Yeast Assimilable Nitrogen in South Moravian Grape Musts and its Effect on Acetic Acid Production during Fermentation

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Abstract

Baroň M. (2011): **Yeast assimilable nitrogen in South Moravian grape musts and its effect on acetic acid production during fermentation**. Czech J. Food Sci., **29**: 603–609.

We observed the content of yeast assimilable nitrogen in grape musts, its consumption by yeasts during fermentation, and acetic acid production. The experiments were performed in the years 2007 and 2008. The experimental variants involved 48 musts and wines originating from Southern Moravia. The data enabled to monitor the contents of yeast assimilable nitrogen in musts and to draw the general conclusion that these musts contain high concentrations of both ammonium ions and yeast assimilable nitrogen (124.4 mg/l and 257.8 mg/l, respectively), thus posing no danger of problems with the nutrition of yeasts in the course of fermentation. Also, the average production of acetic acid was low (215.8 mg/l); this indicated that in the majority of must samples without added nutrients, the course of fermentation was smooth and trouble-free. The results obtained confirm that the method of formaldehyde titration is universal and simple, thus it can be recommended for winemakers as a useful and efficient analytical tool.

Keywords: stuck fermentation; sluggish fermentation; *Vitis vinifela* L.; must; yeast

It is well known that nitrogen strongly influences the growth of grapevine (*Vitis vinifera* L.) annual shoots as well as the colour of its leaves (Kraus *et al*. 1997). Nitrogen compounds stored in berries are very important for the propagation and life activities (metabolism) of yeasts. The yeasts of the species *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, which always predominate in the course of must fermentation, can utilise nitrogen either in the form of ammonia ions or of free amino acids (except proline in anaerobic conditions), and that is why the term yeast assimilable nitrogen (YAN) is generally used.

The content of ammonia ions in grapes has been associated with the processes of their ripening as early as since 1960s. The concentration of ammonia ions in musts is more and more important above all in warm regions and in the case of overripened grapes. Normal levels of ammonia range between tens and hundreds of milligrams. Within the Krebs cycle and during the degradation of sugars, great amounts of α-keto acids are produced and their amination results in the formation of many organic compounds containing nitrogen (above all amino acids).

In ripe grapes, amino acids usually represent 30% to 40% of total nitrogen. These compounds are very beneficial due to their antioxidative, antimicrobial, emulgative, and surface-active properties and make a relatively great part of the sugar-free extract (up to 4 g/l) (Ribéreau-Gayon *et al*. 2006).

It is known that the content of YAN in must is dependent not only on the fertilisation, weather, length of maceration of skins in must but also on the variety, year, origin, and last but not least on the wine making technology (Etiévant *et al.* 1998; Arvanitoyannis *et al.* 2000; Soufleros *et al.* 2003). In addition to all these factors, the utilisation of YAN is greatly dependent also on the metabolism of yeasts and conditions of fermentation. The YAN value of grape juices varies widely between 50 mg/l up to 450 mg/l. The average value of grape must YAN concentration is approximately 200 mg/l. The reported and generally accepted minimum level of YAN required to prevent stuck or sluggish fermentation is considered to be 140–150 mg/l for a 21 Brix clarified must (O'Kennedy *et al.* 2008).

Depending on the availability of the nutritive substances and conditions of nitrogen utilisation, yeasts produce great amounts of substances that significantly influence the quality of the final product, i.e. wine. Some of these metabolites show positive effects on wine but some other may cause problems and organoleptic defects (Jiranek *et al.* 1995).

It is known that a lack of YAN in must is the main cause of a stuck and sluggish fermentation (Boulton *et al.* 1996) as well as of the production of sulphurous compounds (Giudici *et al.* 1994; Ferreira *et al.* 2009). The total content of nitrogen in must may also affect the wine aroma and influence the production of acetic acid (Bely *et al.* 2003), bioamines (Marcobal *et al.* 2005; Costantini *et al.* 2009) and highly carcinogenous ethyl carbamate (Ough *et al.* 1988; Coulon *et al.* 2006). In practice, it is possible to find a lack of YAN above all in unripe grapes and also in the stressed ones originating from the vineyards insufficiently fertilised with nitrogen or produced during dry and/or hot periods. In such cases, the lack of YAN must be compensated by the application of nourishing salts into the must or, possibly, also vitamin B_1 (thiamine). Naturally, grapes with a higher content of sugars require higher amounts of supplied nitrogen for a thorough fermentation.

The aim of this work is the monitoring of YAN, and ammonium ions separately, in grape musts from South Moravia. The second part is focused on the consumption of YAN and acetic acid production according to the level of YAN in the initial point of fermentation.

MATERIAL AND METHODS

The experimental material originated from the viticultural subregions Mikulov (M) and Znojmo and was collected from vineyards with and without grass cover during the period of 2007–2008 (rootstocks Kober 5BB and SO 4, varieties Rhein Riesling and Green Veltliner (Table 1).

Table 1. Sampling scheme (2007–2008)

	Region	Grass	Rootstock	Variety
1	M	G	Kober 5BB	RR
2				GV
3			SO 4	RR
4				GV
5		N	Kober 5BB	**R**
6				GV
7			SO 4	RR
8				GV
9	Z	G	Kober 5BB	RR
10				GV
11			SO 4	RR
12				GV
13		N	Kober 5BB	**RR**
14				**GV**
15			SO 4	RR
16				**GV**

M – Mikulov; Z – Znojmo; G – vineyards with grass cover; N – vineyards without grass cover; RR – Rhein Riesling; GV – Green Veltliner

Variants in picked out bold were not identified

Samples. To assure objectivity, the grapes were sampled from a great number of grapevine plants (more then 20 for each variant). After harvesting, the grapes were manually deprived of stalks and crushed. The minimum weight of berries without stalks was 5 kg per each experimental variant. After a thorough pressing in a mechanical press, must was sulphurised to 40 mg/l SO_2 and spontaneously decanted (12 h in the cold). Subsequently, all musts were frozen to –18°C. After defrosting, the first must samples were taken for fermentation. These defrosted musts were poured into two-litre flasks and inoculated with a pure yeast culture BS Universal White. The fermentation ran at the temperature of ca 14°C. After the end of fermentation, the samples were obtained in the stage of the finished production of carbon dioxide.

Reagents and solvents. Formaldehyde (40%), sodium hydroxide, sulphuric acid, hydrochloric acid, all of them HPLC grade came from Lach-

Ner s.r.o. (Neratovice, Czech Republic). HPMC (hydroxypropyl methylcellulose), 18-crown-6 acid, MES (morfolinethansulfonic acid), and BTP (1,3-bis[tris(hydroxymethyl)methylamino] propane) were purchased from Sigma Aldrich (St. Louise, USA).

Determination of yeast assimilable nitrogen. The Formol titration procedure used was described by Gump *et al*. (2000) and Filipe-Ribeiro *et al*. (2007).

100 ml of sample was poured into a 200 ml beaker and pH was adjusted to 8.0 using 1M NaOH and pH meter. The sample was transferred into a 200 ml volumetric flask, made up to the volume with deionised water and thorough mixed. The solution was filtered through filter paper. 100 ml aliquot of the sample was transferred into a beaker and the pH was readjusted to 8.0 with 1M NaOH, if necessary. 25 ml of neutralised formaldehyde (pH 8.0) was added, the mixture was stirred and titrated to pH 8.0 using 0.1M NaOH.

The results can be calculated using the general equation: mg nitrogen/l = [(vol NaOH) × (conc. NaOH) × 14 × (dilution factor) × 1000]/(sample vol).

Determination of ammonium ions. Ammonium ions were determined by Capillary Isotachophoresis (CITP), Ionosep 2003 (Recman, Ostrava, Czech Republic). Leading electrolyte (LE): 5mM H_2SO_4 + 7mM 18-crown-6 + 0.1% HPMC; terminating electrolyte (TE): 10mM BTP; initial current: 100 μA, final current 50 μA; mode of analysis: cationic.

Determination of acetic acid. Acetic acid was determined by CITP (Ionosep 2003, RECMAN, Ostrava, Czech Republic) – LE 10mM HCl + 5.5mM BTP + 0.1% HPMC (pH 6.2); TE 5mM MES (morfolinethansulfonic acid); initial current 70 μA; final current 30 μA; mode of analysis: anionic.

RESULTS AND DISCUSSION

Yeast assimilable nitrogen in musts

Because no data are available on the levels and consumption of YAN in the Czech Republic, it is necessary to compare the results of this study only with the literature data and the results obtained in other countries. In the period of 2007–2008, altogether 48 musts originating from the viticulture region Moravia were analysed. Regardless of the influencing factors, the mean content of ammonia ions was 124.4 mg/l. This means that even without nutritive supplements, Moravian musts reveal no problems with the initiation and speed of fermentation. According to the available literature data (Dukes *et al*. 1998; Gump *et al*. 2002), these concentrations are higher than the usual (20–60 mg/l). However, it could be expected that in the regions with a colder climate the contents of ammonia ions in grapes would be increased. The contents of ammonia ions and YAN in musts originating from the study region are presented in Table 2 (regardless of the individual years). In the majority of the analysed musts, these concentrations ranged between 100 mg/l and 125 mg/l and in none of the analysed samples was the measured value lower than 75 mg/l (this concentration is critical for the initiation of the fermentation process). The frequencies of the contents of ammonia ions in all samples under study are presented in Figure 1.

As compared with the optimum values of 190 mg/l to 200 mg/l, the mean estimated concentration of YAN (257.8 mg/l) seems to be sufficient even for the musts with an increased content of sugars. Only one sample contained less than 150 mg/l.

Table 2. Results of the statistical analyses of samples collected within the study period, of YAN consumption by yeasts and of acetic acid production during fermentation

Variable	Number of estimations	Mean	Confidence interval	Confidence interval	Median	Minimum	Maximum	Standard deviation
Samples collected within the study period								
NH_4^+	48	124.4	117.3	131.5	121.6	79.5	186.8	24.6
YAN	48	257.8	238.7	276.9	251.7	149.7	402.5	65.7
YAN consumption by yeasts								
YAN	48	162.6	148.7	176.5	163.1	76.6	284.5	47.8
Acetic acid production during fermentation								
Acetic acid	48	215.8	201.0	230.6	216.5	135.0	307.0	50.88

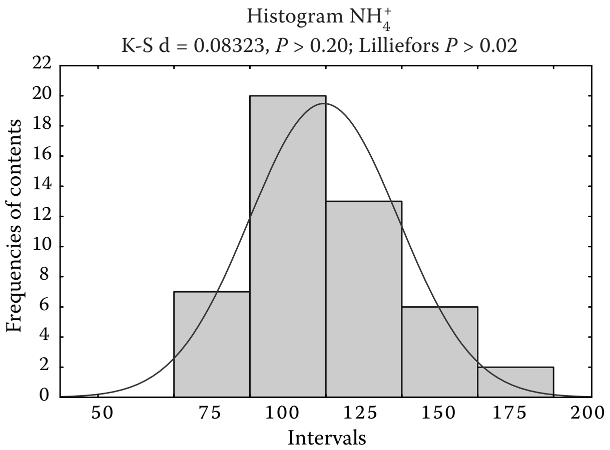

Figure 1. Distribution of frequencies of the content of ammonia ions

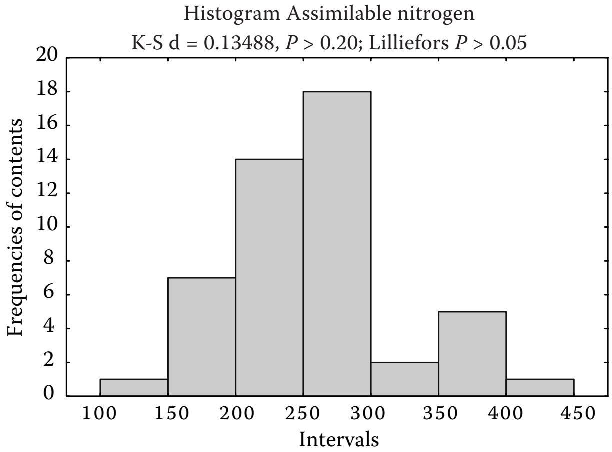

Figure 2. Distribution of frequencies of the content of YAN

Generally speaking, this concentration is too low and the musts with such concentrations of YAN must be supplemented already before the onset of fermentation. On the other hand, however, 8 must samples (i.e. 16.67%) contained more that 300 mg/l YAN and it could be expected that this might cause an increased production of acetic acid in the course of fermentation. Altogether 32 musts (i.e. 66.67%) contained 200–300 mg/l YAN and these levels can be considered as more than sufficient for the onset of fermentation in both normal must and in those with higher contents of sugars. The frequencies of YAN content in all samples under study are presented Figure 2.

Changes in the content of yeast assimilable nitrogen before and after fermentation

The mean value of YAN consumption by yeasts was 162.6 mg/l. With regard to the sugar content, this value fully corresponds with the data published by other authors (Manginot *et al.* 1997; Bely *et al.* 2003). Besides, the highest frequency of YAN consumption (150–200 mg/l) was also corroborated because 25 samples (i.e. 52%) fell into this interval. The results of 48 estimations of YAN content performed before and after fermentation are presented in Table 2. A histogram illustrating the frequencies of YAN consumption is presented in Figure 3.

A very strong positive correlation existed between the concentration of YAN before the onset of fermentation and its consumption in the course of the fermentation processes (Figure 4). In principle, this correlation indicates that, if the level of YAN in the medium is increased, the yeasts are able to utilise it so that they propagate quickly and the speed of fermentation is increased.

Production of acetic acid in the course of fermentation

The relationship between the concentration of YAN and production of acetic acid was not corroborated (these data are not shown). This could be expected above all due to the lack of YAN because only one must out of all those under study contained less than 150 mg/l YAN. This means that yeasts were not stressed due to the lack of nutrition, thus they did not produce increased amounts of volatile acid. On the other hand, higher

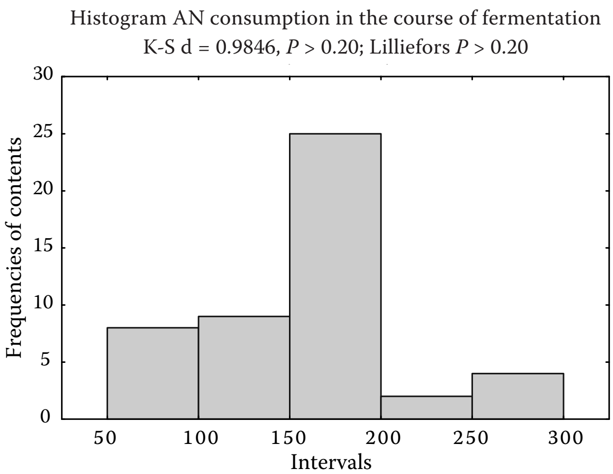

Figure 3. Distribution of frequencies of the YAN consumption in the course of fermentation

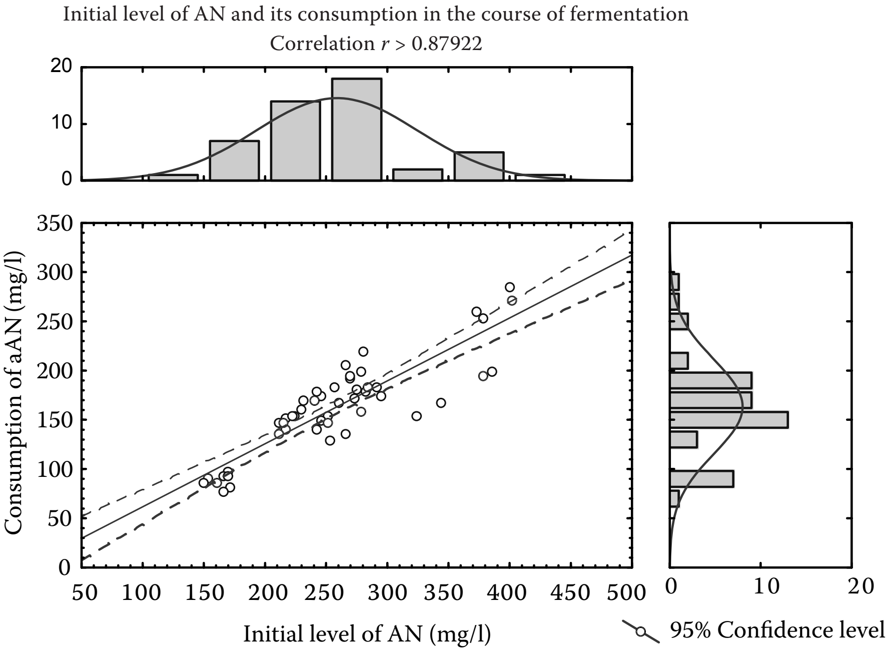

Figure 4. Correlation existing between the initial level of YAN and its consumption in the course of fermentation

concentrations of YAN increased the production of acetic acid; however, this correlation was not strong (correlation coefficient was at the level of 95%, r = 0.193) due to which these data are not presented. To find a similarity of these results with the data published abroad (Vilanova *et al*. 2007), it would be necessary to have a wider range of YAN concentration in musts. The results of 48 estimations of the level of acetic acid after fermentation (i.e. in young wine) are presented in Table 2. The mean value was 215.8 mg/l.

Similarly to Hernandez-Orte *et al.* (2006), also in this study a correlation was observed between the level of ammonium ions in must and the production of acetic acid by yeast (Figure 5). It was also found out that the higher was the concentration of ammonium ions, the lower was the production of acetic acid by yeasts. It can be stated that increased levels of ammonia ions in medium reduce the duration of the so-called "lag" stage of fermentation during which relatively high amounts of acetic acid are usually produced.

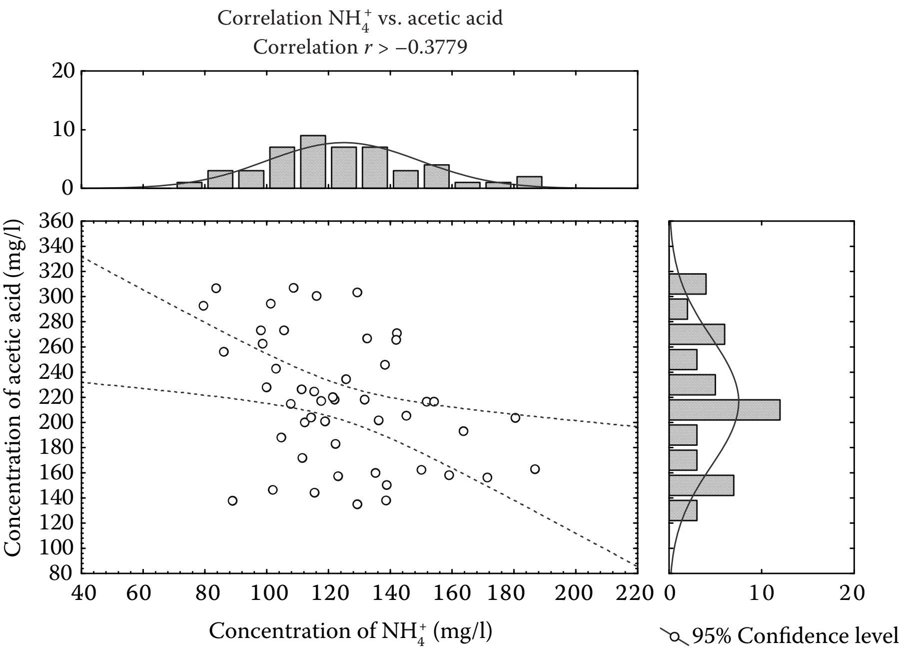

Figure 5. The correlation between the concentration of ammonia ions in must and production of acetic acid in the course of fermentation

CONCLUSIONS

In the viticulture region Moravia, the contents of YAN and ammonia ions in musts are more than sufficient. Based on the results obtained, it can be concluded that in the majority of cases it is not necessary to supplement musts with ammonia salts. However, under the conditions of insufficient nutrition, the risk of sensory defects remains real. For that reason it is recommended to perform the estimation of YAN levels by means of a relatively cheap method of formaldehyde titration directly in practice. This method requires only a simple instrumentation but in spite of this, it enables to predict easily the threat of potential problems in fermentation.

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Received for publication July 13, 2010
Accepted after corrections December 12, 2010

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