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Prediction of the Wine Sensory Properties Related to Grape Variety from Dynamic-Headspace Gas Chromatography−Olfactometry Data

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Wine extracts obtained by a dynamic headspace sampling technique were studied by quantitative gas chromatography−olfactometry (GC−O) to determine the aroma profiles of six young monovarietal Spanish white wines. A partial least-square regression study was carried out to look for models relating wine aroma properties with GC−O scores. Models were validated by sensory analysis. Four out of the five most important sensory descriptors were satisfactorily described by a model, and sensory tests confirmed most of the predictions. The main aroma differences between these wines are due to the ratio linalool/3-mercaptohexyl acetate. Floral, sweet, and muscat are positively related to the concentration of linalool and negatively to that of 3-mercaptohexyl acetate. Tropical fruit is related to the wine content in this last odorant. 2-Phenyl acetate, reinforced by other acetates, can also contribute to floral and sweet notes. Alkyl-methoxypyrazines lessen the tropical fruit note, and acetic acid lessens the muscat nuance.

KEYWORDS: Aroma; flavor; analysis; sensory profile, PLSR models, GC−olfactometry

INTRODUCTION

The intensity and quality of the aroma constitutes the primary quality factor in a white wine, and a substantial part of such quality is related to the variety of grape from which the wine was made. The importance of the grape variety explains the interest that this issue has aroused among researchers. Previous studies have shown that some odorants are related to the aroma characteristics of a given variety. The first discovery, made more than 30 years ago, was about the role of linalool and other terpenols in the aroma of wines made with muscat-related grapes (*1*−*4*). Other significant findings about the role of some odorants in the aroma of specific varieties came later, including works on the role of methoxypyrazines in Sauvignon varieties (*5*−*7*), *o*-aminoacetophenone in *Vitis labrusca* or *Vitis rotundifolia* varieties (*8*, *9*), *cis*-rose oxide in Gewürzttraminer (*10*−*12*), 4-methyl-4-mercaptopentanone in Sauvignon blanc (*13*, *14*) and in Schereube (*11*), and 3-mercaptohexanol in Grenache, Merlot, and Cabernet rosé wines (*15*, *16*). However, the finding of new molecules that explain the particular nuances of a wine made with some type of grapes seems to have come close to an end, at least in the case of dry table wines. In fact, recent researches using GC−olfactometry (GC−O) have not revealed the presence of any other specific odorant in wines from different grape varieties (*17*−*20*). It is not clear, however, whether this is due to a failure in the GC−olfactometry strategy or whether this is a definitive fact and aroma varietal differences are just a question of the existence of specific aroma profiles. A second question is whether GC−O data can be directly used to predict aroma characteristics or whether we should continue developing and using more and more sophisticated and expensive analytical methodology (*21*, *22*). These two questions are addressed in the present paper, of which the main objectives are to analyze the GC−O profiles of six monovarietal wines from the North of Spain and to determine whether their characteristic aroma nuances can be explained and predicted with those GC−O data.

To overcome the limitations of previous researches, the strategy used in the present study involves a novel GC−O technique of which the main features are that the extract is prepared by a sensitive dynamic headspace sampling technique and that the GC−O combines measurements of intensity and of frequency of detection.

MATERIAL AND METHODS

Reagents and Standards. Dichloromethane, HPLC quality, was from Fisher Scientific (Loughborough, U.K.), methanol of LiChrosolv quality was from Merck (Darmstadt, Germany), absolute ethanol (ACS quality) was purchased from Panreac (Barcelona, Spain), and pure water was obtained from a Milli-Q purification system (Millipore, Billerica, MA). LiChrolut EN resins and polypropylene cartridges were obtained from Merck (Darmstadt, Germany). The chemical standards were supplied by Aldrich (Gillingham, U.K.), Fluka (Buchs, Switzerland), Sigma (St. Louis, MO), Lancaster (Strasbourg, France), PolyScience (Niles, IL), ChemService (West Chester, PA), Interchim (Monluçon, France), International Express Service (Allauch, France), and Firmenich (Geneva, Switzerland).

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Table 1. Aroma Reference Standards and Their Composition[a]

term	composition	definition
tree fruit	5 mL of apple juice and 10 mL of apricot nectar	apple, apricot
tropical fruit	10 mL of Pascual Biofrutas	passion fruit
herbaceous	six pieces of fresh and dried grass	vegetative, fresh, green
citric	1 cm × 1 cm piece of grapefruit peel + 6 drops of lemon juice	lemon, orange, grapefruit
floral	crushed petals of one rose	rose, floral, blossom
muscat	0.5 mg of linalool	floral, linalool
aromatic herbs	1 drop of anise extract	licorice, anise
sweet	1 mL of prune juice and 5 mL of brine from canned figs	caramel-like, dried fruit
oxidized	10 mL of sherry	acetaldehyde, oxidized
fermentation	sample 33 (yeast) of "Le Nez du Vin" *(Jean Lenoir)*	yeasty

[a] Standards were prepared in 50 mL of Maccabeo wine.

Wine Samples. The wines selected for this study were six monovarietal young white wines from 2001 vintage: Albariño (Santiago Ruiz) (12% v/v), Godello (Guitián) (12% v/v), Malvasía (Estación Enológica de Castilla y León) (12.5% v/v), Parellada (Freixenet) (12% v/v), Treixadura (Villa Mein) (12% v/v), and Verdejo (Palacio de Bornos) (12.5% v/v). The Malvasía and Parellada wines were taken directly from the cellars whereas the rest were purchased from a wine-retailer in Zaragoza. They were chosen by five experts of the laboratory staff attending to the quality and representativeness of the aroma of the corresponding variety. The sensory study, the GC−O analysis, and the quantitative determination were carried out in the 4 months after the selection of the wines. During this period, the bottles were stored at 4 °C in the dark.

Dearomatized Wine. A wine from Maccabeo, Villalta 2002 from Bodegas San Valero, was used as the matrix for preparing synthetic mixtures of aromas. It was previously dearomatized by adding 4 g of Licholut-EN resins to 750 mL of wine and stirring during 12 h. The aroma of this dearomatized wine was of very low intensity and of neutral character.

Wine Sensory Analysis. The sensory panel was composed of six females and two males, 23−40 years of age, all of them belonging to the laboratory staff and with a long experience in sensory analysis. Five specific 1-h training sessions were carried out. In the first one, judges generated descriptive terms for the six wines. In sessions two and three, different aroma standards were presented and discussed by the panel. From these discussions, the 10 aroma terms and standards shown in **Table 1** were selected for further descriptive analysis. In training sessions four and five, panelists scored the intensity of each attribute using a 4-point scale (0 = not detected, 1 = weak, hardly recognizable note, 2 = clear but not intense note, 3 = intense note). After the training period, wine samples were evaluated in duplicate along three formal sessions (four samples per session). In all cases, wines (20 mL at 20 °C) were presented in coded, black, tulip-shaped wine glasses covered by glass Petri dishes. Samples were presented in a random order. The data processed was a mixture of intensity and frequency of detection (what we labeled as "modified frequency", MF), which was calculated with the formula proposed by Dravnieks (*23*):

$$\mathrm{MF}(\%) = \sqrt{F(\%)I(\%)}$$

where $F(\%)$ is the detection frequency of an aromatic attribute expressed as percentage and $I(\%)$ is the average intensity expressed as percentage of the maximum intensity.

Descriptive analysis data was analyzed by correlation analysis, χ^2 analysis, and two-way analysis of variance (ANOVA), in which wine varieties and judges were considered as the factors. All analyses were carried out using StatView (SAS Institute Inc.) for Windows, version 5.0.

Gas Chromatography−Olfactometry. *Preparation of Extracts.* The volatiles of the wine were collected using a purge-and-trap system. The trap was formed by a standard polypropylene SPE tube (0.8 cm internal diameter, 3 mL internal volume) packed with 400 mg of Lichrolut EN resins. Such resins were selected because of their excellent ability to extract aroma compounds (*24*). The bed was washed with 20 mL of dichloromethane and dried by letting air pass through (negative pressure of 0.6 bar, 10 min). The tube was placed on the top of a bubbler flask containing a mixture of 80 mL of wine and 20 mL of artificial saliva (*25*). The mixture was continuously stirred with a magnetic stir bar and kept at a constant temperature of 37 °C by immersion in a water bath. A controlled stream of nitrogen (100 mL/min) was passed through the sample during 200 min. Volatile wine constituents released in the headspace were trapped in the cartridge containing the sorbent and were further eluted with 3.2 mL of dichloromethane. The extract was kept at −30 °C for 2 h to eliminate any water content by freezing and further decantation. After this, the extract was concentrated under a stream of pure N_2 to a final volume of 200 μL.

Sniffings were carried out in a Thermo 8000 series GC equipped with a flame ionization detector (FID) and a sniffing port (ODO-1 from SGE) connected by a flow splitter to the column exit. The column used was a DB-WAX from J&W (Folsom, CA), 30 m × 0.32 mm with 0.5 μm film thickness. The carrier was H_2 at 3 mL/min. One microliter was injected in splitless mode, 1 min being the splitless time. Injector and detector were both kept at 250 °C. The temperature program was the following: 40 °C for 5 min, then raised at 4 °C/min up to 100 °C and at 6 °C/min up to 200 °C. To prevent condensation of high-boiling compounds on the sniffing port, this was heated sequentially using a laboratory-made rheostat. A panel of eight judges, six women and two men, carried out the sniffings of the extracts. Sniffing time was approximately 30 min, and each judge carried out one session per day. The panelists were asked to rate the intensity of the eluted odor using a 4-point category scale (0 = not detected; 1 = weak, hardly recognizable odor; 2 = clear but not intense odor, 3 = intense odor), half values being allowed. Four members of the panel already had extensive experience with GC−O while the rest, novices, followed a two-month training period during which they became familiar with the scale, the system, and the kinds of aromas found in wine extracts. The quantitative ability of this technique has been already proved (*26*). On this occasion, because some of the odorants in these extracts were much diluted, the olfactometric signal finally processed was not the mean of the olfactometric scores given by the different sniffers but the modified frequency (MF(%)), calculated with the formula previously given.

The identification of the odorants was carried out by comparison of their odors, chromatographic retention index in both DB-WAX and DB-5 columns, and MS spectra with those of pure reference compounds.

Quantitative Analysis. Wine aroma components were determined by GC−FID or GC−MS by using the instruments and methods described in the literature (*22*, *24*, *27*).

Data Treatment. To check the existence of significant differences between the GC−O scores of a given odorant from the different wine samples, two different strategies were carried out: first an analysis of variance with block design (the judges were the blocks and the wines the factors) on the individual intensity scores and second a χ^2 test on the frequency of detection data. Both analyses were carried out using SPSS (SPSS Inc., Chicago, IL) for Windows, version 11.5.

To explore the relationship between the olfactometric data and a single sensory attribute, partial least-square regression (PLSR) 1 was carried out using the Unscrambler 7.5 (CAMO A/S, Trondheim, Norway). A first initial model was built for a given sensory descriptor using all the discriminant X variables (GC−O scores). Different iterations excluding the least important variables were further run to look for the simplest model with the best prediction ability measured by cross-validation. The quality parameters studied to evaluate the prediction ability of the models were the slope of the regression curve between real and predicted Y variables (m), the root mean square error for the prediction (RMSEP), and the percentage of variance explained by the model (%EV).

Validation of the Models by Sensory Analysis. *Sensory Panel.* The test panel that carried out the different sensory experiments described in this work was composed of 12 subjects (eight women and

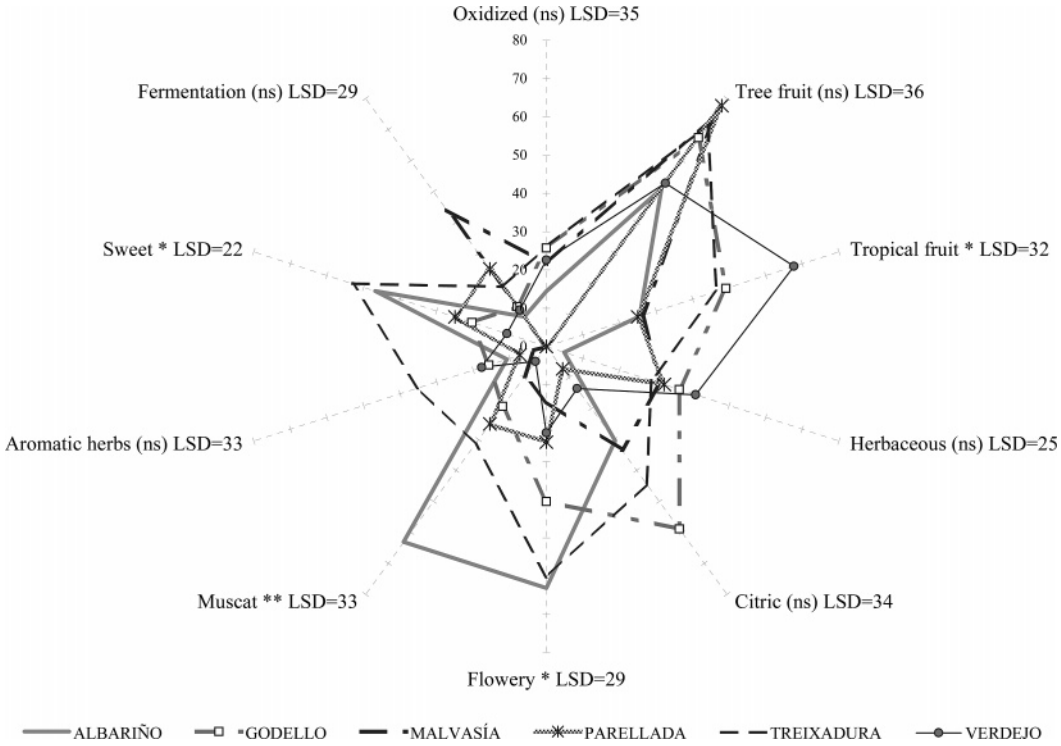

Figure 1. Graph of the mean sensory MF(%) ratings of the six wines (eight judges, two reps). Least significant difference (LSD) at $p < 0.05$ for each term is shown. Notations ns, $*$, and $**$ indicate no significance and significance at $p < 0.05$ and 0.01, respectively (ANOVA).

four men, ranging from 23 to 45 years of age) belonging to the laboratory staff. All of them participated regularly in sensory tests. Triangular tests 1−12 were performed by eight assessors and triangular tests 13−19 were run by 12 individuals. All sensory quantification tests were performed by eight assessors whereas ranking tests were made by seven or eight assessors as indicated in **Table 8**. In all cases, samples (20 mL, 20 °C) were presented in a random order in coded black tulip-shaped wine glasses covered with a Petri dish.

Triangular Tests. The potential sensory effect of a given odorant at a given concentration in the aroma of wine was first studied by triangular tests (*28*). The samples confronted in the test were, on one hand, a dearomatized wine containing or not other odorants and, on the other hand, the same sample to which the targeted odorant was added as detailed in **Table 7**. The tests were carried out following the order given in such table.

Quantification Tests. The relationship between an odorant and the intensity of a given odor was studied by using a sensory panel specifically trained to quantify such descriptor. In the case of the floral−sweet descriptor, judges were trained by making them rank wine samples containing different amounts of linalool, selected as reference for these two descriptors. References contained 20, 50, 100, and 200 μg L^{-1} of linalool in dearomatized wine. These same references were used as anchors for the sensory analysis. A 10 cm structured scale with anchors every 2 cm was used. A similar strategy was used for the tropical fruit descriptor, for which references were prepared by adding 2%, 5%, 10%, and 20% of a soft drink with tropical fruit flavor (Pascual Biofrutas, Spain). The reference for the muscat flavor was prepared by dilution (5%, 10%, 20%, and 50%) of a sweet muscat wine (Moscatel de Ainzón, Spain) with dearomatized wine.

Each sample was quantified in duplicate. The experiments were carefully randomized and were carried out in a tasting room in different sessions. Only three samples were examined per session. The panel was asked to rate the intensity of nuances using the 10 cm structured scale. Intensity ratings were analyzed by analysis of variance (ANOVA) using a mixed model (data not shown).

Ranking Tests (Page Test). The sensory panel was composed of eight and seven subjects as shown in **Table 8**. Each ranking test was performed in duplicate. The effect of the addition of acetic acid or alkyl-methoxypyrazines on the perception of muscat or tropical fruit, respectively, was measured by the summation of the ranks (weakest odor = 1, second least intense odor = 2, second most intense odor = 3, most intense odor = 4) as described for the Page test in ref *29*.

RESULTS AND DISCUSSION

Sensory Analysis. The aroma of six different white wines was described by the sensory panel using 10 different aroma descriptors. Results of the sensory analysis are shown in **Figure 1**. As can be seen, tree fruit is the descriptor in which all the samples scored high. In contrast, there are other descriptors, such as oxidized, fermentation, herbaceous or aromatic herbs, for which none of the samples reached important scores. The terms tropical fruit, citric, floral, muscat, and sweet reached high scores in some of the samples. The χ^2 analysis revealed that all terms, except tree fruit, varied significantly among wines ($p < 0.001$). This result indicates that tree fruit would be a general descriptor for this set of wines with no discriminant power. On the other hand, ANOVA analysis showed that five descriptors, oxidized, fermentation, herbaceous, aromatic herbs, and citric, presented nonsignificant differences between samples ($p < 0.05$). Taking into account the MF(%) values and both χ^2 and ANOVA analysis, the terms tropical fruit, floral, muscat, sweet, and citric seem to be the most important descriptors for defining these wines and for explaining their differential aroma characteristics. Therefore, these five terms were considered as the most interesting to be modeled in the subsequent PLSR analysis.

As can be seen in **Figure 1**, wines from Albariño and Treixadura were the richest in floral, muscat, and sweet notes, the wine from Godello was the richest in citric character, while the wine made with Verdejo got the highest score in tropical fruit notes. The wine from Malvasía was the most neutral in character, and its scores in most of the descriptors were quite low. It can be also seen that the notes sweet, muscat, and floral are correlated, since the rank of samples attending to their corresponding scores is quite similar. The correlation coefficient between muscat and floral notes was 0.827 ($p < 0.05$), between

Table 2. Odorants Found in Young White Wines from Several Spanish Varieties: Gas Chromatographic Retention Data, Olfactory Description, Chemical Identity, and Modified Frequency Percentage (MF(%))[a]

LRI DB-WAX	LRI DB-5	odor description	identity	ALB	GOD	MAL	PAR	TRE	VER
973		fruity	*b*	26	44	45	47	41	48
1012	<800	butter, cream	2,3-butanodione[c]	75	74	72	54	84	78
1035	<800	solvent	isobutyl acetate[c]	23	52	20	63	40	54
1056	801	fruity	ethyl butyrate[c]	69	74	76	76	80	80
1070	849	fruity	ethyl 2-methylbutyrate[c]	60	72	68	54	65	60
1084	853	fruity, anise	ethyl 3-methylbutyrate[c]	65	68	65	72	70	71
1116	<800	bitter, green	isobutanol[c]	44	42	53	41	41	37
1137	875	banana	isoamyl acetate[c]	79	71	80	80	70	80
1204		fruity	*b*	0	7	20	0	0	36
1225	<800	fusel	isoamyl alcohol[c]	82	73	83	81	80	80
1248	996	fruity, anise	ethyl hexanoate[c]	82	76	78	83	87	82
1259		green, flowery	*b*	34	0	9	0	0	0
1286	1008	banana	hexyl acetate[c]	14	0	0	7	23	31
1317	861	onion, meaty	2-methyl-3-furanthiol[d]	55	28	40	27	75	44
1386	942	box tree	4-mercapto-4-methyl-2-pentanone[d]	40	20	40	10	10	22
1398	849	grass	(*Z*)-3-hexenol[c]	20	46	29	55	52	39
1444	1093	pepper, earthy	3-isopropyl-2-methoxypyrazine[d]	62	56	57	16	49	54
1461	<800	vinegar	acetic acid[c]	13	59	52	39	49	54
1514	1173	pepper, earthy	3-*sec*-butyl-2-methoxypyrazine[d]	41	14	22	5	0	0
1537	1181	pepper, earthy	3-isobutyl-2-methoxypyrazine[d]	56	63	41	23	63	56
1562	1100	flowery, muscat	linalool[c]	46	7	0	7	66	20
1636	1022	toasty, burnt	2-acetylpyrazine[d]	48	34	26	22	47	47
1660	1045	flowery, green	phenylethanal[c]	13	0	14	16	13	38
1683	878	cheese	2-/3-methylbutyric acid[c]	49	46	50	41	57	53
1732	1252	basil, box tree	3-mercaptohexyl acetate[d]	0	24	18	0	0	63
1746		honey, liqueur	*b*	20	26	25	32	6	0
1838	1258	roses	2-phenylethyl acetate[c]	35	25	7	28	50	28
1842	1386	baked apple	β-damascenone[c]	56	63	49	48	60	68
1871		nutty	*b*	0	32	0	7	5	0
1944	1116	roses	β-phenylethyl alcohol[c]	40	60	48	43	62	44
2140		leather, urine	*m*-cresol[c]	18	32	18	0	14	23

[a] Abbreviations: LRI, linear retention index; ALB, Albariño; GOD, Godello; MAL, Malvasía; PAR, Parellada; TRE, Treixadura; VER, Verdejo. [b] Not identified. [c] Identification based on coincidence of gas chromatographic retention and mass spectrometric data with those of the pure compounds available in the lab. [d] Identification based on coincidence of chromatographic retention data and on the similarity of odor with standards. The compound did not produce any clear signal in the mass spectrometer because of its low concentration.

muscat and sweet notes was 0.799 and, finally, between floral and sweet notes was 0.939 ($p < 0.01$).

Gas Chromatography−Olfactometry. The GC−O experiment was carried out on extracts obtained in a dynamic headspace system. The proposed headspace strategy made it possible to obtain simpler and cleaner olfactograms than those obtained in previous studies, in which extracts were obtained by solid-phase extraction of wine (*20*, *22*). Despite that, more than 90 different odorants were detected during the experiment, but differences in GC−O scores were much higher than those previously observed, which facilitates the ranking of odorants attending to their potential importance. In the present case, the recorded GC−O signal takes into account not only the evaluation of intensity, but also the frequency of detection of an odorant. This can be done now because a large number of odorants are at concentrations near the threshold, and the differences in individual sensitivity between members of the tasting panel become very important.

For the sake of simplicity, those odorants not reaching a maximum GC−O score of 30% in any of the six studied wines were eliminated and considered as noise. After this operation, the number of odorants was reduced to 31. Results of the study are presented in **Table 2**. As can be seen, only five odorants remain unknown, and none of them reaches high GC−O scores. Most of the compounds present in the table are well-known wine aroma components and have been detected in previous GC−O studies, although there are some points that should be commented on. As can be seen, the GC−O table is enriched in the most volatile and least polar compounds, and only two acids, one phenol, and one aldehyde are found, which contrasts with the GC−O lists obtained by direct extraction (*20*, *22*). The high scores of the three alkyl-methoxypyrazines were also a surprise, since these components were hardly detected in any of the previous GC−O experiments (*20*, *22*).

GC−O data were ranked and processed by statistical analysis. The results of the study are presented in **Table 3**. The odorants can be classified into two main categories attending to the average GC−O score they reach. The first category is formed by all those odorants with high scores in all the wine samples. These odorants constitute the base of the aroma of these wines. Leaving aside β-damascenone, methoxypyrazines, and (Z)-3-hexenol, all these compounds are byproducts of alcoholic fermentation. The second category is formed by odorants with low average GC−O scores. Here compounds from very different origins are found. However, a second and most important classification criterion is the potential ability of the odorant to introduce sensory differences between samples. There are different indicators of such ability, such as the olfactometric range (max−min), or the significance of the effect of the factor wine measured through ANOVA or χ^2 tests. The three parameters are included in the table, and the three lead to more or less similar conclusions. As can be seen, the most discriminant compounds are at the lower part of the table, and the least discriminant ones can be found at the top. The table also shows that χ^2 statistics are more sensitive to detect differences in this set of data, particularly for compounds near the threshold.

Whichever the indicator used, there are two compounds showing outstanding potential discriminant power: linalool and 3-mercaptohexyl acetate. Both components have relatively low average GC−O score but can reach very high scores (more than

Table 3. Ranking of the Odorants by Average Modified Frequency Percentage[a]

compound	mean	max	max−min	χ^2	ANOVA
A. Odorants with Average GC–O Score Higher Than 40					
ethyl hexanoate	81	87	10	*e*	*e*
isoamyl alcohol	80	83	10	*e*	*e*
isoamyl acetate	77	80	11	*e*	*e*
ethyl butyrate	76	80	11	*e*	*e*
2,3-butanodione	73	84	30	*e*	*c*
ethyl 3-methylbutyrate	68	72	7	*e*	*e*
ethyl 2-methylbutyrate	63	72	18	*e*	*e*
β-damascenone	57	68	19	*e*	*e*
3-isobutyl-2-methoxy-pyrazyne	50	63	39	*d*	*b*
3-isopropyl-2-methoxy-pyrazyne	49	62	46	*d*	*b*
β-phenylethyl alcohol	49	62	22	*e*	*e*
2-/3-methylbutyric acid	49	57	16	*e*	*e*
2-methyl-3-furanthiol	45	75	48	*d*	*e*
acetic acid	44	59	46	*d*	*b*
isobutanol	43	53	16	*e*	*e*
isobutyl acetate	42	63	43	*d*	*b*
unknown 973	42	48	22	*e*	*e*
(*Z*)-3-hexenol	40	55	35	*d*	*e*
B. Odorants with Average GC–O Score Lower Than 40					
acetylpyrazine	37	48	26	*c*	*e*
2-phenylethyl acetate	29	50	43	*d*	*e*
linalool	24	66	66	*d*	*d*
4-mercapto-4-methyl-2-pentanone	23	40	29	*d*	*e*
3-mercaptohexyl acetate	18	63	63	*d*	*d*
m-cresol	18	32	32	*d*	*b*
unknown 1746	18	32	32	*d*	*e*
2-phenylethanal	16	38	38	*d*	*e*
3-*sec*-butyl-2-methoxy-pyrazine	14	41	41	*d*	*c*
hexyl acetate	12	31	31	*d*	*e*
unknown 1204	11	36	36	*d*	*c*
unknown 1259	7	34	34	*d*	*c*
unknown 1871	7	32	32	*d*	*d*

[a] Statistically significant differences measured by ANOVA or χ^2 tests. [b] Significance at $p < 0.05$. [c] Significance at $p < 0.01$. [d] Significance at $p < 0.001$. [e] No significant difference.

60%) in some wine samples. As can be seen in **Table 2**, there are some wines in which such compounds were not even detected. Other odorants with important potential discriminant power are 2-methyl-3-furanthiol, acetic acid, isobutyl and 2-phenylethyl acetates, and the 3-alkyl-methoxypyrazines. Many other compounds at the bottom of the table also seem to be very discriminant. However, in these cases GC−O scores are low for all samples, which means that, most likely, the effective contribution of such compounds to the sensory differences between samples will not be substantial.

Modeling Sensorial Descriptors from Olfactometric Composition. Models were built on the basis of the algorithm PLSR1, which deals with only one *Y* variable at a time, for the five most important sensory descriptors (tropical fruit, citric, floral, muscat, sweet). The models were carefully built following an iterative process. First, the 31 odorants listed in **Table 2** were considered. In a second step, the least discriminant odorants (attending to data in **Table 3**) were eliminated, and the models were run again. In subsequent iterations, the models were again run, looking for the best prediction ability with the minimum number of variables. The best results were obtained by direct correlation between the modified frequency of the sensory attribute and the modified frequency of the olfactometric data without any transformation other than centering. Satisfactory models could be built for four of the five more important descriptors. The quality parameters of such models can be seen in **Table 4**, and the loading weights of the different odorants finally included in the models are shown in **Table 5**. As can be seen in **Table 4**, in all the cases the explained variance (always calculated by cross-validation) is higher than 70%. It is noteworthy that only seven compounds were introduced in the models and that, in fact, only linalool and 3-mercaptohexyl acetate, previously identified as the potentially most discriminant odorants, have high loading weights. Three other compounds identified by GC−O as potentially discriminant are also present in the models (2-phenethyl acetate, 3-*sec*-butyl-2-methoxypyrazine, and acetic acid). It may be observed that there is a close similarity between the compounds in **Table 5** and the compounds discussed in the previous paragraph.

Table 4. Quality Parameters of the PLSR Models Relating Discriminant Sensory Notes with GC–O Scores

sensory attribute	%EV[a]	RMSEP[b]	*m*[c]	CC[d]	no. *X*[e]	no. PC[f]
muscat	87	8.3	0.95	0.92	4	3
sweet	84	8.9	0.80	0.89	3	1
floral	79	10.4	0.71	0.84	4	2
tropical fruit	74	9.8	0.55	0.79	3	1

[a] Percentage of variance explained by the model. [b] Root-mean-square prediction error. [c] Slope of the regression curve between real and predicted *Y* variables. [d] Correlation coefficient between real and predicted *Y* variables. [e] Number of *X* variables in the model. [f] Number of principal components in the model.

Table 5. Loading Weights of the Odorants Included in the Different PLSR Models Explaining a Sensory Attribute as a Function of GC–O Scores

compound	tropical fruit	floral	sweet	muscat
unknown 1260		0.29		0.39
acetic acid				–0.51
3-*sec*-butyl-2-methoxy-pyrazine	–0.37			
linalool		0.77	0.76	0.53
3-mercaptohexyl acetate	0.84	–0.42	–0.50	–0.56
unknown 1746	–0.41			
2-phenylethyl acetate		0.34	0.42	

A second observation is that the models for floral, sweet, and muscat show a similar structure, since in all of them linalool is the most important positive contributor and 3-mercaptohexyl acetate is the most important negative contributor. This result is not surprising, given the similarity of these three aroma nuances and the high correlation found between their sensory scores. In any case, the models suggest that the intensity of the floral, sweet, and muscat notes of a wine are directly correlated to the wine content in linalool and inversely correlated to the wine content in 3-mercaptohexyl acetate. These three sensory descriptors are somehow opposed to the tropical fruit descriptor. The model for this last term suggests that the intensity of this odor nuance in a wine depends primarily on the content of the wine in 3-mercaptohexyl acetate.

Sensory Evaluation−Validation of PLSR models. The results obtained by PLSR evidence the existence of a strong correlation between the sensory attributes and the GC−O scores. However, such correlation may be by chance, and therefore, the results must be validated by sensory analysis. In the present work, different kinds of sensory tests have been carried out. First, a series of triangle tests were run to verify whether the addition of a given amount of odorant causes a detectable sensory change in the aroma of wine. In a second stage, the effect of the addition of a given amount of odorant on the

Table 6. Concentrations of the Odorants Taking Part in the PLSR Models in the Six Monovarietal Wines

compound	ALB	GOD	MAL	PAR	TRE	VER
acetic acid (mg L^{-1})	119	400	380	215	290	370
3-*sec*-butyl-2-methoxy-pyrazine (ng L^{-1})	4	*a*	*a*	*a*	*a*	*a*
linalool (μg L^{-1})	94	6	17	4	104	5
3-mercaptohexyl acetate (ng L^{-1})	31	115	43	25	20	750
2-phenylethyl acetate (μg L^{-1})	528	405	212	324	673	314

[a] Lower than the detection limit.

sensory score of the different aroma nuances was measured by a specifically trained panel.

Floral−Sweet Model. Both models are very similar, although an unknown compound has a small loading in the model for the floral note. As the role of such unknown compound cannot be evaluated, both models can be considered to be equivalent for validation and will be discussed together. The models suggest that both sensory notes are positively related to the presence of linalool and 2-phenylethyl acetate and negatively affected by the presence of 3-mercaptohexyl acetate. The main aim of the triangle tests carried out was to demonstrate whether these three odorants at the concentration found in the set of studied wines (see **Table 6**) can effectively cause any sensory effect.

The results of these tests are shown in **Table 7**. The effect of linalool was clear at the highest concentration (104 μg L^{-1}, experiment 1), whereas its effect was not appreciated by the assessors at the lowest concentration assayed (experiment 2). In contrast, the addition of 2-phenylethyl acetate did not bring about any sensory change (experiments 3, 4, and 5). The combined addition of the acetate and linalool (at lowest concentration) did not have any effect either (experiment 6). However, because 2-phenylethyl acetate is correlated with another odorant of the same family, isoamyl acetate, we decided to check whether the addition of the two odorants had any effect. The level of isoamyl acetate added (570 μg L^{-1}) corresponds to the smallest amount of this odorant found in the set of wines, and at this concentration, its characteristic banana aroma was not recognized. Results in the table indicate that the simultaneous addition of 2-phenylethyl acetate and isoamyl acetate was significantly detected by the test panel but only if the level of linalool is low (experiments 7 and 8), which shows that, at high

Table 7. Triangular Tests to Check the Potential Importance of a Given Odorant at a Given Concentration

experiment	sample 1	sample 2	significance
	Effect of Linalool		
1	base **(1)**	**(1)** + [104] linalool	*b*
2	base **(1)**	**(1)** + [10.4] linalool	*e*
	Effect of Acetates		
3	base **(1)**	**(1)** + [673] 2-phenylethyl acetate	*e*
4	base + [104] linalool **(2)**	**(2)** + [673] 2-phenylethyl acetate	*e*
5	base + [10.4] linalool **(3)**	**(1)** + [673] 2-phenylethyl acetate	*e*
6	base **(1)**	**(1)** + [10.4] linalool + [673] 2-phenylethyl acetate	*e*
7	base + [10.4] linalool **(3)**	**(3)** + [673] 2-phenylethyl acetate + [570] isoamyl acetate	*b*
8	base + [104] linalool **(2)**	**(2)** + [673] 2-phenylethyl acetate + [570] isoamyl acetate	*e*
	Effect of 3-Mercaptohexyl Acetate		
9	base + [104] linalool + [673] 2-phenylethyl acetate **(4)**	**(4)** + [0.750] 3-mercaptohexyl acetate	*d*
10	base + [104] linalool + [673] 2-phenylethyl acetate **(4)**	**(4)** + [0.115] 3-mercaptohexyl acetate	*d*
	Effect of Acetic Acid		
11	base + [104] linalool + [673] 2-phenylethyl acetate + **(4)**	**(4)** + 400 mg/L acetic acid	*b*
12	base + [104] linalool + [673] 2-phenylethyl acetate + **(4)**	**(4)** + 100 mg/L acetic acid	*e*
	Effect of 3-*sec*–Butyl-2-methoxypyrazine		
13	base + [0.058] 3-mercaptohexyl acetate **(5)**	**(5)** + [0.004] *sec*-butyl pyrazine	*e*
14	base + [0.058] 3-mercaptohexyl acetate **(5)**	**(5)** + [0.010] *sec*-butyl pyrazine	*c*
15	base + [0.500] 3-mercaptohexyl acetate **(6)**	**(6)** + [0.010] *sec*-butyl pyrazine	*e*
16	base + [0.500] 3-mercaptohexyl acetate **(6)**	**(6)** + [0.040] *sec*-butyl pyrazine	*c*
	Effect of Alkyl-methoxypyrazines		
17	base + [0.058] 3-mercaptohexyl acetate **(5)**	**(5)** + [0.025] mixture pyrazines	*c*
18	base + [0.500] 3-mercaptohexyl acetate **(6)**	**(6)** + [0.025] mixture pyrazines	*e*
19	base + [0.500] 3-mercaptohexyl acetate **(6)**	**(6)** + [0.040] mixture pyrazines	*d*

[a] Concentrations, expressed as μg L^{-1}, are presented in brackets. [b] Significance at $p < 0.05$. [c] Significance at $p < 0.01$. [d] Significance at $p < 0.001$. [e] No significant difference.

Table 8. Ranking Tests (Page Test) Carried Out To Check the Potential Negative Influence of a Given Odorant in the Perception of a Sensory Note

1. Muscat Character ($n = 8$)[a]

original sample	additions			*p*-value
base + 104 μg L^{-1} linalool + 673 μg L^{-1} 2-phenylethyl acetate	+0 mg L^{-1} acetic acid	+100 mg L^{-1} acetic acid	+400 mg L^{-1} acetic acid	
summatory of ranks	20	18	10	<0.001

2. Tropical Fruit Character ($n = 7$)[a]

original sample	additions				*p*-value
base + 500 ng L^{-1} 3-mercaptohexyl acetate	+0 ng L^{-1} mixture pyrazines	+10 ng L^{-1} mixture pyrazines	+20 ng L^{-1} mixture pyrazines	+40 ng L^{-1} mixture pyrazines	
summatory of ranks	22	22	15	11	<0.01
base + 50 ng L^{-1} 3-mercaptohexyl acetate	+0 ng L^{-1} mixture pyrazines	+10 ng L^{-1} mixture pyrazines	+20 ng L^{-1} mixture pyrazines	+40 ng L^{-1} mixture pyrazines	
summatory of ranks	26	23	12	9	<0.001

[a] *n* indicates the number of assessors.

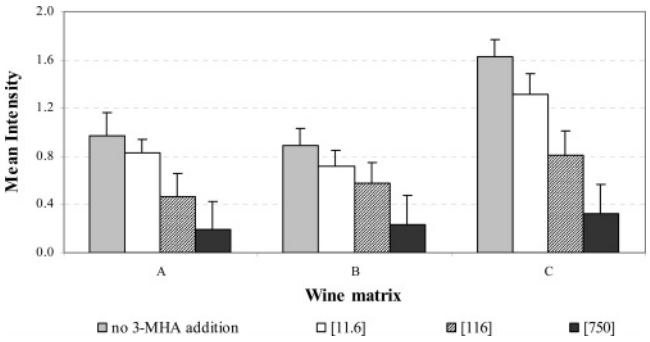

Figure 2. Effect of 3-mercaptohexyl acetate addition in the intensity of the floral–sweet note perception in three wine matrixes (A, B, and C) containing different linalool and 2-phenylethyl acetate concentrations. Concentrations of 3-mercaptohexyl acetate, expressed as ng L^{-1}, are presented in brackets. Error bars are calculated as $s/(n)^{1/2}$; s = standard deviation; n = number of responses.

concentrations, linalool is able to mask the sensory effect of both acetates. This set of experiments demonstrates that linalool is a key odorant, while 2-phenylethyl acetate must be associated with other compounds with sweet aroma to be an effective contributor. Finally, to check the sensory effect of 3-mercaptohexyl acetate, two different concentrations of this compound were added to a wine containing linalool and 2-phenylethyl acetate. The addition of the thiol at any concentration was easily detected by the panel, as can be seen in **Table 7** (experiments 9 and 10).

In the following experiment, a panel specifically trained to quantify the intensity of the floral−sweet note evaluated its odor intensity in 12 different samples containing different amounts of the three compounds involved in the PLSR model: linalool, 2-phenylethyl acetate, and 3-mercaptohexyl acetate. The experiment was designed in such a way that four different levels of thiol concentration were added to three different wine matrixes (A, B, and C) containing different concentrations of linalool and 2-phenylethyl acetate. Matrix C had the maximum content of linalool (104 μg L^{-1}) and of 2-phenylethyl acetate (673 μg L^{-1}) found in the set. Matrix B contained 10 times and 2 times less linalool and 2-phenylethyl acetate, respectively. Matrix A was the dearomatized wine used for the experiment.

The mean intensities of the floral−sweet note quantified in the 12 wines are shown in **Figure 2**. The results are in accordance with the PLSR model. The floral−sweet note increased with the level of linalool and 2-phenylethyl acetate (but only at the highest level) and decreased in all the cases with increasing amounts of 3-mercaptohexyl acetate. As is shown in **Figure 2**, the mean intensity of the floral−sweet note in the matrix C sample decreased to 50% with the addition of 116 ng L^{-1} of 3-mercaptohexyl acetate and to 80% when 750 ng L^{-1} was added. In all the cases, the changes in intensity were significant, as the ANOVA study revealed (data not shown).

Muscat Model. This model is very similar to the two previously analyzed ones but, apparently, the muscat note is negatively affected by the presence of acetic acid. This was checked by adding different amounts of acetic acid to model solutions containing linalool and 2-phenylethyl acetate (experiments 11 and 12 in **Table 7**). The addition of 400 mg L^{-1} of acetic acid (the level found in Godello) was significantly detected by the panel, but the addition of 100 mg L^{-1} did not have any effect. In another test, the panel was asked to rank these same samples (without and with 100 or 400 mg L^{-1} acetic acid) attending to the intensity of the muscat aroma. The results of the test, presented in **Table 8**, confirmed that the intensity of muscat aroma is lower in the samples containing high levels of acetic acid, as was suggested by the model.

Tropical Fruit Model. The tropical fruit model suggests that the intensity of this nuance is positively related to the wine content in 3-mercaptohexyl acetate and negatively related to its content in 3-*sec*-butyl-2-methoxypyrazine and in the unknown 1746. In an aforementioned experiment, it was possible to establish that the addition of 3-mercaptohexyl acetate had a highly significant effect. In addition, the intensity of the tropical fruit note was found to increase as the concentration of this compound increased, as is shown in **Figure 3**. These two results confirm the positive relationship between this note and 3-mercaptohexyl acetate.

The role played by 3-*sec*-butyl-2-methoxypyrazine was studied in different tests. First, triangle tests similar to those previously described were carried out. As it is shown in **Table 7**, the addition of the level of this compound found in wine, 4 ng L^{-1}, did not bring about any sensory change (experiment 13). The addition of 10 ng L^{-1} of 3-*sec*-butyl-2-methoxypyrazine exerted a clear effect only if the concentration of 3-mercaptohexyl acetate is low (experiments 14 and 15), and the addition of 40 ng L^{-1} exerted the effect independently of the concentration of the thiol (experiments 16 and 17). These experiments do not completely confirm the influence of this pyrazine as suggested by the model, since the levels assayed exceeded by far the levels found in wine. However, because an additive or synergic effect of the three alkyl-methoxypyrazines seems likely, the experiment was repeated with a mixture of

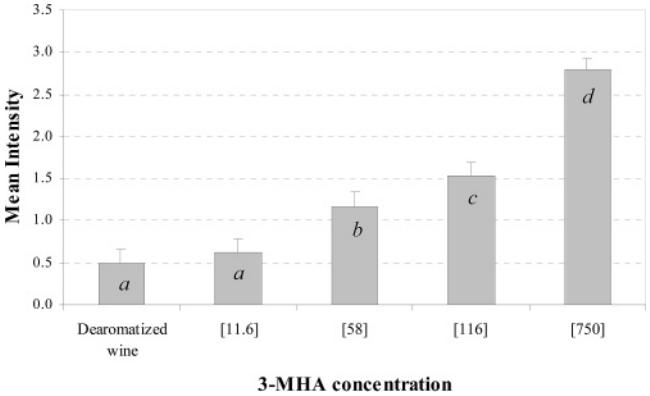

Figure 3. Sensory quantification results for tropical fruit quantification. Concentrations of 3-mercaptohexyl acetate, expressed as ng L^{-1}, are presented in brackets. Error bars are calculated as $s/(n)^{1/2}$; s = standard deviation; n = number of responses. Means marked with different letters are significantly different ($p < 0.05$).

the three pyrazines at the concentrations found in Albariño wine (4, 3, and 18 ng L^{-1} of the *sec*-butyl, isopropyl, and isobutyl isomers, respectively). The results now confirm that the amount of alkyl-methoxypyrazines found in the wine is enough to alter the aroma of the wine if the concentration of 3-mercaptohexyl acetate is not high (experiments 17 and 18). Interestingly, at these levels, the typical peppery-earthy odor of pyrazines was not noted. At 500 ng L^{-1} of the thiol, the amount of pyrazines must be increased more than 50% to exert a clear effect (experiment 19). To verify whether such effects were exerted specifically on the tropical fruit note, a ranking test was proposed in which the panel was asked to rank, attending to the intensity of the tropical fruit note, different solutions containing 3-mercaptohexyl acetate and alkyl-methoxypyrazines. The results are shown in **Table 8** and clearly show that the higher the level of these compounds, the lesser the tropical fruit note.

SUMMARY AND CONCLUSIONS

The dynamic headspace GC−O technique used in this work makes it possible to easily rank the odorants of wines attending to intensity or discriminant-ability criteria. The GC−O profiles of six Spanish monovarietal white wines have been defined, and two odorants, linalool and 3-mercaptohexyl acetate, have been found to have the maximum potential discriminant ability. Models based on PLSR make it possible to explain and even predict the intensity of four sensory descriptors of these wines as a function of GC−O scores. The tropical fruit character is related to the presence of 3-mercaptohexyl acetate and is negatively affected by the presence of alkyl-methoxypyrazines. The sweet, floral, and muscat characters are due mainly to linalool and secondarily to the presence of 2-phenylethyl acetate but are negatively affected by the presence of 3-mercaptohexyl acetate. Acetic acid also decreases the intensity of the muscat note. These results make it possible to understand the varietal characteristics of the aroma of these wines. A fifth important sensory descriptor of these wines, the term citric, could not be modeled with our GC−O data set, a limitation that will have to be further investigated.

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