				extension units			
		(+)-cat	(−)-epicat	(−)-epiG	(−)-epig	(+)-cat	(−)-epicat	(−)-epiG	(−)-epig
carbonic maceration	mean	4.7***a***	1.4***a***	0.2***b***	0.0	9.1***a***	69.9***a***	8.1***b***	6.5***a***
	SD	0.2	0.1	0.0		0.5	1.8	0.5	1.0
stem-contact	mean	5.3***ab***	1.9***b***	0.2***b***	0.0	10.0***a***	69.6***b***	7.0***b***	6.0***a***
	SD	0.2	0.0	0.0		0.9	0.6	0.5	0.2
non-stem-contact	mean	5.8***b***	3.0***c***	0.1***a***	0.0	7.9***a***	69.5***a***	5.7***a***	8.0***b***
	SD	0.4	0.1	0.0		1.0	0.4	0.1	0.3

[a] Abbreviations: (+)-cat, (+)-catechin; (−)-epicat, (−)-epicatechin; (−)-epiG, (−)-epicatechin 3-*O*-gallate; (−)-epig, (−)-epigallocatechin. Means ($n = 2$) followed by the same letter in a column are not significantly different (LSD, 5%).

Table 4. Structural Composition (Percent in Moles) of Total Proanthocyanidins[a]

type of winemaking technology		terminal units				extension units			
		(+)-cat	(−)-epicat	(−)-epiG	(−)-epig	(+)-cat	(−)-epicat	(−)-epiG	(−)-epig
carbonic maceration	mean	8.1***a***	3.2***a***	0.2***b***	0.0	9.6***a***	69.2***b***	4.7***b***	5.0***a***
	SD	0.1	0.1	0.0		0.1	0.1	0.3	0.1
stem-contact	mean	9.7***b***	3.4***a***	0.2***b***	0.0	9.2***a***	68.5***ab***	4.0***b***	5.1***a***
	SD	0.4	0.1	0.0		0.3	1.5	0.1	0.8
non-stem-contact	mean	10.1***b***	6.1	0.1***a***	0.0	8.4***a***	62.2***b***	2.7***a***	10.3***b***
	SD	0.1	0.0	0.0		1.8	3.4	0.3	1.6

[a] Abbreviations: (+)-cat, (+)-catechin; (−)-epicat, (−)-epicatechin; (−)-epiG, (−)-epicatechin 3-*O*-gallate; (−)-epig, (−)-epigallocatechin. Means ($n = 2$) followed by the same letter in a column are not significantly different (LSD, 5%).

Table 5. Characteristics of Proanthocyanidin Fractions from the Tinta Miúda Red Wines Made by Various Winemaking Technologies[a]

type of winemaking technology		oligomers				polymers				total (oligomers+polymers)			
		mDP	aMM	C:T	%G	mDP	aMM	C:T	%G	mDP	aMM	C:T	%G
carbonic maceration	mean	5.0***c***	1455.6***c***	0.3***a***	3.3***c***	15.6***c***	4560.9***c***	0.4***a***	8.3***c***	8.7***c***	2525.6***c***	0.4***a***	4.9***c***
	SD	0.02	7.4	0.0	0.0	0.1	14.7	0.1	0.5	0.2	59.7	0.0	0.3
with stem-contact	mean	4.5***b***	1291.7***b***	0.3***a***	2.2***b***	13.5***b***	3939.0***b***	0.4***a***	7.2***b***	7.6***b***	2201.9***b***	0.4***a***	4.2***b***
	SD	0.1	36.1	0.0	0.0	0.3	88.2	0.0	0.5	0.2	62.2	0.0	0.1
without stem-contact	mean	3.7***a***	1076.4***a***	0.4***a***	1.8***a***	11.1a	3245.8***a***	0.5***a***	5.9***a***	6.1***a***	1780.4***a***	0.7***a***	2.8***a***
	SD	0.2	46.0	0.1	0.1	0.3	92.5	0.2	0.1	0.0	10.9	0.3	0.3

[a] Abbreviations: mDP, mean degree of polymerization; aMM, average molecular mass; C:T, cis:trans ratio; %G, percentage of galloylation. Means ($n = 2$) followed by the same letter in a column are not significantly different (LSD, 5%).

oligomeric proanthocyanidins and total polymeric proanthocyanidins, followed by the stem-contact wine. The non-stem-contact wine had the lowest concentrations of all these compounds.

Structural Composition of Oligomeric and Polymeric Proanthocyanidins. The data on structural composition of oligomeric and polymeric proanthocyanidins obtained by toluene-α-thiolysis are respectively presented in Tables 2 and 3.

The structural compositions of both oligomeric and polymeric proanthocyanidins in carbonic maceration wine were generally not significantly different (LSD, 5%) from those in stem-contact wine, but were significantly different from those of non-stem-contact wine. The higher relative percentage of (−)-epicatechin gallate units in both carbonic maceration and stem-contact wines than non-stem-contact wine is probably due to the contribution of stem proanthocyanidins to the former. Furthermore, the higher percentage of (−)-epigallocatechin units in non-stem-contact wine was owing to the skins which contain much higher amounts of prodelphinidins ((−)-epigallocatechin units) than the stems.

As expected, similar results were also obtained by analysis of the structural composition of total proanthocyanidins (which were not separated into oligomers and polymers) (Table 4). Table 4 also shows the higher percentage of (−)-epicatechin gallate units in carbonic maceration and stem-contact wines, and the higher percentage of extension units of (−)-epigallocatechin in non-stem-contact wine.

From Table 2 to Table 4, the structural characteristics, i.e., mDP and average molecular mass (aMM), cis:trans ratio, and percentage of galloylation (%G) could be calculated. These results were given in Table 5.

Either for oligomeric, polymeric, or total (oligomeric plus polymeric) proanthocyanidin fractions, the highest mDP or aMM value was given by the carbonic macera-

Table 6. Major Individual and Total Anthocyanin Contents (mg/L) and Indexes of Total Phenolics in Tinta Miúda Wines Made by Various Winemaking Technologies[a]

anthocyanin[b]		type of winemaking technology		
		carbonic maceration	stem-contact	non-stem-contact
delphinidin 3-GLC	mean	3.01***a***	5.19***b***	5.73***c***
	SD	0.04	0.23	0.03
cyanidin 3-GLC	mean	1.43***a***	1.86***b***	1.89***c***
	SD	0.01	0.01	0.00
petunidin 3-GLC	mean	4.73***a***	5.93***b***	6.59***c***
	SD	0.02	0.15	0.04
peonidin 3-GLC	mean	8.10***a***	13.94***b***	17.51***c***
	SD	0.07	0.20	0.07
malvidin 3-GLC	mean	54.41***b***	51.52***a***	62.96***c***
	SD	0.47	0.68	0.07
delphinidin 6″-*O*-acglc	mean	1.65***a***	3.09***b***	3.40***c***
	SD	0.01	0.06	0.00
petunidin 6″-*O*-acglc	mean	1.56***a***	1.83***b***	1.56***a***
	SD	0.03	0.03	0.01
peonidin 6″-*O*-acglc	mean	1.56***a***	1.67***b***	1.74***c***
	SD	0.03	0.03	0.02
malvidin 6″-*O*-acglc	mean	5.18***a***	5.22***a***	6.07***b***
	SD	0.04	0.13	0.02
delphinidin 6″-*O*-*p*-cmglc	mean	1.81***a***	2.38***b***	2.69***c***
	SD	0.00	0.04	0.04
peonidin 6″-*O*-*p*-cmglc	mean	3.48***a***	3.61***a***	4.18***b***
	SD	0.07	0.04	0.02
malvidin 6″-*O*-*p*-cmglc	mean	9.10***c***	7.16***a***	8.33***b***
	SD	0.09	0.12	0.00
sum of all individual anthocyanins	mean	96.04***a***	103.38***b***	122.64***c***
	SD	0.49	0.79	0.12
total anthocyanins[c]	mean	123.75***a***	129.72***b***	148.77***c***
	SD	0.39	0.04	0.39
index of total phenolics	mean	37.86***c***	32.19***b***	26.47***a***
	SD	0.01	0.06	0.08

[a] Means ($n = 2$) followed by the same letter in a row are not significantly different (LSD, 5%). [b] Abbreviations: GLC, glucoside; acglc, acetylglucoside; cmglc, coumarylglucoside. [c] Quantified by the spectrophotometric method (*7*) using malvidin-3-glucoside as reference standard.

Table 7. Colors of the Different Types of Tinta Miúda Wines Measured by the CIELAB 76 Convention

type of winemaking technology	*L**	*C**	*h*	*a**	*b**
carbonic maceration	89.0	13.9	3.0	13.9	0.7
stem-contact	84.1	22.0	359.7	22.0	−0.1
non-stem-contact	83.3	24.3	359.8	24.2	−0.1

tion wine, followed by the stem-contact wine and non-stem-contact wine. Similar results were also observed for percentage of galloylated proanthocyanidins. In other words, the carbonic maceration wine contained the highest percentage of galloylated proanthocyanidins followed by the stem-contact wine, whereas the non-stem-contact wine contained the lowest percentage of these galloylated compounds. However, no significant difference in the cis:trans ratios was observed within the different types of wines.

Individual and Total Anthocyanins and Total Phenolics. The concentrations of major individual anthocyanins (HPLC analysis) and total anthocyanins (spectrophotometric method) and the index of total phenolics (A_{280}) in the three types of wines are presented in Table 6.

The major individual anthocyanin of all three types of wines was malvidin 3-glucoside which represented about 50% of the sum of all individual anthocyanins. The non-stem-contact wine contained the highest amounts of nearly all individual anthocyanins and total anthocyanins, followed by the stem-contact wine, whereas the carbonic maceration wine contained the lowest amounts of all these compounds except the major individual anthocyanin malvidin 3-glucoside and malvidin 6″-*O*-*p*-coumarylglucoside. On the other hand, the carbonic maceration wine contained the highest amount of total phenolics, followed by the stem-contact wine, and the non-stem-contact wine which contained the lowest of total phenolics.

Table 8. General Compositions of the Tinta Miúda Red Wines Made by Different Winemaking Technologies

chemical composition	type of winemaking technology		
	carbonic maceration	stem-contact	non-stem-contact
Ca (mg/L)	95.9	104.0	79.2
Cu (mg/L)	0.2	0.1	0.1
Fe (mg/L)	2.6	2.0	2.4
Mg (mg/L)	96.0	96.0	88.0
Na (mg/L)	25.7	20.0	12.4
K (mg/L)	901.8	1088.6	1065.8
density (ρ_{20}; g/cm^3)	0.9977	0.9997	0.9984
ethanol (% v/v)	8.1	7.7	8.4
residual sugar (g/L)	trace	trace	trace
total acidity (g/L tartaric acid)	6.9	8.5	8.6
volatile acidity (g/L acetic acid)	0.8	0.7	0.8
pH	3.1	3.0	3.0
free SO_2 (mg/L)	11	3	9
total SO_2 (mg/L)	141	106	152

As expected, for all these wines, the content of total anthocyanins obtained by the spectrophotometric method was higher than the sum of all the individual anthocyanins analyzed by HPLC. This difference should be mainly due to the contribution of polymerized pigments (*42*).

Color of the Wines. Table 7 lists the values of psychometric lightness (*L**), hue-angle (*h*), and psychomet-

ric chroma (C^*), and also the axes of a three-dimensional color space a^* and b^* of the three types of wines.

From these results, the color of carbonic maceration wine is markedly different from the other two types of wines, but there is no marked color difference between the stem-contact wine and the non-stem-contact wine.

General Composition of Wines. The general compositions of three types of wines are presented in Table 8.

DISCUSSION

Tinta Miúda is a traditional Portuguese grapevine variety. Although this variety is generally difficult to ripen, it is often used, together with other varieties, to make high-quality red wines by traditional wine-making technologies (fermentation with or without stem contact). The wines made in this manner permit an aging period until 10 years or more. One of the main reasons for this may be because of its high concentration of catechins and proanthocyanidins. Furthermore, the high concentration of catechins and proanthocyanidins in this variety makes it very interesting for us to study these phenolic compounds. In fact, some our previous works concerning wine catechins and proanthocyanidins were realized with this grapevine variety (*43*, *44*).

Table 1 and Figure 1 show, respectively, individual catechin and procyanidin contents, and total amounts of catechins, and oligomeric and polymeric proanthocyanidins in Tinta Miúda wines made by different winemaking technologies. Because grape stems are an important source of both monomeric and polymeric flavan-3-ols for wines (*4*, *44*), it is not surprising to find from Table 1 and Figure 1 that the stem-contact wine contained higher amounts of (+)-catechins, di- and trimeric procyanidins, and oligomeric and polymeric proanthocyanidins than the non-stem-contact wine. The fact that there was no significant difference in (−)-epicatechin concentration between the two types of wines should be due to lack of this compound in the stems. However, it is very interesting to note that in our winemaking conditions, the carbonic maceration wine contained higher amounts of catechins, oligomeric and polymeric proanthocyanidins: even higher than those found in stem-contact wine. The reason for this might be explained by the following reasons:

(1) Using the carbonic maceration technique, phenolic compounds released from solid parts of the grape cluster were well-protected against oxidation or other physicochemical reactions during intracellular fermentation/maceration.

(2) A long maceration time was used for the carbonic maceration wine (17 days) in this work. This fact undoubtedly favored better release of catechins and proanthocyanidins from the grape cluster into the wine because an increase of maceration time increased catechin and proanthocyanidin concentration in wine (*43*). As compared, the maceration time for the stem-contact wine was only 6 days until alcoholic fermentation was finished. These results suggested that the carbonic maceration performed in our experimental conditions might be of interest to produce proanthocyanidin-rich wines.

According to the structural composition of proanthocyanidins (Tables 2−4), the non-stem-contact wine contained a higher percentage of extension units of (−)-epigallocatechin. The reason for this may be explained by the fact that grape skins are major source of (−)-epigallocatechin (*46*). Furthermore, although grape stem proanthocyanidins are also composed of small amounts of (−)-epigallocatechin units as confirmed by Souquet et al. (*47*), grape stems could contribute considerable amounts of proanthocyanidins to wine (*4*, *44*) and thus undoubtedly reduced the relative percentages of (−)-epigallocatechin units in wines.

From Table 5, it can be seen that either for oligomeric, polymeric, or total (oligomeric plus polymeric) proanthocyanidin fractions, the highest mDP and highest relative percentage of galloylated unit values were given by the carbonic maceration wine, followed by the stem-contact wine, and the non-stem-contact wine. This would indicate that the carbonic maceration wine contained a higher percentage of more polymerized proanthocyanidins, as compared with stem-contact wine and non-stem-contact wine.

On the other hand, analysis of individual and total anthocyanins has shown that the concentrations of total anthocyanins and nearly all individual anthocyanins in the carbonic maceration wine were lower than those in the stem-contact wine and in the non-stem-contact wine. These results indicate that although carbonic maceration wine could retain higher amounts of catechins and proanthocyanidins, the carbonic maceration technique did not favor retaining or stablizing anthocyanins in wine. Moreover, color measurements (Table 7) also indicated that the color of stem-contact wine was similar to that of non-stem-contact wine, locating at the *red* region, whereas the carbonic maceration wine was much less colored and more brown than the two skin fermentation wines, although its anthocyanin levels were not so markedly different from the latter. These results were in agreement with those obtained by Timberlake and Bridle (*27*), who observed that carbonic maceration wine was less colored than traditional wine, but the concentrations of anthocyanins were not significantly different between the two wines.

It has been known that total phenolic contents in red wine were mainly contributed by proanthocyanidins and anthocyanins. However, it has been demonstrated that the total amounts of oligomeric and polymeric proanthocyanidins were much higher than those of anthocyanins (*43*). Furthermore, the evolution of polymeric proanthocyanidins is very similar to that of total phenolics during fermentation and post fermentation of red wine, which suggested that polymeric proanthocyanidins might be predominant phenolic compounds in red wines (*43*). So it is not surprising to find that, for the three types of wines studied, the total phenolic indexes are positively related to the concentrations of proanthocyanidins, but not to those of anthocyanins.

As already mentioned, the Tinta Miúda variety is generally difficult to ripen. The Tinta Miúda grapes used in this work gave total sugar of 138.0 g/L of juice, and total acidity of 12.6 g/L juice (tartaric acid equivalent), and potential alcohol 8.1°. In other words, the ripening index (total sugar/total acidity) was 10.9. From these data, it would not be surprising to note that the three types of wines had a low alcoholic content and a high acidity (Table 8). Generally, the red wines made with Tinta Miúda grapes might present disqualification in tasting at its young state, but as long as aging time increases, the taste of these wines becomes better and better. So the Tinta Miúda can be used not only for making high-quality wines, providing there is a long time of aging, by traditional winemaking technologies,

it might also be used, according to the results obtained by the present work, to produce proanthocyanidin-rich wines or healthy wines using carbonic maceration technique in our winemaking conditions. If only the traditional technologies are, as usual, preferred for this variety, fermentation with stem contact can also lead to a wine relatively rich in catechins and proanthocyanidins, although the concentration of catechins and proanthocyanidins in such wine would not be so high as that made by carbonic maceration technique. The monitoring of changes in proanthocyanidins and sensory properties of these types of wines in their future several years of aging will demonstrate the advantages and disadvantages of the different winemaking technologies studied, from the viewpoint of human health and from the viewpoint of wine quality.

ABBREVIATIONS USED

aMM, average molecular mass; %G, percentage of galloylation; (+)-cat, (+)-catechin; (−)-epicat, (−)-epicatechin; (−)-epiG, (−)-epicatechin 3-*O*-gallate; (−)-epig, (−)-epigallocatechin; C:T, cis:trans ratio; GLC, glucoside; acglc, acetylglucoside; cmglc, coumarylglucoside.

LITERATURE CITED

(1) Arnold, R. M.; Noble, A. C. Bitterness and astringency of grape seed phenolics in a model wine solution. *Am. J. Enol. Vitic.* **1978**, *29*, 150−152.

(2) Arnold, R. M.; Noble A. C.; Singleton V. L. Bitterness and astringency of phenolic fractions in wine. *J. Agric. Food Chem.* **1980**, *28*, 675−678.

(3) Powers, J. R.; Nagel, C. W.; Weller, K. Protein removal from a wine by immobilized grape proanthocyanidins. *Am. J. Enol. Vitic.* **1988**, *39*, 117−120.

(4) Singleton, V. L. Tannins and the qualities of wines. In *Plant Polyphenols: Synthesis, Properties, Significance*; Hemingway, R. W., Laks, P. S., Eds.; Plenum Press: New York, 1992; pp 859−880.

(5) Oszmianski, J.; Sapis, J. C.; Macheix, J. J. Changes in grape seed phenols as affected by enzymic and chemical oxidation in vitro. *J. Food Sci.* **1985**, *50*, 1505−1506.

(6) Cheynier, V.; Osse, C.; Rigaud, J. Oxidation of grape juice phenolic compounds in model solutions. *J. Food Sci.* **1988**, *53*, 1729−1732.

(7) Cheynier, V.; Basire, N.; Rigaud, J. Mechanism of transcaffeoyl tartaric acid and catechin oxidation in model solutions containing grape polyphenoloxidase. *J. Agric. Food Chem.* **1989**, *37*, 1069−1071.

(8) Cheynier, V.; Prieur, C.; Guyot, S.; Rigaud, J.; Moutounet, M. The structures of tannins in grapes and wines and their interactions with proteins. In *Proceedings of ACS Symposium Series 661, Wine: Nutritional and Therapeutic Benefits*; Watkins, T. R., Ed.; American Chemical Society: Washington, D.C., 1997; pp 81−93.

(9) Lee, C. Y.; Jaworski, A. Phenolics and browning potential of white grapes grown in New York. *Am. J. Enol. Vitic.* **1988**, *39*, 337−340.

(10) Jouve, C.; Cabanis, J. C.; Bourzeix, M.; Heredia, N.; Rosec, J. P.; Vialatte, C. Teneurs en catéchines et procyanidols de vin blancs et rosé; effects du collage par la caséine. *Rev. Fr. Oenol.* (*Cahier Scientifique*) **1989**, *117*, 14−20.

(11) Oh, H. I.; Hoff, J. E. Effect of condensed grape tannins on the in vitro activity of digestive proteases and activation of their zymogens. *J. Food Sci.* **1986**, *51*, 577−580.

(12) Sarni-Manchado, P.; Deleris, A.; Avallone, S.; Cheynier, V.; Moutounet, M. Analysis and characterization of wine condensed tannins precipitated by proteins used as fining agent in Enology. *Am. J. Enol. Vitic.* **1999**, *50*, 81−86.

(13) Yokotsuka, K.; Singleton, V. L. Interactive precipitation between grape peptides from gelatin and specific grape tannin fractions in wine-like model solutions. *Am. J. Enol. Vitic.* **1987**, *38*, 199−206.

(14) Timberlake, C. F.; Bridle, P. Interaction between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97−105.

(15) Ribéreau-Gayon, P. The anthocyanins of grapes and wines. In *Anthocyanins as Food Colors*; Markakis, P., Ed.; Academic Press: New York, 1982; pp 209−244.

(16) Ribéreau-Gayon, P.; Pontallier, P.; Glories, Y. Some interpretation of color changes in young red wines during their conservation. *J. Sci. Food Agric.* **1983**, *34*, 505−516.

(17) Masquellier, J. l'alimentation et la consommation de vin. *Proceedings, C. R. Symposoum Int.*; Verona, Italy, 1982; pp 147−155.

(18) Masquellier, J. Physiological effects of wine. His share in alcoholism. *Bull. O. I. V.* **1988**, *61*, 554−578.

(19) Ricardo-da-Silva, J. M.; Darmon, N.; Fernández, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991**, *39*, 1549−1552.

(20) Teissedre, P. L.; Waterhouse, A. L.; Walzem, R. L.; German, J. B.; Frankel, E. N.; Ebeler, S. E.; Clifford, A. J. Composés phenoliques du raisin et du vin et santé. *Bull. O. I. V.* **1996**, *69*, 252−277.

(21) Terencio, M. C.; Sanz, M. J.; Paya, M. Antihypertensive action of a procyanidin glycoside from *Rhamnus lycioides*. *J. Ethnopharmacol.* **1991**, *31*, 109−114.

(22) Bourzeix, M.; Weyland, D.; Heredia, N. Étude des catéchines et des procyanidols de la grappe de raisin, du vin et d'autres dérivés de la vigne. *Bull. O. I. V.* **1986**, *59*, 1171−1254.

(23) Kovac, V.; Bourzeix, M.; Herdia, N.; Ramos, T. Étude des catechines et proanthocyanidols de raisin et vins blancs. *Rev. Fr. Oenol. (Cahier Scientifique)* **1990**, *30*, 7−14.

(24) Ricardo-da-Silva, J. M.; Rosec, J. P.; Bourzeix, M.; Mourgues, J.; Moutounet, M. Dimer and trimer procyanidins in Carignan and Mourvèdre grape and red wines. *Vitis* **1992**, *31*, 55−63.

(25) Sun, B. S.; Ricardo-da-Silva, J. M.; Spranger, M. I. Proanthocyanidin content of several grapevine varieties from Portugal. In *Proceedings of the 78th general Assembly of the O. I. V.: XXIII World Congress on the Vine and Wine*, Lisbon, 1998; Vol. II, pp 651−656.

(26) Kantz, K.; Singleton, V. L. Isolation and determination of polymeric polyphenols in wines using Sephadex LH-20. *Am. J. Enol. Vitic.* **1991**, *42*, 309−315.

(27) Timberlake, C. F.; Bridle, P. The effect of processing and other factors on the color characteristics of some red wines, *Vitis* **1976**, *15*, 37−42.

(28) Auw, J. M.; Blanco, V.; O'keefe, S. F.; Sims, C. A. Effect of processing on the phenolics and color of Cabernet Sauvignon, Chambourcin, and Noble wines and juices. *Am. J. Enol. Vitic.* **1996**, *47*, 279−286.

(29) Kovac, V.; Alonso, E.; Revilla, E. The effect of adding supplementary quantities of seeds during fermentation on the phenolic composition of wines. *Am. J. Enol. Vitic.* **1995**, *46*, 363−367.

(30) Kovac, V.; Nemanic, J.; Revilla, E. Influencia de la relación fase sólida/fase líquida durante la vinificación en Tinto sobre las características analíticas y sensoriales de los vinos. In *Proceedings of the 78th general Assembly of the O. I. V.: XXIII World Congress on the Vine and Wine*; Lisbon, 1998; Vol. 2, pp 197−202.

(31) Ricardo-da-Silva, J. M.; Cheynier, V.; Samsom, A.; Bourzeix, M. Effect of pomace contact, carbonic maceration, and hyperoxidation on the procyanidin composition of Grenache blanc Wines. *Am. J. Enol. Vitic.* **1993**, *44*, 168−172.

(32) Sun, B. S.; Belchior, G. P.; Ricardo-da-Silva, J. M.; Spranger, M. I. Isolation and purification of dimeric and trimeric procyanidins from grape seeds, *J. Chromatogr. A* **1999**, *841*, 115−121.

(33) Sun, B. S.; Leandro, M. C.; Ricardo-da-Silva, J. M.; Spranger, M. I. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1998**, *46*, 1390−1396.

(34) Sun, B. S.; Ricardo-da-Silva, J. M.; Spranger, M. I. Critical factors of vanillin assay for catechins and proanthocyanidins, *J. Agric. Food Chem.* **1998**, *46*, 4267−4274.

(35) Spranger, M. I.; Belchior, A. P.; Leandro, M. C.; Carvalho, E. C. Influence du systeme de cuvaison sur la diffusion des anthocyanines. In*Proceeding of the 16th international conference of Group Polyphenols*; 1992, Tome II Vol. 16; pp 222−225.

(36) Ribéreau-Gayon, P.; Stonestreet, E. Le dosage des anthocyanes dans le vin rouge. *Bull. Soc. Chim.* **1965**, *9*, 2649−2652.

(37) Ribéreau-Gayon, P. Le dosage des composés phénoliques totaux dans les vins rouges. *Chim. Anal.* **1970**, *52*, 627−631.

(38) McLaren, K. Food colorimetry. In *Developments in Food Colours*; J. Walford, Ed.; Applied Science: 1980; Vol. 1, pp 27−45.

(39) IVP. Triest 1.0 program. *Instituto do Vinho do Porto (The Port Wine Institute)*, Porto, Portugal, 1992.

(40) Prieur, C.; Rigaud, J.; Cheynier, V. Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *3*, 781−784.

(41) O. I. V. *Recueil des méthodes internationales d'analyse des vins*; Office International de la Vigne et du Vin: Paris, 1990.

(42) Rivas-Gonzalo, J. C.; Gutierrez, Y.; Hebrero, E.; Santos-Buelga, C. Comparisons of methods for the determination of anthocyanins in red wines. *Am. J. Enol. Vitic.* **1992**, *43*, 210−214.

(43) Spranger, M. I.; Sun, B. S.; Leandro, M. C.; Carvalho, E. C.; Belchior, A. P. Changes in anthocyanins, catechins and proanthocyanidins during fermentation and early post-fermentation of red grapes. In *Proceedings of XXIII World Congress on Vine and Wine*, Lisbon, 1998; Vol. II, pp 183−189.

(44) Sun, B. S.; Pinto, T.; Leandro, M. C.; Ricardo-da-Silva, J. M.; Spranger, M. I. Transfer of catechins and proanthocyanidins from grape solids into wine. *Am. J. Enol. Vitic.* **1999**, *50* (2), 179−184.

(45) Moutounet, M.; Rigaud, J.; Souquet, J. M.; Cheynier, V. Caracterisation structurale des tannins de la baie de raisin. Quelques exemples de l'incidence du cepage, du terroir et du mode de conduite de la vigne. In *Proceedings of the 75th general Assembly of the O. I. V.*: *XXI World Congress on the Vine and Wine*, Punta del Este, Uruguay, 1995; pp 13−24.

(46) Souquet, J.; Cheynier, V.; Moutounet, M. Phenolic composition of grape stems. In *Proceeding of the 19th International Conference of Group Polyphenols*, Lille, France, 1998; Vol. 2, pp 359−360.

(47) Souquet, J.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43* (2), 509−512.

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