Cercetări Agronomice în Moldova
Vol. XLV , No. 1 (149) / 2012

THE INFLUENCE OF SOME PH VALUES AND SUBSTRATUM ON SUNFLOWER SEEDS LIPASE ACTIVITY

M. AVRAMIUC[1]*

*E-mail: avramiucm@fia.usv.ro

Received December 24, 2011

ABSTRACT. The activity of sunflower seeds lipase on its own substratum (sunflower oil), and on other substrata, belonging to seven plant species, searched under certain conditions of temperature and pH, was the purpose of this work. Dried sunflower seeds (SMC =10%) – used as lipase source, and refined oils from seeds of eight plant species (sunflower, pumpkin, soya bean, sesame, almond, maize, walnut and peanut) – used as substratum for enzyme, has represented the biological material in this work. The lipase activity, expressed as µm oleic acid/gram product/min., was determined at 20ºC and three pH values (5.5, 7.4 şi 9.5), by means of titrating of fatty acids (released from oils by enzyme, in a certain time interval) with a solution of KOH. At pH 5.5, the highest enzyme activity was registered on soya been oil, followed, in order, by walnut, maize, peanut and sunflower oils (very close values for the last three ones). In this pH value, the lowest lipase activities was on pumpkin, almond and sesame oils (all very close values). At pH 7.4, the gratest lipase activity value was registered on walnut oil, followed by peanut and maize oils, and the lowest ones on soya bean and sunflower oils (close values). At pH 9.5, the highest lipase activities have been on sunflower oil, followed by walnut, pumpkin, soya bean and maize oils (close values), and the least ones on sesame and almond oils (close values). The analyse of the sunflower seeds lipase activity on the eigth substrata (sunflower, pumpkin, soya bean, sesame, almond, maize, walnut and peanut oils) at 20ºC, has highlighted the highest values at pH 5.5 and the lowest ones at pH 9.5.The sunflower seeds lipase activity, at 20ºC, was influenced both by pH values and substratum nature.

Key words: Sunflower; Lipase; Enzyme; Substratum; pH; Oils.

REZUMAT. Influenţa unor valori ale pH-ului şi a substratului asupra activităţii lipazei din seminţe de floarea-soarelui. Scopul acestei lucrări a fost cercetarea activităţii lipazei din seminţele de floarea-soarelui pe propriul substrat (ulei de floarea-soarelui) şi pe alte substraturi, aparţinând la şapte specii de plante, în anumite condiţii de temperatură şi pH. Materialul biologic a fost reprezentat de seminţe de floarea-soarelui uscate (U=10%),

[1] „Ştefan cel Mare" University of Suceava, Faculty of Food Engineering, Romania

folosite ca sursă de lipază, şi uleiuri rafinate, provenite de la opt specii de plante (floarea-soarelui, dovleac, soia, susan, migdale, porumb, nucă şi arahide), utilizate ca substrat pentru enzimă. Activitatea lipazei, exprimată ca µmoli (µm) acid oleic/gram produs/minut, a fost determinată la 20º C şi la trei valori de pH (5,5, 7,4 şi 9,5) şi a constat, ca principiu, în titrarea cu o soluţie de hidroxid de potasiu a acizilor graşi, eliberaţi din uleiuri de către enzimă, într-un anumit interval de timp. La pH 5,5, cea mai mare activitate a enzimei s-a înregistrat la uleiul de soia, urmat de uleiurile de nuci, porumb, arahide şi floarea-soarelui (valori foarte apropiate pentru ultimele trei). La acest pH, cele mai mici valori ale activităţii lipazei au fost la uleiurile de dovleac, migdale şi susan (valori foarte apropiate). La pH 7,4, cea mai mare valoare a activităţii lipazei s-a înregistrat la uleiul de nucă, urmat de uleiurile de arahide şi porumb, iar cele mai mici la uleiurile de floarea-soarelui şi soia. La pH 9,5, cea mai mare activitate a enzimei s-a înregistrat la uleiul de floarea-soarelui, urmat de uleiurile de nucă, dovleac, soia şi porumb (valori apropiate), iar cele mai mici activităţi au fost la uleiurile de migdale şi susan. Analiza activităţii lipazei din seminţele de floarea-soarelui la opt uleiuri diferite (floare-soarelui, dovleac, soia, susan, migdale, porumb, nuci şi arahide), la 20ºC, a evidenţiat cele mai mari valori la pH 5,5 şi cele mai mici la pH 9,5. Activitatea lipazei din seminţele de floarea-soarelui, la 20ºC, a fost influenţată atât de valorile pH-ului, cât şi de natura substratului.

Cuvinte cheie: seminţe de floarea-soarelui, lipază; activitate enzimatică; substrat; pH; uleiuri.

INTRODUCTION

The lipolytic enzymes are hydrolases (lipasese and phospho-lipases) involved into the lipids metabolism and demoting. Technologically, the lipolytic enzymes accomplish a controled hydrolisis (favourable) of fats from foods during maturation, or an uncontroled fats hydrolisis (harmful) leading to foods spoiling and to apperance of pronounced rancid taste and odour (Bârnescu *et al.,* 1986).

As microorganisms have a short growth cycle, they were used to obtain lipolytic enzymes, and some scientific works have highlighted some aspects related to lipases isolated from *Candida antarctica* (Anderson *et al.,* 1998; Kirk and Christensen, 2002; Domínguez de María *et al.,* 2005) or *Candida rugosa* (Akoh *et al*., 2004; Domínguez de María *et al.,* 2006). Last time, the vegetable lipases isolated from plants belonging to some families as *Euphorbiaceae* (Giordani *et al*., 1991; Moulin *et al*., 1994; Palocci *et al.*, 2003; Villeneuve *et al.,* 2005), *Brassicaceae* (Hills *et al.,* 1990), *Caricaceae* (Dhuique-Mayer *et al.,* 2003) were used in many scientific researches.

The purpose of this work was to search the activity of the lipase from sunflower seeds on its own substratum (sunflower oil) and on other substrata, at 20ºC and various pH values, to see if pH, substratum or the both of them influence the activity of this enzyme.

MATERIALS AND METHODS

The experiment materials have been represented by ungreased (with petroleum ether) and dried sunflower seeds, used as enzyme source (lipase), and refined oils of

sunflower, pumpkin, soya bean, almond, maize, walnut and peanut, as substratum for enzyme.

The lipase activity has been determined at 20ºC and at three pH values (5.5, 7.4 and 9.5) and has consisted (as principle) in titrating (with a solution of KOH 0.01 N) of fatty acids released from oils by enzyme, in a certain time interval (Bordei *et al*., 2007)**.** The lipase activity (*AL*) was expressed by fatty acid micromols (μm), represented by oleic acid, formed (as result of enzyme action) from a gram of product, in one minute:

$$AL = \frac{(V_p - V_m) \cdot 0.00282}{282 \cdot 10^{-6} \cdot G \cdot T},$$

where:

V_p – the volume of KOH 0.01 N used for sample titrating where the enzyme acted (according to fatty acids released by enzyme and to those ones existing within substratum), ml;

V_m – the volume of KOH 0.01 N used for blank sample titrating (according to fatty acids existing within substratum), ml;

0.00282 – the oleic acid titre, according to KOH 0.01 N (g/ml);

$282 \cdot 10^{-6}$ – 1 micromol (μm) of oleic acid;

G – the product amount (g) used in experiments;

T – thermostating interval (minutes).

The data of experiments (consisting in four replicates for each determination) were statistically processed, using the mean values and standard deviations.

RESULTS AND DISCUSSION

Figs. 1- 4 reproduces the values of the lipase activity from sunflower seeds, determined on eight substrata (oils), belonging to eight plant species, at 20°C and pH 5.5, 7.4 and 9.5.

As seen in the *Fig. 1*, at pH 5.5 the highest values were registered on soya bean oil (8.66 μm oleic acid/gram product/min), followed, in order, by walnut oil (7.23 μm oleic acid/gram product/min) and maize, peanut and sunflower oils (all these having close values: 6.66-6.40 μm oleic acid/gram product/min. Pumpkin, almond and sesame oils were the substrata with the most reduced lipase activity (between 5.16 and 5.33 μm oleic acid/gram product/min).

In the *Fig. 2*, reproducing the lipase activity at pH 7.4, it can observe that, in this pH value, the highest enzyme activity was on walnut oil (5.66 μm oleic acid/gram product/min), followed by peanut and maize oils (4.9-4.33 μm oleic acid/gram product/min), the least values being registered on soya bean oil (0.16 μm oleic acid/gram product/min) and sunflower oil (0.33 μm oleic acid/gram product/min).

In the *Fig. 3*, reproducing the lipase activity at pH 9.5, the greatest enzyme activity was on sunflower oil (2.6 μm oleic acid/gram product/min), followed, in order, by walnut, pumpkin, maize and soya been oils (between 1.66 and 1.33 μm oleic acid/gram product/min). The least lipase activity values were registered on sesame oil (0.33 μm oleic acid/gram product/min) and almond oil (0.56 μm oleic acid/gram product/min).

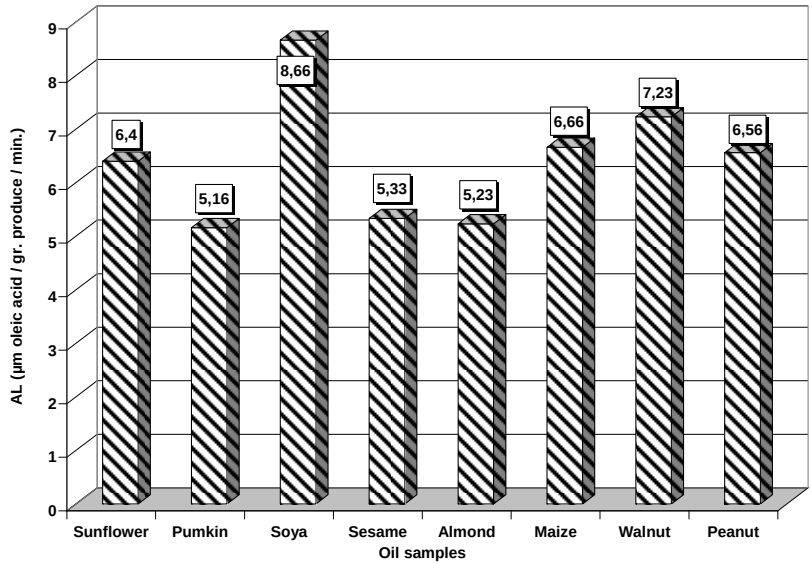

Figure 1 - **The lipase activity values from sunflower seeds on various substratum (oils) at 20ºC and pH=5.5**

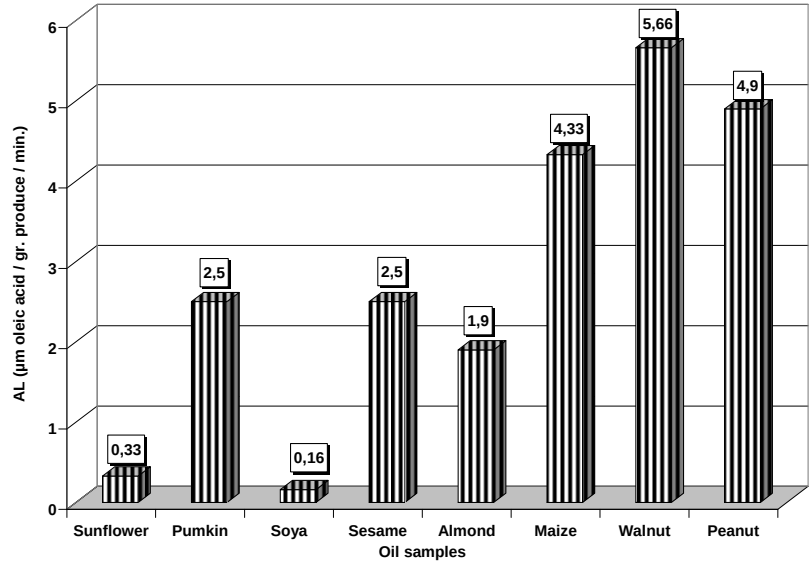

Figure 2 – The lipase activity values from sunflower seeds on various substratum (oils) at 20ºC and pH=7.4

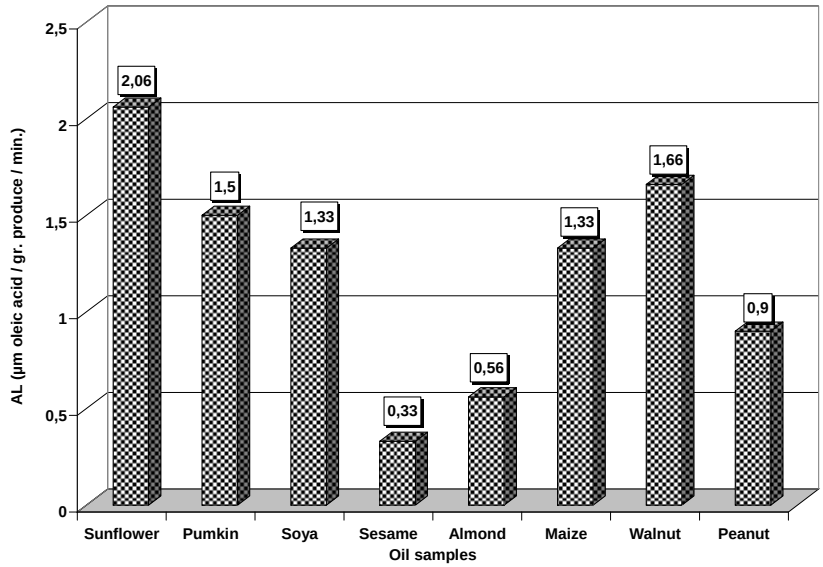

Figure 3 – The lipase activity values from sunflower seeds on various substratum (oils) at 20ºC and pH=9.5

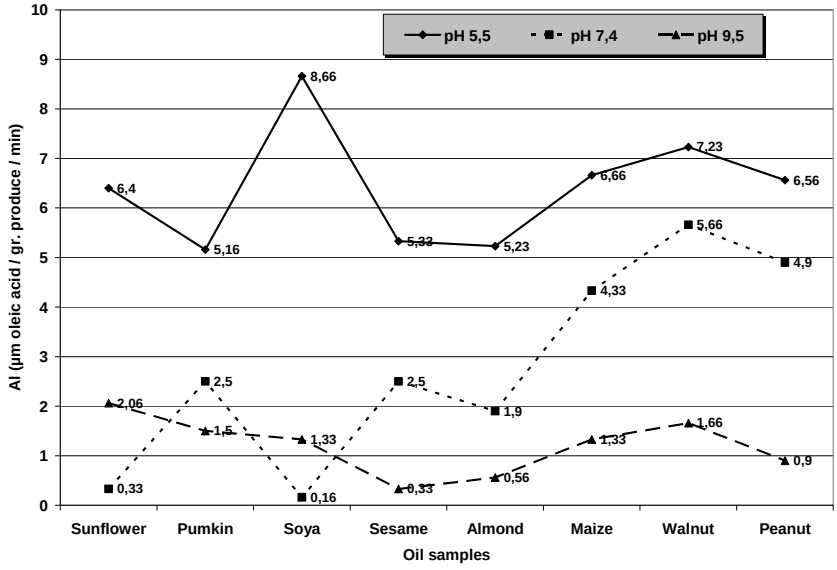

Figure 4 – The comparative evolution of the lipase activity sunflower seeds at 20ºC and pH 5.5 and 9.5

In the *Fig. 4* it can observe the comparative evolution of the lipase activity from sunflower seeeds on the eight oil types, at 20°C and three pH values. As seen, the highest enzyme activity values were registered at pH 5.5, and the least ones at pH 9.5.

Except pH 9.5, where on the same substratum (sunflower oil) the lipase has registered the greatest value, in the other analysed pH the enzyme has registered superior values on other substrata, as, for example, on soya bean, maize, walnut and peanut oils (at pH 5.5), and on pumpkin, sesame, almond, maize, walnut and peanut oils (at pH 7.4).

According to Marangoni (2002), there are three main types of lipase specificity: positional, substrate, and stereospecificity. As to substrate, for example, the porcine pancreatic lipase is specific toward shorter-chain fatty acids, the lipase from *Penicillium cyclopium* is specific toward long-chain fatty acids, and the lipases from *Aspergillus* (*niger* and *delemar)* are specific toward both medium-chain and short-chain fatty acids (Desnuelle, 1972; Stamatis *et al.,* 1993). Other lipases are specific toward fatty acids of varying lengths (Villeneuve and Foglia, 1997).

Referring to this work, it seems that sunflower seed lipase was more specific toward fatty acids from other oils then sunflower oil, at 20°C and pH 5,5 and 7,4.

CONCLUSIONS

The determination of the lipase activity from sunflower seeds, at 20°C and various pH, has shown distinct values of the enzyme activity, as follows:

- at pH 5.5 the highest lipase activity values were registered on soya bean and walnut oils, and the least ones on pumpkin, almond and sesame oils;

- at pH 7.4 the highest enzyme activities were registered on walnut, peanut and maize oils and the least ones on soya bean and sunflower oils;

- at pH 9.5 the greatest sunflower seed lipase activity was registered on its own substratum (sunflower oil) and the least activities on sesame and almond oils.

The comparative analyse of the sunflower seeds lipase activity on various substrata, at 20°C, has evidenced the highest values at pH 5.5, and the least ones at pH 9.5.

The sunflower seeds lipase activity at 20ºC was influenced both by pH values and substratum nature.

REFERENCES

Akoh C.C., Lee G.C., Shaw J.F., 2004 - Protein engineering and applications of *Candida rugosa* lipase isoforms. Lipids 39, 513–526.

Anderson E.M., Larsson K.M., Kirk O., 1998 - One biocatalyst-many applications: the use of Candida antarctica B-lipase in organic synthesis. Biocatal Biotransform 16, 181–204.

Bârnescu R. *et al.* , 1986 - Acțiunea lipolitică şi proteolitică a unor preparate din specii de *Aspergillus* asupra deşeurilor de piele (Lipolytic and proteolytic action of some preparations of *Aspergillus* species on leather waste). Microbiologie industrială şi biotehnologie, Iaşi.

Bordei Despina (coord.) *et al.,* 2007 - Controlul calității în industria panificației. Metode de analize (Quality control in bakery industry. Methods of analysis), Edit. Academica, Galați.

Desnuelle P. in: PD Boyer, ed., 1972 - The Lipases. The Enzymes. Volume VII. 3rd ed. New York: Academic Press, pp 575–616.

Dhuique-Mayer C., Villarreal L., Caro Y., Ruales J., Villeneuve P., Pina M., 2003 - Lipase activity in alcoholysis and esterification reactions of crude latex from babaco fruit (*Carica pentagona*). Oléagineux Corps gras Lipides 10**,** 232–234.

Domínguez de María P., Carboni-Oerlemans C., Tuin B., Bargeman G., A. van der Meer R. van Gemert, 2005 - Biotechnological applications of *Candida antarctica* lipase A: state-of-the-art. J Mol Catal B Enzym 37, 36–46.

Domínguez de María P., Sánchez-Montero J.M., Sinisterra J.V., Alcántara A.R., 2006 - Understanding *Candida rugosa* lipases: an overview. Biotechnol Adv 24, pp. 180–196.

Giordani R., Moulin A., Verger R., 1991 - Tributyroylglycerol hydrolase activity in *Carica papaya* and other lattices. Phytochemistry 30, 1069-1072.

Hills M.J., Kiewitt I., Mukherjee K.D., 1990 - Lipase from *Brassica napus* L. discriminates against cis-4 and cis-6 unsaturated fatty acids and secondary and tertiary alcohols. Biochim Biophys Acta 1042**,** 237–240.

Kirk O., Christensen M.W., 2002 - Lipases from *Candida antarctica:* unique biocatalysts from a unique origin. Org Process Res Dev 6, 446–451.

Marangoni A.G., 2002 - Lipases: Structure, Function and Properties. In: Lipid Biotechnology ed. by Tsung Min Kuo and Harold W. Gardner, Marcel Dekker, Inc., 270 Madison Avenue, New York, NY 10016, 360-386.

Moulin A., Teissere M., Bernard C., Pieroni G., 1994 - 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, 11328–11332.

Palocci C., Soro S., Cernia E., Fiorillo F., Belsito C., Monacelli B. *et al.*, 2003 - Lipolytic isoenzymes from *Euphorbia latex*. Plant Sci 165, 577–582.

Stamatis H., Xenakis A., Provelegiou M., Kolisis F.N., 1993 - Biotechnol Bioeng 42:103–110.

Villeneuve P., Foglia T.A., 1997 - INFORM 8:640–650.

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