

1 **IMPACT OF USING NEW COMMERCIAL GLUTATHIONE**
2 **ENRICHED INACTIVE DRY YEAST OENOLOGICAL**
3 **PREPARATIONS ON THE AROMA AND SENSORY PROPERTIES**
4 **OF WINES**

5
6 **I. Andújar-Ortiz ^a, C. Chaya ^b, P. J. Martín-Álvarez ^a, M. V. Moreno-Arribas ^a,**
7 **M.A. Pozo-Bayón ^{a*}**

8
9 ^a Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM). C/
10 Nicolás Cabrera, 9, Campus de la Universidad Autónoma de Madrid, Cantoblanco,
11 28049 Madrid, Spain.

12 ^b Escuela Técnica Superior de Ingenieros Agrónomos de la Universidad Politécnica de
13 Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain

14
15
16 **Running title:** Glutathione inactive dry yeast preparations in wines

17
18 **ABSTRACT**

19
20 The effect of the addition of a commercial enriched glutathione Inactive Dry Yeast
21 (GSH-IDY) oenological preparation in the volatile and sensory properties of industrially
22 manufactured rosé Grenache wines was evaluated during their shelf-life. In addition,
23 triangle tests were performed at different times during wine aging (among 1 and 9
24 months) to determine the sensory differences between wines with and without GSH-

* corresponding author: mdelpozo@ifi.csic.es phone: + 34 91 0017900 ext: 961

25 IDY preparations. Descriptive sensory analysis with a trained panel was carried out
26 when sensory differences in the triangle test were noticed. In addition, consumer tests
27 were performed in order to investigate consumers' acceptability of wines. Results
28 revealed significant sensory differences between control and GSH-IDY wines after 9
29 months of aging. At that time, GSH-IDY wines were more intense in *fruity* aromas
30 (*strawberry, banana*) and less intense in *yeast* notes than control wine. The impact of
31 the GSH-IDY in the aroma might be the consequence of different effects that these
32 preparations could induce in wine composition: modification of yeast byproducts
33 during fermentation, release of volatile compounds from IDY, interaction of wine
34 volatile compounds with yeast macromolecules from IDY and a possible antioxidant
35 effect of the glutathione released by the IDY preparation on some specific volatile
36 compounds.

37

38 **Key words:** Wine, Glutathione, Inactive Dry Yeast Preparations, aroma, sensory analysis

39

40 INTRODUCTION

41

42 Oxidation processes constitute a serious problem during winemaking and especially in
43 the case of young wines. In general terms, oxidation of young wines, is associated with
44 a rapid loss of the pleasant sensory characteristics of wine, particularly affecting the
45 floral and fruity notes, and the formation of unpleasant new aromas of typical aged
46 wine, as well as atypical aromas associated with wine spoilage ^[1-3]. Wine oxidation also
47 produces wine browning, which results from the oxidation of phenols to quinones,
48 which in turn polymerise to form macromolecules with a typical yellow-brown hue ^[4].

49 The exogenous addition of γ -L-glutamyl-L-cysteinylglycine, named as glutathione
50 (GSH), a tripeptide of non-proteic origin of known antioxidant properties ^[5], is now
51 being studied by the OIV (International Organisation of Vine and Wine) since it has
52 been shown that it prevents the enzymatic browning of white wines ^[6,7], and also
53 preserves varietal aroma compounds, reducing the occurrence of aged off-flavor
54 compounds ^[5]. However, the use of this compound during winemaking is not allowed so
55 far.

56

57 In contrast, from the different types of Inactive Dry Yeast (IDY) preparations allowed
58 for different applications during winemaking ^[8], some of them are claimed to prevent
59 wine oxidation because of their higher content in GSH. Recently, new research
60 performed in our laboratory, has shown a higher level of GSH released into synthetic
61 wines by GSH enriched IDY preparations (GSH-IDY) compared to other non-GSH IDY
62 preparations ^[9]. In addition, it has been shown that these preparations might reduce
63 terpene oxidation in synthetic wines submitted to accelerated aging conditions ^[10].
64 Nevertheless, the impact of glutathione enriched IDY preparations to preserve and/or to

65 improve the sensory characteristics of wines industrially manufactured has not been
66 studied so far. Only the effect of the addition of an IDY preparation in the overall
67 sensory perception of finished wines and their impact on the mouthfeel and taste
68 properties have been studied ^[11,12]. Keeping these antecedents in mind and taking into
69 consideration the importance of contributing to a better knowledge in the use of these
70 preparations during winemaking, the objective of the present research was to evaluate
71 the effect of a glutathione enriched commercial IDY preparation (GSH-IDY) on the
72 volatile and sensory properties of an industrially manufactured rosé Grenache wine
73 during its shelf-life.

74

75 **MATERIAL AND METHODS**

76

77 **Description of the wines**

78

79 Two different types of monovarietal Grenache rosé wines from the 2008 vintage, a
80 control wine and a GSH-IDY wine, were industrially manufactured in a winery from the
81 O.D. Navarra, Spain. To do so, 10,000 L tanks were filled with the same must. GSH-
82 IDY wine was prepared by adding the advised dosage (20 g HL⁻¹) of a commercial
83 glutathione enriched IDY preparation from a yeast autolysate (*Saccharomyces*
84 *cerevisiae*) specially recommended by the manufacturers to prevent wine aroma
85 oxidation. A control wine was also made from the same must without GSH-IDY
86 addition. To carry out the alcoholic fermentation, the same active dry yeast was
87 inoculated in both types of wines. All the wines were stabilised and clarified in the
88 winery, and sent to our laboratory for the instrumental and sensory analysis. Wines were
89 kept at 12 °C during 10 months.

90 General parameters during winemaking (probable alcohol degree in musts, total acidity,
91 volatile acidity and alcohol degree in wines) were determined according to the official
92 methods of wine analysis ^[13]. From these determinations, it can be concluded that
93 fermentation performance was similar in both types of wines and finished wines had
94 values considered in the normal range for this type of wines (**Table 1**).

95

96 **Volatile compounds**

97

98 To determine the effect of GSH-IDY on the volatile profile and its evolution over time,
99 wine volatiles were analyzed after 1, 2, 3 and 9 months of wine aging. To do so, 8 mL
100 of wine spiked with 50 μ L of a solution of methyl nonanoate (5 mg L^{-1}) used as internal
101 standard were placed in a 20 mL headspace vial and sealed with a PTFE/Silicone
102 septum (Supelco, Bellefonte, PA). Vials were kept at 40 °C for 10 min to reach
103 equilibrium before the extraction. The extraction was performed during 20 minutes at
104 40 °C under constant stirring (500 rpm), using a StableFlex 85 μ m carboxen-
105 polydimethylsiloxane, CAR-PDMS fibre (Supelco). The same fibre was used
106 throughout the study and its performance was periodically checked. After the extraction,
107 the fibre was removed from the sample vial and desorbed in the GC injector port in
108 splitless mode for 10 min. An Agilent 6890N GC system (Agilent, Palo Alto, CA) with
109 a split/splitless injector and interfaced with an Agilent 5973 mass spectrometer was
110 used for sample analysis. The injector was set at 280 °C. An Agilent MSD ChemStation
111 Software (D.01.02 16 version) was used to control the system. Separation was
112 performed on a Carbowax 10M column (30 m x 0.25 mm i.d. x 0.5 μ m). The oven
113 temperature was programmed as follows: 40 °C as initial temperature, held for 5
114 minutes. In a first ramp the temperature increased to 60 °C at $1 \text{ }^\circ\text{C min}^{-1}$ and, in the

115 second, to 160 °C at 5 °C min⁻¹, then held for 1 minute. In a third ramp the temperature
116 increased to 180 °C at 20 °C min⁻¹, then held for 2 minutes. Helium was the carrier gas
117 (7 psi and 1mL min⁻¹). For the MS system, the temperatures of the manifold and transfer
118 line were 150 and 230 °C respectively; electron impact mass spectra were recorded at 70
119 eV ionization voltages and the ionization current was 10 µA. The acquisitions were
120 performed in scan mode (from 35 to 450 m/z). Analyses were made in duplicate. The
121 identification was carried out by comparison of the mass spectra of the peaks in the
122 samples with those reported in the mass spectrum libraries, and using the reference
123 compounds when possible. Moreover, linear retention indexes were experimentally
124 calculated with an n-alkane mixture (C5-C30) and compared with those available in the
125 literature. For quantification purposes, the relative area was obtained as the TIC signal
126 of each aroma compound divided by the area of the internal standard. For those
127 compounds whose standards were available, calibration curves in synthetic wines with
128 each of the reference compounds (5 levels of concentration x 2 repetitions) were used,
129 after checking the absence of significant matrix effects for most of the volatile analyzed
130 by the comparison of the slopes of the regression curves obtained in the synthetic and
131 real wines following the same methodology described by Rodriguez-Bencomo and
132 collaborators ^[14]. A Semiquantification, considering that the response factor of the
133 compound had the same value that the internal standard, was carried out when the
134 reference standards were not available.

135

136 **Triangle tests during the shelf-life of the wines**

137

138 Triangle tests were carried out by a panel of 12 judges (6 men, 6 women, aged from 28
139 to 68) belonging to the staff of the Technical University of Madrid. They were

140 previously trained in detection and recognition of tastes and odours, in the use of scales
141 and in difference and ranking assessments according to the International Organization
142 for Standardization ISO 8586-1 ^[15].

143

144 Three wine samples were presented to the judges identified by three-digit random
145 codes. The order of presentation was randomly assigned for each judge, verifying that
146 for the whole panel, presentation order of the samples was balanced. Wine (25 mL) was
147 served in tulip-shaped ISO tasting glasses at a constant temperature of 12 °C, and
148 covered with plastic Petri dishes to allow the volatiles to equilibrate in the headspace.
149 Tests were performed in a sensory lab provided with 16 individual booths and
150 complying with usual requirements such as proper light and temperature control and
151 isolation from noises and odours. No information about the aim of the study or about
152 wine samples was given to the judges prior to the tests. Judges were asked to evaluate
153 samples from left to right, looking for differences in aroma and taste. Judges were
154 informed that two samples were identical and one sample was different. They had to
155 select the odd sample. Judges rested between samples, rinsed their mouth with water
156 and ate breadsticks when necessary. Triangle tests were performed throughout the shelf-
157 life of wines, specifically, after 1, 2, 3 and 9 months of wine aging. Judges were given
158 rewards and provided with positive feed back, as motivated judges are more focused
159 and have better performance.

160

161 **Descriptive analysis**

162

163 The panel was composed by 3 men and 7 women aged from 24 to 68, belonging to the
164 Technical University of Madrid. All conditions were identical to those described before.

165 Descriptive analysis of the two types of rosé wines was carried out in three 2-h sessions
166 divided in training, training evaluation and wine evaluation.

167

168 *Training.* In the first training session, 12 representative attributes of Grenache wines
169 were prepared at the highest concentration described in **Table 2** and presented to the
170 judges. During this first training session, judges were first asked to smell the standards
171 corresponding to the 12 attributes to familiarize themselves, and then, they were asked
172 to rate the intensity of the wines for each attribute in an unstructured 15 cm line scale
173 anchored at 1.5 cm from the end points of the line with the words “low” and “high”. In
174 this step, judges were introduced to the score card, the rating scale and procedure
175 protocol of evaluation. This training period allowed choosing the attributes most
176 representatives in both wines. At the conclusion of the first training period, 6 attributes
177 were selected (*strawberry, peach, banana, floral, yeast, acidity*) (**Table 2**). The second
178 and third sessions were focused on refining the standards and training the judges in
179 using the terms consistently. To do so, aromas were presented at random at low and/or
180 high concentration (**Table 2**), together with a form containing an unstructured 15 cm
181 line scale as described before where the corresponding intensity was rated.

182

183 *Training evaluation.* Booths with 2 wine tasting glasses containing each of the 6
184 standard references at two concentrations (low and high) were prepared as explained
185 before, and properly coded and covered with aluminium paper to avoid the influence of
186 sample colour in the wine tasting evaluation. Judges were asked to determine the
187 attribute and to rate the intensity of the standard in the same unstructured 15 cm line
188 scale as described before. Training evaluation was done in duplicate, therefore each
189 judge rated the 6 attributes at two concentrations twice, with the exception for acidity,

190 for which judges had been previously trained for different sensory studies. Statistical
191 evaluation of performance of the panel was done by two-way ANOVA, in order to
192 discard attributes scores from judges not consistent with the whole panel for the
193 subsequent sessions.

194

195 *Wine evaluation.* Wine evaluation was carried out after training and training evaluation.
196 Both wines were identified by three digit random codes and the presentation order of
197 the samples was randomly assigned and balanced for the whole panel. Judges rated each
198 of the 6 attributes using the same unstructured 15 cm line explained before. First, they
199 were asked to rate the intensity of each aroma attribute in both wines by the orthonasal
200 way. Finally, they were asked to taste the wine and to rate the acidity for both wines.

201

202 **Consumer tests**

203

204 Hedonic evaluation of both types of wines (control wine and GSH-IDY wine) were
205 investigated by a panel of consumers (n=64) belonging to the staff of our research
206 institution (CIAL). The selection criteria were focused on consumers who generally
207 enjoy rosé wines, with no ethical or medical reasons for not consuming alcohol. For this
208 study consumers were recruited taking into consideration a balanced distribution by sex
209 (56% male and 44% women). In addition most of them were aged from 21-34 (56%),
210 while consumers aged from 35-49, 50-65 and older than 65 years old represented the
211 20, 17 and 6%, respectively. No specific information about the samples was given to
212 consumers prior the study. As described before, samples were identified by three-digit
213 random codes at constant serving temperature, using a randomised and balanced serving
214 order across consumers. Consumers were asked to rate each wine for overall liking on a

215 9 point hedonic scale from “dislike extremely” to “like extremely”. Paper score-sheets
216 were used for data collection.

217

218 **Statistical analysis**

219

220 Results corresponding to the concentration of volatile compounds in both types of wines
221 throughout wine shelf-life were submitted to cluster analysis to provide a general view
222 of the main factors involved on data variation (addition of GSH-IDY and aging time). In
223 addition, one-way ANOVA was made to test the effect of aging time in each type of
224 wine. Triangle tests results were analysed as described in ISO 4120 ^[16]. Data from the
225 training evaluation for each sensory attribute were submitted to two-way ANOVA to
226 determine the effect of the two studied factors (concentration and judges). Consistency
227 of scores among judges was assessed by the interaction concentration x judge in order
228 to guarantee that each attribute was perceived by the whole panel similarly. Data from
229 the wine evaluation were submitted to one way ANOVA, using the t-test when
230 differences in both wines were found. Data from the consumer tests were analysed by a
231 mixed model, considering wines as fixed effect and consumers as random effect ^[17].
232 STATISTICA 7.1 (www.statsoft.com) and STATGRAPHICS Plus 5.0
233 (www.statgraphics.com) were used for data processing.

234

235 **RESULTS AND DISCUSSION**

236

237 **Evolution of the volatile profile during the shelf-life of the wines**

238

239 To determine the effect of the IDY-preparation on the volatile profile of the wines, we
240 focused on the evolution of a wide range of volatile compounds (**Table 3**) belonging to
241 different chemical classes: esters (ethyl esters of fatty acids and higher alcohol acetates),
242 alcohols, terpenes, and terpenes derivatives, volatile fatty acids and other compounds
243 such as the norisoprenoids β -damascenone and the aldehyde furfural. Most of them have
244 a fermentative origin, although some terpenes were chosen because of their varietal
245 origin. The concentration, calculated for the volatile compounds, was in agreement with
246 other studies focused on the aroma of Grenache rosé wines ^[18-20]. As can be seen in
247 **Table 3**, the concentration of many volatile compounds in wines aged 1 month was very
248 similar in both types of wines. However, some esters, such as isoamyl, hexyl and 2-
249 phenyl ethyl acetates and some long chain ethyl esters (octanoate, decanoate,
250 dodecanoate) showed higher concentration values in the GSH-IDY-wine. In addition,
251 the concentration of the three fatty acids (hexanoic, octanoic and decanoic) also showed
252 higher concentration in the wines supplemented with the preparation.

253

254 To know if there was a natural grouping of the wine samples based on the addition of
255 GSH enriched IDY during winemaking, a cluster analysis was performed with the data
256 corresponding to the concentration of volatile compounds in both types of wines during
257 their shelf-life (1, 2, 3 and 9 months old wines). The results are shown in **Figure 1**. As
258 can be seen, the dendrogram is showing two separated groups of wines. The first one
259 corresponded to wines of 3 and less than 3 months old, and the second one, included all
260 the wines of 9 months. In addition, within each of these two large groups of samples,
261 the figure is revealing a clear separation between wines depending on the addition or not
262 of the GSH-IDY preparation. These results are showing a major influence of the aging

263 time on wine volatile composition, but also an effect of the addition of the GSH-IDY
264 preparation.

265

266 Taking into account these results, one-way ANOVA was made to test the effect of time
267 in the volatile composition in each type of wine (**Table 3**). As can be seen, differences
268 in the evolution of the volatile compounds during the shelf-life of both types of wines
269 were found. Most of the esters decreased during shelf-life in both type of wines, which
270 might be associated to their slow hydrolysis at wine pH ^[21]. In addition, specific
271 interactions between some esters with some components from the IDY preparations
272 (glycopeptides) have been shown ^[22,23]. However, the higher concentration of esters in
273 the 9 moth GSH-IDY wine compared to the 9 month control wines, might be related to
274 the higher pool of these compounds available, because of the promotion of their
275 production during the alcoholic fermentation due to the extra supplementation in
276 nitrogen compounds by the IDY preparation ^[8,23,24]. In fact, the sum of free amino acids
277 recently determined in the same wines after the alcoholic fermentation was two times
278 higher in the GSH-IDY wine compared to the Control wine ^[9].

279

280 Moreover, the concentration of some terpenes, associated to citric and flowery notes,
281 remained unchanged or even showed a slight increase during the aging of wines.
282 Although during wine aging a slow oxidation of these compounds could have been
283 accounted for, an increase in their concentration may also be possible as a consequence
284 of their spontaneous synthesis from precursors naturally occurring in wines, as has been
285 previously hypothesized ^[25] or, as in the case of linalool, because it can be formed from
286 other monoterpenoids ^[26]. The slight increase of linalool during the shelf-life in wines
287 supplemented with the GSH-IDY preparation compared to the control wines may

288 indicate a lower oxidation of these compounds in these wines compared to the control
289 wines. Recent research has also shown the antioxidant properties of the <5000 Da
290 fraction isolated from GSH-IDY against some terpenes in synthetic wines submitted to
291 accelerated aging conditions ^[10].

292

293 Contrary to most of the studied volatile compounds, fatty acids (octanoic and decanoic)
294 increased in the control wines during aging, while remained practically unchanged in
295 the GSH-IDY wines. In addition, significant differences were found between the two
296 types of wines regarding the alcohol content. The concentration of all the alcohols,
297 except benzenemethanol remained constant during shelf life in the GSH-IDY wines,
298 while decreased in the control wines. This could be due to their oxidation to the
299 corresponding aldehydes. Although the role of GSH-IDY preparations on the volatile
300 compounds have not been studied so far, different authors have shown that the addition
301 of glutathione to wines just before bottling at concentration above 20 mg L⁻¹ might
302 prevent the decrease of terpenic alcohols such as linalool ^[27,28] and aromatic esters ^[28,29]
303 during the storage of wines. Previous research performed with the same wines ^[9]
304 reported higher concentration of GSH in the GSH-IDY wines compared to the control
305 wines. In fact, GSH-IDY wines showed a concentration of GSH about 16 mg L⁻¹, which
306 was higher than the concentrations of GSH reported to have an antioxidant effect in
307 synthetic wine ^[28]. However, in the above cited work, it has been showed that most of
308 the GSH released from IDY is rapidly oxidized, so the protective effect of GSH on
309 some volatile compounds might be very limited in winemaking conditions. Nonetheless,
310 GSH released by the IDY preparations may also have had an effect in the must,
311 protecting it from oxidation in the first steps during winemaking. In this case, wines
312 might have a longer shelf-life due to the higher concentration of odour active esters and

313 a better preservation of varietal aromas ^[30]. However it will be necessary in future
314 works to check this hypothesis by systematically sampling during the fermentation step.
315 Besides the differences noticed in the volatile profile between GSH-IDY and control
316 wines, it was very important to know if these changes are also relevant for the sensory
317 properties of the wines.

318

319 **Triangle tests during the shelf-life of wines**

320

321 Triangle tests were performed to find out if there were sensory differences between
322 GSH-IDY and control wines during their shelf-life. Therefore, they were periodically
323 performed (at 1, 2, 3 and 9 months) until sensory differences were perceived. The
324 numbers of correct answers in each triangle test were five, six, four and eight for the 1,
325 2, 3 and 9 months wines respectively. Therefore, control and GSH-IDY wines were not
326 perceived as different in the just finished wine (1 month wine) ($p \leq 0.05$) and neither
327 during the early shelf-life of the wines (2 and 3 months) ($p \leq 0.05$). This is evidencing a
328 slow evolution in the sensory characteristics of the wines during the first months of
329 aging, which is in agreement with the little evolution of the volatile profile found during
330 the three firsts months of aging (**Figure 1**). These results are indicating that in spite of
331 the supplement in GSH and mainly in nitrogen compounds due to the addition of GSH-
332 IDY preparations into the must ^[9], the impact of these preparations in the sensory
333 characteristics of wines during the first stages of their shelf-life is relatively low.
334 Different authors have shown that supplementation in nitrogen compounds to the must
335 may affect the production of sulfur compounds ^[31], medium-chain fatty acid esters and
336 acetic acid ^[32], whereas other authors claimed that must supplementation with
337 ammonium brings about a decrease in sulphur notes and an increase in the citric flavour

338 ^[33]. Although the addition of GSH-IDY may slightly increase the volatile acidity of
339 wines (**Table 1**), it did not provoke sensory differences among IDY wine and control
340 wine after winemaking nor in wines aged 2 and 3 months. Wines were, however,
341 perceived as different after 9 months of aging ($p \leq 0.05$), which also is in agreement
342 with the highest differences found in their volatile profile.

343

344 **Descriptive analysis**

345

346 To determine which sensory attributes of Grenache wines were the most affected by the
347 addition of the GSH-IDY preparation into the must, descriptive analysis was performed
348 in the 9-month old wines (since, as was evidenced in the triangle test only after 9
349 months differences between the control and GSH-IDY wines were statistically
350 significant). To do so, 12 sensory attributes of Grenache wines were selected on the
351 basis of previous studies performed on the sensory characteristics of Grenache wines
352 ^[34,18,19] and accordingly to the opinion of eight wine sensory experts. All the attributes
353 were typical of rosé young Grenache wines, and they belonged to the fruity (*strawberry*,
354 *peach*, *banana*, *apple* and *lemon* aromas), floral and vegetative (*grassy*) aromas. In
355 addition, other attributes were chosen to evoke sweet aromas, such as *raisin*, *toffee* and
356 *honey* aromas, since they can be found in some oxidized young wines ^[1,2,35,36]. *Yeast*
357 aroma was also included because it has been associated to wines supplemented with
358 IDY in a previous work ^[11]. Finally, *acidity* was also evaluated as a taste attribute
359 because is a typical characteristic of young wines.

360

361 After the first training session, only those attributes marked above 4, in the 15 cm-scale
362 at least in one of the wines under study were selected. These attributes were *strawberry*,

363 *peach, banana, yeast and floral* aromas, and acid taste. The fact that judges did not
364 score higher than 4 the attributes *honey, toffee* or *raisin*, indicated the low presence of
365 sweet-aroma-related notes and therefore, the low grade of oxidation in these wines.

366

367 Once the first training session was concluded, a specific training in the selected
368 attributes at two concentrations was carried out, as has been recommended by Noble
369 and Lesschaeve ^[37]. A training evaluation was carried out in order to verify the correct
370 training of the panel, and also to detect those judges who were using an inconsistent
371 term respect to the other subjects. All the data from the training evaluation were
372 submitted to analysis of variance (two-way ANOVA). Interaction plot revealed that
373 judges 1 and 10 did not properly rate the intensity of *strawberry* and *banana* aromas,
374 and consequently, their scores for these attributes were removed from the training and
375 wine evaluations. **Table 4** showed the F-ratios of concentration, judge and
376 concentration x judge of the ANOVA without taking into account the scores of judges 1
377 and 10 in the attributes *strawberry* and *banana*, respectively. As can be seen, the
378 concentration was significantly different for all the studied attributes, whereas,
379 practically no significant effect was found for judges and concentration x judge.
380 Concentration x judge was not obtained for acidity as the judges evaluate it only once.
381 Therefore, it can be concluded that in general, the two concentrations for each attribute
382 were perceived as different and all the judges used the same part of the scale and rated
383 the attributes in a similar way. Then, the panel was considered as reliable and consistent
384 with respect to all the attributes, thus well-trained in these descriptors to carry out the
385 wine evaluation.

386

387 The wine evaluation was performed once (in both types of wines) in a single session
388 once the consistence of the panel was tested. Analysis of variance (ANOVA) was
389 performed in each attribute to determine if wines were perceived as different, and least
390 significant differences between wine means were computed by a t-test. **Table 5** shows
391 F-ratios and p-values of each attribute, discarding the scores for *strawberry* and *banana*
392 of judges 1 and 10. The attributes significantly different in both wines are presented in
393 bold in the table. In addition, the mean intensity rating for control and GSH-IDY wines
394 have been plotted in a cobweb graph to get a sensory profile of each type of wine
395 (**Figure 2**). In this diagram, the centre of the figure represents the lowest intensity with
396 respect to each descriptor increasing to an intensity of 15 at the end of the axes
397 (corresponding to the maximum rating in the 15 cm unstructured scale). As can be seen
398 in **Table 5**, acidity was rated the same in the control and GSH-IDY wine. As it can be
399 expected, acidity had the same intensity in both wines, as there was no evidence that the
400 GSH-IDY addition may modify the acidity of wines. In spite of having different
401 concentrations in volatile compounds typically associated to flowery notes, such as 2-
402 phenylethyl acetate ^[18, 38], both wines presented similar intensities in floral aroma.
403 Regarding fruit attributes, GSH-IDY wine exhibited almost the double intensity in
404 *strawberry* notes (1.98 times more) and also in the *banana* attribute (1.58 times more)
405 than the control wine. These attributes can be related to a higher concentration of esters
406 related to fruity aroma in the 9 months GSH-IDY wine compared to the control wine.
407 For instance, the concentration of isoamyl acetate, a volatile compound typically
408 associated to *banana* flavour was 446 mg L⁻¹ in the 9-month GSH-IDY wine while it
409 was of 189 mg L⁻¹ in the control wine. However, control wines were more intense in
410 *peach* aroma. The *yeast* aroma attribute was included in this study because it has been
411 previously shown that the sensory profile of IDY preparations might include odorant

412 compounds with *yeast-like* notes ^[11]. In the above mentioned work, authors showed that
413 *yeast-like* notes may mask some typical varietal aromatic notes in wines. Therefore, its
414 presence in young wines may decrease the aroma quality. However, in the present work,
415 GSH-IDY wines were rated lower in *yeast-like* notes compared to the control wine. The
416 possible release of other odorant molecules, such as pyrazines present in these
417 preparations ^[11,39] and typically associated to *roasted, toasted, popcorn* aromatic notes
418 may have masked the characteristic typical yeast odour associated to fermentation yeast,
419 although in this work, the amount of IDY added to the musts was not very high (2 mg L⁻¹)
420 ¹) and it has been shown that the appearance of the *yeast-like* notes is associated to a
421 higher dose of IDY in wines (150-600 mg L⁻¹) ^[11]. Finally, it is important to emphasize
422 that during the training, the panel identified the *yeast* aroma as an off-flavor, being
423 related to sulphur-like aroma. Therefore, the higher intensity in *yeast* aroma in the
424 control wine might have been perceived by the panel as a symptom of lower aroma
425 quality compared to the GSH-IDY wine.

426

427 **Consumer tests**

428

429 Finally, consumer tests were carried out in order to determine if wine consumers could
430 perceive preferences towards some of the wines. On a 9 point hedonic scale, consumers
431 rated their liking of the control and GSH-IDY wines in 6.12 and 5.92 respectively,
432 which indicated that the acceptability for both types of wines was in general good.
433 However, no significant differences in consumer preferences were found between both
434 types of wines, and neither when the sex or the age of the consumers were taken into
435 consideration (data not shown). These results showed that consumers did not evidence
436 preference patterns towards wines made with GSH-IDY addition. Nevertheless, a

437 greater consumers sample size could improve both, an increase of discrimination power
438 between wines and the representativeness of the consumers population, indicating a
439 future line of research to be explored.

440

441 **CONCLUSIONS**

442

443 The addition of glutathione enriched IDY preparations into Grenache musts during
444 winemaking has an impact on the volatile profile of young rosé wines during aging that
445 can be responsible for sensory differences in the later stages of wine shelf-life (above 9
446 months). In general, wines supplemented with a glutathione enriched IDY preparation
447 are more intense in typical fruity attributes of young rosé wines (*banana, strawberry*),
448 which could be related at least in part by the protection of some aroma compounds
449 against oxidation, likely in the first steps during winemaking. However, the changes in
450 the sensory profile could be also related to other effects linked to the addition of IDYs
451 into wines, such as the release of volatile compounds and/or the effect of yeast
452 macromolecules on aroma volatility. In addition, the influence of IDY in the
453 fermentation might have change yeas metabolic by-products inducing changes in wine
454 sensory characteristics. Nonetheless, the sensory effect is not evident enough to show
455 consumer preferences towards GSH-IDY wines. Finally, although the use of industrial
456 manufacturing conditions has allowed to us a valuable study of the use of GSH-IDY
457 preparations in real winery conditions, new research, using more wine samples with
458 other GSH-IDY preparations and industrially manufactured is necessary, in order to
459 fully understand the chemistry beyond the use of these preparations, during
460 winemaking.

461 **ACKNOWLEDGMENTS**

462

463 This work forms part of the project PET 2007-0134 funded by the Ministry of Science
464 and Innovation of Spain. Authors are grateful to Virginia Fernandez-Ruiz for her
465 assistance on panel preliminary training and to judges and consumers for their
466 participation in the sensory analysis. IAO greatly acknowledge the Comunidad de
467 Madrid for her research contract.
468

469 **REFERENCES**

- 470 1. Bueno M, Cullere. L., Cacho, J., Ferreira, V. Chemical and sensory characterization
471 of oxidative behaviour in different wines. *Food Research International*, 2010,
472 43,1423-1428
- 473 2. Escudero A, Asensio E, Cacho J, Ferreira V. Sensory and chemical changes of young
474 white wines stored under oxygen. An assessment of the role played by aldehydes
475 and some other important odorants. *Food Chemistry*, 2002, 77 (3), 325-331
- 476 3. Escudero A, Cacho J, Ferreira V (2000) Isolation and identification of odorants
477 generated in wine during its oxidation: a gas chromatography-olfactometric study.
478 *European Food Research and Technology* 2000, 211 (2), 105-110
- 479 4. Singleton VL. Oxygen with Phenols and Related Reactions in Musts, Wines, and
480 Model Systems - Observations and Practical Implications. *American Journal of*
481 *Enology and Viticulture* 1987, 38 (1), 69-77
- 482 5. Lavigne-Cruege V, Pons, A., Chone, X. & Dubordieu, D. Rôle du glutathion sur
483 l'évolution aromatique des vins blancs secs. In: *Oenologie VII symposium*
484 *International d'oenologie*. Tec&Doc, 2003, 385-388
- 485 6. El Hosry L, Auezova L, Sakr A, Hajj-Moussa E. Browning susceptibility of white
486 wine and antioxidant effect of glutathione. *International Journal of Food Science*
487 *and Technology* 2009, 44 (12), 2459-2463.
- 488 7. Vaimakis V, Roussis IG (1996) Must oxygenation together with glutathione addition
489 in the oxidation of white wine. *Food Chemistry*, 1996, 57 (3), 419-422
- 490 8. Pozo-Bayon MA, Andujar-Ortiz I, Moreno-Arribas MV. Scientific evidences beyond
491 the application of inactive dry yeast preparations in winemaking. *Food Research*
492 *International*, 2009, 42 (7), 754-761.
- 493 9. Andujar-Ortiz I, Pozo-Bayon MA, Moreno-Arribas MV, Martin-Alvarez PJ,
494 Rodriguez-Bencomo JJ. Reversed-phase high-performance liquid-chromatography-
495 fluorescence detection for the analysis of glutathione and its precursor γ -glutamyl-
496 cysteine in wines and model wines supplemented with oenological inactive dry
497 yeast preparations, *Food Analytical Methods*, 2012, 5, 154-161.
- 498 10. Rodriguez-Bencomo JJ, Andujar-Ortiz I, Moreno-Arribas MV, Pozo-Bayon MA
499 (Effect of isolated fractions from glutathione enriched-inative dry yeast on the
500 oxidation of wine terpenes. In: *Proceedings from the II Wine Active Compounds*
501 *(WAC) Congress*, 2011, Beaune (France).

- 502 11. Comuzzo P, Tat L, Tonizzo A, Battistutta F. Yeast derivatives (extracts and
503 autolysates) in winemaking: Release of volatile compounds and effects on wine
504 aroma volatility. *Food Chemistry*, 2006, 99 (2), 217-230.
- 505 12. Guadalupe Z, Palacios A, Ayestaran B. Maceration enzymes and mannoproteins: A
506 possible strategy to increase colloidal stability and color extraction in red wines.
507 *Journal of Agricultural and Food Chemistr*, 2007, 55 (12), 4854-4862.
- 508 13. International Organisation of Wine (OIV). Recueil des méthodes internationales
509 d'analyse des vins et des mouts. In: Complément n°1 à l'édition officielle de juin
510 1990, 1990, OIV, Paris.
- 511 14. Rodriguez-Bencomo JJ, Muñoz-Gonzalez C, Andujar-Ortiz I, Martin-Alvarez PJ,
512 Moreno-Arribas MV, Pozo-Bayon MA Assessment of the effect of the non-volatile
513 wine matrix on the volatility of typical wine aroma compounds by HS-SPME-GC-
514 MS analysis. *Journal of the Science of Food and Agricultural*, 2011, 91, 2484-2494
- 515 15. ISO 8586-1 (1993) Sensory analysis-General guidance-Part 1: selected assessors.
- 516 16. ISO 4120 (2004) Sensory analysis-Methodology-Triangle test.
- 517 17. O'Mahony Sensory evaluation of food: statistical methods and procedures. 1986
518 Marcel Dekker, Inc., New York
- 519 18. Ferreira V, Lopez R, Escudero A, Cacho JF. The aroma of Grenache red wine:
520 Hierarchy and nature of its main odorants, *Journal of the Science of Food and*
521 *Agricultural*. 1998, 77 (2) 259-267
- 522 19. Ferreira V, Ortin N, Escudero A, Lopez R, Cacho J. Chemical characterization of
523 the aroma of Grenache rose wines: Aroma extract dilution analysis, quantitative
524 determination, and sensory reconstitution studies, *Journal of Agricultural and Food*
525 *Chemistry*. 2002, 50 (14), 4048-4054.
- 526 20. López R, Ezpeleta, E., Sánchez, I., Cacho, J., Ferreira, V. Analysis of the aroma
527 intensities of volatile compounds released from mild acid hydrolysates of odourless
528 precursors extracted from Tempranillo and Grenache grapes using gas
529 chromatography-olfactometry, *Food Chemistry*. 2004, 88, 95-103
- 530 21. Rapp A, Mandery H. Wine Aroma. *Experientia*. 1986, 42 (8), 873-884
- 531 22. Lubbers S, Voilley A, Feuillat M, Charpentier C. Influence of Mannoproteins from
532 Yeast on the Aroma Intensity of a Model Wine. *Food Science and Technology-*
533 *LWT*, 1994, 27 (2), 108-114
- 534 23. Pozo-Bayon MA, Andujar-Ortiz I, Alcaide-Hidalgo JM, Martin-Alvarez PJ,
535 Moreno-Arribas MV. Characterization of commercial inactive dry yeast

- 536 preparations for enological use based on their ability to release soluble compounds
537 and their behavior toward aroma compounds in model wines, *Journal of*
538 *Agricultural and Food Chemistry* 2009, 57 (22), 10784-10792
- 539 24. Feuillat M, Guerreau, J. Les nouveaux activateurs de la fermentation alcoolique.
540 *Bulletin OIV*. 1996, (789-790), 987-998
- 541 25. Jarauta I, Cacho J, Ferreira V. Concurrent phenomena contributing to the formation
542 of the aroma of wine during aging in oak wood: An analytical study, *Journal of*
543 *Agricultural and Food Chemistry* 2005, 53 (10), 4166-4177.
- 544 26. Pedersen DS, Capone DL, Skouroumounis GK, Pollnitz AP, Sefton MA.
545 Quantitative analysis of geraniol, nerol, linalool, and alpha-terpineol in wine,
546 *Analytical and Bioanalytical Chemistry*. 2003, 375 (4), 517-522.
- 547 27. Papadopoulou D, Roussis IG. Inhibition of the decline of linalool and alpha-
548 terpineol in muscat wines by glutathione and n-acetyl-cysteine, *Italian Journal of Food*
549 *Science*. 2001, 13 (4), 413-419
- 550 28. Papadopoulou D, Roussis IG. Inhibition of the decrease of volatile esters and
551 terpenes during storage of a white wine and a model wine medium by glutathione and
552 N-acetylcysteine, *International Journal of Food Science and Technology* 2008, 43 (6),
553 1053-1057.
- 554 29. Roussis IG, Lambropoulos I, Tzimas P. Protection of volatiles in a wine with low
555 sulfur dioxide by caffeic acid or glutathione, *American Journal of Enology and*
556 *Viticulture*. 2007, 58 (2), 274-278
- 557 30. Moio L, Ugliano M, Genovese A, Gambuti A, Pessina R, Piombino P. Effect of
558 antioxidant protection of must on volatile compounds and aroma shelf life of
559 falanghina (*Vitis vinifera* L.) wine, *Journal of Agricultural and Food Chemistry* 2004,
560 52 (4), 891-897.
- 561 31. Ugliano M, Fedrizzi B, Siebert T, Travis B, Magno F, Versini G, Henschke PA.
562 Effect of Nitrogen Supplementation and Saccharomyces Species on Hydrogen Sulfide
563 and Other Volatile Sulfur Compounds in Shiraz Fermentation and Wine, *Journal of*
564 *Agricultural and Food Chemistry* 2009, 57 (11), 4948-4955.
- 565 32. Vilanova M, Ugliano M, Varela C, Siebert T, Pretorius IS, Henschke PA.
566 Assimilable nitrogen utilisation and production of volatile and non-volatile
567 compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts.
568 *Applied Microbiology Biotechnology*. 2007, 77, 145-157.

- 569 33. Hernandez-Orte P, Ibarz MJ, Cacho J, Ferreira V. Effect of the addition of
570 ammonium and amino acids to musts of Airen variety on aromatic composition and
571 sensory properties of the obtained wine. Food Chemistry. 2005, 89 (2), 163-174.
- 572 34. de Andres-de Prado R, Yuste-Rojas M, Sort X, Andres-Lacueva C, Torres M,
573 Lamuela-Raventos RM. Effect of soil type on wines produced from *Vitis vinifera* L.
574 Cv. Grenache in commercial vineyards. Journal of Agricultural and Food Chemistry.
575 2007, 55, 779-786.
- 576 35. Esti M, Airola RLG, Moneta E, Paperaio M, Sinesio F. Qualitative data analysis for
577 an exploratory sensory study of grechetto wine, Analytical Chimica Acta. 2010, 660
578 (1-2), 63-67.
- 579 36. Gonzalez-Vinas MA, Perez-Coello MS, Cabezudo MD, Martin-Alvarez PJ. Sensory
580 analysis of aroma attributes of young Airen white wines during storage in the bottle,
581 Journal of Food Quality. 1998, 21 (4), 285-297
- 582 37. Noble AC, Lesschaeve, I. Sensory methods of flavour analysis. In: Taylor AJ,
583 Linforth, R.S.T. (ed) Food Flavour Technology 2010. Blackwell, Chichester (United
584 Kingdom),
- 585 38. Campo E, Ferreira V, Escudero A, Cacho J. Prediction of the wine sensory
586 properties related to grape variety from dynamic-headspace gas chromatography-
587 olfactometry data, Journal of Agricultural and Food Chemistry. 2005, 53 (14), 5682-
588 5690.
- 589 39. Pozo-Bayon MA, Andujar-Ortiz I, Moreno-Arribas MV. Volatile profile and
590 potential of inactive dry yeast-based winemaking additives to modify the volatile
591 composition of wines. Journal of the Science of Food and Agricultural. 2009, 89
592 (10), 1665-1673.

593

594 C-1: Include complete journal titles in all cases?

595 Done

596 C-2: Include couple of recent references (last 2 years) from IJFP?

597 Sorry, but we did not find any recent article published in the IJFP related with the topic
598 of the present manuscript.

599

600 **Table 1.** Evolution of global composition in the must, control wine and in the wine
 601 supplemented with the glutathione enriched IDY preparation.

602

		pH	TA ^a	PAD ^b	AD ^c	VA ^d
Must		3.2	3.7	13.9	-	-
Cont-W	After alcoholic fermentation	3.13	4.2	-	13.8	-
	Stabilized and clarified wine	3.15	3.4	-	13.75	0.16
GSH-IDY-W	After alcoholic fermentation	3.18	4	-	13.6	-
	Stabilized and clarified wine	3.2	3.25	-	13.5	0.22

Cont-W: Control wine; GSH-IDY-W: Wine supplemented with the glutathione enriched IDY preparation.

603

604 ^a: Total acidity (g. sulphuric acid/L)

605 ^b: Probable alcohol degree (% v/v)

606 ^c: Alcohol degree (% v/v)

607 ^d: Volatile acidity (g acetic acid/L)

608

609 **Table 2.** Reference standard composition of aroma and taste attributes

610

Attributes	Reference standard composition ^a	
	Low concentration	High concentration
Strawberry	1.5 g of crushed fresh strawberries	6 g of crushed fresh strawberries
Peach	2 mL of peach nectar	7.5 mL of peach nectar
Banana	¼ 10 mm slice fresh banana	10 mm slice fresh banana
Apple	-	Slice fresh apple, 5 mL apple juice
Lemon	-	5 mL lemon juice, and small peel piece of fresh fruit
Floral	0.2 ml of linalool solution (150 mg/L)	1.5 mL of a linalool solution (150 mg/L)
Grassy	-	1 mL of a cis-3-hexen-1-ol solution (100 mg/L)
Toffee	-	1 toffee candy
Raisin	-	2-3 crushed fresh raisins
Honey	-	8 mL honey
Yeast	0.25 g baker yeast	1 g baker yeast
Acidity	0.2 g/L citric acid in water	0.8 g/L citric acid in water

611 ^a: references were prepared in tasting glasses filled with 25 mL of rosé base wine, covered by
 612 petri dishes, with the exception for acidity that was prepared in water. Attributes in bold were
 613 finally selected for the study.

614 **Table 3.** Concentration of volatile compounds (mean \pm standard deviation, $\mu\text{g L}^{-1}$) determined in the control wines (Cont-W) and in the wines supplemented
 615 with the G-IDY preparation (GSH-IDY-W) at 1, 2, 3 and 9 months of aging (1m, 2m, 3m and 9m, respectively)

				Cont-W				GSH-IDY-W				
	Compounds	Rlexp [†]	Rilit [‡]	Id [§]	1 m	2 m	3 m	9 m	1 m	2 m	3 m	9 m
Esters	Ethyl propanoate	920	903	S, R, M	43.9 ^b \pm 2.6	46.3 ^b \pm 5.3	39.5 ^{b,a} \pm 6.9	26.8 ^a \pm 2.6	26.5 ^a \pm 0.6	29.3 ^a \pm 1.2	33 ^a \pm 0.1	30.9 ^a \pm 5.1
	Isobutyl acetate	975	953	S, R, M	4.5 ^b \pm 0.4	4.1 ^b \pm 0.94	3.3 ^b \pm 0.7	1.4 ^a \pm 0.2	5.0 ^b \pm 0.3	4.5 ^b \pm 0.0	4.6 ^b \pm 0.1	2.7 ^a \pm 0.3
	Ethyl butanoate	1010	1010	S, R, M	240.7 ^b \pm 12.6	225.2 ^b \pm 41.6	200.7 ^b \pm 38.8	103.2 ^a \pm 22.6	237.8 ^b \pm 7.2	229.9 ^b \pm 4.9	242.0 ^b \pm 2.1	173.2 ^a \pm 18.9
	Ethyl 2-methylbutanoate	1026	1031	S, R, M	2.5 ^b \pm 0.1	2.4 ^b \pm 0.4	2.7 ^b \pm 0.5	2.5 ^a \pm 0.2	1.7 ^a \pm 0.1	2 ^{a,b} \pm 0.1	2.2 ^{b,c} \pm 0.1	2.6 ^c \pm 0.2
	Isoamyl acetate	1115	1117	S, R, M	573.7 ^b \pm 16.6	479 ^b \pm 75.1	390.1 ^b \pm 79.5	188.6 ^a \pm 27.0	811 ^c \pm 22.5	786.2 ^c \pm 1.2	730.6 ^b \pm 3.7	445.9 ^a \pm 17.3
	Ethyl hexanoate	1229	1230	S, R, M	710.3 ^b \pm 6.8	582 ^b \pm 70.8	574.6 ^b \pm 106.8	310.7 ^a \pm 28.6	706 ^b \pm 13.1	722.4 ^b \pm 7.4	716.6 ^b \pm 7.5	467.1 ^a \pm 28.3
	Hexyl acetate	1267	1269	S, R, M	130.7 ^b \pm 2.06	110.1 ^b \pm 14.3	97.7 ^b \pm 19.1	44.9 ^a \pm 4.7	219.6 ^c \pm 6.3	213 ^c \pm 0.5	194.6 ^b \pm 1.9	114.5 ^a \pm 3.5
	Ethyl heptanoate	1327	1332	R, M	2.1 ^b \pm 0.1	1.8 ^b \pm 0.2	1.9 ^b \pm 0.4	1.1 ^a \pm 0.2	1.4 ^b \pm 0.1	1.5 ^b \pm 0.0	1.4 ^b \pm 0.1	0.8 ^a \pm 0.1
	Ethyl octanoate	1429	1431	S, R, M	1678.8 ^b \pm 306.8	1745.1 ^b \pm 146.2	1788.4 ^b \pm 145.8	666.1 ^a \pm 31.7	2097.7 ^b \pm 8.4	2104.3 ^b \pm 9.1	2197.4 ^c \pm 14.7	1046.1 ^a \pm 13
	Ethyl nonanoate	1530	1541	S, R, M	1.9 ^a \pm 0.7	3.8 ^a \pm 0.2	4.6 ^a \pm 0.2	4.40 ^a \pm 2.4	2.9 ^a \pm 0.1	3.6 ^b \pm 0.0	4.1 ^b \pm 0.2	2.4 ^a \pm 0.4
	Ethyl decanoate	1634	1634	S, R, M	511.9 ^{a,b} \pm 253.0	883.5 ^c \pm 37	864.3 ^{b,c} \pm 47.1	270 ^a \pm 15	931.6 ^b \pm 55	960.3 ^b \pm 12.7	1045.2 ^b \pm 56.9	398.4 ^a \pm 44.3
	Diethyl succinate	1673	1694	S, R, M	515.3 ^a \pm 62.7	492.4 ^a \pm 5.8	788 ^b \pm 97.4	1035.8 ^b \pm 150.8	279.1 ^a \pm 17.1	300 ^a \pm 21.3	436.4 ^a \pm 33.6	800.2 ^b \pm 230.2
2-Phenyl ethyl acetate	1809	1752	S, R, M	49.4 ^b \pm 1.3	53.3 ^c \pm 0.4	53.6 ^c \pm 0.4	42.6 ^a \pm 1.9	89.4 ^a \pm 5.8	84.2 ^a \pm 3.7	95.6 ^a \pm 0.6	63.7 ^a \pm 23.3	
Ethyl dodecanoate	1840	1833	S, R, M	36.8 ^a \pm 15.0	72 ^a \pm 1.8	49.9 ^a \pm 5.9	97.1 ^a \pm 40.8	82.3 ^a \pm 15.5	65.7 ^a \pm 8.5	52.4 ^a \pm 4.9	63.5 ^a \pm 12.7	
Alcohols	1-Butanol	1141	1157	S, R, M	394.8 ^b \pm 9.9	380.6 ^b \pm 65.7	343.1 ^{a,b} \pm 39.2	226.9 ^a \pm 37.5	333.7 ^a \pm 8.3	310.7 ^a \pm 10.5	361.5 ^a \pm 10.5	322.4 ^a \pm 66.9
	1-Hexanol	1353	1356	S, R, M	1255.6 ^b \pm 100.6	1122.7 ^{a,b} \pm 170.9	1102.7 ^{a,b} \pm 215	756.4 ^a \pm 116.8	864.6 ^a \pm 17.3	718.7 ^a \pm 15.4	877.9 ^a \pm 22.4	893.6 ^a \pm 211.8
	Cis-3-hexenol	1361	1370	S, R, M	44.4 ^b \pm 3.4	40.7 ^{a,b} \pm 5.1	40.3 ^{a,b} \pm 6.9	28.4 ^a \pm 2.5	38.4 ^a \pm 1.1	31.2 ^a \pm 0.2	39.7 ^a \pm 0.3	37.3 ^a \pm 8.8
	Trans-3-hexenol	1378	1370	S, R, M	58.6 ^b \pm 2.2	61.5 ^b \pm 1.0	57.2 ^b \pm 6.6	39.3 ^a \pm 5.8	69 ^a \pm 0.1	59.6 ^a \pm 1.3	73.0 ^a \pm 1.2	68.6 ^a \pm 15.7
	Benzenemethanol	1880	1834	S, R, M	79.6 ^{a,b} \pm 7.0	68.4 ^a \pm 0.9	83.6 ^{a,b} \pm 9.7	86 ^b \pm 3.4	77.8 ^a \pm 3.2	71 ^a \pm 6.7	97.4 ^a \pm 8.0	96.9 ^a \pm 33.5
Terpenes	Limonene	1179	1180	S, R, M	0.4 ^a \pm 0.0	0.3 ^a \pm 0.0	0.4 ^a \pm 0.0	1.1 ^a \pm 0.6	0.5 ^a \pm 0.2	0.3 ^a \pm 0.0	0.4 ^a \pm 0.0	0.5 ^a \pm 0.1
	α -terpinene	1494	-	M	1.1 ^a \pm 0.1	1.2 ^a \pm 0.1	1.40 ^{a,b} \pm 0.0	1.6 ^b \pm 0.2	0.8 ^a \pm 0.0	0.7 ^a \pm 0.1	1.0 ^{a,b} \pm 0.1	1.3 ^b \pm 0.2
	Linalool	1547	1541	S, R, M	3.3 ^a \pm 0.7	3 ^a \pm 0.3	3.6 ^a \pm 0.5	3.3 ^a \pm 0.5	2.6 ^a \pm 0.2	2.6 ^a \pm 0.0	3.3 ^{a,b} \pm 0.1	4.3 ^b \pm 0.8
	Citronellyl acetate	1657	1666	R, M	1.9 ^{a,b} \pm 0.5	2.2 ^b \pm 0.2	2.1 ^{a,b} \pm 0.2	1.4 ^a \pm 0.2	2.3 ^b \pm 0.0	2.1 ^{a,b} \pm 0.6	2.0 ^{a,b} \pm 0.5	1.2 ^a \pm 0.1
	β -Citronellol	1767	1781	S, R, M	4.8 ^a \pm 1.2	4 ^a \pm 0.1	4.5 ^a \pm 0.6	4.8 ^a \pm 0.1	3.9 ^a \pm 0.3	3.3 ^a \pm 0.2	4.2 ^a \pm 0.2	4.0 ^a \pm 0.9
Isopropyl myristate	2035	2040	R, M	0.3 ^a \pm 0.3	0.3 ^a \pm 0.1	0.3 ^a \pm 0.0	0.1 ^a \pm 0.0	0.2 ^{a,b} \pm 0.0	0.4 ^c \pm 0.0	0.3 ^{b,c} \pm 0.1	0.1 ^a \pm 0.0	
Fatty acids	Hexanoic acid	1859	1789	S, R, M	4821.8 ^a \pm 643.4	3411.1 ^a \pm 91.7	4812.9 ^a \pm 683.2	3689.1 ^a \pm 527.2	5097.7 ^a \pm 117.6	4988.2 ^a \pm 152.8	5125.4 ^a \pm 1016	6153.9 ^a \pm 1545.1
	Octanoic acid	2078	1998	S, R, M	2383.2 ^a \pm 188.4	2247.1 ^a \pm 39.7	2858.2 ^b \pm 57.9	3393.4 ^c \pm 191.2	3240.5 ^a \pm 194.5	3335.9 ^a \pm 87.7	3289.6 ^a \pm 226.0	3731.0 ^a \pm 1280.8
	Decanoic acid	2289	2279	S, R, M	438 ^a \pm 4.2	509.5 ^{a,b} \pm 47.4	585.6 ^b \pm 32.2	739.5 ^a \pm 29.1	679.9 ^a \pm 4.6	720.3 ^a \pm 67.0	802 ^a \pm 16.7	597.3 ^a \pm 281.9
Others	2,3 butanedione	937	949	S, R, M	258.7 ^a \pm 51.6	309.1 ^a \pm 61.4	280.8 ^a \pm 59.8	198.1 ^a \pm 17.8	390.2 ^c \pm 1.6	400.3 ^c \pm 21.0	310.5 ^b \pm 24.2	92.8 ^a \pm 21.9
	Furfuraldehyde	1459	1449	S, R, M	3 ^a \pm 0.3	4.5 ^{a,b} \pm 0.4	5.6 ^b \pm 0.1	10.7 ^c \pm 1.3	2.9 ^a \pm 0.4	3.3 ^a \pm 0.3	3.3 ^a \pm 0.6	4.0 ^a \pm 0.9
	γ -butyrolactone	1613	1595	S, R, M	5644.3 ^b \pm 400.4	3625.8 ^a \pm 401.9	5561.9 ^b \pm 997.3	3579.8 ^a \pm 486.7	3411.7 ^a \pm 433	2785.5 ^a \pm 339.5	3252.8 ^a \pm 552.7	3074.3 ^a \pm 807.8
	Methionol	1709	1714	S, R, M	774.9 ^a \pm 15.4	613.3 ^a \pm 7.7	804.5 ^a \pm 217.4	606.2 ^a \pm 15.7	380.2 ^a \pm 42.7	324.5 ^a \pm 97	493.2 ^a \pm 64.8	381.9 ^a \pm 201.0
	β -damascenone*	1809	1752	S, R, M	6 ^a \pm 0.3	6.5 ^{a,b} \pm 0.4	7.4 ^b \pm 0.3	7.3 ^b \pm 0.7	6.5 ^a \pm 0.4	7 ^a \pm 0.4	8.6 ^a \pm 0.1	7.9 ^a \pm 2.2

616 † Retention index calculated by SPME with an alkane mixture (C5-C30)

617 ‡ Retention index reported in the literature from Flavornet database: <http://www.webbook.nis.gov/chemistry>

618 § Identification method: S, identification by comparison with standard compounds; RI, identified by retention index; MS, identified by mass spectra (NIST
619 libraries)

620 Different superscripts denote statistical differences ($p < 0.05$) in the values in the same row for each type of wine

621
622

623
624
625
626
627
628

Table 4. Results from the two-way ANOVA (concentration, judge, concentration x judge) and F-ratios of the sensory terms evaluated by the 10 judges during training in 6 attributes at 2 different concentrations.

Attributes	Concentration	Judge	Concentration x judge
Acidity	162.00***	0.22	-
Banana	1699.54***	1.05	1.53
Floral	1077.5***	1.26	1.68
Peach	98.92***	0.20	1.98
Strawberry	2366.46***	2.78*	9.5***
Yeast	116.55***	1.02	2.28

629 * and ** *denote significance at $p < 0.05$ and $p < 0.001$ respectively
630
631

632 **Table 5.** Results obtained on the descriptive analysis by the panel of judges (n=10) of the 6
 633 sensory attributes evaluated in the control (Cont-W) and GSH-IDY wines (GSH-IDY-W) after
 634 9 months of aging
 635
 636

Attributes	F-ratio	p-value	Mean	
			Cont-W-9m	GSH-IDY-W-9m
Acidity	0.00	0.9944	7.72	7.71
Banana	3.23	0.0911	4.51	7.16
Floral	0.17	0.6875	7.59	8.24
Peach	4.07	0.0589	7.65	4.81
Strawberry	8.13	0.0116	4.02	7.87
Yeast	11.46	0.0038	4.31	1.91

637
 638 Judges 1 and 10 not consistent with the whole panel were excluded from data analysis
 639 of strawberry and banana attributes. Attributes in bold were significantly different
 640 between wines.
 641

642 **Figure Captions**

643

644 **Figure 1.** Dendrogram resulting from the application of cluster analysis to the data
645 corresponding to the concentration of volatile compounds determined in the wines of
646 different aging time (1, 2, 3 and 9 months) made with or without the addition of a
647 glutathione enriched IDY preparation (G-IDY-W and Cont-W, respectively)

648

649 **Figure 2.** Aroma profiles of Grenache rosé wines in the control wine (Cont-W) and in
650 the wine supplemented with a glutathione enriched IDY preparation (GSH-IDY-W)

651

652