1	Influence of ethanol content on sweetness and bitterness
2	perception in dry wines
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21 Abstract:

Ethanol is the second major component of wine after water and exhibits important sensory 22 properties. Previous studies suggested that its bittersweet taste varies according to the alcohol 23 content and the matrix. However, the organoleptic impact of ethanol on wine remains largely 24 ambiguous. Various sensory tests were carried out with a trained panel and the results were 25 statistically analyzed. Tastings revealed that variations of ethanol content usually observed in 26 27 dry wines have no direct effect on sweet taste of wine. The role of ethanol in white wine bitterness was also studied, revealing its ability to impart the perception of bitterness due to 28 sensory interactions with other constituents. Moreover, a threshold effect was observed between 29 7 and 10% alc. vol.. These results underline the importance of sensory interactions in the 30 perception of taste and illustrate the role of matrix effects. 31

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33 Keywords: Wine, Ethanol, Bitterness, Sweetness, Sensory analysis

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35 Highlights

36 Sweetness and bitterness intensity were evaluated in dry wine after panel training.

37 The panel was able to discriminate intensity modulations of sweet and bitter taste.

38 Sweetness of dry wines was not affected by usual variations of ethanol content.

39 Ethanol had an indirect effect on white wine taste by increasing the bitterness perception.

40 These results highlighted the importance of matrix on the perception of wine taste.

41 **1. Introduction**

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Ethanol is a primary metabolite produced by yeasts during alcoholic fermentation of 43 grape sugars present in must. Its content generally varies between 12 and 14% alc. vol. in most 44 dry wines (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). The presence of ethanol 45 entails the moderate intake of wine due to its psychophysiology (Allen et al., 2014, Lanier et 46 al., 2005, Nolden and Hayes, 2015) and health (Baum-Baicker, 1985, Cao et al., 2015) effects. 47 However, it also significantly contributes to the physico-chemical properties and to the 48 microbiological stability of the wine. Moreover, ethanol is the most abundant volatile 49 50 compound in wine and might therefore, depending on its concentration, modify the aromatic 51 perception (Guth & Seis, 2002). For instance, some studies described that the intensity of wine fruitiness decreases with the amount of ethanol (Escudero et al., 2007, Goldner et al., 2009, Le 52 53 Berre et al., 2007). In addition to ethanol impact on wine aromas, the effects of ethanol level on oral sensation have been investigated (DeMiglio et al., 2002, Nurgel and Pickering, 2005). 54 Studies have shown the minimizing effect of ethanol on red wine astringency (Fontoin, Saucier, 55 Teissedre, & Glories, 2008). This observation has been attributed to the interference of ethanol 56 57 with hydrophobic interactions between proteins and tannins, leading to a reduction of tannin precipitation and a decreased astringent sensation (Gawel, 1998, McRae et al., 2015). Finally, 58 ethanol is also a taste-active compound. Various authors have described the sweet taste of 59 ethanol in aqueous solution containing low levels of ethanol (0-4% alc. vol.) (Blizard, 2007, 60 Scinska et al., 2000, Wilson et al., 1973) as well as the bitter taste and the burning characteristics 61 associated with higher levels of ethanol (10-22% alc. vol.) (Bartoshuk et al., 1993, Blizard, 62 2007, Mattes and DiMeglio, 2001, Scinska et al., 2000, Thorngate, 1997, Wilson et al., 1973). 63 Neurophysiological and genetic studies have explained the link between sweet taste perception 64 and ethanol consumption by a similar gustatory neural pathway response (Blednov et al., 2008, 65 Lemon et al., 2004). Sour and salty attributes have also been cited to describe ethanol taste, but 66 with much lower intensities than bitter or sweet taste (Fischer and Noble, 1994, Mattes and 67 DiMeglio, 2001, Scinska et al., 2000). 68

Ethanol has been established as bittersweet, and it appears that its taste-properties vary according to its content. This raises the question of its real contribution to wine sweetness and bitterness. Concerning its sweet taste, ethanol has been described to enhance wine sweetness directly through its own sweet taste (Jackson, 1994). However, recent studies showed that ethanol does not influence sweet taste of model dry wines and Australian Riesling base wines

(Gawel et al., 2007, Jones et al., 2008). Thus, the impact of ethanol on red and white dry wine 74 sweet taste was not clearly established. According to several authors, increasing ethanol content 75 through the range of those encountered in wine results in an elevation of its bitter taste. Fischer 76 and Noble described the enhancing role of ethanol between 8 and 14% alc. vol. in a model wine 77 solution containing 100 mg/L of catechin (Fischer & Noble, 1994) while other studies 78 demonstrated a similar enhancing effect by quinine (Martin & Pangborn, 1970), epicatechin 79 and catechin (Noble, 1994, Thorngate, 1992) and grape seed tannin oligomers (Fontoin et al., 80 2008). A more recent study (Gawel, Van Sluyter, Smith, & Waters, 2013) has investigated the 81 effects of a complex mixture of phenolics on white wine bitterness under various pH and 82 alcohol levels. Its results have also supported a direct effect of ethanol on wine bitterness 83 84 perception. The results of these studies suggest the influence of ethanol on wine taste. However, the question remains whether ethanol affects wine flavor directly or indirectly in the 85 86 concentration range encountered in wine.

For this reason, this study was conducted in order to better understand the role of ethanol 87 88 content on the sweet and bitter taste of wine. Despite the clearly stated bittersweet taste of ethanol, its overall contribution to dry wine taste is still unclear. The first part of this work was 89 90 aimed at studying the influence of variations of ethanol content in quantities generally encountered in wines (12-14% alc. vol.) on the sweet taste of a red and a white wine. Then, the 91 influence of ethanol content on bitter perception was comparatively studied in two wines of 92 differing levels of bitterness (low and high). This approach sought to determine whether the 93 impact of ethanol on wine bitterness was direct or indirect and to demonstrate the importance 94 95 of matrix effects on taste perception.

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97 2. Materials and methods

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99 2.1. Chemicals

Quinine sulfate and tartaric acid were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier,
France). Neohesperidin dihydrochalcone (NHDC) was purchased from Extrasynthese (Genay,
France). The water used for solution preparation was pure and demineralized (eau de source de
Montagne, Laqueuille, France).

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- 105 **2.2. Wines**
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107 2.2.1. Study of ethanol effect on sweetness perception

- 108 One white wine (WW) and one red wine (RW) were chosen for their relatively low ethanolic 109 content. The white wine was a Bordeaux 2008 (12% alc. vol.; pH 3.1; 7.2 g/L of titratable
- acidity; 0.5 g/L of glucose + fructose) and the red wine was a Bordeaux 2008 (12.5% alc. vol.;
- 111 pH 3.6; 5.6 g/L of titratable acidity; 0.2 g/L of glucose + fructose).
- 112

113 2.2.2. Study of ethanol effect on bitterness perception

- During a preliminary sensory analysis, two white wines were selected on the basis of their taste by five experts strongly experienced in wine tasting, The first white wine ("wine A"), chosen for its very low bitterness, was a white Bordeaux 2011 (12.2% alc. vol.; <2 g/L of glucose +
- 117 fructose; pH 3.1). The second white wine ("wine B"), chosen for its strong bitterness, was a
- 118 Pessac-Léognan 2011 (12.5% alc. vol.; <2 g/L of glucose + fructose; pH 3.1).
- 119

120 2.3. Sensory analysis

- Tastings sessions took place in a specific room equipped with individual booths and airconditioned at 20 °C (ISO 8589:2007). Normalized glasses were used (ISO 3591:1977). All
- panelists (15 men and 15 women aged from 25 to 65 years) were wine tasting specialists. They
- 124 were informed of the nature and risks of the present study and were asked to give their consent
- to participate in the sensory analyses.
- 126 Due to saturation and persistence of the bitter taste as well as palate fatigue of the panel, training
- and test sessions were spread over one week. Panelists were asked to rinse mouth with waterand wait one minute between each sample.
- For all evaluations, samples were labeled with random three-digit codes and presented incounterbalanced order to avoid bias.
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132 2.3.1. Panel training

- 133 Panelists attended four sessions to train in recognition and discrimination of taste perception.
- During the first two sessions, different concentrations of reference standard solutions representative of taste were presented to the panel: NHDC (0-4 mg/L) for sweetness and
- 136 quinine sulfate (1.5–12 mg/L) for bitterness. Also, different concentrations of quinine sulfate
- 137 (1.5–12 mg/L) with 3 g/L of tartaric acid were presented to the panel to train them to perceive
- 138 bitterness independent of acidity.
- 139 The last two sessions were used to improve the panel's ability to discriminate sweetness and
- bitterness. Three series were presented to the panel: quinine sulfate (1.5, 3, 6 and 12 mg/L) with

- and without 3 g/L tartaric acid and NHDC (0, 1, 2 and 4 mg/L) (Table 1). Panelists were asked
 to sort the samples by increasing order of bitterness and sweetness for each series.
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144 2.3.2. Sensory experiments on sweetness

To study the effect of ethanol content on wine sweetness, distilled ethanol was added to the 145 white Bordeaux 2008 (12% alc. vol.) to provide ethanol levels of 12.5, 13 and 13.5% alc. vol., 146 and in the same manner, distilled ethanol was added to the red Bordeaux 2008 (12.5% alc. vol.) 147 to provide ethanol levels of 13, 13.5 and 14% alc. vol. as presented in Table 2. The difference 148 149 between the lower and higher alcohol percentage (1.5% alc. vol.) was based on the ethanol content range generally encountered in wine. The alcoholic strength by volume in wine was 150 measured with a FOSS Winescan (Hillerød, Danmark) and by the O.I.V. official Gibertini 151 method (O.I.V., 2015). 152

153 The addition of ethanol leads to a maximal dilution of 1.5%, which is considered as negligible.

The four samples of each wine were presented to the panelists, who were first asked to rate the sweetness intensity on an eight-point scale (0 = "absence" to 7 = "very high"). This test was chosen based on the panel's familiarity with the intensity scale for profile description. In a

second phase, panelists were asked to sort the wines by increasing order of sweetness.

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159 **2.3.3. Sensory experiments on bitterness**

To study the effect of ethanol content on bitterness perception, two white wines were chosen.
The first wine had an imperceptible bitterness ("wine A") while the second wine was
representative of a bitter wine ("wine B").

Both wines were first dealcoholized by evaporation under vacuum to obtain a white wine concentrate of 5% alc. vol. Then, for each wine, addition of distilled ethanol and pure and demineralized water provided wine samples with ethanol levels of 4, 7, 10 and 12.5% alc. vol. as presented in Table 2 (the final volume was the same for all samples and similar to the initial volume of wine before dealcoholization). As described for sweetness experiments, the levels of alcohol were determined after sample preparation.

- The panelists were asked to rate the bitterness intensity of these wines on an eight-point scale (0 = "absence" to 7 = "very high"). For this experiment, the panel was not asked to sort the wines by increasing order of bitterness, as the difficulty of this exercise increases with the number of samples (8 wines).
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174 **2.4. Statistical analysis**

All statistical analyses were carried out using the software, R Statistical (Foundation forStatistical Computing, Vienna, Austria).

According to the international organization for standardization (ISO 8587:2007), results 177 obtained from ranking tests are interpreted by Page's trend test, used to test the hypothesis of a 178 predicted order of levels. Indeed, the order of samples for each wine is predetermined by the 179 increasing concentrations of reference standard (Section 3.1. Panel Training) and ethanol 180 (Section 3.2. Sensory Experiments on Sweetness). For each judge, a value between 1 and 4 was 181 attributed to each sample (1 = "less intense sample" and 4 = "more intense sample"). The sums 182 of the ranks Ri were obtained for each sample, then the parameters L and L' were calculated as 183 described below and L' was compared to threshold values in order to determine whether the 184 result of the test was significant or not for the factor concerned. 185

186
$$L = \sum_{i=1}^{p} i.Ri$$
 $L' = \frac{12L - 3n.p.(p+1)^2}{p.(p+1).\sqrt{n.(p-1)}}$

- 187 *n*, number of panelists (n = 30)
- 188 p, number of modalities (p = 1 to 4)
- 189

A one-way ANOVA (analysis of variance) with a 0.1% risk error was used to statistically interpret and discriminate wine samples on their sweetness or bitterness intensity. ANOVA was performed on normalized panel's rates. Levene's test for homogeneity of variance and Shapiro Wilk's test for normality were previously applied to prove, respectively, that variances were equal and that the population had a normal distribution as assumed by the ANOVA. When the ANOVA showed significant inequality of means (P < 0.001), the Tukey's "post-hoc" pairwise comparison was used to determine the groups of samples that were significantly different.

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3. Results and discussion

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200 **3.1. Panel evaluation**

To evaluate the panel's ability to recognize and discriminate taste perception, various levels of reference standard solutions were displayed as presented in Table 1. NHDC and quinine sulfate were used as they exhibit sweet and bitter taste, respectively. Panelists were asked to sort the samples following the increasing perception of the taste. Data presented in Table 3 showed that for both quinine sulfate series (with or without tartaric acid), panelists were able to classify the samples according to the intensity of the bitterness perception. Indeed, we observed that the

- sum of the ranks was increasing from R1 to R4 in coherence with the addition of quinine sulfate concentrations. Values of L' were distinctly higher to the critic value of 3.09 for a 0.1% risk error establishing the significance of the test.
- 210 In the same manner for the NHDC series, we observed that the sum of the ranks increased with
- the NHDC concentrations, meaning that the panel was able to significantly sort the samples
- according to the intensity of the sweetness perception, with a 0.1% risk error (L > 3.09).
- 213 These results demonstrated the efficiency of the panel to detect sweetness and bitterness and to
- 214 discriminate samples according to the intensity of these tastes.
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3.2. Effect of ethanolic content on wine sweetness

The base wines used in these experiments displayed low concentrations of glucose + fructose (0.5 and 0.2 g/L for the white and red wine, respectively), far below their taste threshold (Amerine et al., 1965, Hladik and Simmen, 1996, Simmen and Hladik, 1998). Thus, these were actually dry wines.

221 To determine if varying ethanol levels in dry wines could affect their sweet perception, different quantities of distilled ethanol were added to these red and white wines as described in Table 2. 222 The choice of using distilled ethanol from wine was made in order to avoid any taste bias from 223 the addition of analytical grade ethanol in the samples. Panelists then rated the intensity of the 224 perceived sweet taste of the four independent samples for the red wine as well as for the white 225 wine on a linear eight point scale. The results were statistically interpreted by ANOVA. The 226 Levene's test and Shapiro Wilk's test proved the homogeneity of variance (p-value RW = 227 0.3517; p-value WW = 0.2537) and the normal distribution (p-value RW = 0.6547; p-value 228 WW = 0.6281) assumed by the ANOVA. Then, the ANOVA was performed on the sensory 229 data (*p*-value RW = 0.487; *p*-value WW = 0.229) and revealed that the test was not significant, 230 indicating that panelists did not perceive differences in sweetness of samples. Even if the panel 231 proved to be efficient with discriminating variations of sweetness, the perception of a variation 232 of 2% alc. vol. might be difficult to rate on an linear scale (Sauvageot, 2009b). In order to 233 234 compel the panel to make a stand on samples' sweetness, they were asked in a second phase to sort the wines in ascending order of sweetness. Thus, a rank test was used here, which was a 235 more intuitive test for the panel (Sauvageot, 2009a). As presented in Table 4, the results of the 236 Page test were not significant for either wine. The progression of rank sums were not in 237 agreement with the ethanol content for the red or the white wine. Also, the L' value was lower 238 than the threshold value of 1.645 for a 5% risk error. 239

These results demonstrated that for both the red wine and white wine, the modality containing 240 1.5% alc. vol. more than the control wine was not perceived as sweeter. More generally, 241 although the ethanol levels presented here covered a range of alcoholic strength characteristic 242 of most French dry wines (12.5%-14% alc. vol. for RW and 12%-13.5% alc. vol. for WW), it 243 appeared that variations of ethanol content did not result in increase of sweet taste of wine. 244 These results were in accordance with previous works carried out on both white and red wines 245 (Gawel et al., 2007, Jones et al., 2008, Marchal et al., 2011). However, some tasters have 246 frequently pointed out a higher sweet sensation in wines containing high levels of alcohol. This 247 phenomenon could be due to the release of grape or yeast compounds, increasing sweetness. 248 For instance, the heat shock protein Hsp12p is responsible for a gain of sweetness of dry wines 249 250 during yeast autolysis and it has been shown that the expression of the gene HSP12 increased with the progression of alcoholic fermentation (Marchal, Marullo, et al., 2015). Thus, the 251 252 impact of ethanol on sweetness could be indirect through the release of taste-active compounds from yeast lees. Moreover, it is possible that matrix effects could occur, and that these results 253 254 might be slightly different using alternative base wines. However, the small differences in ethanol concentration met in dry wines compared with a clear disparity of sweetness perceived 255 256 suggest that the role of ethanol in the perception of sweetness of dry wines remains very limited. Compounds released from yeast lees autolysis and oak wood (Marchal et al., 2015, Marchal et 257 al., 2011) have been recently proposed to give a molecular insight on the sweet taste of dry 258 259 wines.

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261 **3.3.** Effect of ethanol content on bitterness perception of a white wine

The wine with a very low bitterness ("wine A") and the bitter wine ("wine B") were both processed to display ethanol levels of 4, 7, 10 and 12% alc. vol. After dealcoholization of both wines under vacuum, different quantities of the distilled ethanol and demineralized water were added to obtain these ethanol levels.

Aromas of the four wine samples for both wines were judged by five experts as being consistent with the aroma of both selected wines. Also, the pH levels of these four samples were measured

- 268 for both wines and remained consistent.
- 269 Panelists were then asked to rate the intensity of the perceived bitter taste on a linear eight point
- scale for these eight independent samples (Samples S1 to S4 for "wine A" and S1 to S4 for"wine B").
- 272 The Levene's test and Shapiro Wilk's test were applied and proved the homogeneity of variance
- 273 (p-value = 0.5513) and the normal distribution (p-value = 0.7685) of the dataset. A one-way

ANOVA was performed and revealed significant differences (*p*-value = $5.7 \times 10-13$). This result established that at least one of the samples was perceived different from the others, with a 0.1% risk error.

The Tukey's "post-hoc" pairwise comparisons allowed for sorting of the samples in 277 significantly different groups. This multiple means comparison showed that samples S1 to S4 278 of "wine A" and S1 to S2 of "wine B" were significantly different from sample S3 of "wine B", 279 with a 5% risk error, and moreover, highly significantly different from sample S4 of "wine B", 280 with a 0.1% risk error. In this way, the multiple means comparison highlighted the affiliation 281 282 of the samples to two significantly different groups. The group « a » was characterized by its very low bitterness and the group « b » was significantly distinguished as more bitter (Fig. 1). 283 At 12.5% alc. vol., both wines were perceived as different regarding their bitterness, which 284 confirms that the preliminary choice of these two wines was relevant: "wine B" actually 285 286 exhibited a stronger bitter taste. For "wine A", there was no distinction between the sample at 4% and 12.5% alc. vol. For "wine B", the 4% alc. vol. sample was distinguished from the one 287 288 at 12.5% alc. vol. and exhibited a much lower bitterness. This experiment highlighted the role of ethanol. At a 12.5% alc. vol., the "wine B" was distinguished from "wine A" by its higher 289 290 bitterness, while there was no difference perceived between these two wines at 4% alc. vol. Moreover, increasing the ethanol level in "wine A" did not result in any gain of bitterness. 291 Jointly, these results suggest that ethanol was not directly responsible for the perceived 292

bitterness but allowed for its revelation.

Moreover, samples with 4, 7, 10 and 12.5% alc. vol. of "wine A" were not distinguished, and 294 samples with 4 and 7% alc. vol. of "wine B" were not distinguished from "wine A" samples, 295 forming the group « a » (very low bitterness). Nevertheless, samples with 10 and 12.5% alc. 296 vol. of "wine B" were judged to be significantly and highly significantly more bitter, 297 respectively. In this way, a threshold effect for the "wine B" was observed. Indeed, this wine 298 bitterness was not perceived at 4 and 7% alc. vol. while it appeared bitter at 10 and 12.5% alc. 299 vol. Moreover, even if there was no significant difference between the 10 and 12.5% alc. vol. 300 samples of "wine B", we observed a slight increase of the bitterness. The value of this threshold 301 might depend on the wine composition and more particularly the levels of phenolic or other 302 taste active compounds. 303

These results were in agreement with Fontoin et al. (Fontoin et al., 2008). and Fischer and Noble

305 (Fischer & Noble, 1994), who have reported the increasing effect of ethanol on the bitterness

of some phenolic compounds, even if they did not use the same matrices and grade of ethanol.

307 A more recent study (Gawel et al., 2013) has highlighted the complexity of ethanol influence

on wine taste. They have established that higher amounts of ethanol and phenolics
supplemented from various grape varieties are linked with a stronger bitterness in Riesling and
Chardonnay base wines.

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312 4. Conclusions

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The present research focused on impact of ethanol level on wine sweetness and its role in wine bitterness. Sensory tests were carried out with a trained panel and the results were interpreted using statistical analysis.

First, ethanol was added into red and white wines to cover a range generally observed in dry wines (from 12 to 14% alc. vol.). The tasting revealed no modification of the perception of wine sweetness suggesting that ethanol has no direct effect on sweet taste of wine.

The results of the second part of this study showed that ethanol was not directly responsible for 320 the perceived bitterness in white wine. Indeed, increasing the alcohol level did not provide more 321 bitterness in a non-bitter wine contrary to a bitter wine. This supports the hypothesis that, in 322 wine, ethanol allows for the perception of bitterness due to sensory interactions with other 323 324 constituents. Moreover, a threshold effect was observed between 7 and 10% alc. vol. as well as a slight increase in bitterness between 10 and 12.5% alc. vol. These results clearly underline 325 326 the importance of sensory interactions in the perception of taste and illustrate the role of matrix effects. For the past few years, some research studies have aimed at identifying molecular 327 determinants of taste with an inductive approach. Such studies were based on a taste-guided 328 approach and implied a tasting after each step of the purification protocol to select the relevant 329 fractions (Glabasnia and Hofmann, 2006, Hufnagel and Hofmann, 2008, Marchal et al., 2011). 330 The results of the present work demonstrated that the matrix used to taste the fraction has to be 331 chosen very carefully to ensure the relevance of the approach, in particular if bitter compounds 332 are targeted. 333

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Tables

Compounds	Sample 1	Sample 2	Sample 3	Sample 4	
Panel evaluation					
NHDC effect	0 mg/L	1 mg/L	2 mg/L	4 mg/L	
Quinine sulfate effect	1.5 mg/L	3 mg/L	6 mg/L	12 mg/L	
Quinine sulfate + Tartaric acid effect	1.5 mg/L + 3 g/L	3 mg/L + 3 g/L	6 mg/L + 3 g/L	12 mg/L + 3 g/L	

 Table 1. Samples used for the panel evaluation.

 Table 2. Samples used for sensory tests on sweetness and bitterness perception.

Factor studied	Test	Sample 1 S1	Sample 2 S2	Sample 3 S3	Sample 4 S4
Effect on sv	veetness				
Ethanol effect	Intensity notation and Ranking	RW (12.5% alc.vol.)	RW + 0.5% (13% alc. vol.)	RW + 1% (13.5% alc.vol.)	RW + 1.5% (14% alc.vol.)
		WW (12% alc.vol.)	WW + 0.5% (12.5% alc.vol.)	WW + 1% (13% alc.vol.)	WW + 1.5% (13.5% alc.vol.)
Effect on bi	tterness				
Ethanol effect	Intensity notation	Wine A 4% alc. vol.	Wine A 7% alc. vol.	Wine A 10% alc. vol.	Wine A 12.5% alc. vol.
		Wine B 4% alc. vol.	Wine B 7% alc. vol.	Wine B 10% alc. vol.	Wine B 12.5% alc. vol.

 Table 3. Results of rank tests for panel evaluation.

Ranks sum	R 1	R ₂	R 3	R 4	L	L'
NHDC	53	68	82	9 7	823	4.62***
Quinine sulfate effect	42	61	83	114	869	7.53***
Quinine sulfate + Tartaric acid effect	57	69	76	98	815	4.11***

(***) significant at 0.1%.

Table 4. Results of rank tests to study the effect of ethanolic content on wine sweetness.

Ranks sum	<i>R</i> 1	R_2	R 3	R 4	L	L'
Red wine	79	66	75	80	756	0.38 ns
White wine	63	80	77	80	774	1.52 ns

ns, not significant.

Figures

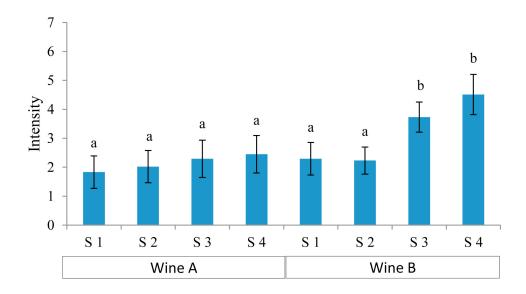


Fig. 1. Bitterness intensities of the samples and group affiliation.