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Analytical characteristics of wild and cultivated strawberries (spring & autumn harvests) of Southern Bulgaria & Northern Greece

S. I. Papanov[1]*, Ek. G. Petkova[2], K. V. Ivanov[1], St. D. Ivanova[1], V. V. Grudeva[3]

[1]*Medical University Plovdiv, Faculty of Pharmacy, 15A V. Aprilov Bul., 4000 Plovdiv, Bulgaria*
[2]*Medical University Plovdiv, Medical College, 120 Br. Bakston Str., 4004 Plovdiv, Bulgaria*
[3]*Hospital Sveta Ekaterina, 52A Pencho Slaveykov Bul., 1431 Sofia, Bulgaria*

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Strawberries with their aroma and sweet taste are the most popular fruit in the world. There are more than 600 sorts of strawberries that differ in taste, size and texture. An analysis of pH, total phenols, antioxidant activity (radical trapping activity) total anthocyanin of wild growing and cultivated varieties of strawberries with origin North Greece and South Bulgaria. A methodology has been developed for determining of common phenols, antioxidant activity, common anthocyanins. The average value of pH index ranges from 3.26 for strawberry samples from northern Greece to 3.34 and 3.55 for strawberry samples from southern Bulgaria. Total phenols: 4.58±0.02 mg GAE/g fresh weight for Wild Strawberries; 1,34±0,02 mg GAE/g fresh weight for strawberry sample from Northern Greece; 1.36±0.03 mg GAE/g fresh weight and 1.41±0.01 mg GAE/g fresh weight strawberry sample from southern Bulgaria. Total anthocyanins: 0.34±0.01 mg/g fresh weight for strawberry sample from Northern Greece; 0,21±0,01 mg/g fresh weight and 0,22±0,01 mg/g fresh weight for strawberry sample from southern Bulgaria. Antioxidant activity: Wild strawberries - 41,38±0,20 m.mol TE/g fresh weight; 7,35±0,30 m.mol TE/g fresh weight for strawberry sample from Northern Greece; 8,99±0,20 m. mol TE/g fresh weight and 10,20±0,20 m.mol TE/g fresh weight for strawberries from southern Bulgaria. There is a direct correlation between antioxidant activity and total phenols. There is an inverse relationship between antioxidant activity / phenols and anthocyanins.

Keywords: strawberries; antioxidant; phenols; anthocyanins.

INTRODUCTION

The strawberry is a genus of plants of the *Rosaceae* family.

Strawberries with their aroma and sweet flavor are the most popular fruit in the world. There are more than 600 varieties of strawberries that differ in taste, size and texture [1-5]. Strawberries are rich in both mineral substances and vitamin C, carotene, vitamin E and vitamins of group B. Vitamin C is predominant with 58,80 mg. per 100 grams of strawberries - the recommended daily dose [1, 4-8]. About 90% of mature strawberry is water but contains sugars, about 80% of which are glucose and fructose.

The color of strawberries is due to anthocyanins, mainly to peargonidin-3-glucoside. Anthocyanins are water-soluble pigments found in plant cells. In addition, anthocyanins can be used as pH indicators- they are pink in acidic, purple in neutral and yellow in alkaline solutions [9].

According to a study published in Journal of Nutritional Biochemistry, strawberries help lower total cholesterol (by 8,78%), bad cholesterol (13,72%), and triglycerides (by 20,8% .).

Studies suggest that consumption of strawberries, may have beneficial effects against oxidative stress mediated diseases such as cancer. Berries contain multiple phenolic compounds, which are thought to contribute to their biological properties [10].

Flavonoids in strawberries have excellent antioxidant and anti-cancer properties [1, 11].

Important phenolic acids in strawberries are the ellagitannins and ellagic acid glucosides, which break down to pure ellagic acid, also present in the fruit [12].

Ellagic acid is valuable to human health because it is antimutagenic and has anticarcinogenic activity against chemical-induced cancers [13].

Ellagitannins in strawberries are primarily located in the achenes [14].

Scope: Determination and analysis of pH, total phenols, antioxidant activity (radical trapping activity) total anthocyanin of wild growing and cultivated varieties of strawberries.

MATERIAL AND METHODS

Object of the study was the antioxidant characteristics of wild growing and cultivated varieties of strawberries grown at different altitudes:

Wild strawberries (Velingrad, Bulgaria) - 755m. altitude.

* To whom all correspondence should be sent: stoyan.papanov@abv.bg

Caparoso variety, (Northern Greece) - 115m altitude.

Zeenger variety (Belomorski-Plovdiv, Bulgaria) - 164m altitude.

Asia variety (Byaga village, Bulgaria) - 374m altitude.

Temptation variety (Gotse Delchev, Bulgaria) - 540m altitude.

Albion variety (Sofia, Bulgaria) - 598m altitude.

The study was conducted from May to September 2017 in laboratories of the University of Food Technologies Plovdiv and Laboratory of Pharmaceutical Analysis of the Medical University of Plovdiv.

The following research methods were used for the study:

- Systematic approach and critical analysis of the available scientific periodicals;
- Spectrophotometric method for determination of adsorption and standard Gallic acid;
- DPPH method for determining the antioxidant activity (radical trapping activity).

The study was carried out using the following apparatus:

1. Analytical scale KERN ABJ 220-4M;
2. Spectrophotometer Camspec M107, UK; pH-determiner;
3. Standardized pH determiner with pH 4,0 and 7,0 standard buffer solutions (WTW inoLab pH 7110, Germany);
4. Standardized pH meter with pH 4,0 and 7,0 standard buffer solutions, pH meter Denver Instrument Ultra Basic.

For the purposes of the study, the following reagents and solutions were used.

- To determine common phenols:

7,5% Na_2CO_3 (weighing 7,5 g Na_2CO_3 and dissolving in 100 ml dH_2O) and Folin-Ciocalteus phenol reagent (Sigma) - a 5 times diluted reagent is used (1 ml + 4 ml dH_2O)

- To determine antioxidant activity by DPPH method:

0,1 M DPPH reagent: 10 mg.DPPH (2,2–Diphenyl–1-picrylhydrazil) dilutes in 250 ml methanol

- To determine common anthocyanins:

Buffer with pH 1.0 (potassium chloride, 0,025 M)

Buffer with pH 4.5 (sodium acetate, 0,4 M)

HC1 (to adjust pH)

All reagents and solutions are of analytical purity.

Samples of fresh strawberries were cut into pieces and then homogenized with a blender to a homogeneous mass. Weighed 8 grams (analytical scale KERN ABJ 220-4M) from the sample and quantitatively transfer it into a 50 ml volumetric flask with ethanol and volume is brought to the mark. The sample was homogenized and stored at room temperature for 15 minutes.

Analysis path to determine common phenols

We placed 0,2 ml of sample solution in a cuvette then we placed 1,0 ml of Folin-Ciocalteus phenol reagent (diluted 5 times) and 0,8 ml 7,5% solution of Na_2CO_3. For control sample, the same reagents were prepared, but instead of 0,2 ml sample there were placed 0,2 ml solvent. The samples thus prepared were allowed to stand for 20 minutes at room temperature [15-18]. Adsorption of the sample was measured spectrophotometrically against a control with wavelength λ=765 nm (Camspec M107, UK). The amount of common phenols was reported according to a pre-set standard gallon acid right: Y=12,557x - 0.0871.

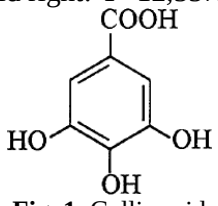

Fig. 1. Gallic acid

Calculations:

$$\text{mg GAE/g f.s} = (V \times C) / M \quad (1)$$

where:

GAE – gallic acid equivalent

f.s – fresh substance, g

V – volume of the extract, ml

C - concentration of phenols in the extract read by standard straight, mg/ml

M - weight of extracted plant material, g

Path of the method for determining the antioxidant activity (radical trapping activity) using DPPH method [19-22].

We placed in a cuvette 2,85 ml of 0,1 M DPPH after that we placed 0,15 ml of sample. For the control, the same reagents were prepared but a solvent was placed instead of sample. Thus, prepared samples are kept 15 min at 37°C. Adsorption of the sample was measured spectrophotometrically against a control (solvent methanol) at a wavelength of λ=517 nm (Camspec M107, UK). Antioxidant activity was reported according to a pre - established standard straight Trolox ® (Sigma).

Calculation:

$$I, \% = \frac{A\ control\ sample - A\ sample}{A\ sample} * 100 \quad (2)$$

$$C, \text{m.mol TE/ml} = 102.06.\ I\% + 0{,}7954 \quad (3)$$

$$\text{m.mol TE/g. fresh weight} = (V \times C) / M \quad (4)$$

where:

- C- concentration, mM TE-TROLOX equivalents
- I – percentage inhibition
- A – Absorption
- M – Weight of the sample, g
- V – Volume of the sample, (100 ml)

Path of the analysis to determine common anthocyanins

Buffer with pH 1.0 (potassium chloride, 0,025 M). – was weighed 1,86 g KCl in a cup and add distilled water around 980 ml. We measured pH and corrected pH to 1.0 (± 0,05) with HC1 (approximately 6,3 ml). We transferred to a 1 liter volumetric flask and dilute to the mark with distilled water;

Buffer with pH 4.5 (sodium acetate, 0.4 M). – was weighed 54.43 g CH_3C0_2Na x $3H_20$ in a cup and add distilled water up to 960 ml. We measured pH and corrected pH to 4.5 (± 0,05) with HCI (approximately 20 ml). We transferred to a 1 liter volumetric flask.

Determine the absorbance of the sample diluted with pH 1.0 (6 times) with buffer, (A) and buffer (B) pH 4,5, as well with 520 nm, as with 700 nm. Diluted test samples were scored against a blank sample filled with distilled water [22-26].

Calculation of the concentration of anthocyanin pigments is expressed as cyanidin-3-glucosidic equivalents as follows:

$$\text{Anthocyanin pigments (mg/L)} = A \times MW \times DF \times 10^3/\varepsilon \quad (5)$$

where:

- A = (A520 nm - A700 nm) pH 1.0 - (A520nm - A700nm) pH 4.5;
- MW (molecular weight) = 449,2 г/mol for cyanidin-3-glucosidic;
- DF = dilution factor
- ε = 26900 molar extinction coefficient in Л x mol^{-1} and cm^{-1}
- 10^3 = conversion factor from g in mg [27-31].

RESULTS AND DISCUSSION

The results presented are the arithmetic mean of at least three parallel studies.

Important phenolic acids in strawberries are the ellagitannins and ellagic acid glucosides, which break down to pure ellagic acid, also present in the fruit [11].

Information on the content of the total phenols in the samples tested is given in Table 1. The values of the total phenols ranged from 4,58 to 1,34.

Table1: Common phenols of wild growing and cultivated varieties of strawberries with origin Northern Greece and South Bulgaria.

Variety	Common phenols(mg GAE/g fresh weight)
Wild Strawberries, (Velingrad, Bulgaria)	4,58 ± 0,02
Caparoso variety, (Northern Greece)	1,34 ± 0,02
Zeenger variety, (Belomorski, Plovdiv, Bulgaria)	1,36 ± 0,03
Asia variety, (Byaga village, Bulgaria)	1,41 ± 0,01
Albion variety, (Sofia, Bulgaria)	1,71 ± 0,01
Temptation variety (Gotse Delchev, Bulgaria)	2,16 ± 0,02

The highest content of common phenols is observed in wild strawberries 4,58 mg GAE/g fresh weight, followed by a variety of Temptation – 2,16 mg GAE/g fresh weight, Albion variety – 1,.71 mg GAE/g fresh weight, Asia variety – 1,41 mg GAE/g fresh weight, Zhenger variety – 1,36 mg GAE/g fresh weight and Caparoso variety – 1,34 mg GAE/g fresh weight.

The anthocyanins are a group of flavonoids with exceptionally good scavenging activities. Pelargonidin-based anthocyanins such as pelargonidin 3-glucoside, pelargonidin 3-rutinoside, and pelargonidin 3-glucoside–succinate are the predominant anthocyanins in cultivated strawberry fruit [29].

The common anthocyanins of the samples we studied are within the range from 0,35 to 0,19 mg/g of fresh weight are presented in Table 2.

The conducted study of anthocyanins gives us reason to arrange the varieties we examine as follows: Temptation variety, 0,35 mg/g fresh weight, Caparoso variety 0,34 mg/g fresh weight, Albion variety 0,25 mg/g fresh weight, Asia variety 0,22 mg/g fresh weight, Zeenger variety 0,21 mg/g fresh weight.

Table 2: Common anthocyanins of wild growing and cultivated varieties of strawberries with origin Northern Greece and South Bulgaria.

Variety	Common anthocyanins (mg/g fresh weight)
Wild Strawberries, (Velingrad, Bulgaria)	0,19 ± 0,01
Caparoso variety, (Northern Greece)	0,34 ± 0,01
Zeenger variety, (Belomorski, Plovdiv, Bulgaria)	0,21 ± 0,01
Asia variety, (Byaga village, Bulgaria)	0,22 ± 0,01
Albion variety, (Sofia, Bulgaria)	0,25 ± 0,01
Temptation variety, (Gotse Delchev, Bulgaria)	0,35 ± 0,01

The DPPH method for determining the antioxidant activity of strawberries is also used by and the results obtained are highly accurate [30].

The results for the antioxidant activity of our samples, determined by the DPPH method is shown in Table 3.

Table 3. Antioxidant activity of wild growing and cultivated varieties of strawberries with origin Northern Greece and South Bulgaria.

Variety	Antioxidant activity (m mol TE/g fresh weight)
Wild strawberries, (Velingrad, Bulgaria)	41,38 ± 0,20
Caparoso variety, (Northern Greece)	7,35 ± 0,30
Zeenger variety, (Belomorski, Plovdiv, Bulgaria)	8,99 ± 0,20
Asia variety, (Byaga village, Bulgaria)	10,20 ± 0,20
Albion variety, (Sofia, Bulgaria)	19,32 ± 0,20
Temptation variety, (Gotse Delchev, Bulgaria)	22,48 ± 0,20

Antioxidant activity ranges from 41,38 to 7,35 m mols TE/g fresh weight.

It is greatest with Wild Strawberries next in place we could put Temptation variety with 22,48 m mols TE/g fresh weight and Albion variety with 19,32 m mols TE/g fresh weight.

Grouped based on the antioxidant activity indicator, we can place Azia variety, sort Zeenger and sort Caparoso, respectively, with 10,20; 8,99; 7,35 m mols TE/g fresh weight.

www.truehealth.ru for the ph indicator, of different strawberry varieties gives values in the range of 3 to 3.9. The results obtained by us for ph of the varieties under consideration are presented in Table 4.

Table 4. pH of wild growing and cultivated varieties of strawberries with origin North Greece and South Bulgaria.

Variety	Ph
Wild strawberries, (Velingrad, Bulgaria)	3,55
Caparoso variety, (Northern Greece)	3,32
Zeenger variety, (Belomorski, Plovdiv, Bulgaria)	3,34
Asia variety, (Byaga village, Bulgaria)	3,55
Albion variety, (Sofia, Bulgaria)	3,74
Temptation variety, (Gotse Delchev, Bulgaria)	3,85

As can be seen from Table 4, the values we obtained for ph are in the range of 3.32 to 3.85, which confirms the objectivity of the methodology used by us.

CONCLUSIONS

1. An absolute favorite in the varieties studied by us on the indicator common phenols are Wild Strawberries followed by Temptation variety. Approximately the same indicator is for Zeenger variety and Caparoso variety.

2. The highest anthocyanin value is seen in Temptation variety and Caparoso variety.

In wild strawberries, the lowest anthocyanin value is observed. This can be explained by approximately the same soil composition.

3. The antioxidant activity of Wild Strawberries is approximately twice as high as that of the Temptation variety and Albion variety strains and three times bigger than that of Asia variety and Zeenger variety and six times greater than that of Caparoso variety.

4. There is a direct correlation between antioxidant activity and total phenols.

5. There is an inverse relationship between antioxidant activity/phenols and anthocyanin.

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АНАЛИТИЧНИ ХАРАКТЕРИСТИКИ НА ДИВИ И КУЛТИВИРАНИ ЯГОДИ (ЛЯТНА И ЕСЕННА РЕКОЛТА) В ЮЖНА БЪЛГАРИЯ И СЕВЕРНА ГЪРЦИЯ

Ст.И. Пъпанов[1]*, Ек.Г. Петкова[2], К.В. Иванов[1], Ст.Д. Иванова[1], В.В. Грудева[3]

[1]Медицински университет в Пловдив, Факултет по фармация, 4000 Пловдив, България

[2] Медицински университет в Пловдив, Медицински колеж, 4004 Пловдив, България

[3]Болница „Св. Екатерина“, 1431 София, България

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(Резюме)

Ягодите със своя арамат и сладък вкус са най-популярният плод на света. Съществуват повече от 600 разновидности ягоди, които се различават по вкус, размер и текстура. Целта на проучването е анализ на pH, общи феноли, антиоксидантна активност (радикал улавяща активност) и общи антоциани на диворастящи и култивирани сортове ягоди с произход Северна Гърция и Южна България. Разработена е методология за определяне на общи феноли, антиоксидантна активност и общи антоцианини.Средната стойност на показателя ph е в границите от 3,26 за проба ягоди от Северна Гърция до 3,34 и 3,55 за проба ягоди от Южна България. Общи феноли: 4,58 ± 0,02 мг GAE/г свежо тегло при Диворастящи ягоди; 1,34+ - 0,02 мг GAE/г свежо тегло за проба ягоди от Северна Гърция; 1,36+_0,03 мг GAE/свежо тегло и 1,41+_0,01мг GAE/г свежо тегло проба ягоди от Южна България.Общи антоциани:0,34+_0,01 мг /г свежо тегло за проба ягоди от Северна Гърция; 0,21+_0,01 мг /г свежо тегло и 0,22+_0,01мг/г свежо тегло за проба ягоди от Южна България. Антиоксидантна активност: Диворастящи ягоди – 41,38± 0,20 м мол ТЕ/г свежо тегло; 7,35 +_ 0,30 м мол ТЕ/г свежо тегло за проба ягоди от Северна Гърция; 8,99+_0,20 м мол ТЕ/г свежо тегло и 10,20 +_0,20 м мол ТЕ/г свежо тегло за прба ягоди от Южна България. Наблюдава се правопропорционална зависимост между антиоксидантната активност и общите феноли и обратнопропорционална зависимост между антиоксидантна активност/ феноли и антоциани.