# Change of Thiamine concentration and amino acid composition during sparkling wine base production

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***Key words:*** amino acid, a-keto glutaric, pyruvic, acetic acid content, acetic aldehyde amount, free SO2 content, thiamine addition, amino acid analyser, HPLC measurements

***Summary:*** It has been established, that thiamin content added in 3 mg/l concentration to musts before fermentation resulted in the enhanced storability of sparkling wine base at lower SO2 levels. Fermentation rate is not increased by this concentration and it causes no "thiamine taste" in organoleptic evaluation.

## Introduction

The focus has been on the problem of sulphurisation (fumigation and stumming) according to the raising pressure of the World Health Organisation (WHO), other health care societies and to the strict demands on up-to-date enological technology. Obeying the rules has strongly reinforced the tendencies towards excluding certain chemical substances: referring to the harmful and unhealthy effects of sulphurising agents (*e.g.* inhibition of sugar metabolism, increase of $B_1$ avitaminosis, blood and stomach poisons). Despite of these all, their application by wine production technologies can not be avoided at well-defined moments, in fixed quantities.

Our work aimed at the main purposes as follows:

- Different amounts of thiamine have been added to fermented musts of healthy grapevine using a control sample as well. Occurrence of sulphur binding compounds has been measured and compared in consequence of thiamine concentration added.
- Chemical composition and sensory features of wines have been examined as a function of thiamine concentration.

## Theoretical

Recently in the fermentation of musts, thiamine (Vitamin $B_1$) became widespread as a component of fertiliser salts and as an individual substance as well. German wine-law permits its use in an amount of 0.6 mg/L. In Hungary the artificial thiamine fortification of botrytised thiamine deficient musts is commonly used (in the form of thiamine-chloride) and is allowed by the wine-law too. By this solution alcohol yield increases, but as a main advantage the sulphurous-acid demand decreases and can be reduced even to the 50 mg/l concentration. Thiamine addition increases the fermentation rate and the specific sugar elimination (g/l*h) but it is frequently accompanied by higher fermentation temperature, that can cause a great loss of aroma substances.

Results achieved and the demands of wine production practice both stimulated the continuation, thus investigations started. Sulphurous-acid level of musts used for base wine production is of great importance because problems might occur during processing and sparkling wine production (its sulphurous acid content must be low).

Thiamine (aneurine, vitamin-$B_1$) is a water soluble vitamin. It is an intrinsic factor of yeasts' growth and affects other substantial features (*e.g.* fermentation ability). Healthy and intact grapes and their musts are rather rich in thiamine (160–450 g/l). In its pyrophosphate form it is the coenzyme of the yeasts' decarboxylase-enzymes and plays a key role in the oxidative decarboxylation of 2-ketoacids. In fermentations and biosyntheses it is essential to the function of several enzymes (*e.g.* pyruvic acid decarboxylase, 2-keto-glutaric-acid decarboxylase), its lack increases the amounts of the matching substrate ketoacids in the musts. Since sulfurous acid is bound strongly by ketoacids, their formation during fermentation should be (Scholten G. 1995) depressed.

Free amino acids play an important role in wine making. From one hand they represent a source of nutrients for the fermenting microorganisms (yeasts, malic acid bacteria *etc.*), on the other hand they are starting materials of certain volatile aroma compounds of wine. Besides they are precursor substances of bacterial biogenic amine formation (Zee et al. 1983).

## Material and methods

Samples derived from the Etyek-Buda wine-district. The must was pressed from the healthy intact grapes of *Olaszrizling, Zenit, Irsai Oliver* varieties, other samples were the young wines of the same cultivars.

Fermentation experiments have been carried out in microvinification scale (volumes of 3–50 l) at regulated temperature. In every cases must was sulphurized with 100 mg/l $SO_2$ according to manufacturing practice. Different amounts of thiamine in concentrations of 0.0 mg/l (control), 0.6 mg/l, 3.0 mg/l, 6.0 mg/l and special sparkling-wine yeasts were added to the must that was filled into glass containers of 50 l capacity. The fermentation has been performed applying a natural free fermentation space at an optimal cellar temperature (11 C) in two parallel experimental rows.

Contents of $SO_2$ , alcohol (EtOH), titrable acids, lactic acid, acetic acid and aldehyde, α-keto-glutaric acid, pyruvic acid and pH were determined both at the beginning and at the end of fermentation. Alcohol, $SO_2$ , titrable acid, acetic acid contents and pH were measured according to the prescriptions of the Hungarian Standards. The α-keto-glutaric acid, pyruvic acid and lactic acid contents were measured at 340 nm; acetic aldehyde content was determined at 570 nm enzymatically by spectrophotometer (BOEHRINGER type enzymes have been applied). Amino acids were measured by ion exchange column chromatography and HPLC (Amerine et al. 1980). The measurements have been performed by an AMINOCHROM II. OE 914 type amino acid analyzer (Simon-Sarkadi-Holzapfel, 1994).

## Results

Our research work was started in September of 1996. Must pressed only from the whole, intact, healthy grapes were used in the experiments. The alcohol, $SO_2$ , acetic aldehyde, α-keto-glutaric acid, pyruvic acid contents of the samples were determined at the end of fermentation, the averages are shown in column diagrams.

- Averages of the *Olaszrizling* wine, vintage 1996: the acetic aldehyde content is the highest in the control sample, it decreases as a function of thiamine concentration added. The amount of α-keto-glutaric acid is the smallest in the control sample, it increases as a function of added thiamine concentration. The concentration of the pyruvic acid is the greatest in the control, its level is the lowest at 6.0 mg/l thiamine added. The free $SO_2$ content is the highest at 3,0 mg/l, in the control and at 6,0 mg/l thiamine concentration it is the smallest. *(Figure 1)*
- Averages of the *Zenit* wine, vintage 1996: the acetic aldehyde content is the highest in the control sample, at 3,0 mg/l thiamine added concentration its level is the lowest. The amount of α-keto-glutaric acid is the smallest in the control sample, it increases as a function of added thiamine concentration. The concentration of the pyruvic acid is the greatest in the control, its level is the lowest at 6.0 mg/l thiamine added. The free $SO_2$ content is the highest at 3,0 mg/l, at 6,0 mg/l thiamine concentration it is the smallest. *(Figure 2)*
- Averages of the *Irsai Olivér* wine, vintage 1996: the acetic aldehyde content is the highest in the control sample, at 3.0 mg/l added thiamine concentration its level is the lowest. The amount of α-keto-glutaric acid is the smallest in the control sample, it increases as a function of added thiamine concentration. The pyruvic acid content is the highest at 0.6 mg/L added thiamine and at 3.0 mg/l it is the lowest. Free $SO_2$ content is the greatest at 3.0 mg/l added thiamine. *(Figure 3)*
- Averages of the *Olaszrizling* wine, vintage 1997: the acetic aldehyde content is the highest in the control. The amount of α-keto-glutaric acid is the smallest in the control sample, it increases as a function of added thiamine concentration. The pyruvic acid concentration is the greatest in the control sample, its level is the lowest at 6.0 mg/L thiamine added. Free $SO_2$ content is the greatest at 3.0 mg/l added thiamine. *(Figure 4)*
- Averages of the *Zenit* wine, vintage 1997: the acetic aldehyde content is the highest in the control sample. The amount of α-keto-glutaric acid is the smallest in the control sample, it increases as a function of added thiamine concentration. The pyruvic acid concentration is the greatest in the control sample, its level is the lowest at 3.0 mg/l thiamine added. The free $SO_2$ content is the greatest at 3.0 mg/l added thiamine, in the control it is the smallest. *(Figure 5)*
- Averages of the *Irsai Olivér* wine, vintage 1997: the acetic aldehyde content is the highest in the control sample, it decreases as a function of added thiamine concentration. The amount of α-keto-glutaric acid is the smallest in the control sample, it increases as a function of thiamine added. The pyruvic acid concentration is the greatest in the control sample, its level is the lowest at 6.0 mg/l added thiamine level. The free $SO_2$ content is the greatest at 3.0 mg/l added thiamine and it is the smallest in the control sample. *(Figure 6)*

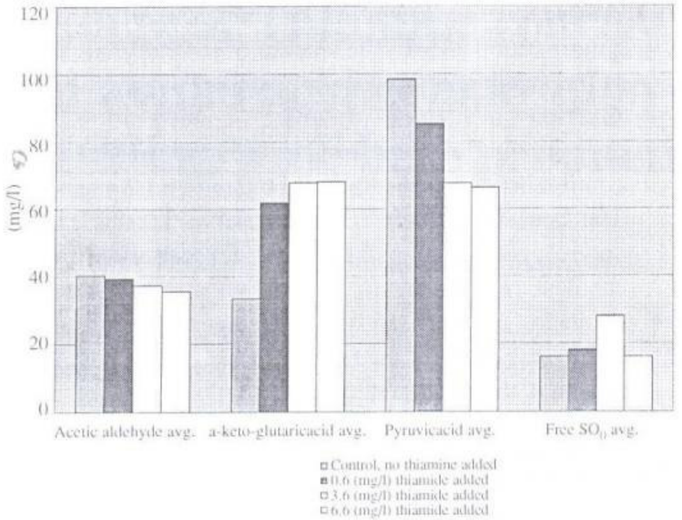


***Figure 1*** Averages of the *Olaszrizling* wine, vintage 1996

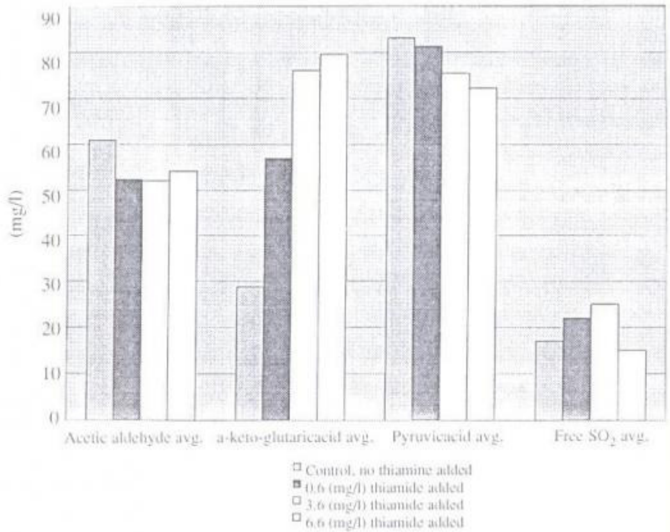


***Figure 2*** Averages of the *Zenit* wine, vintage 1996

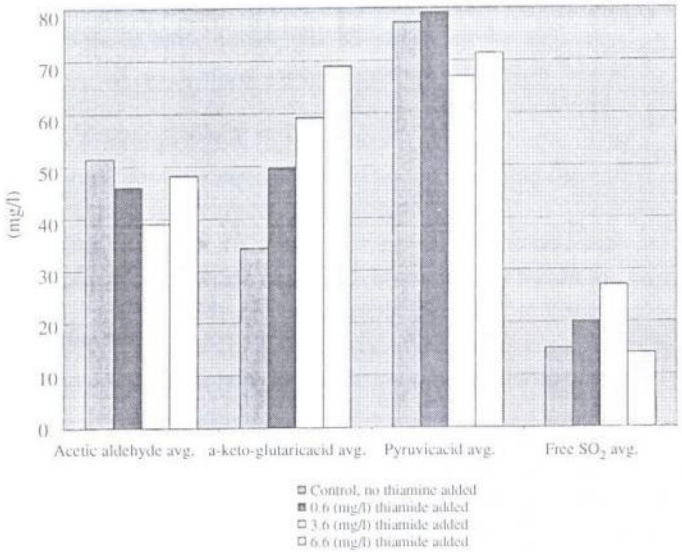


***Figure 3*** Averages of the *Irsai Oliver* wine, vintage 1996

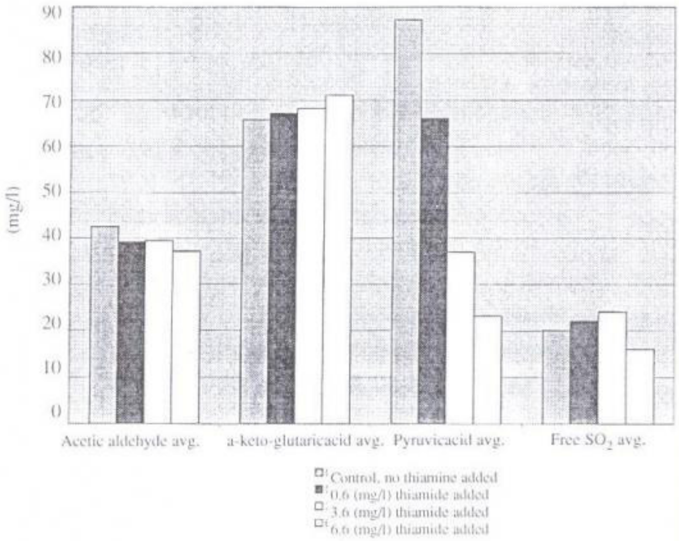


***Figure 4*** Averages of the *Olaszrizling* wine, vintage 1997

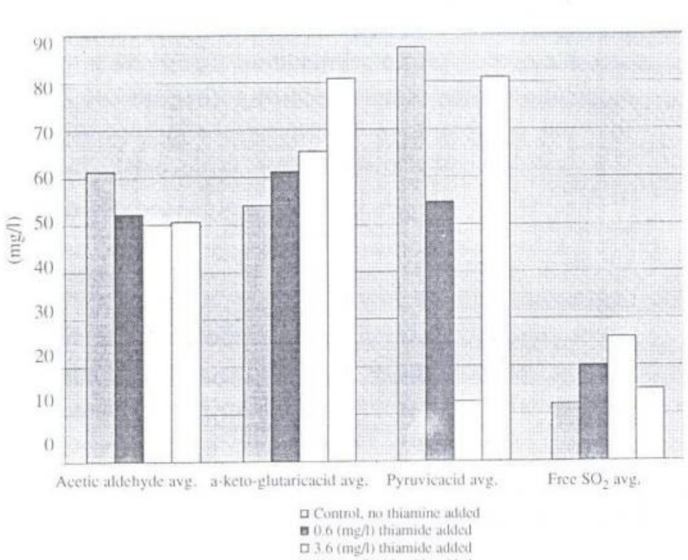


***Figure 5*** Averages of the *Zenit* wine, vintage 1997

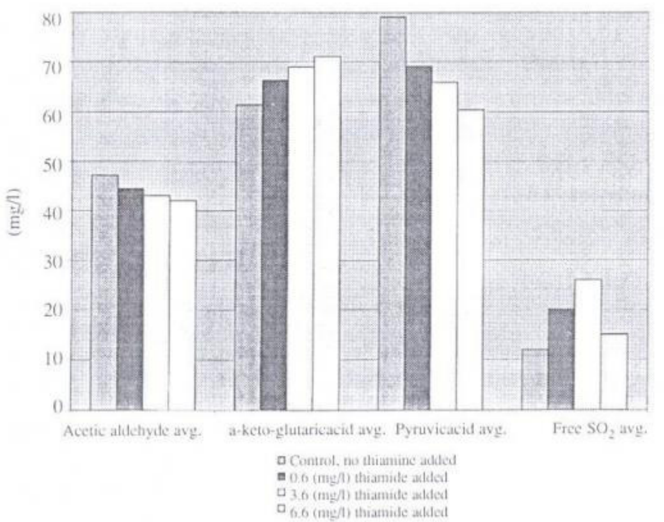


***Figure 6*** Averages of the *Irsai Oliver* wine, vintage 1997

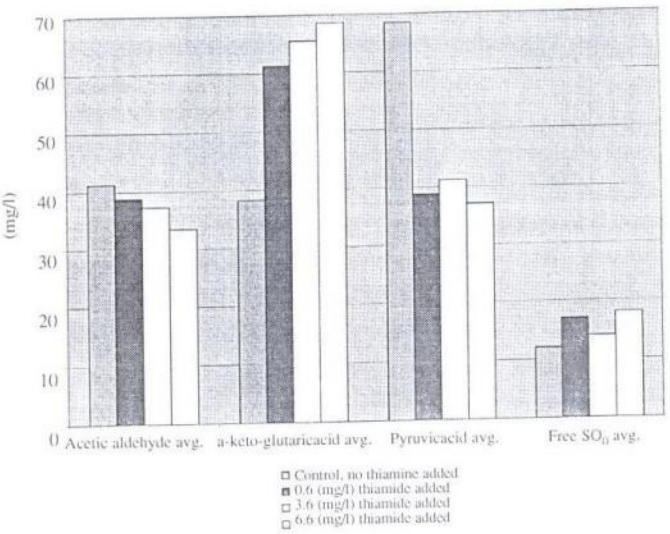


***Figure 7*** Averages of the *Olaszrizling* wine, vintage 1998

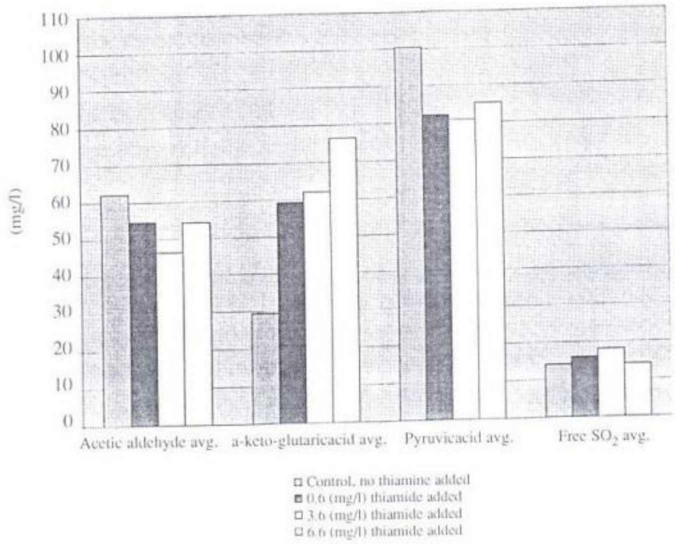


***Figure 8*** Averages of the *Zenit* wine, vintage 1996

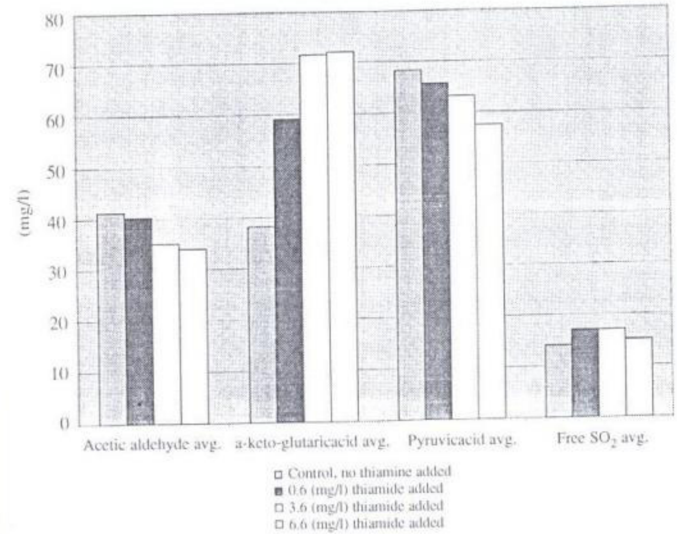


***Figure 9*** Averages of the *Irsai Oliver* wine, vintage 1997

- Averages of the *Olaszrizling* wine, vintage 1998: the acetic aldehyde content is the highest in the control sample, it decreases as a function of thiamine concentration added. The amount of α-keto-glutaric acid is the smallest in the control sample; it increases as a function of added thiamine concentration. The pyruvic acid concentration is the greatest in the control sample; its concentration is the lowest at 6.0 mg/l added thiamine level. Free $SO_2$ content is the greatest at 6.0 mg/l added thiamine. *(Figure 7)*
- Averages of the *Zenit* wine, vintage 1998: the acetic aldehyde content is the highest in the control sample, at 3.0 mg/l added thiamine concentration its level is the lowest. The amount of α-keto-glutaric acid is the smallest in the control sample; it increases as a function of added thiamine concentration. The pyruvic acid concentration is the greatest in the control sample; its level is the lowest at 3.0 mg/l added thiamine level. The free $SO_2$ content is the greatest at 3.0 mg/l added thiamine. *(Figure 8)*
- Averages of the *Irsai Olivér* wine, vintage 1998: the acetic aldehyde content is the highest in the control sample, at 6.0 mg/l added thiamine concentration its level is the lowest. The amount of α-keto-glutaric acid is the smallest in the control sample; its concentration is the highest at 6.0 mg/l added thiamine level. The pyruvic acid concentration is the greatest in the control sample; its level is the lowest at 6.0 mg/l thiamine added. The free $SO_2$ content is the greatest at 3.0 mg/l and 6.0 mg/l added thiamine, its level is lowest in the control sample. *(Figure 9)*

In our measurements the question of α-keto-glutaric acid increase compared to the decrease of other sulphur binding substances was intended to be answered. The samples (musts and fermented wines) containing different amounts of thiamine have been analysed for their amino acid content. Our hypothesis presumed that amino acids occurring in consequence of the decomposition of proteins somehow degrade further. They might take part in the biosynthesis of proteins, might convert into other important metabolic products and during their decay they might connect with different other metabolic pathways of great significance (Glycolysis, Citric acid (Krebs) cycle, Lipid metabolism) or might be consumed by the syntheses of nitrogen containing substances. The conclusion can be drawn that at the end of fermentation among sulphur binding compounds beside acetic aldehyde, pyruvic acid decreases mostly as a function of thiamine concentration, independently of the cultivated variety and vintage. Pyruvic acid binds sulphur the best converting into α-keto-glutaric acid, that is a transitional product. Acetic aldehyde dehydrogenase enzyme also works intensely and converts acetic aldehyde into alcohol with a proper "yield" by the end of fermentation. *The right added thiamine amount at 3.0 mg/L concentration results in those*

***Table 1*** Concentration of amino acids as a function of thiamine content added (mg/L)

| thia-mine | ASP | | **GLU** | | ASN | SER | | **GLN** | GLY | | THR | | ALA | | **ARG** | | ***HIS ammin*** | CY Sammin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (1) | (2) | *(I)* | (1) | (2) | *(I)* | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) | *(I)* | *(I)* |
| 0 | 77.051 | 62.20 | *126.953* | 98.20 | 65.559 | 45.226 | 40.25 | 32.572 | *35.365* | 23.75 | 32.205 | 55.00 | 240.702 | 158.45 | *242.903* | 446.90 | *148.55* | 31.85 |
| 0.6 | 55.931 | 57.85 | *95.445* | 107.30 | 38.598 | 29.829 | 39.30 | 25.363 | *22.689* | 23.75 | 21.514 | 58.60 | 86.295 | 86.10 | *385.947* | *433* | *139.30* | 31.45 |
| 3.0 | 48.483 | 51.55 | *82.601* | **67.65** | 34.957 | 25.822 | 38.25 | 21.200 | *20.232* | **22.75** | 18.962 | 50.30 | 79.551 | **81.85** | 364.856 | ***230.30*** | ***92.70*** | **9.60** |
| 6.0 | **46.440** | **51.00** | ***78.698*** | 96.05 | **32.918** | **25.348** | **37.25** | **21.021** | ***18.565*** | 23.05 | **17.879** | **50.00** | **69.312** | 84.65 | ***223.607*** | *355.65* | *132.00* | 21.65 |

| thia-mine | TYR | | VAL | | MET | | TRP | PHE | | ILE | | LEU | | LYS | | **PRO** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (1) | (2) | (1) | (2) | *(I)* | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) |
| 0 | 55.598 | 51.20 | 52.912 | 37.00 | 32.582 | 28.00 | 8.079 | 77.306 | 63.20 | 41.222 | 30.10 | 125.273 | 89.50 | 166.714 | 113.15 | *1467.530* | *1144.85* |
| 0.6 | 36.613 | 51.40 | 35.441 | 32.70 | 20.544 | 28.05 | 7.515 | 49.733 | 51.80 | 27.682 | 28.00 | 82.262 | 82.05 | 111.770 | 108.10 | *1053.456* | *1090.10* |
| 3.0 | 32.805 | **23.65** | 30.303 | **18.35** | 18.065 | **12.50** | **5.622** | 43.318 | **28.40** | **23.842** | **12.15** | 71.861 | **39.50** | **98.994** | **65.95** | *956.655* | ***805.05*** |
| 6.0 | **30.658** | 47.30 | **29.251** | 30.75 | **17.130** | 22.45 | 8.269 | **42.714** | 56.85 | 24.056 | 25.20 | **69.779** | 75.95 | 99.330 | 101.10 | ***637.833*** | *1013.30* |

(1) results of HPLC measurements
(2) results of amino acid analyser measurements
*(I) unseparable by amino acid analyser*
Arginine, glutamic acid, glutamine, histidine, proline-keto-glutaric acid

*appropriate conditions under that the sulphurous acid content of wines can be kept at lower levels.* It is of great importance in sparkling wine production partly from the prescriptions', partly from the sensory evaluations' point of views ($CO_2$ and $SO_2$ "expelled").

Results gained by amino acid analyser and HPLC supported our suggestion concerning the prolonged decomposition of GLU, GLN, ARG, HIS, PRO amino acids in function of thiamine content added (*see* Table 1.). The highest free $SO_2$ content is at 3.0 mg/L added thiamine concentration and the lowest is in the control sample. *The amino acid content is the lowest at 3.0 mg/l thiamine level measured by amino acid analyser and the smallest is at 6.0 mg/l thiamine concentration measured by HPLC.*

The tenfold concentration of thiamine (6,0 mg/L) allowed by the wine-law *caused no occurrence of the faulty "thiaminic" taste.*

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