Chasing after Minerality, Relationship to Yeast Nutritional Stress and Succinic Acid Production

Mojmír BAROŇ[1] and Jaromír FIALA[2]

[1]Department of Viticulture and Oenology, Faculty of Horticulture, Mendel University in Brno, Lednice, Czech Republic; [2]Department of Fermentation Chemistry and Bioengineering, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague, Prague, Czech Republic

Abstract

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Minerality is certainly one of the most mysterious and most valuable tones of wine taste and it is very often associated with the concept of *terroir*. The isotachophoresis was used for determination of cations – minerals in two wines from vineyards with different soil conditions, with and without exceptional "minerality". However, it was found that it has nothing to do with minerals. More attention was paid to the relationship between the nutritional stress of yeasts and succinic acid production, which can result in a final difference in the taste of wine. In addition, sensory evaluation was used to reveal differences between wines with increasing levels of succinic acid.

Keywords: wine; GABA; yeast assimilable nitrogen; nitrogen catabolite repression; proline utilisation; ammonium ions

Flowers, fruits or wood are surely the tones which have their place in great wines, but the minerality probably occupies the first place among all wine attributes. For all its exclusivity and status, minerality has been defined only vaguely and has not been well described yet. Mineral, salty, flinty, chalky, and also dusty are the terms which can be used to describe one of the most popular and the most valuable characteristics of wine called "minerality". But what is it really and where does it come from? This question is certainly of interest not only for innumerable lovers of wine but also for experts and scientists in the field. Each of us can surely recall a lot of analogies that characterise this special sensory (organoleptic) characteristic, which is often called minerality.

It is expected that above all cations (or alkaline metals) are responsible for the taste of minerality. Minerals are natural components of grape musts and wines. They originate either from the soil in vineyards or from processes associated with wine making and storing. Ashes, as some minerals are also called, must be present in amounts of 2–5 g/l. Musts originating from some southern countries may contain up to 10 g/l of ashes. In dry years (like e.g. in 2003), the content of minerals is usually lower whereas in years with plenty of precipitation it can be relatively high. As far as the berries are concerned, the distribution of minerals is as follows: approximately 40% of them are present in the skin, 50% in the pulp and 10% in seeds. Of cations, the most frequent are potassium, calcium, and magnesium (Ribéreau-Gayon *et al.* 2006). These elements are involved in neutralisation of acids in grapes, musts, and ultimately also in wine. Potassium as a predominating element shows the greatest effect on changes in acidity. Under normal conditions, the percentage of magnesium is the lowest. However, this is a pity because, in contradistinction to potassium and calcium, magnesium salts do not precipitate in wine (magnesium salts show a high solubility at each temperature and pH).

But are they identical with those minerals that we appreciate in wine? Probably not, as can be read in the report presented by Alex Maltman at the annual meeting of the American Geological Society in 2009. The author of this report claimed that a frequently mentioned connection between the geological composition of soil in vineyards and the expression of wine taste was scientifically impossible. He concluded that such a connection was a romantic myth and claimed that no taste of minerality in wine was caused by minerals present in the vineyard. This means that the transport of minerals from the vineyard through roots and stems into grapes and thereafter into the glass is irrelevant.

In essence, it would be very simple if the results were positive and confirmed the hypothesis of cations. But this is not true and for that reason another explanation is possible. Maybe we are all wrong and the mythic minerality is not related to soil conditions, at least not directly, as it might seem at the first sight. The first doubts about it were published by Peynaud (1984), who mentioned organoleptic properties of succinic acid. Its taste was described as salty, slightly bitter, and very desiccating the mouth. Batch *et al.* 2009 examined possibilities of an addition of this acid into wine. Succinic acid is naturally present in wine in relatively high concentrations ranging from 0.5 g/l to 1.5 g/l. Succinic acid can be produced biochemically via the oxidative branch of the Krebs cycle due to mutation of succinate dehydrogenase (Arikawa *et al.* 1999; Camarasa *et al.* 2003) or via the glyoxylate cycle (Kamzolova *et al.* 2009b). Succinic acid can also be produced chemically by the decarboxylation of α-ketoglutaric acid under the action of oxidizing agents, such as hydrogen peroxide (Kamzolova *et al.* 2009a).

MATERIAL AND METHODS

Wines used for the comparison of cation contents (cultivars Italian Riesling and Grüner Veltliner, both 2007, $n = 6$) originated from the Mikulov wine-growing subregion, vineyards Ořechová hora (Březí wine-growing community) and Pod Děvínem (Bavory wine-growing community). Grapes were harvested in two vineyards with different soil conditions. Both varieties were selected on the basis of availability and traditions in this locality. Ořechová hora vineyard is situated near the village of Březí. Soil in this vineyard can be described as moderate, predominantly muck with calcium content up to 10%. Pod Děvínem vineyard is situated in the cadastre of the village of Horní Věstonice. In this locality, the soil is described as loamy, mostly dry soil with a high content of calcium (up to 25%).

For an experiment with succinic acid addition, the neutral wine Malverina, pH 3.3, titratable acids 5.8 g/l, residual sugar 6.2 g/l was used.

Reagents and solvents. Hydrochloric acid (HPLC grade; Lach-Ner, Brno, Czech Republic); HPMC (hydroxypropylmethylcellulose), caproic acid, and BTP (1,3-bis[tris(hydroxymethyl)methylamino] propane) were purchased from Sigma Aldrich (St. Louis, USA). Hydrochloric acid (c = 0.1 mol/dm^3; Penta, Prague, Czech Republic), α-aminobutyric acid (Sigma Aldrich, St. Louis, USA), sodium borate buffer (AccQ Taq Eluent; Waters Corp., Illinois, USA); Methanol, acetonitrile both HPLC grade (Lach-Ner, Brno, Czech Republic); derivatising agent Waters AccQ Fluor (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate; Waters Corp., Illinois, USA).

Determination of cations. Cations were determined by capillary isotachophoresis (CITP), Ionosep 2003 (Recman, Ostrava, Czech Republic). Leading electrolyte (LE) 5 mM H_2SO_4 + 7 mM-18-crown-6 + 0.1% HPMC; Terminating electrolyte (TE) 10 mM BTP; initial current 100 μA; final current 50 μA; mode of analysis cationic.

Determination of individual amino acids. Column AccQ Tag Amino Acid Analysis (4 μm Nova-Pak C18, 3.9 mm × 150 mm). Detector fluorescent (exc. wavelength 250 nm, em. 395 nm), mobile phase: A – 100 ml AccQ Tag eluent (acetate-phosphate buffer) in 1 l demineralised water, B – acetonitrile, C – demineralised water (18 MΩ); flow 1 ml/min, injection 10 μl, temperature 37°C, max. pressure 4000 psi (26 MPa), time of analysis 53 min, retention time of solvent 20 minutes.

RESULTS AND DISCUSSION

Wines from the Pálava Hills, in this case from the Pod Děvínem vineyard site, and especially the cultivars Italian Riesling, Riesling, and Chardonnay, belong to the pearls of Moravian wines and recently they were awarded numerous medals at international wine competitions. Indeed, many experts argue that these wines are absolutely rec-

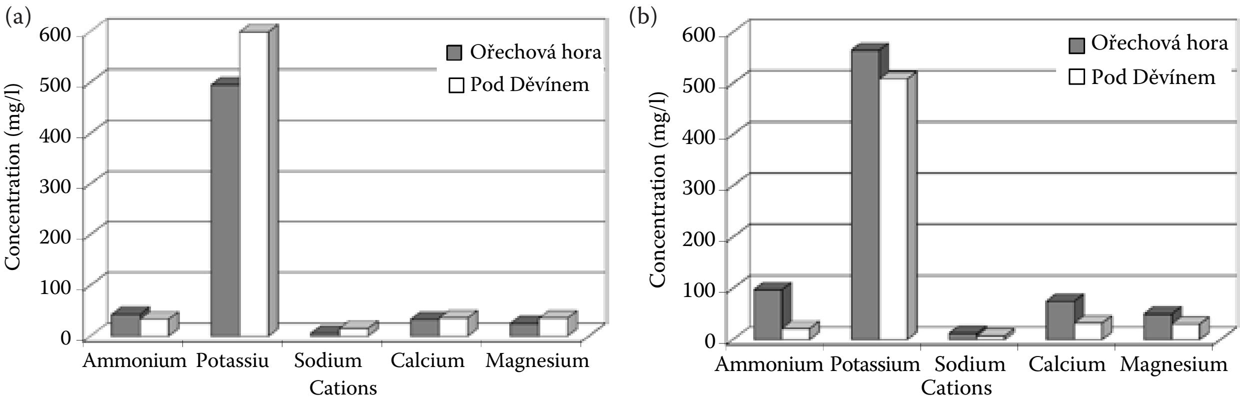

Figure 1. Average content of cations in cv. Italian Riesling (a) and cv. Grüner Veltliner (b) wines from different growing sub-regions (n = 6)

ognisable because of their exceptional minerality. The working hypothesis was to confirm a higher mineral content in wines originating from the Pod Děvínem vineyard site.

Compared were contents of cations in wines made of the varieties Italian Riesling and Grüner Veltliner (Figure 1). It was found out that they had nothing to do with minerals (Figure 1). While the cv. Italian Riesling from the Pod Děvínem vineyard site was in line with our expectations of a higher content of minerals, results obtained with cv. Grüner Veltliner from the Ořechová hora vineyard site were exactly the opposite. Measurements did not reveal a clear trend and showed that the mineral content is not only the characteristic of the locality and that also other factors play a significant role, in this case at least the variety. However, this indicates that the content of minerals is either just a matter of some varieties (which would not be a logical explanation) or it is probably really different. Moreover, differences in their concentrations were rather low, and despite all the respect for the sensory capabilities of trained experts, probably unrecognisable.

In principle it works as follows: if the nitrogen source is poor or if it is simply easier to use GABA, yeasts can do this (Coleman *et al.* 2001). This means that, during fermentation, as many as several hundred or even more mg/l of succinic acid can be produced, which can result in a final difference in the taste of wine. Succinic acid can be esterified and emerging ethyl ester or diethyl ester can contribute to the bouquet of wine.

This expectation can also be further supported by several minor synergistic factors. Firstly, the quantity of easily assimilable nitrogen source is always lower for musts from lighter and less humic soil. This means that, due to a nutritional stress, yeasts are able to use those resources that are not commonly consumed and for that reason they sometimes produce unusual substances.

Secondly, it is known that the cultivars such as Chardonnay, Riesling, and Sauvignon Blanc contain higher amounts of proline, which cannot be assimilated without oxygen (Stines *et al.* 2000; Mauricio *et al.* 2001; Herbert *et al.* 2005). Thus, if a large proportion of nitrogen is present in the form of proline, yeasts will prefer GABA (Huang *et al.* 2000; Batch *et al.* 2009).

Although for many laboratory strains of *S. cerevisiae* proline is a less preferred source of nitrogen, it is very often the most abundant in berries. When better sources of nitrogen such as ammonia, asparagine and glutamine are not available, *S. cerevisiae* can degrade the proline to glutamate in mitochondria (Soufleros *et al.* 2003). This degradation is catalysed by proline oxidase, which is nuclearly encoded by the genes PUT1 and PUT2. This phenomenon occurs only in the presence of proline and in the absence of preferred sources of nitrogen. The presence of better sources of nitrogen inhibits this route, which is known as the effect of nitrogen catabolite repression (NCR) (Wiame *et al.* 1985; Ter Schure *et al.* 2000). This phenomenon may explain an unexpectable behaviour of proline during fermentation.

In our experiment, free amino acid profiles occurring in the course of fermentation in four different musts were obtained by means of HPLC analysis (Figure 2). The next step was to determine the sensory activity of succinic acid in wine. In a blind wine tasting, the increasing additions of succinic acid were detected by all seven assessors (holders of a licence issued by CAFIA – Czech Agriculture

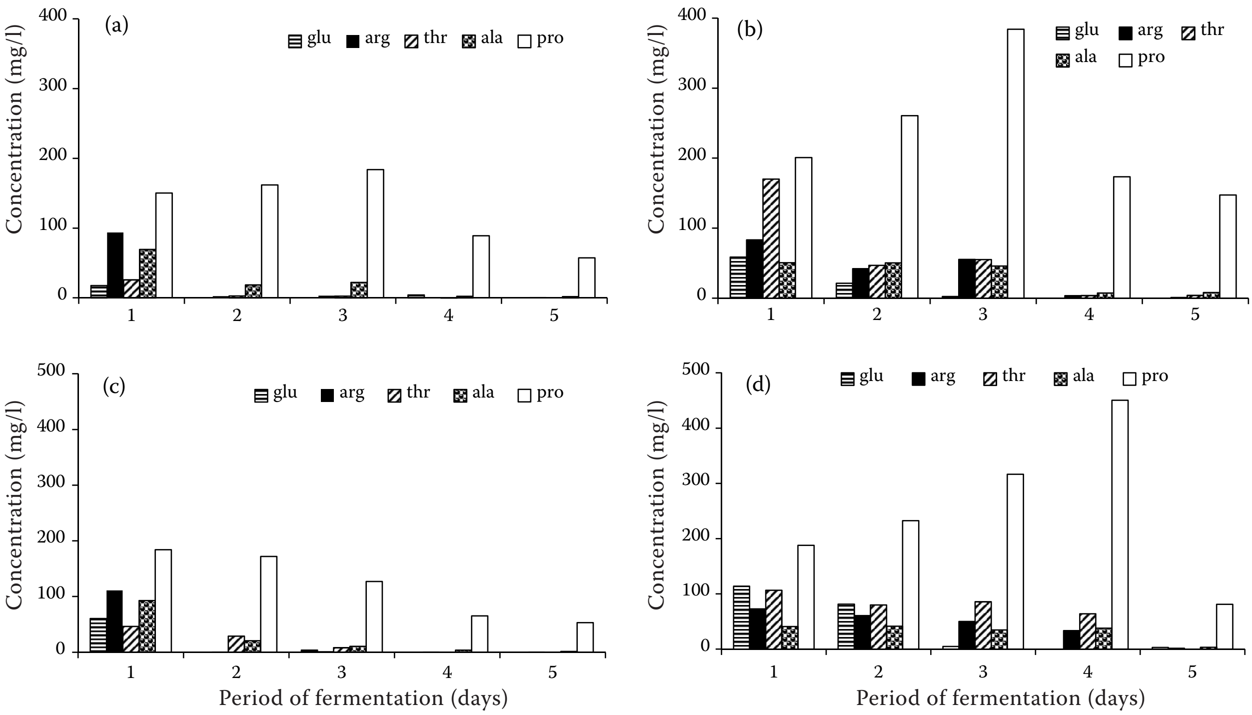

Figure 2. Evolution of free amino acids during fermentation of four grape musts (a, b, c, d) (cv. Grüner Veltliner, vintage 2007)

and Food Inspection Authority) already at the concentration of 100 mg/l as compared with the control wine without succinic acid addition. Higher concentrations were found to be vinous, sapid, salty and sometimes even bitter. Concentrations of 500 mg/l were evaluated as a rough, evocating an impression of very strong dry, unnatural taste in the mouth. Reaction of assessors to increasing concentrations of succinic acid in a blind test is

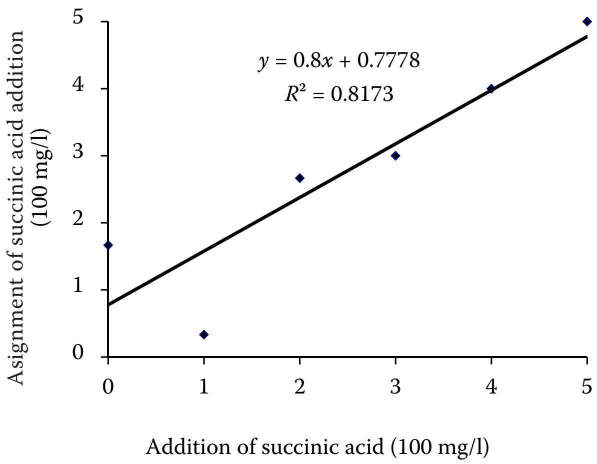

Figure 3. Assignment of wine samples with increasing addition of succinic acid in blind wine tasting

illustrated in Figure 3 (average of five values). Tested samples were served in a different order and the assessors assigned their taste to added concentrations of succinic acid on the basis of their previous experience. It was clearly demonstrated that an addition of succinic acid was very distinct even in the randomised order of samples. The coefficient of reliability of the resulting linear regression was $R^2 = 0.8173$ and this confirmed a substantial compliance.

In this context, it is necessary to emphasise that these experimental results do not undermine the concept of terroir; quite on the contrary, they support it significantly because only in vineyards with truly exceptional properties the ratio between moisture and amounts of nitrogen in berries is really well balanced.

Mineralty need not be influenced only by succinic acid or its ethyl esters (i.e. ethyl and diethyl succinate) but also by a complex of substances generated under conditions of nutritional stress. Especially the unpredictable proline content and its behaviour during the fermentation of musts with different assimilable nitrogen levels is of great interest. Proline can also become a source of glutamic acid, which (after deamination) increases the level of α-ketoglutaric acid and thus

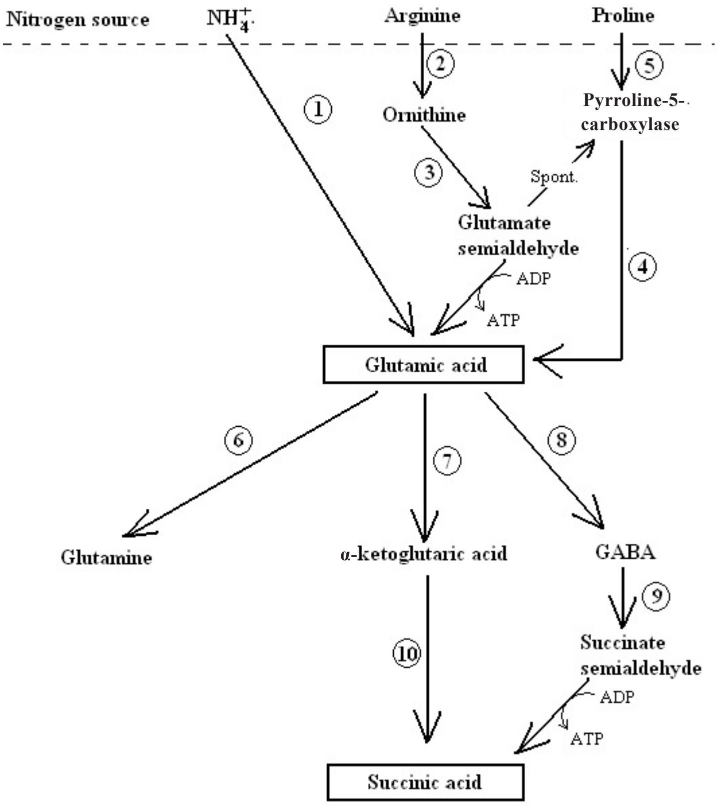

Figure 4. Biochemical pathway of succinic acid in wine

1 – glutamate dehyrogenase
2 – arginase
3 – ornithine aminotransferase
4 – pyrroline-5-carboxylate dehydrogenase
5 – proline oxidase (aerobic)
6 – glutamine synthetase
7 – glutamate dehyrogenase
8 – glutamate decarboxylase
9 – GABA transaminase
10 – via Krebs cycle

also (via the Krebs cycle) the amount of succinic acid (Figure 4).

CONCLUSIONS

It can be concluded that although many people may argue that if this "new" opinion were true, then any added source of nutrition could reduce the so-called minerality. This may be true but the problem of nutrition and particularly ammonium ions is rather complex and requires further research. For winemakers, the chase after minerality can involve another hidden risk because it can even result in nutritional deficits and difficulties associated with fermentation and final quality of produced wine.

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Corresponding author:

Ing. Mojmír Baroň, Ph.D., Mendelova univerzita v Brně, Zahradnická fakulta, Ústav vinohradnictví a vinařství, Valtická 337, 691 44 Lednice, Česká Republika
tel. + 420 519 367 252, e-mail: mojmirbaron@seznam.cz