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Influence of fertilization with foliar urea on the content of amines in wine

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3 **1 Influence of fertilization with foliar urea on the content of amines in**
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6 **2 wine**
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25
26 **10 Abstract**
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29 Amines are substances that could cause toxic effects in the consumer. The concentration
30
31 of amines in wine depends on different factors such as grape variety, vinification
32
33 conditions and nitrogen fertilization of the vines. The aim of this work was to study the
34
35 influence of the application of foliar urea on the concentration of amines in wine. To
36
37 carry out the study, grapevines from the Tempranillo variety were used. These
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39 grapevines were treated with foliar urea at two different concentrations (2 kg N ha⁻¹ and
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41 4 kg N ha⁻¹). Treatment with foliar urea significantly increased (p < 0.05) the
42
43 concentration of histamine in the wines compared to the control sample (65% in the
44
45 treatment with 2 kg N ha⁻¹ and 93% in the treatment with 4 kg N ha⁻¹), reaching higher
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47 concentrations than the threshold level where it could provoke toxic effects in the
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49 consumer (8–20 mg l⁻¹). On the other hand, treatment with foliar urea did not increase
50
51 the concentrations of other amines which could be toxic such as tyramine or
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53 phenylethylamine, nor amines such as putrescine which could enhance the toxic effect
54
55 of histamine. In the case of the volatile amines containing secondary amine groups, the
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25 concentration of pyrrolidine increased by 37% after treatment with 2 kg N ha⁻¹ and 61%
26 after treatment with 4 kg N ha⁻¹.

27
28 **Keywords:** foliar urea fertilization; amines; wine; Tempranillo must.

29 Introduction

30 Nitrogen is the most abundant soil-derived macronutrient for the grapevine and has a
31 fundamental role in fermentation kinetics and on wine quality. The greater part of the
32 geographical zones given over to grape-cultivar in Europe are in areas of soil poor in
33 nitrogen. Habitually, the application of nitrogen comes about in the form of organic or
34 inorganic fertilizers on the vineyard soil which is later absorbed by the roots and so
35 brings about a correct development in both the vine and the grape itself. The problem
36 with this fertilization is that the nitrate salts are very soluble so that the possibility of
37 producing anion leaching is high. Furthermore, most soils have scant absorption of
38 particles that are negatively charged. Nitrate leaching through the sub-soil could
39 contaminate the underground aquifers. If water rich in nitrates is consumed, serious
40 health problems could arise; nitrates could be transformed into nitrites due to the
41 participation of some bacteria that exist in the stomach and urinary bladder. The foliar
42 fertilization does not substitute the traditional fertilization of grape production, but it
43 does serve to help to complete and guarantee the nutritional requirements of a cultivar
44 which is often difficult to supply only through soil fertilization (Xia and Cheng 2004).
45 In addition, this practice permits the application of relatively small amounts of the
46 product in relation to the quantities which need to be supplied to the soil.

47 Biogenic amines in wines are formed through the action of decarboxylase enzymes
48 produced by microorganisms from the corresponding amino acid precursor (ten Brink et

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2
3 49 al. 1990). There are also volatile amines in wine that, with the exception of
4
5 50 phenylethylamine, appear to come from the amination of non-nitrogenous compounds
6
7
8 51 such as aldehydes and ketones (Ough et al. 1981). Amines are formed in wine during
9
10 52 alcoholic fermentation due to the metabolism of yeast strains (Torrea and Ancín 2002),
11
12 53 and during malolactic fermentation due to the proliferation of lactic acid bacteria
13
14 54 (Gerbaux and Monamy 2000) and its concentration is also modified during wine ageing
15
16 55 in barrel (Jiménez-Moreno and Ancín-Azpilicueta 2004). Furthermore, a great
17
18 56 variability in the concentration of amines exists between different wines as there are
19
20 57 many factors (raw materials, amino acid concentration in the must, winemaking,
21
22 58 vinification conditions...) which have an influence, to a greater or lesser extent, in the
23
24 59 formation of these substances. Among these factors, the content of nitrogenous
25
26 60 compounds in the grape stands out and depends, among other factors, on the level of
27
28 61 maturation and on the nitrogenous fertilization. Another important factor is the
29
30 62 winemaking method employed, since red wines show a larger concentration of amines
31
32 63 compared to rosé or white wines (Zee et al. 1983; Radler and Fäth 1991).
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40 64 The main biogenic amines implicated in food poisoning are: histamine, tyramine, and
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42 65 phenylethylamine. Histamine is found naturally in blood and the concentration of this
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44 66 amine in the blood flow is normally at between 25 to 130 mg l⁻¹ (Taylor 1983).
45
46 67 Histamine is the most studied biogenic amine and it is usually taken as an indicator of
47
48 68 freshness and quality in many foods and has traditionally been the amine most related to
49
50 69 the so-called wine intolerance that some sensitive people have to this product.
51
52 70 Symptoms of intoxication by histamine are vomiting, diarrhoea, headaches, facial
53
54 71 flushes, itching and throat inflammation (Silla-Santos 1996). Tyramine and
55
56 72 phenylethylamine form part of the group of vasoconstrictor amines and so they can
57
58 73 provoke hypertensive crisis and they are also responsible for alimentary migraine.
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3 74 Tyramine also reacts with drugs that inhibit the monoamine oxidase (MAOI) giving
4
5 75 place to hypertensive crisis (Mariné–Font 1978). The use of drugs that inhibit the MAO
6
7 76 for the treatment of mental depression interferes in the detoxification process of these
8
9 77 amines and can produce hypertensive crisis in patients that accumulate tyramine in their
10
11 78 blood (Blackwell 1963; Smith and Durack 1978). Putrescine and cadaverine, although
12
13 79 they do not have any toxic activity, can inhibit the action of the monoamine oxidase
14
15 80 (MAO) and diamine oxidase (DAO), enzymes which are responsible for the degradation
16
17 81 of histamine, tyramine and phenylethylamine and so, they can reinforce the toxic
18
19 82 activity of these amines. In experiments with animals, it has been proven that toxicity of
20
21 83 histamine is increased by a factor of 10 when this amine is administered 40 min after
22
23 84 the ingestion of putrescine (Parrot and Nicot 1966) and cadaverine (Bjedanes et al.
24
25 85 1978; Hui and Taylor 1985). On the other hand, those amines with secondary amino
26
27 86 groups (spermine, spermidine, dimethylamine, pyrrolidine...) can react with the nitrous
28
29 87 acid and its salts to form nitrosamines, compounds with carcinogenic, mutagenic and
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31 88 theratogenic activity (Glória et al. 1997).

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39 89 The impact of different factors related to vinification on the concentration of amines in
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41 90 wine has been studied. However, we have found no literature about the influence of the
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43 91 foliar fertilization of the vineyard on the concentration of these compounds in wines.
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45 92 For these reasons outlined above, the aims of this research were to study a) the influence
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47 93 of the application of foliar urea on the concentration of amines in wine and b) the
48
49 94 relation between the amine concentration with the concentration of its precursor amino
50
51 95 acids and with the nitrogenous fractions of the must. To carry out the study, grapevines
52
53 96 from the Tempranillo variety were used and these were treated with two different
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55 97 concentrations of foliar urea (2 kg N ha⁻¹ and 4 kg N ha⁻¹).

98 **Material and methods**99 ***Samples and vinification***

100 The grapes used in this study were *Vitis vinifera* var. Tempranillo (2008 vintage) from
101 three adjacent plots within the same vineyard, located in The Rioja region, in the North
102 of Spain. Vine plantation dates from 1990 while the planting density was 2.70 x 1.50 m.
103 Gobelet pruning of the grapevine was carried out. The pH of the vine soil is 8.3
104 (diluting the soil in water 1/5 p/v). Nitrogen fertilizer of 21 kg N ha⁻¹ was added to the
105 soil of the whole vineyard. Two of the plots were treated with foliar application of urea,
106 in one of the plots 2 kg N ha⁻¹ was applied while in the other plot 4 kg N ha⁻¹ was
107 applied. The third plot was not treated with urea and this was taken as the control plot.
108 Nitrotecnia-20[®] was used for foliar urea application with 20% p/p of total N from the
109 urea. The urea treatment was first applied on the 31st of July and subsequently continued
110 until the 9th of October at seven day intervals. This treatment was started near to
111 veraison as the aim was for the urea nitrogen to pass directly to the grape and not to
112 increase the foliage of the plant. The application was always carried out either early in
113 the morning or late in the evening with the aim that the absorption would be maximized.
114 The grapes were collected from the three plots, that is to say, the control plot and the
115 two plots treated with foliar application of urea. After the harvest, the grapes were
116 destemmed and crushed and afterwards they underwent pressing. Potassium
117 metabisulphite (Aldrich, Gillingham, UK) was added to the must up to a concentration
118 of 50 mg l⁻¹ of total SO₂. Fermentation took place in stainless steel tanks with a capacity
119 of 25 liters at controlled temperature (27 °C). All wines were fermented to dryness
120 (reducing sugar concentration < 2.5 g l⁻¹). Figure 1 shows a flow chart for the life cycle
121 of the wine production steps. A study of amines was made in the must at the beginning
122 of the alcoholic fermentation (M), must at 6 days after the start of fermentation (M6),

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3 123 must at 8 days after the start of fermentation (M8) and wine at the end of alcoholic
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5 124 fermentation (W). Fermentation lasted a total of 11 days. Those samples coming from
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8 125 the grapevines treated with 2 kg N ha⁻¹ of foliar urea were given the name U2; while the
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10 126 samples coming from the grapevines treated with 4 kg N ha⁻¹ of foliar urea were given
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12 127 the name U4 and the samples from the grapevines not treated with foliar urea were the
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14
15 128 control samples.

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18 129 ***Preparation of sample and HPLC analysis of amines***

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20 130 Amine determination was carried out by the method described by Torrea and Ancín
21
22 131 (2001). Samples were passed through a Millipore Ultrafree MC filter (Millipore, Cork,
23
24 132 Ireland) to eliminate molecules of a size higher than 10,000 Da. Afterwards the
25
26 133 precolumn derivatization of the amines was carried out through the addition of 6–
27
28 134 aminoquinolyl–N–hydroxysuccinimidyl carbamate (AQC) from AccQ–Fluor reagent kit
29
30 135 (Waters Chromatography Div., Milford, MA, USA). The chromatography analysis was
31
32 136 made with a Waters high–performance liquid chromatograph equipped with a 474
33
34 137 fluorescence detector, using 250 and 395 nm as the excitation and emission
35
36 138 wavelengths, respectively. A Pico-TagTM reverse phase column (300 mm x 3.9 mm i.d.)
37
38 139 with a stationary phase of dimethyloctadecylsilyl bonded to amorphous silica was used.
39
40 140 For chromatographic control, Empower[®] 2.0 software was used. The sample injected
41
42 141 was 10 µl. The amines under study were histamine, tyramine, putrescine, cadaverine,
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44 142 phenylethylamine, spermidine, spermine, dimethylamine, diethylamine, ethylamine,
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46 143 isobutylamine, isopropylamine, amylamine and pyrrolidine. It was not possible to
47
48 144 separate phenylethylamine and spermidine and consequently they were quantified as
49
50 145 one single peak. Two derivatizations were made of each sample and the samples were
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52 146 analyzed in duplicate (n=4).
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3 147 ***Preparation of sample and HPLC analysis of free amino acids***
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5 148 The Pico-Tag method used for amino acids determination was described by Ancín et al.
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7 149 (1996). Analysis was performed with a Waters high-pressure liquid chromatograph
8
9 150 equipped with two 510 pumps, a U6K injector, and a 996 photodiode array detector
10
11 151 used at 254 nm. A Pico-Tag™ reverse phase column (300 mm x 3.9 mm i.d.) with a
12
13 152 stationary phase of dimethyloctadecylsilyl bonded to amorphous silica was used.
14
15 153 Empower® 2.0 software was employed for chromatographic control. Derivatization was
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17 154 performed using a Telstar lyophilizer, model Cryodos-50 (Telstar Industrial, Barcelona,
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19 155 Spain). Internal standards were L-2-aminohexanoic acid (L-norleucine) and L-2-
20
21 156 amino-4-[methylsulfonyl] butanoic acid (L-methionine sulfone), both from Sigma
22
23 157 (Gillingham, UK). Two mobile phases A and B were used with the following
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25 158 composition: phase A, acetonitrile (2.5 %) and sodium acetate (97.5 %); phase B, a
26
27 159 mixture of acetonitrile, water and methanol (45:40:15). All these reagents were from
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29 160 Scharlau HPLC quality (Barcelona, Spain). The mobile phases used were always
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31 161 purified through an HA 0.45 µm Millipore filter. The amount of sample injected was 10
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33 162 µl. Two derivatizations were made of each sample and the samples were analyzed in
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35 163 duplicate (n=4).
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44 164 ***Nitrogenous fractions and general enological parameters***
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46 165 Ammonium nitrogen and general parameters were measured using WineScan 79000
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48 166 Auto (Foss Analytical, Denmark) with Fourier Transform Infrared Spectroscopy
49
50 167 technology. The wavelength range of 240–1295 nm was used for these analyses.
51
52 168 Yeast assimilable nitrogen (YAN) was determined by adding amino nitrogen plus
53
54 169 ammonium nitrogen and taking away the nitrogen from proline.
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59 170 ***Statistical analysis***
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171 The statistical study of the amine content in must and wine with different fertilization
172 treatments was carried out with analysis of variance (ANOVA), and the means were
173 compared with the Scheffé test. The probability level was 0.05. For data analysis, the
174 software SPSS[®] v.17.0 was used (Chicago, Illinois, USA).

175 **Results and Discussion**

176 *General parameters*

177 The application of foliar urea caused a significant increase ($p < 0.05$) in the pH of the
178 must and wines (Table 1). Bertrand et al. (1991) also found an increase in the pH in
179 Merlot wine from nitrogen-treated vines in comparison with the same wine from non
180 nitrogen-treated vines. Total acidity was also significantly higher ($p < 0.05$) in the
181 Tempranillo must coming from the grape with foliar urea fertilization compared to the
182 control must. Fertilization with foliar urea decreased the alcohol level and the total
183 acidity of the wine (Table 1). Volatile acidity of wines was inferior in all cases to the
184 threshold found by Peynaud (1993) as undesirable for wine aroma (0.6 g HAc l^{-1}).

185 *Nitrogen compounds in must and its relation with the total biogenic amines in wines*

186 When foliar urea was applied, the concentration of amino nitrogen and YAN (yeast
187 assimilable nitrogen) significantly increased ($p < 0.05$) in the must (Figure 2a). In the
188 control Tempranillo must, the concentration of amino nitrogen (201 mg l^{-1}) was similar
189 to the average value (199 mg l^{-1}) found by Henschke and Jiranek (1993) for seven
190 Australian varieties of grapes. It was observed that foliar urea application increased the
191 concentration of amino nitrogen in must by 29% when 2 kg N ha^{-1} was applied and by
192 104% when 4 kg N ha^{-1} was applied (Figure 2a). Yeasts assimilable nitrogen was also
193 much higher in the must from the grape treated with 4 kg N ha^{-1} of foliar urea (500 mg
194 l^{-1}) and in the must from the grape treated with 2 kg N ha^{-1} of foliar urea (300 mg l^{-1})

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3 195 than in the control must (242 mg l⁻¹). These results indicate that must treated with urea
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5 196 accumulated a greater quantity of arginine in relation to proline than in the control must.
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8 197 Furthermore, the consumption both of amino nitrogen as well as of YAN during
9
10 198 fermentation was significantly higher in the musts treated with foliar urea than in the
11
12 199 control must (Figure 2b). The concentration of total biogenic amines did not show any
13
14 200 significant differences ($p < 0.05$) between the three wine samples (control, 33.5 mg l⁻¹;
15
16 201 U2, 34.7 mg l⁻¹; U4, 30.9 mg l⁻¹). Consequently, no correlation was found between the
17
18 202 concentration of yeasts assimilable nitrogen in the must and its consumption, with the
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20 203 concentration of total biogenic amines in the wine.
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25 204 *Biogenic amines in wines*

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27
28 205 In the Tempranillo must used in this study, small quantities of histamine, tyramine,
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30 206 putrescine and spermine were found. The rest of the amines were formed above all in
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32 207 the second half of alcoholic fermentation, although the concentration of cadaverine
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34 208 decreased at the end of fermentation (Figure 3). Some amines are normal constituents of
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36 209 grapes, and the amounts varied with grape variety, soil type, fertilization and climate
37
38 210 conditions (Millán et al. 2007).
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41

42 211 The concentrations of histamine, cadaverine and spermine (Figures 3a, 3d and 3e) were
43
44 212 significantly higher ($p < 0.05$) in the wine coming from the must treated with the higher
45
46 213 concentration of foliar urea (U4 wine) than in the control wine as well as in the U2
47
48 214 wine. The concentrations of histamine and spermine also increased with the application
49
50 215 of 2 kg N ha⁻¹ of urea in comparison with the control sample. The histamine
51
52 216 concentration in both wines from musts that had been treated with urea is within the
53
54 217 range of concentrations (8–20 mg l⁻¹) where this amine could provoke toxic effects in
55
56 218 the consumer (Soufleros et al. 1998). The toxicity of histamine on the general
57
58 219 population is very difficult to establish since in some cases toxic effects have been
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3 220 associated with even small amounts of this amine (Wantke et al. 1996), as there are
4
5 221 some individuals who are especially sensitive to the action of histamine. Generally,
6
7 222 these individuals show a deficiency of DAO or are being treated with MAOI drugs.
8
9 223 There also exist strengtheners of the toxicity of histamine, such as other biogenic
10
11 224 amines which are present in food (tyramine, phenylethylamine, tryptamine, putrescine
12
13 225 or cadaverine) which compete for the same endogen metabolic pathways and strengthen
14
15 226 the absorption of histamine in the intestine (Veciana-Nogués and Vidal-Carou 2000).
16
17 227 Likewise, it has been observed that some compounds present in food and drinks such as
18
19 228 alcoholic ones or the acetaldehyde of wine or the polyphenols from tea all strengthen
20
21 229 the toxic action of histamine. Bertrand et al. (1991) found that histamine, putrescine and
22
23 230 cadaverine concentrations in wines from nitrogen-treated vines were 1.3 to 3.8 times
24
25 231 higher than those of wines from untreated vines. Concentration of putrescine (Figure 3c)
26
27 232 was significantly inferior ($p < 0.05$) in the wine from the must treated with the higher
28
29 233 concentration of foliar urea (U4 wine) than in the control and in the U2 wines. The
30
31 234 concentration of phenylethylamine+spermidine (Figure 3f) also decreased in the wine
32
33 235 on applying foliar urea. The yeasts have a mechanism known as general amino acid
34
35 236 control (Hinnessbuch 1988). The precursor amino acids of histamine, cadaverine and
36
37 237 putrescine (histidine, lysine and arginine respectively) are all under general amino acid
38
39 238 control, so that their metabolic pathways are corelated and depend on the concentration
40
41 239 of each of them. High concentration of arginine stimulates arginase activity, this
42
43 240 regulatory strategy allows a rapid switch from arginine biosynthesis to arginine
44
45 241 catabolism. However, *S. cerevisiae* uses the nitrogen compounds at the start of
46
47 242 fermentation without modification. This yeast, once it has covered its specific demands
48
49 243 for each amino acid, assimilates those that are in excess and uses them as nitrogen
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51 244 sources (Bisson 1991). In our case, the concentration of arginine was very high (Table
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1
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3 245 2) in the U4 must. In this sample, arginine would have been preferably used as a
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5 246 nitrogen source and, to a lesser extent than in the other samples, its decarboxylation
6
7 247 would have been produced to form putrescine. Thus, the concentration of putrescine in
8
9 248 the U4 wine is significantly lower ($p < 0.05$) than in the control wine and in the U2
10
11 249 wine. On the other hand, in the U4 must, where the yeasts had higher amounts of a good
12
13 250 nitrogen source such as arginine, lysine and histidine would not have followed the
14
15 251 biosynthesis pathways and they would have decarboxylated in greater measure to
16
17 252 produce their corresponding biogenic amines (cadaverine and histamine) than in the
18
19 253 other samples.

20 254 The concentration of tyramine (Figure 3b) did not show any significant differences ($p <$
21
22 255 0.05) between the control sample and the wine samples from grape with foliar urea
23
24 256 fertilization. The concentration of the precursor amino acid of this amine, tyrosine, did
25
26 257 not show any significant differences ($p < 0.05$) either between any of the three must
27
28 258 samples (Table 2). In all the samples, the concentrations of tyramine were found to be
29
30 259 below the range at which wines could have toxic effects for the consumer (10–80 mg
31
32 260 l^{-1}), so that these wines do not present any toxic risk due to this amine.
33
34 261 Phenylethylamine+spermidine were formed during alcoholic fermentation and its
35
36 262 concentration was significantly lower ($p < 0.05$) in the wines coming from the must
37
38 263 treated with urea than in the control sample (Figure 3f). The precursor amino acid of
39
40 264 phenylethylamine (phenylalanine) disappeared in the must coming from the grapes
41
42 265 treated with foliar urea (Table 2). In all the wine samples, the concentration of
43
44 266 phenylethylamine+spermidine was so low that it would be necessary to drink more than
45
46 267 two litres of these wines to ingest a dose of 3 mg, above which phenylethylamine causes
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48 268 migraines (Sandler et al. 1974).

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269 ***Volatile amines***

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3 270 Pyrrolidine, ethylamine and diethylamine were found in all the musts (Figure 4). The
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5 271 concentration of these three amines in the must was significantly higher ($p < 0.05$) in
6
7 272 the U4 must than in the other two musts. The rest of the volatile amines were formed
8
9 273 during alcoholic fermentation (Figure 4). Pyrrolidine and diethylamine (Figures 4d and
10
11 274 4f) were found in greater concentration in the U4 wine. In the case of dimethylamine
12
13 275 (Figure 4a), its concentration decreased in the wines coming from grape treated with
14
15 276 urea. Dimethylamine, pyrrolidine and diethylamine contain secondary amino groups in
16
17 277 their molecular structure and so they can react with nitrous acid and their salts to form
18
19 278 nitrosamines, substances that have carcinogenic action (Glória et al. 1997)
20
21 279 Within the volatile amines which do not show secondary amine groups, the
22
23 280 concentrations of isobutylamine and amylamine (Figures 4c and 4g) hardly showed any
24
25 281 significant differences ($p < 0.05$) after the treatment with foliar urea. Only the
26
27 282 concentration of isobutylamine was significantly lower ($p < 0.05$) in the U2 wine than in
28
29 283 the control wine. The concentrations of ethylamine and hexylamine (Figure 4e and 4h),
30
31 284 both decreased after foliar fertilization. On the contrary, the concentration of
32
33 285 isopropylamine (Figure 4b) increased with foliar fertilization, while no significant
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35 286 differences ($p < 0.05$) were observed between the different doses of treatment with
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37 287 foliar urea.
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47 **Conclusions**

48
49 289 Treatment with foliar urea (2 kg N ha^{-1} and 4 kg N ha^{-1}) increased significantly ($p <$
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51 290 0.05) the concentrations of histamine and spermine in the wine. The concentrations of
52
53 291 histamine in the wines coming from the grape treated with urea were above the
54
55 292 threshold of concentration where this amine could provoke toxic effects in the
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57 293 consumer. On the other hand, treatment with foliar urea did not increase the
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59 294 concentrations of tyramine, nor putrescine nor phenylethylamine+spermidine. As for the
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3 295 volatile amines with secondary amino groups, the concentrations of pyrrolidine and
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5 296 diethylamine increased to a significant degree after treatment with foliar urea.
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8 297 Consequently, from the results obtained in this study, it may be concluded that wines
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10 298 coming from grapevines treated with foliar urea could present toxic problems due to
11
12 299 histamine in a part of the general population. The exact impact is difficult to establish as
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14
15 300 poisoning from this amine in wine would depend on personal factors such as the
16
17 301 presence of other substances in the diet that could strengthen the toxic action of
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19
20 302 histamine.
21

22 23 303 **Acknowledgements**

24
25 304 We would like to express our gratitude to the Marqués de Murrieta winery for their
26
27 305 collaboration in fertilizing the vineyard with foliar urea so that this study could be
28
29
30 306 carried out.
31

32 33 307 **References**

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Table 1. Enological parameters of musts and wines. All parameters listed with standard deviation, n=4. Means within the same row followed by different letters are significantly different ($p < 0.05$).

Musts	control	U2 (2 kg N ha ⁻¹)	U4 (4 kg N ha ⁻¹)
pH	3.57 ± 0.00 ^a	3.66 ± 0.00 ^b	3.78 ± 0.00 ^c
Glucose + fructose (g l ⁻¹)	223.3 ± 0.1 ^a	223.5 ± 0.2 ^a	212.9 ± 0.2 ^b
Total acidity (g l ⁻¹)	2.80 ± 0.05 ^a	2.96 ± 0.04 ^b	3.15 ± 0.04 ^c
Malic acid (g l ⁻¹)	3.5 ± 0.1 ^a	3.8 ± 0.1 ^b	3.6 ± 0.0 ^{ab}
Wines	control	U2 (2 kg N ha ⁻¹)	U4 (4 kg N ha ⁻¹)
pH	3.74 ± 0.00 ^a	3.90 ± 0.00 ^b	3.96 ± 0.00 ^c
Alcohol content (% v/v)	12.75 ± 0.01 ^a	12.65 ± 0.00 ^b	11.82 ± 0.02 ^c
Total acidity (g l ⁻¹)	5.50 ± 0.02 ^a	5.35 ± 0.03 ^b	5.27 ± 0.01 ^c
Volatile acidity (g l ⁻¹)	0.26 ± 0.01 ^a	0.30 ± 0.00 ^b	0.28 ± 0.01 ^a

Note: Total acidity expressed as tartaric acid.

Table 2. Concentration of amino acids precursors of biogenic amines. All data listed with standard deviation, n=4. Means within the same row followed by different letters are significantly different ($p < 0.05$).

Musts	control	U2 (2 kg N ha ⁻¹)	U4 (4 kg N ha ⁻¹)
Histidine	119.2 ± 3.8 ^a	192.6 ± 2.6 ^b	266.8 ± 0.9 ^c
Arginine	242.9 ± 21.9 ^a	507.6 ± 67.25 ^b	735.0 ± 63.0 ^c
Tyrosine	17.0 ± 2.9 ^a	16.2 ± 4.5 ^a	15.6 ± 1.5 ^a
Phenilalanine	5.8 ± 0.4 ^a	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b
Lysine	2.5 ± 0.3 ^a	36.0 ± 2.9 ^b	36.1 ± 2.4 ^b

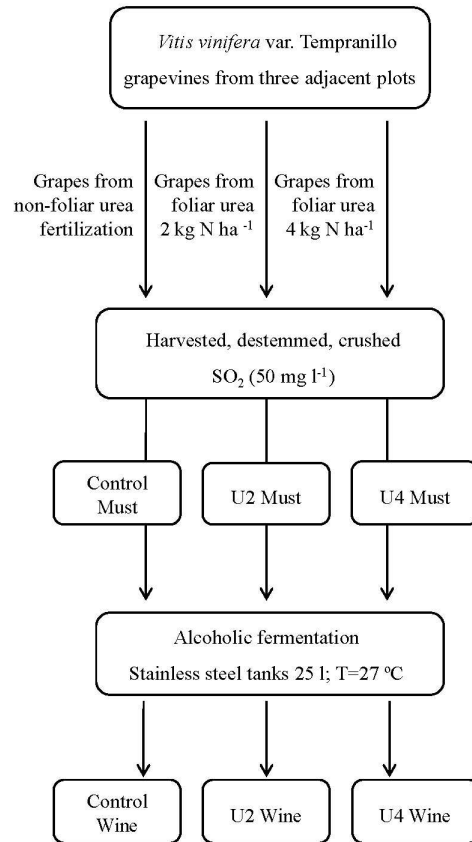


Figure 1. Flow chart for the life cycle of the wine production steps.
190x254mm (200 x 200 DPI)

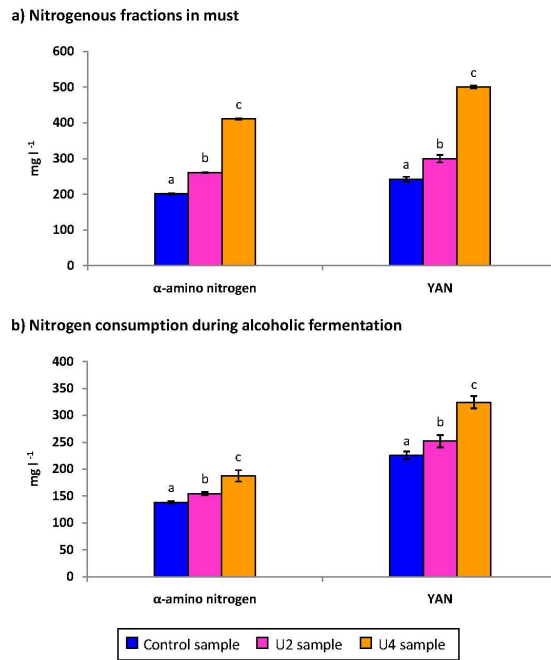


Figure 2. Nitrogenous fractions in must and nitrogen consumption during alcoholic fermentation. Bars with different letters are significantly different ($p < 0.05$). Note: Yeast Assimilable Nitrogen (YAN).
210x297mm (200 x 200 DPI)

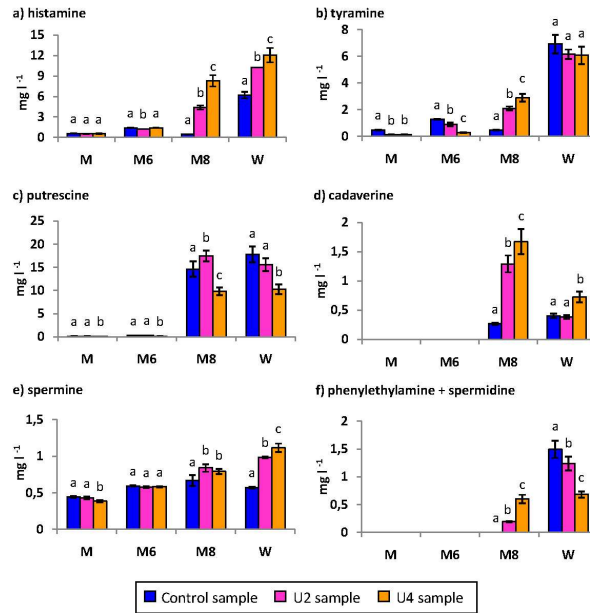


Figure 3. Evolution of the concentration of biogenic amines during the alcoholic fermentation. Bars with different letters are significantly different ($p < 0.05$).
210x297mm (200 x 200 DPI)

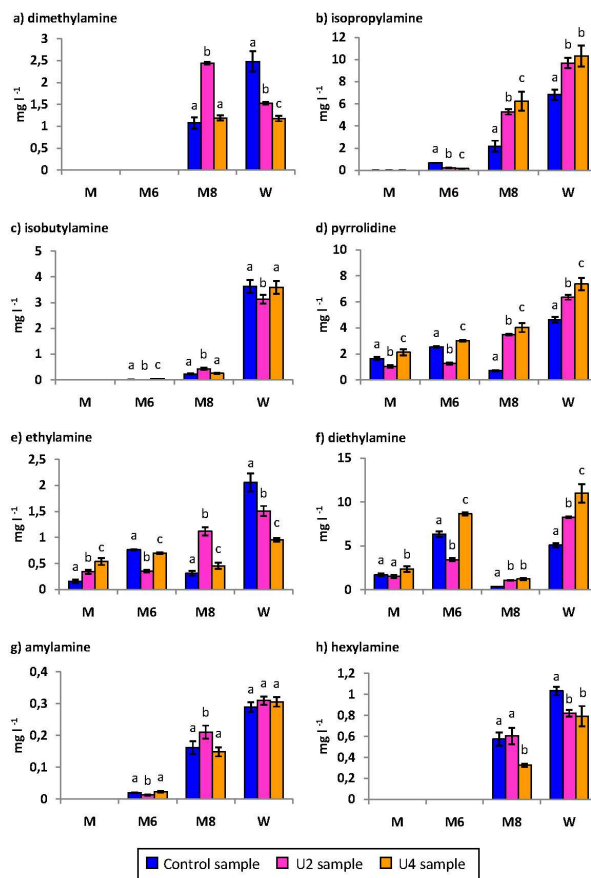


Figure 4. Evolution of the concentration of volatile amines during the alcoholic fermentation. Bars with different letters are significantly different ($p < 0.05$).
210x297mm (200 x 200 DPI)