



1 Article

2 **Vive la difference! Effects of natural and**
3 **conventional wines on blood alcohol concentrations:**
4 **a randomized, triple-blind controlled study.**

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15 **Abstract:** Different alcoholic beverages can have different effects on blood alcohol
16 concentrations (BAC) and neurotoxicity even if equalized for alcohol content by
17 volume. Anecdotal evidence suggested that natural wine is metabolized differently
18 from conventional wines. This triple-blind study compared the BAC of 55 healthy
19 male subjects after consuming the equivalent of 2 units of alcohol of a natural or
20 conventional wine over 3 mins in two separate sessions one week apart. BAC was
21 measured using a professional breathalyzer every 20 mins after consumption for 2
22 hrs. The BAC curves in response to the two wines diverged significantly at twenty
23 minutes, at forty minutes and also at their maximum concentrations (peaks), with
24 the natural wine inducing a lower BAC than the conventional wine (T20 0.40 vs.
25 0.46 [p<0.0002], T40 0.49 vs. 0.53 [p<0.0015], peak 0.52 vs. 0.56 [p<0.0002]). These
26 differences are likely related to the development of different amino acids and
27 antioxidants in the two wines during their production. This in turn may affect the
28 kinetics of alcohol absorption and metabolism. Other contributing factors may also
29 include pesticide residues, differences in dry extract content and the use of
30 indigenous or selected yeasts. Further studies are needed to fully understand why
31 natural wines are metabolized differently from conventional wines.

32 **Keywords:** alcohol; natural wine; blood alcohol content; breathalyzer; pesticides

33

34 **1. Introduction**

35 In recent decades, wine consumption has been the subject of intense debate
36 within the scientific community. On the one hand, wine has been linked to reduced
37 risk for several chronic illnesses, such as cardiovascular diseases, osteoporosis and



38 diabetes [1]. On the other hand, international guidelines for cancer prevention
39 emphasize the direct correlation between alcohol intake and cancer risk [2, 3]. The
40 positive health benefits provided by wine come primarily from compounds called
41 polyphenols, which are natural antioxidants that help fight inflammation and
42 improve plasma lipid profiles [4]. When consumed regularly and moderately,
43 ethanol, the main alcohol component in wine, confers cardioprotective effects by
44 acting directly on cardiomyocytes, blood circulation and platelet aggregation [5].
45 However, ethanol and its metabolite acetaldehyde are also responsible for adverse
46 neurological, hepatic and oncological consequences secondary to alcohol
47 consumption [6, 7]. Because of its potentially beneficial and harmful effects, many
48 scientific organizations recommend that alcohol consumption be limited to lower-
49 alcohol beverages, such as wine [8], and that such beverages be consumed
50 moderately and responsibly, if at all [9, 10].

51 In Italy, the Research Centre for Food and Nutrition (CREA-AN) of the Council
52 for Agricultural Research and Economics has adopted guidelines issued by the
53 National Institute for Research on Food and Nutrition (INRAN), which define
54 moderate alcohol consumption as an average daily allowance of no more than 2-3
55 units of alcohol for men, and 1-2 units for women. The standard value of a unit of
56 alcohol in Italy is 12 g of ethanol [11].

57 As reported by the Italian National Institute for Research on Food and Nutrition,
58 there is a well-known linear correlation between blood alcohol concentration BAC
59 and the deleterious effects of alcohol, particularly those involving the central
60 nervous system [11]. The short-term neurotoxic effects of elevated BAC include a
61 state of euphoria or inebriation, slowed reflex and reaction times, diminished
62 peripheral vision, and cognitive impairment [12].

63 The relationship between the amount of alcohol consumed and BAC is
64 influenced by numerous factors, including the individual's sex, age, body weight,
65 liver volume and function, drinking habits, use of medications, medical conditions,
66 and fasting or non-fasting state [13].

67 It has also been established that, when equalized for alcohol content by volume,
68 different beverages are absorbed at different rates and lead to different maximum
69 concentration (peaks) in BAC [14, 15]. This study set out to determine whether the
70 absorption of ethanol from two wines produced from the same grape (with similar
71 alcohol and sugar content) might be affected by differences in the farming and
72 winemaking techniques used in their production. The approach is therefore to
73 compare the evolution of BAC of healthy male subjects after their consumption of 2
74 units (24 g of alcohol) of a natural wine (i.e., cultivated without pesticides and
75 agrochemicals, fermented with wild yeasts, unfiltered and with no fining; see
76 Materials and Methods) and after their consumption of the same amount of a
77 conventional wine, a week apart and under the same experimental conditions.

78 2. Materials and Methods

79 2.1. Selection of the natural and conventional wines

80 In the absence of clear national or international legislation on the definition of
 81 natural wine, it was decided for the purpose of this study to compare the effects of
 82 consuming two near-identical wines differing only in the farming management and
 83 vinification protocols adopted in their production. Over three hundred wines were
 84 purchased for testing by an independent laboratory specialized in alimentary
 85 analysis to identify those suitable for comparison. Our intention was to find a pair
 86 of wines, one natural and one conventional, with near-perfect (or the best-possible)
 87 correspondence in grape variety, proximity of the area of production, age of
 88 harvesting, alcoholic strength by volume, and low sugar content (<1.5 g/L). Two
 89 wines satisfied the inclusion criteria. Both were whites made from Cortese grapes
 90 grown in vineyards located within ten kilometers of each other in Piedmont, Italy.
 91 They were of the same vintage and aged in bottles for twelve months. Table 1 shown
 92 the main characteristics of the two wines selected for the trial.

93 **Table 1.** Characteristics of the natural and conventional wines tested.

	Natural wine	Conventional wine
Actual alcoholic strength by volume (vol%)	13.19	13.03
Volatile acidity (mEq/L)	20	4.5
Total sugar content (g/L)	< 1.5	< 1.5
Total dry extract (g/L)	24.93	18.23
Total sulfur dioxide (g/L)	0.025	115
Pesticides ¹	n.d.	present

94 ¹Over 200 pesticides were analyzed. Traces of Iprovalicarb (45 µg/kg) and Fenhexamid (120 µg/kg)
 95 were found in the conventional wine.

96 As can be seen from the table, the two wines had the same percent of alcohol by
 97 volume and a low sugar content (<1.5 g/L), and both were made from the same
 98 variety of grape grown in the same geographic location. The grapes used for making
 99 the natural wine, however, were cultivated without pesticides or agrochemicals
 100 other than those approved for organic farming by EC Reg. 834/2007 [16]. The wine
 101 was fermented without the use of selected yeasts or fining processes. The wine was
 102 also left unfiltered and no sulfites were added. Instead, the grapes for making the
 103 conventional wine were grown using regulated synthetic pesticides and
 104 agrochemicals, and fermented with selected yeasts, and the entire winemaking
 105 process was based on conventional methods permitted by Italian law, including
 106 filtration and the addition of sulfur dioxide. The wines were subjected to additional
 107 tests for pesticides, confirming traces of Iprovalicarb (45 µg/kg) and Fenhexamid
 108 (120 µg/kg) in the conventional wine.

109 2.2. Study design

110 The study was a randomized, triple-blind, controlled trial. Each phase of the trial
 111 was conducted in triple-blind, meaning that at no time were any of the three research
 112 teams aware of the identity of the wines being administered to the subjects or being
 113 analyzed. The first team designed the study, selected the volunteers and set up the

114 samples for testing. The second administered the doses of wine to the subjects and
115 recorded the resulting data, and the third conducted the final data analysis. Among
116 the 167 eligible subjects, 55 subjects were drawn by a random number generator. In
117 addition, at the start of the test, the matrices containing the matching between the
118 subjects and the bottles were generated by a random number generator, as was the
119 assignment one of the two types of wine to each subject in the first or second week.

120 2.3. Subjects

121 All participants in the study were university student volunteers, screened using
122 a questionnaire to collect data on their height, weight, body mass index (BMI),
123 dietary habits, and use of prescription medicines. Ethical approval was provided by
124 the Polytechnic University of Turin (1037/2018, 01-30-2018) in compliance with the
125 Helsinki Declaration. Recruitment was limited to male subjects, as the study required
126 consumption of 2 units of alcohol, which exceeds the maximum recommended daily
127 amount for women (INRAN, 2003). At the end of the study, an information and
128 awareness campaign was carried out to promote alcohol awareness and responsible
129 use among the entire student body at the Polytechnic University of Turin. Inclusion
130 criteria: Males aged 18 to 30 with a BMI between 18.5 kg/m² and 25 kg/m² who could
131 understand the purpose of the study and thus provide written informed consent.
132 Exclusion criteria: Use of prescription medicine for chronic conditions; any
133 pathology that might interfere with alcohol metabolism; habitual consumption of
134 more than 4 units of alcohol per day.

135 2.4. Administering the two wines

136 The test took place over two sessions held one week apart. At the first session,
137 each subject was given 3 min to drink a single, unlabeled and randomly selected 248
138 ml dose of either the natural wine or the conventional wine (the equivalent of 2 units,
139 or 24 g of alcohol). At the second session, seven days later, the subjects had to repeat
140 the experiment, this time being administered the other type of wine to drink.

141 Table 1 summarizes the characteristics of the two wines tested. While similar in
142 alcohol strength by volume and sugar content, they showed significant differences
143 in volatile acidity, total dry extract, and sulfur dioxide concentrations, which can be
144 attributed to the different farming and vinification processes used in their
145 production.

146 After providing breath samples at the beginning of each session to verify a zero
147 BAC, the subjects underwent a series of breathalyzer tests to measure their blood
148 alcohol levels at 20-min intervals for a total of 2 h after ingesting the sample (time
149 intervals T0, T20, T40, T60 T80, T100, T120). A professional AlcoTrue M breathalyzer
150 (bluepoint MEDICAL, Selmsdorf, Germany) was used in order to infer the BAC.

151 The subjects were required to abstain from drinking alcohol for 7 days, from
152 smoking for 8 h and from eating for at least 4 h prior to both sessions for minimizing
153 the possible interference of assuming alcohol, smoke or food during the previous
154 week and the previous hours. They were deliberately provided with no information

155 about the wines being tested, and the wines were served at 21°C and the same
156 temperature was maintained in the testing room throughout the test to prevent any
157 temperature-dependent interference on the BAC [17]. The room had artificial
158 lighting and the two tastings took place at the same time of day to minimize any
159 possible interference. The wines were distributed in plain black wine tasting glasses.
160 The labels on the bottles were masked, and the wines were identified strictly by a 4-
161 digit code number. The first two digits indicated the day on which the test was
162 performed, while the second two indicated the bottle index randomly assigned to
163 each bottle.

164 2.5. Pharmacokinetic analysis

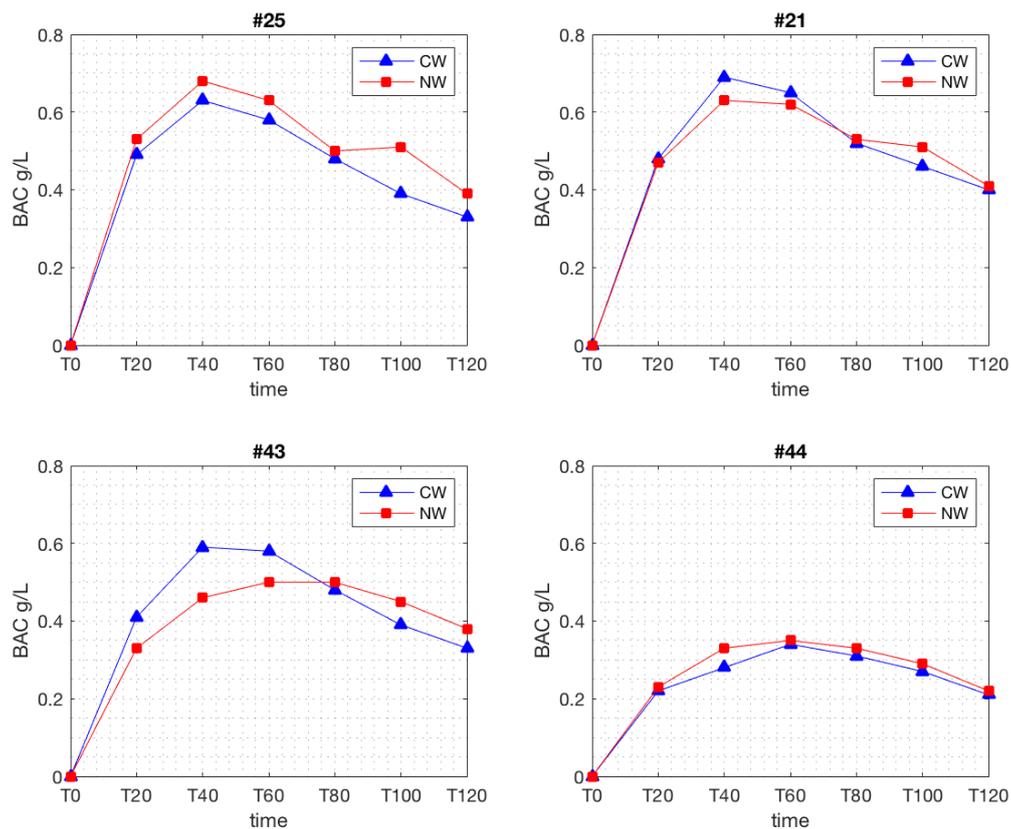
165 A professional breathalyzer was used to estimate the following pharmacokinetic
166 parameters: BACs expressed in g/L at time intervals T0, T20, T40, T60, T80, T100,
167 T120; maximum BAC (peak)-versus time, the ethanol's area under curve (AUC)
168 calculated using the trapezoidal rule.

169 2.6. Statistical analysis

170 The sample size was calculated on the basis of the main expected outcome,
171 defined as the difference between BAC after drinking a fixed dose of natural wine
172 and after drinking the same dose of conventional wine. Using data in the literature
173 on subjects similar to those participating in our study, it was calculated that for an
174 effect size of 0.67 and two tailed alpha-error of 0.05, 50 subjects could be needed to
175 obtain 90% of power. As a precautionary measure, the sample size was set at 55
176 subjects. Continuous variables were expressed as medians and interquartile ranges
177 (IQR), and categorical variables as percentages and absolute frequencies. The
178 Student's t-test for paired samples was used to detect differences in BAC at each of
179 the time intervals (T0, T20, T40, T60 T80, T100, T120) and to detect differences in
180 AUC, too. The resulting data was graphically represented using box-and-whisker
181 plots. The level of significance was set at $p \leq 0.05$. All statistical analysis was
182 performed with the MedCalc Statistical Software version 18.9 (Ostend, Belgium).

183 3. Results

184 The 55 male subjects recruited for the study were of median age 23 (p25: 21 years,
185 p75: 24 years), median weight 69 kg (p25: 65 kg, p75: 78 kg), median height 178 cm
186 (p25: 174 cm, p75: 183 cm), and median BMI 22 kg/m² (p25: 20.8 kg/m² cm, p75: 22.9
187 kg/m²). Breathalyzer measurements obtained at regular twenty-minute intervals
188 were used to plot concentration-time curves of each subject's BAC response to the
189 natural wine and to the conventional wine. These can be seen in Appendix A,
190 Figures A1 to A5. Superimposition of the pairs of curves reveals that each subject
191 had its own distinct pattern of alcohol pharmacokinetics to both wines, as is evident,
192 for example, for subjects 21, 25, 43 and 44, randomly extracted from the sample
193 (Figure 1).



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Figure 1. Examples of blood alcohol concentration (BAC) levels measured every 20 mins after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L), subjects #21, #25, #43 and #44.

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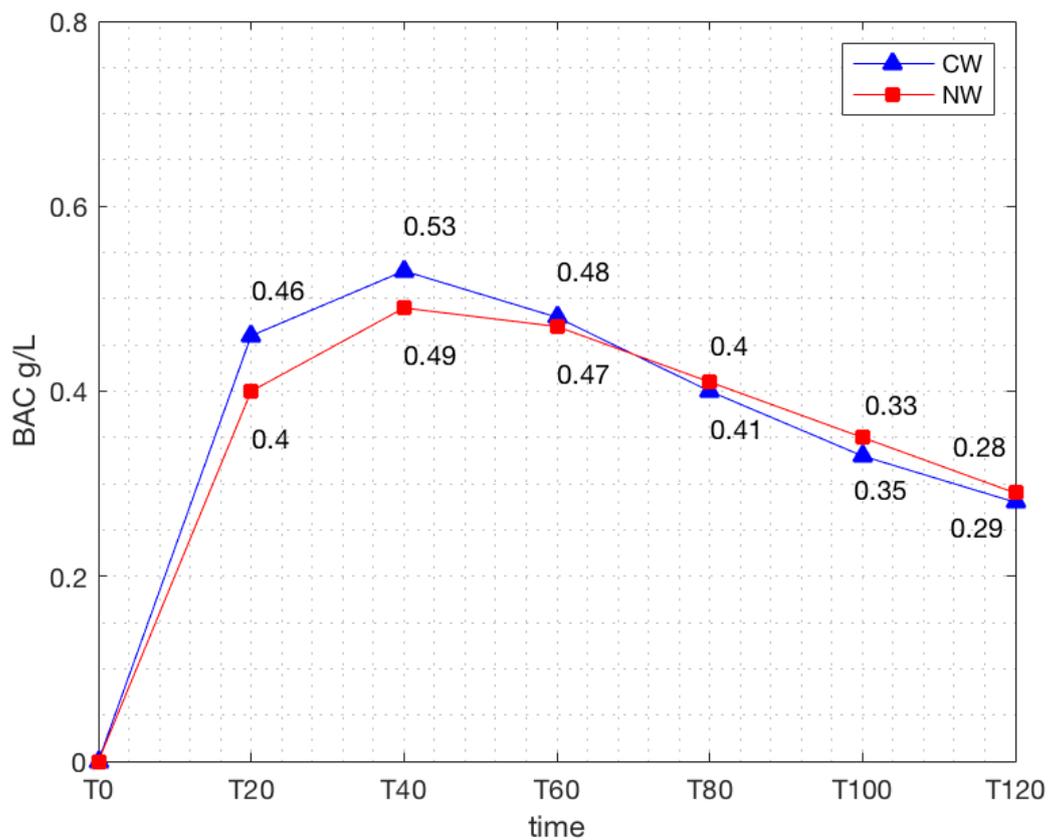
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By summing all of the subjects' BAC values at the different time intervals and dividing by the number of subjects, the average BAC curves were calculated. Figure 2 shows the differences in the average BAC levels registered after the ingestion of the natural vs. the conventional wine.

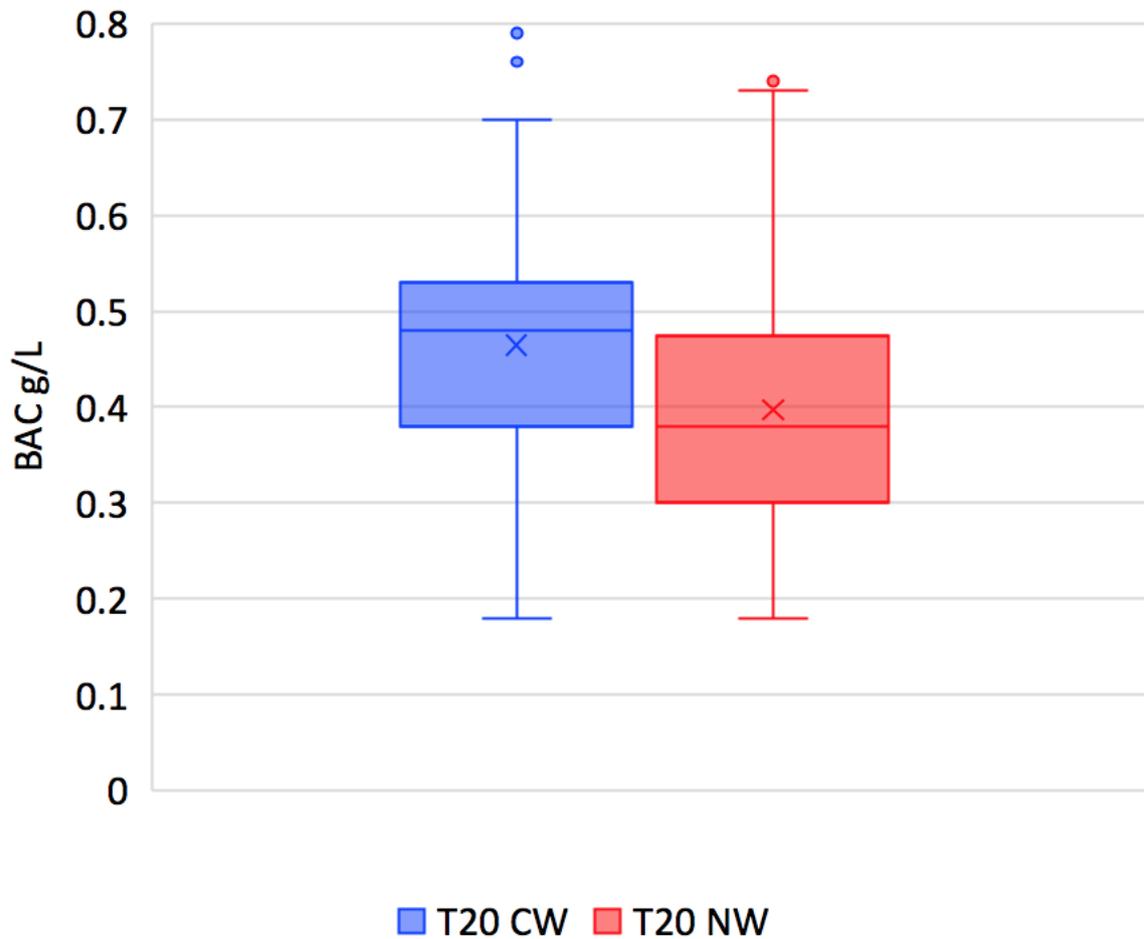


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205 **Figure 2.** Comparison of the average blood alcohol concentration (BAC) levels measured every 20
206 mins after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L).

207 It can be seen that the rate of increase in BAC in response to the two wines
208 diverges significantly at the T20 mark, with natural wine inducing lower levels than
209 conventional wine: 0.40 vs. 0.46 ($p < 0.0002$) (Figure 3); this discrepancy persists at
210 significant levels up to T40: 0.49 vs. 0.53 ($p < 0.0015$) (Figure 4).
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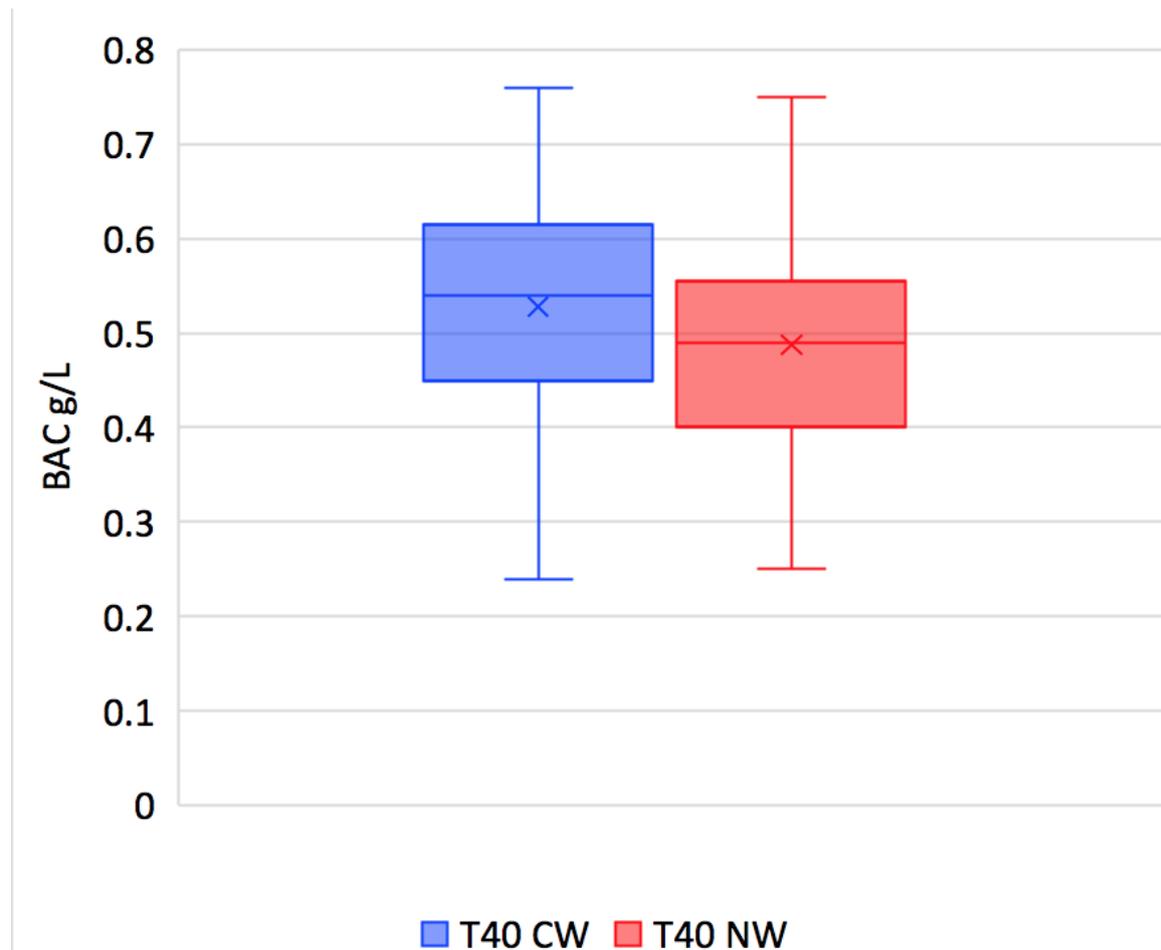


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Figure 3. Box and whiskers diagram of the blood alcohol concentration (BAC) levels at T20 after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L) (T20 $p < 0.0002$).

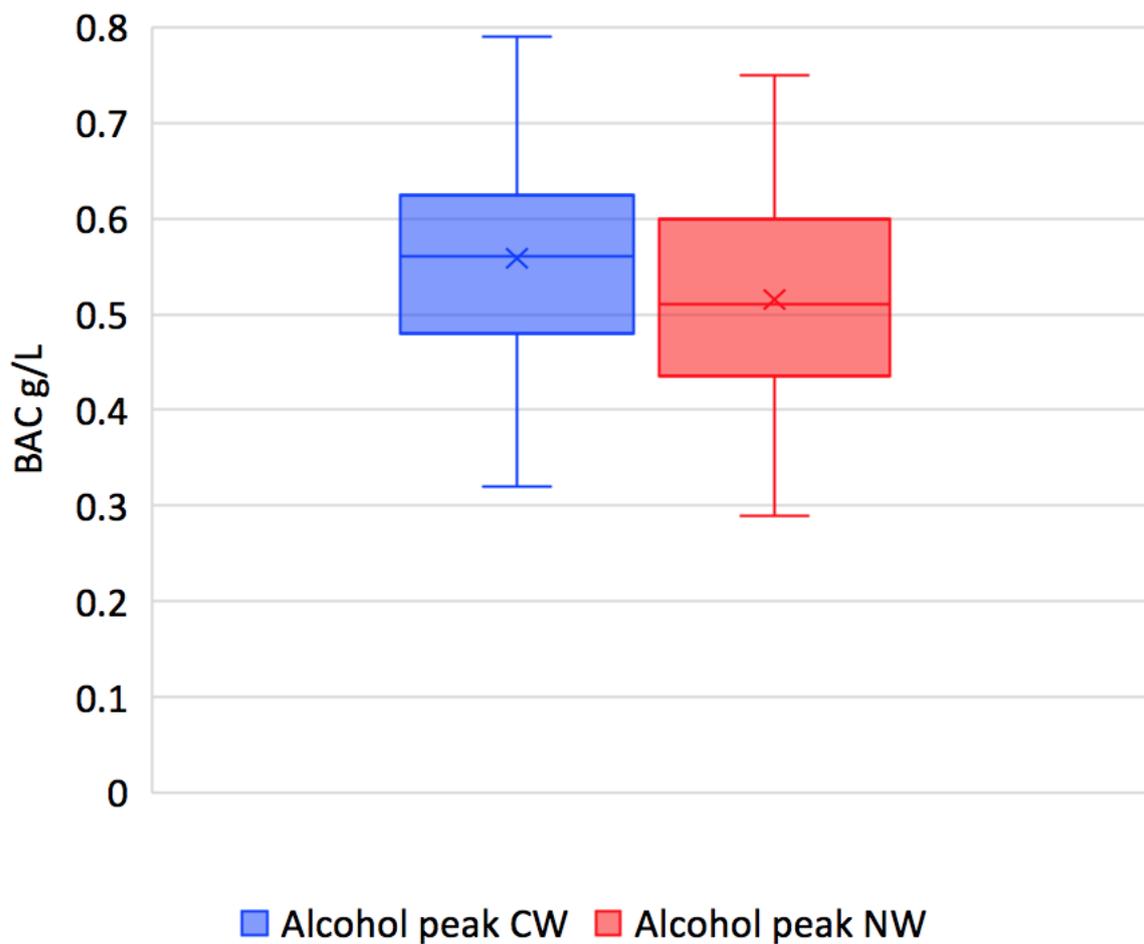


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217 **Figure 4.** Box and whiskers diagram of the blood alcohol concentration (BAC) levels at T20 after the
218 ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L) ($p < 0.0015$).

219 The BAC peaks occur between T40 and T60 for both wines. The difference in
220 values of the maximum BAC levels after the ingestion of the natural wine (NW)
221 is significantly lower than after the ingestion of conventional wine (CW) 0.52 vs. 0.56
222 ($p < 0.0002$) (Figure 5). The curves continue to approach each other until the T80 mark
223 and then intersect. After this point the conventional wine is associated with a slightly
224 lower BAC and the curves gradually converge and largely overlap toward the end.
225



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Figure 5. Box and whiskers diagram of the maximum blood alcohol concentration (peak) levels after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L). ($p < 0.0002$).

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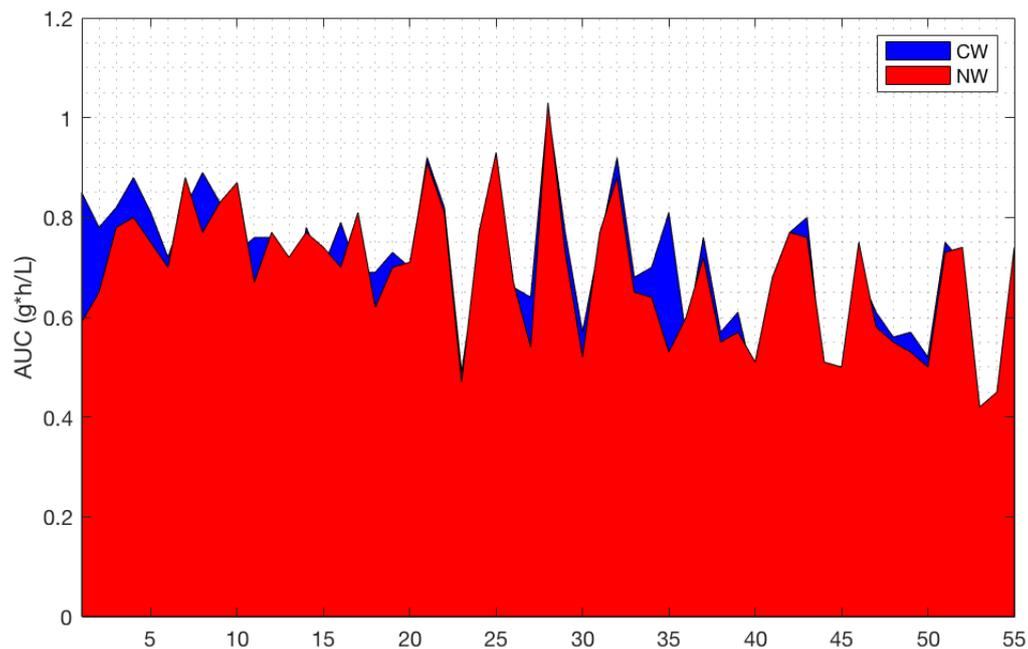
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The AUC was calculated from T0 to T120 using the trapezoidal method. This parameter proved not to be significant ($p = 0.13$). This means that although the increasing BAC in response to the two wines differs at specific points along the curve, the overall variation does not reach significance when the curve is considered as a whole (Figure 6).



234

235

236 **Figure 6.** Area under the curve (AUC) calculated for the conventional wine (CW) and the natural wine
237 (NW). ($p=0.13$).

238 It is interesting to note that, with the exception of the peak, the median BAC
239 measured in response to the natural wine is consistently below 0.5 g/L, the
240 maximum legal drink driving limit in many countries. In contrast, the BAC in
241 response to conventional wine not only exceeds the legal driving limit at its
242 maximum peak, but also approaches it at T20 and exceeds it at T40.

243 4. Discussion

244 Our findings indicate that the peak BAC reached after drinking a natural wine
245 is significantly lower than after drinking the same amount of a conventional wine
246 with a similar total alcohol strength by volume. The alcohol in natural wine is
247 absorbed more slowly than that in conventional wine, as can be seen by the
248 discrepancy between the BAC measurements at T20 and at T40 (Fig. 2). Ethanol is
249 absorbed into the blood stream mainly through the jejunum via passive diffusion,
250 and down the concentration gradient between the small intestine and the capillaries
251 [13]. Numerous factors can influence the absorption rate of alcohol: the type of
252 beverage and manner of ingestion (total alcohol content, the concentration of
253 alcohol, whether or not it is consumed as a single dose or as multiple smaller doses),
254 as well as the intrinsic characteristics of the subject (mucosal integrity of the
255 intestine, efficient blood flow, the presence or absence of food in the stomach, and
256 alcohol dehydrogenase activity in the gastric mucosa) [13]. This study was designed
257 to rule out possible causes for differences related to the mode of consumption or the

258 intrinsic characteristics of the subjects. The causes can thus be attributed to
259 differences in the non-alcoholic component of the two wines.

260 Chemico-physical analysis of the samples revealed substantial differences in the
261 total dry extract of the two wines. This is a direct consequence of differences in
262 farming and winemaking practices, with the absence of filtration processes in
263 natural wine likely to be a key factor. The total dry extract of a wine contains all of
264 its non-volatile substances, such as sugars, polyphenols, fibers and minerals. The
265 total dry extract contained in the dose of natural wine was 1.67 g higher than in the
266 conventional wine used for testing. This may affect gastric emptying time and,
267 consequently, the absorption rate of ethanol [18].

268 The total sulfur dioxide content in the two wines also differed, with conventional
269 wine containing the larger share. Sulfur dioxide has antioxidant and antiseptic
270 properties that inhibit the growth of certain strains of yeast and bacteria during the
271 various phases of winemaking [19]. Although the *in vivo* metabolic effects of sulfur
272 dioxide have been widely studied [20], there have been no reports on its
273 involvement in the absorption or metabolism of alcohol.

274 Another important distinction between natural and conventional wines lies in
275 the vinification process. Natural wine is the product of spontaneous fermentation by
276 indigenous yeasts naturally found on the grapes, while conventional wines are
277 produced using mixtures of laboratory-selected microorganisms. The presence of
278 various strains of bacteria and yeasts during fermentation results in the
279 development of different metabolites [21, 22]. At present, the results of chemico-
280 physical analysis of the samples used in this study are unable to provide precise
281 information about these differences. Additional data may emerge thanks to the use
282 of new technologies. In recent years, for example, high-field ¹H nuclear magnetic
283 resonance (¹H-NMR) spectroscopy has allowed detailed investigation of wine
284 metabonomics [23] and has demonstrated that the vinification protocol is one of the
285 chief factors determining the amino acid, alcohol and polyphenol make-up of two
286 wines from the same geographic location [24]. Another study established that
287 different production chains determine variations in the amount and type of
288 antioxidants found in organic and biodynamic wines [25]. Comparable data on
289 wines produced using the natural winemaking process are not yet available.

290 Besides producing wines with different amino acid and polyphenolic profiles,
291 differences in the natural and conventional fermentation pathways may also
292 generate other molecules that interact with absorption or with specific isoforms of
293 alcohol dehydrogenase (ADH), the enzyme involved in breaking down alcohol. This
294 would in turn lead to differences in the rates of metabolism of natural wine and
295 conventional wine.

296 The polyphenolic content of wine has been found to alter the intestinal
297 microbiota by stimulating growth of bifidobacteria and lactobacilli and decreasing
298 the numbers of clostridia and enterobacteria [26, 27]. Prolonged alcohol abuse, on
299 the other hand, can produce a state of intestinal dysbiosis with overgrowth of
300 proteobacteria [28]. It is unlikely, however, that these differences affect the
301 absorption and metabolism of alcohol in the short term.

302 It seems reasonable to expect the polyphenolic profile of two differently
303 produced wines to have dissimilar effects on the individual's microbiota. In any
304 case, the wines used in this study were white, meaning they were not as rich in
305 antioxidants as reds and rosés, so any variability due to the total content of
306 antioxidants (particularly of resveratrol) was minimized [29].

307 A further possibility is that pesticide residues (Tab. 1) might interfere with the
308 absorption, metabolism and pharmacokinetics of alcohol in conventional wines,
309 where contaminant analysis has revealed traces of the fungicides iprovalicarb and
310 fenhexamid. Both are present within legal limits [30, 31] and there have been no
311 reports of acute intoxication or known effects on liver metabolism caused by their
312 presence in wine [32, 33]. However, understanding the toxicity of pesticides and
313 their interaction with metabolic processes *in vivo* is extremely complex, given the
314 vast number of simultaneously interacting molecules [34]. Therefore, it cannot be
315 ruled out that synergistic interactions among the different contaminants might
316 influence the absorption or metabolism of ethanol.

317 The different kinetics observed for natural wine and conventional wine may
318 have important clinical implications. Acute alcohol intoxication is one of the leading
319 causes of emergency room visits [35], and approximately 5% of deaths from acute
320 poisoning are attributable to alcohol [36]. Systemic toxic effects are proportional to
321 BAC, and levels above 0.5 g/L are enough to impede normal daily activities.
322 Concentrations above 4 g/L cause hypoventilation, which, if untreated, can lead to
323 coma and death [37]. Our findings show that the peak BAC in response to natural
324 wine is lower than that to conventional wine, meaning that natural wine is less likely
325 to lead to alcohol intoxication.

326 Despite numerous mass media campaigns to promote responsible drinking,
327 alarming epidemiological evidence shows that they are largely ineffective in causing
328 a reduction in alcohol consumption [38]. According to 2017 data from the Italian
329 National Institute of Statistics (ISTAT), regular daily consumption of alcohol with
330 meals is slightly on the decline, whereas occasional or irregular drinking outside of
331 meals and binge drinking, particularly among youths below the age of 25, has
332 increased dramatically [39].

333 It is estimated that 35% of road fatalities are linked to alcohol. Because of the
334 dangers of alcohol-induced cognitive impairment, most European countries have
335 passed laws making it an offense to drive with a BAC in excess of 0.5 g/L. The fact
336 that in our study, among those subjects who drank 2 units of natural wine, only 56%
337 exceeded the legal blood alcohol limit of 0.5 g/L, as opposed to 67% of those who
338 drank the same amount of conventional wine, suggest further investigations.

339 A preliminary study by Bassani et al. (Marco Bassani, personal communication,
340 September 11th, 2018) compares the behavior of subjects who consumed natural wine
341 or conventional wine prior to completing a simulated driving task. Subjects who
342 drank conventional wine before the simulation tended to drive more aggressively
343 than those who consumed an equal amount of natural wine. In particular, the
344 natural wine drinkers drove consistently slower and committed fewer traffic
345 violations than those who drank conventional wine.

346 5. Conclusions

347 To our knowledge, this is the first scientific study to compare the
348 pharmacokinetics of alcohol of a conventional wine with that of a nearly identical
349 natural wine. It analyzed the effects on BAC of drinking a natural wine or of an equal
350 amount of conventional wine, both from the same *terroir* and containing the same
351 percent of alcohol by volume and total sugars. The BAC level 20 mins and 40 mins
352 after drinking the natural wine was lower than that after drinking the conventional
353 wine, and the peak blood alcohol response to drinking natural wine was also lower
354 than the peak response to drinking conventional wine. This supports the hypothesis
355 that natural and conventional wines are metabolized differently.

356 The key strengths of this study are its randomized, triple-blind, controlled
357 design and its careful selection of the wines, both of which came from the same
358 variety of grape and were virtually identical in many of their physical and chemical
359 characteristics. Recruitment of a homogeneous group of subjects reduced the
360 variability in individual kinetics and alcohol metabolism. A limitation of the study
361 is the brief period (2 h) allotted to measuring the subjects' BAC, and future trials
362 may wish to extend the time used for testing.

363 More work is needed to fully understand the relationship between natural wine
364 and BAC. Currently, little data is available on other types of wine (red and rosé) and
365 other segments of the population (women and the elderly). However, studies using
366 higher doses of wine would pose ethical challenges related to exposing subjects to
367 more alcohol than is considered safe. Additional studies using new technologies
368 such as ¹H-NMR will make it easier to pinpoint differences in the chemical
369 composition of natural and conventional wines.

370 In the absence of specific laws and more precise laboratory data, the differences
371 between natural wine and conventional wine must be imputed to differences in
372 agricultural methods, winemaking processes, and preservation techniques, and
373 their description is mainly relegated to expression of the consumers' sensorial
374 experience of the final product. The present study has been able to confirm that there
375 are indeed objective differences in the absorption of natural wine and conventional
376 wine.

377 Further research would be useful with a view to developing a universal
378 legislative framework for the regulation of natural wines and for shedding light on
379 differences in the public health implications of natural wine and conventional wine.
380 Because it leads to a lower peak BAC than conventional wine, natural wine may be
381 linked to a lower risk for alcohol intoxication. If future studies confirm this
382 hypothesis, every effort should be made to include this information in public
383 awareness and educational campaigns about responsible drinking. Given the
384 growing international interest in natural wines [40, 41] as well as consumers'
385 increasing demand for "natural" alcoholic beverages with a low environmental
386 impact, further research should be undertaken to better understand the potential
387 health benefits provided by natural wines.

388 **Author Contributions:** conceptualization, Federico Francesco Ferrero; data curation, Marco Barbeta; formal
 389 analysis, Maurizio Fadda and Luca De Carli; methodology, Marco Barbeta; supervision, Federico Francesco
 390 Ferrero and Andrea Pezzana; validation, Maurizio Fadda; writing—original draft preparation, Federico
 391 Francesco Ferrero, Maurizio Fadda and Luca De Carli; writing—review and editing, Federico Francesco Ferrero,
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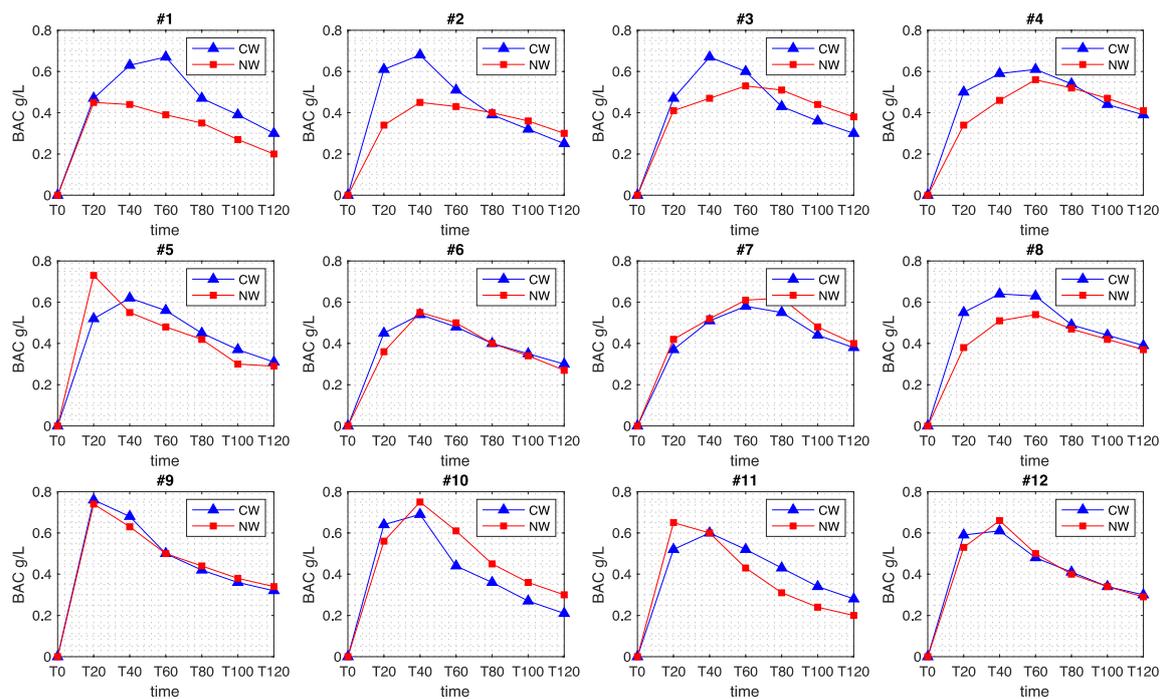
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 400 of the manuscript, or in the decision to publish the results.

401

402 **Appendix A.** Concentration-time curves of each subject's BAC response to the
 403 natural wine and to the conventional wine (Figures A1 to A5).

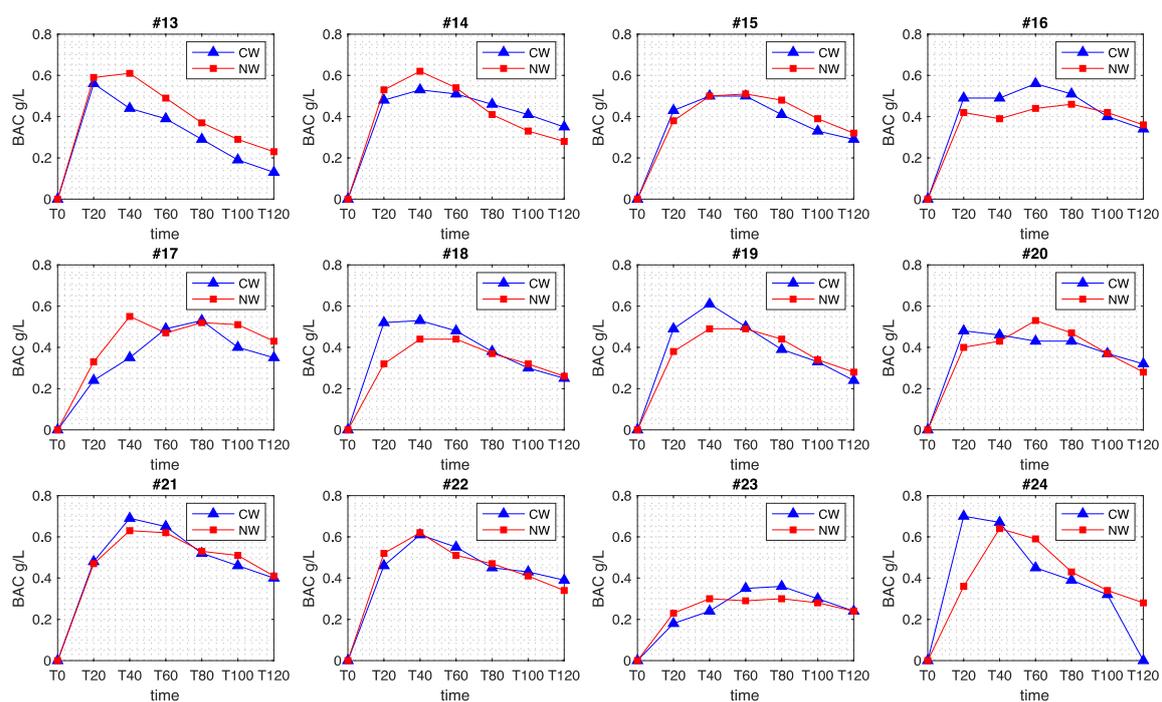


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Figure A1. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #1-#12.

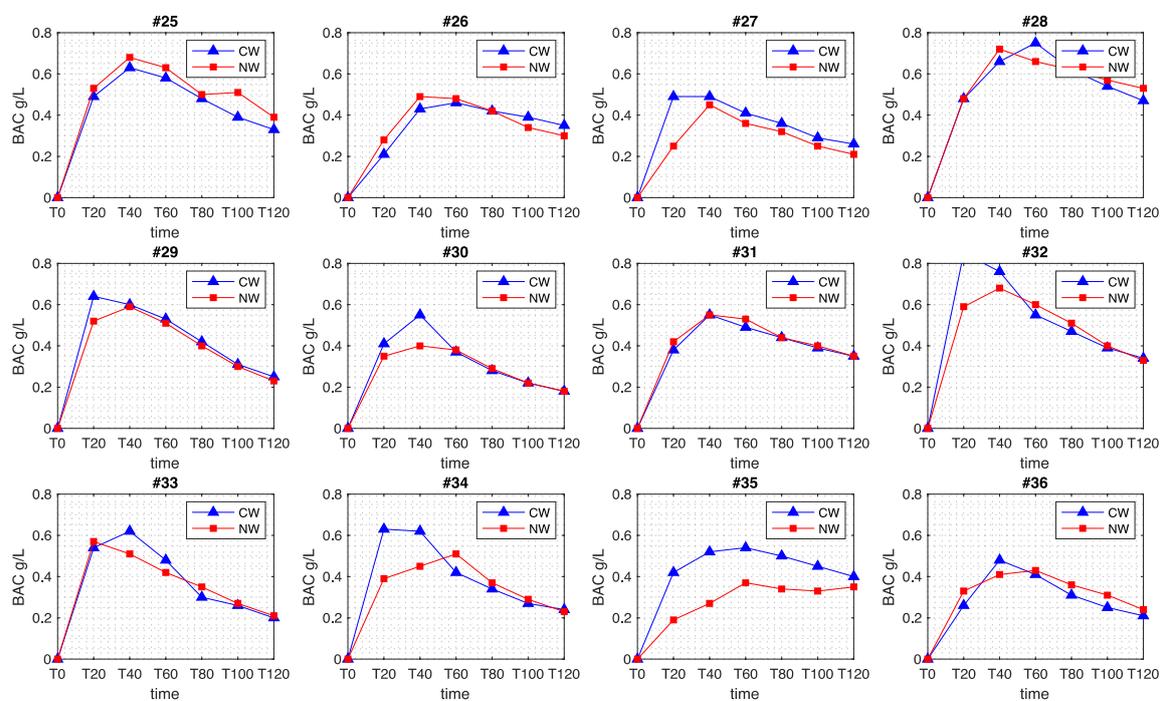


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Figure A2. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #13-#24.

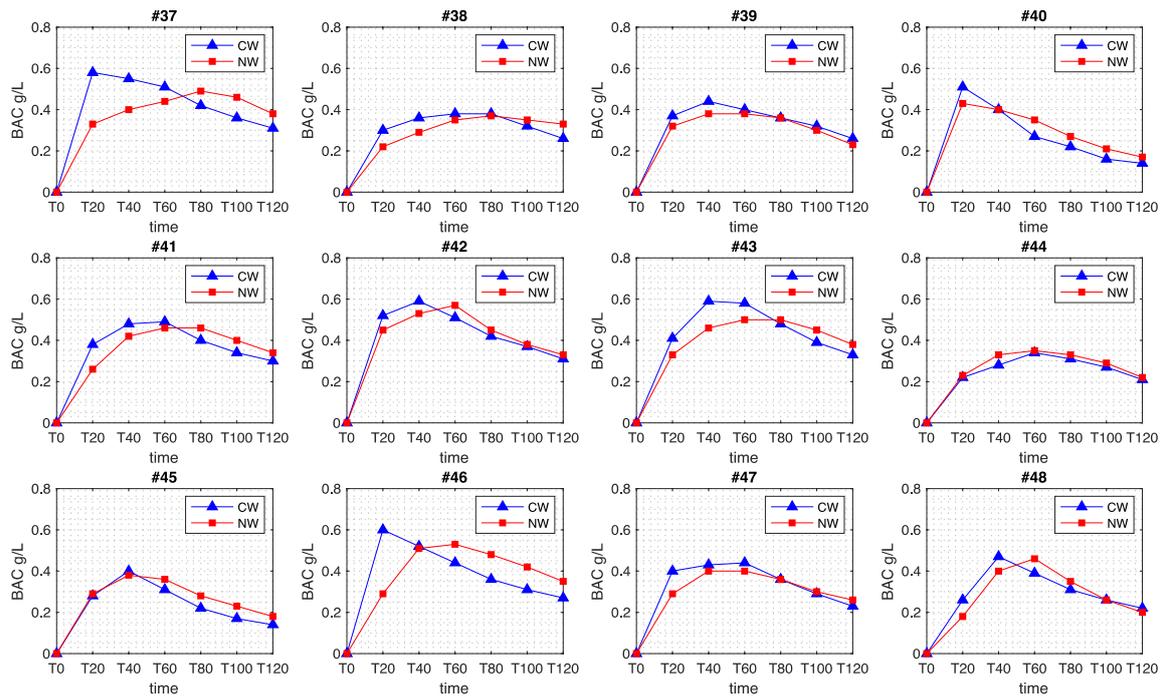


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Figure A3. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #25-#36.

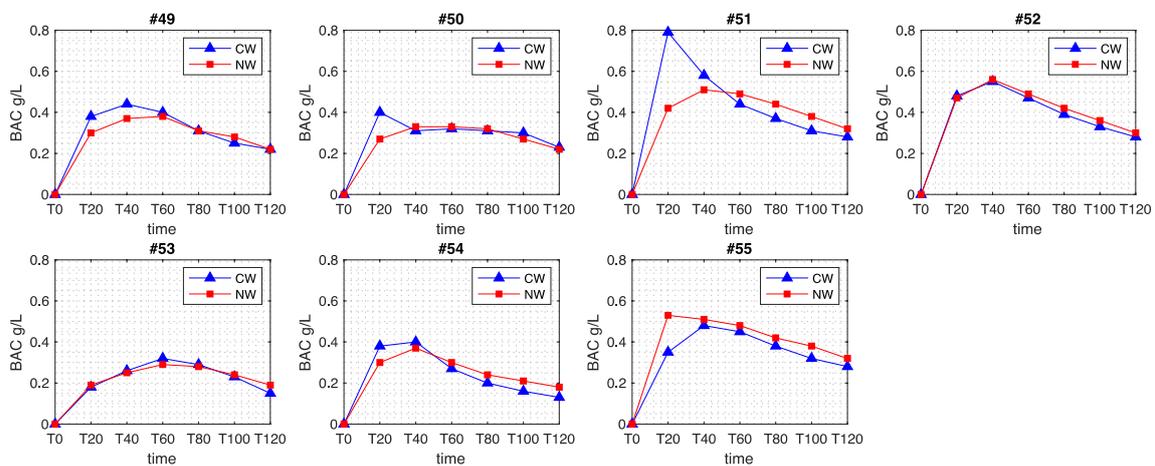


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Figure A4. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #37-#48.



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Figure A5. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #49-#55.

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