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# COMPARATIVE STUDY ON THE LIPASE ACTIVITY FROM PLANT SOURCES, UNDER VARIOUS CONDITIONS OF PH, TEMPERATURE AND SUBSTRATE

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**Abstract.** *The activity of the lipase from four different plant sources, under various conditions of pH, temperature and substrate, was analyzed* in *this paper to see which of these sources shows the highest enzyme activity. The lipase sources were represented by seeds, belonging to four plant species: sunflower, corn, pumpkin and soy, and as substrates for enzyme activity, the following refined oils were used: sunflower, pumpkin, soy bean, corn, peanut, walnut, almond and sesame. The activity of lipase was determined at 20ºC and 40ºC, at three different pH values (5.4, 7.4 and 8.2) for each temperature, and consisted in titrating (with a solution of KOH 0.01 N) fatty acids released from oils by lipase, in a certain time interval. According to the experimental data, the lipases deriving from sunflower, corn, soy and pumpkin seeds, registered the highest values of activity at pH 5.4 (at 20°C or 40°C). As compared to the other three sources, the values of sunflower seed lipase activity were significantly higher ($P<0.05$) in the following oils: sunflower, soybean, peanut, corn and walnut. At the same pH 5.4, the corn caryopse lipase recorded the highest activity on walnut oil, at 20°C, and on corn oil, at 40°C, the pumpkin seed lipase on walnut oil, at 40°C, and the soy bean lipase on walnut, at 40°C. A high content of oleic acid, but especially of linoleic and linolenic acids within oils used as substrate, caused an increased activity of lipase in sunflower seeds.*

**Keywords**: *lipase, oil, seed, source, pH, temperature, substrate*

## 1. Introduction

Lipases act at the organic-aqueous interface, catalyzing the hydrolysis of ester-carboxylate bonds and releasing fatty acids and organic alcohols [1, 2, 3, 4].
According to some authors [5, 6], in water-restricted environments, the reverse reaction (esterification) or even various transesterification reactions can occur.
Lipases are used in many sectors such as food, pharmaceutical, fine chemical, oil chemical, biodiesel and industrial detergent industries [6, 7], and they will acquire importance comparable to that of the peptidases, which currently represent 25 to 40% of industrial enzyme sales [8].
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 [9]. According to some authors [6, 10, 11] ct. by [9], 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
The origin of lipases can be microbial (bacterial, fungal, etc.), vegetal (oilseeds, pulses, cereals) or animal (gastric, hepatic, pancreatic, adipose tissue).
According to [12], 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.

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 [12, 13, 14, 15, 16].

Considering that in recent years, the lipases from seeds arouses an increasing interest, due to the many advantages they have as compared to the microbial and animal ones, in this paper there was conducted a study of the lipases activity from four different plant sources, in terms of various pH, temperatures and substrates (oils), to see which of these sources shows increased activity and under what conditions.

## 2. Experimental

The experimental materials, 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: sunflower (*Helianthus annuus* L., hd. Rapid), corn (*Zea mays* L., hd. F 376), pumpkin (*Cucurbita maxima* L., local variety) and soy (*Glycine max* L., var. Turda 6114). 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 [17].

*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 an 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, and formed, as result of enzyme action, from a gram of product, in one minute (Eq.1):

$$AL = \frac{(V_p - V_m) \cdot 0.00282}{282 \cdot 10^{-6} \cdot G \cdot T} \qquad (1)$$

where:

$V_p$ – 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;
$V_m$ – 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)
$282 \cdot 10^{-6}$ – 1 micromols (µmol) of oleic acid;
G – the product amount (g) used in experiments;
T – thermostating interval (60 minutes).

The data of experiments, consisting in four replicates for each determination, were statistically processed using SAS Version 8.02 [18]**.** In order to analyze the significance of differences among samples, generalized linear model analysis was carried out, and for multiple comparisons was used Duncan's multiple range test ($P < 0.05$).

## 3. Results and discussion

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

**Table 1**

**Lipase activity mean values (±SD) of sunflower seeds on different substrates**

| Lipase source | | Sunflower seeds | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Refined oils | | | SF | PK | SB | PN | CN | WN | AL | SE |
| LA (µmol oleic acid/ g/min. | 20°C | pH = 5.4 | 6.4± 0.7ab* | 5.16± 0.43c* | 8.66± 0.72a | 6.56± 0.45ab | 6.66± 0.09ab | 7.33± 1.08ab | 5.23± 0.61c | 5.33± 0.44c |
| | | pH = 7.4 | 0.33± 0.02ef | 2.5± 0.11de | 0.16± 0.01ef | 2.5± 0.15de | 1.9± 0.22ef | 4.33± 0.28c | 5.66± 0.47c | 4.9± 0.35c |
| | | pH = 8.2 | 2.06± 0.31de | 1.5± 0.24ef | 1.33± 0.15ef | 0.9± 0.08ef | 1.33± 0.27ef | 1.66± 0.18ef | 0.56± 0.07ef | 0.33± 0.04ef |
| LA (µmol oleic acid/ g/min. | 40°C | pH = 5.4 | 7± 0.9ab | 5.66± 0.44c | 7± 1.02ab | 7.33± 0.88ab | 6.33± 0.59ab | 6.66± 0.76ab | 5.83± 0.52c | 6± 0.63c |
| | | pH = 7.4 | 3.33± 0.57cd | 3.66± 0.42cd | 3± 0.39de | 1.33± 0.11ef | 1.16± 0.13ef | 4± 0.77cd | 4.33± 0.38c | 2± 0.32de |
| | | pH = 8.2 | 2.33± 0.24de | 2.33± 0.48de | 2.66± 0.37de | 2.33± 0.31de | 3± 0.42de | 2.83± 0.33de | 2.5± 0.27de | 2.66± 0.29de |

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 ($P < 0.05$).

At 20°C and pH 5.4 sunflower lipase had the highest activity on soybean oil, followed by activity on walnut, corn, peanuts and sunflower oils (with close values). Between the activity on soybean oil, on one hand, and on walnut, corn, peanuts and sunflower oils, on the other hand, were significant differences ($P < 0.05$). Also at 20°C, but at pH 7.4, sunflower seeds lipase had the highest activity on walnut, almond and sesame oils (with no significant differences between values), followed by activity on pumpkin and peanut oils ($P < 0.05$). At the same temperature, but at pH 8.2, the enzyme activity was low on all oil samples - the largest one being registered on sunflower oil.

At 40°C, and pH 5.4 the highest values of sunflower lipase activity were recorded on peanut, sunflower, soybean, corn and walnut oils (with close values), followed by pumpkin, almond and sesame oils ($P < 0.05$). At pH 7.4 the lipase had the highest activity on almond oil, followed by walnut oil, while at pH 8.2 the activity of enzyme registered low values with no significant differences between oil samples ($P < 0.05$). From Tab. 1 it can notice that at 20°C and 40°C the lipase derived from sunflower seeds 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 activity of lipase was registered on soybean oil at 20°C, and pH 5.4.

The Table 2 reproduces the corn caryopses lipase activity on analyzed substrates (oils). From the Tab. 2 it can see that, at 20°C, and pH 5.4, the lipase from corn caryopses had the highest activity on the oils of: walnut, sunflower, soybean, peanut, sesame and on its own substrate (corn oil), where has recorded significantly higher values than on pumpkin and almond oils ($P < 0.05$). Also at 20°C, at pH 7.4 and pH 8.2 the corn lipase has shown significantly lower activities, compared to pH 5.4. At pH 7.4 the highest activity of enzyme was recorded on almond oil, and at pH 8.2 on peanut oil ($P < 0.05$).

**Table 2**

**Lipase activity mean values (±SD) of corn caryopses on different substrates**

| Lipase source | | Corn caryopses | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Refined oils | | | SF | PK | SB | PN | CN | WN | AL | SE |
| LA (µmol oleic acid/ g/min. | 20°C | pH = 5.4 | 4.73± 0.51c | 3.83± 0.37cd* | 4.66± 0.42c* | 4.56± 0.53c | 4.33± 0.28c | 5.33± 0.55c | 3.9± 0.31cd | 4.5± 0.39c |
| | | pH = 7.4 | 0.73± 0.66fg | 1.16± 0.19ef | 1± 0.23ef | 1.66± 0.19ef | 0.9± 0.08fg | 1.33± 0.12ef | 3± 0.37de | 0.9± 0.08fg |
| | | pH = 8.2 | 0.73± 0.06fg | 0.83± 0.08fg | 1± 0.25ef | 3.23± 0.34cd | 1± 0.16ef | 1.33± 0.2ef | 0.56± 0.04fg | 1.33± 0.15ef |
| LA (µmol oleic acid/ g/min. | 40°C | pH = 5.4 | 5± 0.61c | 4.66± 0.39c | 4± 0.41cd | 4.66± 0.55c | 5.33± 0.62c | 3.66± 0.38cd | 3.5± 0.41cd | 5± 0.47c |
| | | pH = 7.4 | 3± 0.28de | 1± 0.15ef | 1.66± 0.18ef | 2± 0.24de | 1.33± 0.17ef | 1.33± 0.21ef | 4.66± 0.39c | 2± 0.18de |
| | | pH = 8.2 | 0.5± 0.06fg | 0.5± 0.04fg | 0.33± 0.04fg | 1.66± 0.22ef | 0.33± 0.03fg | 3.66± 0.41cd | 0.83± 0.07fg | 0.5± 0.04fg |

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 ($P < 0.05$).

At 40°C the corn lipase had the highest activity also at pH 5.4, on its own substrate (corn oil) and on oils of sunflower, pumpkin, peanut and sesame (with close values), followed by oils of soybean, walnut and almond ($P < 0.05$). At pH 7.4 the corn lipase had the largest activity on almond oil, and at pH 8.2 on walnut oil.

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

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

From the Tab. 3 one can see that at 20°C, and pH 5.4, the lipase from pumpkin seeds had the highest activity on walnut and peanut oils, followed by corn, sunflower, pumpkin (own substrate), soy bean and sesame oils, with no significant differences between samples ($P < 0.05$).

Also at 20°C, but at 7.4 and pH 8.2 the lipase from pumpkin seeds has recorded significantly lower activities, compared to pH 5.4. Both at pH 7.4, and at pH 8.2 the highest activity of enzyme was recorded on walnut and soybean oils ($P < 0.05$).

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

**Table 3**

**Lipase activity mean values (±SD) of pumpkin seeds on different substrates**

| Lipase source | | | Pumpkin seeds | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Refined oils | | | SF | PK | SB | PN | CN | WN | AL | SE |
| LA (µmol oleic acid/ g/min. | 20°C | pH = 5.4 | 4.4± 0.35c | 4.33± 0.47c | 4.33± 0.29c | 5.23± 0.47c | 4.66± 0.42c | 5.33± 0.65c* | 3.9± 0.41cd* | 4.16± 0.49c |
| | | pH = 7.4 | 1.73± 0.22ef | 1.16± 0.18ef | 2± 0.25de | 1.16± 0.16ef | 1.73± 0.12ef | 2± 0.17de | 1.66± 0.18ef | 1.23± 0.1ef |
| | | pH = 8.2 | 0.4± 0.03fg | 0.56± 0.39fg | 1.66± 0.17ef | 0.56± 0.04fg | 0.33± 0.02fg | 1± 0.08ef | 0.56± 0.04fg | 0.33± 0.04fg |
| LA (µmol oleic acid/ g/min. | 40°C | pH = 5.4 | 4.66± 0.37c | 3.66± 0.29cd | 5± 0.43c | 4± 0.51cd | 5± 0.42c | 6.33± 0.55ab | 4.5± 0.37c | 4.33± 0.45c |
| | | pH = 7.4 | 2.33± 0.16de | 2.33± 0.21de | 1.33± 0.11ef | 1.8± 0.16ef | 1.33± 0.12ef | 1± 0.11ef | 1.33± 0.15ef | 1± 0.08ef |
| | | pH = 8.2 | 0.33± 0.02fg | 0.33± 0.04fg | 0.33± 0.02fg | 0.66± 0.05fg | 0.66± 0.06fg | 1.66± 0.12ef | 1.5± 0.13ef | 0.98± 0.09fg |

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 ($P < 0.05$).

Analyzing the data of Tab. 3, it results that the lipase from pumpkin seeds had the highest activity at pH 5.4, on walnut oil (at 40°C), and the lowest one at pH 8.2 at both temperatures. In the Table 4 is reproduced the soy beans lipase activity on analyzed substrates (oils).

**Table 4**

**Lipase activity mean values (±SD) of soy beans on different substrates**

| Lipase source | | | Soy bean | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Refined oils | | | SF | PK | SB | PN | CN | WN | AL | SE |
| LA (µmol oleic acid/ g/min. | 20°C | pH= 5.4 | 3.73± 0.31cd* | 3.83± 0.27cd | 3.33± 0.24cd | 4.56± 0.39c | 4± 0.28cd | 4± 0.41cd | 3.5± 0.36cd | 3.5± 0.3cd |
| | | pH= 7.4 | 2.4± 0.18de* | 2.16± 0.22de | 2± 0.15de | 2± 0.14de | 1.73± 0.12ef | 2± 0.19de | 3.33± 0.36cd | 1.23± 0.09ef |
| | | pH= 8.2 | 2.06± 0.21de | 0.5± 0.04fg | 0.66± 0.04fg | 1.9± 0.2ef | 0.33± 0.29fg | 1.66± 0.18ef | 0.56± 0.04 fg | 0.66± 0.68fg |
| LA (µmol oleic acid/ g/min. | 40°C | pH= 5.4 | 4± 0.39cd | 4.16± 0.35c | 3.5± 0.29cd | 3.33± 0.36cd | 4.66± 0.42c | 5± 0.56c | 4.16± 0.51c | 4± 0.3cd |
| | | pH= 7.4 | 2.66± 0.28de | 2.33± 0.19de | 2.33± 0.25de | 1.33± 0.1ef | 1.5± 0.12ef | 2± 0.22de | 2.33± 0.18de | 2± 0.23de |
| | | pH= 8.2 | 3.16± 0.34cd | 0.66± 0.05fg | 0.83± 0.09fg | 0.66± 0.04fg | 0.33± 0.02fg | 1± 0.09ef | 0.83± 0.07fg | 1± 0.12ef |

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 ($P < 0.05$).

At 20°C and pH 5.5, the soy bean lipase had the greatest activity on peanut oil, followed by the other seven oil analyzed (including its own substrate), which registered close values, but significantly lower ($P < 0.05$). Compared to pH 5.5, at pH 7.4 and pH 8.2 the lipase from soy bean had activities significantly reduced, with a higher value (at pH 7.4) on almond oil ($P < 0.05$).

At 40°C the soybean lipase had the highest values at pH 5.4, on oils of: walnut, corn, pumpkin and almond, followed by oils of: sunflower, sesame, soybean and peanut, with values significantly lower ($P < 0.05$).

At pH 7.4 the soybean enzyme had a greater activity on oils of: sunflower,

pumpkin, soybean, walnut, almond and sesame, and at pH 8.2 on sunflower oil ($P < 0.05$).

The data of Tab. 4 show that the soybean lipase recorded the highest activity at pH 5.4, on peanut oil (at 20°C), on walnut, corn, pumpkin and almond oils (at 40°C), the lowest values being registered at pH 8.2, at the both temperatures.

Comparing the data from the four tables, one can see that lipases coming from sunflower, corn, soy and pumpkin seeds, have registered, on the eight refined oils, the highest values of activity at pH 5.4 (at 20°C or 40°C).

The Fig. 1 renders the evolution of lipase activity from sunflower, corn, soy and pumpkin seeds, at pH 5.4, on the eight oils.

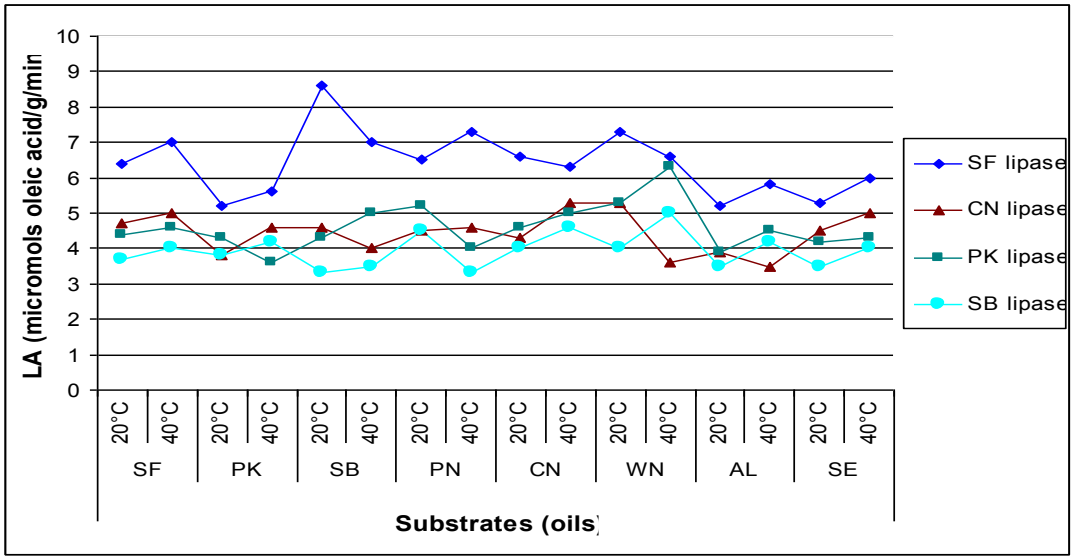


**Fig. 1. The evolution of lipase activity values from sunflower, corn, soy and pumpkin seeds, at pH 5.4, on the eight substrates (oils) analyzed**

SF = sunflower; PK = pumpkin; SB = soy bean; PN = peanut; CN = corn; WN = walnut; AL = almond; SE = sesame

As seen from Tables 1-4 and Fig. 1, at pH 5.4, compared to the other three sources, the values of sunflower seeds lipase activity were higher (at 20°C or 40°C) on all eight oils analyzed, but significantly higher activities were recorded on the oils of: sunflower, soybean, peanut, corn and walnut – the highest one being on soy bean oil, at 20°C ($P < 0.05$). At the same pH (5.4), the corn caryopses lipase recorded its highest activity on walnut oil, at 20°C, and on corn oil, at 40°C, the pumpkin seed lipase on walnut oil, at 40°C, and the soy bean lipase on walnut, at 40°C. On their own substrate, the highest activity was registered by sunflower seed lipase, at 40°C, followed by corn caryopses lipase at 40°C, by pumpkin seed lipase at 20°C, and by soy bean lipase at 40°C (Fig. 1). With some exceptions, oilseed lipases are generally more active with triacylglycerols containing short chain fatty acids [9, 15, 19]. Studying the physical-chemical properties of purified sunflower seed lipase, Sagiroglu and Arabaci [20] observed that the enzyme showed a preference for triacylglycerols with mono-unsaturated fatty acids, a high temperature of 50ºC and a high pH value of 7.5. After some data published in scientific papers and some methods and standards for vegetable oils [21, 22, 23, 24, 25, 26, 27], the oleic acid content of various refined oils ranges between 17 and 67 (wt%), with higher values within oils of: peanut, sesame and pumpkin, followed by corn, sunflower, almond, soybean and walnut. According to the same sources, the linoleic acid content 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, followed by soybean and sunflower oils. In this paper, higher values of lipase activity in sunflower seeds, as compared to the other three sources, could be correlated with the chemical composition of the analyzed oils. Thus, an increased content of linoleic and linolenic acid, but a lower content of oleic acid was the substrate (soybean oil) that sunflower seeds lipase had the highest activity at pH 5.4 and 20°C. A high content of linoleic and linolenic acid, but lower of oleic acid (walnut oil), and a high content of oleic acid but less of linoleic and linolenic acid (peanut oil), made sunflower seeds lipase to have also an increased activity, at pH 5.4 and 20°C (on walnut oil) or 40°C (on peanut oil).

According to Hilditch and Williams [28] ct. by [9], cereal grains contain from 2 to 10% of lipids, depending on the species and variety, and about 80 to 90% of the triacylglycerol fatty acids are oleic and linoleic. Corn lipase presented greater activity with the triacylglycerols containing oleic and linolenic acids, which are the main constituents of corn oil [29, 30, 31]. Compared with the other oils analyzed, it seems that sunflower seeds oil has a high content of linoleic acid, average of oleic acid, and lower of linolenic acid [21, 22, 23, 24, 25, 26, 27].

## 4. Conclusions

Analyzing the activity of lipases coming from four plant sources (sunflower seeds, pumpkin, seeds, corn caryopses and soy bean), on eight refined oils (sunflower, pumpkin, soy bean, peanut, corn, walnut, almond and sesame), the highest activity of those enzymes were at pH 5.4, at 20°C or 40°C.

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

A high content of oleic acid, but especially of linoleic and linolenic acids within oils used as substrate, caused an increased activity of sunflower seeds lipase.

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

## 5. Acknowledgments

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