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Enzymatic Analysis Of Activity Of Metal Ions On Soybean(*Glycine Max*) lipase

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Abstract:*Considering the fundamental and practical aspect regarding the lipase activity,we studied the action of various pH and metal ions (Ca^{+2}, Mg^{+2}, Na^{+}) on the activity of catalyzing property of crude lipase extracted from germinating soya bean. The pH 8.0 was found to yield the maximumlipase activity when compared with other fractionsof pH.Theaddition of divalent cations improved the overall activity, when compared to the control and other metal ions.It was concluded that the pH 8.0and divalent cation as activator improved the lipase activity.*

Keywords – *activators, enzyme kinetics, lipase, metal ions, soya-bean lipase*

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I. Introduction

1.1. Soya bean
Kingdom: Plantae
Sub-kingdom: angiosperms
Order: Fabales
Family: Fabaceae
Subfamily: Faboidae
Genus: Glycine
Species: *G. max*

Soya bean or soybean is a species of legume native to Eastern Asia. It is a terrestrial plant found in tropical regions. It is classified as oil seed rather than a pulse due to its high oil content and its more popular use as a source of vegetable oil and industrial application such as biodiesel. Soya bean contains 20% of total fat (rich in omega 6), 36% protein, 30% carbohydrates and 9% water. It is one of the leading crop commodity produced traded and utilized globally.Lipase enzymes have become more and more prominent on the enzyme biotechnology scenario due to their versatility for hydrolysis and synthesis, their catalytic reactions often being

chemo-selective, region-selective or enantio-selective. Lipases are used in many sectors such as the food, pharmaceutical, fine chemical, oil chemical, biodiesel and industrial detergent industries (Freire and Castilho 2008, Alonso et al. 2005). The participation of lipases in the worldwide enzyme industry market has grown significantly and it is believed that, in the future, they will acquire importance comparable to that of the peptidases, which currently represent 25 to 40% of industrial enzyme sales (Hasan et al. 2006).

1.2. Lipase

Lipases are widespread in nature and have been found in animals, higher plants and microorganisms. In plants lipase activity has been identified in various tissues but relatively high concentration is found in seeds. Seeds are generally rich in triacylglycerols, which serve as compact source of energy for the newly emerging plant. During germination of the seed, the reserved triacylglycerols are disappeared since the fatty acids can't be oxidized to provide energy until they are released from the triacylglycerol. Lipases are probably rate controlling during germination and the activity of the lipase is high during germination (Brockerhoff and Jensen, 1974; Ejedegba et al., 2007). Recently, seed lipases have been the focus of much attention as biocatalysts. In some cases, these enzymes 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 (Barros et al., 2010).

Lipase (triacylglycerol acylhydrolase EC 3.1.1.3.) is a versatile hydrolase that uses triglycerides as its natural substrate. Soybean lipases are well known and characterized. They are generally more active at pH levels close to neutrality, with an optimum temperature of 30ºC and specificity for short and medium chain fatty acids. Triacylglycerols are the main substrates for lipases. Lipases are used in various industries like detergent industry as additives in washing powder, in textile industry to increase the absorbency of the fabrics also for synthesis of biodegradable polymers and for different trans esterification reactions. Lipase enzyme has also found use as a catalyst for production of different products used in cosmetic industry in pulp and paper industry in synthesis of biodiesel degreasing of leather and also in pharmaceutical industry.Inthe present project we report the effect of cations on the activity of Lipase.

1.3. ACTIVATORS AND INHIBITORS

For many enzymes (such as those which actively cleave other molecules) it would be wasteful or even dangerous for them to be active constantly. To avoid this, they are often produced in an inactive state in which they cannot interact with their substrate. They become active once their enzyme activator binds to the enzyme (at a different point to the active site), and thus alters the structure of the whole enzyme. This alters the structure of the active siteand allows it to bind to its substrate and act. Thus, it initiates or increases the activity of the enzyme. Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. There are a variety of types of inhibitors including: nonspecific, irreversible, reversible - competitive and non-competitive. Both these substances can be organic or inorganic in nature. In this case, we will focus on the inorganic ones, particularly metal ions. This study involved effect of three metal ions namely, Ca^{+2}, Na^{+} and Mg^{+2} on the activity of lipase.

1.4. PRACTICAL APPLICATION

The aim of the present article is to study the optimum pH and metallic ion activators of the crude lipase extracted from germinating soya bean. These data can have practical application in food industries, pharmaceutical industries as well as in detergent industries.

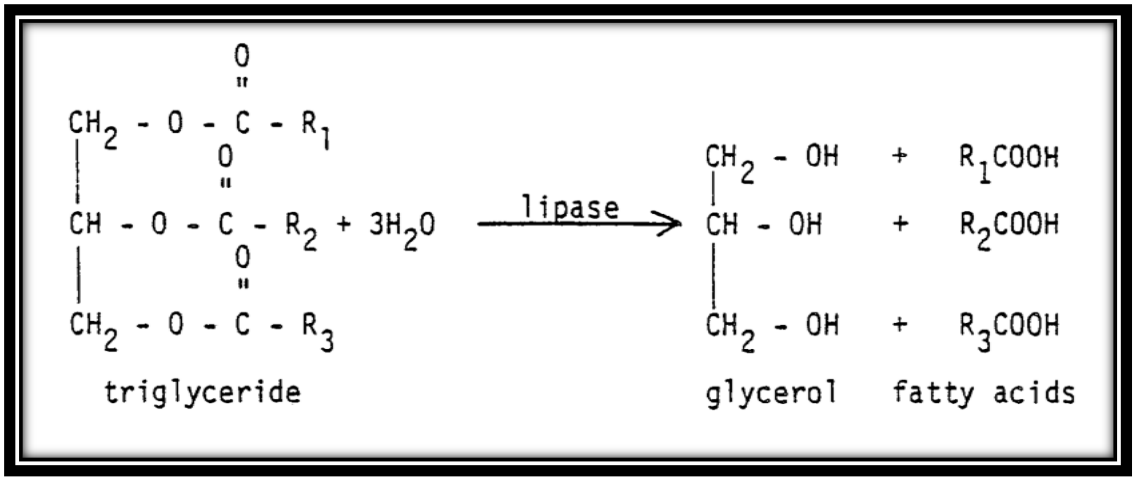
II. Material And Methods

a. Extraction of crude Lipase enzyme from Germinating Soybean by Acetone Precipitation Method

1. The endosperm was removed and weighed for 20.0 gm and washed with distilled water.
2. The endosperm was then crushed in mortar and pestle with Tris buffer of pH 8.0.
3. The suspension was then filtered and enzyme was precipitated with cold acetone.
4. The suspension was then centrifuged at 2700 rpm for 3 mins. Discard supernatant and repeat this step.
5. The pellet obtained is suspended in Tris buffer of pH 8.

b. ESTIMATION OF LIPASE ACTIVITY

It catalyzes the hydrolysis of triacylglycerols to free fatty acids and glycerol as follow:



The release of fatty acids in the solution will cause decrease in the pH.
The liberated free fatty acids at different enzyme concentrations will be titrated with 0.05 N NaOH. Since we are using oil as substrates,various salt is used as emulsifying agent for two reasons:

1- To increase the surface area
2- To decrease the surface tension, thus the oil drop is effetely attacked with the enzyme.

c. ESTIMATION OF OPTIMUM pHOF CRUDE LIPASE

Materials:

1- Lipase – 1g% (as extracted before)
2- Fresh oil (olive oil) as the substrate
3- Calcium chloride / Sodium chloride / Magnesium chloride
4- Sodium hydroxide 0.05 N
5- Tris-Cl buffer (pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0)

Procedure:

Tube no.	Oil Substrate (ml)	Tris-Cl Buffer (1 ml) pH	$CaCl_2$/ NaCl / $MgCl_2$ (g)	D.W (ml)	Lipase (ml)		Enzyme concentration
1	2.0	4.0	0.1	8.0	2.0	Mix	0.02
2	2.0	5.0	0.1	6.0	4.0	Well &	0.04
3	2.0	6.0	0.1	4.0	6.0	Incubate	0.06
4	2.0	7.0	0.1	2.0	8.0	in a	0.08
5	2.0	8.0	0.1	0.0	10.0	Water- bath	0.10
B	2.0	9.0	0.1	10.0	0.0	at 37° C	0.00

d. ESTIMATION OF ENZYME (CRUDE LIPASE) ACTIVITY

Materials:

1- Lipase – 1g% (as extracted before)
2- Fresh oil (olive oil) as the substrate
3- Calcium chloride / Sodium chloride / Magnesium chloride
4- Sodium hydroxide 0.05 N
5- Tris-Cl buffer (pH 8.0)

Procedure:

Prepare 6 tubes which contain the following:
Titrate the liberated fatty acids with NaOH noting the time of the titration should not exceed 10 mins

Tube no.	Oil Substrate (ml)	Tris-Cl Buffer (ml)	$CaCl_2$/ NaCl / $MgCl_2$ (g)	D.W (ml)	Lipase (ml)		Enzyme concentration
1	2.0	1.0	0.1	8.0	2.0	Mix	0.02
2	2.0	1.0	0.1	6.0	4.0	Well &	0.04
3	2.0	1.0	0.1	4.0	6.0	Incubate	0.06
4	2.0	1.0	0.1	2.0	8.0	in a	0.08
5	2.0	1.0	0.1	0.0	10.0	Water- bath	0.10
B	2.0	1.0	0.1	10.0	0.0	at 37° C	0.00

e. Enzyme Activity Calculation:

Calculate the quantity of fatty acids liberated in each subsample based on the equivalents of NaOH used to reach the titration end point, accounting for any contribution from the reagent, using the following equation:

Units/ml = Volume of NaOH(ml) X Molarity of NaOH X1000 X 4*

Volume of enzyme(ml)

*4 because time conversion from 15 minutes to 1 hour (60 minutes)

f. Lipase Kinetics

The activity of the lipase catalyzed reaction were analysed in triplicate using olive oil solution as substrate, diluted with tris-HCl. The activity of the lipase was determined by the increase in concentration of lipase keeping the substrate volume and concentration same. The results obtained were compared with the blank.

One lipase unit has been defined as the amount of the enzyme that releases one µmol fatty acid per ml under standard assay conditions (U=µmol of fatty acid released/ml)

III. Observations And Results

i. Optimum pH

pH	Vol. Of NaOH(ml)	Units/ml enzyme
4.0	1.8	61.0
5.0	2.0	65.0
6.0	2.2	74.5
7.0	2.4	80.5
8.0	2.8	93.0
9.0	2.1	69.0

ii. Enzyme activity of crude lipase extracted one the same day using $CaCl_2$as activator.

Sample	Vol. Of NaOH(ml)	Units/ml enzyme
1	0.6	60.0
2	1.2	60.0
3	2.8	93.5
4	3.5	87.5
5	3.5	70.0

iii. Enzyme activity of crude lipase after storage period of 24 hours at 4^o C using$CaCl_2$as activator.

Sample	Vol. Of NaOH(ml)	Units/ml enzyme
1	0.6	60.0
2	1.0	50.0
3	1.1	36.7
4	2.7	67.5
5	3.5	70.0

iv. Enzyme activity of crude lipase extracted one the same day using $MgCl_2$as activator.

Sample	Vol. Of NaOH(ml)	Units/ml enzyme
1	0.4	49.0
2	1.0	51.0
3	2.5	82.0
4	**3.5**	**88.0**
5	**3.1**	**62.0**

v. Enzyme activity of crude lipase extracted one the same day usingNaCl as activator.

Sample	Vol. Of NaOH(ml)	Units/ml enzyme
1	0.5	50.0
2	1.2	60.0
3	1.8	60.0
4	**2.2**	**55.0**
5	**2.5**	**50.0**

vi. Enzyme activity of crude lipase after storage period of 24 hours at 4^o C using NaCl as activator.

Sample	Vol. Of NaOH(ml)	Units/ml enzyme
1	0.5	50.0
2	1.1	55.0
3	1.4	46.67
4	2.0	50.0
5	2.4	48.0

3.2.1. pH profile:

The enzyme activity as a function of pH, using linoleic acid as substrate. Activity was seen at all pH values tested ranging from 4.0 to 9.0. The optimum pH was found to be 8.0

3.2.2. Effect of metal ions on activity:

A comparison of effect of metal ion activators was done on crude lipase activity using Ca^{+2}, Mg^{+2}, Na^{+}. The results suggestthat divalent cations are more potent activators than monovalent cations. The Ca^{+2} ions increase the activity of Lipase. The same can be said for the Mg^{+2} ions. Na^{+} ions have no considerable effect on the lipase. But in comparison of the three cations, theCa^{+2} ions are more effective activators of Lipase.

IV. Conclusion

Our studies suggest the crude lipase activity at various pH along with metallic cations as activators. pH 8.0 was found to be its optimum pH.the enzyme activity was reduced after one day of storage at 4^o C suggesting the enzyme to be very unstable. According to the activity properties, it is seen that the Ca^{+2} ions increase the activity of Lipase. Thus, they act as the Activator of the Lipase. The same can be said for the Mg^{+2} ions. If comparison is done between these two metal ions, then Ca^{+2} ions are more effective activators of Lipase. Na^{+} ions have no considerable effect on the lipase. But if compared with Ca^{+2}, they lower the activity of the enzyme. Thus, the divalent cations seem to enhance the enzyme activity while monovalent cation seems to lower the enzyme activity. Hence Ca^{+2}has the maximum capacity to act as activator whereas Mg^{+2}andNa^{+}have relatively less capacity to act as activator respectively.

Reference

[1]. Chougule, V.P. Zambare and M.V. Padul,*Biochemical Studies ofLipase from Germinating Oil Seeds (Glycine max). American Journalof Biochemistry and Biotechnology 7 (3): 141-145, 2011.*
[2]. Plummer, D. *An introduction to practical biochemistry.*
[3]. Harvey, R and Champe, P.Lippincott*biochemistry, London.2005*
[4]. Boyer, R. *Concepts in biochemistry. John Wiley and sons, New York.2002*
[5]. https://en.wikipedia.org/wiki/Soybean
[6]. https://en.wikipedia.org/wiki/lipase
[7]. Image was taken from google image

Dr. Jyoti D. Vora. "Enzymatic Analysis Of Activity Of Metal Ions On Soybean (Glycine Max) LIPASE." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 4.3 (2018): 47-51.