Phenolic Compounds of Seed Coats of White and Coloured Varieties of Pea (*Pisum sativum* L.) and Their Total Antioxidant Activity

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Abstract

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The purpose of this study was to compare the composition and contents of phenolic acids and condensed tannin in the seed coats of white and coloured varieties of pea and to examine the antioxidant properties of methanol and acetone extracts containing these phenolic compounds. The contents of phenolic acids were quantified by the HPLC analysis. The sum of free phenolic acids, those liberated from soluble esters and those liberated from soluble glycosides, was higher for coloured seed coat (78.53 g per g dry matter) than for the white seed coat (17.17 g/g dry matter). Protocatechuic, gentisic and vanillic acids were found dominant in the coloured seed coat, while ferulic and coumaric acids in the white seed coat. The content of condensed tannins was 1560 mg of catechin equivalent/100 g of coloured seed coat as determined by a vanillin assay. No condensed tannins were detected in the white seed coat. The antioxidant activity of extracts was measured by the oxidation of phosphatidylcholine to hydroxyperoxidephosphatidyl choline in the liposome system. Strong antioxidant properties were observed in a crude tannin extract from the coloured seed coat. These properties were slightly changed after the seed coat was cooked in water for 30, 60 and 90 min.

Keywords: *Pisum sativum* L.; seed coat; phenolic acids; condensed tannins; antioxidant activity

Legume seeds are rich in many nutrient components including protein, starch, dietary fibre, certain fatty acids and micronutrients (vitamins, trace minerals). They are also a rich source of many bioactive non-nutrient compounds including phenolic antioxidants (ANDERSON *et al*. 1999; MESSINA 1999; SHAHIDI & NACZK 1995). Proanthocyanidines (i.e. condensed tannins) are the predominant phenolic compounds found in legume seeds. They are located mainly in the seed coats (hulls) and play an important role in the defence system of seeds that are exposed to oxidative damage by many environmental factors such as light, oxygen, free radicals and metal ions. The antioxidant activity of tannins from "tannin-rich" medicinal plants (OKUDA 1993; OKUDA *et al*. 1991), green tea (MURAMATSU *et al*. 1986; YEN & CHEN 1995), grape seed (TEBIB *et al*. 1994a, b, 1997), buckwheat hulls (WATANABE *et al*. 1997), rapeseed and canola hulls (AMAROWICZ *et al*. 2000) has been reported. They are known to prevent lipid oxidation as reducing agents, free-radical scavengers and chelators of pro-oxidant catalytic metals. HAGERMAN *et al*. (1998) reported that tannins were 15–30 times more effective in the quenching of peroxyl radicals than simple phenolics. Therefore tannins are considered as important natural antioxidants and can be an alternative to synthetic antioxidants used in food systems that have been implicated in the promotion of carcinogenesis (ITO *et al*. 1996).

The antioxidant properties of tannins present in legume seeds, unlike those of tannins mentioned above, have not been well known yet. Our study indicated that the extracts obtained from some species of legumes with coloured seed coat: pea (*Pisum sativum* L.), faba bean (*Vicia faba* var. *minor* Harz.), lentil (*Lens culinaris* Medik.) and broad bean (*Vicia faba* var. *major* Harz.) were characterized by a high antioxidant activity measured by the oxidation rate of phosphatidyl choline (PC) in the liposome system, whereas with white coat seeds: bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.) and everlasting pea (*Lathyrus sativus* L.) they were distinctly less active (TROSZYŃSKA *et al*. 2001a). There are variations in the content of tannins in legumes depending on the colour of seed coats (ELIAS *et al*. 1979). The white varieties of

legumes contain usually tannins in lower concentrations than red, black or bronze seed coats. We infer from these results that tannins present in the seed coat can be responsible for the antioxidant activity of legume seeds. Legumes also contain phenolic acids (hydroxybenzoic and hydroxycinnamic acids) (BARTOLOME *et al.* 1997; SHAHIDI & NACZK 1995; SOSULSKI & DĄBROWSKI 1984) which, similarly like condensed tannins, may contribute to the antioxidant activity of seeds; however, this phenomenon still remains undiscovered.

Pea (*Pisum sativum* L.) is among the major legumes grown in almost all countries of the world (SALUNKHE & KADAM 1999). In various countries, including Poland, seeds of pea are dehulled. Thus, the use of seed coats after dehulling as a source of natural antioxidants may be one of the ways of their utilization.

The objectives of this study were: (a) to compare the composition of phenolic acids, including free, esterified and glycosylated forms and condensed tannin contents in the seed coats of white and coloured varieties of pea, (b) to evaluate the total antioxidant activity of extracts containing these phenolic compounds by means of the phosphatidylcholine (PC) liposome system as an experimental model. The heat stability of antioxidants will also be the subject of investigations.

MATERIALS AND METHODS

Reagents: Vanillin (Merck), (+)-catechin (Sigma), 4-(dimethylamino)cinnamaldehyde (Merck), all standards of phenolic acids (Sigma and Merck), L-α-phosphatidylcholine (type III-E, PC; Sigma), diethylenetriaminepentoacetic acid (DTPA; Sigma), tris(hydroxymethyl)aminomethate (Sigma), 2,6-di-*tert*-butyl-4-methylphenol (BHT; Aldrich Chem. Co), 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH; Wako Pure Chemical Industries, Ltd., Osaka, Japan). All other reagents of analytical reagent-grade, and of HPLC grade were purchased from POCh (Gliwice, Poland).

Plant material: Pea (*Pisum sativum* L.) of Kwestor variety with white seed coat and of Fidelia variety with coloured seed coat were grown in the same environmental conditions at the Wiatrowo National Agriculture Experiment Station. The seed coats were separated from the cotyledons by cracking the seeds, sieving and manual cleaning. Clean seed coats were stored in airtight containers for further analysis. The pure seed coats were finely ground, extracted with hexane for 12 h using a Soxhlet apparatus, and dried at a room temperature.

Extraction and analysis of phenolic acids: The white and coloured varieties of seed coats were extracted with 80% aqueous methanol for 30 min at the ratio of 1:10 (w/v) in a shaking incubator. Solids were separated by centrifugation at 4000 rpm for 15 min and extracted twice longer with the same solution. The combined filtrates were evaporated under vacuum at 40°C to remove the organic solvent, and then the aqueous solutions were lyophilized.

Phenolic acids (i.e. free and those liberated from soluble esters and from soluble glycosides) were isolated from methanol extract according to the previously described method (KRYGIER *et al*. 1982; WEIDNER *et al*. 1999). An aqueous suspension of the extract (0.5 g in 20 ml water) was adjusted to pH 2 (6M HCl), and free phenolic acids were extracted 3 times into 20 ml diethyl ether using a separating funnel. The ether extract was evaporated to dryness under vacuum at a room temperature. The dry residue was stored for the HPLC analysis of free phenolic acids. The aqueous solution left after extraction of free phenolic acids was neutralized and evaporated to dryness. The dry residue was dissolved in 20 ml of 2M NaOH and hydrolyzed for 4 h under nitrogen atmosphere at a room temperature. Phenolic acids released from soluble esters were extracted 3 times from the hydrolyzate into 30 ml diethyl ether after acidification to pH 2, using a separating funnel. The dry residue was stored for the HPLC analysis of phenolic acids liberated from esters. Aqueous solution was supplemented with 15 ml of 6M HCl and subsequently placed under nitrogen atmosphere and hydrolyzed for 1 h in a water bath at 100°C. Phenolic acids released from soluble glycosides were separated from the hydrolyzate 3 times into 45 ml diethyl ether. After ether evaporation, the dry residue was stored for the HPLC analysis of phenolic acids liberated from glycosides. The samples obtained in the above-mentioned manner were redissolved in 10 ml methanol and filtered through a nylon filter (0.45 µm) before the HPLC analysis. Phenolic acids were analysed by the HPLC method using a Shimadzu system (Japan), including a 290 nm-fixed UV SPD-10 A detector, LC-10 AD pump and C-R 6A recorder. A 25 µl sample was injected into an HPLC system. Separation was performed with a LiChrospher 100 RP-18 column (5 µm, 4 × 250 mm) and a water/acetonitrile/acetic acid mixture (88:10:2, v/v/v) as a mobile phase. Phenolic acids were identified and quantified by comparison with authentic compounds and expressed in µg/g dry matter.

Extraction and analysis of soluble tannins: Condensed tannins were extracted from seed coats of white and coloured varieties with 70% aqueous acetone for 30 min at the ratio of 1:10 (w/v) in a shaking incubator. After centrifugation, the residues were extracted twice with the same solvent system. The combined filtrates were evaporated under the vacuum and the aqueous solutions were lyophilized. In the obtained samples, condensed tannins were assayed colorimetrically according to the modified vanillin method of PRICE *et al*. (1978) and the 4-(dimethylamino)cinnamaldehyde method as described by NACZK *et al*. (1994). These methods were also used for the determination of tannin content in extracts obtained with 80% aqueous methanol.

Effect of cooking on antioxidant activity: Coloured seed coats were cooked in water for 30, 60 and 90 min at the water ratio of 1:10 (w/v), and then homogenized (together with water) and lyophilized. Phenolic compounds from lyophilizates were extracted with 80% aqueous methanol and 70% aqueous acetone (in the manner described above), and then the antioxidant properties of the obtained extracts were determined.

Total antioxidant activity of extracts: The antioxidant activity of all methanol and acetone extracts obtained from the seed coats of white and coloured pea was evaluated by the liposome method according to TERAO *et al*. (1994). An aliquot (50 µl) of extracts in methanol (2.5 mg/ml) was added to the solution (0.7ml) of peroxide-free egg-yolk PC dissolved in chloroform. After removing the solvents in a stream of nitrogen followed by the vacuum, the residue was dissolved in (0.7ml) Tris-HCl buffer (0.01 M, pH 7.4) containing 0.5 mM diethylenetriaminepentoacetic acid (DTPA), vortexed and exposed to ultrasonic waves for 30 s. Liposomes were standardized by extruding the sample (0.6 ml) 21 times in a Liposo Fast-Basic (Avestin, Canada) apparatus with a polycarbonate membrane of 100 nm pore size. The liposomes (0.5 ml) were suspended in 0.5 ml Tris-HCl buffer and placed in a light-protected shaker water bath at 37°C. After 5 min 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was added to the sample as an initiator of radicals. The amount of phosphatidylcholine peroxides (PC-OOH) was analyzed during 5 h of incubation at 72min intervals. Then it was determined at 235 nm by the HPLC method using the Shimadzu system, C8 Nova Pak column and methanol-water mixture (96:4 v/v) as a mobile phase. The amount of peroxides was calculated from the standard curve prepared with PC-OOH according to the method described by TERAO *et al*. (1985).

RESULTS AND DISCUSSION

The contents of phenolic acids in white and coloured seed coat varieties of pea are shown in Table 1. A typical HPLC chromatogram of phenolic acids released from ester bonds is shown in Fig. 1. The phenolic acids found in both investigated seed coats were protocatechuic, gentisic, vanillic, caffeic, syringic, *p*-coumaric, ferulic and *o*-coumaric. The concentration of phenolic acids in the seed coats depended on the variety. The sum of free phenolic acids, those liberated from soluble esters and those liberated from soluble glycosides, was higher for the coloured seed coat (78.53 g/g dry matter) than for the white seed coat (17.17 g/g dry matter). The benzoic acids protocatechuic, gentisic and vanillic were dominant phenolic acids detected in the coloured seed coat while in the white seed coat the hydroxycinnamic acids ferulic and coumaric were the most abundant phenolic acids. In both seed coats investigated, the phenolic acids were found in a free as well as ester-bound form. Only protocatechuic acid was found in a glycoside-bound form. The concentration of free phenolic acids was about six times higher (46.36 µg/g) in the coloured seed coat than in the white one (7.75 µg/g) while that of ester-bound acids was about three times higher (16.45 µg/g) in the coloured seed coat than in the white one (5.31 µg/g).

Generally, it can be pointed out that the contents of phenolic acids in seed coats were low. A comparison of our results with those from literature is difficult because the concentrations of phenolic acids in the seed coats of pea have not been sufficiently reported. SOSULSKI and DĄBROWSKI (1984) fractionated phenolic compounds of pea into free acids, soluble ester, and residue components followed by alkaline hydrolysis. The seed coats contained *p*-hydroxybenzoic, protocatechuic, syringic, gallic, *p*-cou-

Table 1. Concentration of phenolic acids (µg/g d.m.) in the seed coats of white and coloured varieties of pea

Phenolic acids	White seed coat form phenolic acids			Coloured seed coat form phenolic acids		
	Free	Ester bond	Glycoside bond	Free	Ester bond	Glycoside bond
Benzoic						
Protocatechuic	3.36	0.54	4.11	13.29	2.42	15.72
Gentisic	n.d.	0.23	n.d.	24.93	8.44	n.d.
Vanillic	n.d.	0.43	n.d.	5.44	1.13	n.d.
Syringic	0.82	1.23	n.d.	0.54	1.75	n.d.
Cinnamic						
Caffeic	0.35	n.d.	n.d.	0.55	n.d.	n.d.
Ferulic	1.13	1.64	n.d.	0.40	0.94	n.d.
p-Coumaric	1.32	0.69	n.d.	0.81	0.83	n.d.
o-Coumaric	0.77	0.55	n.d.	0.40	0.94	n.d.
Total	7.75	5.31	4.11	46.36	16.45	15.72

n.d. = below detection limit

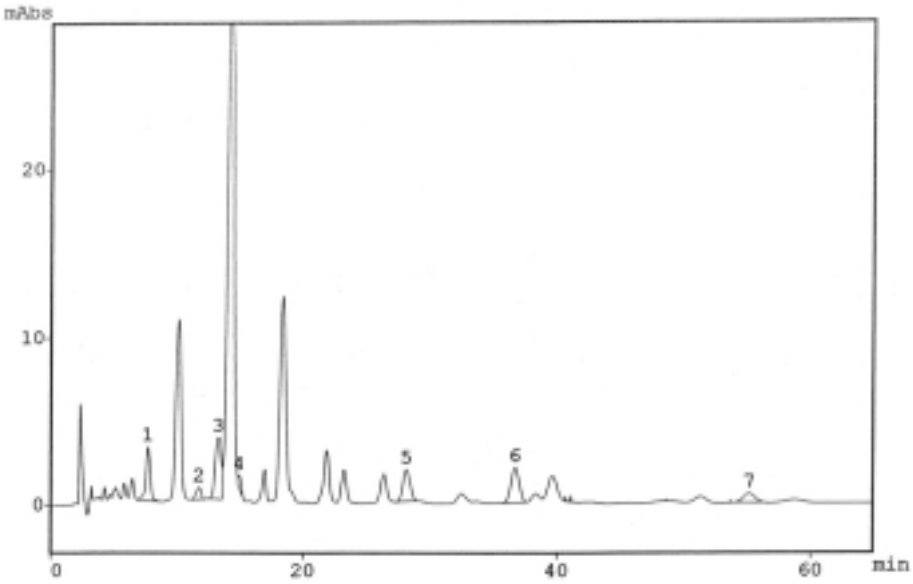

Fig. 1. Typical chromatogram (HPLC) of phenolic acids released from ester bonds

1 = Protocatechuic, 2 = Gentisic, 3 = Syringic, 4 = Vanillic, 5 = *p*-Coumaric, 6 = Ferulic, 7 = *o*-Coumaric

maric and ferulic acids in the soluble ester fraction and to a lesser extent in the insoluble residue analyzed by the capillary GLC.

The contents of condensed tannins in pea seed coats, as determined by the vanillin assay and 4-(dimethylamino) cinnamaldehyde (DC) reagent, are given in Table 2. Different reagents giving coloured reaction products are used for the determination of condensed tannins. The vanillin reagent is used most frequently due to its specificity for flavonols with which it possess a single bond at the 2,3-position of the pyran ring and free OH groups at positions 5 and 7 of the benzene ring (SHAHIDI & NACZK 1995; WATERMAN & MOLE 1994). The DC reagent is often recommended for the analysis of proanthocyanidins as a more sensitive reagent in comparison with vanillin (NACZK *et al*. 1994; SARKAR & HOWARTH 1976). The extracts obtained from the white seed coat did not give a coloured reaction with vanillin and DC reagent, which indicates that this seed coat did not contain any tannins. The contents of tannins in the coloured seed coat were expressed both as catechin equivalents per 100 g of sample and as absorbance values per gram. The content of tannins extracted with acetone as determined by the vanillin assay was 1560 mg of catechin equivalent per 100 g of seed coat, but when determined by the DC reagent it was 513 mg of catechin equivalent/100 g of seed coat. On the other hand, the absorbance values obtained using the DC reagent were 2.8 times higher (DA_{635} = 26.2) than those obtained using the vanillin reagent (DA_{500} = 9.31). Thus, due to its higher sensitivity to tannins, the DC reagent seemed to be more suitable than the vanillin reagent for the determination of tannins in the seed coat of pea. Similar results were obtained by NACZK *et al*. (1994), who determined the content of condensed tannins in canola hulls by the vanillin and DC reagent method. It can be explained by the fact that tannins isolated from plant materials are a mixture of oligo and polymeric compounds that differ in their sensitivity to the reagents used for their determination.

Table 2. Content of condensed tannins in seed coats of pea as determined by vanillin and DC assays

Sample	Vanillin assay		DC assay		Degree of polymerization[b]
	mg/100g of seed coat[a]	DA_{500}/g of seed coat	mg/100g of seed coat[a]	DA_{635}/g of seed coat	
Coloured seed coat					
Acetone extraction	1560 ± 48	9,31 ± 0,42	513 ± 5	26,2 ± 1,3	3,04 ± 0,02
Methanol extraction	682 ± 32	4,12 ± 0,23	364 ± 8	18,4 ± 0,6	1,87 ± 0,01
White seed coat					
Acetone extraction	–	n.d.	–	n.d.	
Methanol extraction	–	n.d.	–	n.d.	

[a] expressed as catechin equivalents; [b] vanillin assay/DC assay ratio; n.d. = below detection limit

The kind of solvent used for the extraction of phenolic compounds, including tannins, is very important and this issue is often discussed in literature (WATERMAN & MOLE 1994). Aqueous acetone is an excellent solvent for the extraction of condensed tannins although these compounds can also be extracted with methanol. The content of tannins extracted with methanol as determined by the vanillin assay was 682 mg of catechin equivalent per 100 g of seed coat, but when determined by the DC reagent it was 364 mg of catechin equivalent per 100 g of seed coat. The absorbance values obtained using the DC reagent were 4.5 times higher (DA_{635} = 18.4) than those obtained using the vanillin reagent (DA_{500} = 4.12). OSZMIAŃSKI and BOURZEIX (1996) showed that in the determination of tannin content as catechin equivalents, the vanillin reagent gives more correct values than the DC reagent. Their results proved that the determination of tannins with the DC reagent was dependent on the degree of polymerization. The oligomer fractions of tannins expressed by catechin equivalents yielded about twice higher values with the vanillin reagent than with the DC reagent and over 5 times higher for the polymer fractions. These authors suggest that the ratio of values obtained with vanillin and DC can indicate an approximate degree of tannin polymerization. The degree of polymerization calculated in this way reached 3.04 for the acetone extract and 1.87 for the methanol extract (Table 2). It indicated that condensed tannins of the seed coat can differ in molecular weight.

The acetone and methanol extracts from white and coloured seed coats of pea were used as antioxidants for the oxidation of PC to PC-OOH in the liposome system. Numerous methods and their modifications have been proposed for evaluation of the antioxidant activity of potential antioxidants. The liposome method is often used as a model for studying the antioxidant activity *in vitro* because liposomes can be related to the lamellar structures of biological membranes found *in vivo* (CHATTERJEE & AGARWAL 1988). In our experiment, AAPH – 2,2'-azobis(2-amidinopropane) dihydrochloride – was used to initiate peroxidation. This azo compound has frequently been used as an initiator of lipid peroxidation and the pathway of lipid peroxidation in liposomal membranes was well defined (NIKI 1987). The final concentration of AAPH in the liposome system was 10 mM according to TERAO *et al*. (1994). The final concentration of the samples investigated in this system was 119 µg/ml. The results of the antioxidant properties of extracts mea-

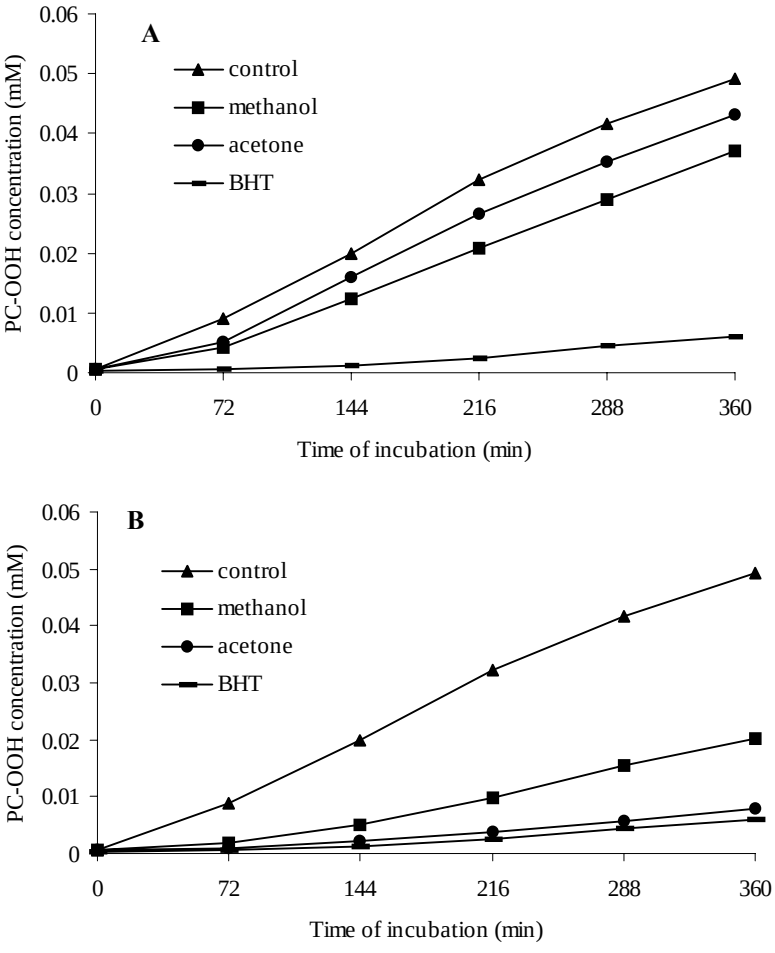

Fig. 2. Time-related changes in peroxide content (PC-OOH) during incubation of phosphatidylcholine with methanol and acetone extracts of white (A) and coloured (B) seed coats of pea. Extract concentration in the liposome system was 119 µg/ml. AAPH (10mM) was used as an oxidant

sured by the PC-OOH concentration in the liposome system are presented in Fig. 2. In general, the oxidative activity of PC was inhibited by all the extracts compared with the control assay. The control did not contain any added antioxidants. The strongest antioxidant properties were revealed by the acetone extract from the coloured seed coats. The effect of the remaining extracts on the peroxidation of PC was lower than that of those mentioned above and decreased in the order: methanol extract from coloured seed coat > methanol extract from white seed coat > acetone extract from white seed coat. These results clearly indicate that crude tannin extract from the coloured seed coat exhibited strong antioxidant activity at the concentration analyzed under AAPH-induced lipid peroxidation in the PC liposome system. Methanol extract from the coloured seed coat contained phenolic acids and tannins, however its activity was much lower if compared with crude tannin acetone extract. It can be explained by the fact that the biological activity of phenolic compounds, including antioxidant activity, is strictly connected with their chemical structure (RICE-EVANS *et al*. 1996). Our results indicated that tannins present in methanol and acetone extracts were characterized by a different degree of polymerization; therefore they could also exhibit different activities. The antioxidant activity of tannin-free extracts from the white seed coat can be related with phenolic acids, especially with cinnamic acids (ferulic, caffeic and coumaric), which are very potent and interesting antioxidants. Their antioxidant capacity appears to be mediated through their ability to react with free radicals and so to break the chain propagation cycle associated with peroxidation reactions. However the contents of phenolic acids in the investigated seed coats were low, ranging from 0.23 to 3.36 µg/g. Apart from phenolic acids, pea seeds also contain other non-tannin antioxidants, e.g. kaempferol, quercetin (MENDEZ & LOJO 1971), which may affect the antioxidant capacity of extracts under investigation. However, there is a lack of information whether these compounds occur in seed coats.

We also used BHT as a reference standard in this study because it has a phenolic structure and was used in various food systems (AMAROWICZ *et al*. 2000). The results (Fig. 2) indicate that the concentration of PC-OOH and the time of oxidation inhibition were similar for acetone extract from the coloured seed coat and the results obtained in BHT assays. It should be mentioned, however, that BHT is a pure compound and the investigated extracts contained various phenolic compounds.

Depending on the quantitative and qualitative composition of antioxidants, pea varieties (similarly like the varieties of other legume seeds) can display different antioxidant activity. The following model used in the study: a tannin-free and a tannin-containing variety of pea, can be considered as an experimental model that makes it possible to confirm previous assumptions (TROSZYŃSKA *et al*. 2001b) that condensed tannins located in the seed coats can be the main antioxidants of pea.

Food is subjected to different technological and culinary processes before consumption, and for this reason the effect of cooking on antioxidants present in the coloured seed coat was also determined (Figs. 3 and 4). It was found that methanol extracts obtained from seed coats cooked for 30 and 60 exhibited a slight antioxidant activity at the concentration analyzed under AAPH-induced lipid peroxidation in the liposome system. The concentration of PC-OOH and the time of oxidation inhibition were similar for the sample cooked for 90 min and the results obtained in the control assay (not containing antioxidants) whereas acetone extracts displayed strong antioxidant properties. The activity of samples cooked for 30, 60 and 90 min was similar to that of the uncooked sample. It indicated that the compounds extracted from the coloured seed coat with aqueous acetone were heat-stable antioxidants.

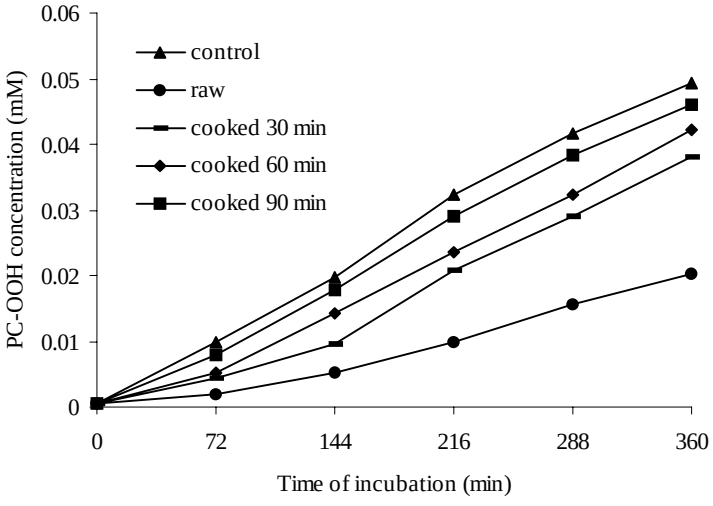

Fig. 3. Time-related changes in peroxide content (PC-OOH) during incubation of phosphatidylcholine with methanol extracts from coloured seed coats cooked for 30, 60, and 90 min. Extract concentration in the liposome system was 119 µg/ml. AAPH (10mM) was used as an oxidant

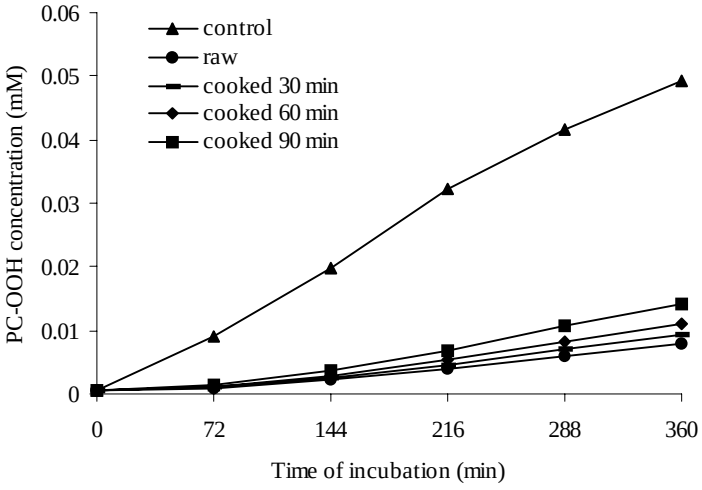

Fig. 4. Time-related changes in peroxide content (PC-OOH) during incubation of phosphatidylcholine with acetone extracts from coloured seed coats cooked for 30, 60, and 90 min. Extract concentration in the liposome system was 119 µg/ml. AAPH (10mM) was used as an oxidant

CONCLUSION

In conclusion, we found that the crude tannin acetone extract of coloured pea exhibited a pronounced antioxidant activity. These properties were slightly changed after the seed coat was cooked in water for 30, 60, and 90 min. The results indicate that condensed tannins occurring in the coloured seed coat of pea can be considered as heat-stable natural antioxidants which can be effectively employed in food systems. Further investigations are needed to characterize these heat-stable antioxidants.

References

AMAROWICZ R., NACZK M., SHAHIDI F. (2000): Antioxidant activity of crude tannins of canola and rapeseed hulls. J. Am. Oil Chem. Soc., **77**: 957–961.

ANDERSON J.W., SMITH B.M, WASHNOCK C.S. (1999): Cardiovascular and renal benefits of dry bean and soybean intake. J. Clin. Nutr., **70**: 464S–474S.

BARTOLOME B., ESTRELLA I., HERNANDEZ T. (1997): Changes in phenolic compounds in lentils (*Lens culinaris*) during germination and fermentation. Z. Lebensm.-Unters. u. Forsch., A **205**: 290–294,

CHATTERJEE S.N, AGARWAL S. (1988): Liposomes as membrane model for study of lipid peroxidation. Free Rad. Biol. Med., **4**: 51–72.

ELIAS L.G, FERNANDEZ D.G., BRESSANI R. (1979): Possible effects of seed coat polyphenolics on the nutritional quality of bean protein. J. Food Sci., **44**: 524–531.

HAGERMAN A.E., RIEDL K.M., JONES A., SOVIK K.N., RITCHARD N.T., HARTZFELD P.W., RIECHEL T.L. (1998): High molecular weight plant polyphenolics (tannins) as antioxidants. J. Agric. Food Chem., **46**: 1887–1892.

ITO N., HIROSE M., FUKUSHIMA S., TSUDA H., SHIRAI T., TATEMATSU M. (1996): Studies on antioxidants: their anticarcinogenic and modifying effects on chemical carcinogenesis. Food Chem. Toxicol., **24**: 1099–11022.

KRYGIER K., SOSULSKI F.W., HOGGE L. (1982): Free, esterified and insoluble phenolic acids. 2. Composition of phenolic acids in rapeseed flour and hulls. J. Agric. Food Chem., **30**: 330–334.

MENDEZ J., LOJO M.I. (1971): Phenolic and indole constituents of edible peas. J. Food Sci., **36**: 871–872.

MESSINA M.J. (1999): Legume and soybeans: overview of their nutritional profiles and health effects. J. Clin. Nutr., **70** (Suppl.): 439S–449S.

MURAMATSU K., FUKUYO M., HARA Y. (1986): Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. J. Nutr. Sci. Vitaminol., **32**: 613–632.

NACZK M., NICHOLS T., PINK D., SOSULSKI F. (1994): Condensed tannins in canola hulls. J. Agric. Food Chem., **42**: 2196–2200.

NIKI E. (1987): Antioxidants in relation to lipid peroxidation. Chem. Phys. Lipids, **44**: 227–253.

OKUDA T. (1993): Natural polyphenols as antioxidants and their potential use in cancer prevention. In: SCALBERT A. (ed.): Polyphenolic Phenomena. INRA, Paris: 222–235.

OKUDA T., YOSHIDA T., HATANO T. (1991): Chemistry and biological activity of tannins in medicinal plants. In: WAGNER H., FARNSWORTH N.R. (eds): Economic and Medicinal Plant Research. Acad. Press, London: 129–165.

OSZMIAŃSKI J., BOURZEIX M. (1996): Comparison of methods for determining the content and polymerization of proanthocyanidins and catechins. Pol. J. Food Nutr. Sci., **2**: 43–50.

PRICE M.L., VAN SCOYOC S., BUTLER L.G. (1978): A critical evaluation of the vanillin reaction as an assay for tannins in sorghum grain. J. Agric. Food Chem., **26**: 1214–1218.

RICE-EVANS C., MILLER N.J., PAGANDA G. (1996): Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med., **20**: 933–956.

SALUNKHE D.K., KADAM S.S. (1999): CRC Handbook of World Food Legumes: Nutritional Chemistry. In: Proc. Technology and Utilization. CRC Press, Boca Raton FL: 215–251.

SARKAR S.K., HOWARTH R.E. (1976): Specificity of the vanillin test for flavanols. J. Agric. Food Chem., **24**: 317–320.

SHAHIDI F., NACZK M. (1995): Phenolic compounds in cereals and legumes. In: Food Phenolics: Sources, Chemistry, Effects, Applications. Technomic Publ. Co. Inc., Lancaster PA: 13–18.

SOSULSKI F.W., DĄBROWSKI K.J. (1984): Composition of free and hydrolysable phenolic acids in the flours and hulls of ten legume species. J. Agric. Food Chem., **32**: 131–136.

TEBIB K., BESANCON P., ROUANET J.M. (1994b): Dietary grape seed tannins affect lipoproteins, lipoprotein lipases and tissue lipids in rats fed hypercholesterolemic diets. J. Nutr., **124**: 2451–2457.

TEBIB K., BITRI L., BESANCON P., ROUANET J.M. (1994a): Polymeric grape seed tannins prevent plasma cholesterol changes in high cholesterol-fed rats. Food Chem., **49**: 403–406.

TEBIB K., ROUANET J.M, BESANCON P. (1997): Antioxidant effects of dietary polymeric grape seed tannins in tissue of rats fed a high cholesterol-vitamin E-deficient diet. Food Chem., **59**: 135–141.

TERAO J., ASANO I., MATSUHITA S. (1985): Preparation of hydroperoxy and hydroxy derivatives of rat liver phosphatidyl choline and phosphotidylethanolamine. Lipids, **20**: 312–331.

TERAO J., PISKUIA M., YAO Q. (1994): Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch. Biochem. Biophys., **308**: 278–284.

TROSZYŃSKA A, HONKE J, KOZŁOWSKA H. (2001a): Antioxidant properties of legumes. In: AMADO R., LAIRON D., GERBER M., MAIANI G. (eds): Cost 916 Action on Bioactive Plant Cell Wall Components in Nutrition and Health. Eur. Commun., Luxembourg: 59–61.

TROSZYŃSKA A., ESTRELLA I., LOPEZ-AMOROS M., HERNANDEZ T. (2001b): Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. Lebensm.-Wiss. u.-Technol., (in press).

WATANABE M., OHSHITA Y., TSUSHIDA T. (1997): Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Möench.) hulls. J. Agric. Food Chem., **45**: 1039–1044.

WATERMAN P.G., MOLE S. (1994): Method in Ecology. Analysis of Phenolic Plant Metabolites. Blackwell Sci. Publ., London: 66–103.

WEIDNER S., AMAROWICZ R., KARAMAĆ M., DĄBROWSKI G. (1999): Phenolic acids in caryopses of two cultivars of wheat, rye and triticale that display different resistance to preharvest sprouting. Eur. Food Res. Technol., **210**: 109–113.

YEN G.C., CHEN H.Y. (1995): Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Sci. Food Agric., **67**: 415–420.

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Souhrn

TROSZYŃSKA A., CISKA E. (2002): **Fenolové látky v osemení bílých a barevných odrůd hrachu (***Pisum sativum* L.**) a jejich celková antioxidační aktivita**. Czech J. Food Sci., **20**: 15–22.

Porovnávali jsme složení a obsah fenolových kyselin a kondenzovaných taninů v osemení u bílých a barevných odrůd hrachu a zjišťovali antioxidační vlastnosti extraktů získaných pomocí metanolu a acetonu, které obsahují fenolové látky. Obsah fenolových kyselin byl stanoven analýzou HPLC. Hodnota sumy volných fenolových kyselin, fenolových kyselin uvolněných z rozpustných esterů a z rozpustných glykosidů byla vyšší u barevných osemení (78,53 g/g sušiny) než u bílých (17,17 g/g sušiny). Převládajícími kyselinami v barevném osemení byly kyselina protokatechová, gentisinová a vanilová, zatímco kyselina ferulová a kumarová byly nejhojněji zastoupeny v bílém osemení. Obsah kondenzovaných taninů stanovený vanilinovým testem dosahoval 1560 mg katechinového ekvivalentu na 100 g barevného osemení. V bílém osemení jsme taniny nezjistili. Antioxidační aktivitu extraktů jsme měřili v liposomovém systému na základě oxidace fosfatidyl cholinu na hydroxyperoxidfosfatidyl cholin. U extraktu surového taninu z barevného osemení jsme zjistili silné antioxidační vlastnosti, které se mírně změnily po varu osemení ve vodě po dobu 30, 60 a 90 minut.

Klíčová slova: *Pisum sativum* L.; osemení; fenolové kyseliny; kondenzované taniny; antioxidační aktivita

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