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## Levels of histamine and other biogenic amines in high quality red wines.

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## Abstract

Biogenic amines in wine may impair sensory wine quality and cause adverse health effects in susceptible individuals. In this study, histamine and other biogenic amines were determined by HPLC after amine derivatization to dansyl chloride conjugates in 100 selected high quality red wines made from seven different cultivars. Amine levels varied considerably between different wines. The most abundant amines were putrescine (median 19.4 mg/L, range **2.9-122**), histamine (7.2, 0.5-26.9), and tyramine (**3.5**, 1.1-10.7), whereas lower levels were found for isoamylamine (median 0.25 mg/L), phenylethylamine (0.16 mg/L), cadaverine (0.58 mg/L), spermidine (**1.8** mg/L), and tryptamine (0.06 mg/L). Positive correlations were observed between isoamylamine and phenylethylamine, and between histamine, putrescine and tyramine levels. Amine concentrations were similar in all wine cultivars except Pinot noir and St. Laurent wines which showed significantly higher tryptamine and cadaverine levels. The results indicate that levels of histamine and other biogenic amines may vary considerably between red wines independent of grape variety and that high amounts can be found also in high-rated wines. Adopting a legal histamine threshold level of 10 mg/L in the EU, as formerly introduced in other countries, would have excluded 34% of the investigated wines from the market.

**Keywords:** biogenic amines; histamine; HPLC; phenylethylamine; red wine; tyramine; wine intolerance

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4 **Abbreviations:**

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6 MLF malolactic fermentation

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9 OPA ortho-phthaldialdehyde

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For Peer Review Only

## Introduction

Biogenic amines are low molecular substances generated by decarboxylation of amino acids acting as regulators of growth, neuronal transmitters or inflammatory mediators. The interest for biogenic amine formation during wine production arises from the necessity to better control and understand the complexity of the wine-making process as well as from concerns related to human health since some amines commonly found in wines, such as histamine, tyramine and phenylethylamine, may cause unwanted side effects (Wantke 1993; Kalač 2005). High histamine levels are acutely toxic and implicated in scombroid fish poisoning (Lehane 2000). In experimental settings, a single oral dose of 75 mg histamine, a quantity reasonably ingestible during a meal composed of histamine-rich food, provoked acute diarrhea in as much as 50% of healthy subjects (Wöhrl 2004). However, there might exist a population of susceptible individuals reacting to even much lower quantities of biogenic amines (Wantke 1993).

Wine is a common cause for food adverse reactions in the population and may elicit a range of allergy-like symptoms including flushing, itching, headaches, rhinitis, meteorism, diarrhea, as well as urticaria and asthma (Wantke 1993; Wantke 1996; Jarisch 1996; Vally 2003). The pathomechanisms underlying wine intolerance appear to be manifold. Symptoms may arise from hypersensitivity to sulphites added to wines (Wüthrich 1989; Vally 2007) or, in rare cases, represent true allergic reactions to grape lipid transfer proteins or other allergens surviving in low amounts in the fermented product (Pastorello 2003; Schad 2005). Other studies identified acetaldehyde, a metabolite of ethanol with the capacity to release histamine from lung mast cells, as a major cause for wine-induced asthma in Japanese patients (Kawano 2004; Vally 2003).

Anyhow, biogenic amines are considered the most important reason for wine intolerance. Due to the predominant generation of biogenic amines during malolactic

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3 fermentation (MLF) (Vidal-Carou 1990b; Vidal-Carou 1990a; Radler 1991; Soufleros 1998;  
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6 Romero 2002), amine levels are usually higher in red wines than in white wines (Zee 1983;  
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8 Maxa 1992; Romero 2002). This fits well to the observation that among alcoholic beverages  
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10 red wine is most often accused for eliciting adverse reactions (Linneberg 2008). Although red  
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12 wines contain still moderate amine levels in comparison to cheese (up to 2500 mg/kg), salami  
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14 (up to 600 mg/kg) and fish products (up to 4000 mg/kg) (Diel 1997), red wine intolerance  
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16 might be a typical marker symptom and thus has been proposed as a model for histamine  
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18 intolerance (Wantke 1994; Wantke 1999). Possibly, the pharmacologic properties of biogenic  
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20 amines in wine are potentiated by some bystander effects of ethanol such as augmented gut  
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22 permeability or potential interference with amine-metabolising pathways (Draper 1983; Sessa  
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24 1984).

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30 However, according to clinical experience not all red wines are able to elicit adverse  
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32 reactions to the same degree. This might be reasonably explained by the variable amine  
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34 content of different products and has prompted some attentive winemakers to specialize in the  
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36 production of low-histamine wines expected to be tolerated even by wine-sensitive subjects  
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38 (Bodmer 1999). Because high amine concentrations in wines may reflect poor hygienic  
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40 conditions during wine-making (Bodmer 1999; Lüthy 1983), we wondered whether carefully  
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42 processed high-quality wines from top wineries will show below-average amine  
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44 concentrations. The aim of the present study was to determine levels of histamine and other  
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46 common biogenic amines in 100 high-quality red wines from Austria randomly selected from  
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48 a set of wine samples submitted to professional wine tasters for enrolment in a well-  
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50 acknowledged national wine guide.  
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## Materials and methods

### *Wine Samples*

One hundred Austrian red wines from 77 wineries made from seven different grape varieties grown locally or worldwide were analysed (Zweigelt n=25, Blaufränkisch n=25, Merlot n=10, St. Laurent n=10, Pinot noir n=10, Shiraz n=10, Cabernet-Sauvignon n=10). All wines were high quality red wines submitted to a national wine challenge and rated with a mean Parker score of  $89.5 \pm 1.69$  (range 85-94). 90% of the wines reached a score of 88 or higher. Most wines were from vintage 2004 (n=84), 10 from 2005, and 6 from 2003.

### *Derivatization of wine samples*

Biogenic amines were detected by HPLC after pre-column derivatization to fluorescent dansyl chloride conjugates (**Figure 1**). 1 mL of wine was mixed in a glass vial ( $\leq 5$  mL) with 100  $\mu$ L diaminoheptane (80 mg/L) as internal standard, 30  $\mu$ L NaOH and 1 mL of Merck Titrisol<sup>®</sup> boric acid/potassium chloride/sodium hydroxide buffer giving a final pH of 8. If pH was below 8 at the start, derivatization of some amines (e.g. histamine) was incomplete, leading to a striking loss of sensitivity and altered elution times.

To finish the reaction, 2 mL dansyl chloride were added, the tubes shaken vigorously and placed into a water bath at 55°C for 1 hour. After cooling for 10 min on ice, samples were centrifuged for 5 minutes and the supernatants were ready for analysis which was performed within the following 24 hours. All Chemicals were purchased from Sigma-Aldrich (Vienna, Austria).

### *Preparation of amine standards*

10 mL stock solutions with a concentration of 10,000 mg/L were made from the following eight amines: isoamylamine, tryptamine, phenylethylamine, putrescine, cadaverine, tyramine (all from Sigma-Aldrich, Vienna, Austria) and from histamine and spermidine (FLUKA, Vienna, Austria). For tryptamine and tyramine, methanol was added (1 part methanol + 9 parts H<sub>2</sub>O) to improve solubility. Dansyl chloride (FLUKA, Vienna, Austria) was prepared by dissolving 400 mg in 100 mL acetone and subsequent filtration through a 0.45 µm nylon filter to remove unsolved particles.

A standard mix containing all eight amines was prepared by mixing 1 mL of each stock solution together with 2 ml of water giving a final volume of 10 mL and a concentration of 1000 mg/L for each amine. From this concentration a 6-step dilution series was prepared. To enable identification of individual biogenic amines and their potential matrix effects in real wine samples, selected wines were spiked with the standard mix.

After the first results obtained from spiked samples, the standard mix was optimized by changing amine concentrations to values close to those found in wines ("optimized STD"). For the different amines, the following maximum calibration points were determined: isoamylamine 10 mg/L, tryptamine 10 mg/L, phenylethylamine 10 mg/L, cadaverine 15 mg/L, tyramine 30 mg/L, spermidine 30 mg/L, spermine 30 mg/L, histamine 40 mg/L, and putrescine 150 mg/L. The results obtained from the two standard mixes established excellent linearity between area and concentration.

### ***Recovery rates and detection limits***

Recovery rates were assessed by spiking two selected red wines with the standard mix dilution series. Recovery rates were calculated separately from each of the two wines and expressed as the average of the recoveries obtained from the set of dilution experiments.

Detection limits were calculated only for histamine because it represents the most interesting amine with respect to effects on human health. To test the limits of quantification, histamine standard solutions with concentrations of 1.0, 0.5, 0.1, 0.05, 0.01 and 0.005 mg/L were injected. The limit of detection (LOD) was obtained by measuring the peak area from 6 blanks at the position of histamine, calculating the difference between the highest and the lowest value, and multiplying this difference by 3. For limit of quantification (LOQ), the difference between the highest and lowest blank was multiplied by 10.

### ***HPLC running conditions***

Derivatized samples were subjected to RP-HPLC chromatography (Shimadzu, Korneuburg, Austria). Samples were loaded onto a 20 mm guard column (Phenomenex, Aschaffenburg, Germany) followed by a Phenomenex Synergi 4u polar RP 80A column 150 x 4.60 mm employing a stepwise gradient within 43 minutes at room temperature. Mobile phase A consisted of 200 mL ethanol (Merck, Vienna, Austria), 300 mL acetonitrile (Sigma-Aldrich, Vienna, Austria), 470 mL water, and 30 mL tris buffer *pH* 8 (0.1 M trishydroxymethylaminomethan plus 0.1 M acetate plus water, 2+1+2). Mobile phase B consisted of 450 mL ethanol, 450 mL acetonitrile, 98 mL water, and 2 mL tris buffer *pH* 8. Gradient elution started with 5% phase B and was gradually increased to 13% over 15 min and subsequently to 100% at 28 min. After staying at 100% for 3 min, the column was regenerated to 5% within 13 min. The flow rate was 1mL/min and the injection volume was 50  $\mu$ L. The time of analysis was 43 minutes.

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4 UV detection at 220 nm was chosen for best results. An advantage of this simple and  
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6 fast method was that the derivatized samples were stable enough to be put in the autosampler  
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8 for a day without cooling and there was no need for a column oven.  
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#### 10 11 *Determination of histamine by radioimmune assay* 12

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14 Histamine was determined in all wines also by a commercial radioimmune assay  
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16 (Immunotech, Marseille, France).  
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## Results

### *Recovery rates and detection limits*

Mean recovery rates for the different biogenic amines obtained from spiking experiments in two different red wines are summarized in **Table 1**. For very high amine concentrations the recovery rates were often lower. Table 1 also shows minimum amine concentrations found in the investigated 100 red wine samples. Injecting histamine standards down to 0.005 mg/L, LOD and LOQ for histamine were calculated as 0.07 and 0.22 mg/L, respectively. The lowest histamine concentration found in our wine samples was 0.53 mg/L indicating that all histamine measurements were clearly above the calculated LOQ.

### *Biogenic amine levels in red wine samples*

Median values and range of amine concentrations found in the 100 included red wines are depicted in **Table 2**. Putrescine, histamine and tyramine were the most abundant biogenic amines. All amines showed substantial variability without consistent differences between different cultivars. One striking exception was found for tryptamine which was significantly elevated in Pinot noir wines ( $0.542 \pm 0.42$  mg/L; Mann-Whitney U-test  $p < 0.0001$ ) and, to a lesser extent, also in St. Laurent wines ( $0.100 \pm 0.07$  mg/L;  $p < 0.05$ ) as compared to wines from the other varieties ( $0.056 \pm 0.06$  mg/L) (**Figure 2**). Furthermore, also cadaverine levels were significantly higher in Pinot noir ( $1.25 \pm 0.39$  mg/L;  $p < 0.0001$ ) and St. Laurent wines ( $1.55 \pm 0.95$  mg/L;  $p < 0.0001$ ) as compared to wines made from other cultivars ( $0.57 \pm 0.05$ ).

Histamine levels were lowest on average in wines prepared from the local grape varieties Zweigelt and Blaufränkisch, but the difference was not statistically significant due to the pronounced variability of amine levels in both cultivars. Results for histamine correlated very well between HPLC (mean  $8.50 \pm 6.66$  mg/L, range 0.52 - 26.97) and radioimmune assay (mean  $8.44 \pm 7.49$  mg/L, range 0.45 - 27.54) with a correlation coefficient of  $r = 0.86$ .

### *Correlation between different biogenic amines*

Each biogenic amine was analysed for a possible positive or negative correlation with any of the other amines (**Table 3**). A strong positive correlation was evident between phenylethylamine and isoamylamine levels ( $r = 0.87$ ) whereas otherwise these two amines were not linked up with any of the other amines. Positive correlations were observed also between histamine, putrescine and tyramine ( $r = 0.55-0.66$ ). All of the latter also correlated moderately with cadaverine ( $r = 0.36-0.42$ ). No correlation with any other amine was found for spermidine and tryptamine.

### *Influence of vintage year on amine levels*

To study the possible influence of the vintage year on amine production, wines from 2004 ( $n=84$ ) and 2005 ( $n=10$ ) were compared. Levels of isoamylamine ( $p<0.001$ , Mann-Whitney U-test) and phenylethylamine ( $p<0.00001$ ) were significantly higher in wines from 2005 (a year delivering average red wine quality) than in wines from 2004 (a vintage with very good red wines), whereas histamine, putrescine and tyramine levels were higher in 2004 than in 2005 ( $p<0.05$ ) (**Table 4**). The higher mean tryptamine concentration in 2004 wines was due to the higher number of Pinot noir wines in the 2004 group.

### **Discussion**

The present study was undertaken to examine high-quality red wines for the presence of histamine and other potentially harmful biogenic amines. The dansyl chloride method used by us ensured very good selectivity and reproducibility for eight biogenic amines commonly found in wine, i.e. isoamylamine, phenylethylamine, histamine, putrescine, tyramine, cadaverine, spermidine and tryptamine. Due to optimised conditions (adjustment of pH) and the establishment of a high throughput method with low running and disposable costs highly

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4 reproducible amine values were obtained. An improvement of the dansyl chloride method as  
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6 compared to the frequently used ortho-phthaldialdehyde (OPA) method is the higher stability of  
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8 derivatized samples which may be stored for 24 hours without significant loss in signal  
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10 intensity. As another practical advantage, the method does not require handling with toxic  
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12 mercaptoethanol and therefore may be used also on systems without autosampler.  
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14 Furthermore, OPA derivatization does not allow measurement of the polyamines spermidine  
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16 and spermine.  
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20 Putrescine, histamine and tyramine were identified as the most abundant biogenic  
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22 amines in this study which is consistent with the results from previous studies (Vidal-Carou  
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24 1990a; Maxa 1992; Gloria 1998; Bodmer 1999; Herbert 2005; Landete 2005; Garcia-Villar  
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26 2007). Contrasting with our expectations, histamine values were remarkably high in the tested  
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28 red wines with a mean of 8.5 mg/L and maximum concentrations up to 27 mg/L. Although  
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30 wines with histamine levels above 20 mg/L have been reported in the past (Herbert 2005),  
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32 most studies found much lower levels with upper limits rarely exceeding 10 mg/L (Subden  
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34 1979; Mafra 1999; Romero 2002; Hernández-Orte 2006; Garcia-Villar 2007). Also in two  
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36 previous studies of Austrian red wines, mean histamine levels were below 2 mg/L (Maxa  
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38 1992; Eder 2002). However, the good correlation between the histamine values obtained from  
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40 HPLC and from the commercial radioimmunoassay strongly supports the reliability of our  
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42 results. It must remain open for the time being if the observed above-average histamine levels  
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44 are a real feature of these selected high quality red wines, e.g. resulting from longer aging in  
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46 oak barrels which may be associated with increased histamine formation (Garcia-Villar 2007),  
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48 or if the discrepancies are rather due to methodological differences.  
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56 In agreement with earlier papers (Gloria 1998; Mafra 1999; Landete 2005; Garcia-  
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58 Villar 2007) we could not observe consistent differences in major biogenic amines between  
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60 different grape varieties. This supports the view that the strong variability in final amine

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3 levels primarily depends on factors such as crop health, fermenting yeasts and bacterial strains  
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5 involved in MLF which together override possible minor variety-specific differences. With  
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7 regard to histamine, wines from the locally prevailing cultivars Zweigelt and Blaufränkisch  
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9 showed the lowest mean histamine levels, but there was substantial overlap with other grape  
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11 varieties and some representatives contained up to 20 mg/L histamine, so that these cultivars  
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13 cannot generally be recommended to wine-sensitive persons as a convenient alternative.  
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18 An interesting exception concerning cultivar-specific amine profiles concerns Pinot  
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20 noir and St. Laurent. High amounts of tryptamine were solely found in Pinot noir wines. This  
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22 fits well to studies in Oregon wines where tryptamine was detected only in Pinot noirs but not  
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24 in Cabernets (Gloria 1998). Remarkably, moderately elevated tryptamine concentrations were  
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26 also found in St. Laurent, and both Pinot and St. Laurent contained significantly more  
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28 cadaverine than other cultivars. Considering that St. Laurent has been recently identified as a  
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30 descendent of the Pinot family (Regner 2000), these observations might suggest some genetic  
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32 influence on the aberrant tryptamine and cadaverine profile in these two cultivars.  
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37 Analysing possible relationships between different biogenic amines revealed a marked  
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39 positive correlation between histamine, putrescine and tyramine. Concordant associations  
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41 have been previously described for histamine-tyramine (Soufleros 1998), putrescine-tyramine  
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43 (Herbert 2005) or all three amines (Romero 2002). We could not confirm a close linkage  
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45 between phenylethylamine, tryptamine and cadaverine (Garcia-Villar 2007). High putrescine  
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47 levels have been suggested to indicate poor hygiene conditions during wine production  
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49 (Radler 1991). However, the red wines studied by us were high quality wines submitted by  
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51 winemakers for being awarded by well-acknowledged wine connoisseurs. It seems reasonable  
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53 to assume that particular care has been taken concerning selection and processing of grapes.  
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55 All in all, the close correlation between putrescine, histamine and tyramine found by us and  
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57 other investigators suggests a common origin of these amines primarily during MLF, possibly  
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4 generated by the same set of bacteria. Taking into account that decarboxylase activity in  
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6 *Oenococcus oeni* and other bacteria participating in MLF is highly variable and largely strain-  
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8 dependent (Coton 1998; Guerrini 2002; Moreno-Arribas 2003), high amine levels may be  
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10 essentially determined by the specific local microflora participating in MLF and not  
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12 necessarily indicate poor hygienic conditions.  
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16 An even stronger linkage was found between phenylethylamine and isoamylamine  
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18 levels ( $r = 0.86$ ). This is consistent with the findings of Eder and co-workers who made  
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20 similar observations also in white wines (Eder 2002). In their study, high phenylethylamine  
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22 and isoamylamine levels were evidently associated with wine or must spoilage but not with  
23  
24 MLF. Interestingly, phenylethylamine and isoamylamine were the amines showing the most  
25  
26 striking differences between vintage years in our study. We speculate that the elevated levels  
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28 in the 2005 wines were related to unfavourable weather conditions (cool and rainy summer)  
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30 promoting bursting, infection and subsequent spoilage of crops. In fact, red wines from the  
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32 vintage 2005 were of inferior quality (median Parker score 88) compared to the much better  
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34 rated wines from 2004 (median Parker score 90). Vintage year might thus have little influence  
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36 on histamine and other MLF-associated biogenic amines but a significant effect on  
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38 phenylethylamine and isoamylamine levels, although admittedly the number of 2005 wines  
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40 investigated in this study was very low. With a clinical view to wine intolerance,  
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42 phenylethylamine and isoamylamine might be expected to occur in comparable amounts in  
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44 red and in white wines, whereas amines associated with MLF appear to be more typical for  
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46 red wines. It remains to be elucidated whether patient-reported intolerance to red wines alone  
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48 vs. intolerance to all types of wine (including sparkling wines) reflects susceptibility to  
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50 different biogenic amines.  
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60 Currently, histamine levels in foodstuffs are regulated in the USA and in Europe only  
for certain sea fish products but not for wine, cheese and other histamine-containing foods.

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4 No thresholds at all exist for other pharmacologically active amines like tyramine and  
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6 phenylethylamine. While this may suggest a need for action for public health care authorities  
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8 concerning enhanced regulation, it has to be emphasized that the toxicological knowledge  
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10 about critical amine levels in foodstuffs and the scientific evidence demonstrating a  
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12 significant health effect of moderate biogenic amine doses as found in red wines is still  
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14 insufficient and often contradictory (Jansen 2003; Panconesi 2008). For instance, patients  
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16 with phenylethylamine-induced headaches, confirmed by double-blind placebo-controlled  
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18 provocation test, paradoxically reacted more often to commercial wines samples with low  
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20 amine content than to those with high concentrations (Lüthy 1983). Likewise, in a more  
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22 recent French study in wine-intolerant subjects, no clear relationship was found between the  
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24 amine content of test wines and the frequency and severity of symptoms elicited by these  
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26 wines (Kanny 2001) suggesting that wine components other than biogenic amines might be  
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28 even more important in wine intolerance.  
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35 To our knowledge, Switzerland is the only country having temporarily introduced a  
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37 legal histamine threshold of 10 mg/L for wines (abandoned in 2008 when adjusting  
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39 regulations to current EU standards). Legal limits down to 2 mg/L have been recommended in  
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41 early papers and been reclaimed during recent European COST research programs (Lüthy  
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43 1983). Applying these recommendations to the red wines investigated by us, as much as 84%  
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45 would have been excluded from the market in case of a 2 mg/L limit, and still 34% when  
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47 sticking to the 10 mg/L threshold. For comparison, the maximum allowed histamine  
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49 concentration in fish products according to EU regulation 2073/2005 is 200 mg/kg. In the  
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51 USA, the corresponding toxicity level defined by the FDA is 500 mg/kg. A recent risk  
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53 assessment study based on simulated real life situations proposed a possible threshold of 500  
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55 mg/kg for cheeses, and 400 mg/kg for fermented sausages (Rauscher-Gabernig 2009). In view  
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57 of the substantially higher limits for solid foods, the justification of the recommended  
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59 threshold levels for wines must be challenged. However, once reliable pharmacological data  
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4 on critical amine levels in wine are available, mandatory food labelling of biogenic amine  
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6 content may be helpful for consumers suffering from wine intolerance. It is evident from this  
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8 and previous studies that the production of wines largely devoid of biogenic amines is  
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10 technically possible.  
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## Legends

**Figure 1:** Derivatization of biogenic amines to dansyl chloride conjugates. Dansyl chloride reacts in buffered basic environment with amines to stable covalently bound sulfonamides, resulting in a fluorescent compound.

**Figure 2:** Tryptamine (left panel) and cadaverine levels (right panel) in Pinot noir and St. Laurent wines compared to other grape varieties.

**Table 1:** Mean recovery rates for the analysed biogenic amines according to spiking experiments and minimum amine concentrations found in the tested 100 red wine samples. *n.a.* not analysed.

<b>Biogenic amine</b>	<b>Recovery rates (%)</b>	<b>Lowest value measured in red wines samples (mg/L)</b>
Isoamylamine	94.5 - 110.1	0.02
Tryptamine	82.3 - 102.1	0.004
Phenylethylamine	80.6 - 102.5	0.04
Putrescine	80.4 - 98.4	2.93
Cadaverine	67.6 - 90.4	0.03
Histamine	89.4 - 104.6	0.52
Tyramine	65.6 - 110.8	1.07
Spermidine	69.4 - 108.5	0.03
Spermine	n.a.	n.a.

**Table 2:** Levels of biogenic amines (mg/L) in 100 Austrian high quality red wines assessed by HPLC. Figures refer to median values, minimum and maximum levels are indicated in parenthesis.

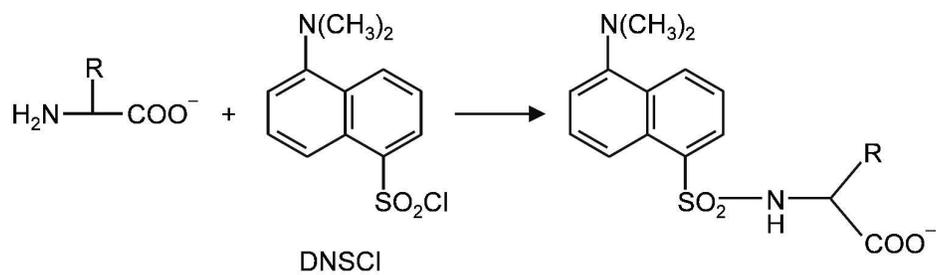
Grape variety	Isoamyl-amine	Phenylethyl-amine	Histamine	Putrescine	Tyramine	Cadaverine	Spermidine	Tryptamine
Zweigelt (n=25)	0.42 (0.09-4.34)	0.25 (0.09-1.74)	4.96 (0.52-17.3)	17.0 (4.95-77.6)	2.99 (1.07-9.35)	0.33 (0.00-2.08)	1.71 (0.41-3.24)	0.03 (0.00-0.09)
Blaufränkisch (n=25)	0.30 (0.02-3.79)	0.14 (0.00-0.92)	6.54 (0.73-20.2)	19.6 (4.22-108)	3.77 (1.14-9.35)	0.42 (0.09-2.13)	1.99 (2.15-4.31)	0.04 (0.00-0.27)
Merlot (n=10)	0.16 (0.04-0.37)	0.14 (0.07-0.27)	8.37 (5.38-26.4)	12.6 (2.93-44.3)	3.50 (1.42-6.33)	0.27 (0.03-1.00)	1.42 (0.21-1.96)	0.06 (0.00-0.16)
St. Laurent (n=10)	0.30 (0.06-0.93)	0.15 (0.06-0.31)	8.74 (1.18-26.4)	29.5 (7.11-68.7)	5.00 (1.48-10.2)	1.11 (0.73-3.27)	1.84 (1.04-3.29)	0.09 (0.00-0.18)
Pinot noir (n=10)	0.23 (0.12-0.37)	0.11 (0.06-0.19)	9.33 (2.37-26.3)	24.8 (6.53-68.6)	3.99 (1.81-8.95)	1.23 (0.78-2.20)	1.09 (0.37-2.49)	0.44 (0.08-1.59)
Shiraz (n=10)	0.42 (0.09-2.76)	0.22 (0.10-0.73)	10.9 (2.13-27.0)	28.0 (9.18-122)	4.16 (2.32-10.7)	0.59 (0.13-2.51)	2.5 (0.61-4.96)	0.07 (0.00-0.25)
Cabernet-Sauvignon (n=10)	0.13 (0.06-0.76)	0.15 (0.11-0.42)	7.66 (1.79-22.0)	20.0 (7.21-36.1)	2.25 (1.28-8.32)	0.69 (0.38-1.09)	1.76 (0.03-2.81)	0.05 (0.00-0.31)
All wines (n=100)	0.25 (0.02-4.34)	0.16 (0.00-1.74)	7.20 (0.52-27.0)	19.4 (2.93-122)	3.52 (1.07-10.7)	0.58 (0.00-3.27)	1.79 (0.03-4.96)	0.06 (0.00-1.59)

**Table 3:** Correlation between different biogenic amines in the tested red wines.

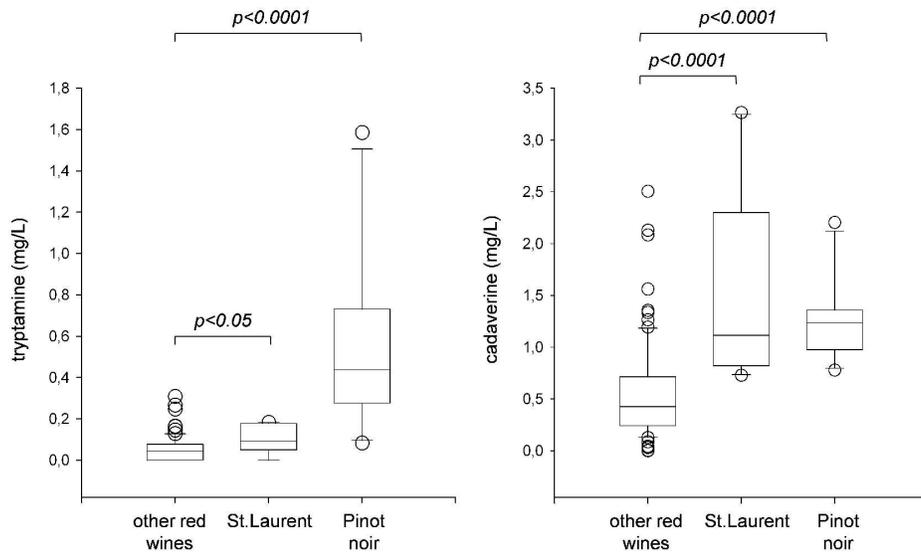
	Isoamyl-amine	Phenylethyl-amine	Histamine	Putrescine	Tyramine	Cadaverine	Spermidine	Tryptamine
Isoamylamine		<b>0.868</b>	-0.055	-0.032	0.000	-0.190	-0.095	-0.089
Phenylethylamine	<b>0.868</b>		-0.055	0.045	0.000	-0.195	0.000	-0.161
Histamine	-0.055	-0.055		<b>0.628</b>	<b>0.655</b>	<b>0.415</b>	-0.071	0.118
Putrescine	-0.032	0.045	<b>0.628</b>		<b>0.555</b>	<b>0.362</b>	0.268	0.105
Tyramine	0.000	0.000	<b>0.655</b>	<b>0.555</b>		<b>0.379</b>	0.000	0.089
Cadaverine	-0.190	-0.195	<b>0.415</b>	<b>0.362</b>	<b>0.379</b>		0.187	0.195
Spermidine	-0.095	0.000	-0.071	0.268	0.000	0.187		-0.285
Tryptamine	-0.089	-0.161	0.118	0.105	0.089	0.195	-0.285	

**Table 4:** Comparison of biogenic amine levels in red wines from vintages 2004 and 2005.

	<b>2004 (n=84)</b>	<b>2005 (n=10)</b>	<b>p (U-test)</b>
Isoamylamine	0.42 ± 0.57	1.29 ± 1.39	0.001
Phenylethylamine	0.20 ± 0.17	0.71 ± 0.54	<0.00001
Histamine	8.8 ± 6.8	4.3 ± 4.8	<0.05
Putrescine	26.6 ± 22.5	17.3 ± 13.9	n.s.
Tyramine	4.5 ± 2.6	2.5 ± 0.8	<0.05
Cadaverine	0.75 ± 0.62	0.64 ± 0.8	n.s.
Spermidine	1.9 ± 0.9	1.7 ± 0.9	n.s.
Tryptamine	0.12 ± 0.22	0.05 ± 0.07	n.s.



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