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Changes in the catechin and epicatechin content of grape seeds on storage under different water activity (a_w) conditions

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Abstract

Storage effect on antioxidant content and capacity of grape seeds under different a_w conditions (a_w 0.33; 0.53; 0.75/50 days, 25 °C) was examined. Total phenol content (determined by the Folin–Ciocalteu method) decreased during storage though changes were trivial for samples stored at 33% or 53% RH. High level of humidity (75%) accelerated degradation and resulted in a ~50% reduction of total phenol content. Minor loss of the DPPH radical scavenging activity (%RSA) of the extracts was observed. Catechin and epicatechin content monitored by RP-HPLC was reduced during storage, particularly at 75% RH. Epicatechin content proved to be less sensitive to water activity conditions than catechin content. Results of various *in vitro* assays (Folin–Ciocalteu, FRAP, DPPH, ABTS, CBA, ORAC and copper induced liposome oxidation) did not support difference in terms of resistance to oxidation. Based on the continuous release of gallic acid, our finding was related to hydrolytic reactions. Control of a_w of grape seeds can be of practical importance for the wine industry.

Keywords: Grape seeds; Catechin; Epicatechin; Degradation; Water activity; Antioxidant activity

1. Introduction

Among the different parts of the grape, seeds accumulate the majority (60–70%) of phenolic compounds (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2000), and have for that reason been thoroughly studied for more than 10 years (Shi, Yu, Pohorly, & Kakuda, 2003).

Fractionation, characterization and identification of low and high molecular weight grape seed phenolics (Escribano-Bailón, Gutiérrez-Fernández, Rivas-Gonzalo, & Santos Buelga, 1992; Labarbe, Cheynier, Brossaud, Souquet, & Moutounet, 1999; Oszmianski & Sapis, 1989), revealed that monomeric flavan-3-ols (catechin, epicatechin and epigallocatechin) and their acylated forms (esters with gallic acid) (epicatechin gallate and epigallocatechin gallate), phenolic acids (gallic acid in particular) and dimeric B1, B2 procyanidins are the characteristic phenolic constituents of grape seeds (De Freitas and Glories, 1999; Fuleki and Da Silva, 1997; Guendez, Kallithraka, Makris, & Kefalas, 2005a,2005b; Palma and Taylor, 1999; Shi et al., 2003; Yilmaz and Toledo, 2004b). The presence of dimeric B3, B4, B5, B6, B7, B8, trimeric C1, tetrameric and polymeric procyanidins has also been reported (Escribano-Bailón et al., 1992; Oszmianski & Sapis, 1989; Santos-Buelga, Francia-Aricha, & Escribano-Bailón, 1995).

Based on quantitative information catechin–epicatechin monomers seem to be the two principal compounds, at about equal amounts, depending on the grape variety. Their levels vary from a few to some hundreds of mg/100 g of dry seeds. Epicatechin content is slightly higher in red varieties.

During vinification, only a portion of catechins and procyanidins is extracted from seeds and is diffused to the

Abbreviations: FRAP, ferric reducing antioxidant power; DPPH, 1,1-Diphenyl-2-picrylhydrazyl; ABTS, 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt; CBA, crocin bleaching assay; ORAC, oxygen radical absorbance capacity; TPTZ, tripyridyltriazine.

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wine (González-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004). The high remaining content renders them a rich source of antioxidants (~4 g/kg of grape seeds). Due to accepted health benefits of the above compounds (Ahmad & Mukhtar, 1999; Wang, Provan, & Helliwell, 2000; Yilmaz & Toledo, 2004a) the interest in adding value to grape seeds is increasing (Guendez et al., 2005a; Kim et al., 2006; Yilmaz & Toledo, 2006).

Grape seeds can be recuperated either during first transfer of new wine at the end of maceration or from the grape marc after pressing. Once collected, grape seeds are directly treated or stored and gradually manipulated. In the second case, storage conditions (temperature, relative humidity) are expected to exercise an important impact on endogenous constituents such as antioxidants since drying is not yet an inherent part of the overall process.

The aim of the present study was to examine the effect of storage at room temperature and different water activity conditions (a_w 0.33; 0.53; 0.75, 25 °C), on the phenolic content and antioxidant potential of grape seeds (whole and ground), by-products of red wine vinification. Monitoring of changes on storage was achieved through colorimetric total phenol content determination and estimation of DPPH radical scavenging activity of the methanol extracts. Emphasis was paid on changes in the content of the two major compounds, catechin and epicatechin, as determined by RP-HPLC. HPLC findings were further highlighted by studying the antioxidant activity of the two monomers under the conditions of various *in vitro* assays (Folin–Ciocalteu, FRAP, DPPH, ABTS, CBA, ORAC and copper induced liposome oxidation). Formation of gallic acid, as an indication of hydrolytic processes was also followed chromatographically.

2. Materials and methods

2.1. Samples and standards

Grape seeds were from the red hellenic *Vitis vinifera* variety Xinomavro, cultivated in the region of Naoussa (northern Greece). Samples were obtained from the Agricultural Viticultural Winemaking Cooperative of Naoussa and collected after alcoholic fermentation (1 week, 28–30 °C) during transfer of fresh wine from the fermentor through a mechanical drain. The seeds were transported to the laboratory, manually separated from remaining skins and stems and stored frozen (−20 °C) until used. For the study, 5 g portions of seeds were placed in weighing dishes and stored over saturated solutions in desiccators, at 25 °C. Saffron red stigmas were donated by Saffron Cooperative of Kozani (Greece). Gallic acid (99.5%) and (+) catechin (98%) were from Sigma Chemical Co., (−) epicatechin (90%) was purchased from Fluka, Bio Chemika (Switzerland).

2.2. Solvents and reagents

The salts ($MgCl_2 \cdot 6H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, NaCl, all reagent grade) and anhydrous Na_2CO_3 were from Riedel-de Haën (Seezle, Germany). Methanol (97% and UV–IR-HPLC), acetic acid (99.7%, ACS-ISO) and acetonitrile (HPLC) were from Panreac Quimica S.A. 2,2-Azobis (2-amino-propane) dihydrochloride (AAPH) was purchased from Fluka Chemie (Buchs, Switzerland). *L*-a-phosphatidylcholine (lecithin, ~40%) from soybean, TPTZ, ABTS, DPPH, were from Sigma Chemical Co. (St. Louis, MO). Folin–Ciocalteu, fluorescein sodium salt and $FeCl_3 \cdot 6H_2O$ were from Panreac Quimica S.A. (Barcelona, Spain). Water used in HPLC was obtained by a MilliQ water system with a minimum resistance of 17.2 MΩ/cm.

2.3. Apparatus

A Shimadzu UV-1601 spectrophotometer (Kyoto, Japan), accompanied with UVPC-1601 software, was used for all UV–Vis absorbance measurements. For the CBA measurements, the system was thermostated at 39.5 (±0.5 °C) with the aid of an outer water-circulating bath. A Shimadzu RF 1501 spectrofluorometer equipped with a stirrer and a temperature-controlled cell holder at 37 °C was used for fluorescence measurements. Calculations were carried out by means of the RF 1501-PC software. Adjustment of pH was achieved using a Consort 5231 model portable pH-meter (Turnhout, Belgium).

2.4. Sample storage over saturated salt solutions

Portions of approximately 5 g of grape seeds were spread in weighing dishes and kept over saturated salt solutions in desiccators. Three saturated salt solutions were used to achieve the different relative humidity values: $MgCl_2 \cdot 6H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$ and NaCl, which give a_w values (25 °C) of 0.33, 0.53 and 0.75, respectively (Labuza, Acott, Tatini, & Lee, 1976). The closed desiccators were stored in a temperature-controlled chamber (GRW-500D, CDR) set at the experimental temperature (25 °C), for 50 days, in the dark. The saturated salt solutions were kept for 2 days before use at the above temperature.

2.5. Moisture determination

The moisture content was determined gravimetrically by weight difference after heating the grape seeds at 130 °C for 1 h.

2.6. Extraction of phenolic compounds

Whole or ground grape seeds (1 g) were extracted with 10 mL of methanol in a shaker incubator (140 rpm) for 24 h at room temperature. The operation was done in duplicate. The methanolic extracts were filtered through a Whatman paper filter, the filtrates joined in a pre-weighed 50 mL flask and the solvent evaporated to dryness in a Büchi rotavapor (35 °C). The flask was weighed and the yield in dry extract calculated. The solid residue was

re-dissolved in 10 mL methanol HPLC, further diluted if needed, and directly analysed.

2.7. Total phenol content determination

Total phenol content was measured by the method of Singleton and Rossi (1965) used in wine, slightly changed: in a 10 mL volumetric flask, 6 mL of water, 0.1 mL of diluted methanol grape seed extract and 0.5 mL Folin–Ciocalteu reagent were mixed. After exactly 3 min, 1.5 mL of saturated $Na_2(CO_3)$ solution (27%) was added and the mixture was agitated. The volume was adjusted with water and the flask left in the dark for 2 h at room temperature. The absorbance was measured at 750 nm against a blank prepared in the same way with distilled water in the place of the extract. Measurements were performed in triplicate. Results were expressed as mg gallic acid equivalents/g dry extract.

2.8. Determination of individual phenols by HPLC

A Shimadzu high performance liquid chromatograph consisting of a LC-10AD VP pump, a Rheodyne model 7725i injection valve with a 20 μL loop and a UV–Vis SPD-10AV detector was used. The separation of catechin, epicatechin and gallic acid was performed on a 250 mm × 4.6 mm i.d., 5 μm, Nucleosil 100-5 C_{18} column (Macherey–Nagel, Duren, Germany). The data from the UV–Vis detector were stored and processed with EZChrom chromatographic software (Scientific Software Inc., San Ramon, CA). The eluting system, consisted of 3%, v/v, acetic acid (solvent A) and acetonitrile (solvent B) in isocratic conditions: 90% A:10% B, was a slight modification of the protocol suggested by Tsanova-Savova, Ribarova, and Gerova (2005). The flow rate was 1 mL/min and the injection volume 5 μL. Detection was at 280 nm. Peak identification was based on retention times and spiking with standard solutions. Seven-point calibration curves of the three phenolic compounds were used for quantification.

2.9. Radical scavenging activity of seed extracts

The radical scavenging activity of the extracts was determined as follows: 2900 μL of DPPH· methanolic solution (0.1 mM), were thoroughly mixed with 100 μL of diluted extract (each time appropriate dilute solutions of extract were prepared in order to obtain a [total phenols expressed as moles of gallic acid]/[DPPH·] = 0.25). Absorbance was measured after 30 min at 515 nm against methanol. Measurements were performed in triplicate. Results are expressed as DPPH·% scavenging = $[[abs_{t=0}] - [abs_{t=30}]/[abs_{t=0}]] \times 100$, where $abs_{t=0}$ is the absorbance of the 0.1 mM DPPH· methanolic solution at $t = 0$ min and $abs_{t=30}$ is the absorbance of the mixture at $t = 30$ min. The DPPH activity of the extracts was determined after 10, 35 and 50 days of storage.

2.10. Evaluation of antioxidant activity of catechin and epicatechin

2.10.1. FRAP assay

The assay was performed according to the protocol of Benzie and Strain (1996). The reaction was monitored at the absorption maximum (593 nm) for up to 4 min. For each compound and each concentration measurements were made in triplicate with suitable blank solutions each time. Graphs of antioxidant concentration *vs* ΔA_{593} at 4 min ($\Delta A_{593} = A_{AH} - A_{Cont}$) were then constructed. As FRAP values were considered those of the slopes of the linear curves ($\times 10^3$) derived from the constructed graphs. Results were expressed as gallic acid equivalents.

2.10.2. Folin–Ciocalteu assay

The assay was performed according to Nenadis, Boyle, Bakalbassis, and Tsimidou (2003). For each compound and each concentration (0.5, 1.0, 1.5 and 2.0 mM), measurements were carried out in triplicate using suitable blank solutions each time. Graphs of antioxidant concentration *vs* absorbance at 725 nm were then constructed. As F–C values were considered those of the slopes of the linear curves derived from the constructed graphs. Results were expressed as gallic acid equivalents.

2.10.3. $ABTS^{\cdot+}$ assay

Measurements were carried out as described by Re et al. (1999) in a phosphate buffer solution pH 7.4 (PBS) and with initial absorbance value of the radical solution of 0.7 ± 0.05 at 734 nm. The decrease in absorbance was recorded at 0 and after 6 min in the presence of antioxidants at 0–15 μM final concentration. For each compound and each concentration measurements were made in triplicate with suitable blank solutions each time. Graphs of antioxidant concentration *vs* %inhibition were then constructed. ABTS values were those of the slopes of the linear curves derived from the constructed graphs. Results were expressed as gallic acid equivalents.

2.10.4. DPPH· assay

An aliquot (2.9 mL) of a 0.1 mM ethanolic DPPH· solution was transferred in a glass cuvette (10 mm) and then mixed with 0.1 mL of an antioxidant solution at different ratios [AH]/[DPPH·] depending on the activity of the tested compound. The decrease of the DPPH· concentration was monitored at the 516 nm until the steady state was reached. Based on the data obtained the amount of antioxidant necessary to decrease the initial [DPPH·] by 50% known as EC_{50}, T_{EC50} and the antiradical efficiency, $AE = 1/EC_{50} \times T_{EC50}$ were calculated (Nenadis et al., 2003). All measurements were performed in triplicate. Results were expressed as gallic acid equivalents.

2.10.5. CBA kinetic study

Estimation of crocin bleaching was carried out according to Ordoudi and Tsimidou (2006). In brief, 0.1 mL of

AHs from a 0.5 mM solutions along with suitable amount of crocin methanol solution was transferred in 5 mL volumetric flask so that a [AH]/[C] = 1 to be achieved. The reaction started with the addition of 250 μL (0.25 M) AAPH ($t = 0$ min). After dilution to 5 mL (total volume) with PBS and stirring for ca. 30 s, the test solution was transferred into a 3 mL quartz cell and absorbance monitoring (440 nm) started at exactly 1 min after the addition of initiator. For each compound measurements were made in triplicate with suitable blank solutions each time. Percent inhibition of crocin bleaching value (% In h) was calculated as % In $h = [(\Delta A_0 - \Delta A)/(\Delta A_0) \times 100]$ where ΔA_0 and ΔA is the difference in absorbance during the bleaching in the absence and the presence of the AH, respectively. Results were expressed as gallic acid equivalents.

2.10.6. ORAC assay

In a 5 mL volumetric flask, 4 mL of a 8.6 nM fluorescein solution (pre-incubated at 37 °C for 15 min) and daily prepared from a 0.11 mM stock, were introduced. Then, 250 μL of the antioxidant solution (final concentration 0.5 or 1 μM for all compounds) or phosphate buffer for the control reading, were added. The reaction started with the addition of 120 μL of a 125 mM AAPH solution. All the referred solutions were diluted with a 75 mM phosphate buffer (PB) pH 7.0. Then the volume was brought to 5 mL with buffer solution (pH 7.0) and the reaction mixture was vortexed for 0.5 min. The fluorescence was recorded every 0.5 min (excitation 490 nm, emission 515 nm) until zero fluorescence occurred. During the whole experimental procedure the temperature was maintained at 37 °C and the reaction mixture was stirred. For each compound and each concentration measurements were made in triplicate. The net area under the curve (AUC) was obtained by subtracting the area under the curve (AUC) of blank (AUC_{blank}) from that of the tested compound (AUC_{Test}), respectively. Calculations were carried out using PC-1501 software (Shimadzu, Sci. Inst., Inc.). Results were expressed as gallic acid equivalents.

2.10.7. Phosphatidylcholine liposome oxidation

Liposomes were prepared in 100 mL Erlenmeyer flasks (100 mL) so that a final lecithin concentration of 0.8%, w/w, to be achieved. Antioxidants were tested at 60 μM final concentration. Cupric acetate (3 μM final concentration) was used to initiate oxidation at 37 °C in the dark. The course of oxidation was monitored through measurement of conjugated diene formation at 234 nm (Nenadis et al., 2003). All measurements were performed in triplicate.

2.10.8. Calculation of partition coefficient (log P)

Calculation of the log P values, simulating partitioning of tested compounds in an n-octanol:water (1:1, v/v) system, was based on Broto's fragmentation method (Broto, Moreau, & Vandyke, 1984) and was accomplished using the CS Chem Draw Ultra 5.0 software (CambridgeSoft-Corporation, MA, USA).

2.11. Statistical analysis

Statistical comparisons of the mean values for total phenols and radical scavenging activity of seed extracts were performed by one-way analysis of variance (ANOVA), followed by the multiple Duncan test ($p < 0.05$ confidence level).

3. Results and discussion

3.1. Preliminary work

Grape seeds contain a relatively high amount of water. Introduced to a controlled relative humidity environment, they will exchange water to equilibrium. In a preliminary attempt the time necessary for equilibrium establishment was determined. As physical form of samples (whole or ground) is being demonstrated to affect extraction and activity of the extracts (Bonilla, Mayen, Merida, & Medina, 1999; Kim et al., 2006; Pekić, Kovač, Alonso, & Revilla, 1998), both whole and ground grape seeds (5 g) were placed under three different relative humidity (RH) conditions (33%, 53% and 75%) and 25 °C in order to investigate the influence of this parameter under the experimental conditions. Daily determination of moisture content (Fig. 1) showed that seeds with 34.7% mean initial moisture and consequently, water activity (a_w) between 0.8 and 1 (Rockland & Beuchat, 1987), lost a great percentage of their water in a rather slow process during the first days of storage. Equilibrium between a_w of the samples and RH of the controlled environment was established between 4 and 8 days after seed introduction in the desiccators for the most dry and humid environment, respectively. There was no particular difference in the behavior of whole

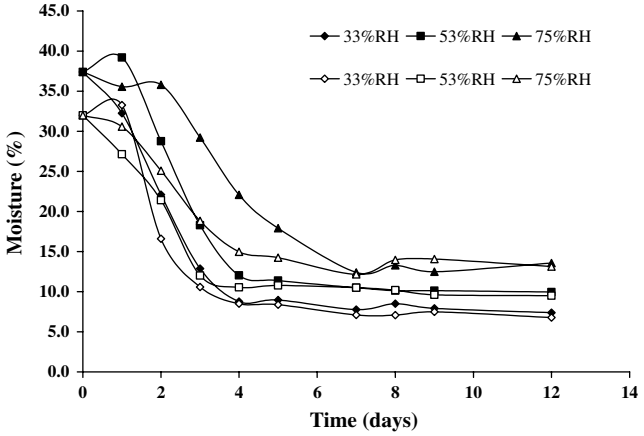

Fig. 1. Moisture content (%) evolution of whole (♦,■,▲) and ground (◇,□,△) grape seeds during storage at different relative humidity environments.

and ground seeds with regards to the determined moisture content.

The second trial was undertaken using several portions of seeds placed in individual weighing dishes stored in the desiccators at the three different RH conditions. At the 8th day of storage, moisture and total phenol level were then determined (Fig. 2). Methanol was chosen as an extracting polar solvent providing maximum extraction yields of total phenolics (Moure et al., 2001) and the best quantitative extraction of the phenols of interest (Kallithraka, Garcia-Viguera, Bridle, & Bakker, 1995). Once more, no difference was observed in the equilibrium values of moisture content between stored whole and ground seeds. Moisture content of 6.7% obtained by storage at 33% RH corresponds to almost complete removal of water type II and is considered rather prohibitive for fungal growth. Indeed, a maximum moisture content of 8% is suggested for maintaining quality of seeds during storage (Jordan, 2002), whilst seed drying to a level of a_w below 0.6 is recommended in order to minimise the risk of moulding and heating during storage. Moisture content of 11.5% obtained after storage at 75% RH, corresponds to removal of water type III while 8.5% obtained by storage at 53% RH to partial removal of water type II.

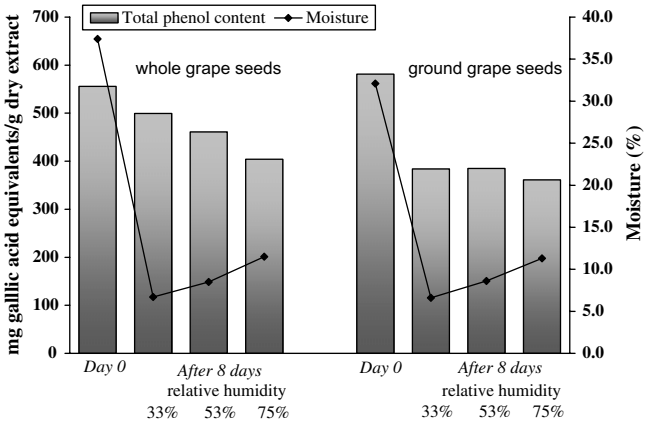

Fig. 2. Moisture content (%) and total phenol content (mg gallic acid equivalents/g dry extract) in methanolic extracts of grape seeds stored at different relative humidity conditions, at equilibrium.

On the contrary, total phenol content of ground seeds was significantly lower (60–64% of the initial amount) compared to that in whole seeds (75–91% of the initial amount). Nevertheless, differences in the total phenol content among samples of each physical form stored under the mentioned experimental conditions were rather insignificant (Fig. 2). Taking into account the experimental findings we considered work on whole seeds to be a more realistic approach for studying the effect of moisture on the content and activity of the antioxidants present in these by-products.

Since no significant changes in total phenols level were observed till the 8th day, monitoring of changes in the antioxidants started after that day.

3.2. Influence of storage under various conditions of relative humidity on the phenolic compounds (content and activity)

Measurements for total phenol content evolution were taken after 10, 20, 35 and 50 days of storage representing possible and extreme cases of storage period (Table 1). Total phenol content was determined for the methanolic extracts using the Folin Ciocalteu method. The changes observed were not dramatic in size when expressed as mg gallic acid equivalents/g dry extract for samples stored at 33% or even at 53% RH. Definitely, length of time is a negative factor but it cannot be considered a limiting one, if the amount of waste available is taken into account. High level of humidity accelerates degradation so that, even a slight aeration or drying of grape seeds is expected to be beneficial as, recently, was pointed out by Kim et al. (2006).

Promising were also the results for the radical scavenging activity of the extracts (%RSA) towards the DPPH radical. No loss of practical importance was found whilst lower water activity coincided with higher values of radical scavenging activity.

Since we had a particular interest in the fate of the two major monomeric components, changes in their content on storage were monitored using a convenient isocratic HPLC system. All samples were analysed using two calibration curves constructed with catechin and epicatechin standard solutions, respectively. Initial amounts of catechin and

Table 1
Effect of storage at different relative humidity conditions on moisture content, total phenol content and radical scavenging activity towards the DPPH radical (%RSA), of grape seeds

Time (days)	Moisture content (%)			Total phenol content (mg gallic acid equivalents/g dry extract)			%RSA		
	33%RH	53%RH	75%RH	33%RH	53%RH	75%RH	33%RH	53%RH	75%RH
0	39.0	39.0	39.0	$438 \pm 9^{a,C}$	$438 \pm 9^{a,C}$	$438 \pm 9^{a,C}$	$61.0 \pm 1.7^{a,A}$	$61.0 \pm 1.7^{a,A}$	$61.0 \pm 1.7^{a,B}$
10	8.6	10.1	15.2	$525 \pm 24^{b,D}$	$449 \pm 19^{a,C}$	$513 \pm 12^{b,D}$	$72.0 \pm 0.3^{c,B}$	$63.3 \pm 1.7^{b,A}$	$57.7 \pm 2.2^{a,AB}$
20	7.9	9.5	14.0	$367 \pm 18^{c,B}$	$286 \pm 24^{b,A}$	$231 \pm 28^{a,A}$	–	–	–
35	7.5	9.3	12.2	$358 \pm 17^{b,B}$	$349 \pm 4^{b,B}$	$279 \pm 12^{a,B}$	$86.8 \pm 1.8^{b,C}$	$85.4 \pm 3.3^{b,C}$	$72.0 \pm 2.0^{a,C}$
50	7.7	9.4	12.1	$327 \pm 12^{b,A}$	$344 \pm 4^{b,B}$	$234 \pm 20^{a,A}$	$72.5 \pm 2.0^{b,B}$	$70.7 \pm 1.3^{b,B}$	$57.0 \pm 0.2^{a,A}$

[a–c]Different lower case letters within a row are significantly different at $P < 0.05$.
[A–C]Different uppercase letters within a column with same % relative humidity are signifficantly different; $n = 3$.
–, Not measured.

epicatechin were 83.2 mg and 106.2 mg/100 g dry seeds, respectively. Our values were of the same order with those of other authors (Fuleki and Da Silva, 1997; Yilmaz & Toledo, 2004b) who determined them in Merlot and Chardonnay grape seeds, but much higher than those reported by Guendez et al. (2005b), namely 37, and 17.5 mg/100 g seeds for Xinomavro samples. Grape seeds phenolic content may vary according to grape variety, climatic conditions and grape maturity (Shi et al., 2003) and that's probably the reason of such differences. Final catechin and epicatechin level was ~57%, 68%, 51% and 85%, 77%, 67% of the initial amount, respectively (Fig. 3). Epicatechin proved to be less sensitive to different humidity conditions than catechin, yet storage at 75% RH seriously affected levels of both flavan-3-ols.

Using the simple isocratic elution system it was not possible to measure changes in the complex forms of phenols present in the grapes. Nevertheless, the continuous increase in gallic acid content was a clear indication of hydrolytic processes in the system on storage (Fig. 3). Free gallic acid level is generally extremely low in seeds from different varieties (Escribano-Bailón et al., 1992; Fuleki and Da Silva, 1997; Guendez et al., 2005a, 2005b; Santos-Buelga et al., 1995; Yilmaz & Toledo, 2004b).

To highlight whether epicatechin is less sensitive to oxidation processes than its counterpart catechin we carried out a series of experiments using different *in vitro* antioxidant activity assays. The results were expressed as gallic acid equivalents and are presented in Table 2 and Fig. 4.

The results obtained did not support a significant difference in the behavior of the two compounds that could justify greater degradation of catechin on storage with comparison to that of epicatechin. The higher susceptibility of catechin to oxidation cannot be supported even in terms of oxidation potential as either experimentally (Hotta et al., 2002; Pietta, 2000) or theoretically (Wilson-Mendoza & Mitnik-Glossman, 2006) the reported values are almost the same. However, based on the computational study in the gas phase of Wilson-Mendoza and Mitnik-Glossman (2006), the twisting of B ring in (−) epicatechin (−45.77°) is greater than that of (+) catechin (−31.37°). On the basis of this finding and those of Burton et al. (1985) who concluded that the dihedral angle is an important factor in the orbitals overlapping in aromatic rings and consequently activity of compounds, the authors stated that catechin should be expected to oxidize more easily. Apart from such a finding, it is obvious, based on HPLC data, that though the content of the two flavan-3-ols decreased

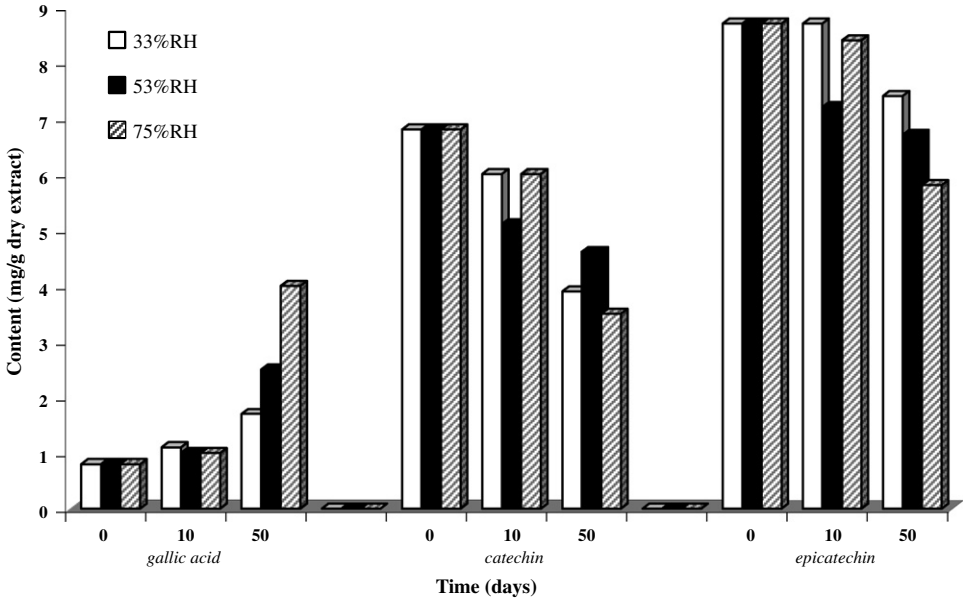

Fig. 3. Effect of storage at different relative humidity conditions on gallic acid, catechin and epicatechin concentration (mg/g extract) of grape seeds.

Table 2
Antioxidant activity of catechin and epicatechin using different redox and radical scavenging assays expressed in gallic acid equivalents

Antioxidants	FRAP[a]	Folin[a]	ABTS[a]	DPPH[a]		Crocin[a]	ORAC[a,d]	
				(EC_{50})[b]	(AE)[c]			
Catechin	0.59	1.69	1.09	0.72	0.14	0.77	4.24	2.64
Epicatechin	0.60	1.74	1.08	0.72	0.13	0.66	4.08	2.42

[a] Each value is the mean of triplicate determination.
[b] Efficient [AH] for scavenging the 50% of [DPPH·].
[c] AE values [$AE = 1/(EC_{50} \times TEC_{50})$].
[d] Results are presented for 0.5 and 1.0 μM final concentration.

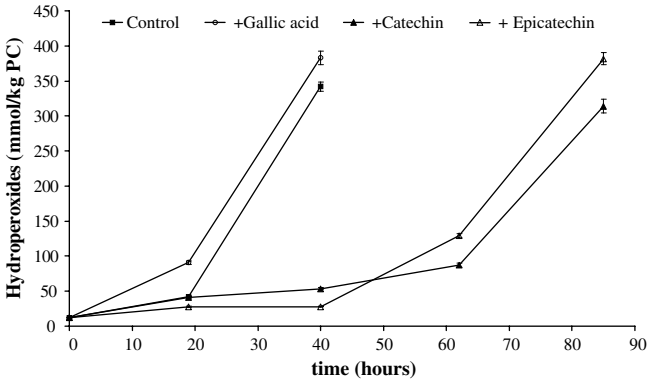

Fig. 4. Copper-induced liposome oxidation in the absence or presence of antioxidants (60 μM final concentration) at 37 °C. Each value is the mean ± SD ($n = 3$).

with time, formation of gallic acid is evident as a result of hydrolysis of more complex compounds present in grape seeds (e.g., epicatechin gallate). Gallic acid is also a very efficient antioxidant as it can be deduced from the results of Table 2, although it may act as a pro-oxidant in dispersed systems as liposomes. Such a behavior is attributed to the high polarity of the acid ($\log P = 0.06$) in comparison to that of the flavonoids ($\log P = 0.80$). For this reason, the acid is expected to be located mainly in the aqueous phase where its high reducing capacity may promote the pro-oxidative action of metal ions. Such a behavior however is changed at higher concentrations (data not shown). It seems therefore, that under the aforementioned storage conditions, the formation of gallic acid may compensate the loss in catechins and therefore maintain the antioxidant potential of the seeds. Epicatechin gallate is the third, from a quantitative point of view, compound present in grape seeds. We can assume that under conditions of increased moisture (75% RH), hydrolytic reactions favored release of the gallic acid and consequently of epicatechin.

It is of practical importance to reconsider the waste management operations concerning grape seeds by-products of red wine vinification. The installation of a waste management enterprise in the proximity of main viticultural areas is, thus, suggested. Effective drying or provision of controlled relative humidity environment can assist storability of seeds. The challenge remains to economically optimize and realize the proposed process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2007.05.032.

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