RESEARCH REGARDING LIPASE ACTIVITY FROM SOME PLANT SPECIES UNDER THE INFLUENCE OF SOME EXTERNAL FACTORS

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Abstract

The aim of this paper was to search the lipase coming from four plant sources, to see to what extent pH, temperature or/and substrate influence the activity of this enzyme, and which of these sources shows a higher enzyme activity. The lipase sources were represented by seeds (fruits) of: peanut, walnut, almond, and sesame, and as substrates were used refined oils of: sunflower, pumpkin, soy bean, corn, peanut, walnut, almond and sesame. The activity of lipase was determined at 20°C and 40ºC, at three pH values (5.4, 7.4 and 8.2) for each temperature, and has consisted in titrating of fatty acids released from oils by lipase, in a certain time interval. The lipases coming from peanut, walnut, almond and sesame have registered, on the eight refined oils, the highest values of activity at pH 5.4 (at 40°C). Compared to the other three sources, the activity of walnut lipase was significantly higher on the oils of: sunflower, pumpkin, soy bean and walnut, the highest activity being recorded on walnut oil, at 40°C. Both the temperature, but especially pH and chemical composition of substrate (oil) have influenced the activity of lipases derived from the four plant sources.

Key words: lipase, oil, source, pH, temperature, substrate

INTRODUCTION

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are enzymes belonging to hydrolases class, esterases which catalyze hydrolysis of glycerides, leading to fatty acids and organic alcohols. The origin of lipases can be animal (gastric, hepatic, pancreatic, adipose tissue, vegetal (oilseeds, pulses, cereals) or microbial (bacterial, fungal, etc.)

Due to their versatility to catalyze different kinds of reactions, as well as their different specificities make lipases to have an important and vast application potential in: foods, detergents, oleochemicals, pharmaceuticals, fine chemistry, biodisel, cosmetics and fragrances, paper pulp, leather, biosensors and lipid-rich wastewater treatment, [2, 8, 9, 11, 12, 14, 16, 20, 21, 22, 24, 28].

According to some authors [3, 6, 17, 25], commercial lipases are generally produced from animals (pancreatic and pregastric tissues of ruminants) or microorganisms (*Penicillium spp., Geotrichum spp., Aspergillus spp., Rhizomucor spp., Candida spp. or Pseudomonas spp.*).

Despite the extensive range of microbial lipases, the use of these enzymes on an industrial scale is still restricted due to high production costs, favoring the search for other sources of these enzymes [20].

Seed lipases present advantages over animal and microbial lipases due to some quite interesting features such as: specificity, low cost, availability and ease of purification, representing a great alternative for potential commercial exploitation as industrial enzymes [9, 15, 20, 22, 27].

As last years, due to their many advantages, the seeds lipases arouses an increasing interest, in this paper there was conducted a search on the lipases coming from four plant sources, to see to what extent pH, temperature and/or substrate (oil) influence the activity of this enzyme, and which of these sources shows an increased activity.

MATERIALS AND METHODS

The biological material, used as lipase sources, and provided by Suceava Genebank and by Suceava Agricultural Research and Development Station, have been represented by seeds (with moisture content of 10-12%), belonging to the following plant species:

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peanut (*Arachis hypogaea* L., var. Dabuleni), walnut (*Juglans regia* L., var. Bratia), almond (*Amygdalus communis* L., var. Mărculesti 2/1), and sesame (*Sesamum indicum* L.). As substrates for enzyme activity, were used the following refined oils: sunflower, pumpkin, soy bean, corn, peanut, walnut, almond and sesame, purchased from supermarkets.

The lipase activity has been determined at 20ºC and 40ºC, at three different pH values (5.4, 7.4 and 8.2) for each temperature, and has consisted in titrating (with a solution of KOH 0.01 N) of fatty acids released from oils by lipase, in a certain time interval [4].

In order to obtain the enzyme preparation skimmed and dried, in a glass bottle with a stopper it has mixed some seeds finely divided with two sides ether, then let stand for 2-3 hours for oil extraction, stirring periodically. It has separated ether, and they have introduced again 5 parts ether over the product partly skimmed, for one hour, after which it was separated ether. The skimmed seeds were dried in a oven with fan, at a temperature of 30°C. There were obtained defatted seeds containing lipase.

For lipase activity determination, in an Erlenmeyer flask were introduced: 1 g of refined oil, 2 ml phosphate buffer pH 5.4, then 1 g of defatted seeds finely ground and 3 ml of distilled water at temperature of 20ºC. The flask was closed with a stopper and it was stirred gently for 30 minutes, then they were added 15 ml of alcohol 96% (v) and 15 ml of petroleum ether (v), and the content was stirred again for 10 seconds. Finally, the fatty acids present in the sample were titrated with 0.01N KOH in the presence of phenolphthalein, as indicator. In the same way, it was done using phosphate buffers pH 7.4 and pH 8.2, creating each three working variants for temperature 20ºC, and 40ºC respectively.

In parallel, they were done two control samples (for each working sample), consisting of the mixture of all reaction components, except the oil, which were well stirred and heated for 5 minutes in a water bath at boiling, to inactivate the enzyme. After cooling, it has added to each 1 ml oil and it has proceeded like at the investigated samples

The lipase activity (AL) was expressed as fatty acid micromols (µmol), represented by oleic acid, formed, as result of enzyme action, from a gram of product, in one minute:

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

whereas:

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

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

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

282 x 10^{-6} – 1 micromol (µmol) of oleic acid;

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

T – thermostating interval (60 minutes).

The data of experiments, consisting in 4 replicates for each determination, were statistically processed using SAS Version 8.02 [23]. In orer to analyse the significance of differences among samples, generalised linear model analysis was carried aut, and for multiple comparisons was used Duncan's multiple range test ($P<0.05$).

RESULTS AND DISCUSSIONS

Table 1 reproduces the peanut seeds lipase activity on the eight various substrates (oils).

At 20°C and pH 5.4 peanut seeds lipase has registered significant higher values of its activity on walnut, peanut and pumpkin oils, as compared to corn, soybean, sunflower, sesame and almond oils ($P<0.05$). At 20°C, but at pH 7.4, the lipase from peanut seeds had the highest activity on almond oil, followed by activity on pumpkin oil ($P<0.05$). At pH 8.2, the enzyme activity was very low on all oil samples (0,33-1µmol oleic acid/g/min.).

At 40°C, and pH 5.4 the highest value of peanut lipase activity was recorded on peanut oil, followed by pumpkin, sunflower, corn and almond oils (P<0.05). At pH 7.4 the peanut lipase had the highest activity on sesame oil, and at pH 8.2 its activity was very low on all oil samples analyzed (0.16-0,66 µmol oleic acid/g/min.).

From Tab. 1 results that, at 20°C and 40°C, the lipase derived from peanut seeds has recorded the highest values of its activity at pH 5.4, and the lowest ones at pH 8.2. Also, one can see that the largest lipase activity was registered on peanut oil at 40°C, and pH 5.4 (P<0.05).

The Table 2 reproduces the almond seeds lipase activity on analyzed substrates (oils).

Table 1 Lipase activity mean values (±SD) of peanut seeds on different substrates

Lipase source		Peanut seeds								
Substrate (oil)		pH	SF	PK	SB	PN	CN	WN	AL	SE
LA (µmol oleic acid/ g/min.	20°C	pH= 5.4	4.23 ±0.42 cd*	4.83 ±0.38 c*	4.33 ±0.51 cd	5.23 ±0.47 c	4.66 ±0.43 cd	5.33 ±0.59 c	3.9 ±0.28 cd	4 ±0.33 cd
		pH= 7.4	2.4 ±0.17 ef	2.5 ±0.21 de	2.33 ±0.28 ef	2 +0.18 ef	1.9 ±0.11 ef	2 ±0.22 ef	3.66 ±0.36 cd	1.9 ±0.2 ef
		pH= 8.2	0.73 ±0.09 fg	0.83 ±0.08 fg	0.66 ±0.05 fg	0.9 ±0.08 fg	0.83 ±0.09 fg	1 ±0.12 ef	0.56 ±0.04 fg	0.33 ±0.03 fg
LA (µmol oleic acid/ g/min.	40°C	pH= 5.4	5 ±0.42 c	5.33 ±0.65 c	4.66 ±0.48 cd	6 ±0.72 ab	5 ±0.39 c	4.33 ±0.44 cd	4.83 ±0.45 c	4.33 ±0.57 cd
		pH= 7.4	1.33 ±0.16 ef	2 ±0.18 ef	2 ±0.23 ef	0.83 ±0.06 fg	1.16 ±0.1 ef	1.33 ±0.12 ef	1.33 ±0.09 ef	2.5 ±0.14 de
		pH= 8.2	0.66 ±0.05 fg	0.5 ±0.04 fg	0.33 ±0.02 fg	0.5 ±0.06 fg	0.33 ±0.04 fg	0.5 ±0.06 fg	0.66 ±0.06 fg	0.16 ±0.02 fg

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soy bean; PN=peanut; CN=corn; WN=walnut; AL=almond; SE=sesame; *Means with different letters are statistically different (P<0.05).

Table 2 Lipase activity mean values (±SD) of walnut seeds on different substrates

Lipase source		Walnut fruits								
Substrate (oil)		pH	SF	PK	SB	PN	CN	WN	AL	SE
LA (µmol oleic acid/ g/min.	20°C	pH= 5.4	6.06 ±0.7 ab*	6.16 ±0.48 ab	5.33 ±0.5 c*	4.9 ±0.38 C	5.66 ±0.67 C	6.33 ±0.55 ab	5.23 ±0.38 c	4.33 ±0.33 Cd
		pH= 7.4	2.06 ±0.18 ef	2.16 ±0.24 ef	3 ±0.41 de	3 ±0.32 de	3.56 ±0.4 cd	3.66 ±0.38 cd	4 ±0.4 cd	2.9 ±0.14 de
		pH= 8.2	2.06 ±0.15 de	0.5 ±0.04 fg	1 ±0.07 ef	1.23 ±0.12 ef	0.33 ±0.29 fg	0.66 ±0.53 fg	0.9 ±0.07 fg	0.83 ±0.09 fg
LA (µmol oleic acid/ g/min.	40°C	pH= 5.4	6.16 ±0.65 ab	6.66 ±0.48 ab	5.83 ±0.63 c	6 ±0.49 ab	6.33 ±0.65 ab	7.33 ±0.62 a	5.83 ±0.46 c	5.33 ±0.57 c
		pH= 7.4	3.33 ±0.35 de	2.33 ±0.28 ef	3 ±0.27 de	2 ±0.16 ef	1.83± 0.2 ef	3.33 ±0.41 de	3.66± 0.29cd	3.33 ±0.3 de
		pH= 8.2	1.66 ±0.14 ef	1.33 ±0.09 ef	0.83 ±0.09 fg	1.66 ±0.19 ef	0.66± 0.07 fg	1 ±0.08 ef	1.16 ±0.2 ef	1.33 ±0.17 ef

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soy bean; PN=peanut; CN=corn; WN=walnut; AL=almond; SE=sesame; *Means with different letters are statistically diferent (P<0.05)

From Tab. 2 it can be seen that, at 20°C, and pH 5.4, the lipase from walnut (core) had the highest activity on: walnut, pumpkin and sunflower seed oils, where it has recorded significantly higher values, compared to corn, soy, almond and peanut seed oils (P<0.05). At 20°C, and pH 7.4 the walnut lipase had its highest activity on almond, walnut and corn oils (with close values), and at pH 8.2 on sunflower oil (P<0.05).

At 40°C the walnut lipase had the highest values of activity at pH 5.4, on its own substrate (walnut oil), followed by a significant lower activity on: pumpkin, corn, sunflower and peanut oils (P<0.05). At pH 7.4 the walnut lipase had the largest activity on almond oil, followed by activity on: walnut, sesame, sunflower and soybean oils, while at pH 8.2 higher activities were registered on: sunflower, peanut, pumpkin and sesame (P<0.05).

According to Tab. 2, at 20°C and 40°C the lipase derived from walnut has registered the highest activity at pH 5.4, and the lowest one at pH 8.2.

In the Table 3 is rendered the almond seeds lipase activity on analyzed substrates (oils).

Table 3 Lipase activity mean values (±SD) of almond seeds on different substrates

Lipase source		Almond seeds								
Substrate (oil)			SF	PK	SB	PN	CN	WN	AL	SE
LA (µmol oleic acid/ g/min.	20°C	pH= 5.4	3.73 ±0.21 cd*	4.5 ±0.48 cd	4.33 ±0.35 cd	4.56 ±0.44 cd	5.33 ±0.39 c*	5.16 ±0.52 c	3.56 ±0.4 cd	4.66 ±0.51 cd
		pH= 7.4	2.23 ±0.19 ef	1.73 ±0.2 ef	2.33±0. 25 ef	2 ±0.15 ef	1.9 ±0.22 ef	1.5 ±0.1 ef	4 ±0.39 cd	1.56 ±0.17 ef
		pH= 8.2	1.4 ±0.18 ef	1.33 ±0.13 ef	1.33 ±0.21 ef	1.23 ±0.16 ef	0.66 ±0.07 ef	1.5 ±0.12 ef	0.9 ±0.06 ef	1.16 ±0.18 ef
LA (µmol oleic acid/ g/min.	40°C	pH= 5.4	5 ±0.53 c	5.16 ±0.47 c	5.33 ±0.59 c	4.83 ±0.41 C	6.33 ±0.55 ab	6 ±0.72 ab	4.16 ±0.38 dc	5.5 ±0.44 C
		pH= 7.4	2.33 ±0.25 ef	3 ±0.26 de	1.83 ±0.22 ef	2.66 ±0.31 de	2.5 ±0.16 de	2 ±0.19 ef	2.66 ±0.3 de	1.5 ±0.12 ef
		pH= 8.2	2.66 ±0.18 de	3.66 ±0.31 cd	0.66 ±0.04 fg	0.66 ±0.05 fg	1.66 ±0.14 ef	1.33±0. 15 ef	0.66 ±0.07 fg	1.2 ±0.17 ef

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soy bean; PN=peanut; CN=corn; WN=walnut; AL=almond; SE=sesame; *Means with different letters are statistically different (P<0.05).

As seen in the table 3, at 20°C, and pH 5.4, the lipase from almond seeds had an activity significant higher on corn and walnut oils, then on the other six oils, where the enzyme registered close values (P<0.05). Also at 20°C, compared to pH 5.4, both at pH 7.4 and pH 8.2 the almond seeds lipase recorded significantly lower activities on all oils, except the almond oil at pH 7.4 (P<0.05).

At 40°C the lipase from almond seeds had the highest rates of activity, at pH 5.4, on corn and walnut oils, followed by: sesame, soybean, pumpkin and sunflower oils, with significant lower values (P<0.05). At pH 7.4 the almond seeds lipase had a greater activity on pumpkin, peanut, almond and corn oils, and at pH 8.2 on pumpkin oil (P<0.05).

Analyzing the data of Tab. 3, it results that the almond seeds lipase had the highest activity at pH 5.4, on corn and walnut oils (at 40°C), and the lowest one at pH 8.2 at both temperatures.

In the Table 4 is reproduced the sesame seeds lipase activity on analyzed substrates (oils).

At 20°C and pH 5.5, the sesame seeds lipase recorded close values of its activity on all the eight oils analyzed. Compared to pH 5.5, at pH 7.4 and pH 8.2 the lipase from sesame seeds had activities significantly reduced, with higher values on almond oil, both at pH 7.4 and pH 8.2 (P<0.05).

Table 4 Lipase activity mean values (±SD) of sesame seeds on different substrates

Lipase source		Sesame seeds								
Substrate (oil)			SF	PK	SB	PN	CN	WN	AL	SE
LA (µmol oleic acid/ g/min.	20°C	pH= 5.4	4.4 ±0.56 cd	4.66 ±0.39 cd	3.83 ±0.42 cd	3.73 ±0.28 cd	4 ±0.47 cd	4.66 ±0.35 cd	4.56 ±0.51 cd	4.33 ±0.33 cd
		pH= 7.4	0.73 ±0.08 fg*	1.16 ±0.1 ef*	1.16 ±0.09 ef	1 ±0.13 ef	1.23 ±0.11 ef	0.66 ±0.05 fg	3.33 ±0.38 de	0.93 ±0.08 ef
		pH= 8.2	1.06 ±0.07 ef	0.5 ±0.06 fg	1.16 ±0.1 ef	0.56 ±0.06 fg	0.83 ±0.09 fg	0.83 ±0.06 fg	1.56 ±0.12 ef	1 ±0.07 ef
LA (µmol oleic acid/ g/min.	40°C	pH= 5.4	5.33 ±0.47 c	5.5 ±0.51 c	4.5 ±0.55 cd	4.66 ±0.38 cd	5 ±0.42 c	5.16 ±0.49 c	4.83 ±0.52 c	5.66 ±0.29 c
		pH= 7.4	1.66 ±0.15 ef	1.5 ±0.19 ef	2 ±0.16 ef	1.66 ±0.09 ef	2.16 ±0.27 ef	1.83 ±0.21 ef	2.33 ±0.1 ef	1.33 ±0.14 ef
		pH= 8.2	0.83 ±0.05 fg	1 ±0.09 ef	0.5 ±0.04 fg	1.66 ±0.2 ef	1.33 ±0.17 ef	1.33 ±0.09 ef	1.5 ±0.12 ef	1.16 ±0.3 ef

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soy bean; PN=peanut; CN=corn; WN=walnut; AL=almond; SE=sesame; *Means with different letters are statistically diferent (P<0.05).

At 40°C the lipase from sesame seeds had the highest activity at pH 5.4, on oils of: sesame (own substrate), pumpkin, sunflower, walnut, corn, and almond, followed by peanut and soybean oils with values significantly lower (P<0.05). Compared to pH 5.4, at pH 7.4 and pH 8.2, the sesame seeds lipase recorded lower values of its activity on all the eight oils analyzed.

The data of Tab. 4 show that the sesame seeds lipase recorded the highest activity at pH 5.4, on its own substrate (sesame oil), at 40°C, the lowest activity being registered at pH 8.2, at the both temperatures used.

Comparing the data from the four tables, one can see that lipases coming from peanut, walnut, almond and sesame seeds, have registered, on the eight refined oils, the highest values of activity at pH 5.4 (at 40°C).

The Fig. 1 renders the evolution of lipase activity from peanut, walnut, almond and sesame seeds, at pH 5.4, on the eight oils.

As seen from Tables 1-4 and Fig. 1, at pH 5.4, compared to the other three sources, the values of walnut lipase activity were higher (at 20°C and/or 40°C) on the oils of: sunflower, pumpkin, soy bean, walnut and almond, but significantly higher activities were recorded on the oils of: sunflower, pumpkin, soy bean and walnut - the highest one being recorded on walnut oil, at 40°C (P<0.05).

At the same pH (5.4), the peanut seeds lipase recorded the highest activity on peanut oil, the almond seeds lipase on corn and walnut oils, and the sesame seeds lipase on sesame and pumpkin oils - in all cases at 40°C.

On their own substrate, the highest activity was registered, at 40°C, by walnut lipase, followed, in order, by peanut seeds lipase, by sesame seeds lipase, and by almond seeds lipase (Fig. 1).

According to some authors [5, 9, ct. by 2], with some exceptions, oilseed lipases are generally more active with triacylglycerols containing short chain fatty acids.

Within various refined oils, the oleic acid content ranges between 17 and 67 (wt%), with higher values in oils of: peanut, sesame and pumpkin, followed by corn, sunflower, almond, soybean and walnut, the linoleic acid varies between 14 and 74 (wt%), with higher values in oils of: sunflower, walnut, soybean, corn and pumpkin seeds, and lower ones in sesame, almond and peanut oils, and the linolenic acid ranges between 0,5 to 14 (wt%), being present in a higher quantity in walnut oil, and less in soybean and sunflower oils [1, 7, 10, 13, 18, 19, 26].

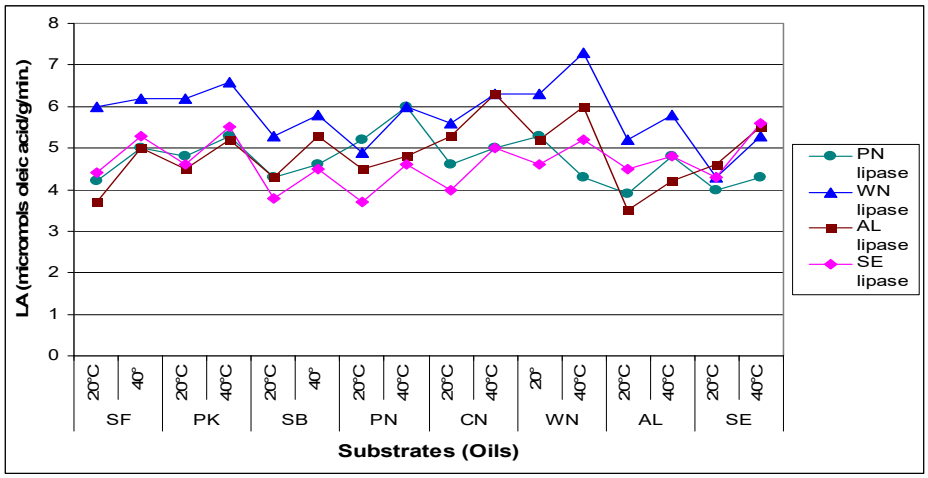

Fig. 1 The evolution of lipase activity values from peanut, walnut, almond and sesame seeds, at pH 5.4, on the eight substrates (oils) analyzed
SF=sunflower; PK=pumpkin; SB=soy bean; PN=peanut; CN=corn; WN=walnut; AL=almond; SE=sesame

In this paper, higher values of lipase activity from walnut, as compared to the other three sources (peanut, almond and sesame), could be correlated with the chemical composition of the analyzed oils. Thus, an increased content of linoleic and linolenic acid, but lower of oleic acid was the substrate (walnut oil) that walnut lipase had the highest activity at pH 5.4 and 40°C.

A high content of linoleic acid (sunflower and soybean oils), on one hand, and a high content of oleic acid (pumpkin oil), on the other hand, made walnut lipase to have, an increased activity on sunflower and soybean oils (at pH 5.4 - 20°C and 40°C), respectively on pumpkin oil (at pH 5.4 - 20°C and 40°C).

CONCLUSIONS

Analyzing the activity of lipases coming from four plant sources (peanut, walnut, almond and sesame seeds), on eight refined oils (sunflower, pumpkin, soy bean, peanut, corn, walnut, almond and sesame), the highest activity of those enzymes was at pH 5.4, at 40°C.

As compared to lipase activity from peanut, almond and sesame seeds, the values of walnut lipase activity were significantly higher at pH 5.4 (at 20°C and 40°C), on the oils of: sunflower, pumpkin, soy bean and walnut - the highest activity being recorded on walnut oil at 40°C.

Of the four types of lipase analyzed, the highest activity on its own substrate was registered, at 40°C, by walnut lipase.

Both the temperature, but especially pH and chemical composition of substrate (oil) have influenced the activity of lipases derived from peanut, walnut, almond and sesame.

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