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## KÜLÖNBÖZŐ EREDETŰ FURMINTOK ÖSSZPOLIFENOL- TARTALOM NYOMONKÖVETÉSE AZ ERJEDÉS SORÁN

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### Összefoglalás

*A borban előforduló polifenoloknak fontos hatása van a bor érzékszervi tulajdonságaira (összehúzó hatás, kesernyőség, testesség, bársonyosság), így a borászati termékek fenolos éréseinek tanulmányozása sok szempontból figyelmet érdemelnek. A bor polifenoljainak jelentős része a szőlőből származik, és a bor feldolgozása, erjesztése és érlelése során különböző átalakulásokon megy keresztül. Mindamellet, hogy a bor fenolos érettsége érzékszervi szempontból fontos paraméter, mégis, a mustok polifenol-tartalmának az erjedés alatt bekövetkezett változásáról adatot nem találtunk. Jelenleg a piacon kedveltebbek a friss, könnyed fehér borok, amelyek alacsonyabb összpolicenol-tartalommal rendelkeznek, ezért figyelmünk az e kategóriába sorolható egyik fajtaborra irányult. Munkánk céljául tűztük ki, hogy megfigyeljük a magyar furmint minták összespolifenol-tartalmának változásait a 15 napos erjedés során, így eldönteni, hogy a fermentációs folyamat nyomon követhető-e egy viszonylag olcsó, egyszerű fotometriás méréssel.*

*Adaptáltuk a Magyar Borkódexben található, Folin-Ciocalteu-index meghatározására szolgáló módszert, úgy, hogy azzal az összpolicenol-tartalmat számszerűen mérni tudjuk, valamint minta-és reagensigényét méretcsökkentéssel redukáltuk. Az összes mintában a kiinduló értékek az irodalom alapján várható 300–500 mg/l galluszsav-egyenérték (GAE) között mozogtak. Az 5-6. napon minden mintában hirtelen változást észleltünk az összpolicenol-tartalomban. A hátralévő időben az összpolicenol-tartalom átmeneti hullámzásokkal, de végig csökkenő tendenciát mutatott. A megfigyelt ingadozások háttere – a polifenol-összetétel változása, átrendeződések az erjedés folyamán – további vizsgálatokkal felderítendő.*

**Kulcsszavak:** Furmint, Folin-Ciocalteu, galluszsav-egyenérték, összpolicenol, erjedés

## FOLLOWING CHANGES OF TOTAL POLYPHENOL CONTENT OF FURMINT SAMPLES DURING FERMENTATION

### Abstract

*Polyphenols occurring in wine have important contributions to the sensory properties (astringency, hardness, bitterness) thus the study of phenolic maturity of oenological products deserve attention. A major portion of wine polyphenols are derived from the grape (others from the material of the wooden barrels). Nevertheless, data dealing with the polyphenol content of grape juice during fermentation were not found. Nowadays the white wines with fresh character, less astringency, and lower total polyphenol content (TPC) are more popular on the Hungarian market thus the study is focusing to the Furmint wine which represents this category. The aim of the study is to observe changes of TPC in Hungarian Furmint grape juices during the 15-day fermentation period and thus decide if the fermentation is follow-able via a simple and relatively low-cost spectrophotometric method. Furthermore the standard method of determination of Folin-Ciocalteu indices in white wine referred to in the Hungarian Code of Wine was adapted to perform quantitative measurements of total polyphenol content in gallic acid equivalents (GAE). The sample and reagent demand of the method is reduced with keeping the original concentrations and reaction conditions thus it may be applicable to large sample numbers.*

*The initial TPC values were 300-500 mg GAE/L in the examined Furmint samples. A sudden decrease of TPC values was observed at the 5-6<sup>th</sup> day of fermentation, and after this a decreasing tendency was observable with temporary fluctuations. The background of these processes (changes and rearrangements of polyphenol composition during fermentation) needs to be further investigated by chromatographic methods.*

**Keywords:** *Furmint, Folin-Ciocalteu, gallic acid equivalents, total polyphenolcontent, fermentation*

## **Introduction**

Polyphenols occurring in wine deserve attention from many viewpoints because of their influence of the sensory properties of wines. Astringency, bitter aroma, hardness, colour and in a lesser proportion the scent, the bouquet of it are in connection with the presence of wine polyphenols [1][2]. Like many other plant polyphenols, they also can influence the metabolism of low density lipoproteins [3] and in in case of some flavonoid type compounds the permeability of capillaries (vitamin P activity) [4], thus may have role in prophylaxis of atherosclerosis and/or other cardiovascular diseases [1]. A significant proportion of wine polyphenols is derived from the grape and may undergo different transformations during the processing, fermentation and ageing of wine. Another phenolic compounds become present via dissolution from the material of the barrel. Most of wine polyphenols can be classified into different subclasses of products of the shikimate pathway, a fundamental process of plant metabolism. Nevertheless some phenolics are produced by yeasts. Groups of the most important wine polyphenols are summarized in Table 1 [1] [2] [4] together with their direct sensory character and other traits which may influence these properties of wine (e.g. browning by oxidation of polyphenolic antioxidant/antiradical agents).

Table 1. Legends A = aged wine, G = grape, G-S = grapeseed, W = wood, Y = yeast

| Phenolic acids and related compounds (plant metabolites, shikimate pathway) |        |   |                                   |                     |  |
|---|--------|---|-----------------------------------|---------------------|--|
| Subclass  | Origin | Examples  | Presence in wine                  | Sensory function    | Other character                        |
| benzoic acid derivatives  | G/W    | hydroxybenzoic, salicylic, gallic acids             | General                           | Astringency         | Antiradical activity, protein binding* |
| hydroxybenzoic acid polymers  | W      | ellagic, hexaoxidiphenic acids                      | Wines stored in barrel            | Astringency         | Antiradical activity, protein binding* |
| hydroxycinnamic acids   | G      | caffeic, ferulic acids and their tartrates          | General                           | Astringency         | Antiradical activity, protein binding* |
| cinnamic aldehydes  | W      | sinapyl-, coniferyl aldehyde                        | Wines stored in barrel            | Scent and aroma     |  |
| Flavonoids (plant metabolites, shikimate pathway)                           |        |   |                                   |                     |  |
| Subclass  | Origin | Examples  | Presence in wine                  | Sensory function    | Other character                        |
| flavonols/kaempferol glycosides   | G      | kaempferol-3-O-glucoside, -3-O-glucuronide          | General                           | Color - pale yellow | Chelator activity**                    |
| flavonols/quercetin glycosides  | G      | isoquercitrin, quercetin-3-O-glucuronide, rutin     | General                           | Color - yellow      | Antiradical and chelator activity **   |
| catechins   | G      | catechin, epigallocatechin and their gallate esters | Higher concentration in red wines | Astringency         | Antiradical activity                   |
| anthocyanins  | G      | malvidin-3-O-glucoside, malvidin-3-O-glucuronide    | Red wines only                    | Color - red, purple | Antiradical and chelator activity **   |

|   |               |                               |  |                          |  |
|---|---------------|-------------------------------|--|--------------------------|--|
| pyranoanthocyanins  | A             | pyranomalvidin glycosides     | Red wines only<br>If grape seeds break; ageing red wine                | Color - deep red         | Antiradical activity, protein binding* |
| procyanidins  | G-S, A        | procyanidin A and B oligomers |  | Bitterness, undesired    | Antiradical activity, protein binding* |
| <b>Stilbenoids (plant metabolites, shikimate pathway)</b> |               |                               |  |                          |  |
| <b>Subclass</b>   | <b>Origin</b> | <b>Examples</b>               | <b>Presence in wine</b>  | <b>Sensory function</b>  | <b>Other character</b>                 |
| aglycones   | G (skin)      | cis and trans-resveratrol     | Higher concentration in red wines<br>Higher concentration in red wines | Astringency              | Antiradical activity                   |
| glycosides  | G (skin)      | piceid                        |  | Astringency              |  |
| <b>Others (yeast metabolites)</b>                         |               |                               |  |                          |  |
| <b>Subclass</b>   | <b>Origin</b> | <b>Examples</b>               | <b>Presence in wine</b>  | <b>Sensory function</b>  | <b>Other character</b>                 |
| phenolic volatiles  | Y             | phenylethanol                 | General  | Scent and aroma (floral) |  |

\*Polyphenols having the ability to precipitate proteins, alkaloids and polysaccharides called collectively *tannins*. [2]. These are strong astringents when ingested. \*\*Chelator ability of flavonols may influence the color, e.g. ferric ion chelates of flavonols can turn it to stronger/reddish from pale yellow. Chelation also can delay ferric/ferrous ion catalyzed oxidative deterioration.

The total polyphenol content and polyphenol profile of wines are mostly determined by the type of the grape. White grape and wines, lacking anthocyanins (and pyranoanthocyanins) [5], poorer in stilbenoids show much lesser TPC than red types. Steps of processing (contact of the musts with grape skins, occasionally with broken seeds), storage (maturation in oak barrels) and ageing also influence it.

There are many studies on polyphenols of mature wine [1], [6], or on following some wine ageing processes from the viewpoint of polyphenol contents [7] or polyphenol composition. Data dealing with the polyphenol content of grape juice during fermentation were not found. However, it would deserve attention as the phenolic maturity of the wine is important from the sensory viewpoints. White wines with high total polyphenol contents are less demanded in the Hungarian market than the ones bearing lower TPC and being fresher, less astringent. Therefore the present study aimed the followings

- 1) to observe the changes in the total polyphenol content of Hungarian Furmint samples during fermentation, thus decide if fermentation process is follow-able with a relatively inexpensive, simple photometric method.
- 2) adaptation of the method established to determination of Folin-Ciocalteu indices by the Hungarian Codex of Wine, to determine total polyphenol contents on wines, partially in the case of high sample numbers and/or restricted quantity of samples.

## Materials and Methods

### *Sampling*

Furmint grape samples were collected in duplicate. Vineyard plots (fields) were Betsek, Király, Nyúlászó, Szt. Tamás, Dancka at Tokaj-Hegyalja region. Grapes were crushed and pressed, must was tempered in cellar.  $K_2S_2O_5$  was added (1ml/L) at inoculation with yeast (20 g/hL) (Mycroferm Arom, Interker-Wein Kft.). The fermentation passed in glass balloon. The must undergoing fermentation was sampled on the 1-15<sup>th</sup> days daily. These samples were filtered on Millipore 0.22  $\mu$ m, 47 mm, sterile filter and stored in deep freezer till analyses.

### *Reagents and instrumentation*

Folin and Ciocalteu's reagent, 2N, AnalAR Normapur,  $Na_2CO_3$  anhydrous a. r., gallic acid, a. r. (VWR) Instrumentation: double-beam spectrophotometer (Shimadzu UV-VIS 1800).

### Analyses

The original EU standard method to determine Folin-Ciocalteu indices on wine [8], referred in the Hungarian Codex of Wine [9], prescribes the followings. 1 ml white wine, 50 ml distilled water, 5 ml Folin and Ciocalteu's and 20 ml 20 m/V% aqueous solution of  $\text{Na}_2\text{CO}_3$  should be filled to 100 ml with distilled water in a volumetric flask. After 30 min at room temperature, absorption at 750 nm is immediately to measure in a 1 cm cuvette. The downsized method keeps these reaction conditions and concentration ratios of sample and reagents but can be processed with one tenth of all the required quantities. Thus to 100  $\mu\text{l}$  sample, 5 ml distilled water, 500  $\mu\text{l}$  Folin-Ciocalteu's and 2 ml  $\text{Na}_2\text{CO}_3$  were filled to 10 ml in a volumetric flask. The samples are homogenous solutions thus downsizing may not increase margin of error. To convert the absorption to total polyphenol contents (which is usually given in gallic acid equivalents, GAE [10]), a calibration made with aqueous solution of gallic acid in 0-1000 mg/l interval. All sample measurements were performed in duplicate, here means are provided. The expected total polyphenol contents for white wine are 200-700 mg GAE/L [6] [7].

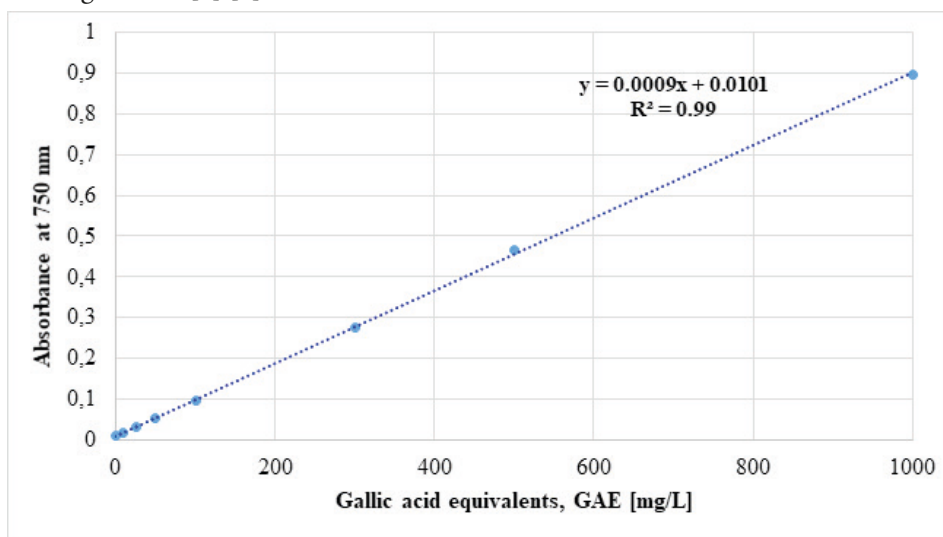


Figure 1. Gallic acid calibration used at the analyses

### Results and discussion

The TPC of all samples during the whole fermentation time was found to be in the interval expected based on the literature data. Figures 2A-E and Table 2 shows the



TPCs in dependence of time, arranged as sample pairs of the five different vineyard plots.

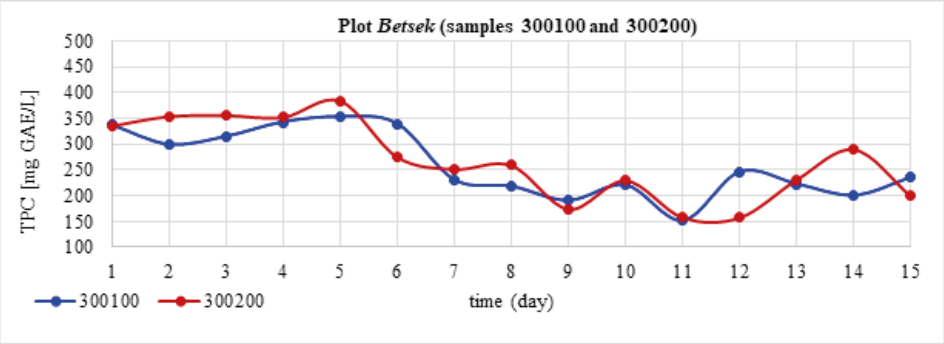


Figure 2A. Total polyphenol content (TPC) in the samples of Betsek vineyard plot

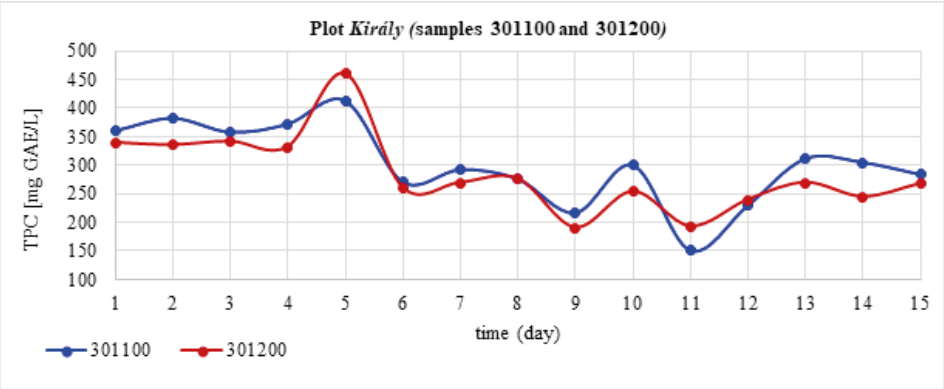


Figure 2B. Total polyphenol content (TPC) in the samples of Király vineyard plot

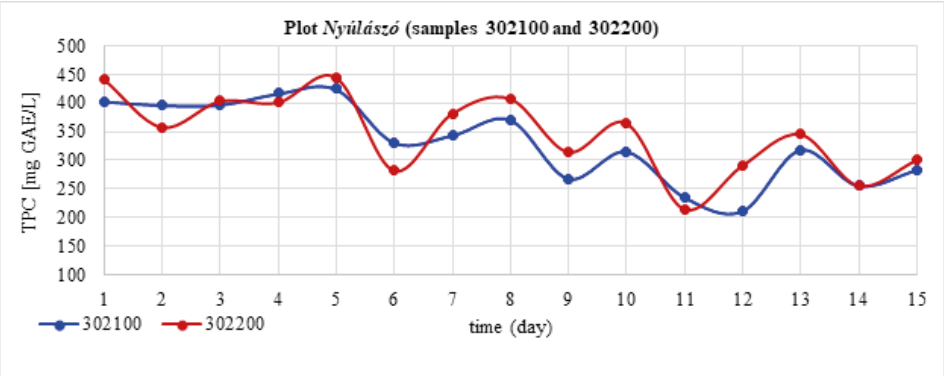
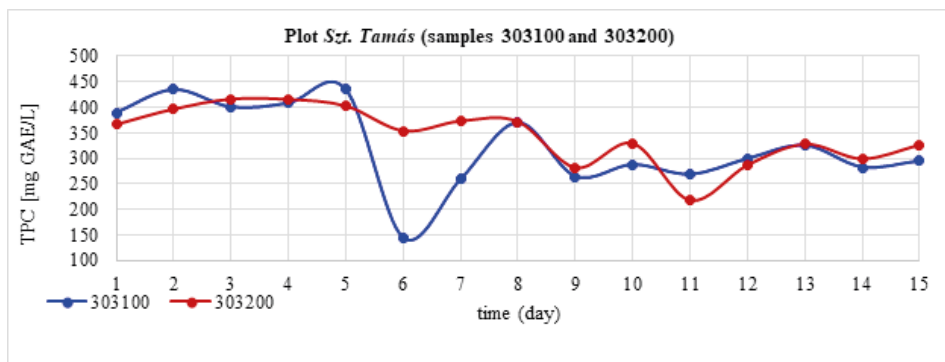
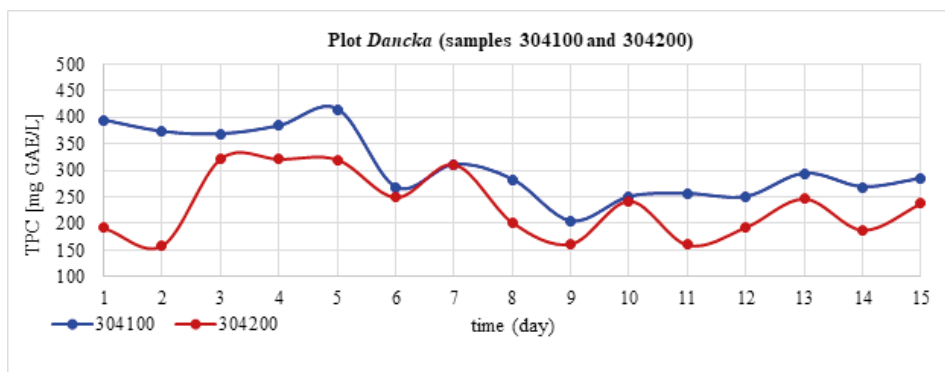


Figure 2C. Total polyphenol content (TPC) in the samples of Nyúlászó vineyard plot



**Figure 2D.** Total polyphenol content (TPC) in the samples of Szt. Tamás winery plot



**Figure 2E.** Total polyphenol content (TPC) in the samples of Dancka winery plot

Table 3 The total polyphenol and its changes in the five sample pairs of Furmint

| Plot       | Sample ID | TPC [mg GAE/L] |       |       |       |       |       |       |       |       |        |        |        |        |        |        | Changes in TPC* |            |
|------------|-----------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|-----------------|------------|
|            |           | Day 1          | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 | Day 15 | %*              | Tendency   |
| Betsék     | 300100    | 337            | 299   | 314   | 342   | 352   | 339   | 230   | 218   | 191   | 220    | 152    | 246    | 223    | 201    | 235    | -30,1           | decreasing |
|            | 300200    | 336            | 354   | 356   | 352   | 384   | 275   | 252   | 259   | 174   | 230    | 158    | 158    | 232    | 290    | 199    | -40,7           |            |
| Király     | 301100    | 360            | 382   | 357   | 372   | 412   | 271   | 292   | 276   | 217   | 300    | 152    | 230    | 311    | 305    | 284    | -21,1           | decreasing |
|            | 301200    | 339            | 335   | 341   | 332   | 460   | 260   | 269   | 276   | 191   | 253    | 193    | 239    | 269    | 244    | 268    | -21,1           |            |
| Nyúlászó   | 302100    | 403            | 396   | 397   | 416   | 424   | 331   | 343   | 371   | 268   | 315    | 235    | 211    | 318    | 256    | 283    | -29,9           | decreasing |
|            | 302200    | 442            | 358   | 404   | 401   | 445   | 282   | 382   | 408   | 315   | 365    | 215    | 291    | 346    | 256    | 300    | -32,2           |            |
| Szt. Tamás | 303100    | 390            | 434   | 400   | 408   | 434   | 143   | 259   | 370   | 264   | 287    | 268    | 299    | 325    | 282    | 295    | -24,4           | decreasing |
|            | 303200    | 366            | 396   | 416   | 415   | 402   | 354   | 373   | 372   | 281   | 329    | 217    | 286    | 327    | 298    | 326    | -10,9           |            |
| Dancka     | 304100    | 394            | 373   | 368   | 384   | 415   | 268   | 311   | 282   | 204   | 250    | 256    | 250    | 294    | 269    | 284    | -27,8           | ambiguous  |
|            | 304200    | 192            | 160   | 322   | 321   | 320   | 250   | 311   | 202   | 162   | 242    | 160    | 193    | 247    | 188    | 238    | 24,1            |            |

\*Change of TPC in percentage is calculated as: [TPC(day 1)-TPC(day 15)/TPC(day 15)]\*100

As it can be seen, in days 1-5 there is no major change in TPCs. In all of the samples values were between 300 and 500 mg GAE/L with one exception (Dancka 2 sample, ID: 304200, initial TPC = 192 mg/L). On day 5-6, in every sample a sudden change of TPC was observable. After this, decrease of TPC was follow-able (except Dancka 2 sample, ID: 304200). Contrary to the fluctuations, to day 15 (the end of fermentation), this loss in polyphenol content has become significant, reaching 10-30 percent of the initial TPC.

The sudden decrease in the TPC around day 5 may be connected with the changes in the composition of the microbiota of the samples under fermentation and thus with emission of yeast metabolites. According to literature data [10] a must (or juice under the beginning of fermentation) has an own flora. This, called apiculate microbes contains low,  $10^6$ - $10^8$  cfu/ml germ count including other taxa than of *Saccharomyces* genus, namely e. g. *Kloeckera* and *Hanseniospora* spp. (Otherwise, apiculate microbes have important role in formation of volatile composition of the wine). These microbes has lower tolerance towards ethanol as of *Saccharomyces* thus the latter becomes dominant around the 4-6<sup>th</sup> days of fermentation. Intensified metabolism of *Saccharomyces* may increase the quantity of reactive oxygen species (ROS) in the juice thus consume a part of the polyphenols of wine through oxidative processes. [7].

Regarding the data of the sample pairs as duplicates of the grape harvested at same time and conditions, our observations are the followings: The data series of Brix values (20.4 -22.6, detailed data not shown) and pH values (3.12-3.31, data not shown) of the 5×2 grape samples contained no outliers and they have shown the optimal interval to harvest [4], thus their initial oenological parameters did not differ significantly. As it was expectable their fermentation is also shows similar tendencies regarding TPCs. Nevertheless there are two exceptions which may be noteworthy to mention. The sample Szent Tamás 1 (ID: 303100) at the 5-6 days has shown a temporary relapse of TPCs from 434 mg GAE/L to 143 mg GAE/L which in part has restored to day 8 (TPC = 370 mg GAE/L) then slowly decreased till day 15. The parallel sample has not shown this behavior. (Fig 2D) Dancka 2 sample (ID: 304200) may deserve more attention as its fermentation is totally different than the Dancka 1 (ID: 304100) when TPC is regarded. The data suggests differences in its polyphenol composition but this background in this study is not investigated yet. There are deviation in the TPCs of Dancka 1 (ID: 304100) and its parallel Dancka 2 (ID: 304200). It may originate in some differences in the fermentation process of these two samples.

This phenomenon may make probable if some types of deviations or faults in the fermentation process of a grape juice may be follow-able via measurement of TPC. But this problem have got to perform further investigations by all means for to make the answer clear, because our observations origin in only a lesser number of samples.

## Concluding remarks. Proposals

The described (downsized) method to determine total polyphenol content of grape juice under fermentation resulted in TPC data inside the interval of literature data of white wines. We would like emphasize that the TPC measurement has no international or domestic standard and in this case the method can be connected/ deduced to a standard spectrophotometric determination accomplished with a gallic acid calibration. The reduction of the requested quantities of reagents and sample (in comparison with the original Folin-Ciocalteu method may make it to applicable large sample series with low costs. In long term, the follow-up of TPC, the establishing of phenolic maturity in a white wine may be performed using this method if needed. Based on our examinations, phenolic maturity of white wines can be characterized via continuous monitoring of TPC levels during fermentation, however further studies are needed to improve the method.

The tendency of changes in TPC during fermentation is accordance with expectations. But the background of it, partially of the temporary fluctuations need further investigations. They are probably connected to redox reactions and rearrangements in the polyphenol composition of the samples, which composition may clarify with high performance liquid chromatography HPLC investigations on the polyphenol profile.

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