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# Changes in contents of phenolic compounds during maturing of barrique red wines

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**Abstract**

Concentrations of derivatives of benzoic and cinnamic acids and furaldehyde were studied during maturing of a red wine (a mixture of *Cabernet Sauvignon* and *Merlot*) in barrique barrels (*Quercus robur*). Samples were taken at three-week intervals over 6 months. The influence of degree of toasting of the wood on the amount of phenolic compounds in barrique wine was also investigated. The samples were pre-separated using a solid-phase extraction on an RP 105 polymeric sorbent and analysed by high-performance liquid chromatography with UV-DAD detection. Gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, benzoic and ellagic acids and *p*-hydroxybenzaldehyde, vanillin, 2-furaldehyde, 5-methoxy-2-furaldehyde and 5-methyl-2-furaldehyde were identified in the extracts of natural and toasted wood chips and in the extracts of the wine. Syringaldehyde was identified only in the extracts of the toasted wood chips. Ellagic acid can be regarded as a characteristic compound of barrique wine ageing and its constant level during some periods could become a marker of maturity of barrique wines. Due to the absence of furaldehydes in natural wines, these compounds can be considered as typical components of barrique wines and so they can serve as a marker of authenticity of barrique wines.


*Keywords:* Barrique wines; Phenolic compounds; Benzoic acid derivatives; Cinnamic acid derivatives; Furaldehydes; Authenticity markers

## 1. Introduction

The production of barrique wines is a modern way of a further processing of natural red and white wines. The maturing of the wines in toasted oak-barrels changes them and emphasises some of their characteristics. The wines exhibit a taste typical of barrique wines.

Phenolic compounds are secondary metabolites naturally present in wine grapes and/or produced during the winemaking process. The characteristic spectrum of phenolic compounds in barrique wines depends on the origin of the natural wine, the species and origin of wood, the characteristics of the barrel, and on the circumstances and duration of the winemaking process (Afonso, 2002; Cadahia, Munoz, de Simon, & Garcia-Vallejo, 2001; Chatonnet & Dubourdieu, 1998; Mosedale & Puech, 1998; de Simon, Cadahia, Conde, & Garcia-Vallejo, 1996).

Derivatives of benzoic and cinnamic acids are the main phenolic compounds that give the typical character to the barrique wines. Further compounds found in the wines are 2-furaldehyde and its derivatives, 5-methoxy-2-furaldehyde and 5-methyl-2-furaldehyde, the products of the degradation of sugars during toasting of the wood (Goldberg, Hoffman, Yang, & Soleas, 1999; Ho, Hogg, & Silva, 1999; Kadim & Mannheim, 1999; Laszlavik, Gól, Misik, Erdei, & L K, 1995). The presence of products of lignin hydrolysis (derivatives of 2- furaldehyde and vanillin) in wine is at sub-threshold concentration due to chemical transformation with no significant

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contribution to the wine aroma (Singleton, 1995; Spillman, Pollnitz, Liacopoulos, Skouroumounis, & Sefton, 1997, 1998). However, maturing of wine in oak casks, and mainly in toasted barrels, changes the content of phenolic compounds and so these become some of the most important compounds of wine ageing in toasted casks (Cutzach, Chatonnet, & Dubourdoeu, 2000; Laszlavik et al., 1995; Pisarnitskii, 1995; Tesfaye, Morales, Garcia-Parrilla, & Troncoso, 2002).

Phenolic compounds contribute directly or indirectly to colour, astringency, bitterness, and aroma of barrique wines. Recently, more attention has been paid to these substances because of their antioxidant properties (Flesch, Schwarz, & Bohm, 1998; Hollman, 2001; Shrikhande, 2000). Determination of this group of compounds is important since they can characterize variations in wine types and styles and differences in winemaking and maturation processes.

Traditional separation techniques include liquid–liquid extraction (Laszlavik et al., 1995; Malovaná, García Montelongo, Pérez, & Rodríguez-Delgado, 2001) with water-immiscible solvents (diethylether, ethylacetate). Solid-phase extraction (SPE) has become the preferential technique used for isolation, purification and preconcentration. Sorbents based on alkylated silica gel $C_{18}$ (Andrade, Mendes, Falco, Valentao, & Seabra, 2001; Escalona, Birkmyre, Piggott, & Paterson, 2002; Malovaná et al., 2001) or combinations of two cartridges with different sorbents ($C_{18}$ and quaternary amine-SAX) are the most popular (Guillén, Barroso, & Pérez-Bustamante, 1996, 1997; Merello, Guillén, Barroso, & Pérez-Bustamante, 1997). Polymeric sorbents have been shown to be preferable for extraction of phenolic compounds (Chilla, Guillén, Barroso, & Pérez-Bustamante, 1996; Klejdus & Kubáň, 2000; Samanidou, Antoniou, & Papadoyannis, 2001). The advantages of SPE compared to liquid–liquid extraction are that SPE is faster and more reproducible and cleaner extracts are obtainable.

In this study, time-dependent changes in the spectrum of phenolic compounds and furaldehydes in one natural red wine (an 80:20 v/v mixture of *Cabernet Sauvignon* and *Merlot*) matured in middle- and highly toasted oak-barrels were investigated. Two oak species (*Quercus robur* and *Quercus alba*) were compared in a model experiment in order to characterize the kinetics of extraction of these compounds from the toasted wood to wine.

## 2. Materials and methods

### 2.1. Reagents

Phenolic compounds – gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, benzoic acid, ferulic acid, sinapic acid, cinnamic acid, ellagic acid, *p*-hydroxybenzaldehyde, syringaldehyde, vanillin, 2-furaldehyde, 5-methyl-2-furaldehyde and 5-methoxy-2-furaldehyde were purchased from Fluka (Fluka Chemie, Buchs, Switzerland). Stock standard solutions of each of the compounds ($c = 0.02$ mg ml$^{-1}$) were prepared in mobile phase (except of ellagic acid, that was prepared in methanol) and stored in a refrigerator.

Acetic acid and methanol of gradient-grade purity, supplied by Merck (Darmstadt, Germany), were used as solvents for chromatography. De-mineralised water, obtained using an Aqua-Dem 2 (Aqua-Osmotic, Tišnov, Czech Republic) was further purified using a MILLI-Q RG (Millipore, Bedford, MA, USA). Diethylether was obtained from Lach-Ner a.s. (Neratovice, Czech Republic).

Polymeric sorbent (polystyrene–divinylbenzene copolymer) RP-105 Resin (200 mg packing in cartridge, Applied Separations, Allentown, IL, USA) was used for solid phase extraction procedure.

### 2.2. Samples of wines

Samples of the red barrique wine (a mixture 80:20% v/v of *Cabernet Sauvignon* and *Merlot)* collected at various stages of its aging in toasted oak barrels of *Q. robur* (Nadalie, France) were obtained from ZNOVIN Znojmo a.s. (Czech Republic) and stored in a refrigerator at 5 °C prior to analyses. Wine samples were filtered through a 0.45-μm nylon membrane filter prior to analyses. Samples were taken from five barrels marked 5, 10, 15 (middle-toasted barrels) and 20 HT, 25 HT (highly-toasted barrels). The barrels were once re-passed. The wooden chips were collected after toasting the barrels just before filling with the wine.

### 2.3. Wood analysis

Toasted-wood chips (1–5 mm) from two oak species (*Q. robur* and *Q. alba*), toasted under the same conditions, and chips (1–5 mm) of natural wood (*Q. robur*) were examined (all from Nadalie, France). Extraction of the species for the determination of phenolic compounds and furaldehydes was performed using a "fexIKA® Werke 50" extractor (Redeker, 1961), a computer-controlled device related to the Soxhlet apparatus (IKA-Werke GmbH & Co., Staufen KG, Germany). One gramme of wooden chips was extracted with 40 ml 2 M HCl or 10% ethanol (v/v) adjusted to pH 3.5 with acetic acid for 1 h in the extractor. The temperature of the cooling/heating block was kept at 50% higher than the boiling point of a given solvent for 30 min; the temperature was lowered to 40 °C in the next 5 min and the same steps were repeated in the second run.

Two milliliters of an extract was further purified using solid-phase extraction on RP 105 polymeric sorbent. The equilibrated RP 105 cartridge was washed with 3 ml of a mixture of 0.1 M hydrochloric acid:methanol (9:1, v/v), and the retained phenolic compounds and furaldehydes were eluted with 3 ml of a mixture of 0.1 M hydrochloric acid:methanol (1:4, v/v). The solvent was removed using a vacuum rotary evaporator at 40 °C; the residue was dissolved in 500 μl of the mobile phase, filtered through a 0.45-μm nylon membrane filter and analysed.

A model test was carried out for the determination of time-dependent changes of phenolic compounds and furaldehydes released from wooden barrels to wine. One gramme of toasted wooden chips from a highly-toasted barrel of *Q. robur* and *Q. alba* was extracted in 100 ml 12% (v/v) ethanolic solution adjusted to pH 3.5 with acetic acid. The mixtures were stored at 9 °C in stoppered glass flasks and 100 μl of sample was taken at various time intervals and analysed without further sample treatment. The chromatographic conditions were identical to those wine samples.

### 2.4. Solid phase extraction procedure

$C_{18}$ silica (Applied Separations Alletown, II, USA) and six polymeric sorbents (styrene–divinylbenzene copolymers – RP-101, RP-105, polyamide – Amide 2 (Applied Separations Allentown, I1, USA) and polyvinylpyrrolidone–divinylbenzene copolymers – Oasis HLB (Waters, Milford, MA, USA), Strata X (Phenomenex, Torrance, CA, USA), Abselut Nexus (Varian, Harbour City, CA, USA)) were tested.

The application of a $C_{18}$ column was not suitable for the derivatives of benzoic and cinnamic acids due to low recoveries of individual compounds (14.2% for gallic acid, 24.5% for protocatechuic acid, 9.8% for 2-furfural, 17.2% for 5-methoxyfufrural and 38.6–81.2% for the others) when 0.1 M HCl:MeOH (1:4, v/v) was used for elution and 0.1 M HCl for washing.

The polymeric sorbents allow direct application of wine samples (up to 15% of ethanol in wine samples) to the SPE cartridge without any preliminary treatment. A washing solvent, containing 10% (v/v) of methanol, can be applied for removing ballast substances after applying the sample to the RP-105 polymeric sorbent. Application of 0.1 M HCl is preferable for all other sorbents since no elution of the analytes was observed.

Only more polar ballast compounds were removed using the washing solvents. Application of a water-immiscible solvent (diethylether) instead of acidified methanol eliminated elution of anthocyanins, and methanol in the washing solvent seriously reduces the number of co-eluted ballast compounds, i.e., less strict chromatographic conditions are necessary for the final chromatographic separations. Elution of other substances was reduced while the analytes of interest were eluted with the same yield (82.1–95.8%), as when HCl:methanol (1:4, v/v) was used (83.8–98.9%). When diethylether was used for the elution, 14 compounds were identified in the wine samples. Only 10 analytes of interest were identified when methanol was used.

The optimised SPE method was compared to a classical liquid–liquid extraction (LLE) with diethylether. Lower recoveries (73.2–88.9%) and lower repeatabilities of results (RSDs 6.5–12.3% compared to 1.8–3.2% for SPE) were obtained in the case of LLE. In addition, only 8 compounds were identified and quantified in this case.

Extraction of the phenolic compounds and furaldehydes from the wine was carried out according to the following procedure: the RP-105 SPE cartridge was first conditioned with 3 ml of methanol and followed by 3 ml of water. Then, 2 ml of the untreated wine were applied to the cartridge. Co-extracted substances were removed from the sorbent with a mixture of 0.1 M hydrochloric acid:methanol (9:1, v/v). The column was dried by nitrogen gas for 10 min. All retained compounds of interest were eluted with 10 ml of ethylether. The solvent was removed using a rotary evaporator at 20 °C, the residue was dissolved in 500 μl of the mobile phase, filtered through a nylon membrane filter, and 10 μl aliquots were injected into the HPLC system (Matějíček, Mikeš, Klejdus, Stěrbová, & Kubáň, 2003).

### 2.5. HPLC analysis

The analyses were performed using an HP 1100 liquid chromatograph (Hewlett–Packard, Wilmington, DE, USA) with a diode array detector working at 280 nm (100 SBW). UV spectra were automatically acquired at all peaks (range 190–400 nm, 2 nm step). Separation was carried out with a Hypersil BDS $C_{18}$ column (4.6 mm × 100 mm, 3 μm particle sizes, Hewlett–Packard, Palo Alto, CA, USA).

The resolution ($R$) of *p*-hydroxybenzaldehyde/vanillic acid/caffeic acid and syringic acid/vanillin were the crucial problems in analyses. The gradient elution profile was optimised using different ratios of acetonitrile to methanol, applying different modifiers (acetic or formic acids) at different concentrations (0.1–0.5% v/v), changing the flow rate of mobile phase (0.5–1.0 ml $min^{-1}$) and the column temperature (20–30 °C) to determine the highest resolution of analytes ($R > 1.5$) and the shortest time of analyses (less than 30 min).

A linear gradient profile of mobile phase, consisting of 0.3% acetic acid (solvent A) and methanol (solvent B), 7–20% B (0–7 min), 20–30% (7–12 min), 30% (12–18 min), 30–60% (18–20 min) 60–100% (20–23 min) and 100–7% (23–28 min) was applied at a flow rate of 0.6 ml $min^{-1}$. The column was equilibrated for 7 min under initial conditions prior to injection of the next sample. The column temperature was 25 °C.

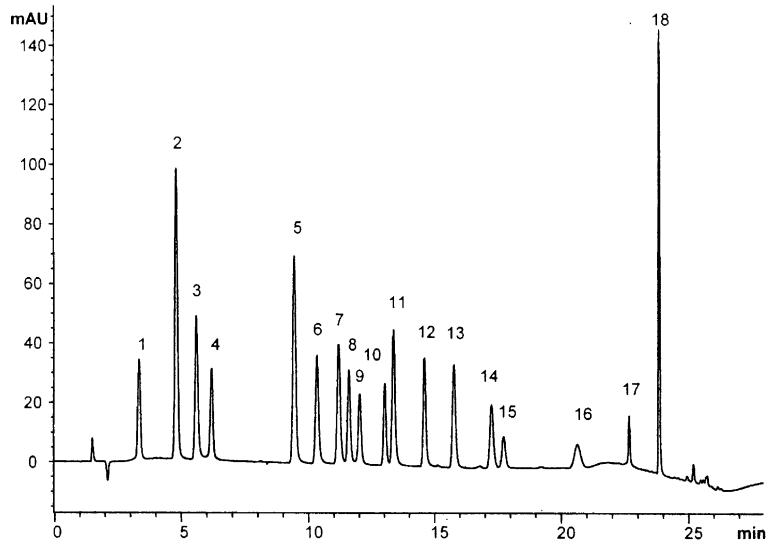


Fig. 1. Chromatographic separation of phenolic compounds using Hypersil BDS $C_{18}$column. Key to peak identification: 1. gallic acid; 2. 5-methoxy-2-furaldehyde; 3. 2-furaldehyde; 4. protocatechuic acid; 5. *p*-hydroxybenzoic acid; 6. 5-methyl-2-furaldehyde; 7. *p*-hydroxybenzaldehyde; 8. vanillic acid; 9. caffeic acid; 10. syringic acid; 11. vanillin; 12. syringaldehyde; 13. *p*-coumaric acid; 14. ferulic acid; 15. sinapic acid; 16. benzoic acid; 17. ellagic acid; 18. cinnamic acid. Chromatographic conditions see Section 2.5.

The peak identity was confirmed by comparing the retention times and UV-spectra of individual compounds (match factors greater than 995) stored in a user's library. All compounds were well resolved with $R > 1.7$ (see Fig. 1). Calibration curves were strictly linear ($r^2 > 0.9998$) for each compound in the concentration range from 0.1 to 100 μg $ml^{-1}$. Limit of detection (LOD = 3 $S_{bl}$) of the compounds were from 0.13 to 4.53 ng, i.e., 5.2–181 μg $l^{-1}$.

## 3. Results and discussion

### 3.1. Analysis of toasted wood material

The concentrations of phenolic compounds and furaldehydes are given in Table 1. The typical chromatograms of the extracts of toasted *Q. robur* and *Q. alba* are presented in Fig. 2. Hydrochloric acid and 10% ethanol adjusted to pH 3.5 with acetic acid were tested as extraction agents for the isolation of extractable phenolic compounds.

Application of hydrochloric acid can even cause extraction of bound phenolic compounds, but the amounts of phenolic compounds and furaldehydes were lower than when applying ethanol as extraction agent. This was probably due to decarboxylation of phenolic compounds; therefore results of extraction with 10% ethanol were considered. One order of magnitude higher concentrations of ellagic acid were obtained when ethanol was used as an extraction agent for toasted wooden chips. Similar results were obtained for all other compounds. In contrast, twice higher concentrations of ellagic acid and other compounds (except of gallic and vanillic acids) were found using HCl as extraction agent in the case of natural woods. This phenomenon can be explained by changes in chemical composition of the two types of samples, in the character of sample matrices and, mainly, in the processes of toasting. The procedures influence the physical characteristics of the surface, the content of individual analytes and, of course, the matrix.

Significant differences between the toasted oak species were found. The contents of 5-methoxy-2-furaldehyde, 2-furaldehyde and ellagic acid were noticeably higher in *Q. robur*. On the other hand, the concentrations of vanillin and syringaldehyde were twice higher in *Q. alba*; 5-methyl-2-furaldehyde and sinapic acid were detected only in *Q. robur*.

### 3.2. Effect of toasting degree of the oak-barrels

The effect of maturing of natural wine, in toasted wooden barrels, on the contents of phenolic compounds and furaldehydes was estimated in a model experiment. The chips of toasted wood of two oak species (*Q. robur* and *Q. alba*) were tested. The composition of the extracting agents corresponded to the wine matrix in pH and alcohol content. Changes in concentration of phenolic compounds and furaldehydes released from the toasted woods of both species are shown in Fig. 3. Syringaldehyde, vanillin, 2-furaldehyde, 5-methoxy-2-furaldehyde and 5-methyl-2-furaldehyde were the most representative compounds of both woods; vanillic acid,

Table 1
Contents (μg $g^{-1}$) of furaldehydes and phenolic compounds in natural and toasted *Q. robur* and toasted *Q. alba*

| | Natura *Q. robur* | | Toasted *Q. robur* | | Toasted *Q. alba* | |
|---|---|---|---|---|---|---|
| | 2 M HCl | 10% EtOH | 2 M HCl | 10% EtOH | 2 M HCl | 10% EtOH |
| Gallic acid | 25.2[a] | 661 | 52.9 | 70.7 | N.D.[b] | N.D. |
| 5-Methoxy-2-furaldehyde | 54.7 | N.D. | 44.9 | 179 | 25.1 | 81.8 |
| 2-Furaldehyde | 46.4 | N.D. | 65.8 | 73.7 | 49.2 | 49.1 |
| 5-Methyl-2-furaldehyde | N.D. | N.D. | N.D. | 13.2 | N.D. | N.D. |
| *p*-Hydroxybenzaldehyde | N.D. | N.D. | N.D. | N.D. | N.D | 10.8 |
| Vanillic acid | 10.4 | 15.6 | 27.0 | 111 | 19.1 | 110 |
| Vanillin | 12.1 | 8.75 | 55.7 | 192 | 70.4 | 437 |
| Syringic acid | 27.5 | 17.1 | 48.6 | 253 | 52.6 | 287 |
| Syringaldehyde | N.D. | N.D. | 97.2 | 553 | 130 | 1149 |
| Sinapic acid | N.D. | N.D. | N.D. | 51.5 | N.D. | N.D. |
| Ellagic acid | 1005 | 590 | 105 | 1881 | 97.7 | 1131 |

Extraction with 2 M HCl and 10% ethanol (v/v) adjusted to pH 3.6 with acetic acid.
[a] Relative standard deviations (RSDs) were 3.4–6.8%.
[b] N.D., not detected.

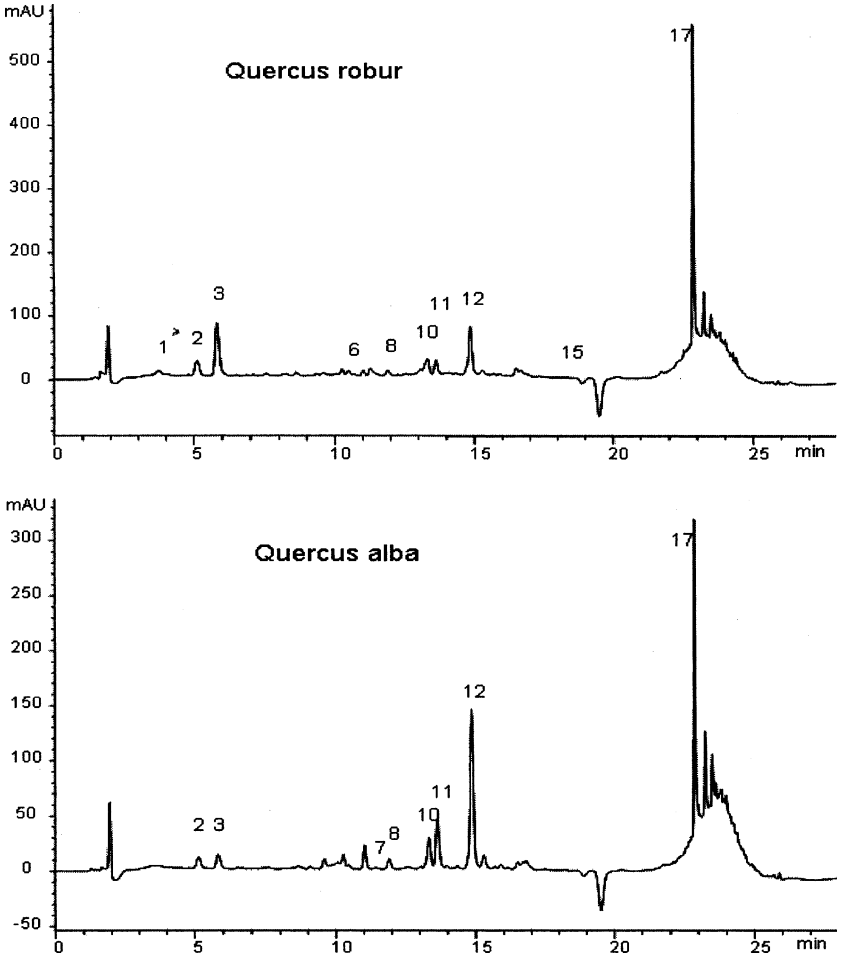


Fig. 2. Chromatographic separation of phenolic compounds in *Quercus robur* and *Quercus alba* wood using Hypersil BDS $C_{18}$column after solid-phase extraction on RP 105 sorbent. For peak identification see Fig. 1. Chromatographic conditions see Section 2.5.

syringic acid, and gallic acid, were further determined in *Q. robur*. Ellagic acid was determined in both species after 100 days of the experiments.

The most significant increase in contents of phenolic compounds was observed during the first 30 days of the experiments. In contrast, the concentration of ellagic

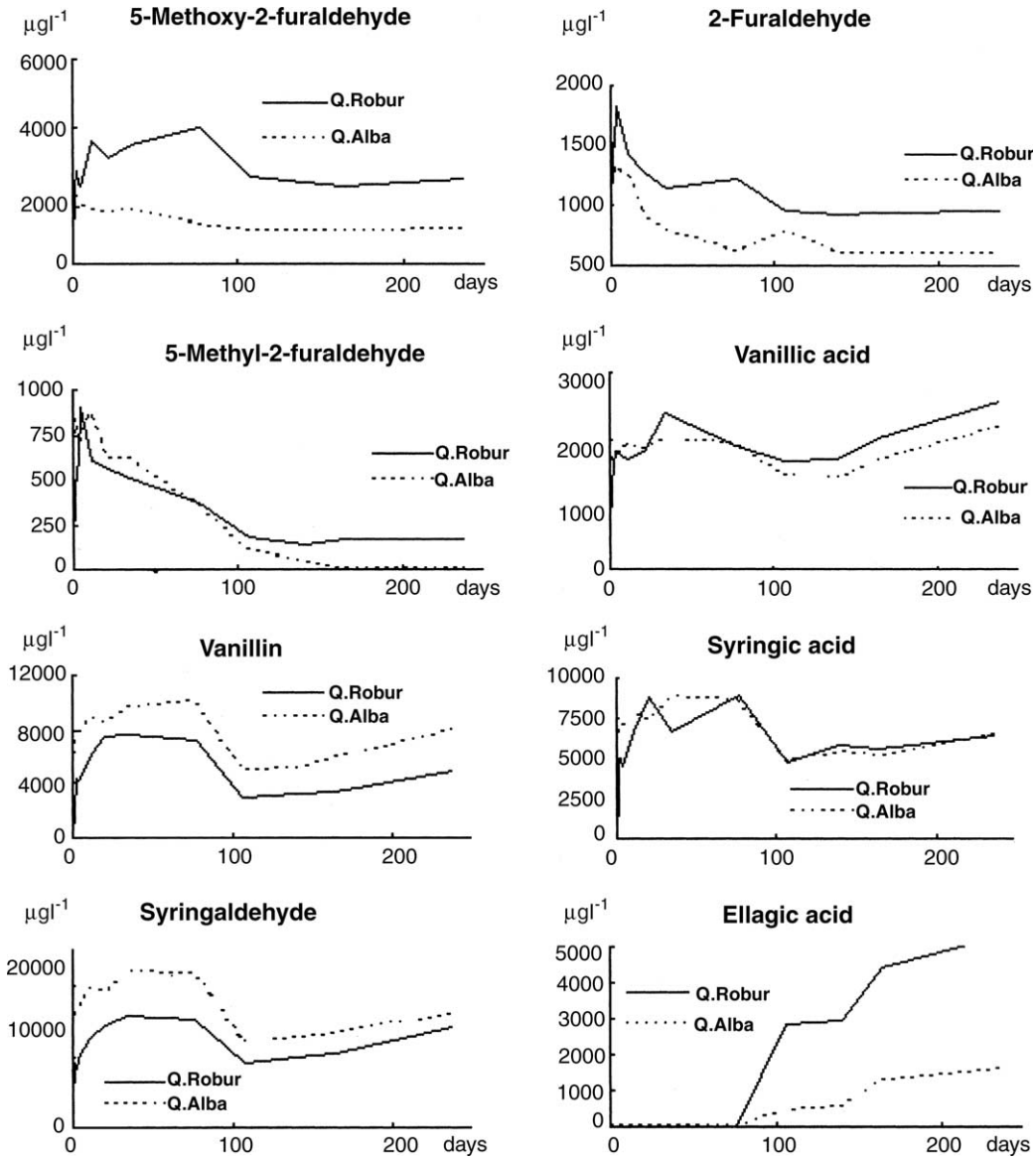


Fig. 3. Changes in phenolic compound and furaldehyde contents in two oak species (*Q. robur* and *Q. alba*) in the model experiments (238 days interval).

acid increased constantly in the time that corresponded to hydrolysis of wood constituents. The kinetics of extraction of furaldehydes differed from those of other compounds. The concentrations of these compounds increased in the first 10 days and then started to decrease constantly, and even 5-methyl-2-furaldehyde was not detected after 160 days in *Q. alba*.

The most striking difference was found between the furaldehydes and vanillin contents, although the extraction kinetics were very similar. Amounts of 5-methoxy-2-furaldehyde and 2-furaldehyde were 1.6- and 2.4-times higher in the *Q. robur*, respectively. On the other hand, the content of vanillin in *Q. alba* was 1.6 times higher than that in *Q. robur*. The differences correspond to concentrations of phenolic compounds and furaldehydes described below. Syringaldehyde was determined at concentrations up to 11 mg $l^{-1}$ (e.g. 1.1 mg $g^{-1}$ of wood), but was not found in any real sample of barrique wines.

### 3.3. Analysis of wine samples

Results of routine wine analyses obtained at the beginning and at the end of ageing process are shown in Table 2. Fig. 4 shows a characteristic chromatogram of the wine aged in toasted oak-wood barrels.

Changes in contents of phenolic compounds and furaldehydes in barrique wines ageing in toasted oak barrels were controlled over a period of 200 days, when the maturing of wines was finished (Table 3, Figs. 5 and 6). Gallic acid, protocatechuic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid and ellagic acids were the most representative compounds. The

Table 2
Wine constituents at the beginning (0 days) and at the end (207 days) of ageing of wines (a mixture *of Cabernet Sauvignon* and *Merlot)* in *Quercus robur* barrels

| Components | 0 day | 207 days |
|---|---|---|
| Density ($g\,cm^{-3}$) | 0.99385 | 0.99477 |
| Alcohol (%) | 11.5 | 11.3 |
| PH | 3.31 | 3.40 |
| Total acid-calculated as tartaric acid ($g\,l^{-1}$) | 5.3 | 5.0 |
| Volatile acid-calculated as acetic acid ($g\,l^{-1}$) | 0.64 | 0.72 |
| Sugar free extract ($g\,l^{-1}$) | 21.7 | 22.1 |
| Sugar ($g\,l^{-1}$) | 3.9 | 3.3 |
| Free $SO_2$ ($mg\,l^{-1}$) | 27 | 11 |
| Total $SO_2$ ($mg\,l^{-1}$) | 97 | 48 |

derivatives of 2-furaldehyde were not detected in the natural wine at the beginning of ageing. Sinapic acid, cinnamic acid and Syringaldehyde were not detected at any time of wine ageing.

Co-sinusoid time-dependences of concentration were found for each target compound during the barrique process, except for furaldehydes. The beginning of ageing of this sort of wine was characterized by decreasing concentrations of phenolic compounds during the first 30 days. There was a slow increase to approximately the original concentration (in the case of gallic acid). A rapid increase of level of phenolic components of wine was observed after 100 days. The nearly linear increase of the content of derivatives of 2-furaldehyde during the ageing period was a characteristic feature. The decrease of the concentration of 5-methyl-2-furaldehyde after 100 days of ageing in highly-toasted barrels (20 HT, 25 HT) corresponded to the results of the model experiments.

The decrease of the concentrations of phenolic compounds in the first 30 days can be explained by equilibration of concentrations of phenolic compounds between wine and oakwood, respectively. An adsorption on barrel walls and/or a very slow penetration of phenolic compounds into the wood barrels was observed in the first stage of the ageing of the wine. The repeated rise of the concentrations after 100 days could be explained by hydrolysis of bound phenolic compounds in wood and their subsequent release into the barrique wine.The same elevation of the concentration of phenolic compounds was observed after 100 days in the model experiments. The potential further decrease of their concentration can be attributed to their enzymatic or chemical degradation, and also to a possible sorption of the phenolic compounds on new products of polymerisation of wine.

Changes in concentrations of gallic, protocatechuic, vanillic, caffeic, syringic and ellagic acids are shown in Fig. 5. The concentration of gallic acid differed seriously over the ageing period, but it seems that its concentration was slightly higher in the wine samples aged in middle-toasted barrels, probably due to the higher content of hydrolysed tannins. The profiles of changes of concentrations of protocatechuic acid, as well as of vanillic, caffeic and syringic acids, were very similar and no measurable effect of toasting intensity was observed.The changes in ellagic acid concentration were in an agreement with the results of the model experiments. Its concentration constantly increased after 100 days. Thus, ellagic acid can be regarded as the characteristic compound of barrique wine ageing. Its constant level during some periods could become a marker of maturity of a barrique wine. In our case, the ageing of the wine was finished after 200 days and the wine was ready to be sold. This period corresponds to the constant concentration of ellagic acid for about 40 days.

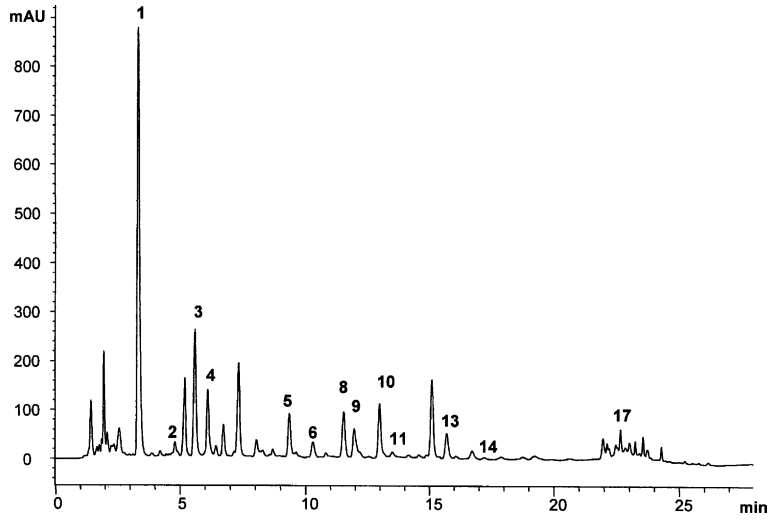


Fig. 4. Chromatographic separation of phenolic compounds in barrique wine using Hypersil BDS $C_{18}$ column after solid-phase extraction on RP 105 sorbent. For peak identification see Fig. 1. Chromatographic conditions see Section 2.5.

Table 3
Concentration of phenolic compounds and furaldehydes at the beginning (0 day) and at the end (207 days) of ageing of wine (a mixture *of Cabernet Sauvignon* and *Merlot)* in toasted *Q. robur* barrels

| Compounds | Concentration ($mg\,l^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | BRW[a] | 207 day | | | | |
| | | 5 | 10 | 15 | 20 HT | 25 HT |
| Gallic acid | 21.5[b] | 20.4 | 18.6 | 19.9 | 19.5 | 18.6 |
| 5-Methoxy-2-furaldehyde | N.D.[c] | 0.016 | 0.016 | 0.018 | 0.031 | 0.027 |
| 2-Furaldehyde | N.D. | 0.098 | 0.104 | 0.091 | 0.094 | 0.115 |
| Protocatechuic acid | 2.38 | 2.48 | 2.45 | 2.51 | 2.22 | 2.36 |
| *p*-Hydroxybenzoic acid | 0.037 | 0.032 | 0.032 | 0.028 | 0.029 | 0.031 |
| 5-Methyl-2-furaldehyde | N.D. | 0.0097 | 0.0095 | 0.0084 | 0.0096 | 0.0085 |
| *p*-Hydroxybenzaldehyde | 0.034 | 0.031 | 0.027 | 0.032 | 0.027 | 0.035 |
| Vanillic acid | 1.74 | 1.92 | 1.65 | 1.90 | 2.02 | 1.94 |
| Caffeic acid | 2.55 | 2.69 | 2.17 | 1.92 | 2.54 | 1.83 |
| Vanillin | 0.066 | 0.118 | 0.099 | 0.103 | 0.142 | 0.123 |
| Syringic acid | 1.95 | 1.91 | 1.50 | 1.94 | 2.27 | 1.54 |
| *p*-Coumaric acid | 0.96 | 2.39 | 0.88 | 1.11 | 0.95 | 0.72 |
| Ferulic acid | 0.23 | 0.21 | 0.20 | 0.19 | 0.22 | 0.14 |
| Benzoic acid | 0.165 | 0.155 | 0.152 | 0.158 | 0.156 | 0.156 |
| Ellagic acid | 0.62 | 0.77 | 0.76 | 0.77 | 0.74 | 0.74 |

[a] Basic red wine (BRW) in 0 day.
[b] Relative standard deviations (RSDs) were 2.1–5.1%.
[c] N.D., not detected.

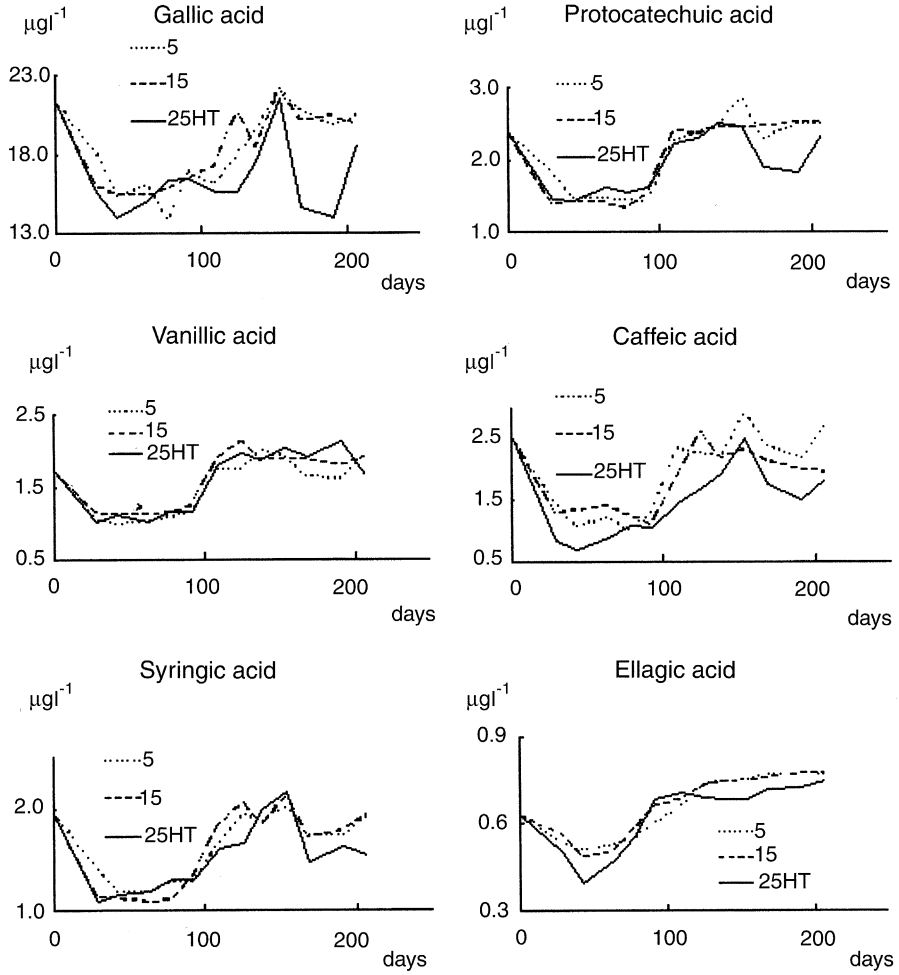


Fig. 5. Changes in gallic, protocatechuic, vanillic, caffeic, syringic and ellagic acids concentrations during the maturing of red wine in *Quercus robur* barrels.

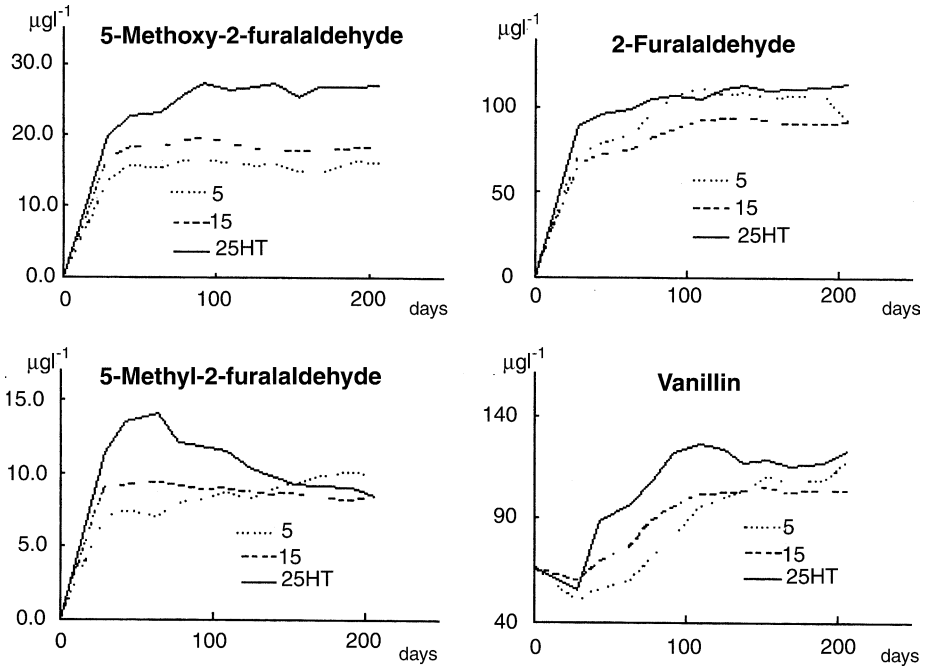


Fig. 6. Changes in concentrations of 5-methoxy-2-furaldehyde, 2-furaldehyde, 5-methyl-2-furaldehyde and vanillin during the maturing of red wine in *Quercus robur* barrels.

Changes in furaldehydes and vanillin contents are shown in Fig. 6. The concentrations of 2-furaldehyde were much higher than its methyl- and methoxyderivatives. No marked differences were noticed in the kinetics of their extraction. After the very rapid growth of their concentrations in the first 50 days, only small changes in their concentrations were observed during the remaining period. The effect of the toasting intensity was appreciable on 5-methoxy-2-furaldehyde. Its concentration in the wine ageing in the highly toasted oak barrels was approximately 1.5-times higher than in the case of middle-toasted barrels (samples 5, 10 and 15). The differences in the concentration of 5-methyl-2-furaldehyde were observed until 100 days when the concentration of this compound started to decrease and no differences among the wine samples were obvious in its content. The concentration of 2-furaldehyde was slightly higher in the highly toasted barrel (25 HT). Because of the absence of furaldehydes in natural wines, these compounds can be considered as typical components of a barrique wine. They can serve as a marker of the authenticity of barrique wines.

The presence of vanillin can strongly influence wine aroma and thus it is the important phenolic compound of ageing of beverages in toasted-wood barrels although, in the case of wines, this is the subject of some dispute. Vanillin can be subject to biological reduction and further transformation and so a constant decrease of vanillin over time can be observed (Spillman et al., 1997, 1998). In our study, its content constantly increased in time after the initial equilibration and so our results confirm the results found for cognac (Velíšek, 1999).

Ferulic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *p*-coumaric acid and benzoic acid were further compounds determined in the wine (data not shown). Their contents and the extraction kinetics were very similar in all the barrels; no significant changes were observed.

## 4. Conclusion

Changes of abundance and amounts of derivatives of benzoic and cinnamic acids and derivatives of 2-furaldehyde during maturation of the barrique wine were studied. A co-sinusoidal time-dependence of concentration was found for the target phenolic compounds during the barrique process. A constant rise was found in the content of 2-furaldehyde derivatives. The degree of toasting enormously increased the content of phenolics and furaldehydes in the samples. The influence of toasting intensity of the wood on the amount of phenolic compounds in barrique wine was negligible; only content of 5-methoxy-2-furaldehyde differed noticeably between middle- and highly-toasted barrels. Vanillin, ellagic acid and derivatives of 2-furaldehyde were the most notable compounds. Ellagic acid can be regarded as the characteristic compound of barrique wine ageing and possibly its constant level during some periods could become a marker of the maturity of barrique

wines. Due to the absence of furaldehydes in natural wines, these compounds can be considered as typical components of barrique wines and so they can serve as markers of authenticity.

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## References

Afonso, V. L. G. (2002). Sensory descriptive analysis between white wines fermented with oak chips and in barrels. *Journal of Food Science, 67*, 2415–2419.

Andrade, P. B., Mendes, G., Falco, V., Valentao, P., & Seabra, R. M. (2001). Preliminary study of flavonols in port wine grape varieties. *Food Chemistry, 73*, 397–399.

Cadahia, E., Munoz, L., de Simon, B. F., & Garcia-Vallejo, M. C. (2001). Changes in low molecular weight phenolic compounds in Spanish, French and American oak woods during natural seasoning and toasting. *Journal of Agricultural and Food Chemistry, 49*, 1790–1798.

Chatonnet, P., & Dubourdieu, D. (1998). Comparative study of the characteristics of American white oak (*Quercus alba*) and European oak (*Q. petrea*, *Q. robur*) for production of barrels used in barrel ageing of wines. *American Journal of Enology and Viticulture, 49*, 79–85.

Chilla, C., Guillén, D. A., Barroso, C. G., & Pérez-Bustamante, J. A. (1996). Automated in-line solid phase extraction high-performance liquid chromatography diode array detection of phenolic compounds in sherry wine. *Journal of Chromatography A, 750*, 209–214.

Cutzach, I., Chatonnet, P., & Dubourdoeu, D. (2000). Influence of storage conditions on the formation of some volatile compounds in white fortified wines (vins doeux naturels) during the ageing process. *Journal of Agricultural and Food Chemistry, 48*, 2340–2345.

Escalona, H., Birkmyre, L., Piggott, J. R., & Paterson, A. (2002). Effect of maturation in small oak casks on the volatility of red wine aroma compounds. *Analytica Chimica Acta, 458*, 45–54.

Flesch, M., Schwarz, A., & Bohm, M. (1998). Effect of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arterium. *American Journal of Physiology of Heart, 44*, H1183–H1190.

Goldberg, D. M., Hoffman, B., Yang, J., & Soleas, G. J. (1999). Phenolic constituents, furanes and total antioxidative status of distilled spirits. *Journal of Agricultural and Food Chemistry, 47*, 3978–3985.

Guillén, D. A., Barroso, C. G., & Pérez-Bustamante, J. A. (1996). Automation as a sample preparation as a preliminary stage in the high-performance liquid chromatographic determination of polyphenolic compounds in sherry wines. *Journal of Chromatography A, 730*, 39–46.

Guillén, DA., Barroso, C. G., & Pérez-Bustamante, J. A. (1997). Automated solid phase extraction followed by HPLC DAD for the monitoring of phenolic compounds during fermentation of sherry must. *Quimica Analytica, 16*, 21–25.

Ho, P., Hogg, T. A., & Silva, M. C. M. (1999). Application of a liquid chromatographic method for the determination of phenolic compounds and furans in fortified wines. *Food Chemistry, 64*, 115–122.

Hollman, P. C. H. (2001). Evidence for health benefits of plant phenolics: local or systemic effects?. *Journal of the Science, Food and Agriculture, 81*, 842–849.

Kadim, D., & Mannheim, C. H. (1999). Kinetics of phenolics extraction during ageing of model wine solutions and white wine. *American Journal of Enology and Viticulture, 50*, 33–39.

Klejdus, B., & Kubáň, V. (2000). High Performance liquid chromatographic determination of phenolic compounds in seed exudates *of Festuca arundinacea* and Festuca pratense. *Phytochemical Analysis, 11*, 375–379.

Laszlavik, M., Gól, L., Misik, S., Erdei & L K (1995). Phenolic compounds in Hungarian red wines maturated in *Quercus robur* and *Quercus petrea* barrels- HPLC analysis and diode-array detection. *American Journal of Enology and Viticulture, 46*, 67–74.

Malovaná, S., García Montelongo, F. J., Pérez, J. P., & Rodríguez-Delgado, M. A. (2001). Optimisation of sample preparation for the determination of trans-resveratrol and other polyphenolic compounds in wines by high-performance liquid chromatography. *Analytica Chimca Acta, 428*, 245–253.

Matějíček, D., Mikeš, O., Klejdus, B., Stěrbová, D., & Kubáň, V. (2003). Evaluation of a solid-phase extraction for the determination of phenolic and other compounds in barrique wines. *Analytical and Bioanalytical Chemistry, 377*, 340–345.

Merello, F., Guillén, D. A., Barroso, C. G., & Pérez-Bustamante, J. A. (1997). Solid phase extraction for sample preparation in the HPLC analysis of polyphenolic compounds in fine sherry wine. *Journal of Agricultural and Food Chemistry, 45*, 403–406.

Mosedale, J. R., & Puech, J. L. (1998). Wood maturation of distilled spirits. *Trends in Food Science and Technology, 9*, 95–101.

Pisarnitskii, A. F. (1995). Aldehydes as a factor of lignin degradation in the seasoning of spirits. *Applied Biochememistry and Microbiology*, 31,126–128.

Redeker, J. (1961). Patent Application, DEP 1961, 12037.3, DEG 8411672.

Samanidou, V. F., Antoniou, C. V., & Papadoyannis, I. N. (2001). Gradient RP-HPLC determination of the phenolic acids in wine vinegar samples after solid phase extraction with photodiode array detector identification. *Journal of Liquid Chromatography, RT 24*, 2161–2176.

de Simon, B. F., Cadahia, E., Conde, E., & Garcia-Vallejo, M. C. (1996). Molecular weight phenolic compounds in Spanish oak woods. *Journal of Agricultural and Food Chemistry, 44*, 1507–1511.

Singleton, V. L. (1995). Maturation of wines and spirits – comparisons, facts and hypotheses. *American Journal of Enology and Viticulture, 46*, 98–115.

Spillman, P. J., Pollnitz, A. P., Liacopoulos, D., Skouroumounis, G. K., & Sefton, M. A. (1997). *Journal of Agricultural and Food Chemistry, 45*, 2584–2589.

Spillman, P. J., Pollnitz, A. P., Liacopoulos, D., Skouroumounis, G. K., & Sefton, M. A. (1998). *Journal of Agricultural and Food Chemistry, 46*, 657–663.

Shrikhande, A. J. (2000). Wine by-products with health benefits. *Food Research International, 33*, 467–475.

Tesfaye, W., Morales, M. L., Garcia-Parrilla, M. C., & Troncoso, A. M. (2002). Evaluation of phenolic compounds during an experimental ageing in wood of sherry vinegar. *Journal of Agricultural and Food Chemistry, 50*, 7053–7061.

Velíšek, J. (1999). *Food Chemistry* [OSSIS Tábor], 2, 264 (in Czech).