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Comparative assessment of Blaufränkisch wines from Central Europe: Antioxidant activity and polyphenolic study

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Abstract: This model comparative study evaluated 16 monovarietal Blaufränkisch red wines from the Czech Republic, Hungary, Austria, and Slovakia focused on five parameters related to their antioxidant properties: total polyphenol content (TPC), total anthocyanin content (TAC), antioxidant activity assessed by the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radical scavenging assay and the ferric reducing antioxidant power (FRAP) assays, and wine colour intensity. Multivariate statistics were applied. The FRAP assay showed a very strong positive correlation with TPC (r = 0.968), supporting the central role of polyphenols in wine antioxidant activity. The highest TAC (285.6 mg·L^{-1}) was observed in the Czech sample, while the lowest value (48.0 mg·L^{-1}) was found in the Hungarian sample (overall mean 159.61 mg·L^{-1}). FRAP results ranged from 13.6 to 21.53 mmol TE·L^{-1}, with the highest value in the Austrian wine and the lowest in the Hungarian wine. DPPH activity showed a strong negative correlation with wine colour (r = –0.924) and a moderate negative correlation with TPC (r = –0.606). Statistically significant differences among countries were found for DPPH, FRAP and colour (Austria and Czech Republic for colour, P = 0.003 226). FRAP method, which aligns with TPC, appears to be better suited for characterising Blaufränkisch antioxidant activity than DPPH.

Keywords: total polyphenol content; total anthocyanin content; wine colour; DPPH; FRAP; wine origin

Blaufränkisch is a traditional Central European red wine grape variety originating from the region between Austria and Slovakia. Slovakia, the variety has been officially registered since 1941 (Ailer 2016).

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It is a late-ripening variety, typically harvested in the second decade of October (Poklar Ulrih et al. 2020). The high-quality red wines are generally produced when grapes are harvested at sugar levels exceeding 20.5 °Bx (85 °Oe). One of the key attributes of wine is its antioxidant potential, mainly derived from biologically active polyphenolic compounds, which represent the major phytochemicals in grapes and wine and are associated with healthpromoting effects (Onache et al. 2023). Grapes and wines contain phenolic acids, flavonoids, anthocyanins, proanthocyanidins, and stilbenes, which significantly contribute to antioxidant activity and wine stability (Buljeta et al. 2023). According to Fusco et al. (2023), the region of grape origin includes environmental, soil, climatic, cultural, and traditional factors that influence a wine's uniqueness and quality. This concept is associated with terroir, reflecting the combined effects of natural conditions and human practices (Aurier et al. 2005). Winegrowing regions are characterised by specific cultivation practices, such as vine training systems, labour intensity, and planting density, as well as by technological approaches including barrel ageing, the use of local yeasts, and fermentation and maceration techniques (Artem et al. 2016; Regecová et al. 2024).

The aim of this model study was to screen Blaufränkisch wines, based on their selected chemical properties. It is focused on the antioxidant potential and polyphenol content of wines important for consumer, which originated from four Central European regions and different vintages. The main part of the research was focused on advanced statistical approaches applied in order to assess data reflecting antioxidant properties of consumeravailable wines and contribute to the limited knowledge on one of the most important monovarietal red wine in the Central Europe.

MATERIAL AND METHODS

Wine samples

Samples of Blaufränkisch varietal wines originated from four Central European countries. Their selection was based on their availability at the Slovak market. All wines included in the study were obtained at the same time from the Slovak retail network; therefore, the sample set represents Blaufränkisch wines classified as dry to semi-dry. The study therefore focuses on the assessment of quality parameters relevant to consumers of this varietal wine. In these samples, total antioxidant activity (TAA – using the DPPH and FRAP methods), total polyphenol content (TPC), total anthocyanin content (TAC), and wine colour were determined. The wines originated from the Czech Republic (F1–F4), Hungary (F5–F8), Austria (F9–F12), and Slovakia (F13–F16). The samples are described based on data provided by producers in Table 1.

Chemicals, laboratory material and apparatus employed

For the determination of total polyphenol content, the following chemicals were used: Folin–Ciocalteu phenolic reagent (p.a., Centralchem, Slovakia), anhydrous sodium carbonate (p.a., 99%; Centralchem, Slovakia), 3,4,5-trihydroxybenzoic acid (gallic acid monohydrate, p.a., 99%; Alfa Aesar Thermo Fisher (Kandel) GmbH, Germany), and distilled water.

Antioxidant activity methods employed: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich, Merck KGaA, Germany), methanol (CHROMASOLV™, ≥ 99.9%, Riedel-de Haën, Switzerland), ferric chloride ($FeCl_3 \cdot 6H_2O$, p.a., Centralchem, Slovakia), glacial acetic acid (extra pure, min. 99.8 %, Centralchem, Slovakia), sodium acetate (p.a., Centralchem, Slovakia), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (≥ 98%, Supelco, Merck KGaA, Germany), and catechin (98 %, Supelco, Merck KGaA, Germany).

For the determination of total anthocyanin content, hydrochloric acid (p.a., 35%, Centralchem, Slovakia), sodium metabisulphite (p.a., Centralchem, Slovakia), malvidin-3-monoglucoside chloride (Merck KGaA, Germany), and McIlvaine buffer (pH = 3.5) were used. Instruments employed in the analyses were the UV/VIS spectrophotometer (Shimadzu UV/VIS–1240, Shimadzu, Japan), pH meter (3510 pH Meter, Jenway, Cole-Parmer, USA), and a laboratory scale (BP 110 S, Sartorius, Germany).

Method used

Determination of total antioxidant activity (TAA) using the DPPH method. Antioxidant activity was analysed by the modified method based on Brand-Williams et al. (1995) employing the DPPH radical. Precisely 3.9 mL of the DPPH methanolic solution (25 mg DPPH·L^{-1}) was pipetted directly into a glass cuvette. The initial absorbance of the DPPH solution was measured spectrophotometrically at a wavelength of 515.6 nm. Subsequently, 0.1 mL of the wine sample was added, mixed thoroughly, and incubated in the dark for 10 min. Absorbance was then measured again at 515.6 nm, and antioxidant activity was expressed

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Table 1. Description of Blaufränkisch varietal wine samples

Sample designation	Vintage	Type of wine	Viticultural region/country	Residual sugars ($g \cdot L^{-1}$)	Alcohol content (vol. %)	Total acidity as TA ($g \cdot L^{-1}$)
F1	2022	S-D	Morava/CZ	N	12.50	N
F2	2022	D	Morava/CZ	N	11.50	N
F3	2022	S-D	Morava/CZ	14.20	12.00	4.80
F4	2022	D	Morava/CZ	N	11.50	N
F5	2022	D	Eger/HU	N	12.00	N
F6	2022	D	Duna-Tisza közi/HU	N	11.50	N
F7	2021	D	Dunántúl –Tolna, Mőcsény/HU	0.80	13.50	4.80
F8	2018	D	Eger/HU	1.00	13.50	5.96
F9	2019	D	Thermenregion/AT	N	13.00	N
F10	2022	S-D	Neusiedlersee/AT	N	13.50	N
F11	2021	D	Burgenland – Rabenkropf/AT	1.10	13.00	5.40
F12	2020	D	Burgenland/AT	2.70	13.00	4.50
F13	2020	D	SCVR/SK	5.50	11.50	5.67
F14	2020	D	SCVR/SK	2.50	12.00	5.60
F15	2020	D	CSVR, Bušince – Čomor/SK	N	12.50	N
F16	2023	D	MVR/SK	4.60	12.50	5.70

D – dry wine; S-D – semi-dry wine; N – not specified; TA – tartaric acid; SCVR – Small Carpathian Viticultural Region; CSVR – Central Slovak Viticultural Region

as percentage inhibition of the DPPH radical according to Equation 1:

$$\%\ inhibition\ of\ DPPH = \frac{(A_0 - A_s) - (A_t - A_s)}{(A_0 - A_s)} \times 100 \quad (1)$$

where: A_0 – absorbance of DPPH radical solution before sample addition; A_s – absorbance of methanol; A_t – absorbance of DPPH radical solution after sample addition and 10 min of incubation.

For calculation purposes, the absorbance of the methanol as a solvent was also measured at the same wavelength. All analyses were performed in triplicate.

Determination of total antioxidant activity (TAA) using the FRAP method. The procedure followed the modified protocol described by Paulová et al. (2004). The FRAP reagent solution is prepared by mixing TPTZ with ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). The acetate buffer (pH = 3.6) is prepared by mixing 400 mL of 0.1 $mol \cdot L^{-1}$ acetic acid with 200 mL of 0.1 $mol \cdot L^{-1}$ sodium acetate. Calibration curve for Trolox standard was in the range 0–50 mmol $Trolox \cdot L^{-1}$. Using an automatic pipette, 6 mL of the FRAP reagent solution is dispensed into test tubes, to which 25 µL of the wine sample was added. After vortex mixing, the tubes are placed in a water bath at 37 °C for 30 min in the dark. Absorbance was then measured spectrophotometrically (Shimadzu UV-1800 spectrophotometer) at the wavelength of 593 nm after 30 min. Results are expressed as the Trolox equivalents (TE) (mmol $TE \cdot L^{-1}$).

Determination of total polyphenol content (TPC). The total polyphenol content was determined by modified method (Jakabová et al. 2021) based on Singleton and Rossi (1965), employing: 20% Na_2CO_3 solution, Folin–Ciocalteu reagent, and distilled water. Diluted red wine sample (1: 10), 1 mL was pipetted into 50 mL volumetric flask and diluted with 25 mL of distilled water. Next, 2.5 mL of Folin–Ciocalteu reagent (diluted 1 : 1 with distilled water) was added, and after 3 min, 1.5 mL of 20% Na_2CO_3 solution was added. The sample was then made up to 50 mL with distilled water, mixed thoroughly, and left to stand at room temperature for 2 h. Blank sample was prepared by adding all the reagents listed above and filling up to volume 50 mL with distilled water. Calibration solutions of gallic acid were prepared in the same manner in the range of 50–500 $mg \cdot L^{-1}$ in 50 mL volumetric flasks. The absorbance of the sample solutions was measured against the blank at the wavelength of 765 nm. Total polyphenol content in the wines was calculated as gallic acid equivalent (GAE) in mg per litre of wine (mg $GAE \cdot L^{-1}$). All analyses were performed in triplicate.

Determination of total anthocyanin content (TAC). The total anthocyanin content was deter-

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mined by modified method following Laporník et al. (2005). Wine samples were diluted fivefold and adjusted to yield absorbance values between 0.5 and 0.8 at 520 nm. The appropriately diluted sample was pipetted into two test tubes in 1 mL volumes, with 1 mL of HCl in ethanol (0.01 %) added to each one. Next, 10 mL of aqueous HCl solution (2%) was added to the first test tube, and 10 mL of McIlvaine buffer (pH = 3.5) to the second one. Two variants of the blank were prepared similarly using distilled water instead of diluted wine. Samples were sealed and mixed in the test tubes and, after transfer to cuvettes, absorbance was measured at 520 nm against the blank. All analyses were performed in triplicate. Total anthocyanin content was calculated as malvidin-3-glucoside equivalents (Ma-3-gl) by the following equation:

$$TAC = f_z \times 12 \times \Delta A \times M \times 1\,000 / d / e \quad (2)$$

where: TAC – total anthocyanin content (mg Ma-3-gl·L^{-1}); f_z –the wine dilution factor, ΔA –the difference in absorbance of diluted wine solutions measured at 520 nm; d – the path length of the macro-cuvette (1 cm), M – the molar mass of malvidin-3-glucoside (493.44 g·mol^{-1}); ε – the molar absorptivity of malvidin-3-glucoside (28 000 dm^3·mol^{-1}·cm^{-1}).

Determination of wine colour. Wine colour reflects its anthocyanin content, with colour intensity recorded at wavelengths of 420 nm and 520 nm by modified method of Sudraud (1958). The absorbance of red wine samples was measured in a 0.2 cm path length cuvette against distilled water. Samples were diluted with dilution factor Df = 5, and absorbances at 520 nm and 420 nm were measured. Colour intensity was calculated as the sum of both absorbance values (Equation 3). All analyses were conducted in triplicate, with results expressed in absorbance units (AU).

$$Wine\ colour = A520 + A420 \quad (3)$$

Statistical analysis of results

Basic descriptive statistics, including calculation of the means and standard deviations, were applied at the total polyphenol content, total antioxidant activity, total anthocyanin content, and wine colour. Boxplot visualisations were employed in order to evaluate the distribution, central tendency, and variability of the chemical parameters at the Blaufränkisch wine samples. In the study, boxplots revealed the dispersion of data points. Analysis of variance for non-parametric data was performed using the Kruskal–Wallis test with Dunn's post hoc test, which was subsequently applied to perform pairwise comparisons and determine homogeneous sub-groups. Correlations between parameters were analysed using Pearson's correlation test. Pearson's linear correlation analysis was performed to measure the direction and strength of linear relationships between pairs of continuous chemical variables, using uncorrected P-values to evaluate statistical significance ($P < 0.05$). Samples were classified by hierarchical cluster analysis that was implemented to group wine samples based on overall chemical similarity without *a priori* assumption of regional origin. Dissimilarity between samples was calculated using Euclidean distance matrices, and clusters were agglomerated using Ward's minimum variance method to minimise intra-cluster variance. The hierarchical structure was visualised via a dendrogram. Principal component analysis (PCA) was applied as an unsupervised multivariate dimensionality-reduction technique to transform the correlated chemical parameters into orthogonal, uncorrelated variables termed principal components (F1, F2). This approach captured the maximum variance within the multi-parameter dataset to map the spatial distribution of the wines. Data analysis and processing were prepared using Excel, XLSTAT (v. 2023.1.2, Addinsoft, France), and Past4.03 software (2001).

RESULTS AND DISCUSSION

Antioxidant activity (DPPH and FRAP methods) of wines

Antioxidant activity by the DPPH and FRAP assays is presented in Figure 1 using boxplots. Although both methods are based on an electrontransfer mechanism (Apak et al. 2013), differences in overall trends among wine samples were observed.

The highest antioxidant activity measured by the DPPH method was recorded in sample F4 from Moravia, Czech Republic (87.5%), while the lowest value was found in sample F14 from the Small Carpathian region, Slovakia (79.8%). The mean DPPH antioxidant activity of all samples was 84.44%. At the country level, the greatest difference in DPPH antioxidant activity was observed between Czech (mean 85.87%) and Austrian wines (mean 82.97%). Among Czech samples, F3 exhibited significantly lower DPPH antioxidant activity, whereas sample F1 showed values comparable to the remaining Czech wines. Within Austrian samples, the highest DPPH antioxidant activ-

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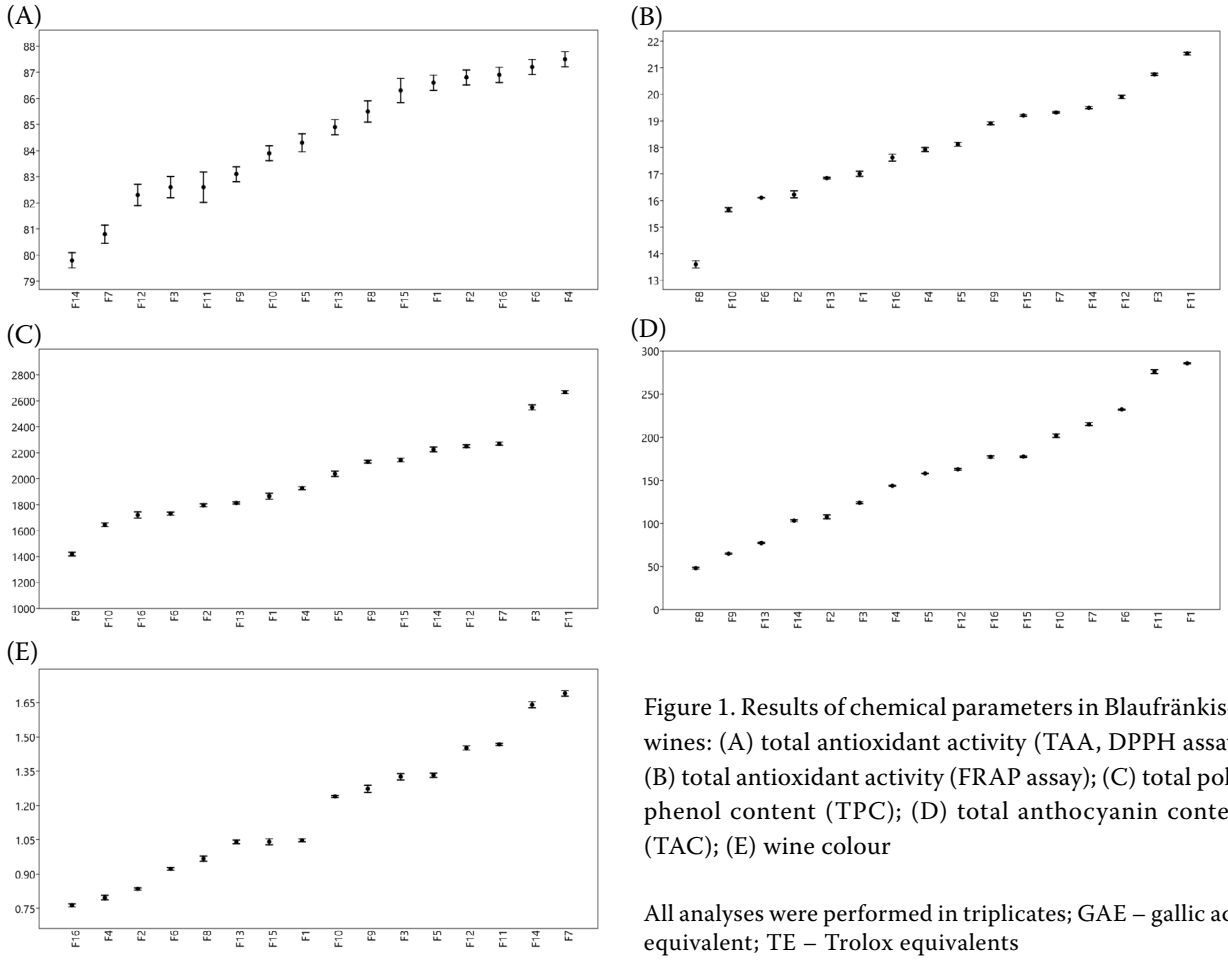

Figure 1. Results of chemical parameters in Blaufränkisch wines: (A) total antioxidant activity (TAA, DPPH assay); (B) total antioxidant activity (FRAP assay); (C) total polyphenol content (TPC); (D) total anthocyanin content (TAC); (E) wine colour

All analyses were performed in triplicates; GAE – gallic acid equivalent; TE – Trolox equivalents

ity was observed in sample F10 from the Neusiedlersee region. Using the FRAP assay, the highest antioxidant activity was recorded in sample F11 from Austria (Rabenkropf, 21.53 mmol TE·L^{-1}), while the lowest value was observed in sample F8 from Hungary (Eger, 13.6 mmol TE·L^{-1}). Mean FRAP antioxidant activity across all samples was 18.02 mmol TE·L^{-1}. At the country level, the greatest difference was observed between Austrian (mean 19 mmol TE·L^{-1}) and Hungarian wines (mean 16.78 mmol TE·L^{-1}). Among Czech samples, F3 exhibited higher FRAP antioxidant activity; however, this trend was not confirmed by the DPPH assay. Sample F1 showed values comparable to the remaining Czech wines. In contrast to the DPPH results, sample F10 exhibited the lowest antioxidant activity among Austrian wines by the FRAP method.

Boxplot analysis indicated wider value ranges for the DPPH assay, particularly in samples F11 and F15, whereas FRAP results showed generally narrower distributions, with higher variability observed for samples F8, F2, and F16. Statistical evaluation using the Kruskal–Wallis test followed by Dunn's post hoc test revealed two homogeneous groups for both DPPH and FRAP assays, with statistically significant differences between groups ($P < 0.05$).

Earlier studies reported DPPH inhibition values ranging from 69% to 86%, confirming that Blaufränkisch belongs among red wine varieties with high antioxidant activity.

Čeryová et al. (2021) demonstrated a strong positive correlation between total polyphenol content and antioxidant activity assessed by DPPH and FRAP methods, suggesting that wines richer in polyphenols and anthocyanins exhibit higher antioxidant potential. Similar findings were reported by Poklar-Ulrih et al. (2020), who observed increased antioxidant activity in longer-macerated Blaufränkisch wines produced in Slovenia. DPPH inhibition ranged from 70% to 85%, while FRAP values varied between 15 and 30 mmol TE·L^{-1}, with higher antioxidant activity associated with higher polyphenol content. The absolute antioxidant values observed in this study (FRAP ranging from 13.60 to 21.53 mmol TE·L^{-1}) align closely with the upper limits reported for thermomacerated

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Blaufränkisch and Saint Laurent musts, where thermal extraction at 80 °C for 20 min enhanced antioxidant capacity up to tenfold (reaching 19.9–24.0 mmol Trolox·L^{-1}) (Tříska et al. 2016). This substantial radical scavenging potential is primarily driven by the thermal disruption of cellular membranes in grape skins, facilitating the release of key stilbenes – such as *trans*-resveratrol and *trans*-piceid – alongside hydrophilic monomeric flavonoids (Tříska et al. 2016). Moreover, the antioxidant response in red wine matrixes is governed not solely by total polyphenolic yield, but also by non-phenolic constituents and specific monomeric phenolic profiles, which explains why raw musts occasionally exhibit weak or non-significant correlations between phenolic content and total antioxidant capacity prior to vinification (Balík et al. 2008).

Total polyphenol content (TPC) of wines

Phenolic compounds are key constituents of wine and important indicators of quality and authenticity related to grape variety, terroir, and winemaking practices (Semjon et al. 2025). As shown in Figure 1C, the highest total phenolic content (TPC) was recorded in sample F11 from Austria (Rabenkropf region; 2 667 mg GAE·L^{-1}), while the lowest in sample F8 from Hungary (Eger region; 1 419 mg GAE·L^{-1}). Boxplot analysis indicated generally low variability in TPC values, with higher dispersion observed in samples F1 and F3 (Czech Republic, Moravia) and F16 (Slovakia). Among Czech wines, the semidry sample F3 exhibited higher TPC, whereas sample F1 showed values comparable to the remaining Czech samples. In contrast, the semidry Austrian sample F10 displayed one of the lowest TPC values. Evaluation of semidry wines (F1, F3, F10) did not reveal systematic differences, indicating that residual sugar content did not substantially influence TPC determination using the Folin–Ciocalteu method. Kruskal–Wallis analysis followed by Dunn's post hoc test identified two homogeneous groups with statistically significant differences between them ($P < 0.05$). Extended maceration of Blaufränkisch grapes has been shown to increase total phenolics, flavonoids, anthocyanins, and resveratrol, while maturation leads to a general decline in phenolic parameters, except for resveratrol (Poklar Ulrih et al. 2020). Similarly, Branco et al. (2025) demonstrated that when grape variety, region, and vintage are constant, fermentation technique is the dominant factor affecting phenolic extraction and antioxidant capacity.

TPC values for Blaufränkisch wines show wide variability across regions, including Slovakia, Moravia, Macedonia, and Austria (Eder et al. 2023). These ranges are consistent with our results, where TPC values ranged from 1 645 mg GAE·L^{-1} (sample F10) to 2 667 mg GAE·L^{-1} (Austrian sample). Phenolic content and antioxidant activity are primarily shaped by vinification and maturation processes, with some compounds, such as resveratrol, exerting a disproportionate influence on antioxidant capacity despite their low concentrations (Hou et al. 2023; Božović et al. 2025). The marked variability in TPC observed across the analysed Central European samples (1 419 to 2 667 mg GAE·L^{-1}) reflects the profound impact of pre-fermentative and clarification operations on phenolic extraction and retention (Balík et al. 2008; Garrido and Borges 2013; Tříska et al. 2016). Pre-fermentative thermomaceration (80 °C for 20 min) has been demonstrated to increase total polyphenols by up to 5.4-fold in organic Blaufränkisch (reaching 4 023 mg GAE·L^{-1}) (Tříska et al. 2016). However, subsequent technological procedures can severely deplete specific bioactive fractions (Balík et al. 2008; Garrido and Borges 2013). For instance, fining treatments using polyvinylpolypyrrolidone (PVPP) induce drastic losses of *trans*-resveratrol (72–94%), whereas proteinaceous clarifiers like egg white selectively precipitate flavan-3-ols, reducing (+)-catechin and (–)-epicatechin concentrations by 71–73% (Balík et al. 2008). These technological interventions alter the natural C6-C3-C6 flavonoid skeleton ratio and hydroxyl substitution patterns (Garrido and Borges 2013), thereby modulating the net polyphenolic profile independently of geographical origin.

Total anthocyanin content (TAC) of wines

TAC is a key parameter of red wine quality and is influenced by grape variety, ripeness, health status, and winemaking practices (Balga et al. 2022). In our study (Figure 1D), TAC ranged widely, with the highest value observed in the Czech Blaufränkisch from Moravia (F1; 285.6 mg·L^{-1}) and the lowest in the Hungarian sample from Eger (F8; 48 mg·L^{-1}). Higher dispersion was observed in Czech and Austrian wines. Nonparametric ANOVA followed by Dunn's posthoc test revealed two homogeneous groups based on total anthocyanin content. Samples F8, F9, F13, F14, F2, F3, F4, F5, F12, F16, and F15 formed the first group without significant internal differences, while samples F10, F7, F6, F11, and F1 constituted the second group. Statistically significant differences were observed between these two groups (P <0.05).

Reported TAC values for Blaufränkisch wines in the literature generally range from 70 to 450 mg·L^{-1}, de-

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pending on origin, vintage, and wine age (Bajčan et al. 2011; Čeryová et al. 2021; Eder et al. 2023). Slovak, Austrian, Hungarian, and Slovenian studies report mean values comparable to those obtained in this study, confirming that Blaufränkisch typically belongs to varieties with moderate to lower anthocyanin content.

Wine colour

Wine colour is a key sensory attribute affected mainly by anthocyanin content and technological factors. The boxplot of wine colour (Figure 1E) shows the highest variability in the Austrian sample F9 (Gumpoldskirchen) and the Czech sample F3 (Moravia). The highest colour value was observed in the Hungarian sample F7 (Tolna; 1.69), while the lowest occurred in the Slovak sample F16 from the Small Carpathian Viticultural Region (0.76). The mean colour value across all samples was 1.18, with Austrian wines showing higher average colour intensity (1.35) than Czech wines (1.00). Nonparametric Kruskal–Wallis ANOVA followed by Dunn's posthoc test classified the samples into three homogeneous groups with significant differences between them ($P < 0.05$), while no significant differences were observed within individual groups.

Wine colour values in the literature correspond with our results. Bajčan et al. (2011) reported colour values ranging from 0.75 to 2.78 (mean 1.39). Wine colour intensity is affected by anthocyanin concentration, pH, aging, and vinification practices, including fermentation and storage conditions, which simultaneously influence phenolic composition and antioxidant properties (Lachman et al. 2009; Cave and Waterhouse 2019). The wide spectrum of TAC (48.0 to 285.6 mg·L^{-1}) and colour intensity (0.76 to 1.69 AU) across the evaluated Blaufränkisch wines can be mechanistically attributed to anthocyanin equilibrium shifts and structural degradation pathways occurring during fermentation and aging (Garrido and Borges 2013). Monomeric anthocyanins (predominantly malvidin-3-O-glucoside) are highly susceptible to nucleophilic attack, autoxidation, and enzymatic cleavage, yielding chalcone intermediates and smaller degradation products such as syringic acid (Garrido and Borges 2013). During wine maturation, free anthocyanins undergo condensation reactions with flavan-3-ols – either directly or mediated by acetaldehyde bridges – as well as cycloaddition with pyruvic acid or vinylphenols, giving rise to pyranoanthocyanins (e.g. vitisins and portisins) (Garrido and Borges 2013). These polymeric pigments exhibit enhanced thermodynamic stability against bisulphite bleaching and pH-induced discolouration (Garrido and Borges 2013). Furthermore, enological fining agents differentially modulate colour parameters: while egg white treatment reduces total anthocyanins by 32–34%, it paradoxically enhances colour intensity and advances the chemical age of the wine by promoting the proportion of polymeric pigment complexes (Balík et al. 2008).

Statistical evaluation of the results

Differences among individual parameters across countries of origin were observed. The results of non-parametric ANOVA (Kruskal–Wallis test), followed by Dunn's post hoc test, revealed statistically significant differences related to the country of origin. The outcomes of the statistical analysis, including the calculated *P*-values, are presented in Table 2.

For antioxidant activity, significant differences were observed among countries, with the largest differences between Czech and Austrian wines for the DPPH and between Austrian and Hungarian wines for FRAP. No statistically significant differences in total polyphenols and total anthocyanins were detected among samples with respect to country of origin ($P > 0.05$). Regarding wine colour, significant differences were recorded between samples originating from Austria and the Czech Republic ($P = 0.003\,226$), indicating differences in co-

Table 2. Statistical evaluation of Blaufränkisch varietal wines by countries of origin

Parameter	Origin	HU	AT	SK
TAA (DPPH)	CZ	0.148 8	0.001 439*	0.207 1
	HU		0.081 36	0.855 3
	AT			0.054 21
TAA (FRAP)	CZ	0.339 6	0.212 5	0.620 1
	HU		0.027 69*	0.146 8
	AT			0.452 7
TPC	CZ	0.565	0.712 5	0.969 1
	HU		0.098 17	0.828 4
	AT			0.437 1
TAC	CZ	0.999 9	0.956 9	0.676 2
	HU		0.938 5	0.717 9
	AT			0.371 4
Wine colour	CZ	0.089 39	0.003 226*	0.418 4
	HU		0.212 5	0.373 8
	AT			0.032 67*

*significance at 0.05 level; TAA (DPPH) – total antioxidant activity by DPPH method; TAA (FRAP) – total antioxidant activity by FRAP method; TPC – total polyphenol content; TAC – total anthocyanin content

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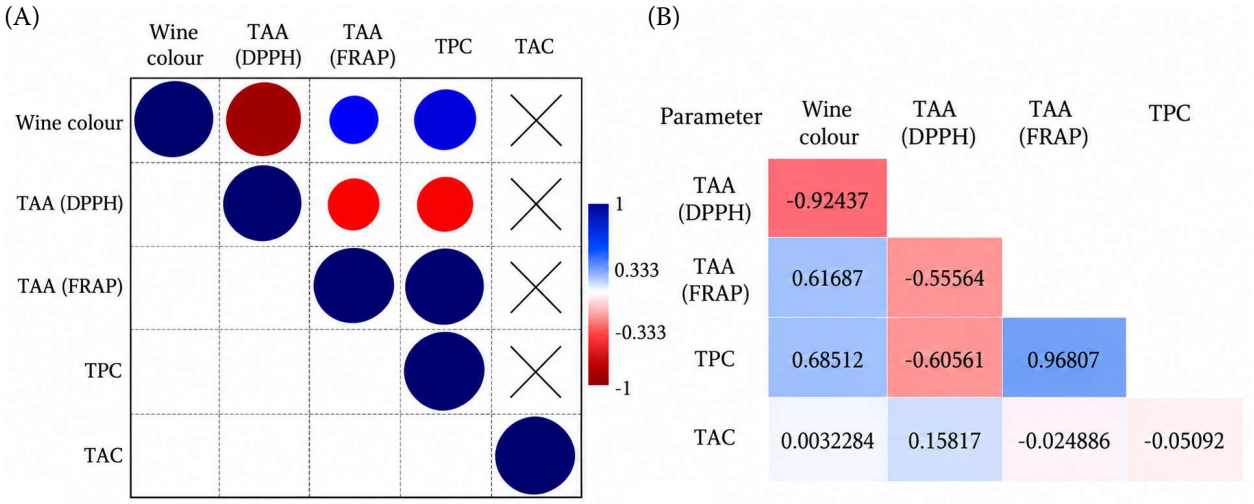

Figure 2. Graphical representation of linear r correlation with (A) uncorrected *P*-values and (B) Pearson correlation coefficients

TAA (DPPH) – total antioxidant activity by DPPH; TAA (FRAP) – total antioxidant activity by FRAP; TPC – total polyphenol content; TAC – total anthocyanin content

louring properties. Wines originating from Austria differed statistically from wines of other origins in three parameters: both antioxidant activity parameters (DPPH and FRAP) and wine colour.

Pearson's linear correlation coefficient (r) with uncorrected *P*-values was applied to evaluate correlations among the monitored chemical properties. Positive correlations are displayed in blue, while negative correlations are shown in red. The diameter of each circle corresponds to the strength of the correlation, with larger diameters indicating stronger correlations. Correlations with very low coefficients were not statistically significant ($P > 0.05$) and are marked by a cross in Figure 2.

The strongest positive correlation was observed between the FRAP method and TPC ($r = 0.968$), indicating a close association between these parameters. Similar strong positive corelations between FRAP and TPC also been reported by Fotakis et al. (2012), with a slightly lower correlation observed for DPPH. A very strong negative correlation was found between wine colour and antioxidant activity measured by the DPPH method ($r = –0.924$), which is consistent with the findings of Bajčan et al. (2017). FRAP assay – which demonstrated an exceptionally strong positive correlation with TPC ($r = 0.968$) is in agreement with the robust linear relationships ($r = 0.8881$) reported for thermomacerated red musts (Tříska et al. 2016). FRAP assay provides a superior reflection of the true reducing potential of Blaufränkisch wines, as it directly measures the redox transformation of hydrophilic polyphenolic antioxidants without being confounded by pigment condensation state or steric constraints (Balík et al. 2008; Garrido and Borges 2013; Tříska et al. 2016).

In addition, a moderate positive correlation was observed between TPC and wine colour ($r = 0.685$). A strong negative correlation was observed between the DPPH method and TPC ($r = –0.606$), which is consistent by Čeryová et al. (2021). In addition, a negative correlation was found between the DPPH and FRAP methods ($r = –0.556$).

The DPPH assay is widely used for rapid screening of antioxidant activity, particularly for low-molecular-weight phenolic compounds. In contrast, the FRAP assay is suitable for assessing TAA in hydrophilic systems such as wines, although it does not directly evaluate radical scavenging activity (Apak et al. 2013).

Based on the correlation analysis, the FRAP assay proved to be more suitable for wine evaluation. Stratil et al. (2008) reported higher values obtained by the FRAP method compared to the DPPH assay using the same standards.

The strongest correlation was found between TPC and FRAP, in agreement with Fotakis et al. (2012). Wine colour showed a strong negative correlation with antioxidant activity by the DPPH method, which is sensitive to sample pH and may be influenced by pH during colour measurement.

Multivariate analysis of origin and antioxidant properties. By principal component analysis (PCA) used, the first two principal components (F1 and F2) explain 87.45% of the total variability. Component F1 (65.06%) is

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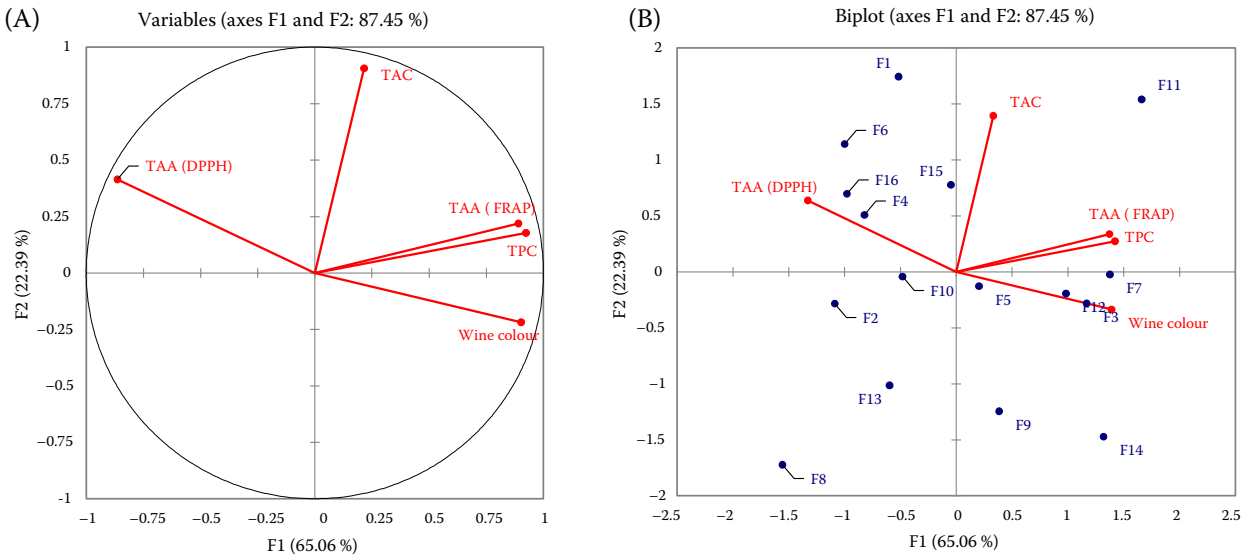

Figure 3. PCA biplot illustrating the relationships between wne colour, antioxidant activity, total polyphenol content, and total anthocyanin content: (A) PCA variables and (B) PCA biplot map

TAA (DPPH) – antioxidant activity by DPPH method, TAA (FRAP) – antioxidant activity by FRAP method, TPC – total polyphenol content, TAC – total anthocyanin content

mainly associated with antioxidant activity by the FRAP assay, total polyphenols, and wine colour, indicating a close relationship among these variables. Component F2 (22.39%) is affected primarily by total anthocyanins and DPPH method. The PCA loadings show that FRAP, total polyphenols, and wine colour are strongly correlated, whereas DPPH and total anthocyanins exhibit a different behaviour. Total anthocyanins contribute minimally to F1 but dominate component F2, while the remaining variables have only minor influence on F2.

The PCA biplot (Figure 3B) reveals a clear distribution of wine samples by their chemical characteristics. Samples located on the right side of the biplot are mainly associated with higher FRAP antioxidant activity, total polyphenol content, and wine colour, whereas samples on the left are characterised by higher DPPH antioxidant activity and lower FRAP values. Samples positioned in the lower left quadrant show generally lower antioxidant activity, wine colour intensity, and total anthocyanin content.

Hierarchical cluster analysis. Ward's method and the Euclidean distance was applied to evaluate similarities among wine samples and visualised by a dendrogram (Figure 4). Potential classification of wines by selected chemical parameters and country of origin, clustering into two (K = 2) and four (K = 4) groups was evaluated.

At K = 2, the samples were divided into two major clusters; however, wines originating from different countries were mixed within both groups. This indicates that the selected chemical properties do not allow discrimination of wines by country of origin, reflecting substantial variability of chemical composition within individual countries.

At K = 4, four clusters were obtained, but each consisted of samples from multiple countries. Cluster 1 comprised Hungarian (F5) and Slovak wines (F14, F15), cluster 2 included Austrian (F9, F12, F11), Hungarian (F7), and Czech (F3) samples, cluster 3 grouped Slovak (F16), Hungarian (F6), and Austrian (F10) wines, and

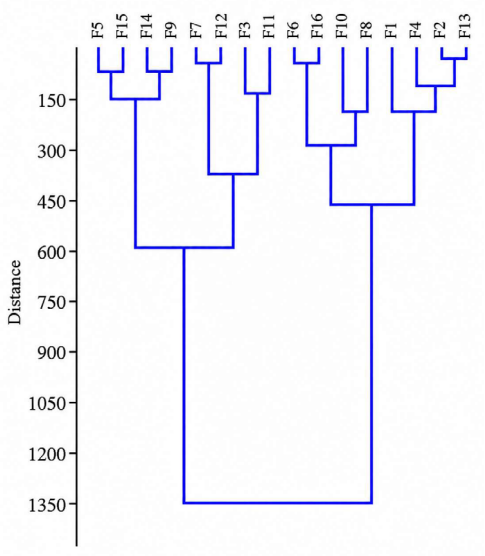

Figure 4. Hierarchical cluster analysis of wines

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cluster 4 contained Hungarian (F8), Czech (F1, F2, F4), and Slovak (F13) samples.

Dendrogram reflects similarities in chemical composition that occur across different regions rather than regional specificity. In contrast, HerasRoger et al. (2025) reported successful classification of monovarietal red wines based on organic acid profiles, achieving classification accuracies of up to 88.3% by cultivar and 83.4% by island of origin, indicating that other compositional markers and multivariate approaches may provide higher discriminative power. Alcoholic beverages are economically and culturally important and susceptible to fraud, isotopic analyses could by valuable tool for geographical authentication (Roncone and Bontempo 2025).

CONCLUSION

In this model study significant differences in TPC, TAA, and wine colour were observed among Blaufränkisch wines originating from four European countries, both at the level of individual samples and by country of origin. Czech and Austrian wines exhibited higher antioxidant activity compared to Slovak and Hungarian samples. Multivariate statistical approaches (PCA and HCA) were applied to test the hypothesis that selected chemical parameters could support wine classification by geographical origin. Although samples were grouped by similarities in chemical properties, the variability of the measured parameters did not allow reliable discrimination by country of origin, indicating limited robustness of the dataset for geographical classification. The integration of additional analytical approaches, such as detailed compositional profiling or the combination of chemical and sensory data, may provide a more comprehensive assessment of wine typicity.

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