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Antioxidant activity of musts from Pedro Ximénez grapes subjected to off-vine drying process

Juan Moreno [a], Jose Peinado [b], Rafael A. Peinado [a,*]

[a] Departamento de Química Agrícola y Edafología, Edificio Marie Curie, Universidad de Córdoba, Campus Universitario Rabanales, 14014 Córdoba, Spain
[b] Departamento de Bioquímica y Biología Molecular, Edificio Severo Ochoa, Universidad de Córdoba, Campus Universitario Rabanales, 14014 Córdoba, Spain

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Abstract

Free radical-scavenging activity of Pedro Ximénez must obtained in different stages of the off-vine grape drying process have been evaluated by using 2,2′-azinobis(3-ethylbenzothiazolinesulfonic acid) radical cation ($ABTS^{+\cdot}$). Significant increases in the total antioxidant activity (TAA) of must obtained during the drying process were obtained, highlighting TAA at 12 days (11.2 mM trolox). High correlation coefficients between TAA-sugar concentrations ($r = 0.970$), TAA-absorbance at 420 nm ($r = 0.915$) and TAA-absorbance at 520 nm ($r = 0.932$) were also obtained. Similar results were obtained with synthetic samples, subjected to heat treatment, containing an amino acid extract and increasing concentrations of sugars. Increases obtained in the absorbances and in the TAA, in the synthetic samples, can be explained by the formation of Maillard reaction products. Therefore, it is possible that the Maillard reaction contributes in a significant way to the formation of brown pigments, as well as to the increase in the TAA of the musts from sun-dried grapes. This could enhance the beneficial effects, for human health, of a moderate intake of sweet wines obtained from off-vine sun-dried grapes.

Keywords: Antioxidant activity; Off-vine drying process; Maillard reaction products; White grape must; Wine

1. Introduction

The off-vine grape-drying process, by direct exposure to sun, is a post-harvest treatment which is only feasible in some warm grape-growing regions, such as those of the southern part of Spain. Particularly, in the Montilla-Moriles region, grapes are harvested within the first 15 days of August. The drying process begins with placing mature and healthy grapes over 0.75 m × 10 m length plastic meshes, which are extended in smoothly sloped soil parcels, called "paseras", which receive the maximum hours of sunshine. Average values of 28 ± 2 °C and 32 ± 9% for temperature and ambient humidity, respectively, are obtained in the grape-growing area. Under these conditions, bunches reach temperatures above 50 °C during all the drying process. Montedoro and Bertuccioli (1986) described the drying process and Franco, Peinado, Medina, and Moreno (2004) characterized the must from sun-dried grapes.

The Maillard reaction is a non-enzymatic browning reaction, which involves a reducing sugar and an amino acid. This reaction has usually been studied in model systems because of the complexity of the intermediate and final reaction products. Taking into account that the Maillard reaction proceeds at 50 °C and it is favoured at pH 4–7, values within the range for food (Morales & Jiménez-Pérez, 2001), and that the caramelisation requires high temperatures and pH values between 3 and 9 (Kroh, 1994), it is feasible that the Maillard reaction takes places during the off-vine drying process, contributing to the browning and antioxidant activity of must obtained from dried grapes. In a recent paper, Frank, Gould, and Millikan (2005)

* Corresponding author. Tel.: +34 957218534; fax: +34 957212146.
E-mail address: qe2peamr@uco.es (R.A. Peinado).

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establish that browning in sultana grapes occurs as result of the Maillard reaction between sugars and amino acids, excluding the involvement of oxidation of phenolic compounds such as *trans*-caftaric acid.

Frankel and Meyer (2000) reviewed the factors affecting measurement of antioxidant activity when the food matrix is critically important. Notably, most of the molecules present in grapes and wines exert their biological activity in the human body in a hydrophilic matrix, and it is desirable to study their antioxidant activity in an aqueous medium. However, enzymatic activities in intestine and liver can modify the absorbed molecules and the active metabolites can differ from that originally present in the diet (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005).

The aim of this paper is to study the total antioxidant activity as trolox equivalents in must obtained from grapes at different stages of the off-vine grape drying process, used for the elaboration of special sweet Pedro Ximénez wines.

2. Material and methods

2.1. Grape samples and must preparation

Healthy white grapes of the Pedro Ximénez variety were taken during the sun-drying process at 0, 4, 8 and 12 days. Grape samples were crushed in a laboratory press and the resulting must was centrifuged at 5000*g* for 10 min. Sugars were measured as °Brix with a hand refractometer. Absorbances at 280, 420, and 520 nm were measured, after dilution by 1/20 in distilled water, in a Beckman DU-640 spectrophotometer.

2.2. Preparation of synthetic samples

Different solutions (250, 350, 500 and 600 g/l) containing equimolecular concentrations of glucose and fructose, and 1.5 g/l of amino acid extract were used to simulate the off-vine drying process and to study the contribution of Maillard reaction products. The amino acids extract contain the more frequently quantified amino acids in grape-musts (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2000) and was prepared by mixing glutamic acid (13.5%), valine (6.0%), leucine (6.0%), methionine (14.5%), alanine (24.0%), proline (24.0%) and threonine (12.0%). All the amino acids were supplied by Sigma–Aldrich. Two additional solutions were also prepared, the first containing only 350 g/l of glucose and fructose (control 1) and the second containing 350 g/l of glucose and fructose and 3 g/l of amino acids extract (control 2). Samples were adjusted to pH 4 and autoclaved at 100 °C for 1 h.

2.3. Determination of total antioxidant activity

The antioxidant activity was estimated by following the decolourization of $ABTS^{+\cdot}$ chromophore, which was previously generated by oxidation of 7 mM ABTS with 2.45 mM potassium persulfate (Re, Pellegrini, Pannala, Yang, & Rice-Evans, 1999). The $ABTS^{+\cdot}$ solution was diluted in 20 mM potassium phosphate buffer, pH 7.4, to an absorbance of 0.7 ± 0.05 at 734 nm (Beckman DU-640 spectrophotometer). Trolox was used as an antioxidant standard. Trolox (5 mM, as stock solution) was prepared in the above buffer and then diluted to 0.5 mM. 1 ml of $ABTS^{+\cdot}$ solution was added to 25 μl of diluted must samples (1/20 with distilled water) or standard trolox solution (0–800 μM), and the absorbance of the mixture was determined after exactly 4 min of incubation at 37 °C. The extent of inhibition of the absorbance at 734 nm of the $ABTS^{+\cdot}$ against the concentration of trolox in the standard samples was used to calculate the total antioxidant activity (TAA) of the must grapes.

2.4. Statistical analysis

Homogeneous group analysis was carried out to determine whether the differences observed during the drying process in the analyzed variables were significant at $p \leqslant 0.05$. Controls and synthetic sample 2 (see Table 2) were compared by analysis of variance. Simple regression analysis was used to establish relationships between the analyzed variables, both in must from dried grapes and in the synthetic samples. All statistical analyses were conducted with the statistical software package Statgraphics Plus v. 2, from STSC, Inc. (Rockville, MD).

3. Results and discussion

Visual observation during the off-vine drying process showed a progressive shrinking and browning of the grapes. A significant increase of the sugar concentration of the corresponding musts was also observed as a consequence of the grape dehydration (Table 1). Absorbance at 280 nm is usually related to the content of total polyphenols in must and wines whereas absorbances at 420 and 520 nm are related to wine browning particularly in white wines (Peinado et al., 2004). On the other hand, Turkmen, Sari, Poryzoglu, and Velioglu (2006) related the absorbance at 420 nm to the brown pigment formation due to the Maillard reaction. Absorbances at 280, 420 and 520 nm increased significantly during the drying process (Table 1). High correlation coefficients were obtained after plotting the data for absorbances at 280, 420 or 520 nm vs the sugar concentrations during the drying process, which suggests a high correlation between the variables (Table 2). Table 1 shows the antioxidant activity of the must obtained at different stages of the drying process; high correlation was also observed between the antioxidant activity and the sugar concentration (Table 2).

It is known that polyphenols present in fruits and vegetables, and in beverages, such as tea and wine, are potent antioxidants. Several authors have correlated the antioxidant activities of wines with their total contents of phenolic compounds (Landraut et al., 2001; Minussia et al., 2003; Rice-Evans, Miller, & Paganga, 1997). Gallic acid, cate-

Table 1
Sugar concentration, absorbance and antioxidant activity in must from Pedro Ximénez grape-must during the off-vine drying process

Days	Sugars (g/l)	Absorbance			TAA[a]
		280 nm	420 nm	520 nm	
0	251 ± 2	12.3 ± 0.1	1.26 ± 0.01	0.58 ± 0.01	4.3 ± 0.1
4	218 ± 2	23.4 ± 0.2	3.06 ± 0.03	1.54 ± 0.02	5.4 ± 0.6
8	333 ± 3	24.2 ± 0.2	3.19 ± 0.03	1.74 ± 0.02	7.1 ± 0.3
12	611 ± 5	37.4 ± 0.3	6.03 ± 0.05	3.42 ± 0.03	11.2 ± 0.5
Homogeneous group[b]	ABCD	ABCD	ABCD	ABCD	ABCD

[a] TAA (total antioxidant activity) as mM trolox equivalents measured using the $ABTS^{+\cdot}$ scavenging assay.
[b] From the analysis of variance. Different letters indicate significant differences at 95% confidence level.

Table 2
Regression equations and correlation coefficients obtained in musts from Pedro Ximénez sun-dried grapes during the off-vine drying process

	Regression equations	Correlation coefficient
Absorbance 280 vs sugars	$y = 6.925 + 0.049x$	0.881
Absorbance 420 vs sugars	$y = -0.109 + 0.010x$	0.915
Absorbance 520 vs sugars	$y = -0.318 + 0.006x$	0.932
Antioxidant activity vs sugars	$y = 1.243 + 0.016x$	0.970

chin, epicatechin, caffeic acid and resveratrol represent 18% of the polyphenols of wine and account for 25% of its antioxidant activity in trolox equivalents (Frankel, Waterhouse, & Teissedre, 1995; Miller & Rice-Evans, 1995; Rice-Evans et al., 1997).

Other phenolic compounds, such as quercetin–galactoside, isorhamnetin and peonidin–glucoside, and different extracts obtained by ultrafiltration techniques, definitely contribute to the total antioxidant capacity of wines. Monomeric phenolic compounds and procyanidin B1 explain 11–24%, while large molecular weight unknown compounds (>50 kDa) explain 46%, and oligomeric, polymeric phenols compounds and unknown compounds (<50 kDa) explain 34% of the total antioxidant capacity in Pinotage wines (De Beer, Joubert, Marais, & Manley, 2006).

According to Leighton, Urquiaga, and Diez (1997) anthocyanins contribute 55%, tannins 25%, flavonols 15% and phenolic acids 5% of the antioxidant activity of red wines. The colorant intensity (calculated as the sum of absorbances at 420 and 520 nm), is also correlated with the antioxidant capacity ($r = 0.99$), which supposes that monomeric and polymeric anthocyanins play an important role in the total antioxidant activities of red wines.

Fernández-Pachón, Villaño, García-Parrilla, and Troncoso (2004) related 50% of total red wine radical-scavenging activity to polymeric phenolic compounds. For the remaining 50%, anthocyanins and flavan-3-ol were the most active followed by phenolic acids and flavonols.

Evidence for the role of grapefruit juice and wine in the prevention of degenerative diseases is recognized (Williamson & Manach, 2005). The biological activities of both are mainly due to the polyphenols; as micronutrients, their bioavailability differs greatly from one polyphenol to another, so that the most abundant polyphenols in our diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach et al., 2005). In grape, gallic acid is the best absorbed polyphenol, followed by catechins, flavanones, and quecetin glucosides, but with different kinetics. In general, glycoside forms are absorbed to a lesser extent than are aglycones (Meng, Maliakal, Lu, Lee, & Yang, 2004). Once absorbed, the metabolites present in blood, resulting from digestive and hepatic activity, usually differ from the native compounds, and modification reactions of polyphenols include oxidation, sulfation and glucuronation (Spencer, Abd-el-Mohsen, & Rice-Evans, 2004).

Anthocyanins are absent in white grapes, which can explain the low antioxidant activity in white wines, although this does not explain the remarkable antioxidant activity of musts obtained from dried grapes. In fact, the Pedro Ximénez must sample at 0 day showed similar antioxidant values to those observed in white wines, whereas the sample at 12 days showed a comparable antioxidant value to those obtained in some red wines (Landraut et al., 2001).

Dehydration of grapes probable favours the Maillard reaction, and increases the concentration of phenolic compounds, explaining the increased antioxidant activity of the musts (Table 1). Brown pigment formation occurs during the dehydration of grapes, which could be also explained by the increase in concentration and polymerization of phenolic compounds and/or by the formation of Maillard reaction products. Table 2 shows the high correlation between the antioxidant activity and absorbances at 280 (related to the phenolic compound concentration) and 420 and 520 nm (related to the brown pigment formation). The influence of different post-harvest treatments on the formation of Maillard reaction products, related to brown pigment formation, has been recently published (Frank, Gould, & Millikan, 2004a, 2004b; Gonzalez-Barrio, Salmenkallio-Marttila, Tomas-Barberan, Cantos, & Espin, 2005), and the antioxidant activity of melanoidins in sweet wines has also been studied (Morales, Fernández-Fraguas, & Jiménez-Pérez, 2005; Rivero-Pérez, Pérez-Magariño, & González-San José, 2002).

Several authors have studied the antioxidant properties of Maillard reaction products using different model systems (Dittrich et al., 2003; Monti et al., 1999; Morales

Table 3
Compositions of synthetic samples, absorbances and antioxidant activities after heating at 100 °C

	Sugar (g/l)	Amino acids (g/l)	Absorbance			TAA[a]
			280 nm	420 nm	520 nm	
Sample 1	250	1.5	69 ± 4	4.1 ± 0.2	0.7 ± 0.1	5.1 ± 0.1
Sample 2	350	1.5	115 ± 7	6.3 ± 0.4	1.3 ± 0.1	8.6 ± 0.1
Sample 3	500	1.5	161 ± 10	8.2 ± 0.5	1.4 ± 0.1	13 ± 1
Sample 4	600	1.5	232 ± 14	11.1 ± 0.7	1.8 ± 0.1	14.0 ± 0.3
Control 1	350	0	127 ± 8	1.3 ± 0.1	0.0 ± 0.0	2.9 ± 0.3
Control 2	350	3.0	105 ± 6	6.9 ± 0.4	1.3 ± 0.1	8.3 ± 0.1
ANOVA[b]	Control 1 vs sample 2		ns	***	***	***
	Control 2 vs sample 2		ns	ns	ns	ns

Analysis of variance (ANOVA) to establish differences between the controls and sample.
[a] TAA (total antioxidant activity) as mM trolox equivalents measured using the $ABTS^{+\cdot}$-scavenging assay.
[b] From the analysis of variance; ns: no significant differences. *** = $p \leqslant 0.001$.

& Babbel, 2002; Wagner, Derkits, Herr, Schuh, & Elmadfa, 2002; Wijewickreme, Kitts, & Durance, 1997; Yilmaz & Toledo, 2005; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997). Synthetic samples, having sugar concentration similar to those obtained during the off-vine drying process were prepared to study the effect of the Maillard reaction on the formation of brown coloured compounds and on the antioxidant activity (Table 3). All the samples were supplied with an extract containing the more frequent amino acids quantified in grape musts (Ribéreau-Gayon et al., 2000).

Maillard reaction produces coloured or colourless products which depend on the reaction status and factors, such as reactants (Wijewickreme et al., 1997; Wijewickreme, Krejpcio, & Kitts, 1999), temperature, pH (Monti, Bailey, & Ames, 1998), water activity, intermediate products (Vasiliauskaite & Wedzicha, 1997) and availability of oxygen. All these factors can strongly affect the formation and properties of the obtained products.

Sugars by themselves show similar reactions (caramelisation) to those produced between sugars and amino acids (Nursten, 1981). Control 1 corresponds to a synthetic sample without amino acids and has a relatively high antioxidant activity (Table 3), spanning the values of sherry and white wines (Fernández-Pachón et al., 2004) and some brown coloured compounds are formed, as can be seen from the absorbances at 420 nm. No differences in antioxidant activity were detected between solutions with different sugar concentrations subjected to different heat treatments (data not show); the values obtained for these samples were quite similar to those for control 2 (3.0 ± 0.4). No significant differences in the absorbance at 280 nm between control 1 and sample 2 were observed. Both samples have similar sugar concentrations, so the values obtained for this absorbance are due to compounds other than those formed by the Maillard reaction.

However, the presence of amino acids (sample 2 vs control 1) promoted the formation of the brown pigment due to the Maillard reaction; in this sense, significant differences in absorbances at 420 and 520 nm (Table 3) were obtained. The formation of brown pigment is also related to sugar concentration (Table 4). In the same way, a significant difference ($p \leqslant 0.001$) in the antioxidant activity was obtained when control 1 and sample 2 were compared (Table 3). Such difference is due to Maillard reaction products formed. The antioxidant activity is also highly correlated with the sugar concentration (Table 4). Similar results were described in Sultana grapes by Frank et al. (2005).

Table 4
Regression equations and correlation coefficients obtained in synthetic samples

	Regression equations	Correlation coefficient
Absorbance 420 vs sugars	$y = -0.567 + 0.019x$	0.977
Absorbance 520 vs sugars	$y = 0.128 + 0.003x$	0.944
Antioxidant activity vs sugars	$y = -0.780 + 0.226x$	0.982

Control 2 has twice the amino acid concentration of sample 2, although this fact does not contribute to a higher antioxidant capacity (control 2 vs sample 2). Neither were significant differences observed in the absorbances at 280, 420 and 520 nm between control 2 and sample 2 (Table 3).

According to the results, it could be assumed that the changes observed in the antioxidant activity during the drying process in grapes, and in the synthetic samples were similar. So, the brown pigment formation and the increase in the antioxidant activity of must from sun-dried grapes and in the synthetic samples can be explained through the Maillard reaction. This could enhance the beneficial effects for human health of a moderate intake of sweet wines obtained from off-vine sun-dried grapes.

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