1. Introduction

Grapevine belongs to a few plants whose final product of processing, wine, can bear imprint of land and place fully and sensitively. Individual grapevine varieties differ in their typical look and features. Their characteristics are given in literature [1]. Composition and wine grape berries fresh weight are under the control of complex interactions among genotype, environmental factors, and viticulture practice. All the factors affect not only the mean values but also the ranges of variation. Both values play a role in wine grapes quality and, finally, wine typicity [2-9]. Veraison is critical a metabolically stage during grape berry development, and marks the beginning of ripening. The position of the berries within the bunch mainly influences the metabolic profile of berries. Growing area and clone seem to be less significant factors influencing metabolic profile.

After blossoming and fertilization, the berries start their growth by increasing volume and weight; it takes 40-45 days in average [2]. Significant variability of organic acids and amino acids characterizes the rapid rearrangements of the metabolic profile. Comparatively high organic acids contents are present in the initial stages. Due to intensive breathing, the content of acids grows during ripening and it often reaches values up to 35 g L⁻¹. Glucose and fructose accumulate in the later stages.

Malic and tartaric acids are the principal acids found in grapes; nevertheless, they also contain lower levels of succinic, oxalic and citric acids [3-9]. Among all these acids, malic acid demonstrates the highest growth of its
content during ripening of grapes. The level of tartaric acid rises during that period, too; however, part of the acid is bound with potassium in the form of potassium hydrogen tartrate. Tartaric acid is found specifically in grapes; it does not occur in other fruits in substantial amounts. It is found in all parts of bunches of grapes; grapevine leaves contain 13 to 16 g kg\(^{-1}\) of tartaric acid. Tartaric acid is considered a significant acid of must and wine.

During ripening, major qualitative changes occur in grape berries; however, their size and weight grow only moderately. Softening is an apparent symptom of maturing accompanied by grapes becoming transparent [2-9]. Two inverse processes occur in grapes during their ripening: on one hand, concentrations of both free and bound acids in berries increase, and, on the other hand, the acids are decomposed due to less intensive breathing and oxidation. The amount of acids decomposed during ripening is higher than the amount of acids synthesized throughout the process. Only free unbound acids such as malic acid are degraded; its level decreases significantly during maturing. However, the content of bound malic acid remains unchanged. The amount of free unbound tartaric acid decreases during ripening; nevertheless, the content of bound tartaric acid increases. Form of occurrence and changes happening with acids during ripening are also effected by mineral substances such as potassium, calcium, or sodium that form salts with acids. Mineral substances penetrate into grape berries from vineyard soil. Decrease of acid content during ripening is also attributed to growth of cation amounts, especially to higher potassium levels; potassium binds with tartaric acid to make potassium hydrogen tartrate.

Left unpicked, the berries maturing in sunny vineyards over-ripen under proper weather conditions. Water evaporates from grapes and, at the same time, the grape juice starts to be more concentrated. When leaves become red and peduncles and stems lignify, the nutrient and water intakes to berries are stopped. Water evaporation causes them to become smaller, which results in juice becoming more concentrated. Because of various biochemical changes, the amounts of acids and tannins in grapes decrease [2-9].

Beneficial effects of grapes, must, and wine are attributed to phenolic substances such as flavonoids, phenolic acids and others that are naturally formed in them. Besides other factors, the content of phenolic substances depends on grapevine variety, degree of grape maturation and, especially, on technological processing applied. The grape seeds and skin contain the highest amounts of phenolic substances; their lowest levels are found in pulp.

Phenolic substances are regarded important because of their ability to demonstrate antioxidant effects [9-10]. Cells of living organisms produce continuously free radicals whose reactions cause major damages to cells and tissues. Thus, the protective effect of antioxidants represents one possible way of human body defense against negative influence of free radicals because antioxidants decrease activity of oxygen radicals.

We studied changes of total content of phenolic substances, alteration in total titratable acidity and differences in tartaric acid content in grapes of four white and two blue grapevine varieties throughout their growth, ripening and maturing (July – November). The following white varieties were subjected to research: Müller-Thurgau (MT), Pinot Blanc (Rulandské bílé in Czech, RB), Sauvignon (Sg), and Muscat Ottonel (Muškát Ottonel in Czech, MO). They were grown in the Bzenec wine growing village, namely in the Maršálky and Grefty (MO) vineyards. Two blue varieties were studied: Dornfelder (Df) and blue Frankish (Frankovka in Czech, Fr), both of them grown in the Maršálky vineyard. Bzenec is located in the wine growing subregion Slovácko found in Moravian wine producing region, Czech Republic.

2. Experimental procedure

2.1. Chemicals and instruments

A pH meter Level 1 with a combined pH electrode (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) that was regularly calibrated by a set of pH buffers of pH 4.01, 7.00 and 9.23. All spectrophotometric data were measured with a UV-VIS spectrophotometer Helios Delta (Spectronic Unicam, Cambridge, UK) in quarts covets of 10 mm optical path. A Folin-Ciocalteau reagent (10-times diluted) and gallic acid at c\(_{\text{ag}}\) = 0.08 g L\(^{-1}\) (Sigma-Aldrich, St. Louis, MO, USA), Na\(_2\)CO\(_3\), 10 H\(_2\)O at c\(_{\text{ag}}\) = 200 g L\(^{-1}\), NaOH at c\(=0.1\) mol L\(^{-1}\), acetate buffer solution (20 mL concentrated CH\(_3\)COOH and 80 mL of 270 g L\(^{-1}\) CH\(_3\)COONa diluted up to 250 mL with distilled water), reagent solution (5 g NH\(_4\)VO\(_3\) dissolved in 75 mL 1 mol L\(^{-1}\) NaOH and after addition of 100 mL 270 g L\(^{-1}\) CH\(_3\)COONa diluted up to 250 mL with distilled water), tartaric acid (c\(_{\text{ag}}\) = 10 g L\(^{-1}\)), charcoal (all Pliva- Lachema, Brno, Czech Republic).

Four white varieties Müller-Thurgau (MT), Pinot Blanc (Rulandské bílé in Czech, RB), Sauvignon (Sg), and Muscat Ottonel (Muškát Ottonel in Czech, MO) were subjected to research. They were grown in the Bzenec wine-growing village, namely in the Maršálky and Grefty (MO) vineyards. Two blue varieties were studied: Dornfelder (Df) and blue Frankish (Frankovka in Czech, Fr).
2.2. Methods of determination

Grape berries (see Fig. 1) were picked in the year 2009, 2010 and 2011 at 8 – 11 different stages of their growing and ripening. After blossoming, the berries start their growth by increasing volume and weight; it takes 40-45 days in average, thus their diameter was 30 – 60 mm at the beginning of the study and 90 – 180 mm at the end of the study. The berries were pressed and the samples of grape juice were analyzed immediately after their picking.

2.2.1. Titratable acids in grapes

Potentiometric titration \[11,12]\ was applied for the determination. Usually 3 – 10 mL of grape juice was gently pressed using hydraulic press depending on the maturation stage. The grape juice was diluted up to 20 mL with distilled water and titrated with NaOH solution at \(c_{NaOH} = 0.1\) mol L\(^{-1}\). Total content of acids was expressed as tartaric acid equivalents in g L\(^{-1}\).

2.2.2. Tartaric acid in grapes

Spectrophotometric method according Rebelein \[11\] based on the formation of a colored complex of ammonium metavanadate and tartaric acid was used. Calibration curve was constructed using standard solutions of tartaric acid at \(c_{H_2A} = 1, 2, 3, 4\) and 5 g L\(^{-1}\). Absorbance was measured after 10 min at \(\lambda = 530\) nm against blank solution. Usually 25 mL buffer solution, 1 mL analyzed juice (volume of juice and necessary dilution was adjusted in relation to the ripening), 4 mL distilled water and 0.8 – 1.0 g charcoal were transferred in volumetric flask and the mixture was gently mixed and filtered. Ten mL of the filtrate was transferred into a 50 mL volumetric flask and 5 mL of the reagent and finally filled up to mark with distilled water. Absorbance of the solution was measured at \(\lambda = 530\) nm after 10 minutes of incubation.

2.2.3. Total content of phenolic substances in grapes \[9-10\]

A spectrophotometric method based on reduction of phosphomolybdato-tungsten complex in alkaline solution using Folin-Ciocalteau reagent was applied for determination of total content of phenolic substances (TCP). Calibration curve was constructed using standard solutions of gallic acid at \(c_{H_2A} = 1, 2, 4, 6, 8\) and 10 mg L\(^{-1}\). Absorbance of the blue solution was measured at \(\lambda = 765\) nm after 60 minutes incubation. The total content of phenolic substances was expressed in gallic acid equivalents (GAE) in mg L\(^{-1}\).

2.3. Mathematical - statistic methods of evaluation

2.3.1. Titratable acids in grapes

Always 3 parallel titrations were carried out for each of the vine variety at all stages of growing and ripening. The first and the second derivative of titration curve \((\delta pH/\delta V) ev. \delta^2 pH/\delta V^2 = f(V)\) was used for exact determination of equivalence points using POTIK program in Microsoft Excel. Relevant standard deviations and confidence
Changes of organic acids and phenolic compounds contents in grapevine berries during their ripening

Intervals $L_{1-2} (\alpha = 0.05)$ were calculated (see Fig. 2) from founded values the average values.

Repeatability of determination (expressed as the relative standard deviation $s_r$) for all varieties of vine was determined in the beginning of picking – $s_r$(July) and at the end of picking in November – $s_r$(Nov.) always from 10 parallel determinations.

2.3.2. Tartaric acid in grapes

Parameters of calibration curves (slope – $b$, intercept – $a$, standard deviation about regression – $s_{y,x}$, power of correlation coefficient – $r^2$, determination limit – $c_{lim}$) for five standard solutions of freshly prepared tartaric acid for $c_m = 1, 2, 3, 4$ and $5$ g L$^{-1}$, and concentrations of tartaric acid were determined using the SKAZA program in Microsoft Excel in samples of all varieties of vine at all different stages of growing and ripening.

Repeatability of determination (expressed as the relative standard deviation $s_{r}$) was determined using 10 parallel determinations at two levels of concentrations of the tartaric acid (low – $c_m = 1$ g L$^{-1}$ and high – $c_m = 4$ g L$^{-1}$) in the beginning of picking – $s_{r}$(July, low), $s_{r}$(July, high) and at the end of picking in November – $s_{r}$(Nov., low), $s_{r}$(Nov., high).

Always 3 parallel determinations of individual vine samples were carried out for each of the variety of vine and at all different stages of growing and ripening. The average values, relevant standard deviations and confidence intervals $L_{1-2} (\alpha = 0.05)$ were calculated (see Fig. 4) from founded values.

2.3.3. Total content of phenolic substances in grapes

The six points calibration curves $A = f(c_m)$ were constructed measuring six standard solutions of freshly prepared gallic acid and their parameters (slope – $b$, intercept – $a$, standard deviation about regression – $s_{y,x}$, power of correlation coefficient – $r^2$, determination limit – $c_{lim}$) and concentrations of total content of phenolic compounds in samples of all varieties of vine were determined using the SKAZA program in Microsoft Excel at all different stages of growing and ripening.

Figure 2. Changes of titratable acidity in Muscat Ottonel (MO) and blue Frankish (Fr) grapes in 2009, 2010, and 2011.

Figure 3. Comparison of titratable acidity changes in grapes of individual varieties in 2010 and 2011: Muscat Ottonel (MO), Müller-Thurgau (MT), Pinot Blanc (RB), Sauvignon (Sg), Dornfelder (Df), and blue Frankish (Fr).
Repeatability of determination (expressed as the relative standard deviation $s_r$) was determined using 10 parallel determinations at two levels of concentrations of the gallic acid (low – $c_m = 2$ mg L$^{-1}$ and high – $c_m = 8$ mg L$^{-1}$) in the beginning of picking – $s_r$(July, low), $s_r$(July, high) and at the end of picking – $s_r$(Nov., low), $s_r$(Nov., high).

Always 3 parallel determinations of individual vine samples were carried out for each of the variety of vine and at all different stages of growing and ripening. The
Changes of organic acids and phenolic compounds contents in grapevine berries during their ripening

relevant standard deviations and confidence intervals \(L_{1,2}(\alpha = 0.05)\) were calculated (see Fig. 7) for the average values.

3. Results and discussion

3.1. Determination of titratable acidity

Determination of total titratable acidity was implemented three times: Muscat Ottonel, Pinot Blanc and blue Frankish varieties were analyzed in 2009; and Muscat Ottonel, Müller-Thurgau, Pinot Blanc, Sauvignon, Dornfelder, and blue Frankish varieties were studied in 2010 and 2011 by the means of alkalimetric titration with potentiometric indication of the equivalence point. After recalculation, titratable acidity was expressed in g L\(^{-1}\) of tartaric acid.

Repeatabilities of determination of titratable acidity in individual varieties of vine in the year 2009, 2010 and 2011 are given in Table 1.

The values of standard relative deviations (1.29% – 3.25%) show a satisfactory repeatability of the method of determination (see Table 1).

During growth and maturation of grapes, the values of titratable acidity increased mostly due to rise of malic acid content at first. Later on, they decreased with grape ripening, which is in accord with literature [3-9]. The comparison of titratable acidities analyzed in 2009, 2010, and 2011 in Muscat Ottonel and blue Frankish varieties (Fig. 2) suggests that in 2010, grapes ripening occurred one or two weeks later and that all the studied grape varieties demonstrated higher values of titratable acidity, partly thanks to lack of sunshine. In the studied wine-growing village of Bzenec, the 2010 yield of grapes was 20% lower compared to the average amount harvested in previous years. Fig. 3 depicts titratable acidity values found in the six studied varieties in 2010 and 2011. In 2009, 2010, and 2011, ripened grape berries of individual varieties contained between 3.80 (MO, 2009) and 12.3 (Sg, 2010) g L\(^{-1}\) of titratable acidity.

3.2. Determination of tartaric acid content

In 2010 and 2011, the content of tartaric acid was determined throughout the growth and maturation of grapes every fortnight in the period of five months. The confidence intervals \(L_{1,2}(\alpha = 0.05)\) of parameters of calibration curves and repeatability (relative standard deviations) of determinations are in Table 2.

The calibration curves have satisfactory rate of linearity \((L_{1,2}(s_y,x) = 0.009 – 0.014 (2010) and 0.004 – 0.011 (2011), L_{1,2}(r^2) = 0.990 – 0.996 (2010) and 0.996 – 0.999 (2011))\) and values of standard relative deviations (2.57% – 4.99% (2010) and 2.42 % – 4.87% (2011)) show a good repeatability of the method of determination (see Table 2). The confidence intervals of tartaric acid contents in grapes of Muscat Ottonel (MO) and blue Frankish (Fr) varieties at all different stages of growing and ripening in 2010 and 2011 are compared in Fig. 4. The 2010 and 2011 contents of tartaric acid in all studied grapevine varieties are shown in Fig. 5.

As apparent from results, tartaric acid content moderately increased at the beginning of grapes growth and then it gradually decreased until they got fully

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Changes of total contents of phenolic compounds in Muscat Ottonel (MO) and blue Frankish (Fr) grapes.

**Table 1.** Repeatability (relative standard deviations) of determinations for all varieties of vine in years of 2009, 2010 and 2011.

| Variety | 2009 | 2010 | 2011 |
|---------|------|------|------|
| MO      | July | Nov. | July | Nov. | July | Nov. |
| MO      | 2.04 | 1.29 | 1.88 | 1.56 | 2.12 | 2.17 |
| MT      | -    | -    | 2.33 | 2.35 | 2.98 | 2.57 |
| RB      | -    | -    | 2.12 | 2.04 | 2.24 | 2.09 |
| Sg      | -    | -    | 2.66 | 1.74 | 1.89 | 1.44 |
| Df      | -    | -    | 3.25 | 2.87 | 3.24 | 2.89 |
| Fr      | 2.23 | 1.79 | 3.02 | 2.08 | 2.45 | 2.42 |
Table 2. Confidence intervals of parameters of calibration curves and repeatability (relative standard deviations) of determinations.

| Parameters of calibration curves | 2010          | 2011          | Repeatability $s_i$ (%) | 2010          | 2011          |
|--------------------------------|---------------|---------------|-------------------------|---------------|---------------|
| $L_{1,2}(b)$                   | 0.066 – 0.070 | 0.058 – 0.065 | $s_i$ (%)                | 4.99          | 4.87          |
| $L_{1,2}(a)$                   | -0.018 – -0.011 | -0.005 – -0.001 | (July, low)              | 3.57          | 3.46          |
| $L_{1,2}(s_y,s_x)$             | 0.009 – 0.014 | 0.004 – 0.011 | (July, high)             | 4.38          | 4.02          |
| $L_{1,2}(r^2)$                 | 0.990 – 0.996 | 0.996 – 0.999 | (Nov., low)              | 2.57          | 2.42          |
| $L_{1,2}(c_{min})$ [g L$^{-1}$] | 0.106 – 0.111 | 0.111 – 0.158 | Appeals (%)              | 3.34          | 3.34          |

*a* calculated from all calibration curves prepared in individual dates of picking  
*b* calculated in the beginning of picking (July) and at the end of picking (November), using 10 parallel determinations at two levels of concentrations (low and high).

Table 3. Confidence intervals of parameters of calibration curves and repeatability (relative standard deviations) of determinations.

| Parameters of calibration curves | 2010 | 2011 | Repeatability $s_i$ (%) | 2010 | 2011 |
|--------------------------------|------|------|-------------------------|------|------|
| $L_{1,2}(b)$                   | 0.062 – 0.066 |      | $s_i$ (%)                | 3.43          |      |
| $L_{1,2}(a)$                   | -0.017 – -0.004 | (July, low) | 2.18          |      |      |
| $L_{1,2}(s_y,s_x)$             | 0.008 – 0.032 | (July, high) | 2.14          |      |      |
| $L_{1,2}(r^2)$                 | 0.987 – 0.994 | (Nov., low) | 3.34          |      |      |
| $L_{1,2}(c_{min})$ [mg L$^{-1}$] | 0.138 – 0.148 | (Nov., high) | 2.14          |      |      |

*a* calculated from all calibration curves prepared in individual dates of picking  
*b* calculated in the beginning of picking (July) and at the end of picking (November), using 10 parallel determinations at two levels of concentrations (low and high).

Figure 8. Total content of phenolic compounds in grape juice of the grapevine varieties: Muscat Ottonel (MO), Müller-Thurgau (MT), Pinot Blanc (RB), Sauvignon (Sg), Dornfelder (Df), and blue Frankish (Fr).

3.3. Determination of the total content of phenolic substances

The confidence intervals $L_{1,2}$ ($\alpha = 0.05$) of parameters of calibration curves and repeatability (relative standard deviations) of determinations are in Table 3.

The calibration curves have satisfactory rate of linearity ($L_{1,2}(s_y,s_x) = 0.008 – 0.032$, $L_{1,2}(r^2) = 0.987 – 0.994$) and values of relative standard deviations (2.14% – 3.43%) show a good repeatability of the method of determination (see Table 3). The confidence intervals of the concentrations of total contents of phenolic compounds in Muscat Ottonel (MO) and blue Frankish (Fr) grapes at all different stages of growing and ripening are shown in Fig. 7.

Total content of phenolic substances decreased gradually with passing time of the experiment in all the studied varieties (see Fig. 8). The analyzed juice from unripened berries picked at the beginning of the collection period in June and July, that contained also phenolic substances from seeds and skin, showed the highest levels of phenolic substances ranging between 2.28 – 5.00 g L$^{-1}$. Later, juice of grapes collected gradually during their ripening contained phenolic ripened, which is in accord with literature [4-9]. Later, when over-ripening occurred, tartaric acid content slightly rose (see Figs. 4 and 5), or it remained almost unchanged. In 2010, the highest content or tartaric acid was found in the blue Frankish grapes and it was ranging between the values of 19.2 – 7.83 g L$^{-1}$. The lowest content of tartaric acid between 15.1 and 6.20 g L$^{-1}$ was shown by the Müller-Thurgau variety. In 2011, the highest content of tartaric acid ranging between 21.4 – 5.98 g L$^{-1}$ was revealed again in the blue Frankish variety and the lowest one dropping to 16.6 – 3.24 g L$^{-1}$ was detected in the Muscat Ottonel grapes.
matters originating mostly from pulp. These grapes demonstrated substantially lower contents of phenolic compounds between 104 – 163 mg L\(^{-1}\) at their picking. The above decrease proves considerable differences in contents of phenolic substances in various parts of berries, namely in seeds, skin and pulp.

### 4. Conclusions

The content of titratable acids in the time of grapes growing and ripening at first increases (up to 40 g L\(^{-1}\)) for individual varieties of vine, then with progressive ripening the content rapidly decreases (down to 3.8 g L\(^{-1}\)) and in the end is constant. The unripped grapes represent a valuable source of organic acids (containing up to 40 g L\(^{-1}\) acids and other bioactive substances, i.e., polyphenolic substances, polysaccharides etc.). The verjuice (from Middle French verjus “green juice”) is a highly acidic juice made by pressing the unripe grapes [13].

The content of the tartaric acid in the time of grapes growing and ripening at first also increases (up to 24 g L\(^{-1}\)) for individual varieties of vine, then with progressive ripening the content rapidly decreases (down to 3 g L\(^{-1}\)) and in the end is constant. For vines of the year 2010 (as a result of the adverse of weather conditions in period of August – October 2010) is evident that ripening of grapes in this year was going on about 1 – 3 weeks later and additionally the content of titratable acids and the tartaric acid was noticeably higher (2 – 3 g L\(^{-1}\)) than in vines of the years 2009 and 2011. The percentage ratio of the content of the tartaric acid to the content of titratable acids rapidly decreases to the values of 34% – 46% during ripening at first in individual varieties. It gradually increases in next progress of ripening to the values of 67% – 89% in the end of the monitored period.

The total content of phenolic compounds for individual varieties of vine during the monitored time period decreases from 5 000 mg L\(^{-1}\) (first dates of picking – unripe grapes) to 104 mg L\(^{-1}\) (the period of grapes harvest). This fact confirms the content of phenolic compounds in various parts of grapes (seeds, skins and pulp). There are not the principal differences among individual varieties of vine and also between white and red (blue) varieties of vine.

Results of mathematical-statistic processing show, that differences and dependences in values of contents of individual components cause not deviations in statistical evaluations, but changes in the chemical composition of vine grapes and the representation of individual components during their growing and ripening.

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