Role of Oak Coumarins in the Taste of Wines and Spirits: Identification, Quantitation, and Sensory Contribution through Perceptive Interactions

Delphine Winstel, Eric Gautier, Axel Marchal

Université Bordeaux, Unité de Recherche Œnologie, EA 4577, USC 1366 INRA, ISVV, 33882 Villenave d'Ornon Cedex, France

Corresponding author:
Axel Marchal
axel.marchal@u-bordeaux.fr

Abstract:

During barrel aging, wines and spirits undergo sensory changes as a result of the release of aroma and taste molecules. Among the nonvolatile compounds, various coumarins have already been identified in oak wood but their sensory role remained unclear. In this study, the presence of coumarins in oak wood extract, wine, and spirits was first assessed by targeted screening. Fraxetin was identified for the first time in these matrices. After development and validation of a liquid chromatography–high-resolution mass spectrometry quantitation method, esculetin, scopoletin, fraxetin, umbelliferone, 4-methylumbelliferone, and coumarin were assayed in various wines and spirits. The concentrations measured were generally below the gustatory detection thresholds determined in wines and spirits. Nevertheless, by adding a mixture of coumarins in wines and spirits, a significant increase in bitterness was observed, thus demonstrating their potential contribution to the taste of wines and spirits through perceptive interactions.

Keywords: coumarins, bitterness, taste-active compounds, quantitation, perceptive interaction

INTRODUCTION

Oak barrels have long been used to transport beverages. Nowadays, their use has evolved and is mainly limited to the production process during winemaking and/or aging for most of the great wines and during aging only for several spirits, such as cognac, armagnac, rum, and whiskey. This contact significantly modifies the sensory properties of the beverages, and the compounds responsible for changes in color, aromas, tactile sensations, and taste have been studied in recent decades.[1,2] While the key aromatic compounds released from oak wood in wines and spirits are now well known,[1,3] modifications in gustatory properties have been only partially explained. A gain in sweetness is frequently observed during aging[4] that can be explained by the release of sweet triterpenoids from oak wood.[5,6] On the other hand, aging in barrels can sometimes increase the perception of bitterness in wines and spirits and negatively impacts their value. This phenomenon has been widely attributed to ellagitannins, whose bitter characteristics have been suggested.[7] However, Glabasnia and Hofmann showed that the detection thresholds of the main hydrolysable tannins, in bottled water at pH 4.5, were significantly higher than their concentrations in wines, suggesting their limited influence on wine bitterness.[8] In addition to ellagitannins, other oak polyphenols have been studied. In particular, oak wood contains various lignans with taste properties.[9] For example, (+)-lyoniresinol has the strongest bitterness.[10] Oak wood also contains coumarins,[11] which are secondary metabolites originating from the phenylpropanoid pathway *via t*-cinnamic acid.[12] These molecules, which are widespread in the plant kingdom, result from the lactonization of *ortho*-hydroxycinnamic acid. They are classified into four categories: simple coumarins, formed from a benzene ring and a lactone nucleus (benzo-α-pyrone); furocoumarins, formed from a furan ring and a simple coumarin core; pyranocoumarins, formed from a pyran ring and a simple coumarin core; and phenylcoumarins, formed from a phenyl ring and a simple coumarin core. Variable hydroxylations, *O*-methylations, and glycosylations can affect the aglycons, resulting in a high diversity of compounds, of which more than 700 have already been characterized in 30 plant families and more than 150 species.[12] Coumarins and furanocoumarins are generally present in all parts of the plant but especially in seeds, fruits, leaves, and roots.[13] They are considered as phytoalexins because they enhance the defense of the plant against attacks by various pathogens.[14] Furthermore, they have potentially valuable biological activities for human health, such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, and anticancer properties, and they also have neuroprotective effects.[15]

The influence of cooperage parameters, such as the seasoning or the toasting of the staves[16,17] on coumarin concentrations in oak wood extracts, has received attention. From a gustatory point of view, the coumarins in the glycosylated form are described as bitter while the aglycone forms are perceived as slightly sour.[1,18] In one of these studies, their detection thresholds were estimated at 2 µg/L in a red wine,[18] which seems surprisingly low. However, the methodology used, and in particular the number of tasters, was not reported; so the robustness of this finding is questionable. If the detection thresholds for coumarins were indeed to be this low, they could have a significant effect on taste. Nevertheless, this hypothesis has been contested by other authors given the very low concentrations measured for these molecules and their sensory properties.[11,16] In view of these contradictions and the lack of knowledge in the literature, the real contribution of oak coumarins to the taste of wines and spirits remains unclear.

The present study aimed to clarify this issue. For this purpose, a targeted screening of coumarins was first performed in oak wood. Then, a liquid chromatography–high-resolution mass spectrometry (LC-HRMS) quantitation method was developed and validated to assay the targeted compounds in wines and spirits. The gustatory detection threshold of the main coumarins was established, and interactive perceptions between these compounds were studied in order to compare their sensory contribution with the levels found in wines and spirits.

MATERIALS AND METHODS

Chemicals. For sample preparation, ultrapure water (Milli-Q purification system, Millipore, France) was used. Acetonitrile (ACN) and water, used for chromatographic separation, and formic acid, used for acidification of solvents, were of liquid chromatography-mass spectrometry (LC–MS) grade and were purchased from Fisher Chemical (Illkirch, France). The coumarin standards were purchased from different companies: esculetin (≥98%), scopoletin (≥98%), coumarin (≥99%), and 4-methylumbelliferone (≥98%) from Sigma-Aldrich (Saint-Quentin Fallavier, France); umbelliferone (≥98%) from Fluka (Seelze, Allemagne) and fraxetin (≥99%) from Extrasynthese (Genay, France).

Samples and Calibration Solution. *Preparation of Samples.* For the LC-HRMS targeted screening, an oak wood extract (100 g/L) prepared in a hydro-alcoholic solution (50:50 H_2O/EtOH) at room temperature for three days under an inert atmosphere, and protected from

light, a red wine aged 16 months in French oak barrels (Margaux, 2011, 12.5% vol. alc.) and a commercial spirit (Cognac XO) were used. Coumarins were quantitated in 90 commercial wines, 44 red wines (24 from Bordeaux, 6 from Burgundy, 2 from Languedoc-Roussillon, 2 from Rhône Valley, 1 from Loire Valley, 1 from Beaujolais, 7 from Spain, and 1 from Germany) and 46 white wines (13 from Burgundy, 11 from Alsace, 9 from Bordeaux, 5 from Loire Valley, 4 from Languedoc-Roussillon, and 4 from Rhône Valley) with vintages from 1995 to 2017. Two series of spirits were used in this study. Coumarins were assayed in 28 commercial spirits aged in oak wood (including 12 cognacs, 4 grape brandies, 3 rums, 5 whiskies, and 4 bourbons). The second set of spirits, supplied by Rémy Martin, consisted of 10 vintages of "eau-de-vie" of cognac from 1970 to 2015, with five replicates for each year. The samples came from the same distillery and had been aged in similar conditions. They were matured in used barrels (350 L coarse grain oak barrels). All concentrations were expressed in µg/L of wine or spirits. For quantitative analysis, the wine samples were diluted with water by a factor 5, and the spirit samples were reduced to 8% alcohol and then filtered at 0.45 µm.

Preparation of Calibration Solution. A stock solution of esculetin, scopoletin, coumarin, umbelliferone, 4-methylumbelliferone, and fraxetin (1 g/L for each compound) was prepared in ethanol. One range of calibration was prepared by successive dilutions of this solution in ultrapure water in order to supply calibration samples (10 mg/L, 5 mg/L, 2 mg/L, 1 mg/L, 500 µg/L, 200 µg/L, 100 µg/L, 50 µg/L, 20 µg/L, 10 µg/L, 5 µg/L, 2 µg/L, and 1 µg/L).

LC Analysis. The high-performance LC (HPLC) appliance consisted of an HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and an Accela U-HPLC system with quaternary pumps. For analyses, a C18 column (Hypersil Gold 2.1 × 100 mm, 1.9 µm particle size, Thermo Fisher Scientific) was used with water containing 0.1% of formic acid (eluent A) and ACN with 0.1% of formic acid (eluent B) as mobile phases. The flow rate was set at 600 µL/min, and the injection volume was 5 µL. For screening analysis, eluent B varied as follows: 0 min, 10%; 1.0 min, 10%; 5.0 min, 50%; 5.3 min, 98%; 6.0 min, 98%; 6.15 min, 10%; 7 min, 10%. For quantitative analysis, eluent B varied as follows: 0 min, 15%; 1.6 min, 15%; 5.0 min, 20%; 9.0 min, 25%; 9.3 min, 98%; 10.3 min, 98%; 10.4 min, 15%; 12.0 min, 15%.

HRMS. An Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization (ESI) (HESI II) probe (both from Thermo Fisher Scientific, Les Ulis, France) was used. The mass analyzer was calibrated each week using PierceESI Positive Ion Calibration solutions (Thermo Fisher Scientific). The ionization and spectrometric parameters were not the

same for screening and quantitative analysis. Mass acquisitions were performed and optimized in the positive HRMS ionization mode. Table 1 summarizes the main parameters for both methods. All data were processed using the Qual Browser and Quan Browser applications of Xcalibur version 3.0 (Thermo Fisher Scientific). Detection of each coumarin was based on the theoretical exact mass of their protonated molecular ion ($[M + H]^+$) and their retention time (Table S1). Peak areas were determined by automatic integration of extracted ion chromatograms (XICs) built in a 3 ppm window around the exact mass of the $[M + H]^+$ ion.

Method Validation for Quantitation. The quantitation method was validated by studying sensitivity, linearity, specificity, intraday repeatability, and trueness.

Sensitivity. Given the high selectivity of the mass measurement, the notion of signal-to-noise is not relevant to evaluate sensitivity for this technique. The limit of detection (LOD) of a molecule is defined as the lowest concentration of this molecule for which a reliable and reproducible signal is observed. In addition, the signal must be different from a blank made under the same conditions. In this study, the method described by De Paepe et al.[19] was used. The lowest levels of the calibration curve (from 1 to 20 µg/L) were injected into five replicates. Limit of quantitation (LOQ) is defined as the lowest concentration of the molecule that can be quantitatively determined by the method, with a precision lower than, for example, 10% and an accuracy (recovery of back-calculated concentrations) higher than, for example, 90%. They were obtained for each compound.

Linearity and Accuracy. The working range was based on the LOQ determined previously. A calibration curve was established by plotting the areas for each concentration level versus the nominal concentration. Quadratic regression was used with a 1/x statistical weight for each coumarin. Linearity was evaluated by the correlation coefficient (R^2) and by deviations of each back-calculated standard concentration from the nominal value.

Repeatability and Trueness. To determine intraday precision, five replicates of three intermediate calibration solutions (10 µg/L, 200 µg/L, and 10 mg/L) were injected, and the relative standard deviation (RSD %) was calculated. Trueness was checked by calculating the recovery ratio (between measured and expected areas) from three samples (a red wine, a white wine, and a spirit). They were chosen among the analyzed samples and were spiked with calibration solution corresponding to an addition of 10 µg/L, 200 µg/L, and 10 mg/L of the six coumarins. Interday repeatability was estimated by injections of the same standard solutions for five successive days.

Specificity. Specificity was assessed by evaluating the mass accuracy and retention time repeatability. These parameters were determined concomitantly with the precision and trueness analysis described above.

Sensory Analyses. Tasting sessions took place in a specific air-conditioned room at 20 °C equipped with individual booths and normalized glasses. The panel consisted of 22 wine tasters, 12 women and 10 men, aged from 20 to 45 years, also trained to taste a matrix with a higher alcohol concentration (40%, v/v). Because coumarin has a strong odor, nose clips were used for each session. Wines used for sensory analyses were a nonoaked white "Pays d'Oc" (wine A, 12% vol. alc.; 3.6 g/L of titratable acidity; pH 3.4) and a nonoaked red "Blaye Côtes-de-Bordeaux" (wine B, 12.7% vol. alc.; 3.2 g/L of titratable acidity; pH 3.7). The "eau-de-vie" used for sensory analysis was a nonoaked spirit adjusted to 40% v/v of ethanol with pure and demineralized water (eau de source de Montagne, Laqueuille, France). The absence of these coumarins in these three matrices was checked by LC-HRMS analysis.

Preliminary Gustatory Characterization. Pure compounds were first tasted by five experts in winetasting. Each molecule was dissolved at 1 mg/L in a 12% vol. alc. hydroethanolic solution. Experts described the gustatory perception (bitterness, sourness, sweetness, and saltiness) of each coumarin using the vocabulary of winetasting and were asked in particular to evaluate the bitterness intensity on a scale from 0 (not detectable) to 5 (strongly detectable).

Determination of Gustatory Detection Threshold of Three Coumarins in Wines and Spirits. One session for each coumarin (esculetin, scopoletin, and 4-methylumbelliferone) was performed in wine A. For each molecule, five concentrations, following a geometric progression of ratio 4, were presented in ascending order to the tasters: 10, 40, 160, 640, and 2560 μg/L.

Concerning spirits, the gustatory detection threshold of each coumarin (esculetin, scopoletin, and 4-methylumbelliferone) was also evaluated in a nonoaked "eau-de-vie" adjusted to 40% v/v. Owing to the higher alcohol concentration present in this matrix and the remanence of the bitter taste, two different sessions were planned to avoid tiredness among the panelists. In the first session, three concentrations (200, 400, and 800 μg/L) were presented in ascending order. Each concentration was displayed according to the triangular test described by ISO.[20] Concentrations presented in the second session depended on the results from the first session for each taster. For a given panelist, if all the answers were correct in the first session, then two lower concentrations (40 and 160 μg/L) and one higher (640 μg/L) were presented following a geometric progression of ratio 4, starting with the lowest. Conversely, tasters who gave only

one correct answer (the last concentration) or who did not give any correct answers during the first session received two higher concentrations (1 and 2 mg/L) and one lower (500 μg/L) in the other session.

Individual thresholds were estimated for the two matrices as the geometrical mean between the lowest concentration of a continuous series of three correct answers and the concentration just below this level. The group threshold was estimated as the geometrical mean between all the individual thresholds.

Gustatory Profiling of Wines and Spirits Added with Mixed Coumarins. Gustatory profiling was performed in wine A, wine B, and nonoaked "eau-de-vie." For each matrix, five different modalities were presented to the tasters. The control modality corresponded to the matrix without addition of molecules and the other four to increasing concentrations of coumarins. The values were based on the results of the quantitation in wines and spirits. Thus, the concentrations added for each modality corresponded to the 1st quartile (M1), the median (M2), the 3rd quartile (M3), and the maximum (M4) calculated for each coumarin. Panelists were asked to create a sensory profiling by rating the bitterness and the sweetness intensities on a 10-point scale (0 = "absence" to 10 = "very high") for control glass and supplemented glass. For all evaluations, samples were labeled with random three-digit codes and presented in counterbalanced order to avoid bias.

Statistical Analysis. All statistical analyses were carried out using the software XL-STAT version 2019.1.1.56334 (Addinsoft, Paris, France). According to the international organization for standardization,[21] sensory profiling results were interpreted by the Friedman test.

RESULTS AND DISCUSSION

Search for Oak Coumarins in Red Wine and Spirit Aged in Barrels. To determine whether coumarins have a sensory impact, their presence in an oak wood extract, in a wine aged in oak barrels and a spirit aged for several years was first investigated. Thanks to its mass measurement accuracy and separative performances, LC-HRMS is a powerful technique to screen complex matrices. In a preliminary study (data not shown), the $[M + H]^+$ ions corresponding to empirical formulas of various coumarins frequently observed in plants were targeted. The screening of an oak wood extract had suggested the presence of esculetin,

scopoletin, umbelliferone, 4-methylumbelliferone, and coumarin, which was concordant with previous studies.[11,17,22] A signal corresponding to the ion of fraxetin, a widespread coumarin in plants and especially in the genus *Fraxinus*,[23] had also been detected, whereas this compound had never been identified in oak wood. Consequently, the commercial standards of these six coumarins were injected in LC-HRMS (Figure 1). XICs were built for each coumarin by targeting the protonated $[M + H]^+$ ions within a window of 5 ppm around their theoretical *m/z*. These XICs obtained for the blend of the pure standards and for an oak wood extract were compared and showed peaks at the same retention times. Doping the oak wood extract with the standards led to an increase in the peak area. In addition, the HRMS/MS spectra revealed in both matrices the same fragment signals at the retention times of each compound. These data established the presence of the six coumarins in the oak wood extract, which confirmed previous studies for esculetin, scopoletin, umbelliferone, 4-methylumbelliferone, and coumarin. However, fraxetin has never been described in the *Quercus* genus until now. Then, the same investigation was performed on a red wine and a cognac both aged in oak barrels. XICs were obtained for the standards (Figure S2A) and for the two matrices (Figure S2B,C). Their comparison highlighted signals of significant intensity, meaning a signal-to-noise ratio (S/N) greater than 3, at the retention times of esculetin, fraxetin, umbelliferone, coumarin in the oaked red wine, and of esculetin, fraxetin, scopoletin, coumarin, and 4-methylumbelliferone in oaked "eau-de-vie" of cognac. Furthermore, the same fragment ions were obtained following the analysis of the MS^2 fragmentation spectra for these retention times. These results demonstrated the presence of most of the targeted coumarins in the red wine and cognac injected. Scopoletin and 4-methylumbelliferone were not detected in red wine nor was umbelliferone in cognac (S/N < 3). As for oak wood, five of these compounds, including esculetin, umbelliferone, scopoletin, 4-methylumbelliferone, and coumarin, were previously described in wines and spirits[11,17,24,25] but never fraxetin. Apart from their presence, the sensory contribution of coumarins to the taste of wines and spirits remained largely unclear.

Sensory Characterization of Coumarins from Oak Wood. *Assessment of the Gustatory Properties of Coumarins by a Preliminary Tasting.* First, coumarins were tasted individually by a panel of five expert tasters at a concentration of 1 mg/L in a hydroalcoholic solution to accentuate their sensory properties. If the perception of bitterness, sweetness, saltiness, or sourness of the spiked solution differed from the blank hydro-alcoholic solution, the intensity of this perception was evaluated on a scale from 1 to 5 (Table 2).

Five of the six targeted coumarins were described as bitter. 4-Methylumbelliferone was the most intense, followed by esculetin, scopoletin, then coumarin and umbelliferone. The perceived bitterness for coumarin confirmed previous sensory observations.[26] Furthermore, Meyerhof et al. showed that coumarin could activate two bitter taste receptors, Tas2R10 and Tas2R14, which confirms our tasting.[27] Fraxetin developed no bitterness but a slight acidity. Sensory analysis showed taste differences depending on the chemical structure of the targeted coumarins. In particular, a significant difference was observed between umbelliferone and 4-methylumbelliferone. Although this observation is too isolated to establish a general structure–activity relationship for coumarins, the presence of a methyl group at the 4 position strongly increased the bitterness of this molecule. This preliminary tasting highlighted the bitterness of certain coumarins. Esculetin, scopoletin, and 4-methylumbelliferone were the bitterest; therefore, their gustatory detection threshold was established.

Determination of Gustatory Detection Threshold of Three Coumarins in Wines and Spirits. Previous studies have shown that the nature of the matrix can significantly affect the taste properties of a compound, as demonstrated for (±)-lyoniresinol.[9,28] For this reason, the gustatory detection thresholds of esculetin, scopoletin, and 4-methylumbelliferone were determined in a nonoaked wine A and in a nonoaked "eau-de-vie" (Table 3). The values were in the range of a few hundred μg/L, which is relatively low for nonvolatile compounds but higher than reported in a previous study.[18] However, the methodology used in that study was not described, which significantly affected the robustness of the results and limited the interest of the comparison. In general, the findings showed that the detection thresholds for the three targeted coumarins in a nonoaked wine were lower than those measured in an "eau-de-vie" of cognac. The threshold for scopoletin and 4-methylumbelliferone did not seem to be significantly influenced by the nature of the matrix. Conversely, esculetin had a very low detection threshold in wines, whereas it was almost nine times higher in spirits. These results underline the importance of measuring a detection threshold for each compound in each matrix in order to determine its real taste impact. In addition, for each molecule, a high interindividual variability in detection thresholds was observed. The same trends have been described for other molecules in wine and spirits, such as lyoniresinol.[9,28] Other studies on these interindividual differences in sensitivity showed that, for certain volatile compounds, a factor of 1000 was frequently observed between the most sensitive and the least sensitive tasters.[29] This testifies to the importance of using a large well-trained panel to determine the detection threshold of an odorous or taste-active compound as thoroughly as possible.

Thereafter, the gustatory detection thresholds had to be compared to quantitative values of esculetin, scopoletin, and 4-methylumbelliferone measured in wines and spirits by LC-HRMS in order to assess their sensory impact.

Development of a LC-HRMS Method to Quantitate Coumarins in Wines and Spirits. Previous studies have shown the relevance of using UHPLC-MS to quantitate coumarins in natural products such as plants[30] and leaves[31] but also in wines and spirits.[24] By combining the separation power of UPLC and the specificity of Fourier transform mass spectrometry (FTMS), LC-HRMS appears to be a reliable technique to quantitate compounds of low abundance in complex matrices.

The chromatographic conditions used for the quantitative method were optimized in order to improve the separation of coumarins. The spectrometric parameters were also adapted to enhance sensitivity for the six molecules. Optimization of gas values, voltages, and temperatures applied for ionization and ion transfer was carried out in the positive mode by direct injection of standards and by using as the reference the signal intensities of *m/z* 147.0441; 193.0495; 179.0339; 209.0445; 177.0546; and 163.0390, corresponding to coumarin, scopoletin, esculetin, fraxetin, 4-methylumbelliferone, and umbelliferone, respectively.

Method Validation. Absolute quantitation was carried out by preparing calibration solutions of pure coumarins in ultra-pure water. Indeed, preliminary accuracy tests had shown that there was no significant matrix effect. In this study, the LOD and LOQ were established at 2 and 5 µg/L, respectively, for each coumarin. The sensitivity was sufficient regarding the concentrations estimated in wines and spirits.

For all compounds, a quadratic calibration curve (1/x statistical weight) was obtained with a good correlation coefficient (R^2 of 0.999) in the range from 5 µg/L to 10 mg/L. The recovery of back-calculated concentrations was higher than 90% at each method calibration level, thus establishing the accuracy.

Intraday repeatability (RSD %) for each molecule and each concentration was lower than 7%. Two wines and a spirit spiked with stock solutions were also injected. Recovery ratios ranged from 85 to 114%, which remained in accordance with common specifications.[32] Consequently, these results established the repeatability and the trueness of the method applied to wines and spirits. Interday repeatability was estimated by injections of the same standard solutions for five successive days. As usually observed for LC–ESI–MS analysis, the RSD values were quite high. To overcome this issue, all the calibration solutions were injected for each quantitative analysis of an unknown sample.

Analysis of the above-mentioned samples revealed very small variations in retention time (<0.07 min) and a mass deviation lower than 2 ppm for all compounds at various concentrations, guaranteeing the specificity of the method.

All these results validated the LC-HRMS method to quantitate each targeted coumarin in wines and spirits (Table 4).

Application of Method to Quantitate Coumarins in Wines and Spirits. *Content of Coumarins in Various Commercial Wines.* In total, 90 commercial wines were analyzed to assess the range of coumarin concentrations in white and red wines using the LC-HRMS method previously validated. For each coumarin, different concentrations were observed depending on the samples. In general, higher concentrations of coumarins were obtained in red wines, ranging from a few µg/L to more than a hundred of µg/L (Figure 2). This can be explained by the amount of new oak barrels used for aging, which is generally higher for red wines than for white wines.

4-Methylumbelliferone could not be quantitated in wines because all the values obtained were below the LOQ calculated previously. This was consistent with the results of Salagoity-Auguste et al. who identified and quantitated 4-methylumbelliferone by fluorescence in French red wines, with values ranging from 0.4 to 0.8 µg/L.[11] More recently, higher concentrations up to 180 µg/L were surprisingly found in wines from the Tokaj region.[33] Nevertheless, in the wines of the present study, 4-methylumbelliferone was present at trace level, far below its detection threshold; therefore, it appeared to have no direct impact on the taste of wine.

For umbelliferone, Salagoity-Auguste et al. already reported its concentrations in red wines to range from 1.5 to 1.7 µg/L.[11] In our study, most of the results obtained in wines were close but with greater variations (from 0 to 94.6 µg/L). This can be explained by the number of samples analyzed: 2 versus 90.

Coumarin has already been identified in wines[25] but never quantitated. The values measured in red wines were much higher than those in white wines, 143.4 versus 9.4 µg/L on average. Red wines are generally aged for a longer period and with a higher percentage of new barrels than white wines, which could explain the significant variations observed between the two matrices. Moreover, coumarin is well known for its characteristic odor reminiscent of vanilla pod but also of almond, together with the scent of cut hay. Its olfactory detection threshold has been established in water at 11 µg/L.[34] Consequently, it could contribute to wine aroma. The determination of its olfactory and gustatory detection thresholds in wine could

clarify its impact, but its slight bitterness described above suggested that its direct impact on wine taste might be very weak or inexistent.

Fraxetin had never been quantitated in wines. Its average value was 9.8 µg/L in white wines (3.3–36.6 µg/L) and almost twice as much in red wines, 20.1 µg/L, with values ranging from 3.4 to 77.0 µg/L. Moreover, of the 15 red wines above the average measured, 13 came from the Bordeaux region. This observation could be explained by a higher percentage of new oak barrels in Bordeaux cellars compared to those in other regions. Regarding these low concentrations and the taste properties of fraxetin, this compound does not likely affect the taste of wines.

The average concentration of esculetin and scopoletin in the red wines analyzed was 96.6 µg/L (from 12.3 to 268.9 µg/L) and 13.1 µg/L (from 0 to 307.2 µg/L), respectively. In general, lower values were found for white wines with an average content of 41.4 µg/L for esculetin and 15.5 µg/L for scopoletin. These results are consistent with the previous studies.[11,24] For scopoletin, concentrations were far below its gustatory detection threshold, which demonstrated its lack of impact. Esculetin levels below its threshold were observed in all white wines and in some red wines. However, concentrations above the detection threshold were found for 12 red wines (Table S3), suggesting a sensory contribution of esculetin to the bitterness of these wines.

By comparison with the gustatory detection thresholds, these quantitative results suggested that each coumarin might not individually affect the taste of wine, except esculetin in certain red wines. However, additive and synergistic effects have already been described between taste-active compounds.[35] It is thus conceivable that coumarins influence the sensory perception of aged wines through perceptive interactions.

Content of Coumarins in Various Commercial Spirits. The average contents obtained for esculetin, fraxetin, scopoletin, coumarin, and 4-methylumbelliferone in 28 commercial spirits were 131.1, 31.5, 363.8, 127.6, and 87.4 µg/L, respectively, with large variations from one reference to another (Figure 3). The signals observed for umbelliferone were lower than the LOQ, except for two spirits. In general, the quantitation results showed higher contents of coumarins in spirits than in wines. This could be explained by the aging time in barrels, which is generally longer for spirits than for wines. Moreover, the extraction of the compounds can be influenced by the alcoholic degree of the matrix, which is higher in spirits.

The gustatory detection thresholds of esculetin and 4-methylumbelliferone in spirits were estimated at 1.1 mg/L and 397 µg/L, respectively. The measured contents of these two

molecules in commercial spirits were below their detection threshold. Although these compounds have a strong bitter taste, individually they might not affect the taste of spirits.

Regarding scopoletin, huge variations in concentrations were observed from one spirit to another, ranging from 40.1 µg/L to 1.5 mg/L. The contents varied also according to the nature of the "eau-de-vie," with higher values in whiskeys, rums, and bourbons than in cognacs and brandies. These differences could be related to the botanical origin of the wood used for aging. Indeed, cognacs and brandies are generally aged in French oak barrels, sessile, or pedunculate, while bourbons are aged in American oak barrels. The rum sample (noted R-3), where the concentration of scopoletin was 1.5 mg/L, was also aged in American white oak barrels that had previously contained American whiskey. Indeed, previous studies have shown a higher scopoletin content in American oaks than in French oaks.[36] This coumarin appears to be a chemical marker for this oak species.[37,38] A comparison with sensory data revealed that scopoletin concentrations were above its gustatory detection threshold (789 µg/L) in the five spirits aged in American oak barrels (Table S4). These results established the sensory relevance of scopoletin, which seemed to contribute to the bitterness of these spirits.

Content of Coumarins in Various Vintages of the Same Spirits. Coumarins were quantitated in a series of "eau-de-vie" of cognac of 10 different vintages from the same distillery and using similar aging conditions (Table S5). The samples, which were collected in the distillery, were not commercial cognac but "eau-de-vie" still aging in barrels. For each vintage, a sample was collected from five different barrels to limit variations between casks. The concentrations presented in Figure 4 correspond to the mean values of these five replicates.

The esculetin and scopoletin contents were higher in old spirits, reaching 338 and 264 µg/L for the 1970 vintage, respectively, which is consistent with the previous studies.[39] Moreover, some authors have already shown higher scopoletin concentrations during barrel aging.[11,40]

The coumarin contents are also higher in old spirits, reaching 209 µg/L for the 1995 vintage. This molecule had been studied previously only in cachaça[41] and in spirits produced from cane sugar,[22] but the values were consistent.

Fraxetin has never been described in cognac. Its concentration seemed to follow a bell-shaped curve; low in the 2015 sample (19 µg/L), maximal in the 1995 sample (204 µg/L), and lower in older vintages (e.g., 118 µg/L for the 1973 vintage). The same trends were observed for 4-methylumbelliferone. Indeed, from 2015 (69 µg/L) to 2008 (307 µg/L), the results showed that the older the "eau-de-vie", the higher the level of 4-methylumbelliferone, thereby confirming a previous study.[42] Conversely, lower concentrations were found for older vintages,

for example, 27 µg/L for the 1973 vintage. Such variations might suggest a degradation of these compounds after long aging in barrels. However, this hypothesis needs further study because the results could also have been due to the differences in aging practices in the distillery or to changes in barrel manufacturers over the past 50 years.

Umbelliferone was detected at low concentrations (from 5.5 to 67.8 µg/L) in four old vintages (1995, 1993, 1990, and 1973). Rodríguez Dodero et al. have already quantitated this molecule by fluorescence in several spirits, with values up to 4 µg/L.[39] This low level might be due to the occurrence of the molecule only in small amounts in oak wood and to its slow extraction kinetics. It might also be produced during aging by degradation of other compounds.

Esculetin, scopoletin, and 4-methylumbelliferone contents in these spirits samples were below their gustatory detection threshold, which corroborated the results observed for commercial spirits. Individually, each molecule did not seem to contribute to the taste of the spirits. However, as previously mentioned for wines, coumarins might be involved in perceptive interactions likely to influence the taste of wines and spirits.

Influence of Mixed Coumarins on Taste Balance in Wines and Spirits. To determine the contribution of the six coumarins in the mixture on the taste balance of wines and spirits, three gustatory experiments were organized: in a white wine (wine A), in a red wine (wine B), and in an "eau-de-vie." For each matrix, the different coumarins were added at four levels corresponding to the 1st quartile (M1), the median (M2), the 3rd quartile (M3), and the maximum (M4) concentrations obtained from quantitative analysis (Table 5). The control and these four spiked modalities were presented to the panelists, who were asked to assess the bitterness and the sweetness intensity of the wines and spirits presented.

For the first two sessions, the results of the statistical test showed similar trends between white wines and red wines (Table 5). For these two matrices, only the bitter descriptor presented significant differences between the control and supplemented modalities. Indeed, all the *p*-values associated with the sweet descriptor were considerably higher than 0.05. In addition, this study highlighted significant changes in bitterness for two modalities, M3 and M4, with *p*-values equal to 0.032 and 0.025 for white wines and p-values equal to 0.033 and 0.039 for red wines, respectively. M1 and M2 were not distinguishable from the control modality (Table 6).

For the third session, no significant differences of sweetness intensity were perceived between the modalities, as for wine. However, for the bitter descriptor, the Friedman test highlighted a distinction between the samples. The tasters were able to differentiate the M2,

M3, and M4 modalities from the control modality, with *p*-values of 0.039, 0.033, and 0.028 respectively, whereas M1 and control remained undistinguishable (Table 6).

In conclusion, the statistical tests showed that the addition of coumarins had no impact on the sweetness of wines or spirits, which was consistent with the properties of each individual coumarin. For bitterness, significant differences were observed in the two matrices at concentrations present in wines and spirits. These concentrations were below the individual detection threshold for all coumarins, which suggested perceptive interactions.

The differences of perceptive interactions between the first two sessions (wine) and the third one (spirit) could be due to several factors. For instance, the addition of 4-methylumbelliferone in this latter matrix could play a role. It is also possible that these differences were due to the higher concentrations added in the "eau-de-vie" compared to the wines. Moreover, variations of the ethanol content between these two matrices could have an impact on interactions with some coumarins.

The results of this study demonstrated that although coumarins did not contribute individually to the taste of wines and spirits, they nevertheless played a role through their synergistic or additive effects by increasing the bitter perception of wines and spirits. In enology, such a phenomenon has already been observed for aromatic compounds[43,44] but only rarely for taste-active molecules. Previous studies have reported that the detection threshold of a single taste-active compound can be reduced when it is mixed with other taste-active compounds, which might imply neuronal integration during perception.[45,46]

By using analytical and sensory techniques, this work provides new insights into the role played by coumarins in wines and spirits. Fraxetin was identified and quantitated for the first time in these two matrices. Despite their strong bitterness, coumarins were detected at concentrations lower than their detection thresholds in wines and spirits. While they had no impact on taste individually, sensory analysis of wines and spirits spiked with a mixture of coumarins revealed significant modifications of bitterness intensity. Therefore, these findings demonstrate the sensory importance of coumarins in wines and spirits through perceptive interactions. Complementary studies will be necessary to better characterize the nature of this phenomenon (additivity or synergism). From a more practical point of view, it would be interesting to try to limit the content of coumarins in the wood given their influence on taste. In this way, the influence of cooperage parameters such as the botanical origin of oak wood or the toasting of staves on coumarin concentrations could be studied. A better control of these parameters might improve the monitoring of oak wood aging and its sensory effect.

SUPPORTING INFORMATION

Spectrometric data of six targeted coumarins used for quantitation; positive LC–ESI–FTMS–XIC of standards (A, on the left), a red wine (B, in the middle), and a spirit (C, on the right), corresponding to $[M + H]^+$ ions of six targeted coumarins; individual concentrations of esculetin, fraxetin, scopoletin, umbelliferone, and coumarin for 90 commercial wines; individual concentrations of esculetin, fraxetin, scopoletin, umbelliferone, coumarin, and 4-methylumbelliferone for 28 commercial spirits; and individual concentrations of esculetin, fraxetin, scopoletin, umbelliferone, coumarin, and 4-methylumbelliferone for 10 vintages of the same spirit.

ABBREVIATIONS

LOD: limit of detection

LOQ: limit of quantitation

Acknowledgments

D.W.'s grant is supported by Remy Martin and Seguin Moreau. Analytical techniques were funded by Biolaffort. The authors are also grateful to J.-C. Mathurin and L. Urruty for providing samples. We thank Dr. Ray Cooke for proofreading the manuscript.

References

(1) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. Traité d'œnologie. Chimie du vin. Stabilisation et Traitements, 7th ed.; Dunod, 2017; Vol. 2.

(2) Garde-Cerdán, T.; Ancín-Azpilicueta, C. Review of Quality Factors on Wine Ageing in Oak Barrels. Trends Food Sci. Technol. 2006, 17, 438– 447, DOI: 10.1016/j.tifs.2006.01.008

(3) Piggott, J. R. Flavour of Distilled Beverages: Origin and Development; Ellis Horwood Limited, 1983.

(4) Marchal, A.; Pons, A.; Lavigne, V.; Dubourdieu, D. Contribution of Oak Wood Ageing to the Sweet Perception of Dry Wines: Effect of Oak Ageing on Wine Sweetness. Aust. J. Grape Wine Res. 2013, 19, 11– 19, DOI: 10.1111/ajgw.12013

(5) Marchal, A.; Waffo-Téguo, P.; Génin, E.; Mérillon, J.-M.; Dubourdieu, D. Identification of New Natural Sweet Compounds in Wine Using Centrifugal Partition Chromatography–Gustatometry and Fourier Transform Mass Spectrometry. Anal. Chem. 2011, 83, 9629–9637, DOI: 10.1021/ac202499a

(6) Gammacurta, M.; Waffo-Teguo, P.; Winstel, D.; Cretin, B. N.; Sindt, L.; Dubourdieu, D.; Marchal, A. Triterpenoids from Quercus Petraea: Identification in Wines and Spirits and Sensory Assessment. J. Nat. Prod. 2019, 82, 265– 275, DOI: 10.1021/acs.jnatprod.8b00682

(7) Quinn, M. K.; Singleton, V. L. Isolation and Identification of Ellagitannins from White Oak Wood and an Estimation of Their Roles in Wine. Am. J. Enol. Vitic. 1985, 36, 148

(8) Glabasnia, A.; Hofmann, T. Sensory-Directed Identification of Taste-Active Ellagitannins in American (*Quercus Alba* L.) and European Oak Wood (*Quercus Robur* L.) and Quantitative Analysis in Bourbon Whiskey and Oak-Matured Red Wines. J. Agric. Food Chem. 2006, 54, 3380– 3390, DOI: 10.1021/jf052617b

(9) Marchal, A.; Cretin, B. N.; Sindt, L.; Waffo-Téguo, P.; Dubourdieu, D. Contribution of Oak Lignans to Wine Taste: Chemical Identification, Sensory Characterization and Quantification. Tetrahedron 2015, 71, 3148– 3156, DOI: 10.1016/j.tet.2014.07.090

(10) Cretin, B. N.; Sallembien, Q.; Sindt, L.; Daugey, N.; Buffeteau, T.; Waffo-Teguo, P.; Dubourdieu, D.; Marchal, A. How Stereochemistry Influences the Taste of Wine: Isolation, Characterization and Sensory Evaluation of Lyoniresinol Stereoisomers. Anal. Chim. Acta 2015, 888, 191– 198, DOI: 10.1016/j.aca.2015.06.061

549 (11) Salagoity-Auguste, M.-H.; Tricard, C.; Sudraud, P. Dosage Simultané Des Aldéhydes
550 Aromatiques et Des Coumarines Par Chromatographie Liquide Haute Performance:
551 Application Aux Vins et Eaux-de-Vie Vieillis En Fŭt de Chĕne. J. Chromatogr. A 1987, 392,
552 379– 387, DOI: 10.1016/s0021-9673(01)94281-5
553 (12) Bourgaud, F.; Hehn, A.; Larbat, R.; Doerper, S.; Gontier, E.; Kellner, S.; Matern, U.
554 Biosynthesis of Coumarins in Plants: A Major Pathway Still to Be Unravelled for
555 Cytochrome P450 Enzymes. Phytochem. Rev. 2006, 5, 293– 308, DOI: 10.1007/s11101-
556 006-9040-2
557 (13) Ehlers, D.; Pfister, M.; Bork, W.-R.; Toffel-Nadolny, P. HPLC Analysis of Tonka Bean
558 Extracts. Z. Lebensm.-Unters. Forsch. 1995, 201, 278– 282, DOI: 10.1007/bf01193004
559 (14) Beier, R. C.; Oertli, E. H. Psoralen and Other Linear Furocoumarins as Phytoalexins in
560 Celery. Phytochemistry 1983, 22, 2595– 2597, DOI: 10.1016/0031-9422(83)80173-3
561 (15) Venugopala, K. N.; Rashmi, V.; Odhav, B. Review on Natural Coumarin Lead
562 Compounds for Their Pharmacological Activity. BioMed Res. Int. 2013, 2013, 1– 4, DOI:
563 10.1155/2013/963248
564 (16) Chatonnet, P.; Boidron, J.-N.; Dubourdieu, D.; Pons, A. Evolution Des Composés
565 Polyphénoliques Du Bois de Chêne Au Cours de Son Séchage. Premiers Résultats. OENO
566 One 1994, 28, 337, DOI: 10.20870/oeno-one.1994.28.4.1135
567 (17) Fernández de Simón, B.; Cadahía, E.; Conde, E.; García-Vallejo, M. C. Evolution of
568 Phenolic Compounds of Spanish Oak Wood during Natural Seasoning. First Results. J.
569 Agric. Food Chem. 1999, 47, 1687– 1694, DOI: 10.1021/jf9805855
570 (18) Vivas, N. Recherches Sur La Qualité Du Chêne Français de Tonnellerie (*Q. Petraea*
571 Liebl. , Q. Robur L.) et Sur Les Mécanismes d'oxydoréduction Des Vins Rouges Au Cours
572 de Leur Élevage En Barriques. PhD Thesis, Bordeaux 2, 1997.
573 (19) De Paepe, D.; Servaes, K.; Noten, B.; Diels, L.; De Loose, M.; Van Droogenbroeck, B.;
574 Voorspoels, S. An Improved Mass Spectrometric Method for Identification and
575 Quantification of Phenolic Compounds in Apple Fruits. Food Chem. 2013, 136, 368– 375,
576 DOI: 10.1016/j.foodchem.2012.08.062
577 (20) AFNOR. ISO 4120:2007. Sensory Analysis–Methodologie–Test Triangulaire Analyse
578 Sensorielle: Recueil, Normes, Agroalimentaire; AFNOR: Geneva, 2007.
579 (21) AFNOR. NF ISO 8587:2007. Analyse Sensorielle–Méthodologie–Classement Par
580 Rangs. Analyse Sensorielle: Recueil, Normes, Agroalimentaire; AFNOR, 2007.

(22) da Silva, A. A.; do Nascimento, E. S. P.; Cardoso, D. R.; Franco, D. W. Coumarins and Phenolic Fingerprints of Oak and Brazilian Woods Extracted by Sugarcane Spirit. J. Sep. Sci. 2009, 32, 3681– 3691, DOI: 10.1002/jssc.200900306

(23) Tsukamoto, H.; Hisada, S.; Nishibe, S. Coumarins from Bark of Fraxinus Japonica and F. Mandshurica Var. Japonica.. Chem. Pharm. Bull. 1985, 33, 4069– 4073, DOI: 10.1248/cpb.33.4069

(24) Barnaba, C.; Dellacassa, E.; Nicolini, G.; Nardin, T.; Malacarne, M.; Larcher, R. Identification and Quantification of 56 Targeted Phenols in Wines, Spirits, and Vinegars by Online Solid-Phase Extraction – Ultrahigh-Performance Liquid Chromatography – Quadrupole-Orbitrap Mass Spectrometry. J. Chromatogr. A 2015, 1423, 124– 135, DOI: 10.1016/j.chroma.2015.10.085

(25) Bi, H.; Xi, M.; Zhang, R.; Wang, C.; Qiao, L.; Xie, J. Electrostatic Spray Ionization-Mass Spectrometry for Direct and Fast Wine Characterization. ACS Omega 2018, 3, 17881– 17887, DOI: 10.1021/acsomega.8b02259

(26) Burdock, G. A.; Fenaroli, G. Fenaroli's Handbook of Flavor Ingredients, 5th ed.; CRC Press: Boca Raton, Fla, 2005.

(27) Meyerhof, W.; Batram, C.; Kuhn, C.; Brockhoff, A.; Chudoba, E.; Bufe, B.; Appendino, G.; Behrens, M. The Molecular Receptive Ranges of Human TAS2R Bitter Taste Receptors. Chem. Senses 2010, 35, 157– 170, DOI: 10.1093/chemse/bjp092

(28) Winstel, D.; Marchal, A. Lignans in Spirits: Chemical Diversity, Quantification, and Sensory Impact of (±)-Lyoniresinol. Molecules 2019, 24, 117, DOI: 10.3390/molecules24010117

(29) Tempere, S.; Cuzange, E.; Malak, J.; Bougeant, J. C.; de Revel, G.; Sicard, G. The Training Level of Experts Influences Their Detection Thresholds for Key Wine Compounds. Chemosens. Percept. 2011, 4, 99, DOI: 10.1007/s12078-011-9090-8

(30) Durand-Hulak, M.; Dugrand, A.; Duval, T.; Bidel, L. P. R.; Jay-Allemand, C.; Froelicher, Y.; Bourgaud, F.; Fanciullino, A.-L. Mapping the Genetic and Tissular Diversity of 64 Phenolic Compounds in Citrus Species Using a UPLC–MS Approach. Ann. Bot. 2015, 115, 861– 877, DOI: 10.1093/aob/mcv012

(31) Bhatt, V.; Sharma, S.; Kumar, N.; Sharma, U.; Singh, B. Simultaneous Quantification and Identification of Flavonoids, Lignans, Coumarin and Amides in Leaves of *Zanthoxylum Armatum* Using UPLC-DAD-ESI-QTOF–MS/MS. J. Pharm. Biomed. Anal. 2017, 132, 46– 55, DOI: 10.1016/j.jpba.2016.09.035

(32) Guidance for Industry. Bioanalytical Method Validation; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM): Rockville, MD, 2018.

(33) Hroboňová, K.; Sádecká, J. Coumarins content in wine: application of HPLC, fluorescence spectrometry, and chemometric approach. J. Food Sci. Technol. 2020, 57, 200–209, DOI: 10.1007/s13197-019-04048-2

(34) Wagner, J.; Granvogl, M.; Schieberle, P. Characterization of the Key Aroma Compounds in Raw Licorice (*Glycyrrhiza Glabra* L.) by Means of Molecular Sensory Science. J. Agric. Food Chem. 2016, 64, 8388– 8396, DOI: 10.1021/acs.jafc.6b03676

(35) Keast, R. S. J.; Breslin, P. A. S. An Overview of Binary Taste–Taste Interactions. Food Qual. Prefer. 2003, 14, 111– 124, DOI: 10.1016/s0950-3293(02)00110-6

(36) Puech, J.-L.; Moutounet, M. Liquid chromatographic determination of scopoletin in hydroalcoholic extract of oak wood and in matured distilled alcoholic beverages. J. Assoc. Off. Anal. Chem. 1988, 71, 512– 514, DOI: 10.1093/jaoac/71.3.512

(37) Canas, S. Phenolic Composition and Related Properties of Aged Wine Spirits: Influence of Barrel Characteristics. A Review. Beverages 2017, 3, 55, DOI: 10.3390/beverages3040055

(38) Baldwin, S.; Black, R. A.; Andreasen, A. A.; Adams, S. L. Aromatic Congener Formation in Maturation of Alcoholic Distillates. J. Agric. Food Chem. 1967, 15, 381– 385, DOI: 10.1021/jf60151a012

(39) Rodríguez Dodero, M. C.; Guillén Sánchez, D. A.; Rodríguez, M. S.; Barroso, C. G. Phenolic Compounds and Furanic Derivatives in the Characterization and Quality Control of Brandy de Jerez. J. Agric. Food Chem. 2010, 58, 990– 997, DOI: 10.1021/jf902965p

(40) Otsuka, K.-i.; Zenibayashi, Y. On the Determination of Scopoletin in Aged Distilled Liquors. Agric. Biol. Chem. 1974, 38, 1079– 1080, DOI: 10.1080/00021369.1974.10861285

(41) Rodrigues, L. M. A.; das Graças Cardoso, M.; Santiago, W. D.; Soares, L. I.; Passamani, F. R.; de Andrade Santiago, J.; De Azevedo Lira, N.; Nelson, D. L.; Batista, L. R. Phenolic Extracts of Cachaça Aged in Different Woods and Quantifying Antioxidant Activity and Antifungal Properties: Phenolic Extracts of Cachaça Aged in Different Woods. J. Inst. Brew. 2016, 122, 644– 652, DOI: 10.1002/jib.375

(42) Santiago, W. D.; Cardoso, M. d. G.; Santiago, J. d. A.; Gomes, M. S.; Rodrigues, L. M. A.; Brandão, R. M.; Cardoso, R. R.; d'Avila, G. B.; Silva, B. L. d.; Caetano, A. R. S. Comparison and Quantification of the Development of Phenolic Compounds during the

Aging of Cachaça in Oak (*Quercus* Sp) and Amburana (*Amburana Cearensis*) Barrels. Am. J. Plant Sci. 2014, 05, 3140– 3150, DOI: 10.4236/ajps.2014.521330

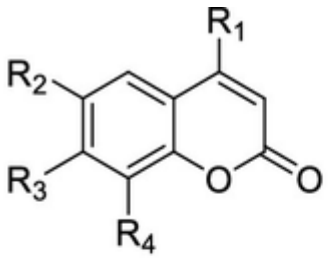
(43) Lytra, G.; Tempere, S.; Le Floch, A.; de Revel, G.; Barbe, J.-C. Study of Sensory Interactions among Red Wine Fruity Esters in a Model Solution. J. Agric. Food Chem. 2013, 61, 8504– 8513, DOI: 10.1021/jf4018405

(44) Pineau, B.; Barbe, J.-C.; Van Leeuwen, C.; Dubourdieu, D. Which Impact for β-Damascenone on Red Wines Aroma? J. Agric. Food Chem. 2007, 55, 4103– 4108, DOI: 10.1021/jf070120r

(45) Stevens, J. C. Detection of Very Complex Taste Mixtures Generous Integration Across Constituent Compounds. Physiol. Behav. 1997, 62, 1137– 1143, DOI: 10.1016/s0031-9384(97)00270-9

(46) Dalton, P.; Doolittle, N.; Nagata, H.; Breslin, P. A. S. The Merging of the Senses: Integration of Subthreshold Taste and Smell. Nat. Neurosci. 2000, 3, 431– 432, DOI: 10.1038/74797

Figures



	R1	R2	R3	R4
Esculetin	H	OH	OH	H
Fraxetin	H	OCH_3	OH	OH
Umbelliferone	H	H	OH	H
Scopoletin	H	OCH_3	OH	H
4-Methylumbelliferone	CH_3	H	OH	H
Coumarin	H	H	H	H

Figure 1. Chemical structures of six targeted coumarins.

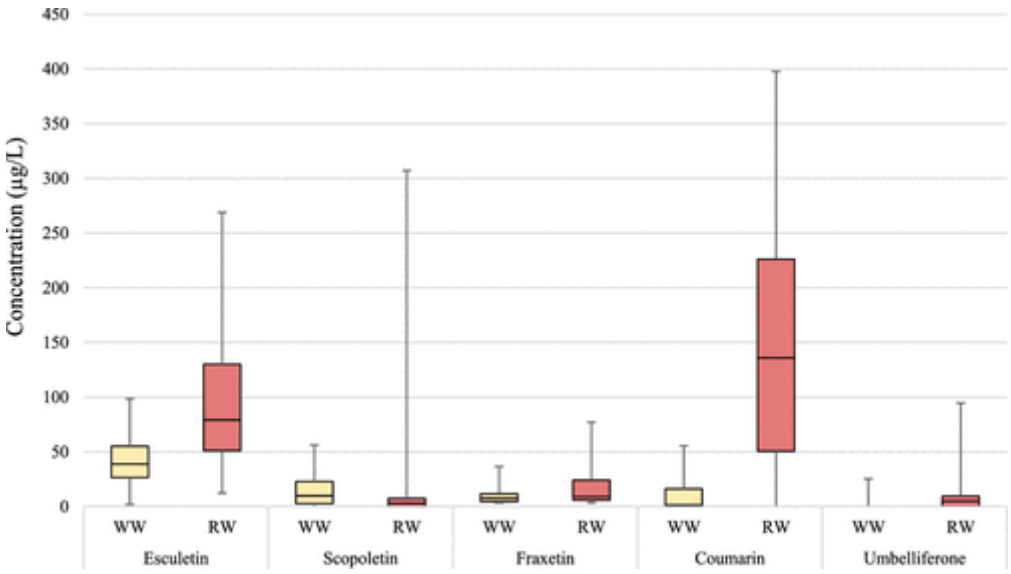


Figure 2. Variations in concentrations of esculetin, scopoletin, fraxetin, coumarin, and umbelliferone in 90 commercial wines (46 white wines and 44 red wines). The boxes represent values comprised between 1st and 3rd quartile. Error bars indicate minimum and maximum values.

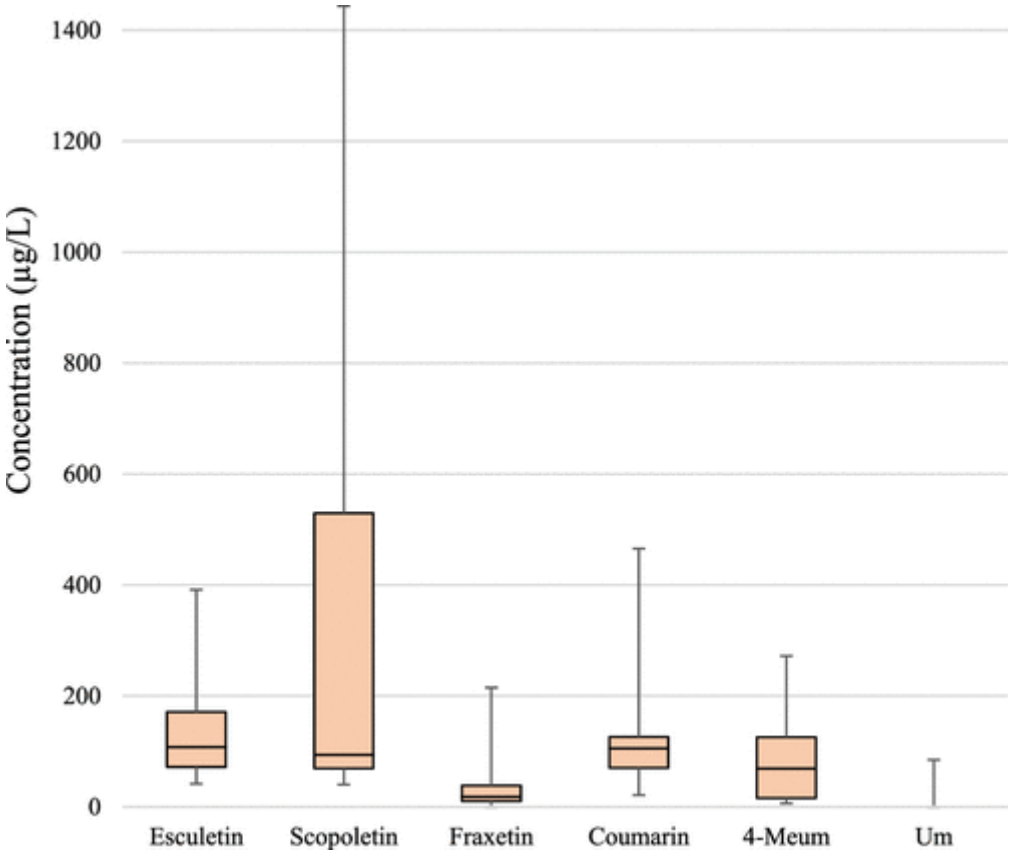


Figure 3. Variations in concentrations of esculetin, scopoletin, fraxetin, coumarin, 4-methylumbelliferone, and umbelliferone in 28 commercial spirits. The boxes represent values comprised between 1st and 3rd quartile. Error bars indicate minimum and maximum values.

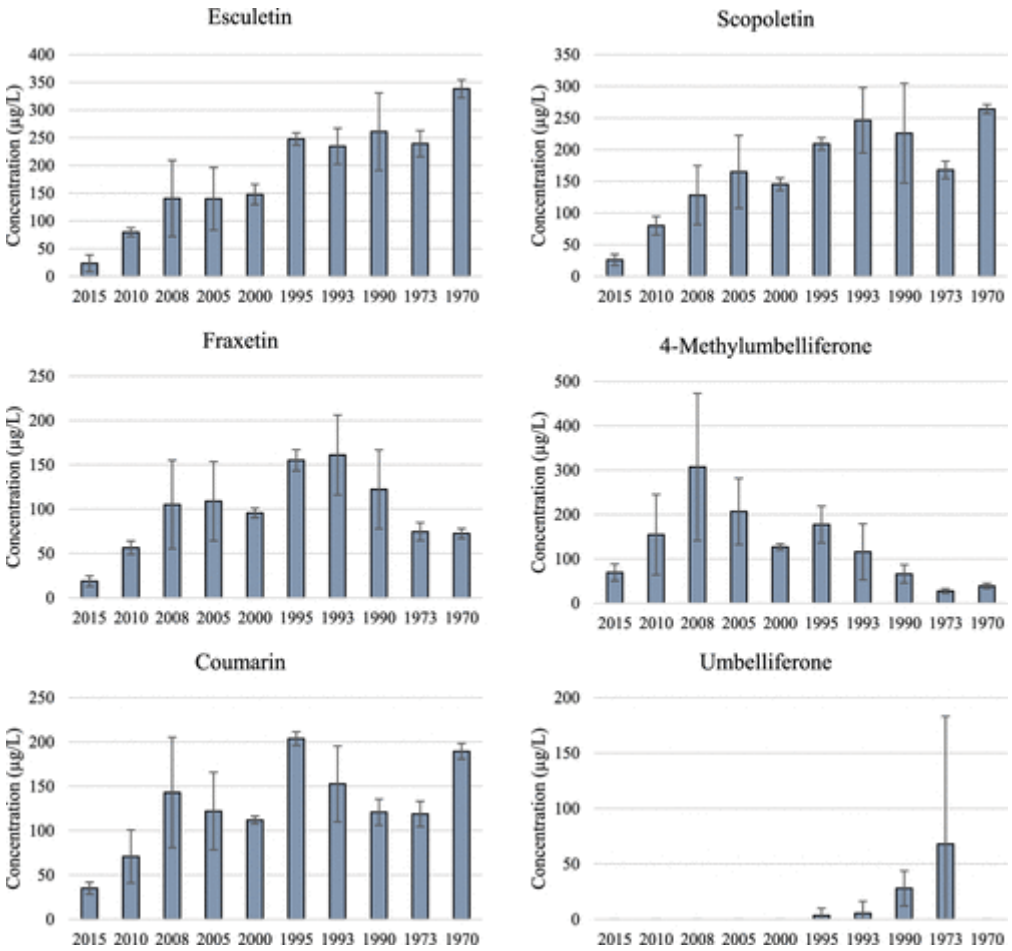


Figure 4. Concentrations of esculetin, scopoletin, fraxetin, 4-methylumbelliferone, coumarin, and umbelliferone (from left to right and from top to bottom) in 10 vintages of "eaux-de-vie" of cognac from the same distillery.

Tables

Table 1. Ionization and Spectrometric Conditions for HRMS Analyses

mass spectrometer	**exactive**	
use ionization mode	**LC-HRMS screening positive**	**LC-HRMS quantitation positive**
sheath gas flow[a]	70	68
auxiliary gas flow[a]	15	15
HESI probe temperature	320 °C	305 °C
capillary temperature	350 °C	310 °C
electrospray voltage	3.5 kV	4.5 kV
capillary voltage	25 V	52.5 V
tube lens voltage offset	120 V	110 V
skimmer voltage	20 V	30 V
mass range (in Th)	100–1000	100–400
resolution[b]	25,000	10,000
AGC value[c]	10^6 ions	3×10^6 ions

[a]Sheath gas and auxiliary gas flows (both nitrogen) expressed in arbitrary units.

[b]Resolution m/Δm, fwhm at m/z 200 Th.

[c]Automatic gain control.

Table 2. Gustatory Characterization of Six Coumarins (1 mg/L) in 12% vol. alc. Hydroethanolic Solution

compounds	**taste in hydroethanolic solution**	**bitterness intensity[a]**
esculetin	bitter	3/5
fraxetin	sour	
umbelliferone	bitter	1/5
scopoletin	bitter	2/5
4-methylumbelliferone	bitter	5/5
coumarin	bitter	1/5

[a]Bitterness intensity rated by experts on a 1–5 scale.

Table 3. Gustatory Detection Thresholds of Esculetin, Scopoletin, and 4-Methylumbelliferone in NonOaked White Wine and NonOaked "Eau-de-vie"

	gustatory detection threshold[a] (μg/L)	
compounds	**nonoaked wine A**	**nonoaked "eau-de-vie"**
esculetin	217	1108
scopoletin	702	789
4-methylumbelliferone	320	397

[a]Based on the geometric mean of all individual detection thresholds.

Table 4. Validation Parameters for HRMS Quantitation of Six Coumarins in Wines and Spirits

	sensitivity		linearity and accuracy		specificity		repeatability and trueness											
							intraday repeatability			recovery white wine			recovery red wine			recovery "eau-de-vie"		
compounds	LOD[a] (µg/L)	LOQ[b] (µg/L)	working range	R^2	t_R variation (min)	mass accuracy (ppm)	10 µg/L (%)	200 µg/L (%)	10 mg/L (%)	10 µg/L (%)	200 µg/L (%)	10 mg/L (%)	10 µg/L (%)	200 µg/L (%)	10 mg/L (%)	10 µg/L (%)	200 µg/L (%)	10 mg/L (%)
coumarin	2	5	5 µg/L–10 mg/L	1	0.06	0.52	6.9	3.6	2	95	108	98	98	105	92	86	95	100
scopoletin	2	5	5 µg/L–10 mg/L	1	0.05	0.03	1.3	4.5	1.1	91	99	105	91	86	111	85	86	97
esculetin	2	5	5 µg/L–10 mg/L	1	0.05	0.02	6.2	5.5	2.4	105	87	97	91	85	90	85	91	99
fraxetin	2	5	5 µg/L–10 mg/L	0.999	0.07	0.72	6	5.4	3.9	95	91	92	90	97	89	87	98	99
umbelliferone	2	5	5 µg/L–10 mg/L	1	0.05	1.12	3.1	4.3	1.3	85	86	110	86	88	110	87	98	100
4-methylumbelliferone	2	5	5 µg/L–10 mg/L	1	0.07	0.68	2.3	5.7	1.9	85	113	114	89	85	100	85	95	86

[a]LOD: limit of detection.

[b]LOQ: limit of quantitation.

Table 5. Concentrations in Coumarins Used for Sensory Profiling in White Wine A, Red Wine B, and "Eau-de-vie" for Each Modality[a]

		1st quartile (M1)	median (M2)	3rd quartile (M3)	maximum (M4)
white wine A	esculetin	26.6	38.9	55.1	98.5
	scopoletin	2.7	10	22.9	56.3
	coumarin	0	1.4	16.2	55.6
	fraxetin	4.5	7.6	11.7	36.6
	umbelliferone	0	0	0	25.3
	4-methylumbelliferone	0	0	0	0
red wine B	esculetin	51	79.2	130.1	268.9
	scopoletin	0	2.9	7.5	307.3
	coumarin	50.6	135.1	226.1	397.8
	fraxetin	5.7	9	24.1	77
	umbelliferone	0	5	9.5	94.6
	4-methylumbelliferone	0	0	0	0
"eau-de-vie"	esculetin	83.9	148.9	268.6	407.6
	scopoletin	81.3	172.9	265.6	1640.9
	coumarin	80.5	111.2	141.4	465.6
	fraxetin	22.1	62.9	101.9	269.1
	umbelliferone	0	0	0	249.5
	4-methylumbelliferone	52	102.9	201.5	690.2

[a]All concentrations expressed in (μg/L).

Table 6. *p*-Values Associated with Bitter Descriptor for Four Modalities in White Wine A, Red Wine B, and "Eau-de-vie" of Cognac in Comparison with Control

p-value	1st quartile (M1)[b]	median (M2)[b]	3rd quartile (M3)[b]	maximum (M4)[b]
white wine A	1	0.347	0.032[a]	0.025[a]
red wine B	0.083	0.499	0.033[a]	0.039[a]
"eau-de-vie"	0.074	0.039[a]	0.033[a]	0.028[a]

[a]Results considered as significant (*p*-value < 0.05).

[b]The values of M1, M2, M3, and M4 are presented in Table 5.