

Changes of malic acid content in wines of Eger

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Összefoglaló

Egri borok almasav-tartalmának változásai. A borok savtartalma és a bor minősége között szoros kapcsolat van. A savra, mint alapízre vagy „gerinc-re” épülnek rá a zamatok, sőt egyes illatok is, amelyek együttesen megszabják a bor individuális tulajdonságait. A borok savtartalma igen változatos, amelyek: borkősav, citromsav, almasav, borostyánkősav, ecetsav, fumársav stb. formákban, $\approx 0\text{-}10$ g/l koncentrációtartományban találhatók. Az **almasav** a legmarkánsabb ízű, ugyanakkor pedig biokémiai szempontból a legkönnyebben átalakuló és a leginstabilabb sav. A szőlőben keletkezik, így koncentrációja nagymértékben függ a szőlő érettségi állapotától. Lényeges, hogy a borban lehetőleg ne legyen számottevő mennyiségben, különösen a vörösborokban célszerű értéket minimalizálni kell. A borban lévő almasav **fizikai-kémiai**, ill. **mikrobiológiai** eljárással távolítható el vagy csökkenthető. A fizikai-kémiai eljárás az ún. kettős sós savtompítás CaCO_3 segítségével történik. A mikrobiológiai technológia **a) élesztőtörzsek** segítségével, – az erjedés során a jelenlévő almasavat alkohollá képesek alakítani tejsavon keresztül. **b) a tejsavbaktériumok** alkalmazásával 1) természetes eljárással, ami nem más, mint a seprőn tartás és annak gyakori felkeverése. 2) tejsavas erjedést biztosító baktériumok adagolásával. Az Egri Borvidéken egyre többen ismerik fel az almasav a minőséget befolyásoló hatását. Az almasav lebontását az egri vörösborokban egyre többen a **baktériumtörzsek felhasználásával** végzik.

„It is almost impossible to express experience given by the flavour of wine by words” (Hugh Johnson: History of Wine)

What is it, that the flavour of wine comes from? It is the usual question often asked. Flavour of wine is assured by its chemical components. The very first one is acid content, acid components, so acid content and quality of wine are in closely related to each other. Flavour, bouquet are based on acid as basic taste giving inner, special characteristic features to wine. In this respect acid can stay in the background this is when we speak about harmonic flavour. It can also be harsh, dominant providing disharmony. This approach is rather subjective, the

limit of harmony and disharmony cannot be measured by either subjective or objective methods.

Acid content of wine is varied: tartaric acid, citric acid, malic acid, succinic acid, acetic acid, fumaric acid, etc. They are the ones that give complexity and cannot be distinguished individually, subjectively. Exemptions are malic and acetic acid. Acetic acid-content is maximised by various regulations (standards) since they irreversibly influence harmony of bouquet and flavour of the given wine.

Situation of malic acid is different. It has the most characteristic taste yet it is the most instable, transmutes the earliest, so it determines the storability of the wine. Its concentration largely depends on the maturity, that is on outside conditions – climatic changes depending on year –. Thus it is important not to have it in large quantity in wine, mostly in red wine so that the wine get stabil and velvety flavoured.

Malic acid in wine can be extracted or decreased by physico-chemical or microbiological process.

Physico-chemical method is the so called dual salty acid weakening with finely ground CaCO_3 when Ca tartarat-malate is formed. (figure 1.)

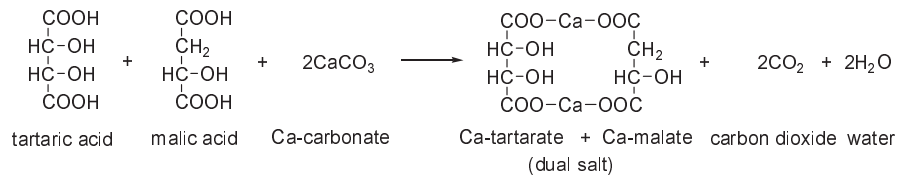


Figure 1. Dual salty acid weakening

The method means that pH must be higher than 4,5 when the dual salt is formed, so all the CaCO_3 is given to 10% of the wine, when it is not dissolved completely and 50% of the rest of wine is mixed to it. The salt extracted during the chemical reaction is removed by filtering, then the wine is mixed with the 40% rest.

The microbiological process can be of 2 types:

Certain yeast stocks can transform malic acid into alcohol through pyruvate (malo alcoholic fermentation) (figure 2)

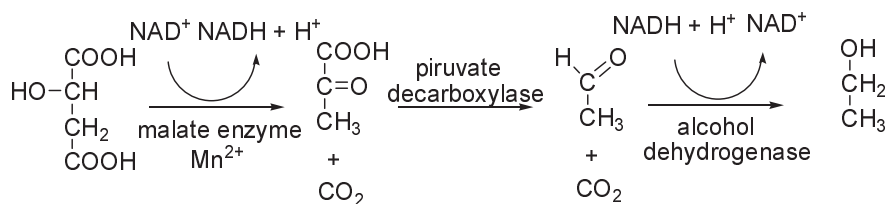


Figure 2. Biochemical mechanism of maloalcoholic fermentation

The most common yeast stock used when fermenting wine is *Saccharomyces cerevisiae*, malic acid dissolving ability of which is little, malic acid content is 10-20%. It is much bigger in case of *Zygosaccharomyces bailii* and *Schizosaccharomyces*, certain stocks of which can dissolve malic acid in 100%

Malic acid dissolution is different because malic acid intake of different yeast cells is of different mechanism:

Sach. cerevisiae: simple diffusion

Sch. pombe: active transport

Zyg. Bailii: passive transport through carrier

Experiments are going on and there is even practical use of *Schizosaccharomyces pombe*. Its advantage is its good acid and sulfuric acid resistance. The regulation has been advantageous since there has been possibility to use so called registering technology (immobilized methods). It is important because quality of wine made with these yeast stocks is below *Saccharomyces* stocks.

It is important to have a little sugar in the wine for the yeast. There are experiments with gene technology to create *Sach. cerevisiae* in which genes of *Sch. pombe* were planted to achieve decrease of malic acid by the most fermenting yeast stock.

The wine produced this way can have unfavourable flavour though. It becomes „empty” because of the missing lactic acid.

b) Use of lactic acid bacteria has been the most common recently, which can be of natural and artificial process. The natural process means to keep the liquid on wine lees and mix it frequently (Old wine makers of Eger used to put their wine into barrels only at Christmas). Artificial method means giving bacteria in right conditions. It is good if the process is connected to so called directed fermentation.

Considering these it is understandable why awareness of malic acid concentration is important in grape and wine analysis and in certain phases of the technology. Yet there are different conditions of practical realization of malic acidification in spite of the fact that lactic acid bacteria are the ones best adapted to wine after yeast fungus.

Their energy providing metabolism is anaerobic; they ferment sugar - also the ones with five carbons - to become lactic acid completely or partly. This way they can be homo-fermentative and hetero-fermentative ones. They are usually acid resistant, pH optimum of most stocks is between 5 and 6, but the lowest limit of their reproduction is 3-4 pH, which is just that of the pH value of wine.

Hetero-fermentative stocks produce several dangerous side products, e.g. mannitol by reduction of fructose. This is the so called mannitol fermentation. Favourable condition for decomposition of malic acid is when wine has just a little source of carbohydrate for bacteria, thus they get energy for reproduction through decomposition of malic acid. This must be taken into consideration when making wine. From stereo-chemical aspect it is interesting that lactic acid

coming from sugar contains all three optical isomers (D,L and DL) while lactic acid bacteria transform malic acid into just L-lactic acid.

So when analysing the configuration we can depict the unfavourable fermentation conditions from large proportion of D-lactic acid, while in opposite case bacterial decomposition of malic acid and directed fermentation.

From morphological aspect there are coccus shape (homo-fermentative, e.g. *Pediococcus* stock) and also rod shape (hetero-fermentative, e.g. *Lactobacillus*) stocks as well.

Bacteria take in malic acid with the help of a specific enzyme, permease and dissolution is done by malic acid decarboxylase or malolactic enzyme. The enzyme contains Mn^{2+} ion and NAD^+ coenzyme, yet no $NADH+H^+$. L lactic acid is produced (figure.3).

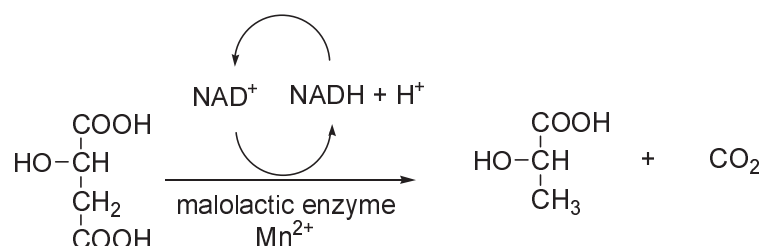


Figure 3. Biochemical mechanism of malolactic fermentation

In case piropogrape acid is produced as intermediate product, then any configuration can appear just like when sugar is dissolved.

It would be optimal if only lactic acid was produced during dissolution of malic acid but it does not work in practice. This can mostly be achieved by use of *Leuconostoc oenos* stock. So in practice use of this stock is the most common.

The effect of the importance of degradation of malic acid on quality is more and more realised in Eger Wine Region. More and more wine makers prefer use of bacteria stocks from applicable technologies.

Analytical research methods to determine malic acid are also very different. Preference when choosing them is speed, precision and cost.

The following methods are used:

- thin layer chromatography (TLC)
- reflexion photometry
- enzyme researches
- HPLC method.

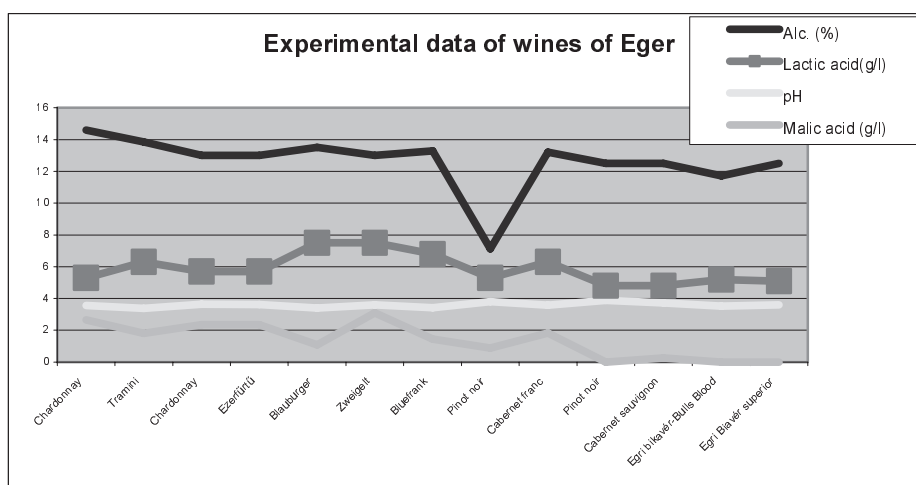
Precision and cost increases from TLC to HPLC, but not proportionally. Our research covered 13 wine samples. Four white and red wines made from nine blue grapes.

Experimental data of wines of Eger

No	Sort	Year	Alc. (%)	Lactic acid(g/l)	pH	Malic acid (g/l)	
1.	White	Chardonnay	2006	14,6	5,3	3,56	2,65
2.		Tramini	2006	13,85	6,3	3,37	1,8
3.		Chardonnay Ezerfürütü	2006		5,7	3,64	2,36
4.	Red	Blauburger	2006	13,5	7,5	3,39	1,08
		Zweigelt	2006	13,0		3,6	3,1
5.		Bluefrank	2006	13,29	6,8	3,42	1,44
6.		Pinot noir	2006	12,7	5,3	3,8	0,89
7.		Cabernet franc	2006	13,2	6,3	3,58	1,83
8.		Pinot noir	2005	12,5	4,8	3,89	0
9.		Cabernet sauvignon	2005	12,5	4,8	3,72	0,26
10.		Egri bikavér-Bulls Blood	2005	11,72	5,2	3,52	0
13.		Egri Biavér superior	2003	12,5	5,1	3,6	0

Wines of 2005 are considered to be ready. Wines of 2006 need technological action. pH values can be the clue, e.g. at No. 2 and 4. it is advisable to combine acid weakening with bacteria treatment.

Analytical data of examined wines very well show degradation of malic acid in time and also formation of lactic acid.



Bibliography

- Agouridis, N., Bekatorou, A., Nigam, P., Kanellaki, M., 2005. Malolactic fermentation in wine with *Lactobacillus casei* cells immobilized on delignified cellulosic material. *J. Agr. Food Chem.* 53, 2546–2551.
- Argiriou, T., Kaliafas, A., Psarianos, K., Kanellaki, M., Voliotis, S., Koutinas, A.A., 1996. Psychrotolerant *Saccharomyces cerevisiae* strains after an adaptation treatment for low temperature wine making. *Process Biochem.* 31, 639–643.
- Colagrande, O., Silva, A., Fumi, M.D., 1994. Recent applications of biotechnology in wine production. *Review. Biotechnol. Progr.* 10, 2–18.
- Crapisi, A., Spetolli, P., Nuti, M.P., Zamorani, A., 1987. Comparative traits of *Lactobacillus brevis*, *Lactobacillus fructivorans* and *Leuconostoc oenos* immobilized cells for the control of malolactic fermentation in wine. *J. Appl. Bacteriol.* 63, 513–521.
- Kosseva, M., Beschkov, V., Kennedy, J.F., Lloyd, L.L., 1998. Malolactic fermentation in chardonnay wine by immobilized *Lactobacillus casei* cells. *Process Biochem.* 33, 793–797.
- Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* 76, 317–331.
- Maicas, S., Gil, J.V., Pardo, I., Ferrer, S., 1999. Improvement of volatile composition of wines by controlled addition of malolactic bacteria. *Food Res. Int.* 32, 491–496.
- Maicas, S., Pardo, I., Ferrer, S., 2001. The potential of positively-charged cellulose sponge for malolactic fermentation of wine, using *Oenococcus oeni*. *Enzyme Microb. Tech.* 28, 415–419.
- Bioconversion of L-malic into L-lactic acid using a high compacting multiphasic reactor (HCMR). *J. Chem. Technol. Biot.* 51, 81–95.
- Rossi, J., Clementi, F., 1984. L-malic acid catabolism by polyacrylamide gel entrapped *Leuconostoc oenos*. *Am. J. Enol. Viticult.* 35, 100–102.
- Spetolli, P., Nuti, M.P., Crapisi, A., Zamorani, A., 1987. Technological improvement of malolactic fermentation in wine by immobilized cells in a continuous flow reactor. *Annals NY Acad. Sci.* 501, 386–389.
- Zoecklein, W.B., Fugelsang, C.K., Gump, H.B., Nury, S.F., 1995. In *Wine Analysis and Production*. Chapman & Hall, NY (pp. 399–402, 192, 240, 101, 221–222).