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

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

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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[1]. Among strategies employed for evaluating product quality, categorization task has been successfully applied to identify quality exemplars based on expert´s judgements[2]. 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[3]. 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[4,5]. 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[6]. These methods allow consumers to choose and use their own vocabulary without being trained in the use of descriptors[7], which deem faster, and more cost-effective[4,5] 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[7]. Among alternative methods, flash profiling[8] 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 can 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[7].

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[9]. 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[10]. 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[11,12], acetates[13] or varietal aromas such as norisoprenoids, terpenoids or mecaptans (released from their odorless precursor due to β-glucosidase and β-liase activity of yeasts)[14,15] 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[16], acetic acid[17], ethyl acetate[18], higher alcohols[12], diacetyl (by oxidation of acetoin)[19] or negative sulfur compounds such as hydrogen sulfide[20,21]. 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[22-24]. 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 complexity[25-29].

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 concentration[12,30-32]. 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 wines[12,30] 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 flavor[33,34]. 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[35]. 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 10^6 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 SO_2 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[36] 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

158 both sessions). These control samples were presented in both sessions for examining panel
159 performance. LQ_W was a neutral white wine spiked with 70 mg L^{-1} of acetaldehyde to generate a
160 low-quality wine (acetaldehyde is related to wine oxidation), while HQ_W was a commercial wine
161 expected to have higher aroma quality than LQ_W. A total of fifty-four white samples (sessions 1
162 and 2) were evaluated in terms of aroma quality by the panel of wine experts. The seventeen
163 participants had to examine in each session 27 samples exclusively in terms of orthonasal aroma
164 quality and sort them in five quality groups: ‘‘very high’’, ‘‘high’’, “average”, ‘‘low’’ or ‘‘very
165 low’’. These five categories were easily interpretable by participants. Once they had formed the
166 groups on the table, participants were provided with a pencil and a sheet in order to write down
167 their responses. Then, participants were asked to associate to each of the five groups a maximum of
168 2-3 attributes. Participants were presented with the following instructions:

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

173 *Red samples.* Fifteen Spanish wine experts (wine-science researchers and teaching staff of LAAE)
174 (40% men and 60% women from 25 to 74 years, median = 36.8) living in Zaragoza area took part in
175 the study. Fifty-one samples were categorized in terms of aroma quality by the 15 wine experts.
176 Two sessions within the same day (one at 10 a.m. and the second at 16 p.m.) were devoted to this
177 task. Twenty-five and 26 samples were included in the first and second sessions, respectively.
178 Within each session the same two control wines (HQ_R, LQ_R) were included to control panel
179 performance. LQ_R was a red wine spiked with 70 mg L^{-1} of acetaldehyde to generate a red wine
180 lower in quality than HQ_R (commercial red wine).

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

183 “average”, ‘‘low’’ or ‘‘very low’’. Then, participants were asked to associate to each of the five
184 groups a maximum of 2-3 attributes.

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

191 *Descriptive analysis. Flash profiling (FP)*

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

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

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

205 *Procedure.* Flash profiling (for both white and red wines) involved two sessions separated by an
206 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 nonstructured 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).

232 The standards used for identifications were supplied by Aldrich (Steinheim, Germany),Merck
233 (Darmstadt,Germany), ChemService (West Chester, PA), Fluka (Buchs, Switzerland), Sigma (St.
234 Louis, MO), PolyScience (Niles, IL), Lancaster (Strasbourg, France), Alfa Aesar (Karlsruhe,
235 Germany), Panreac (Barcelona, Spain), SAFC (Steinheim, Germany), and Oxford Chemicals
236 (Hartlepool, U.K.). β-Damascenone was a gift from Firmenich (Geneva, Switzerland),.

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

238 Total wine extracts were obtained by direct solid phase extraction (SPE) as described by Lopez,
239 Aznar[37] with some modifications. Therefore, 100 mL of sample was passed through commercial
240 cartridges of 100 mg of resin LiChrolut EN. Aroma extracts were obtained by elution with 1 mL of
241 ethanol. Extracts were stored at -20ºC until GC-O analyses.

242 *Reconstitution of aroma extracts.*

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

246 *Preparation of wine extracts.*

247 A dynamic headspace sampling technique designed to obtain representative extracts for
248 olfactometry analysis was used to capture wine aroma[38]. Therefore, a standard SPE cartridge (0.8
249 cm internal diameter, 3 mL internal volume) filled with 400 mg of LiChrolut EN resins was first
250 washed with 20 mL of dichloromethane and then dried by letting air pass through (negative
251 pressure of 0.6 bar, 10 min). The Lichrolut EN cartridge was placed on the top of a bubbler flask
252 near the liquid surface (80 mL of reconstituted wine), which was continuously stirred with a
253 magnetic stir bar and kept at a constant temperature of 37 ºC by immersion in a water bath. A
254 controlled stream of nitrogen (500 mL min^{-1}) was passed through the sample for 100 min. The
255 volatile wine constituents released in the headspace were trapped in the cartridge containing the

256 sorbent. After 100 min, the cartridge was removed and dried by letting N_2 pass through; then,
257 analytes were eluted with 3.2 mL of dichloromethane with 5% methanol. After this, the extract was
258 concentrated under a stream of pure nitrogen to a final volume of 200 µL.

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

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

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

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

279 Olfactometric analyses were also performed on a blank. This blank was prepared by boiling 80 mL
280 of synthetic wine (5 g L^{-1} of tartaric acid and 9% ethanol, pH 3.2 and 3.5 for white and red wines,

281 respectively) in the system described in the sample preparation section and the headspace extraction
282 was performed in the same way as for the samples.

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

285 **Data analysis**

286 *Categorization task (CT)*

287 The number of times each wine was classified by participants in each of the five quality groups was
288 counted. Three categories were finally considered in data analysis for simplifying the presentation
289 of results. The "very high" and "high" as well as "low" and "very low" quality categories were
290 jointly considered. Data were encoded in a wine (50) x quality level (3) contingency table, in which
291 each cell represented the frequency of the categorization of a wine in one category level.
292 Correspondence Analysis (CA) was performed on the contingency table. Hierarchical Cluster
293 Analysis (HCA) with the Ward criteria was finally applied to all the factors derived from CA. The
294 quality category ("very high/high", "average" and "low/very low") best defining the resulting
295 clusters were identified by computing their probability of characterizing a cluster[39]. Analyses were
296 carried out with SPAD software (version 5.5).

297 *Flash profiling (FP)*

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

303 *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[40]:

$$\%MF = \sqrt{\%F \; x \; \%I}$$

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

351 quality cluster (57%), while only 13% of samples formed part of the very high/high quality category
352 (R45, R24, R22, R27, R47, R19_sacch, R20_sacch).

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

357 Attributes. After categorization task, participants were instructed to cite a maximum of three terms
358 describing samples belonging to each of the quality categories, which allowed having a raw
359 association of quality categories and aroma descriptors. Results showed that most cited (>20% of
360 the panel) attributes for the highest quality category of white wines were *fruit* (41%), *tropical fruit*
361 (41%) and *floral* (35%) and for red wines, *lactic* and *caramel* (both cited by 33% of experts),
362 followed by *fresh fruit*, *red fruit*, *strawberry*, *banana*, *floral* and *toffee* (all of them cited by 27% of
363 the panel). On the contrary, *dirty aroma* (41%), followed by *sewer* (24%) and *reduction* (24%) were
364 mainly used for characterize white wines within the lowest quality category and *rotten eggs-*
365 *hydrogen sulfide* (40%), *reduction* (33%) and *sewer* (27%) for red samples. These results are
366 supported confirm the suggested that the presence of reductive-related aroma were common in low
367 quality exemplars of both white and red sample sets.

368 *Flash profiling (FP)*

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

372 Generation of attributes. In the first session, wine experts quickly generated their own list of
373 discriminant attributes (in less than 30 min in all cases) given their familiarity with wine aroma
374 description, citing between 3 and 15 attributes for both white and red wines. For the second session,
375 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

401 white wines, and 54% and 27% for described red wines. Control samples presented in duplicate
402 (white wines: MR_1/MR_2 and LMW_1/LMW_2; red wines: BJ_1/BJ_2 and LMR_1/LMR_2) are
403 plotted close together in the map, which suggests that the panel can be globally considered as
404 repeatable.

405 Hierarchical cluster analysis (HCA) calculated on all the GPA dimensions yielded two main groups
406 of white samples: Cluster 1 and Cluster 2. The first component (PC1) opposed both groups (Figure
407 5a). Cluster 1 (positive values of PC1) was formed by the four commercial wines (MR_1, MR_2,
408 LMW_1, LMW_2), two samples fermented with commercial *S.cerevisiae* yeasts (W38, W39) and
409 one sample fermented with *non-saccharomyces* (W20). According to Figure 3b, the panel agreed in
410 mainly attributing a *citrus fruit* aroma character to this group of samples. Cluster 2 (negative values
411 of PC1) was composed of exclusively samples fermented with *non-saccharomyces* yeasts (W47,
412 W33, W36, W50, W12 and W34). According to Figure 3b, these samples were consensually
413 characterized by the following terms: *fruit in syrup* and *tropical fruits*. Even if samples belonging to
414 cluster 2 shared these aroma attributes, they were spread along PC2, being sample W47 opposed to
415 W34 and W12. Sample W47 was described with less sweet aromas such as *fruit in syrup*, while
416 with more fresh character such as *box tree-vegetal* (J12) and *green-herbal* (J1). Contrary, W34 and
417 W12 would have sweeter nuances related to *fruit in syrup*. Samples W33, W36 and W50, which
418 were plotted close in the map (Figure 3a) were also characterized by the term *wet grain* as can be
419 observed in Figure 3b.

420 The projection of red wine samples on the first two dimensions of the GPA and the projection of
421 most cited terms are shown in Figures 4a and 4b, respectively. Cluster analysis yielded three groups
422 of samples. Cluster 1 was formed by the four commercial wines used as control samples:
423 BJ_1/BJ_2 and LM_1/LM_2. Attending to the descriptions shown in Figure 4b, the panel agreed in
424 describing samples BJ_1/BJ_2 as *spicy*, which seems logical as these samples were aged in oak
425 barrels which could contribute to this nuance. On the contrary, sample LM_1/LM_2 did not seem to

426 be clearly associated to any of the generated descriptors as no term is located close to it (Figure 4b).
427 This supports the idea that this wine was selected to be a quite neutral sample in terms of aroma
428 properties. The second cluster, formed by two samples fermented with commercial *S.cerevisiae*
429 yeasts (R19_sacch and R20_sacch), was plotted on the top part of the map (positive values for PC2)
430 and associated to attributes such as *strawberry yogurt*, *red fruits* and *toffee* (Figure 4b). The third
431 cluster, which is formed by five samples fermented with *non-saccharomyces* yeasts (R22, R24,
432 R47, R45, R27), presented common aroma nuances related to *fruit in syrup*, *white fruits* and
433 *banana*. Within this cluster of samples, sample R22 acquired the lowest value of PC1, which would
434 suggest that this last sample was less intense in these sweet aromas. Besides, it is the unique sample
435 within the cluster that was located in the positive direction of PC2, suggesting that it was richer in
436 aromas related to *red fruits* and *strawberry yogurt* than the rest of samples belonging to this cluster.
437 All this showed that among the *non-saccharomyces* samples, R22 would yield the most different
438 aroma profile showing intermediate characteristics between *saccharomyces* and *non-saccharomyces*
439 samples.

440 **GC-O analysis**

441 Most different aroma profiles yielded by the yeasts object of study were further characterized by
442 GC-O. Among white samples, five exemplars were analyzed: W39_sacch (*citrus fruit*), W20 (*citrus*
443 *fruit*), W47 (*box tree* and *tropical fruits*), W36 (*fruit in syrup*, *tropical fruit* and *wet grain*) and W12
444 (*fruit in syrup*). The following red samples were submitted to GC-O analyses: R20_sacch
445 (*strawberry yogurt*, *red fruit* and *toffee*), R22 (*red fruit*, *strawberry yogurt*, *fruit in syrup*, *white*
446 *fruits* and *banana*), R24, R45 and R27 (*fruit in syrup*, *white fruits* and *banana*).

447 A summary of the results from the GC-O analysis of both white and red wines can be seen in Tables
448 3 and 4, respectively. Twenty-six odorants for white wines and 31 for red wines have been
449 identified. Eight compounds for white and eleven for red samples presented %MF significantly
450 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 wines[41]. The second PC, explaining 22% of variability, was mainly driven by 3-

476 mercaptohexyl acetate-3MHA- (Figure 5), which could explain the fact that sample W47 (higher
477 value on PC2) was described with a more fresh character (boxtree-vegetal or green-herbal) than
478 samples W36 and especially W12. This volatile thiol was already demonstrated to be responsible of
479 the tropical fruit and box tree character[42] of white and rosé wines at concentrations above 50 ng L^{-1}.
480 In line with these results, differences in concentration of 3MHA have been already attributed to
481 different yeasts[43].

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

493 **CONCLUSIONS**

494 Categorization task followed by flash profiling and GC-O analysis has revealed to be a fast and
495 effective sensory-directed methodology for the selection of high quality aroma wines. This method
496 allowed identifying seven Verdejo and five Tempranillo samples fermented with different *non-*
497 *saccharomyces* yeasts and producing high quality aroma profiles according to a panel of Spanish
498 wine professionals. Among quality exemplars, different aroma profiles could be identified such as
499 citrus, fruit in syrup, boxtree/vegetal, tropical or wet grain aromas for Verdejo and red fruit or fruit
500 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.

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631 fermentation temperature on volatile thiols concentrations in Sauvignon blanc wines.
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633

634

635 **Figure captions**

636 **Figure 1.** Projection of the three quality categories (low/very low, average and very high/high) and
637 52 white samples on the bi-dimensional CA map yielded from the categorization task based on
638 orthonasal aroma quality perception of a panel of experts. Cluster 1: low/very low quality
639 represented by a dot; cluster 2: average quality represented by a triangle and cluster 3: very
640 high/high quality.

641 **Figure 2.** Projection of the three quality categories (low/very low, average and very high/high) and
642 51 red samples on the bi-dimensional CA map yielded from the categorization task based on
643 orthonasal aroma quality perception of a panel of experts. Cluster 1: low/very low quality
644 represented by a dot; cluster 2: average quality represented by a triangle and cluster 3: very
645 high/high quality.

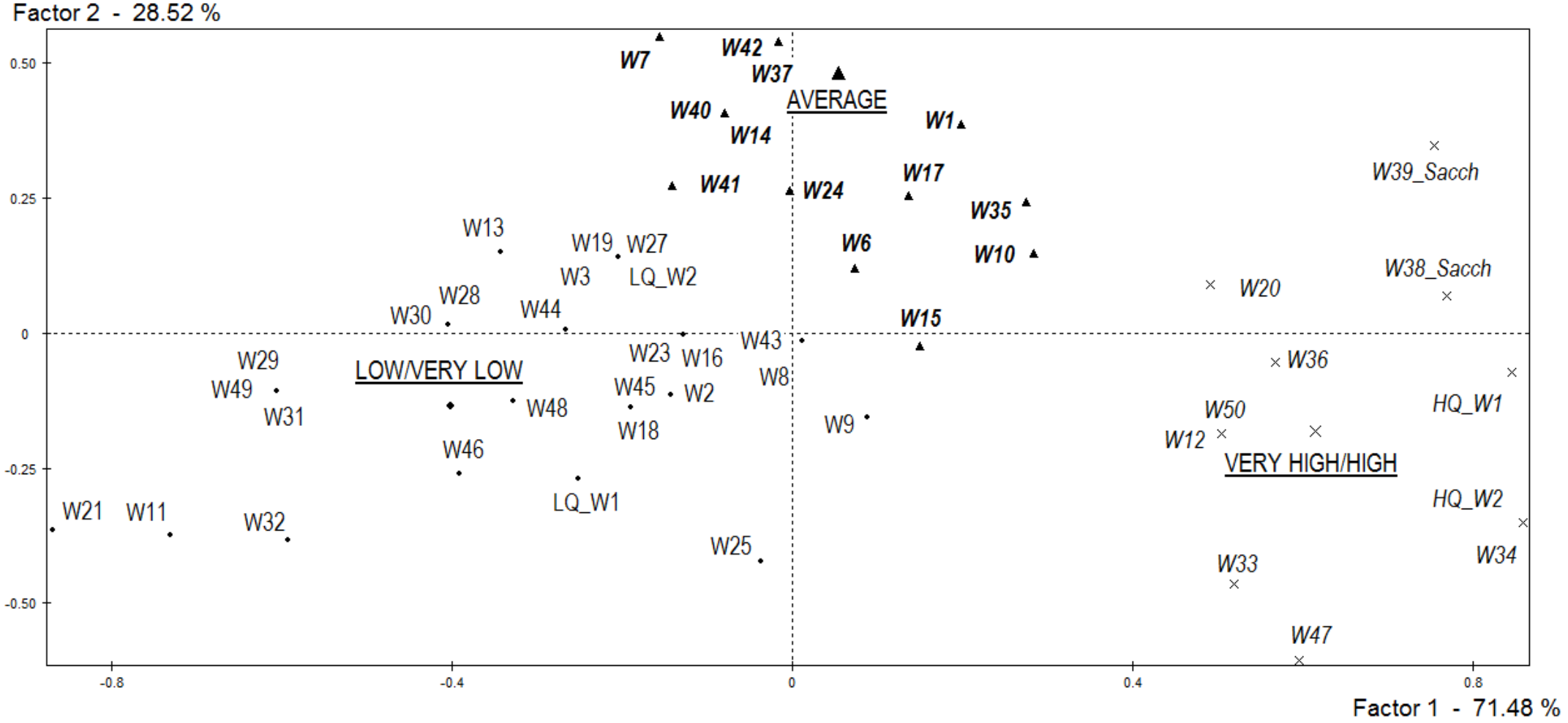
646 **Figure 3.** Projection of a) white samples (samples belonging to Cluster 1 and Cluster 2 are
647 represented with different symbols) and b) individual descriptors (given by each of the 15 judges:
648 J1-J15) on the consensus space obtained using Generalised Procruster Analysis (GPA) over the
649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

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

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

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

656


Factor 2 - 28.52 %
0.50
0.25
0
-0.25
-0.50
-0.8
-0.4
0
0.4
0.8
Factor 1 - 71.48 %
W7
W42
W37
AVERAGE
W40
W14
W1
W41
W24
W17
W35
W6
W10
W15
W39_Sacch
W38_Sacch
W20
W36
HQ_W1
HQ_W2
W34
W50
W12
VERY HIGH/HIGH
W33
W47
W13
W19
W27
W3
LQ_W2
W28
W30
W44
W43
W23
W16
W8
W45
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W29
W49
W31
LOW/VERY LOW
W21
W11
W32

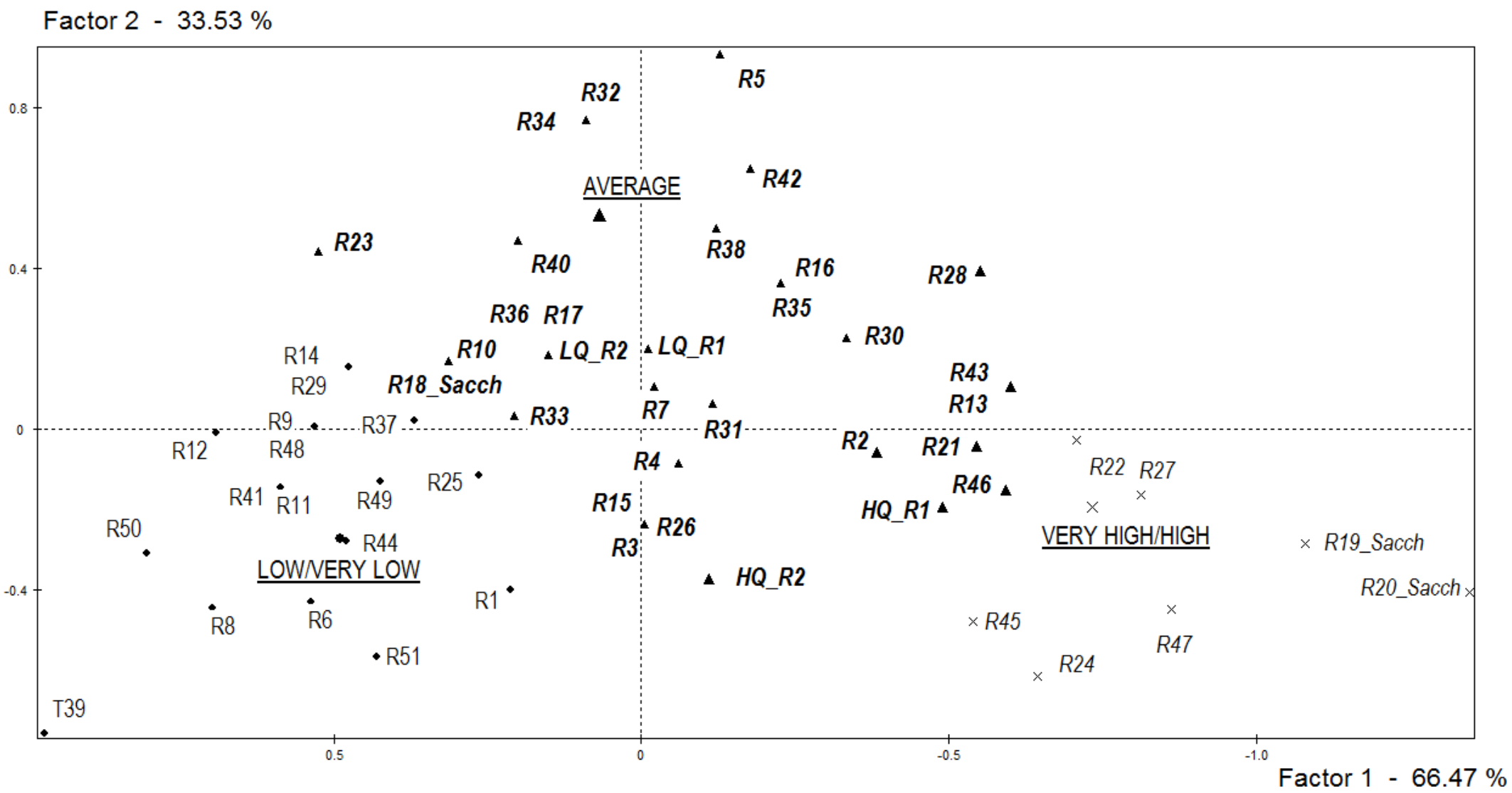

Factor 2 - 33.53 %
0.8
0.4
0
-0.4
0.5
0
-0.5
-1.0
Factor 1 - 66.47 %
R5
R32
R34
R42
AVERAGE
R23
R38
R40
R16
R28
R35
R36
R17
R30
R14
R10
LQ_R2
LQ_R1
R29
R18_Sacch
R43
R13
R9
R37
R33
R7
R31
R12
R48
R2
R21
R4
R22
R27
R25
R46
R41
R11
R49
R15
HQ_R1
R50
R26
R44
R3
VERY HIGH/HIGH
R19_Sacch
LOW/VERY LOW
HQ_R2
R20_Sacch
R1
R45
R8
R6
R47
R51
R24
T39

Figure 3.

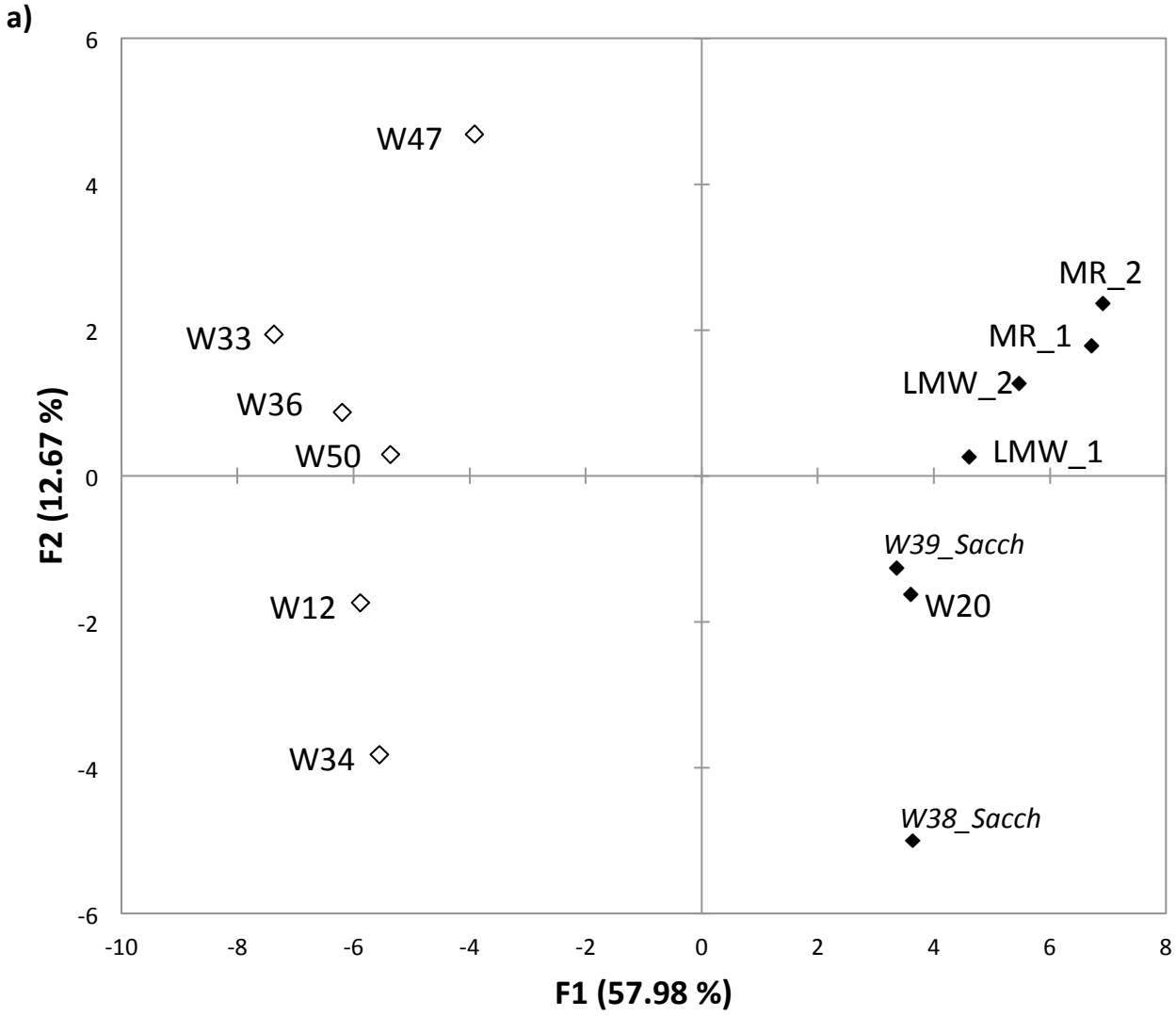

a)
W47
W33
W36
W50
W12
W34
MR_2
MR_1
LMW_2
LMW_1
W39_Sacch
W20
W38_Sacch
F2 (12.67 %)
F1 (57.98 %)
6
4
2
0
-2
-4
-6
-10
-8
-6
-4
-2
0
2
4
6
8

Figure 4

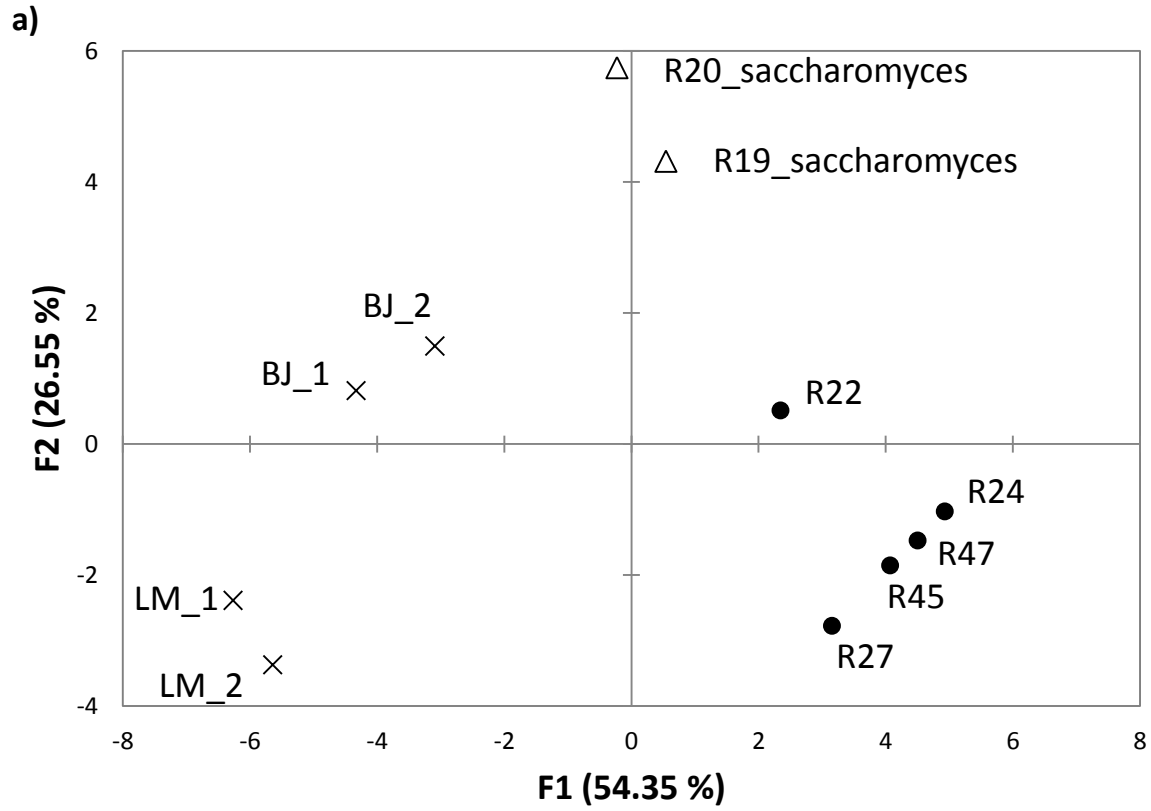

a)
R20_saccharomyces
R19_saccharomyces
BJ_2
BJ_1
R22
R24
R47
R45
R27
LM_1
LM_2
F2 (26.55 %)
F1 (54.35 %)
6
4
2
0
-2
-4
-8
-6
-4
-2
0
2
4
6
8

Figure 5

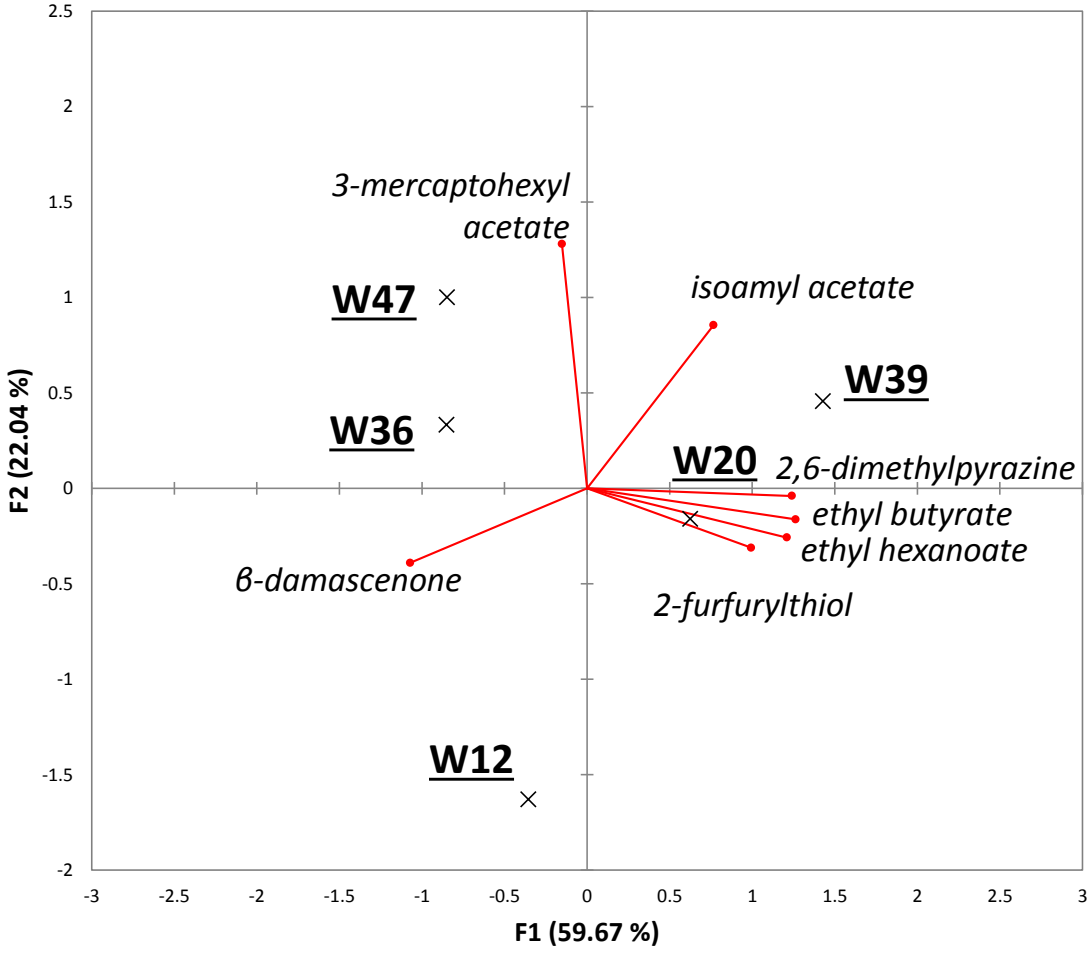
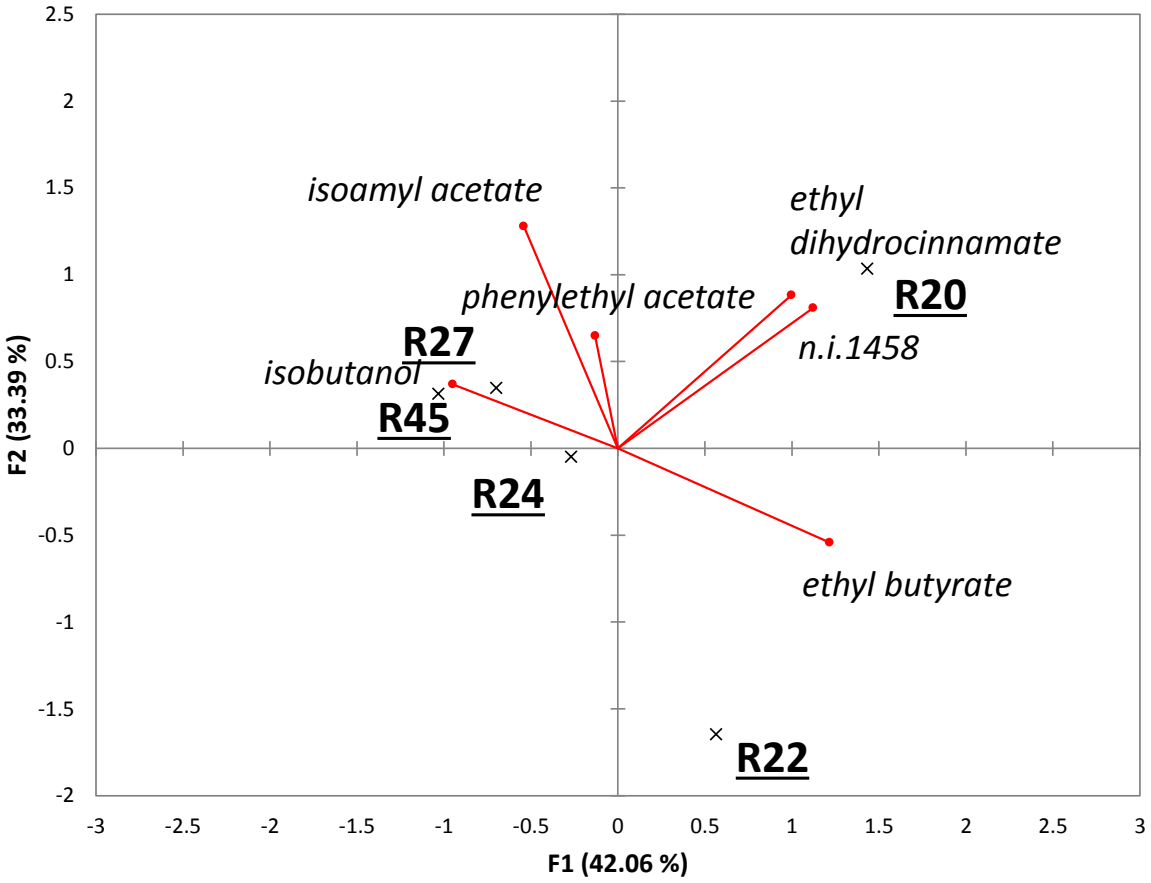
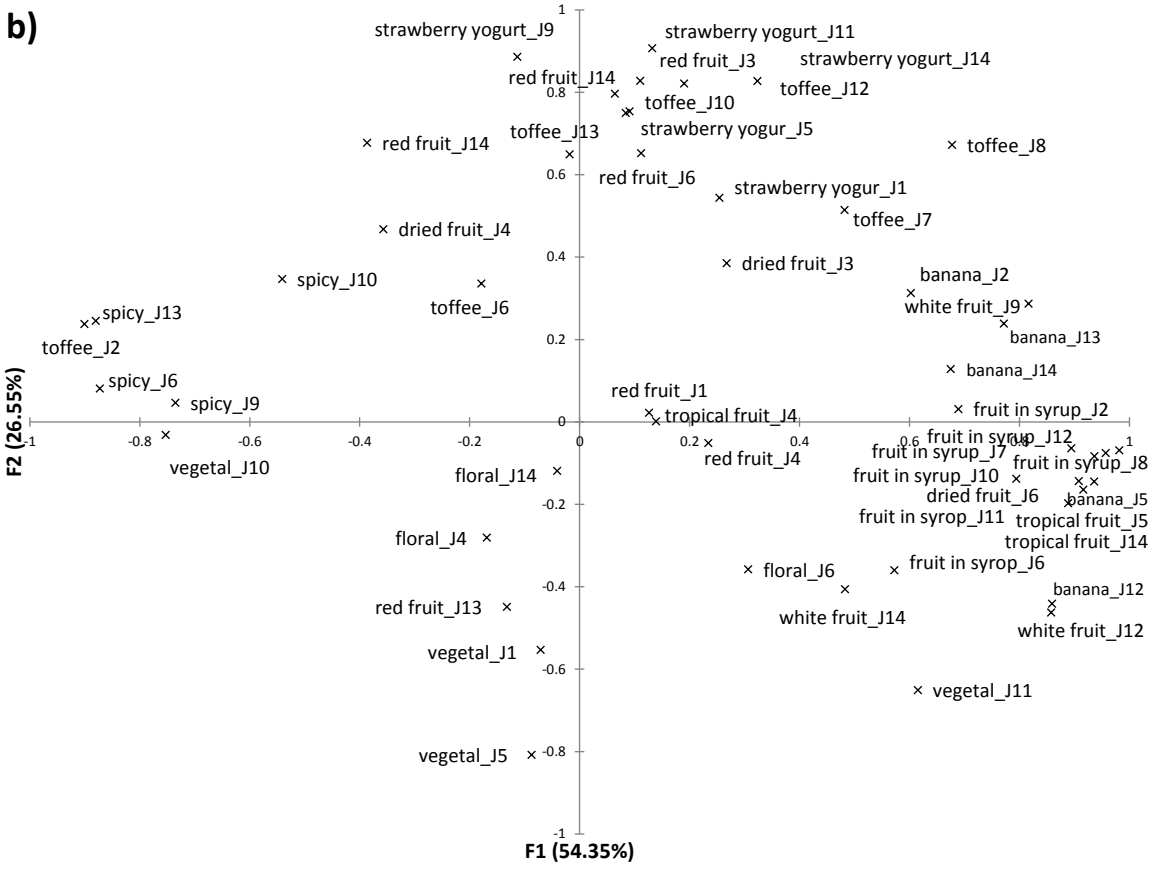

3-mercaptohexyl acetate
isoamyl acetate
W47
W39
W36
W20
2,6-dimethylpyrazine
ethyl butyrate
ethyl hexanoate
β-damascenone
2-furfurylthiol
W12
F1 (59.67 %)
F2 (22.04 %)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3

Figure 6


isoamyl acetate
ethyl dihydrocinnamate
phenylethyl acetate
R20
R27
n.i.1458
isobutanol
R45
R24
ethyl butyrate
R22
F2 (33.39 %)
F1 (42.06 %)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3

b)


strawberry yogurt_J9
strawberry yogurt_J11
red fruit_J3
strawberry yogurt_J14
red fruit_J14
toffee_J12
toffee_J10
toffee_J13
strawberry yogur_J5
red fruit_J14
toffee_J8
red fruit_J6
strawberry yogur_J1
toffee_J7
dried fruit_J4
dried fruit_J3
banana_J2
spicy_J10
white fruit_J9
toffee_J6
spicy_J13
banana_J13
toffee_J2
banana_J14
spicy_J6
red fruit_J1
spicy_J9
fruit in syrup_J2
tropical fruit_J4
fruit in syrup_J12
red fruit_J4
fruit in syrup_J7
fruit in syrup_J8
vegetal_J10
fruit in syrup_J10
floral_J14
dried fruit_J6
banana_J5
fruit in syrop_J11
tropical fruit_J5
floral_J4
tropical fruit_J14
floral_J6
fruit in syrop_J6
banana_J12
red fruit_J13
white fruit_J14
white fruit_J12
vegetal_J1
vegetal_J11
vegetal_J5
F2 (26.55%)
F1 (54.35%)

b)

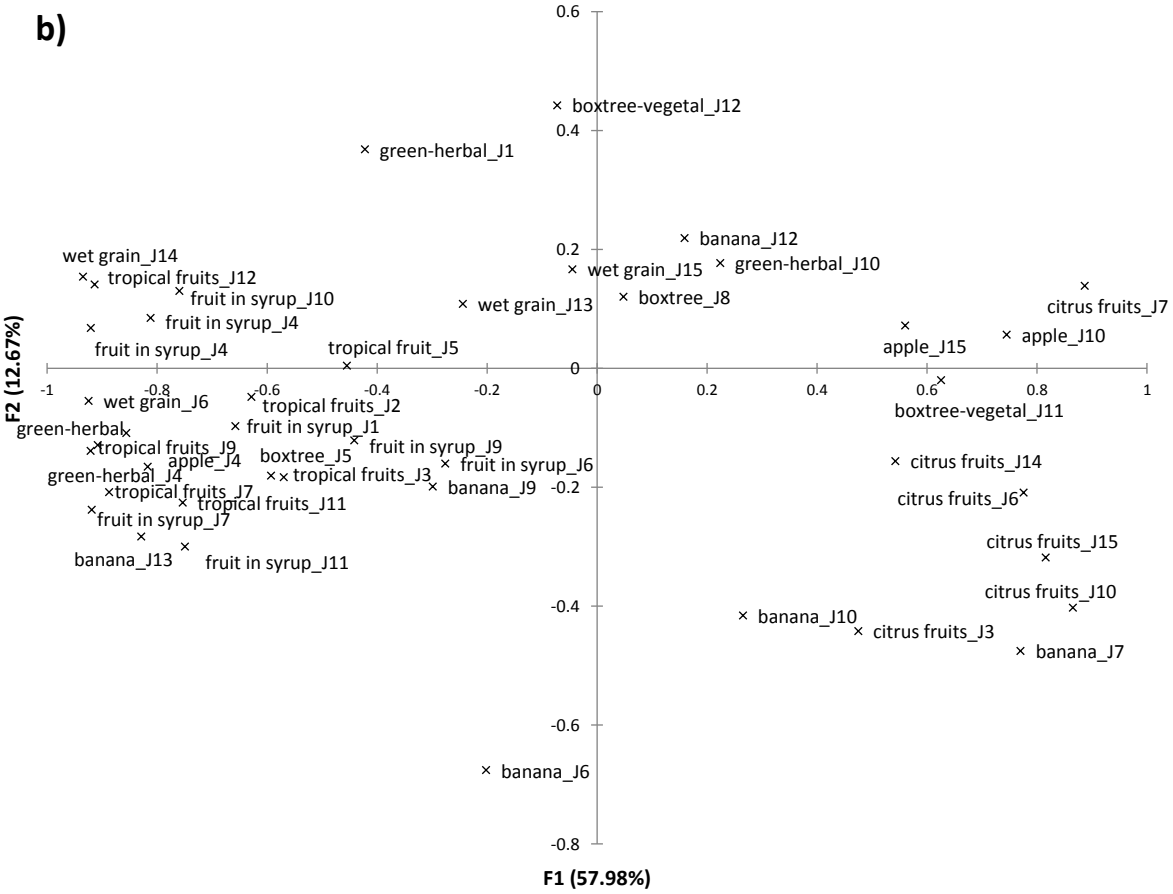

0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
F2 (12.67%)
F1 (57.98%)
boxtree-vegetal_J12
green-herbal_J1
banana_J12
wet grain_J14
tropical fruits_J12
wet grain_J15
green-herbal_J10
fruit in syrup_J10
boxtree_J8
citrus fruits_J7
wet grain_J13
fruit in syrup_J4
apple_J15
apple_J10
fruit in syrup_J4
tropical fruit_J5
wet grain_J6
tropical fruits_J2
boxtree-vegetal_J11
green-herbal
fruit in syrup_J1
tropical fruits_J9
fruit in syrup_J9
apple_J4
boxtree_J5
fruit in syrup_J6
citrus fruits_J14
green-herbal_J4
tropical fruits_J3
banana_J9
tropical fruits_J7
citrus fruits_J6
tropical fruits_J11
fruit in syrup_J7
citrus fruits_J15
banana_J13
fruit in syrup_J11
citrus fruits_J10
banana_J10
citrus fruits_J3
banana_J7
banana_J6

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

attribute	frequency of citation (%)
fruit in syrop	53%
tropical fruits	47%
citrus fruits	40%
banana	40%
boxtree-vegetal	27%
wet grain-hay	27%
green-herbal	27%
apple	20%

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 red wines selected in the categorization task

attribute	frequency of citation (%)
red fruit	47%
fruit in syrop	47%
caramel-toffee	47%
strawberry yogurt	33%
banana	33%
spicy	27%
vegetal-green	27%
white fruit	27%
floral	20%
tropical fruit	20%
alcohol	20%
dried fruit	20%

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.

LRI[a]	Odour description	Chemical identity	W39	W20	W47	W36	W12	*P*	max-min
978	Butter, fruity, strawberry	diacetyl+ ethyl isobutyrate	24	40	0	7	20	ns	40
1015	Sweet, fruity, solvent	isobutyl acetate	10	55	62	58	53	ns	52
1043	**Fruity, strawberry**	**ethyl butyrate**	**62^{a}**	**67^{a}**	**15^{b}**	**10^{b}**	**24^{b}**	**<0.01**	**57**
1078	Sweet, strawberry	ethyl 3-methylbutyrate	30	0	0	0	0	ns	30
1119	Tabacco, green, herbal	1-hexen-3-one	55	10	10	0	0	ns	55
1136	**Fruity, banana**	**isoamyl acetate**	**83^{a}**	**65^{b}**	**75^{b}**	**10^{c}**	**7^{c}**	**<0.001**	**75**
1218	Solvent	isoamyl alcohol	75	65	66	20	74	ns	55
1248	**Fruity, strawberry**	**ethyl hexanoate**	**64^{b}**	**75^{b}**	**0^{a}**	**7^{a}**	**28^{a}**	**<0.1**	**75**
1285	Fruity, anise	hexyl acetate	7	7	19	0	34	ns	34
1320	Earthy, musty, roasted	2,5-dimethylpirazine	33^{ab}	0^{b}	19^{ab}	40^{b}	26^{ab}	<0.1	40
1333	**Roasted, spicy, bready, barbecue**	**2,6-dimethylpyrazine**	**69^{a}**	**29^{b}**	**0^{b}**	**0^{b}**	**25^{b}**	**<0.01**	**69**
1445	Floral, green, medicinal	*E*-2-octenal	37	27	0	18	17	ns	37
1454	Green, tabacco, earthy	2-isopropyl-3-methoxypyrazine	0^{b}	0^{b}	49^{a}	0^{b}	0^{b}	<0.001	49
1455	Green, earthy, dusty	3,5-dimethyl-2-methoxypyrazine[*]	0^{b}	0^{b}	0^{b}	43^{a}	0^{b}	<0.01	43
1456	**Roasted, coffee**	**2-furfurylthiol**	**71^{a}**	**7^{b}**	**0^{b}**	**0^{b}**	**37^{b}**	**<0.001**	**71**
1559	Wet cardboard, dusty	2-methylpropanoic acid	18^{b}	17^{b}	0^{b}	47^{a}	0^{b}	<0.1	47
1658	Burnt fur, roasted	2-acetylpyrazine	48	44	50	54	57	ns	13
1741	**Tropical, citrus, grapefruit**	**3-mercaptohexyl acetate**	**71^{a}**	**48^{b}**	**81^{a}**	**76^{a}**	**25^{b}**	**<0.05**	**57**
1753	Spicy, saffron	n.i. 1753	8	0	0	0	31	0.333	31
1842	**Sweet, cooked apple**	**β-damascenone**	**12**	**33**	**65**	**38**	**64**	**<0.1**	**53**
1847	Green, fruity, sulfury	3-mercaptohexanol	22	0	33	0	45	0.265	45
1881	Spicy, sweet, medicinal, smoke	guaiacol	7	10	25	59	24	0.166	42
1933	Floral, roses	β-phenylethanol	24	30	10	10	30	0.252	20
2025	Metalic, green, caustic	n.i. 2025	0	0	0	35	0	0.118	35
2055	Caramel, sweet, strawberry	furaneol	10	7	29	0	37	0.353	37
2217	Spicy, clove, curry	sotolon	47	40	14	58	0	0.146	58

[a]LIR 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 fix factor and judges as random factor). Different letters indicate significant differences according to Fischer post-hoc test.

LRI[a]	Odour description	Chemical identity	R20	R22	R24	R45	R27	*P*	max
978	Butter, fruity, strawberry	diacetyl+ethyl isobutyrate	40	74	14	25	10	0.175	40
1017	Sweet, fruity, solvent	isobutyl acetate	52	25	54	29	56	0.510	54
1043	**Fruity, strawberry**	**ethyl butyrate**	**65^{ab}**	**76^{a}**	**23^{bc}**	**0^{c}**	**31^{bc}**	**<0.05**	**76**
1064	Fruity, anise, strawberry	ethyl 2-methylbutyrate	14	14	0	14	40	0.375	40
1112	**Solvent**	**isobutanol**	**13^{b}**	**13^{b}**	**0^{b}**	**65^{a}**	**47^{a}**	**<0.01**	**65**
1134	**Banana**	**isoamyl acetate**	**83^{a}**	**18^{b}**	**80^{a}**	**91^{a}**	**87^{a}**	**<0.05**	**91**
1219	Solvent	isoamyl alcohol	75	84	68	41	64	0.797	84
1248	Fruity, strawberry	ethyl hexanoate	65	52	32	0	29	0.249	65
1306	**Floral, green, medicinal**	***E*-2-octenal**	**0^{b}**	**0^{b}**	**43^{a}**	**14^{ab}**	**0^{b}**	**<0.05**	**43**
1309	**Roasted, barbecue**	**2-methyl-3-furanthiol**	**0^{b}**	**43^{a}**	**0^{b}**	**0^{b}**	**0^{b}**	**<0.01**	**43**
1319	Mushroom, solvent	n.i.1319	0	59	32	35	50	0.287	59
1453	Earthy, green pepper	3-isopropyl-2-methoxypyrazine	14	68	53	18	71	0.126	71
1458	**Meaty, cardboard**	**n.i.1458**	**53^{a}**	**0^{b}**	**0^{b}**	**0^{b}**	**0^{b}**	**<0.01**	**53**
1470	Fruto seco, cartón, alcachofa, tierra	n.i.1470	54	10	0	35	0	0.236	54
1516	**Floral, soap**	**decanal**	**43^{a}**	**0^{b}**	**0^{b}**	**0^{b}**	**0^{b}**	**<0.01**	**43**
1560	Green, carboard, rancid	*E-2*-nonenal	35	38	50	38	61	0.729	61
1605	**Mouldy, cooked vegetable**	**n.i.1605**	**48^{a}**	**0^{b}**	**0^{b}**	**29^{a}**	**0^{b}**	**<0.05**	**48**
1610	**Grass, green, fresh**	**acetaldehyde**	**0^{b}**	**0^{b}**	**0^{b}**	**43^{a}**	**0^{b}**	**<0.05**	**43**
1654	Burnt fur, roasted	2-acetylpyrazine	68	29	47	74	35	0.478	74
1832	Rancid, floral, green	*E*,*E*-2,4-decadienal	35	0	59	50	10	0.138	59
1839	**Floral, rose**	**phenylethyl acetate**	**35^{ab}**	**0^{b}**	**53^{a}**	**0^{b}**	**68^{a}**	**<0.05**	**68**
1847	Sweet, cooked apple	**β-damascenone**	50	19	29	29	14	0.852	50
1880	Spicy, sweet, medicinal, smoke	guaiacol	14	74	74	41	40	0.420	74
1907	**Sweet, floral**	**Ethyl dihydrocinnamate**	**52^{a}**	**0^{b}**	**0^{b}**	**10^{b}**	**0^{b}**	**<0.05**	**52**
1934	Floral, rose	β-phenylethanol	48	23	45	10	14	0.495	48
1964	Caramel, spicy	n.i.1964	14	41	38	0	14	0.269	41
2023	Caramel, solvent, vegetal	n.i. 2023	20	10	40	14	25	0.591	40
2053	Caramel, sweet, strawberry	furaneol	52	37	29	0	20	0.553	52
2155	Sweet, floral	ethyl cinnamate	0	0	46	0	14	**<0.1**	46
2206	**Animal, leather**	**4-ethylphenol**	**13**	**0**	**47**	**0**	**29**	**<0.1**	**47**
2219	**Spicy, clove, curry, burnt**	**sotolon**	**14**	**0**	**46**	**20**	**0**	**<0.1**	**46**

[a]LIR Linear retention index on polar capillary column (DB-WAX)

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