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HPLC determination of phenolic acids in the underground part of carrots of “Nantska Kharkivska” and “Yaskrava” varieties.

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ABSTRACT

The composition of phenolic acids in the roots of carrots of “Nantska Kharkivska” and “Yaskrava” varieties was determined using the HPLC method. All the samples studied were established to contain chlorogenic acid which quantitatively dominated in the roots of the first year of “Nantska Kharkivska” variety.

Keywords: carrot, phenolic acids, HPLC

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INTRODUCTION

Daucus carota L. subsp. *sativus* (family Apiaceae) is a biennial herbaceous plant which is cultivated in many countries of the world [1]. As the researches show carrot show potent antioxidant and antitumor activity [2].

Italian scientists have established that phenolic acids, chlorogenic, caffeic and ferulic in particular, show antioxidant activity [3,4,5]. Besides, Chinese scientists have proven that chlorogenic acid has antimutagenic, antitumor and anti-inflammatory activity aside from the antioxidant one [6].

Thus, the study of phenolic acids in carrot plant material is rational. Carrot has a great number of varieties with “Nantska Kharkivska” and “Yaskrava” varieties being the most wide-spread in Ukraine.

The purpose of this work was to determine the qualitative composition and quantitative content of phenolic acids by the means of HPLC in the roots of carrot of the abovementioned varieties.

EXPERIMENTAL

The carrot plant material was collected in summer in Kharkiv region (Ukraine) in 2016.

Phenolic acids in the underground organs of carrots of “Nantska Kharkivska” and “Yaskrava” varieties were determined by the means of HPLC using the method described below [7].

0,5 g (accurate weight) of crushed plant material were placed into 100 ml conical flask, equipped with a reflux condenser, where 25 ml of 50% ethanol were added, and kept on a boiling water bath for 45 min. After that the extract was cooled to the room temperature and then filtered through a “red ribbon” filter into a 25 ml measuring flask. The volume of the extract was made up to the mark with 50% ethanol. Chromatographic study of the samples was carried out using an LC system with photodiode array detector Shimadzu HPLC system, ser. 20 under the following conditions: Phenomenex Luna C 18 (2) column of 250 x 4,6 mm size, particle size – 5 µm, column temperature – 35°C, detection wavelength – 330 nm, mobile phase flow rate – 1 ml/min, sample volume – 5 µl, mobile phase – eluent A (0,1% solution of trifluoroacetic acid in water) and eluent B (0,1% solution of trifluoroacetic acid in acetonitrile).

Chromatography time (min)	Eluent A, %	Eluent B, %
0-5	95	5
5-35	95→75	5→25
35-40	75	25
40-60	75→50	25→50
60-65	50→20	50→80
65-70	20	80
70-85	95	5

The identification of the components was carried out according to the retention time and correspondence of the UV-spectra to the ones of standard samples.

RESULTS AND DISCUSSION

Chromatograms are given in the fig.. 1-3.

Results of the study are shown in the table 1.

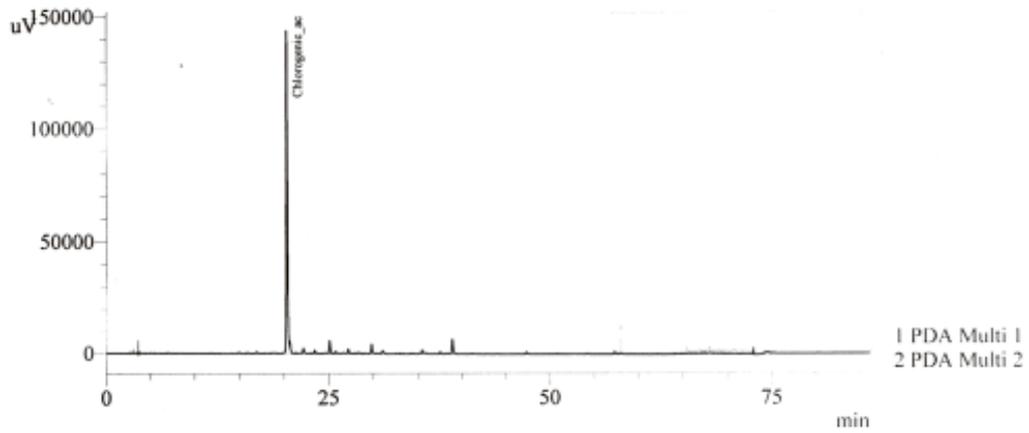


Fig1: HPLC of phenolic compounds in carrot roots of “Nantska Kharkivska” variety

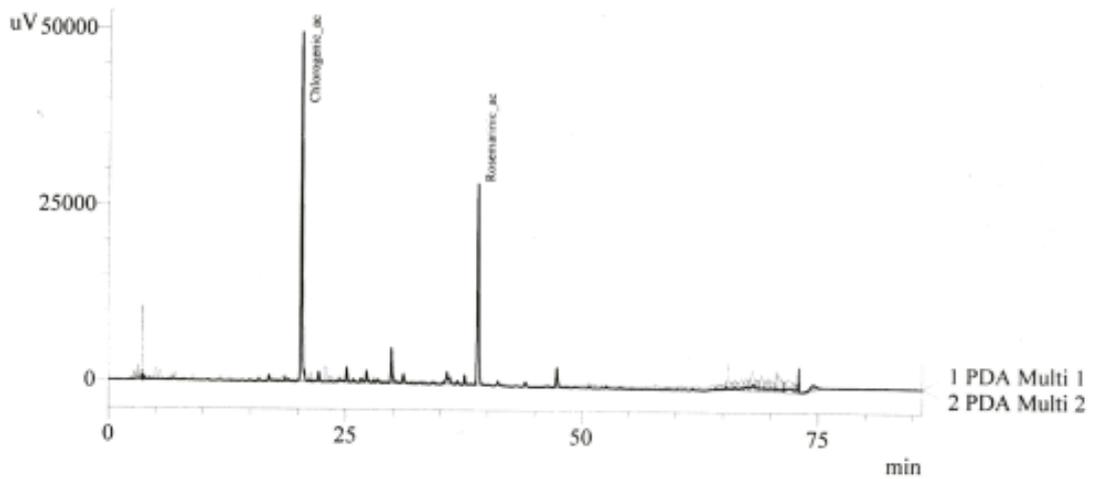


Fig 2: HPLC of phenolic compounds in 1 year carrot roots of “Yaskrava” variety

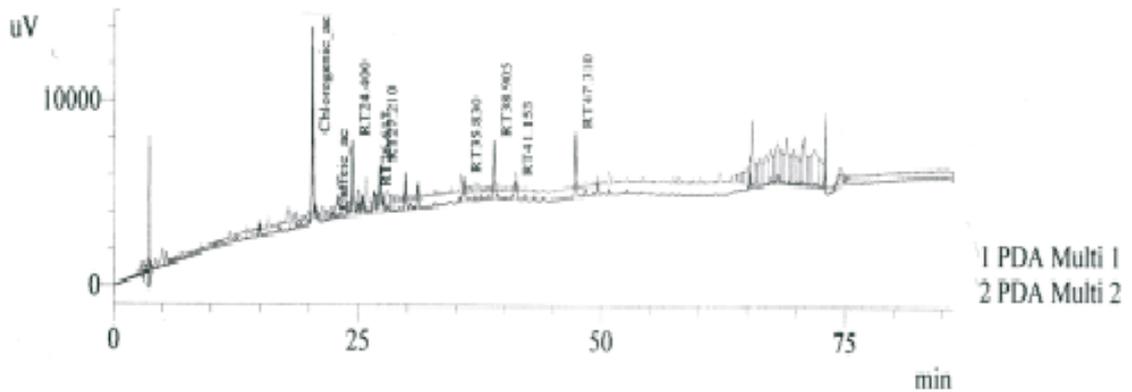


Fig 3: HPLC of phenolic compounds in 2 year carrot roots of “Yaskrava” variety

Table 1: Results of quantitative determination of phenolic acids content in carrot roots of “Nantska Kharkivska” and “Yaskrava” varieties

Acid	“Nantska Kharkivska” variety		“Yaskrava” variety			
	Roots of the 1 st year		Roots of the 1 st year		Roots of the 2 nd year	
	Content, %	Retention time, min	Content, %	Retention time, min	Content, %	Retention time, min
Chlorogenic	0,24	20,325	0,08	20,342	0,02	20,334
Rosmarinic	-	-	0,06	38,947	-	-
Caffeic	-	-	-	-	0,0003	22,198

As a result of the experiment carried out the presence and quantitative content of chlorogenic, rosmarinic and caffeic acid in carrot roots was determined. The content of chlorogenic acid in the 1st year carrot roots of “Nantska Kharkivska” amounted to 0,24%, the content of chlorogenic and rosmarinic acids in the 1st year carrot roots of “Yaskrava” variety was 0,08% and 0,06% respectively, and the content of chlorogenic and caffeic acids in the 2nd year carrot roots of “Yaskrava” variety comprised 0,02% and 0,0003% respectively.

As the obtained data have shown, all the samples studied contain chlorogenic acid. The highest content of this compound was found in the 1st year carrot roots of “Nantska Kharkivska” variety, while the 2nd year carrot roots of “Yaskrava” variety contained the smallest amount of chlorogenic acid.

On comparing the carrot roots of the 1st and 2nd year of “Yaskrava” variety it is worth mentioning that the 1st year roots contain rosmarinic acid, and the 2nd year roots tend to contain caffeic acid.

CONCLUSION

The HPLC method has allowed to identify 1 phenolic acid in the 1st year carrot roots of “Nantska Kharkivska” variety, and in the 1st and 2nd carrot roots of “Yaskrava” variety – 2 phenolic acids per sample. All the samples studied have shown the presence of chlorogenic acid which quantitatively dominated in the 1st year carrot roots of “Nantska Kharkivska” variety.

The data obtained can be further used in new medicines on the basis of carrot underground organs working out and obtaining.

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