Food Chemistry 116 (2009) 906–910

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Characterisation of lipoxygenase from pea seeds (*Pisum sativum* var. *Telephone* L.)

Urszula Szymanowska, Anna Jakubczyk, Barbara Baraniak *, Agnieszka Kur

Department of Biochemistry and Food Chemistry, University of Life Sciences, ul. Skromna 8, 20-704 Lublin, Poland

ARTICLE INFO

Article history:
Received 7 October 2008
Received in revised form 10 March 2009
Accepted 11 March 2009

Keywords:
Lipoxygenase
Pisum sativum
Purification
Characterisation
Linoleic acid

ABSTRACT

Lipoxygenase (LOX) from pea seeds (*Pisum sativum* var. *Telephone* L.) was extracted and studied of biochemical properties. The molecular mass of purified lipoxygenase was 93 kDa. The effects of substrate specificity, pH, and sensibility to various inhibitors: caffeic acid, ferulic acid, benzoic acid, catechin, quercetin and kaempferol of LOX were investigated. Lipoxygenase showed the highest activity toward linoleic acid and the lowest toward oleic acid as substrates. Kinetic studies indicated that V_{max} of the LOX activity was 151.5 U/min and corresponding K_m value of 0.44×10^{-3} M. Optimum pH of lipoxygenase was reported at 5.5. Caffeic acid was the most effective inhibitor and kaempferol was the least effective.

1. Introduction

Lipoxygenase (LOX EC 1.13.11.12; linoleate: oxygen oxidoreductase) is an iron containing dioxygenase which catalyzes the hydroperoxidation of linoleic acid and other polyunsaturated fatty acids and their esters, as well as glycerides containing a *cis,cis*-1,4-pentadiene double bond system, originating a hydroperoxide that possesses a chain with a *cis, trans* conjugated double bond (Grechkin, 1998; López-Nicolás, Pérez-Gilabert, & Gracía-Carmona, 2001).

Products formed during the enzymatic reaction are very important for food quality. Hydroperoxides derivatives and products of their degradations can react with proteins, peptides and amino acids, which can result in off – flavors production and lowering nutritional values of the products. The action of lipoxygenase generates very reactive compounds like free radicals, which can react with chlorophyls, carotenoids, ascorbic acid, phenols and also α-tocopherol (vit. E) (Gomboeva, Shumaev, Gessler, & Lankin, 2001).

LOX plays an essential role in forming biologically active substances. It is engaged in the biosynthesis of controllers such as jasmonic acid, his methyl ester, abescisic acid, traumatic acid, which plays an important role in growth of plants and response to biotic and abiotic stress (Babitha, Prakash, & Shetty, 2004; Blée, 1998; Porta & Rocha-Sosa, 2002; Robinson, Wu, Domoney, & Casey, 1995).

Lipoxygenases are widely distributed in plants, animals and microorganisms. Plant lipoxygenases have been investigated since the early 1930s (Shibata & Axelord, 1995). These enzymes have been found in: soybean (Christopher, Pistorius, & Axeelrod, 1970; Márczy, Simon, Mózsik, & Szajáni, 1995), pea seeds (Busto et al. 1999; Liagre, Battu, Riagaud, & Beneytout, 1996; Yoon & Klein, 1979), beans (Bahceci, Serpen, Gökmen, & Acar, 2005), lupinus (Olias & Valle, 1988; Yoshie-Stark & Wäsche, 2004), barley (Hugues et al., 1994), tomatoes (Todd, Paliyath, & Thompson, 1990), olives (Lorenzi, Maury, Casanova, & Berti, 2006), bananas (Kuo et al., 2006), sunflower (Rodriguez-Rosales, Kerkeb, Ferrol, & Donaire, 1998), wheat, cucumber, potatoes, broccoli florets, pears and other plants (Robinson et al., 1995).

Green pea is very popular and available vegetable, recommended by dieticians because it contains proteins with essential amino acids, macro and microelements: phosphorus, calcium, magnesium, iron and vitamins: C, B_1, B_2, PP, E and carotene (Orłowski, Kołota, & Szczecin, 1999).

There are reports of pea seeds lipoxygenase, but publishers concentrate on genetic aspects or molecular properties of lipoxygenase isozymes. For dieteticians, food quality or food processing, it is more important to know more about properties of purified LOX. For this reason we have focused on the physical and biochemical properties of lipoxygenase that can be useful during food preparation and storage (Robinson et al., 1995). On the other hand lipoxygenases play an important role in the baking industry. They are quite effective as bleaching agents, increase mixing tolerance and improve dough rheology (Cumbee, Hildebrand, & Addo, 1997).

The aim of this work was to characterise the activity of LOX from pea seed var. *Telephone* taking pH optima, substrate specificity, kinetic studies and the inhibitory effect of some phenolic compounds under consideration.

* Corresponding author. Tel.: +48 81 4623323; fax: +48 81 4623324.
E-mail address: barbara.baraniak@up.lublin.pl (B. Baraniak).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.03.045

2. Materials and methods

2.1. Materials

Dried pea seeds var. *Telephone* were used. Linoleic acid, linolenic acid, linoleic acid methyl ester, arachidonic acid, Bradford reagent, caffeic acid, ferulic acid, benzoic acid, catechin, quercetin and kaempferol were purchased from Sigma–Aldrich, USA. Any other chemical was of analytical grade.

2.2. Enzyme extraction

Pea seeds were ground to a fine powder, then 10 g of it was suspended in 50 ml M/15 phosphate buffer pH 5.5 and lipoxygenase was extracted on magnetic stirrer for 3 h at 4 °C. The homogenate was filtered through three layers of cheesecloth and centrifuged at 1000*g* for 15 min. at 4 °C. Supernatant was used to purification.

2.3. Purification of lipoxygenase

The purification of lipoxygenase was initiated by the addition of solid ammonium sulfate at 80% saturation. The suspension was left for 30 min and then centrifuged (1000*g*, 15 min, 4 °C). The precipitated proteins were dissolved in a minimum amount of M/15 phosphate buffer (pH 5.5) and dialysed against the same buffer for 12 h at 4 °C (MW cut off. 12000). The buffer was changed three times during dialysis. The dialysate was loaded into ion-exchange chromatography on DEAE-Sephadex A-50 column (2 × 35 cm) previously equilibrated with M/15 phosphate buffer, pH 5.5. The column was eluted with the same buffer at the rate of flow 18 ml/h and next with linear gradient of NaCl concentration from 0 to 0.8 M. Three milli litre fractions were collected and protein concentration and LOX activity were monitored. Peak with LOX activity was pooled, concentrated and then dissolved in 3 ml of M/15 phosphate buffer pH 5.5. The combined fractions were transferred to a column (2 × 35 cm) filled with Sephadex G-50 gel. The column was eluted with the same buffer and 2 ml fractions were collected. Absorbance at 280 nm (protein content) and lipoxygenase activity were measured on each 2 ml fraction.

2.4. Substrate preparation and enzyme assay

The substrate solution was prepared by mixing 157.2 μl of pure linoleic acid, 157.2 μl of Tween-20 and 10 ml deionized water. The solution was clarified by adding of 1 ml 1 M NaOH and diluted to 200 ml M/15 phosphate buffer (pH 5.5) (final concentration of linoleic acid 2.5 mM). Lipoxygenase activity was determined spectrophotometrically at room temperature by measuring the increase absorbance at 234 nm over a 2 min (Axelroad, Cheesborough, & Laakso, 1981). The reaction mixture contained 2.97 ml phosphate buffer, 20 μl substrate solution and 10 μl enzyme solution. The blank sample contained 2.98 ml phosphate buffer (pH 5.5) and 20 μl substrate solution. One unit of LOX activity was defined as an increase absorbance of 0.001 per minute at 234 nm.

2.5. Protein estimation

The protein content was determined according to the dye-binding method of Bradford (1976) using bovine serum albumin as a standard.

2.6. Determination of molecular weight

The molecular weight of the purified enzyme was estimated by SDS–PAGE and gel permeation. SDS–PAGE was performed according to the method of Laemmli (1970). Proteins were dissolved in a 12% polyacrylamide gel and visualised with colloidal Coomassie staining.

2.7. Characterisation of LOX

2.7.1. Effect of pH on enzyme activity

Optimum pH was determined by measuring lipoxygenase activity in the pH range 4.0–9.0. The substrate solutions were prepared using the following buffering system: mixing appropriate amounts of M/15 KH_2PO_4 and Na_2HPO_4 for the range 5.0–8.0; 1 M CH_3COOH and CH_3COONa for pH 4 and M/15 Na_2HPO_4 and 1 M NaOH for pH 9.0.

2.7.2. Effect of enzyme dilution on lipoxygenase activity

To determine the optimal concentration of pea seeds lipoxygenase, different solutions of enzymatic extract (3, 5, 7 and 10× diluted) with phosphate buffer pH 5.5 were prepared. Activity of lipoxygenase was measured under standard conditions.

2.7.3. Kinetic study and substrate specificity of lipoxygenase

Linoleic acid solutions (0.156 mM, 0.312 mM, 0.625 mM, 1.25 mM, 2.5 mM, 5,0 mM, 7.5 mM) were prepared and used to determine the Michaelis constant (K_m) and the maximum velocity (V_{max}) of lipoxygenase. The kinetic parameters were evaluated by Lineweaver–Burk's method. The specificity of pea lipoxygenase was investigated for different possible substrates: linolenic acid, arachidonic acid, oleic acid and methyl linoleate at concentration 2.5 mM.

2.7.4. Effect of inhibitors on LOX

The inhibitory effect of caffeic acid, ferulic acid, benzoic acid, catechin, kaempherol and quercetin in different concentrations (0.5; 1 and 2 mM) on the LOX activity were determined. Lipoxygenase was incubated with ethanolic solution of inhibitor for 10 min. The reaction was started by the addition of substrate. The corresponding control contains the same concentration of enzyme in the absence of inhibitor.

3. Results and discussion

3.1. Enzyme assay and purification of lipoxygenase

The extraction and purification of LOX was described in Section 2. Ammonium sulphate precipitated protein (0–80% saturation) was loaded in to ion-exchange chromatography column. Elution profile on DEAE-Sephadex A-50 is shown in Fig. 1a. One peak containing LOX activity was collected, concentrated and further purified by gel filtration using Sephadex G-50 bed (Fig. 1b). As summarised in Table 1, lipoxygenase was purified about 47 times with protein recovery at about 22%. Purification procedures were allowed to get results comparable with another found in the literature. Babitha et al. (2004) applied DEAE–Sephadex A-50 and Sephadex G-200 gel-filtration after ammonium sulfate precipitation to purify lipoxygenase from pearl millet. They obtained three isozymes of LOX (with purification fold 46.5, 73.6 and 115.7). Results of our studies correspond well with those obtained by Regdel, Schewe, and Rapoport (1985) where also one izosyme was indicated. On the other hand Yoon and Klein (1979) found four isozymes with another physical and chemical properties. In should be noted that these enzymes were isolation from different various of green pea. Additionally, differences in the amounts of LOX isozymes could be bound with cultivation conditions and further purification procedures. According to Bowsher et al. (1992) obtained 49-fold purified microsomal lipoxygenase from tomato.

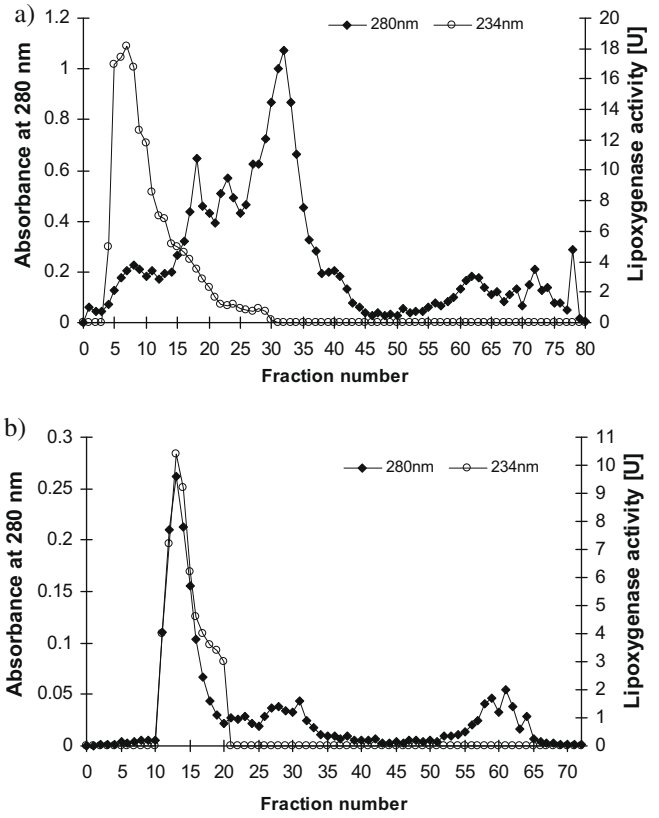

Fig. 1. Purification of LOX from pea seeds by liquid chromatography (a) separation on DEAE Sephadex A 50, (b) separation on Sephadex G-50.

65-fold purification and 18% protein recovery were obtained in the olives LOX case by Lorenzi et al. (2006) using differential centrifugation and hydrophobic interaction chromatography.

3.2. Determination of molecular weight

Using SDS–PAGE the molecular mass of lipoxygenase was found to be 93 kDa (Fig. 2). Generally, plants contain lipoxygenases with molecular mass in the range 94–104 kDa (Coffa, Schneider, & Brash, 2005). It was already reported, that purified English pea lipoxygenase had a molecular weight of 72 and 78 kDa (Eriksson & Svensson, 1970). According to Babitha et al. (2004) pearl millet lipoxygenase isozymes had a molecular masses 83 kDa, 77 kDa and 73 kDa for LOX 1, 3, 6, respectively. The molecular weight of purified LOX from banana leafs was 85 kDa (Kuo et al., 2006). Lipoxygenase from eggplant was a monomer with molecular mass 95 kDa (Nakayama, Takeura, & Ueda, 1995). According to Lorenzi et al. (2006), olive LOX had a molecular mass of 95 kDa.

3.3. Characterisation of LOX

3.3.1. Effect of pH on enzyme activity

The optimum pH for activity of lipoxygenase from dried green pea seeds was 5.5, however, enzyme exhibited also high activity

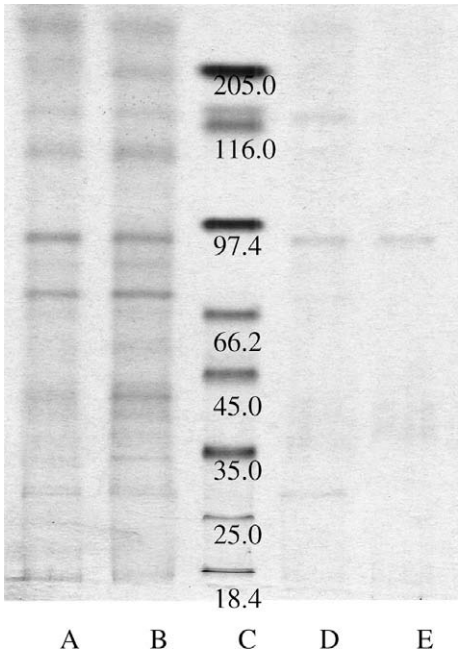

Fig. 2. SDS–Page of purified LOX from pea seeds. A – crude extract, B – dialysate, C – molecular weight markers, D – DEAE - Sephadex A-50, E – Sephadex G-50.

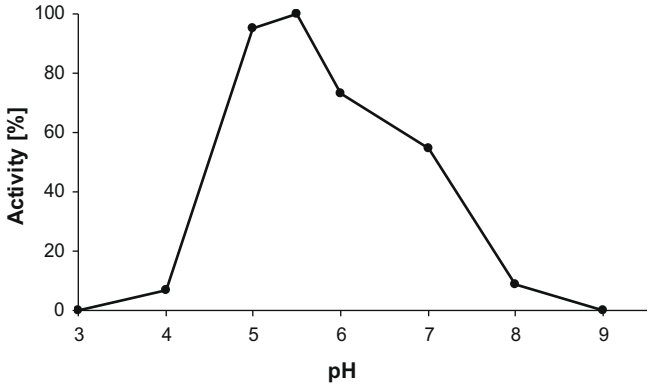

Fig. 3. Determination of optimum pH of green pea LOX activity.

at pH 5 and 6. Significantly, lower activity was observed at pH 4.0 and 8.0 (less than 10%), whereas at pH below 4.0 and at pH above 8.0, enzyme had no activity (Fig. 3).

Optimum pH for lipoxygenase activity depends on plant species: eggplant – pH 7.0 (López-Nicolás et al., 2001), sweet corn germ – pH 6.0–7.0 (Theerakulkait & Barrett, 1995), lupinus – pH 6.0–8.0 (Yoshie-Stark & Wäsche, 2004), soybean LOX-1 – pH 9, LOX-2 – pH 6.5 (Sheu & Chen, 1991). Gökmen, Bahceci, and Acar (2002) have reported that optimum pH for LOX obtained from green pea was at 6.0. Definitely, lower activity was detected at pH 7.0, enzymes were very stable at pH range 5.0–6.5. Different optimal pH for green pea lipoxygenase action was also determined

Table 1
Purification of green pea lipoxygenase.

Fraction	Total protein [mg]	Specific activity [U/$mg_{protein}$]	Purification fold	Recovery [%]
Crude extract	17.6	431.8	1	100.0
Amonium sulfate precipitation	11.85	819.8	1.89	52.1
DEAE – Sephadex A-50	0.363	10413.22	24.11	39.79
Sephadex G-50	0.104	20365.38	47.16	22.29

by another scientists: Regdel et al. (1985) – pH 5.9; Chen and Whitaker (1986) – pH 6.5; Hardy, Violana-Gallegos, and Gaunt (1991) – pH 7.0. It should be noted, that differences in the protein concentration and enzymes activity within the same species and varieties of *Pisum sativum*, may be due to the climate, cultivation and store conditions (Liagre et al., 1996).

3.3.2. Effect of enzyme dilution on lipoxygenase activity

The influence of extract dilution on LOX activity toward 2.5 mM linoleic acid was measured. Fig. 4 shows the directly proportional correlation consistent with correct enzymatic reaction course. The literature on this subject is scarce. Christopher et al. (1970) found the third isoenzyme of soybean lipoxygenase (LOX – 3), that showed deviation from a linear relationship between the enzyme quantity and the reaction velocity (measured as the rate of oxygen consumption) in contrast to LOX – 1 and LOX – 2.

3.3.3. Kinetic study and substrate specificity of lipoxygenase

The Lineweaver–Burk method was used to determine the effect of substrate concentration on the initial velocities of reaction catalysed by lipoxygenase from dried pea seeds. Fig. 5 shows the double-reciprocal plot of initial velocities versus different concentration of linoleic acid. K_m value for LOX was determined to be 0.44 mM, whereas the V_{max} was 151.5 U/min (15150 U/ml/min). Similar graph was obtained by Gökmen et al. (2002) in the crude extract from fresh green pea case, but the kinetic values were slightly different: (K_m = 2.33 mM, V_{max} = 1666 U/mg/min). Our results correspond well to those reported in the available literature. The K_m values obtained toward linoleic acid was: 0.24 mM for lupin seeds (Olias & Valle, 1988), 0.06 mM for LOX 1 and 0.18 mM for LOX 2 from barley (Hugues et al., 1994).

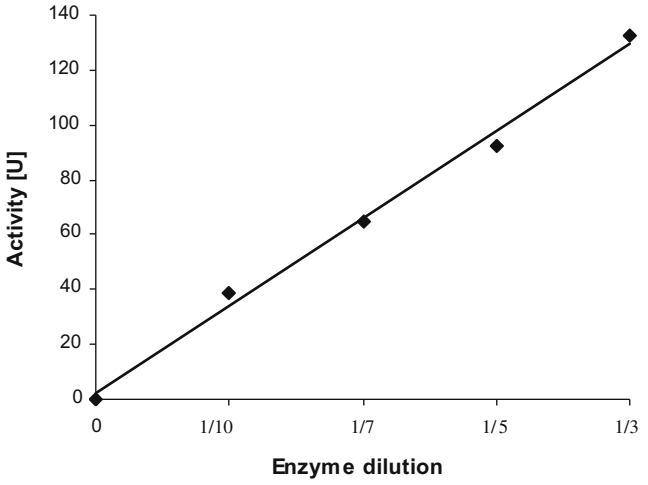

Fig. 4. Enzyme dilution profile for LOX.

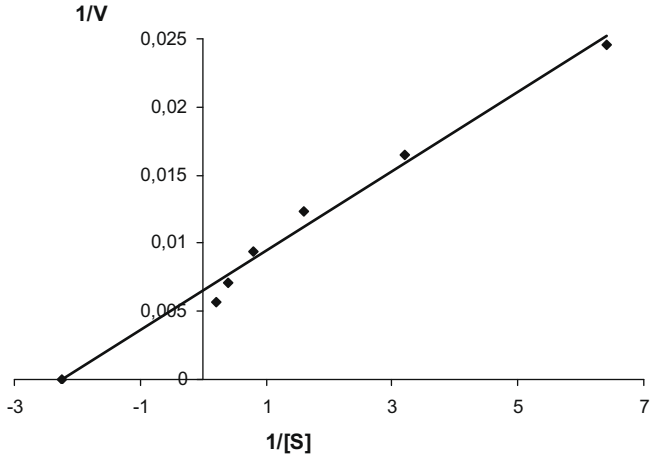

Fig. 5. The Lineweaver–Burk plot for the lipoxygenase activity.

The activity of lipoxygenase on variety of substrates was showed in the Table 2. Linoleic acid was the best substrate for this enzyme. Considerably lower activities were recorded for arachidonic acid, linolenic acid and methyl linoleate. No activity was found towards oleic acid. Lipoxygenases isolated from plants are the most effective toward linoleic acid, so this is the most often applied substrate for determination LOX activity. Olias and Valle (1988) measured the activity of lipoxygenase from lupinus seeds using linoleic acid and linolenic acid, and obtained similar results. Tomato lipoxygenase exhibited the highest activity toward linoleic acid and no activity toward oleic acid (Todd et al., 1990). Similar results obtained Babitha et al. (2004) for LOX from pearl millet. LOX isolated from eggplant did not metabolize arachidonic acid at all and showed high affinity to linoleic acid. On the other hand, arachidonic acid is the best substrate for potato lipoxygenase (López-Nicolás et al., 2001), but generally, it is the main substrate for animal lipoxygenases (Grechkin, 1998).

3.3.4. Effect of inhibitors

The conducted research proved that activity of lipoxygenase from pea seeds could be effectively inhibit by some phenolic compounds (Table 3). The most effective inhibitor is caffeic acid (about 57% of inhibition). Flavonoids like catechin and quercetin considerably inhibit the LOX activity. Inhibitors used for investigation in this study were placed in the following order: caffeic acid > quercetin > catechin > benzoic acid > ferulic acid > kaempferol. The same results were obtained by Redrejo-Rodriguez, Tejeda-Cano, del Carmen Pinto, and Macías (2004) in those studies all phenols inhibited lipoxygenase from horse bean and barley. Liagre et al. (1996) proved that inhibition degree is dependent on hydroxylic groups

Table 2
Substrate specificity of LOX.

Substrate (2,5 mM)	Relative activity [%]
Linoleic acid	100.0 ± 0.115
Arachidonic acid	47.1 ± 0.08
Linolenic acid	40.0 ± 0.076
Methyl linoleate	37.9 ± 0.042
Oleic acid	0

Table 3
Effect of various inhibitors on LOX activity.

Inhibitor	C [mM]	Inhibition [%]
Caffeic acid	0.5	14.1 ± 0.1
	1	30.3 ± 0.14
	2	57.5 ± 0.191
Ferulic acid	0.5	2.9 ± 0.09
	1	7.7 ± 0.12
	2	36.1 ± 0.32
Benzoic acid	0.5	3.2 ± 0.04
	1	10.8 ± 0.126
	2	37.9 ± 0.166
Kaempferol	0.5	5.6 ± 0.088
	1	12.4 ± 0.097
	2	34.8 ± 0.142
Quercetin	0.5	7.2 ± 0.04
	1	22.6 ± 0.06
	2	45.6 ± 0.13
Catechin	0.5	11.3 ± 0.02
	1	19.7 ± 0.15
	2	44.3 ± 0.171

position (ortho, meta, para) and these properties are the evidence of their role as antioxidants inhibits oxidation processes.

It can be concluded, that LOX isolated from dried pea seeds had an activity similar to another leguminous plants. The enzyme is a dioxygenase active towards polyunsaturated fatty acids and had the greatest specificity toward linoleic acid among the substrates tested. Moreover, the examined LOX was sensitive to some phenolic compounds known as lipoxygenase inhibitors, especially to caffeic acid. The complete characterisation of this enzyme gives possibility to avoid destructive influence during food processing and storage. Inhibitors studies of LOX from pea showed that phenolic antioxidant components were effective and can be used to protect food lipids against oxidation.

References

Axelroad, B., Cheesborough, T. M., & Laakso, S. (1981). Lipoxygenases in soybeans. *Methods in Enzymology, 71*, 441–451.

Babitha, M. P., Prakash, H. S., & Shetty, H. S. (2004). Purification and properties of lipoxygenase induced in downy mildew resistant pearl millet seedlings due to infection with *Sclerospora graminicola*. *Plant Science, 166*, 31–39.

Bahceci, K. S., Serpen, A., Gökmen, V., & Acar, J. (2005). Study of lipoxygenase and peroxidase as indicator enzymes in green beans: Change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. *Journal of Food Engineering, 66*, 187–192.

Blée, E. (1998). Phytooxylipins and plant defense reactions. *Progress in Lipid Research, 37*, 33–72.

Bowsher, C. G., Ferrie, B. J. M., Ghosh, S., Todd, J., Thompson, J. E., & Rothstein, S. J. (1992). Purification and partial characterization of a membrane-associated lipoxygenase in tomato fruit. *Plant Physiology, 100*, 1802–1807.

Bradford, M. M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Busto, M. D., Owusu Apenten, R. K., Robinson, D. S., Wu, Z., Casey, R., & Hughes, R. K. (1999). Kinetics of thermal inactivation of pea seed lipoxygenases and the effect of additives on their thermostability. *Food Chemistry, 65*, 323–329.

Chen, A. O., & Whitaker, J. R. (1986). Purification and characterization of a lipoxygenase from immature English peas. *Journal of Agricultural and Food Chemistry, 34*, 203–211.

Christopher, J. P., Pistorius, E. K., & Axeelrod, B. (1970). Isolation of a third isoenzyme of soybean lipoxygenase. *Biochimica and Biophysica Acta, 12*, 54–62.

Coffa, G., Schneider, C., & Brash, A. R. (2005). A comprehensive model of positional and stereo control in lipoxygenases. *Biochimical and Biophysical Research Communications, 338*, 87–92.

Cumbee, B., Hildebrand, D. F., & Addo, K. (1997). Soybean flour lipoxygenase isozymes effects on wheat flour dough rheological and breadmaking properties. *Journal of Food Science, 62*, 281–283.

Eriksson, C. G., & Svensson, S. G. (1970). Lipoxygenase from peas: Purification and properties of the enzyme. *Biochimica and Biophysica Acta, 198*, 449–452.

Gökmen, V., Bahceci, S., & Acar, J. (2002). Characterization of crude lipoxygenase extract from green pea using a modified spectrophotometric method. *European Food Research Technology, 215*, 42–45.

Gomboeva, S. B., Shumaev, K. B., Gessler, N. N., & Lankin, V. Z. (2001). The mechanism of oxidation of β-carotene and polyunsaturated fatty acids. *Biochemistry, Biophysics and Molecular Biology, 377*, 402–405.

Grechkin, A. (1998). Recent developments in biochemistry of the plant lipoxygenase pathway. *Progress in Lipid Research, 37*, 317–352.

Hardy, D. J., Violana-Gallegos, M. A., & Gaunt, J. K. (1991). Extracts of *Pisum sativum* metabolize phospholipid via a lipoxygenase-like mechanism. *Phytochemistry, 30*, 2889–2894.

Hugues, M., Boivin, P., Gauillard, F., Nicolas, J., Thiry, J. M., & Richard Forget, F. (1994). Two lipoxygenases from germinated barley – heat and killing stability. *Journal of Food Sciences, 59*, 885–889.

Kuo, J. M., Hwang, A., Yeh, D. B., Pan, M. H., Tsai, M. L., & Sun Pan, B. (2006). Lipoxygenase from banana leaf: Purification and characterization of an enzyme that catalyzes linoleic acid oxygenation at the 9-position. *Journal of Agricultural and Food Chemistry, 54*, 3151–3156.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 68012–68015.

Liagre, B., Battu, S., Riagaud, M., & Beneytout, J. L. (1996). Effectiveness of talc as adsorbent for stabilization and expression of *Pisum sativum hortense* Cv. Solara lipoxygenase-lyase coupled activities. *Journal of Agricultural and Food Chemistry, 44*, 2057–2062.

López-Nicolás, J. M., Pérez-Gilabert, M., & Gracía-Carmona, F. (2001). Eggplant lipoxygenase (*Solanum melogena*): product characterization and effect of physicochemical properties of linoleic acid on the enzymatic activity. *Journal of Agricultural and Food Chemistry, 49*, 433–438.

Lorenzi, V., Maury, J., Casanova, J., & Berti, L. (2006). Purification, product characterization and kinetic properties of lipoxygenase from olive fruit (*Olea europaea* L.). *Plant Physiology and Biochemistry, 44*, 450–454.

Márczy, J. S., Simon, M. L., Mózsik, L., & Szajáni, B. (1995). Comparative study on the lipoxygenase activities of soybean cultivars. *Journal of Agricultural and Food Chemistry, 43*, 313–315.

Nakayama, T., Takeura, Y., & Ueda, T. (1995). Visible spectrophotometric assay, purification, and molecular properties of a lipoxygenese from eggplant (*Solanum melongena* linne) fruits. *Biochemical and Biophysical Research Communications, 214*, 1067–1072.

Olias, J. M., & Valle, M. (1988). Lipoxygenase from lupin seed: purification and characterization. *Journal Sciences of Food and Agricultural, 45*, 165–174.

Orłowski, M., & Kołota, E., Uprawa warzyw, Brasika, Szczecin, 1999, [in Polish].

Porta, H., & Rocha-Sosa, M. (2002). Plant lipoxygenases. Physiological and molecular features. *Plant Physiology, 130*, 15–21.

Redrejo-Rodriguez, M., Tejeda-Cano, A., del Carmen Pinto, M., & Macías, P. (2004). Lipoxygenase inhibition by flavonoids: Semiempirical study of the structure-activity relation. *Journal of Molecular Structure, 674*, 121–124.

Regdel, D., Schewe, T., & Rapoport, S. M. (1985). Enzymatic properties of the lipoxygenase from pea seeds. *Biomedica and Biochimica Acta, 44*, 1411–1428.

Robinson, D. S., Wu, Z., Domoney, C., & Casey, R. (1995). Lipoxygenases and a quality of foods. *Food Chemistry, 54*, 33–43.

Rodriguez-Rosales, M. P., Kerkeb, L., Ferrol, N., & Donaire, J. P. (1998). Lipoxygenase activity and lipid composition of cotyledons and oil bodies of two sunflower hybrids. *Plant Physiology and Biochemistry, 36*(4), 285–291.

Sheu, S. C., & Chen, A. O. (1991). Lipoxygenase as blanching indicator for frozen vegetable soybeans. *Journal of Food Sciences, 56*, 448–451.

Shibata, D., & Axelord, B. (1995). Plant lipoxygenases. *Journal of Lipid Mediators and Cell Signalling, 12*, 213–228.

Theerakulkait, C., & Barrett, D. M. (1995). Lipoxygenase in sweet corn germ: Isolation and phytochemical properties. *Journal of Food Sciences, 5*, 1029–1032.

Todd, J. F., Paliyath, G., & Thompson, J. E. (1990). Characteristic of a membrane-associated lipoxygenase in tomato fruit. *Plant Physiology, 94*, 1225–1232.

Yoon, S., & Klein, B. P. (1979). Some properties of pea lipoxygenase isoenzymes. *Journal of Agricultural and Food Chemistry, 27*, 955–962.

Yoshie-Stark, Y., & Wäsche, A. (2004). Characteristics of crude lipoxygenase from commercially de-oiled lupin flakes for different types of lupins (*Lupinus albus*, *Lupinus angustifolius*). *Food Chemistry, 88*, 287–292.