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(+)-Dihydrorobinetin: a Marker of Vinegar Aging in Acacia (*Robinia pseudoacacia*) Wood

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The use of acacia wood for the aging of vinegars is increasing because the efficient air transfer through the pores permits a good acetification rate. In this study, vinegars aged in acacia (*Robinia pseudoacacia*) wood barrels were analyzed and found to contain a characteristic compound, which increased during the aging process. This so far unknown compound was isolated by semipreparative LC and structurally identified by NMR spectroscopy. ^{1}H and ^{13}C NMR chemical shifts and optical rotation revealed its structure to be (+)-dihydrorobinetin, a dihydroflavonol identified for the first time in vinegars as a marker of aging in this kind of wood. This study also reports for the first time the complete assignment of ^{13}C NMR data for this compound. Moreover, it revealed a longer contact time with acacia wood results in higher concentrations of (+)-dihydrorobinetin found in vinegars. Another finding was that the vinegars aged with nontoasted acacia chips showed significantly higher concentrations of (+)-dihydrorobinetin than found in vinegars aged with toasted acacia chips (384.8 and 23.5 mg/L, respectively). The in vitro antioxidant activity (DPPH$^{\bullet}$ and ORAC assays) of (+)-dihydrorobinetin was also determined. (+)-Dihydrorobinetin is reported here for the first time as a chemical marker of vinegars aged in acacia wood and can be used for authenticity purposes.

KEYWORDS: Acacia; wood; vinegar; antioxidant; NMR; (+)-dihydrorobinetin; *Robinia pseudoacacia*.

INTRODUCTION

Sherry vinegar and traditional balsamic vinegar (ABT) are high-quality vinegars that are famous all over the world. Sherry wine vinegar is produced in a series of wooden oak butts in which acetification and aging take place simultaneously. ABT is obtained by a traditional method that ferments the cooked must and ages the product in a set of wooden barrels. The barrels are made in decreasing sizes (from 60 to 20 L) and of different woods (oak, chestnut, cherry, ash, juniper, and mulberry) (*1*−*4*). Both elaboration processes of high-quality vinegars require an aging period in wooden butts. During this period, the finished product develops the desired organoleptic properties that make it highly appreciated (*5*).

At present, oak is the most commonly used wood in enology for aging wines, spirits, and vinegars. Its contribution to the quality and chemical composition of the product is well-known (*6*−*9*). Nowadays, other woods such as acacia, cherry, chestnut, and mulberry are increasingly being used to make products with other sensory properties. The impact that these woods have on chemical composition and sensorial properties still needs to be evaluated (*10*−*12*).

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The EU-funded WINEGAR project (ref. COOP-CT-2005-017269) proposed using four different kinds of woods (acacia, chestnut, cherry, and oak) for wine vinegar production. The two main purposes of the project were to choose the wood with the air transfer that best fulfilled the acetification oxygen requirements and to obtain a high-quality product. Thus, the vinegars were produced by traditional means in barrels especially made for the project, and the quality and chemical composition were checked throughout the process (*13*). The chemical characterization of vinegar can be used for a variety of purposes including authentication and product classification according to quality criteria. The analysis of the vinegars aged in acacia (*Robinia pseudoacacia*) revealed a compound that was not present in vinegars aged in the other woods. This compound was likely to serve as a chemical marker of acacia wood for authenticity purposes.

The aims of the study described herein have been, first, to isolate and identify this compound; second, to evaluate its release from acacia wood; and, finally, to explore the influence of wood treatments on its concentration in vinegars. Last but not least, the antioxidant activity of this compound has been determined by DPPH and ORAC assays. To the best of our knowledge, this is the first time that this compound has been described in products aged in wood and its antioxidant activity presented.

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MATERIAL AND METHODS

Samples. Three wines (two red Garnacha variety wines, groups G1 and G2; and one white Trebbiano variety wine, group T) were acetified and subsequently aged in acacia barrels (*R. pseudoacacia*) of 60 L capacity. The barrels were made especially for this experiment and were not toasted. Surface culture acetification took place until the acetic degree reached 6°. After filtration to remove the acetic acid bacteria, the aging period started. Samples were taken at 0, 1.5, 6, and 12 months.

One red wine vinegar (Cabernet sauvignon variety, group C), fermented with submerged culture in a laboratory acetator, was aged with acacia wood chips (*R. pseudoacacia*). Toasted and nontoasted chips (5−10 mm) were tested in different proportions (0.5 and 1%, w/v). The samples were analyzed at 0, 15, and 30 days of aging with chips.

Isolation. *Sample Preparation for Semipreparative LC.* An Amberlite XAD7 column (Fluka; 43 cm × 22 mm) was first conditioned with 200 mL of methanol and then with 500 mL of Milli-Q water. A total of 700 mL of diluted vinegar sample with water (1:1) was loaded onto the column and cleaned with 500 mL of water to remove sugars, proteins, organic acids, and minerals. Elution was performed with 300 mL of methanol/water (70:30). The flow rate was 1 drop/s. The eluate was concentrated with a rotary evaporator under vacuum, frozen, and freeze-dried to obtain 1.6 g of extract from 1 L of vinegar.

Semipreparative LC. The unknown compound was isolated using a preparative LC system equipped with a binary pump (Knauer K-1001) and a Knauer injection valve with a 500 μL loop. It was detected with an UV detector (Knauer K-2600), and data were processed by Eurochrom 2000 V.2.05. The column was a Luna 5 μm C18 (2), 250 × 10 mm (Phenomenex). Samples were filtered through a Chromatofil PET 0.45 μm membrane filter before injection. Two different solvents were used as the mobile phase: solvent A (glacial acetic acid in water at pH 2.65) and solvent B (20% solvent A mixed with 80% acetonitrile), at a flow rate of 4 mL/min and a the following linear gradient: 0 min, 10% B; 32 min, 10% B; 36 min, 100% B; 41 min, 10% B.

Identification. *ESI-MS and MS/MS Analysis.* Negative mode ESI-MS and MS/MS were performed using a QTRAP LC-MS/MS system equipped with an electrospray ion source. A sample dissolved in acetonitrile/water (50:50 v/v) containing 0.1% formic acid was infused at 10 μL min^{-1}. The capillary voltage was −4500 V, the declustering potential was −30 V, and the collision energy was set at −30 V.

NMR Analysis. NMR spectroscopy was used to examine the isolated compound in a solution (10 mg/mL) of 99.6% DMSO-d_6. Spectra were recorded at 303 K on a Bruker Avance-500 spectrometer operating at 500.13 MHz (^{1}H) and 125.75 MHz (^{13}C), respectively. Chemical shifts are given in parts per million (ppm), using the DMSO signals (2.49 and 39.5 ppm for ^{1}H and ^{13}C, respectively) as references. The 2D homonuclear proton double-quantum filtered correlation experiment (DQF-COSY) (*14*) was performed in the phase-sensitive mode using the Bruker standard pulse sequence. The 2D heteronuclear one-bond proton−carbon correlation experiment (*15*) was recorded in the ^{1}H detection mode (inverse detection) via single-quantum coherence (HSQC). ^{13}C decoupling was achieved by the GARP scheme. This experiment was slightly modified by implementing an editing block in the sequence. The long-range proton−carbon correlation experiment (HMBC) (*16*) was collected in the ^{1}H detection mode. The delay time was 80 ms between the first and second pulses, and there were 96 scans per increment. The pure absorption 2D NOESY experiment was performed using a mixing time of 400 ms.

Polarimetric Analysis. Polarimetric analysis was performed to check the enantiomeric nature of the compound. Optical rotation ($[\alpha]_D$) was determined with a Perkin 341 polarimeter at 25 °C. The dihydrorobinetin concentration was 0.45 g/100 mL; Me_2CO/H_2O (1:1) was used as solvent.

Vinegar Samples Analysis. *LC-DAD.* LC analysis of the unknown phenolic compound was performed using an Agilent series 1100 system equipped with a quaternary pump (series 1100 G1311A), automatic injector (series 1100 G1313A), and degasser (series 1100 G1379A). Detection was accomplished using an UV−vis diode detector (series 1100 G1315B) coupled to a Chemstation HP A.10.02 (HP/Agilent). The column was a Merck LiChroCART 250-4 Superspher 100 RP-18 1.16056.0001. Duplicate samples were filtered through a Millex-LCR 13 mm filter before injection. The chromatographic conditions had previously been used for vinegar analysis (*17*). The method uses a binary gradient, A (glacial acetic acid/water pH 2.65) and B (20% A + 80% acetonitrile), programmed in the following gradient: 0 min, 100% A; 5 min, 98% A + 2% B; 10 min, 96% A + 4% B; 15 min, 90% A + 10% B; 30 min, 80% A + 20% B; 35 min, 70% A + 30% B; 40 min, 100% B; 45 min, 100% A; 60 min, 100% A. The sample volume injected was 50 μL. The flow rate was 1.5 mL min^{-1}, and the temperature was set at 40 °C. Quantification was performed by external calibration at 280 nm.

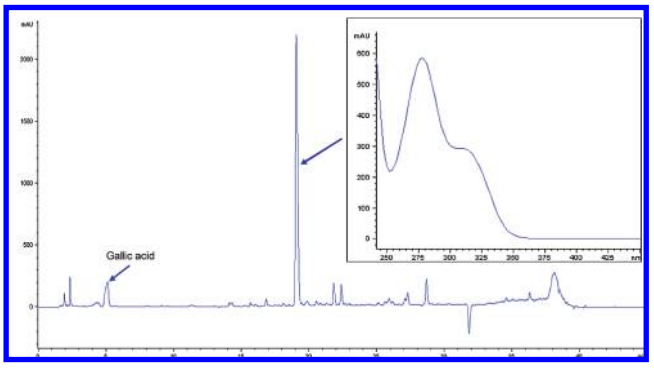

Figure 1. LC-DAD chromatogram at 280 nm of red wine vinegar (Garnacha variety) aged in an acacia wood barrel for 12 months.

Antioxidant Activity of (+)-Dihydrorobinetin. *ORAC Assay.* The ORAC assay is based on a previously reported method with slight modifications (*18*). Briefly, it is as follows: 50 μL of sample or Trolox is mixed with 100 μL of fluorescein (45 nM) and 50 μL of AAPH (15 mM). Fluorescence is recorded for 60 min (the excitation wavelength is set at 485 nm and the emission wavelength at 528 nm). Measurements were taken in triplicate in a multidetector microplate reader (Synergy HT, Biotek). Trolox was used as a calibration standard (0.5−9.5 μM).

The results were calculated as ORAC values from the differences between the blank and the sample areas under the fluorescein decay curve. They are expressed as Trolox equivalents.

DPPH Method. A total of 0.1 mL of (+)-dihydrorobinetin (0.328−0.164 mM) or sample (1:50, v/v) or Trolox (0.000−1.000 mM) was added to 3.9 mL of DPPH$^\bullet$ (0.063 mM), all in methanolic solution. Absorbance was measured at 515 nm after 60 min (when the reaction reached equilibrium). The blank reference cuvette contained methanol. The initial absorbance was close to 0.700 in all cases. All measurements were performed in triplicate. A linear curve was obtained by plotting four concentrations of (+)-dihydrorobinetin against the respective Trolox concentrations. The Trolox value corresponding to a 1 mM concentration of (+)-dihydrorobinetin is, by definition, its TEAC value.

RESULTS AND DISCUSSION

Peak Isolation and Identification. Figure 1 shows the LC-DAD chromatogram of a vinegar aged in acacia wood for 12 months. As can be seen, there is a major peak at t_R = 14.4 min and 280 nm, which was previously reported in vinegars aged in acacia wood, and its area increases with aging (*19*).

To isolate this still unknown compound from the vinegar extract, semiprepartive LC was applied. After concentration and freeze-drying, 264.1 mg of compound/L vinegar was obtained. Its MS spectrum showed a molecular ion $[M]^{\bullet+}$ at m/z 304, and the MS/MS spectrum showed the following fragmentation pattern of m/z: 275, 168, 149, 139, 137, 121.

NMR analysis was carried out to make a complete structural determination. Hence, ^{1}H and ^{13}C NMR chemical shifts were unequivocally assigned by 2D DQF-COSY, NOESY, HSQC, and HMBC experiments (**Table 1**). A relative 2,3-trans configuration was evident from the homonuclear coupling constant for protons in these positions ($J_{2,3}$ = 11.1 Hz), which indicated a trans-diaxial disposition between them. Moreover, this fact was further confirmed by the existence of an intense NOE effect between H-2′ and H-3, as observed in the 2D-NOESY spectrum

Table 1. ^{1}H and ^{13}C NMR Data of Isolated Compound [a]

position	δ_H	δ_C [b]	HMBC [b,c]
2	4.89 (d, 11.1)	83.7	3, 4, *1′*, *2′*
3	4.31 (d, 11.1)	72.6	2
4		192.1	
5	7.61 (d, 8.6)	128.6	*4*, 7
6	6.51 (dd, 1.9)	110.8	8, 10
7		164.9	
8	6.28 (d, 1.9)	102.3	6, 10
9		162.7	
10		112.0	
1′		127.4	
2′	6.40 (s)	106.9	*2*, 3′, 4′
3′		145.6	
4′		133.3	
OH in 3	5.44 (bs)		
phenolics OH	8.89 (bs)		

[a] Recorded in DMSO-d_6; chemical shifts are expressed as δ values in ppm; signal multiplicities and coupling constants (Hz) are shown in parentheses. [b] Carbons showing long-range couplings to proton, $^{n}J_{CH}$ ($n \geq 2$). [c] Inter-ring couplings are shown in *italics*.

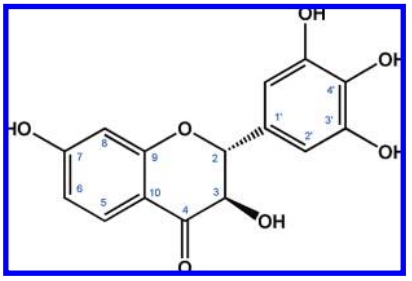

Figure 2. Molecular structure of (+)-dihydrorobinetin.

Table 2. (+)-Dihydrorobinetin Concentration (Milligrams per Liter) in Vinegar Samples Aged in Nontoasted Acacia Wood Barrels

	substrates[a]		
aging time (months)	G1	G2	T
0	65.34 ± 0.20	129.7 ± 0.3	36.7 ± 1.9
1.5	124.7 ± 4.8	325.86 ± 0.17	109.1 ± 0.7
6	300.64 ± 0.18	412.10 ± 0.11	214.4 ± 2.7
12	304.7 ± 0.6	438.58 ± 2.16	266.3 ± 0.9

[a] G1 and G2, different substrates of Garnacha variety; T, Trebbiano variety.

(not shown). Our ^{1}H NMR data agreed with data obtained previously by Saleh et al. (*20*) for dihydrorobinetin (**Figure 2**). Nevertheless, as far as we know, this is the first study to include the complete assignment of ^{13}C NMR data for this compound. The confirmation of these assignments (including quaternary carbon) was obtained from the $^{1}H-^{13}C$ long-range correlation NMR experiment (HMBC) (**Table 1**). Of significance was the appearance of cross-peaks corresponding to the long-range couplings of H_2 (C ring) with $C_{1'}$ (B ring), of $H_{2'}$ (B ring) with C_2 (C ring), and of H_5 (A ring) with C_4 (C ring). Dihydrorobinetin has been previously described in the stemwood of *R. pseudoacacia* L. (*21*) and in the leaf extracts of some *Cordia* spp. (*22*).

Optical rotation analysis revealed an $[\alpha]_D$ value of +12.6° (C 0.45, 1:1 Me_2CO/H_2O), which is in agreement with the data obtained by Weinges for (+)-dihydrorobinetin (*23*). Our work reveals that (+)-dihydrorobinetin can be released from the acacia wood to vinegar during aging.

Quantification of (+)-Dihydrorobinetin. Table 2 shows the concentration of (+)-dihydrorobinetin in vinegars throughout the aging in acacia barrels. As aging is considered to start after the acetification in acacia wood was complete, there must be a period of 1.5−5 months of wood contact before aging begins. It was found that the longer the contact with acacia wood was, the higher the concentration of (+)-dihydrorobinetin. The concentration of (+)-dihydrorobinetin after 12 months of aging was far higher (266.3−438.58 mg/L) than concentrations reported during vinegar aging for other phenolic compounds such as aldehydes, which can reach concentrations of up to 39 mg/L (*9*, *24*). The concentration of (+)-dihydrorobinetin was higher in red vinegars than white ones because the contact period with acacia wood was longer during the acetification process (**Table 2**).

Table 3. (+)-Dihydrorobinetin Concentration (Milligrams per Liter) in Vinegar Samples (Group C) Aged with Different Percentages (w/v) and Thermal Treatment of Acacia Wood Chips for Different Aging Times

		treatment	
% (w/v)	aging time (days)	nontoasted	toasted
0.5	15	234.6 ± 0.9	11.1 ± 0.4
	30	246.8 ± 1.4	11.6 ± 0.5
1	15	388.5 ± 4.4	20.06 ± 0.04
	30	384.8 ± 2.5	23.50 ± 0.13

The concentration of (+)-dihydrorobinetin in vinegar aged with nontoasted acacia chips was 20 times higher the concentration in vinegar aged with toasted acacia chips (**Table 3**). The thermal treatment of the wood, then, is crucial for the release of (+)-dihydrorobinetin into the vinegar. As expected, the higher the content of wood chips was, the greater the release of (+)-dihydrorobinetin (**Table 3**). Also, 15 days of aging is enough to obtain the highest increases of (+)-dihydrorobinetin (84−100%), and the delay of 15 days more has no significant effects (**Table 3**). This agrees with the data obtained by Tesfaye et al. (*17*) about the release of other phenolic compounds in vinegars aged with chips.

Antioxidant activity of (+)-Dihydrorobinetin. The TEAC and ORAC values of (+)-dihydrorobinetin were 1.57 ± 0.03 mM and 0.51 ± 0.06 μmol of Trolox/μmol of (+)-dihydrorobinetin. The main characteristics of the molecular structure influencing the antioxidant activity are the number of −OH groups in the B ring and the double bond in the C ring, which increase electron delocalization. (+)-Dihydrorobinetin is a dihydroflavonol with three −OH groups in the B ring but no conjugated double bond in the C ring. As a consequence, its antioxidant activity was slightly lower than that of myricetin (*25*). However, it should be taken into account as its concentration in the finished product is high. Indeed, we assessed the antioxidant value of the vinegar without (+)-dihydrorobinetin (group C) and this vinegar aged for 30 days with a (+)-dihydrorobinetin concentration at 384.8 mg/L (**Table 3**). The TEAC values were 11.64 ± 0.41 mM Trolox equivalents for vinegar and 14.65 ± 0.35 mM Trolox equivalents for aged vinegar. The ORAC results were 5912.6 ± 707.8 and 7466.8 ± 1281.7 μM Trolox equivalents. If we consider the concentration of (+)-dihydrorobinetin in the final product and the antioxidant activity values for this compound, the (+)-dihydrorobinetin explains 13.65 or 8.6% for the TEAC or ORAC value. This contribution to overall antioxidant activity of the product is higher than that of other phenolic compounds described in wines (*26*).

Conclusions. (+)-Dihydrorobinetin is released into products aged in barrels made of acacia. Therefore, it might be useful as a chemical marker of products aged in acacia. Nontoasted wood releases a higher amount of (+)-dihydrorobinetin than toasted wood. The in vitro antioxidant activity of (+)-dihydrorobinetin is

also reported because the high amounts of the compound in the product mean that aging in acacia wood increases its overall antioxidant activity, contributing to the functional properties of vinegars.

ABBREVIATIONS USED

ABT, traditional balsamic vinegar; EU, European Union; NMR, nuclear magnetic resonance; ORAC, oxygen radical absorbance capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; AAPH, 2,2′-diazobis(amidinopropane) dihydrochloride; DPPH•, 1,1-diphenyl-2-picrylhydrazyl; TEAC, Trolox equivalent antioxidant capacity.

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LITERATURE CITED

(1) Llaguno, C.; Polo, Ma. C. Definición y tipos de vinagre. In *El Vinagre de Vino*; CSIC: Madrid, Spain, 1991; p 143.
(2) Plessi, M.; Bertelli, D.; Miglietta, F. Extraction and identification by GC-MS of phenolic acids in traditional balsamic vinegar from Modena. *J. Food Compos. Anal.* **2006**, *19*, 49–54.
(3) Sanarico, D.; Antonelli, A.; Giudici, P. Simulation model of concentration of traditional Balsamic vinegar. *Ind. Bev.* **2002**, *31* (182), 526–532.
(4) Zeppa, G.; Giordano, M.; Gerbi, V.; Meglioli, G. Characterisation of volatile compounds in three acetification batteries used for the production of "Aceto Balsamico Tradizionale di Reggio Emilia". *Ital. J. Food Sci.* **2002**, *14* (3), 247–266.
(5) Tesfaye, W.; Morales, M. L.; García-Parrilla, M. C.; Troncoso, A. M. Optimising wine vinegar production: fermetation and ageing. *Appl. Biotechnol., Food Sci. Policy* **2003**, *1* (2), 109–114.
(6) Bosso, A.; Petrozziello, M.; Santini, D.; Motta, S.; Guaita, M.; Marulli, C. Effect of grain type and toasting conditions of barrels on the concentration of the volatile substances released by the wood and on the sensory characteristics of Montepulciano d'Abruzzo. *J. Food Sci.* **2008**, *73* (7), 373–382.
(7) Cadahía, E.; Fernandez De Simón, B.; Vallejo, R.; Sanz, M.; Broto, M. Volatile compound in Spanish oak wood (*Quercus petraea* and *Quercus pyrenaica*) during natural seasoning. *Am. J. Enol. Vitic.* **2007**, *58* (2), 163–172.
(8) Fernandez de Simon, B.; Cadahía, E.; Sanz, M.; Poveda, P.; Perez-Magarino, S.; Ortega-heras, M.; González-huerta, C. Volatile compounds and sensorial characterization of wines from four Spanish denominations of origin, aged in Spanish rebollo (*Quercus pyrenaica* Willd.) oak wood barrels. *J. Agric. Food Chem.* **2008**, *56*, 9046–9055.
(9) Tesfaye, W.; Morales, M. L.; García-Parrilla, M. C.; Troncoso, A. M. Evolution of phenolic compounds during an experimental aging in wood of Sherry vinegar. *J. Agric. Food Chem.* **2002**, *50*, 7053–7061.
(10) De Rosso, M.; Cancian, D.; Panighel,. A.; Vedova, A. D.; Flamini, R. Chemical compounds released from five different woods used to make barrels for aging wines and spirits: volatiles compounds and polyphenols. *Wood Sci. Technol.* **2009**, *43* (5−6), 375–385.
(11) Fernandez de Simón, B.; Esteruelas, E.; Muñóz, A. M.; Cadahía, E.; Sanz, M. Volatiles compounds in acacia, chestnut, cherry ash and oak woods, with a view to their use in cooperage. *J. Agric. Food Chem.* **2009**, *57* (8), 3217–27.
(12) Flamini, R.; Dalla Vedova, A.; Cancian, D.; De rosso, M. GC/MS-positive ion chemical ionization and MS/MS study of volatile benzene compounds in five different woods used in barrel making. *J. Mass Spectrom.* **2007**, *42*, 641–646.
(13) Cerezo, A. B.; Tesfaye, W.; Torija, M. J.; Mateo, E.; Garcia-Parrilla, M. A.; Troncoso, A. M. The phenolic composition of red wine vinegar produced in barrels made from different woods. *Food Chem.* **2008**, *109* (3), 606–615.
(14) States, D. J.; Haberkorn, R. A.; Ruben, D. J. A two-dimensional nuclear Overhauser experiment with pure absorption phase in four quadrants. *J. Magn. Reson.* **1982**, *48* (2), 286–292.
(15) Bodenhausen, G.; Ruben, D. J. Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy. *Chem. Phys. Lett.* **1980**, *69* (1), 185–9.
(16) Frey, M. H.; Leupin, W.; Sorensen, O. W.; Denny, W. A.; Ernst, R. R.; Wuethrich, K. Sequence-specific assignment of the backbone proton- and phosphorus-31-NMR lines in a short DNA duplex with homo- and heteronuclear correlated spectroscopy. *Biopolymers* **1985**, *24* (12), 2371–80.
(17) Tesfaye, W.; Morales, M. L.; Benítez, B.; García-Parrilla, M. C.; Troncoso, A. M. Evolution of wine vinegar composition during accelerated aging with oak chips. *Anal. Chim. Acta* **2004**, *513* (1), 239–245.
(18) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.
(19) Cerezo, A. B.; Tesfaye, W.; Soria-Díaz, M. E.; Torija, M. J.; Mateo, E.; Garcia-Parrilla, M. C.; Troncoso, A. M. Wood impact on phenolic profile and sensory properties of wine vinegars during ageing. *J. Food Compos. Anal.* **2009**, in press.
(20) Saleh, M. M.; Hashem, F.; El-Megeed, Abd; Glombitza, K. W. Study of Citrus taitensis and radical scavenger activity of the flavonoids isolated. *Food Chem.* **1998**, *63* (3), 397–400.
(21) Mogel, E.; Jay-Allermand, C.; Ziegler, H. Formation of heartwood substances in the stemwood of *Robinia pseudoacacia* L.: II. Distribution of nonstructural carbohydrates and wood extractives across the trunk. *Trees* **1994**, *8*, 165–171.
(22) Ficarra, R.; Ficarra, P.; Tommasini, S.; Calabro, M. L.; Rogusa, S.; Barbera, R.; Rapisard, A. Leaf extracts of some *Cordia* species: analgesic and anti-inflammatory activities as well as their chromatographic analysis. *Farm., Ed. Sci.* **1995**, *50*, 245–256.
(23) Weinges, K. Catechins and their formation from leucoanthocyanidin hydrates. *Justus Leibigs Ann. Chem.* **1958**, *615*, 203–209.
(24) García-Parrilla, M. C.; Heredia, J. F.; Troncoso, A. M. Sherry wine vinegars: phenolic composition changes during aging. *Food Res. Int.* **1999**, *32*, 433–440.
(25) Villaño, D.; Fernández-Pachón, M. S.; Troncoso, A. M.; García-Parrilla, M. C. Comparison of antioxidant activity of wine phenolic compounds and metabolitos in vitro. *Anal. Chim. Acta* **2005**, *538*, 391–398.
(26) Fernandez-Pachón, M. S.; Villaño, D.; Troncoso, A. M.; García-Parrilla, M. C. Determination of the phenolic composition of sherry and table white wines by liquid chromatography and their relation with antioxidant activity. *Anal. Chim. Acta* **2006**, *563*, 101–108.

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