BIOGENIC AMINES AND GRAPES: EFFECT OF MICROBES AND FINING AGENTS

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SUMMARY
Biogenic amines are believed to cause allergy-like reactions in sensitive people after uptake. In this study, the focus was laid on the content of biogenic amines in grapes produced under different conditions and it was also investigated how fining agents might lower the concentration of amines in the resulting wines.

Biogenic amines are produced by the grape vines in response to stress factors like heat, salt, water deficiency etc. Therefore, it is not possible to produce amine-free grapes in the vineyard.
Intensive nitrogen fertilization, especially in conjunction with *Botrytis* infections, increase significantly the concentration of biogenic amines in grapes. Histamine, as key amine, may increase up to 2 mg/L. Rotten grapes show higher levels of aliphatic amines, which act as enhancers of negative effects of cyclic amines. Fining agents react preferably with aliphatic amines. Histamine is adsorbed onto bentonite but not completely.

Our results show that stress factors like vineyard fertilization management and mould infections of grapes display significant impacts on the initial content of biogenic amines in grape must, already before any alcoholic fermentation by yeasts or malolactic fermentation by bacteria has taken place. Fining agents, that are currently available for the wine industry, do not efficiently reduce the levels of biogenic amines.

INTRODUCTION
Biogenic amines are organic low molecular substances which are the result of decarboxylation of amino acids in the metabolisms of microbes, plants, animals and humans. They occur naturally in nearly every food in our daily nutrition. Higher concentration are sometimes reported in fish, chocolate, yeast extract and fermented products like alcoholic drinks, cheese, soy products, sauerkraut and processed meat (Askar and Treptow, 1986).

A number of adverse effects on human health like headache, urticaria, rhinitis, respiratory problems and digestive problems have been ascribed to the uptake of biogenic amines by sensitive people.
Although there is a wide-spread believe that high contents of biogenic amines in food might cause negative reactions after oral uptake in sensitive persons, a remarkable study based on a literature survey by Jansen et al., published in 2003, came to the conclusion that the current literature (until 2002) showed no relation between the oral uptake of biogenic amines and food intolerance reactions of humans.

Biogenic amines are important regulating substances in humans. Some are actively produced like histamine and serotonine and act for example as neurotransmitters (neuro-active) or as vaso-active substances on blood pressure.

Biogenic amines can be grouped from a chemical point of view into cyclic and aliphatic amines, where the latter are believed to act as enhancers of the effects induced by cyclic amines like histamine.

At present, only Switzerland among the European countries has a legal regulation for the amine content in wine which is 10 mg/l for histamine, addressed as key amine.

RESULTS AND DISCUSSION

EFFECTS OF VINEYARD MANAGEMENT

The origins of biogenic amines are sound grapes, infected (rotten) grapes, alcoholic fermentations, malolactic fermentations and microbial activities during wine storage. This presentation will concentrate on amine content in sound and rotten grapes as well as on the impact of fining agents to reduce the level of amines.

Nicolini et al. (2003) determined the concentrations of various amines in sound and ripe grapes of different red and white varieties, native to the Trentino region. Table 1 clearly demonstrates that biogenic amines are always present and can vary in a vast range when different varieties are compared. Phosphoethanolamine and ethanolamine only showed minor variations.

Biogenic amine	Concentrations (mg/L)
Putrescine	1.3 ± 0.7
Methylamine	0.17 ± 0.14
Histamine	0.13 ± 0.16
cadaverine	0.03 ± 0.02
2-Phenylethylamine	< 0.02
Ethylamine	0.31 ± 0.27

Table 1: Variation of biogenic amines in grapes

With Riesling as the typical cultivar for the wine producing region “Rheingau”, located on the slopes of the Rhine River in Germany, we investigated the effects of nitrogen fertilization, green cover of the vineyard soil and rottenness (fungal diseases).

It could be shown for vintage 1994 and 1995 that with increasing nitrogen fertilization, also the total content of polyamine increased. This effect was much more pronounced in 1995 and was most probably due to the fact that higher rainfalls enabled higher uptake of nitrogen from the soil (Table 2).

Variant kg N/ha	Total polyamines (µg/l)	
	1994	1995
00/00	793	987
00/60	795	1356
90/00	796	1402
30/60	833	1557

Table 2: Impact of fertilization on amine concentrations in grapes (Bleser, 2000)

Mechanisation of vineyard work resulted in growing green cover in every second row or in all rows to ease usage of tractor. As the green cover also impacts the mineralisation of organic material and also the uptake of inorganic nitrogen, we checked the eventual impact on biogenic amines in grapes.

It turned out that the impact on the vintage was higher than the impact by green cover in every second row (Table 3).

	1994		1995	
Variant	Putrescine (µg/l)	Spermidine (µg/l)	Putrescine (µg/l)	Spermidine (µg/l)
No cover	131	519	481	351
Green cover	95	491	340	442

Table 3: Impact of green cover on biogenic amines in grapes (Bleser, 2000)

The influence of mould infections on amine concentrations was checked by Eder et al. (2002). Some biogenic amines like ethylamine and isopentylamine were present in much higher concentrations in rotten grapes and a further increase was observed in the final wines too. In a more general approach, Scholten and Friedrich (1998) analysed commercially available wines which had been produced from grapes that had undergone spontaneous *Botrytis*infections.
Histamine, tyramine, putrescine and cadaverine were measured in low concentrations in quite narrow range. On the other hand, concentrations of ethanolamine, 2- phenyethylamine and isopentylamine varied in a wide range (Table 4).

Wine	Ethanolamine mg/l	Histamine mg/l	Tyramine mg/l	Phenylethylamine mg/l	Isopentylamine mg/l	Putrescine mg/l	Cadaverine mg/l
1	3.5	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1
2	21.2	0.2	0.2	10.1	14.6	0.2	0.1
3	6.2	0.3	< 0.1	6.9	7.9	0.1	< 0.1
4	11.2	0.2	< 0.1	1.3	5.4	1.1	< 0.1
5	6.3	< 0.1	< 0.1	0.2	0.2	0.5	0.1
6	7.1	< 0.1	< 0.1	0.3	0.2	0.5	0.1
7	36.2	1.1	0.2	6.4	12.3	0.3	0.1
8	41.2	0.8	0.1	7.1	14.1	0.4	0.1
9	38.2	0.4	0.1	6.5	14.0	0.4	0.1
10	7.5	< 0.1	< 0.1	< 0.1	0.1	0.3	< 0.1
11	11.8	< 0.1	< 0.1	< 0.1	0.2	0.5	0.1

Table 4: Amine concentration in Botrytis wines

In an experimental set-up, where grapes where treated either with botrytizides or actively infected with *Botrytis* spores and then compared with an untreated control, grapes from each variant were

harvested and their respective amine profile analysed. Figure 1 shows the impact of the infection degree of *Botrytis* on the amount of different biogenic amines.

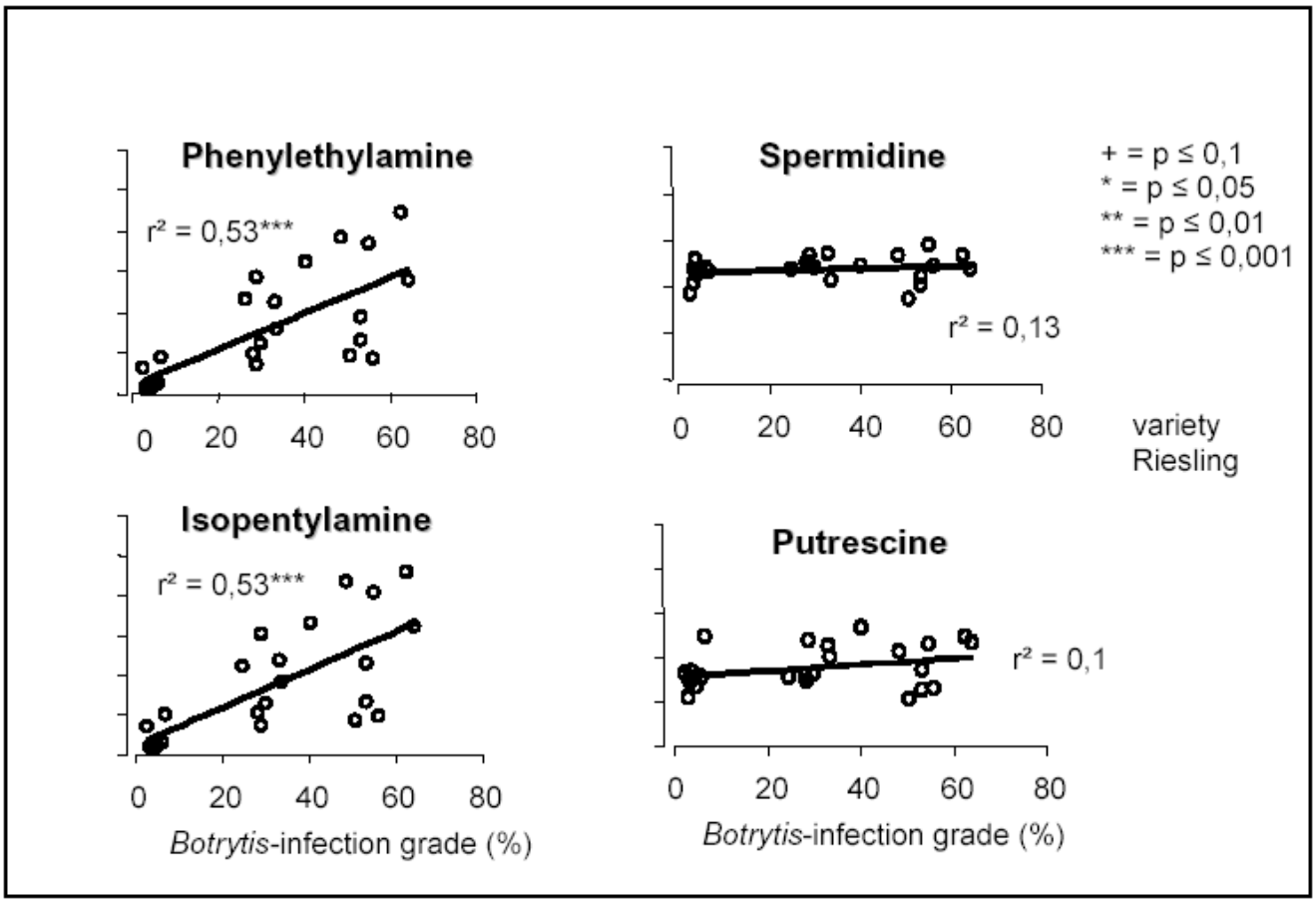

Figure 1: Effect of vine rottenness – grape must 2006

In reaction to the different infection degrees different amines increased in concentrations (phenylethylamine, isopentylamine) while others did not (spermidine, putrescine). These results confirmed the findings of Scholten and Friedrich with commercial wines and demonstrated that their infected wines were actually based on the *Botrytis* noble rot infection.

In another experimental set in vintage 2006, we looked for correlation between the amount of nitrogen fertilization per hectar and potential influences on *Botrytis* infection and amine production. The correlation coefficient ranged between 0.57 and 0.72 for pheynethylamine, isopentenylamine and putrescine, while spermidine gave 0.05.

EFFECTS OF FINING AGENTS

As the amount of biogenic amines in grape must or wine might be in a critical range, it was already tried in the last 70's of the last century to eliminate or at least to reduce them by the action of finings. It was shown in those days that only the bentonite treatment showed adsorptive capacities, mainly when in contact with histamine (Mayer and Pause, 1978).
We chose fining agents which are currently available on the market: potassium caseinate, gelatine, enological carbon, sodium bentonite, sodium calcium bentonite and calcium betonite.

Finings were applied either in the musts or in the latter wines. As it is shown in Figure 2 with Riesling, vintage 2005, must treatment was much more effective than the treatments in the resulting wines. Nearly all fining agents decreased the concentrations of putrescine, spermidine and spermine when applied to must. Significant effects in wine treatments could only be seen with bentonite. It also became obvious that the pre-treatment of bentonite either as sodium, sodium-calcium or calcium bentonite stayed without any impact.

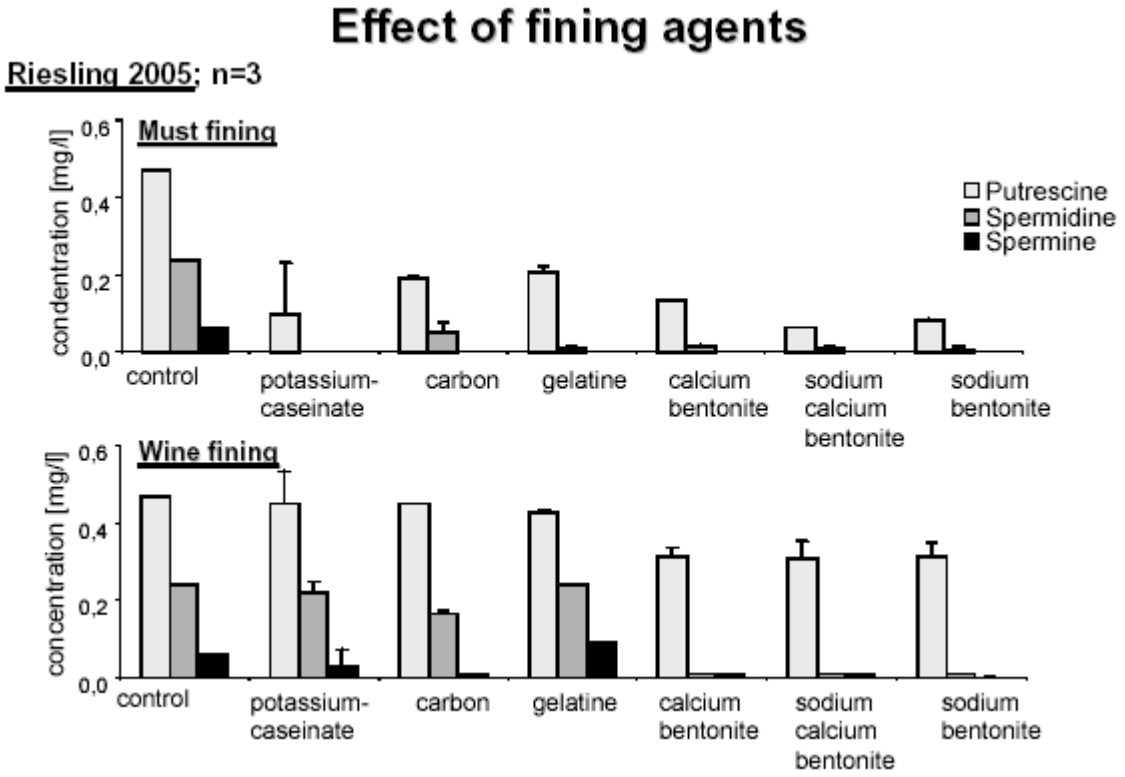

Figure 2: Effect of fining agents

To conclude, biogenic amines are naturally occurring substances in all grapes. Their concentrations vary with the amount of nitrogen fertilizers used in the vineyard and the amount of *Botrytis* infections. It was also clearly shown that the higher the nitrogen fertilization the higher the risk for mould infections which result in significantly increased concentrations of different amines.
When it comes to the necessity of applying fining agents, their use should be preferably already in the must.

Last but not least, a remarkable publication from 2003 should be mentioned. Jansen and coworkers did a large literature review and examined those medical publications where oral challenge studies with biogenic amines were performed with randomized, double-blind, placebo-controlled design. In total, 13 studies were found. Among these, 5 showed positive results and 8 negative. 3 (all with positive results) of the 13 studies proved to be ineligible.

From the remaining 10 studies, 6 were considered inconclusive. The 4 conclusive studies all reported negative results. One conclusive study showed no relation between biogenic amines in red wine and wine intolerance. Two conclusive studies found no effect of tyramine on migraine. One conclusive study demonstrated no relation between the amount of phenylethylamine in chocolate and headache attacks in individuals with headache. The authors concluded that the scientific literature (until 2003) showed no scientific basis for dietary recommendations concerning biogenic amines in such patients (Jansen et al., 2003).

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