Physicochemical Model To Interpret the Kinetics of Aroma Extraction during Wine Aging in Wood. Model Limitations Suggest the Necessary Existence of Biochemical Processes

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A model based on physicochemical parameters has been developed and applied to the study of the kinetics of extraction of aroma compounds from oak casks having been used a variable number of times. Two major deviations from the model can be observed: a strong seasonal dependence of extraction (unexpected in a thermostated cellar); and higher yields with a smaller number of longer extractions. 4-Propylguaiacol follows both behaviors, and its levels have been found to be closely related to those of 4-ethylguaiacol and to the number of uses of the barrel, which suggests that this compound may be formed by the same microorganisms forming ethylphenols from a precursor found in wood. Although the extraction kinetics of all aroma compounds appear to be more or less mediated by the action of microorganisms, those of whiskeylactones, eugenol, and 4-allyl-2,6-dimethoxyphenol are more in accordance with the physicochemical model, whereas those of vanillin, syringaldehyde, furfuryl alcohol, and, of course, 4-propylguaiacol are much more coherent with a microbiological extraction/formation.

KEYWORDS: Wine; aroma; flavor; extraction; aging; American oak; French oak; GC-MS; modeling

INTRODUCTION

Aging in oak barrels is a very old tradition used to improve the sensory characteristics of wine and, as has been recently shown (*1*), there are at least five different phenomena influencing the aroma evolution and formation during the process of aging in wood. One of these phenomena, and the best documented, is that of aroma extraction from wood (*2*−*18*). It is well-known that oak wood releases significant amounts of some aroma compounds, which have deep impact on the aroma profile and on the sensory characteristics of the wine (*2*, *3*, *18*, *19*). Particularly important are the sensory effects caused by *Z*-whiskeylactone, eugenol, and vanillin (*2*, *3*, *6*, *18*, *20*, *21*). Many factors affecting the release of these compounds from the barrel have been investigated, such as the effect of the origin of oak wood (American or French) (*1*, *5*−*13*, *22*−*25*), the place and type of seasoning and cooperage (*6*, *10*, *26*), the toasting process (*7*, *10*), the extraction time (*3*, *6*), the level of sulfur dioxide (*16*), the size of the barrel (*12*, *14*), the temperature (*5*, *27*), or the previous history (number of uses) of the barrel (*2*, *3*, *5*, *11*, *12*). Due to the difficulties and cost linked to the experiments, most of the research has focused on finding general trends rather than measuring the extraction kinetics, and only in a few works are the extraction curves calculated and commented on (*5*, *6*, *8*, *17*); however, to our knowledge, there have been no previous attempts to fit such curves to the

mathematical functions governing the physicochemical process of extraction in contrast to the case of phenolics (*28*).

In addition to its obvious practical interest, the study of the extraction curves has a high scientific interest, because extraction curves are related to the extraction mechanism(s). For instance, Spillman and Sefton suggest that vanillin is extracted from wood by two different mechanisms (*8*): one fast (direct extraction from vanillin already present in the wood) and one slow (formation of vanillin by ethanolysis or oxidation of lignin and further extraction). To get the maximum amount of information from a kinetic curve, it would be convenient to have a mathematical model, based exclusively on the simple expected processes taking part in aroma extraction. This would determine if the observed kinetic curve follows the model. On the other hand, large enough deviations from the "ideal" behaviors would reveal that processes not yet considered are governing the extraction. It would also be convenient to include in the model a factor that should take into account the previous history of the barrel, so that the model could be applied to barrels used different numbers of times. This would make it possible, at least ideally, to design the most efficient aging strategy in a cellar and to optimize the life of a barrel.

The main goals of the present paper are to develop a model based exclusively on physicochemical parameters, to apply it to the study of the extraction of aroma compounds from oak casks with various numbers of uses, and to evaluate the deviations found between the expected and observed extraction

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curves. This would reveal any other parameters governing aroma extraction from wood during the aging of wine in barrels.

MATERIALS AND METHODS

Wine. Three different red wines were used in this study. The first was made in the year 2000 with grapes from the varieties Tempranillo (63%), Cabernet Sauvignon (17%), and Merlot (17%). After fermentation, its pH was 3.55 and its alcoholic degree 13.0% (v/v). The second wine was made with the same varieties in 2001, its pH was 3.53, and its alcoholic degree was 13.34% (v/v). The third wine was made in 2002 and had an alcoholic degree of 14.35% (v/v) and a pH of 3.41. The total volume of homogeneous wine used in the experiment was 15000 L per year. The wines were made following standard winemaking practices.

Oak Barrels. The 225 L barrels used in the experiment were made with American oak (*Quercus alba* from Missouri) or with French oak (*Quercus sessilis* from Allier). The casks were made by Toneleria Aitona, owned by Caja Rural de Navarra, one of the organizers of the experiment. The species of oak were determined by the suppliers but were not independently confirmed by us. The seasoning of the wood was carried out by storing the wood outdoors for 3 years. The barrels were toasted to a medium intensity (190−200 °C for 10 min).

Wine Aging Experiment. The purpose of the experiment was to investigate the effect of the number of uses of the barrel on the extraction kinetics. Twelve (4 × 3 subsets) new 225 L barrels of American oak and another 12 of French oak were filled with the wine (taken from a 15000 L stainless steel vat) in April 2001 and stored in a conditioned cellar. Each 12-barrel set was divided into 4 subsets. The barrels from the first subset were emptied and refilled with wine every 3 months. For those from the second subset this was done every 6 months, for those from the third subset every year, and for those from the last subset after 2 years of aging. The experiment lasted 3 years, so that at the end of the third year we had 3 barrels × 4 wines/ year × 3 years = 36 wines aged for 3 months in American barrels with different numbers of uses and another 36 in French barrels from the first subset of barrels; 3 barrels × 2 wines/year × 3 years = 18 wines aged for 6 months in American barrels with different numbers of uses and the same number aged in French barrels from the second subset; 3 barrels × 1 wine/year × 3 years = 9 wines aged for 1 year from the third subset; and 3 barrels × 2 wines = 6 wines aged for 2 years or 1 year from the fourth subset. Samples for analysis were taken from each barrel immediately before it was emptied. The wine for filling the casks was renewed every year with new wine (see above).

Three 1.5 L samples were taken from each subset, one from each one of the barrels, and analyzed separately. All samples were analyzed immediately after removal from the barrels. The three subsamples from each subset were analyzed immediately and separately for major and trace volatiles (analytical methods A and B).

Analytical Reagents and Chemical Standards. The chemical standards were supplied by Aldrich (Gillingham, U.K.), Fluka (Buchs, Switzerland), Sigma (St. Louis, MO), Lancaster (Strasbourg, France), PolyScience (Niles, IL), Chemservice (West Chester, PA), Interchim (Monlucon, France), International Express Service (Allauch, France), and Firmenich (Geneva, Switzerland).

LiChrolut EN resins, prepacked in 200 mg cartridges (6 mL total volume) or in bulk, were obtained from Merck (Darmstadt, Germany). Dichloromethane of HPLC quality was obtained from Fisher Scientific (Loughborough, U.K.); methanol of LiChrosolv quality was from Merck; absolute ethanol (ACS quality) and ammonium sulfate were from Panreac (Barcelona, Spain) and were ARG; pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

The 3-*tert*-butyl-4-hydroxyanisole (BHA) solution contained 10 mg of this compound per gram of ethanol. Semiautomated solid-phase extraction was carried out with a VAC ELUT 20 station from Varian (Walnut Creek, CA).

Analysis. Two different analytical methods were used. Both have been developed and validated in our laboratory and have undergone extensive testing before their use. Such testing included the definition of acceptance criteria for the system suitability and for the determination of the validity of the analysis.
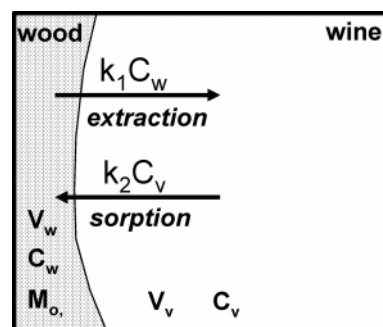
Figure 1. Scheme showing the basic mass transferences of odorant between wine and wood.

Major Compounds (Microextraction and GC-FID Analysis). Quantitative analysis of major compounds was carried out using the method proposed and validated by Ortega et al. (*29*). In accordance with this method, 3 mL of wine and 7 mL of water were salted with 4.5 g of ammonium sulfate and extracted with 0.2 mL of dichloromethane. The extract was then analyzed by GC (Hewlett-Packard 5890 series II) with flame ionization detection using the conditions described elsewhere. Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs built by the analysis of synthetic wines containing known amounts of the analytes. 2-Butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol were used as internal standards and for quality control purposes. For quality control, acceptance regions for both absolute areas of each of the internal standards and for relative areas to 4-methyl-2-pentanol were predetermined.

Minor Compounds (SPE and GC−Ion Trap−MS Analysis). This analysis was carried out using the method proposed and validated by López et al. (*30*). In accordance with the method, 50 mL of wine, containing 25 μL of BHA solution and 75 μL of a surrogated standard solution (surrogates were isopropyl propanoate, 3-octanone, heptanoic acid, and β-damascone), was passed through a 200 mg LiChrolut EN cartridge at ∼2 mL/min. The sorbent was dried by letting air pass through (−0.6 bar, 10 min). Analytes were recovered by elution with 1.3 mL of dichloromethane. An internal standard solution (2-octanol and 4-hydroxy-4-methyl-2-pentanone in dichloromethane) was added to the eluted sample. The extract was then analyzed by GC (Star 3400CX from Varian) with ion trap MS detection (Saturn 4 from Varian) under the conditions described in the reference. For quality control, acceptance regions for absolute areas of the internal standards and for the relative areas of the surrogates to 2-octanol were predefined.

RESULTS

Model: General Relationships and Main Equations. There are two different stages in the extraction of compounds from wood. During the first one, the wine penetrates slowly into the wood, and the extraction kinetics are influenced by the wine penetration rate, as was shown by Kadim and Mannheim (*28*). During this period, the surface of the wood wetted by wine steadily increases, which can make the extraction kinetics vary depending on the particular physicochemical characteristics of the compound. The duration of this period can be indirectly estimated from the findings by Kadim and Mannheim as around 4 months. From this point on, the surface of the wood wetted by wine will remain approximately constant and a steady state will be reached. During this period, it can be considered that the extraction rate coefficients will be constant. The basic relationships for this stage are illustrated in **Figure 1**. The final process of extraction is the result of two kinetically opposed processes, one of extraction and another of sorption. The amount

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of odorant extracted into the wine at time t (dC_v) is proportional to the concentration at that time in wood (C_w):

$$\frac{dC_v}{dt} = k_1 C_w \tag{1}$$

Similarly, the amount of odorant absorbed by the wood will be proportional to the concentration of the odorant at that time in the wine (C_v):

$$\frac{dC_v}{dt} = -k_2 C_v \tag{2}$$

Combining both expressions

$$\frac{dC_v}{dt} = k_1 C_w - k_2 C_v \tag{3}$$

If we assume that the odorant is not further degraded or transformed, then the following mass balance has to be fulfilled

$$M_o = V_w C_w + V_v C_v = C_w^0 V_w \tag{4}$$

where M_o is the total mass of odorant; V_w and V_v are the volumes of wood and wine, respectively; C_w and C_v are the concentrations of the odorant at that moment in the wood and wine, respectively; and C_w^0 is the initial concentration of such odorant in the new wood before any extraction takes place.

Therefore

$$C_w = \frac{M_o - V_v C_v}{V_w} = C_w^0 - \Phi C_v \tag{5}$$

where Φ is the phase ratio (V_v/V_w).

Replacing this in eq 3, we have

$$\frac{dC_v}{dt} = k_1 C_w^0 - (k_2 + k_1 \Phi) C_v \tag{6}$$

Rearranging, we find

$$\frac{dC_v}{\dfrac{k_1 C_w^0}{(k_2 + k_1 \Phi)} - C_v} = (k_2 + k_1 \Phi)\, dt \tag{7}$$

If we make

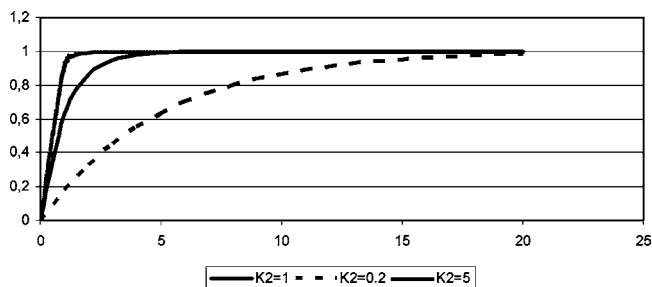$$\frac{k_1 C_w^0}{(k_2 + k_1 \Phi)} = K_1 \tag{8}$$

and

$$k_2 + k_1 \Phi = K_2 \tag{9}$$

the equation becomes

$$\frac{dC_v}{K_1 - C_v} = K_2\, dt \tag{10}$$

the integral of which is

$$\ln\left(\frac{K_1}{K_1 - C_v}\right) = K_2 t \tag{11}$$

Figure 2. Expected extraction profiles (extracted concentration vs time), according to eq 12, of three aroma compounds with $K_1 = 1$ (K_1 is the concentration of aroma at equilibrium) and three different K_2 (K_2 determines the extraction rate).

Finally

$$C_v = K_1(1 - e^{-K_2 t}) \tag{12}$$

Such function is represented in **Figure 2**, which shows the practical meaning of K_1 and K_2. K_1 is the concentration of the odorant found in the wine at equilibrium in a single extraction carried out on a new barrel. This parameter is a constant characteristic of the odorant and the barrel. In the figure this parameter takes the arbitrary value 1. K_2 is related to the speed of the extraction. A compound reaching the equilibrium concentration quickly will have a high K_2 and vice versa. Experimental curves for compounds not suffering degradations (Z-whiskeylactone, eugenol, and 4-propylguaiacol) have more or less this shape (see ref *1* and **Figure 5**), which confirms the previous reasoning. However, in most cases, the amount of odorant extracted during the first months after the barrel is filled with wine for the first time is smaller than predicted; that is, the first extraction is less efficient (*1*). This is because the extraction rate is proportional to the surface of wood in contact with wine, and this surface slowly increases during the first 4 months as the wine slowly penetrates into the wood (*28*). On average, the extraction efficiency in such first 4 months amounts to half of the maximum efficiency, that is, the amount extracted is roughly equal to that extracted in 2 months. Thus, the effect can be easily simulated by subtracting 2 months from the time in eq 12, so that the final main equation becomes

$$C_v = K_1(1 - e^{-K_2(t-2)}) \tag{13}$$

As will be shown later, this function satisfactorily fits the experimental behavior of compounds not suffering degradations.

Relationships To Study the Release of Compounds along Successive Extractions. The barrel is used several times before it is discarded, and its capacity to release aroma compounds changes according to the number and extent of previous uses. This has deep influence on the practical life of the barrel and becomes a critical parameter to control and to model.

Equation 13 must be slightly modified to take into account the previous history of the barrel if it has been previously used. In particular, we need an estimate of the total amount of odorant that the barrel can release. We will call this parameter K_3, and we will show later how it can be estimated. K_3 is defined as the concentration of odorant that a wine aged in the barrel would have if 100% of the odorant was released into the wine. Its dimensions are, therefore, those of a concentration. This parameter, again, is a constant characteristic of an odorant and
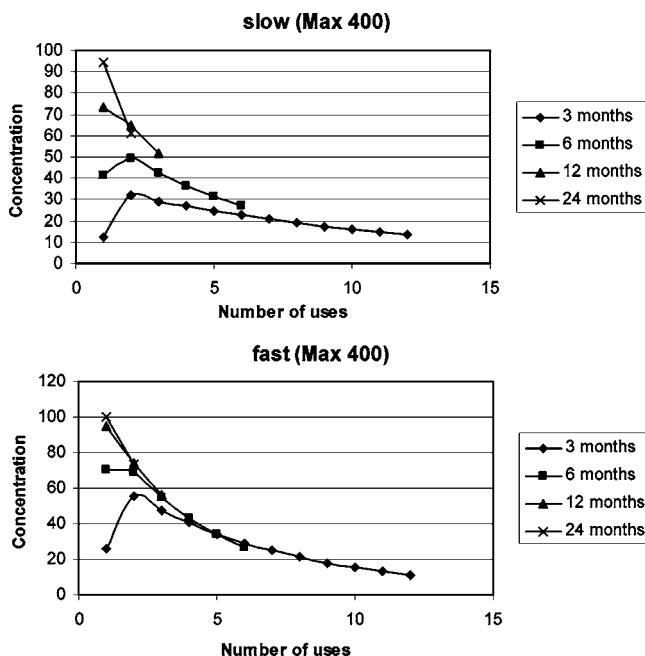
Figure 3. Concentration of odorant (in micrograms per liter) released from the wood into the wine as a function of the extraction time and the number of previous uses of the cask. Plots have been obtained from eq 15 using the following parameters: (**a**, slow extraction) $K_1 = 100$ μg/L, $K_2 = 0.15$ μg/L, and $K_3 = 400$ μg/L; (**b**, fast extraction) $K_1 = 100$ μg/L, $K_2 = 0.33$ μg/L, and $K_3 = 400$ μg/L.

a given barrel. If in the first extraction the amount extracted is C_1, the proportion remaining in the barrel will be

$$1 - \frac{K_3 - C_1}{K_3}$$

and the kinetics of a second extraction will be defined by the expression

$$C_{v2} = K_1\left(1 - \frac{K_3 - C_1}{K_3}\right)(1 - e^{-K_2(t-2)}) \qquad (14)$$

Similarly, for the ith extraction, the kinetics will be driven by

$$C_{vi} = K_1\left(1 - \frac{K_3 - \sum\limits_{j=1}^{j=i-1} C_j}{K_3}\right)(1 - e^{-K_2(t-2)}) \qquad (15)$$

All of these expressions make it possible to obtain different graphical representations of the expected release of odorants from the barrel depending on the aging conditions. For instance, plots in **Figure 3** show the expected concentrations of odorant released from a barrel in successive extractions of different duration. The plots show something usually observed in practice: for short extractions, the first wine extracts less. Another interesting observation is that, for a compound slowly released from the barrel, the differences linked to the duration of the extraction time are important (**Figure 3a**), whereas for a compound released slightly more quickly (2 times faster), the differences are less important (**Figure 3b**).

It can be observed that K_3 is the integral of the functions represented in **Figure 3** if they are extrapolated to a very high number of extractions. This graphical solution is the approach used to get in practice a reasonably good estimate for this
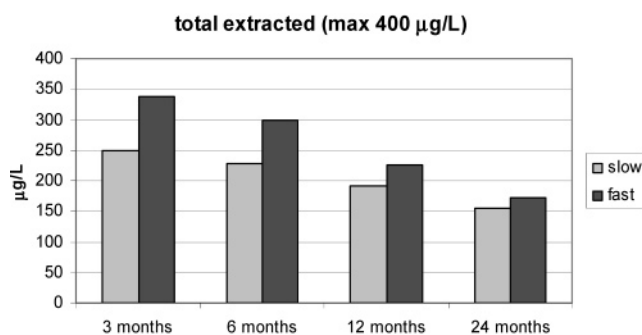
Figure 4. Total amount of odorant extracted in a three-year cycle of uses of the barrel, using different extraction times (3, 6, 12, and 24 months) and two different kinetic parameters: slow, $K_1 = 100$ μg/L, $K_2 = 0.15$ μg/L, and $K_3 = 400$ μg/L; fast, $K_1 = 100$ μg/L, $K_2 = 0.33$ μg/L, and $K_3 = 400$ μg/L. Estimates have been obtained by using eq 15.

Figure 5. Fitting of experimental data "concentration of *Z*-whiskeylactone extracted" versus time (a single extraction) with eq 13 for two different sets of barrels (American and French). Original data are from ref *1*. For American oak $K_1 = 1200$ μg/L and $K_2 = 0.12$ μg/L. For French oak $K_1 = 650$ μg/L and $K_2 = 0.25$ μg/L.

parameter. Plots similar to those shown in **Figures 3** are built with experimental data and extrapolated, and the total area under the curve is then calculated.
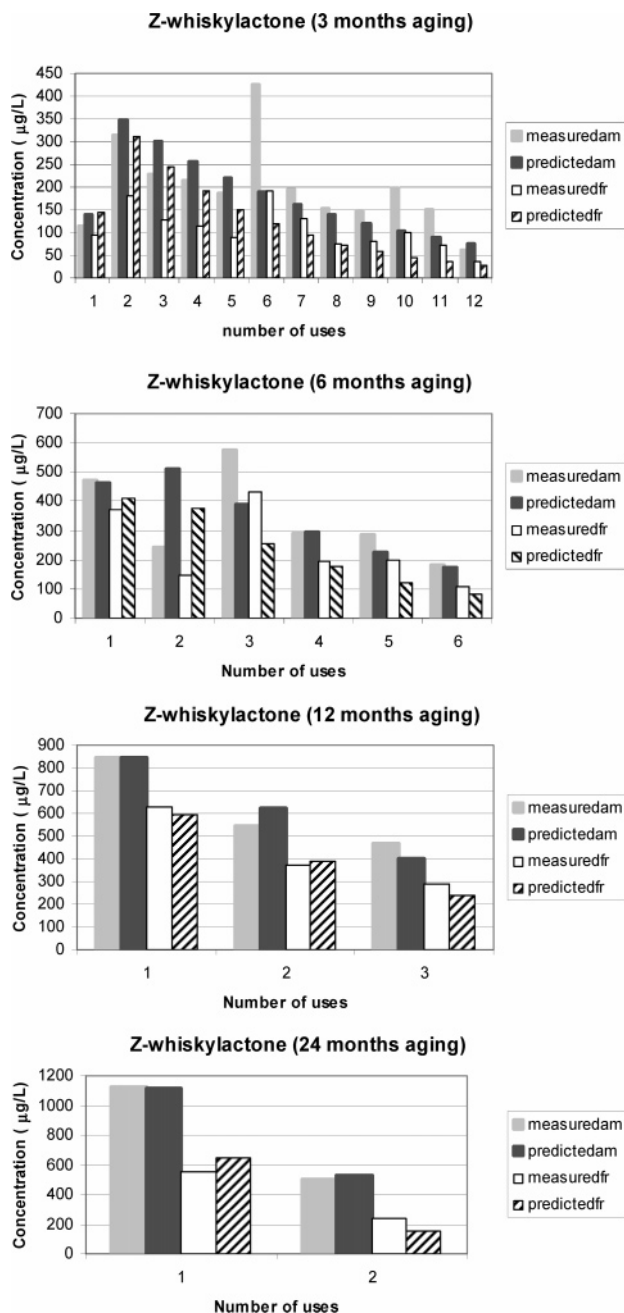
A third plot that can be obtained from the model is the total amount of odorant extracted during a given time in which the barrel has kept different cycles of wine. For instance, **Figure 4** shows the total amount of a hypothetical odorant extracted over 3 years depending on the kind of odorant and the length of the extraction time. Obviously, the model predicts that the maximum yields will be obtained with a large number of short extractions, particularly for compounds with a fast extraction. This plot has a great potential to diagnose deviations from the model.

Practical Use of the Model: Z-Whiskeylactone as a Case Study. The model can be used with two purposes. A primary one would be to study the differences in yields linked to different aging options and to select those conditions leading to an optimum yield in desirable odorants, such as *E*-whiskeylactone or eugenol. All of this has an obvious practical role. A secondary purpose, which is of paramount importance from a scientific point of view, is to use the model for the detection of anomalous behaviors. The model is based on very simple physicochemical considerations and, if a given odorant follows a trend (defined by plots such as those shown in **Figures 2−4**) not in accordance with the model, this will evidence the existence of factors with effect different from those included in the premises of the model.
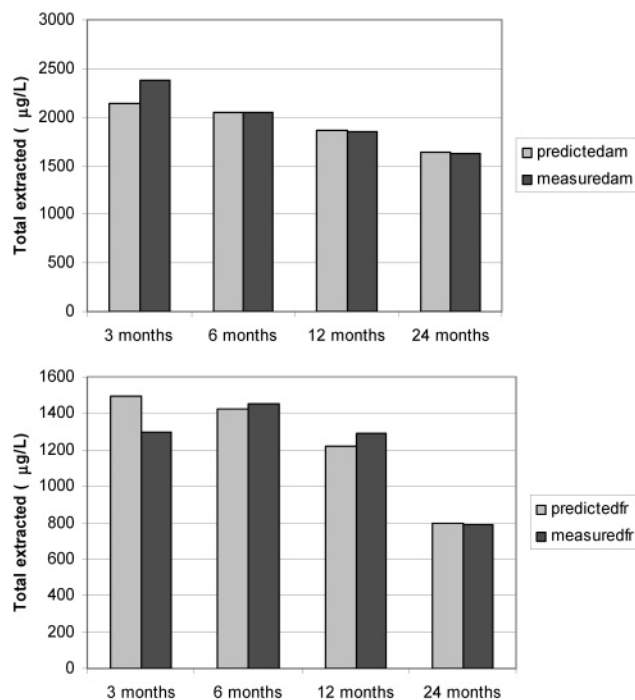
We will examine the application of the model to the study of the extraction kinetics of Z-whiskeylactone. **Figure 5** shows the fit of eq 13 to the basic curves of release of the compound observed in two different types of barrel (data are means from three equivalent barrels and can be seen in ref *1*). The fit in the

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Figure 6. Fitting with eq 15 the experimentally determined concentration extracted of *Z*-whiskeylactone from American or French barrels with different numbers of uses and using different extraction times (3 months in **a**; 6 months in **b**; 12 months in **c**; 24 + 12 months in **d**). For American oak $K_1 = 1200$ μg/L, $K_2 = 0.12$ μg/L, and $K_3 = 2600$ μg/L. For French oak $K_1 = 650$ μg/L, $K_2 = 0.25$ μg/L, and $K_3 = 1600$ μg/L.

Figure 7. Fitting with eq 15 the experimentally determined total amount of *Z*-whiskeylactone extracted from different sets of American or French barrels in a three-year cycle using different extraction times. For American oak $K_1 = 1200$ μg/L, $K_2 = 0.12$ μg/L, and $K_3 = 2600$ μg/L. For French oak $K_1 = 650$ μg/L, $K_2 = 0.25$ μg/L, and $K_3 = 1600$ μg/L.

before. The estimates obtained for this parameter were 2600 μg/L (American oak) and 1600 μg/L (French oak).

With K_1, K_2, and K_3 known, it is possible to predict the amount of *Z*-whiskeylactone extracted from the wood as a function of both the number of uses of the barrel and the duration of each extraction (3, 6, 12, or 24 months) and to evaluate the quality of the predictions. The series of plots is shown in **Figure 6** and the total amount of odorant extracted in 3 years of extractions are shown in **Figure 7**.

Comparison between the measured and predicted values shown in the different plots reveals the existence of strong disagreements, particularly in the cases of short extractions (3 and 6 months, as shown in **Figure 6a,b**). **Figure 6a** shows the existence of three maxima in the experimental plots at 2, 6, and 10 uses. The three maxima correspond to the wines kept in the barrel over the three summers of the three consecutive years of the experiment. Similarly, three less clear maxima (at 1, 3, and 5 uses) are also seen in the experimental plots in **Figure 6b**. Again, those maxima correspond to the wines kept in the barrel during the summers.

From a quantitative point of view, this implies that the "effective" extraction coefficients of *Z*-whiskeylactone can vary by factors as high as 3 or 4, depending on the season and the number of uses of the barrel. These shocking disagreements between expected and observed contrast with the more or less satisfactory agreement between expected and observed achieved when extraction times are 12 or 24 months (**Figure 6c,d**) or between the expected and observed total amounts extracted (**Figure 7a,b**). It must also be noted that any other combination of model parameters (K_1, K_2, and K_3) produced higher prediction errors. In all of the cases, the fit for the set of data obtained from French barrels was worse, in agreement with the poorer adjustment obtained for this set of barrels for the data in **Figure 5**. The anomalous low yield of *Z*-whiskeylactone for 3-month

case of the American barrels is perfect, whereas it is slightly worse for the French barrels because in the latter case a decrease in the amount of odorant at higher times is observed, an effect that cannot be modeled with our set of equations. This does not pose a problem for modeling extraction cycles with extraction times of 3, 6, or 12 months. In our case, the fitting of experimental data in **Figure 5** by a least-square fitting method gave the following estimates of these parameters: $K_1 = 1200$ μg/L and $K_2 = 0.12$ μg/L (American oak); $K_1 = 650$ μg/L and $K_2 = 0.25$ μg/L (French oak). These data reveal that the extraction rate in French oak is faster. K_3 was estimated from the experimental curves shown in **Figure 6**, as was explained
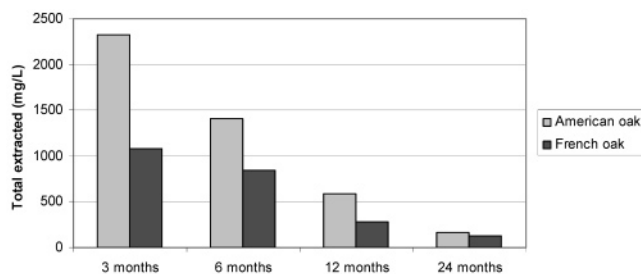
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Figure 8. Total amount of furfural extracted from wine in a three-year extraction cycle using different extraction times and types of wood.

Figure 9. Total amount of 4-propylguaiacol extracted from wine in a three-year extraction cycle using different extraction times and types of wood.

extractions in the case of the French barrels (**Figure 7b**) should also be pointed out. This result is particularly surprising, because it apparently goes against the physicochemical principle of successive extractions, according to which the higher the number of extractions, the higher the yield.
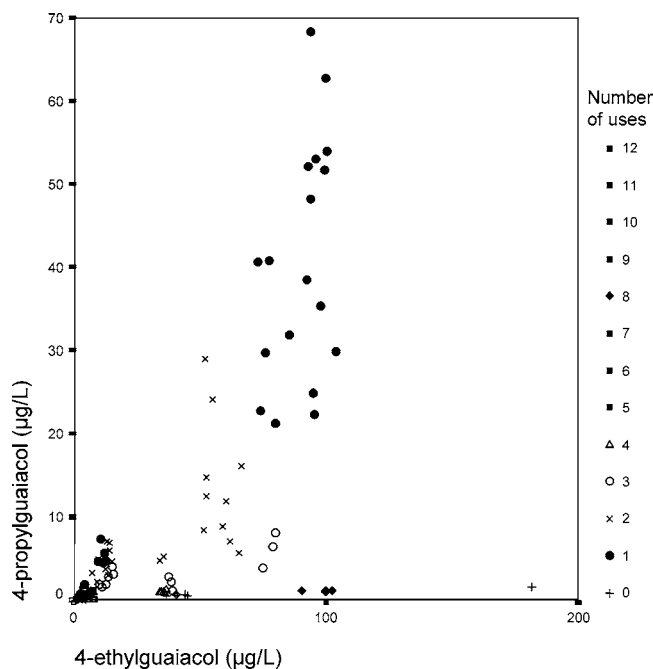
In conclusion, although the model seems to explain the general pattern of extraction of *Z*-whiskeylactone, there are some deviations that imply the concourse of other phenomena in the extraction. These deviations are as follows: (1) a seasonal dependence according to which the extraction is much more efficient during the summer and (2) an anomalously low yield of short extractions in the case of the French barrel.

The behavioral pattern exemplified by *Z*-whiskeylactone can also be found in the case of other important wood extractables, such as eugenol, 4-allyl-2,6-dimethoxyphenol, and *E*-whiskey-lactone.

Second Case Study: Furfural. Furfural represents the paradigm of compound suffering strong degradations during the aging process (*2, 17, 31*). Its extraction kinetics cannot be modeled with our simple model, because its extraction curve shows one or even two maxima. Plots similar to those shown in **Figure 6** are also chaotic. The plot of total amount extracted reveals, however, a very clear trend as shown in **Figure 8**.

At first glance, it could be thought that the plot shows a structure coherent with the physicochemical laws of extraction, because a major yield is obtained by using a longer number of short extractions. However, a closer look will reveal that the amount finally recovered at longer extractions is too low. For instance, the total amount recovered in two long extractions (2 years for the first and 1 year for the second) from American oak barrels is 167 mg/L, which is 14 times less than the amount recovered in 12 3-month long extractions, well above the theoretical limit (6 times). Therefore, for compounds suffering degradation, the plot of total amount extracted will show a trend similar to this observed for furfural. Other compounds following this behavior are 5-methylfurfural and *Z*-isoeugenol.

Third Case Study: 4-Propylguaiacol. This compound shows a very interesting behavior because its basic plots (amount extracted vs time and amount extracted vs number of uses) give the impression that its extraction is regulated exclusively by the physicochemical laws beneath the model and that its behavior can be, therefore, perfectly modeled. So far, in our first approach to data treatment, we selected this compound as representative of a purely physical extraction. However, the plot of total amount extracted versus extraction time reveals the surprising fact that, for this compound, the yield achieved with numerous short extractions is lower than that obtained with a few long extractions, as can be seen in **Figure 9**. This trend is absolutely incompatible with an extraction regulated by simple physicochemical principles, and a plot like the one shown in **Figure 9** will be evidence of the existence of other processes, as will be discussed later. There are no other compounds
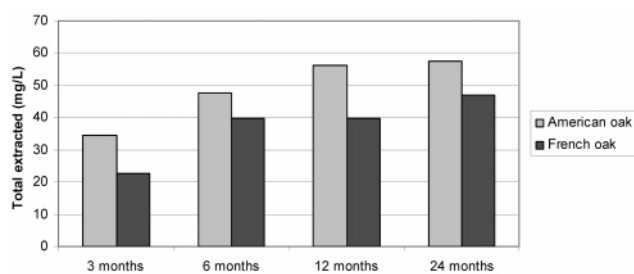
following exactly the same trend, but the extraction behavior of some important wood constituents, such as vanillin, syring-aldehyde, and furfuryl alcohol, can be interpreted as intermediate to those observed in the cases of 4-propylguaiacol and furfural.

DISCUSSION

The most important result related to the proposed model is that the extraction kinetics of wood constituents into wine cannot be completely explained by a physicochemical-based model. No single compound behaves as the model predicts. In the best of cases, such as the compounds behaving like *Z*-whiskey-lactone, important seasonal variations appear. In the worst of cases, the model simply does not work, as was previously shown. Of course, a first plausible explanation for the seasonal variations would be a change in temperature. However, the experiment was carried out in a fully thermostated cellar, and temperature changes were minimal (18 °C maximum in summer hot peaks and 14 °C minimum in winter) to explain the huge variations observed. It must be noted that the extraction speed more than doubles between the 5th and 6th uses and between the 9th and 10th or between the 2nd and 3rd and between the 4th and 5th (**Figure 6**). These changes will surely require a major increment of temperature. A second possibility would be the existence of an undetected bias in the experiment. However, such possibility is definitively remote, because the same behavior has been observed over different years and in different cellars in independent experiments and affects only a limited number of compounds. A third possibility would be the concourse of microorganisms in the extraction. In the summer time there is a huge explosion of microbiota around the cellars, and some microorganisms can reach the barrels. Our hypothesis is that some of them, even if they do not form big colonies or big biomasses or produce major changes in the wine, can alter the extraction kinetics. We do not have direct evidence for this, because the presence of small colonies living at the wood/wine interface is very difficult to demonstrate. However, we cannot find a more plausible hypothesis, and we have more indirect evidence supporting this.

The most important evidence comes from the third case study: 4-propylguaiacol. This compound has often been reported as one of the wood constituents released into wine. However, it has several peculiarities because it is structurally related to 4-ethylguaiacol, and we have found that its levels in wine are correlated to the wine content in 4-ethylphenol and 4-ethyl-guaiacol (*20*). In the present case, if we represent the levels of this compound versus those of 4-ethylguaiacol and segregate the samples by the number of uses, a surprising relationship appears, as shown in **Figure 10** and **Table 1**.

The trend disclosed in **Figure 10** reveals that the existence of 4-ethylguaiacol is a necessary but not sufficient condition for the existence of 4-propylguaiacol: high levels of 4-propyl-

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Figure 10. Relationship between the wine content of 4-propylguaiacol and 4-ethylphenol. Wines are marked with different symbols corresponding to the number of previous uses of the barrel in which they were aged.

Table 1. Straight-Line Regression Analysis of 4-Propylguaiacol (Y Variable) and 4-Ethylguaiacol (X Variable): Correlation Coefficient, Slope, and Statistical Significance[a]

	R	slope	slope error	P
first use	0.945	0.454	0.064	0.0004
second use	0.709	0.186	0.076	0.0487
third use	0.897	0.0477	0.012	0.0153
fourth–sixth uses	0.717	0.0140	0.0043	0.0087
seven or more uses	0.897	0.0092	0.0014	0.0001
stainless steel vat	0.957	0.0092	0.0027	0.0092

[a] Samples were segregated by the number of previous uses of the barrel.

guaiacol imply high levels of 4-ethylguaiacol, but the opposite is not true. Furthermore, the levels of 4-propylguaiacol are extremely dependent on the number of previous uses of the barrel as can be observed in the figure and in **Table 1**. This table reveals that the amount of 4-propylguaiacol is always significantly related to that of 4-ethylguaiacol but that the slope of the regression strongly decreases as the number of previous uses of the barrel increases. New barrels in their first use produce 0.45 mass unit of 4-propylguaiacol by mass units of 4-ethylguaiacol. In the second use, only 0.186 mass unit of the first per unit of the second is produced, and in the third this figure comes down to 0.048 (10% of the figure obtained with new barrels); between the fourth and sixth uses it is only 0.014, and for barrels used seven or more times, this figure is 0.0092, exactly the same as the slope observed between these compounds in wines stored in stainless steel vats. All of this suggests that this compound may be formed microbiologically by the same microorganisms forming 4-ethylguaiacol (most likely *brettanomyces/deckkera*), but acting this time on a precursor exclusively present in the new wood. A second observation, coherent with the concourse of microorganisms in the extraction/ production of this compound, is the fact that higher yields are obtained with a limited number of long extractions, as can be seen in **Figure 9**. If the extraction is mediated by microorganisms, these should form more stable colonies in barrels kept
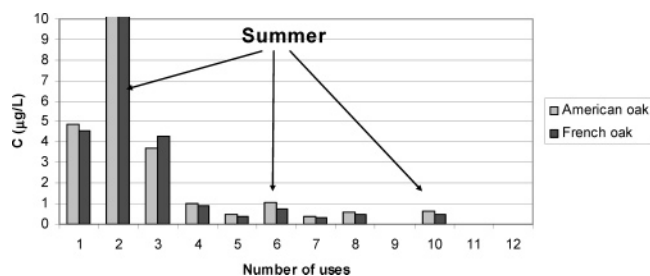
Figure 11. Concentrations of 4-propylguaiacol extracted from barrels with different number of uses (extraction time was 3 months).
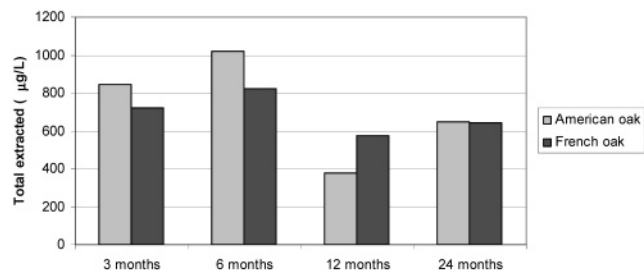
Figure 12. Total amount of vanillin extracted from wine in a three-year extraction cycle using different extraction times and types of wood.

unaltered longer periods. Finally, a third observation is the seasonal dependence (**Figure 11**), although in this case this effect is less apparent because the level of this compound seems to be extremely dependent on the level of precursor, and this seems to quickly decay after a couple of extractions.

If the previous hypothesis was true, then we should conclude that the extraction kinetics of most wood aroma compounds are affected by the microbiological activity but to a very different extent. The least affected compounds would be those behaving similarly to Z-whiskeylactone (E-whiskylactone, eugenol, and 4-allyl-2,6-dimethoxyphenol), for which only the seasonal dependence is noticeable (although some of the other effects are also slightly apparent, as was discussed in the case of Z-whiskeylactone). The extraction kinetics of these compounds can be roughly modeled with our model based on physico-chemical parameters, but a season correction factor should be included (for short extraction times). On the other hand, we found 4-propylguaiacol, the experimental behavior of which violates some of the basic principles of physicochemical extraction. The extraction kinetics of this compound would be entirely driven by the microbiological activity. The extraction kinetics of vanillin, syringaldehyde, and furfuryl alcohol share some similarities with those of 4-propylguaiacol, although they are further complicated by the fact that these compounds are unstable (*6, 31*). The extraction of these compounds follows the same seasonal dependence (except for furfuryl alcohol) observed in the other cases, and their yields tend to increase with a smaller number of longer extractions. The plots observed are a mixture of those observed for 4-propylguaiacol and those observed for furfural. As an example, the plot of total extracted amount versus extraction time for the case of vanillin is shown in **Figure 12**. As can be seen, 12 6-month extractions are more efficient than 24 3-month extractions and 2 24/12 extractions more than 3 12-month extractions, as observed for 4-propyl-guaiacol. However, the yields at longer extraction times are lower than those obtained at shorter extraction times, as observed for furfural and as expected from the low stability of this compound (*31*). All of this would suggest that the extraction of these compounds is also mediated by microorganisms. This would be in accordance with the hypothesis of Spillman and

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Sefton about the existence of a slow extraction mechanism of vanillin, although our hypothesis suggests that the transformation of the lignin would be caused by a microorganism.

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