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EVALUATION OF CONTENT AND COMPOSITION OF PHENOLIC ACIDS AND TANNINS IN LEAF DRY MATTER OF TWO CELERY CULTIVARS (*APIUM GRAVEOLENS* L. VAR. *DULCE* MILL. PERS.)

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ABSTRACT

Content and composition of free phenolic acids fraction as well as tannins in leaves (blades and leaf stalk) of two celery cultivars (Helios and Zefir) were evaluated in studies. It was found that tannin levels in both raw materials were from 2.09 to 7.42%, but leaf stalks contained about 3-fold more than blades. Also content of free phenolic acids sum was determined in raw material. Studies revealed that blades contained about 3-fold more phenolic acids than leaf stalks. Such results could point that celery might be of chemopreventive properties.

Key words: *Apium graveolens*, phenolic acids, chemoprevention, antioxidants, high-performance liquid chromatography (HPLC)

INTRODUCTION

However celery is the most popular plant widely cultivated in USA, France, Italy and Netherlands; in Poland, it is still poorly known vegetable [3]. As it known from the literature data [8], both celeriac and celery have rich mineral and vitamin composition. Among vitamins, complex of vitamins B and C as well as β -carotene are worth mentioning. Potassium, calcium, phosphorus and iron are important mineral elements occurring in celery. This vegetable contains nutritional fiber, essential oils and other secondary metabolites numbered to polyphenols among which flavonoids, phenolic acids, coumarins, furanocoumarins and sterols can be distinguished [6, 8].

Due to the progress in the studies upon the influence of reactive forms of oxygen (RFO), so-called free oxygen radicals exert negative effects and may cause the acceleration and development of many civilization disorders, including cancer and arteriosclerosis [2]. The interests on natural antioxidants contained in vegetables and fruits, continuously increases [7].

The aim of study was to evaluate the content and composition of secondary metabolites, i.e. phenolic acids and tannins as well as water content in leaves of two celery cultivars.

MATERIALS AND METHODS

Leaves of two celery cultivars (Helios and Zefir) originating from experimental fields of Department of Vegetable and Medical Plants Cultivation, University of Agriculture, (Lublin – Poland) were the objectives of the studies. Seedlings of both cultivars were set on 20 May 1999; harvest was done after 80, 100 and 120 days of plant's vegetation. Water content [4] and free phenolic acids sum using two methods: Folin-Ciocaltu' – FC [8] and Arnov – Ar [4] were determined in plants collected. Moreover, percentage of tannins according to pharmacopoeia [4] was recorded in raw material. Isolation and purification of free phenolic acids for chromatographic analyses were made applying earlier described procedure [5]. High-performance liquid chromatography (HPLC) was used for qualitative and quantitative determination of free phenolic acids from extracts obtained. HPLC equipment consisted of liquid chromatograph with dosing loop of 20 μ l volume by Merck (LaChrom), diode detector DAD (L-7450) and pump array (L-7100). The separation of phenolic compounds was performed using steel column LiChrospher RP-18 (250 \times 4 mm) filled with stationary phase of $d_p = 5 \mu$ m mesh.

All analyses were carried out at ambient temperature. Mobile phase consisted of methanol + water (25:75, v/v) with 1% v/v addition of acetic acid. Flow rate was adjusted to 0.8 ml/min. Qualitative analysis was based on a comparison of the retention times for substances under study with electron spectra of standard phenolic acids. Percentage of particular phenolic acid is an absolute value in relation to the sum of surface area under peak for a sample.

RESULTS

[Table 1](#) presents the percentage of humidity and tannins in raw material studied. This trait depended on the harvest date and amounted as follows: 9.15-11.05% for Helios cv. and 10.06-11.29% for Zefir cv. Leaf stalks were characterized with about 3 times higher for Helios cv. and 2 times higher content of tannins for Zefir cv. as compared to leaf blades.

Table 1. Percentage of humidity and tannins in raw material depending on harvest date

Crop time	Part of plant	Water content (%)		Tannins (%)	
		Helios	Zefir	Helios	Zefir
80 days	blades	9.39	10.26	2.40	3.49
	stalks	11.05	11.29	7.42	7.36
100 days	blades	9.15	10.06	2.37	3.38
	stalks	10.94	11.21	7.32	7.21
120 days	blades	9.17	10.10	2.09	3.21
	stalks	10.98	11.24	7.11	7.19

[Table 2](#) illustrated the percentage of free phenolic acids determined by means of two methods (FC and Ar). It was found that FC method resulted in slightly higher percentage of phenolic acids than Ar. As it follows from

the data (tab. 2), leaf blades of Helios cv. depending on harvest date contained 0.77-0.87%, and leaf stalks 0.24-0.26% of free phenolic acid sum, those values for Zefir cv. amounted to 0.57-0.70% and 0.24-0.25%, respectively. Regardless of the celery variety, the leaf blades contained more free phenolic acids than leaf stalks; blades in Helios cv. had over 3 times more quantity of the phenolic acids as those in Zefir cv. which did not exceed these values.

Table 2. Percentage of total free phenolic acids determined by means of Folin-Ciocaltu's (FC) and Arnov's (Ar) methods in raw material

Crop time	Part of plant	Methods			
		Folin-Ciocaltu' (%)		Arnova (%)	
		Helios	Zefir	Helios	Zefir
80 days	blades	0.77	0.57	0.68	0.51
	stalks	0.24	0.24	0.20	0.21
100 days	blades	0.78	0.61	0.69	0.57
	stalks	0.24	0.25	0.21	0.22
120 days	blades	0.87	0.70	0.74	0.61
	stalks	0.26	0.25	0.23	0.22

The percentage of free phenolic acids occurring in extracts and determined by means of HPLC technique is presented in Table 3. Five free phenolic acids were identified in leaf blades and stalks: protocatechuic, p-hydroxybenzoic, caffeic, p-coumaric and ferulic.

Table 3. Retention time of standards of phenolic acids correlation coefficients with standards

Phenolic acids	Retention time tr (min)	Corelation cooificents (%)			
		Helios		Zefir	
		blades	stalks	blades	stalks
protocatechuic	6.25	0.997	0.989	0.993	0.988
p-hydroxybenzoic	10.44	0.997	0.997	0.995	0.992
caffeic	12.98	0.999	0.998	0.996	0.996
p-coumaric	24.36	0.993	0.997	0.990	0.990
ferulic	36.05	0.997	0.993	0.993	0.991

DISCUSSION

The phenols, polyphenols, phenolic acids and tannins have a very important antioxidants meaning in food of plant origin (including vegetables and fruits).

As it follows from present study, the leaves of two cultivars celery are abundant in tannins containing about 10% of those substances. Leaf blades contained about 3 times higher percentage of free phenolic acids than leaf stalks. The total level of free phenolic acids in the celery leaves was about 1% by dry weight.

Evaluation the content and composition of extracts from celery leaves made by means of HPLC technique revealed the lack of gallic and elagic acids. It may suggest that tannins occurring in raw material studied do not belong to hydrolizing (gallo- and elagotannins) but to not-hydrolizing i.e. catechinic ones. This group of compounds can be converted into catechins and epigallocatechins showing high antioxidation activity in animal organisms. Cancers are actually one of most civilization illnesses. Chemopreventive drugs are the new strategy in prophylactic and therapy of cancers.

According to Wolski [8] celery and celeriac, both are the medical raw materials and are applied for treatment of blister and kidneys distortions. They are components for herbal diuretic and antirheumatic mixtures as well as

improving digestion and improving appetite in the animal organisms. Own studies revealed our leaves (blades + stalks) of celery contained great amounts of tannins and fraction of free phenolic acids. In our opinion, this raw material can have valuable chemopreventive features properties apart from dietetic vitaminizing and remineralizing meaning [1].

CONCLUSIONS

Percentage of tannins in leaves (blades + stalks) amounted 9.02-9.82% for Helios cv. and 10.4-10.85% for Zefir cv. and did not depend on harvest date but leaf stalks contained about three times more tannins than leaf blades.

Percentage of free phenolic acids determined by means of FC was slightly higher than Ar method. Sum of free phenolic acids in leaves (blades + stalks) was slightly higher for Helios cv. as compared to Zefir cv. Percentage of free phenolic acids was three times higher in leaf blades than in leaf stalks.

Applying HPLC technique five phenolic acids were identified in extracts from raw material: protocatechuic, p-hydroxybenzoic, caffeic, p-coumaric and ferulic. Their percentage was about three times higher in leaf blades comparing to leaf stalks.

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