

Antioxidant activity of technical grapes harvested in the Samara Region in 2013

Nadezhda Viktorovna Makarova, Dinara Fanisovna Valiulina, Irina Andreevna Batkova

The Samara State Technical University, Str. Molodogvardeyskaya, 244, Main Building, Samara, 443100, Russian Federation

Abstract. The following data are the results of a comparative study of the content of the functional directivity substances affecting the chemical composition and antioxidant activity of the grape varieties: Druzhba, December, Tashkent, Cabernet of the North, Zhuravlik, Strelets, Isabella. The study involved the analysis of the content of the total amount of phenolic compounds using the Folin-Ciocalteu method, the content of the total amount of tannins, flavonoids, the total amount of anthocyanins, the antioxidant capacity using ABTS reagent (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), measuring the level of free DPPH radical scavenging (2,2'-diphenyl-1-picrylhydrazyl).

[Makarova N.V., Valiulina D.F., Batkova I.A. **Antioxidant activity of technical grapes harvested in the Samara Region in 2013.** *Life Sci J* 2014;11(12s):797-801] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 172

Keywords: technical grapes, antioxidant activity, Folin-Ciocalteu, tannins, flavonoids, anthocyanins

Introduction

Grapes are a widely used crop due to the unique chemical composition. Grapes contain sugar (mainly glucose and fructose), enzymes, vitamins, microelements, organic acids, nitrogenous, phenolic and other substances very important for human health [1].

Grapes are distinguished from other crops by a variety of useful properties. Grapes are a nutritious, dietary and therapeutic product. One kilogram of fresh grapes provides about 30% of calories of the daily human diet. Grapes contain 14-30% of sugar, a significant amount of organic acids (tartaric, malic, citric, etc.), which increase appetite and improve digestion and prevent the formation of kidney stones [2]. Grapes and grape juice are used as therapeutic agents for feeding retarded children, for the rehabilitation of the patients after cardiac and gastrointestinal diseases, polyarthritis, after surgeries. Muscatel grape varieties contain antibiotic aromatics. Pectins contained in the skin of dark-coloured grapes, bind radioactive metals to insoluble salts and thus remove them from the body [3]. Due to the presence of biologically active substances, grapes help to treat anemia and nervous system disorders. Grapes are a source of a whole vitamin complex: A, B₁, B₂, B₆, B₇, C, K, and P. Grapes contain many microelements, including manganese, zinc, rubidium, fluorine, vanadium, iodine, titanium, cobalt, etc. Dark-coloured grapes contain very useful tannins and colorants, having bactericidal and antiradiation action [4].

The aim of our research was to study the chemical composition and antioxidant power of seven varieties of wine grapes: six varieties grown in the Samara Region: Druzhba (Friendship), December, Tashkent, Cabernet of the North, Zhuravlik (Crane),

Strelets (Sagittarius) and one comparative variety from Abkhazia: Isabella.

Wine grapes are distinguished by the indicators of acidity and sugar content, which determine the type of produced wine. They are rated in terms of mechanical characteristics and chemical composition, which in turn depend on the conditions of grape cultivation and its biological characteristics. Grape varieties belonging to this group can be cultivated in almost all areas where winegrowing is possible, which affects the quality of the crop. Therefore, when grown in different climatic and soil conditions the same wine grape varieties can be used for a different industrial purpose [5].

Druzhba is a universal grape variety with increased resistance to disease. Druzhba refers to the very early ripening varieties. Its bunches are of medium size, cylinder-conic, of moderate density. The average weight of a bunch is 280 grams. The grapes are white, rounded, large, 22 by 23 mm, the average weight of a grape is 4 grams. The pulp is sappy and juicy, of a pleasant harmonious taste with a muscatel aroma. The average yield of this variety is up to 150-180 metric centners. Druzhba grape variety is characterized by high resistance to mildew, botrytis cinerea and depending on the area of cultivation requires 1-2 sprayings against mildew. Against oidium it is treated in the usual time for all grape varieties.

December is a wine grape variety with a medium ripening period. The period from budbreak to full ripening is 165 days with accumulated favourable temperatures of 2800°C. December grape variety is resistant to mildew, botrytis cinerea, spider mites. The bunches are of medium density and good structure, providing increased resistance to grape-berry moths. Their average weight is 220 grams. The grapes are black, weighing 3-3.5 grams, with the size of 2.3by1,5

cm. The leaves are medium-sized, slightly oval, with three blades, whole or slightly divided, slightly grooved, smooth, shiny or slightly reticulate-rugose, partly pubescent beneath. The blossoms are monoecious. The taste is simple, but harmonious [6].

Tashkent is a wine grape variety with a middle-late ripening period. The period from budbreak to full ripening is 155 days with accumulated favourable temperatures of 2900°C. The growth strength of the bushes is above average. The leaves are medium-sized, rounded, with five blades, multi-divided, reticulate-rugose, glabrous beneath. The petiolar cut is open, lyrate. The blossoms are monoecious. The bunches are medium-sized, conical, of medium density. The grapes are medium-sized, rounded, black, covered with a thick wax coating. The skin is thin. The pulp is sappy and juicy. The ripening of sprouts is good. The yield is 150-300 metric centners per hectare. This variety is relatively frost resistant.

Cabernet of the North is a wine grape variety with a medium ripening period. Ripens in the first half of September. The bunches are small, weighing 90-100 grams, cylinder-conic, dense. The grapes are small, weighing 1.4 grams, rounded, black, with a thick wax coating. The skin is strong. The pulp is juicy, with a flavor of nightshade. The juice is not colored. The ripening period is 140 days. The bushes are of medium growth strength and low-growing. The ripening of sprouts is good. The fruiting coefficient is 1.8, tends to be overloaded with the harvest. The frost resistance is increased up to -25 -26 °C. The resistance to mildew is average. This grape variety is recommended for semi-covered or uncovered cultivation. Cabernet grapes are used for making table and dessert wines.

Zhuravlik is a wine grape variety with a medium-late or late ripening period. The biological ripeness of the grapes comes in the second half of September, and the wine ripeness comes in early October. The bushes are full-grown and similar in habitus. The bunches are medium-sized, weighing 170 grams, conical, often with alae, of medium density. The grapes are small, rounded, dark blue, pruinose. Red table wines produced from this grape variety are intensely colored, of alcohol strength, whole and extractive, with a pleasant taste.

Strelets is a wine grape variety with a very late ripening period. The period from budbreak to full ripening is 169 days with accumulated favourable temperatures of 3490 °C. The growth strength of the bushes is above average. The leaves are medium-sized, rounded, with five blades, with distinct extra blades, deeply and medium divided, wide-reticulate-rugose, glabrous beneath. The petiolar cut is open, lyrate, with a sharp bottom. The blossoms are

monoecious. The bunches are medium-sized, cylinder-conical, of medium density. The grapes are medium-sized, rounded and ovoid, black. The skin is thick. The pulp is juicy. The ripening of sprouts is good. The frost resistance is high. This variety is susceptible to mildew, botrytis cinerea and oidium.

Isabella refers to the group of table-wine grape varieties, which are characterized by late ripening period. The bushes are full-grown, the ripening of sprouts is medium. The bunches weigh 140 grams on average, have a cylinder or cylinder-conical shape, their density is middle or lax. The grapes are round or oval, medium-sized, with a strong thick skin, black, covered with a wax coating, which gives them a blue-grey tint. The sugar content of this variety is 16-18 % with the acidity of 6-7 g / l. The pulp is mucilaginous, with a distinct aroma of strawberries. The ripening period is 150-180 days. The variety is resistant to phylloxera and various fungous diseases. This variety is characterised by high frost resistance. It grows best on fertile subcalcareous soils. The variety does not withstand drought well, but easily withstands high humidity [7].

The pulp [8], the skin and the grape seeds [9] were taken as objects for the analysis. For the analysis of the chemical composition and antioxidant activity determination, the following methods of analysis were used: measurement of total phenolics content [10], the total flavonoid content, the total content of tannins, the total content of anthocyanins [11], measuring of the level of free DPPH radical scavenging (2,2' - diphenyl picrylhydrazyl 1), the ability to scavenge ABTS radicals (2,2' - azino -bis (3- ethylbenzthiazoline -6-sulfonic acid)), the total antioxidant power by the FRAP method (ferric reducing antioxidant power with the reagent 2,4,6- tripyridyl -s- triazine), antioxidant activity in a linoleic acid system.

Materials and methods

Chemicals and reagents

Folin-Ciocalteu reagent in sodium carbonate, gallic acid, catechin, ABTS (2,2' -azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) were purchased from Fluka (Germany). DPPH (2,2-diphenyl- 1-picrylhydrazyl), Tween 40, hydrogen peroxide, sodium nitrite, aluminum chloride, thiobarbituric acid, trichloroacetic acid were purchased from Sigma-Aldrich Chem. mp. (USA).

Fruit collection

The grapes collected on the territory of Samara region in Kinel'skiy and Bolsheglushitskiy areas in the period of early maturing in 2013.

Determination of total phenols

Total phenolic content of methanolic fruit extracts was assessed using a modified version of the Folin-Ciocalteu assay [12]. Gallic acid was used as a

standard and the aqueous gallic acid solution (200 mg l⁻¹) was di-luted with distilled water to give appropriate concentrations for a standard curve. For the analysis, 100 μ l of methanolic fruit extract or gallic acid standard, 100 μ l of methanol, 100 μ l of Folin–Ciocalteu reagent and 700 μ l of Na₂CO₃ were added into 1.5 ml micro-centrifuge tube. The samples were vortexed immediately and the tubes were incubated in the dark for 20 min at room temperature. After incubation all samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was then measured at 735 nm in 1 ml plastic cuvettes using evolution 200 Series spectrophotometer. The results were expressed in mg gallic acid equivalent/100 g dry weight.

Determination of total flavonoids

The flavonoid content of the methanolic extracts were measured using a assay [13]. A known volume (0.5 ml) of the extract or standard solution of quercetin was added to a 10 ml vol-umetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v NaNO₂ was added to the flask. After 5 min, 0.6 ml of 10% w/v AlCl₃ was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was read at 350 nm against the blank (water) and flavonoid content was expressed as mg querce-tin equivalents in 100 g of fresh material.

Determination of the anthocyanin profile

The concept of determining the amount of anthocyanin present in a material by measuring the change in absorbance at 2 different pH values (3.4 and 2.0) [14 and 15]. Researchers have proposed using the pH values of 1.0 and 4.5 (2–5). Monomeric anthocyanins undergo a reversible structural transformation as a function of pH (colored oxonium form at pH 1.0 and colorless hemiketal form at pH 4.5. Thus, the difference in absorbance at the vis-max (520 nm) of the pigment is proportional to the concentration of pigment. Degraded anthocyanins in the polymeric form are resistant to color change with change in pH. Therefore, polymerized anthocyanin pigments are not measured by this method because they absorb both at pH 4.5 and 1.0.

Determination of condensed tanins

In presence of concentrated H₂ SO₄, condensed tanins were transformed by the reaction with vanillin to anthocyanidols [16]. 50 μ l of the methanolic seed extract appropriately dilute was mixed with 3 ml of 4% methanol vanillin solution and 1.5 ml of H₂ SO₄. After 15 min, the absorbance was measured at 500 nm. Con-densed tannin contents of seeds (three replicates per treatment) were expressed as mg catechin equivalents (CE) per gram of dry

weight through the calibration curve with catechin. The calibration curve range was 50–600 mg ml⁻¹.

DPPH radical scavenging activity

The scavenging activity of samples was measured in accordance with the method [17]. The method was based on the reduction of methanolic DPPH in the presence of a hydrogen-donating antioxidant. DPPH solution was an intense violet colour and showed an absorption band at 515 nm. Adsorption and colour lowered when DPPH was reduced by an antioxidant compound. The remaining DPPH corresponded inversely to the radical-scavenging activity of the antioxidant. DPPH (2 mg) was dissolved in 54 ml of MeOH. Aliquots of investigated extract (50, 100, 200, 300, 500 and 1000 μ g) were dissolved in 2 ml of MeOH. Then 1.0 ml of each solution was added to 2.0 ml of DPPH solution at room temperature. The absorbance at 515 nm was measured against a blank (2 ml MeOH in 2.0 ml of DPPH solution) using evolution 200 Series spectrophotometer. The results were expressed as percent-age of reduction of the initial DPPH adsorption by test samples:

$$\% \text{ of reduction of the initial DPPH adsorption} = \frac{\text{ADPPH}(t)_{\text{Asample}}(t) / \text{ADPPH}(t)_{100}}{\text{ADPPH}(t)_{\text{Asample}}(t)}$$

ADPPH(t) is absorbance of DPPH at time t and Asample (t) is absorbance of sample at t the same time.

FRAP assay

The FRAP assay was carried [18]. The FRAP solution was freshly prepared on the day of use, by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively. Following this the FRAP solution was heated, while protected from light, until it had reached a temperature of 37 LC. Appropriate dilutions of methano-lic fruit extracts were prepared. One hundred microlitres of the di-luted sample extract (or for blank 100 μ l methanol and for Trolox standard curves 100 μ l Trolox of appropriate concentration) and 900 μ l of FRAP solution were added into a micro-centrifuge tubes. The tubes were vortexed and left at 37 LC for exactly 40 min, and the absorbance was measured at 593 nm. The Trolox standard curves were used to calculate the antioxidant activity of the sam-ples in relation to Trolox and were expressed as mg Trolox equivalent/100 g dry weight sample (mg TE 100 g 1 DW).

ABTS free radical decolorization assay

The total antioxidant capacity assay conducted using evolution 200 Series spectrophotometer. The procedure was based on a method [19] with some modification. ABTS₊ was generated by reacting ABTS (7.4 mM) with potassium persulphate (2.6 mM). The solution was diluted to

obtain an absorbance of 1.4 units at 414 nm (molar extinction coefficient $E=3.6_{104} \text{ mol}^{-1} \text{ cm}^{-1}$, Forni, Morla-Arellano, Packer, & Willison 1986) with 50 mM glycine-HCl buffer (pH 4.5) before use. Three millilitres of the solution were added to 20–80 ml of AA, trolox, hydroquinone, pyrogallol and fruit extracts separately. The changes in absorbance at 414 nm were recorded at 1, 3, 6, 10, 20, 30, 40, 60 and 90 min after mixing and until the absorbance reached a plateau. The antioxidant capacities, obtained by comparing the absorbance change at 414 nm in a test reaction mixture containing extract of fruit with that containing AA, were expressed as mg of AA equivalents per 100 g of homogenate (AEAC)..

Determination of Antioxidant Activity in a Linoleic Acid System

The total antioxidant activity of FEHP was carried out by use of a linoleic acid system [20]. The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL of phosphate buffer (0.2 M, pH 7.0), and then the mixture was homogenized. A 0.5-mL ethanol solution of different concentration of FEHP (50-500 $\mu\text{g/mL}$) was mixed with linoleic acid emulsion (2.5 mL, 0.2 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0). The reaction mixture was incubated at 37 °C in the dark to accelerate the peroxidation process. The levels of peroxidation were determined according to the thiocyanate method by sequentially adding ethanol (5 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 20 mM in 3.5% HCl). After the mixture was left for 3 min, the peroxide value was determined by reading the absorbance at 500 nm on a spectrophotometer.

Results

Determination of phenolic compounds

Based on data from Table 1, we can say that by the total content of phenols the undisputed leaders are Zhuravlik variety seeds. By the total content of phenols in the skin, the first place belongs to Strelets variety, the second place belongs to Tashkent variety. The lowest rates by the content of phenols in the skin belong to Druzhba variety. The pulp of all the varieties contains the least amount of phenolic compounds compared to the skin and seeds.

Flavonoids content

The seeds of Cabernet of the North variety contain the highest amount of flavonoids. The first place by the content of flavonoids in the skin belongs to Strelets variety.

Determination of condensed tannins

By the total content of tannins in the seeds the leading variety is December. The highest rates for the skin belong to Strelets variety. The seeds of

Isabella variety do not differ significantly from them. The last place by the total content of tannins in the skin belongs to Druzhba variety. The pulp of all the considered grape varieties has low levels of the total content of tannins.

Determination of the anthocyanin profile

By the total content of anthocyanins in the skin, the first place belongs to Zhuravlik variety, with Cabernet of the North following. The final place belongs to Druzhba variety which has the lowest rates.

Total DPPH radical scavenging activity

Analyzing the experimental data in Table 2, we can see that the undisputed leaders by the ability to scavenge free DPPH radicals are the seeds of the Strelets variety, the second place belongs to Druzhba seeds. Among the grape skin, by the ability to scavenge free DPPH radicals, the first place belongs to Druzhba skin.

Total antioxidant capacity of fruits by ABTS

By the ability to scavenge ABTS radicals, the seeds of all the studied varieties grades are undisputed leaders. Among the grape skin, by the ability to scavenge ABTS radicals, the first place belongs to December skin and the last place belongs to Tashkent variety. The ability of the pulp to scavenge ABTS radicals is approximately the same for all the varieties.

FRAP assay

In terms of FRAP, Isabella seeds can be singled out. Among the skin, the first place in terms of FRAP belongs to Zhuravlik variety, the last place belongs to Cabernet of the North.

Determination of Antioxidant Activity in a Linoleic Acid System

By the antioxidant activity in a linoleic acid system, the best results belong to Cabernet of the North seeds, the skin and pulp of Zhuravlik variety.

Discussion

The book “A New Approach to Wine-growing” by Stetcenko V.M. and Derzhakov N.V. [21] describes the chemical composition and useful properties of grapes. The value of the grapes is determined primarily by a favourable combination of the taste of the fruit with its nutritional and dietary advantages.

Grapes contain digestible sugars, glucose and fructose, organic acids: tartaric, malic, citric, oxalic, etc. Besides organic acids, grape juice contains up to 1.5 % of mineral substances: potassium, sodium, phosphorous, iron, aluminum, iodine, bromine, boron and many other macro- and microelements. Grapes are rich in vitamins A (carotene), B₁ (thiamine), B₂ (riboflavin), B₆ (adermin), C (ascorbic acid).

Grape seeds have a powerful antioxidant effect, help body cleansing and health improvement, cell renewal and skin rejuvenation. Alcoholic grape

seed extract reduces cholesterol levels, regulates blood pressure, protects and restores blood vessels, affects sleep beneficially, strengthens the immunity system. Grape seed oil is rich in vitamins E, A, B, C and PP, microelements and a unique fatty acid composition. It has a beneficial effect on the immune system, strengthens the walls of the blood vessels and makes them elastic, helps reduce cholesterol levels, affects the kidneys positively, prevents oncological diseases.

Conclusions

Based on these results, we can say that all grape seeds contain the maximum values by the antioxidant activity and the chemical composition and the grape pulp contains the minimum values.

Among the studied grape varieties we can distinguish three: December, Strelets, Zhuravlik as the varieties with a maximum content of tannins, phenols, as well as with a high antioxidant activity.

Thus, it can be concluded that by the chemical composition the grape seeds and skin contain the highest number of phenols, tannins, anthocyanins, they also have the highest antioxidant effect and are excellent materials for the production of extracts with antioxidant activity.

Corresponding Author:

Dr. Makarova Nadezhda Viktorovna
The Samara State Technical University
Str. Molodogvardeyskaya, 244, Main Building,
Samara, 443100, Russian Federation

References

1. Gluschenko, V.T., Y.S. Berezovsky, 2008. Grapes. Moscow: AST.
2. Litvinov, P.I., 1980. Viticulture Kiev: Vintage, pp: 360.
3. Potebnya, A. B., Z.X. Skrobishevsky, 2010. Wine-growing Guide. Fiton, pp: 448.
4. Stogova, N., 2006. Grapes against a Hundred Diseases. St. Petersburg.: Peter, pp: 96.
5. Solomonov, S.S., 2011. New Resistant Varieties of Grapes. Donbass, pp: 66.
6. Troshin, L.P. and N.A. Sviridenko, 1988. Resistant Varieties of Grapes. Simferopol: Tavriya, pp: 208.
7. Dokuchaeva, E.S., E.S. Komarova and N.N. Pilipenko, 1986. Grape Varieties. Kiev: Urozhay, pp: 272.
8. Pooja, D., A. Pangurang and B. Kaushik, 2006. Phenolic composition and antioxidant activity in grapevine parts and berries cv. Kismish black during maturation. *Int. J. Food Sci. and Technol*, 1 (41): 1-9.
9. Panasyuk, A.L., V.V. Zhirova, I.O. Mikhailov, N.M. Romanyuk and E.A. Nikulin, 2003. Extraction of Phenolic Compounds from Grape Seeds. *Winemaking and Wine-growing*, 1: 36-37.
10. Baraboy, V.A., 1976. Biological Effects of Plant Phenolic Compounds. Kiev: Nayk. dumka, pp: 260.
11. Chub, B., 2008. What are Anthocyanins Needed for. *Floriculture*, 6: 22-25.
12. Rockenbach, I.I., E. Rodrigues, L.V. Gonzaga, V. Caliar, M.I. Genovese, G. Schmidt and R. Fett, 2011. Phenolic compounds content and antioxidant activity in pomace from selected red grapes (*Vitis vinifera* L. and *Vitis Labrusca* L.) widely produced in Brazil. *Food Chem*, 1(127): 174-179.
13. Skerget, M., P. Kotnik, M. Hadolin, A.R. Hras, M. Simoncic and Z. Knez, 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem*, 1(89): 191-198.
14. Harbourne, N., J. Christophe, D.J. Morgan and J.G. Lyng, 2008. Determination of the degradation kinetics of anthocyanins in a model juice system using isothermal and non-isothermal methods. *Food Chem*, 2(111): 204-208.
15. Obradovic, D., M. Oatey and M. Schulz, 2005. Enhanced stabilisation of anthocyanin compounds. *Austral. N.Z. Grapegrow and Winemaker*, 498: 51-53.
16. Crespy, A., 2002. Tannins de pepins de raisin: possibilites de stabilisation de la couleur, de protection contre l'oxydation et d'amelioration de la tenue en boushe sur vins rouges et roses. *Rev. fr. oenol*, 195(30): 23-27.
17. Rufino, M.S.M., F.A.N. Fernandes, R.E. Alves and E.S. de Brito, 2009. Free radical-scavenging behavior of some north-east Brazilian fruits. *Food Chem*, 4(114.): 693-695.
18. Nilsson, J., R. Stegmark and B. Akesson, 2004. Total antioxidant capacity in different pea (*Pisum sativum*) varieties after blanching and freezing. *Food Chem*, 3(86): 501-507.
19. Mariken, J.T.J., Dallinga J. Sebastiaan, V. Hans-Peter, R.M.M. Guido, B. Aalt, 2004. A new approach to assess the total antioxidant capacity using the TEAC assay. *Food Chem*, 4(88): 567-570.
20. Qing, L. and Y. Huiyuan, 2007. Antioxidant activities of barley seeds extracts. *Food Chem*, 3(102): 732-737.
21. Stetcenko, V.M. and N.V. Derzhakov, 2011. A New Approach to Wine-growing. Moscow: AST: Poligrafizdat, pp: 415.

7/29/2014