THE SUNFLOWER SEEDS LIPASE ACTIVITY ON VARIOUS SUBSTRATA UNDER THE INFLUENCE OF SOME OUTER AND INNER FACTORS

***Marcel AVRAMIUC[1]**
[1]„Stefan cel Mare" University of Suceava, Faculty of Food Engineering,
Str. Universităţii, no. 13, 720229, Suceava, România, e-mail: avramiucm@fia.usv.ro
*Corresponding author
Received 20 February 2011, accepted 25 April 2011

Abstract: *In this work the sunflower seeds lipase activity on its own substratum (sunflower oil), as well as on other substrata, of 7 plant species (pumpkin, soy bean, sesame, almond, maize, walnut and peanut), at various temperatures and pH values, has been analyzed.*
The experiment materials consist of dried sunflower seeds degreased (with petroleum ether), used as enzyme (lipase) source, and refined oils from 7 plant seeds above mentioned – as substratum for enzyme.
The lipase activity was determined at 20ºC and 40ºC as well as at 3 pH values (5.5; 7.4 and 9.5). The method principle consists in titrating fatty acids (released from oils by enzyme, in a certain time interval) with a solution of KOH 0.01n.
The determination of the lipase activity from sunflower seeds, at 20°C, 40°C and various pH, has shown distinct values of the enzyme activity depending on substratum nature, temperature and pH values. The enzyme activity on various substrata has registered:
- at 20°C the highest values in oils of: soy and walnut (pH 5.5), walnut and peanut (pH 7.4), sunflower and walnut (pH 9.5);
- at 40°C the highest values in oils of: peanut, soy and sunflower (pH 5.5), walnut and maize (pH 7.4), maize and walnut (pH 9.5).
The comparative analysis of the sunflower seeds lipase activity on various substrata has shown, both at 20°C and at 40ºC, the highest values at pH 5.5, and the lowest ones at pH 9.5.

Keywords: *lipase, substratum, pH, oil, seeds.*

1. Introduction

From the technological point of view, lipolytic enzymes, which are hydrolases involved in lipids' metabolism and demoting, make a controlled hydrolysis (favorable) of fats in foods during maturation, or an uncontrolled hydrolysis of fats (harmful) leading to foods' spoilage and occurrence of a pronounced rancid taste and odor [1].

Due to their short outgrowth cycle, microorganisms are used to obtain lipolytic enzymes and some scientific works have analyzed some aspects related to lipases isolated from *Candida antarctica* [2, 3, 4] or *Candida rugosa* [5, 6]. In the last decades, the vegetable lipases isolated in plants belonging to some families such as *Euphorbiaceae* [7, 8, 9, 10], *Brassicaceae* [11], *Caricaceae* [12] were used in various scientific research studies. In this work the activity of the lipase in sunflower seeds on their own substratum (sunflower oil), and on

various substrata, at different temperatures and pH values, has been analyzed.

2. Experimental

The experiment materials consist of dried sunflower seeds, degreased (with petroleum ether) used as lipase source, and refined oils of: sunflower, pumpkin, soy bean, almond, maize, walnut and peanut – as substratum for enzyme. The sunflower seeds have been obtained from Suceava Gene bank collection and the refined oils from supermarkets. The lipase activity has been determined at 20ºC, 40ºC and at 3 pH values (5.5, 7.4 and 9.5) and consisted (as principle) in titrating (with a solution of KOH 0.01 n) fatty acids released from oils by enzymes, in a certain time interval [13]**.**

The lipase activity was expressed by fatty acid micro molls, represented by oleic acid, formed (as result of enzyme action) from a gram of product, in a minute. The data, consisted in 4 replicates for each sample (determination), were statistically processed, using the mean values and standard deviations.

3. Results and Discussion

Figure 1 shows the comparative evolution of lipase activity in sunflower seeds of the 8 oil types, at 20°C temperature and at the 3 pH values.

At pH 5.5 the highest values were registered on soy bean oil, followed, in order, by walnut, maize, peanut and sunflower oils. The lowest ones were registered in sesame, almond and pumpkin oils.

At pH 7.4 the highest values of lipase activity were registered, in order, in walnut, peanut and maize oils, and the lowest ones in sunflower and soy bean oils.

At pH 9.5 there were not so high differences between samples as before, the highest value being registered by sunflower oil and the lowest ones by sesame and almond oils.

Except for the pH 9.5, where on the same substratum (sunflower oil) the lipase has registered the greatest value, whereas for the other analyzed pH, the enzyme has registered superior values on other substrata, for example, in soy bean, maize, walnut and peanut oils (at pH 5.5), and in pumpkin, sesame, almond, maize, walnut and peanut oils (at pH 7.4). At pH 5.5 the highest values were registered, in the following order: in peanut, soy bean and sunflower oils, and the lowest ones in almond and pumpkin oils.

At pH 7.4 the highest values of lipase activity were registered, in order, in walnut and maize oils, and the lowest ones in almond and sesame oils.

At pH 9.5 there were not so high differences between samples as in the case of pH 5.5 and 7.4, the highest values being registered by maize and walnut oils, and the lowest ones by sunflower, pumpkin and peanut oils.

As can be seen in this figure**,** for all pH values analyzed, the enzyme has registered superior values on other substrata than on its own one, as follows: on peanut oil (at pH 5.5), on pumpkin, maize, walnut (at pH 7.4) and on soy, sesame, almond, maize and walnut (at pH 9.5).

Comparing the lipase activity in the two thermal thresholds analyzed (20ºC and 40ºC) it can be observed that, except for the pH 9.5, at pH 5.5 and 7.4, superior values were registered at 20ºC.

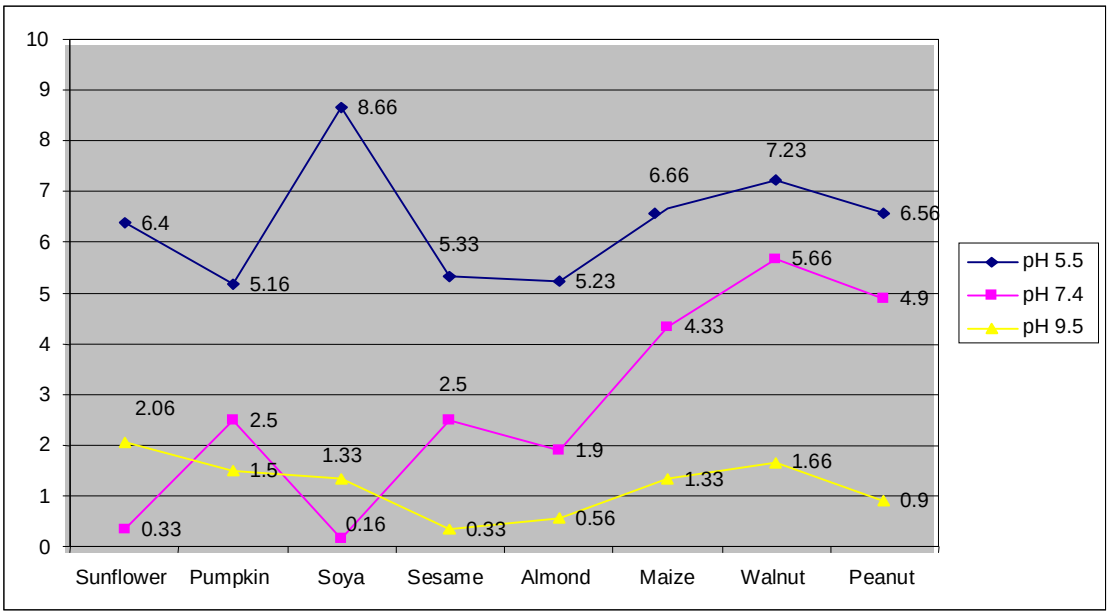

Fig. 1. The lipase activity of sunflower seeds on various substrata (oils), and various pH values, at 20ºC

Fig. 2 reproduces the comparative evolution of lipase activity of sunflower seeds in the 8 oils types, at 40°C temperature and at the 3 pH values.

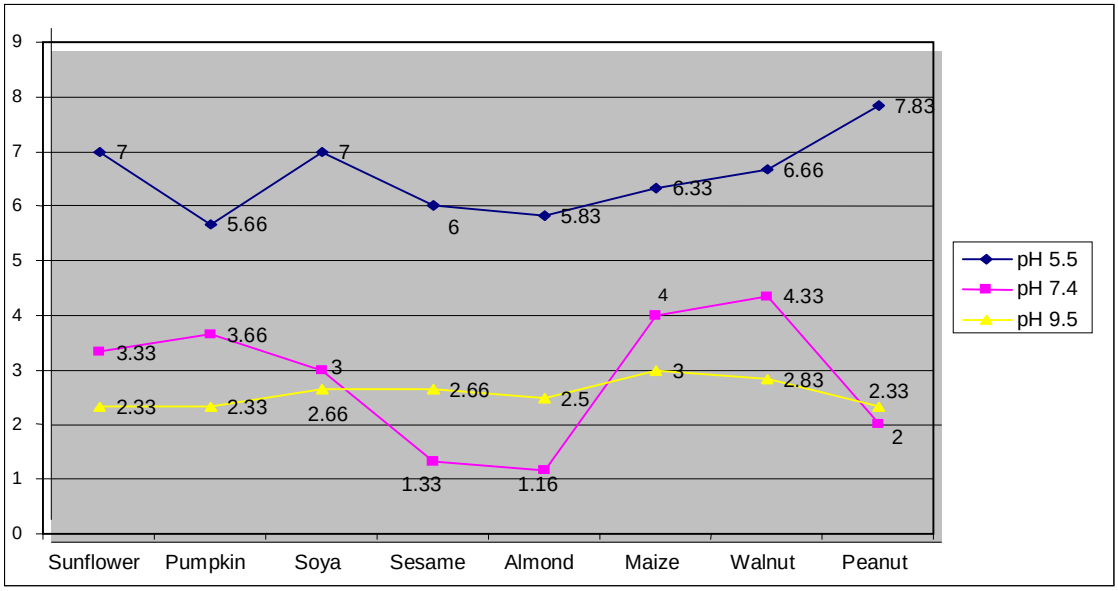

Fig. 2. The lipase activity of sunflower seeds on various substrata (oils), and various pH values, at 40ºC

4. Conclusions

The determination of the lipase activity of sunflower seeds, at 20°C, 40°C and various pH, has shown distinct values of the enzyme activity depending on the substratum nature, temperature and pH values.

The enzyme activity on various substrata has registered:

- at 20°C the highest values in oils of: soy and walnut (pH 5.5), walnut and peanut (pH 7.4), sunflower and walnut (pH 9.5);
- at 40°C the highest values in oils of: peanut, soy and sunflower (pH 5.5), walnut and maize (pH 7.4), maize and walnut (pH 9.5).

Comparing the lipase activity in two thermal thresholds (20° and 40°C) it can be observed that, except for the pH 9.5, at pH 5.5 and 7.4, superior values were registered at 20°C.

The comparative analysis of the sunflower seeds lipase activity on various substrata has shown, both at 20°C and at 40ºC, the highest values at pH 5.5, and the lowest ones at pH 9.5.

5. References

1. BÂRNESCU R. et al. - *Acţiunea lipolitică şi proteolitică a unor preparate din specii de Aspergillus asupra deşeurilor de piele.* Microbiologie industrială şi biotehnologie. Iaşi, 1986.

2. ANDERSON E.M., LARSSON K.M., KIRK O. - *One biocatalyst-many applications: the use of Candida antarctica B-lipase in organic synthesis,* Biocatal Biotransform 16 (1998), 181–204.

3. KIRK O., CHRISTENSEN M.W. - *Lipases from Candida antarctica: unique biocatalysts from a unique origin,* Org Process Res Dev 6 (2002), 446–451.

4. DOMÍNGUEZ DE MARÍA P., CARBONI-OERLEMANS C., Tuin B., Bargeman G., A. van der Meer, R. van Gemert - *Biotechnological applications of Candida Antarctica lipase A: state-of-the-art,* J Mol Catal B Enzym 37 (2005), 36–46.

5. AKOH C.C., LEE G.C., SHAW J.F. - *Protein engineering and applications of Candida rugosa lipase isoforms,* Lipids 39 (2004), 513–526.

6. DOMÍNGUEZ DE MARÍA P., SÁNCHEZ-MONTERO J.M., SINISTERRA J.V., ALCÁNTARA A.R. - *Understanding Candida rugosa lipases: an overview,* Biotechnol Adv 24 (2006), pp. 180–196.

7. GIORDANI R., MOULIN A., VERGER R. - *Tributyroylglycerol hydrolase activity in Carica papaya and other latices,* Phytochemistry 30 (1991), 1069-1072.

8. MOULIN A., TEISSERE M., BERNARD C., PIERONI G. - *Lipases of the Euphorbiaceae family: purification of a lipase from Euphorbia characias latex and structure-function relationships with the B chain of ricin,* Proc Natl Acad Sci 91 (1994), 11328–11332.

9. PALOCCI C., SORO S., CERNIA E., FIORILLO F., BELSITO C., MONACELLI B. et al. - *Lipolytic isoenzymes from Euphorbia latex,* Plant Sci 165 (2003), 577–582.

10. Villeneuve P., Turon F., Caro Y., Escoffier R., Baréa B., Barouth B. et al. - *Lipase-catalyzed synthesis of canola phytosterols oleate esters as cholesterol lowering agents,* Enzyme Microb Technol 37 (2005), 150–155.

11. HILLS M.J., KIEWITT I., MUKHERJEE K.D. - *Lipase from Brassica napus L. discriminates against cis-4 and cis-6 unsaturated fatty acids and secondary and tertiary alcohols,* Biochim Biophys Acta 1042 (1990), 237–240.

12. DHUIQUE-MAYER C., VILLARREAL L., CARO Y., RUALES J., VILLENEUVE P., PINA M. - *Lipase activity in alcoholysis and esterification reactions of crude latex from babaco fruit (Carica pentagona*), Oléagineux Corps gras Lipides 10 (2003), 232–234.

13. BORDEI D (coord.)., et al. - C*ontrolul calităţii în industria panificaţiei. Metode de analize.* Ed. Academica, Galaţi, 2007;