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Extraction of Polyphenols from Vine Shoots of *Vitis vinifera* by Superheated Ethanol−Water Mixtures

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A study of the nonvolatile fraction of extracts from vine shoots obtained by superheated ethanol−water mixtures is presented. The influence of the temperature, extraction time, and percentage of ethanol on extraction was investigated by a multivariate experimental design to maximize the yield of total phenolic compounds, measured by using the Folin−Ciocalteu method. The best values found for these variables were 80% (v/v) ethanol, 240 °C, and 60 min. Under these conditions, the effect of pH was also investigated, and a strong improvement of yield was observed by decreasing the pH. The extracts were subject to liquid−liquid extraction with *n*-hexane. The remaining polar phase was dried in a rotary evaporator and then reconstituted in 10 mL of water. The insoluble residue was dissolved in 10 mL of methanol. Both fractions (aqueous and methanolic) were analyzed by HPLC, and the differences in composition according to the extraction conditions were studied. Compounds usually present in commercial wood extracts were identified (mainly benzoic and hydroxycinnamic acids and aldehydes); the most abundant were quantified, and the stability of the identified phenolic families under different extraction conditions was also investigated. Finally, the superiority of the superheated liquid extraction over conventional solid−liquid extraction was demonstrated.

KEYWORDS: Phenolic compounds; vine shoots; superheated liquid extraction; wood extracts; agricultural byproducts

INTRODUCTION

Vine shoots are an agricultural byproduct very abundant in some Spanish regions, such as Castilla-La Mancha, where their production is estimated between 600 000 and 1 000 000 Tm every year (*1*). At present, vine shoots do not have any significant economic value as they are traditionally used as a heating source or cast upon the ground to rot. During the past 15−20 years, the possibility of employing this material for paper production has been studied, although some shortcomings for this application in comparison with other agricultural byproducts were found (*2*, *3*). An alternative for exploitation of this residue, which would increase considerably its economic value, would be its use as a source of polyphenols.

The distribution of phenolic compounds in vine shoots depends on several factors such as grapevine variety, age, growth conditions, or exposition to fungus and mold infections, among others. They are found as nonstructural components (extractives) or forming part of lignin, constitute about 20% of the dry weight of vine shoots (*3*), and present a three-dimensional amorphous structure produced by polymerization of coniferyl, sinapyl, and *p*-coumaryl alcohol units (*4*). In general, their degradation yields phenolic aldehydes, ketones, acids (*5*), and phenols (*6*). These products have potential industrial application, such as tanning and dyeing of leather (*7*) or production of chemical intermediaries in the pharmaceutical and agricultural industries (*8*). Due to their allelopathic properties, they could also be used for controlling weed growth, thus reducing the use of herbicides (*9*), and their antioxidant and free radical scavenger activities make them substances with potential health benefits (*10*−*12*).

Stilbenes have also healthy effects (*13*). Studies carried out with vineatrol, a fraction enriched in stilbenes isolated specifically from young grapevine shoots extracts, have shown anticancer effect (even higher than that of resveratrol) (*14*) and promising results for epilepsy treatment (*15*).

Polyphenolic extracts from vine shoots can have a key application in the winemaking industry. The aging of wine is traditionally performed mainly in oak, but also chestnut, wood barrels. During the process, ethanol reacts with lignin and leachs out aromatic aldehydes (vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde, fundamentally), which are partially oxidize to the corresponding acids (*16*). The phenolic content of wine is also enriched with hydrolyzable tannins and their hydrolysis products (gallic and ellagic acids) present in these woods (*17*). Some countries not integrated in the International Organisation of Vine and Wine (OIV) authorize the addition of wood extracts (from oak wood, principally) to wines with the aim of modifying their color and flavor and/or accelerating aging processes. In this way, the wine remains in barrel for

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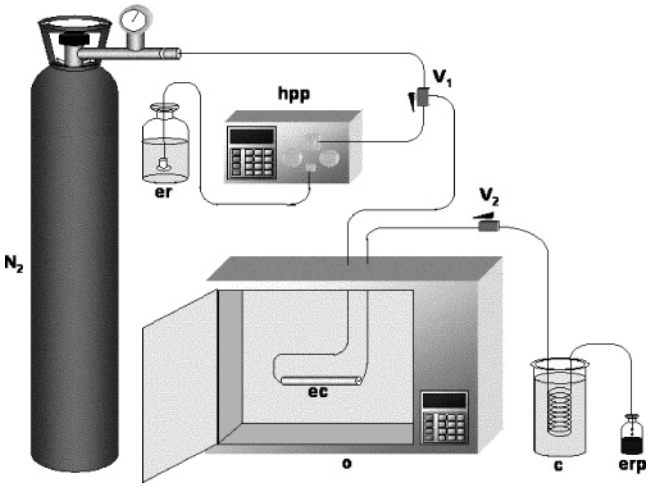

Figure 1. Extraction system used: hpp, high-pressure pump; er, extractant reservoir; ec, extraction cell; o, oven; c, cooler; V_1, selection valve; V_2, restriction valve; erp, extract receptacle

shorter times and the production cost is reduced. Nevertheless, European legislation only authorizes this addition for accelerating the aging of certain brandies (*18*) and only allows the use of hydroalcoholic extracts with this aim (*19*).

Our research group has checked and patented (*20*) the feasibility of using superheated ethanol−water mixtures to obtain phenolic compounds from oak wood. In superheated liquid extraction, a pressure high enough to maintain the extractant in liquid state above its boiling point is applied; under these conditions the extractant polarity decreases, and its contact with the sample increases as a result. These facts accelerate the physical−chemical processes of cleaving of bonds, mass transfer, and solubilization of the resulting compounds, increasing the efficiency of the process. Also, it offers the possibility of performing extractions in an inert atmosphere protected from light, which represents a great advantage as phenolic compounds are very sensitive to these two factors. All of these characteristics, together with both time and solvent consumption reduction, make superheated liquid extraction enormously more effective than conventional solid−liquid extraction (*21*, *22*). In the present work, this technique has been applied to obtain rich-in-polyphenols extracts from vine shoots, and the nonvolatile fractions have been studied in depth by HPLC to determine the differences in composition depending on the extraction conditions. The stability of the identified phenolic families has also been studied.

MATERIALS AND METHODS

Apparatus. Vine shoots were milled with a grinder (Moulinex D56, Barcelona, Spain).

Superheated liquid extractions were performed with the approach depicted in **Figure 1**, which consists of the following units: (a) an extractant reservoir; (b) a high-pressure pump (Shimadzu LD-AC10), which propels the extractant through the system; (c) a selection valve (V_1) located next to the pump, which allows the extract to be flushed out with dry N_2 after extraction; (d) a stainless steel cylindrical extraction chamber (200 mm × 10 mm i.d., 15 mL internal volume), where the sample is placed [this chamber is closed at both ends with screws having caps that contain stainless steel filter plates (1 mm thick, 12 mm diameter) to ensure the sample is not carried away by the extractant]; (e) a restriction valve (V_2) to maintain the preset pressure in the system; (f) a cooler made of stainless steel tubing (1 m length, 0.4 mm i.d.) and refrigerated with water; (g) a gas chromatograph oven (Konix, Cromatix KNK-2000) where the extraction chamber is placed and heated.

Shaking and centrifugation of the extracts were carried out by means of an MS2 Minishaker (IKA, Germany) Vortex and a Mixtasel (Selecta, Barcelona, Spain) centrifuge, respectively.

Absorbance of the extracts was measured by an Agilent 8453E UV−visible spectrometer (Waldbronn, Germany), and the extracts were analyzed by a modular 1100 Hewlett-Packard liquid chromatograph (Pittsburgh, PA), consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, a 7725 Rheodyne high-pressure manual injection valve (HPIV), and a G1315A diode array detector.

Statgraphics plus v. 5.1 for Windows was used for the multivariate studies.

Reagents. Vine shoots were from Manzanares (Ciudad Real, Spain). They were dried for 24 h at 105 °C and then milled to get a 40-mesh particle size (<0.42 mm diameter). Ethanol 96% (v/v) PA from Panreac (Barcelona, Spain) and distilled water were used for preparing the different ethanol−water mixtures, and *n*-hexane (LiChrosolv, Merck, Darmstadt, Germany) was used to perform liquid−liquid extraction; methanol and acetic acid (both of HPLC grade and supplied by Merck) solutions were the mobile phases. Ultrapure water was obtained from a Millipore (Bedford, MA) Milli-Q plus system. (+)-Catechin, vanillin (4-hydroxy-3-methoxybenzaldehyde), syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), coniferaldehyde (4-hydroxy-3-methoxycinnamaldehyde), sinapaldehyde (4-hydroxy-3,5-dimethoxycinnamaldehyde), acetovanillone [1-(4-hydroxy-3-methoxyphenyl)ethanone], acetosyringone [1-(4-hydroxy-3,5-dimethoxyphenyl)ethanone], furfural (2-furancarboxaldehyde), 5-hydroxymethylfurfural (5-hydroxymethyl-2-furancarboxaldehyde), pyrogallol (1,2,3-trihydroxybenzene), pyrocatechol (1,2-dihydroxybenzene), phenol, guaiacol (2-methoxyphenol), syringol (2,6-dimethoxyphenol), and gallic (3,4,5-trihydroxybenzoic acid), protocatechuic (3,4-dihydroxybenzoic acid), *p*-hydroxybenzoic, vanillic (4-hydroxy-3-methoxybezoic acid), syringic (4-hydroxy-3,5-dimethoxybenzoic acid), *p*-coumaric (4-hydroxycinnamic acid), ferulic (4-hydroxy-3-methoxycinnamic acid), sinapic (4-hydroxy-3,5-dimethoxycinnamic acid), and ellagic acids used as chromatographic standards were from Sigma-Aldrich (St. Louis, MO), as was *p*-cresol (1-hydroxy-4-methylbenzene), used as external standard. The nitrogen for dragging the extract from the extraction cell was supplied by Carburos Metálicos (Barcelona, Spain).

Superheated Ethanol−Water Extraction (SEWE). One gram of vine shoots was placed in the extraction cell and the cell in the oven; then, the pump was turned on with a high flow rate (5 mL/min) to fill the cell quickly (3 min). To ensure the absence of air in the system, valve V_2 was kept open until the first drop of extractant appeared. At that moment, valve V_1 was closed, the pump was turned off, and the oven was turned on. During the rise of the temperature, valve V_2 had to be opened at short intervals to prevent the pressure from surpassing the working value. When the selected temperature and pressure values were reached, valve V_1 was also closed and static extraction was performed for the preset time. Finally, the oven was turned off, the chamber was cooled to below the boiling point of ethanol before valve V_2 was opened, and valve V_1 was switched to enable dry nitrogen to flow through the cell and flush out the extract.

Study of Stability. Four standard solutions were prepared by dissolving gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic, and sinapic acids, as well as vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, furfural, and 5-hydroxymethylfurfural (100 ppm each) in 80% (v/v) aqueous ethanol, pH 3; 80% (v/v) aqueous ethanol, pH 11; 20% (v/v) aqueous ethanol, pH 3; and 20% (v/v) aqueous ethanol, pH 11. Aliquots of each solution were superheated for 60 min at 100 bar and 120, 180, and 240 °C, for which they were pumped into the extraction cell and subjected to the procedure described in the previous section.

Conventional Solid−Liquid Extraction (SLE). For the study of the extraction kinetics at room temperature, 3-g vine shoot samples were extracted in triplicate with 45 mL (1 g/15 mL) of 80% (v/v) aqueous ethanol at pH 3. The extractions were performed for 1, 2, 3, 4, 6, 24, and 48 h, in the absence of light and with drastic stirring.

Determination of Total Phenolics According to the Folin−Ciocalteu Method. The amount of total phenolics was measured by means of the Folin−Ciocalteu method using gallic acid as standard, for which a calibration curve was carried out with solutions of 100,

200, 300, 400, 500, and 600 mg/L of this compound ($y = 0.0025x + 0.0405$, $R^2 = 0.9998$). A 0.5-mL aliquot of dilute extract (all of the extracts were diluted with distilled water to adjust the absorbance within the calibration limits), 10 mL of distilled water, 1 mL of Folin−Ciocalteu reagent, and 3 mL of Na_2CO_3 (20%, w/v) were mixed in this order, made up to 25 mL by distilled water, and heated at 50 °C for 5 min. After 30 min, the absorbance was measured at 765 nm against a blank similarly prepared, but containing distilled water instead of extract. The concentration of phenolics thus obtained was multiplied by the dilution factor of the extract volume and divided by the amount of vine shoots used. In this way, the results were expressed as the equivalent to milligrams of gallic acid per gram of vine shoots (mg of GAE/g).

Treatment of Extracts and Standard Solutions. The extracts from SEWE were subjected to two liquid−liquid extractions with *n*-hexane (2 × 10 mL, 5 min of shaking, and 5 min of centrifugation at 4000 rpm) to remove nonpolar compounds, which could complicate the subsequent chromatographic separation. The hydroalcoholic extracts were dried in a rotary evaporator and then reconstituted in 10 mL of water. The residue insoluble in water was dissolved in 10 mL of methanol. In this way, the original extract was divided into two fractions: an aqueous fraction (AF), which contained the most polar compounds, and a methanolic fraction (MF), which contained the less polar ones. The aim of this step was to facilitate the separation, identification, and quantification of the compounds extracted. Finally, each fraction was filtered using a 0.45-μm filter before injection into the chromatograph.

The conventional SLE extracts were subjected to the process described above to obtain the different fractions using 100 mL of *n*-hexane (2 × 50 mL).

Standard solutions for the study of stability were also liquid−liquid extracted such as those from SEWE, although they were not divided into fractions.

HPLC Analysis. The separation of analytes both from the extracts and from the standard solutions was performed on an Ultrabase C-18 column (250 mm × 4.6 mm i.d., 5-μm particle, Análisis Vínicos, Tomelloso, Ciudad Real, Spain), using an injection volume of 20 μL and a flow rate of 1 mL/min. Mobile phase A consisting of 6% (v/v) acetic acid in 2 mM sodium acetate aqueous solution (pH 2.5) and mobile phase B consisting of methanol were used. For AF and standard solutions a linear gradient from 0 to 1% B in 10 min, from 1 to 4% B in 3 min, from 4 to 11% B in 6 min, from 11 to 21.5% B in 11 min, from 21.5 to 59.5% B in 13 min, and from 59.5 to 100% B in 10 min was used. For MF a linear gradient from 10 to 28% B in 7 min, from 28 to 46% B in 10 min, from 46 to 64% B in 30 min, and from 64 to 100% B in 20 min was used. The analytes were identified by comparing both their retention times and UV spectra with those of the corresponding standards. The absorption wavelengths were set at 260 nm for monitoring ellagic acid, at 280 nm for hydroxybenzoic acids, catechin, and phenolic aldehydes; at 320 nm for hydroxycinnamic acids; and at 360 nm for hydroxycinnamic aldehydes.

RESULTS AND DISCUSSION

Study of the Extraction Variables. The influence of the percentage of ethanol, temperature, and time on the extraction was studied to maximize the yield of phenolic compounds extracted from wood in a time as short as possible. The tested ranges and the selected values are shown in **Table 1**, as well as detailed information about the designs used and the results obtained. The applied pressure was enough high (100 bar) to guarantee the liquid state of the ethanol−water mixtures in all instances. The response measured in each extract to know the efficiency of the extraction in each experiment was the total amount of phenols using the Folin−Ciocalteu method.

A complete factorial design was selected for the first approach. The results showed that the three variables had a significant positive effect. Therefore, the highest value of each variable was chosen as the lowest value in a second complete factorial design. Data analysis of this showed that the only significant variable was the temperature; the percentage of ethanol and extraction time had negative and positive effects, respectively. According to its effect, 80% ethanol (v/v) was selected. However, in the case of the time, the most reasonable option was selecting the shortest (60 min), because the increase of efficiency was not significant. Under these conditions, higher temperatures (270 and 300 °C) were tested, thus increasing the amount of total phenolics extracted. Nevertheless, two trends were also detected, which made inadvisable the use of temperatures above 240 °C, as discussed in the following sections: the strong increase of the burnt wood smell of the extract and

Table 1. Description of the Experimental Designs Used and Results Obtained

design	variable	tested range: first screening	tested range: second screening	selected conditions
multivariate	ethanol (%)	20–80	80–100	80
	temperature (°C)	120–180	180–240	240
	time (min)	20–60	60–90	60
univariate	pH	1–13		3

First Complete Factorial Design

run order	ethanol	temperature	time	mg of GAE/g of vine shoots
1	0	0	0	17
2	1	1	–1	20
3	–1	1	1	18
4	0	0	0	16
5	0	0	0	17
6	–1	1	–1	13
7	1	–1	1	15
8	1	–1	–1	9
9	–1	–1	1	10
10	1	1	1	25
11	–1	–1	–1	8

effect	sum of squares	DF	mean square	*F* ratio	*P* value
ethanol	50.0	1	50.0	18.72	0.0124
temperature	144.5	1	144.5	54.11	0.0018
time	40.5	1	40.5	15.17	0.0176
total error	10.6818	4	2.67045		
total (corr)	256.182	10			

$R^2 = 95.8304\%$ R^2 (adj for DF) $= 89.5759\%$

Second Complete Factorial Design

run order	ethanol	temperature	time	mg of GAE/g of vine shoots
1	0	0	0	30
2	1	–1	1	24
3	–1	–1	–1	25
4	–1	1	–1	38
5	1	–1	–1	22
6	0	0	0	27
7	–1	–1	1	27
8	1	1	1	34
9	1	1	–1	31
10	–1	1	1	41
11	0	0	0	35

effect	sum of squares	DF	mean square	*F* ratio	*P* value
ethanol	50.0	1	50.0	6.05	0.0124
temperature	264.5	1	264.5	32.02	0.0018
time	12.5	1	12.5	1.51	0.0176
total error	33.0455	4	8.26136		
total (corr)	368.545	10			

$R^2 = 91.0335\%$ R^2 (adj for DF) $= 77.5839\%$

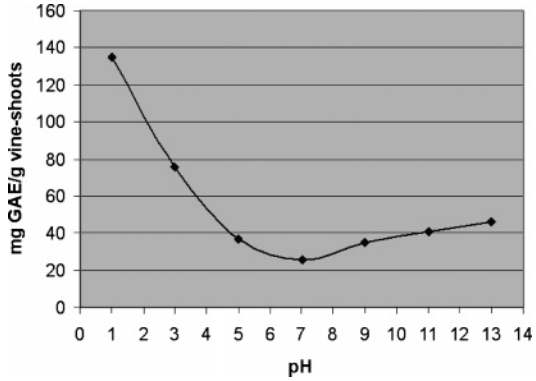

Figure 2. Influence of pH on the total amount of phenolic compounds in SEWE at 240 °C (80% ethanol, 60 min).

the decrease in the concentration, or even the disappearance, of potentially healthy compounds. Therefore, 240 °C was the temperature selected.

Influence of pH. The influence of pH on the yield of the process under the selected conditions was also investigated by a univariate approach. Thus, extractions were carried out adjusting the pH of the extractant at 1, 3, 5, 7, 9, 11, and 13. **Figure 2** shows that the yield of phenolic compounds enormously increased when the pH was decreased. This result agrees with bibliographic data, which report that the dilute acidified hot water processing of lignocellulosic materials facilitates the breakage of ether linkages in lignin, especially under high-temperature conditions, generating a great number of low molecular weight phenols (*23*, *24*). Nevertheless, an enormously fast corrosion of capillary tubes of the system, which caused even their perforation several times, was produced after only a few extractions at pH 1. Consequently, a minimum pH of 3 was used, for which no trace of corrosion was detected after numerous extractions.

Precision of SEWE. The precision of the SEWE, expressed as repeatability and reproducibility, was calculated for total phenolics. Within-day assays (intraday) and between-day assays (interday) were developed over a 7-day period. Two extractions under the selected working conditions were carried out every day, one in the early morning and another in the evening. The intraday assay variability (RDS) was 9.7%, and the interday assay variability was 10.4%.

Stability Study. This study was carried out with the aim of clarifying the processes responsible of the different compositions of the SEWE extracts depending on the working conditions. Solutions of 14 compounds identified by HPLC in SEWE extracts (see the following section) were selected as models. They were dissolved in the two more different media used as extractants and at an acid and basic pH (viz., 80 and 20% aqueous ethanol and pH values of 3 and 11, respectively). Aliquots of these solutions were subjected to 120, 180, and 240 °C for 60 min. The results obtained are shown in **Table 2**.

At 120 °C, the recoveries were very similar for all of the solutions, except 20% aqueous ethanol and pH 11. All of the aldehydes and benzoic acids (except gallic acid) were practically stable; hydroxycinnamic and gallic acids were partially degraded, particularly in acid and basic media, respectively. In 20% aqueous ethanol and pH 11, only benzoic aldehydes and acids provided recoveries >90%, whereas gallic, ferulic, and sinapic acids and furfural were widely degraded.

At 180 °C, aldehydes hardly suffer degradation, except in 20% ethanol and basic medium. In this case, hydroxycinnamic aldehyde recoveries decreased dramatically, whereas vanillin and syringaldehyde recoveries increased significantly as compared with those under the other working conditions. This behavior is probably due to the oxidative cleavage of the C=C bond of coniferaldehyde and sinapaldehyde, yielding vanillin and syringaldehyde, respectively, which also explains the disappearance of *p*-coumaric, ferulic, and sinapic acids. As the increase in benzoic aldehydes did not correspond to the decrease of hydroxycinnamic aldehydes and acids, vanillin and syringaldehyde must suffer other reactions, such as ethyl hemiacetal formation (favored by an acid medium) or oxidation to the corresponding acids (favored by a basic medium). The recoveries of acids decreased in all instances with respect to those obtained at 120 °C, particularly in 20% aqueous ethanol and pH 11. These decreases could be caused by the formation of the corresponding ethyl esters, in both acid and basic media (*25*). Also, pyrogallol, pyrocatechol, phenol, guaiacol, and syringol were detected in 20% aqueous ethanol solutions, a fact previously described in the bibliography (*26*), which denotes decarboxylation of gallic, protocatechuic, *p*-hydroxybenzoic, and vanillic acids and syringaldehyde, respectively.

At 240 °C the highest recoveries for acids were obtained in 80% aqueous ethanol and pH 3, which explains that an acid medium is more influential than the presence of ethanol for preserving these compounds at this temperature. On the other hand, the amounts of pyrogallol, pyrocatechol, phenol, guaiacol, and syringol increased with respect to those obtained at 180

Table 2. Stability after Superheating for 60 min of the Main Phenolic Compounds Identified in SEWE Extracts (Data Expressed as Percent of Recovery)

	80% (v/v) aqueous ethanol						20% (v/v) aqueous ethanol					
	pH 3			pH 11			pH 3			pH 11		
compound	120 °C	180 °C	240 °C	120 °C	180 °C	240 °C	120 °C	180 °C	240 °C	120 °C	180 °C	240 °C
gallic acid	84 ± 3	79 ± 3	<5	49 ± 2	29 ± 1		88 ± 3	53 ± 2		29.4 ± 0.3	25 ± 1	
protocatechuic acid	96 ± 3	95 ± 3	14 ± 1	98 ± 3	62 ± 4		96 ± 6	75 ± 3		95 ± 6	80 ± 3	
p-hydroxybenzoic acid	98 ± 4	98 ± 1	60 ± 1	99 ± 1	98 ± 2	29 ± 1	98 ± 2	94 ± 4	11.0 ± 0.1	100 ± 2	81 ± 3	
vanillic acid	91 ± 2	82 ± 2	57 ± 2	93 ± 2	99 ± 4	26 ± 1	100 ± 4	89 ± 2	<5	80 ± 3	60 ± 2	
syringic acid	99 ± 3	104 ± 4	44 ± 1	104 ± 4	108 ± 3	33 ± 1	98 ± 3	87 ± 3	5.5 ± 0.2	96 ± 3	63 ± 2	
p-coumaric acid	80 ± 2	68 ± 2	<5	98 ± 3	83 ± 3		92 ± 4	48 ± 1		62 ± 2	7.4 ± 0.2	
ferulic acid	85 ± 4	68 ± 3	<5	89 ± 4	63 ± 1		77 ± 1	23 ± 1		26 ± 0.4		
sinapic acid	58 ± 2	50 ± 1	7.4 ± 0.2	62 ± 2	47 ± 1		51 ± 1	9.7 ± 0.4		5.7 ± 0.2		
vanillin	101 ± 2	99 ± 3	91 ± 3	101 ± 3	103 ± 4	101 ± 2	101 ± 4	100 ± 2	98 ± 3	103 ± 4	150 ± 2	146 ± 5
syringaldehyde	105 ± 3	102 ± 3	90 ± 3	102 ± 3	100 ± 3	96 ± 2	102 ± 4	101 ± 3	102 ± 3	92 ± 3	148 ± 4	145 ± 5
coniferaldehyde	97 ± 3	96 ± 3	58 ± 2	98 ± 3	96 ± 3	88 ± 3	99 ± 3	98 ± 3	84 ± 3	77 ± 2	6.0 ± 0.2	
sinapaldehyde	96 ± 4	96 ± 4	55 ± 2	101 ± 4	98 ± 3	85 ± 3	97 ± 3	96 ± 4	81 ± 3	75 ± 2	6.1 ± 0.2	
furfural	101 ± 4	96 ± 3	93 ± 3	98 ± 3	98 ± 3	98 ± 3	100 ± 3	101 ± 4	102 ± 3	44 ± 1	<5	
5-hydroxymethylfurfural	103 ± 4	101 ± 4	86 ± 3	101 ± 4	102 ± 3	100 ± 3	100 ± 3	98 ± 3	92 ± 4	69 ± 2	16 ± 1	

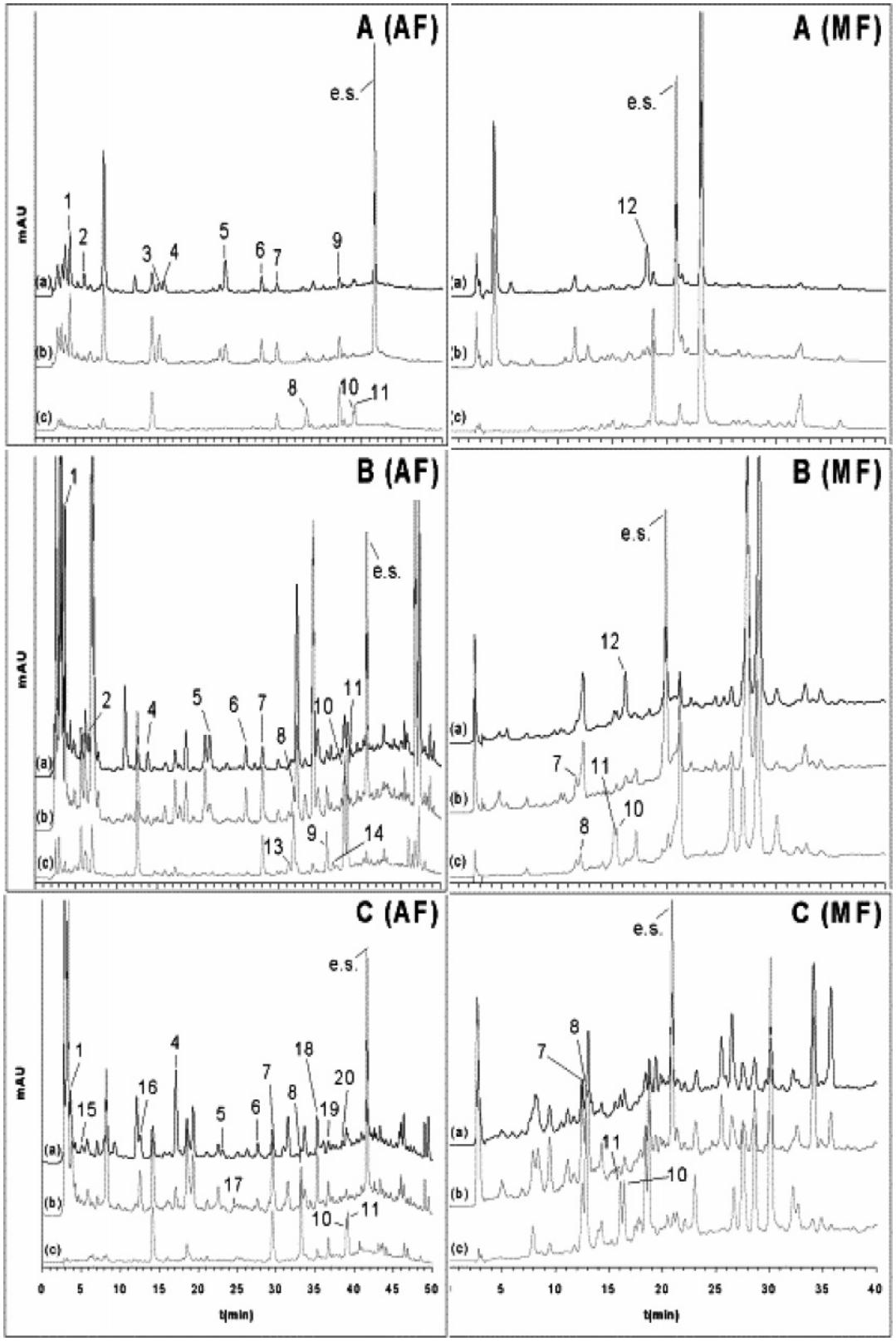

Figure 3. Chromatograms at 260 (**a**), 280 (**b**), and 320 nm (**c**) of the AF and MF of the extracts obtained under the following conditions: (**A**) 80% ethanol, room temperature (25 °C), 24 h; (**B**) 80% ethanol, pH 3, 180 °C, 60 min; (**C**) 80% ethanol, pH 3, 240 °C, 60 min. Peaks: 1, gallic acid; 2, protocatechuic acid; 3, catechin; 4, *p*-hydroxybenzoic acid; 5, vanillic acid; 6, syringic acid; 7, vanillin; 8, syringaldehyde; 9, ferulic acid; 10, coniferaldehyde; 11, sinapaldehyde; 12, ellagic acid; 13, *p*-coumaric acid; 14, sinapaldehyde; 15, pyrogallol; 16, furfural; 17, 5-hydroxymethylfurfural; 18, acetovanillone; 19, acetosyringone; 20, syringol; e.s., *p*-cresol.

°C, from which a higher decarboxylation is inferred. The behavior of the aldehydes was different depending on the type. Benzoic aldehydes increased dramatically with respect to the behavior at 180 °C in 20% aqueous ethanol at pH 11. Under the highest temperature of these experiments and 80% aqueous ethanol, pH 3, the decline was gradual, whereas in 80% aqueous

Table 3. Yields of the Main Identified Phenolic Compounds Extracted from Vine Shoots by 80% (v/v) Aqueous Ethanol, pH 3 (Expressed as Micrograms per Gram of Vine Shoots)

	SEWE[a]			conv SLE[b]
compound	120 °C	180 °C	240 °C	25 °C
gallic acid	470 ± 41	508 ± 37	85 ± 6	50 ± 3
ellagic acid	53 ± 2	57 ± 5		17.9 ± 0.4
protocatechuic acid	379 ± 29	23 ± 2		94 ± 4
vanillic acid	73 ± 4	96 ± 8	57 ± 5	72 ± 4
syringic acid	110 ± 8	113 ± 9	53 ± 5	64 ± 2
ferulic acid	28 ± 3	32 ± 3	11 ± 1	9.1 ± 0.7
vanillin	101 ± 10	133 ± 9	153 ± 13	52 ± 2
syringaldehyde	61 ± 4	108 ± 10	145 ± 16	32 ± 1
coniferaldehyde	82 ± 6	133 ± 6	107 ± 8	8.9 ± 0.2
sinapaldehyde	91 ± 8	161 ± 10	121 ± 6	6.8 ± 0.2

[a] Extraction time = 60 min. [b] Extraction time = 24 h.

ethanol, pH 11, and 20% aqueous ethanol, pH 3, similar recoveries were obtained. Hydroxycinnamic aldehydes greatly decreased in 80% ethanol, pH 3, and completely disappeared at 20% aqueous ethanol, pH 11. Furanic aldehydes were practically stable except at 20% aqueous ethanol, pH 11, at which they were not detected.

In summary, the data show that benzoic acids probably disappear by esterification with ethanol (mainly) and decarboxylation, whereas hydroxycinnamic acids are esterified or degraded to the corresponding benzoic aldehydes. All of these changes are higher above 180 °C and in basic medium. The reactions for aldehydes probably included ethyl hemicetal formation (favored in acid media), oxidation to acids (basic medium), and hydroxycinnamic aldehyde degradation to the corresponding benzoic aldehydes. The influence of ethanol percentage and pH is higher when the temperature increases, and 80% aqueous ethanol and pH 3 are the conditions under which these compounds suffer fewer alterations.

Identification and Quantification of Phenolic Compounds in SEWE Extracts. The AF and MF fractions of all the extracts from the experiments in the optimization study (analyzed by using the Folin−Ciocalteu method) were analyzed by HPLC to know the evolution of extracts composition as a function of the working conditions of SEWE.

The analysis of both multivariate designs showed that the temperature was the most influential variable on the composition of the extracts, whereas the percentage of ethanol and extraction time had only an influence on the amount of each compound. Compounds usually present in both wines aged in oak barrels and commercial wood extracts can be identified (*27*, *28*). Compounds such as gallic, *p*-hydroxybenzoic, vanillic, syringic, ferulic, and sinapic acids and vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde were found in all AF extracts; protocatechuic and *p*-coumaric acids were detected in only the extracts obtained at 180 °C or below this temperature; acetovanillone, acetosyringone, furfural, pyrogallol, and syringol were detected only above 180 °C; and 5-hydroxymethylfurfural was identified only at 240 °C. In the MF fraction, only vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, and ellagic acid were identified: the first four appeared in all extracts and the fifth only in those obtained at temperatures below 180 °C (**Figure 3**). On the other hand, the extracts obtained in the study of pH showed similar chromatographic profiles, differing only in the magnitude of the signals.

The most relevant phenolic compounds (viz., gallic, ellagic, protocatechuic, vanillic, syringic, and ferulic acids, vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde) were quantified using *p*-cresol as external standard (**Table 3**). The

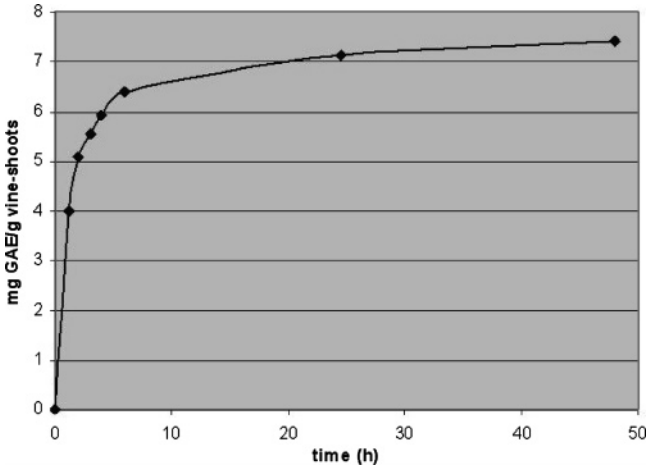

Figure 4. Conventional SLE kinetics with 80% (v/v) aqueous ethanol, pH 3.

results, together with those from the stability study, allowed the following conclusion: (a) the presence of gallic and ellagic acids demonstrates that vine shoots contain hydrolyzable tannins, which were hydrolyzed under superheated conditions; (b) the highest yields of these compounds were obtained at 180 °C; (c) above this temperature, degradation starts and pyrogallol is generated; (d) the amount of gallic acid at 240 °C (both in acid and in basic medium) was higher than expected according to the stability study, which suggests that hydrolysis of tannins occurs simultaneously with esterification and decarboxylation reactions of gallic acid.

Other phenolic compounds identified were mainly from lignin degradation products generated by breaking of β-aryl ether bonds of syringyl and guaiacyl nuclei. The yield of syringic and vanillic acids was increasing up to 180 °C, above which the yield decreased very slightly, indicating that the production rate was similar to their disappearance rate. The yield for all aldehydes increased with temperature. These results enable one to conclude that raising the temperature under the working conditions increases the lignin degradation rate, but the differences observed within extracts are due to secondary reactions of the resulting phenolic compounds (aldehydes) and not to changes of the degradation processes.

On the other hand, furanic derivatives result from sugar degradation. Pentoses (main constituents of hemicellulose) yield furfural, and hexoses from cellulose generate 5-hydroxymethylfurfural. The lower temperature at which furfural is detected as compared with 5-hydroxymethylfurfural can be ascribed to the lower stability of hemicellulose to hydrothermal treatment than cellulose (*29*).

Comparison with Conventional SLE. Figure 4 shows the conventional SLE kinetics carried out with 80% (v/v) aqueous ethanol, pH 3. All compounds in these extracts, except catechin, were also in the SEWE extracts; the latter extracts had very much higher contents of quantifiable phenolic compounds and total phenolic contents (**Table 3**), which indicates that the extraction of the nonvolatile phenolic compounds from vine shoots under superheated conditions was enormously more effective.

Conclusions. The capability of superheated ethanol−water mixtures to extract nonvolatile phenolic compounds from vine shoots has been tested, and a study of the stability for several phenolic families under these working conditions has been carried out. The following conclusions have been reached: (a) There is a dramatic yield enhancement and shortening of the

extraction time over conventional SLE. (b) Nonvolatile phenolic acids and aldehydes usually found in vegetables, fruits, and wood extracts are present in the SEWE extracts from vine shoots, and the most relevant have been quantified. (c) The composition of the extracts is highly dependent on the extraction conditions; extracts obtained under the working conditions that provided a higher total phenolic content are especially rich in low molecular mass phenolic compounds from lignin degradation (e.g., vanillin and syringaldehyde); extracts obtained under gentler working conditions are enriched in phenolic acids, particularly those from the hydrolysis of tannins. Therefore, superheated liquid extraction allows manipulation of the extracts' composition (e.g., enrichment in one or several families of phenolic compounds) by changing the extraction variables.

These results lead one to conclude that industrial exploitation of this agricultural byproduct to obtain extracts rich in polyphenols can be an excellent alternative to commercial wood extracts traditionally used in wineries; to alimentary additives used to modify the flavor and color properties of foods or avoid their oxidation; or as a cheap source of chemicals for nutraceutical industries.

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