

## Profiling of Primary Metabolites in Grapes of Interspecific Grapevine Varieties: Sugars and Organic Acids

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### Abstract:

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The quality of grapes is determined above all by the contents of the primary and secondary metabolites. The primary metabolites involve sugars and organic acids, and just these compounds are dealt with in this study. Its objective was to analyse and critically evaluate the primary metabolites in new interspecific varieties and, based on a comparison with European varieties of grapevine (*Vitis vinifera* L.), to find out the similarities and also possible differences between them. The study evaluates and compares 4 conventional varieties of *Vitis vinifera* with 11 new interspecific cultivars. The contents and compositions of the individual sugars and acids were estimated by means of the HPLC method. Most of these varieties belong to the group with either medium or low content of malic acid, i.e. with a medium to high  $\beta$  ratio. This corroborates the similarity of interspecific varieties to those of *V. vinifera*. The cluster analysis identified the existence of two interesting groups of varieties: the first one involved the varieties Riesling, Nativa, Marlen, and Kofranka while the other group consisted of varieties Blaufränkisch, Blauer Portugieser, and Laurot. This observation also indicates similarity between *Vitis vinifera* L. varieties and interspecific cultivars and demonstrates that the contents of the primary metabolites (i.e. sugars and organic acids) are also comparable.

**Keywords:** grapevine; interspecific variety; sugars; organic acid; profiling

From the economic point of view, the grapevine is the most important fruit species in the world. The genus *Vitis* L. involves more than 50 species. Of them, the most widespread and cultivated is the European grapevine, *Vitis vinifera* L. Approximately 70% of the total harvest of grapes are used for making wine, 27% for direct consumption as table grapes, 2% for drying to raisins, and less than 1% for making musts and/or distillates.

The invasion of dangerous grapevine pathogens to Europe began in the 2<sup>nd</sup> half of the 19<sup>th</sup> century and this caused profound changes in the growing of grapevine cultivars and induced the establishment of a new specialisation in the domain of plant breeding. To the most significant fungal pathogens and pests that appeared in European vineyards in the second half of the 19<sup>th</sup> century do

belong the following: *Plasmopara viticola* (Berk & Curt.) Berl. & de Toni, disease agent of downy mildew, *Erysiphe necator* Schwein, disease agent of powdery mildew, and phylloxera (*Dactulosphaira vitifoliae* Fitch.).

For this reason, the grapevine breeders focused not only on the stable yields, good quality of grapes, good quality of wine, and adaptability to soil and climatic conditions that are characteristic for European grapevine cultivars but also on taking advantage of good resistance, which is a characteristic of wild species belonging to the genus *Vitis* spp. (ALLEWELDT 1970).

Breeding efforts focused on the creation of hybrids showing not only the positive characteristics of European grapevine cultivars (i.e. quality of wine) but also those which are typical for Ameri-

can species (i.e. high resistance to fungal diseases and phylloxera). The resulting new grape cultivars can be classified in view of their genealogic origin – as new interspecific cultivars.

BREIDER and WOLF (1966) studied qualitative properties of interspecific hybrids and found out that the olfactorial quality of wine is determined by the genetic characteristics of individual cultivars, involving also the contents of sugars and organic acids.

The quality of grapes is determined by the contents of the primary metabolites (i.e. sugars and organic acids) and of the secondary ones (i.e. phenolic compounds and aromatic substances). The contents and proportions of primary metabolites are decisive for the quality of grapes (RUSJAN *et al.* 2008; ALI *et al.* 2009).

Photosynthesis is a fundamental physiological process that determines the contents of sugars in grapes. Photosynthetic processes take place in leaves and also in green berries. The sugars synthesised are transported to berries via phloem. In the grapevine (*Vitis vinifera* L.) cultivars, the major transported sugar is saccharose which is enzymatically hydrolysed to glucose and fructose in berries (SHIRAISHI 2000).

Glucose, fructose, malic acid, and tartaric acid are primarily stored in the vacuoles of mesocarp cells; however, some glucose and fructose may be found also in the exocarp (LUND & BOHLMANN 2006).

A sufficient and harmonic content of acids in grapes plays a critical role as far as the suitability of grapes for wine making is concerned (DE BOLT *et al.* 2007). Acids also help to maintain the colour of wine and influence esterification and wine aroma (FOWLES 1992).

Although the wine acidity is generally expressed in equivalents of tartaric acid or sulphur acid, there are several free organic acids and their salts in grapes. Of them, tartaric acid and malic acid are the most important.

In general, tartaric acid and malic acid represent 69% to 82% of all organic acids present in berries and leaves of grapevine (KLIEWER 1966).

Malic acid accumulates in the pulp cells towards the end of the 1<sup>st</sup> growth stage and its maximum level can be detected just before the softening of berries. Tartaric acid and malic acid are synthesised in leaves and unripe green berries. Photosynthesis, which takes place in green berries, produces approximately 50% of all acids. A decrease in the content of organic acids starts at the beginning of

ripening and is associated with the transformation of malic acid to glucose. Malic acid may be either transformed to fructose and glucose or utilised as a source of carbon and energy for respiration (CONDE *et al.* 2007).

Acidity of grapes and wine is associated also with pH value. Although pH is generally indirectly proportional to the content of acids, no simple relation exists between pH and titratable acids or between pH and total acids (SMITH & RAVEN 1979).

The value of pH increases in the course of the berries ripening due to a decrease in the level of organic acids and also due to an increase in the content of metallic cations. Normally, the pH values range from 3.0 to 3.5 but in overripened berries they can be as high as 4.0 (KELLER 2010).

The contents and compositions of sugars and organic acids present in grapevine berries were evaluated in several studies (AMERINE & THOUKIS 1958; KLIEWER 1965; SHIRAISHI 1993, 1995; LAMIKANRA *et al.* 1995). These two substance groups are important also in the breeding and selection of new cultivars (LIU *et al.* 2007).

Liquid chromatography represents the most important technique used for the estimation of the contents of organic acids in grapes and wine (VEREDA *et al.* 1998). The separation and quantification of organic acids can be performed either by high performance liquid chromatography (HPLC) or by ion exchange chromatography (IC) (MATO *et al.* 2005).

Because the introduction of new interspecific cultivars into the breeding work and growing practice has always been associated with various opinions about the quality of grapes and wine, it is necessary to say that the research, analysis, and explanation of the basic quality parameters are very important.

The aim of this study was to analyse and critically evaluate the primary metabolites occurring in new interspecific cultivars of grapevine and compare them with the data on the cultivars of European grapevine *Vitis vinifera* L. Some of these parameters may be used for the evaluation of authenticity of individual grapevine cultivars. The profiles of primary metabolites are therefore very important for grapevine breeding and the introduction of new cultivars into the grapegrowing practice.

## MATERIAL AND METHODS

**Site description.** The experiments and evaluation were performed in the laboratories of the Depart-

ment of Viticulture and Oenology of the Faculty of Horticulture (Mendel University in Brno) in the years 2006, 2008, and 2009. All cultivars under study were grown in the experimental vineyard of the aforementioned faculty. This vineyard is situated in the vineyard site called “V Mendeleu” (In Mendeleum) in the wine village Lednice na Moravě. The spacing of vines was  $2.2 \times 1.0$  m and the plants were trained using Guyot pruning with 10 buds per vine. This vineyard was established in 1993 and all cultivars were grafted on the rootstock Teleki 5C.

**Samples.** Within the framework of this study, altogether 4 cultivars of *Vitis vinifera* L. – Müller Thurgau (Blanc – B), Riesling (B), Blaufränkisch (Noir – N), and Blauer Portugieser (N) were evaluated and compared with 11 interspecific cultivars: Erilon (B), Hibernál (B), Malverina (B), Merzling (B), Savilon (B), Cerason (N), Kofranka (N), Laurot (N), Marlen (N), Nativá (N), and Regent (N). Basic information on these cultivars is presented in Table 1. These cultivars are maintained and evaluated within the framework of a collection of genetic resources of grapevine (for more detailed information see [www.evigez.cz](http://www.evigez.cz)). The grapes were sampled using the method described by ILAND *et al.* (2000).

**Parameters under study.** The following parameters were evaluated in this study: pH value (pH), titratable acids (TA), total acids (TOA), tartaric acid (TARA), malic acid (MALA), ratio of tartaric acid to malic acid ( $\beta$  ratio), citric acid (CITA), glucose (GLU), fructose (FRU), ratio of glucose to fructose (GLU/FRU), and total sugar

(TS). Total acid (TOA) was calculated as all acids determined by HPLC method and expressed as tartaric acid. Total sugar (TS) is the sum of glucose and fructose.

**Reagents and solutions.** Malic acid was manufactured by Merck KGaA (Darmstadt, Germany). All other chemicals were supplied by Czech manufacturers (Lachema, Neratovice, Penta, Chrudim, Czech Republic).

**Estimation of titratable acids.** The estimation of all titratable acids contained in must was performed with 0.1M solution of NaOH in an automatic Schott TitroLine easy titrator (SI Analytics GmbH, Mainz, Germany) with a preset potentiometric point of equivalence of pH 7.0. The reference factor of NaOH solution was determined with potassium hydrogen phthalate (KPH). The results are expressed in equivalents of tartaric acid (g/l).

**Estimation of pH-value.** pH-value was estimated by means of a table pH-meter WTW (Weilthelm, Germany) with a combined glass and argent chloride gel electrode.

**HPLC estimation of acids and sugars.** Must samples were centrifuged ( $3000 \times g$ ; 6 min) and diluted with  $10 \times$  demineralised water. The estimation was performed by means of IC in the Shimadzu LC-10A system plus the thermostat (column oven) CTO-10ACvp set at  $60^\circ\text{C}$ . The manual injection Rheodyne valve had a loop with the volume of 20  $\mu\text{l}$ . The separation was performed in an isocratic regime with the mobile phase of 2mM sulphuric acid at the flow rate of 0.75 ml/min in the column Watrex Polymer IEX H form 10  $\mu\text{m}$ ;  $250 \times 8$  mm

Table 1. Interspecific varieties, their pedigree and origin of variety

| Cultivar  | Pedigree                                                  | Origin of variety                                                 |
|-----------|-----------------------------------------------------------|-------------------------------------------------------------------|
| Erilon    | (Lemberger $\times$ Cabernet franc 6/76) $\times$ Merlan  | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Hibernál  | (Seibel 7053 $\times$ Riesling Gm 239) F2                 | Forschungsanstalt Geisenheim                                      |
| Malverina | Rakisch $\times$ Merlan                                   | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Merzling  | Seyve Villar 5276 $\times$ (Riesling $\times$ Pinot gris) | Weinbauinstitut Freiburg                                          |
| Savilon   | Rakisch $\times$ Merlan                                   | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Cerason   | Merlan $\times$ Fratava                                   | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Kofranka  | Merlan $\times$ Fratava                                   | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Laurot    | Merlan $\times$ Fratava                                   | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Marlen    | Merlan $\times$ Fratava                                   | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Nativá    | Merlan $\times$ Fratava                                   | Vilém Kraus, Miloš Michlovský <i>et al.</i>                       |
| Regent    | Diana $\times$ Chambourcin                                | Julius Kühn (Institut, Institut für Rebenzüchtung, Geilweilerhof) |

with 10 × 8 mm. Spectrophotometric detection was performed by the DAD detector SPD-MAvp. Sugars and organic acids were measured at 190 nm and 210 nm, respectively. The quantification of the individual analyses was performed on the basis of external calibration.

**Statistical evaluation.** The results obtained were statistically analysed using the statistical package UNISTAT. Evaluated were the means and standard deviations using ANOVA with subsequent Tukey's test at significance levels of  $P > 0.95$  a  $P > 0.99$ . These data were further analysed by means of a hierarchical cluster analysis with the objective to find the groups of cultivars showing the most possible similarities in primary metabolites profiles.

## RESULTS AND DISCUSSION

When evaluating the contents of sugar and organic acids, attention was paid to the effect of vintage and it was found out that the year of harvest influenced significantly the differences in the mean values of all cultivars under study.

Statistically significant differences ( $P > 0.95$ ) were found out in pH-value and the contents of titratable

and total acids (Table 2). The values of titratable and total acids ranged from 7.96 g/l to 9.55 g/l and from 10.84 g/l to 12.89 g/l, respectively. Similarly significant effects of the year of harvest on the levels of tartaric acid and malic acid were also observed (Table 3). The contents of tartaric acid ranged from 6.85 g/l to 8.24 g/l and those of malic acid from 2.68 g/l to 3.72 g/l. The effect of year on the content of citric acid was not significant.

A highly significant effect of the year was demonstrated also in the case of the  $\beta$  ratio after SHIRAI-SHI (1995). In 2008 and 2009, the measured values of  $\beta$  ratio were 2.03 and 2.96, respectively. This ratio is a very important qualitative parameter of grapes and for that reason is it very important to pay attention to this parameter when harvesting grapes and making wine.

A highly significant effect of the year of harvest was demonstrated also with the parameters associated with the contents and profiles of sugars in must (Table 4), this concerned above all the contents of glucose, fructose, and total sugars. The content of glucose ranged from 97.00 g/l to 112.49 g/l, that of fructose from 95.90 g/l to 113.22 g/l, and that of total sugars from 192.90 g/l to 225.71 g/l (Table 3). The GLU/FRU ratio was not influenced by the year

Table 2. Values of pH, titratable acids, and total acids as estimated in individual years

|          | pH                 |      | Titratable acid (g/l) |      | Total acid (g/l)    |      |
|----------|--------------------|------|-----------------------|------|---------------------|------|
|          | $\bar{x}$          | SD   | $\bar{x}$             | SD   | $\bar{x}$           | SD   |
| 2006     | 3.24 <sup>ab</sup> | 0.15 | 9.55 <sup>a</sup>     | 2.39 | 12.89 <sup>a</sup>  | 3.20 |
| 2008     | 3.17 <sup>a</sup>  | 0.12 | 9.01 <sup>ab</sup>    | 2.08 | 11.49 <sup>ab</sup> | 1.93 |
| 2009     | 3.32 <sup>b</sup>  | 0.16 | 7.96 <sup>b</sup>     | 1.72 | 10.84 <sup>b</sup>  | 2.29 |
| <i>F</i> | *                  |      | *                     |      | *                   |      |

The results were statistically analysed by the ANOVA method and Tukey test. The letters indicate statistically significant differences determined by Tukey's test; \* $P > 0.95$

Table 3. Contents of tartaric acid, malic acid and citric acid, and the  $\beta$  ratio as estimated in individual years

|          | Tartaric acid (g/l) |      | Malic acid (g/l)  |      | Citric acid (g/l) |      | $\beta$ ratio      |      |
|----------|---------------------|------|-------------------|------|-------------------|------|--------------------|------|
|          | $\bar{x}$           | SD   | $\bar{x}$         | SD   | $\bar{x}$         | SD   | $\bar{x}$          | SD   |
| 2006     | 8.24 <sup>a</sup>   | 1.64 | 3.72 <sup>a</sup> | 1.66 | 0.22              | 0.11 | 2.45 <sup>ab</sup> | 0.70 |
| 2008     | 6.85 <sup>b</sup>   | 0.87 | 3.69 <sup>b</sup> | 1.21 | 0.24              | 0.08 | 2.03 <sup>a</sup>  | 0.60 |
| 2009     | 7.36 <sup>ab</sup>  | 1.66 | 2.68 <sup>b</sup> | 0.92 | 0.23              | 0.09 | 2.96 <sup>b</sup>  | 0.89 |
| <i>F</i> | *                   |      | *                 |      | n.s.              |      | **                 |      |

The results were statistically analysed by the ANOVA method and Tukey test. The letters indicate statistically significant differences determined by Tukey's test; n.s. = non-significant; \* $P > 0.95$ ; \*\* $P > 0.99$

Table 4. The glucose/fructose ratio and contents of glucose, fructose, citric acid, and total acids as estimated in individual years

|          | Glucose (g/l)       |       | Fructose (g/l)      |       | Glucose/Fructose |      | Total sugars (g/l)  |       |
|----------|---------------------|-------|---------------------|-------|------------------|------|---------------------|-------|
|          | $\bar{x}$           | SD    | $\bar{x}$           | SD    | $\bar{x}$        | SD   | $\bar{x}$           | SD    |
| 2006     | 112.49 <sup>a</sup> | 12.65 | 113.22 <sup>a</sup> | 13.78 | 1.00             | 0.06 | 225.71 <sup>a</sup> | 25.25 |
| 2008     | 97.00 <sup>b</sup>  | 7.36  | 95.90 <sup>b</sup>  | 9.24  | 1.01             | 0.05 | 192.90 <sup>b</sup> | 16.08 |
| 2009     | 104.48 <sup>c</sup> | 5.63  | 106.36 <sup>c</sup> | 7.70  | 0.98             | 0.05 | 210.85 <sup>c</sup> | 12.19 |
| <i>F</i> | **                  |       | **                  |       | n.s.             |      | **                  |       |

The results were statistically analysed by the ANOVA method and Tukey test. The letters indicate statistically significant differences determined by Tukey's test; \*\* $P > 0.99$

of harvest. This observation therefore corroborates only the effect of the year (and especially of weather conditions existing in the individual years) on the contents of the individual sugars in grapes, not on the ratios between them.

Both the contents and profiles of the individual sugars and organic acids were more significantly influenced by the cultivar.

The effect of cultivar on the pH-value of must was highly significant. The highest average pH values were found out in the cultivars belonging to the species *Vitis vinifera* (Table 5). For the cvs Müller Thurgau and Blauer Portugieser, the estimated pH-values were 3.41 and 3.56, respectively; the latter cultivar showed the absolutely highest values in 2006 and 2009 (3.62 and 3.69, respectively). On

Table 5. The average pH value and contents of titratable and total acids in grapes of individual varieties as estimated in years 2006, 2008, and 2009

| Variety            | pH                 |      | Titratable acid (g/l) |      | Total acid (g/l)      |      |
|--------------------|--------------------|------|-----------------------|------|-----------------------|------|
|                    | $\bar{x}$          | SD   | $\bar{x}$             | SD   | $\bar{x}$             | SD   |
| Erilon             | 3.18 <sup>a</sup>  | 0.06 | 10.04 <sup>de</sup>   | 2.18 | 14.30 <sup>ef</sup>   | 4.14 |
| Hibernal           | 3.22 <sup>ab</sup> | 0.10 | 8.38 <sup>bcd</sup>   | 0.86 | 10.53 <sup>bcd</sup>  | 0.76 |
| Malverina          | 3.16 <sup>a</sup>  | 0.19 | 9.16 <sup>cde</sup>   | 1.47 | 11.67 <sup>cde</sup>  | 1.67 |
| Merzling           | 3.24 <sup>ab</sup> | 0.15 | 5.58 <sup>a</sup>     | 0.42 | 7.80 <sup>ab</sup>    | 0.26 |
| Müller Thurgau     | 3.41 <sup>bc</sup> | 0.17 | 6.82 <sup>ab</sup>    | 0.62 | 11.10 <sup>cd</sup>   | 1.37 |
| Riesling           | 3.13 <sup>a</sup>  | 0.10 | 11.45 <sup>e</sup>    | 0.99 | 15.13 <sup>f</sup>    | 0.60 |
| Savilon            | 3.31 <sup>ab</sup> | 0.06 | 7.45 <sup>abc</sup>   | 0.71 | 10.63 <sup>bcd</sup>  | 1.00 |
| Blaufränkisch      | 3.22 <sup>ab</sup> | 0.11 | 10.36 <sup>de</sup>   | 1.56 | 12.43 <sup>cd</sup>   | 0.42 |
| Blauer Portugieser | 3.56 <sup>c</sup>  | 0.17 | 6.63 <sup>ab</sup>    | 0.16 | 10.20 <sup>abc</sup>  | 0.46 |
| Cerason            | 3.13 <sup>a</sup>  | 0.06 | 10.80 <sup>e</sup>    | 0.76 | 13.47 <sup>def</sup>  | 0.76 |
| Kofranka           | 3.22 <sup>ab</sup> | 0.09 | 10.24 <sup>de</sup>   | 1.64 | 12.53 <sup>cdef</sup> | 2.91 |
| Laurot             | 3.24 <sup>ab</sup> | 0.22 | 9.63 <sup>cde</sup>   | 1.78 | 12.20 <sup>cdef</sup> | 0.36 |
| Marlen             | 3.29 <sup>ab</sup> | 0.13 | 10.19 <sup>de</sup>   | 2.45 | 14.57 <sup>ef</sup>   | 3.23 |
| Nativa             | 3.15 <sup>a</sup>  | 0.08 | 9.58 <sup>cde</sup>   | 1.17 | 11.97 <sup>cde</sup>  | 0.15 |
| Regent             | 3.17 <sup>a</sup>  | 0.08 | 6.27 <sup>ab</sup>    | 1.75 | 7.57 <sup>a</sup>     | 1.88 |
| <i>F</i>           | **                 |      | **                    |      | **                    |      |

The results were statistically analysed by the ANOVA method and Tukey test. The letters indicate statistically significant differences determined by Tukey's test; \*\* $P > 0.99$

the other hand, the lowest average values of pH were estimated for the following cultivars: Cerason (3.13), Riesling (3.13), Nativa (3.15), Malverina (3.16), Regent (3.17), and Erilon (3.18). The cv. Riesling showed the absolutely lowest pH-value in 2008, namely 3.03. In no case did the value drop below 3.0. It seems that, due to the global warming and the increase in the average air temperatures, the pH-values of musts are also increasing and this may show not only a positive but also (and above all) negative influence on the wine quality. As far as all interspecific cultivars under study are concerned, the pH-value fluctuated around 3.25 and it can be said that this is very positive with regard to the quality of grapes.

During the grapes ripening, pH-values range from 2.8 to 3.5 (or even more); this is dependent on the cultivar, year, and macroclimate (AMERINE *et al.* 1965). This observation was corroborated also in our study.

The pH-values above 3.6 are undesirable; the reason is that they cause a low intensity of colour,

impair microbial stability, raise the spoilage potential, and increase the susceptibility to oxidation of the wine produced (KELLER 2010). A tendency to higher pH-values of must was observed only in cultivars Müller Thurgau and Blauer Portugieser. As far as the new interspecific cultivars are concerned, pH-values did not exceed the risk limit values and this fact is important from the viewpoint of making good and quality wines.

Titrateable acids in must belong to the parameters that are routinely used for the grapes quality estimation (OLIVEIRA & SOUSA 2009).

Good knowledge of the content of titrateable acids and its distribution among the individual cultivars is very important. Unfortunately, no similar screening of these new cultivars has been performed until now. The average content of titrateable acids ranged from the lowest value of 5.58 g/l (cv. Merzling) to the highest one of 11.45 g/l (cv. Riesling). However, the absolutely highest contents of titrateable acids were found out in 2006; the cv. Marlen showed the maximum content of 13.01 g/l and was followed

Table 6. The contents of tartaric acid, malic acid and citric acid, and the  $\beta$  ratio in grapes of individual cultivars as estimated in years 2006, 2008, and 2009

| Cultivar           | Tartaric acid (g/l) |      | Malic acid (g/l)       |      | Citric acid (g/l)    |      | $\beta$ ratio         |      |
|--------------------|---------------------|------|------------------------|------|----------------------|------|-----------------------|------|
|                    | $\bar{x}$           | SD   | $\bar{x}$              | SD   | $\bar{x}$            | SD   | $\bar{x}$             | SD   |
| Erilon             | 8.69 <sup>de</sup>  | 1.31 | 4.47 <sup>cdef</sup>   | 2.48 | 0.41 <sup>e</sup>    | 0.15 | 2.30 <sup>abcd</sup>  | 1.06 |
| Hibernal           | 6.91 <sup>bcd</sup> | 0.20 | 2.78 <sup>abc</sup>    | 0.60 | 0.26 <sup>abcd</sup> | 0.08 | 2.57 <sup>abcde</sup> | 0.54 |
| Malverina          | 7.57 <sup>cde</sup> | 0.99 | 3.27 <sup>abcd</sup>   | 0.90 | 0.31 <sup>de</sup>   | 0.08 | 2.42 <sup>abcde</sup> | 0.60 |
| Merzling           | 5.71 <sup>ab</sup>  | 0.43 | 1.59 <sup>a</sup>      | 0.09 | 0.15 <sup>ab</sup>   | 0.02 | 3.62 <sup>e</sup>     | 0.47 |
| Müller Thurgau     | 7.16 <sup>bcd</sup> | 0.52 | 2.97 <sup>abc</sup>    | 0.71 | 0.19 <sup>abcd</sup> | 0.06 | 2.47 <sup>abcde</sup> | 0.38 |
| Riesling           | 9.05 <sup>e</sup>   | 1.43 | 5.04 <sup>def</sup>    | 1.21 | 0.24 <sup>abcd</sup> | 0.06 | 1.90 <sup>ab</sup>    | 0.64 |
| Savilon            | 6.36 <sup>abc</sup> | 0.21 | 3.44 <sup>bcdef</sup>  | 0.98 | 0.22 <sup>abcd</sup> | 0.01 | 1.97 <sup>abcde</sup> | 0.65 |
| Blaufränkisch      | 8.09 <sup>cde</sup> | 1.04 | 3.38 <sup>abcdef</sup> | 1.07 | 0.19 <sup>abcd</sup> | 0.10 | 2.59 <sup>abc</sup>   | 0.95 |
| Blauer Portugieser | 5.73 <sup>ab</sup>  | 0.80 | 3.32 <sup>abcd</sup>   | 0.64 | 0.27 <sup>bcd</sup>  | 0.13 | 1.79 <sup>abc</sup>   | 0.56 |
| Cerason            | 8.29 <sup>de</sup>  | 1.08 | 4.14 <sup>cdef</sup>   | 0.63 | 0.28 <sup>cd</sup>   | 0.10 | 2.05 <sup>abcd</sup>  | 0.51 |
| Kofranka           | 8.38 <sup>de</sup>  | 2.44 | 3.35 <sup>abcdef</sup> | 0.46 | 0.23 <sup>abcd</sup> | 0.06 | 2.46 <sup>abcde</sup> | 0.40 |
| Laurot             | 8.61 <sup>de</sup>  | 0.70 | 2.96 <sup>abc</sup>    | 0.87 | 0.14 <sup>a</sup>    | 0.04 | 3.15 <sup>cde</sup>   | 1.24 |
| Marlen             | 8.27 <sup>de</sup>  | 0.82 | 5.19 <sup>f</sup>      | 2.12 | 0.22 <sup>abcd</sup> | 0.03 | 1.72 <sup>a</sup>     | 0.49 |
| Nativa             | 8.39 <sup>de</sup>  | 1.02 | 2.77 <sup>abc</sup>    | 0.63 | 0.17 <sup>abc</sup>  | 0.04 | 3.22 <sup>de</sup>    | 1.22 |
| Regent             | 5.02 <sup>a</sup>   | 1.02 | 1.75 <sup>ab</sup>     | 0.61 | 0.15 <sup>ab</sup>   | 0.04 | 2.99 <sup>bcde</sup>  | 0.54 |
| F                  | **                  |      | **                     |      | **                   |      | **                    |      |

The results were statistically analysed by the ANOVA method and Tukey test. The letters indicate statistically significant differences determined by Tukey's test; \*\* $P > 0.99$

Table 7. Classification of grapevine varieties on the basis of  $\beta$  ratio according to KLIEWER *et al.* (1967)

| $\beta$ ratio | Variety                                                                                     |
|---------------|---------------------------------------------------------------------------------------------|
| < 1.20        |                                                                                             |
| 1.21–1.75     | Marlen                                                                                      |
| 1.76–2.50     | Erilon, Malverina, Müller Thurgau, Riesling, Savilon, Blauer Portugieser, Cerason, Kofranka |
| > 2.51        | Hibernal, Merzling, Blaufränkisch, Laurot, Nativa, Regent                                   |

by Riesling (12.48 g/l) and Erilon (12.48 g/l). The lowest contents of titratable acids were estimated for the varieties Regent (4.82 g/l) and Merzling (5.32 g/l) in 2009. A significant divergence of the content of titratable acids from all other varieties under study was demonstrated for the varieties Merzling, Cerason, and Riesling.

Cultivars Merzling, Müller Thurgau, Blauer Portugieser, and Regent may be classified as varieties with low contents of titratable acids while the varieties Erilon, Blaufränkisch, Kofranka, Marlen, Cerason, and Riesling show high contents of them. Red interspecific varieties are more frequent among the varieties with high contents of acids, thus it is necessary to pay increased attention to the technologies of their growing and processing to wine.

A similar trend as in the case of titratable acids may be observed also in the content of total acids (Table 5). The highest average content of total acids was found out in the cvs Erilon (14.30 g/l) and Marlen (14.57 g/l) compared to the cvs Regent (7.57 g/l) and Merzling (7.80 g/l) where the contents of total acids were the lowest. This means that the blue must cv. Marlen and the white must cv. Erilon may be classified as those with the highest contents of total acids. Both of them belong to the group of interspecific cultivars.

The results of the evaluation of the individual organic acids and  $\beta$  ratio are presented in Table 6. The average content of tartaric acid ranged from 5.02 g/l (cv. Regent) to 9.05 g/l (cv. Riesling). These cultivars markedly differed from all other cultivars under study. The highest content of tartaric acid was found out in the cvs Kofranka (10.90 g/l), Riesling (10.55 g/l), and Erilon (10.19 g/l) in 2006. However, the results obtained corroborated the finding that the interspecific blue cultivars showed high contents of tartaric acid: cvs Marlen (8.27 g/l), Cerason (8.29 g/l), Kofranka (8.38 g/l), Nativa (8.39 g/l), and Laurot (8.61 g/l).

Their genotypes are very similar and this fact was markedly manifested also in the content of tartaric acid in must.

The content of malic acid is also a very important qualitative parameter of must and wine. Similarly as in the case of tartaric acid, the lowest values were estimated in the cvs Merzling (1.59 g/l) and Regent (1.75 g/l). The content of this acid in the cultivar Merzling was significantly different from all other cultivars. The highest level of malic acid was found out in the cv. Marlen (5.19 g/l). In 2006, the content of malic acid was even 7.61 g/l!

The content of citric acid ranged from 0.14 g/l (cv. Laurot) to 0.41 g/l (cv. Erilon). These two cultivars were significantly different from all other cultivars under study. Significant differences between the individual cultivars are presented in Table 6.

KLIEWER (1967a) studied the concentrations of acids in 25 grapevine cultivars cultivated in California. They found out that the contents of tartaric and malic acids ranged from 7.95 g/l to 4.70 g/l and from 5.0–1.6 g/l, respectively.

SOYER *et al.* (2003) evaluated the contents of organic acids in grapes of white cultivars and found out the following: the content of citric acid ranged from 0.30 g/l to 0.16 g/l, that of tartaric acid from 4.98 g/l to 7.48 g/l, and that of malic acid from 1.43 g/l to 3.40 g/l.

In this study, interspecific cultivars showed mostly higher contents of tartaric acid and comparable average contents of malic acid.

After the softening of berries, the content of malic acid rapidly decreases, while the pH-value increases. VOLSCHENK *et al.* (2006) mentioned that the pH-value is influenced by the profile of organic acids; this observation was corroborated also in our study.

The evaluation of the contents and profiles of organic acids demonstrated a significant effect of cultivar on the parameters under study. RUSJAN *et al.* (2008) obtained similar results in their evaluation of 11 cultivars of table grapes. This means that the contents and profiles of organic acids may be considered as a cultivar-specific property.

The ratio of tartaric to malic acids, which is called  $\beta$  ratio, can be used to differentiate grapevine cultivars (SHIRAISHI 1995).

As far as the quality of grapes and selection of an adequate technology of wine-making are concerned,  $\beta$  ratio is very important. The results obtained demonstrate a significant effect of the individual cultivars on this parameter. The range of the measured values was relatively wide and

Table 8. The contents of glucose, fructose, total sugars, and the glucose/fructose ratio in grapes of individual varieties as estimated in years 2006, 2008, and 2009

| Variety            | Glucose (g/l)        |       | Fructose (g/l)         |       | Glucose/Fructose     |      | Total sugars (g/l)   |       |
|--------------------|----------------------|-------|------------------------|-------|----------------------|------|----------------------|-------|
|                    | $\bar{x}$            | SD    | $\bar{x}$              | SD    | $\bar{x}$            | SD   | $\bar{x}$            | SD    |
| Erilon             | 108.42 <sup>ab</sup> | 10.45 | 107.15 <sup>abcd</sup> | 8.51  | 1.01 <sup>bcd</sup>  | 0.02 | 215.57 <sup>ab</sup> | 18.95 |
| Hibernal           | 122.30 <sup>b</sup>  | 15.79 | 122.19 <sup>d</sup>    | 8.76  | 1.00 <sup>abcd</sup> | 0.06 | 244.49 <sup>b</sup>  | 24.54 |
| Malverina          | 102.51 <sup>a</sup>  | 20.22 | 109.52 <sup>abcd</sup> | 21.37 | 0.94 <sup>ab</sup>   | 0.01 | 212.03 <sup>ab</sup> | 41.57 |
| Merzling           | 111.98 <sup>ab</sup> | 14.40 | 112.76 <sup>bcd</sup>  | 8.60  | 0.99 <sup>abcd</sup> | 0.05 | 224.74 <sup>ab</sup> | 22.87 |
| Müller Thurgau     | 102.12 <sup>a</sup>  | 8.29  | 106.33 <sup>abcd</sup> | 10.46 | 0.96 <sup>abc</sup>  | 0.02 | 208.44 <sup>ab</sup> | 18.71 |
| Riesling           | 95.73 <sup>a</sup>   | 9.07  | 94.57 <sup>ab</sup>    | 10.24 | 1.01 <sup>bcd</sup>  | 0.02 | 190.31 <sup>a</sup>  | 19.29 |
| Savilon            | 106.17 <sup>ab</sup> | 11.33 | 105.39 <sup>abcd</sup> | 11.79 | 1.01 <sup>abcd</sup> | 0.02 | 211.56 <sup>ab</sup> | 22.98 |
| Blaufränkisch      | 104.74 <sup>ab</sup> | 10.51 | 100.01 <sup>abc</sup>  | 13.29 | 1.05 <sup>d</sup>    | 0.04 | 204.75 <sup>a</sup>  | 23.70 |
| Blauer Portugieser | 106.50 <sup>ab</sup> | 8.66  | 106.00 <sup>abcd</sup> | 10.24 | 1.01 <sup>abcd</sup> | 0.03 | 212.50 <sup>ab</sup> | 18.77 |
| Cerason            | 104.37 <sup>ab</sup> | 12.77 | 101.93 <sup>abc</sup>  | 12.94 | 1.02 <sup>cd</sup>   | 0.02 | 206.29 <sup>a</sup>  | 25.65 |
| Kofranka           | 99.44 <sup>a</sup>   | 5.80  | 100.06 <sup>abc</sup>  | 6.12  | 0.99 <sup>abcd</sup> | 0.03 | 199.49 <sup>a</sup>  | 11.61 |
| Laurot             | 104.09 <sup>a</sup>  | 4.36  | 106.06 <sup>abcd</sup> | 4.16  | 0.98 <sup>abcd</sup> | 0.01 | 210.16 <sup>ab</sup> | 8.50  |
| Marlen             | 98.64 <sup>a</sup>   | 5.82  | 97.12 <sup>abc</sup>   | 11.05 | 1.02 <sup>bcd</sup>  | 0.07 | 195.76 <sup>a</sup>  | 16.42 |
| Nativa             | 97.79 <sup>a</sup>   | 6.64  | 92.58 <sup>a</sup>     | 11.24 | 1.06 <sup>d</sup>    | 0.06 | 190.37 <sup>a</sup>  | 17.83 |
| Regent             | 105.11 <sup>ab</sup> | 2.81  | 115.71 <sup>cd</sup>   | 19.31 | 0.92 <sup>a</sup>    | 0.14 | 220.83 <sup>ab</sup> | 21.99 |
| F                  | **                   |       | **                     |       | **                   |      | **                   |       |

The results were statistically analysed by the ANOVA method and Tukey test. The letters indicate statistically significant differences determined by Tukey's test; \*\* $P > 0.99$

fluctuated within the limits of 1.72 (cv. Marlen) and 3.62 (cv. Merzling).

KLIEWER *et al.* (1967) defined the following four categories for the evaluation of  $\beta$  ratio: (1) high content of malic acid ( $\beta$  ratio below 1.20); (2) moderate content of malic acid (1.21–1.75); (3) average content of malic acid (1.76–2.50); (4) low content of malic acid ( $\beta$  ratio above 2.51).

The classification of the grapevine cultivars into individual groups is presented in Table 7. Most of them belong to groups (3) and (4), i.e. with average and high  $\beta$  ratios.

In most cultivars, higher values of  $\beta$  ratio contribute to a better stability of wine (LIU *et al.* 2007); in this study, most of the cultivars showed a higher  $\beta$  ratio.

Among the individual grapevine cultivars,  $\beta$  ratio shows a great variability and, for that reason, it enables to differentiate between the individual cultivars; this was demonstrated also in this study (Figure 1). The greatest difference was found out between the cvs Merzling and Marlen.

$\beta$  ratio is dependent on the genetic characteristics of the individual cultivars (KLIEWER 1967a; KANELIS & ROUBELAKIS-ANGELAKIS 1993; SHIRAIISHI 1995); this observation was corroborated also in the interspecific cultivars under study.

From the viewpoint of wine quality, higher values of  $\beta$  ratio show positive effects above all on white cultivars grown under cooler climatic conditions (KASERER *et al.* 1996).

The contents of glucose and fructose as well as their ratio (GLU/FRU) belong to the parameters which are significantly influenced by the cultivar (JAIN *et al.* 2002). The average content of glucose ranged from 95.73 g/l (cv. Riesling) to 122.30 g/l (cv. Hibernal). The highest content of glucose (140.36 g/l) was found out in the cv. Hibernal in 2006. The results of statistical analysis indicated the differences existing between the individual varieties (Table 8). The average content of fructose ranged from 92.58 g/l (cv. Nativa) to 122.19 g/l (cv. Hibernal). Both of them significantly differed from all other cultivars under study.

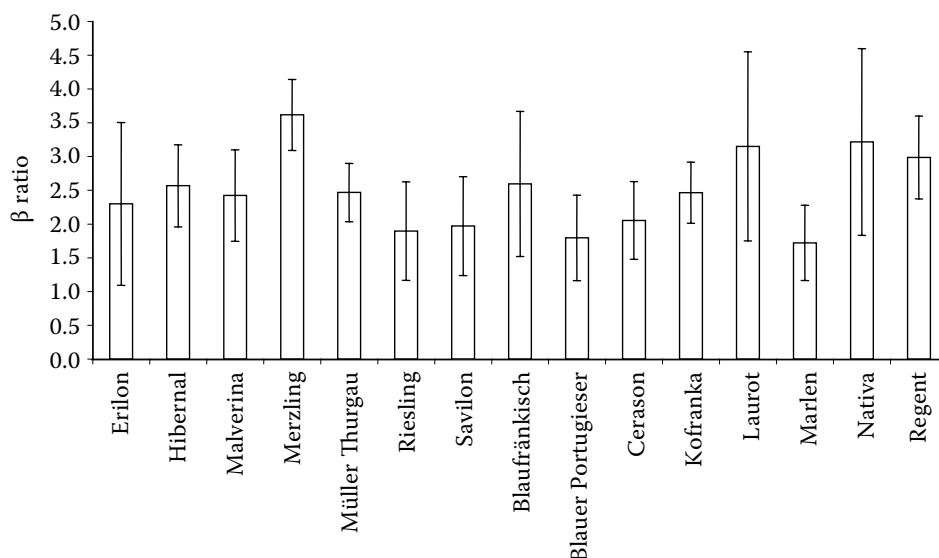


Figure 1. Differentiation of grapevine cultivars on the basis of  $\beta$  ratio. The results were statistically analysed by the Tukey Test in  $P > 0.95$

The contents of glucose and fructose corresponded to the content of total sugars. The lowest and the highest contents of total sugars were found out in the cvs Riesling and Hiberna (190.31 g/l and 244.49 g/l, respectively).

LIU *et al.* (2006) found out in 98 cultivars the range of glucose content within the limits of 45.86 g/l and 122.89 g/l while the content of fructose ranged from 47.64 g/l to 131.04 g/l.

The GLU/FRU ratio is also an important varietal property. KLIEWER (1966) and RICE (1974) mention that the berries contain glucose and fructose as primary sugars in the ratio 1:1. Similar results were obtained also by SHIRAISHI (1993) who studied 259 cultivars, hybrids, rootstocks, and wild species. In our study, average values ranged from 0.92 (cv. Regent) to 1.06 (cv. Nativa). Both of them

were different from all other cultivars. The value of 1.00 was found out only in the case of Hiberna. The lowest value of GLU/FRU was estimated in cv. Regent (0.88) in 2009 while the highest one in cv. Marlen (1.07) in 2008.

A wider GLU/FRU ratio range corresponds with the results published by AMERINE and THOUKIS (1958) who found out GLU/FRU ratios ranging from 0.72 (cv. Riesling) to 1.20 (cv. Gamay) and by KLIEWER (1967a) who mentioned that this ratio ranged from 0.47 to 1.12.

KLIEWER (1967b) studied the contents of sugars and organic acids in 78 cultivars of *Vitis vinifera*. He observed a great variability of these parameters and divided the cultivars into groups showing high and low contents of glucose and a low content of tartaric acid.

Table 9. Correlations existing between the individual parameters under study

|               | TA      | TOA                   | TARA                  | MALA                  | CITA                  | $\beta$ ratio        | GLU                   | FRU                   | GLU/FRU               | TS                    |
|---------------|---------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| pH            | -0.52** | -0.27 <sup>n.s.</sup> | -0.26 <sup>n.s.</sup> | -0.22 <sup>n.s.</sup> | -0.08 <sup>n.s.</sup> | 0.05 <sup>n.s.</sup> | 0.26 <sup>n.s.</sup>  | 0.27 <sup>n.s.</sup>  | -0.10 <sup>n.s.</sup> | 0.27 <sup>n.s.</sup>  |
| TA            |         | <b>0.87**</b>         | <b>0.76**</b>         | <b>0.74**</b>         | 0.34 <sup>n.s.</sup>  | -0.40**              | -0.24 <sup>n.s.</sup> | -0.33*                | 0.27 <sup>n.s.</sup>  | -0.30 <sup>n.s.</sup> |
| TOA           |         |                       | <b>0.84**</b>         | <b>0.86**</b>         | 0.43 <sup>n.s.</sup>  | -0.46**              | -0.20 <sup>n.s.</sup> | -0.35**               | 0.36**                | -0.29 <sup>n.s.</sup> |
| TARA          |         |                       |                       | 0.45**                | 0.14 <sup>n.s.</sup>  | 0.04 <sup>n.s.</sup> | -0.04 <sup>n.s.</sup> | -0.18 <sup>n.s.</sup> | 0.28 <sup>n.s.</sup>  | -0.12 <sup>n.s.</sup> |
| MALA          |         |                       |                       |                       | <b>0.53**</b>         | <b>-0.79**</b>       | -0.31*                | -0.40**               | -0.28 <sup>n.s.</sup> | -0.37**               |
| CITA          |         |                       |                       |                       |                       | -0.53**              | 0.08 <sup>n.s.</sup>  | -0.06 <sup>n.s.</sup> | 0.09 <sup>n.s.</sup>  | -0.03 <sup>n.s.</sup> |
| $\beta$ ratio |         |                       |                       |                       |                       |                      | 0.29 <sup>n.s.</sup>  | 0.29 <sup>n.s.</sup>  | -0.11 <sup>n.s.</sup> | 0.30*                 |
| GLU           |         |                       |                       |                       |                       |                      |                       | 0.87**                | -0.04 <sup>n.s.</sup> | <b>0.96**</b>         |
| FRU           |         |                       |                       |                       |                       |                      |                       |                       | -0.52**               | <b>0.97**</b>         |
| GLU/FRU       |         |                       |                       |                       |                       |                      |                       |                       |                       | -0.31*                |

Significant correlations are in bold; n.s. = non-significant; \* $P > 0.95$ ; \*\* $P > 0.99$

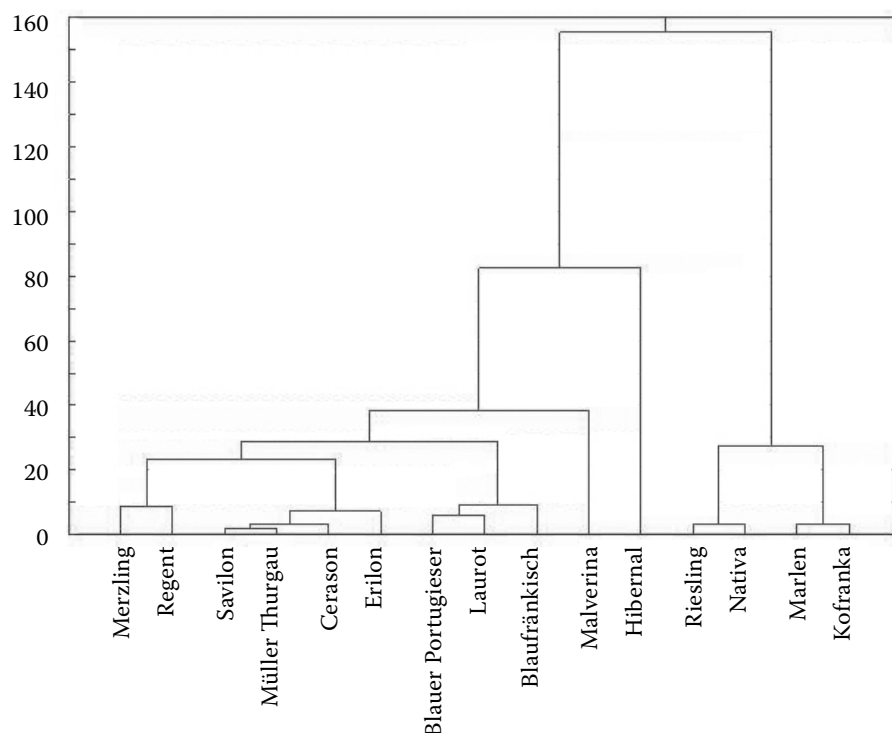


Figure 2. Hierarchical tree plot showing the classification of individual cultivars on the basis of cluster analysis of profiled primary metabolites

Our study also demonstrated significant and highly significant effects of the cultivar and year on the parameters under study.

The method of Hierarchical Cluster Analysis was used with the aim to classify the individual cultivars into groups showing similar properties and parameters; the contents of organic acids and sugars were used as variables. The dendrogram presented below shows the classification of all cultivars into several groups (Figure 2). The group involving the cvs Riesling, Nativ, Marlen, and Kofranka markedly differs from all others. In this group, the white cv. Riesling, which belongs to the species *Vitis vinifera* L., resembles the interspecific varieties used for the production of red wine (cvs Nativ, Marlen, Kofranka). Another group involves the cvs Merzling and Regent, which show a great similarity in the parameters under study. As far as the quality of grapes is concerned, the similarity of the cvs Blaufränkisch, Blauer Portugieser, and Laurot is also very interesting. Hiberna occupies a separate position, above all due to its markedly different contents of sugars. The dendrogram enabled a relatively good comparison of the grapevine (*Vitis vinifera* L.) cultivars with the interspecific hybrids; this comparison demonstrated that also

the basic qualitative parameters of grapes (i.e. sugars and acids) are comparable.

The results of the correlation analysis involving the individual parameters under study are presented in Table 9. A significant correlation exists between the contents of glucose, fructose, and total sugars. Also, the correlation existing between the value of titratable acids on one hand and the contents of tartaric ( $r = 0.76$ ) and malic ( $r = 0.74$ ) acids is relatively high. A markedly negative relationship exists between the content of malic acid and  $\beta$  ratio ( $r = -0.79$ ); this demonstrates that this ratio is influenced above all by the content of malic acid in the grapes.

However, we were not able to find out any marked relationship between the content of acids and pH-value (RÜHL *et al.* 1988); these authors found out a positive correlation between pH-values of must and the content of tartaric acid and  $\beta$  ratio.

## CONCLUSION

It can be concluded that this study presents the first complete overview of the contents and profiles of sugars and organic acids in new interspecific

cultivars of grapevine. A marked similarity exists between many interspecific cultivars evaluated within the framework of this study and the conventional cultivars of *Vitis vinifera* L., and for that reason their quality is mutually comparable.

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