Oenological characterisation of white wines produced from some Georgian grape varieties using Kakhetian winemaking methods

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Abstract

Introduction. The aim of the study was to highlight the quality parameters (oenological characterisation and content of bioactive compounds) of wines based on their varietal origin and winemaking methods without environmental, soil, viticulture, and industrial environment.

Materials and methods. Four white grape varieties Rkatsiteli, Mtsvane Kakhuri, Kisi, and Khikhvi were used to make twelve wine samples. "Kakhetian" winemaking technique was applied by spontaneous must fermentation with grape's skins, seeds, peduncles, pips, and stalk. The content of organic acids was determined by the HPLC method. The total tannin content was analysed by the titration method. Spectrometric methods were used to measure the total phenolic content and total antioxidant activity.

Results and discussion. The research demonstrated that the quality characteristics of Kisi and Khikhvi wines were better than those of Rkatsiteli and Mtsvane Kakhuri. However, Khikhvi showed higher results related to the technological parameters: the content of alcohol ranged between 13.6 and 13.7%; the content of reducing sugars was between 3.7 and 4.0 g/L. Those values were predictable due to the high sugar concentration in Khikhvi and Kisi's grape juice. Concentrations of volatile acids (VA) depend on intracellular metabolism during vinification and can cause differences in their values, which varied from 0.40 to 0.46 g/L. In addition, concentrations of malic, citric, and succinic acids varied from 1.72 to 1.85 g/L, from 0.007 to 0.72 g/L, and from 1.05 to 1.5 g/L, respectively. Mtsvane Kakhuri differed by the composition of the organic acids and revealed the highest tartaric acid content, 1.42–1.95 g/L, within the studied wine samples. Both grape variety and yeast strain can cause variations in the content of organic acids during spontaneous fermentation. The content of bioactive compounds was higher in the Kisi wine samples than in the rest of the analysed white wines. Total tannin content ranged from 0.123 to 0.155%, total phenolic content varied from 636.4–743.7 mg/L gallic acid equivalents and possessed a total antioxidant activity of 651.2–2629.8 mg/L in the Kisi samples. Therefore, it seems possible that the grape cultivar also played a significant role in the content of phenolic compounds and tannins. Furthermore, a high positive correlation was found between total tannins content and antioxidant activity (R^2 =0.8871), which was stronger than the correlation between total phenolic content and antioxidant activity with R^2=0.8324. This could be explained by the different chemical structures of bioactive compounds, particularly the quantitative content of the OH group.

Conclusion. The "Kakhetian" winemaking method is advantageous by enhancing wine with oenological and bioactive compounds and ensures obtaining high-quality wine. Additionally, the quality of the wines is highly correlated with the grape cultivar.

Introduction

Wine contains various chemical compounds, including plant secondary metabolic products, which affect the nutritional quality of food, wine, and other beverages (Morata and Loira, 2016). The quality parameters of wine correlates with its chemical composition and content of bioactive compounds, which get into the wine from different parts of the grape such as skin, pulp, seeds, pips, and stalks (Bora et al., 2016). The composition and quantity of bioactive compounds depend on the grape variety, winemaking and post-fermentation methods (Luna-Guevara et al., 2018). “Kakhetian” technique is an oenological practice based on fermenting and maturating the grape must with all solid parts of the grape: skins, seeds, peduncles, pips, and stalk (Glonti, 2020). In contrast, the classical white winemaking method is described by must fermentation without solid parts of the grape (Ribéreau-Gayon et al., 2006). Characterising Georgian varieties and winemaking methods is vital due to the increasing popularity in the domestic and international market (Kharaishvili et al., 2014; Rytkönen et al., 2021). Rkatsiteli (R), Mtsvane Kakhuri (MK), Kisi (K), and Khikhvi (KH) are one of the autochthonous and widely planted grapevines in Georgia. These grape wines have some of the most commercial relevance for Georgian white wine production (National Statistics Office of Georgia, 2016). Numerous studies have been conducted on the correlation between chemical and bioactive compounds and the antioxidant properties of wine. However, access is limited to the most commercially available Georgian white grape varieties fermented by the traditional winemaking method. Especially white wines according to potential chemical descriptions without the influence of terroirs and viticulture methods (Gurgenidze et al., 2019; Sordia, 2020; Tauchen et al., 2015).

Therefore, the research aims to assess the wine quality based on grape varieties and winemaking methods. For this purpose, the following objectives were established: (1) to determine the major chemical component and organic acids composition (i.e., tartaric, malic, citric, succinic, fumaric acids), total phenolic and total tannin content and (2) to find a correlation, if any, between content of bioactive compounds and antioxidant activity in studied wines (Rkatsiteli, Mtsvane Kakhuri, Kisi, and Khikhvi varieties).

Materials and methods

Wine samples

Four individual white grape varieties were selected to make wine, which were as follows- Rkatsiteli (R), Mtsvane Kakhuri (MK), Kisi (K), and Khikhvi (KH). For each grape variety, three simultaneous micro-vinification was carried out. As a result, twelve wine samples were collected and used for further analysis (Table 1).

The grapes were obtained from the experimental base of perennial crops at Georgian Scientific Research Centre of Agriculture, Jigaura, Mtskheta. All-wine samples were produced by “Kakhetian” methods, which included spontaneous must fermentation with skins, seeds, peduncles, pips, and stalks of the grape. Each wine was stirred several times a day. Vinification was carried out approximately at 20 °C. After completed fermentation, maceration was extended for about five months. Later wine samples were filtered and bottled into 750 ml glass vessels, and samples were stored at 4 °C before analyses.

Table 1

Wine samples of each micro-vinification

Grape variety	Wine sample	Grape variety	Wine sample
Rkatsiteli	R1 R2 R3	Kisi	K1 K2 K3
Mtsvane Kakhuri	MK1 MK2 MK3	Khikhvi	KH1 KH2 KH3

Grape juice sugar concentration was determined refractometrically. The main chemical wine indicators, alcohol content (AC), pH, residual sugars (RS), total acidity (TA) and volatile acidity (VA), were assessed according to the methods of the International Organization of Vine and Wine (2009, 2015, 2011).

Total tannins content by titration method

Total tannin content (TTC) was determined by the potassium permanganate titration method (Khudbudin al., 2016). Concisely, back (A) and blank (B) titration was conducted for the wine samples. Consumed quantity for the back (A) and blank (B) titration were recoding for later calculations. The back titration: 5 mL wine with 10 mL distilled water was heated until the volume was decreased to 5 mL. Another 10 mL of water with Indigo carmine (2 mL, 0.5%) was added, and titration was carried out by Potassium permanganate (0.004 M) until the golden yellow colour was noted. For blank (B) titration – charcoal (1g) was mixed with a 25 mL wine sample and was kept at room temperature for a quarter of an hour. For titration, the back titration producer was carried out. The presence of tannin in wine can be calculated using the following equation:

$$TTC = 0.01664 \cdot (A - B) \quad (1)$$

where A is a volume of 0.004 M $KMnO_4$ consumed by the back sample in mL;

B is a volume of 0.004 M $KMnO_4$ consumed by the blank sample in mL;

The standard tannin solution for which 1 mL of 0.004 M KMnO4 = 0.0832 mg of tannin, therefore, percent of tannins in wine is $0.01664 \cdot (A - B)$

Total phenolic content

Total phenolic content (TPC) was measured by methods (Singleton et al., 1999) with slight modification. Concisely, the total phenolic content was determined using the Folin-Ciocalteu reagent and spectrophotometric method and expressed as mg/L gallic acid equivalent. First, 1 mL of 20 times diluted wine sample was mixed with ten times diluted 5 mL Folin-Ciocalteu reagent and was kept at room temperature. Then, about 8 minutes later solution was mixed with sodium carbonate (4 mL) and was measured at 765 nm wavelength after an hour delay again at room temperature.

The correlation of gallic acid standard solution concentration (10–50 µg/mL) and absorbance was used to calculate TPC (R^2=0.983). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per litre of wine.

Total antioxidant activity

A quantitative equivalent of ascorbic acid was used to measure total antioxidant activity (AOA) (Benzie and Strain, 1996) with minor variation. For AOA determination ferric reducing ability of plasma (FRAP) solution was prepared by the concentration 1:1:10–0.01M 2,4,6-tripyridyl-s-triazine (TPTZ) diluted with 0.04 M hydrochloric acid, 0.02 M Iron (III) chloride, and 0.3 M acetate buffer (pH 3.6). The resulting solution and 1mM ascorbic acid were placed in a water bath at 37°C for a quarter of an hour. 1000 µmol/L solution of $FeSO_4 \cdot 7H_2O$ was used to calibrate the spectrophotometer. First, the 100 µL wine sample was added to 3 mL of working solution; later, the absorbance was determined by 593 nm wavelength. The result was fixed after 4 minutes. A working solution was used for control, and ascorbic acid was used for comparison.

Determination of organic acid by HPLC

Organic acid determination was carried out according to the International Organization of Vine and Wine method (OIV, 2020). Concisely, the sample was filtered by nylon membrane (0.45 mm), and a volume of 10 µL was injected into the C18 column (4 X 250:5mm) of HPLC (Varian Prostar 500, Walnut Creek, California, USA). The organic acid was detected at 210 nm. The eluent was KH_2PO_4, and an ammonium sulphate solution with pH 2.1. Identification was performed by calculating pure compound retention times. Calibration curves were applied to determine quantification.

Statistical analysis

Three replicates were performed for all data and were expressed as mean values ±Standard Deviation (SD). Pearson correlation between two variables was determined. In addition, the ANOVA was used to represent variance among chemical data in individual wines produced by different grapevines. All statistical analyses were performed with Microsoft Excel (Microsoft 365, 2021).

Results and discussion

Oenological composition

The physicochemical composition of the studied wines is presented in Table 2. Regarding the alcoholic content, no significant differences were observed between the analysed samples. The average alcohol content among samples was 13.3% (12.6–13.7%). All studied samples had higher residual sugar concentrations than Georgian dry wines, which were predictable due to the sugar content in grape juice. Residual sugar concentration (1.6–4.0 g/L) indicated complications of the fermentation process. Sugar content in grape juice was as follows: Rkatsiteli – 21.2 g/100 mL; Mtsvane Kakhuri – 21.8 g/100 mL; Kisi – 24.0 g/100 mL; Khikhvi – 28.7 g/100 mL. For white wines maximum limit of volatile acids is1.0 g/L and total acidity should be not less than 4 g/L (Resolution No. 3 of 2014 of Georgian Government on General Rules for Production of Grape Wines). All sample studied herein corresponded to the local regulations and had the content ranged: TA from 5.03±0.03 to 6.08±0.00g/L, and VA from 0.26±0.01 to 0.46±0.05g/L. Khikhvi wine exhibited the highest content of VA 0.46±0.05 g/L and pH 4.2 and the lowest content of TA 5.03±0.06 g/L.

Table 2

Physicochemical composition of studied wines

Wine sample	Reducing sugar, g/L	Alcoholic content,% vol.	pH	Volatile acidity, g/L	Total acidity, g/L
R1	1.63±0.01	13.00±0.09	4.1	0.26±0.01	5.55±0.05
R2	1.72±0.01	13.20±0.01	4.1	0.33±0.01	5.70±0.05
R3	1.72±0.02	12.60±0.01	4.1	0.33±0.00	5.40±0.05
MK1	1.82±0.01	13.00±0.03	4.0	0.33±0.00	5.18±0.07
MK2	1.58±0.05	13.30±0.00	4.0	0.33±0.03	5.33±0.05
MK3	1.92±0.09	13.20±0.00	4.0	0.33±0.01	5.25±0.03
K1	3.78±0.17	13.20±0.00	4.1	0.33±0.04	5.93±0.02
K2	2.54±0.23	13.50±0.00	4.1	0.40±0.01	6.08±0.00
K3	3.99±0.16	13.00±0.03	4.1	0.33±0.00	6.00±0.01
KH1	3.99±0.14	13.60±0.05	4.2	0.46±0.05	5.18±0.05
KH2	3.68±0.2	13.70±0.00	4.2	0.40±0.07	5.03±0.06
KH3	3.17±0.07	13.70±0.05	4.2	0.40±0.02	5.18±0.04
Mean	2.63	13.25	4.1	0.35	5.48
Max	3.99	13.7	4.2	0.46	6.08
Min	1.58	12.6	4.0	0.26	5.03

Organic acid composition

The content of tartaric, malic, citric, succinic, and fumaric acids were determined in studied white wines (Table 3).

Table 3

Organic acids concentration of the studied wines

Wine sample	Tartaric acid, g/L	Malic acid, g/L	Citric acid, g/L	Succinic acid, g/L	Fumaric acid, g/L
R1	1.38±0.02	0.29±0.05	0.085±0.01	N/A*	N/A
R2	1.63±0.07	0.3±0.29	0.058±0.02	N/A	N/A
R3	1.44±0.01	0.32±0.12	0.052±0.01	N/A	N/A
MK1	1.95±0.03	0.09±0.04	0.001±0.01	N/A	1.3±0.23
MK2	1.48±0.02	0.33±0.09	0.66±0.12	0.46±0.04	N/A
MK3	1.42±0.09	0.13±0.12	0.65±0.10	0.9±0.09	N/A
K1	1.51±0.04	0.6±0.04	0.006±0.00	N/A	N/A
K2	1.63±0.06	0.94±0.14	0.007±0.00	N/A	N/A
K3	1.49±0.02	1.18±0.08	0.092±0.01	N/A	N/A
KH1	1.37±0.01	1.78±0.07	0.095±0.01	1.23±0.16	0.1±0.01
KH2	1.18±0.03	1.72±0.05	0.72±0.2	1.05±0.18	N/A
KH3	1.38±0.08	1.85±0.13	0.007±0.01	1.5±0.25	N/A
Mean	1.488	0.794	0.203	1.328	0.655
Min	1.180	0.090	0.001	0.460	0.100
Max	1.950	1.850	0.720	1.500	1.300

*Not determined.

The tartaric acid concentration varied from 1.18 g/L to 1.95 g/L with a mean of 1.49 g/L. Tartaric acid has an essential role in wine sensory perception (Volschenk et al., 2006). Thus, tartaric acid content in Mtsvane Kakhuri wines was in a range from 1.95±0.03 to 1.42±0.09 g/L with mean 1.62 g/L, in Kisi wines varied from 1.49±0.02 to 1.63±0.06 g/L with mean 1.54 g/L, in Rkatsiteli wines the tartaric acid ranged from 1.38±0.02 to 1.63±0.07 g/L with mean 1.48 g/L, and in Khikhvi wine its content varied from 1.18±0.03 to 1.38±0.08 g/L with mean 1.31 g/L. According to Waterhouse et al. (2016), tartaric acid concentration changes during ageing through the different physicochemical processes.

Different concentration variations were reported for malic acid, g/L, based on the average value. They were as followed: in Khikhvi wine it was 1.72±0.05 – 1.85±0.13 g/L (mean 1.78 g/L), Kisi wines contained 0.60±0.04 – 1.18±0.08 g/L (mean 0.90 1/L), in Rkatsiteli wines this acid ranged from 0.29±0.05 to 0.32±0.12 g/L (mean 0.30 g/L), in Mtsvane Kakhuri wines it was in a range from 0.09±0.0 to 40.33±0.09 g/L (mean 0.18 g/L).

Similar results were reported by Whiting (1976). Furthermore, according to his study, malic acid content can vary due to spontaneous fermentation.

Presence of citric acid has an essential effect on the sensory profile of the wine and its stability (Mendes Ferreira and Mendes-Faia, 2020). The highest concentration of citric acid was observed in KH2, 0.72±0.2 g/L, and the lowest was found in MK1, 0.001±0.01 g/L. The average content of citric acid among the studied samples was 0.203 g/L.

The succinic acid was detected only in the second and third samples of Mtsvane Kakhuri: 0.46±0.04 and 0.9±0.09 g/L, respectively, and in all samples of Khikhvi wines – 1.05±0.18 – 1.5±0.25 g/L, with an average mean of 1.328 g/L. According to Chidi et al. (2018), succinic acid concentration depends on several factors, especially from yeast strain.

The fumaric acid was detected only in KH1, 0.1±0.01 g/L, and in MK1, 1.3±0.23 g/L. According to Ough and Kunkee (1974), fumaric acid may be metabolised by yeast and therefore were not represented in other samples.

The organic acid composition differs based on the terroirs, grape variety, microbial diversity, and winemaking methods (Chidi et al., 2018). In our study, grape variety strongly effect succinic acid and fumaric acid content. All organic acids were found only in the first sample of Khikhvi wine, and as mentioned above, fumaric acid was observed in MK1 and KH1. Khikhvi wine samples contained a higher average content of all organic acids. However, it is worth underlining that tartaric acid content was lowest compared to the other wine sample. The results of total acid contents, tartaric, malic, and citric acids, were compared to other white wines reported in publications. The average content of tartaric, citric, and succinic acids was similar to results reported by Chahine et al. (2019), meanwhile, the fumaric acid content was higher and malic acid lower in the studied Georgian wine than reported in the above research.

Total tannin and total phenolic contents

Bioactive components are present in much smaller quantities in white wines than in red wines (Pérez-Navarro et al., 2020). Even though, the content of bioactive compounds is low in white wines, studied wines contained relatively high values of total tannin content (TTC) ranged from 0.067±0.001% to 0.155±0.002% with a mean of 0.098% . The highest value of tannins was observed in Kisi, 0.123±0.002 – 0.155±0.002%, followed by Khikhvi, 0.093±0.00 – 0.108±0.001%, and Rkatsiteli, 0.077±0.002 – 0.083±0.002%. The lowest TTC content was found in Mtsvane Kakhuri, 0.067±0.001 – 0.078±0.001% (Table 4). This difference is due to the varietal properties, and in the same wine, the variation may be caused by grape skins and seeds tannins ability to bind with proteins and cell wall material (Watrelot and Norton, 2020).

Table 4

Bioactive components and antioxidant activity of the studied wines

Wine sample	Total tannin content,%	Total phenolic content, mg GAE /L	Antioxidant activity, mg AAE /L,
R1	0.083±0.002	233.171±8.31	661.247±63.455
R2	0.077±0.002	225.041±7.424	777.155±83.681
R3	0.087±0.002	241.301±3.415	725.852±89.452
MK1	0.067±0.001	174.634±3.032	418.030±13.764
MK2	0.078±0.001	176.260±3.031	317.323±27.925
MK3	0.072±0.001	203.902±6.163	321.123±23.705
K1	0.123±0.002	636.423±31.621	1651.217±19.489
K2	0.155±0.002	743.740±35.111	2460.675±148.209
K3	0.135±0.002	670.569±28.455	2629.787±129.095
KH1	0.103±0.001	504.715±1.849	1548.610±93.637
KH2	0.093±0.002	478.699±8.31	765.754±81.132
KH3	0.108±0.001	486.829±7.424	965.269±79.420
Mean	0.098	397.940	1103.504
Min	0.067	174.634	317.323
Max	0.155	743.740	2629.787

Total phenolic content in the studied wine samples showed the same varietal characteristics as was observed in the case of total tannin quantity: Kisi, 636.423±31.621 – 743.740±35.111 mg GAE/L, followed by Khikhvi, 504.715±1.849 – 478.699±8.31 mg GAE/L, and Rkatsiteli, 225.041±7.424 – 241.301±3.415 mg GAE /L. The lowest content of TP was showed by Mtsvane Kakhuri, 174.634±3.032 – 203.902±6.163 mg GAE/L. According to Sordia (2020) the fermentation and maturation during winemaking by the Kakhetian method ensure phenolic compounds' migration into wine. The same results were shown by Shalashvili et al. (2010) while studying white wine obtained by the Kakhetian method.

The total antioxidant activity presents one of the essential attributes of wine quality and may determine consumer buying behaviour (Droli et al., 2019). In this research, all wines showed relatively high level of AOA ranging between 317.323 – 2629.787 mg AAE/L with average: 721.418 mg AAC/L for Rkatsiteli, 352.1587 mg AAC /L for Mtsvane Kakhuri, 2247.226 mg AAE/L for Kisi and 1093.211 mg AAE/L for Khikhvi. The highest total antioxidant activity, 2629.787±129.095 mg AAE/L, was observed in the third sample of Kisi, which was almost eight time higher than the lowest antioxidant activity in the MK2 sample, 317.323±27.925 mg AAC /L.

High total tannin content and antioxidant activity were found in samples R2 and R3: the total antioxidant activity was 77.16–725.85 mg AAC/L and the content of tannin was 0.08–0.09%. It was similar to KH2: the total antioxidant activity was 765.75 mg AAC/L and the content of tannin was 0.09%. This may be explained by spontaneous fermentation (Vejarano et al., 2019). As a result, in Rkatsiteli, wine bioactive components were ultimately transformed from the grape into wine, while a minimum of them was migrated into the Khikhvi wine sample. The MK1 sample had the lowest total tannin and total phenolic contents. However, the highest total antioxidant activity was found for MK1 among the Mtsvane Kakhuri wine samples. The MK3 showed higher total phenolic content than the MK

2 sample, however content of total tannin showed the opposite; KH1 had lower total tannin content, and higher total antioxidant activity compared to the KH3 sample, having higher TTC and lower AOA. The R2 sample had a lower TTC and TPC than the R3 sample, but AOA was higher for the R2 sample. Similarities were observed between the K2 sample (higher TTC and TPC) and the K3 sample (higher AOA).

Pearson correlation was used to quantify the correlation between bioactive parameters and antioxidant activity. Among all samples, a strong relationship was observed between the TPC-AOA and TTC-AOA. The correlation coefficient for antioxidant activity and total tannin content was 0.832 (Figure 1) and for antioxidant activity and total phenolic content was equal 0.8871 (Figure 2).

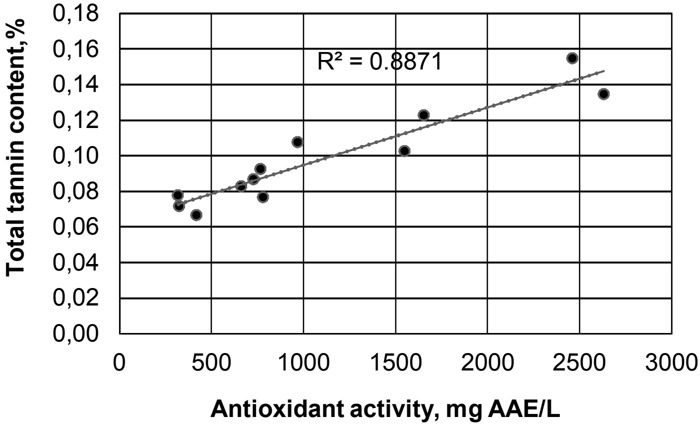

Figure 1. Correlation between the total tannin content and antioxidant activity of Kakhetian style wine samples

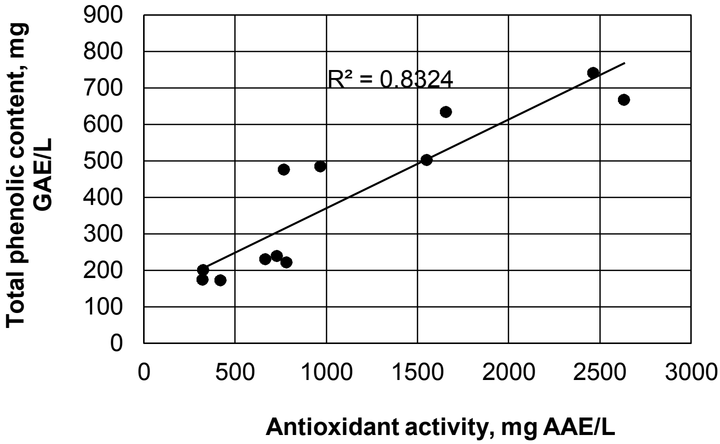

Figure 2. Correlation between the antioxidant activity and total phenolic content of Kakhetian style wine samples

An incomplete correlation and some exemption between TTC-AOA and TPC-AOA can be caused by different chemical structures of bioactive compounds, particularly the quantitative content of the OH group (Bendary et al., 2013). The high correlation between TPC and AOA within Kakhetian style Georgian white wines was described by Khatchapuridze et al., (2021). It is worth mentioning that the present study was focused on micro-vinification, and further studies are needed to observe the same parameters in a large-scale wine production.

Conclusions

1. The technological parameters of the wine produced by the "Kakhetian" method using the Khikhvi grape variety had the highest concentration of the studied technological indicators compared to the rest of the studied white wines. Kisi had the highest content of some bioactive compounds, and the Mtsvane Kakhuri was distinguished by tartaric acid content.
2. The studied wines were rich in bioactive compounds and possessed high antioxidant activity.
3. A high positive correlation was found between total phenolic content and antioxidant activity and between total tannins content and antioxidant activity. It should be mentioned that antioxidant activity and total tannin content were better correlated than antioxidant activity and total phenolic content.

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