THE BIOGENIC AMINE HISTAMINE: PHYSIOLOGICAL EFFECT AND CONCENTRATIONS IN WINE

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SUMMARY

Wine samples were collected from German wineries during a two-year study (2007/2008) and their histamine concentration was determined. The data from 2007 showed that between two wineries either no histamine or a low concentration of it was found when with starter cultures (*Oenococcus oeni*) were used or maximum values of 1,01 up to 7,30 mg/l were detected when spontaneous MLF (malolactic fermentation) occurred. In the 2008 survey maximum histamine concentrations ranged between 1,85 and 2,24 mg/l when the musts were inoculated with starter cultures and 2,77 to 3,37 mg/l in the case of spontaneous MLF. The two-year study showed that inoculation with starter cultures reduced concentrations of histamine in wine but if no spontaneous MLF-bacteria were present, there was no histamine in the wines.

Key words: amines, histamines, health, wine quality.

INTRODUCTION

The term biogenic amines covers a group of 20-30 compounds of biological origin that are formed in human, animal and plant tissues and may occur in food containing protein. Some of these amines play a role in biological functions, are important precursors of vitamins or other substances, and may trigger physiological responses. In food, they originate from microbial breakdown of amino acids. High contents of biogenic amines can therefore be found especially in spoiled food, in some cases also in fermented food such as cheese, cold meats, wine or sauerkraut. Biogenic amines ingested with food are normally harmless, since they are broken down by enzymes in the small intestine mucosa (Gappmaier, 2000). Negative effects are only expected in special situations, e.g. in cases of very high intake of histamine and tyramine through food, individual hypersensitivity, or in conjunction with medicines (Askar and Treptow, 1986). Biogenic amines are physiologically active substances that perform important functions, although in higher concentrations they may trigger adverse health effects or even toxic effects. From a nutrition-toxicological perspective the main issues are histamine intoxications and risk of high blood pressure caused by tyramine. The best-known representative of biogenic amines is histamine (Eder, 2003). It has numerous functions in the human organism. Particularly noteworthy is its role in defensive reactions. Histamine is released whenever a cell in the body is damaged. This results in increased permeability of the blood vessels, enabling immune defence cells (e.g. white blood cells) to enter damaged tissue from the blood supply to counteract a possible infection. In addition to these desired effects, the immunological response to alien proteins (allergens) also results in a release of histamine, which, in the case of allergies, causes undesired symptoms such as itching, swelling or a contraction of the respiratory tract (asthma). A similar effect is caused by the venoms of bees, wasp and hornets, which also contain histamine, in addition to other substances (Eder, 2002). Although various barriers limit the intake of orally supplied biogenic amines, food with a high content of such amines can lead to health problems. In particular, this may be the case if the body's regulatory systems are impaired by medication or a chronic intestinal disease. Symptoms can usually be explained by the effects of histamine and tyramine. Little is known about adverse health effects of other biogenic amines (Beutling, 1996). Histamine can also be classified as a poison, since in high concentrations it can trigger a state of shock and therefore have a life-threatening effect. The tolerance limit is around 10 mg, although this may fluctuate widely. In terms of health disorders a distinction is made between histamine intolerance and acute histamine intoxication. Histamine intolerance occurs when histamine as a tissue hormone is produced naturally in the body and as a messenger substance causes allergic reactions. In approxymately 1% of the human population (mainly women) the consumption of even small quantities of food containing histamine can lead to pseudo-allergic responses due to intolerance. These health problems are

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due to increased histamine levels in the plasma or tissue (Biegantski *et al.*, 1983). The symptoms may vary between different individuals and can include abdominal cramps, diarrhoea, flatulence, feverishness, reddening of the skin, skin rashes, itching, runny nose, headache, exhaustion, fatigue, asthma, mental aberration or even aggressive behaviour. The symptoms usually start around 45 min after intake and may require hours to subside. The normal histamine level in the blood is 1 ng/ml. Higher levels indicate increased histamine intake or reduced histamine breakdown (Gappmaier, 2000). Histamine intoxication can be caused by intake of large histamine quantities (100-1000 mg) and can lead to acute symptoms of poisoning within 30-60 min, such as nausea, vomiting, diarrhoea, migraine, asthma, low blood pressure, giddiness, dizziness and circulatory collapse. In persons with histamine intolerance the reactions can be particularly severe, while good histamine tolerance results in weaker reactions (Taylor *et al.*, 1984; Hui *et al.*, 1985). Children are more vulnerable than adults, since their enzyme system is not yet fully developed. Histamine intoxications are most frequently caused by fish and seafood, but can also by triggered by cheese, poultry, sauerkraut, cold meats, beer or wine (Beutling, 1996).

There is currently no limit value for histamine in wine. Various European studies (Straub *et al.*, 1993; Masqué *et al.*, 2007; Herbert *et al.*, 2005) and research projects are currently examining a wide range of aspects, which are discussed in the international technical literature. These projects investigate the formation and occurrence, as well as the avoidance of biogenic amines in wine. Researchers study the viticultural factors (nitrogen fertilization, grape variety, water availability, grape health) and the oenological conditions (spontaneous fermentation, pure yeast fermentation, microbiological contamination of the must, spontaneous MLF, MLF starter cultures, wine aging). In terms of histamine formation and occurrence the focus is on microbiological conversion during malolactic fermentation. As early as 1985, Davis *et al.* (1985) showed that there are significant differences with regard to the formation of biogenic amines within the family Lactobacteriaceae, within the genera that are relevant for wine production, i.e. *Lactobacillus*, *Pediococcus* and *Oenococcus oeni*. The highest amine levels, particularly histamine, were observed with representatives of the genera *Pediococcus* and *Lactobacillus*. In addition to genetic constitution, the occurrence of amino acids, i.e. the precursors of the amines, is a further significant factor. Which species will develop from which genus during wine production depends mainly on the pH value of the wine. Bacteria from the genera *Pediococcus* and *Lactobacillus* (spontaneous MLF) usually multiply at pH values of 3.4 or higher and therefore represent a particular risk in wine production.

The aim of this work was to determine the concentration of histamine in German wines in the autumn 2007 and 2008 either inoculated with MLF starter cultures or undergoing spontaneous MLF.

MATERIALS AND METHODS

Determination of biogenic amines was according to Smit and Ansorge (2005). HPLC with fluorescence detection was done with HP Agilent 1100 Series. The wine samples (15 µl) were injected under a pressure of 220-240 bar and 35°C column temperature on a PerfectSil Target column (250 × 3 mm). Eluent A was 100% acetontrile and eluent B was diluted (1:3) in tris buffer at pH 8.5. The detection was at 254 nm and the emission at 51 nm with a fluorescence detector. HPLC validations of histamine concentration were done at 0.5%, 1%, 2.5%, 5%, 7.5% and 10% standards (dilution with 0.2 N perchloric acid). The wine sample preparation was done in three steps: an extraction step with 10 mM diamino heptane and 0.2 N perchloric acid in relation 1/3 mixed during 60 min, followed by incubation at 4°C for 20 min. The second step was a derivatisation: 300 µl of extract from the extraction step were added to 200 ml Na_2CO_3 and 400 µl dansyl chloride and mixed at 60°C for 1 h in a thermocycler. The cleaning step was a solid phase extraction with the J.T. Baker SPE-System. Washing in the Baker SPE-System was done twice with 3 ml methanol and twice with 3 ml bidistilled water; elution was finally done with 2 ml methanol.

In autumn 2007, 42 wine samples were taken after MLF in the winery GI and 42 samples in GII. The 42 wine samples of each winery consisted of 21 white and 21 red wine samples. Samples (100 ml) were collected at 2/3 height of the tank. Controlled MLF in white wines was done with the commercial product Viniflora CH35 LS and in red wines with Viniflora Oenos LS.

In autumn 2008, 42 samples, 21 of white and 21 of red wine .were colleted in each winery (GI, GII, GIII, GIV, GV, GVI) after MLF, in the same quantity and with the same modality as above. Controlled MLF was also as specified above.

RESULTS

In winery I (GI) 1 million litres of wine were treated with direct inoculation of starter cultures after alcoholic fermentation. MLF was spontaneous in the whole wine batch. In winery II (GII) 1.4 million litres were treated with direct inoculation of starter cultures and in 4.2 million litres MLF was spontaneous. In wines inoculated with starter cultures histamine were either not detected or occurred only in low concentrations. The highest

Table 1. Histamine concentrations at two large wineries (GI & GII) in autumn 2007 with sampling after MLF. Maximum, minimum and mean values are shown. "Controlled" indicates the samples that were treated with direct inoculation of starter culture. "Spontaneous" means that the MLF occurred without addition of starter cultures.

Wineries	G I (controlled)	G I (spontaneous)	G II (controlled)	G II (spontaneous)
Histamine max. value (mg/l)	1.11	7.30	0.00	1.01
Histamine min. value (mg/l)	0.00	0.89	0.00	0.07
Histamine mean value (mg/l)	0.63	2.75	0.00	0.49

concentration (maximum value) in GI was 1.11 mg/l, whereas in GII no histamine was found in any of the analysed wine samples (Table 1). In GI the mean histamine value in spontaneously fermented samples was 2.75 mg/l, but in samples with controlled malolactic fermentation the histamine level was only 0.63 mg/l. The same was observed in GII since samples with spontaneous MLF had a mean histamine level of 0.49 mg/l, whereas no histamine was found in any of samples with controlled MLF. It is interesting to note that in spontaneous samples from GI the maximum histamine level was 7.30 mg/l, while the maximum value for GII was 1.01 mg/l.

Table 2 shows the histamine concentrations in autumn 2008 detected in six large wineries with a total volume of 5.5 million litres. On the average, the wines with starter cultures showed somewhat lower mean and maximum values (Table 2). This is illustrated by the mean histamine values of 1.12 mg/, 1.17 mg/l and 1.41 mg/l for GI, GIII and GIV respectively. Slightly higher histamine concentrations were found in the wines where spontaneous MLF had occurred. The values were 1.38 mg/l, 1.58 mg/l and 1.60 mg/l from GII, GV and GVI, respectively.

DISCUSSION

In both studies (autumn 2007 and 2008) the addition of direct inoculation with starter cultures reduced histamine development. On the other hand, an addition of starter cultures is no guarantee for histamine-free wines. One of the main causes for histamine formation is likely the natural wine flora, which is present in any winery. Only pasteurization or flash pasteurization of the grape mash can reduce the natural flora sufficiently so as to eliminate histamine in wines with direct inoculation of starter cultures. In any case, it should be noted that in smaller and medium-sized wineries fluctuations in histamine concentrations were significantly larger.

To conclude, in the absence of MLF, histamine-free wine can be produced through application of direct inoculation of starter cultures. Our studies have shown that the use of direct inoculation of starter cultures results in lower histamine concentrations than in wines with spontaneous MLF. These wines exhibit larger variations (minimum/maximum values).in histamine content.

Table 2. Histamine concentrations at six large wineries (GI-GVI) in autumn 2008 with sampling after MLF. Maximum, minimum and mean values are shown. "Controlled" indicates samples that were treated with a direct inoculation of starter culture. "Spontaneous" means that the MLF occurred without addition of starter cultures.

Wineries	G I (controlled)	G II (spontaneous)	G III (controlled)	G IV (controlled)	G V (spontaneous)	G VI (spontaneous)
Histamine max. value (mg/l)	1.85	2.95	2.24	1.87	3.37	2.77
Histamine min. value (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00
Histamine mean value (mg/l)	1.12	1.38	1.17	1.41	1.56	1.60

REFERENCES

Askar A., Treptow H., 1986. Biogene Amine in Lebensmitteln – Vorkommen, Bedeutung und Bestimmung, 1st Ed. Eugen Ulmer Verlag, Stuttgart, Germany.

Beutling D.M., 1996. Biogene Amine in der Ernährung, 2nd Ed. Springer-Verlag, Berlin, Germany.

Biegantski T., Kusche J., Lorenz W., Hesterberg R., Stahlknecht C.D., Feussner K.D., 1983. Distribution and properties of human intestinal diamine oxidase and its relevance for histamine catabolism. *Biochemica and Biophysica Acta* **756**: 196-203.

Davis C.R., Wibowo D., Eschenbruch R., Lee T.H., Fleet G.H., 1985. Pratical implications of malolactic fermentation: a review. *American Journal of Enology and.Viticulture* **36**: 290-301.

Eder R., 2002. Biogene Amine im Wein – ein aktueller "Evergreen" ("Dauerbrenner"). *Mitteilungen Klosterneuburg* **52**: 206.

Eder R., 2003. Weinfehler, 2nd Ed. Österreichischer Agrarverlag, Vienna, Austria.

Gappmaier S., 2000. Einfluss des Säureabbaus auf den biogenen Amingehalt in österreichischen Weinen, Bachelor Thesis, University of Vienna, Austria.

Herbert P., Cabrita M.J., Ratola N., Laureano O., Alves A., 2005. Free amino acids and biogenic amines in wines and musts from the Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. *Journal of Food Engeneering* **66**: 315-322.

Hui Y.L., Taylor S.L., 1985. Inhibition of *in vivo* histamine metabolism in rats by foodborne and pharmacologic inhibitors of diamine oxidase, histamine N-methyl-transfersase and monoamine oxidase. *Toxicology and Applied Pharmacology* **81**: 241-249.

Masqué M.C., Romero S.V., Rico S., Elórduy X., Puig A., Capdevila F., Suárez C., Heras J.M., Palacios A.T., 2007. Coinoculation of yeasts and lactic acid bacteria for the organoleptic improvement of wines and for the reduction of biogenic amine production during malolactic fermentation. *Microsafety Wine Conference*, *Vilafranca del Penedes 2007*: 28-31.

Smit I., Ansorge A., 2005. Biogenic amines and grapes: effect of microbes and fining agents. *Bulletin OIV* **80**: 245-250.

Straub B.W., Schollenberger M., Kicherer M., Luckas B., Hammes W.P., 1993. Extraction and determination of biogenic amines in fermented sausages and other meat products using reversed-phase HPLC. *Zeitschrift fuer Lebensmittel-Untersuchung und Forschung* **197**: 230-232.

Taylor S.L., Hui J.Y., Lyon D.E., 1984. Toxicology of scombroid poisoning. In: Ragelis E.P. (ed.). Seafood Toxins, pp 417-430, American Chemical Society. Washington DC, USA.