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Antioxidant content and antioxidant activity in raisins from seedless hybrid vine varieties with coloured grape juice

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Abstract: A study has been conducted on the antioxidant content and antioxidant activity in raisins from seedless hybrid varieties of vines with coloured grape juice. It has been found that the amounts of trans-resveratrol and quercetin as well as their antioxidant activity in raisins from the seedless coloured hybrid forms almost always mathematically exceed the levels of these indicators in the raisins of the Gamay Freaux, Black Corinth and Sangiovese grape varieties. It has been shown that 54% of the change in antioxidant activity is due to changes in the content of trans-resveratrol and 42% of quercetin. The applied mathematical models enable the theoretical study of the chemical composition of raisins of different grape varieties through the analytical forms of the proven relationships between them.

Keywords: antioxidants and antioxidant activity; seedless coloured hybrid vine forms; raisins

The taste qualities of grapes are determined to a large extent by the number of phenolic substances that affect the colouring of the skin, while they are involved in the processes of breathing and photosynthesis in the leaves, in the immunity building of the vine and in neutralising the oxygen radicals. They are important for the ageing and the physicochemical stability of red wines as well as for their antioxidant properties. Antioxidant content and antioxidant activity are indicators that characterise the health potential of foods. Today it is assumed that over 100 types of human diseases are related to the action of reactive oxygen species in the body (Shahidi 1997). There are many studies on the phenol content and antioxidant activity in wine, but publications on the influence of these ingredients on grapes and raisins are scarce (Jang et al. 1997; Sato et al. 1997; Careri et al. 2003; Pineiro et al. 2006; Williamson & Carughi 2010). The stilbene trans-resveratrol and the flavonol quercetin are phytoalexins produced de novo in plant cells and their synergistic effect as antioxidants have long been known (Mikstacka et al. 2010; Khandelwal et al. 2012; Arias et al. 2016). When grown under the same biotic and abiotic conditions, the content of resveratrol in grapes is influenced by the vine variety, variation or clone (Bavaresco et al. 2007; Gatto et al. 2008; Gatti et al. 2014). Table grapes and their raisins have great health potential due to their high content of various bioactive phenols (Cantos et al. 2002; Lutz et al. 2011). Comparing the antioxidant capacity and phenolic content of two types of raisins of Thompson seedless variety, Parker et al. (2007) found that pre-

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processed raisins had the highest antioxidant capacity and phenolic content. In contemporary scientific viticultural literature, there is no information on the antioxidant content and the level of antioxidant activity of raisins obtained from seedless hybrids with coloured grape juice.

MATERIAL AND METHODS

Data description. Raisins of 26 seedless hybrids with coloured grape juice obtained as a result of sexual hybridisation between the seeded coloured grape variety Alicante Bouschet x seedless Mermaid 1 and the ones accepted as a conditional control ‒ seedless Corinthian Black and dried grapes (raisins) from two seeded grape varieties Gamay Freaux (coloured) and Sangiovese (red, wine, uncoloured grape juice) were used in the experimental work.

Methodology. The grapes were harvested during two vegetation periods, a total of six repetitions for each variant (hybrid form, variety). Grapes were dried in an electric dryer without pre-treatment with a hot alkaline solution and dipping in a solution of sulphuric acid. The temperature, humidity and speed of the drying agent were within 65–70 °C, 50–60% RH and 2 ± 0.2 m s^{-1}. Until the analysis was performed, the raisins were stored in a refrigerator at 4–6 °C. Only in the Gamay Freaux variety the grapes used and dried for the comparative study were produced in 1965.

For the quantification of the phytoalexins trans-resveratrol (t-RVT) and quercetin (QU) in raisins, the method of Tzanova & Peeva (2018), was applied: a sample of 10 g whole grapes raisin was weighed to the nearest ± 0.0001 g, ground and homogenised with a mechanical homogeniser in 60 mL of 1% solution of HCl in methanol for 5 min in a dark room. The dispersed samples were left in the dark at room temperature for three days and then filtered through a 0.45 µm membrane filter (Whatman, Merck, Darmstadt, Germany). The solid residue was washed twice with 10 mL of 1% HCl in methanol. The extracts were collected and brought to 100 mL with the solvent and stored at –12 °C until initiation of the HPLC analysis. A small amount of each extract was placed in the autosampler of the HPLC system. Five-point calibration graphs (0.05, 0.50, 1.0, 2.0, and 5.0 mg L^{-1}) were made in advance using the reference materials trans-resveratrol HPLC grade (not less than 99%) and quercetin HPLC grade (not less than 98%), purchased from Sigma-Aldrich (St. Louis, MO, USA). An excellent linear dependence with regression coefficients (r^2) 0.9991 for RVT and 0.9999 for QU was achieved. Analytical HPLC was performed with a Hypersil Gold C18 column (5 µm; 150 mm × 4.6 mm) on a Thermo system composed of a Surveyor LC Pump Plus, Surveyor Autosampler Plus, and Surveyor photodiode array detector PDA Plus. Chromatograms were recorded at 289 nm for quercetin and 306 nm for trans-resveratrol in a 7-minute single run, with retention times of approximately 4.7 min for RVT and 5.6 min for QU (Figure 1).

The antioxidant activity (TE) in raisins was established by determining the radical scavenging capacity by the DPPH (1,1›-diphenyl-2-picrylhydrazyl radical) method. A sample preparation methodology was used to measure TE of the extracts obtained as described above and brought to a final concentration of 1 mg mL^{-1} (Serpen et al. 2007). To 3.9 mL of a 100 µM solution of DPPH in methanol, 0.1 mL of methanol extract (1 mg mL^{-1}) was added. Three parallel samples of each extract were analysed. Since the composition of the extracts is complex, the results for the radical scavenging capacity of the extracts were compared with those of Trolox (water-soluble analogue of vitamin E) and calculated by regression analysis from the linear dependence between the concentration of Trolox and absorption at 517 nm measured on a Thermo Scientific Evolution 300 spectrophotometer. The results were expressed as mmol of Trolox equivalent (TE) in 1 kg of starting material.

Statistical methods of analysis. In order to determine the genetic distance of the tested seedless coloured hybrids and varieties of vines according to the defined indicators t-RVT, QU and TE, a hierarchical cluster analysis was applied and for a qualitative description of the obtained clusters; a cluster analysis using the K-means

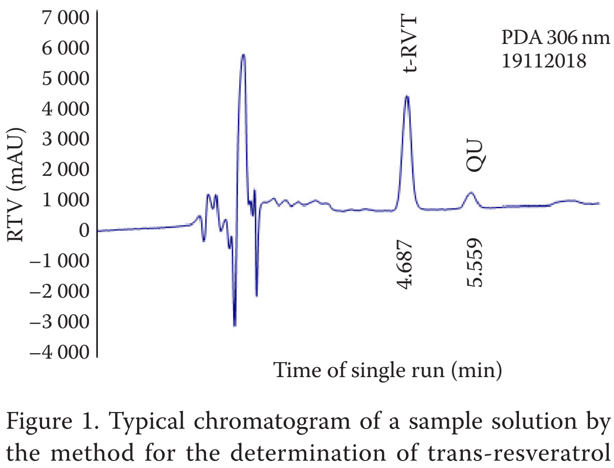

Figure 1. Typical chromatogram of a sample solution by the method for the determination of trans-resveratrol (t-RVT) and quercetin (QU)

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method and two-step clustering. As an agglomeration method, the method of intergroup coupling was chosen with a measure of similarity; the quadratic Euclidean distance. The choice of this method is determined by the maximum value of its corresponding contingency coefficient compared to other clustering methods. Comparative assessment using a single-factor dispersion analysis and Duncan's test was carried out on the experimental data obtained. The magnitude and direction of the dependence between the studied indicators was determined via a correlation analysis by the Bravais-Pearson coefficients. A single-factor and a multifactorial regression analysis was carried out, which gave the opportunity to present the already proven dependences in analytical form through their mathematical modelling. Mathematical processing of experimental data was performed using the statistical software IBM Statistics SPSS 24 and Microsoft Excel (Meyers et al. 2013; Hilton & McMurray 2014; Aldrich & Conningham 2016).

RESULTS AND DISCUSSION

As a result of the applied clustering procedure, it was found that the twenty-nine studied seedless hybrids and seeded varieties of vines were grouped into three summarised clusters (Figure 2). The first consists of 21 hybrid forms and varieties of vines grouped into three sub-clusters. The forms with low values of the three indicators t-RVT, QU and TE: 30/10, 31/13, 29/57, 30/6, 31/41, and 31/36 are in the first sub-cluster, the ones characterised by similar low values of QU and relatively high in TE: 26/49, 31/22, 29/13, 30/3, 31/40, 32/9, 31/9, 31/15, 31/26, 30/2, and 29/68 in the second sub-cluster, the forms with a proven similarity to the QU indicator, whose values are higher than those of the previous sub-clusters: 29/27, 29/60, 32/2 and 29/62 are in the third sub-cluster. The second summarised cluster includes 29/56, 31/58, 29/24 hybrids and the Corinthian Black and the Gamay Freaux varieties which have very low antioxidant ac-

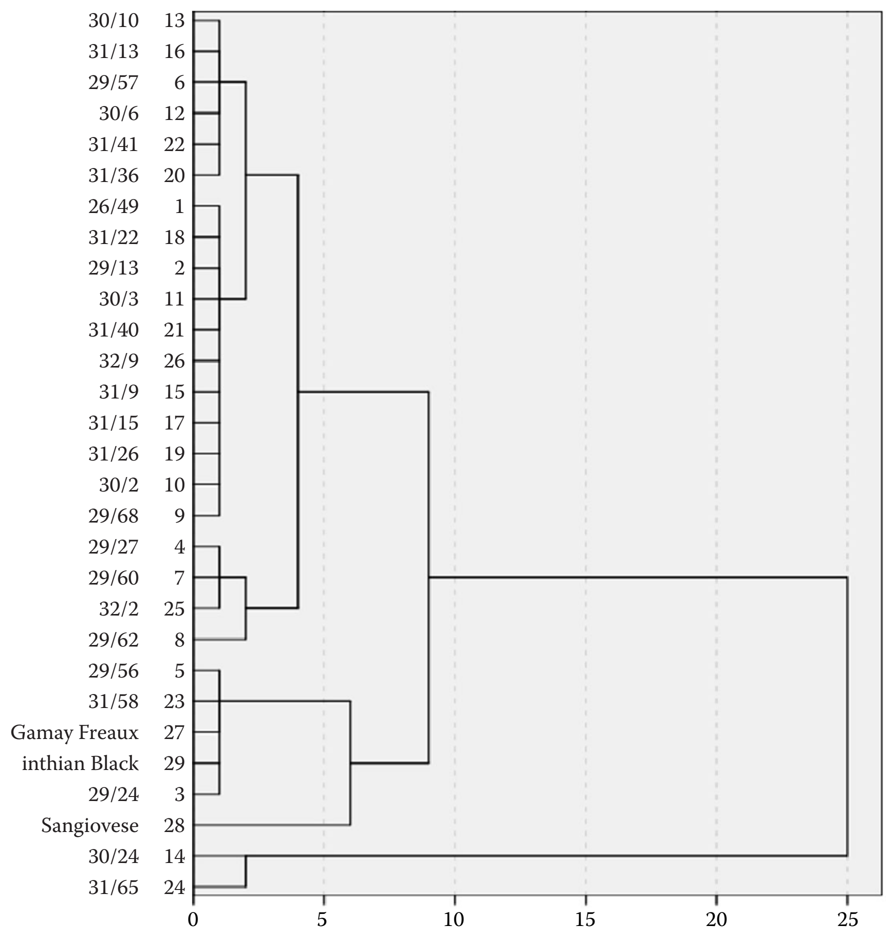

Figure 2. Dendrogram: Clusterisation of the studied seedless coloured hybrid forms and varieties of vines depending on the content of trans-resveratrol, quercetin and their antioxidant activity in raisins

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tivity. The Sangiovese variety is genetically the most remote according to these indicators from all the hybrid forms studied, which determines its separation into a separate sub-cluster. Its difference from the other forms is due to the reported lowest values of trans-resveratrol and quercetin and to the weakest antioxidant activity. The 30/24 and 31/65 hybrid forms, which are characterised by a statistically proven similarity due to the maximum content of quercetin in raisins and the highest antioxidant activity are in the third cluster. The descriptive analysis and the *F*-test conducted to assess the differences showed that all three indicators have a statistically proven effect on the distribution of hybrid forms and varieties in clusters.

According to the results of the cluster analysis, in the selection of the vine for the production of varieties with increased antioxidant activity by the sexual hybridisation method, the 30/24 and 31/65 hybrid forms, which have more t-RVT, QU, and higher TE, should be used. For this purpose, the Sangiovese variety is not suitable due to the lowest content of trans-resveratrol and quercetin in its grapes, which will negatively affect the antioxidant properties of the wine.

Data from the application of the single-factor analysis of variance and Duncan's multiple range test

Table 1. Comparative assessment of the values of the tested parameters in raisins from seedless coloured hybrid forms and varieties of vines via single-factor analysis of variance and Duncan's test

Cluster	Hybrid form/variety	t-RVT			QU			TE		
		Average value	SE	Duncan	Average value	SE	Duncan	Average value	SE	Duncan
I	30/10	11.85	1.19	defg	89.98	5.39	defghi	38.84	0.82	bcdef
	31/13	12.04	0.43	defg	89.73	6.31	defghi	38.49	1.00	bcdef
	29/57	13.77	1.60	cdef	92.70	3.57	defghi	36.01	1.11	efgh
	30/6	10.65	0.31	fg	86.10	7.29	efghi	34.02	0.47	ghij
	31/41	12.74	0.73	cdefg	86.35	5.55	defghi	36.51	0.76	defg
	31/36	8.97	0.39	gh	87.18	3.96	defghi	37.30	0.35	cdefg
	26/49	10.90	0.73	fg	97.90	1.13	defgh	35.83	0.74	fgh
	31/22	10.68	1.62	fg	98.52	7.05	cdefgh	34.51	2.38	fghij
	29/13	14.48	0.27	bcdef	95.02	9.06	defgh	40.82	1.08	abcd
	30/3	13.58	0.46	cdef	96.55	9.04	defgh	40.39	1.19	abcde
	31/40	11.98	0.25	defg	95.77	9.04	defgh	41.62	1.18	abc
	32/9	16.22	0.65	abc	96.57	8.23	defgh	42.70	0.81	ab
	31/9	15.12	0.20	abcde	91.85	10.31	defghi	41.99	1.09	ab
	31/15	12.89	0.37	cdef	100.33	8.12	cdefg	42.11	1.04	ab
	31/26	14.91	0.35	bcde	100.93	11.81	cdefg	40.33	2.16	abcde
	30/2	11.95	1.19	defg	103.05	1.19	cdefg	40.27	0.79	abcde
	29/68	17.69	2.78	ab	99.00	2.95	cdefgh	37.36	2.12	cdefg
	29/27	10.91	0.50	fg	108.72	3.92	cde	42.06	0.27	ab
	29/60	13.26	0.91	cdef	110.47	7.08	cd	41.99	2.36	ab
	32/2	11.29	1.13	efg	107.18	4.56	cde	35.18	1.49	fghi
	29/62	15.31	0.37	abcd	121.03	1.43	bc	41.98	0.47	ab
II	29/56	11.71	1.90	defg	70.78	6.46	ij	31.95	1.89	hij
	31/58	11.73	2.17	defg	75.50	4.93	hij	31.91	1.39	hij
	Gamay Freaux	6.81	0.15	hi	77.53	0.84	ghij	30.61	0.27	j
	Corinthian Black	5.59	0.22	i	80.27	0.95	fghij	34.58	0.22	fghij
	29/24	11.79	1.84	defg	82.02	4.11	fghi	30.91	1.90	ij
	Sangiovese	1.97	0.35	j	58.87	2.06	j	21.7	1.19	k
III	30/24	18.62	2.08	a	134.85	9.15	ab	41.88	2.11	ab
	31/65	13.94	0.96	cdef	144.42	16.25	a	44.54	1.69	a

t-RVT – trans-resveratrol, QU – quercetin; TE – antioxidant activity; SE – standard error; a–k – degree of evidence at a level of significance $\alpha = 0.05$

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show that the richest in trans-resveratrol are raisins from 30/24 (18.62 mg kg^{-1}), followed by 29/68 (17.69 mg kg^{-1}), and the lowest content is in Sangiovese (1.97 mg kg^{-1}) (Table 1). In these hybrid forms, this indicator is unstable due to the high value of their standard error, at the level of which 32/9 (16.22 mg kg^{-1}) stands out with the most persistent t-RVT content. The greatest amount of quercetin was reported in 31/65 (144.42 mg kg^{-1}) and in 30/24 (134.85 mg kg^{-1}), and the lowest in Sangiovese (58.87 mg kg^{-1}). Due to the high error value in 31/65, the 30/24 form should be considered the best for this indicator. Antioxidant activity is most pronounced in 31/65 (44.54 mmol kg^{-1}), followed by 32/9 (42.70 mmol kg^{-1}) and 31/15 (42.11 mmol kg^{-1}) and the lowest in Sangiovese (21.70 mmol kg^{-1}). Considering the stability of the hybrids with higher TE, it can be assumed that 29/27 has the most significant value of this indicator (42.06 mmol kg^{-1} and SE = 0.27). The amounts of trans-resveratrol and quercetin and their antioxidant activity in raisins from seedless hybrid forms with coloured grape juice almost always exceed the levels of these indicators in raisins of the Gamay Freaux, Corinthian Black and Sangiovese varieties. The existing differences in the magnitudes of the studied indicators between these two conditional groups of variants are mathematically proven for all for t-RVT and with few exceptions for QU and TE.

Yang et al. (2009) studied 14 grape varieties. They found that Baco Noir had the highest resveratrol content among the tested varieties, Cabernet Franc possessed the highest antioxidant activity.

As a result of the applied correlation analysis, statistically significant correlations between t-RVT, QU on the one hand, and TE on the other (Table 2) were obtained. Both indicators t-RVT (0.732**) and QU (0.652**) also have a strong, positive influence on the antioxidant activity of raisins. A moderate positive effect of t-RVT on QU (0.370**) has also been demonstrated. Existing reliable correlations determine the need for an application of regression analysis, the results of which present in an analytical form the already established correlations between the content of trans-resveratrol and quercetin and the antioxidant activity. After a check, normal distribution of experimental data on antioxidant activity was established. The linear regression model showing the positive influence of the t-RVT and QU indicators on TE is represented by the Equation 1:

$$y = 17.58 + 0.776x_1 + 0.11x_2$$

where: x_1 – the t-RVT value; x_2 – the QU value are independent variables; y – the TE value is a dependent variable.

Table 2. Correlation coefficients between the studied indicators in raisins from seedless coloured hybrid forms and varieties of vines

Indicators	TE	t-RVT	QU
Antioxidant activity (TE)	1	0.732**	0.652**
Trans-resveratrol (t-RVT)		1	0.370**
Quercetin (QU)			1

**Statistical significance of correlation coefficients at a significance level of α = 0.01

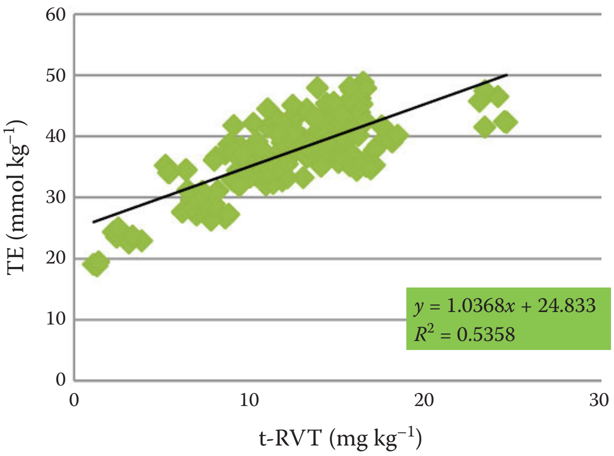

Figure 3. Effect of trans-resveratrol (t-RVT) content on antioxidant activity (TE) in raisins from the studied seedless coloured hybrid forms and varieties of vines

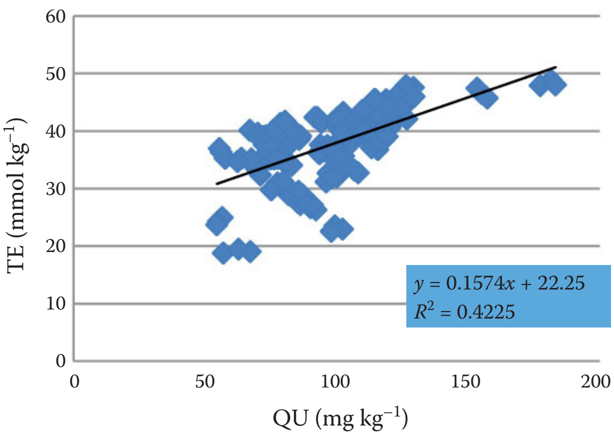

Figure 4. Effect of quercetin (QU) content on antioxidant activity in raisins from the studied seedless coloured hybrid forms and varieties of vines

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The established model, as well as all regression coefficients involved, are statistically significant (sig. = 0.000). The β coefficient values confirm again the stronger positive effect of t-RVT (β = 0.568) on TE compared to that of QU (β = 0.442).

Figures 3 and 4 demonstrate the positive effect of trans-resveratrol and quercetin content on antioxidant activity, respectively. It is observed that the experimental data seen as points in the two-dimensional plane are located around a straight line, suggesting the formation of a linear regression model. The respective coefficients of determination are indicated. It has been shown that 54% of the TE change is due to changes in the content of t-RVT and 42% of QU. The presented models are statistically significant.

The comparison of the studied indicators in dried grapes of the Gamay Freaux variety obtained in 1965 and in 2017-2018 was performed in order to analyse information on the impact of the storage period on the change in the content of t-RVT, QU and TE. The results obtained show that the amounts of both phytoalexins differ in absolute values: 1965/2017–2018: t-RVT 3.1/6.81 mg kg^{-1}; QU 99.8/77.50 mg kg^{-1}, and TE 22.99/30.61 mmol kg^{-1}. In dried grapes obtained in 1965, the content of t-RVT and TE is higher than that in 2017–2018, and in QU; just the opposite.

According to the results of the correlation analysis, the degree of influence of t-RVT and QU on TE (Table 3) changes during the prolonged storage of dried grapes. In the 1965 variant, trans-resveratrol had a significant negative effect on the antioxidant activity (–0.630*), which not only decreased in 2018 but was also unproven (–0.107). The effect of quercetin is opposite. Its quantity in 2018 affects the antioxidant activity very positively (0.930**). This probably means that the shelf life of the raisins does not change the direction of influence of the phenolic compounds on TE but only enhances or decreases it. Since these data are quite insufficient for a reliable analysis and more definite conclusions in terms of the experimental work, we consider it acceptable to suppose that this model or trends in the change of these indicators may be close to the real ones.

Table 3. Effect of trans-resveratrol and quercetin on antioxidant activity in dried Gamay Freaux grape variety

Year	Indicator	TE	t-RVT	QU
	TE	1	–0.630*	0.148
1965	t-RVT		1	0.674**
	QU			1
	TE	1	–0.107	0.930**
2018	t-RVT		1	–0.466
	QU			1

TE – antioxidant activity; t-RVT – trans-resveratrol, QU – quercetin; *statistical significance of correlation coefficients at a significance level of α = 0.05; **statistical significance of correlation coefficients at a significance level of α = 0.01

CONCLUSION

Depending on the content of the phytoalexins trans-resveratrol and quercetin and their antioxidant activity in raisins and dried grapes, the twenty-nine seedless coloured hybrids and seeded varieties of vines were grouped into three summarised clusters consisting of a different number of sub-clusters. The antioxidant activity had the most statistically significant influence on the distribution of hybrid forms and varieties in clusters.

The amounts of trans-resveratrol and quercetin, as well as their antioxidant activity in raisins from seedless coloured hybrids almost always mathematically exceeded the levels of these indicators in the raisins of the Gamay Freaux, Corinthian Black, and Sangiovese grape varieties. The highest content of trans-resveratrol was found in 30/24 (18.62 mg kg^{-1}) and in 29/68 (17.69 mg kg^{-1}), but the most stable was 32/9 (16.22 mg kg^{-1}) while the highest content of quercetin was in 31/65 (144.42 mg kg^{-1}) and it was more stable in 30/24 (134.85 mg kg^{-1}).

The highest antioxidant activity was found in the raisins of 31/65 (44.54 mmol kg^{-1}), 32/9 (42.7 mmol kg^{-1}) and 31/15 (42.11 mmol kg^{-1}), but 29/27 had a higher stability indicator (42.06 mmol kg^{-1}). The two phytoalexins t-RVT (0.732**) and QU (0.652**) had a strong positive effect on the antioxidant activity of the raisins. It was proved that 54% of the TE change was due to changes in t-RVT and 42% in QU.

The established models enable the theoretical study of the chemical composition of raisins of different vine varieties through the analytical appearance of the reliable relations between them.

The formed regression equations allow determining the theoretical value of the corresponding dependent variable without conducting the corresponding scientific experiment, while preserving the objective factors influencing the dependent variable. Due to the high statistical significance of each model, a minimal difference between the experimental and theoretical value of the individual studied indicators can be assumed.

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