Chemical diversity within strawberry (*Fragaria vesca* L.) species

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S u m m a r y

The chemical diversity within wild strawberry (*Fragaria vesca* L.) species was studied depending on its origin (natural *vs* cultivated). The herb was collected at the full flowering stage. Following items were determined in collected material: flavonoids, phenolic acids, tannins, anthocyanins, as well as anti-oxidation activity (%) was evaluated by means of ability to neutralize the DPPH radicals. Contents of studied biologically active substances depended on the plant origin. Extracts made from examined raw materials showed no differences in the ability to reduce DPPH radicals to diphenylopicrylohydrazine.

Key words: *Fragaria vesca* L.*, flavonoids, phenolic acids, tannins, anthocyanins, anti-oxidation activity, DPPH, phenotypic variability, genetic variability*

INTRODUCTION

Wild strawberry (*Fragaria vesca* L.) growing in a natural habitat, is a plant from rose family (*Rosaceae*). All varieties cultivated nowadays are derived from [1, 2]. During the selection process aiming at improving the cultivated plant species quality and creating the new cultivars, biologically and economically important features are lost instead of other ones.

Wild growing and old cultivated varieties have many positive traits: resistance to diseases, frost or drought, but also possess genes responsible for good fruit storage or high content of secondary metabolites.

Recently, the nutritional and health features of strawberry and wild strawberry have been emphasized more often, which can be associated with the presence of polyphenols in their organs. As it is well known, these compounds, particularly flavonoids, form an anti-oxidative potential and protect against chronic diseases such as tumors or heart disorders [3-5].

Phenolic compounds are a widespread group of secondary metabolites. They have a wide spectrum of biochemical activities, among others: anti-oxidative, anti-mutagenic, anti-carcinogenic activity, as well as ability to modify the gene expression [6]. Great number of researches confirmed that they had a positive influence on human health. Along with carotenoids, tocopherols and vitamin C, phenolic compounds belong to natural nutrients of antioxidant character [7, 8].

The anti-oxidative properties of phenolic compounds consist of eliminating the reactive oxygen forms, blocking (sweeping) the free radicals, inhibiting the enzymes from oxidase group and chelating the metal ions (iron, copper) [7].

Taking into account the structure of their principle carbon sceleton, they can be divided into phenolic acids (hydroxybenzoic and hydroxycinnamic acids derivatives) and flavonoids, among which many sub-divisions can be made depending on heterocyclic ring structure [9].

Vegetables, fruits, seeds, some cereals, red wine, green tea, coffee, fruit juices, and spices are abundant sources of phenolic compounds.

It was also found that phenolic compounds are also present in wild strawberry herb. Polyphenols present in leaves of *Fragaria orientalis* species plants are a main group of biologically active substances known for their medicinal properties. It can be supposed that polyphenols play a major role in pharmacological activity of *Fragaria orientalis* species [10]. These compounds can also be found in roots and fruits of many species from *Fragaria* genus [11].

Therefore, the aim of present study was the comparative analysis of secondary metabolites contained in wild strawberry herb grown in natural (*Fragaria vesca* L.) and cultivated (*Fragaria vesca* 'Regina') habitats, which could exert a significant influence on anti-oxidation activity of these materials.

MATERIAL AND METHODS

Studied material consisted of the herb (whole leaves) of naturally growing wild strawberry (*Fragaria vesca* L.) collected from wild habitats near Nowy Sącz and its cultivated form (*Fragaria vesca* 'Regina') achieved from own agrotechnical experiments. Wild and cultivated forms of wild strawberry plants were collected once at the end of June 2007, then all material was cleaned and dried at 30ºC.

Total flavonoids estimation

Studied material was investigated for total content of flavonoids, using modified Christ and Müller method, calculated for quercetin QE [12].

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

$$X = \frac{0.875 \times A}{m}$$

where m (g) was the amount of dry material.

Total phenolic acids estimation

Total phenolic acids estimation was carried out according to Arnov method [13]. One milliliter 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 completed to 10 ml with distilled water.

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

Tannin estimation

The amount of tannin estimation was determined using Pharmacopoeia procedure [13]. The content of tannins was expressed as dry weight percentage.

Anthocyanins estimation by means of colorimetry [14]

Samples of dry weight whole leaves (1.0 g) extracted with 50 ml HCl (1 mol/dm^3) and heating in water bath for 1 hour. The obtained extract hydrolised with 20 ml n-butanol and than add two times 10 ml n-butanol as a solution. Anthocyanin extracts rinse the 50 ml flask with n-butanol. The absorbance was measured immediately at 533 nm.

Calculation of the percentage content of anthocyanins, as delphynidyn chloride, from the expression:

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

where:
P – total anthocyanins (%);
A – absorbance at 533 nm;
V - value of butanol phase (50 ml);
F – coefficient for delphinidyn chloride (2.6);
m – mass of sample to be examined (mg).

Antioxidant activity

Antioxidant activity (%) was evaluated on a base of the ability to neutralize the DPPH radicals by means of spectrophotometry according to Chen and Ho [15]: in order to do this, water extracts were prepared from herbs of both wild strawberry forms, then evaporated till dry and lyophilized. Analyses were performed for 20 $\mu g \cdot ml^{-1}$ concentration. The absorbance measurements were made at $\lambda = 517$ nm wavelength using spectrophotometer UVIKON 932 (Canberra Packard).

Statistical analysis

Achieved results from laboratory experiments were statistically processed by means of variance analysis method and Tukey's confidence intervals at 5% confidence level.

RESULTS AND DISCUSSION

Numerical data describing general parameters, i.e. dry matter and water contents in studied forms of wild strawberry are presented in table 1. It was found that analyzed materials were characterized by great content of dry matter (20.5%, on average), although, *Fragaria vesca* L. contained slightly more dry matter (21.4%). Air-dried material had low amounts of water – about 8.5%, on average. Such low value, i.e. below 10%, has a significant influence on further storage and stability of biologically active substances contained in studied materials.

Table 1.

General parameters of studied wild strawberry forms

form	dry matter %	moisture content %
Fragaria vesca L.	21.4b	8.7a
Fragaria vesca 'Regina'	19.7a	8.4a
mean	**20.5**	**8.5**
$LSD_{0.05}$	1.03	1.24

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

There are no detailed data in available literature references on quantitative contents of phenolic compounds groups present in *Fragaria vesca* 'Regina' and *Fragaria vesca* L. herbs.

Flavonoids are an important group of biologically active compounds that occur in fruits, vegetables and herbs [16-20].

Achieved results (tab. 2) indicated that cultivated material contained more flavonoids (4.0%) as compared to herb collected from natural habitats (3.03%, on

average). Content of phenolic acids varied from 1.3% to 1.8%, although wild form was characterized by slightly higher concentration of these compounds.

Contents of flavonoids and phenolic acids in both studied forms of wild strawberry were many times higher compared to results reported by Wolski et al. [21] for summer lilac (*Buddleja davidii* Franch.) (0.38% vs. 0.20%, respectively). Rosłon and Suchorska-Tropiło [22] found that different species from the *Ericaceae* family and growing in natural habitats might be a valuable source of phenolic compounds. The authors confirmed that among three studied species (lingonberry, bilberry, bearberry), regardless of the harvest date, the largest amounts of flavonoids were present in lingonberry leaves (0.62%), while phenolic acids were present most abundantly in bilberry leaves (4.63%, on average).

Studies performed by Wardziak and Osińska [23] proved that woodruff (*Galium odoratum* L.) was the abundant source of phenolic compounds. The authors examined the developmental and chemical differentiation of three woodruff populations under natural and cultivated conditions. It was recorded that flavonoids and phenolic acids contents depended on the origin and development stage of a plant reaching 0.23% and 1.19% (at plants from natural habitats) and 0.54% and 1.25% (at cultivated plants). Our study confirmed the dependence between analyzed biologically active compounds contents and the material origin.

Tannins are an important fraction of phenolic acids; their contents in studied materials ranged from 4.3% (at plants growing in natural habitats) to 5.4% (at cultivated plants). The tannin content in summer lilac herb was about 10.2% which was almost twice as high as for wild strawberry herb [21].

Comparison of anthocyanins concentrations in both studied wild strawberry form revealed similar levels (tab. 2).

Table 2.

Contents of selected secondary metabolites (% dry weight) in herb of two wild strawberry forms as well as anti-oxidation activity expressed as the ability to neutralize the DPPH radical in water extracts made of studied materials

form	flavonoids	phenolic acids	tannins	anthocyanins	antioxidant activity
Fragaria vesca L.	3.0a	1.8b	4.3a	11.2a	20.9a
Fragaria vesca 'Regina'	4.0b	1.3a	5.4b	11.2a	20.3a
Mean	**3.5**	**1.5**	**4.8**	**11.2**	**20.6**
$LSD_{0.05}$	0.04	0.03	0.74	n.s.	n.s.

* - means followed by the same letter are not significantly different at p=0.05
n.s. – non-significant

Considering the anti-oxidative activity, it was observed that water extracts made of both forms of wild strawberry showed similar abilities to reduce DPPH radical to diphenolopicrylohydrazine at extract concentration of 20 $\mu g \cdot ml^{-1}$ (tab. 2).

The tendency to search new forms and products abundant in secondary metabolites, namely flavonoids, anthocyanins, and phenolic acids can be observed

in available literature [21, 23-30]. Here achieved results allow for concluding that studied forms of wild strawberry contain considerable quantities of secondary metabolites showing anti-oxidation properties that make that material biologically very valuable.

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CHEMICZNE ZRÓŻNICOWANIE POZIOMKI POSPOLITEJ (*FRAGARIA VESCA* L.)

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S t r e s z c z e n i e

Badano zróżnicowanie chemiczne poziomki pospolitej (*Fragaria vesca* L.) w zależności od pochodzenia surowca (ze stanu naturalnego i uprawy). Ziele zebrano w pełni kwitnienia. W pozyskanym surowcu oznaczono zawartość flawonoidów, kwasów fenolowych, garbników, antocyjanów oraz określono aktywność antyoksydacyjną (%) poprzez zdolność znoszenia rodnika DPPH. Zawartość badanych związków biologicznie czynnych zależała od pochodzenia surowca. Nie wykazano różnicy w zdolności redukowania rodnika DPPH do difenylopikrylohydrazyny przez ekstrakty z badanych surowców.

Słowa kluczowe: *Fragaria vesca L., flawonoidy, kwasy fenolowe, garbniki, antocyjany, aktywność antyoksydacyjna, DPPH, zmienność genotypowa, zmienność fenotypowa*