RAPID SENSORY-DIRECTED METHODOLOGY FOR THE SELECTION OF HIGH QUALITY AROMA WINES

María-Pilar Sáenz-Navajas, Yohanna Alegre, Arancha de-la-Fuente, Vicente Ferreira, David García, Sara Eizaguirre, Iosu Razquin and Purificación Hernández-Orte

"Laboratorio de Análisis del Aroma y Enología (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), Associate unit to Instituto de las Ciencias de la Vid y el Vino (ICVV) (UR-CSIC-GR), c/ Pedro Cerbuna 12, 50009 Zaragoza, Spain

“Lev2050. Poligono Mocholi Plaza CEIN, 5 Nave B13 31110 Noáin, Navarra, Spain

Running title: Sensory-directed screening methodology for the selection of wine yeasts

*corresponding author: Purificación Hernández-Orte (puhernan@unizar.es)
Abstract

BACKGROUND: The present work contributes by developing a rapid sensory-directed methodology for the screening and selection of high quality wines with different sensory profiles. Therefore, Verdejo and Tempranillo musts were fermented with 50 different yeasts each under controlled laboratory conditions. Resulting samples were firstly categorized according to five levels of quality by a panel of wine professionals. Higher quality samples were described by flash profiling by a semi-trained panel and most distinctive samples were screened by gas chromatography-olfactometry (GC-O).

RESULTS: Seven Verdejo and five Tempranillo samples were classified in the highest quality category, presenting different aroma profiles such as citrus, fruit in syrup, boxtree/vegetal, tropical or wet grain aromas for Verdejo and red fruit or fruit in syrup for Tempranillo. β-damascenone, 3-mercaptohexyl acetate and ethyl butyrate appeared as distinctive quality compounds linked to dried, tropical and red fruit aromas, respectively.

CONCLUSIONS: Categorization task followed by flash profiling and GC-O analysis has revealed to be a rapid and effective sensory-directed methodology for the screening of distinctive and quality wine aroma profiles in a case study of yeast selection. Wine industry could benefit from the use of this methodology as a complementary tool for optimizing technical processes along elaboration.

Key words: sensory, categorization task, flash profiling, GC-O
INTRODUCTION

Sensory science works at the service of food industry in that it aims at developing methodologies able to characterize and measure product quality towards product features. Among strategies employed for evaluating product quality, categorization task has been successfully applied to identify quality exemplars based on expert’s judgements. This task consists in classifying samples in different predetermined categories and measures distances between expert’s quality prototypes (stored in their memory and based on previous experience), and the exemplars tasted. Besides the identification of products linked to quality perception, finding the sensory drivers of quality is essential for the food industry in general and the wine industry in particular. Descriptive sensory methodologies are the most powerful tools used in sensory discipline as they generate descriptive data explaining sensory differences among samples and thus the distinctive sensory character of the final product. Traditional descriptive methods are time and money-consuming mainly due to the long training period that is usually needed for developing vocabulary, references and reaching consensus in the use of descriptors. Thus, there is a trend in food sensory science to develop less time-consuming and more flexible methodologies. These methods tend to replace trained panelists by non-trained consumers based on the assumption that panelists do not differ in their perceptions but solely in the way they describe them. These methods allow consumers to choose and use their own vocabulary without being trained in the use of descriptors, which deem faster, and more cost-effective than classical conventional descriptive analysis. Another advantage of carrying out descriptive analysis with non-trained consumers is that the vocabulary they generate is often easily interpreted, which facilitates communication between marketing and scientific departments. Among alternative methods, flash profiling is able to provide a product map in a very short time; however the interpretation of sensory terms describing samples is sometimes difficult as they are freely generated by consumers and no consensus in their definition is reached. This absence of consensus can be partly overcome by carrying out the task with semi-trained
panelists instead of naïve consumers, which in most cases provide descriptive profiles similar to non-trained consumers and thus easier to understand and interpret than those of trained panels, and at the same time they are likely to generate descriptions easier to interpret than the consumer panel. In wine industry, the intrinsic quality of the product, which is related to its organoleptic properties, is dependent on both grape composition and technology used during wine making. During this process, the selection of the suitable fermentation yeast strain is one of the most important factors that affect the flavor quality of the final product. In the present time, there is a widely spread tendency among winemakers to inoculate musts with industrial Saccharomyces yeasts. This practice has the advantages of assuring reliable and rapid fermentations and reducing the risks of spoilage and unpredictable changes of wine flavor. However, the massive culture of commercial yeasts during winemaking can led to the loss of characteristic aromas attributed to certain spontaneous fermentations. The dominance of spontaneous non-saccharomyces yeasts during the early stages of alcoholic fermentation has been associated with the generation of both positive distinctive aromas and negative off-odors. Positive odor compounds such as fruity esters, acetates or varietal aromas such as norisoprenoids, terpenoids or mecaptans (released from their odorless precursor due to β-glucosidase and β-liase activity of yeasts) have been related to the presence of non-saccharomyces yeasts. At the same time, certain strains appearing in uncontrolled fermentations have also been found to produce undesirable off-odors such as acetaldehyde, acetic acid, ethyl acetate, higher alcohols, diacetyl (by oxidation of acetoin) or negative sulfur compounds such as hydrogen sulfide. Hence, the selection of non-saccharomyces yeasts producing positive aromas under controlled conditions deems important. The low tolerance of this nonconventional yeasts to alcohol concentration, usually leads to stuck fermentations. For ensuring complete alcoholic fermentation mixed inoculations of selected strains of these species with S. cerevisiae are usually employed. Hence, inoculating mixed cultures of yeasts generating quality aromas has
been revealed as an interesting tool in the wine industry for limiting the potential uniformity of aromatic characteristics of final wine and gaining in sensory complexity25-29.

Selection criteria of non-saccharomyces yeasts producing quality aromas are usually based on their capacity to produce individual volatile compounds with positive aroma descriptors. Therefore, a limited number of individual volatiles with known either positive sensory activity, such as esters or acetates, or negative such as acetaldehyde, volatile acids or negative such as higher alcohols, are usually quantified and their contribution to overall wine flavor is discussed based on their concentration12,30-32. This methodology is bound to lose important information related to impact aroma compounds, because it is limited to the study of a reduced list of volatiles ignoring others. Besides, the sensory role of individual compounds based on their concentration is often misinterpreted. As an example, the rose-like higher alcohol β-phenylethanol has been suggested to contribute positively to the floral aroma of wines12,30 and thus yeasts producing higher amounts of it are reported to be superior exemplars. However, studies carried out in our laboratory in complex matrices, have demonstrated that the presence of this compound at concentrations (of even 300 mg L\(^{-1}\)) higher than their sensory threshold (14 mg L\(^{-1}\)) do not have any significant sensory role in the overall wine flavor33,34. This suggests that most usual methodologies aimed at finding quality aromas based on the quantification of a limited number of volatiles would either misinterpret or lose valuable information, especially when optimizing any technical process (such as the selection of the appropriate fermentative yeast) during wine elaboration. Thus, the implementation of sensory strategies able to directly measure the sensory impact of the product on consumer perception can improve the identification of quality exemplars and provide valuable information to producers.

In this context, the present work aimed at developing a rapid sensory-directed methodology for the screening and selection of wine samples, with diverse aroma profiles, generated by a wide range of yeasts based on their capacity to generate quality and distinctive aromas. For this purpose, the methodological approach combined categorization task for the selection of quality exemplars.
followed by descriptive flash profiling with GC-O analysis for identifying chemical odorants driving main sensory differences among wine samples and related to quality aroma profiles.

**MATERIAL AND METHODS**

**Yeast strains**

Forty-eight *non-Saccharomyces* strains from the yeast culture collection of LEV2050 (Pamplona, Spain) were used for red and white grape fermentation. All of them were non-commercial. Along with *non-saccharomyces* yeasts, two *Saccharomyces* strains were used as reference for red (R18-R20) and white musts (W38, W39). Thus, a total of one-hundred yeast strains were studied (50 for red and 50 for white musts).

**Microvinification process**

The study was carried out with Tempranillo red grapes and Verdejo white grapes collected during October 2013 from DOCa Rioja and DO Rueda regions in Spain, respectively. Tempranillo grapes were frozen at -20ºC until the fermentation process was carried out. Verdejo grapes were removed from the stems, crushed, and pressed. Then, the must was sulfited (3 g hL⁻¹), racked off and stored at -20 ºC until winemaking.

Tempranillo red grapes and Verdejo white musts were defrosted at room temperature during 48h. Tempranillo whole clusters were pressed, sulfited with *potassium metabisulfite* (4 g hL⁻¹) and distributed to the different tanks. Red must was firstly supplemented with *diammonium phosphate* to reach 180 mg L⁻¹ of yeast *assimilable* nitrogen content to avoid nitrogen deficiencies during alcoholic fermentation as recommended in literature. White must was not supplemented as it already contained 245 mg L⁻¹. Then, musts were distributed to 2-liter-containers equipped with vent bungs. Prior to inoculation, they were pasteurized and controlled (inoculation in YM-agar plates during 48h at 28ºC) to assure the dominance during fermentation of the strains object of study. Pasteurized musts were inoculated at the rate of 10⁶ cfu mL⁻¹.
A total of one hundred fermentations in duplicate (50 with red must and the same number for whites) were carried out at 20ºC and 16ºC for red and white wines, respectively. Fermentations were controlled by measuring the content in reducing sugars by refractometry. Alcoholic fermentation took place in the range of 4-8 and 3-11 days, for red and white musts, respectively. Once fermentation concluded (no variation in refractive index in two consecutive days), samples were stored at 4 ºC during 48h to permit the sedimentation of gross lees and then were racked off again. The sulfur dioxide content was adjusted to reach 30 mg L⁻¹ of free SO₂ and samples were stored at 5 ºC to favor the sedimentation of fine lees. Finally, duplicated samples were mixed before being bottled and stored at 4 ºC until sensory and chemical analyses.

Sensory analysis

Experimental conditions.

White and red wine samples were separately submitted to two different sensory tasks. Firstly a categorization task was carried out to select exemplars with higher aroma quality according to a panel of wine experts. These samples were further sensory described (flash profiling) by a panel of semitrained panelists. All assessments were conducted in individual tasting booths. Sensory analysis tasks were carried out in January 2015 in Zaragoza (Spain) at Laboratorio de Análisis del Aroma y Enología (LAAE).

Pre-selection of yeast strains. Categorization task (CT)

White samples. Seventeen Spanish wine experts (51.8% men and 48.2% women from 19 to 67 years, median = 39.5) living in Zaragoza area took part in the study. They fitted the category of wine-science researchers and teaching staff who were regularly involved in wine-making and/or wine evaluation. They were all considered wine experts according to Parr, Heatherbell specifications. The seventeen wine experts participated in two sessions within the same day (one at 10 a.m. and the second at 16 p.m.). Twenty-seven white samples were included in each session: 25 different samples together with two control commercial wines (HQ_W and LQ_W presented in
both sessions). These control samples were presented in both sessions for examining panel performance. LQ_W was a neutral white wine spiked with 70 mg L$^{-1}$ of acetaldehyde to generate a low-quality wine (acetaldehyde is related to wine oxidation), while HQ_W was a commercial wine expected to have higher aroma quality than LQ_W. A total of fifty-four white samples (sessions 1 and 2) were evaluated in terms of aroma quality by the panel of wine experts. The seventeen participants had to examine in each session 27 samples exclusively in terms of orthonasal aroma quality and sort them in five quality groups: “very high”, “high”, “average”, “low” or “very low”. These five categories were easily interpretable by participants. Once they had formed the groups on the table, participants were provided with a pencil and a sheet in order to write down their responses. Then, participants were asked to associate to each of the five groups a maximum of 2-3 attributes. Participants were presented with the following instructions:

“Twenty-seven glasses of young white wines are presented on the table. Each glass is coded by a three-digit number. You are asked to orthonasally smell the twenty-seven wines firstly from left to right and then to form five groups (according to the following categories: very high, high, average, low or very low) on the table according to your perceived aroma quality”.

Red samples. Fifteen Spanish wine experts (wine-science researchers and teaching staff of LAAE) (40% men and 60% women from 25 to 74 years, median = 36.8) living in Zaragoza area took part in the study. Fifty-one samples were categorized in terms of aroma quality by the 15 wine experts. Two sessions within the same day (one at 10 a.m. and the second at 16 p.m.) were devoted to this task. Twenty-five and 26 samples were included in the first and second sessions, respectively. Within each session the same two control wines (HQ_R, LQ_R) were included to control panel performance. LQ_R was a red wine spiked with 70 mg L$^{-1}$ of acetaldehyde to generate a red wine lower in quality than HQ_R (commercial red wine).

As for white samples, participants had to examine in each session 25 or 26 samples exclusively in terms of orthonasal aroma quality and sort them in five quality groups: “very high”, “high”,

Red samples. Fifteen Spanish wine experts (wine-science researchers and teaching staff of LAAE) (40% men and 60% women from 25 to 74 years, median = 36.8) living in Zaragoza area took part in the study. Fifty-one samples were categorized in terms of aroma quality by the 15 wine experts. Two sessions within the same day (one at 10 a.m. and the second at 16 p.m.) were devoted to this task. Twenty-five and 26 samples were included in the first and second sessions, respectively. Within each session the same two control wines (HQ_R, LQ_R) were included to control panel performance. LQ_R was a red wine spiked with 70 mg L$^{-1}$ of acetaldehyde to generate a red wine lower in quality than HQ_R (commercial red wine).
“average”, “low” or “very low”. Then, participants were asked to associate to each of the five groups a maximum of 2-3 attributes.

In both cases, one hour before formal tasting, samples were removed from the 5 °C cold room and twenty-mL of samples were served in dark approved wine glasses (ISO 3591, 1977) labelled with 3-digit random codes and covered by plastic Petri dishes according to a random arrangement different for each assessor. All samples were served at room temperature and were evaluated in individual booths. Panelists were not informed about the nature of the samples to be evaluated. They were only told that they were either young white or young red wines.

**Descriptive analysis. Flash profiling (FP)**

Samples mostly included in both “high” and “very high” quality categories in the categorization task (nine white and seven red samples) were further submitted to descriptive analysis by means of flash profiling methodology.

**Participants.** A total of fifteen staff members (51.8% men and 48.2% women from 19 to 67 years, median = 39.5) from the Laboratory for Analysis of Aroma and Enology (LAAE) completed two sessions in different days (one for white and one for red wine analysis). They were semi-trained assessors with experience in sensory description of wine.

**Samples.** For white samples, the descriptive task was carried out with a total of 13 samples: nine selected in the categorization task and two blind control samples (RS_W and LM_W both commercial white wines elaborated with Verdejo) in duplicate for examining panel performance. For red wines, descriptive task was carried out with a total of 11 red samples: seven selected in the categorization task and two blind commercial control samples (BJ_R and LM_R elaborated with Grenache and Tempranillo, respectively) in duplicate for examining panel performance.

**Procedure.** Flash profiling (for both white and red wines) involved two sessions separated by an inter-session. In the first session the 13 white samples (or the 11 samples for red samples) were
presented simultaneously to each assessor. They were firstly given an explanation about the procedure. Then, they were asked to individually generate the aroma descriptors that differentiated the wine set. They were asked to avoid hedonic terms and to use exclusively descriptive terms. They were free to generate as many attributes as they wanted and to take as much time as needed.

During the inter-session, the experimenter pooled all the generated attributes to form a global list that was provided to the assessors in the second session. This global list was presented as an aid tool to allow assessors to update their own list if desired but it was not aimed at reaching a consensus. With this global list they could either add to their list a few terms they thought were relevant but did not generate themselves or replace some of their own terms by terms they thought were more adapted. In the second session, assessors were asked to rank order the 13 white samples (or the 11 red samples) on each of their chosen attributes. Sensory attributes were evaluated using a non-structured 10 cm continuous length scale anchored with the words “absence” and “high intensity” on the left and right ends, respectively, being ties allowed. All samples were presented simultaneously attending to a random order different for each assessor. Twenty-mL samples were presented in dark approved wine glasses (ISO 3591, 1977) labelled with 3-digit random codes and covered by plastic Petri dishes. All samples were served at room temperature and evaluated in individual booths. Panelists were not informed about the nature of the samples to be evaluated.

**GC-O study**

Wines selected in the categorization task (9 white and 7 red wines) were submitted to GC-O analysis.

**Samples, reagents and standards**

Dichloromethane, HPLC quality was from Fisher Scientific (Loughborough, UK), methanol was LiChrosolv quality from Merck (Darmstadt, Germany), absolute ethanol, ACS quality, was purchased from Panreac (Barcelona, Spain), pure water was obtained from a Milli-Q purification system (Millipore, USA). LiChrolut EN resins were obtained from Merck (Darmstadt, Germany).
The standards used for identifications were supplied by Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany), ChemService (West Chester, PA), Fluka (Buchs, Switzerland), Sigma (St. Louis, MO), PolyScience (Niles, IL), Lancaster (Strasbourg, France), Alfa Aesar (Karlsruhe, Germany), Panreac (Barcelona, Spain), SAFC (Steinheim, Germany), and Oxford Chemicals (Hartlepool, U.K.). β-Damascenone was a gift from Firmenich (Geneva, Switzerland).

Solid phase extraction (SPE): Direct extraction of wine aroma

Total wine extracts were obtained by direct solid phase extraction (SPE) as described by Lopez, Aznar with some modifications. Therefore, 100 mL of sample was passed through commercial cartridges of 100 mg of resin LiChrolut EN. Aroma extracts were obtained by elution with 1 mL of ethanol. Extracts were stored at -20ºC until GC-O analyses.

Reconstitution of aroma extracts.

Total wine aroma extracts were reconstituted in synthetic wine (5 g L⁻¹ of tartaric acid and 9% ethanol, pH 3.2 and 3.5 for white and red wines, respectively) by adding one mL of extract to 99 mL of synthetic wine.

Preparation of wine extracts.

A dynamic headspace sampling technique designed to obtain representative extracts for olfactometry analysis was used to capture wine aroma. Therefore, a standard SPE cartridge (0.8 cm internal diameter, 3 mL internal volume) filled with 400 mg of LiChrolut EN resins was first washed with 20 mL of dichloromethane and then dried by letting air pass through (negative pressure of 0.6 bar, 10 min). The Lichrolut EN cartridge was placed on the top of a bubbler flask near the liquid surface (80 mL of reconstituted wine), which was continuously stirred with a magnetic stir bar and kept at a constant temperature of 37 ºC by immersion in a water bath. A controlled stream of nitrogen (500 mL min⁻¹) was passed through the sample for 100 min. The volatile wine constituents released in the headspace were trapped in the cartridge containing the
sorbent. After 100 min, the cartridge was removed and dried by letting N\textsubscript{2} pass through; then, analytes were eluted with 3.2 mL of dichloromethane with 5% methanol. After this, the extract was concentrated under a stream of pure nitrogen to a final volume of 200 µL.

**Gas chromatography-olfactometry (GC-O)**

GC-O analyses with the extracts prepared were carried out with a Trace GC gas chromatograph (ThermoQuest, Milan, Italy) with a flame ionization detector (FID) and a sniffing port ODO-I from SGE (Ringwood, Australia). The capillary column used was a DB-WAX (polyethylenglycol) supplied by J&W (Folsom, CA, USA), 30 m x 0.32 mm i.d. x 0.5 mm film thickness, and a deactivated precolumn (3 m x 0.32 mm i.d.) from Supelco (Bellefonte, PA). Hydrogen was used as carrier gas at a constant flow rate of 3.5 mL min\textsuperscript{-1}.

The injection was conducted in splitless mode (60 s splitless time). The injection volume was 1 µL. The injector and detector temperature was 250 °C. The sniffing port was heated using a thermostat made in the laboratory to prevent the condensation of high boiling point compounds, and it was equipped with a humidifier of deionized water. The temperature program used for analysis of the sample was 40 °C for 2 min, increased by 20 °C min\textsuperscript{-1} to 130 °C and then 4 °C min\textsuperscript{-1} to 220 °C, maintaining this temperature for 10 min.

The olfactometric analysis was carried out by a panel of 6 trained judges (66% women and 34% men from 23 to 29 years, median = 26 years) belonging to the laboratory staff. Each olfactometry was performed in one 25-min session (within the range of 3-28 min of the GC-O). Sniffers indicated the time, description and odor intensity when an aroma was detected. A 7-point structured category scale was used for measuring perceived odor intensity (anchored with 0 = not detected; 1 = weak odor, 2 = clear odor; 3= extremely strong odor), and allowing intermediate values (0.5, 1.5 and 2.5).

Olfactometric analyses were also performed on a blank. This blank was prepared by boiling 80 mL of synthetic wine (5 g L\textsuperscript{-1} of tartaric acid and 9% ethanol, pH 3.2 and 3.5 for white and red wines,
respectively) in the system described in the sample preparation section and the headspace extraction was performed in the same way as for the samples.

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

Data analysis

Categorization task (CT)

The number of times each wine was classified by participants in each of the five quality groups was counted. Three categories were finally considered in data analysis for simplifying the presentation of results. The “very high” and “high” as well as “low” and “very low” quality categories were jointly considered. Data were encoded in a wine (50) x quality level (3) contingency table, in which each cell represented the frequency of the categorization of a wine in one category level. Correspondence Analysis (CA) was performed on the contingency table. Hierarchical Cluster Analysis (HCA) with the Ward criteria was finally applied to all the factors derived from CA. The quality category (“very high/high”, “average” and “low/very low”) best defining the resulting clusters were identified by computing their probability of characterizing a cluster. Analyses were carried out with SPAD software (version 5.5).

Flash profiling (FP)

Individual assessors’ rank data were firstly collected in a matrix built for each participant (wines in rows and terms in columns). The global data matrix formed by the individual matrices generated by the 15 assessors was submitted to Generalized Procruster Analysis (GPA). Descriptors mentioned by at least three assessors (20% of the panel) were used to visualize the relationships between samples and attributes. Analyses were carried out with XLSTAT software (version 2014.2.02).

GC-O data
The GC-O data were processed taking into account the frequency of citation (F) and the intensity of each odor zone (I), obtaining the modified frequency percentage (% MF) from the formula given by Dravnieks:

\[ \%MF = \sqrt{\%F \times \%I} \]

where F(\%) is the detection frequency of an aromatic stimulus expressed as a percentage and I(\%) is the average intensity expressed as percentage of the maximum intensity. For the sake of simplicity, those odorants not reaching a maximum GC-O score of 30% MF in any of the studied samples were eliminated and considered as noise.

A two-way analysis of variance (ANOVA) in which sample was the fix factor and judges random factor was performed on the intensity scores of each of the olfactory areas for assessing their discrimination ability. Further Fischer’s post-hoc pairwise comparisons (95\%) were carried out for significant effects.

RESULTS AND DISCUSSION

Sensory analysis

Categorization task (CT)

Panel control. Control samples (for whites: HQ_W1/HQ_W2 and LQ_W1/LQ_W2; for reds: HQ_R1/HQ_R2 and LQ_R1/LQ_R2) were included in the categorization task aimed at (i) evaluating panel reproducibility and (ii) covering a relatively wide range of aroma quality for evaluating panel discrimination ability. Concerning reproducibility, in both cases, duplicated samples (for whites: HQ_W1/HQ_W2 and LQ_W1/LQ_W2; for reds: HQ_R1/HQ_R2 and LQ_R1/LQ_R2) presented in different sessions were projected close together in the maps (Figures 1 and 2), which suggests that the panel was globally reproducible. With regard to discrimination ability of the panel, LQ_W1/LQ_W2 for white and LQ_R1/LQ_R2 for red sample sets were wines spiked with 70 mg L\(^{-1}\) of acetaldehyde to decrease aroma quality, while samples HQ_W1/HQ_W2...
and HQ_R1/HQ_R2 were commercial wines in absence of defaults. For white wines, samples LQ_W1/LQ_W2 were included in the low/very low quality category, while HQ_W1/HQ_W2 in the high/very high quality group, which would demonstrate the discrimination ability in terms of aroma quality of the panel of experts. For red wines, both pairs of samples (LQ_R1/LQ_R2 and HQ_R1/HQ_R2) were mainly classified in the average quality group, but they were differently perceived in terms of quality as HQ_R1/HQ_R2 were included by 47% of experts in the highest quality category, while LQ_R1/LQ_R2 by 23% of participants. Thus, even if control samples did not show important quality differences, the discrimination ability of the panel can be confirmed as there were samples such as T39 (included by 100% of the panel in the very low/low quality category) and R20_sacch (included by 93% of the panel in the high/very high quality category) which were clearly classified in different quality categories (Figure 2). This suggests that the panel of experts was able to classify in different quality categories both white and red wines with different aroma quality, which confirmed the discrimination ability of the panel for both sample sets.

Categorization task. In both sample sets (white and red wines), the first dimensions of the CA maps (Figures 1 and 2), which represent most variability, could be interpreted as the quality perceived by experts. Wines mostly included in the highest quality category (very high/high) are projected on the right part of the plot and just in the opposite side are samples categorized in the lowest quality group (very low/low). In the middle of the plot are samples belonging to the “average” quality category. According to hierarchical cluster analysis (HCA) calculated on all the CA factors, 54% of white wine samples belonged to the lowest quality category, 26% to “average” and 20% were categorized in the highest quality group (W20, W50, W12, W33, W36, W47, W39_sacch, W38_sacch, W34 and two control samples: HQ_W1, HQ_W2) as it can be observed in Figure 1. For red wines (Figure 2), less number of samples than for white wines was included in the lowest quality group (30% for red vs 54% for white wines). Most red samples were included in the average
quality cluster (57%), while only 13% of samples formed part of the very high/high quality category (R45, R24, R22, R27, R47, R19_sacch, R20_sacch).

It is worth mentioning, that for white wines, both reference samples fermented with *S. cerevisiae* (W39_sacch, W38_sacch) were mainly included in the highest quality category. Similarly, for red wines, two out of the three samples (R19_sacch, R20_sacch) inoculated with *S. cerevisiae* formed part of the high/very high quality group.

Attributes. After categorization task, participants were instructed to cite a maximum of three terms describing samples belonging to each of the quality categories, which allowed having a raw association of quality categories and aroma descriptors. Results showed that most cited (>20% of the panel) attributes for the highest quality category of white wines were *fruit* (41%), *tropical fruit* (41%) and *floral* (35%) and for red wines, *lactic* and *caramel* (both cited by 33% of experts), followed by *fresh fruit*, *red fruit*, *strawberry*, *banana*, *floral* and *toffee* (all of them cited by 27% of the panel). On the contrary, *dirty aroma* (41%), followed by *sewer* (24%) and *reduction* (24%) were mainly used for characterize white wines within the lowest quality category and *rotten eggs-hydrogen sulfide* (40%), *reduction* (33%) and *sewer* (27%) for red samples. These results are supported confirm the suggested that the presence of reductive-related aroma were common in low quality exemplars of both white and red sample sets.

*Flash profiling (FP)*

Leaving aside control samples used in the previous sensory task, nine white and seven red wines categorized in the highest quality group were further submitted to orthonasal descriptive analysis by means flash profiling with a panel of semi-trained assessors.

Generation of attributes. In the first session, wine experts quickly generated their own list of discriminant attributes (in less than 30 min in all cases) given their familiarity with wine aroma description, citing between 3 and 15 attributes for both white and red wines. For the second session, assessors retained between 3 and 10 attributes in both cases. For a total of 99 and 83 sensory terms,
49 and 33 terms were semantically different for white and red wines, respectively. Among them, 8 for white wines (Table 1) and 12 for red wines (Table 2) were used by at least three assessors (20% of the panel), being fruit in syrup (53% of assessors), tropical fruits (47%), citrus fruits (40%), and banana (40%) the attributes mostly cited for white samples and red fruit (47%), fruit in syrup (47%) and caramel-toffee (47%) for red samples. Interestingly, the term fruit in syrup appeared in both sample sets.

It is interesting to point out that most attributes cited in categorization task were further used in flash profiling, even if lower number of descriptive terms, and less specific, were generated in CT than in FP. This difference was especially important for white sample set. Thus, in categorization only three terms were cited by at least 20% of the panel (fruit, tropical fruit and floral), while eight in flash profiling (Table 1). Among them, the fruity category, which involved exclusively fruity and tropical fruit in white sample categorization, it was unfolded into five different terms in flash profiling (fruit in syrup, tropical fruit, citrus, banana and apple). In both sample sets, new attributes appeared in flash profiling, which were not cited in categorization. This could be attributed to the fact that in FP the sensory space was more specific (only samples included in the highest quality category) than in CT, where samples ranging from very low to very high quality were evaluated.

Ranking. It is noteworthy that for the sample set of white wines, participants declared that ranking the samples for all attributes was difficult. However, for the group of red wines (carried out with the same participants and one week later), they stated that the task was easier mainly because they had already develop their own strategy for performing flash profiling. This could illustrate the difficulty of performing this sensory task for the first time. Notwithstanding all participants finished the ranking task in less than 60 minutes for both white and red sample sets, suggesting that it is a feasible task for describing wine samples by semitrained judges.

Figures 3a and 4a show the projection of white wine samples on the first and second principal components of the GPA maps representing, respectively, 58% and 13% of the original variance for
white wines, and 54% and 27% for described red wines. Control samples presented in duplicate (white wines: MR_1/MR_2 and LMW_1/LMW_2; red wines: BJ_1/BJ_2 and LMR_1/LMR_2) are plotted close together in the map, which suggests that the panel can be globally considered as repeatable.

Hierarchical cluster analysis (HCA) calculated on all the GPA dimensions yielded two main groups of white samples: Cluster 1 and Cluster 2. The first component (PC1) opposed both groups (Figure 5a). Cluster 1 (positive values of PC1) was formed by the four commercial wines (MR_1, MR_2, LMW_1, LMW_2), two samples fermented with commercial *S.cerevisiae* yeasts (W38, W39) and one sample fermented with *non-saccharomyces* (W20). According to Figure 3b, the panel agreed in mainly attributing a *citrus fruit* aroma character to this group of samples. Cluster 2 (negative values of PC1) was composed of exclusively samples fermented with *non-saccharomyces* yeasts (W47, W33, W36, W50, W12 and W34). According to Figure 3b, these samples were consensually characterized by the following terms: *fruit in syrup* and *tropical fruits*. Even if samples belonging to cluster 2 shared these aroma attributes, they were spread along PC2, being sample W47 opposed to W34 and W12. Sample W47 was described with less sweet aromas such as *fruit in syrup*, while with more fresh character such as *box tree-vegetal* (J12) and *green-herbal* (J1). Contrary, W34 and W12 would have sweeter nuances related to *fruit in syrup*. Samples W33, W36 and W50, which were plotted close in the map (Figure 3a) were also characterized by the term *wet grain* as can be observed in Figure 3b.

The projection of red wine samples on the first two dimensions of the GPA and the projection of most cited terms are shown in Figures 4a and 4b, respectively. Cluster analysis yielded three groups of samples. Cluster 1 was formed by the four commercial wines used as control samples: BJ_1/BJ_2 and LM_1/LM_2. Attending to the descriptions shown in Figure 4b, the panel agreed in describing samples BJ_1/BJ_2 as *spicy*, which seems logical as these samples were aged in oak barrels which could contribute to this nuance. On the contrary, sample LM_1/LM_2 did not seem to
be clearly associated to any of the generated descriptors as no term is located close to it (Figure 4b). This supports the idea that this wine was selected to be a quite neutral sample in terms of aroma properties. The second cluster, formed by two samples fermented with commercial \textit{S.cerevisiae} yeasts (R19\textsubscript{sacch} and R20\textsubscript{sacch}), was plotted on the top part of the map (positive values for PC2) and associated to attributes such as \textit{strawberry yogurt}, \textit{red fruits} and \textit{toffee} (Figure 4b). The third cluster, which is formed by five samples fermented with \textit{non-saccharomyces} yeasts (R22, R24, R47, R45, R27), presented common aroma nuances related to \textit{fruit in syrup}, \textit{white fruits} and \textit{banana}. Within this cluster of samples, sample R22 acquired the lowest value of PC1, which would suggest that this last sample was less intense in these sweet aromas. Besides, it is the unique sample within the cluster that was located in the positive direction of PC2, suggesting that it was richer in aromas related to \textit{red fruits} and \textit{strawberry yogurt} than the rest of samples belonging to this cluster. All this showed that among the \textit{non-saccharomyces} samples, R22 would yield the most different aroma profile showing intermediate characteristics between \textit{saccharomyces} and \textit{non-saccharomyces} samples.

**GC-O analysis**

Most different aroma profiles yielded by the yeasts object of study were further characterized by GC-O. Among white samples, five exemplars were analyzed: W39\textsubscript{sacch} (\textit{citrus fruit}), W20 (\textit{citrus fruit}), W47 (\textit{box tree and tropical fruits}), W36 (\textit{fruit in syrup, tropical fruit} and \textit{wet grain}) and W12 (\textit{fruit in syrup}). The following red samples were submitted to GC-O analyses: R20\textsubscript{sacch} (\textit{strawberry yogurt, red fruit} and \textit{toffee}), R22 (\textit{red fruit, strawberry yogurt, fruit in syrup, white fruits} and \textit{banana}), R24, R45 and R27 (\textit{fruit in syrup, white fruits} and \textit{banana}).

A summary of the results from the GC-O analysis of both white and red wines can be seen in Tables 3 and 4, respectively. Twenty-six odorants for white wines and 31 for red wines have been identified. Eight compounds for white and eleven for red samples presented %MF significantly different (P<0.05) among wines. Besides, two compounds for white and three for red samples were
close to significance, showing a trend (P<0.1). The difference between the maximum MF and the minimum (max-min) can be taken as a criterion for differentiability. Compounds reaching values above 50% in this parameter and presenting significant (P<0.05) or close to significance (P<0.1) differences among the studied wines are marked in bold letters in the corresponding column of Tables 3 and 4. Seven compounds for white (three fruity esters: ethyl butyrate, isoamyl acetate and ethyl hexanoate; one pyrazine: 2,6-dimethylpyrazine; two sulphur-derived compounds: 2-furfurylthiol and 3-mercaptohexyl acetate and one norisoprenoid: β-damascenone) and six for red wines (ethyl butyrate, ethyl hydrocinnamate, phenyl acetate, isoamyl acetate, isobutanol, and one non-identified compound: n.i. 1458). Two-dimensional PCA plots calculated with the average %MF (of scores given by the 6 sniffers) for each compound and sample are shown in Figures 3 and 4, for white and red samples, respectively.

For white samples, Figure 5 shows that data derived from GC-O were able to differentiate samples (similar projection of samples) as did sensory profiling (Figure 3a). The first PC of Figure 5, which explained almost 60% of variability, confronted samples W39_sacch and W20 (positive values on PC1) from samples W47, W36 and W12 (negative values on PC1). The first group of samples (W39 and W20), which were mainly characterized with fresh fruity aroma (citrus fruit) according to flash profiling, were richer in two linear fruity-like esters (ethyl butyrate and hexanoate), 2,6-dimethylpyrazine (described with terms such as roasted, spicy, bready and barbecue by the panel of sniffers) and 2-furfurylthiol (roasted/coffee-like odor). It is difficult to explain the citrus character of these samples by the presence of exclusively these compounds, which would indicate that more complex sensory interactions are involved in the formation of such fresh character. The sweet character (such as fruity in syrup) attributed to samples plotted on the left part of Figure 5 (W47, W36, W12) could be explained by the presence of β-damascenone. This norisoprenoid has been demonstrated to be involved in the formation of the sweet-fruity aroma (and contrary to fresh aroma) of wines41. The second PC, explaining 22% of variability, was mainly driven by 3-
mercaptohexyl acetate-3MHA- (Figure 5), which could explain the fact that sample W47 (higher value on PC2) was described with a more fresh character (boxtree-vegetal or green-herbal) than samples W36 and especially W12. This volatile thiol was already demonstrated to be responsible of the tropical fruit and box tree character of white and rosé wines at concentrations above 50 ng L⁻¹. In line with these results, differences in concentration of 3MHA have been already attributed to different yeasts.

For red samples, as for white wines, Figure 6 shows that data derived from GC-O were able to differentiate samples (similar projection of samples) as did sensory profiling (Figure 4a). The first PC of Figure 6, which retained more than 42% of variability, separated samples R20 (Saccharomyces yeast) and R22 (non-saccharomyces) from the rest of samples (R27, R45, R24), all of them being the result of fermentation with non-saccharomyces yeasts. In Table 4 it can be observed that samples R20_sacch and R22 presented higher MF values for ethyl butyrate (strawberry aroma), which could explain their distinctive red-fruitly aroma described by the panel of experts. These samples were confronted to the sweet aroma (fruit in syrup) characterizing the other three non-saccharomyces samples (R27, R45, R24). These samples presented high MF values for the sweet-like compounds such as isoamyl acetate and phenylethyl acetate (R24 and R27), which could the responsible for their fruit in syrup aroma.

CONCLUSIONS

Categorization task followed by flash profiling and GC-O analysis has revealed to be a fast and effective sensory-directed methodology for the selection of high quality aroma wines. This method allowed identifying seven Verdejo and five Tempranillo samples fermented with different non-saccharomyces yeasts and producing high quality aroma profiles according to a panel of Spanish wine professionals. Among quality exemplars, different aroma profiles could be identified such as citrus, fruit in syrup, boxtree/vegetal, tropical or wet grain aromas for Verdejo and red fruit or fruit in syrup for Tempranillo. GC-O analyses identified β-damascenone, 3-mercaptohexyl acetate and
ethyl butyrate as distinctive quality compounds linked to dried, tropical and red fruit aromas, respectively.

This sensory-directed methodology is presented as an effective and rapid tool in the screening and characterization of quality aroma profiles. The wine industry could benefit from the use of this methodology as a complementary tool for identifying and characterizing quality exemplars obtained under different technical procedures.

Acknowledgements

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References


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34. Escudero A, Gogorza B, Melus MA, Ortin N, Cacho J, Ferreira V. Characterization of the aroma of a wine from Macabeo. Key role played by compounds with low odor activity values.


Figure captions

Figure 1. Projection of the three quality categories (low/very low, average and very high/high) and 52 white samples on the bi-dimensional CA map yielded from the categorization task based on orthonasal aroma quality perception of a panel of experts. Cluster 1: low/very low quality represented by a dot; cluster 2: average quality represented by a triangle and cluster 3: very high/high quality.

Figure 2. Projection of the three quality categories (low/very low, average and very high/high) and 51 red samples on the bi-dimensional CA map yielded from the categorization task based on orthonasal aroma quality perception of a panel of experts. Cluster 1: low/very low quality represented by a dot; cluster 2: average quality represented by a triangle and cluster 3: very high/high quality.

Figure 3. Projection of a) white samples (samples belonging to Cluster 1 and Cluster 2 are represented with different symbols) and b) individual descriptors (given by each of the 15 judges: J1-J15) on the consensus space obtained using Generalised Procruster Analysis (GPA) over the aroma profile derived from flash profiling.

Figure 4. Projection of a) red samples (samples belonging to Cluster 1 and Cluster 2 are represented with different symbols) and b) individual descriptors (given by each of the 15 judges: J1-J15) on the consensus space obtained using Generalised Procruster Analysis (GPA) over the aroma profile derived from flash profiling.

Figure 5. Projection of selected white samples and compounds derived from GC-O analysis

Figure 6. Projection of selected red samples and compounds derived from GC-O analysis
Figure 3.
Figure 4

(a)

- R20_saccharomyces
- R19_saccharomyces
- BJ_1
- BJ_2
- R22
- LM_1
- LM_2
- R24
- R47
- R45
- R27

F1 (54.35 %)
F2 (26.55 %)
Figure 5

- 3-mercaptohexyl acetate
- 6-damascenone
- Isoamyl acetate
- 2,6-dimethylpyrazine
- Ethyl butyrate
- Ethyl hexanoate
- 2-furfurylthiol

F1 (59.67 %)
F2 (22.04 %)
Figure 6

- isoamyl acetate
- ethyl dihydrocinnamate
- phenylethyl acetate
- ethyl butyrate
- isobutanol
- n.i.1458
- R20
- R24
- R27
- R45
- R22

F1 (42.06 %)

F2 (33.39 %)
Table 1. Frequency of citation (expressed as %) of attributes rated by at least 20% of judges in the flash profiling task carried out with the 13 white wine wines selected in the categorization task

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<thead>
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<td>tropical fruits</td>
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<td>citrus fruits</td>
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</tr>
<tr>
<td>banana</td>
<td>40%</td>
</tr>
<tr>
<td>boxtree-vegetal</td>
<td>27%</td>
</tr>
<tr>
<td>wet grain-hay</td>
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</tr>
<tr>
<td>green-herbal</td>
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<td>apple</td>
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Table 2. Frequency of citation (expressed as %) of attributes rated by at least 20% of judges in the flash profiling task carried out with the 11 red wines selected in the categorization task

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</tr>
<tr>
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<td>banana</td>
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<td>vegetal-green</td>
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<td>white fruit</td>
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<td>alcohol</td>
<td>20%</td>
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<tr>
<td>dried fruit</td>
<td>20%</td>
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Table 3. Odorants identified by GC-O in the five white wines selected. Gas chromatographic data, olfactory description, chemical identity, modified frequency (MF) expressed as %, significance (P) and maximum %MF for each compound. Compounds in bold letters present significant differences (P<0.1) according to two-way ANOVA (samples as fix factor and judges as random factor) and maximum minus minimum values >50% (max-min >50%). Different letters indicate significant differences according to Fischer post-hoc test.

<table>
<thead>
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<th>LRI</th>
<th>Odour description</th>
<th>Chemical identity</th>
<th>W39</th>
<th>W20</th>
<th>W47</th>
<th>W36</th>
<th>W12</th>
<th>P</th>
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</table>

* LRI Linear retention index on polar capillary column (DB-WAX)

n.i. Not identified (compound did not produce any clear signal in the mass spectrometer)
Table 4. Odorants identified by GC-O in the five red wines selected. Gas chromatographic data, olfactory description, chemical identity, modified frequency (MF) expressed as %, significance (P) and maximum %MF for each compound. Compounds in bold letters present significant differences (P<0.1) according to two-way ANOVA (samples as fixed factor and judges as random factor). Different letters indicate significant differences according to Fischer post-hoc test.

<table>
<thead>
<tr>
<th>LRI</th>
<th>Odour description</th>
<th>Chemical identity</th>
<th>R20</th>
<th>R22</th>
<th>R24</th>
<th>R45</th>
<th>R27</th>
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<tbody>
<tr>
<td>978</td>
<td>Butter, fruity, strawberry</td>
<td>diacetyl+ethyl isobutyrate</td>
<td>40</td>
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<td>14</td>
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*LRI Linear retention index on polar capillary column (DB-WAX)

n.i. Not identified (compound did not produce any clear signal in the mass spectrometer)