POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, SI 1, pp. 147–150

ANTIOXIDATIVE POTENTIAL OF LENTIL SEED COAT TOWARDS LIPOXYGENASE ACTIVITY AND β-CAROTENE OXIDATION

Ewa Kubicka, Agnieszka Troszyńska*

Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Legume seeds - apart from protein, carbohydrates, micronutrients - contain phytochemicals including phenolic compounds, which are mainly present in seed coats. The aim of this work was to determine the antioxidative properties of the lentil seed coat towards lipoxygenases activity and in β-carotene-linoleate system. It has been found that the total phenolics content in lentil seed coat makes up to 53.14 mg/g dry matter and flavanols (tannins) up to 35.92 mg/g dry matter. It was found that type-1 and type-2 lipoxygenases were effectively inhibited by phenolics extracted from lentil seed coat. Crude tannin extract from lentil seed coat also showed strong antioxidative properties in β-carotene-linoleate system. It can be concluded that phenolic constituents of lentil seed coats can counteract lipid peroxidation catalyzed by lipoxygenase and β-carotene oxidation and can be utilized as potent antioxidants.

INTRODUCTION

Legume seeds play an important role in human diet. They are recognized as a rich source of proteins, carbohydrates, micronutrients and phytochemicals, including high phenolic content. In legume seeds, the most abundant group of phenolic compounds are tannins. They are present mainly in seed coats and are considered to play a very important role in plant defensive system.

Recent interest in tannins has been focused on beneficial to health aspects. Tannins have been shown as potent inhibitors of tumorigenicity [Wang *et al.*, 1989]. It has been found that water extract obtained from coconut husk fiber rich in condensed tannins revealed antimicrobial activity against *Staphylococcus aureus* [Esquenazi *et al.*, 2002]. Tannins have been shown to have inhibitory effect - stronger than that exerted by synthetic inhibitors - on protease ex *Aspergillus* oryzae (PAO) *in vitro*, and thus it can be considered as a potent suppressor of PAO-induced inflammation and tail oedemization in mice [Sutiak *et al.*, 2002] as well suppression of the immune response in antigen challenged mice when fed with tannins has been observed (unpublished data). The beneficial effects to urinary tract health [Howell, 2002], lipid metabolism [Yugarani *et al.*, 1993] and insulin enhancing capacities have also been reported [Anderson & Polansky, 2002].

Tannins have been shown to be strong inhibitors of oxidative enzymes present in foodstuffs and many of these studies reported the inhibitory effect of especially phenolics on lipoxygenase activity. Lipoxygenase is an important enzyme in genesis of flavour of food. It can also contribute to lowering sensory and nutritional value of foods. Therefore a number of studies have been devoted to inactivation and inhibition of lipoxygenase in such products.

The aim of this work was to determine the antioxidative properties of the crude tannins extract obtained from lentil seed coat and their antioxidative effect measured towards lipoxygenase activity and in β-carotene-linoleate system.

MATERIAL AND METHODS

Material. Seeds of lentil (*Lens culinaris*) grown in the Podlasie region were investigated. Material was obtained from the Agricultural University in Lublin, Poland. The seed coat was separated from the seeds of lentil by cracking the seeds and sieving, and subsequently subjected to further analysis.

The chemical composition of lentil seed coat. Ash, protein and fat contents were determined according to the Official Methods of Analysis [1990]. The dietary fibre content (both soluble and insoluble fractions) was determined by the enzymatic assay according to Asp [1983]. Total phenolic compounds content was determined according to the method of Julkunen-Tiitto [1985]. Flavanols content was determined according to the method of Price *et al.* [1978].

Preparation of crude tannin extract. The crude tannin extract was prepared as previously described [Troszyńska *et al.*, 2002]. The separated seed coats were powdered and then subjected to extraction with 70% acetone at the ratio of 1:7 (w/v) in a shaking incubator for 30 min. After 30 min, the mixture was centrifuged, supernatant collected and pellets extracted twice more with the same solution. The resultant extract was evaporated under vacuum at 40°C and lyophilized. The extract obtained was analysed for total phenolics and flavanols contents according to the methods mentioned above.

*Author's address for correspondance: Ewa Kubicka, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima Street 10, PO Box 55, 10-747 Olsztyn 5, Poland; tel. (48 89) 523 46 88; fax: (48 89) 524 01 24; e-mail: ewa@pan.olsztyn.pl

Lipoxygenase extraction. Lipoxygenases were extracted from soybean with 0.05 mol/L phosphate buffer, pH 7.0, at the ratio of 1:20 (w/v) [Funk *et al.*, 1985; Fauconnier *et al.*, 1995]. The extraction was carried out for 1 h with mixing every 10 min at a temperature of 0-4°C. Then, the mixture was centrifuged (3 500 rpm) for 20 min. The obtained supernatant (enzymatic extract) was subjected to further analysis of lipoxygenases inhibition.

Lipoxygenase inhibition. The extract of lipoxygenases was incubated with crude tannins extract in concentration range from 0 to 5 mg for 15 min and then the enzyme activity was assayed.

Lipoxygenase activity. Determination of lipoxygenase activity was proceeded by the preparations of substrate and reaction mixture emulsion. Linoleic acid emulsion (substrate) was prepared according to the modified Surrey's method [1964] in nitrogen atmosphere from: 0.1 mL cis, cis-9,12-octadecadienoic acid, 2.5 mL phosphate buffer (pH 7.4), 0.12 mL Tween 20 and 0.32 mL 1.0 mol/L solution of NaOH. Volume of the prepared emulsion was filled up to 50 mL with phosphate buffer (pH 7.4).

The reaction mixture contained 2.5 mL of reaction buffer (phosphate buffer pH 7.0 while measuring the type-2 lipoxygenase activity and glycine buffer pH 9.0 while measuring the type-1 lipoxygenase activity), 0.09 mL of substrate emulsion and the addition of enzymatic extract incubated with phenolic compounds. Lipoxygenase activity was determined according to the Zimmerman & Vick's spectrophotometric method [Zimmerman & Vick, 1970] at a wavelength of 234 nm at 25°C.

β-Carotene oxidation. β-Carotene oxidation was determined according to the method of Miller [1971] in β-carotene-linoleate system. Emulsion (5 mL) was incubated with 4 mg crude tannin extract for 120 min. At regular intervals the absorbance was read at 470 nm.

RESULTS AND DISCUSSION

The chemical composition was presented in Table 1. It has been found that the main component of the seed coat is fibre with its insoluble fraction (Table 1). The seed coat is characterized by high content of total phenolic compounds. Flavanols (tannins) make up to 67.59% of total phenolics, which indicates that apart from tannins the seed coat also contains other phenolic compounds.

It was found that lipoxygenase activity was effectively inhibited by crude tannin extract from lentil seed coat (Figure 1). The total inhibition of type-1 and type-2 lipoxygenases was observed when they were incubated with 3 mg of phenolic compounds extract (Figure 1). The phenolics are known for their antioxidative properties and their capacity to inhibit autoxidation of linolenic acid is contributed to their radical scavenging activity (to form stable free radicals) [Okuda, 1993]. The phenolic compounds have been shown to effectively inhibit lipoxygenases in a number of studies [Kohoyama *et al.*, 1997; Khalyafa *et al.*, 1990; Tamagawa *et al.*, 1999] and their antioxidative potential have been considered as free radical traps. Their capacity to inhibit the oxidation of linolenic

TABLE1. Characteristics of lentil seed coat.

Component	Composition
Seed coat share in the whole seed weight [g/100 g dry matter]	7.8 ± 0.2
Basic chemical composition [g/100 g dry matter]:	
Crude protein	4.8 ± 0.1
Crude fat	0.1 ± 0.0
Ash	2.6 ± 0.1
Dietary fibre	
Total (TDF)	83.4
Soluble (SDF)	11.5
Insoluble (IDF)	71.9
Polyphenols [mg/g dry matter]:	
Total phenolics	53.10 ± 0.01
Flavanols	35.90 ± 0.01

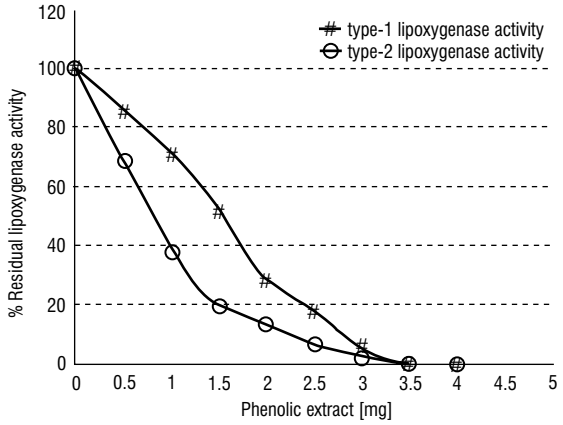

FIGURE 1. Inhibition of type-1 and type-2 lipoxygenase activity by phenolic extract from lentil seed coat.

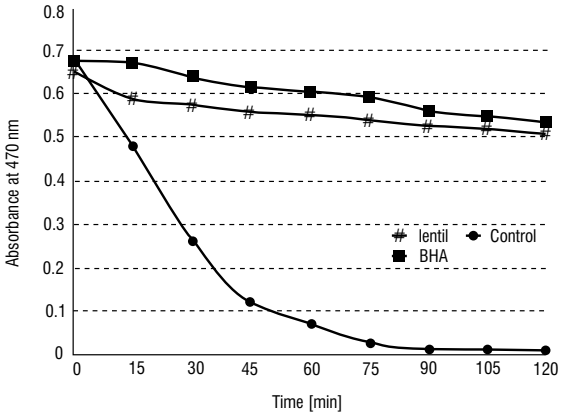

FIGURE 2. Antioxidative properties of phenolic extract from lentil seed coat in β-carotene-linoleate system.

acid in reaction catalysed by lipoxygenase could also be contributed to their non-specific binding to proteins, however as it has been found by Okuda *et al.* [1993] the inhibitory effect of polyphenols was not correlated with their protein binding capacity, thus it could be supposed that also other inhibitory mechanism is involved in the inhibition process. It has been found that curcumin, a polyphenolic phytochemical isolated from the powdered rhizome of the plant *Curcuma longa,* can act as a

lipoxygenase substrate. It can bind to lipoxygenase in a noncompetitive manner, thus inhibiting the lipid peroxidation. When it is trapped in the catalytic site of the enzyme it is photodegraded in the X-rays, forms the peroxy complex Enz-Fe-O-O-R as 4-hydroperoxy-2-methoxy-phenol, that later is converted into 2-methoxycyclohexa-2,5--diene-1,4-dione [Skrzypczak-Jankun, 2003].

Tannins extracted from lentil seed coat showed strong antioxidative properties in β-carotene-linoleate system (Figure 2). Their antioxidative potential was similar to that showed by BHT used as a reference standard in the experiment. After 60 and 120 min of incubation of β-carotene with seed coat extract, it was found that only 15-25% of β-carotene was oxidised. Carotenoids are highly susceptible to oxidative degradation and the nature of this degradation in foods appears to be complex. In the studies on oxidation of pigments the peroxy radicals mechanism is considered to be involved. It can also be partly contributed to lipoxygenases. There are two types of lipoxygenases distributed in plant kingdom: type-1 lipoxygenases (with pH optima of 9 and showing weak tendency to participate in cooxidation reactions) and type-2 lipoxygenases (with pH optima of 6.5 and showing strong tendency to participate in cooxidation reactions) [Grossman, 1969; Pourplanche *et al.*, 1993]. As type-1 lipoxygenases convert most peroxy radicals into hydroperoxides and type-2 lipoxygenases release the radicals more readily, the oxidation of pigments by lipoxygenases is considered to be mediated by the peroxy radicals. In our β-carotene-linoleate system the lentil seed coat phenolics have been proven to strongly inhibit the oxidation reaction, and their antioxidative role can be contributed to free peroxy radical scavenging activity.

Lentil seed coat phenolics have been shown to be effective inhibitors of lipoxygenase activity and β-carotene oxidation in β-carotene-linoleate system thus it appears that phenolics from lentil seed coat are characterised by strong antioxidative properties and their activity in inhibition of oxidative changes is complex and can be contributed to different mechanisms.

CONCLUSIONS

It can be concluded from the results obtained that phenolic constituents of lentil seed coats show strong antioxidative potential and can counteract lipid peroxidation catalyzed by lipoxygenases and β-carotene oxidation.

REFERENCES

1. Asp N., Johansson C., Hallmer H., Siljestrom M., Rapid enzymatic assay of insoluble and soluble dietary fiber. J. Agric. Food Chem., 1983, 31, 476–482.
2. Anderson R.A., Polansky M.M., Tea enhances insulin activity. J. Agric. Food Chem., 2002, 50, 7182–7186.
3. Esquenazi D., Wigg M.D., Miranda M.M.F.S., Rodrigues H.M., Tostes J.B.F., Rozental S., da Silva A.J.R., Alviano C.S., Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res. Microbiol., 2002, 153, 647–652.
4. Fauconnier M.L., Vanzeveren E., Marlier M., Lognay G., Wathelet J.P., Severin M., Assessment of lipoxygenase activity in seed extracts from 35 plant species. Grasas y Aceites, 1995, 46, 1, 6–10.
5. Funk M.O., Whitney M.A., Hausknecht E.C., O'Brien E.M., Resolution of isozymes of soybean lipoxygenase using isoelectric focusing and chromatofocusing. Anal. Biochem., 1985, 146, 246–251.
6. Grossman S., Enzymic oxidation of carotene and linoleate by alfalfa: extraction and separation of active fractions. Phytochem., 1969, 8, 2287–2293.
7. Howell A.B., Cranberry proanthocyanidins and the maintenance of urinary tract health. Crit. Rev. Food Sci. Nutr., 2002, 42, Suppl. S, 273–278.
8. Julkunen-Tiitto R., Phenolic constituents in the leaves of northern willows: Methods for analysis of certain phenolics. J. Agric. Food Chem., 1985, 33, 213–217.
9. Khalyafa A., Kermasha S., All I., Partial purification and characterization of lipoxygenase of canola seed (*Brassica napus* var. Westar). J. Agric. Food Chem., 1990, 38, 2003–2008.
10. Kohoyama N., Nagata T., Fujimoto S., Sekiya K., Inhibition of arachidonate lipoxygenase activities by 2-(3, 4-dihydroxyphenyl) ethanol, a phenolic compound from olives. Biosci. Biotech. Biochem., 1997, 61, 347–350.
11. Miller H.E., A simplified method for the evaluation of antioxidants. J. Amer. Oil Chem. Soc., 1971, 45, 91.
12. Official Methods of Analysis, 1990, (ed. K. Helrich), Association of Official Analytical Chemists, Inc. Arlington.
13. Okuda T., Natural polyphenols as antioxidants and their potential use in cancer prevention. 1993, *In:* Polyphenolic Phenomena. (ed. A. Scalbert). INRA, Paris, pp. 221–235.
14. Pourplanche C., Larreta Garde V., Thomas D., β-Carotene cooxidation by soybean lipoxygenase type 1 in non conventional media. Sciences des Alimentes, 1993, 13, 275–283.
15. Price H.L., Van Scoyoc S., Butler L.G., A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 1978, 26, 1214–1218.
16. Skrzypczak-Jankun E., Zhou K.J., McCabe N.P., Selman S.H., Jankun J., Structure of curcumin in complex with lipoxygenase and its significance in cancer. Int. J. Mol. Med., 2003, 12, 17–24.
17. Surrey K., Spectrophotometric method for determination of lipoxydase activity. Plant Physiol., 1964, 30, 65–70.
18. Sutiak V., Korenek M., Sutiakova I., Korenekova B., Sobekova A., Vasko L., Neuschl J., The induction of inflammation in mouse tails with *Aspergillus oryzae* protease and the inhibitory effects of synthetic and natural substances *in vitro*. Acta Veterinaria-Beograd, 2002, 52, 345–353.
19. Tamagawa K., Iizuka S., Ikeda A., Koike H., Naganuma K., Komiyama Y., Inhibitory effects of proanthocyanidins isolated from barley bran on hyaluronidase activity, soybean lipoxygenase activity and complementary activity. J. Jpn. Soc. Food Sci., 1999, 46, 521–527.
20. Troszyńska A., Estrella I., Lopez-Amores M.L., Hernandez T., Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. Lebensm.- Wiss. u.-Technol., 2002, 35, 158–164.

21. Wang Z.Y., Khan W.A., Bickers D.R., Mukhtar H., Protection against polycyclic aromatic hydrocarbon--induced skin tumor initiation in mice by green tea polyphenols. Carcinogenesis, 1989, 10, 411–425.
22. Yugarani T., Tan B.K.H., Das N.P., The effects of tannic acid on serum lipid parameters and tissue lipid peroxides in the spontaneously hypertensive and Kyoto rats. Planta Medica, 1993, 59, 28–35.
23. Zimmerman N.D., Vick B.A., Hydroperoxide isomerase. A new enzyme in lipid metabolism. Plant Physiol., 1970, 46, 445–453.