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Selection of Potential Impact Odorants and Sensory Validation of Their Importance in Typical Chardonnay Wines

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The aim of the present study was to validate the joint sensory impact of target compounds on the typicality degree of wine. Target compounds were selected from previous gas chromatography−olfactometry analysis. The preliminary experiment consisted in selecting odorants thought to have a positive effect on typical Chardonnay wines. Two sets of target compounds were chosen with regard to expected relationships between their concentrations and typicality scores. Target compounds were quantified in 20 wines. The second experiment was dedicated to the sensory evaluation of aroma models obtained by supplementation in wines. Three Chardonnay wines with intermediate typicality scores were supplemented with 6- or 10-compound combinations. The typicality degree of 24 samples was assessed by expert orthonasal perception. Wines supplemented with the 6-compound combinations were judged to be intermediate, whereas wines including the 10-compound combinations were considered to be quite representative of the Chardonnay concept. Such results confirm the active contribution of the 10 combined target compounds to typical Chardonnay wines.

KEYWORDS: Wine; aroma; GC-O; Chardonnay; typicality; aroma model; key odorants; quantification; sensory concept

INTRODUCTION

Food products, and especially alcoholic beverages such as wine, beer, or spirits, contain a great number of volatiles. Food aromas are therefore the expression of complex mixtures of volatiles. As reported by Lee and Noble (*1*), over 800 volatiles have been identified in wine aroma. For instance, Simpson and Miller (*2*) listed 140 aroma compounds in Chardonnay wines. As precisely indicated by Bult et al. (*3*), a large number of these volatiles are responsible for odors, whereas others might not produce noticeable odors at all. Among hundreds of volatiles, impact odorants are usually detected by sensory analysis after the mixture has been decomposed by gas chromatography. In this context, to investigate both the odor activity and the sensory significance of volatiles, gas chromatography−olfactometry (GC-O) is a highly relevant tool for screening impact odorants, which are significantly involved in food aromas (*1*, *4*, *5*). It is integrated into an overall procedure, as the schedule of a complete analytical approach covers many operations such as producing representative extracts, appropriate selection of panelists, qualitative and quantitative characterization of odorant areas by GC-O, identification of volatile(s) responsible for each odorant area, and finally quantification. The principal drawback of such an approach, however, is that it considers the impact of isolated aroma compounds in the extract, overlooking their joint effects in the original food product. Consequently, GC-O should be considered as an essential but partial procedure. Compounds finally selected by GC-O and subsequently identified and quantified should be regarded as no more than potential active compounds until their real impact has been confirmed. The sensory validation of GC-O is therefore very useful and is to be seen as a critical concluding step in the complete process.

There have been many sensory validation studies of GC-O data. Several food products were examined, including wines (*6*−*8*), roasted coffee and coffee brew (*9*, *10*), dairy products (*11*−*14*), rye bread (*15*), olive oils (*16*), and fresh fruit juices (*17*). Whatever the food product, authors were unanimous that the success of sensory validation tests primarily depended on accurate quantification of target odorants (*18*). Peterson and Reineccius (*14*) reported sensory differences between models and real butter, which they explained by possible aroma compound quantification errors (although their work nevertheless advanced the chemical knowledge of butter aroma). Guth (*4*) advised quantifying the full amounts of recognized odorants to correct approximations due odorant loss during isolation

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procedures. The reliability of subsequent odor activity value (OAV) calculation depends both on measuring concentration accurately and on determining the odor threshold in the food matrix. Stephan and Steinhart (*5*) claimed that precise quantification was a prerequisite for applying the OAV concept. Every subsequent formulation was established on the basis of quantitative odorant data: most authors now recommend stable isotope dilution assay for accurate quantification of volatiles in foods (*10*, *12*, *13*, *15*).

To reconstruct food aromas, some authors implemented the complete model, containing all of the quantified compounds selected by GC-O (*6*, *7*−*9*, *14*), before reducing, if necessary, the number of target odorants. Mostly, when the resulting model mixtures were compared by sensory analysis to the original product, the complete model showed good agreement with the original food product. A preselection of compounds could, however, help focus directly on the primary impact compounds. To minimize the complexity of mixtures, odorants were generally chosen according to their OAV (*5*). Successful applications have shown the OAV concept to be well adapted to selecting potential odorants. Some authors (*13*, *17*) constituted semicomplete models, containing all compounds with OAV >1 or >0.5; others used simplified models composed only of the volatiles with the highest OAVs. Rychlick and Bosset (*13*) demonstrated that semicomplete models comprising 13 volatiles with OAV >1 were sufficient to simulate the odor of real Gruyère cheese samples. Of 54 soybean lecithin odorants, combining the 25 with the highest retronasal OAVs proved likely to reproduce the odor of the original product (*5*). Guth (*4*), however, demonstrated that excessive simplification of the model mixture affected the odor profile: a model composed of the 29 odorants with OAV ≥ 1 was judged to be closer to real Gewurztraminer wine than one comprising the 8 odorants with OAV ≥ 10. Similar findings were reported by Escudero et al. (*8*) for a simplified reconstitution containing only the 23, of 53, compounds with OAV >1. There are few reports of any alternative to the OAV concept for selecting odorants for blending to assess their joint contribution to overall flavor. Kirchhoff and Schieberle (*15*) employed an aroma extract dilution analysis (AEDA) data set, followed by identification experiments to screen for compounds to be used to reconstitute the aroma of rye bread crumbs; the 21 volatiles related to odorant areas exhibiting flavor dilution (FD) factors >128 were selected prior to quantification, OAV calculation, and recombination. Final selection used omission tests to investigate the individual compounds' contributions to overall flavor. As recently reviewed by Grosch (*18*), some authors prepared a series of model mixtures, omitting a single compound to reveal whether the preselected high-OAV odorants were actually key compounds (*4*, *6*, *8*, *15*, *17*, *19*). Omission tests have also been performed to assess change in overall flavor after removal of one or more odorants, usually chosen according to chemical features (*9*, *10*) or odor quality (*17*). Such experiments sought to highlight possible additive effects.

It is easier to prepare model mixtures simulating the odor quality of liquid than of solid food. Three principal types of medium have been used to reconstitute wine aroma models. Basically, target compounds were added in various combinations to a water/ethanol mixture (*6*). Aroma models were also prepared by mixing target compounds in a synthetic wine chosen to simulate a more realistic wine base (*7*). Finally, synthetic mixtures of aromas were prepared from dearomatized wine obtained by 48 h XAD-4 resin treatment (*8*). To our knowledge, model mixtures have never been reconstituted from natural wine. The pre-existence of target compounds in natural media seems to be a real obstacle.

The final step of sensory validation consists of assessing the similarity between the aromas of reconstituted models and the original product. Quantitative descriptive analyses have been conducted (*5*, *10*, *16*, *19*). In the case of orange juice (*17*), a short list of sweet, fruity, grassy, terpene-like, pungent, and citrus-like descriptors was first generated from the original product. Then judges assessed the intensity of each descriptor on a linear scale, for the product and the various aroma models. Sensory profiles were thus established. In other studies, the similarity between aroma models and original food product was based on an overall assessment by means of an uncategorized line scale (*14*). Discriminative tests such as triangular or duo−trio tests were also usually performed (*8*).

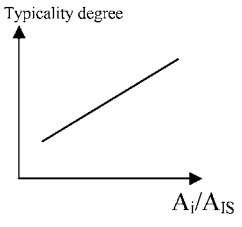
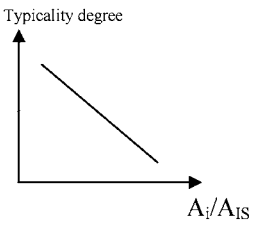
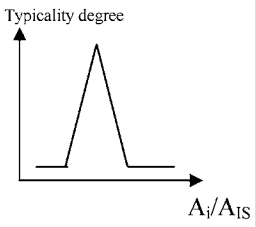
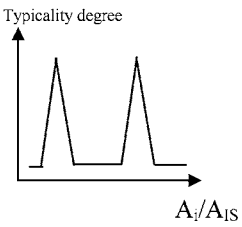
More recently, Ballester et al. (*20*) showed the existence of a sensory concept related to wines produced from the Chardonnay grape variety, based on a consensual mental representation shared by an expert panel. Panelists were asked to assess wine typicality by orthonasal perception. In a pool of 48 wines, including 29 Chardonnay and 19 non-Chardonnay wines, 2 contrasting groups (good and bad examples of the Chardonnay wine concept) were discriminated. Intermediate wines (neither good nor bad examples) were discarded. Then 17 selected wines (9 good and 8 bad examples) were analyzed by GC-O. Seventy-one compounds, common to all 17 wines or not, were identified and quantified by GC-MS-SIM (*21*). These investigations were not extended to the intermediate wines.

The present study sought to extend these earlier results (*20*, *21*), by means of sensory validation of the joint role of certain aroma compounds in the typicality of Chardonnay wines. It was essential to select target compounds among the 71 above-mentioned; so large a field required a selection methodology radically different from all those reported in the literature cited above. Selection criteria such as OAV or FD factors would have been too onerous; rather, target compounds were selected by presumed relationships between typicality scores and the quantitative data for volatiles in the respective wines. Two subsets of 6 and 10 target compounds were thus selected and tested. Then, aroma models were prepared in three intermediate Chardonnay wines (i.e., neither good nor bad examples), which were accurately quantified. The typicality of the supplemented wines was assessed by the expert panel using the recently reported sensory concept methodology (*20*).

PRELIMINARY EXPERIMENT: SELECTION OF POTENTIAL IMPACT ODORANTS

Quantitative data, obtained in triplicate, were expressed as the relative concentration A_i/A_{IS} in the extract, where A_i is the area of the *i*-compound peak ($i = 1-71$) and A_{IS} the area of the internal standard peak (*21*). Target compounds were screened on the basis of the relationship between A_i/A_{IS} values and the typicality scores previously attributed by the expert panel. For each of the 71 compounds, one-way analysis of variance (ANOVA) and subsequent multiple-comparison procedures were performed using Statistica software (version 5.1; Statsoft, Inc., Tulsa, OK). Thus, five categories of compound were distinguished. The first comprised 48 compounds, the amounts of which proved to be uncorrelated to the typicality degree and which were consequently disregarded. The remaining 23 compounds were divided into four categories (positive, negative, single optimum, and double optimum) depending on the link between typicality degree and compound quantity (**Table 1**).

Table 1. Four Categories of Impact Compoundsz: Relationships between Relative Concentrations (A_i/A_{IS}) and Wine's Typicality Degree

Categories	Positive	Negative	Single optimum	Double optimum
Four patterns of relationship between typicality degree and A_i/A_{IS}				
Compounds	ethyl butanoate ethyl hexanoate δ-decalactone 2-hydroxy-4-pyranone 4-vinylphenol 2-methyltetrahydrothiophen-3-one 4-vinyl-2-methoxy-phenol linalool	vanillin α-terpineol ethyl furoate ethyl 2-methyl butanoate diethyl pentanedioate ethyl 3-methyl butanoate 1,1-diethoxy ethane	benzyl alcohol diethyl butanedioate phenylacetic acid ethyl hydrogen succinate	3-methylbutyl acetate phenylacetaldehyde octanoic acid decanoic acid

The relationship between A_i/A_{IS} value and typicality degree was further categorized as follows: 1, no tendency; 2, marked tendency; and 3, very marked tendency (**Table 2**). To focus on those volatiles responsible for the typicality of Chardonnay wines, the negative category was discarded and the remaining 16 of the 23 original compounds were shortlisted: that is, those of the positive (8 compounds), single optimum (4 compounds), and double optimum (4 compounds) categories. The target compounds were then categorized by the odor description made in the previous GC-O analysis (*21*) and/or as indicated in the literature, in 6 classes, according to the standardized terminology given by Noble et al. (*22*): fruity, microbiological, floral, spicy, chemical, and nutty. For the first model, a single compound representative of both the qualitative and quantitative categories was chosen from each class. The six compounds thus selected for the first aroma model were ethyl butanoate (fruity), octanoic acid (microbiological), phenylacetaldehyde (floral), 4-vinylphenol (spicy), 2-methyltetrahydrothiophen-3-one (chemical), and δ-decalactone (nutty). In the second model, four additional compounds (3-methylbutyl acetate, decanoic acid, linalool, and 4-vinyl-2-methoxyphenol) were introduced to reinforce the weight of four odor classes (fruity, microbiological, floral, and spicy) thought to be consistent in Chardonnay wines according to previous studies (*1*, *23*, *24*). The additive effects of odorants of similar odor quality, as suggested by Buettner and Schieberle (*17*), could thus be investigated. Thus, the second model comprised 10 compounds of the positive and double-optimum categories.The experimental design is described in **Table 3**.

MAIN EXPERIMENT: SENSORY VALIDATION OF GC-O DATA

Materials and Methods. *Chemicals.* The 10 reference compounds were ethyl butanoate, 3-methylbutyl acetate, phenylacetaldehyde, decanoic acid, octanoic acid, δ-decalactone (Aldrich, Gilligham, U.K); 4-vinylphenol (Interchim, Montluçon, France); 2-methyltetrahydrothiophen-3-one (Lancaster, Strasbourg, France); linalool (Fluka, Buchs, Switzerland); and 4-vinyl-2-methoxyphenol (Pernod-Ricard, Créteil, France). The internal standard, methyl heptanoate (99%), was purchased from Fluka; dichloromethane and ethanol (99.8%) were from Carlo Erba Reagents (Milan, Italy); sodium sulfate, K_2SO_4 (99.5%), and NaOH (98%) were from Prolabo (Paris, France); tartaric acid (99.5%), glycerol (87%), and $MgSO_4$ (99.8%) were from Merck (Darmstadt, Germany); and malic acid (99%) was from Aldrich (Gilligham, U.K).

Quantitative Analysis of Target Compounds. As in the preliminary experiment, quantitative data for the 17 wines were expressed as relative concentrations (*21*). For each of the 10 target compounds, calibration curves were established by GC-MS, using a dichloromethane dilution series. Correction factors were calculated according to known amounts of volatiles in a synthetic medium, prepared as follows: 120 mL of ethanol, 4 g of tartaric acid, 3 g of malic acid, 3 g of glycerol, 0.1 g of K_2SO_4, and 0.025 g of $MgSO_4$ were added, and the solution was adjusted to pH 3.3 using 5 M NaOH. A 1 L volumetric flask was then filled with distilled water. The supplemented synthetic medium was extracted and analyzed by GC-MS and subsequently described. Calibration curves and correction factors were used to convert relative concentration into quantitative data expressed in milligrams per liter. These calculations were used for the quantification of target compounds in the 17 wines and to carry out their quantification in three intermediate (as yet nonquantified) Chardonnay wines (Cha10, Cha16, and Cha26). These three wines were especially suitable as incorporation media for the preparation of the 6- and 10-aroma models.

Extraction. Odorants were isolated by liquid−liquid extraction. One hundred milliliters of wine or synthetic medium was extracted with 2 × 30 mL and then 20 mL of dichloromethane in appropriate flasks, as reported by Moio et al. (*25*). The combined organic phases were dried over anhydrous sodium sulfate prior to filtration through glass wool. The extract (about 80 mL) was concentrated to 1.5 mL under nitrogen flow (150 mL/min). An aliquot (0.8 mL) was placed in a 2 mL vial. Then 0.8 mL of internal standard dichloromethane solution (503 mg/L) was added. Methyl heptanoate was used as internal standard. The extract was stored at −18 °C prior to analysis. For each sample, extraction was carried out in triplicate.

GC-MS-SIM. Analyses were performed on a Hewlett-Packard 6890 gas chromatograph, equipped with a split/splitless injector and a DB-1701 capillary column (30 m × 0.32 mm i.d., 1 μm film thickness: J&W Scientific, Folsom, CA). A 1 μL sample of each concentrated extract was injected. The injector temperature was held at 250 °C. The splitless time was 0.3 min, and the purge flow to the split vent was 25 mL/min. The helium

Table 2. Multiple Comparison Procedures (Newman–Keuls Test, $\alpha = 0.1$) of Relative Concentrations (A_i/A_{IS}) in Extract for the 16 Preselected Compounds[c]

ethyl butanoate (P: 3) [a, b]

wines [c]	A_i/A_{IS}	LRI = 860
CHA6	0.094	A
cha7	0.105	AB
cha2	0.107	AB
che1	0.108	AB
MEL3	0.108	AB
cha18	0.110	AB
mar1	0.112	AB
cha20	0.114	AB
cha14	0.115	AB
syl2	0.118	AB
CHA15	0.119	AB
CHA28	0.120	AB
CHA9	0.120	AB
CHA27	0.136	B
CHA1	0.136	B
ALI2	0.163	C
MEL2	0.188	D

ethyl hexanoate (P: 1) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1059
CHA6	0.360	A
cha7	0.370	A
mar1	0.370	A
cha18	0.371	A
che1	0.378	A
CHA28	0.388	A
MEL3	0.398	A
CHA15	0.401	A
ALI2	0.417	AB
cha2	0.427	AB
cha14	0.431	AB
CHA9	0.432	AB
cha20	0.466	ABC
syl2	0.481	ABC
CHA27	0.484	ABC
CHA1	0.529	BC
MEL2	0.568	C

2-methyl-tetrahydrothiophen-3-one (P: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1123
cha2	0.003	A
syl2	0.007	AB
che1	0.007	AB
CHA27	0.008	ABC
ma1	0.010	BC
MEL3	0.010	BC
cha20	0.014	CD
CHA15	0.017	DE
MEL2	0.018	DE
cha18	0.022	EF
CHA6	0.025	FG
CHA28	0.025	FG
CHA9	0.028	FG
CHA1	0.030	G
cha7	0.031	G
ALI2	0.039	HI
cha14	0.115	I

2-hydroxy-4-pyranone (P: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1189
syl2	0.21036	A
che1	0.23102	A
mar1	0.28255	AB
MEL3	0.34526	BC
CHA15	0.35616	BCD
cha7	0.36824	BCD
MEL2	0.39288	BCDE
cha18	0.39770	BCDE
CHA9	0.41470	CDE
cha2	0.42977	CDE
ALI2	0.44246	CDE
cha14	0.47803	DEF
CHA28	0.51250	EFG
CHA6	0.55345	FGH
CHA1	0.59110	GH
cha20	0.62766	H
CHA27	0.82310	I

linalool (P: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1195
cha18	0.000	A
cha2	0.000	AB
che1	0.000	AB
MEL3	0.001	AB
CHA6	0.001	ABC
MEL2	0.001	ABC
cha7	0.001	BCD
CHA27	0.001	BCD
mar1	0.001	CD
CHA1	0.001	CD
CHA28	0.001	DE
ALI2	0.002	DE
cha20	0.002	E
cha14	0.003	F
CHA9	0.003	G
CHA15	0.004	H
syl2	0.004	I

4-vinylphenol (P: 3) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1489
cha18	0.017	A
cha7	0.022	A
mar1	0.027	AB
syl2	0.029	AB
che1	0.031	AB
cha14	0.033	AB
CHA6	0.033	AB
cha2	0.043	B
MEL3	0.045	B
CHA9	0.058	C
MEL2	0.060	C
CHA1	0.069	C
ALI2	0.111	D
CHA27	0.112	D
CHA28	0.115	D
CHA15	0.120	D
cha20	0.179	E

4-vinyl-2-methoxy-phenol (P: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1489
cha18	0.008	A
cha7	0.012	AB
cha2	0.012	AB
che1	0.013	AB
cha14	0.013	AB
CHA6	0.016	AB
MEL3	0.019	B
CHA27	0.025	C
CHA9	0.027	CD
CHA1	0.029	CD
MEL2	0.030	CD
mar1	0.035	D
CHA28	0.035	D
ALI2	0.036	D
syl2	0.043	E
cha20	0.046	E
CHA15	0.054	F

δ-decalactone (P: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1746
ALI2	0.003	A
CHA9	0.004	AB
syl2	0.004	AB
cha7	0.004	ABC
ma1	0.005	ABC
CHA15	0.005	ABC
cha18	0.005	ABC
cha14	0.005	BC
che1	0.005	CD
cha20	0.005	CDE
cha2	0.005	CDE
CHA28	0.005	CDE
CHA6	0.006	CDE
MEL3	0.006	DEF
CHA1	0.007	EF
MEL2	0.007	F
CHA27	0.008	G

benzyl alcohol (SO: 1) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1212
CHA27	0.028	A
che1	0.031	AB
MEL2	0.040	ABC
cha7	0.048	BC
CHA9	0.050	BC
ALI2	0.055	C
cha14	0.071	D
cha18	0.080	DE
MEL3	0.081	DE
CHA1	0.085	DEF
CHA6	0.086	DEF
cha2	0.087	DEF
cha20	0.100	EFG
CHA28	0.101	EFG
CHA15	0.106	FG
mar1	0.112	G
syl2	0.174	H

diethyl butanedioate (SO: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1305
syl2	2.147	A
cha20	2.396	A
che1	2.418	A
CHA27	3.140	B
CHA15	3.403	BC
MEL2	3.446	BC
cha14	3.606	BC
CHA9	3.682	BC
cha7	3.967	CD
CHA28	4.345	DE
ALI2	4.581	E
MEL3	4.641	E
CHA6	4.863	E
CHA1	6.037	F
cha18	6.433	F
ma1	8.126	G
cha2	9.086	H

phenylacetic acid (SO: 3) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1505
cha20	0.000	A
CHA9	0.000	A
cha7	0.000	A
CHA27	0.001	A
syl2	0.001	A
CHA15	0.001	A
ALI2	0.001	AB
MEL2	0.001	AB
CHA28	0.001	AB
CHA1	0.001	AB
CHA6	0.001	AB
cha14	0.001	AB
MEL3	0.002	BC
cha18	0.002	CD
che1	0.002	CD
ma1	0.003	CD
cha2	0.003	CD

ethyl hydrogen succinate (SO: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1421
syl2	13.831	A
che1	26.609	B
CHA15	27.143	B
ALI2	27.148	B
cha7	28.354	B
CHA1	29.602	B
CHA9	29.967	B
cha20	30.162	B
CHA6	31.283	B
cha14	31.850	B
MEL2	33.095	B
CHA27	33.251	B
CHA28	33.942	B
MEL3	38.886	C
cha18	40.456	C
cha2	47.414	D
mar1	47.846	D

3-methylbutyl acetate (DO: 3) [a, b]

wines [c]	A_i/A_{IS}	LRI = 941
cha18	0.020	A
cha2	0.021	A
ma1	0.029	ABC
CHA6	0.033	BCD
CHA1	0.036	CD
MEL3	0.038	CDE
syl2	0.043	DEF
che1	0.049	EFG
cha7	0.053	FG
cha14	0.055	FG
ALI2	0.059	G
CHA9	0.063	G
MEL2	0.089	H
CHA15	0.093	HI
CHA28	0.101	I
CHA27	0.137	J
cha20	0.145	J

phenylacetaldehyde (DO: 3) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1189
mar1	0.004	A
cha20	0.005	A
CHA27	0.005	A
CHA15	0.006	A
MEL2	0.010	AB
CHA9	0.012	BC
CHA28	0.016	BCD
cha14	0.016	BCD
cha7	0.016	BCD
syl2	0.018	CD
cha2	0.018	CD
MEL3	0.020	CD
CHA6	0.020	CD
ALI2	0.021	D
cha18	0.028	E
CHA1	0.031	E
che1	0.112	F

octanoic acid (DO: 2) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1351
ALI2	0.658	A
CHA28	0.772	AB
che1	0.848	AB
CHA6	0.851	AB
cha7	0.877	AB
cha18	0.898	AB
CHA9	0.911	AB
CHA15	0.948	ABC
cha20	0.968	BC
ma1	0.970	BC
cha2	1.006	BC
cha14	1.045	BC
syl2	1.049	BC
MEL3	1.080	BC
CHA27	1.214	CD
CHA1	1.313	D
MEL2	1.406	D

decanoic acid (DO: 3) [a, b]

wines [c]	A_i/A_{IS}	LRI = 1541
ALI2	0.072	A
CHA28	0.130	A
CHA9	0.225	B
cha7	0.249	B
CHA15	0.276	B
syl2	0.290	B
cha2	0.339	B
cha18	0.435	C
che1	0.436	CD
cha14	0.453	CD
mar1	0.458	CD
CHA6	0.472	CD
cha20	0.476	CD
CHA27	0.632	E
MEL3	0.641	E
MEL2	0.723	EF
CHA1	0.762	F

[a] P, positive effect; SO, single optimum effect; DO, double optimum effect. Within each category, compounds appear in order of increasing linear retention indices (LRIs). [b] 1, no tendency; 2, marked tendency; 3, very marked tendency. [c] Good examples of Chardonnay wines given in capital letters and bold characters. [d] Same letters (A, B, C, ...) indicate means belonging to the same homogeneous group.

Table 3. Categorization of the 16 Preselected Compounds, According to Their Odor Qualities

			individual odor description					aroma model[d]	
compound[a]	LRI[b]	odor quality	GC-O[c]	literature		category	tendency	1	2
ethyl butanoate	860	fruity	fruity, strawberry	fruity, banana, pineapple, sweet, strawberry candy	*1*, *7*, *27–30*, *35*, *36*	positive	3	X	X
3-methylbutyl acetate	941		banana	fruity, pear, apple, banana	*1*, *7*, *23*, *27*, *28*, *30*, *36*	double optimum	3		X
ethyl hexanoate	1059		apple peel	fruity, strawberry, pineapple, malty, anise, over-ripe fruit	*1*, *23*, *27–31*, *35*	positive	1		
benzyl alcohol	1212		fruity, floral	fruity, sweet, boiled cherry, herbaceous, grass, Paraguay tea, roasted, toasted, moldy	*27*, *28*, *32–35*	single optimum	1		
diethyl succinate	1305	microbiological	caramel	wine, ether, herbaceous grape, fabric, floral	*27*, *28*, *35*	single optimum	2		
octanoic acid	1351		animal, spicy	sweat, acid, cheese, fatty, unpleasant, rancid, goat	*1*, *7*, *27*, *28*, *30*, *34*, *36*	double optimum	2	X	X
decanoic acid	1541		vinegar, animal	wine, dusty, synthetic, fatty, waxy	*1*, *27*, *28*, *30*, *36*	double optimum	3		X
2-hydroxy-4-pyranone	1189	floral	floral, spicy			positive	2		
phenylacetaldehyde	1189		floral, spicy	floral, hawthorn, hyacinth, herbaceous, grassy, honey	*27–31*, *37–39*, *41*	double optimum	3	X	X
linalool	1195		floral, burnt	floral, lemon, citrus, camphor, sweet, fruity, herbaceous	*6*, *23*, *27–29*, *32*, *33*, *35*, *36*, *40– 42*	positive	2		X
ethyl hydrogen succinate	1421		floral, spicy			single optimum	2		
phenylacetic acid	1505		floral, fruity	floral, geranium, honey, pollen, rose	*7*, *27*, *28*, *36*, *39*, *42*	single optimum	1		
4-vinylphenol	1489	spicy	spicy, pharmaceutical	phenolic, cypress, vanilla	*36*, *39*	positive	3	X	X
4-vinyl-2-methoxy-phenol	1489		spicy, pharmaceutical	spicy, clove, smoky, nutty	*1*, *27*, *28*, *37–39*, *41*	positive	2		X
2-methyltetrahydrothio-phen-3-one	1123	chemical	gas, diesel oil	gas, chlorine, wet, ozone	*27*, *28*, *36*, *42*	positive	2	X	X
δ-decalactone	1746	nutty	coconut, floral	nutty, peach, coconut	*7*, *27*, *28*, *31*, *36*, *42*, *43*	positive	2	X	X

[a] Selected compounds in bold characters. [b] Linear retention index of odorant area on DB-1701 capillary column. [c] Major descriptors generated during GC-O analyses developed by Ballester (*21*). [d] 1, 6-compound combination; 2, 10-compound combination.

carrier gas velocity was 32.9 cm/s. The initial oven temperature was 40 °C, programmed to rise by 4 °C/min to 220 °C, at which it was maintained isothermally for 30 min. The detector was a mass spectrometer (HP 5973). Mass spectra were generated at 70 eV and analyzed in the electron impact mode (MS-EI). The source temperature was 240 °C. The HP data analysis ChemStation software (version B.01.00; Hewlett-Packard, Agilent Technologies) was used for peak area integration.

Preparation of Aroma Models. Selected compounds were separately incorporated into 500 mL of the three intermediate wines (Cha10, Cha16, and Cha26) using appropriate volumes of alcoholic solutions to control ethanol content. Prior to incorporation, two quantitites of 750 mL of wine were blended to limit a possible bottle effect. Supplementation was adjusted according to the pre-existent amounts of target compounds in the three wines. Expected concentrations corresponded to the highest level in the good examples of Chardonnay wine (i.e., 6 of the 17 wines). The 6- and 10-aroma models were subsequently labeled as follows: Cha10-6 and Cha10-10; Cha16-6 and Cha16-10; Cha26-6 and Cha26-10. Prior to evaluation, the aroma models were stored at 12 °C under nitrogen for 48 h. The chemical stability of the aroma models was checked after 24, 48, and 72 h.

Sensory Analysis. Twenty-four samples were assessed. The six aroma models and the three original Chardonnay wines, considered as controls, were tasted to assess the effect of supplementations on typicality. The 24 samples were therefore presented along with 15 additional wines, already assessed by the expert panel (*20*), 6 of which had been produced from Chardonnay (labeled Cha7, Cha9, Cha14, Cha20, Cha27, and Cha28), and 9 from other white varieties: Sauvignon Blanc (Sau3 and Sau4), Sylvaner (Syl2), Marsanne (Mar1), Pinot Blanc (Pb3), Chenin (Che1), Aligoté (Ali2), and Melon de Bourgogne (Mel2 and Mel3). For the first sensory evaluation, wines were stored at 10 °C. The panel included 16 (14 men and 2 women) of the 28 experts who had participated in the previous assessment (*20*). All were based in the Burgundy region of France, and most of them exercised professions related to wine and had extensive knowledge of the various expressions of Chardonnay wines produced in the most important wine-producing countries. Each judge assessed the 24 samples by orthonasal perception in a 1 h session. The methodology used in this experiment was that previously developed by Candelon et al. (*26*) and Ballester et al. (*20*).

Statistical Analysis. Raw data were scanned and converted into scores ranging from 0 to 10 using FIZZ Papier software (Biosystems, Couternon, France). A principal component analysis (PCA) was performed with StatBox 3.0 (Grimmer Logiciels, Paris, France) on the sensory scores assigned to each wine by each panelist, with wines considered as observations and panelists as variables. Interjudge consensus was determined by Kendall's coefficient of concordance (w), calculated using Statistica (StatSoft, Inc.) software. Three-way ANOVA (supplementation, wine, judge) with interactions and a multiple-comparison procedure using the Newman−Keuls test were performed using Statistica.

Results and Discussion. *Quantitative Analysis of Target Compounds and Preparation of Aroma Models.* Quantitative data were determined for 20 wines: the 17 wines incompletely quantified by Ballester (*21*) and the 3 intermediate wines used for supplementations (**Table 4**). Correction factors concerned volatile defects occurring during extraction, and especially concentration under nitrogen flow, to enhance the accuracy of the quantitative data, and ranged from 62.5 to 102.2% (2-methyltetrahydrothiophen-3-one and 4-vinylphenol, respectively). For each compound, the expected concentrations of the aroma models were deduced from the highest concentration found in the good example category of Chardonnay wines. When

Table 4. Quantification of the 10 Selected Compounds in the 20 Wines: Average Amounts Expressed in Milligrams per Liter

	compound[a]									
	et-but	oct-ac	phenyl	4vinph	2methy	δ-deca	3mebac	dec-ac	4vin2m	linalo
Good Examples[b]										
Chardonnay[c]										
Cha1	**0.991**	**24.086**	**0.028**	0.237	**0.089**	0.037	0.694	**8.638**	0.159	0.010
Cha6	0.696	16.847	0.019	0.105	0.074	0.028	0.641	6.885	0.090	0.007
Cha9	0.880	17.785	0.013	0.196	0.085	0.014	1.180	5.395	0.149	0.020
Cha15	0.872	18.362	0.008	**0.424**	0.052	0.021	1.706	5.701	**0.297**	**0.023**
Cha27	0.987	22.537	0.006	0.395	0.026	**0.053**	**2.471**	7.853	0.135	0.008
Cha28	0.880	15.604	0.015	0.404	0.076	0.028	1.847	4.819	0.194	0.010
Non-Chardonnay										
Ali2	1.179	13.805	0.020	0.391	0.115	0.011	1.107	4.472	0.194	0.011
Mel2	1.352	26.283	0.011	0.201	0.055	0.039	1.634	8.403	0.167	0.007
Mel3	0.796	20.439	0.019	0.147	0.033	0.036	0.726	7.909	0.102	0.005
Bad Examples[b]										
Chardonnay										
Cha2	0.785	19.268	0.017	0.139	0.011	0.027	0.428	6.085	0.065	0.005
Cha7	0.771	17.250	0.016	0.064	0.093	0.017	0.992	5.540	0.064	0.008
Cha14	0.842	19.891	0.015	0.105	0.336	0.023	1.034	6.773	0.074	0.017
Cha18	0.807	17.574	0.026	0.044	0.067	0.021	0.415	6.664	0.043	0.004
Cha20	0.839	18.672	0.006	0.638	0.044	0.027	2.614	6.911	0.250	0.013
Non-Chardonnay										
Che1	0.793	16.796	0.094	0.097	0.023	0.026	0.932	6.669	0.071	0.005
Ma1	0.821	18.710	0.006	0.083	0.032	0.017	0.570	6.802	0.190	0.010
Syl2	0.864	19.947	0.017	0.089	0.023	0.014	0.926	5.787	0.235	0.027
Intermediate Wines[b,d]										
Cha10	0.271	6.714	0.021	0.282	0.042	0.003	1.037	4.449	0.17	***0.027***
Cha16	0.362	4.648	***0.030***	0.369	0.069	0.012	0.348	4.32	0.166	***0.023***
Cha26	0.482	9.161	0.004	0.457	0.031	0.021	***3.237***	4.191	0.162	0.020

[a] Ethyl butanoate, octanoic acid, phenylacetaldehyde, 4-vinylphenol, 2-methyltetrahydrothiophen-3-one, and δ-decalactone, used for the 6-aroma combination; 3-methylbutyl acetate, decanoic acid, 4-vinyl-2-methoxy-phenol, and linalool, additional compounds used for the 10-aroma combination. [b] According to Ballester et al. (*20*). [c] Expected concentrations in boldface characters: maximum concentration found in good examples of Chardonnay wines. [d] Pre-existent expected concentrations found in intermediate wines given in boldface italic characters.

Table 5. Concentration Ratios between $t = 24$, 48, and 72 h after Supplementation

compound	C48/C24	C72/C48
ethyl butanoate	0.94	0.98
3-methylbutyl acetate	0.93	0.95
2-methyltetrahydrothiophen-3-one	1.00	1.00
phenylacetaldehyde	1.01	0.74
octanoic acid	0.93	1.07
4-vinylphenol	0.98	0.98
4-vinyl-2-methoxyphenol	0.98	1.14
decanoic acid	1.01	0.94
δ-decalactone	0.96	0.97
linalool	0.88	1.02

the expected concentration level pre-existed in the original wine, supplementation was not carried out.

To check chemical stability, added compounds were quantified by GC-MS-SIM at $t = 24$, 48, and 72 h. The aroma model was found to be chemically stable (**Table 5**), and supplementation was carried out 48 h before assessment.

Typicality Assessment of Aroma Models. To check consensus between panelists, a principal component analysis (PCA) was performed on the typicality scores. The first two principal components accounted for 44% of the total variation, with 32 and 12% explained by PC1 and PC2, respectively (**Figure 1**). All of the variables were located on the positive part of PC1. Moreover, Kendall's coefficient of concordance was significant ($w = 0.29$; $p < 0.001$), confirming the consistency between panelists. Thus, individual data could subsequently be averaged. The projection of the 24 individuals on the first two principal components is presented in **Figure 2**. Samples were mainly distributed along the first dimension. The correlation coefficient between mean scores and coordinates on PC1 ($r = 0.99$) confirmed that the first dimension could be considered as the typicality axis. Consequently, samples located to the right of the PCA plot were considered to be good examples, and those to the left to be bad examples. Six of the nine Chardonnay wines were considered to be representative of the concept, including two of the three wines used for supplementation (Cha10 and

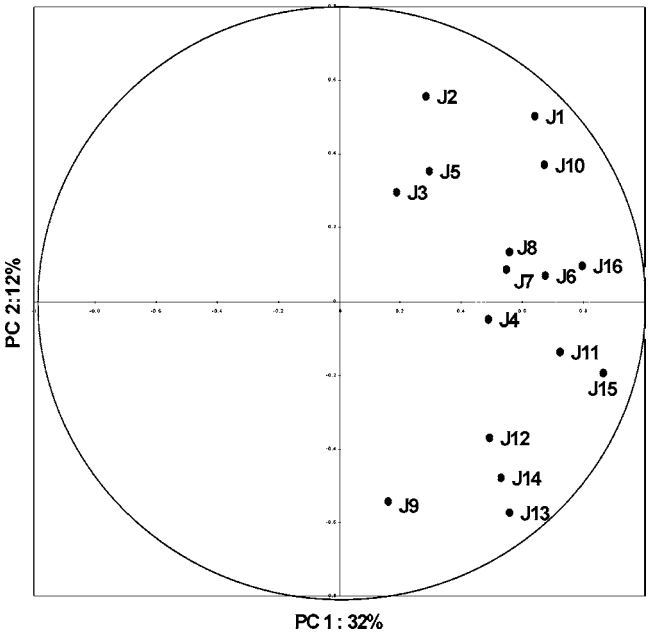


Figure 1. Projection of judges (variables J1–J16) on principal components 1 and 2 of the PCA.

Table 6. Results of the Three-Way Analysis of Variance

variation source	effect	SSE[a]	Df[b]	MS[c]	*F* ratio	*p* value
wine	fixed	81.05	2	40.53	7.01	1.3E–03
supplementation	fixed	49.29	2	24.64	4.26	1.6E–02
judge	random	122.90	15	8.19	1.42	1.5E–01
wine × supplementation		12.76	4	3.19	0.55	6.9E–01
residual		693.50	120	5.78		

[a] Sum of squares. [b] Degree of freedom. [c] Mean square.

Table 7. Multiple Comparison Procedure (Newman–Keuls Test, $\alpha = 0.05$)[a]

aroma model		6	control	10
mean scores		4.05	4.67	5.48
6	a		0.212	0.012
control	ab	0.212		0.101
10	b	0.012	0.101	

[a] Same letters indicate means belonging to the same homogeneous group.

Cha16). The third wine (Cha26) was located centrally. As expected, five of the nine non-Chardonnay wines, especially those derived from Sauvignon Blanc, Sylvaner, and Marsanne, were judged to be bad examples. In agreement with Ballester et al. (*20*), the sole non-Chardonnay wine close to the Chardonnay wine concept was produced from Melon de Bourgogne (Mel3). It was noticeable that the three 10-compound combinations were loaded on the right side of the typicality axis. The distribution of the 6-compound combinations along the typicality axis was less clear. These samples had a rather intermediate position on the low typicality side except for Cha10-6, which was clearly located on the right side. At all events, the supplemented wines remained within the limits of the Chardonnay sensory space. To focus on the 6- or 10-compound supplementation effects and to complete the above descriptive statistics, a three-way ANOVA (wine, fixed; supplementation, fixed; and judge, random) with the three first-order interactions (wine × supplementation, judge × supplementation, and wine × judge) was first conducted on the individual scores of the 9 samples, the 6 aroma combinations, and their controls. The second-order interaction (wine × supplementation × judge) was considered to be residual. The first-order interactions were not significant. Second, the two first-order interactions depending on the random factor were included in the residual, and a new ANOVA with only one first-order interaction (wine × supplementation) was performed according to the following model: score = wine + supplementation + judge + wine × supplementation + residual. The interaction was still not significant (**Table 6**). Wine and supplementation factors were, therefore, separately analyzed. Results showed a significant wine effect on the typicality degree and revealed the significance of the supplementation factor on typicality. However, the ANOVA suggested no more than a trend for the supplementation environment to have an impact on typicality. A multiple-comparison procedure (**Table 7**) distinguished two homogeneous groups: the 6-compound combination and control versus control and the 10-compound combination. As suggested by the previous PCA (**Figure 2**), the only significant difference was observed between the 6- and 10-compound combinations. The multiple-comparison procedure indicated that the typicality degree of the control generally lay between those attributed to their respective 6- and 10-aroma combinations. The typicality degrees of the 10-compound combinations were clearly better than those of the 6-compound combinations. Nevertheless, Guth (*4*) and Ferreira et al. (*7*) have reported that wine aroma could not be restored by a single fraction of odorants. A simplified aroma model, composed of impact compounds with OAV >10 (about 10 compounds), generated an olfactory sensation very different from the original one. The aroma of semicomplete models, consisting of about 20 compounds with OAV >0.5 or >1, was judged to be more illustrative. In the present study, both combinations can be considered as simplified models, and the greater the mixture complexity, the more the olfactory sensation was judged as representative of the sensory concept. Escudero et al. (*8*) underscored the effect of adding restricted compounds on aroma balance: supplementation caused a rupture of aroma balance, which implies an alteration of certain aroma nuances. Consequently, 6-compound supplementation could involve a gap in aromatic harmony that was detected by panelists. Despite their intermediate position, the 6-compound

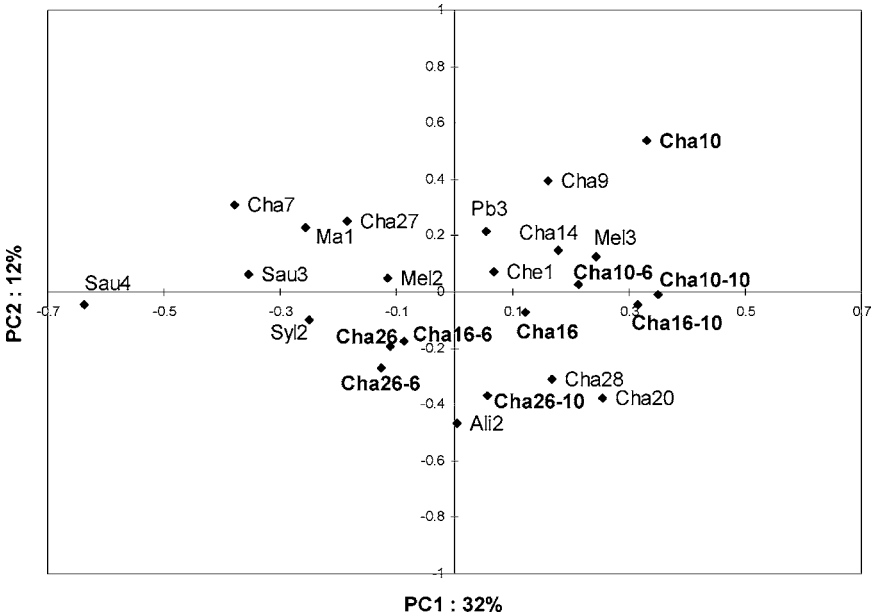


Figure 2. Projection of wines (individuals) on principal components 1 and 2 of the PCA. The 6- and 10-compound combinations and their controls are shown in boldface characters.

combinations were generally not considered to be illustrative of the mental representation shared by the expert panel. In contrast, all of the 10-aroma combinations, although they could also be considered as simplified models, were judged as good examples of the Chardonnay wines concept. Supplementation with four additional compounds was enough to ensure the expected olfactory perception. According to Grosch (*18*) and Peterson and Reineccius (*14*), the reliability of results depends on both appropriate selection of target compounds and the accuracy of their quantification. When such conditions were satisfied, the supplementation with four additional well-chosen compounds had a real incidence on the aromatic balance and, consequently, on typicality. As reported by Buettner and Schieberle (*17*), additive effects could also be suspected for the four reinforced influential categories: fruity, floral, spicy, and animal.

The selection of potent impact odorants by presumed relationships between wine typicality degree and concentration of compounds was a new and original approach to combination and sensory validation. The interest of the sensory approach was to determine whether aroma models restore the expected olfactory representation of typical Chardonnay wines. Typicality seemed to derive from an association of volatiles in specific proportions. An oversimplified model composed of six odorants failed to reproduce good examples of Chardonnay wines, but the addition of only four other potent odorants enabled a model representative of the sensory concept to be constructed. Satisfactory results thus depend on both appropriate selection of potent impact compounds and their accurate quantification. The present results confirmed the active role of the 10 combined target compounds in typical Chardonnay wines: ethyl butanoate, octanoic acid, phenylacetaldehyde, 4-vinylphenol, 2-methyltetrahydrothiophen-3-one, δ-decalactone, 3-methylbutyl acetate, decanoic acid, 4-vinyl-2-methoxyphenol, and linalool. However, these should be considered as preliminary findings, and it remains to be determined whether enhancement of the typicality degree depends on both the complexity of the model and the level of expertise of the panelists. To elucidate the individual impact of potent odorants, omission tests should be performed.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; ANOVA, analysis of variance; FD, flavor dilution; GC-MS, gas chromatography−mass spectrometry; GC-MS-SIM, gas chromatography−mass spectrometry−selected ion monitoring; GC-O, gas chromatography−olfactometry; LRI, linear retention index; MS-EI, mass spectrometry−electron impact; OAV, odor activity value; PCA, principal component analysis.

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