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Automated optical grape sorting: Influence of grape rot on the contents of biogenic amines in musts and wines of the vintages 2010 and 2011

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Automated optical grape sorting was tested concerning grape rot in the vintages of 2010 and 2011 in the Mosel wine region. Different grape varieties, especially 'Riesling', were sorted and the four different fractions, control (unsorted berries), free-run (juice from opened berries, if present), positive (sound and intact berries) and negative (sorted and rotten berries) were analysed for the content of the biogenic amines putrescine and histamine. Since these fractions contain different amounts of rotten grapes, the aim was to determine the influence of grape rot on the contents of biogenic amines in the resulting musts and wines. It could be shown that the infection of the berries by microorganisms resulted in only small contents of these biogenic amines in the range of 0.1 to 1.2 mg/l. With putrescine, the negative fraction occasionally contained slightly higher amounts. No correlation could be found between the corresponding must and wine fractions, e.g. histamine was mainly formed during the alcoholic fermentation. In conclusion, different intensities of rot on grapes in the sorting fractions did not highly influence the content of putrescine and histamine in the resulting musts and wines and so did not affect the wine quality.
Keywords: grape rot, Mosel, histamine, putrescine, optical grape sorting

Vollautomatisch-optische Traubensortierung: Einfluss der Traubenfäule auf die Gehalte biogener Amine in Mosten und Weinen der Jahrgänge 2010 und 2011. *Die vollautomatisch-optische Traubensortierung wurde zur Entfernung von fäulnisbelasteten Beeren aus dem Lesegut mit Trauben verschiedener Rebsorten, vor allem 'Riesling', der Jahrgänge 2010 und 2011 des Weinanbaugebietes Mosel angewendet. Zu jedem Versuch lagen eine Kontrolle (Referenz/unsortierte Trauben) sowie die erhaltenen Sortierfraktionen Saftvorlauf (aus geöffneten, meist faulen Beeren auslaufender Saft), positive Fraktion (gesunde, intakte Beeren) und negative Fraktion (faule Beeren) im Most- und auch im Weinstadium vor. Ziel der Untersuchungen war es, den Einfluss einer Fäulnis auf den Beeren hinsichtlich des Gehaltes an den biogenen Aminen Putrescin und Histamin in den resultierenden Mosten und Weinen zu analysieren. Die Ergebnisse zeigen, dass ein Mikroorganismenbefall auf den Beeren zu insgesamt nur geringen Gehalten an Putrescin und Histamin mit 0,1 bis 1,2 mg/l führte. Putrescin lag vereinzelt in den Negativfraktionen der Versuche in leicht erhöhten Gehalten vor. Histamin wurde überwiegend während der alkoholischen Gärung gebildet. Insgesamt konnte kein Zusammenhang zwischen den korrelierenden Mosten und Weinen einer Fraktion beobachtet werden. Fazit: Die unterschiedlichen Fäulnisintensitäten in den einzelnen Sortierfraktionen hatten insgesamt keinen großen Einfluss auf die Gehalte an Putrescin und Histamin in den untersuchten Mosten und Weinen und somit auch keine Auswirkung auf die diesbezügliche Weinqualität.*
Schlagwörter: Traubenfäule, Mosel, Histamin, Putrescin, optische Traubensortierung

Tri optique entièrement automatique du raisin : l'influence de la pourriture du raisin sur les teneurs en amines biogènes des moûts et des vins des millésimes 2010 et 2011. *Le tri optique entièrement automatique du raisin a été utilisé afin d'éliminer des raisins atteints de pourriture provenant de la vendange de raisins de différents cépages, surtout 'Riesling', des millésimes 2010 et 2011 de la région viticole Moselle. Chaque essai était accompagné d'un contrôle (référence/raisins non triés), et on disposait également des fractions de triage obtenues, soit la tête du jus (le jus s'écoulant de baies ouvertes, pour la plupart pourries), la fraction positive (baies saines, intactes) et la fraction négative (baies pourries) au stade du moût et également au stade du vin. L'étude avait pour objectif d'analyser l'influence d'une pourriture sur les baies en vue de déterminer la teneur des moûts et des vins obtenus en amines biogènes, soit la putrescine et l'histamine. Les résultats montrent qu'une contamination des baies par des microorganismes n'entraîne qu'une faible teneur en putrescine et en histamine qui se situe entre 0,1 et 1,2 mg/l. Des teneurs légèrement élevées en putrescine ont pu être rencontrés, dans des cas isolés, dans les fractions négatives des essais. La formation de l'histamine a essentiellement eu lieu au cours de la fermentation alcoolique. Dans l'ensemble, aucune relation entre les moûts et les vins correspondants d'une fraction n'a pu être observée. Conclusion : globalement, l'influence des différentes intensités de pourriture dans les différentes fractions du tri sur les teneurs en putrescine et en histamine des moûts et des vins examinés n'était pas très importante et, par conséquent, elles n'avaient aucun effet sur la qualité des vins obtenus.*

Mots clés : pourriture du raisin, Moselle, histamine, putrescine, tri optique du raisin

Optical grape sorting of rotten grapes

In some wine producing areas with cool and humid climates the climate change plays an important role because of the increasing hazard of different kinds of bunch rot. Among these, *Botrytis cinerea* is the most important kind of rot on grapes (Dittrich and Grossmann, 2005). In the last years, several secondary and tertiary infections such as acetic acid bacteria and other microorganisms as well as mechanical damage of the berry skins by insects could be increasingly observed in those regions (Rosch et al., 2009). These factors influence the wine quality and represent new challenges for the wine producers. If rotten berries are processed, it can lead to undesirable sensory effects in the wine. On rotten berries, a lot of microorganisms are active. The question rises, if besides known undesirable metabolites like acetic acid or ethyl acetate, metabolites such as biogenic amines can also be formed on the berries. Therefore, it can be important to separate the grapes to ensure and improve the wine quality.

Commonly, such a sorting process is performed manually, which represents a time-consuming and costly method. Optical sorting machines have already been used for years in the food industry. Recently, this technology in terms of optical grape sorters has provided an innovation in the wine industry.

In this study, the optical sorting process concerning rotten grapes has been investigated over two vintages, 2010 and 2011, and the effects of different climatic conditions with different kinds of rot on the grapes could be investigated. The performance of the automated optical grape sorter allowed the sorting of high amounts of grapes, i.e. from a whole vineyard. Consequently, authentic results were obtained with this innovative system. The musts and the corresponding wines of the sorting fractions were analysed for putrescine and histamine.

Biogenic amines

Biogenic amines are present on several fermented foods and beverages as well as on microbially infected foods (Soufleros et al., 1998; Landete et al., 2007; Lonvaud-Funel, 2001). Therefore, they are also considered to be indicators of spoilage (Eisenbrand und Schreier, 1995).

In wine, putrescine, histamine and tyramine represent the most important biogenic amines (Glória et al., 1998; Lonvaud-Funel, 2001; Martín-Álvarez et al., 2006; Landete et al., 2007). They are formed by decarboxylation of the corresponding amino acids (Lonvaud-Funel, 2001; Landete et al., 2007; Beneduce et al., 2010). There are different possibilities for the occurrence of biogenic amines in wine: Some of them, like putrescine, can already occur on the berry (Del Prete et al., 2009). During alcoholic and malolactic fermentation the concentrations of biogenic amines can increase depending on the yeasts and bacteria present (Coton et al., 1998; Goñi and Azpilicueta, 2001; Caruso et al., 2002; Landete et al., 2007; Von Wallbrunn et al., 2011). In general, biogenic amines are more abundant in wines after malolactic fermentation (Ribéreau-Gayon et al., 2006). It could be shown recently that during alcoholic fermen-

tation also yeast species like *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* are able to form and degrade biogenic amines, depending on the yeast species (Caruso et al., 2002; Von Wallbrunn et al., 2011).
Especially histamine is of key importance because it is described to cause headaches and discomfort (Taylor, 1986). Additionally, biogenic amines can influence the sensory properties of the wine. In a white wine, concentrations from 15 to 20 mg/l of putrescine can negatively affect the sensory quality (Arena and Manca de Nadra, 2001). Higher concentrations can lead to an undesirable smell of rotten fruit (Izquierdo Cañas et al., 2008). In contrast, histamine does not seem to influence the sensory quality of a wine (Wantke et al., 2008).
In this study we tried to find out if an infection by rot, especially *Botrytis cinerea*, leads to higher amounts of biogenic amines. Therefore optical grape sorting was used to separate the grapes and to compare the different sorting fractions concerning the content of biogenic amines.

Material and Methods

Sorting process

Grape sorting was performed with the automated optical grape sorter Optyx 3000 (Key Technology, Walla Walla, Washington State, USA) with an integrated high-speed RGB (red, green, blue) camera system (up to 4000 pictures per second) and an infrared laser (765 nm) for the detection of damaged berries and particles that reduce the quality to ensure and improve the wine quality (Fig. 1). Prior to sorting, the machine has to be trained with sound and rotten berries. A computer compares the data from the camera and the laser detection with the given data and sorts the grapes by blowing out "bad" (e.g. rotten) berries by air pressure in flight. Each sorting trial was performed with the optimal calibration depending on the grape variety and the type of rot.
Different steps have to be performed before the optical scanning of the grapes (Fig. 1 (3)). The whole bunches are first destemmed (Fig. 1 (1)) because the berries have to be singularized for detection and sorting. As further preparation steps, there is a spreading and a pressureless pre-straining of the berries on a vibration table where the free-run can drain off through the perforated plate of the table (Fig. 1 (2)). To get representative results, amounts of grapes as obtained in actual practice from a whole vineyard, approximately two tons per experiment, were sorted and processed, thus resulting in authentic conditions.
White grape varieties, especially 'Riesling', can be sorted with an accuracy level of 98 % on average by our system, i.e. 98 % of rotten berries from the grape crop can be separated.
The resulting fractions of such a sorting trial are first the control fraction (unsorted berries), a positive fraction (sound and intact berries), a negative fraction (sorted and rotten berries) and a free-run (juice from opened berries, if present).

Samples

In 2010 and 2011 different grape varieties from different vineyards of the Mosel wine region were studied: in 2010 only 'Riesling' (Rsl) was sorted (6 experiments); in 2011 'Riesling' (Rsl; 4 experiments), 'Müller-Thurgau' (MTh; 2 experiments), 'Pinot Gris' (PGr; 2 experiments) and 'Pinot Noir' (PNr; 1 experiment) were sorted. The experiments are indicated as E1-2010 to E6-2010 from 2010 and as E1-2011 to E9-2011 from 2011. The number of these individual experiments refer to the ascending order of the different harvest dates. The experimental grapes were harvested by hand or machine from 11th of October to 28th of October in 2010 and from 21st of September to 18th of October in 2011. Must and wine samples of the four sorting fractions were analysed. The free-run amount depends on the condition of the berries. During the processing of the grapes from the vineyard up to the sorting process, the perforated skins of botrytized berries can split easily. So the juice can leak from the berries. In 2010, however, frequently dried botrytized berries were harvested leading to only little amounts or the absence of a free-run fraction.
Must and wine preparations were performed in the following standardized manner without any finings: For vinification of the white wines and the free-run of the 'Pinot Noir', the musts were clarified after pressing by sedimentation or filtration below 100 NTU (Nephelometric Turbidity Unit). For alcoholic fermentation, a white wine yeast of the species *Saccharomyces cerevisiae* was used. The fermentation process took 20 days on average at temperatures of 15 °C in the first half of this time and of 18 °C in the second. Afterwards, the wines were stabilized by cooling and sulfurization (adjusted to 40 mg/l free sulfur dioxide). The red wines were fermented on the mash with a red

wine yeast of the species *Saccharomyces cerevisiae*. After alcoholic fermentation, the mash was directly pressed and stabilized by cooling and sulfurization. Wines did not undergo a malolactic fermentation. Wine samples were sterile filtered and bottled. Must samples were frozen to -20 °C until preparing for analysis.

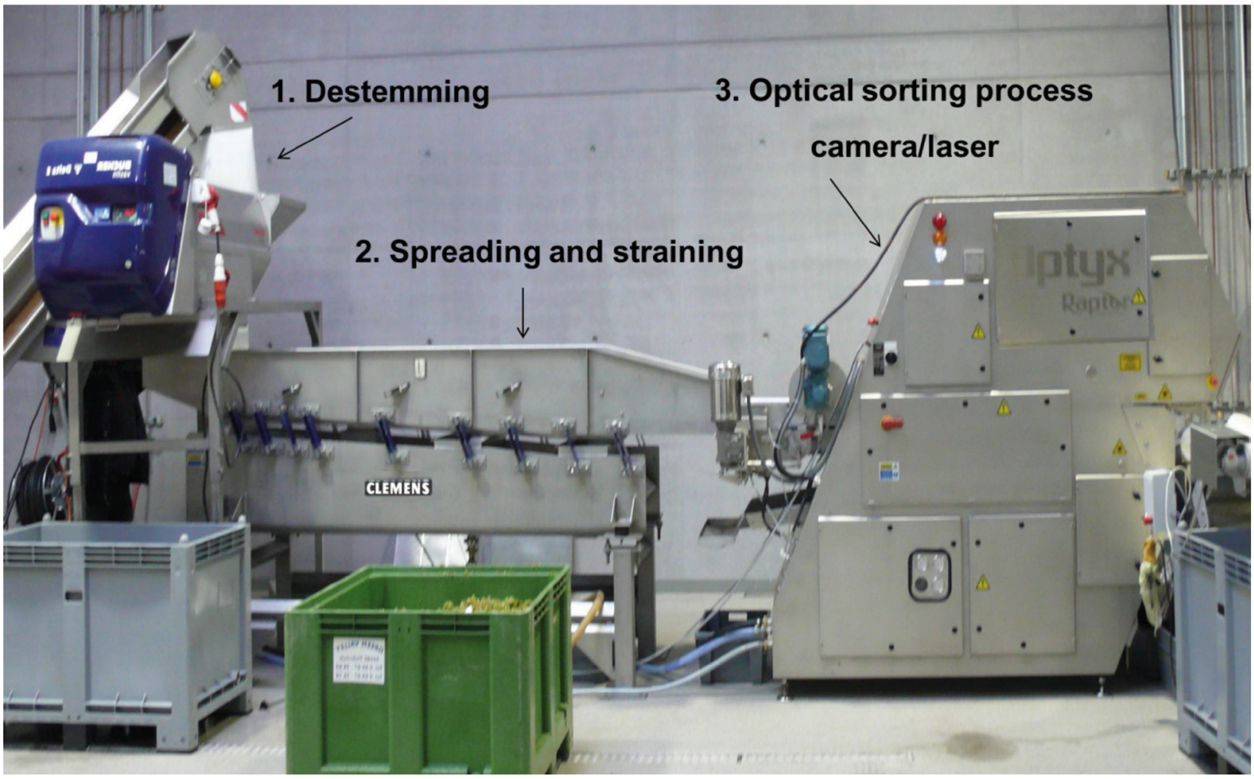

Fig 1 Automated optical grape sorter at the DLR Mosel

Statistics

Statistics were performed using the two-way ANOVA with Tukey's post-hoc test with a significance level of $p \leq 0.05$ (Software: XLStat Version 2013.4.04, Addinsoft, New York, USA). Must and wine samples of the four fractions from each experiment were statistically tested separately. Statistical differences of the must samples were marked with a, b, c and d and the differences of the wine samples with A, B, C and D.

Climate

In autumn of 2010, the climate during harvest in the Mosel wine region was rather cool and foggy in the mornings and dry during the day. In combination with the vegetative development of the grapes, these conditions led to a widespread infection with *Botrytis cinerea*. The amount of rot on the berries varied between 13 and 49 % (on average 33 % by mass), assessed by visual rating. In autumn of 2011 the climate was warm and moist during the day with some tropical nights (over 20 °C) (Agrarmeteorologie Rheinland-Pfalz). As primary infection *Botrytis cinerea* also occurred, but secondary and tertiary infections like sour rot also appeared under these climatic conditions. Despite these subsequent infections, the amount of rot on the berries varied only between 5 and 24 % (on average 13 % by mass) assessed by visual rating.

Chemicals

Histamine dihydrochloride and putrescine dihydrochloride were purchased from Sigma Aldrich (Schnelldorf, Germany). Nitric acid titrisol (concentration 1 mol/l) was purchased from Merck (Darmstadt, Germany), Sulfossol 200 from Sofralab (Epernay Cedex, France) and the standard solution for wine analytics

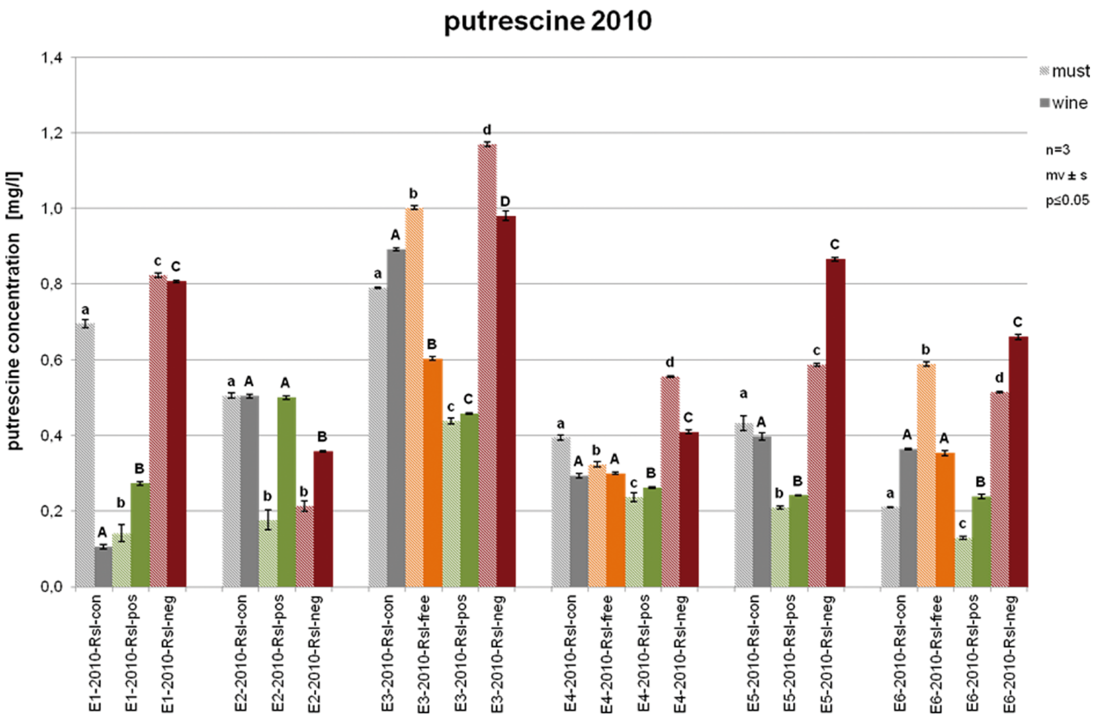

Fig. 2a
Putrescine concentrations in the vintage of 2010

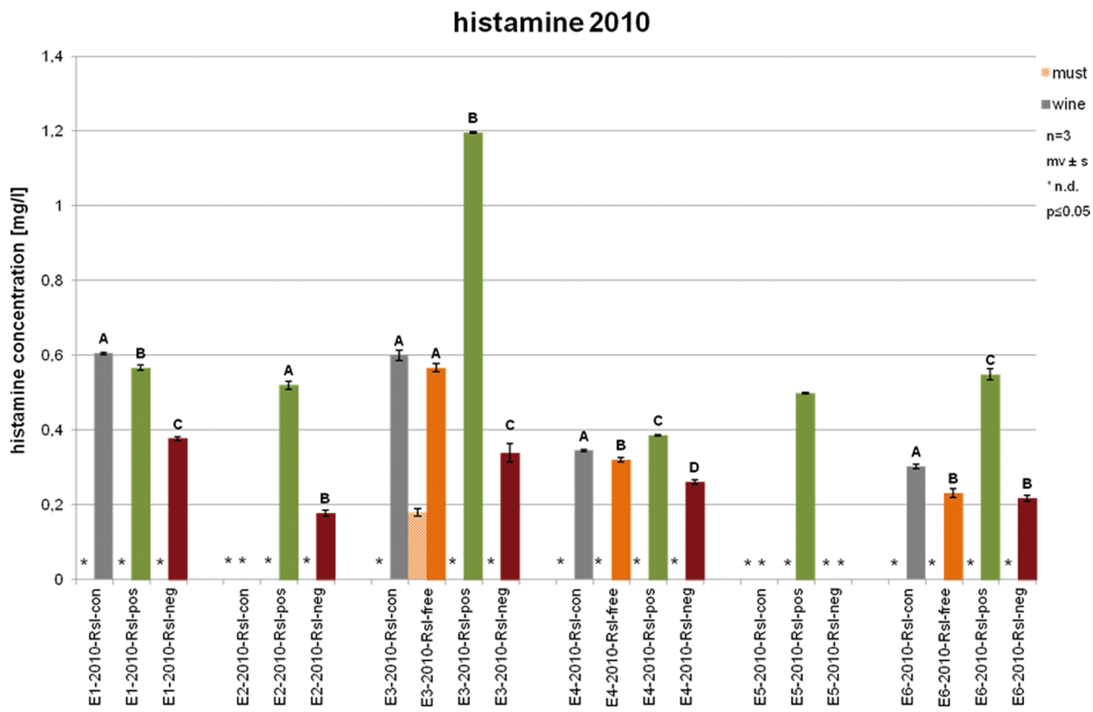

Fig. 3a
Histamine concentrations in the vintage of 2010

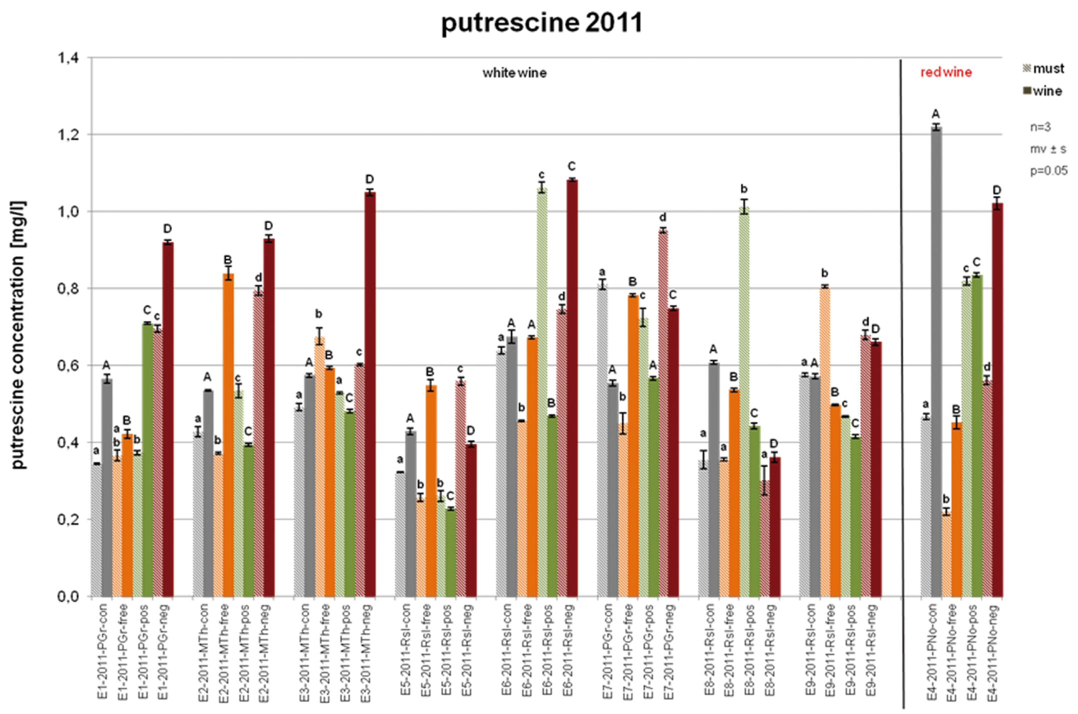

Fig. 2b
Putrescine concentrations in the vintage of 2011

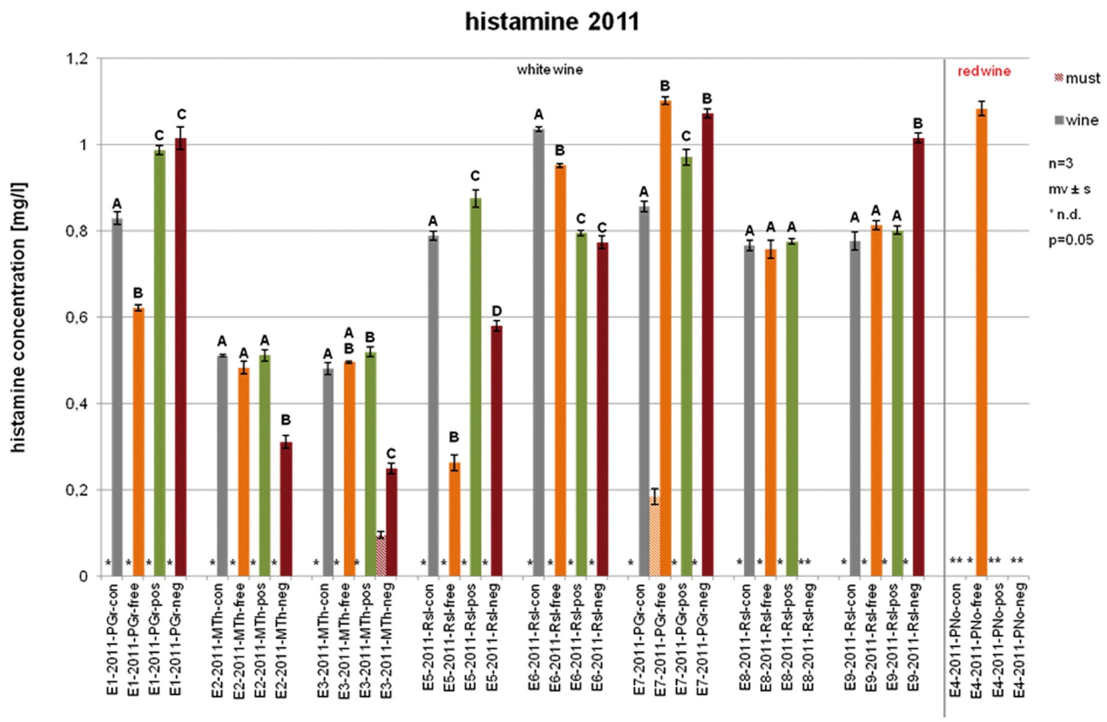

Fig 3b
Histamine concentrations in the vintage of 2011

from Deutsche Weinanalytiker e.V. (Aspisheim, Germany). All solutions and dilution preparations were prepared using demineralised water. All samples were filtered through 0.45 µm cellulose-acetate-membranes (VWR; Darmstadt, Germany).

Fig. 2 and Fig. 3:
E1-2010 to E6-2010: sorting trials in 2010 and E1-2011 to E9-2011: sorting trials in 2011; con = control; free = free-run; pos = positive fraction; neg = negative fraction; PGr = Pinot Gris; MTh = Müller-Thurgau; PNo = Pinot Noir; Rsl = 'Riesling'; mv = mean value; s = standard deviation; n.d. = not detected (Statistics: Must and wine samples of the four fractions from each sorting trial were statistically tested separately; a, b, c, d = significance of must fractions; A, B, C, D = significance of wine fractions, $p \leq 0.05$).

Biogenic amines

Putrescine (1,4-diaminobutane) and histamine (2-imidazol-4-ylethylamine) were determined by ion chromatography with conductivity detection (IC-CD) on a Metrohm Compact IC plus system 882 (Deutsche METROHM, Filderstadt, Germany). Separations were performed on a Metrosep C1 column (silicagel with carboxyl groups, 125 x 4.6 mm, 5 µm; Deutsche METROHM, Filderstadt, Germany). The mobile phase consisted of 5 mmol/l nitric acid and was used isocratically with a flow of 1 ml/min. Injection volume of the samples was 20 µl (Rudy, 2012). Must samples were stabilized with Sulfossol 200 (solution of ammonium bisulphite) to 100 mg/l of total sulphite content and filtered. A volume of 25 ml of the must and wine samples was acidified with 25 µl of HNO_3 (1 mol/l) and membrane-filtered (0.45 µm cellulose-acetate-membranes; VWR, Darmstadt, Germany). All analyses were performed in triplicate (n = 3). Quantitative determinations were made using the external standard calibration method at concentrations ranging from 0.1 to 10 ppm putrescine and histamine, added to the standard solution for wine analytics. The limits of detection (LOD) and the limits of quantification (LOQ) were determined according to DIN 32645. The LOD of putrescine was 0,03 mg/l, the LOQ 0,1 mg/l. The LOD of histamine was 0,02 mg/l and the LOQ 0,08 mg/l.

Results

The results of the different sorting trials in the diagrams were arranged according to the order of harvest date indicated as E1-2010 to E6-2010 from 2010 and E1-2011 to E9-2011 from 2011. The four fractions of each sorting trial were abbreviated as follows: con = control; free = free-run; pos = positive fraction; neg = negative fraction.

Putrescine

The putrescine concentrations of the vintages 2010 and 2011 showed average values between 0.1 and 1.2 mg/l in the must and the wine fractions (Fig. 2a and 2b). Regarding the must fractions of 2010, the negative fractions of the sorting trials E1, E3, E4, E5 and E6 showed the highest concentrations and all positive fractions showed lowest concentrations. In 2011, there was no general trend between the putrescine concentrations and the fractions of all experiments while the negative must fractions of the sorting trials E1, E2, E5 and E7 also showed the highest concentrations compared to the correlating musts of these experiments. Regarding each fraction individually there was also no general trend between the must and the related wine concentration over all experiments.

Histamine

In the vintage of 2010, only the sample E3-2010-Rsl-free and in 2011, only the two samples E3-2011-MTh-neg and E7-2011-Rsl-free showed detectable low histamine concentrations in the must state. Histamine was formed obviously during the alcoholic fermentation and reached average concentrations between 0.2 and 1.2 mg/l in the wine fractions of both vintages (Fig. 3a and 3b). There was no correlation between the histamine concentrations and the sorting fractions over all experiments.

Discussion

The results for the biogenic amines show that different kinds and intensities of rot did not consistently influence the levels of putrescine. Histamine was mainly formed by the yeast species used for alcoholic fermentation. The results also suggest that the occurrence of the biogenic amines does not depend on the grape variety.

The recent findings are consistent with previous reports in literature. Scholten and Friedrich (1998) analysed wines of higher predicates (Auslese, Trockenbeerenauslese and Beerenauslese) with a marked *Botrytis* note. Putrescine concentrations between 0.1 and 0.6 mg/l and histamine concentrations between 0.2 and 0.8 mg/l with values even below 0.1 mg/l were found. The results of Kiss et al. (2006) also confirm the low histamine concentrations on botrytized grapes. The higher putrescine concentrations of up to 1.1mg/l in the negative fractions can be explained by the composition of this fraction. There are more skin and seed portions with higher putrescine contents which could already be shown by Broquedis et al. (1989) and Kiss et al. (2006). The varying trend between the must and the related wine concentrations can be ascribed to the ability of the yeasts to metabolize putrescine during fermentation. Eder et al. (2002) reported that there was only a small average increase in putrescine concentrations in must and wine of rotten berries compared to sound ones.

In literature histamine was described not to be detectable in the must samples (Ladero et al., 2010), but high concentrations of up to 27.6 mg/l with 8.4 ± 7.5 mg/l on average were found in red wines (Wantke et al., 2008). In contrast, the red wine analysed in our study did not contain detectable amounts of histamine. For the fermentation of the red wine fractions, a red wine yeast was used, whereas the free-run of the red wine, containing no skins, was fermented with the standard white wine yeast. Since histamine could also be determined in the free-run wine fraction, the formation of biogenic amines seems to depend on the yeast species used during alcoholic fermentation as described in literature (Goñi and Azplilicueta, 2001; Caruso et al., 2002; Von Wallbrunn et al., 2011). In agreement with this conclusion, Von Wallbrunn et al. (2011) showed that histamine can be formed and degraded by yeasts depending on the species. Further studies have to be done with several yeast species commonly used for alcoholic fermentation to assess their formation/degradation ability of biogenic amines and to derive practical recommendations.

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