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Comparative analysis of secondary metabolites contents in *Fragaria vesca* L. fruits

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Abstract

Fragaria vesca (wild strawberry) belongs to the *Rosaceae* family. Besides the leaves (*Fragariae folium*) and roots (*Fragariae radix*), the aromatic fruits (*Fragariae fructus*) of wild strawberry are also herbal materials used in medicine. The aim of this study was to compare the value of phytochemical and antioxidant activity of wild strawberry fruits (*Fragaria vesca* L.). The fruits were analyzed regarding their secondary metabolites contents (flavonoids, sum of phenolic acids, tannins, anthocyanins, DPPH), depending on the origin of the raw material (from natural habitats vs. cultivation). According to the obtained results, raw material originating from natural habitats contained significantly more flavonoids (0.559 mg·g^{-1}), compared to fruits harvested from cultivation (0.472 mg·g^{-1}, on average). Mean concentration of phenolic acids ranged from 1.648 mg·g^{-1} – 2.348 mg·g^{-1}, although the wild form was characterized by higher levels of examined substances. Tannins are an important fraction of phenolic compounds; their content in studied fruits ranged from 2.2% (from cultivation) – 3.0% (from natural habitats). When comparing the average contents of anthocyanins in the studied materials, it was revealed that remarkably more of these compounds were recorded in wild strawberry fruits harvested from natural habitats vs. those from cultivations: 132 mg·100 g^{-1} vs. 90 mg·100 g^{-1}. A difference was indicated with respect to the ability of DPPH radical reduction to diphenylpicrylhydrazine by extracts made of examined fruits.

Key words

wild strawberry fruits, flavonoids, phenolic acids, anthocyanins, antioxidant activity

INTRODUCTION

Wild strawberry is commonly cultivated in house and allotment gardens. Despite the ease of using agricultural technology and the possibility to reach high yields, large-scale wild strawberry plantations cannot be found anywhere in the world. The excellent scent and unforgettable taste of the species fruits determine their high dessert, dietetic, and medicinal values [1, 2].

The important role of a diet in either promoting or preventing diseases has long been recognized. Global epidemiological studies confirm an inverse relationship between the consumption of fruit and the incidence of cardiovascular, degenerative, and proliferative diseases [3, 4, 5], and there is convincing evidence that the considerable health benefits of fruits are due to their specific chemical compositions, particularly to compounds of nutritional relevance. Edible berries have been part of the human diet for centuries. Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most commonly consumed berries. Together with other soft fruits, it is an important dietary source of fibre and bioactive compounds, both micronutrients and phytochemicals. In particular, strawberries are rich in vitamin C and are among the richest natural food sources of folate [5, 6].

Fragaria vesca (wild strawberry), belongs to the *Rosaceae* family. Besides the leaves (*Fragariae folium*) and roots (*Fragariae radix*), the aromatic fruits (*Fragariae fructus*) of wild strawberry are also herbal materials used in medicine [7]. Plants contain flavonoids, tannins, volatile oils, methyl salicylate, and borneol [8]. The fruits contain salicylic acid and are beneficial in the treatment of liver and kidney complaints, as well as in the treatment of rheumatism and gout [9, 10]. The berries, leaves, and roots of *Fragaria vesca* have all been medicinally used in the past. The root was once a popular household remedy for diarrhea and the stalks for wounds. Antioxidant properties have recently been discovered in the fruit, making them a valuable preventive for cancer. The leaves are gently astringent. Tea can be brewed with the leaves for diarrhea, digestive upsets, and to stimulate the appetite. Fruits of cultivated wild strawberry are appreciated both as fresh and as processed foods [11].

The aroma of wild strawberries is very pleasant and more herbaceous than that of cultivated varieties, and wild-type berries could therefore be a more desirable raw material for the food industry. However, the low productivity and varying annual yields of wild strawberries have restricted their cultivation [12].

In recent years, both wild and cultivated berries have become very attractive for consumers because of potentially beneficial phytochemicals contained in these fruits. Fruit nutritional quality can be described by standard quality parameters (sugars and organic acids), and analysis of the antioxidant capacity influenced by specific related compounds. The importance of flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease.

Taking this into account, the presented study was undertaken to analyze wild strawberry fruits (*Fragaria vesca* L.) in view of secondary metabolites contents depending on raw material origin (from natural habitats vs. cultivation).

The variability in the content of phenolic compounds, as well as the correlations between total phenolic content and antioxidant capacity, can point to genetic differences among wild species and cultivated varieties of *Fragaria vesca*. It may

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provide a better understanding on the wild species' role as an important native genetic source for breeding new cultivars with high levels of phenolic compounds, contributing to both better sensory attributes and their antioxidant capacity [13].

MATERIALS AND METHOD

The studied material consisted of fruits from naturally growing wild strawberry (*Fragaria vesca* L.) and its cultivated form (*Fragaria vesca* cv. 'Rugia'). The study comprised a three-year cycle (2007–2009) of field research, field experiments, and laboratory trials.

Field research. Refers to the selection of natural stands of wild strawberry. The studied area consisted of a Polish region that is important due to a great abundance of natural stands of *Fragaria vesca* L., in Nawojowa village located in Nawojowa Forest Inspectorate near Nowy Sącz in southern Poland (49° 37' N, 20° 43' E). The *Fragaria vesca* L. population covered about 40 m^2 area and was characterized by a strong growth and large number. Six plots of 1.5 m^2 each were randomly defined within the stand. The study was carried out in three replicates. The analyses revealed that soil of that stand contained: 14.7 mg $N \cdot dm^{-3}$, 118.0 mg $P \cdot dm^{-3}$; 137.9 mg $K \cdot dm^{-3}$; and 296.1 mg $Ca \cdot dm^{-3}$, while its pH was 5.6. Salinity reached 0.6g $NaCl \cdot dm^{-3}$.

Field experiment. Conducted in 2006–2009 at the Felin Experimental Station of the University of Life Sciences in Lublin, south-eastern Poland (51° 23' N, 22° 56' E). Seeds of the cultivated form (*Fragaria vesca* cv. 'Rugia') were sown manually on 5 March 2006 into boxes filled with a substrate (peat substrate), and covered with a thin layer of sandw. After emergence and forming 2–3 true leaves, the seedlings were transferred into boxes at 5 × 3.5 cm spacing. Plants were set into their permanent place on 20 June 2006 in a row system at 40 × 25 cm spacing on dusty soil characterized by a good abundance of nutrients and neutral reaction. Soil under wild strawberry cultivation was prepared according to commonly accepted procedures, applying manure (40 kg·ha) for the forecrop (onion). Phosphorus, potassium, and magnesium fertilizers were used before seedling planting at the amounts: 80 kg·ha – P_2O_5; 100 kg·ha – KP_2O. Starter rate (N – 30 kg·ha) was applied when the seedlings were taking root. The wild strawberry plantation was regularly de-weeded manually.

The first harvest occurred in the second growing year. The fruit harvest was carried out at the full fruiting stage in 2007–2009. Fruits were harvested once in the early morning, in June, every year.

Weather conditions during growth and studies on *Fragaria vesca* are presented in Table 1.

Laboratory analyses. Raw material was subject to determinations of dry matter (%) by means of the drier method [14], flavonoids ($mg \times g^{-1}$) [15], sum of phenolic acids ($mg \times g^{-1}$) [16], tannins (%) [15], anthocyanins ($mg \cdot 100g^{-1}$) [17], as well as antioxidant capacity (%) as an ability to neutralize the DPPH radicals [18]. Biochemical analysis was performed in the Laboratory for Vegetable and Herbal Material Quality at The Department of Vegetable and Medicinal Plants, University of Life Sciences in Lublin.

Table 1. Mean monthly air temperatures, amount and total hours of precipitation at ES Felin and in Nawojowa village in the years 2007–2009

Month	ES Felin				Nawojowa village			
	2007	2008	2009	Mean for 1951–2000	2007	2008	2009	Mean for 1981–2000
Temperature °C								
IV	8.8	8.7	9.3	7.5	9.5	10.6	11.7	10.3
V	14.9	15.0	12.8	13.0	16.3	13.9	14.4	14.5
VI	18.1	18.1	17.7	16.5	19.0	18.7	18.2	17.5
VII	19.1	19.2	18.3	17.9	20.0	18.7	19.6	19.5
Amount of precipitation mm								
IV	17.4	17.4	55.8	40.6	16.0	13.9	0.2	24.9
V	80.5	81.5	101.6	58.3	60.4	38.9	57.0	90.0
VI	87.8	87.8	25.9	65.8	95.9	25.2	60.3	107.8
VII	87.0	87.0	77.1	78.0	55.8	168.8	102.1	98.0
Total hours in sunshine hrs.								
IV	238.8	139.6	291.5	156.6	213.7	153.0	276.2	186.1
V	268.6	183.0	274.6	280.9	245.9	153.0	232.7	194.9
VI	272.2	316.6	200.3	228.7	223.3	229.8	256.7	220.1
VII	236.7	242.3	279.6	158.0	239.4	210.1	197.2	219.3

Dry matter. Aliquots of about 1 g (0.0001 g accuracy) of raw and ground fruits were weighed. Samples were placed in a drier and dried at 105°C for 6 hours. The drying process was repeated till the constant weight of samples (difference between two subsequent weighings should not be greater than 0.5 g). The difference of weights before and after drying was the water loss; the result was then recalculated onto the percentage of dry matter. Determinations were made in three replicates.

Total flavonoids estimation. Studied material was investigated for total content of flavonoids, using modified Christ and Müller method, calculated for quercetin QE [15].

Absorbance was measured at 425 nm on a Shimadzu spectrophotometer. The content of flavonoids was calculated from the equation:

$$x = \frac{8.75 \times A}{m}$$ where m (g) was the amount of fresh material.

Total phenolic acids estimation. Carried out according to the Arnov method [16]. One millilitre of sample was mixed with 5 ml of distilled water, 1 ml 0.5 M HCl, 1 ml of Arnov reagent and 1 ml 1M NaOH, and subsequently adjusted to 10 ml with distilled water.

The absorbance was measured at 490 nm. The total phenolic acid content was expressed as caffeic acid equivalent (CAE).

Tannin estimation. The amount of tannins was determined using the Pharmacopoeia procedure [15]. The content of tannins was expressed as fresh weight percentage.

Determination of Total Phenolics (TPH). The Folin-Ciocalteu method [19] was used to determine total soluble phenolics (TPH). Extracts were diluted 1:500 or 1:1,000 before incubation at 40 °C. Absorption was measured at 755 nm. TPH was expressed as mg of gallic acid/100 g of fresh fruit.

Anthocyanins estimation by means of colorimetry. Samples of raw material (1.0 g) were extracted with 50 ml HCl (1mol·dm^3) and heated in a water bath for 1 hour. The obtained extract was hydrolyzed with 20 ml n-buthanol, after which two 10 ml n-buthanol portions were added as a solution. Anthocyanin extracts were rinsed in a 50 ml flask with n-buthanol. Absorbance was measured immediately at 533 nm [17].

The percentage of anthocyanins, as delphynidyn chloride, was calculated from the expression:

$$P = \frac{A \times V \times F}{m}$$

where:
P – total anthocyanins (mg·100g^{-1})
A – absorbance at 533 nm
V – value of buthanol phase (50 ml)
F – coefficient for delphinidyn chloride (2,6)
M – mass of sample to be examined (mg).

Determination of antioxidant capacity. Antioxidant capacity was determined by DPPHAntioxidant capacity (%), evaluated based on the ability to neutralize the DPPH radicals by means of spectrophotometry according to Chen and Ho [18]: to do this, water extracts were prepared from fruits, extracts were then evaporated untill dry and lyophilized. Analyses were performed for 20 μg×ml^{-1} concentration. The absorbance measurements were made at λ = 517 nm wavelength using spectrophotometer (HITACI U-2900).

Statistical analysis. Results obtained from laboratory experiments were statistically processed by means of the variance analysis method and Tukey's confidence intervals at 5% confidence level.

RESULTS AND DISCUSSION

The fruit of wild strawberries is often small, sparse and seedy, while cultivated strawberries are larger, sweeter and of better quality. Wild strawberries ripen in midsummer, while many cultivated forms of wild strawberries have been bred to mature later in the season. Cultivated wild strawberries may be bred for a longer storage life, but wild strawberries are very perishable [2, 20].

The presented results are the continuation of earlier research on the chemical diversity of wild strawberry (*Fragaria vesca* L.) [21]. A remarkable difference exists between the fruit of the diploid wild species and the modern, cultivated species, not only in terms of fruit size and yield, but also in flavour and aroma profile [20, 22, 23, 24, 25, 26]

There were differences in the ratio of dry weight to fresh weight between the two forms, ranging from 28.47% – 34.53%, with an average of 31.50% for all forms (Tab. 2). An almost three-times higher mean dry matter content in wild strawberry fruits was recorded, compared to average dry matter quantity determined for strawberry fruits, [27, 28]. Changes of dry matter contents over particular years of the study are presented in Figure 1.

Tannins are somehow an important fraction of phenolic compounds, the content of which in the studied material oscillated between 2.20% (cultivated plants) – 3.00% (fruits from natural habitats). The graphical variability of tannins quantities in particular years of study is presented in Figure 2.

Table 2. Content of dry matter and tannins in fruits of studied wild strawberry forms (mean values from years 2007–2009)

Raw form	Dry matter	Tannins
	%	
Fragaria vesca L.	28.47a	3.00a
Fragaria vesca 'Rugia'	34.53b	2.20b
Mean	**31.50**	**2.60**
$LSD_{0.05}$	4.194	0.631

* followed by the same letter are not significantly different at α=0.05

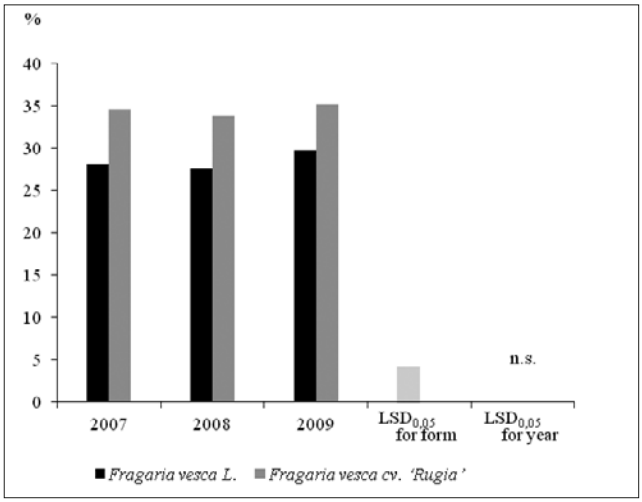

Figure 1. Average content of dry matter in fresh fruits of studied wild strawberry forms in successive years of study

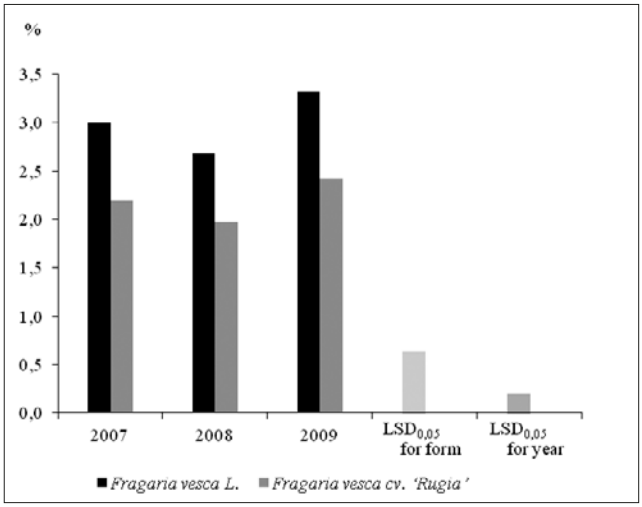

Figure 2. Average content of tannins in fresh fruits of studied wild strawberry forms in successive years of study

Fruits of *Fragaria* species plants are an important source of phytochemicals; in particular, the phenolic composition seems to strongly influence the quality of the fruits, contributing to both their sensory-organoleptic attributes and their nutritional value [5].

Table 3 lists the results related to the contents of selected secondary metabolites, as well as antioxidant capacity of studied material.

Table 3. Content of selected secondary metabolites in fresh fruits of two wild strawberry forms, as well as antioxidant activity expressed as the ability to neutralize the DPPH radical in water extracts made of studied materials (mean values from years 2007–2009)

Raw form	Flavonoids mg · g^{-1}	Phenolic acids mg · g^{-1}	Antho-cyanins mg · 100g^{-1}	Antioxidant activity %
Fragaria vesca L.	0.559a	2.348a	132a	13.07a
Fragaria vesca 'Rugia'	0.472b	1.648b	90b	12.80b
Mean	**0.516**	**1.998**	**111**	**12.94**
LSD $_{0.05}$	0.0271	0.3107	23.7	0.201

* followed by the same letter are not significantly different at p=0.05

On basis of the results obtained, it was found that raw material originating from natural habitats contained significantly more flavonoids (0.559 mg·g^{-1}), compared to fruits harvested from cultivation (0.472 mg·g^{-1}, on average). Mean concentration of phenolic acids ranged from 1.648 mg·g^{-1} – 2.348 mg·g^{-1}, although the wild form was characterized by higher levels of examined substances.

Anthocyanins are quantitatively the most important type of polyphenols in strawberry. The major anthocyanin representative compounds have already been identified [pelargonidin- (Pg) and cyanidin- (Cy) glycosides or acylated forms] [Tulipani et al. 2008], and the presence of the main derivatives seems to be constant in all varieties (i.e., Pg-3-gluc and in smaller proportion Cy-3-gluc). Nevertheless, new anthocyanin-related pigments (also called condensed pigments) are still being detected in small amounts [3, 29, 30], and qualitative and quantitative variations on the anthocyanin profile have been observed among strawberry cultivars [32], as well as within the same variety, depending on the genetic background, the degree of ripeness, post-harvest storage of the fruits, and climatic factors.

When comparing the average contents of anthocyanins in studied materials, it was revealed that remarkably more of these compounds were recorded in wild strawberry fruits harvested from natural habitats vs. those from cultivations: 132 mg·100g^{-1} vs. 90 mg·100g^{-1}.

There are data on anthocyanins contained in strawberry fruits in many publications [3, 32, 33, 34]. The presented study revealed that the mean content of anthocyanins in wild strawberry fruits was higher compared to cultivated strawberry fruits, which was at the level of 111 mg·100g^{-1}. Changes of the secondary metabolites contents in the fruits of both wild strawberry forms are presented on Figures 3–5.

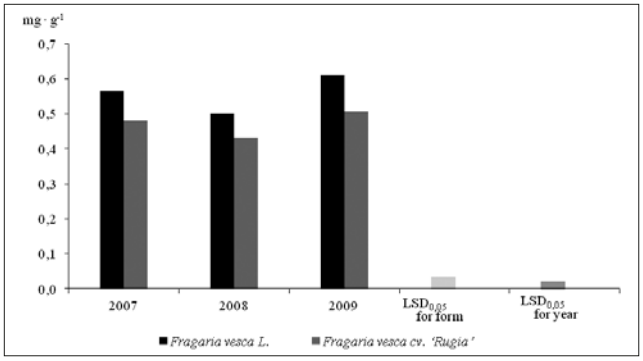

Figure 3. Average content of flavonoids as quercetin in fresh fruits of studied wild strawberry forms in successive years of study

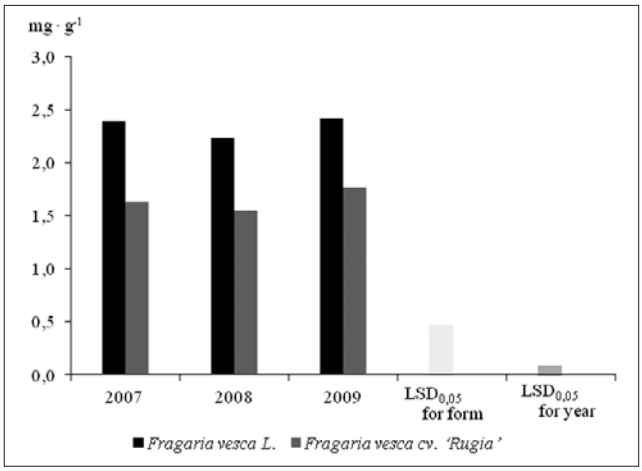

Figure 4. Average content of phenolic acids as caffeic acid in fresh fruits of studied wild strawberry forms in successive years of study

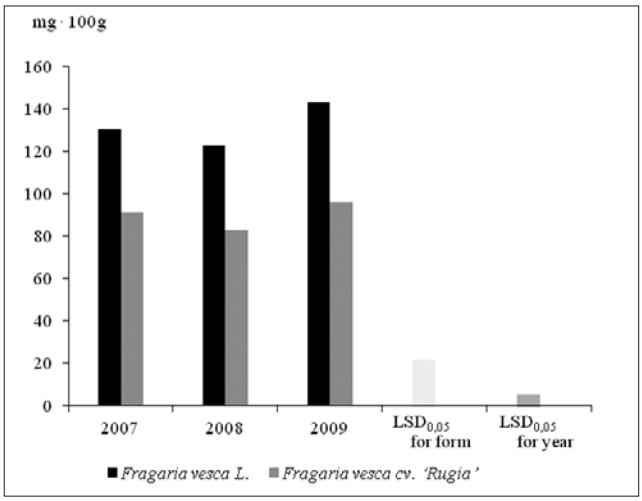

Figure 5. Average content of anthocyanins in fresh fruits of studied wild strawberry forms in successive years of study

Effects similar to those observed for flavonoids, phenolic compounds, and anthocyanins are also revealed by antioxidant activity measurements. Results of the DPPH assay are shown in Table 2 and Figure 6. Statistically significant

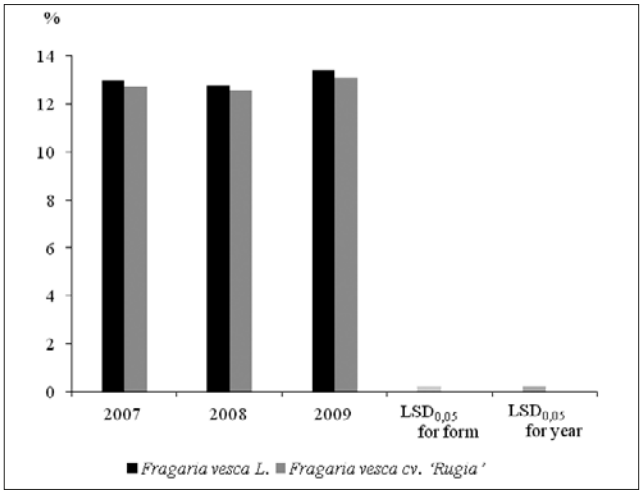

Figure 6. Antioxidant activity expressed as ability to neutralize DPPH radical in water extracts made in fresh fruits of studied wild strawberry forms in successive years of study

differences were confirmed in the ability to neutralize the free radical by water extracts made of the studied material.

CONCLUSIONS

Many authors have described the large diversity of secondary metabolites produced by plants, and the differences observed between wild and cultivated species. The results of the presented study confirm that thesis.

A significant positive relation observed in this study between total phenolics and total antioxidant capacity indicate the need for the use of wild species, especially in the *Fragaria* sp. breeding programmes, in order to increase their nutritional value and health benefits.

REFERENCES

1. Almenar E, Hernández-Muñoz P, Lagarón JM, Catalá R, Gavara R. Controlled Atmosphere Storage of Wild Strawberry Fruit (Fragaria vesca L.). J Agric Food Chem. 2006; 54: 86–91.
2. Dyduch M, Najda A. Contents of secondary metabolites at various anatomical parts of three wild strawberry (*Fragaria vesca* L.) cultivars. Herba Pol. 2009; 55(3): 147–152.
3. Heinonen IM, Meyer AS, Frankel EN. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J Agric Food Chem. 1998; 46: 4107–4112.
4. Yi-Fang C, Jie S, Xianzhong W, Rui HL. Antioxidant and antiproliferative activities of common vegetables. J Agric Food Chem. 2002; 50: 6910–6916.
5. Tulipani S, Mezzetti B, Capocasa F, Bompadre S, Beekwilder J, Ric De Vos CH, Capanoglu A, Bovy A, Battino M. Antioxidants, Phenolic Compounds, and Nutritional Quality of Different Strawberry Genotypes. J Agric Food Chem. 2008; 56: 696–704.
6. Halbwirth H, Puhl I, Haas U, Jezik K, Treutter D, Stich K. Two-Phase Flavonoid Formation in Developing Strawberry (Fragaria x ananassa) Fruit. J Agric Food Chem. 2006; 54: 1479–1485.
7. Wyk B, Wink M. Medicinal plants of the world. MedPharm, Polska, 2007.
8. Agrawal SS, Paridhavi M. Essentials of crude drugs. In: Herbal drug technology. 1st ed. Hyderabad, India, Universities Press, 2007.p.583–587.
9. Phillips R, Foy N Herbs. Plants for a future: Edible, medicinal and useful plants for a healthier world. London, Pan Books Ltd., 1990 www.pfaf.org/index.htm (access: 2008.05.28).
10. Kanonia L, Das S. A comparative study of analgesic property of whole plant and fruit extracts of Fragaria vesca in experimental animal models Bangladesh J Pharmacol. 2008; 4: 35–38.
11. Bombarely A, Merchante C, Csukasi F, Cruz-Rus E, Caballero JL, Medina-Escobar N, Blanco-Portales R, Botella MA, Muñoz-Blanco J, Sánchez-Sevilla JF, Valpuesta V. Generation and analysis of ESTs from strawberry (Fragaria x ananassa) fruits and evaluation of their utility in genetic and molecular studies. BMC Genomics. 2010; 11: 503.
12. Hirvi T, Honkanen E. The Volatiles of Two New Strawberry Cultivars, "Annelie" and "Alaska Pioneer", Obtained by Backcrossing of Cultivated Strawberries with Wild Strawberries, Fragaria vesca, Riigen and Fragaria virginiana. Z Lebensm Unters Forsch. 1982; 175: 113–116.
13. Milivojevic J, Maksimomovic V, Niklonic M. Chemical and antioxidant properties of cultivated and wild fragaria and rubus berries. J Food Quality 2011; 34(1): 1–9.
14. Charłampowicz Z. Analyses of fruit, vegetable, and mushroom products. WPLS, Warszawa, 1966.
15. Polish Pharmacopoeia VII. Wyd. PTF-arm, Warszawa, 2005.
16. Polish Pharmacopoeia VI. Wyd. PTF-arm, Warszawa, 2002.
17. Miłkowska K, Strzelecka H. Flos *Hibisci* – metody identyfikacji i ocena surowca. Herba Pol. 1995; 41(1): 11–16 (in Polish).
18. Chen JH, Ho CT. Antioxidant activities of caffeic acid and its related hydroxycinnaminic acid compounds. J Agric Food Chem. 1997; 45: 2374–2378.
19. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16: 144–158.
20. Aharoni A, Giri AP, Verstappen FWA, Bertea CM, Sevenier R, Sun Z, Jongsma MA, Schwab W, Bouwmeester HJ. Gain and Loss of Fruit Flavor Compounds Produced by Wild and Cultivated Strawberry Species. Plant Cell. 2004; 16(11): 3110–3131.
21. Najda A, Dyduch M. Chemical diversity within wild strawberry (*Fragaria vesca* L.) species. Herba Pol. 2009; 55(3): 140–146.
22. Hanson MA, Gaut BS, Stec AO, Fuerstenberg SI, Goodman MM, Coe EH, Doebley JF. Evolution of anthocyanin biosynthesis in maize kernels: The role of regulatory and enzymatic loci. Genetics1996; 143: 1395–1407.
23. Hartmann T. Diversity and variability of plant secondary metabolism: A mechanistic view. Entomol Exp Appl. 1996; 80: 177–188.
24. Kliebenstein DJ, Lambrix VM, Reichelt M, Gershenzon J, Mitchell-Olds T. Gene duplication in the diversification of secondary metabolism: Tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in Arabidopsis. Plant Cell. 2001; 13: 681–693.
25. Hadacek F. Secondary metabolites as plant traits: Current assessment and future perspectives. Crit Rev Plant Sci. 2002; 21: 273–322.
26. Schwab W. Metabolome diversity: Too few genes, too many metabolites? Phytochemistry 2003; 62: 837–849.
27. Olsson ME, Andersson CS, Oredsson S, Berglund RH, Gustavsson KE. Antioxidant Levels and Inhibition of Cancer Cell Proliferation in Vitro by Extracts from Organically and Conventionally Cultivated Strawberries. J Agric Food Chem. 2006; 54: 1248–1255.
28. Jarosz Z, Dzida K, Bartnik K. Yielding and chemical composition of „honeoye" cultivar strawberries depending on the kind of substratum and nitrogen dose. Acta Sci Pol Hortorum Cultus 2011; 10(1): 95–104.
29. González-Paramás AM, Lopes-da-Silva F, Martìn-Lòpez P, Macz-Pop G, González-Manzano S, Alcalde-Eon C, Pérez- Alonso JJ, Escribano-Bailòn MT, Rivas-Gonzalo JC, Santos-Buelga C. Flavanol-anthocyanin condensed pigments in plant extracts. Food Chem. 2006; 94: 428–436.
30. Aaby K, Skrede G, Wrolstad ER. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). J Agric Food Chem. 2005; 53: 4032–4040.
31. Määttä-Riihinen KR, Kamal-Eldin A, Törrönen AR. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family *Rosaceae*). J Agric Food Chem. 2004; 52: 6178–6187.
32. Vinson JA, Su X, Zubik L, Bose P. Phenol Antioxidant Quantity and Quality in Foods: Fruits. J Agric Food Chem. 2001; 49: 5315–5321.
33. Vinson JA, Zubik L, Bose P, Samman N, Proch J. Dried Fruits: Excellent *in Vitro* and *in Vivo* Antioxidants. Journal of the American College of Nutrition 2005; 24(1): 44–50.
34. Panico AM, Garufi F, Nitto S, Mauro RD, Longhitano RC, Magrě G, Catalfo A, Serrentino ME, Guidi GD. Antioxidant activity and phenolic content of strawberry genotypes from *Fragaria* x *ananassa*. Pharmaceutical Biology 2009; 47(3): 203–208.