Contribution of oak lignans to wine taste: chemical identification, sensory characterization and quantification

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Abstract:

The modification of wine taste during oak ageing is due to the release of non-volatile compounds from wood. Among these molecules, some lignans have been previously described as exhibiting bitterness. However, the lack of knowledge concerning this class of compounds in oak wood led us to explore both their structural diversity and their sensory properties. Nine lignans were isolated from extracts of *Quercus petraea* oak heartwood. Among them, one new compound called quercoresinol was identified and four other molecules were described for the first time in *Quercus* genus. The presence of these lignans in oaked wine was then established and their gustatory properties were evaluated. Lyoniresinol was the bitterest compound with a detection threshold of 1.5 mg/L. An LC–HRMS quantitative method was performed to study the influence of oenological practices on lyoniresinol concentration in wine.

Keywords: Bitter taste, Wine, Oak wood, Lignan, Lyoniresinol

1. Introduction

Vinification is the natural transformation of grapes into wine involving microbiological and chemical mechanisms. Various compounds are released from grapes. Some are bio-transformed by yeasts during alcoholic fermentation and altogether they reveal the taste of wine.[1] These molecules play an essential role as they are responsible for the sensory image of wine and for the pleasure that consumers obtain. In recent decades, advances in oenological science have uncovered many of the molecular determinants of wine colour, smell and flavour.[2] After the crucial vinification steps, wine is generally aged in oak barrels to improve its stability and organoleptic properties. Indeed, both volatile and non-volatile compounds are released from oak wood to wine, which becomes more refined and more complex.[3]

The aromatic modifications consecutive to oak ageing have been well described and explained. The main volatiles migrating from oak wood to wine are vanillin, whisky lactone, eugenol and 2-furanmethanethiol, which are responsible, respectively, for vanilla, coconut, spicy and roasted coffee.[4,5,6]

Oak non-volatile compounds are mainly non-flavonoid polyphenols. They include ellagitannins such as castalagin, vescalagin and roburin, phenolic acids such as ferulic and gallic acids, coumarins such as scopoletin and umbelliferone and polymeric compounds.[2,7,8,9]

Some of these non-volatile compounds have shown sensorial properties such as wine colouration, taste and astringent sensation. In particular, Chassaing et al.[10] explained the red-to-purple change of colour during wine ageing in oak barrels as being caused by the molecular association of the grape anthocyanin oenin (malvidin-3-*O*-glucoside) and the oak-derived ellagitannin vescalagin.[10] Moreover, Marchal et al.[11] discovered new highly sweet triterpenoids extracted from oak wood *Quercus petraea*. These taste-active compounds contribute to explaining the increased wine sweetness observed during oak ageing.[12] Withal, the effect of oak non-volatiles on wine taste is complex. Indeed, tasting of the purified oak ellagitannins grandinin, roburin E, castalagin and vescalagin and quantitative analysis in red wines showed their capacity to contribute to the astringency and bitterness of oak-matured red wines.[13,14,15,16]

Beyond ellagitanins, another oak polyphenol called lyoniresinol has been shown to exhibit a bitter taste.[11] This lignan has been described in oak wood, spirits[17,18,19] and wines[8] but its sensorial influence has never been studied. Other oak wood lignans have also been reported such as lyoniresinol 3α-*O*-β-d-glucopyranoside,[17] isolariciresinol and secoisolariciresinol.[20]

The latter two compounds were observed and quantified by GC/MS but not isolated from the extract.

Lignans are a class of secondary plant metabolites produced from shikimic acid via the phenylpropanoid pathway. They occur in roots, seeds, fruits and wooden part of vascular plants.[21] The biological functions of lignans are not entirely understood, but their antimicrobial, antifungal, antiviral, antioxidant, insecticidal and antifeeding properties[21,22,23,24] indicate that they may be involved in plant defence against diseases and pests. They may also participate in plant growth and development. In addition to their purpose in nature, lignans possess significant biological activities such as anti-cancer, anti-inflammatory, antimicrobial, antioxidant and immunosuppressive activities.[25,26]

Lignans are found in various foods and beverages.[27,28,29,30] In particular, some lignan and neolignan derivatives from lariciresinol, isolariciresinol and secoisolariciresinol have been identified in Riesling wine[31,32] and in red wines.[33]

Furthermore, a study has shown that wine is the beverage with the highest concentrations in lignans[34] with a total amount of secoisolariciresinol, matairesinol, lariciresinol, syringaresinol, and isolariciresinol varying from 354 µg/L to 1.4 mg/L.

Despite the bitter taste developed by lyoniresinol and the significant presence of other lignans in wine, there is a lack of knowledge concerning the chemical diversity and the sensorial role of this class of compounds.

In this study, we focused on the lignans of oak wood and their contribution to wine taste. The structural nature of this class of molecules was investigated by fractionating an oak wood extract. Several separation techniques were used to isolate lignans and their identification was performed by Fourier Transform Mass Spectrometry (FTMS) and Nuclear Magnetic Resonance (NMR). Then, the presence of the isolated lignans was studied in oaked wine and their sensory properties were evaluated. Finally, a new quantification method makes it possible to analyze their sensory impact along with the influence of oenological parameters on their content.

2. Results and discussion

2.1. Isolation and identification of lignans in oak wood

2.1.1. Extraction and purification of lignans from oak wood. To be close to wine ageing conditions, a solid/liquid extraction of heartwood of *Q. petraea* was performed in a hydro-ethanolic solution. After ethanol removal, this crude extract was partitioned successively by liquid/liquid extractions using solvents with increasing polarity (EtOAc and *n*-BuOH) to extract, respectively, non-glycosylated and glycosylated lignans.

These EtOAc and BuOH prepurified extracts were fractionated by CPC to obtain lignan-enriched fractions for further purification. Given the large disparity in polarity of the compounds, two different solvent systems were used: a quaternary ARIZONA-G modified system for non-glycosylated lignans (EtOAc prepurified extract) and a ternary system for glycosylated lignans (BuOH prepurified extract). Each CPC experiment made it possible to fractionate up to 1 g of extract in 45 min with a lower consumption of solvent as compared to other conventional LC techniques. Successive injections were carried out to process the entire extracts.

Fractions enriched in lignans (Table 1) were constituted by pooling CPC tubes on the basis of UPLC–ESI–HRMS analyses. After freeze-drying, these fractions were submitted to preparative HPLC. Adapted gradients I and II were used to separate, respectively, the compounds within the non-glycosylated and glycosylated fraction. Nine lignans were subsequently obtained as white powders.

2.1.2. Identification of lignans present in oak wood. Four non-glycosylated lignans were isolated from EtOAc prepurified extract (Fig. 1).

Compound **1** HRMS spectrum exhibited a quasi-molecular $[M-H]^-$ ion at *m*/*z* 419.1712, suggesting that its empirical formula was $C_{22}H_{28}O_8$. The ^1H and ^{13}C NMR data were found to be similar to those already reported in the literature for lyoniresinol.[35,36,37,38] The presence of lyoniresinol has been established in *Quercus rubra* wood[39] but also in spirits[17,18,19] and wines[8] aged in oak barrels, and it is known for its anti-mutagenic and anti-oxidative activities.[40,41] Vivas[42] claimed that lyoniresinol has a red vermillion colour. More recently, a study of the same group hypothesized that it probably contributes to oak wood brown colour.[35,20] However, the lyoniresinol purified in our study was a white amorphous powder and, to our knowledge, all other studies on it have reported a similar aspect.[43] Moreover, owing to its chemical structure, it seems highly unlikely that lyoniresinol absorbs in the visible range to exhibit red colour. Considering these converging facts, we assume that the red compound described by Vivas is not pure lyoniresinol so its involvement in wood colouration is doubtful.

Compound **2** HRMS spectrum exhibited a quasi-molecular $[M-H]^-$ ion at *m*/*z* 389.1606, suggesting that its empirical formula was $C_{21}H_{26}O_7$. Thus, this molecule contained one methoxy group fewer than lyoniresinol. The interpretation of 2D NMR spectra and comparison with literature ^1H and ^{13}C data showed that compound **2** was 5′-methoxyisolariciresinol (Table 2). This compound has been identified in various species[44,45] but never in *Quercus* genus.

Compound **3** HRMS spectrum exhibited a quasi-molecular $[M-H]^-$ ion at *m*/*z* 571.1823, suggesting that its empirical formula was $C_{29}H_{32}O_{12}$. HCD fragmentation showed an ion at *m*/*z* 419.1710 ($C_{22}H_{27}O_8^-$) corresponding to a neutral loss of 152.0113 ($C_7H_4O_4$). These MS data suggest that compound **3** might be a galloyl-derivative of lyoniresinol. No such compound has been described until now.

Assignments of all ^1H and ^{13}C NMR signals for **3** were made by analyzing 1D and 2D NMR data (Table 2). The ^1H NMR spectrum showed the presence of two sets of characteristic signals. The former, between δ_H 7.05 and δ_H 6.0, was characterized by a singlet at δ_H 7.04 (2H, H-2″ and H-6″) of a gallate unit, a singlet at δ_H 6.62 (H-2) of a 1,2,3,4,5-penta-substituted aromatic ring, and a singlet δ_H 6.40 (2H, H-2′ and H-6′) of a sinapyl unit. The second between δ_H 5.0 and δ_H 2.0 was constituted by three singlets at δ_H 3.39 (3H, 5-OMe), δ_H 3.87 (3H, 3-OMe) and δ_H 3.74 (6H, 3′-OMe, 5′-OMe), two oxymethylene groups δ_H 4.36 dd (10.9; 5.1 Hz; H_a-9) and 4.17 dd (10.8, 7.2 Hz; H_b-9), and δ_H 3.59 dd (11, 4.3 Hz; H_a-9′) and 3.53 dd (11.1; 6.2 Hz; H_b-9′). Therefore, compound **3** could be proposed as a galloyl-derivative of lyoniresinol.

The position of the gallate unit in **3** was determined by HMBC NMR, which showed a long-range correlation between the oxymethylene protons at δ_H 4.36 (H_a-9) and 4.17 (H_b-9) and C-7″ at δ_C 166.9 of the galloyl group. The relative configuration of the stereogenic carbons of **3** (C-8, C-7′ and C-8′) was deduced from the coupling constants $J_{H\text{-}8/H8'}$=5.4 Hz characteristic of a $J_{Hax/Heq}$.[46] The relative configuration was also established by ROESY NMR. The presence of NOE between H-8 and H-7′ indicated that these protons were cofacial. Accordingly, **3** is a new lignan called quercoresinol and its structure was assigned as lyoniresinol 9-*O*-gallate.

Compound **8** HRMS spectrum exhibited a quasi-molecular $[M-H]^-$ ion at *m*/*z* 421.1869, suggesting that its empirical formula was $C_{22}H_{30}O_8$. Thus, this molecule contained one unsaturation fewer than lyoniresinol. The interpretation of 2D NMR spectra and comparison with literature ^1H and ^{13}C data showed that compound **8** was 5,5′-dimethoxysecoisolariciresinol (Table 2). This compound has been identified in various species[47,48] but never in *Quercus* genus.

Five other lignans with glycosyl groups were identified in BuOH prepurified extract (Fig. 1).

Compounds **4** and **5** presented similar HRMS spectra with a quasi-molecular $[M-H]^-$ ion at *m*/*z* 551.2136, suggesting isomers with $C_{27}H_{36}O_{12}$ as empirical formula. HCD fragmentation revealed a fragment ion at *m*/*z* 419.1713 ($C_{22}H_{27}O_8^-$) corresponding to a neutral loss of 132.0423 ($C_5H_8O_4$). Compounds **4** and **5** may therefore be pentosyl derivatives of lyoniresinol. Interpretation of NMR spectra and comparison with data in the literature confirmed this hypothesis. Compounds **4** and **5** were, respectively, lyoniside and nudiposide. Both molecules have already been identified in *Q. petraea* wood.[35,49] They are diastereoisomers and differ only by the absolute stereochemistry of the genin. Indeed, by comparison of NMR data and polarimetric measurement, compounds **4** and **5** have (+) and (−) lyoniresinol, respectively, as genin.

Compound **6** HRMS spectrum exhibited a quasi-molecular $[M-H]^-$ ion at *m*/*z* 581.2241, in accordance with an empirical formula $C_{28}H_{38}O_{13}$ for the molecule. HCD fragmentation revealed a fragment ion at *m*/*z* 419.1711 ($C_{22}H_{27}O_8^-$) corresponding to a neutral loss of 162.0530 ($C_6H_{10}O_5$) and suggesting that the molecule might contain a hexosyl group and lyoniresinol as genin. Interpretation of NMR spectra and comparison with data in the literature confirmed this hypothesis and established compound **6** as lyoniresinol 9′-*O*-β-glucopyranoside. This molecule has already been described in *Q. petraea* wood.[35] Its antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and its antifungal activity against *Candida albicans* were demonstrated by Lee et al. in 2005[50] and its antioxidant activity in *Vitis thunbergii* stems was highlighted by Tung et al. in 2011.[43]

Compound **7** HRMS spectrum exhibited a quasi-molecular $[M-H]^-$ ion at *m*/*z* 703.2248, suggesting that its empirical formula was $C_{34}H_{40}O_{16}$. HCD fragmentation revealed fragment ions at *m*/*z* 571.1812 (neutral loss of $C_5H_8O_4$) and at *m*/*z* 169.0138 ($C_7H_5O_5^-$). Therefore molecule **7** might contain a pentosyl, a galloyl and lyoniresinol as genin. Interpretation of NMR spectra and comparison with data in the literature confirmed this hypothesis and established compound **7** as lyoniresinol 9-*O*-gallate 9′-*O*-β-xylopyranoside (Table 2). Such a structure has been recently described in *Quercus mongolica*[51] with a positive specific optical rotation, whereas compound **7** was laevorotatory, so this is the first time that the latter isomer has been isolated.

Compound **9** HRMS spectrum exhibited a quasi-molecular $[M-H]^-$ ion at *m*/*z* 553.2292, suggesting that its empirical formula was $C_{27}H_{38}O_{12}$. HCD fragmentation revealed a fragment ion at *m*/*z* 421.1870 ($C_{22}H_{29}O_8^-$) corresponding to a neutral loss of 132.0422 ($C_5H_8O_4$). It might therefore contain a pentosyl group and an isomer of compound **8** as genin. Interpretation of NMR spectra (Table 2) and comparison with data in the literature confirmed this hypothesis

and established compound **9** as a xylose derivative of lignan **8** called ssioriside.[52] This molecule has already been reported in various plants[53,54] but never in *Quercus* genus.

Among the nine lignans we isolated from oak wood, one is a new compound (**3**) and four were identified for the first time in *Q. petraea* (**2**, **7**, **8** and **9**). Thus, this study highlights the structural diversity of lignans present in this species and raises the question of their contribution to the taste modification occurring in wine during oak ageing.

2.2. Research of lignans in a white wine aged in oak barrel

Most great wines are aged in contact with oak for several months, which improves their organoleptic properties. During this time, volatile and non-volatile compounds are released from oak wood to wine and play a role in the modification of wine smell and taste. To determine whether lignans have a sensory impact, the first step was to study their potential presence in a wine aged in new oak barrels. Thanks to its mass measurement accuracy, LC–HRMS allowed us to screen samples by targeting characteristic *m*/*z* ions of specified empirical formulae. Fig. 2 presents extracted ion chromatograms (XIC) obtained in an oak wood extract (left) and in an oaked white wine (right) for *m*/*z* ratios specific of lignans **1** to **9**.

Fig. 2 shows that similar signals were detected for both an oak wood extract and a new oak-aged wine. Specificity of mass measurement (<5 ppm) and retention time similarity (<0.04 min) demonstrated that lignans **1**–**9** were present in wine. Whereas we purified the most abundant compound of each XIC, minority isomers also seemed to be present for some $[M-H]^-$ ions. Lyoniresinol has already been described in oak-aged wine,[8] but this work highlights for the first time the presence of lignans **2**–**9** in a wine. Comparing the signal intensity of the various compounds suggested that lyoniresinol was the most abundant lignan.

2.3. Sensory characterization of lignans from oak wood

2.3.1. Gustatory properties of lignans isolated from oak wood. The nine lignans isolated from oak wood were shown to be present in a white wine in various amounts. Among them, lyoniresinol **1**[17,11] and ssioriside **9**[52] have been previously described as bitter, suggesting that this class of molecules could impact the taste of wine. To assess their sensory properties, the lignans were individually tasted at 10 mg/L in a hydro-ethanolic solution (12% alc. v/v) by five experts. They described the gustatory perception of each lignan solution in comparison with the control medium solution. Their ratings are presented in Table 3.

Four lignans exhibited bitterness in model solution. These results do not allow to establish clear structure/taste relationship. In particular, the glycosilation did not seem to have a decisive impact on gustatory properties: among the two bitterest lignans, one is non-glycosylated (**1**) whereas the other one contains a xyloside group (**9**).

Then, the isolated molecules were added to a white wine and tasted in the same conditions. Compounds **1** and **9** again received the highest scores. Altogether, these sensory results and the relative contents of lignans presented in Fig. 2 suggest that lyoniresinol, the most abundant lignan in both oak wood extracts and oaked wines, may have the strongest impact on wine taste. To evaluate its influence, the detection threshold of lyoniresinol must be determined and compared with the amounts found in oaked wines.

2.3.2. Determination of lyoniresinol detection threshold in white wine. To determine the detection threshold of lyoniresinol in white wine, we added it at various concentrations to an unoaked white wine. Two sessions were necessary to avoid sensory tiredness and weariness of the panelists. Lyoniresinol concentrations were followed by a geometric progression with common ratio 2 and samples were assessed using a triangle test.

The lyoniresinol group threshold was calculated to be 1.5 mg/L with strong inter-individual variability. Indeed, individual detection thresholds covered a range from 125 µg/L to 11.3 mg/L, respectively, for the most and least sensitive ones.

2.4. Quantification and sensory impact of lyoniresinol in wine

2.4.1. Development of an LC–HRMS method to assay lyoniresinol in wine. From a chemical point of view, wine is a highly complex matrix so specific and powerful tools are required to study its composition. Owing to its mass measurement accuracy, LC–HRMS appeared to be a choice technique to quantify lyoniresinol in wine. Absolute quantification was performed by preparing calibration solutions in a non-oaked wine. Peak integration was performed from XIC constructed with a 5 ppm accuracy around the theoretical *m*/*z*. Each calibration sample was injected three times and the lowest concentration (50 µg/L) five times to prove the repeatability of the method (RSD<5.8%). A linear calibration curve was obtained with a good correlation coefficient (R^2=0.9995) and RSD<6.2% between back-calculated and nominal concentrations for each level. These results implied that the limit of quantification was lower than 50 µg/L and validated the method for quantifying lyoniresinol in our working range (50 µg/L to 5 mg/L).

All wine samples were diluted two-fold to reduce the ethanol content and to avoid deterioration of the chromatographic separation.

2.4.2. Influence of ageing container on lyoniresinol content in a white wine. Wines obtained from the same must fermented and aged in four different containers (stainless steel tank, SST; new oak tank, NOT; one-year-old oak barrels, 1OB and new oak barrels, NOB) were analyzed to study the influence of the ageing modality on the lyoniresinol content.

Fig. 3 shows that the container strongly influenced the lyoniresinol content in wine. The measured concentrations varied from traces in the stainless steel tank to 1.7 mg/L in new oak barrels, thus confirming that lyoniresinol was released from oak wood to wine. The NOB value was above the detection threshold of lyoniresinol, so this lignan was likely to affect the taste of wine by increasing its bitterness. Moreover, these samples were taken 5 months after the end of alcoholic fermentation, which is a rather short ageing time. This suggests that lyoniresinol levels might increase with longer ageing. Consequently, the use of new oak wood for wine ageing seemed to increase the perception of bitterness. Nevertheless, the link between oak ageing and wine taste is not so obvious since previous studies established that sweet triterpenoids are also released from oak to wine. As oak wood provides both sweet and bitter compounds, its influence on the taste of wine certainly depends on the balance between both classes of sapid molecules.

2.4.3. Content of lyoniresinol in various vintages of the same commercial wine. Lyoniresinol was quantified in a series of vintages of the same white commercial wine aged in barrels (Fig. 4).

Concentrations varied from 1.3 to 2.4 mg/L and all the measured values were above the detection threshold except for three vintages, which were very close. These results suggest that lyoniresinol has a significant impact on wine perception. Moreover, the analyzed wines resulted from the blending of the whole production (ca. 600 barrels with 30% of new oak wood) and the ageing modalities have not been drastically modified since 1994. No correlation was observed between lyoniresinol content and the age of wine, so lyoniresinol seemed to remain stable in wine during bottle ageing, which highlights its sensory importance.

Previous studies[34,33] showed that the total amount of lignans from grapes varies from 155 µg/L to 14 mg/L. The comparison between these data and our results suggest that most of the lignans in an oak-aged wine come from the wood rather than from the grapes.

3. Conclusion

By analyzing both chemical composition and wine sensory perception, this study investigated the diversity and gustatory importance of lignans present in oak wood. Nine lignans were isolated from an oak wood extract; among them, one called quercoresinol, was identified for the first time, another exhibited a novel diastereomeric form and three compounds had never been described in *Quercus* genus. All these molecules were released in wine aged in oak barrels and lyoniresinol was both the most abundant and the bitterest lignan. Its detection threshold was estimated to be 1.5 mg/L. An LC–FTMS quantification method was developed to show that lyoniresinol was above its detection threshold in various oaked wines. The ageing modality strongly influenced its concentration and it appeared to remain stable during bottle ageing.
The present findings therefore highlight the importance of lignans, particularly lyoniresinol, and demonstrate that it is a significant determinant of bitterness in oaked wines. This study focused mainly on white wines, since bitterness especially damages their taste balance, but these compounds are also likely to impact the perception of oaked red wines. Given the stereochemical diversity of the isolated lignans, the relationship between the spatial structure and the organoleptic properties of these compounds now needs to be studied.

4. Materials and methods

4.1. Chemicals

Ultrapure water (Milli-Q purification system, Millipore, France) and HPLC grade solvent (acetonitrile, ethanol, ethyl acetate, *n*-heptane, methanol and propan-2-ol, VWR International, Pessac, France) were used for sample preparation and lignan purification. Acetonitrile and water used for chromatographic separation were LC–MS grade and were purchased from Fisher Chemical (Illkirch, France).

4.2. Purification of lignans from oak wood

4.2.1. Extraction of oak wood. Wood chips from oak heartwood of *Q. petraea* were used in this work. A bunch of wood chips (1.5 kg) was extracted with a hydro-alcoholic solution (50:50 ethanol/water, 6 L) at room temperature for 2 weeks. After a 0.45 µm filtration and

concentration in vacuo to remove ethanol, the aqueous solution (800 mL) was extracted three times with 400 mL of ethyl acetate and five times with 250 mL of butanol. The combined organic layers were evaporated to dryness, suspended in water and freeze-dried to obtain brownish powders of EtOAc (9.342 g) and BuOH (9.462 g) prepurified extracts.

4.2.2. Centrifugal partition chromatography fractionation. The CPC apparatus consisted of a Spot prep II LC system equipped with SCPC-100+1000 (Armen Instrument, Saint-Avé, France). Fractionation was performed on the 100 mL rotor. The solvent was pumped into the column by a 4-way quaternary high-pressure gradient pump. The samples were introduced into the CPC column via an automatic high-pressure injection valve. The system was controlled by Armen Glider Prep V5.0 software. All the experiments were conducted at room temperature.

EtOAc and BuOH prepurified extracts were fractionated using, respectively, a quaternary modified ARIZONA-G system (heptane/ethyl acetate/acetonitrile/water 1:4:1.29:4 v/v) and a ternary system (ethyl acetate/isopropanol/water 2:1:2 v/v). For each injection, 1 g of extract was dissolved in 10 mL of the upper and lower phases (40/60) of the system and 0.45 µm filtered. Experiments were carried out in ascending mode at 2500 rpm with a flow rate of 10 mL/min for 45 min. The Spot prep fraction collector was set to 1 tube/min. Every five CPC tubes, an aliquot (10 µL) was taken, evaporated, dissolved in 1 mL of H_2O/MeOH 95:5 and analyzed by LC–HRMS to constitute fractions enriched in lignans. To obtain such fractions, CPC tubes were pooled, evaporated in vacuo, suspended in water and freeze-dried.

4.2.3. Preparative liquid chromatography. Preparative HPLC analyses were performed using a Waters Prep 150 LC including a 2545 Quaternary Gradient Module, a 2489 UV/visible detector and a Fraction Collector III (Waters, Guyancourt, France).

Separations were obtained using an Atlantis T3 Prep OBD Column (19×250 mm, 5 µm, Waters, Guyancourt, France). The mobile phase was a mixture of water containing 0.05% of trifluoroacetic acid (Eluent A) and acetonitrile (Eluent B). The flow rate was set to 20 mL/min. Two gradients were developed and implemented depending on the injected fraction. Gradient I: 0 min, 17%; 5 min, 17%; 15 min, 23%; 19 min, 23%; 30 min, 38%, 35 min, 80%; 36 min, 100%; 48 min, 100%; 49 min, 17%; 60 min, 17%. Gradient II: 0 min, 15%; 5 min, 15%; 30 min, 20%; 40 min, 30%; 45 min, 80%; 46 min, 100%; 54 min, 100%; 56 min, 15%; 60 min, 15%. Aliquots (20 mg) of CPC fractions were dissolved in methanol (200 µL), filtered and introduced manually into the system. UV detection was carried out at 254 and 280 nm and chromatographic peaks were collected manually just after the detector. Samples obtained after

successive injections were pooled, evaporated in vacuo to remove acetonitrile and freeze-dried twice to obtain white amorphous powders.

4.3. LC–HRMS analysis

The LC–HRMS platform consisted of an HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland), an Accela U-HPLC system with quaternary pumps and an Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization (HESI I) probe (both from Thermo Fisher Scientific, Les Ulis, France). Liquid chromatography separation was performed on a C18 column (Hypersil Gold 2.1 mm×100 mm, 1.9 µm particle size), Thermo Fisher Scientific with water (A) and acetonitrile (B) as mobile phases. The flow rate was 600 µL/min and eluent B varied as follows: 0 min, 14%; 0.5 min, 14%; 1.5 min, 19%; 2 min, 19%; 4.5 min, 38%; 4.6 min, 98%; 6.9 min, 98%; 7 min, 14%; 8.6 min, 14%. The injection volume was 5 µL. Mass acquisitions were performed in negative Fourier transform mass spectrometry (FTMS) ionization mode at a unit resolution of 25,000 ($m/\Delta m$, fwhm at 200 Th). The mass analyzer was calibrated each week using Pierce® ESI Negative Ion Calibration solution (Thermo Fisher Scientific). The sheath and auxiliary gas flows (both nitrogen) were optimized at 75 and 18 arbitrary units, respectively. The HESI probe and capillary temperatures were 320 and 350 °C, respectively. The electrospray voltage was set to −3 kV, the capillary voltage to −60 V, the tube lens voltage offset to −135 V and the skimmer voltage to −26 V. Mass spectra were recorded from 200 to 800 Th, with an AGC value of 10^6. All data were processed using the Qualbrowser and Quanbrowser applications of Xcalibur version 2.1 (Thermo Fisher Scientific).

4.4. NMR experiments

All 1D and 2D NMR experiments were performed on a Bruker Avance 600 NMR spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz) equipped with a 5-mm TXI probe. All NMR spectra were acquired at 300 K in methanol-d_4. ^{1}H and ^{13}C chemical shifts were referenced to solvent signals. Data were processed using TOPSPIN software (Bruker). Molecule assignments were obtained by two-dimensional ^{1}H–^{1}H COSY, ^{1}H–^{1}H ROESY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC experiments.

4.5. Quantification of lyoniresinol in wine

4.5.1. Wines and sample preparation. Two series of wines were used in this study.

First, the same Sauvignon blanc must (Bordeaux, 2008) was fermented and aged in four different kinds of container[12]: a stainless steel tank (400 L, SST), a new oak tank (5000 L, NOT), two one-year-old oak barrels (225 L, 1OB) and two new oak barrels (225 L, NOB). After 5 months of ageing, four different wines were obtained (the barrel modalities were taken equally in both barrels) and analyzed.

The second set consisted of 13 vintages (from 1994 to 2008 except 1999 and 2000) of a white classified growth of Graves (Pessac-Léognan) made from Sauvignon blanc and Semillon and aged in oak barrels (ca. 30% of new oak barrels) for 10 months. The bottles were stored in the estate's cellar until analysis.

For quantitative analysis, wines were diluted with water (50:50) and 0.45 µm filtered. Triplicates of each sample were injected directly in LC–HRMS using the chromatographic and spectrometric parameters described above.

4.5.2. Preparation of calibration solution. A stock solution of lyoniresinol (1 g/L) was prepared in ethanol. Successive dilutions of this solution were performed with non-oaked white wine (SST modality) to provide calibration samples (5 mg/L, 2 mg/L, 1 mg/L, 500 µg/L, 200 µg/L, 100 µg/L, 50 µg/L).

Detection of lyoniresinol was based on theoretical exact mass and retention time. Peaks areas were determined by automatic integration. A calibration curve was obtained by plotting peak areas versus nominal concentration for each concentration level. Linearity was evaluated by correlation coefficient (R^2) and deviation of each back-calculated standard concentration from the nominal value. To determine repeatability, five replicates of the lowest calibration solution (50 µg/L) were injected and the relative standard deviation (RSD%) was calculated. Final concentrations of lyoniresinol in wine were expressed by considering the dilution factor.

4.6. Sensory analyses

All the tasting sessions took place in a specific room equipped with individual booths and air-conditioned at 20 °C. Normalized glasses were used.

4.6.1. Gustatory characterization of the different lignans purified in oak wood. After purification and identification, each lignan was dissolved at 10 mg/L in a 12 vol. % alc. hydro-

ethanolic solution as well as in a white non-oaked wine (Bordeaux 2011). The hydro-ethanolic solution was composed of pure and demineralized water (eau de source de Montagne, Laqueuille, France) and distilled ethanol. The samples were tasted by five experts in wine tasting. They described the gustatory perception of each compound using the vocabulary of wine tasting, and they evaluated in particular the bitterness intensity on a scale from 0 (not detectable) to 5 (strongly detectable).

4.6.2. Determination of lyoniresinol taste threshold in white wine. All the panellists (26 tasters aged from 22 to 61 years) were wine-tasting specialists or winemakers and had been previously informed of the nature and risks associated with the investigation.

The taste threshold of lyoniresinol was evaluated in a white wine (Bordeaux 2009, 12.6% alc. vol.; 5.9 g of glycerol/L; 0.71 g/L of glucose+fructose). Owing to saturation and the persistence of the bitter taste as well as the tiredness of the panel, two sessions were organized to optimize lyoniresinol concentrations for each taster. In the morning session, four concentrations (0.5, 1, 2 and 4 mg/L) were presented in ascending order. Each concentration was displayed according to the triangle test described by the International Organization for Standardization.[55] Concentrations presented in the afternoon depended on results from the first session for each taster. They again tasted the lowest concentration at which they had given a correct answer as well as two lower concentrations following a geometric progression of ratio 2. Tasters who did not give any correct answer during the morning session received two higher concentrations (8 and 16 mg/L) in the afternoon.

Individual thresholds were estimated as the geometrical mean between the lowest concentration of a continuous series of three correct answers and the previous concentration. The group threshold was estimated as the geometrical mean between all the individual thresholds.

4.7. Isolated compounds

The specific optical rotations were determined in methanol at 20 °C on a JASCO P-2000 polarimeter using the sodium emission wavelength (λ=589 nm).

*4.7.1. (±)-Lyoniresinol **1**.* Amorphous white powder; 138 mg; $[\alpha]_D^{20}$ +7.6 (c = 2.06, MeOH); HRMS m/z 419.1712 $[M-H]^-$ ($C_{22}H_{27}O_8^-$, 0.1 ppm); ^{1}H NMR (600 MHz, CD_3OD): δ 6.6 (s, 1, H-2), 6.4 (s, 2, H-2′, 6′), 4.32 (d, 1, J=5.6 Hz, H-7′), 3.87 (s, 3H, 3-OCH_3), 3.75 (s, 6H, 3′, 5′-

OCH_3), 3.6 (dd, 1, *J*=10.7, 5 Hz, H_b-9), 3.5 (dd, 1, *J*=10.3, 6.8 Hz, H_a-9), 3.5 (d, 2, *J*=4.9 Hz, H_a, H_b-9′), 3.39 (s, 3H, 5-OCH_3), 2.71 (dd, 1, *J*=15.1, 4.6 Hz, H_b-7), 2.58 (dd, 1, *J*=15.1, 11.8 Hz, H_a-7), 1.99 (m, 1, H-8′), 1.63 (m, 1, H-8); ^{13}C NMR (150 MHz, CD_3OD): *δ* 147.6 (C-3′, 5′), 147.3 (C-3), 146.3 (C-5), 137.9 (C-1′), 137.5 (C-4), 133.2 (C-4′), 128.8 (C-1), 124.9 (C-6), 106.4 (C-2), 105.5 (C-2′, 6′), 65.4 (C-9), 62.8 (C-9′), 58.7 (5-OCH_3), 55.3 (3-OCH_3), 55.2 (3′, 5′-OCH_3), 47.5 (C-8), 40.9 (C-7′), 39.5 (C-8), 32.1 (C-7).

4.7.2. (±)-5-Methoxyisolariciresinol ***2***. Amorphous white powder; 6.7 mg; $[\alpha]_D^{20}$ −3.6 (*c* = 1.13, MeOH); HRMS *m*/*z* 389.1606 $[M-H]^-$ ($C_{21}H_{25}O_7^-$, 0.1 ppm); ^{1}H and ^{13}C NMR see Table 2 in agreement with Jutiviboonsuk et al.[45]

4.7.3. Quercoresinol ***3*** *((±)-lyoniresinol 9-O-gallate).* Amorphous white powder; 6.5 mg; $[\alpha]_D^{20}$ +5 (*c* = 1.22, MeOH); HRMS *m*/*z* 571.1823 $[M-H]^-$ ($C_{29}H_{31}O_{12}^-$, 0.4 ppm); ^{1}H and ^{13}C NMR see Table 2.

4.7.4. Lyoniside ***4*** *((+)-lyoniresinol 9′-O-β-xylopyranoside).* Amorphous white powder; 7.8 mg; $[\alpha]_D^{20}$ +37.5 (*c* = 1.06, MeOH); HRMS *m*/*z* 551.2136 $[M-H]^-$ ($C_{27}H_{35}O_{12}^-$, 0.4 ppm); ^{1}H NMR (600 MHz, CD_3OD): *δ* 6.58 (s, 1, H-2), 6.43 (s, 2, H-2′, 6′), 4.39 (d, 1, *J*=6.7 Hz, H-7′), 4.23 (d, 1, *J*=7.6 Hz, H-1″), 3.86 (s, 3H, 3-OCH_3), 3.85 (dd, 1, *J*=11.3, 5.3 Hz, H_b-9′), 3.84 (dd, 1, *J*=11.3, 5.3 Hz, H_a-5″), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.66 (dd, 1, *J*=10.9, 4.2 Hz, H_b-9), 3.55 (dd, 1, *J*=10.8, 6.5 Hz, H_a-9), 3.49 (ddd, 1, *J*=10.1, 8.9, 5.4 Hz, H-4″), 3.43 (dd, 1, *J*=9.7, 3.9 Hz, H_a-9′), 3.34 (s, 3H, 5-OCH_3), 3.32 (m, 1, H-3″), 3.23 (dd, 1, *J*=8.9, 7.7 Hz, H-2″), 3.17 (dd, 1, *J*=11.4, 10.4 Hz, H_b-5″) 2.73 (dd, 1, *J*=15.2, 4.7 Hz, H_b-7), 2.64 (dd, 1, *J*=15, 11.5 Hz, H_a-7), 2.07 (m, 1, H-8), 1.72 (m, 1, H-8′); ^{13}C NMR (150 MHz, CD_3OD): *δ* 147.2 (C-3′, 5′), 147 (C-3), 146 (C-5), 139.5 (C-4′), 137 (C-4), 134.3 (C-1), 132.2 (C-1′), 122.7 (C-6), 106.2 (C-2), 105.4 (C-2′, 6′), 104 (C-1″), 76.5 (C-3″), 73.3 (C-2″), 69.5 (C-4″), 69.4 (C-9′), 65.3 (C-5″), 64.2 (C-9), 58.3 (5-OCH_3), 55.1 (3′, 5′-OCH_3), 54.7 (3-OCH_3), 45 (C-8), 41.2 (C-7′), 38.6 (C-8′), 32.1 (C-7).

4.7.5. Nudiposide ***5*** *((−)-lyoniresinol 9′-O-β-xylopyranoside).* Amorphous white powder; 7.9 mg; $[\alpha]_D^{20}$ −64.2 (*c* = 1.30, MeOH); HRMS *m*/*z* 551.2136 $[M-H]^-$ ($C_{27}H_{35}O_{12}^-$, 0.4 ppm); ^{1}H NMR (600 MHz, CD_3OD): 6.58 (s, 1, H-2), 6.42 (s, 2, H-2′, 6′), 4.23 (d, 1, *J*=7 Hz, H-7′), 4.11 (d, 1, *J*=7.5 Hz, H-1″), 3.86 (s, 3H, 3-OCH_3), 3.82 (dd, 1, *J*=10.1, 4.6 Hz, H_b-9′), 3.87 (dd, 1, *J*=11.3, 5.6 Hz, H_a-5″), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.65 (dd, 1, *J*=11.1, 4.6 Hz, H_b-9), 3.62

(dd, 1, *J*=11.1, 6.1 Hz, H_a-9), 3.5 (ddd, 1, *J*=10.1, 8.9, 5.4 Hz, H-4″), 3.59 (dd, 1, *J*=10, 4.8 Hz, H_a-9′), 3.31 (s, 3H, 5-OCH_3), 3.28 (dd, *J*=9.8, 8 Hz, 1, H-3″), 3.2 (dd, 1, *J*=9.7, 7 Hz, H-2″), 3.17 (dd, 1, *J*=11.3, 10.5 Hz, H_b-5″), 2.7 (m, 1, H_b-7), 2.69 (m, 1, H_a-7), 2.04 (m, 1, H-8), 1.72 (m, 1, H-8′); ^{13}C NMR (150 MHz, CD_3OD): *δ* 147.2 (C-3′, 5′), 147 (C-3), 145.9 (C-5), 139.5 (C-4′), 137.6 (C-4), 129 (C-1), 132.5 (C-1′), 124.2 (C-6), 106.3 (C-2), 105.5 (C-2′, 6′), 103.6 (C-1″), 76.3 (C-3″), 73.3 (C-2″), 69.5 (C-4″), 69.5 (C-9′), 65.3 (C-5″), 64.4 (C-9), 58.3 (5-OCH_3), 55 (3′, 5′-OCH_3), 54.7 (3-OCH_3), 45.1 (C-8), 41.5 (C-7′), 38.9 (C-8′), 32.1 (C-7).

4.7.6. (+)-Lyoniresinol 9′-O-β-glucopyranoside ***6***. Amorphous white powder; 17.5 mg; $[\alpha]_D^{20}$ +38.4 (*c* = 2.09, MeOH); HRMS *m/z* 581.2241 $[M-H]^-$ ($C_{28}H_{37}O_{13}^-$, 0.2 ppm); ^{1}H NMR (600 MHz, CD_3OD): *δ* 6.6 (s, 1, H-2), 6.44 (s, 2, H-2′, 6′), 4.44 (d, 1, *J*=6.2 Hz, H-7′), 4.3 (d, 1, *J*=7.8 Hz, glu-H-1), 3.9 (dd, 1, *J*=9.7, 5.6 Hz, H_b-9′), 3.89 (dd, 1, *J*=9.7, 5.6 Hz, H_b-6″), 3.87 (s, 3H, 3-OCH_3), 3.76 (s, 6H, 3′, 5′-OCH_3), 3.67 (dd, 1, *J*=12, 5.6 Hz, H_a-6″), 3.66 (dd, 1, *J*=10.8, 4.3 Hz, H_b-9), 3.56 (dd, 1, *J*=10.9, 6.6 Hz, H_a-9), 3.47 (dd, 1, *J*=9.8, 4 Hz, H_a-9′), 3.36 (s, 3H, 5-OCH_3), 3.38 (t, 1, *J*=8.8, 8.8 Hz, glu-H-3), 3.3 (t, 1, *J*=8.6, 8.6 Hz, glu-H-4), 3.25 (m, 1, glu-H-5), 3.25 (dd, 1, *J*=9.1, 8.1 Hz, glu-H-2), 2.73 (dd, 1, *J*=15.1, 4.6 Hz, H_b-7), 2.63 (dd, 1, *J*=14.8, 11.7 Hz, H_a-7), 2.09 (m, 1, H-8′), 1.72 (m, 1, H-8); ^{13}C NMR (150 MHz, CD_3OD): *δ* 147.1 (C-3′, 5′), 146.9 (C-3), 145.6 (C-5), 137.6 (C-1′), 136.8 (C-4), 132 (C-4′), 124.4 (C-1), 122.7 (C-6), 106.3 (C-2), 105.4 (C-2′, 6′), 103.3 (glu-C-1), 76.5 (glu-C-3), 76.2 (glu-C-5), 73.6 (glu-C-2), 70 (glu-C-4), 69.8 (C-9′), 64.5 (C-9), 61.2 (glu-C-6), 58.3 (5-OCH_3), 54.8 (3-OCH_3), 55.1 (3′, 5′-OCH_3), 44.7 (C-8′), 40.9 (C-7′), 38.9 (C-8), 31.9 (C-7).

4.7.7. (−)-Lyoniresinol 9-O-gallate 9′-O-β-xylopyranoside ***7***. Amorphous white powder; 1 mg; $[\alpha]_D^{20}$ −47.3 (*c* = 0.35, MeOH); HRMS *m/z* 703.2248 $[M-H]^-$ ($C_{34}H_{39}O_{16}^-$, 0.6 ppm); ^{1}H and ^{13}C NMR see Table 2.

4.7.8. 5,5′-Dimethoxysecoisolariciresinol ***8***. Amorphous white powder; 9 mg; $[\alpha]_D^{20}$ +27.3 (*c* = 1.60, MeOH); HRMS *m/z* 421.1869 $[M-H]^-$ ($C_{22}H_{29}O_8^-$, 0.3 ppm); ^{1}H and ^{13}C NMR see Table 2 in agreement with Rahman et al. and Perez et al.[48,47]

4.7.9. Ssioriside ***9*** *(5,5′-dimethoxysecoisolariciresinol 9-O-β-xylopyranoside).* Amorphous white powder; 3 mg; $[\alpha]_D^{20}$ −9.24 (*c* = 0.89, MeOH); HRMS *m/z* 553.2292 $[M-H]^-$ ($C_{27}H_{37}O_{12}^-$, 0.3 ppm); ^{1}H and ^{13}C NMR see Table 2 in agreement with Yoshinari et al.[52]

Supporting Information

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.07.090. These data include MOL files and InChiKeys of the most important compounds described in this article.

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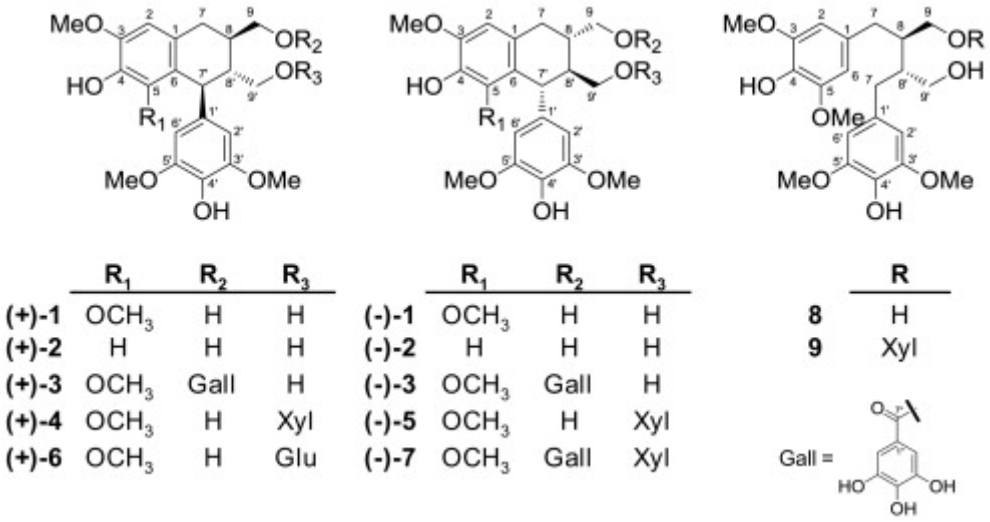
Figures



	R_1	R_2	R_3
(+)-1	OCH_3	H	H
(+)-2	H	H	H
(+)-3	OCH_3	Gall	H
(+)-4	OCH_3	H	Xyl
(+)-6	OCH_3	H	Glu

	R_1	R_2	R_3
(-)-1	OCH_3	H	H
(-)-2	H	H	H
(-)-3	OCH_3	Gall	H
(-)-5	OCH_3	H	Xyl
(-)-7	OCH_3	Gall	Xyl

	R
8	H
9	Xyl

Fig. 1. Chemical structures of lignans **1**–**9**. Xyl and Glu correspond, respectively, to β-xylopyranose and β-glucopyranose.

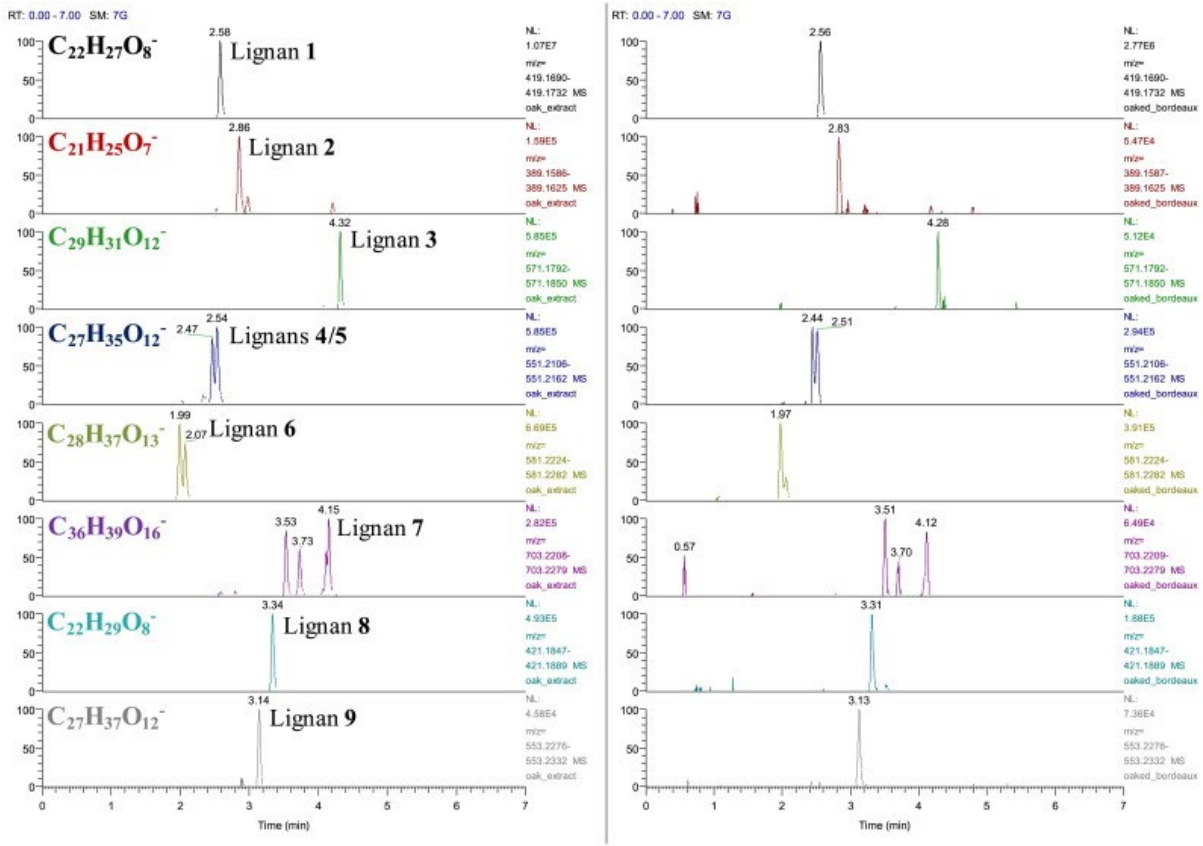


Fig. 2. Negative LC–ESI-FTMS XIC of an oak wood extract (left) and an oaked wine (right) corresponding to $[M-H]^-$ ions of lignans **1**–**9** (from top to bottom).

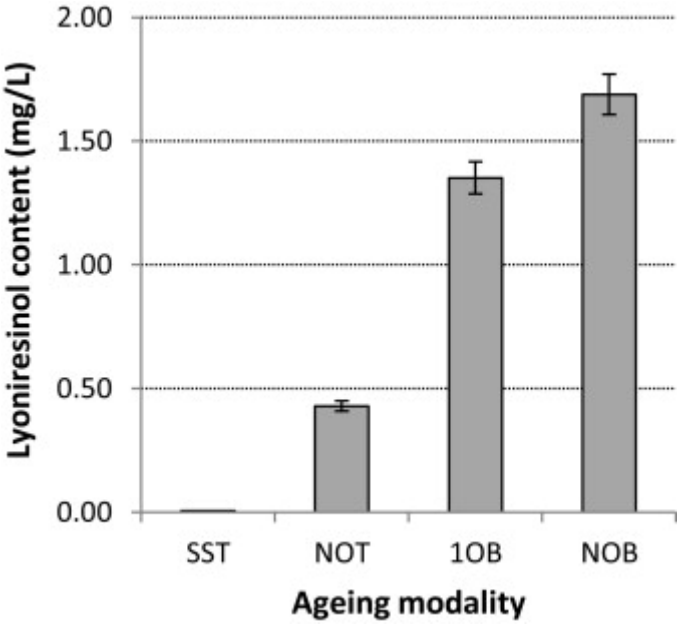


Fig. 3. Influence of ageing conditions on lyoniresinol content in white wines.

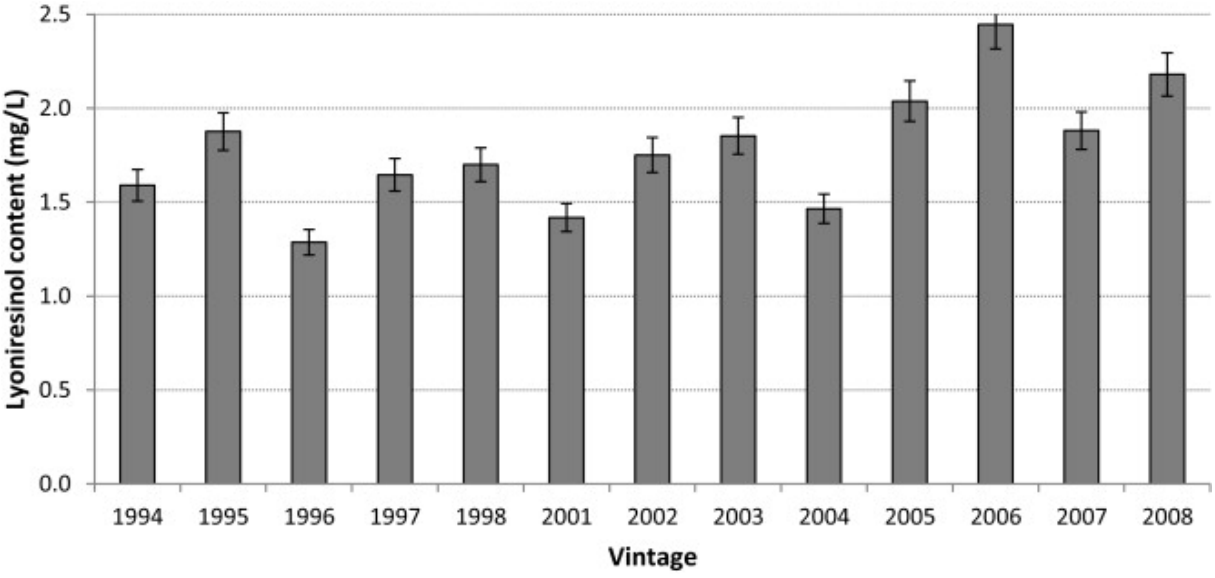


Fig. 4. Variations of lyoniresinol content in different vintages of a commercial wine.

Tables

Table 1. Constitution of CPC fractions enriched in lignans

Injected extract	System	Tubes	Isolated lignans
BuOH extract	Ternary system	22–35	**7**
		36–50	**4**, **5**, **9**
		51–70	**6**
EtOAc extract	Quaternary system	10–13	**3**
		19–23	**2**
		24–33	**1**, **8**

Table 2. ^{1}H and ^{13}C NMR data of compounds **2**, **3**, **7**, **8** and **9** in CD_3OD

Moiety		**2**		**3**		**7**		**8**		**9**	
		δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)
	1	127.5		124		124.4		131.4		131.6	
	2	111	6.67 s	106.2	6.62 s	106.3	6.6 s	105.8	6.33 s	105.8	6.34 s
	3	145.8		147.3		147.1		147.2		147.4	
	4	143.9		137.3		137.5		132.8		132.8	
	5	115.9	6.22 s	146.1		146.1		147.2		147.4	
	6	132.3		127.7		123.6		105.8	6.33 s	105.8	6.34 s
	7	32.2	2.8 m	31.5	2.68 dd (15.1; 10.8) 2.83 dd (15.2; 5)	32	2.73 dd (15.2; 11.1) 2.81 dd (14.7; 4.2)	35.1	2.54 dd (8; 13.3) 2.71 dd (5.3; 13.3)	34.6	2.72 dd (13.7; 7) 2.57 dd (12; 8.2)
	8	38.5	2.02 m	35.6	2.01 m	35.9	2.06 m	42.8	1.92 m	39.7	2.08 m
	9	64.5	3.72 m	66.9	4.17 dd (10.8; 7.2) 4.36 dd (10.9; 5.1)	66.6	4.25 dd (8.2; 6.4) 4.36 dd (11; 4.5)	60.6	3.58 dd (5.5; 10.8) 3.67 m	69.5	3.99 dd (9.6; 5.8) 3.49 m
3-OMe		54.9	3.82 s	54.8	3.87 s	54.8	3.87 s	55.1	3.76 s	54.7	3.76 s
5-OMe				58.3	3.39 s	58.3	3.35 s	55.1	3.76 s	54.7	3.76 s
1′		136.2		137.1		133		131.4		131.6	
2′		106.2	6.45 s	105.1	6.4 s	105.4	6.45 s	105.8	6.33 s	105.8	6.37 s
3′		147.8		147.3		147.4		147.2		147.4	
4′		133.5		132.8		139		132.8		132.8	
5′		147.8		147.3		147.4		147.2		147.4	
6′		106.2	6.45 s	105.1	6.4 s	105.4	6.45 s	105.8	6.33 s	105.8	6.37 s
7′		47.1	3.83 m	40.2	4.39 d (5.4)	41.2	4.44 d (6.3)	35.1	2.54 dd (8; 13.3) 2.71 dd (5.3; 13.3)	34.2	2.65 dd (13.6; 6.9) 2.58 dd (12; 8.5)
8′		46.5	1.8 m	46.9	2.13 m	45.3	2.2 m	42.8	1.92 m	42.2	1.95 m
9′		60.6	3.42 dd (3.9; 11.3) 3.69 m	61.8	3.53 dd (11.1; 6.2) 3.58 dd (11; 4.3)	68.8	3.49 m 3.89 m	60.6	3.58 dd (5.5; 10.8) 3.67 m	61	3.56 dd (10.9; 6.3) 3.7 dd (10.9; 5.6)
3′-OMe		55.3	3.8 s	55	3.74 s	55.2	3.75 s	55.1	3.76 s	54.7	3.77 s
5′-OMe		55.3	3.8 s	55	3.74 s	55.2	3.75 s	55.1	3.76 s	54.7	3.77 s

Xylose	1″			104.2	4.24 d (6.9)	103.9	4.19 d (7.5)
	2″			73.3	3.24 dd (8.6; 7.9)	73.5	3.21 dd (8.8; 7.8)
	3″			76.5	3.32 m	76.5	3.31 m
	4″			69.5	3.49 m	69.4	3.46 m
	5″			65.3	3.15 dd (11.3; 10.5) 3.83 dd (11.6; 5.3)	65.4	3.86 dd (11.5; 5.3) 3.18 dd (11.1; 10.8)
		119.9					
Gallate	1‴	108.4		119			
	2‴, 6‴	145	7.04 s	108.6	7.05 s		
	3‴, 5‴	138		145.2			
	4‴	166.9		138.1			
	7‴	119.9		167.1			

Table 3. Sensory characterization of isolated lignans **1–9**

Compounds	Taste in water	Bitterness intensity in water/EtOH[a]	Bitterness intensity in wine[a]
1	Bitter	5	5
2	—	—	—
3	Slightly sweet	—	—
4	—	—	—
5	—	—	—
6	Bitter	3	2
7	—	—	—
8	Bitter	2	2
9	Bitter	5	5

[a]Intensity was evaluated on a scale from 0 (not detectable) to 5 (strongly detectable).

Graphical abstract