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THE INFLUENCE OF SUBSTRATE AND TEMPERATURE ON LIPASE ACTIVITY IN SOME PLANT SPECIES

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Abstract. *The purpose of this work was to study the lipases coming from six plant sources, to see to what extent the substrate (oil) and/or temperature influence the activity of this enzyme, and which of these sources shows a higher activity. The biological materials, used as lipase sources (provided by Suceava Genebank and by Suceava Agricultural Research and Development Station), have been represented by seeds belonging to the following plant species: sunflower (Helianthus annuus L., hd. Rapid), pumpkin (Cucurbita maxima L., local variety), soy (Glycine max L., var. Turda 6114), peanut (Arachis hypogaea L., var. Dabuleni), corn (Zea mays L., hd. F 376), and walnut (Juglans regia L., var. Bratia). As substrates for enzyme activity, were used the following refined oils: sunflower, pumpkin, soy bean, peanut, corn, and walnut, purchased from supermarkets. The research method 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. At pH 5.4 and 20°C the lipase from sunflower seeds has registered the highest activity on soybean oil. At the same pH, but at 40°C, the lipase from walnut kernels has registered the highest activity on walnut oil. On its own substrate, the highest activity was registered, at 20°C, by walnut and sunflower lipase, while at 40°C by walnut lipase. It seems that a higher content of linoleic and linolenic acids of some oils (used as substrates), as well as the enzyme chemical composition and spatial configuration caused an increased activity of sunflower and walnut lipase, as compared to the other enzyme sources analyzed.*

INTRODUCTION

The lipases (triacylglycerol hydrolases, EC 3.1.1.3) are enzymes of the hydrolase class, esterase subclass, which catalyses the hydrolysis of glycerides releasing, finally, fatty acids and organic alcohols. Glycerides are most widespread in the animal kingdom and vegetable fat. They are substances spare that accumulate in fat tissue of animals and the plants, seeds (sunflower, castor, poppy, cotton, pumpkin) and fruit (olive, peanuts, almonds) (*Oprica, 2011*).

Lipases may have various origins (animal, vegetable or microbial), playing 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 (*Barros et al*., *2010; Donato et al., 2008; Enujiugha et al., 2004; Freire and Castilho, 2008; Gandhi, 1997; Hasan et al., 2006; Isbilir et al., 2008; Paques and Macedo, 2006; Pastore et al., 2003; Polizelli et al., 2008; Sharma et al., 2001; Yesiloglu and Baskurt, 2008*).

According to *Barros et al.* (2010), lipases are employed in food manufacturing to liberate fatty acids into food products by selective hydrolysis of the fats and oils present in many kinds of food. Depending on the carbon chain length and on the degree of unsaturation, the fatty acid obtained provides the food with flavors, colors and unusual smells, playing an important role in the physical-chemical, organoleptic and nutritional properties of many products (*Freire et al., 2008; Gandhi, 1997; Sharma et al.*, 2001) ct. by *Barros et al*. (2010).

The commercial lipases are generally produced from microorganisms (*Penicillium* spp., *Geotrichum* spp., *Aspergillus* spp., *Rhizomucor* spp., *Candida* spp. or *Pseudomonas* spp.) or animals, such as pancreatic and pregastric tissues of ruminants (*Baumann et al., 2000; Cardenas et al., 2001; Kilcawley et al., 2002; Steenkamp and Brady, 2003*).

The use of microbial lipases on an industrial scale is still restricted due to high production costs, favoring the search for other sources of these enzymes (*Paques and Macedo, 2006*). 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 (*Paques and Macedo, 2006, Hellyer et al., 1999; Villeneuve, 2003; Enujiugha et al., 2004; Polizelli et al., 2008*).

Thanks to their many advantages, the seed lipases aroused last years an increasing interest among scientists. That is why, the purpose of this work was to study the lipases coming from six plant sources, to see to what extent the substrate (oil) and/or the temperature influences the activity of this enzyme, and which of these sources shows a higher activity.

MATERIALS AND METHODS

Research materials. The biological materials, used as lipase sources, 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: sunflower (*Helianthus annuus* L., hd. Rapid), pumpkin (*Cucurbita maxima* L., local variety), soy (*Glycine max* L., var. Turda 6114), peanut (*Arachis hypogaea* L., var. Dabuleni), corn (*Zea mays* L., hd. F 376), and walnut (*Juglans regia* L., var. Bratia). As substrates for enzyme activity, were used the following refined oils: sunflower, pumpkin, soybean, peanut, com, and walnut, purchased from supermarkets.

Research methods. Each *enzyme sample* was prepared in glass bottle with stopper where it mixed 2 g seeds finely divided with two sides ether, then let stand for 2 hours for oil extraction, stirring periodically. It has separated ether, and they have introduced again 6 parts ether over the product partly skimmed, for 2 hours, after which it was separated ether. The skimmed seeds were dried in an oven with fan, at a temperature of 28°C. There were obtained defatted seeds containing lipase.

Since many previous attempts to determine the lipase activity, carried out at different pH (5.4, 7.4 and 8.2) and at 20°C and 40°C, showed that at pH 5.4 were obtained the highest values, the lipase activity was determined at 20ºC and 40ºC, and at pH 5.4 for each temperature. The method 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 (*Bordei et al., 2007*). The lipase activity (LA) 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

Statistical analysis. The data of experiments, consisting in 4 replicates for each determination, were statistically processed using SAS Version 8.02 (*SAS Institute, 2005*). 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

Tables 1-6 reproduce the lipase activity of the six seeds species on the six various substrates (oils).

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

SF lipase activity	Temp.	pH	Refined oils					
			SF	PK	SB	PN	CN	WN
LA (µmol oleic acid/ g/min.)	20°C	5.4	6.3± 0.8ab*	5.18± 0.39c*	8.70± 0.69a	6.58± 0.47ab	6.64± 0.08ab	7.35± 1.07ab
LA (µmol oleic acid/g/min.)	40°C	5.4	7.02± 0.83ab	5.64± 0.38c	6.9± 1.05ab	7.25± 0.78ab	6.34± 0.62ab	6.63± 0.71ab

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

As seen in table 1, at 20°C and pH 5.4 sunflower lipase has registered significant differences ($P<0.05$) between its activity on soybean oil (with highest value), and the activity on walnut, corn, peanuts and sunflower oils (with close values).

At the same pH, but at 40°C, the highest values of sunflower lipase activity were registered, in order, on peanut, sunflower and soybean, corn and walnut oils (with close values), followed by pumpkin ($P<0.05$).

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

PK lipase activity	Temp.	pH	Refined oils					
			SF	PK	SB	PN	CN	WN
LA (µmol oleic acid/ g/min.)	20°C	5.4	4.4± 0.32c	4.36± 0.52c	4.35± 0.36c	5.3± 0.42c	4.7± 0.48c	5.37± 0.55c*
LA (µmol oleic acid/g/min.)	40°C	5.4	4.68± 0.41c	3.72± 0.3cd	5.02± 0.45c	4.2± 0.53cd	5.1± 0.45c	6.36± 0.5ab*

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

At 20°C and pH 5.4, the activity of lipase from pumpkin seeds has registered no significant differences between samples, even if the highest activity was on walnut and peanut oils, followed by corn, sunflower, pumpkin (own substrate) and soybean (Table 2).

From the same Table 2, one can see that at 40°C the pumpkin seeds lipase had the highest activity on walnut oil, followed by soybean, corn, and sunflower oils, the lowest activity being on peanut and pumpkin oils (P<0.05).

Table 3. Lipase activity (mean values ± SD) of walnut kernels on different substrates

WN lipase activity	Temp.	pH	Refined oils					
			SF	PK	SB	PN	CN	WN
LA (µmol oleic acid/ g/min.)	20°C	5.4	6.16± 0.68ab*	6.18± 0.5ab	5.35± 0.52c*	4.93 ±0.4c	5.7± 0.65c	6.35± 0.48ab
LA (µmol oleic acid/g/min.)	40°C	5.4	6.2± 0.64ab	6.59± 0.5ab	5.86± 0.63c	6.1± 0.5ab	6.23± 0.55ab	7.98± 0.67a

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soybean; PN=peanut; CN=com; WN=walnut; Temp = temperature ;*Means with different letters are statistically diferent (P<0.05).

In the Table 3. at 20°C, and pH 5.4, the lipase from walnut (kernels) had the highest activity on walnut, pumpkin and sunflower seed oils, where it recorded significantly higher values, compared to corn, soybean and peanut seed oils (P<0.05).

At 40°C the walnut lipase registered the highest activity on its own substrate (walnut oil), followed by a significant lower activity on: pumpkin, corn, sunflower and peanut oils (P<0.05).

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

PN lipase activity	Temp.	pH	Refined oils					
			SF	PK	SB	PN	CN	WN
LA (µmol oleic acid/ g/min.)	20°C	5.4	4.25± 0.47cd*	4.9± 0.42c*	4.36± 0.5cd	5.27± 0.44c	4.7± 0.48cd	5.41± 0.63c
LA (µmol oleic acid/g/min.)	40°C	5.4	5.12± 0.43c	5.36± 0.55c	4.7± 0.44cd	6.14± 0.69 ab	5.15± 0.4c	4.37± 0.42cd

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soybean; PN=peanut; CN=com; WN=walnut; Temp = temperature ;*Means with different letters are statistically diferent (P<0.05).

As seen in Table 4, at pH 5.4 and 20°C the peanut seeds lipase activity has registered significant higher values on walnut, peanut and pumpkin oils, as compared to corn, soybean, and sunflower oils (P<0.05).

At 40°C, the highest value of peanut lipase activity was recorded on peanut oil (own substrate), followed by pumpkin, corn, sunflower, soybean and walnut oils (P<0.05).

Table 5. Lipase activity (mean values ± SD) of corn caryopses on different substrates

CN lipase activity	Temp.	pH	Refined oils					
			SF	PK	SB	PN	CN	WN
LA (µmol oleic acid/ g/min.)	20°C	5.4	4.68± 0.52c	3.82± 0.4cd*	4.7± 0.38c*	4.61± 0.54c	4.38± 0.26c	5.4± 0.63c
LA (µmol oleic acid/g/min.)	40°C	5.4	5.03± 0.58c	4.7± 0.42c	4,1± 0.45cd	4.7± 0.39c	5.36± 0.7c	3.68± 0.4cd

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soybean; PN=peanut; CN=corn; WN=walnut; Temp = temperature ;*Means with different letters are statistically diferent (P<0.05).

Analysing the data of Table 5, one can see that, at 20°C, and pH 5.4, the activity of corn lipase has registered values without significant differences on: walnut, sunflower, soybean, peanut, and corn oils, as compared to pumpkin oil where its activity was lower (P<0.05).

At 40°C and pH 5.4, the corn lipase had the highest activity on corn, sunflower, pumpkin and peanut (with close values), as compared to soybean and walnut oils (P<0.05).

Table 6. Lipase activity (mean values ± SD) of soybean on different substrates

SB lipase activity	Temp.	pH	Refined oils					
			SF	PK	SB	PN	CN	WN
LA (µmol oleic acid/ g/min.)	20°C	5.4	3.75± 0.29cd*	3.8± 0.35cd	3.4± 0.31cd	4.73± 0.44c*	4.1± 0.38cd	4.03± 0.47cd
LA (µmol oleic acid/g/min.)	40°C	5.4	4.02± 0.35cd	4.77± 0.28c	3.6± 0.32cd	3.36± 0.41cd	4.9± 0.51c	5.03± 0.48c

SD=standard deviation; LA=lipase activity; SF=sunflower; PK=pumpkin; SB=soybean; PN=peanut; CN=corn; WN=walnut; Temp = temperature ;*Means with different letters are statistically diferent (P<0.05).

In the Table 6, at 20°C and pH 5.5, the soybean lipase had the greatest activity on peanut oil, followed by the other five oils analyzed (including its own substrate), which registered close values, but significantly lower (P<0.05).

At 40°C the soybean lipase registered the highest activity on walnut, corn and pumpkin oils (with close values), followed by sunflower, soybean and peanut oils with significantly lower values (P<0.05).

Fig. 1 reproduces the comparative evolution, at pH 5.4 and 20°C, of the lipase activity from sunflower, pumpkin, soybean, peanut, corn and walnut on the six analyzed substrates (oils).

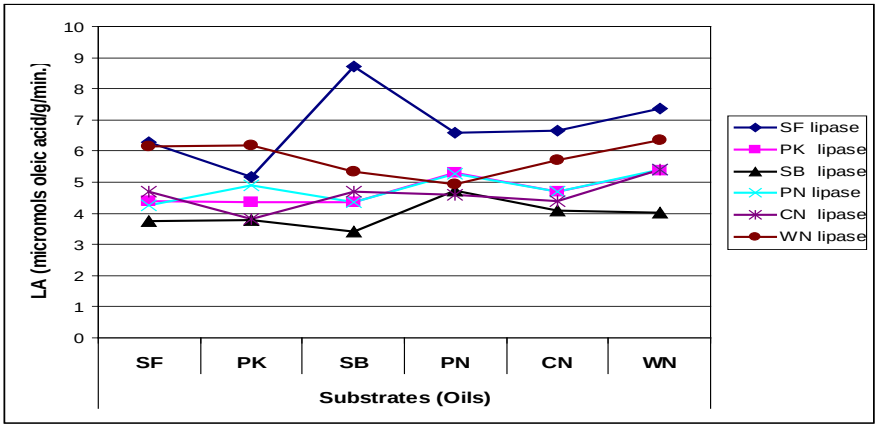

Fig. 1. The evolution of lipase activity values from sunflower, pumpkin, soybean, peanut, corn and walnut, at pH 5.4 and 20°C, on the six analyzed substrates (oils)
SF=sunflower; PK=pumpkin; SB=soybean; PN=peanut; CN=corn; WN=walnut

As seen in Fig. 1, at 20°C, compared to the other enzyme sources, the sunflower lipase (on sunflower, soybean, peanut, corn and walnut oils), and walnut lipase (on sunflower, pumpkin and walnut oils), had higher activities, the highest one being registered (by sunflower lipase) on soybean oil.

On its own substrate, the highest activity was registered by walnut and sunflower lipase, with close values, followed by corn, peanut and pumpkin lipase, also with close values, but significant lower ($P<0.05$).

Fig. 2 reproduces the comparative evolution, at pH 5.4 and 40°C, of the lipase activity from sunflower, pumpkin, soybean, peanut, corn and walnut on the six analyzed substrates (oils).

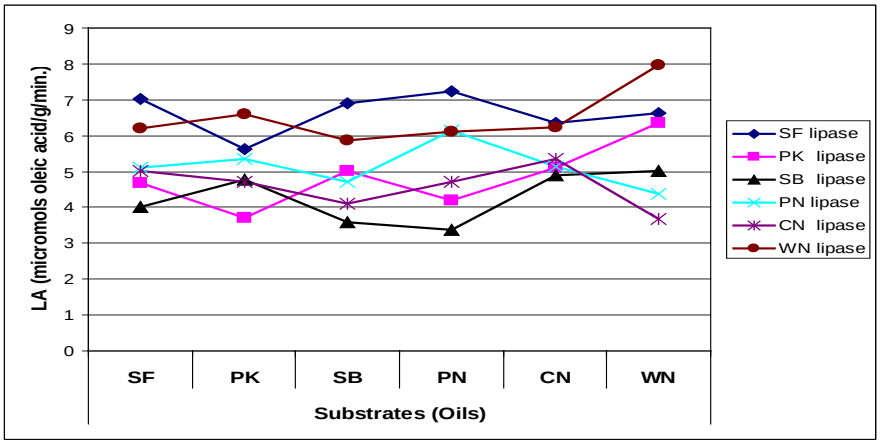

Fig. 2. The evolution of lipase activity values from sunflower, pumpkin, soybean, peanut, corn and walnut, at pH 5.4 and 40°C, on the six analyzed substrates (oils)
SF=sunflower; PK=pumpkin; SB=soybean; PN=peanut; CN=corn; WN=walnut

In the Fig. 2 one can see that, at 40°C, compared to the other enzyme sources, the sunflower lipase (on sunflower, soybean and peanut oils), and walnut lipase (on pumpkin and walnut oils), had higher activities, the highest one being registered by walnut lipase on walnut oil.

On its own substrate, the highest activity was registered by walnut lipase, followed by sunflower and peanut lipase (with close values), but significant lower ($P<0.05$).

Since, under the same conditions of pH, temperature and substrates, the lipases from the other sources (PK, SB, PN and CN) had enzyme activities significantly lower, compared to those of sunflower seeds and walnut kernels, this could be correlated either with chemical composition of analyzed substrates (oils), or with chemical composition and spatial configuration of the enzyme itself, or with the both.

As to chemical composition of substrates, according to some data on vegetable refined oils, published in methods, standards and scientific works (*Orthoefer, 1996; AOCS, 1997; Firestone, 1999; Codex Alimentarius, 1999; Grompone, 2011; Kochlar Prakash, 2011; Tong Wang, 2011*), the linoleic acid content varies between 14 and 74 (wt%), with higher values in sunflower, walnut, soybean, corn and pumpkin oils, and lower ones in peanut, sesame, and almond oils. By the same authors, the linolenic acid ranges between 0,5 to 14 (wt %), with a higher quantity in walnut oil, followed by soybean and sunflower oils, while the oleic acid between 17 and 67 (wt%), with higher values within peanut, sesame and pumpkin oils, and less in corn, sunflower, almond, soybean and walnut.

Thus, an increased content of linoleic and linolenic acid were the substrate (soybean oil) where sunflower seeds lipase had the highest activities at pH 5.4 and 20°C.

It seems that, at 40°C, the chemical composition of oils influenced the lipase activity of the sources analyzed. Thus, the increased content in linoleic and linolenic acids in walnut kernels oil made the lipase of walnut kernels to register the highest enzyme activity.

CONCLUSIONS

Analysing the activity of lipases coming from six plant sources (sunflower seeds, pumpkin seeds, soybean, peanut seeds, corn caryopses and walnut kernels), on six refined oils used as substrates (sunflower, pumpkin, soybean, peanut, corn, and walnut), at pH 5.4, and temperatures of 20°C and 40°C, it could find out a relation between oils chemical composition and the lipase activity.

As compared to the other sources, at pH 5.4 and 20°C the lipase from sunflower seeds has registered the highest activity on soybean oil. At pH 5.4 and 40°C the lipase from walnut kernels has registered the highest activity on walnut oil.

On its own substrate, the highest activity was registered, at 20°C**,** by walnut and by sunflower lipase, and at 40°C by walnut lipase.

It seems that a higher content of linoleic and linolenic acids of some oils (used as substrates), as well as the enzyme chemical composition and spatial configuration caused an increased activity of sunflower and walnut lipase, as compared to the other enzyme sources analyzed.

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