

# Short Paper

## Effect of phenolic compounds extracted from sunflower seeds on native lipoxygenase activity

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### RESUMEN

#### Efecto de los compuestos fenólicos extraídos de semillas de girasol sobre la actividad lipoxigenasa nativa.

Se obtuvieron extractos de lipoxigenasa de semillas de girasol usando 12 mezclas de extracción diferentes, y se determinó la actividad lipoxigenasa y el contenido en compuestos fenólicos. Este último se llevó a cabo mediante medida de la relación de absorbancias a 320/280 nm. El análisis estadístico de los resultados para los extractos mostró una correlación entre la actividad lipoxigenasa y la relación  $OD_{320nm}/OD_{280nm}$  con  $r = 0,619$ . Sin embargo, después de la diálisis de los extractos, no se observó esta correlación.

**PALABRAS-CLAVE:** Compuestos fenólicos - Girasol - Lipoxigenasa (actividad).

### SUMMARY

#### Effect of phenolic compounds extracted from sunflower seeds on native lipoxygenase activity.

Extracts of lipoxygenase were obtained from sunflower seeds using 12 different extraction mixtures, and lipoxygenase activity and content of phenolic compounds in the extracts were determined. The content of phenolic compounds was determined as the ratio of optical densities at 320/280nm. Statistical analysis of the results for the extracts showed a significant correlation between the lipoxygenase activity and the  $OD_{320nm}/OD_{280nm}$  ratio with  $r = 0.619$ . However, after dialysis of the extracts, no correlation was found.

**KEY-WORDS:** Lipoxygenase (activity) - Phenolic compounds - Sunflower.

### 1. INTRODUCTION

Lipoxygenase (E.C. 1.13.11.12) is an enzyme, which catalyzes the oxidation of fatty acid containing *cis, cis* 1,4 - pentadiene moieties into fatty acids hydroperoxides containing a conjugated double bond system. The hydroperoxides can further be degraded either enzymatically or non-enzymatically

(Eskin *et al.*, 1977). Lipoxygenase-generated products contribute to either desirable, fresh flavours arising from metabolic processes or undesirable off-flavours (Sessa, 1979). Lipoxygenase is generally distributed in oilseeds, which are characterized by a high content of phenolic compounds. Some of these compounds are inhibitors of enzymes as lipoxygenases activity (Amarowicz and Fornal, 1995; Zadernowski *et al.*, 1998).

Sunflower seeds are a rich source of phenolic compounds (Leung *et al.*, 1981; Sosulski, 1979) but the interaction of these compounds extracted together with lipoxygenase on the activity of this enzyme has not yet been characterized. Therefore, the aim of this study was to investigate the relationship between phenolics compounds and lipoxygenase activity in crude enzyme extracts from sunflower seeds.

### 2. MATERIALS AND METHODS

Sunflower seeds were obtained from Seeds Central Company in Olsztyn (Poland). Seeds were dehulled and defatted with acetone on MPW-120 homogenizer.

Enzymatic extracts with different lipoxygenase activity and content of phenolics compounds were prepared from the defatted seeds using the following extraction mixtures at a 1:10 (w/v) ratio (Gambor and Zalik, 1958; Leoni *et al.*, 1985; Shiba *et al.*, 1991; Price, 1993).

1. 0.1M Tris-HCl buffer pH7.0 containing 2mM EDTA, 1% (v/v) Triton x-100
2. 0.1M Tris-HCl buffer pH 7.0 mM EDTA, 1% (v/v) Triton X-100, 0.01M  $Na_2S_2O_5$
3. 0.2M phosphate buffer pH 7.0, 1% (w/v) bovine serum albumin (BSA), 1% (w/v) polyvinylpyrrolidone (PVP), 1% (v/v) Triton X-100

4. 0.2M phosphate buffer pH 7.0, 1% (w/v) BSA, 1% (w/v) PVP, 1% (v/v) Triton X-100, 0.01 M Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>
5. 0.2M phosphate buffer pH 7.0, 0.01 M Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>
6. 0.2M phosphate buffer pH 7.0, 0.01 M Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, 1% (v/v) Triton X-100
7. 0.2M phosphate buffer pH 7.0
8. 0.2M acetate buffer pH 5.0, 1% (w/v) BSA, 1% (w/v) PVP, 1% (v/v) Triton X-100
9. 0.2M acetate buffer pH 5.0, 1% (w/v) BSA, 1% (w/v) PVP, 1% (v/v) Triton X-100, 0.01 M Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>
10. 0.2M acetate buffer pH 5.0, 0.01 M Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>
11. 0.2M acetate buffer pH 5.0, 0.01 M Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, 1% (v/v) Triton X-100
12. 0.2M acetate buffer pH 5.0

Extraction was performed at temperatures between 0° C and 4° C for 60 min. The mixture was then centrifuged at 24000 x g for 20 min. Pellets were discarded and supernatants were used for the enzyme assay.

Low molecular compounds, including phenolic compounds, were removed by dialysis against 6 litres of 0.1 M phosphate buffer, pH 7.0 carried out in dialysis bags with 16 kDa cuts off overnight. After dialysis, the extracts were centrifuged at 8000 x g for 20 min.

The relative content of phenolic compounds in the extracts was determined by optical density measurement at 280 and 320 nm (Beckman DU 7500 diode array spectrophotometer) using cuvettes with a 1 cm path length. Results were expressed as the ratio of the optical density at 320 nm/optical density at 280 nm (i.e. OD<sub>320nm</sub>/OD<sub>280nm</sub>). Direct determination of phenolic compounds using colorimetric method was difficult because a Folin-Denis reagent gives also a positive reaction with aromatic amino acids for extracts originated from lipoxygenase or other proteins molecules. Many phenolic compounds, above all phenolic acids, show strong absorption band at 320 nm (Shahidi & Naczki, 1995). In contrast a wavelength 280 nm is used for protein determination.

Lipoxygenase activity in supernatants before and after dialysis was measured spectrophotometrically at 25° C (Zimmerman and Vick, 1970) with linoleic acid as the substrate. An emulsion of linoleic acid was prepared according to Surrey's (1964) method and as modified by Shiiba *et al.*, (1991). Results are presented in activity units per mg protein (A.U./mg protein). One unit of lipoxygenase activity corresponds to the amount of the enzyme that produces one unit change of absorbance at 234 nm per minute. The protein content in extracts was determined according to Bradford's method (1976) and bovine serum albumin (Sigma) was used as a standard.

Regression analysis was performed on «Excel» statistical system.

### 3. RESULTS AND DISCUSSION

Lipoxygenase extracts with specific activities ranging from 2.38 (extraction mixture No 10) to 5.61 (No 11) activity units/mg protein could be selected from the various extracts so obtained (Table I). Extraction mixtures used in investigations contained different amounts of phenolic compounds and values of the OD<sub>320nm</sub>/OD<sub>280nm</sub> index ranged from 1.09 (No 6) to 1.45 (No 12).

Table I  
Lipoxygenase specific activity in crude extracts from sunflower and crude extracts  
OD<sub>320nm</sub>/OD<sub>280nm</sub> ratio

Extraction mixture	Activity units/mg protein	OD <sub>320</sub> /OD <sub>280</sub>
1	3.59	1.40
2	2.90	1.30
3	3.84	1.17
4	3.97	1.15
5	3.56	1.12
6	3.57	1.09
7	3.93	1.18
8	3.86	1.29
9	3.42	1.31
10	2.38	1.46
11	5.61	1.18
12	2.41	1.45

After dialysis of crude lipoxygenase extracts in general, specific enzyme activities increased and ranged from 2.41 (No 8) to 9.18 (11). For instance, almost a 2.5-fold increase in activity was observed for extracts obtained from extraction mixtures No 1, 2, and 6. Furthermore, application of dialysis reduced values of the OD<sub>320nm</sub>/OD<sub>280nm</sub> index, which ranged from 0.17 (No 2) to 0.49 (No 12) (Table II).

A relationship between phenolic compounds present in the extracts and lipoxygenase activity was confirmed by regression analysis. The correlation coefficient for the relationship between the OD<sub>320nm</sub>/OD<sub>280nm</sub> index and lipoxygenase activity was statistically significant, but only for crude extracts ( $\alpha = 0.05$ ) (Fig. 1). For dialysed samples, the statistic dependence disappeared (Fig. 2).

Table II  
Lipoxygenase specific activity in dialysed extracts  
from sunflower and dialysed crude extracts  
OD<sub>320nm</sub>/OD<sub>280nm</sub> ratio

Extraction mixture	Activity units/mg protein	OD <sub>320</sub> /OD <sub>280</sub>
1	8.42	0.18
2	7.45	0.17
3	1.85	0.30
4	2.12	0.21
5	4.69	0.23
6	8.75	0.33
7	5.32	0.28
8	2.41	0.36
9	2.55	0.33
10	4.11	0.46
11	9.18	0.41
12	3.03	0.49

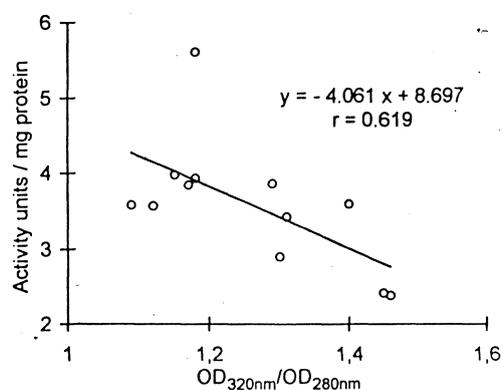


Figure 1  
Correlation between lipoxygenase specific activity and the  
OD<sub>320nm</sub>/OD<sub>280nm</sub> ratio for crude extracts

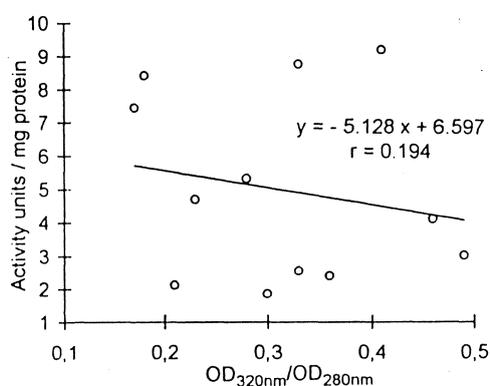


Figure 2  
Correlation between lipoxygenase specific activity and the  
OD<sub>320nm</sub>/OD<sub>280nm</sub> ratio for dialysed extracts

The highest OD<sub>320nm</sub>/OD<sub>280nm</sub> ratio after dialysis of lipoxygenase extracts were observed when an acetate buffer pH 5,0 or an acetate buffer pH 5,0 containing sodium bisulfite were employed. This suggests that the phenolic compounds were probably irreversibly bound to proteins to a great extent at pH 5.0 than at pH 7.0. Phenolic compounds can interact with proteins reversibly by means of hydrogen binding or irreversibly, when covalent binding of their oxidized form to the protein takes place (Sabir *et al.*, 1974; Sosulski, 1979).

The influence of phenolic compounds from the extract on lipoxygenase activity, observed in this study, is in agreement with literature data of other oilseeds. Inhibition of soybean lipoxygenase by chain-breaking antioxidants was noted by Maccarone *et al.*, (1995). Arachidonate lipoxygenase was strongly inhibited by extract from olives. One of the compounds responsible for this inhibition was purified and identified as 2-(3,4-dihydroxyphenyl)ethanol (Kohyama *et al.*, 1997). Caffeic acid phenylethyl ester was found as an inhibitor of lipoxygenase activity (Sud'ina *et al.*, 1993). Soybean lipoxygenase was inhibited in a model system by lipo- and hydrophobic phenolic compounds extracted from rapeseed (Zadernowski *et al.*, 1998).

In conclusion, evidence is presented that phenolic compounds extracted together with lipoxygenase inhibited enzyme activity. Partial identification of these compounds is in progress.

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