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Impact of Perceptive Interactions on Red Wine Fruity Aroma

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ABSTRACT: A preparative HPLC method was applied to aromatic red wine extracts. Twenty-five fractions with various flavors were thus obtained, and several aromatic reconstitutions were produced by mixing some of these fractions. Discriminative tests revealed that the omission of some fractions from the mixture of fruity fractions or the addition of others affected the overall expression of fruity aroma. Sensory profile analyses identified significant differences among aromatic reconstitutions in terms of intensity of black-berry, as well as fresh-, and jammy-fruit descriptors. A fraction with a very low fruity note (fraction 17) had an additive effect on the fresh fruity aroma, while fractions with caramel and lactic notes (fractions 3−5) had a masking effect on this aroma and an additive effect on the jammy-fruit aroma. Further analysis revealed that ethyl 2-hydroxy-4-methylpentanoate was eluted in fraction 17, while diacetyl, acetoin, acetic acid, and γ-butyrolactone were eluted in fractions 3−5. Omissions tests established that ethyl 2-hydroxy-4-methylpentanoate was responsible for enhancing black-berry and fresh-fruit aroma and that a combination of diacetyl, acetoin, acetic acid, and γ-butyrolactone, at levels between 2 and 40% of their perception thresholds, had the same hypoadditive effect on the overall and fresh fruity aroma as fractions 3−5.

KEYWORDS: *red wine, fruity aroma, aromatic reconstitutions, perceptive interactions, aroma enhancer/suppressor*

INTRODUCTION

Wines consist of highly complex mixtures of volatiles derived from grapes, fermentation processes, and aging. To date, only a few of these compounds, from the alcohol, ester, acid, aldehyde, ketone, thiol, lactone, etc. families, present in different concentrations and proportions depending on the wines, have been demonstrated to make a direct contribution to wine aroma.[1−3] Is wine perception just a simple sum of these constituents? According to Francis et al.,[4] it is widely recognized that wine aroma is not the result of any single dominant compound, that confers to a specific aroma on a particular wine or wine type, but that, on the contrary, all wines owe their aromatic character to a multitude of volatiles. In some wine types, a few compounds may have a dominant influence, but even in these special cases several compounds are involved.

In mixtures, the diversity of sensory perceptions reported result from qualitative (odor quality) and quantitative (odor intensity) perceptual interactions between odorants,[5] defined in various ways by different authors.[6,7] However, perceptual interactions between multiple volatile components in combination remain difficult to predict in such a complex matrix as wine, where overall perception cannot be predicted from the sum of perceptions of individual compounds. According to Godinot,[8] while some compounds in an odorous mixture may predominate or impose their qualities on the mixture, others will tend to fade away, becoming unidentifiable. Even simple binary mixtures demonstrate that qualitative and quantitative odor perception is not straightforward. Indeed an increasing number of experiments provide strong evidence to refute Olsson's predictive model, which states that the perceived intensity and quality of a mixture can be predicted from the perceived intensity of its components presented separately.[5,9−12] Given the existence of these perceptive interactions, the standard instrumental approach of gas chromatography-olfactometry[13] alone is incapable of evaluating the organoleptic impact of a compound,[14,15] and further sensory studies in complex matrices such as wine are irreplaceable. Many models have attempted to describe the behavior of different molecules in mixtures, but no current model is capable of explaining how some aromatic compounds interact in mixtures.[6,11,16−18]

Rather than assessing the olfactive behavior of mixtures prepared from pure products, the main goal of this work was to highlight and study the impact of perceptive interactions on wine fruity aroma expression using various aromatic reconstitutions. Samples were prepared from wine using an HPLC method which preserves wine aroma and isolates fruity characteristics in specific fractions. The composition of these fractions of interest was then studied by instrumental methods. The final target was to investigate the impact of fraction components on fruity aroma by preparing aromatic reconstitutions and using sensory reconstitution tests, to assess the role of these compounds on the perceptive interactions previously observed.

MATERIALS AND METHODS

Chemicals. Dichloromethane (Pestipur quality, Carlo Erba, SDS, Italy) and absolute ethanol (analytical grade, 99.97%, Scharlau Chemie S.A, Barcelona, Spain) were 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 compounds were obtained from commercial sources as follows: diacetyl (2,3-butanedione), acetoin, acetic acid and γ-butyrolactone from Sigma−Aldrich, Saint-Quentin-Fallavier, France; *R*-ethyl 2-hydroxy-4-methylpentanoate and *S*- ethyl 2-hydroxy-4-methylpentanoate were synthesized by Hangzhou Imaginechem Co., Ltd., Hangzhou, China.

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Samples. Two red wines were used: a Vin de Pays d'Oc (1) (2010 vintage) and a Margaux appellation wine (2) (2000 vintage). Dilute alcohol solution was prepared using double-distilled ethanol and microfiltered water (12%, v/v).

Aromatic Reconstitution Made from HPLC Fractions (AR). Sample preparation was as optimized by Lytra et al.[19] A 500 mL wine sample was extracted successively using 80, 80, and 50 mL of dichloromethane, with a separatory funnel for 10 min. The organic phases were collected, blended, dried over sodium sulfate, and concentrated under nitrogen flow (100 mL/min) to obtain 1.25 mL of wine extract. Reversed-phase (RP) HPLC was performed on this raw extract using a Nova-Pak C18 column (300 × 3.9 mm i.d., 4 μm, 60 Å, Waters, Saint-Quentin, France), without a guard cartridge. The HPLC system consisted of an L-6200A pump (Merck-Hitachi, Germany). Chromatographic conditions were as optimized by Pineau et al.:[20] flow rate, 0.5 mL/min; injection volume, 250 μL wine extract; program gradient, phase A, water, phase B, ethanol; 0−2 min, 100% A, linearly programmed until 100% B at minute 50. The effluent was collected in 1 mL fractions. Twenty-five fractions with various aromas were obtained in dilute alcohol solution. For aromatic reconstitutions, fractions were retained and added individually or blended together to reproduce the initial concentrations in the original wines, adding double-distilled ethanol and microfiltered water to obtain an ethanol level of 12% (v/v).

HPLC Fractions Extraction. Each fraction was diluted in distilled water to obtain 12% ethanol, then re-extracted by the same method as the wine, but with 4, 2, and 2 mL of dichloromethane (5 min stirring for each extraction). The organic phases were collected, dried over sodium sulfate, and concentrated under nitrogen flow to obtain 100 μL extract.

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 odiferous impurities and to ascertain that the compound considered was responsible for the odor properties identified. Any olfactive impurity was detected by the three judges who performed this analysis. 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, Australie), 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. Less than 0.2 μL of each pure odorant was 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., film thickness: 0.25 μm. The oven was programmed at 40 °C for the first minute, and the temperature was 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.

GC−MS analysis of wine fractions. Two microliter samples of organic extract were injected in splitless-split mode (injector temperature: 250 °C, interface temperature: 280 °C, splitless time: 45 s, split flow: 50 mL/min) using an HP 6890N gas chromatograph (Hewlett−Packard, Wilmington, DE, USA) coupled to a mass spectrometer (HP 5973i). The column was a BP20 (SGE, Ringwood, Australia), 50 m × 0.22 mm i.d., film thickness: 0.25 μm. The oven was programmed at 40 °C for the first minute, then increased at a rate of 3 °C/min up to a final isotherm at 250 °C for 30 min. The carrier gas was Helium N55 (Air Liquide, France) with a column head pressure of 8 psi. The MSD was used in full-scan mode (m/z 40−300, 3.09 scans/sec). MS data were recorded and processed using Chemstation software equipped with NIST 2008 MS library (US National Institute of Standards and Technology, Gaithersburg, MD, USA). The compounds were then characterized by comparing their LRI and mass spectra with those of standards.

Ethyl 2-Hydroxy-4-methylpentanoate Quantitation. A 50 mL sample was spiked with 100 μg/L octan-3-ol as an internal standard. It was then extracted using 4, 2, 2 mL of dichloromethane, with magnetic stirring (700 rpm), for 5 min each and separated in a separatory funnel for 5 min. The organic phases were blended, dried over sodium sulfate, and concentrated under nitrogen flow (100 mL/min) to obtain 250 μL of wine extract. Ethyl 2-hydroxy-4-methylpentanoate was assayed using an HP 6890 gas chromatograph coupled to an HP 5972 mass spectrometer (Hewlett−Packard, Wilmington, DE, USA). Two microliter samples of organic extract were injected in splitless-split mode (injector temperature: 180 °C, interface temperature: 180 °C, splitless time: 0.75 min, split flow: 30 mL/min). The column was a CHIRALDEX Gamma-TA (Astec, Whippany, NJ, USA), 50 m × 0.25 mm i.d., film thickness: 0.12 μm. The oven was programmed at 40 °C for the first minute, raised to 100 °C at 1 °C/min, then programmed at a rate of 4 °C/min up to a final isotherm at 170 °C, maintained for 5 min. The carrier gas was Helium N55 (Air Liquide, France) with a column head pressure of 20 psi. The mass spectrometer was operated in electron impact mode at 70 eV with selected-ion-monitoring (SIM) mode (dwell = 50 ms), using 3 characteristic ions; m/z 69 as quantifier and m/z 87 and m/z 104 as qualifiers. *R*- and *S*-ethyl 2-hydroxy-4-methylpentanoate were injected separately to determine their LRI. The mass spectra of the compounds naturally present in wine were compared with those of the reference products.

Diacetyl Quantitation. Chromatographic conditions and sample preparation were as optimized by de Revel et al.[21] A 50 mL sample was spiked with 50 μL of hexane-2,3-dione (1.2 g/L in dilute alcohol solution 50% vol) as an internal standard and 5 mL an aqueous solution of 1,2-diaminobenzene at 6.5 g/L; pH was adjusted to 8 (NaOH). After 3 h at 60 °C the mixture was acidified with 2 M sulfuric acid to pH 2 and then extracted using 5 and 5 mL of dichloromethane, with magnetic stirring (700 rpm), for 5 min each and separated in a separatory funnel for 5 min. The organic phases were blended and dried over sodium sulfate. Diacetyl was assayed using an HP-6890N gas chromatograph coupled to an HP-5973i mass spectrometer (Hewlett−Packard, Wilmington, DE, USA). Two microliter samples of organic extract were injected in splitless-split mode (injector temperature: 250 °C, interface temperature: 280 °C, splitless time: 30s.), using a HP-5MS capillary column (30 m × 0.25 mm, film thickness: 0.25 μm, SGE, Ringwood, Australia). The oven was programmed at 60 °C for the first minute and then at a rate of 2 °C/min up to a final isotherm at 220 °C, maintained for 20 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 impact mode at 70 eV with selected-ion-monitoring (SIM) mode (dwell = 100 ms). Diacetyl was identified by determining the mass spectra and LRI of quinoxaline derivates.

Acetoin and γ-Butyrolactone Quantitation. Chromatographic conditions and sample preparation were as optimized by de Revel et al.[22] A 1 mL sample was spiked with 50 μL of 3-octanol (400 mg/L in dilute alcohol solution 40% vol) and 50 μL of 1,4-butanediol (1.4 g/L in dilute alcohol solution 40% vol) as internal standards and 2 mL of methanol. Acetoin and γ-butyrolactone were assayed by direct sample injection into an HP 5890 gas chromatograph coupled to a flame ionization detector. Samples (0.5 μL) were injected in splitless-split mode (injector temperature: 250 °C, interface temperature: 250 °C, splitless time: 20 s), using a CP-WAX 57 CB capillary column (50 m × 0.25 mm i.d., film thickness: 0.25 μm, Varian, Agilent Technologies, Palo Alto, CA). The oven was programmed at 80 °C for the first 5 min and then at a rate of 3 °C/min up to a final isotherm at 200 °C. The carrier gas was hydrogen 5,5 (Air Liquide, France) with a column head pressure of 15 psi.

Acetic Acid Quantitation. An enzymatic test kit (Boehringer Mannheim/R-Biopharm, Germany) based on the UV spectrometry (V-530 UV/vis spectrophotometer, JASCO, Japan) was used to quantitate acetic acid in various samples, according to the manufacturer's instructions.

Sensory Analyses. *General Conditions.* Sensory analyses were performed as described by Martin and de Revel.[23] Samples were evaluated at controlled room temperature (20 °C), in individual booths, using covered, black AFNOR (Association Française des Normes) glasses,[24] containing about 50 mL of liquid, coded with three-digit random numbers. Sessions lasted approximately 5 min.

Sensory Panels. Panel 1 consisted of 3 judges, 1 male and 2 females, aged 30.5 ± 7.2 (mean ± SD). Panel 2 consisted of 17 judges, 9 males and 8 females, aged 31.2 ± 6.2 (mean ± SD). Panel 3 consisted of 19 judges, 8 males and 11 females, aged 30,7 ± 5,1 (mean ± SD). Panel 4 consisted of 18 judges, 6 males and 12 females, aged 31.4 ± 8.5 (mean ± SD).

Table 1. Triangular Tests Using Various Aromatic Reconstitutions (AR)[a]

	Samples compared			Wines	Panel
Experiment 1	AR (1-25)	AR (17-22)	*	(1)	2
	AR (1-25)	AR (18-22)	*		
	AR (17-22)	AR (18-22)	-		
	AR (17-22)	AR (17-22 + 3-5)	*		
Experiment 2	AR (18-22)	AR (18-22) + 250 μg/L R- $2OH4MeC_5C_2$	-	(1)	3
	AR (17-22)	RA (18-22)	*	(2)	
	AR (18-22)	AR (18-22) + 550 μg/L R- and S- $2OH4MeC_5C_2$ (R/S:95/5, m/m)	*		
Experiment 3	AR (17-22)	AR (17-22) + 4 mg/L D + 3,2 mg/L A + 25 mg/L Ac + 8,5 mg/L GBL	*	(1)	4
	MS	MS + 4 mg/L D	*	-	
	MS	MS + 3,2 mg/L A	-		
	MS	MS + 25 mg/L Ac	-		
	MS	MS + 8,5 mg/L GBL	-		

[a]AR, aromatic reconstitutions using HPLC fractions were supplemented with: D, diacetyl; A, acetoin; Ac, acetic acid; GBL, γ-butyrolactone; D, A, Ac, and GBL at concentrations found in fractions 3−5 of wine (1); $2OH4MeC_5C_2$, ethyl 2-hydroxy-4-methylpentanoate at concentrations found in fraction 17 of the corresponding wine. MS, model wine solution (dilute alcohol solution). *, 0.1% significant level. −, No significant difference.

Table 2. Aromatic Reconstitutions (AR) Compared by Performing Sensory Profiles[a]

	Samples compared			Wines	Panel
Experiment 1	AR (17-22)	AR (18-22)		(1)	2
	AR (17-22)	AR (1-25)			
	AR (17-22)	AR (17-22 + 3-5)			
Experiment 2	AR (17-22)	AR (18-22)	AR (18-22) + 550 μg/L R- and S- $2OH4MeC_5C_2$ (R/S:95/5, m/m)	(2)	3

[a]AR, aromatic reconstitution made from HPLC fractions supplemented with: $2OH4MeC_5C_2$, ethyl 2-hydroxy-4-methylpentanoate at concentrations found in fraction 17 of the corresponding wine.

All panelists were research laboratory staff at ISVV, Bordeaux University, selected for their experience in assessing fruity aroma 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.

(a) Discriminative Testing Method. Triangular tests were performed for various aromatic reconstitution samples (Table 1). For each triangular test, three numbered samples were presented in random order. Two were identical and the third one was different. Each judge had to identify the sample perceived as different in each test and give an answer, even if s/he was not sure. The results of all the triangular tests were statistically interpreted, according to the tables given in the literature,[23] based on the binomial law corresponding to the distribution of answers in this type of test.

(b) Descriptive Testing Methods. Wine samples, aromatic reconstitutions, and HPLC fractions were evaluated by panel 1. Fractions were selected and preliminary tests were carried out prior to preparing the aromatic reconstitutions.

Experiments 1 and 2. Sensory profiles of aromatic reconstitutions for red-berry, black-berry, fresh-, and jammy-fruit aroma intensity were evaluated by panels 2 and 3 for experiments 1 and 2, respectively, using a 0−7 point structured scale, where 0 indicated that no odor was perceived and 7 indicated high intensity. These aromatic descriptors were shown to be the most specific of red wines from Bordeaux area.[25] Various aromatic reconstitutions made from HPLC fractions of wines (1) and (2) in dilute alcohol solution were presented in different sessions, as shown in Table 2.

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 Shapiro-Wilk Test. All descriptors are mean centered per panelist and scaled to unit variance. The statistically significant level is fixed at 5% ($p < 0.05$).

Experiment 3. Sensory profiles were evaluated by panel 4 in eight sessions, each held on separate days. Two different matrices (dilute alcohol solution and HPLC fruity fractions) were supplemented with diacetyl, acetoin, acetic acid, and γ-butyrolactone (individually or mixed) at the concentrations found in fractions 3−5 in wine (1) (Table 3). Each sample was presented twice during each session. First, the overall aroma intensity of the six samples was evaluated. Afterward, the subjects had to evaluate the intensity of fresh- and jammy-fruit aroma in the same six samples. The samples were presented in identical order in both evaluations, to obtain comparable results and avoid order effects. For each sample, the subject rated the intensity of these descriptors on a 100

Table 3. Compounds and Fractions Added to Two Different Matrices (Dilute Alcohol Solution and HPLC Fruity Fractions) for Sensory Profile Evaluation[a]

	Samples					
Experiment 3	MS	AR (17-22)	MS + D + A + Ac + GBL	MS + F 3 to 5	AR (17-22) + D + A + Ac + GBL	AR (17-22) + F 3 to 5
	MS	AR (17-22)	MS + D + Ac + GBL	MS + A + Ac + GBL	AR (17-22) + D + Ac + GBL	AR (17-22) + A + Ac + GBL
	MS	AR (17-22)	MS + D + A + Ac	MS + D + A + GBL	AR (17-22) + D + A + Ac	AR (17-22) + D + A + GBL
	MS	AR (17-22)	MS + A + GBL	MS + D + GBL	AR (17-22) + A + GBL	AR (17-22) + D + GBL
	MS	AR (17-22)	MS + GBL + Ac	MS + A + Ac	AR (17-22) + GBL + Ac	AR (17-22) + A + Ac
	MS	AR (17-22)	MS + D + A	MS + D + Ac	AR (17-22) + D + A	AR (17-22) + D + Ac
	MS	AR (17-22)	MS + Ac	MS + GBL	AR (17-22) + Ac	AR (17-22) + GBL
	MS	AR (17-22)	MS + D	MS + A	AR (17-22) + D	AR (17-22) + A

Panel 4

[a]MS, model wine solution (dilute alcohol solution); F, fractions; AR (17−22), aromatic reconstitution (AR) made from HPLC fruity fractions (17−22); supplemented with D, diacetyl; A, acetoin; Ac, acetic acid; GBL, γ-butyrolactone. D, A, Ac and/or GBL, at concentrations found in fractions 3−5.

Table 4. Olfactive Description of HPLC Fractions and the Corresponding Wines

		Wine (1)	Wine (2)
		Fresh-fruit aroma (strawberry, cherry, blackberry). Milky aroma, banana.	*Red-berry fruit aroma (cherry, strawberry). Black-berry fruit aroma (blackcurrant). Slight woody notes.*
FRACTIONS	3	strawberry, milky	caramel
	4	strawberry, milky	light caramel
	5	strawberry, milky	light caramel, cheese, butyric
	17	light fresh fruits	light fresh fruits
	18	fruity aroma (red-berry fruit, fresh fruits: cherry - citrus fruits)	fruity aroma (fresh fruits)
	19	fruity aroma (fresh fruits: citrus fruits)	fruity aroma (red-berry fruit)
	20	fruity aroma (black-berry fruit: blackberry) + intense banana	very intense fruit (black-berry fruit: blackberry, red-berry fruit: cherry)
	21	fruity aroma (black-berry fruit: blackcurrant) + intense banana	fruity aroma (fresh blackcurrant)
	22	fruity aroma (fresh blackcurrant)	fruity aroma (blackcurrant liqueur) + spicy

Panel 1

mm scale printed on paper, labeled "no odor perceived" on the left and "very intense" on the right.

Experimental data were reported on a graph based on two parameters [$\sigma = f(\tau)$] introduced by Patte and Laffort.[17] Tau (τ) reflects the ratio of perceived intensity of the aromatic reconstitution made from HPLC fruity fractions alone, to the sum of perceived intensities of mixture's components, prior to mixing: $\tau = I_{AR\ (17-22)}/(I_{AR\ (17-22)} + I_C)$, where $I_{AR\ (17-22)}$ and I_C are the perceived odor intensities of an aromatic reconstitution containing HPLC fruity fractions and a test compound (diacetyl, acetoin, acetic acid, and γ-butyrolactone, individually or mixed) prior to mixing. Sigma (σ) reflects the ratio of perceived intensity of the mixture, to the sum of perceived intensities of its components, prior to mixing: the degree of overall intensity addition in the mixture: $\sigma = I_{mix}/(I_{AR\ (17-22)} + I_C)$, where I_{mix} is the perceived odor intensity of the mixture. Tau and sigma were calculated for the intensity of overall, fresh-, and jammy-fruit aroma. The mean experimental results for the panel were presented using the synthetic representation $\sigma = f(\tau)$. The graph was divided into several parts, according to the interaction level (Figure 2a). The position of experimental points reflects the interaction level.[16] Cain and Drexler[7] referred to mixture interactions in terms of the overall perceived intensity of a mixture compared to the intensities of each separate component. They indicated that the perceived strength of a mixture may be (a) as strong as the sum of the perceived intensities of the unmixed components, exemplifying complete addition ($\sigma = 1$); (b) more intense than the sum of its components, exemplifying hyper-addition ($\sigma > 1$); or (c) less intense than the sum of its components, exemplifying hypoaddition ($\sigma < 1$). Moreover, Frijters[16] distinguished three cases of hypoaddition: the terms 'partial addition', 'compromise', and 'subtraction' are used if the quality intensity of the mixture is greater, intermediate, or smaller than that of the single intensities.

For each sample, the significance of the observed perceptual interaction was statistically tested by calculating the 95% confidence interval on the mean intensity of the 18 subjects for both σ and τ.

RESULTS AND DISCUSSION

Olfactive Description of Wines and Fractions Obtained by HPLC. Applying reversed-phase HPLC to a wine extract resulted in 25 fractions in dilute alcohol medium, and the aromatic characteristics of each fraction were assessed by direct olfaction. Analyses performed by panel 1 on red wines confirmed that fruity characteristics were conserved from wines to fractions (examples shown in Table 4). It was also previously established, by Pineau et al.,[20] that fruity characteristics were well-conserved from wines to fractions for both red and white wines.

Fraction Selection. First results suggested the importance of fractions 17−22, which had intense fruity notes, as already observed by Lytra et al.[19] and Pineau et al.[20] During the preliminary test phase, panel 1 reported that the addition of

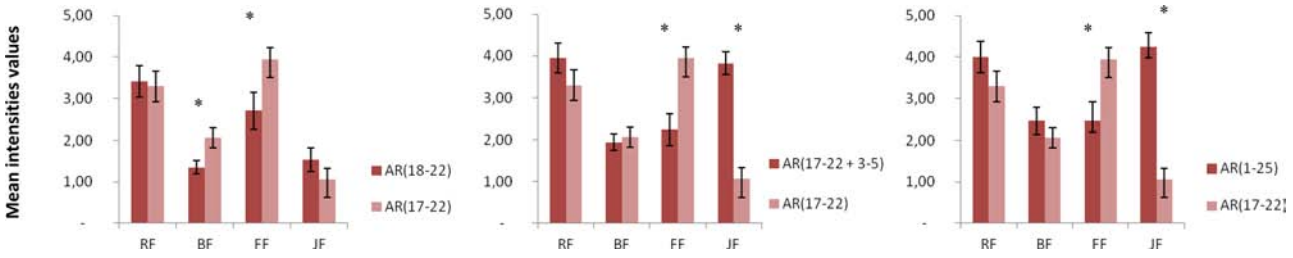

Figure 1. Mean intensities of aromatic descriptors of AR prepared from HPLC fractions of wine (1) in dilute alcohol solution (Experiment 1). *$p < 0.05$; AR, aromatic reconstitution; RF, red-berry fruit; BF, black-berry fruit; FF, fresh fruit; JF, jammy fruit. Error bars indicate standard error deviation.

fractions 3−5 to the fruity mixture (17−22) and the suppression of fraction 17 from the fruity mixture (17−22) had an aromatic impact.

Impact of Some Fractions on Overall Aroma. During tests performed using wine (1) (Table 1), over 80% of the judges distinguished between the total AR (1−25) and less exhaustive aromatic reconstitutions (AR (18−22) and AR (17−22)). This is understandable due to the considerable difference in composition between total AR and simpler mixtures. For wine (1), it was also observed that the absence of fruity fraction 17 from the total fruity AR (17−22) had no impact on overall fruity aroma expression (Table 1). Nevertheless, this was not the case for wine (2), as over 80% of the judges detected the omission of fraction 17 (Table 1). This phenomenon may be explained by a difference in the composition of the fraction 17 obtained from these two wines. Finally, the addition of fractions 3−5 to the AR (17−22) of wine (1) was significantly perceived, indicating that volatiles in these fractions may play an important role (Table 1).

Sensory Profiles of Wine (1) - Experiment 1. As shown in Figure 1, significant differences were found among the intensities of black-berry, fresh-, and jammy-fruit descriptors in AR prepared from HPLC fractions of wine (1). In total AR (1−25), jammy-fruit intensities were significantly higher and fresh-fruit were significantly lower than in fruity AR (17−22). This result suggests that fractions without any fruity character may contribute to overall jammy- and fresh-fruit aroma, as attested by the total AR intensities of these descriptors in model wine solution. The same results were observed when AR (17−22) was spiked with fractions 3−5, confirming that fractions without any clear fruity character may contribute to overall jammy- and fresh-fruit aroma. The mean intensities of descriptors for AR (17−22) spiked with fractions 3−5 and total AR led to statistically identical average scores for fresh- and jammy-fruit intensity, highlighting the importance of fractions 3−5, which seem to have the same aromatic impact as than the rest of the nonfruity fractions taken together on the expression of fresh- and jammy-fruit notes. This modification of fresh-fruit aroma by fractions with caramel and lactic aroma (3−5) suggests that components in these fractions may "mask" the fresh-fruit notes.

Significant differences were found between AR (18−22) and AR (17−22): when fraction 17 was present, the average intensities of black-berry and fresh-fruit aroma were significantly higher than in AR (18−22). These results indicated that fraction 17, which had little fruity character, contributed to overall black-berry and fresh-fruit aroma, suggesting that its constituents may act as an enhancer of these notes.

These finding indicated the importance of fractions 17−22, recently revealed to consist of esters and acetates with fruity notes,[19,20] which, apparently form the basis of the fruity aroma perceived in red wines,[19,20,24] produced by the yeast metabolism during alcoholic fermentation, a phase when red- and black-berry fruit aromas are formed.[27]

Our data also showed that fractions without any clear fruity character may contribute to overall fruity aroma. There is evidence in the literature that other compounds besides esters and acetates, which do not necessarily present fruity aroma, may have an important synergistic or masking effect on the overall fruity aroma of wine. Furaneol and homofuraneol, smelling strongly of caramel, seem to have an enhancing impact on the perception of red-berry fruit aroma.[28] In addition, some thiols (3-sulfanylhexan-1-ol)[29] and other volatile sulfur compounds (dimethyl sulfide) may also affect the perception of fruity aroma in red wines.[30,31] Finally, some C13-norisoprenoids, such as β-damascenone, are generally considered to affect red wine fruity aroma.[32]

Identification of Compounds in HPLC Fractions. Fraction 17 presented a light, fresh-fruit aroma and was relatively simple, from an analytical point of view. Ethyl 2-hydroxy-4-methylpentanoate, recently identified in red wines by Falcao et al.[26] using sensory gas chromatography−olfactometry (GC−O) and two-dimensional gas chromatography analysis (GC−GC−MS), was almost the only compound eluted from this fraction. An evaluation of its organoleptic impact by Lytra et al.[19] revealed that this ester contributed to a synergistic effect, enhancing the perception of fruity character. Fractions 3, 4, and 5, with their caramel and lactic aroma were found to be highly complex, from an analytical standpoint. The main compounds isolated were diacetyl, acetoin, acetic acid, and γ-butyrolactone, products of yeast, as well as lactic and acetic acid bacteria metabolisms.[33−37] The presence of diacetyl and acetoin, byproducts of malolactic fermentation, with a characteristic lactic/buttery aroma,[34] as well as γ-butyrolactone, the main lactone in wine, which has a slightly creamy, caustic odor, may explain the milky/caramel descriptors given for fractions 3−5 (Table 4). Even if the origin and organoleptic properties of these individual compounds have been described in some detail, there was no previous data concerning their impact on fruity aroma expression.

Explanation of Sensory Differences between Aromatic Reconstitutions. Identification and quantitation of ethyl 2-hydroxy-4-methylpentanoate in fraction 17 from wines (1) and (2) revealed a difference in its composition likely to result in different perceptual appreciations (Table 1). Contrary to wine (1), where only the R enantiomer of ethyl 2-hydroxy-4-methylpentanoate was present at a concentration of 250 μg/L, in wine (2), where over 80% of the judges recognized the addition of fraction 17, the concentration of ethyl 2-hydroxy-4-methylpentanoate was almost twice that in wine (1), and the *S*-enantiomeric form was also present (*R*/*S*: 95/5, m/m) (Table 1). Further triangular tests performed by panel 3 (Table 1) confirmed that the addition of 250 μg/L of *R*-ethyl 2-hydroxy-4-methylpentanoate to AR (18−22) was not perceived, whereas

the addition of 550 μg/L of *R*- and *S*-ethyl 2-hydroxy-4-methylpentanoate (*R*/*S*: 95/5, m/m) to AR (17−22) was significantly perceived. These results are in agreement with data previously obtained by Lytra et al.,[19] demonstrating that both enantiomeric forms played a sensory role in wine.

Sensory Profiles of Wine (2) - Experiment 2. As shown in Table 5, the addition of fraction 17 or 550 μg/L *R*- and *S*-ethyl 2-hydroxy-4-methylpentanoate (*R*/*S*: 95/5, m/m) (as observed in fraction 17) to AR of HPLC fruity fractions 18−22 of wine (2) resulted in identical average scores for red-berry intensity and lower average scores for jammy-fruit intensity. Significant differences were found for black-berry and fresh-fruit aroma descriptors between AR (18−22) and both AR (18−22) when supplemented with 550 μg/L *R*- and *S*-ethyl 2-hydroxy-4-methylpentanoate (*R*/*S*: 95/5, m/m) or with fraction 17. Average intensities for black-berry and fresh-fruit aroma were significantly higher than in AR (18−22), revealing that ethyl 2-hydroxy-4-methylpentanoate alone played the same aromatic role as fraction 17 in the expression of black-berry and fresh-fruit notes. These results are in agreement with previous observations by Lytra et al.,[19] demonstrating that ethyl 2-hydroxy-4-methylpentanoate was the only ester eluted from fraction 17 (the others were eluted from fractions 18−22) and that this compound was an active contributor to the black-berry and fresh-fruit notes.

Table 5. Mean Intensities of Aromatic Descriptors of AR Made from HPLC Fractions of Wine (2) in Dilute Alcohol Solution (Experiment 2)

	samples[a]		
descriptors	AR (18−22)	AR (17−22)	AR (18−22) + *R*- and *S*- 550 μg/L ethyl 2-hydroxy-4-methylpentanoate (*R*/*S*:95/5, m/m)
red-berry fruit	3.59 a	3.00 a	3.06 a
black-berry fruit	1.76 a	2.76 b	2.94 b
fresh-fruit	2.64 a	3.64 b	3.82 b
jammy-fruit	3.23 b	2.35 a	2.41 a

[a] $p < 0.05$; values with different letters within each row are significantly different; AR, aromatic reconstitution; supplemented with ethyl 2-hydroxy-4-methylpentanoate at concentrations found in fraction 17 of wine (2).

Experiment 3. Our experimental design, first introduced by Patte and Laffort,[17] was also used by Berglund and Olsson[9] to study odor intensity interactions in binary and ternary mixtures, and it was also recently applied by Atanasova et al.[12] Our experimental data covered not only overall aroma intensity interactions but also odor quality interactions evaluating the intensity of two descriptors (fresh- and jammy-fruits). Changes in overall aroma intensity when one, two, three, or all four compounds (D + A + Ac + GBL), or fractions 3−5, were added to AR (17−22) as well as changes in odor quality (fresh- and jammy-fruits) will be discussed in this article.

Impact of Diacetyl, Acetoin, Acetic Acid, and γ-Butyrolactone, Individually or Mixed, on Overall Aroma. Before sensory analysis evaluation, the triangular test conducted by panel 4 (Table 1) revealed that the simultaneous addition of diacetyl (D), acetoin (A), acetic acid (Ac), and γ-butyrolactone (GBL) (at concentrations found in fractions 3−5 of wine (1)) to AR (17−22) was significantly perceived. Further triangular tests confirmed that the individual addition of A, Ac, and GBL (at concentrations found in fractions 3−5 of wine (1)) to dilute alcohol solution was not perceived. As the concentrations tested were 3.2 mg/L for A, 25 mg/L for Ac and 8.5 mg/L for GBL, these observations were in agreement with high olfactory thresholds reported in the literature: with values of approximately 150 mg/L,[33] 200 mg/L,[38] and 20 mg/L,[39] respectively, in dilute alcohol solution. Finally, triangular tests also revealed that the addition of D (at the concentrations found in fractions 3−5 of wine (1)) to dilute alcohol solution was significantly perceived. Taking into consideration that D was tested at a concentration of 4 mg/L, this observation supported the literature, where the olfactory threshold for D, reported to be dependent on the matrix type, has been determined at between 50 μg/L in model wine solution[40] and 2.8 mg/L in Cabernet Sauvignon wine,[41] considerably lower than the concentrations tested here.

Perception of Individual Compounds. The addition of A, Ac and GBL (at concentrations found in fractions 3−5 of wine (1)) to dilute alcohol solution did not affect the intensity of any descriptors, confirming triangular tests results. A, Ac and GBL may, therefore, be present at subthreshold concentrations. On the contrary, the presence of D caused a significant increase in overall intensity, confirming triangular tests results, as well as jammy-fruit character, demonstrating that our panel associated its buttery/lactic character with jammy fruit.

Impact of Omissions on Overall Aroma Intensity. As shown in Figure 2a, analyses of overall aroma intensity revealed a hypoaddition for both mixtures: AR (17−22) + D + A + Ac + GBL and AR (17−22) + 3 to 5. A decrease in overall aroma intensity was observed when D + A + Ac + GBL or fractions 3−5 were added to AR (17−22), indicating that these mixture had the same effect.

The *omission of one* of the four compounds from the total mixture (AR (17−22) + D + A + Ac + GBL) resulted in the same hypoaddition effect on overall aroma intensity (Figure 2b). A compromise level (σ value < 1) was observed for all mixtures, except RA (17−22) + D + Ac + GBL, where the hypoaddition was not significant as compared to partial-addition. Interestingly, the simultaneous presence of A, Ac and GBL in AR (17−22), at subthreshold concentrations generally considered to have no impact on overall sensory perception, resulted in a marked attenuation of overall aroma intensity, showing that these compounds have a considerable impact on the expression of overall aroma.

As shown in Figure 2c, the *omission of two* of the four compounds from the total mixture, a hypoaddition effect on overall aroma intensity was observed only in one mixture: AR (17−22) + A + Ac. For the rest of the mixtures, the levels of interaction could not be explicated because the large confidence intervals resulted in nonsignificant interaction levels. However, the importance of A and Ac, as adding them in pair to AR (17−22), markedly attenuated the overall aroma intensity.

Hypoaddition effects were also observed for all mixtures following the *omission of three* out of the four compounds from the total mixture (Figure 2d). Adding D, A, Ac, or GBL had a significant attenuating effect on overall aroma intensity. Even the individual presence of A, Ac, or GBL in AR (17−22) resulted in a hypoaddition effect on overall aroma intensity, thus confirming the perceptual role of these compounds on aroma expression, and their marked impact, despite the subthreshold concentrations present. It was observed that the mixture containing D

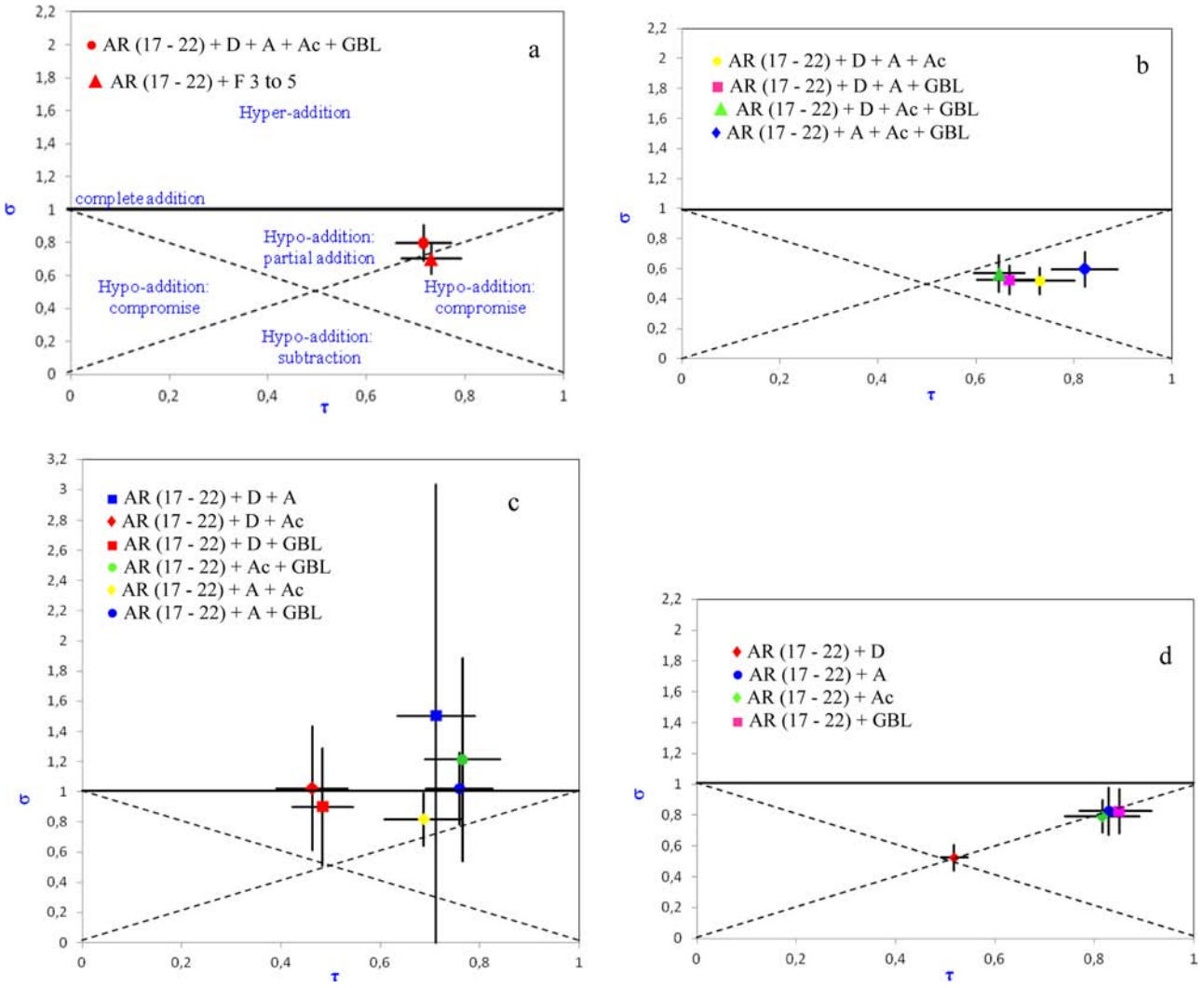

Figure 2. Impact of diacetyl (D), acetoin (A), acetic acid (Ac), and γ-butyrolactone (GBL) addition, individually or mixed, on overall aroma intensity (Experiment 3). Symbols represent σ and τ mean values; error bars indicate the 95% confidence interval on the mean for both τ and σ; F, fractions; AR (17−22), aromatic reconstitution (AR) made from HPLC fruity fractions (17−22).

had lower σ and τ values, highlighting the importance of D in overall aroma intensity.

Our findings revealed that most of the mixtures produced the same result, demonstrating the impact of each of these compounds on overall aroma intensity. D had the greatest direct impact, but A, Ac and GBL also contributed indirectly to the decrease of overall aroma intensity. Their impact was demonstrated conclusively, even at subthreshold concentrations.

Impact of Omissions on Fresh-Fruit Aroma. As shown in Figure 3a, analyses of fresh-fruit aroma intensity revealed a compromise level of hypoaddition for both mixtures: AR (17−22) + D + A + Ac + GBL and AR (17−22) + 3 to 5. A decrease in fresh-fruit intensity was observed when D + A + Ac + GBL or fractions 3−5 were added to AR (17−22), indicating that these mixture had the same effect.

The *omission of one* of the four compounds from the total mixture (AR (17−22) + D + A + Ac + GBL) resulted in the same hypoaddition effect on fresh-fruit aroma intensity (Figure 3b). A compromise level (σ value < 1) was observed for all mixtures. Interestingly, the simultaneous presence of A, Ac and GBL in AR (17−22), at subthreshold concentrations generally considered to have no impact on overall sensory perception, resulted in a marked attenuation of fresh-fruit notes, showing that these compounds have a considerable impact on the expression of fresh-fruit aroma.

As shown in Figure 3c, the *omission of two* of the four compounds from the total mixture produced the same hypoaddition effect on fresh-fruit aroma intensity except AR (17−22) + A + GBL, where the hypoaddition was not significant as compared to hyperaddition and complete addition. It was observed that mixtures containing D had lower sigma (σ) values (Figure 2c), highlighting the importance of D in attenuating fresh-fruit aroma intensity, and also the impact of A, Ac and GBL, as adding them in pairs to AR (17−22) markedly attenuated the fresh-fruit aroma.

Hypoaddition effects were also observed for all mixtures following the *omission of three* out of the four compounds from the total mixture (Figure 3d). However the hypoaddition observed for AR (17−22) + GBL, was not significant as compared to hyperaddition and complete addition). Adding D, A, or Ac, had a significant attenuating effect on fresh-fruit aroma intensity. Even the individual presence of A or Ac in AR (17−22) resulted in a hypoaddition effect on fresh-fruit intensity, thus confirming the perceptual role of these compounds on fresh-fruit

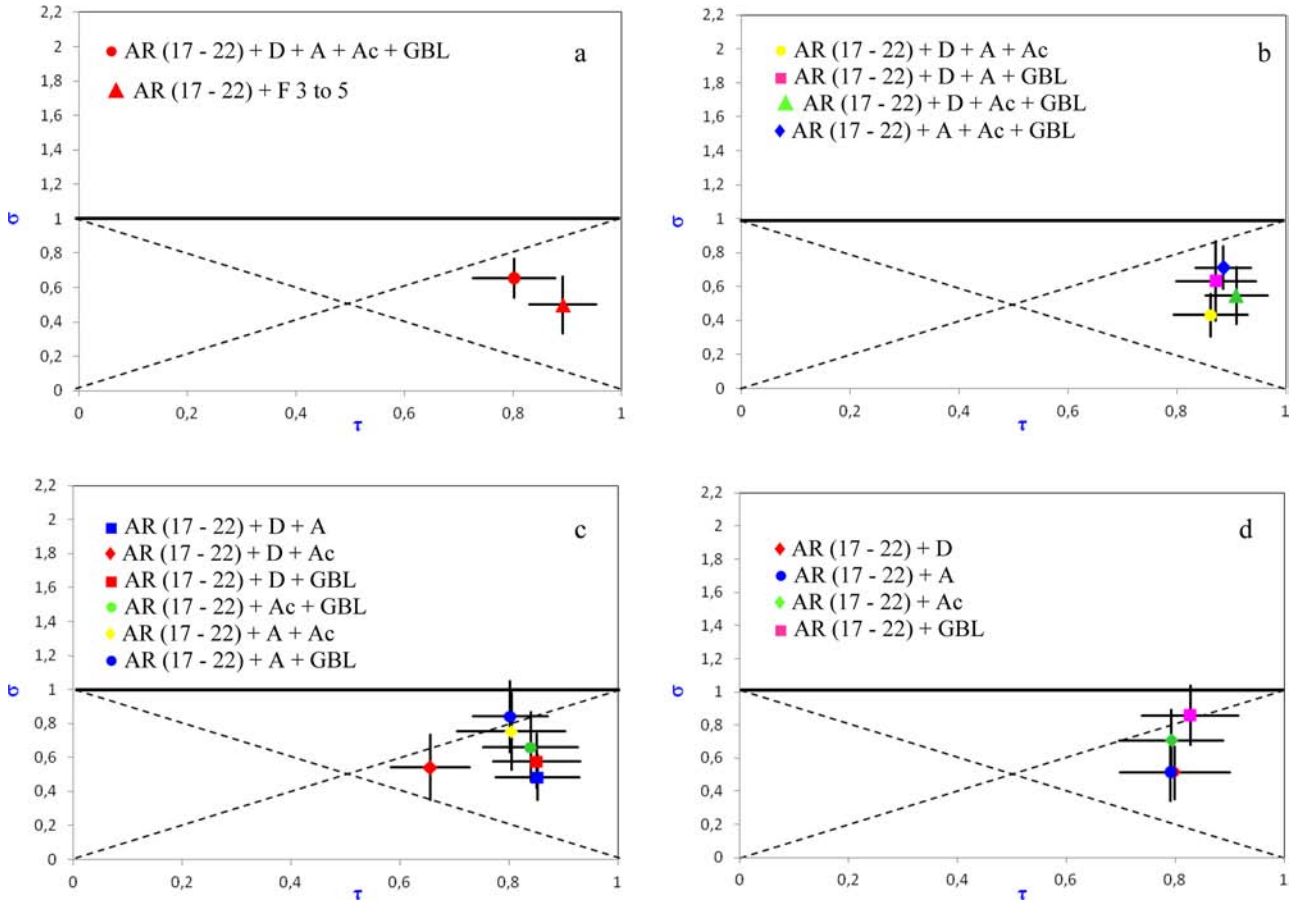

Figure 3. Impact of diacetyl (D), acetoin (A), acetic acid (Ac), and γ-butyrolactone (GBL) addition, individually or mixed, on fresh-fruit aroma (Experiment 3). Symbols represent σ and τ mean values; error bars indicate the 95% confidence interval on the mean for both τ and σ; F, fractions; AR (17−22), aromatic reconstitution (AR) made from HPLC fruity fractions (17−22).

aroma expression, and their marked impact, despite the subthreshold concentrations present.

Our findings revealed that all 16 mixtures produced the same result, demonstrating the impact of each of these compounds on fresh-fruit aroma intensity. D had the greatest direct impact, but A, Ac, and GBL also contributed indirectly to the decrease in fresh-fruit aroma intensity. The impact of A, Ac, and GBL was demonstrated conclusively, even at subthreshold concentrations. These findings highlighted the existence of new remarkable perceptual interactions impacting fresh-fruit aroma perception and also confirmed the results of experiment 1, indicating that constituents of the fractions with caramel and lactic aroma (3−5) had a "masking" effect on the fresh-fruit notes.

A decrease in intensity is the most frequent effect of odor mixtures. According to Laing et al.,[5] suppression or inhibition is by far the most common perceptual effect resulting from component interactions, which varies in magnitude according to their intensity. Berglund and Olsson[9] also observed a compromise level of hypoaddition for 41% of the 51 binary mixtures they studied, whereas the other samples showed subtraction levels of hypoaddition.

Impact of Omissions on Jammy-Fruit Aroma. As shown in Figure 4a−d, concerning jammy-fruit aroma intensity, a hyperaddition effect was observed in three mixtures: AR (17−22) + A + Ac + GBL, AR (17−22) + D + Ac + GBL, and AR (17−22) + Ac + GBL. For the other mixtures, the hyperaddition was not significant as compared to hypoaddition and complete addition. The large confidence intervals resulted in non-significant interaction levels, highlighting the difficulty of evaluating the role of these compounds in jammy-fruity aroma expression.

The hyperaddition effect observed for AR (17−22) + A + Ac + GBL demonstrated the impact of A, Ac, and GBL, simultaneously, on the perception of jammy-fruit aroma (Figure 3d). The presence of A, Ac, and GBL at subthreshold concentration was conclusive in this case, highlighting their perceptual role and sensory impact. These three cases of hyperaddition are in agreement with data from experiment 1, where constituents of fractions with caramel and lactic aroma (3−5) were found to enhance jammy-fruit notes.

This finding is also in agreement with bibliographic data, reporting hyperaddition in mixtures with components at low intensity levels, A, Ac, and GBL in this case. A tendency to hyperaddition was only observed by Atanasova et al.[12] when compounds individually perceived at the lowest intensities were tested in binary mixtures. Cases of hyperaddition were previously reported by Laing et al.[5] in only 3 out of 150 odor mixtures studied, when the perceived intensity and quality of binary mixtures consisting of benzaldehyde, eugenol, propionic acid, and carvon were determinate over a wide range of concentrations.

The marked lack of consensus concerning jammy-fruit aroma made it impossible to draw any conclusions concerning the impact of all compounds studied for jammy-fruit aroma intensity, as intersubject disagreement offset the significant interaction levels of the mixtures studied.

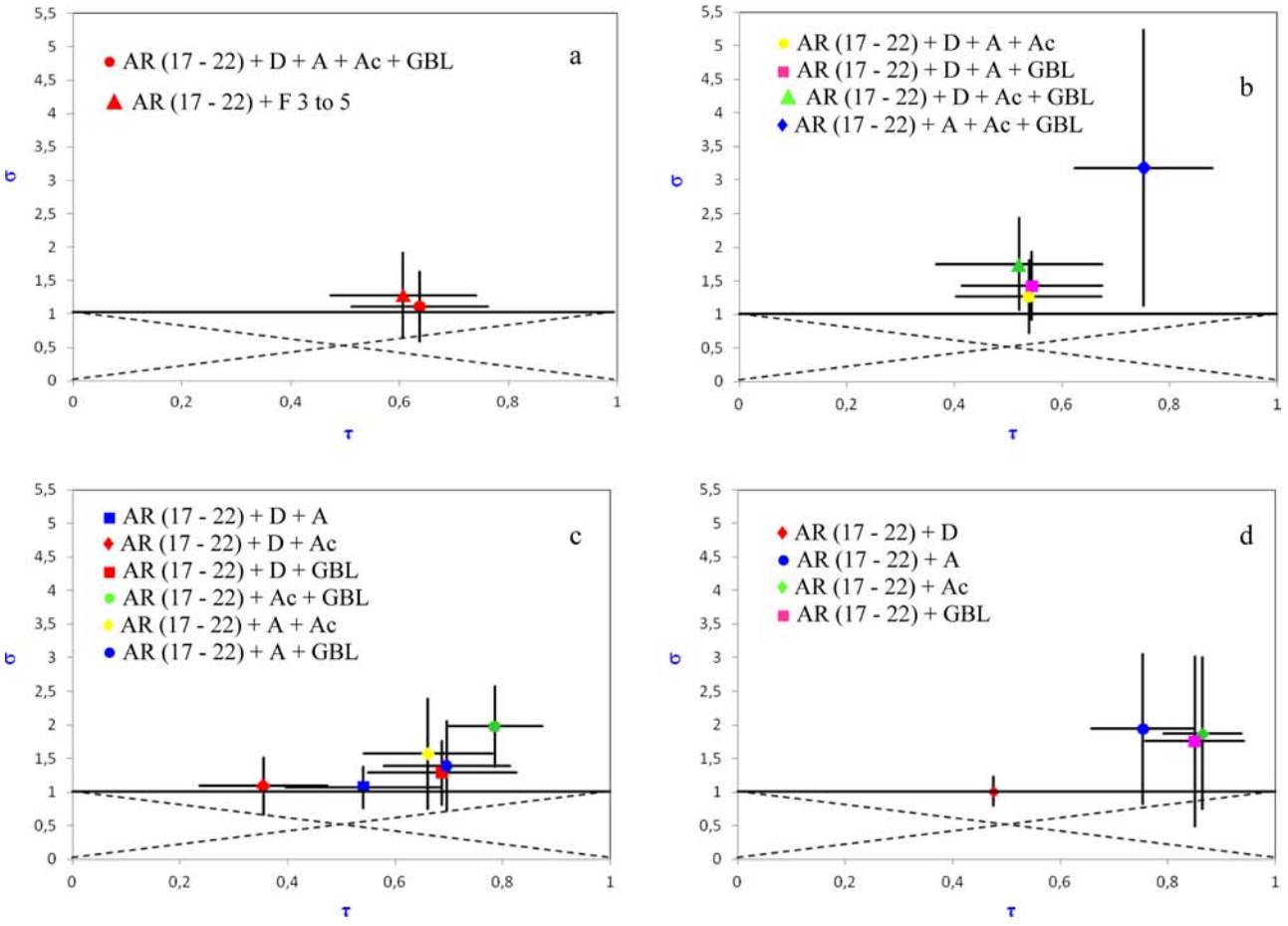

Figure 4. Impact of diacetyl (D), acetoin (A), acetic acid (Ac), and γ-butyrolactone (GBL) addition, individually or mixed, on jammy-fruit aroma (Experiment 3). Symbols represent σ and τ mean values; error bars indicate the 95% confidence interval on the mean for both τ and σ; F, fractions; AR (17−22), aromatic reconstitution (AR) made from HPLC fruity fractions (17−22).

These findings highlighted the direct role of diacetyl, as well as new perceptual interactions, including the indirect impact of acetoin, acetic acid, and γ-butyrolactone at concentrations representing about 2%, 12%, and 40% of their perception thresholds, respectively, on overall and fruity aroma expression. Examples of perceptive interactions in wine tasting have been reported since the early 1970s.[42] Some of them are very striking, such as the impact of very small variations in certain ethyl esters (as little as 1.3% of the olfactory threshold of ethyl 2-methylpropanoate) on fruity aroma in wines.[20]

In light of these data, aroma changes following malolactic fermentation may be explained by the significant impact of diacetyl, acetoin, acetic acid, and γ-butyrolactone, of which concentrations change during this process, on fruity aroma expression. It has been reported that malolactic fermentation resulted in an overall increase,[43] decrease,[44] or even qualitative changes[45] in the fruity aroma of red wines.

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