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PHENOLIC ACID CONTENTS IN FRUITS OF AUBERGINE (*SOLANUM MELONGENA* L.)

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Key words: aubergine, *Solanum melongena* L., phenolic acids, hydroxycinnamic acids, hydroxybenzoic acids

Quantitative and qualitative analyses of phenolic acid fractions (free and liberated after acidic and basic hydrolysis) in three aubergine cultivars (Black Beauty, Solara F_1, Epic F_1) were performed. Qualitative and quantitative HPLC analysis was done using a reversed phase system applying a LaChrom type (Merck) liquid chromatograph equipped with a DAD detector.

Caffeic, p-coumaric, ferulic, gallic, protocatechuic and p-hydroxybenzoic acids were found in all investigated aubergine fruits. Studies suggested that the fruits of the Black Beauty cultivar contained the highest level of phenolic acid (35.14 μg/g fresh matter) with protocatechuic acid (22.36 μg/g fresh matter) predominating. Fruits of other cultivars contained the following amounts of the compounds studied: Solara F_1 24.82 μg/g fresh matter and Epic F_1 18.76 μg/g fresh matter. Moreover, it was shown that free phenolic acids had the highest share in the total sum of aubergine phenolic acids.

INTRODUCTION

Vegetables and fruits are a source of natural antioxidants, among which phenolic acids could be distinguished [Yanishlieva-Maslarova & Heinonen, 2001; Kaur & Kapoor, 2002]. These compounds provided with a diet remove the effects of oxygen reactions which may lead to the so-called "oxidation stress" that can have serious consequences for human health [Bartosz, 1995; Rice-Evans *et al.*, 1997]. It is considered that an increase in reactive oxygen form concentration is associated with the course of many diseases such as some types of cancer, heart diseases, cataracts, diabetes, rheumatism *etc*. Therefore, a diet rich in products with high levels of antioxidant compounds plays an important role in protection against the hazardous action of reactive oxygen forms. Close attention has been paid lately to phenolic acids in phytochemical research as substances having immunostimulation effects. Ester and glycoside derivatives of caffeic acid from the group of hydroxycinnamic acid are of the special significance [Borkowski, 1993; 1996].

Aubergine fruits (*Solanum melongena* L.) can be an important source of natural antioxidants, including phenolic acids. Lately, this vegetable has become quite popular in Polish cuisine. Aubergine fruits are of high dietetic value. They contain (in 100 g of fresh matter) up to 1.4% protein, up to 0.3% fat and up to 4.32% water-soluble sugars [Czikow & Łaptiew, 1983; Cebula, 1999; Gajewski & Gajc-Wolska, 1998; Lawande & Chavan, 1998]. Aubergine fruits deserve attention due to their low energy values (100 kJ/100 g fresh matter) and the presence of crude fiber whose total amount is over 30% of dry matter.

Aubergine fruits are very desirable because of their mineral elements. Experts consider aubergine to be an important natural source of potassium. According to German research, 100 g of fresh fruits contains about 220 mg K. It covers 10% of human daily requirements for potassium. Nutritional experts have shown that consuming the aubergine fruits strengthens the heart and improves water balance through rational fluid excretion from the body. The effect of aubergine fruits on the prevention and even treatment of atherosclerosis is determined by the presence of phytosterols, which are synthesized by aubergine plants [Korszikow, 1991; Procyk, 1993]. The following compounds were found in aubergine fruits: campesterol, -sitosterol, stigmasterol and small amounts of cholesterol [Zimowski & Wojciechowski, 1996]. There are a lot of reports on the composition of anthocyanins present in aubergine [Pifferi & Zamorani, 1969; Tanchev *et al.*, 1970; Fiorini, 1995]; however, few reports describe the composition and content of phenolic acids in aubergine, particularly hydroxybenzoic acids [Herrmann, 1996]. Therefore, the aim of the present study was a qualitative and quantitative evaluation of phenolic acids occurring in aubergine fruits.

MATERIAL AND METHODS

Fruits of three aubergine cultivars (Black Beauty, Solara F_1, Epic F_1) originating from the experimental plantation of the Department of Vegetable and Medical Plants, University of Agriculture in Lublin (August 2000), were preliminarily lyophilised.

Isolation and purification of free phenolic acids (FPA) and those liberated by acidic (AH) and basic (BH) hydroly-

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sis was performed as was described earlier [Kowalski & Wierciński, 2003]. Three extracts of FPA, AH and BH fractions were achieved from each aubergine cultivar.

Particular fractions of FPA, AH and BH were dissolved in 10 mL of methanol and then analysed with the HPLC technique.

Qualitative and quantitative HPLC analyses were performed in a reversed phase system by applying a LaChrom-Merck type liquid chromatograph equipped with a DAD detector (L-7450), pump (l-7100), degasifier (L-7612), 20 μL dosing loop, thermostat (L-7360), injector Rheodyne, steel column LiChrospher 100 RP-C_{18} of 250×4 mm, particle size of 5 μm. The sample analysis was carried out at 25°C. Methanol:water solution (25:75, v/v) with 1% addition (v/v) of acetic acid [Drost-Karbowska *et al.*, 1996] was the mobile phase. The flow rate was 0.8 mL·min^{-1}. Identification of phenolic acids was performed by comparing their retention times (t_r) and spectra in UV (220–400 nm) with those for standards. The contents of particular phenolic acids in fruits were calculated on the basis of calibration

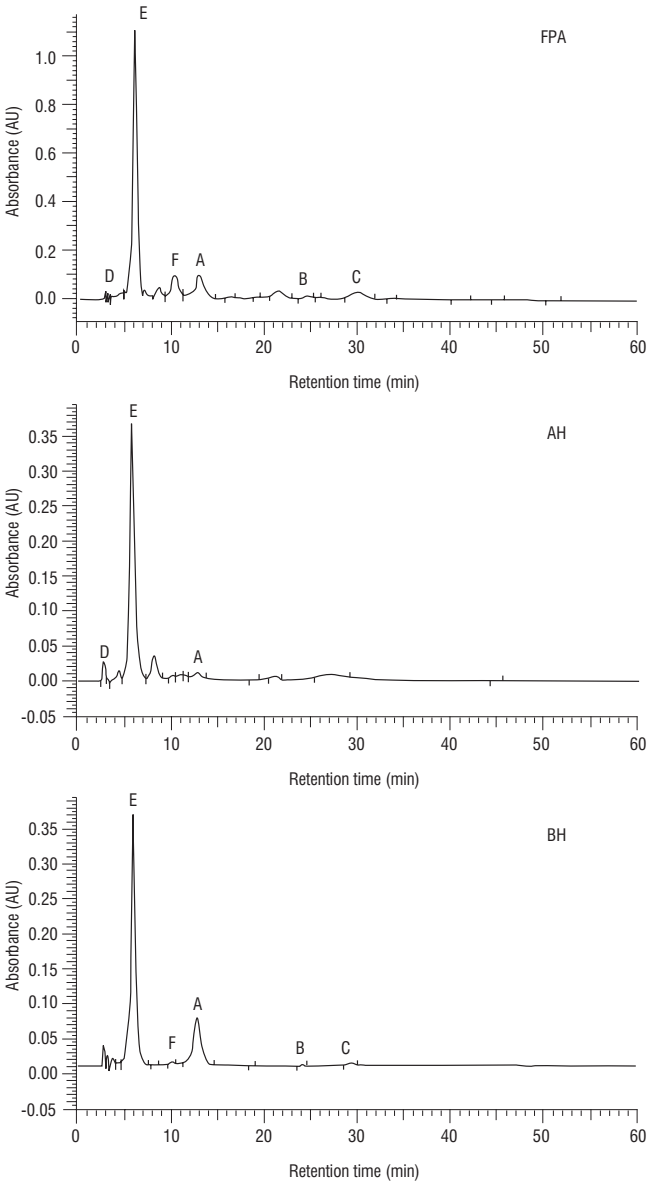

FIGURE 1. HPLC chromatograms of phenolic acids fraction released from aubergine fruits Black Beauty cv.
Explanations: FPA – free phenolic acids, AH – phenolic acids liberated by acid hydrolysis, BH – phenolic acids liberated by basic hydrolysis. Phenolic acids: A – caffeic, B – p-coumaric, C – ferulic, D – gallic, E - protocatechuic, F – p-hydroxybenzoic.

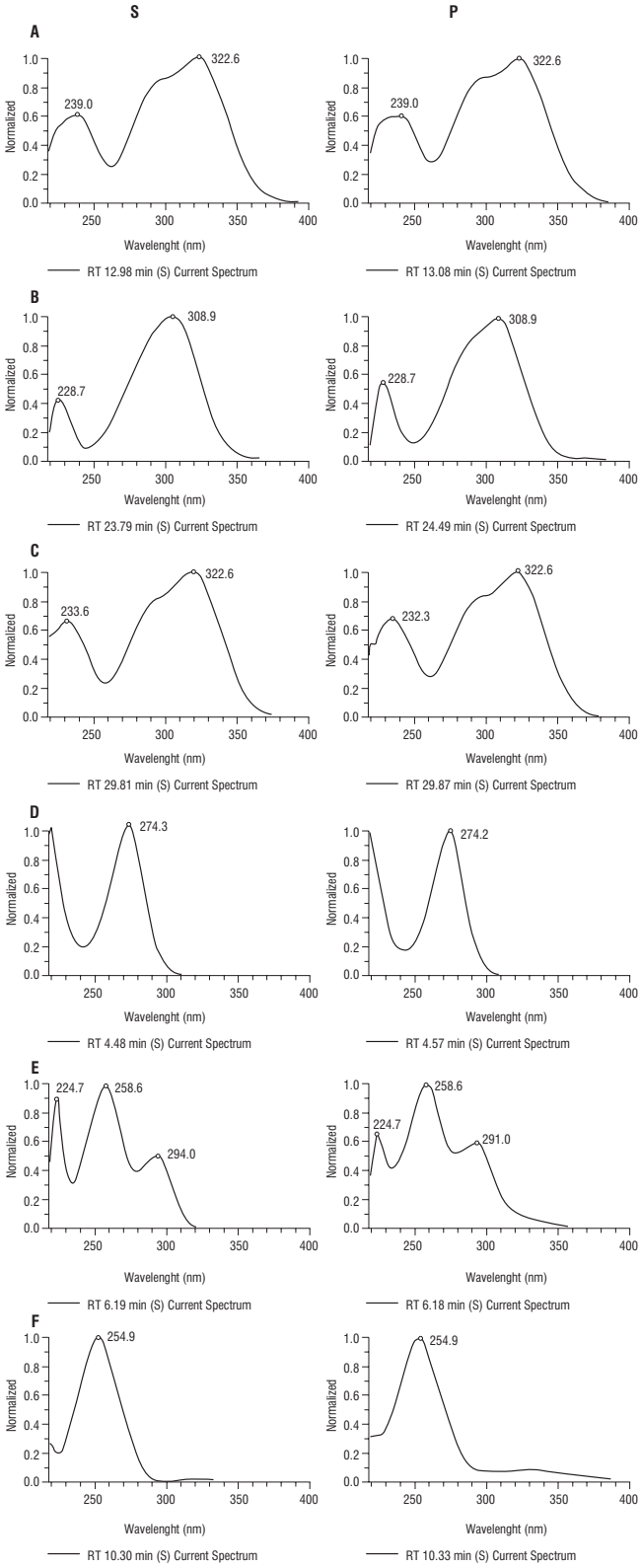

FIGURE 2. UV spectra (220–400 nm) of standard phenolic acids (S) and those isolated from combined fraction of three aubergine cultivars (P).
Explanations: Phenolic acids: A – caffeic, B – p-coumaric, C – ferulic, D – gallic, E - protocatechuic, F – p-hydroxybenzoic.

curves plotted as the dependence of area surface under peaks for standard phenolic acids (methanol solutions) on their concentration in the range of: 5.0–25 mg/100 mL.

All HPLC analyses were made in five replications.

RESULTS AND DISCUSSION

Figure 1 presents examples of HPLC chromatograms for isolated phenolic acid fraction occurring in Black Beauty fruits. Figure 2 shows spectroscopic spectra, allowing the identification of the studied compounds present in aubergine fruits (Black Beauty). Phenolic acid contents in particular fractions (FPA, AH and BH) achieved from aubergine were presented in Table 1. Moreover, Figure 3 presents the percentage of isolated phenolic acids and fraction of phenolic acid groups occurring in the studied vegetable.

The results of the analyses indicate that aubergine fruits were characterized by the occurrence of phenolic acids both in free and bounded forms. Six phenolic acids were identified in the studied fruits: caffeic, p-coumaric, ferulic, gallic, protocatechuic and, p-hydroxybenzoic. Black Beauty fruits contained the highest levels of phenolic acids (35.14 μg/g fresh matter) with protocatechuic acid dominating (22.36 μg/g fresh matter). Fruits of other varieties contained the following amounts of studied compounds: Solara F_1 24.82 μg/g fresh matter and Epic F_1 18.76 μg/g fresh matter. Moreover, it was shown that free phenolic acids had the highest percentage (from 47.4% to 69.8%) in the total sum of phenolic acids of aubergine. Large amounts of phenolic acids released during acidic and basic hydrolysis probably indicate that these compounds may be present in aubergine in ester and heteroside forms bonded, among others, to sugars. It was found that protocatechuic acid is dominant in aubergine fruits. Protocatechuic acid, from the group of hydroxybenzoic acids, occurs in fruits, vegetables and nuts and shows anti-oxidant and anti-tumour properties [Tseng *et al.*, 1998]. It inhibits chemical carcinogenesis at the stage of cancer initiation and post-initiation in animals. However, caffeic and ferulic acids from the group of hydroxycinnamic acids, show choleretic and cholekinetic activity [Borkowski, 1993]. Published data [Rice-Evans *et al.*, 1997] have revealed that 1 mmol/L of hydroxycinnamates (caffeic, ferulic, p-coumaric and chlorogenic acids) occurring in the fruits and vegetables (apple, peach, cherry, white grapes, cabbage, asparagus and tomato) showed antioxidant activity: from 1.3 to 2.2 mmol/L TEAC (Trolox equivalent antioxidant activity). The results of this study revealed that aubergine fruits contained from about 9 μg/g to about 12 μg

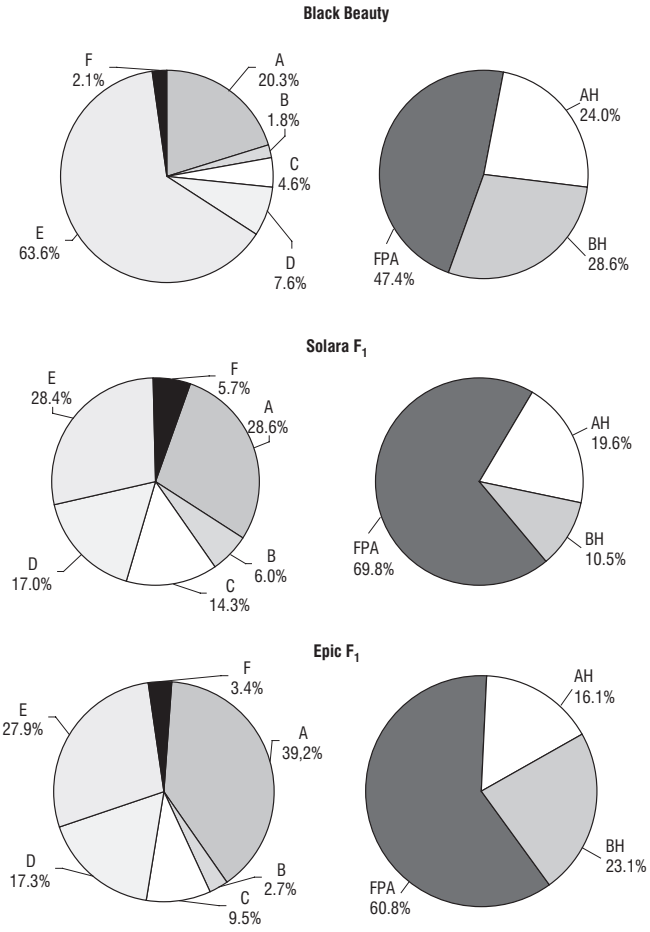

FIGURE 3. The percentage of phenolic acids fraction in aubergine fruits.
Explanations: FPA – free phenolic acids, AH – phenolic acids liberated by acid hydrolysis, BH – phenolic acids liberated by basic hydrolysis. Phenolic acids: A – caffeic, B – p-coumaric, C – ferulic, D – gallic, E - protocatechuic, F – p-hydroxybenzoic.

TABLE 1. The contents of phenolic acids in aubergine fruits determined by HPLC.

Phenolic acid	Content of phenolic acids (μg/g fresh matter)											
	Black Beauty				Solara F_1				Epic F_1			
	FPA	AH	BH	Σ	FPA	AH	BH	Σ	FPA	AH	BH	Σ
X* HYDROXYCINNAMIC ACIDS												
A. Caffeic	3.01±0.33	0.40±0.02	3.72±0.22	**7.13**	4.90±0.24	0.27±0.01	1.93±0.12	**7.10**	3.62±0.20	0.12±0.01	3.62±0.13	**7.36**
B. p-Coumaric	0.53±0.04	-	0.11±0.01	**0.64**	1.48±0.09	-	-	**1.48**	0.50±0.02	-	-	**0.50**
C. Ferulic	1.49±0.03	-	0.11±0.01	**1.60**	3.43±0.11	tr.	0.12±0.04	**3.55**	1.79±0.11	-	-	**1.79**
Σ X*	**5.03**	**0.40**	**3.94**	**9.37**	**9.81**	**0.27**	**2.05**	**12.13**	**5.91**	**0.12**	**3.62**	**9.65**
Y* HYDROXYBENZOIC ACIDS												
D. Gallic	1.19±0.04	1.49±0.03	-	**2.68**	2.02±0.10	2.21±0.10	-	**4.23**	1.24±0.02	2.01±0.11	-	**3.25**
E. Protocatechuic	9.71±0.33	6.53±0.30	6.12±0.21	**22.36**	4.08±0.12	2.38±0.11	0.59±0.02	**7.05**	3.62±0.13	0.89±0.02	0.72±0.02	**5.23**
F. p-Hydroxybenzoic	0.73±0.02	-	tr.	**0.73**	1.41±0.02	-	tr.	**1.41**	0.63±0.01	-	tr.	**0.63**
Σ Y*	**11.63**	**8.02**	**6.12**	**25.77**	**7.51**	**4.59**	**0.59**	**12.69**	**5.49**	**2.90**	**0.72**	**9.11**
Σ (X* + Y*)	**16.66**	**8.42**	**10.06**	**35.14**	**17.32**	**4.86**	**2.64**	**24.82**	**11.40**	**3.02**	**4.34**	**18.76**

FPA – free phenolic acids, AH – phenolic acids liberated by acid hydrolysis, BH – phenolic acids liberated by basic hydrolysis. Phenolic acids: A – caffeic, B – p-coumaric, C – ferulic, D – gallic, E - protocatechuic, F – p-hydroxybenzoic; tr. – trace (<0.01 μg/g); „-" lack

of hydroxycynnamic acids in fresh matter. Winter and Herrmann [1986] found that for aubergine, the contents of depside of hydroxycinnamic derivatives are as follows: caffeic acid depside – 5-O-caffeiloquinic acid (chlorogenic acid) approx. 600 μg/g fresh matter, 4-O-caffeiloquinic acid approx. 8 μg/g; ferulic acid depside – 5-O-feruiloquinic acid approx. 15 μg/g. The content of 5-O-caffeiloquinic acid in aubergine amounted to about 33 μg/g fresh matter according to De Maria *et al.* [1999]. The above-mentioned differences are probably the result of various methods of extraction and determination of the studied components. It is worth mentioning that no other data on the phenolic acid composition of aubergine fruits was found in literature for comparative purposes.

CONCLUSIONS

1. No significant differences in the qualitative composition of phenolic acids were found in the three studied aubergine cultivars.

2. Free phenolic acids had the highest percentage of total phenolic acids in aubergine.

3. The Black Beauty cultivar contained the highest level of phenolic acids (35.14 μg/g fresh matter) with protocatechuic acid (22.36 μg/g) dominating.

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ZAWARTOŚĆ FENOLOKWASÓW W OWOCACH OBERŻYNY *SOLANUM MELONGENA* L.

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W prezentowanej pracy dokonano jakościowej i ilościowej analizy frakcji fenolokwasów (wolnych fenolokwasów i uwolnionych po hydrolizie kwaśnej i zasadowej) owoców trzech odmian oberżyny (Black Beauty, Solara F_1, Epic F_1). Jakościową i ilościową analizę prowadzono metodą HPLC w układzie faz odwróconych, stosując chromatograf cieczowy typu LaChrom – Merck z detektorem DAD.

W owocach trzech analizowanych odmian oberżyny stwierdzono obecność kwasu kawowego, p-kumarowego, ferulowego, galusowego, protokatechowego i p-hydroksybenzoesowego. Wykazano największe zawartości fenolokwasów w owocach odmiany Black Beauty – 35,14 μg/g ś. m. z dominującym kwasem protokatechowym (22,36 μg/g ś. m.). Owoce odmian Solara F_1 i Epic F_1 zawierały mniej fenolokwasów, odpowiednio 24,82 i 18,76 μg/g ś. m. Ponadto wykazano, że wolne fenolokwasy stanowiły największy udział w ogólnej sumie fenolokwasów oberżyny.