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THE ACTIVITY AND IMMUNOREACTIVITY OF LIPASE IN STORED SUNFLOWER SEEDS

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During the storage of sunflower seeds with moisture content adjusted to 6 and 18%, activity (by the diffusion method) and immunoreactivity (by the immunoblotting method) of lipase were examined. The activity of sunflower lipase ranged from 28.4 to 37.5 AU/mg protein and from 24.5 to 31.4 AU/mg protein during storage of sunflower seeds with moisture content adjusted to 6% and 18%, respectively. Protein fraction characterized by molecular weight of 78 kD on electrophoregrams of protein extract from sunflower seeds with moisture content 6% cross reacted with the anti-lipase antibodies. The presence of immunoreactive proteins was not however detected in the extracts from sunflower seeds with 18% moisture content. The enzyme immunoreactive properties appear to be affected by moisture content to a higher extent as compared to its activity, so no relationship between the enzyme activity and immunoreactivity is observed.

Sunflower seeds are rich in lipids which constitute about 42-55% of seed weight. Linoleic acid is the most abundant fatty acid in sunflower oil. It accounts for approx. 55% of all fatty acids. For optimal health a human diet should be provided with "essential fatty acids". They are necessary for proper development of human organisms and play important role in development and function of brain and retina, as well in biosynthesis of eicosanoid hormone-like substances [Linder, 1991]. The plant oils are very susceptible to deteriorative changes, thus the quality and nutritional value of seeds can be lowered. The enzyme starting unfavourable transformation of lipids is of high interest to food scientists, when concerning the raw material rich in lipids.

Lipases are the enzymes, which can essentially contribute to deterioration of lipid fraction of oil seeds. They are considered to begin unfavourable changes of lipid fraction due to releasing fatty acids from glycerides thus increasing the acidity of the product and providing the substrate for oxygenation reaction catalysed by lipoxygenase.

The best known lipases are those originating from microorganisms and their application in bioorganic synthesis and fat modifications is common [Chen & Sih, 1989; Mukherjee, 1990]. The plant lipases have also been intensively studied but are considered to be characterized to a lesser extent as compared to those of microbial origin.

In the examination of lipases, the traditional methods assaying their activities have been generally used and immunological methods have been used to a small extent in the studies on oilseeds lipases. There is a high immunological similarity between lipases of different origins and thus the polyclonal antibodies raised against castor bean lipase have been used to detect the presence of lipase in dry seeds of many young oilseed plants [Hills & Beevers, 1987], and the polyclonal antibodies raised against pancreatic lipase have been used in the studies on rapeseed lipases [Belguith *et al*., 2001].

The objective of this study was to determine the enzymatic activity and immunoreactivity of lipase in stored sunflower seeds of varied moisture content using the immunoblotting method.

MATERIAL AND METHODS

Material. The sunflower seed of Szarobiały variety was subjected to examination.

Conditions of storage. The sunflower seeds with moisture content adjusted to 6% were stored for 42 days and the sunflower seeds with moisture content adjusted to 18% were stored for 14 days. The seeds were stored in jars at a temperature of 0-4°C [Meshehdani *et al.,* 1990]. During the storage, the samples were taken at defined intervals to assay the lipase activity and immunoreactivity.

Moisture content. Moisture content was determined according to the Polish Standard method [PN-62/R-66163].

Extraction of lipases. Lipase extraction was carried out according to the method of Lin and Huang [1983]. Ten mL of extraction solution (0.1 mol/L TRIS-HCl, pH 8.0, 1 mM EDTA, 10 mM KCl, 1 mM $MgCl_2$) were added to 2 g of material. Next the mixture was homogenized on Ultraturax T-25 (IKA Labortechnik) for 1.5 min at 24,000 rev/min and a temperature of 0-4°C, and centrifuged (10 000xg for 20 min). The obtained supernatant was used to determine lipase activity.

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Protein content determination. Protein in extracts was determined by Bradford's method [Bradford, 1976]. Bovine serum albumin (BSA) was used as a standard protein.

Lipase activity. The activity of lipase was determined by the diffusion method in solidified agar according to Lawrence [1967] using 1,2,3 - tributyrylglycerol (Sigma) as a substrate. The substrate was prepared by adding the emulsion of tributyrylglycerol in 0.1 mol/L TRIS-maleate buffer (pH 8.0) to hot solution of agar. One mL of agar mixture was spread on microscopic slide. A well of 5 mm in diameter in the solidified agar mixture was bored and 5 μL of enzymatic extract was added. After incubation at 30°C for 16 h, the diameter of the zone of clearing was measured. Logarithmic increase in clearing zone by one unit was accepted as lipolytic activity unit (AU) according to Lawrence [1967]. The results were given in conversion to the specific activity (AU/mg protein).

Electrophoresis of enzymatic extracts. The electrophoretic separations of the enzymatic extracts were carried out on the 12% SDS-PAGE slabs (6 cm x 10 cm x 1 mm) with the addition of 25% (w/w) urea according to the method of Hills and Murphy [1988]. The electrophoretic separation was run in Tris-glycine buffer, pH 8.3, at 50 V in the electrophoresis apparatus of Kucharczyk's T.E. production (Gdańsk, Poland).

Immunoblotting. After electrophoretic separation, the proteins were transferred to a nitrocellulose membrane, which was then incubated in the solution of rabbit blood serum containing a 1:5 000 dilution of anti-lipase antibodies. The antibody-antigen complexes were detected using specific goat antibodies anti-rabbit-IgG conjugated with horseradish peroxidase [Murphy *et al.*, 1989].

Anti-lipase antibodies were raised against purified rapeseed lipase as previously described [Grabska *et al.*, 1997]. The molecular mass of separated immunostained proteins were determined on the basis of standard proteins (Sigma).

RESULTS AND DISCUSSION

The activity of sunflower lipase ranged from 28.4 to 37.5 AU/mg protein and from 24.5 to 31.4 AU/mg protein during storage of sunflower seeds with moisture content adjusted to 6% (Figure 1) and 18% (Figure 2), respectively.

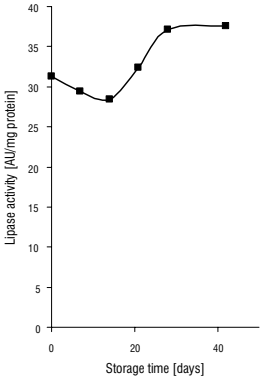

FIGURE 1. Activity of lipase of stored sunflower seeds with moisture content adjusted to 6%.

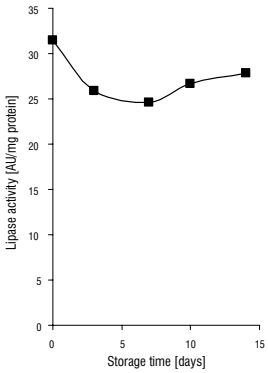

FIGURE 2. Activity of lipase of stored sunflower seeds with moisture content adjusted to 18%.

It was found that after SDS-PAGE separations of crude protein extracts from sunflower seeds (Figures 3A and 4), the antibodies cross reacted with 78 kD protein fraction on electrophoregrams of proteins of crude extract from sunflower seeds with moisture content 6% (Figure 3B). The intensity of the stain was similar for protein fractions obtained from stored sunflower seeds with moisture content 6% during the whole storage period. The presence of immunoreactive proteins was not however detected in the extracts from sunflower seeds with 18% moisture content.

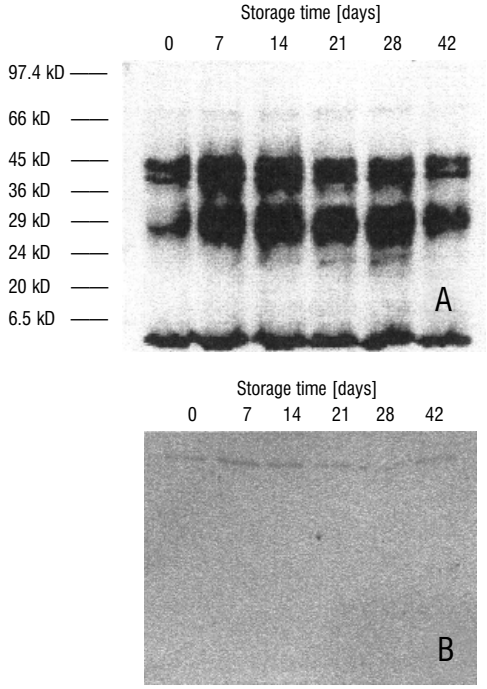

FIGURE 3. Electrophoretic separations (A) and immunoblotting (B) of electrophoretically separated proteins of crude extracts obtained from sunflower seeds with 6% moisture content.

Lipase is the enzyme of high molecular mass as it has been found by gel filtration chromatography in a number of studies [Weselake *et al*., 1989; Lin & Huang, 1983; Grabska *et al.*, 1997]. The molecular mass of the subunits is, however, considered to be 55-62 kD as it has been found in the numerous immunological experiments with the use of antibodies raised against purified lipase from castor bean or pancreatic lipase [Belguith *et al*., 2001; Murphy *et al.*, 1989; Hills & Beevers, 1987]. According to literature, sunflower

seeds are characterized by the presence of the enzyme characterized by fatty acyl-ester hydrolase consisting of one glycosylated polypeptide chain of 45 kDa molecular mass [Teissere *et al.*, 1995]. As in our present experiment the molecular mass of sunflower seed lipase has been found to be 78 kD, it can be supposed that enzyme can form complex protein structures and the moisture content can largely contribute to enzyme immunoreactivity. During storage of sunflower seeds with moisture content much higher than its critical moisture it can be expected that unfavourable changes within proteins and those affecting enzyme activities can occur in stored sunflower seeds. The structural changes within the enzyme molecule can affect epitopes areas thus changing the enzyme immunoraectivity as well can change the enzyme active site avaiability for the substrate as it has been found for soybean lipoxygenase [Di Venere *et al.*, 2003]. During storage there can be observed the protein degradation resulting from enhanced activity of proteolytic enzymes, as well oxidative changes within lipid fraction resulting in higher content of hydroperoxides, which in turn can bind with enzyme proteins, thus affecting protein structures and their immunoreactive properties and activities.

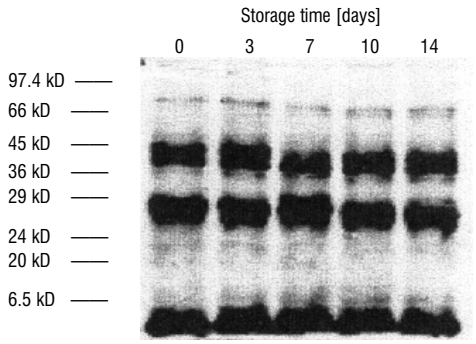

FIGURE 4. Electrophoretic separations of proteins of crude extracts obtained from sunflower seeds with 18% moisture content.

CONCLUSIONS

The results obtained suggest that stored sunflower seeds with different moisture content are characterized by activity of lipase and the enzyme immunoreactive properties appear to be affected by moisture content to a higher extent as compared to its activity, so no relationship between the enzyme activity and immunoreactivity is observed.

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