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Study of Sensory Interactions among Red Wine Fruity Esters in a Model Solution

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ABSTRACT: Our study focused on the impact of 12 red wine esters, in complex mixtures, on the perception of fruity aromas. Aromatic reconstructions were prepared in dilute alcohol solution at the average concentrations found in red wines, using pure commercial products. The impact of ethyl propanoate, ethyl-3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate was detected by omission tests, although they were present at subthreshold concentrations in the fruity mixture. The "olfactory threshold" of the fruity pool, consisting of all of the esters excluding ethyl propanoate, ethyl-3-hydroxybutanoate, butyl acetate, or 2-methylpropyl acetate was calculated in two different matrices: dilute alcohol solution and dilute alcohol solution supplemented with each of the four compounds mentioned above. The presence of ethyl-3-hydroxybutanoate and 2-methylpropyl acetate in the mixture led to a significant decrease in the olfactory threshold of the fruity pool, demonstrating their synergistic effect in increasing the overall intensity. Sensory profiles revealed that besides ethyl-3-hydroxybutanoate, the omission of each of these compounds had a significant attenuating effect on blackberry and fresh-fruit aroma intensity. These compounds with similar chemical structures participate, both quantitatively and qualitatively, in modulating fruity aromas and, specifically, naturally enhancing blackberry and fresh-fruit aromas.

KEYWORDS: *esters, fruity mixtures, perceptive interactions, aromatic reconstitutions, aroma enhancer*

INTRODUCTION

The specific aromas of red wines have been studied less than those of whites. The existence of a typical fruity aroma in red wines, different from that of whites, remained relatively controversial even until the 2000s.[1] Since then, the work of Pineau,[2] characterizing a specific olfactory sensory space, as defined by Ballester,[3] proved the sensory reality of a typical fruity aroma in red wines.

Numerous authors have studied the fruity aroma of red wines, with the aim of indentifying the aromatic compounds responsible for these specific notes.[4−8] However, the fact that the "key" aromatic molecules responsible for this typical fruity aroma were not identified did not necessarily mean that they did not exist.

Studies investigating fruity aromas in red wines over the past decade have revealed a certain number of compounds that are potentially involved. Perceptive interactions have been described involving furanones (furaneol and homofuraneol),[9] C13-norisoprenoids, such as β-damascenone,[9−11] sulfur compounds, such as dimethyl sulfide[12,13] or diacetyl, and acetoin, acetic acid, and γ-butyrolactone,[14] which may contribute indirectly to fruity expression in red wines. These examples emphasize the importance of perceptive interactions on the intensity and quality of red wine fruity aromas. Pineau et al.[15] demonstrated that, in some complex mixtures in dearomatized red wine, very small variations in the concentrations of some ethyl esters were perceived, even at concentrations far below their individual olfactory thresholds, and affected their red- and blackberry aromas. More precisely, they showed that ethyl propanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate were involved in blackberry aromas, whereas ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate impacted red-berry aromas.

According to Berglund et al.,[16] perceptual interactions may have different origins. These authors proposed four levels of possible interactions. The first level of interaction is presensory, involving chemical or physicochemical interactions between components in the mixture, reflecting changes in physical stimuli properties.[17,18] The second level of interaction is peripheral sensory, involving interactions at the receptor level,[19−21] thought to play a major role in processing odor quality.[22] The third level of interaction is electrophysiological, at the peripheral level of the nervous system. Signals from a particular receptor may interact with signals from other receptors on the way to the olfactory bulb. Interactions occur through the convergence of many primary neurons to a specific glomerulus or via lateral connections among neurons.[23−25] The fourth level of interaction occurs in the central nervous system.[26]

Although the importance of the variation of esters concentrations effecting red wine fruity aroma has already been highlighted, [15] previous works did not investigate this further. The goal of this work is to study the qualitative and quantitative impact of several esters, present in a mixture, at the average concentrations found in red wines, especially on fruity character. The 12 esters (ethyl esters or acetate), constituting the fruity pool, highlighted by Pineau et al.[15] form the basis of this work. From omission tests applied to aromatic reconstitutions

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Table 1. Olfactory Impact of the Individual Addition of Various Esters in Dilute Alcohol Solution[a]

	C_3C_2	C_4C_2	C_6C_2	C_8C_2	2Me C_3C_2	2Me C_4C_2	2OH 4MeC_5C_2	3OH C_4C_2	C_2C_4	C_2C_6	C_2iC_4	C_2iC_5	
concn (μg/L)	150	200	200	200	250	50	400	300	10	2	50	250	difference obsd
test 1	x	-	-	-	-	-	-	-	-	-	-	-	=
test 2	-	x	-	-	-	-	-	-	-	-	-	-	***
test 3	-	-	x	-	-	-	-	-	-	-	-	-	***
test 4	-	-	-	x	-	-	-	-	-	-	-	-	**
test 5	-	-	-	-	x	-	-	-	-	-	-	-	***
test 6	-	-	-	-	-	x	-	-	-	-	-	-	***
test 7	-	-	-	-	-	-	x	-	-	-	-	-	***
test 8	-	-	-	-	-	-	-	x	-	-	-	-	=
test 9	-	-	-	-	-	-	-	-	x	-	-	-	=
test 10	-	-	-	-	-	-	-	-	-	x	-	-	***
test 11	-	-	-	-	-	-	-	-	-	-	x	-	=
test 12	-	-	-	-	-	-	-	-	-	-	-	x	***

[a]***, 0.1% significant level; **, 1% significant level; *, 5% significant level; =, no significant difference; x, presence of listed compounds; and -, absence of listed compounds. C_3C_2, ethyl propanoate; C_4C_2, ethyl butanoate; C_6C_2, ethyl hexanoate; C_8C_2, ethyl octanoate; $2MeC_3C_2$, ethyl 2-methylpropanoate; $2MeC_4C_2$, ethyl 2-methylbutanoate; $2OH4MeC_5C_2$, ethyl 2-hydroxy-4-methylpentanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; C_2C_4, butyl acetate; C_2C_6, hexyl acetate; C_2iC_4, 2-methylpropyl acetate; and C_2iC_5, 3-methylbutyl acetate.

elaborated in dilute alcohol solution, the occurrence of interactions and their origins, from chemical, physicochemical, or psychophysical points of views, was investigated.

MATERIALS AND METHODS

Chemicals and Odorant Stimuli. Absolute ethanol (analytical grade, 99.97%, Scharlau Chemie S.A, Barcelona, Spain) was distilled before use. Sodium sulfate (99%) was provided by Scharlau Chemie S.A, Barcelona, Spain. Microfiltered water was obtained using a Milli-Q Plus water system (resistivity: 18.2 MΩ cm, Millipore, Saint-Quentin-en-Yvelines, France). Standard grade purity compounds were obtained from commercial sources as follows: ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl hexanoate, ethyl octanoate, ethyl 3-hydroxybutanoate, 2-methyl-propyl acetate, butyl acetate, and hexyl acetate from Sigma−Aldrich, Saint-Quentin-Fallavier, France; 3-methylbutyl acetate from VWR-Prolabo, Fontenay-sous-Bois, France; D-ethyl leucate and L-ethyl leucate were synthesized by Hangzhou Imaginechem Co., Ltd. (Hangzhou, China).

Samples: Aromatic Reconstitution Using Esters. For aromatic reconstitutions, the various esters were added individually or blended together at the average concentrations found in red wines (Table 1)[15,27] to double-distilled ethanol and microfiltered water to obtain an ethanol level of 12% (v/v) (pH adjusted to 3.5 with tartaric acid). The mixtures containing all 12 esters consisted of the total aromatic reconstitution (TAR).

Gas Chromatography−Olfactometry (GC-O) Analysis of Reference Compounds. GC-O analyses were carried out to ensure that the high-purity reference compounds did not contain any odoriferous impurities and to ascertain that the compound considered was responsible for the odor properties identified. Olfactometry analyses were carried out using an HP-6890 gas chromatograph (Hewlett−Packard, Wilmington, DE, USA), equipped with a flame ionization detector (FID) and a sniffing port (ODO-I SGE, Ringbow, Australia), connected by a flow-splitter to the column exit. GC effluent was combined with humidified N_2 (Air Liquide, France) at the bottom of the glass-sniffing nose (SGE, Victoria, Australia) to avoid nasal dehydration. Samples containing less than 0.2 μL of each pure odorant were directly injected in splitless-split mode (injector temperature, 240 °C; splitless time, 30 s; split flow, 50 mL/min). The column was a BP20 (SGE, Ringwood, Australia), 50 m × 0.22 mm i.d., and film thickness was 0.25 μm. The oven was programmed at 40 °C for the first minute and the temperature increased at a rate of 10 °C/min up to a final isotherm at 220 °C for 10 min. The carrier gas was hydrogen 5.5 (Air Liquide, France) with a column head pressure of 15 psi.

Ethyl Ester and Acetate Analyses. Chromatographic conditions and sample preparation were as optimized by Antalick et al.[27] The fiber (Supelco, Bellefonte, PA) was coated with 100 μm stationary phase polydimethylsiloxane film (PDMS-100). For the quantitative study, 20 mL of a stock solution of internal standards, ethyl-d5 butyrate, ethyl-d5 hexanoate, ethyl-d5 octanoate, and ethyl-d5 cinnamate at about 200 mg/L each in absolute ethanol, was added to 25 mL of the samples. A 10 mL sample was placed in a 20 mL headspace vial, 3.5 g of sodium chloride was added, and the vial was tightly sealed with a PTFE-lined cap. The solution was homogenized in a vortex shaker and then loaded onto a Gerstel (Mülheim an der Ruhr, Germany) autosampling device. The program consisted of swirling the vial at 500 rpm at 40 °C for 2 min, then inserting the fiber into the headspace at 40 °C for 30 min as the solution was swirled again, then transferring the fiber to the injector for desorption at 250 °C for 15 min. Gas chromatography analyses were carried out on an HP 5890 GC system coupled to an HP 5972 quadrupole mass spectrometer (Hewlett-Packard), equipped with a Gerstel MPS2 autosampler. Injections were in splitless mode for 0.75 min, using a 2 mm i.d. nondeactivated direct linear transfer (injector temperature, 250 °C; interface temperature, 280 °C) and a BP21 capillary column (50 m × 0.32 mm, film thickness, 0.25 μm, SGE). The oven temperature was programmed at 40 °C for 5 min, then raised to 220 °C at 3 °C/min, and held at that temperature for 30 min. The carrier gas was Helium N55 (Air Liquide, France) with a column-head pressure of 8 psi. The mass spectrometer was operated in electron ionization mode at 70 eV with selected-ion-monitoring

Table 2. Ions Used for Identification and Quantification of the Tasted Aromatic Compounds

compd	qualitative ions	quantitative ions
Major and Branched Aliphatic Acids Ethyl Esters		
ethyl propanoate	*m/z* 57/75/45	*m/z* 102
ethyl 2-methylpropanoate	*m/z* 43/88/71	*m/z* 116
ethyl butanoate	*m/z* 71/60	*m/z* 88
ethyl 2-methylbutanoate	*m/z* 57/85/74	*m/z* 102
ethyl 3-hydroxybutanoate	*m/z* 71/88/117	*m/z* 87
ethyl hexanoate	*m/z* 60/99	*m/z* 88
ethyl 2-hydroxy-4-methylpentanoate	*m/z* 87/104	*m/z* 69
ethyl octanoate	*m/z* 101/127	*m/z* 88
Higher Alcohol Acetates		
2-methylpropyl acetate	*m/z* 43/73/116	*m/z* 56
butyl acetate	*m/z* 43/73/61	*m/z* 56
3-methylbutyl acetate	*m/z* 55/43	*m/z* 70
hexyl acetate	*m/z* 43/61/84	*m/z* 56

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Table 3. Olfactory Impact of the Omission of Various Esters from Complex Aromatic Reconstitutions[a]

	C_3C_2	C_4C_2	C_6C_2	C_8C_2	2Me C_3C_2	2Me C_4C_2	2OH 4MeC_5C_2	3OH C_4C_2	C_2C_4	C_2C_6	C_2iC_4	C_2iC_5	
concn (μg/L)	150	200	200	200	250	50	400	300	10	2	50	250	difference obsd
test 13	-	-	-	-	x	x	x	x	x	x	x	x	***
test 14	x	x	x	x	-	-	x	x	x	x	x	x	*
test 15	x	x	x	x	x	x	-	-	x	x	x	x	**
test 16	x	x	x	x	x	x	x	x	-	-	x	x	**
test 17	x	x	x	x	x	x	x	x	x	x	-	-	**
test 18	-	x	x	x	x	x	x	x	x	x	x	x	***
test 19	x	-	x	x	x	x	x	x	x	x	x	x	***
test 20	x	x	-	x	x	x	x	x	x	x	x	x	**
test 21	x	x	x	-	x	x	x	x	x	x	x	x	*
test 22	x	x	x	x	-	x	x	x	x	x	x	x	=
test 23	x	x	x	x	x	-	x	x	x	x	x	x	***
test 24	x	x	x	x	x	x	-	x	x	x	x	x	***
test 25	x	x	x	x	x	x	x	-	x	x	x	x	***
test 26	x	x	x	x	x	x	x	x	-	x	x	x	***
test 27	x	x	x	x	x	x	x	x	x	-	x	x	**
test 28	x	x	x	x	x	x	x	x	x	x	-	x	**
test 29	x	x	x	x	x	x	x	x	x	x	x	-	***

[a]***, 0.1% significant level; **, 1% significant level; *, 5% significant level; = no significant difference; x, presence of listed compounds; and -, absence of listed compounds. C_3C_2, ethyl propanoate; C_4C_2, ethyl butanoate; C_6C_2, ethyl hexanoate; C_8C_2, ethyl octanoate; $2MeC_3C_2$, ethyl 2-methylpropanoate; $2MeC_4C_2$, ethyl 2-methylbutanoate; $2OH4MeC_5C_2$, ethyl 2-hydroxy-4-methylpentanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; C_2C_4, butyl acetate; C_2C_6, hexyl acetate; C_2iC_4, 2-methylpropyl acetate; and C_2iC_5, 3-methylbutyl acetate.

Table 4. Composition of Samples Subjected to Olfactory Threshold Determination in Dilute Alcohol Solution[a]

compd tested	test concn (μg/L)
C_3C_2	25/50/100/200/400/800/1600/3200/6400/12800
$3OHC_4C_2$	50/100/200/400/800/1600/3200/6400/12800/25600
C_2C_4	4/8/16/32/64/128/256/512/1024/2048
C_2iC_4	10/20/40/80/160/320/640/1280/2560/5120

[a]C_3C_2, ethyl propanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; C_2C_4, butyl acetate; and C_2iC_4, 2-methylpropyl acetate.

Table 5. Composition of Samples Subjected to Olfactory Threshold Determination in Various Matrices[a]

AR	AR diluted in 50 mL matrix (mL)	matrix
TAR excluding C_3C_2	0.1/0.2/0.4/0.8/1.6/3.1/6.3/12.5/25/50	MS−MS + 150 μg/L C_3C_2 MS + 150 μg/L C_3C_2 in the positive sample
TAR excluding $3OHC_4C_2$	0.1/0.2/0.4/0.8/1.6/3.1/6.3/12.5/25/50	MS + 300 μg/L $3OHC_4C_2$ MS + 300 μg/L $3OHC_4C_2$ in the positive sample
TAR excluding C_2C_4	0.1/0.2/0.4/0.8/1.6/3.1/6.3/12.5/25/50	MS + 10 μg/L C_2C_4 MS + 10 μg/L C_2C_4 in the positive sample
TAR excluding C_2iC_4	0.1/0.2/0.4/0.8/1.6/3.1/6.3/12.5/25/50	MS + 50 μg/L C_2iC_4 MS + 50 μg/L C_2iC_4 in the positive sample

[a]TAR, total aromatic reconstitution (including all reference compounds); AR, aromatic reconstitution; MS, model wine solution (dilute alcohol solution); C_3C_2, ethyl propanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; C_2C_4, butyl acetate; and C_2iC_4, 2-methylpropyl acetate.

(SIM) mode. Monitored ions are listed in Table 2. Esters were characterized by comparing their linear retention indices and mass spectra with those of standards.

Sensory Analyses. *General Conditions.* Sensory analyses were performed as described by Martin and de Revel.[28] Samples were evaluated at controlled room temperature (20 °C), in individual booths, using covered, black ISO glasses,[29] containing about 50 mL of liquid, coded with three-digit random numbers. Sessions lasted approximately 5 min.

Table 6. Aromatic Reconstitutions Compared by Sensory Profiles[a]

	samples	
1	TAR excluding C_3C_2	TAR
2	TAR excluding $3OHC_4C_2$	TAR
3	TAR excluding C_2C_4	TAR
4	TAR excluding C_2iC_4	TAR

[a]TAR, total aromatic reconstitution (including all reference compounds); C_3C_2, ethyl propanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; C_2C_4, butyl acetate; and C_2iC_4, 2-methylpropyl acetate.

Sensory Panels. Panel 1 consisted of 18 judges, 9 male and 9 female, aged 29.7 ± 5.5 (mean ± SD). Panel 2 consisted of 22 judges, 9 male and 13 female, aged 28.6 ± 5.3 (mean ± SD).

All panelists were research laboratory staff at ISVV, Bordeaux University, selected for their experience in assessing fruity aromas in red wines. They attended 3 sessions per week, each lasting 5 min, for 4 weeks. Fresh berry-fruit standards were presented (blueberry, blackberry, blackcurrant, strawberry, cherry, and raspberry). Commercial jams, made from the same fruits, were presented directly as jammy fruit standards.

Discriminative Testing Methods. Triangular tests were performed in a three-alternative by panel 1 for various aromatic reconstitution samples (Table 1). A first set of triangular tests (*tests 1* to *12*) consisted of evaluating the individual perception of each compound in dilute alcohol solution. Each compound, present at the concentrations listed in Table 1, was compared to dilute alcohol solution alone. In the second phase, the same panel was subjected to triangular omission tests (Table 3; *tests 13* to *29*): first the omission of each entire chemical family (Table 3; *tests 13* to *17*) and then the omission of only one compound (Table 3; *tests 18* to *29*) among the 12 esters. Thus, the solution prepared with all tested compounds was compared to a solution containing only some of these compounds. For each triangular test, three numbered samples were presented in random order: two identical and one different. Each judge used direct olfaction to identify the sample perceived as different in each test and gave an answer, even if s/he was not sure. The results of all of the triangular tests were statistically analyzed, according to the tables given in the literature,[28,30] based on the

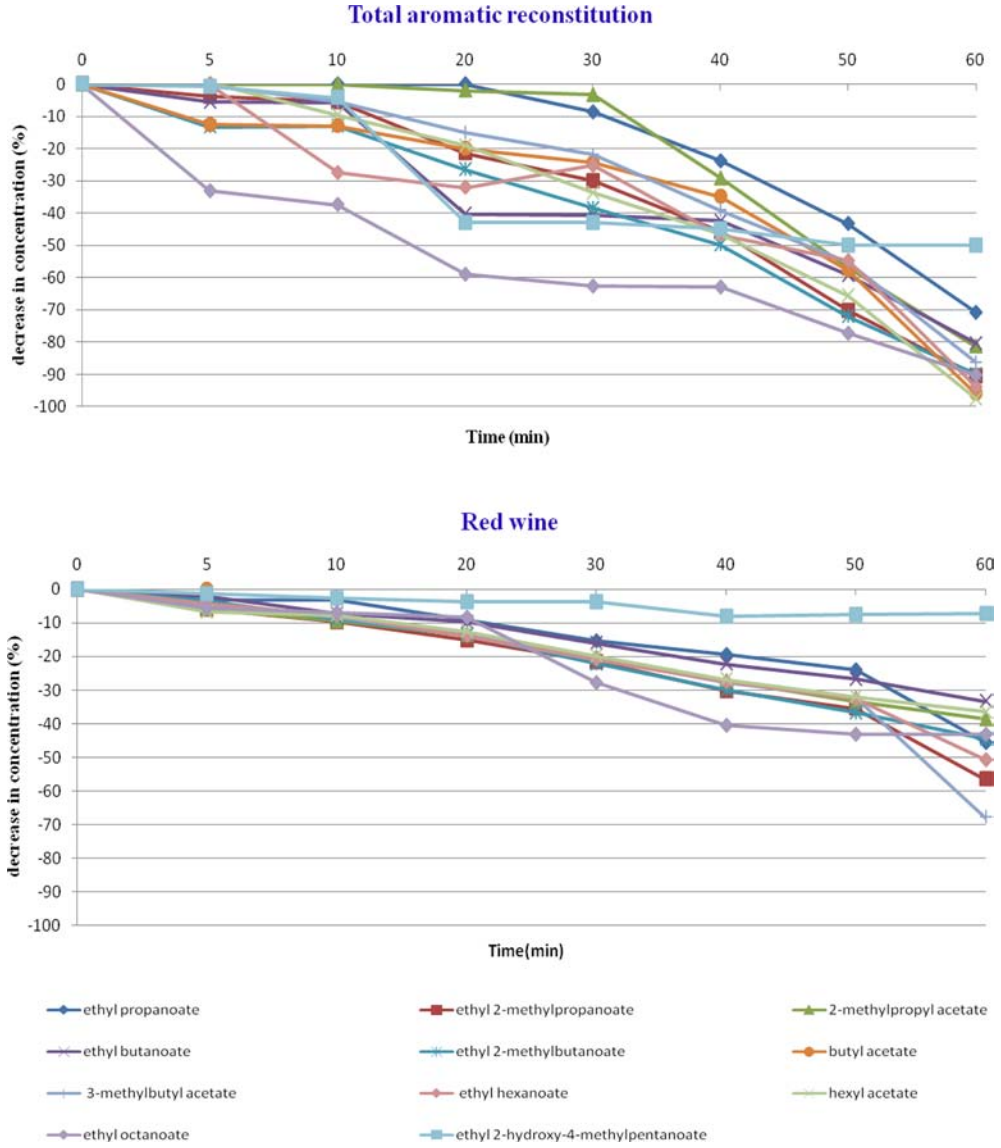

Figure 1. Evolution of ester concentrations in two different matrices during a sensory analysis session.

binomial law corresponding to the distribution of answers in this type of test.

Olfactory thresholds were determined by panel 1, in a three-alternative, forced-choice presentation (3-AFC) in dilute alcohol solution.[31] Each session consisted of 10 forced-choice tests. Each test contained one positive sample supplemented with increasing concentrations of the compound to be evaluated (Table 4). The olfactory thresholds of ethyl propanoate, ethyl 3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate were measured.

The olfactory thresholds of specific mixtures were also established. Olfactory thresholds of aromatic reconstitutions were thus measured in different matrices: dilute alcohol solution, dilute alcohol solution containing a compound of interest, and dilute alcohol solution containing a compound at a fixed concentration only in the positive sample (Table 5). The olfactory thresholds of the following mixtures were measured: total aromatic reconstitution (TAR) excluding ethyl propanoate, TAR excluding ethyl 3-hydroxybutanoate, TAR excluding butyl acetate, and TAR excluding 2-methylpropyl acetate.

The results of all 3-AFC tests were statistically analyzed. The detection threshold was defined as the concentration at which the probability of detection was 50%. This statistical value was determined using an adaptation of the ASTM-E1432 method.[32] The concentration/response function is a psychometric function and fits a sigmoid curve ($y = 1/(1 + e^{(-\lambda x)})$). Detection probability was corrected using the chance factor ($P = (3 \cdot p - 1)/2$, where p = proportion of correct responses for each concentration, and P = proportion corrected by the chance effect, 1/3 for 3-AFC). Sigma Plot 8 (SYSTAT) software was used for graphic resolution and ANOVA transform for nonlinear regression.[33,34]

In addition, interaction effects for certain mixtures were evaluated using Feller's additive model,[35] as developed by Miyazawa et al.[36] Mixture interaction patterns were compared using a simple additive response model. The probability of detecting the mixture p(AB) is defined as follows: p(AB) = p(A) + p(B) − p(A)p(B), where p(A) represents the probability of detecting component A and p(B) that of detecting component B. If the panel's detection performance for the mixture was below the sum of probabilities, some degree of suppression had occurred relative to statistical independence. A performance above the sum of probabilities indicated that some form of mutual enhancement or synergy had occurred. Moreover, if detection performance matched the sum of probabilities, no mixture interaction had occurred.

Descriptive Testing Methods. Sensory profiles of aromatic reconstitutions were evaluated by panel 2 for overall aromatic intensity, red-berry, blackberry, fresh-, and jammy-fruit aroma intensity. These aromatic descriptors were selected as the most typical of red wines from the Bordeaux area.[37] Each sample was presented twice in each session. The samples were presented in identical order in both evaluations, to

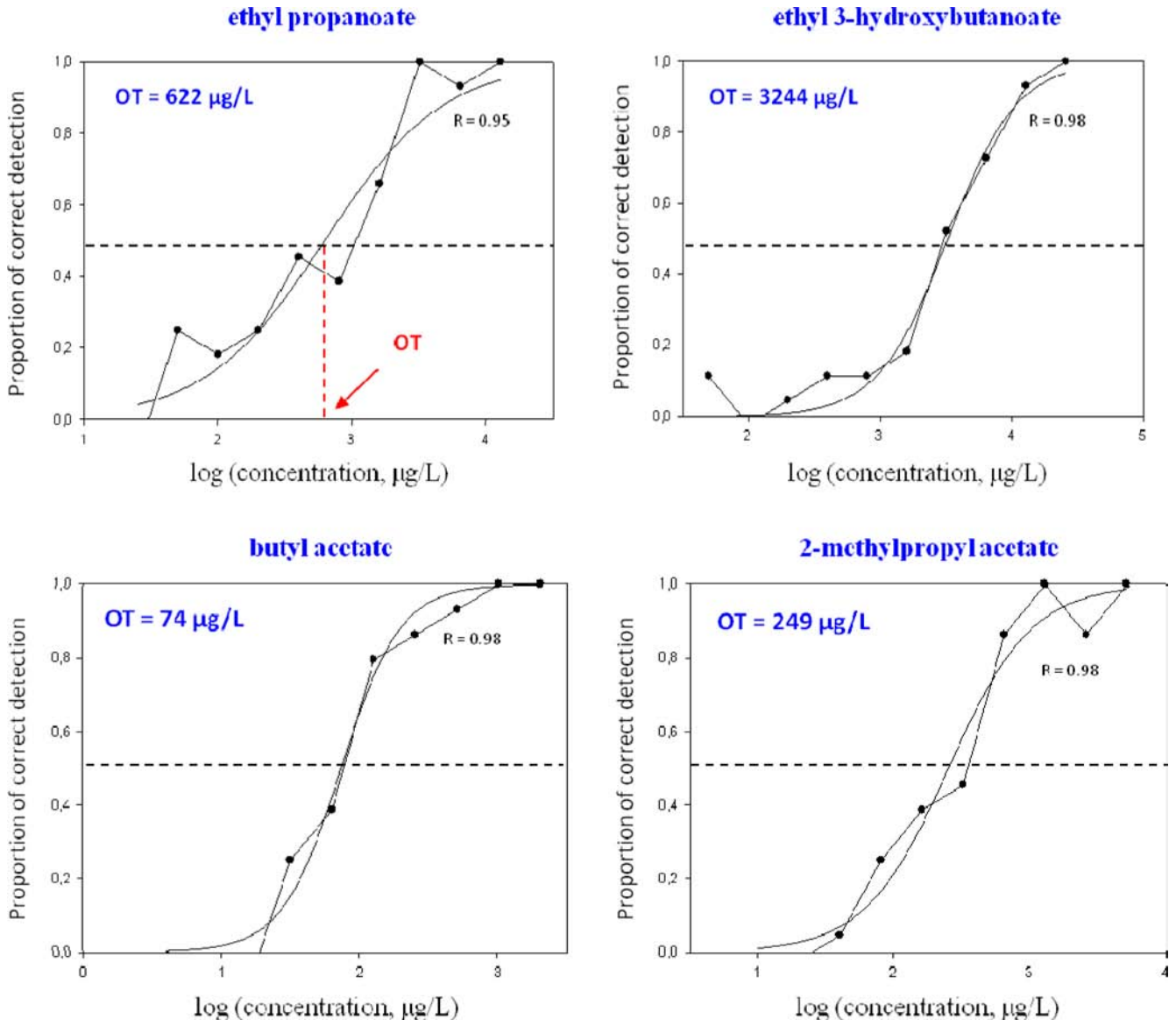

Figure 2. Detection probability of ethyl propanoate, ethyl 3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate in dilute alcohol solution. OT, olfactory threshold. The curves are drawn according to a sigmoid function.

obtain comparable results and avoid order effects. For each sample, the subject rated the intensity of these descriptors on a 100 mm scale printed on paper, labeled "no odor perceived" on the left and "very intense" on the right. The aromatic reconstitutions presented in different sessions are shown in Table 6.

Statistical data were analyzed using R analysis of variance (ANOVA) software: the homogeneity of variance was tested using Levene's Test, and the normality of residuals was tested using the Shapiro−Wilk test. All descriptors were mean-centered per panelist and scaled to unit variance. The statistically significant level was 5% ($p < 0.05$).

RESULTS AND DISCUSSION

Preliminary Verification. *Odorant Stimulus Purity.* GC-O analysis revealed parasite odors in some commercial products. These products were removed, and new ones were purchased. Finally, all compounds used were olfactorily pure, and any olfactory impurities were detected by the three judges who performed this analysis. Moreover, FID analysis confirmed the products' very high purity.

Evolution of Sample Composition during Sensory Analysis. Prior to the first sensory tests, the evolution kinetic of the compounds in dilute alcohol solution was evaluated in order to assess the stability of the composition of the samples submitted to the panel. This kinetic evaluation demonstrated that the esters remained stable for the first 10 min, except for ethyl hexanoate and ethyl octanoate, where concentrations declined by about 30 and 40%, respectively (Figure 1). After 10 min, the concentrations of certain compounds in the solution had decreased by up to 50%. Consequently, the solutions presented to the panel were prepared every 10 minutes. A study of the evolution kinetics of these esters in wine, under the same conditions, revealed that they remained more stable for up to 20 min. However, a decrease of up to 70% was observed for some compounds after 60 min (Figure 1).

These results tend to reinforce the idea that a wine's aromatic evolution under such conditions is due to physicochemical phenomena: the composition of the headspace changes over time as some compounds evaporate, according to their affinity for the matrix. Better stability in wine than in dilute alcohol solution may be explained by interactions involving, for example,

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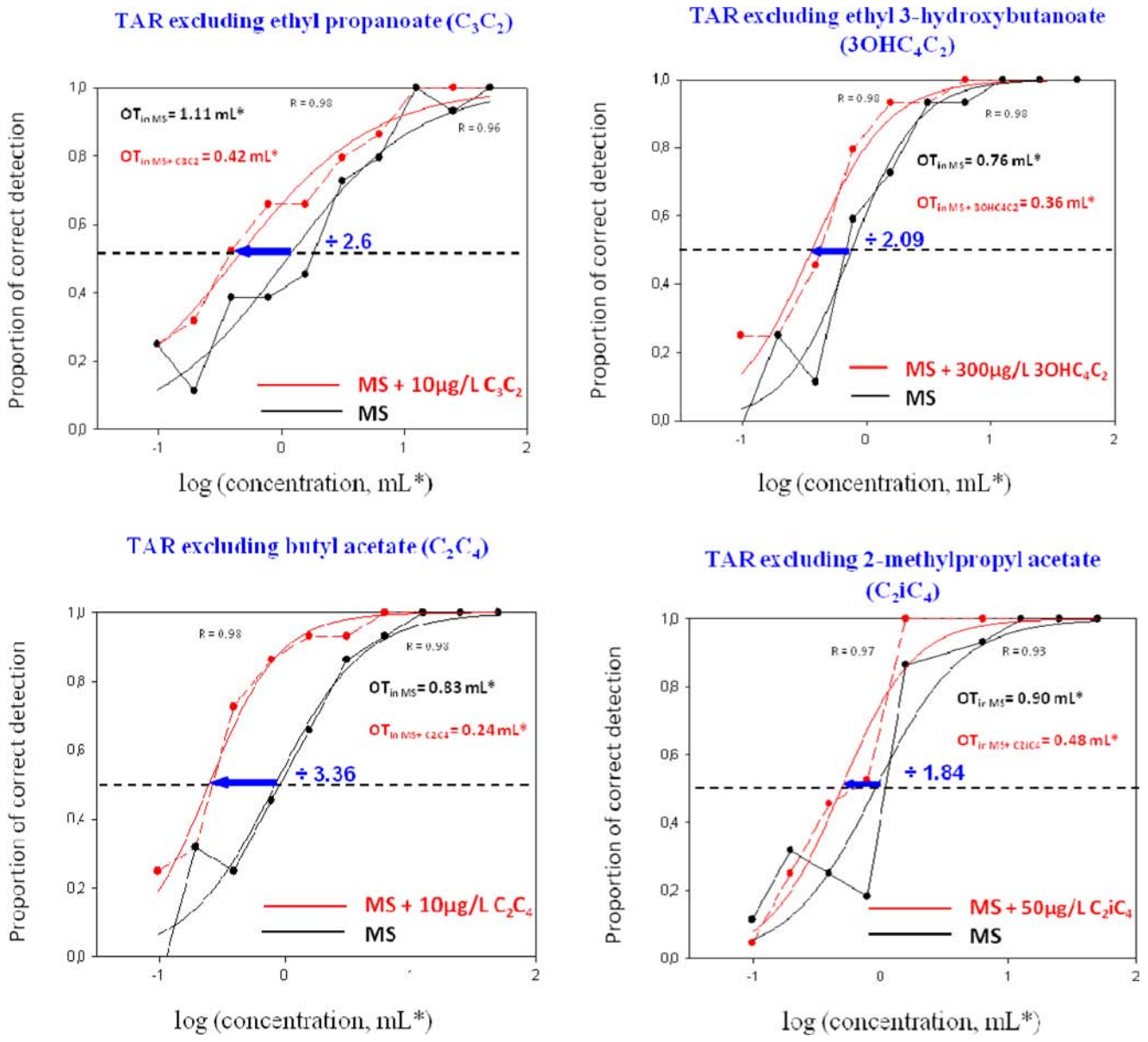

Figure 3. Effect of ethyl propanoate, ethyl-3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate addition on the detection probability of aromatic reconstitutions.*: expressed in mL of total aromatic reconstitution (TAR) diluted in 50 mL of matrix. MS, model wine solution (dilute alcohol solution). OT, olfactory threshold. The curves are drawn according to a sigmoid function.

van der Waals force or hydrogen bonds between aromatic compounds and nonvolatile constituents in the matrix.[38] The nonvolatile components of the matrix are apparently able to modulate the composition of the headspace, thus impacting the aromatic perception of the wine.[38,39]

Analysis of Sample Headspace Composition. In order to evaluate the matrix effect on headspace composition, concentrations of ethyl propanoate, ethyl 2-methylpropanoate, ethyl 2-hydroxy-4-methylpentanoate, butyl acetate, and 2-methylpropyl acetate were analyzed. For each compound, its headspace concentration was compared using dilute alcohol solution and total aromatic reconstitution (TAR).

A statistical nonparametric bilateral test (Wilcoxon test) did not reveal any significant difference between the chromatographic peak areas of the compound analyzed in the headspace of the two matrices. This finding indicated that there was no presensory interaction between these aromatic compounds in the mixture and that the effects observed were not explained by any differential effect due to the matrix.

Impact Hierarchy of the Compounds. *Perception of Individual Compounds.* As indicated in Table 1, all compounds were identified by the panel, except ethyl propanoate, ethyl 3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate (*tests 1*, *8*, *9*, and *11*), revealing that they were present in subthreshold concentrations. These observations are in agreement with the olfactory thresholds previously established for ethyl 3-hydroxybutanoate and 2-methylpropyl acetate in dilute alcohol solution: 1000 µg/L and 870 µg/L, respectively.[2] The olfactory thresholds of ethyl propanoate and butyl acetate in dilute alcohol solution are not available in the literature. Their thresholds reported in dearomatized wine, 2100 µg/L and 1800 µg/L, respectively,[2] are consistent with our observations. For all other compounds, the differences observed by the panel were significant, with a confidence interval of at least 1%. These

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results are in agreement with the bibliographic data on olfactory thresholds.[2]

The olfactory thresholds obtained for ethyl propanoate, ethyl 3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate clearly confirmed that these four compounds in hydroalcoholic solution had no direct olfactory impact (Figure 2). The concentrations of ethyl propanoate, ethyl 3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate tested were considerably lower than their olfactory thresholds under the same experimental conditions (matrix and panel): about 24%, 9%, 13%, and 20% respectively, of their olfactory thresholds.

Effect of the Omission of One or More Compounds. The omission tests results are presented in Table 3. The omission of entire families of compounds resulted in a statistically significant modification in the odor of the aromatic reconstitution. With the exception of ethyl-2-methylpropanoate (*test 22*), the omission of each compound from the TAR was significantly perceived, showing that these compounds contribute to the overall fruity aroma of the complex mixture. Omission of each of the four compounds present at subthreshold concentrations was conclusive (*tests 18*, *25*, *26*, and *28*). These results highlighted the existence of new perceptual interactions. The most notable effects were attributable to ethyl 3-hydroxybutanoate and butyl acetate, which impacted the aromatic reconstitutions at concentrations representing about 9% and 13% of their olfactory thresholds, respectively. Compounds at concentrations below their olfactory thresholds are generally considered to have little or no impact on overall sensory perception. However, some studies have demonstrated that certain compounds, even at levels well below their odor threshold, may play a role in the overall aroma. For example, the recent findings of Lytra et al.[14] highlighted the indirect impact on overall and fruity aroma expression of acetoin, acetic acid, and γ-butyrolactone at concentrations representing about 2%, 12%, and 40% of their perception thresholds, respectively.

Olfactory Properties of Compounds Present at Subthreshold Concentrations. *Quantitative Effect.* As shown in Figure 3, the presence of each compound, whose omission at subthreshold levels could be perceived, resulted in a decrease in the olfactory threshold of the TAR, reflecting an individual quantitative contribution of these four compounds to overall aroma intensity. According to the esters tested, the addition of ethyl propanoate, ethyl-3-hydroxybutanoate, butyl acetate, or 2-methylpropyl acetate led to a 2.6 (confidence interval (CI) <0.01), 2.09 (CI <0.01)-, 3.36 (CI <0.01)-, and 1.84 (CI >0.05)-fold decrease, respectively, in the olfactory threshold of the fruity pool constituted by the other 11 compounds. These results clearly confirmed that these compounds, present at subthreshold concentrations, play an important role as fruity aroma enhancers, via perceptive interactions. Similar phenomena have been reported in wine. Ribéreau-Gayon et al.[40] demonstrated additive effects involving compounds at levels below their olfactory thresholds. In order to verify whether the quantitative contribution of these compounds was due to a simple addition phenomenon or a hyper-addition effect, the impact of the presence of each one on the odor of the mixture was evaluated, and the data obtained were compared with theoretical values calculated according to Feller's additive model.

An example of a calculation of detection probability according to Feller's additive model for ethyl propanoate at 150 μg/L is presented in Table 7. The psychometric curve obtained using the olfactory threshold of each compound of interest was used to calculate the detection probability of each compound individually at the studied concentration: for respective concentrations of ethyl propanoate, ethyl-3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate of 150, 300, 10, and 50 μg/L, the probability of detection was 0.2, 0.019, 0.016, and 0.092, respectively, i.e., 20%, 1.9%, 1.6%, and 9.2% of the panelists, respectively, were able to detect the presence of these compounds.

The experimental detection probability of TAR excluding ethyl 3-hydroxybutanoate and TAR excluding 2-methylpropyl acetate was higher than the value calculated using Feller's additive model (Figure 4), revealing a hyper-addition effect after the addition of each of these compounds to the fruity pool (CI < 0.001). There was no significant difference (CI > 0.05) between the experimental detection probabilities of TAR excluding ethyl propanoate and TAR excluding butyl acetate and those calculated according to Feller's additive model (Figure 4), indicating a simple additive effect for each of these compounds in the fruity pool. Depending on the esters tested, the ratio between the experimental olfactory thresholds and those obtained using Feller's additive model was at a minimum of 1.34 for butyl acetate and up to 11.80 for 2-methylpropyl acetate (Figure 4).

Qualitative Effect. Significant results for the descriptors evaluated are summarized in Table 8. Besides ethyl-3-hydroxybutanoate, which had no qualitative impact, the omission of these subthreshold compounds from the fruity matrix had a significant attenuating effect on blackberry and fresh-fruit aroma intensity and enhanced red-berry fruit aroma intensity.

Taken together, these compounds with similar chemical structures have both quantitative and qualitative effects, modulating the fruity aromas of red wines. This research revealed their role as natural enhancers of blackberry and fresh-fruit aromas and revealed four new perceptual interactions. The behavior of these esters is similar to that of ethyl 2-hydroxy-4-methylpentanoate, recently described by Lytra et al.[41] Therefore, the mainly quantitative hyper-additive effect of ethyl-3-hydroxybutanoate was established. In contrast, ethyl propanoate, butyl acetate, and

Table 7. Detection Probabilities[a]

before mixture		after mixture	
p(C_3C_2) alone	p(TAR excluding C_3C_2) alone	p(TAR excluding C_3C_2 + C_3C_2) experimental	p(TAR) calculated according to Feller's additive model
calculated and fixed	= ((3* proportion of correct responses) − 1)/2		= p(TAR excluding C_3C_2) + p(C_3C_2) − p(TAR excluding C_3C_2) p(C_3C_2)
0.20	0.25	0.39	0.40
0.20	0.11	0.25	0.29
0.20	0.39	0.73	0.51
0.20	0.39	0.66	0.51
0.20	0.45	0.86	0.56
0.20	0.73	0.93	0.78
0.20	0.80	1.00	0.84
0.20	1.00	1.00	1.00
0.20	0.93	1.00	0.95
0.20	1.00	1.00	1.00

[a]Determination of the detection probability (p) of total aromatic reconstitution (TAR) excluding ethyl propanoate (C_3C_2) in dilute alcohol solution supplemented with C_3C_2 (150 μg/L) in the positive sample. Experimental data and values were calculated according to Feller's additive model.

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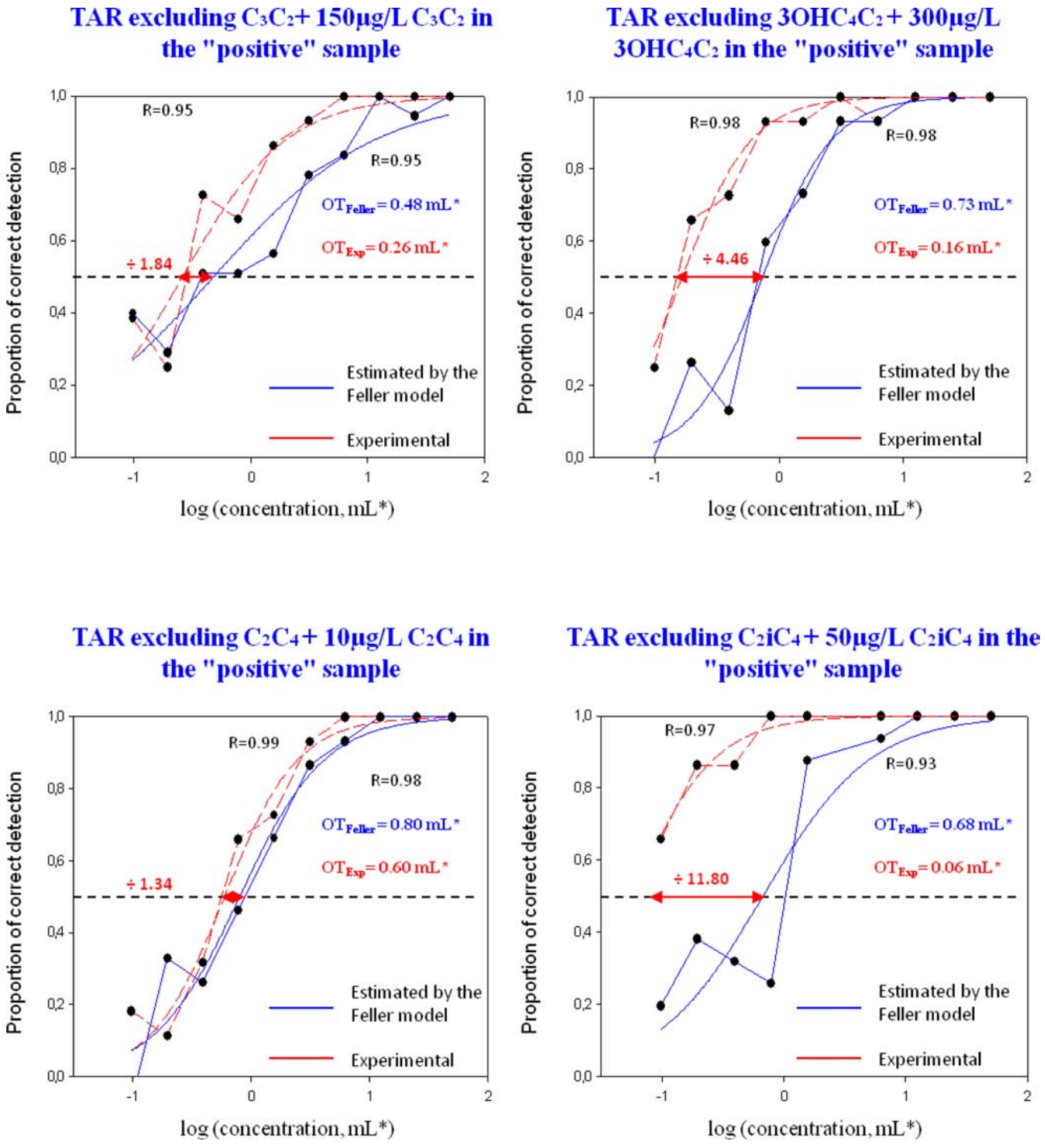

Figure 4. Detection probability of aromatic reconstitutions determined experimentally and calculated according to Feller's additive model. *: expressed in mL of total aromatic reconstitution (TAR) diluted in 50 mL of matrix. C_3C_2, ethyl propanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; C_2C_4, butyl acetate; C_2iC_4, 2-methylpropyl acetate; OT_{Feller}, olfactory threshold estimated using Feller's additive model; and OT_{Exp}, experimental olfactory threshold. The curves are drawn according to a sigmoid function.

2-methylpropyl acetate modified fruity aroma perception both quantitatively and qualitatively.

These findings confirmed the importance of these esters and acetates, produced by the yeast metabolism during alcoholic fermentation, resulting in the red- and blackberry fruit aromas,[42] which form the basis of the fruity aroma perceived in red wines.[15,41,43]

These results revealed the indirect impact of ethyl propanoate, ethyl-3-hydroxybutanoate, butyl acetate, and 2-methylpropyl acetate, present at subthreshold concentrations, on fruity aroma expression. As a whole, these findings highlight the importance of aromatic reconstitution as well as that of omission tests to investigate the aromatic behavior of complex matrices.

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Table 8. Aromatic Impact of the Omission of Certain Esters from the Total Aromatic Reconstitution[a]

		omissions			
		C_3C_2	$3OHC_4C_2$	C_2C_4	C_2iC_4
descriptors	OA	↑	-	↑	↑
	RF	↑	-	↑	↑
	BF	↓	-	↓	↓
	FF	↓	-	-	↓
	JF	↑	-	-	↑
concn (μg/L)		150	300	10	50

[a]↑, intensity increase; ↓, intensity decrease; -, no significant difference. C_3C_2, ethyl propanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; C_2C_4, butyl acetate; C_2iC_4, 2-methylpropyl acetate. OA, overall aroma; RF, red-berry fruit; BF, blackberry fruit; FF, fresh fruit; and JF, jammy fruit.

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Notes

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