Effect of accelerated ageing on the content and composition of polyphenolic complex of wheat (*Triticum aestivum* L.) grains

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ABSTRACT

The influence of accelerated ageing test (AAT), i.e. of higher temperature and humidity, on the content and composition of phenolic compounds in the grains of five cultivars of wheat (*Triticum aestivum* L.) was studied in the years 1998 and 1999. Total polyphenols were determined spectrophotometrically by Folin-Ciocalteau's phenolic reagent and free phenolic acids by an HPLC method in control samples and after deterioration treatment (AAT) in the Ebi, Estica, Nela, Samanta and Šárka cultivars. Statistical significance of deterioration treatment, cultivar, cultivation site, and harvest year was proved. Content of total polyphenols increased during deterioration and levels of the individual free phenolic acid changed depending on their structures. Mean contents of total polyphenols varied from 600 to 960 mg/kg of dry matter. The increase caused by the AAT ranged between 0–20%, the greatest mean increase was observed in the cultivar Nela (by 19%). Sinapic acid (as high as 540 mg/kg of dry matter), 3-hydroxy-benzoic acid and 7-hydroxycoumarin were determined as the major phenolic acids and coumarins. A decrease of free phenolic acids containing methoxy groups in their molecules (sinapic and vanillic acids) and an increase of phenolic acids with free hydroxy groups (caffeic and gallic acids) was observed after deterioration treatment. Statistically significant ($P \leq 0.05$) effect of AAT on the content of free phenolic acids was observed.

Keywords: *Triticum aestivum* L.; wheat grain; polyphenols; phenolic acids; deterioration; accelerated ageing test; effect of higher temperature and humidity

Physical factors and their interactions are important in determining performance and distribution of plants. Temperature and water availability are of the foremost relevance, the other additional interferences derive from abiotic factors such as light intensity, nutrients, organic and inorganic pollutants. Moreover, the importance of biotic factors such as insects, harmful predators, herbs etc. is affected by the same features. Temperature, water, radiation and nutrient stress are responsible for as much as 50% reduction of the potential yield of the main crops.

As far as the quality is concerned, reproductive development is usually severely affected with consequences on the crops, which are important for fruits and seeds. Synthesis, accumulation and storage of dominant compounds (proteins, polysaccharides, lipids) and minor compounds, i.e. secondary metabolites (polyphenols, carotenoids, isoprenoids, glycoalkaloids etc.) are substantially affected by these abiotic stress factors (Lachman et al. 1997, 1999, Hamouz et al. 1999a, b, Orsák et al. 2001).

Polyphenols as the effective antioxidants are classified to several groups according to their structure and attributes. Phenolics possess so called free radical scavenging effect – they reduce the number of free radicals (Richard-Forget et al. 1995), inhibit lipoxygenase and work as uncompetitive inhibitors. They have protective effects against diseases, pests, and physical stress. Regarding the quality of seeds, they work as regulating factors that slower the process of germination and positively affect the level of dormancy in seeds, protect the embryo against UV-irradiation and have allelopathic effects.

In this study we focused on the determination of the effects of increased temperature and humidity using the accelerated ageing test (AAT), cultivar, cultivation year and site on the contents of phenolic compounds and free phenolic acids in wheat grains. Changes of dominant phenolics were investigated.

MATERIAL AND METHODS

Samples of five wheat cultivars (Ebi, Estica, Nela, Samanta and Šárka) were obtained from the harvests in the years 1998 and 1999 from three stations of Central Institute for Supervising and Testing in Agriculture in Jaroměřice n. R., Staňkov and Brno-Chrlice.

Accelerated ageing test (AAT) was performed according to Te Krony (1995). Weighed grains (ca 50 g) were placed in the inner box of an incubator at 41°C and relative humidity > 90% and left there for 72 h.

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Table 1. Increase of total polyphenols (% of the initial level) in the individual cultivars after treatment with test of accelerated ageing (AAT)

Cultivar	1998			1999		
	C (mg/kg)	AAT (mg/kg)	change of TP (%)	C (mg/kg)	AAT (mg/kg)	change of TP (%)
Ebi	783	885	13.1	814	811	–0.3
Estica	888	965	8.7	853	878	2.9
Nela	810	955	18.0	794	955	20.2
Samanta	823	960	16.6	776	861	10.9
Šárka	797	862	8.1	604	723	19.7
Mean	820	926	12.9	768	846	10.0

C – control, AAT – accelerated ageing test

Determination of total polyphenols (TP) with Folin-Ciocalteau's reagent: approximately 12 g of disintegrated grains were extracted in Soxhlet apparatus with 80% ethanol for 18 hours. Extract was quantitatively placed into 250ml volumetric flask and filled to the mark with 80% ethanol. 5ml aliquots were pipetted into 50ml volumetric flasks. About 25 ml of distilled water and 2.5 ml of Folin-Ciocalteau's phenolic reagent (Penta Chrudim, CZ) was added. After 3 minutes 7.5 ml of 20% solution of sodium carbonate was added and the volume was adjusted with distilled water to 50 ml. After thorough agitation and standing for two hours the blue solution was centrifuged at 2000 cycles per minute for 12 minute period (Janetzki T 30, Germany) and absorbance was measured against blank using an UV-VIS spectrophotometer Helios γ (Spectronic Unicam, UK) at $\lambda = 765$ nm. Total polyphenol content was expressed as gallic acid.

Determination of phenolic acids by HPLC: HPLC Waters™ system with gradient elution consisting of Waters™ 600S pump, Waters™ 717 plus autosampler, Waters™ PDA 996 – UV-VIS detector and Watrex 250 × 4 mm Sepharon SGX C18 7 µm column was used. As mobile phases 5% methanol solution in water (phase A) and 40% methanol solution in water (phase B) adjusted with H_3PO_4 to pH 2.5 were used. Flow rate was 1 ml/min, elution time 56 min, injection volume 20 µl, detection at $\lambda =$ 280 nm. The used standards were obtained from Lachema CZ (veratric acid), Fluka AG (2,3-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, ferulic acid, gallic acid, caffeic acid, coumarin, coumarin-3-carboxylic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, *p*-hydroxybenzoic acid, sinapic acid, cinnamic acid, syringic acid), Dr. Theodor Schuchardt, Munich (2,4-dihydroxybenzoic acid), Sigma-Aldrich (2,5-dihydroxybenzoic acid, 3-hydroxybenzoic acid, 4-hydroxycoumarin, 7-hydroxycoumarin, 7-methoxycoumarin, salicylic acid), The British Drug Houses, Ltd., GB (*m*-hydroxybenzoic acid), Heyl & Co. (vanillic acid). Quantitative determination of the individual phenolcarboxylic acid contents was performed by the method of absolute calibration.

Dry matter determination was performed according to Davídek et al. (1977). 2 g of homogenised sample was dried in an oven at 105°C until constant weight was obtained.

Statistical evaluation was performed by Statgraphic programme by *F*-test and *T*-test and multiple range variance analysis from two parallel measurements at the significance level 0.05.

RESULTS AND DISCUSSION

TP contents in wheat grains varied from 600 to 960 mg/kg of dry matter. These levels are approximately twice higher in comparison with the pea levels and by one quarter lower comparing with barley grains (Lachman et al. 1997). It is obvious from the obtained results (Table 1) that the AAT deterioration treatment caused increase of total polyphenol levels. The highest relative increase was found in Nela (+19%) and Samanta cultivars. In general, the AAT caused a mean increase 11.5%. This increase is comparable to values given for pea and barley by Lachman et al. (1997). *F*-test (Table 2) revealed high statistical significance of AAT on TP content ($P = 0.0130$). At the significance level $\alpha = 0.05$ the effect of cultivar on TP content was near the limit ($P = 0.0520$). The influence of site and year of cultivation was observed to be of lower significance ($P = 0.0655$ and 0.0847, respectively).

Among phenolic acids and coumarins in wheat caryopses there were found the highest contents of sinapic acid, 3-hydroxybenzoic acid and 7-hydroxycoumarin (Table 3). 2,3-dihydroxybenzoic, 2,4-dihydroxybenzoic, 3,4-dihydroxybenzoic, ferulic, gallic, caffeic, coumarin-3-carboxylic, *m*-coumaric, *o*-coumaric, *p*-coumaric, *p*-hydroxybenzoic, salicylic, cinnamic, syringic, vanillic and veratric acids and 4-hydroxycoumarin and coumarin were identified from the chromatograms (Figure 1).

From the multiple range variance analysis (Table 4) at $P = 0.05$ could be seen statistically highly significant effect of cultivation site ($P = 0.005$), cultivar ($P = 0.0022$) and AAT ($P = 0.0398$) on free phenolic acids levels. It was found that after AAT the content of sinapic acid decreased (by 10–80%, 0–540 mg/kg of dry matter), as well as that of vanillic acid (by 40–83%, 0–15 mg/kg of dry matter). On the other hand, caffeic acid content increased (by 80–220%, 0–6.9 mg/kg of dry matter). Statistically significant effect of deterioration on three most abundant phenolic acids (sinapic, caffeic and vanillic acid) content

Table 2. Results of multiple range variance analysis of total polyphenols contents in wheat grains

Source of variability	Sum of squares	*d.f.*	Variance	*F*-test	*P*-value
		Main effects			
A – cultivar	61906.2	1	61906.2	5.20	0.0520
B – treatment	120246.0	1	120246.0	10.11	0.0130*
C – year	81238.7	2	40619.3	3.42	0.0847
D – site	162581.0	4	40645.3	3.42	0.0655
		Interaction			
AB	2313.5	1	2313.5	0.19	0.6709
AC	9967.5	2	4983.7	0.42	0.6713
AD	49268.2	4	12317.0	1.04	0.4456
BC	14429.1	2	7214.6	0.61	0.5685
BD	19618.0	4	4904.5	0.41	0.7955
CD	70937.6	8	8867.2	0.75	0.6561
ABC	64309.2	2	32154.6	2.70	0.1268
ABD	13049.2	4	3262.3	0.27	0.8866
ACD	79032.0	8	9879.0	0.83	0.6004
BCD	64481.4	8	8060.2	0.68	0.7026
Inner (residual)	95154.6	8	11894.3		
Total	908532.0	59			

* statistically significant influence ($P \leq 0.05$) on the content of total polyphenols

was demonstrated. From the changes of phenolcarboxylic acids content could be concluded that levels of acids containing in their molecules methoxy groups (sinapic and vanillic acid) decrease and phenolic acids containing in their molecule free hydroxy groups (caffeic and gallic acid) increase. It could be due to demethylation of methoxy groups contained in the molecules of phenolic acids. Phenolic acids as well as other polyphenolic structures could interact and change each other during various physiological processes and could vary regarding

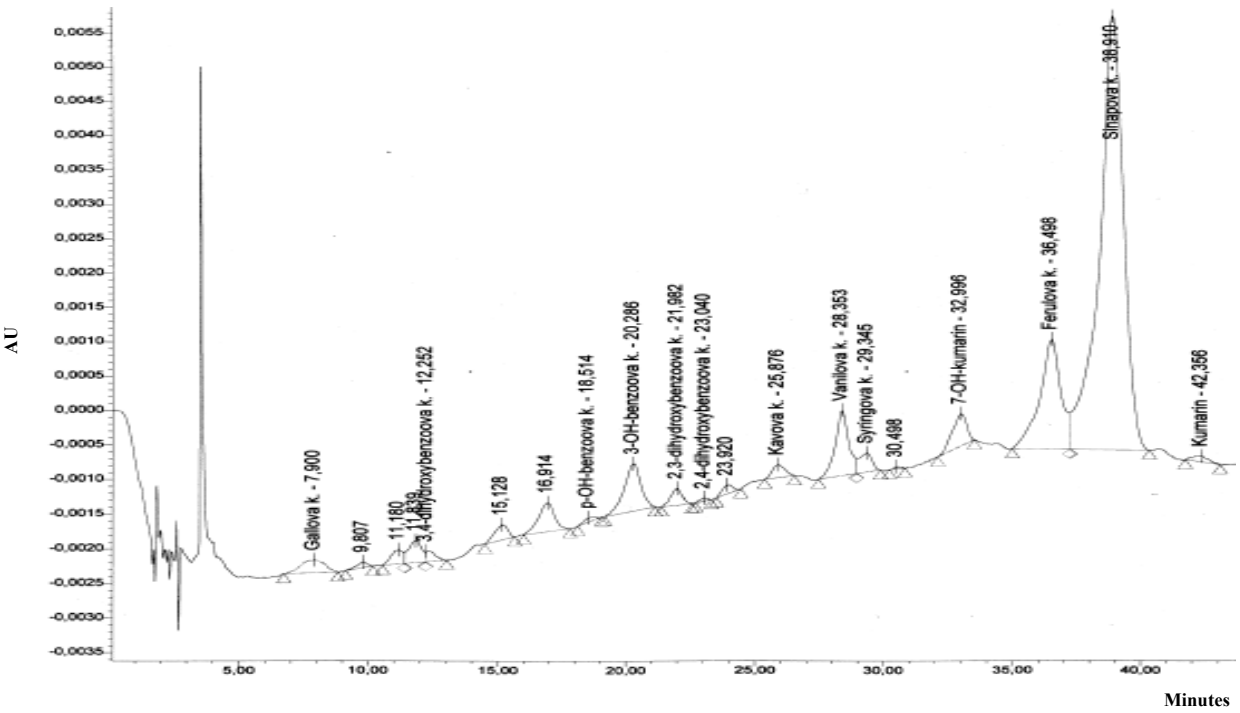

Figure 1. Chromatogram of Šárka cultivar, site Staňkov, 1999, control sample

Peaks sequence: gallová k. = gallic acid; 3,4-dihydroxybenzoová k. = 3,4-dihydroxybenzoic acid; *p*-OH benzoová k. = *p*-hydroxybenzoic acid; 3-OH benzoová k. = 3-hydroxybenzoic acid; 2,3-dihydroxybenzoová k. = 2,3-dihydroxybenzoic acid; 2,4-dihydroxybenzoová k. = 2,4-dihydroxybenzoic acid; kávová k. = caffeic acid; vanilová k. = vanillic acid; syringová k. = syringic acid; 7-OH kumarin = 7-hydroxycoumarin; ferulová k. = ferulic acid; sinapová k. = sinapic acid; kumarin = coumarin

Table 3. Content of phenolcarboxylic acids in wheat grain (mg/kg of dry matter)

Variety	Site	Year	Treatment	Sum of all acids	2,3-dihydroxybenzoic acid	2,4-dihydroxybenzoic acid	3,4-dihydroxybenzoic acid	3-hydroxybenzoic acid	4-hydroxycoumarin	7-hydroxycoumarin	Ferulic acid	Gallic acid	Caffeic acid	Coumarin	Coumarin-3-carboxylic acid	*m*-coumaric acid	*o*-coumaric acid	*p*-coumaric acid	*p*-hydroxybenzoic acid	Salicylic acid	Sinapic acid	Cinnamic acid	Syringic acid	Vanillic acid	Veratric acid
Ebi	CHR	98	C	520	32.8	0.7	3.4	99.5		133	50.7		0.4	0.7			11.5			0.7	163		17.4	5.5	
Ebi	CHR	98	AAT	500	28.2	1.3	6.5	86.3		54.7	6.5	1.9	5.6	0.9					0.5		300		0.3	5.4	1.7
Ebi	CHR	99	C	207		3.3	0.7	49.8	0.1	32.6			0.2	0.5			4.3			0.5	104			10.5	
Ebi	CHR	99	AAT	216	26.7	0.7	26.3	44.4		38.3	14.8		3.2				53.9		0.2		0.4	2E-02	5.2	1.2	
Ebi	JAR	98	C	270	10.3	8.8	2.5	84.5		88.6		6E-04	1.5	0.5			29.2	0.2		1.5	32.2			10.3	
Ebi	JAR	98	AAT	494	17.4	0.9	7.0	68.9		45.1	19.6		2.4					0.8			322		4.1	0.9	5.1
Ebi	JAR	99	C	410	16.6		2.0	59.1		29.7	18.8		2.6	0.8							265		3.5	12.2	
Ebi	JAR	99	AAT	285	18.2	0.6	94.5	43.6		23.3	11.7		1.0			0.2	82.9	0.3	0.2		4.1	2E-02	3.5	0.2	
Ebi	STA	98	C	257	14.8		0.2	36.9		15.2	16.0		0.1	0.6							160		10.7	2.7	
Ebi	STA	98	AAT	190	31.1				0.3	40.6	20.5	1.3	2.1				68.1	0.7			6.3	2E-02	3.5	1.1	14.9
Ebi	STA	99	C	279	17.9		11.1	76.6		24.2	5.2		2.0	1.1				0.2	0.7		128			7.9	3.5
Ebi	STA	99	AAT	294		9.6	7.2	72.1		53.2		3.4	1.4	0.8			6.2		0.6		131	0.1		7.4	1.3
Estica	CHR	98	C	566	25.7		4.8	90.7		92.7	41.3		2.0	0.8			6.9			1.4	287		10.3	2.8	
Estica	CHR	98	AAT	322	26.2	0.2	4.7	88.5		58.8	27.1		1.3	0.7	0.4		109		0.7				4.1	0.6	
Estica	CHR	99	C	447	26.3	0.6	2.2	64.3		28.2	20.2	3.4	0.3	1.1					0.4		286		2.8	11.2	
Estica	CHR	99	AAT	292	30.6	1.3	3.2	63.9		47.0	21.1	4.7	4.8				103	0.5	0.6		6.1	6E-03	0.7	4.3	
Estica	JAR	98	C	688	30.3	0.8		79.3		109	63.5		1.6	1.2							380		11.8	2.8	7.8
Estica	JAR	98	AAT	694	26.2	0.9	10.9	70.7		83.2	24.3		4.4	3.0						1.4	459		0.6	3.2	6.1
Estica	JAR	99	C	633	24.7		4.3	73.4		126	62.6		2.2	1.6	1.1		17.6	0.7			300		3.9	15.2	
Estica	JAR	99	AAT	381	28.7	0.8	4.1	62.3		77.6	30.5		6.9			0.6	153	0.7	0.6		8.1	3E-02	1.0	5.6	
Estica	STA	98	AAT	295	25.8	1.1	1.1	39.4		70.3	21.8		5.2			0.9	117	1.3	0.3		3.1	4E-02	6.0	1.3	
Estica	STA	99	C	824	25.4		8.1	109		104			5.5	1.4			20.6		0.5	1.1	540			9.3	
Estica	STA	99	AAT	650		11.0	7.7	67.0		99.7	27.1		2.4	1.5				0.1			424			5.2	4.3
Nela	CHR	98	AAT	180	31.4	3.1	4.8	110		19.1	20.6	5.4	4.9	0.5			59.5		1.2		3.5	3E-02	5.8	1.0	
Nela	CHR	99	C	270		3.1	7.1	80.3		17.6	16.6		1.2	0.7					1.6		167			6.1	
Nela	CHR	99	AAT	301	43.3	0.5		97.5	2.3	39.4	22.7	0.9	4.8				87.6		1.7	1.0	14.4	3E-02	10.1	1.9	

Nela	JAR	98	C	328		9.0	0.3	108		71.7	35.4		1.0		0.9		9.5				174			11.8	
Nela	JAR	98	AAT	421		5.9	8.2	52.9		88.0	9.4		0.3	3.4				0.2	0.9		122		0.2	4.7	2.8
Nela	JAR	99	C	300		7.1	5.0	154		51.5		0.3	4.9	1.7			9.0		0.6		456			13.5	
Nela	JAR	99	AAT	704	26.9		7.6	93.3		70.8	36.7	33.4	3.3			171		0.7	0.5		16.6	6E-02	7.0	0.4	
Nela	STA	99	AAT	472	109	2.9	6.7	162	14.5	96.5	31.5	1.0	2.4	2.2				2.2	1.3		334	5E-02	14.6	1.6	
Samanta	CHR	98	C	782		4.6	0.8	97.7		42.1			0.4			4,9				0.4	0.8			14.8	
Samanta	CHR	98	AAT	167	25.8	3.0	6.4	134		27.2	8.5	1.5	3.2	0.4				0.9	0.8		103	3E-02	6.7	1.5	
Samanta	CHR	99	C	323		2.4	0.8	43.3		11.2			1.0	0.6			3.1	0.5	0.2		73.2			5.3	
Samanta	CHR	99	AAT	142	27.2	0.8		49.9		22.9	5.9	6.3	4.2		1.0		27.6	0.5	0.5		11.9	6E-02	5.4	1.5	
Samanta	JAR	98	C	167	17.4		2.8	71.1		40.2	20.3		0.4				6.4	0.3		0.3	204			9.4	2.6
Samanta	JAR	98	AAT	385	36.0		3.6	102		46.5	30.8	16.2	4.9		0.7	117		1.5			16.8	0.2	7.1	1.4	
Samanta	JAR	99	C	385	17.7		6.4	96.9		28.6	12.0		0.4	1.9							306		12.1	2.9	
Samanta	JAR	99	AAT	485	18.8	1.2	9.3	95.2		30.3	7.3	2.5	4.5	1.8				2.5	0.2		343	0.3	2.1	5.9	3.9
Samanta	STA	98	C	528	31.2	0.8	1.9	89.1		114	60.3	1.2	1.4			13.3				1.5	271		18.4	4.9	
Samanta	STA	98	AAT	608	39.6	3.4	5.1	68.1		43.8	20.3	3.5	5.5		0.5	103					12.5	0.1	6.1	1.0	
Samanta	STA	99	C	313	18.3	0.1	5.8	54.9		21.3	4.9		3.4	2.2	0.3		3E-02		0.2		279		0.7	7.2	2.8
Samanta	STA	99	AAT	401		14.3	34.1	137		121	8.2	9.0	0.4	2.7					0.7		326			11.5	2.8
Šárka	CHR	98	AAT	292	18.9		44.6	158		24.4	25.2	4.6	6.6					0.6	2.1		255	2E-02	8.3	1.6	
Šárka	CHR	99	C	549	22.7	0.8	1.6	75.6		19.6	19.9		2.2	0.8					0.7		160		2.5	9.2	
Šárka	CHR	99	AAT	316	9.2		0.3	46.1		8.7	12.5	1.2	0.5			39.2			1.0		0.8		4.1	0.8	
Šárka	JAR	98	C	124	22.4		3.3	61.5		73.1	41.9		0.9			9.1			0.3	1.2	279		9.5	2.6	
Šárka	JAR	98	AAT	505	20.4		2.3	73.2		38.7	18.3	5.1	2.8		0.6	94.1		0.4	1.3		10.5	9E-02	4.9	1.0	
Šárka	JAR	99	C	274		3.7	14.6	62.9		33.0			2.1	0.8		23.5			0.3		390			7.8	
Šárka	JAR	99	AAT	539	109		1.6	44.3		20.1	23.9		2.1	0.5		104					0.9		3.7	0.8	
Šárka	STA	98	C	311	19.4		3.8	64.0		15.3	24.8		0.5	1.5					0.5		325		2.4	9.1	
Šárka	STA	98	AAT	466	29.8		4.4	66.8		10.5	24.1	3.5	1.2		0.6	95.8			1.0		12.6	3E-02	4.4	0.8	
Šárka	STA	99	C	256	21.9	0.6	2.5	62.5		20.5	23.4	3.3	1.8	0.8					0.2		236		1.5	5.6	
Šárka	STA	99	AAT	381	42.4	2.7	4.0	62.0		80.8	21.2		6.2	0.7		86.5			0.6		3.2		6.6	1.2	

CHR – Chrlice, JAR – Jaroměřice nad Rokytkou, STA – Staňkov
C – control, AAT – accelerated ageing test

Table 4. Results of multiple range variance analysis of the sum of phenolcarboxylic acid contents

Source of variability	Sum of squares	*d.f.*	Variance	*F*-test	*P*-value
		Main effects			
A – cultivar	301049.00	4	75262.30	5.61	0.0022**
B – treatment	62837.10	1	62837.10	4.68	0.0398*
C – year	5700.23	1	5700.23	0.42	0.5203
D – site	280780.00	2	140390.00	10.46	0.0005**
		Interaction			
AB	95516.50	4	23879.10	1.78	0.1632
AC	127727.00	4	31931.70	2.38	0.0777
AD	239935.00	8	29991.90	2.24	0.0579
BC	761.87	1	761.87	0.06	0.8135
BD	29790.50	2	14895.30	1.11	0.3446
CD	140100.00	2	70050.00	5.22	0.0124*
Inner (residual)	348858.00	26	13417.60		
Total	1615760.00	55			

* statistically significant influence ($P \leq 0.05$) on the sum of all determined phenolcarboxylic acids
** statistically highly significant influence ($P \leq 0.01$) on the sum of all determined phenolcarboxylic acids

cultivar, seed size and other factors (Weidner and Paprocka 1996, Weidner et al. 1996, 1999). Decrease of free phenolcarboxylic acids depends on storage conditions – humidity, temperature and oxygen access (Beweley and Black 1985), factors that were used in accelerated ageing test. At the same time the level of free phenolic acids in live plant cells regarding their potential toxicity is strictly regulated (Harborne 1980). Weidner et al. (1993) showed positive correlation between the levels of dormancy and phenolic content in developing and ripening corn grains supports hypothesis about the role of phenolic acids in dormancy and germination of corn grains control. The obtained results are in accordance with previous data of Lachman et al. (1999) on the isoflavonoid levels in barley grains and pea seeds influenced by their deterioration.

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ABSTRAKT

Vliv urychleného stárnutí na obsah a složení polyfenolického komplexu obilek pšenice (*Triticum aestivum* L.)

Byl studován vliv urychleného stárnutí (vyšší teploty a vlhkosti) na obsah a složení fenolických látek v obilkách pěti odrůd pšenice (*Triticum aestivum* L.). Byly stanoveny obsahy celkových polyfenolů spektrofotometricky s fenolovým Folin-Ciocalteuovým reagens a volných fenolických kyselin pomocí vysokoúčinné kapalinové chromatografie v kontrolních vzorcích a po ošetření testem urychleného stárnutí (AAT) v odrůdách Ebi, Estica, Nela, Samanta a Šárka. Byl zjištěn statisticky významný vliv odrůdy, lokality, ročníku a deteorace (AAT). Obsah celkových polyfenolů se zvyšoval po deteoraci a obsah jednotlivých fenolických kyselin se měnil v závislosti na struktuře kyseliny. Průměrný obsah celkových polyfenolů v letech 1998 a 1999 se pohyboval v rozmezí od 600 do 900 mg/kg sušiny. Nárůst způsobený AAT byl 0–20 %, nejvyšší byl u odrůdy Nela (průměrně o 19 %). Jako hlavní fenolické kyseliny a kumariny byly stanoveny sinapová kyselina (maximum 540 mg/kg sušiny), 3-hydroxybenzoová kyselina a 7-hydroxykumarin. Po ošetření testem urychleného stárnutí (AAT) byl pozorován pokles volných fenolických kyselin obsahujících methoxylové skupiny (kyseliny sinapová a vanilová) a nárůst fenolických kyselin obsahujících volné hydroxylové skupiny (kyseliny kávová a gallová). Byl zjištěn statisticky významný vliv testu urychleného stárnutí na hladině významnosti $P \leq 0,05$ na obsah volných fenolických kyselin.

Klíčová slova: *Triticum aestivum* L.; pšeničná obilka; polyfenoly; fenolické kyseliny; deteorace; test urychleného stárnutí; vliv vyšší teploty a vlhkosti

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