Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2016, Vol. 32, No. (2):
Pg. 1235-1247

Studies on *Ricinus Lipase* Enzyme Isolated from Castor Seeds

ALKA SRIVASTAVA[1]*, RASHMI MOHAN MATHUR[1], RACHNA PRAKASH[1] and SHASHI AGRAWAL[1]

*[1]Department of Chemistry D.G. (PG) College, Kanpur, India.
[2]Department of Chemistry D.B.S College, Kanpur, India.

http://dx.doi.org/10.13005/ojc/320250

(Received: January 19, 2016; Accepted: February 12, 2016)

ABSTRACT

Healthy and bold seeds of castor variety "Aruna" were collected. The de-hulled castor kernels were separated from seed coat and finely grounded in an electric grinder. The powdered kernels were passed through 60 mesh sieve and defatted using n-hexane in a soxhlet extractor. After complete extraction of oil seed meal was made free from solvent at low temperature. The defatted seed meal was pulverized and again passed through 60-mesh sieve to get final product .The RICINUS LIPASE source thus, obtained was stored in a screw capped glass vial and kept in a desiccator. As and when required portions from this preparation were used in further enzymatic studies.

Keywords: Ricinus Lipase, Germinated castor seeds, Cotyledons.

INTRODUCTION

In plant seeds particularly in castor seeds the utilization of the storage fats is initiated by the stepwise hydrolysis of the tri-glycerides to di and mono glycerides and ultimately to free fatty acids and glycerol[1]. These are converted to sucrose by a long glyconeogenic pathway[2,3] These reactions are commonly catalyzed by the enzyme lipase. In almost all oil seeds lipase enzyme catalysed hydrolysis of triglycerides take place during germination as enzyme lipase is activated mainly in the seedling tissues of different plant species[4,5,6]. The castor bean has also been reported to possess powerful lipase activity which is responsible for the hydrolysis of triglycerides .The lipase present in castor bean has a unique behaviour and possess some extraordinary characteristics, in contrast to the other oilseeds in which lipase is activated only during germination, In castor bean lipase is activated during germination in dormant seeds and germinated seeds both[7].

It has been observed that castor bean contains two lipases .one enzyme is associated with the membranes of spherosomes (lipid bodies) and is active in dormant or resting seeds, This lipase

is active only in acid pH, hence is termed as acid lipase and is capable of hydrolyzing triglycerites with maximum activity at pH 4.2-5.0 The activity of the pH decreases sharply during germination[8,9,10].

The other enzyme, alkaline lipase is associated with membrane of glyoxysomes and endoplasmic reticulum. It is active in alkaline pH with optimum activity at pH 9 and is capable of acting only on mono glycerides. This enzyme is inactive in dormant seeds.

MATERIALS AND METHODS

Preparation of Ricinus Lipase source from dormant castor seed

Healthy and bold seeds of castor variety 'Aruna' were collected. The dehulled castor kernels were seperated from seed coat and finally grounded in an electric grinder. The powdered kernels were passed through 60 mesh sieve and defatted using n-hexane in a soxhlet extractor. After complete extraction of oil seed meal was made free from solvent at low temperature. The defatted seed meal was pulverized and again passed through 60-mesh sieve to get final product .The RICINUS LIPASE source thus obtained was stored in a screw capped glass vial and kept in a desiccator. As and when required portions from this preparation were used in further enzymatic studies.

Preparation of Ricinus Lipase Source from germinated castor seeds

Healthy and bold seeds of castor variety of "Aruna" were soaked in a distilled water for twenty four hours. Water was drained off and water soaked seeds were placed on germinating papers in a petri dish and were allowed to germinate for 5 days. Seed coats of five day old germinated castor seeds were carefully removed and cotyledons along with sprouts were subsequently crushed to fine thick paste in a pestle and mortar. Freshly prepared fine thick paste was always used as RICINIUS LIPASE source in subsequent experiments.

Measurement of Rates of Hydrolysis and Lipase Activity using Ricinus Lipase Source from Dormant and Germinating Castor Seeds

Standard Procedure

Unless otherwise specified a simplified, standard procedure as adopted by Longencker and Haley was employed with some modifications for the measurement of rates of hydrolysis of various glycerides and lipase activity using Ricinus Lipase source from both, dormant and germinated castor seeds. A fixed weight of substrate (vegetable oils) (1.0 g) was placed in a hard glass screw capped vial and two drops of toluene was added to it. The mixture was thoroughly mixed followed by the addition of fixed weight of Ricinus Lipase source (0.1 g) enzyme material in case of dormant seeds and 1.6g (on fresh weight basis) in case of enzyme material from 5 day old germinated castor seeds). 1.6g Fresh-weight of lipase source -from germinated castor seeds -was equal to 1 g of lipase source from dormant seeds on dry weight basis. distilled water(2.0 ml) was then added to the exaction mixture followed by short stirring for uniform mixing of the contents of the vial. pH of the reaction mixture was set to 4.8 by the addition of 1N acetic acid solution (.7ml).The contents of the viral were again stirred and incubated at 35°C for a constant period of time (30 min.). After the completion of the incubation period, the reaction mixture was transferred to a conical flask containing 40ml of 95% hot ethanol to stop the reaction .The remains in the vial were rinsed twice with hot alcohol in such a way that the total volume of the alcohol added comes to 50ml contents of the flask containing fatty acids released from glycerides were titrated with .IN sodium hydroxide solution using phenolphthalein as an indicator. A blank experiment without enzyme source was also conducted along with each set of experiment with different substrate.

In the case of experiments containing Ricinus lipase source from germinated seeds an additional blank experiment without substrate was also conducted to determine fatty acids released from endogenous castor oil present in Ricinus Lipase source from germinated seed, so that fatty acid released from the substrate added in the reaction mixture could be calculated accurately. The % lipolysis / hydrolysis of the substrate (glycerides) in each experiment was calculated by the expression.

% lipolysis / hydrolysis= (vol. of 1N alkali (sample))-(vol.of 1N alkali (blank))* 100 Saponification no. of a substrate- Acid value of substrate

Lipase Activity

Measurement of lipase activity of lipase source from dormant acid germinated castor seeds was determined using standard procedure which indicate micro-molar concentration of free fatty acids released from substrate per minute from 10µg lipase source and is expressed in m mole fatty acid released per minute 10mg enzyme per source.

Factor Affecting Ricinus Lipase Catalysed) Hydrolysis of Vegetable Oils used as Substrate

Effect of pH

In both the sets of experiment using Ricinus Lipase source from dormant and germinated seeds the reaction condition of enzyme catalysed hydrolysis of various substrate (vegetable oil) were maintained as per standard procedure and pH of reaction mixture was varied maintaining pH at 4.0, 4.8, 5.0 and 5.5 in different experiments by the addition of 1N acetic acid solution .results on the effect of pH on % lipolysis of glycerides and Ricinus lipase activity were calculated as given in standard procedure

Effect of temperature

In both types of experiment with dormant and germinated seeds as lipase source, the effect of temperature on Ricinus Lipase catalyzed hydrolysis of vegetable oils was determined using the standard procedure with the exception that the incubation temperature of the reaction was maintained at 30, 35, 40, 45 and 50 deg celcius in different experiment.

Effect of reaction time

In order to determine the effect of reaction period on enzyme catalyzed lipolysis of various substrate (vegetable oils) the reaction were conducted at five variable time viz. 15 , 30, 60, 90 and 120 mi Other condition were maintained same as reported in standard procedure

Effect of enzyme Concentration

In both the sets of experiment using lipase source from dormant and germinated castor seeds. effect of enzyme concentration on the rates of hydrolysis of various vegetable oil was determined using four variables of enzyme concentration. In the case of experiments using iipase .source from dormant seeds the concentration of enzyme source was maintained at .05, 0.1, 0.15 and 0.20 g in the whole experiment was maintained at 1.6, 2.1 ,2.6 and 3.1g on fresh weight basis. Rest of reaction condition were same as per in the standard condition

Effect of Substrate concentration

In both sets of experiments employing source from dormant and germinating castor seeds vegetable oils namely castor oil, linseed oil cottonseed oil, olive oil and coconut oil were used as substrate, The concentration of each substrate was maintained at four variables viz. .5, 1.0, 1.5 and 2.0g. The concentration of enzyme ad other components of reaction mixture were same as used in the standard procedure

Effect of Inorganic salts

Effect of series of inorganic salts on the rate of enzyme catalysed lipolysis/hydrolysis of various substrate was determined employing lipase source from dormant seeds only. In each experiment 0.1g of inorganic salt was added additionally to the digestion mixture before the addition of 0.1N acetic acid solution. The concentration of substrate enzyme and other reaction conditions were same as per in the standard procedure. Various inorganic salts used were lead acetate lead chloride, cobalt acetate chromium acetate, chromium chloride, calcium chloride and cobalt chloride

RESULTS AND DISCUSSION

Ricinus lipase **: Measurment of rate of hydrolysis an]) lipase activity**

In plant seeds, particularly iii oleaginous seeds, breakdown and utilization of reserve oil/fat begins with stepwise hydrolysis of triglycerides to di- and mono-glycerides and then to glycerol and fatty acid-, which are then used in the biosynthesis of sucrose through glyconeogenic pathway .The steps involved in the conversion of triacyiglycerides to free fatty acids and glycerol are catalysed by enzyme lipase (glycerol and hydrolase E.C..3.1.1.3). reports are known indicating the activation of lipase enzymes of plant seeds only during germinated phase as the enzyme lipase is activated mainly in seedling tissues of different plant species Like other oilseeds , castor seeds also possess lipase system but -nature amid characteristics ef Ricinus Lipase or castor bean lipase are different than -those of other oleaginous

seeds. In contrast to other oil seed, Ricinus Lipase is active in dormant seeds in addition to germinated seeds .The reason for the activation Ricinus Lipase in both, dormant and germinated stages has been attributed to the presence of two different lipases in castor bean of which one is activated during dormant seeds (at acidic pH). The acid lipase is capable of hydrolysing tri, di- and monoglycerides to fatty acids and glycerol. The enzyme is highly active during germination. However ,the reason of activation of this lipase in dormant seeds and decrease in its activity during germinated seeds has not yet been elucidated. Moreover such a pattern of lipolytic action in dormant seeds has yet not been observed to other oleaginous seeds wherein lipases are activated only in germinated seeds

The alkaline lipase is reported to be inactive during dormant seeds but its activity increases markedly during germination. However, this enzyme is capable of hydrolyzing monoglycerides only. It is not known, how reserve glycerides in castor seeds are converted to monoglycerides during germination as acid lipase present in the seed for purpose becomes inactive during phase.

Table 1: Comparative Status of Rates of Hydrolysis of Different Substrate Catalysed By Ricinus Lipase Source From Dormant and Germinated Castor Seeds Under Standard Conditions

Substrate	Rates of hydrolysis	
	Ricinus Lipase Source from DCS	Ricinus Lipase Source from GCS
Castor oil	45.00	4.00
Linseed oil	34.02	2.16
Cottonseed oil	31.65	1.29
Olive oil	41.32	3.04
Coconut oil	40.29	2.50

*Data given is a mean of triplicate analysis DCS= Dormant Castor Seed; GCS= Germinated castor Seed

Table 2: Effect of germination on the Activation of Ricinus Lipase over Various Substrates under Standard Condition

activity* / Substrate	Ricinus lipase μ mole F.A. released minute 10mg lipase source	
	Ricinus Lipase Source from DCS	Ricinus Lipase Source from GCS
Castor oil	22.1	2.33
Linseed oil	16.7	1.06
Cottonseed oil	15.5	0.62
Olive oil	20.2	1.40
Coconut oil	19.7	1.20

Table 3 A: Effect of Ph on The Rates Of Hydrolysis of Different Substrates Catalyzed by Ricinus Lipase Source from Dormant Castor Seeds

Substrate	Rates of Hydrolysis (%)*			
	pH4.0	pH4.8	pH5.0	pH5.5
Castor oil	10.45	45.00	10.01	8.20
Linseed oil	8.71	34.02	8.09	8.00
Cottonseed oil	10.29	31.65	8.90	8.00
Olive oil	8.09	41.32	9.01	8.90
Coconut oil	9.00	40.29	8,40	7.32

Table 3 B: Effect of pH on the Activity of Ricinus Lipase Source Obtained from Dormant seeds using various substrate

Substrate	Ricinus lipase activity* (μ mole F.A. released minute 10mg lipase source^{-1})			
	pH4.0	pH4.8	pH5.0	pH5.5
Castor oil	5.13	22.1	4.99	4.02
Linseed oil	4.37	16.7	3.97	3.92
Cottonseed oil	3.03	15.5	4.35	2.91
Olive oil	3.95	20.2	4.40	4.30
Coconut oil	4.13	19.7	4.10	3.57

In light of the above information efforts on the measurement of Ricinus acid lipase activity in both dormant and germinated seeds have been made to determine comparative status of acid lipase activity in both the phases in an attempt to contribute to the existing knowledge in the area

In order to achieve this goal two sets of experiments were performed were which involved the use of both dormant and germinated seeds as source of Ricinus lipase to measure its lipolytic activity on various substrate (vegetable oils)

Table 4 A: Effect of P11 on the Rates of Hydrolysis of Different Substrates Catalyzed by Ricinus Lipase Source from Germinated Castor Seeds

Substrate	Rates of Hydrolysis (%)*			
	pH4.0	pH4.8	pH5.0	pH5.5
Castor oil	4.00	4.00	4.00	4.00
Linseed oil	2.14	2.16	2.10	2.12
Cottonseed oil	1.20	1.29	1.22	1.23
Olive oil	2.91	3.04	2.99	2.65
Coconut oil	2.42	2.50	2.48	2.51

Table 4 B: Effect of pH on the Activity of Ricinus LipaseSource Obtained from Germinated seeds using various Substrates

Substrate	Ricinus lipase activity* (µ mole F.A. released minute 10mg lipase source^{-1})			
	pH4.0	pH4.8	pH5.0	pH5.5
Castor oil	2.33	2.33	2.33	2.33
Linseed oil	1.04	1.02	1.03	1.03
Cottonseed oil	0.56	0.63	0.59	0.60
Olive oil	1.42	1.48	1.48	1.26
Coconut oil	0.97	1.20	1.06	1.10

Table 5 A: Effect of Temperature on the rates of Hydrolysis of Different Substrates Catalysed by Ricinus Lipase Source from Dormant Seeds

Substrate	Rate of Hydrolysis (%)*				
	30°C	35°C	40°C	45°C	50°C
Castor oil	25.21	45.00	45.29	42.33	31.00
Linseed oil	30.02	34.02	34.99	33.25	30.69
Cottonseed oil	29.12	31.65	39.50	35.29	28.12
Olive oil	20.19	41.32	39.89	35.00	30.09
Coconut oil	28.51	40.29	39.92	38.10	36.20

Table 5 B: Effect of Temperature on the rates of Hydrolysis of Different Substrates Catalysed by Ricinus Lipase Source from Dormant Seeds

Substrate	Ricinus lipase activity* (µ t mole F.A. released minute-1 10mg lipase source)				
	30°C	35°C	40°C	45°C	50°C
Castor oil	12.3	22.1	22.2	20.7	15.2
Linseed oil	14.6	16.7	17.7	16.2	15.0
Cottonseed oil	15.0	14.2	15.5	19.3	17.2
Olive oil	13.7	9.8	20.2	19.5	17.1
Coconut oil	14.7	13.9	19.7	19.5	18.6

The source of Ricinus lipase material from dormant seeds employed in the experiment was prepared by dehulling and deflating finely powdered castor seeds, while the source Ricinus Lipase from germinated castor seeds was obtained by repeated grinding of five days old germinated seeds to the fine paste. These two experimentally active preparations were used in subsequent experiments to measure the rate of hydrolysis of various substrate and lipase activity

In both the sets of experiments in addition to castor oil (substrate of Ricinus Lipase), linseed, cottonseed, olive and coconut oils were also used as substrate to assess relative substrate specificity of Ricinus acid lipase material obtained from dormant and germinated castor seeds, both.

Results on rate of hydrolysis (percentage fatty acids produced) of various substrate using. Ricinus lipase source from dormant seeds under standard condition are illustrated in table 4.1. It is clear that maximum rate of lipolysis (45%) under 30% of incubation period was recorded with castor oil substrate. Olive oil recorded 41.32% lipolysis followed by coconut oil (40.29%) while the enzyme source showed lowest lipolysis in case of cottonseed oil .Linseed oil was lipolysed to 34.02%.

Although maximum lipolysis was recorded in the case of castor oil, however, the enzyme material lipolysed other substrate also to a considerable extent indicating its broad substrate specificity for vegetable oils as shown in table 1.

Lipase activity expressed as mew moles of free fatty acids released under substrate condition also recorded similar pattern indicating maximum lipase activity of 22.1 µmole per minute. 10 mg enzyme per material when castor oil was used as substrate. Lipase activity with other substrate viz. olive, coconut, linseed and cottonseed oils was found to be in the decreasing order of 20.2, 19.7, 16.7 and 15.5 µ mole per minute 10mg enzyme per material respectively

Table 6 A: Effect of Temperature on the rates of Hydrolysis of Various Glycerides Catalysed by Ricinus lipase Source from Germinated Castor Seeds

Substrate	Rate of Hydrolysis (%)*				
	30°C	35°C	40°C	45°C	50°C
Castor oil	3.99	4.00	4.04	4.1	4.00
Linseed oil	2.15	2.16	2.16	2.14	2.15
Cottonseed oil	1,27	1.29	1.25	1.25	1.24
Olive oil	2,98	3.04	2.95	2.86	2.58
Coconut oil	2.49	2.50	2.56	2.46	2.41

Table 6 B: Activity of Ricinus Lipase Source Obtained from Germinated Castor Seed as Affected by Temperature

Substrate	Rate of Hydrolysis (%)*				
	30°C	35°C	40°C	45°C	50°C
Castor oil	2.21	2.33	2.61	2.61	2.40
Linseed oil	1.04	1.06	1.05	1.04	1.05
Cottonseed oil	0.61	0.62	0.61	0.60	0.61
Olive oil	1.51	1.48	1.43	1.50	1.26
Coconut oil	1.20	1.20	1.24	1.15	1.15

Similar set of experiments under standard condition were also performed using Ricinus lipase source from geminated castor seeds which exhibited drastic release in percentage lipolysis and lipase activity in the case of all the as substrate (Table 1 and 2) as compared to experiments performed with Ricinus lipase source from dormant seeds.

It clearly indicated that in dormant castor beans active acid lipase was present, however, it almost disappeared or inactivated in germination seeds. In the case of castor oil lipase activity decreased from 22.1 to 2.33 µ mole per minute 10mg enzyme per source when germinated seed was used in place of dormant castor seed. Decrease in lipase activity was also observed with all other substrates when lipase source from germinated seeds was used. Similar drastic loss in Ricinus lipase activity in germinated castor seeds has also been observed by Huang and Moreau, Muto and Beevers (1974) and Ory indicating unique behaviour of Ricinus lipase not found in other oilseeds.

Effects of pH

The % lipolysis and Ricinus lipase activity as influenced by pH was also determined using lipase source from dormant and germinated castor seeds (Table 3 A & and 4 A &B).

Rate of hydrolysis and lipase activity using dormant seeds as enzyme Source was recorded at pH 4.0, 4.8, 5.0 and 5.5 on various substrate at 350 C as shown in Table 9 A and B.

When Rienus lipase source from donnant seed was used with all the substrate, it was found that rates of hydrolysis and lipase activity initially increased from pH4 and reached at its maximum at pH4.8, thereafter its pH decreased at 5.5 indicating its pH at 4.8.

The pH optimum of acid lipase of castor bean has been reported to be 4.2 - 4.5 by Ory (1969) while Huang and Moreau (1978) have recorded its

Table 7 A: Effect of reaction time on the Extent of Hydrolysis of Substrates Catalyzed by Ricinus Lipase Source from Dormant Seeds

Substrate	Rate of Hydrolysis (%)* Free fatty acid produced				
	15 min.	30 min.	60 min.	90 min.	120 min.
Castor oil	32.19	45.00	50.50	55.26	72.26
Linseed oil	72.26	21.91	34.02	43.35	45.19
Cottonseed oil	46.22	19.61	3.1.65	40.02	49.25
Olive oil	52.25	29.23	41.32	49.92	59.25
Coconut oil	60.25	26.52	40.29	42.12	44,26

Table 7 B: Effect of reaction time on the Activity of Ricinus Liase Source Obtained from Dormant Castor Seeds using Various Substrates

Substrate	Rate of Hydrolysis (%)* (µ mole F.A. released minute 10mg lipase source-1)				
	15 min.	30 min.	60 min.	90 min.	120 min.
Castor oil	15.8	22.1	24.8	27.1	35.4
Linseed oil	10.7	16,7	21.2	22,1	22.6
Cottonseed oil	9,60	15.5	19.5	24.1	25.8
Olive oil	14.2	20.2	24.4	28.9	29.4
Coconut oil	12.0	19.7	20.5	20.6	21.0

pH to be 5.0 which is almost in agreement with the results recorded by the author

Similar sets of experiment conducted with Ricjpps lipas source from germinated castor beans did pot show any impact of alteration in pH values on the rates of lipolysis and lipase activity various substrate and recorded almost negligible value (table 4.4 A and B) Huang and Moreau (1978) have also recorded negligible activity in 6 day old germinated seeds of castor at ph 5.0.

Effect of Temperature

Effect of varying temperature (30, 35, 40, 45 and 50ºC) on the lipase activity and rates of hydrolysis of different substrate using lipase enzyme source from dormant and germinated castor from dormant and castor seeds was also recorded, other conditions of the reaction were same as in standard condition .The results are depicted in Table 5 A & B and 6 A & B.

Results showed that rates of hydrolysis and lipase activity increased up to 40°Celsius and then showed declining trend at 45 °C recording lowest value at 50°C in almost all the substrates in the case of Ricinus lipase source from dormant seeds as shown in Table 5 A & B.

Similar Trend Indicating increase in lipase activity at 40 deg Celsius compared to 35 deg Celsius in the case of Ricinus lipase activity on castor oil as substrate has also been recorded by Ory *et al*. (1962).

When germinated castor seeds were used as lipase source, incubation at the various temperature did not bring about any noticeable change on the rates of hydrolysis and lipase activity irrespective of any substrate used as shown in Table 6 A and B. The data remained almost static as those observed in the case of experiments conducted under standard condition using germinated castor

Table 8 A: Effect of Reaction Time on the Rate of Hydrolysis of Various Substrates Catalyzed by Ricinus Lipase Source from GCS

Substrate	Rate of Hydrolysis (%)* Free fatty acid produced				
	15 min.	30 min.	60 min.	90 min.	120 min.
Castor oil	0.15	4.00	4.00	4.03	4.03
Linseed oil	0.52	2.16	2.19	2.22	2.19
Cottonseed oil	0.31	1.29	1.29	1.28	1.26
Olive oil	0.26	3.04	3.03	3.02	3.03
Coconut oil	0.01	2.50	2.55	2.55	2.58

Table 8 B: Effect of Reaction Tune on the Activity of Ricinus Lipase Source Obtained from GCS using Various Substrates

Substrate	Ricinus lipase activity* (µ mole F.A. released minute 10mg lipase source^{-1})				
	15 min.	30 min.	60 min.	90 min.	120 min.
Castor oil	0.08	2.33	2.33	2.34	2.34
Linseed oil	0.087	1.06	1.07	1.06	1.06
Cottonseed oil	0.062	0.62	0.63	0.63	0.61
Olive oil	0.090	1.48	1.45	1.44	1.45
Coconut oil	0.089	1.20	1.22	1.20	1.22

seeds as lipase source. It clearly indicated loss of acid lipase activity n germinated seeds as compared to dormant castor seeds as reported by others.

Effects of Reaction Time

Rates of hydrolysis and lipase activity of enzyme material from dormant seeds was measured using various substrates at different reaction time viz 15, 30, 60, 90 and 120 minutes .The rates of showed linear increase with the increase in reaction time when castor oil was used as substrate. Similar trend was observed in linseed, cottonseed olive and coconut oil, however, magnitude of increase in fatty acids released was different with each substrate as depicted in Table 7A. Maximum value of lipase activity in during 120 minute of reaction hue was 35.4, 29.4, 25.8, 22.6. and 21.0 µ.

Moles per minute 10mg enzyme per source in the case of castor oil, olive oil, cottonseed oil, linseed oil and coconut oil substrates respectively (Table 7). Similar type of linear increase in lipase activity with the increase in reaction time also has been reported by Sabders and Pattee (1975) in the case of peanut lipase using tributyrin as substrate and by Longnecker and Haey in the case of Ricunus lipase dormant castor seeds using olive oil as substrate .°w et al (1960) have also obtained a plot of glyceride concentration versus reaction time in straight line in the case of hydrolysis of endogenous castor oil by crude Ricinus lipase donnant seed.

Rate of hydrolysis and lipase activity measured using germinated castor seeds as lipase source with various substrate did not show alterations

Table 9 A: Effect of Ricinus Lipase Concentration (obtained from DCS) on the Extent of Hydrolysis of substrate

Substrate	Rate of Hydrolysis (%)* Ricinus lipase concentration			
	0.05g	0.10g	0.15g	0.20g
Castor oil	42.00	45.00	47.92	50.12
Linseed oil	30.06	34.02	36.16	38.12
Cottonseed oil	29.15	31.65	32.06	34.12
Olive oil	39.13	41.32	43.61	44.00
Coconut oil	37.12	40.29	42.10	44.36

Table 9 B: Effect of Enzyme Concentration on source obtained from DCS using various substrate

Substrate	Ricinus lipase activity* (µ mole F.A. released minute 10mg lipase source) Enzyme concentration			
	0.05g	0.10g	0.15g	0.20g
Castor oil	20.6	22.1	23.5	24.6
Linseed oil	14.7	16.7	17.7	18.7
Cottonseed oil	14.2	15.5	15.7	16.7
Olive oil	19.1	20.2	21.3	21.5
Coconut oil	18.1	19.7	20.5	21.6

Table 10 A: Effect of Ricinus Lipase Concentration (from RCS) on the Rate of hydrolysis of various substrate

Substrate	Rate of Hydrolysis (%)* Ricinus lipase concentration			
	1.6g	2.1g	2.6 g	3.1g
Castor oil	4.00	4.00	5.06	5.10
Linseed oil	2.11	2.16	2.14	2.14
Cottonseed oil	1.28	1.29	1.26	1.27
Olive oil	2.58	3.04	3.05	3.09
Coconut oil	2.47	2.50	2.50	2.50

Table 10 B: Ricinus Lipase Activity as affected by Concentration of Lipase Enzyme Source from GCS

Substrate	Ricinus lipase activity* (µ mole F.A. released minute^{-1} 10mg lipase source^{-1})			
	1.6g	2.1g	2.6 g	3.1g
Castor oil	2.31	2.33	2.37	2.39
Linseed oil	1.07	1.05	1.06	1.06
Cottonseed oil	0.62	0.62	0.61	0
Olive oil	1.41	1.48	1.46	0.91
Coconut oil	1.21	1.20	1.21	1.21

with the in reaction period which may be attributed to the loss of lipase activity in germinated Ricinus beans (Table 8 A and B).

Effects of enzyme concentration

Effect of concentration of enzyme on the rate of hydrolysis of castor oil and other substrate using source from dormant seed as shown in table 9 A. It clearly indicated linear increase in the percent hydrolysis and lipase activity with the increase in enzyme concentration Maximum rate of hydrolysis was recorded to be 50.12% in the case of castor oil as substrate using 0.2 g enzyme material from dormant castor seeds at pH4.8 during 30 min. at 35° C. Maximum rate of hydrolysis of coconut oil and olive oil using .2 g of concentration Of enzyme material from dormant seed under same condition was found to be 44.36 % and 44.00 %.Lowest rate of hydrolysis (34.12%) was recorded when cottonseed oil was used as substrate as shown in Table 9 A. It clearly indicated linear increase in the percent hydrolysis and lipase activity with the increase in the percent hydrolysis and lipase activity with the increase in enzyme concentration .Maximum rates of hydrolysis was recorded to be 50.12% in the case of castor oil as substrate using. enzyme material from dormant castor seeds at pH 4.8 during 30 mm. at 35 deg Celsius . Maximum rate of hydrolysis of coconut oil and olive oil using .2 g of concentration of enzyme material from dormant seed under same condition

Table 11 A: Effect of Substrate Concentration on the Rate of Hydrolysis of various Glycerides using Ricinus lipase from DCS

Substrate	Rate of Hydrolysis (%)* Substrate concentration			
	0.05g	0.10g	0.15g	0.20g
Castor oil	44.28	45.00	48.13	46.70
Linseed oil	33.72	34.02	37.19	39.25
Cottonseed oil	30.40	31.65	33.00	35.12
Olive oil	40,56	41.32	43.65	42.12
Coconut oil	38.71	40.29	42.90	39.65

Table 11 B: Effect of Substrate Concentration on the Activity of Ricinus lipase source obtained from DCS using various substrates

Substrate	Ricinus lipase activity* (μ mole F.A. released minute^{-1} 10mg lipase source^{-1}) Substrate concentration			
	0.5g	1.0g	1.5g	2.0g
Castor oil	21.6	22.1	23.6	22.9
Linseed oil	16.5	16.7	18.2	19.2
Cottonseed oil	14.8	15.5	16.1	17.1
Olive oil	19.8	20.2	21.3	20.5
Coconut oil	18.9	19.7	20.9	19.3

* Mean of three measurements, FA fatty acids

Table 12 A: Effect of Substrate Concentration on the Rate of Hydrolysis of Various Substrates Catalyzed by Ricinus Lipases Source from OCS

Substrate	Rate of Hydrolysis (%)* Substrate concentration			
	0.05g	0.10g	0.15g	0.20g
Castor oil	4.01	4.00	4.00	3.96
Linseed oil	2.14	2.16	2.10	2.13
Cottonseed oil	1.26	1.29	1.29	1.26
Olive oil	3.05	3.04	2.99	2.96
Coconut oil	2.51	4.00	2.42	2.37

Table 12 B: Effect of Substrate Concentration on the Activity of Ricinus lipase source obtained from DCS using various substrates

Substrate	Ricinus lipase activity* (μ mole F.A. released minute^{-1} 10mg lipase source^{-1}) Substrate concentration			
	0.5g	1.0g	1.5g	2.0g
Castor oil	2.28	2.33	2.37	2.37
Linseed oil	1.0	1.06	1.00	1.00
Cottonseed oil	0.61	0.62	0.62	(.61
Olive oil	1.48	1.48	1.42	1.36
Coconut oil	1.24	1.20	1.20	1.15

Table 13: Effect of Inorganic Salts on the rate of hydrolysis on various Substrates catalyzed by Ricinus Lipase Source from DCS

Substrate	Rate of Hydrolysis (%)*				
	Castor oil	Linseed oil	Cotton Seed Oil	Olive oil	Coconut oil
Nil (Control)	45.00	34.02	31.65	41.32	40.29
Lead acetate	20.12	27.22	31.20	25.12	22.29
Manganese acetate	22.25	20.25	21.92	19.15	21.21
Calcium acetate	25.00	20.12	23.29	24.29	24.01
Lead chloride	15.29	12.21	13.12	14.29	16.29
Cobalt acetate	15.22	14.19	20.25	13.25	14.52
Chromium acetate	14.32	12.25	14.00	14.25	13.56
Chromium chloride	13.12	15.51	16.29	14.25	13.25
Calcium chloride	10.22	12.25	12.12	9.25	11.92
Cobalt Chloride	10.2	10.00	15.55	11.52	9.25

* Mean of two replication, DCS=Dormant castor seed

was found to be 44.36% and 44.00%. Lowest rate of hydrolysis (34.12%) was recorded when cottonseed oil was used as substrate as shown in Table 9 A.

Activity of lipase from dormant seed expressed as μ molar concentration of ay released also revealed similar pattern of linear increase with the increase in enzyme concentration with all the substrate indicating maximum lipase activity of 24.6, 18.7, 16.7, 21.5 and 21.6 t mole per minute 10 mg lipase per source when castor, linseed, cottonseed, olive and coconut oils, respectively ,were used as substrate (Table 9 B)

Data on the effect of enzyme concentration on the rate of hydrolysis of various substrate clearly indicated broad specificity of Ricinus lipase from dormant seed .Data obtained by the author is in agreement with those reported by Wetter and Lin and Huang on lipases from rape and mustard and Hassanien and Mukharjee on lipases from rape ,mustard and lupine seedlings. Findings of Ory *et al* (1962) on the effect of enzyme concentration of acid lipase from castor bean on the rate of hydrolysis of castor oil is almost in accordance with the results obtained by the author.

Effect of enzyme concentration on the activity of Ricinus lipase source from genninated castor seeds were also recorded as shown in Table 10 A and B. Rates of Hydrolysis of various oils used as substrate remained almost unaffected with the increase in enzyme concentration. Moreover rates of hydrolysis of different vegetable oils were very poor as compared to the rates of hydrolysis recorded with lipase from dormant seed under similar reaction conditions indicating loss of lipase activity in germinated castor beans. Such type of behaviour in germinated castor seeds has also been reported by Huang and Moreau. Same trend was observed when lipase activity from germinated seed was measured in a mew molar concentration of free fatty acid released

Effect of substrate concentration

Effect of increasing the substrate concentration on the rates of hydrolysis of various vegetable oils (substrate) at 35^0 Celsius using Ricinus Lipase source from dormant seed showed two different pattern in Table 11 A & B

Data on lipase activity expressed as mew moles of free fatty acid released from triglycerides (substrates) as influenced by increasing the concentration of respective substrates in illustrated in Table 11.

Increasing the concentration of castor oil as substrate from 0.5g to 1.5 g increased lipase activity to its maximum,there after it decreased. Same

pattern was recorded when olive oil and coconut oil were used as substrates. Cottonseed and olive oils recorded linear increase in lipase activity with the increasing concentration of respective substrates (Table 11 B)

The Lipase activity and rates of hydrolysis of various substrate as affected substrate concentration was also determined using lipase source from d day old germinated caster seeds (Table 12 A & B),however, it did not produce any meaningful results

Effect of Inorganic Salts on the rate of Hydrolysis on Different Substrates

Effect of certain inorganic salts which included acetates and chlorides of lead calcium cobalt, chromium and acetate of manganese (µg each) on the Ricinus lipase catalyzed hydrolysis of various substrates using lipase source from dormant castor seeds was also studied to examine the inhibitory or circulatory action of added salts on catalytic activity of lipase. Results on the rates of hydrolysis of each substrate as compared to control are shown in table 1.

Each added salt inhibited lipase catalysed hydrolysis of substrates but the magnitude of inhibitory action varied depending on inorganic salts and substrate used. Least inhibitory effect was noticed in acetates of lead, manganese and calcium in almost all substrates while moderate inhibitory action was recorded in the case of all substrates when lead chloride, cobalt acetate, chromium acetate and chromium chloride were added to reaction mixture. Maximum inhibition was exhibited by calcium and cobalt chloride in the case of all substrates. Similar inhibitory action of inorganic salts on Ricinus lipase activity have also been observed by Longnecker and Haleyand Ory *et al.* the results of which are in agreement with the findings recorded by author.

REFERENCES

1. Stumpf.P.K**.** Lipid metabolism In : plant Biochemistry (Bonner, J, and vemer, J.F., Editors), *Academic Press,* New York, **1976** ,427.
2. Beevers.H**.** Glyoxysomes of castor bean endosperm and their nation to gluconeogenesis Ann. N.Y. *Acad. Sci.* **1969,***168*,323,
3. Beevers.H Organelles from castor bean seedlings: Biochemical roles in gluconeogenesis and phospholipid biosynthesis In: Recent Advances in the Chemistry and Biochemistry of Plant Lipids (Galliard, T. and Mercer, E.I., Editors), *Academic Press* New York, ,**1975,**287
4. Bamann.E. and Ullrnann.E**.** Lipases In: Encyclopedia of plant physiollogy. (Ruhland, W.B.,Editor), *Springer Berlin Heidelberg,***1957** *7*, l09
5. Butt.V.S. and Beevers.H. The plant lipids; In: Plant Physiology (F.C. Steward, Editor) *Academic Press, New York*, ,**1966,***4*,465
6. **Matite.P.** The lytic compartments of plant cells IN : cell Biology Monographs. (Alfert *et et al*., Editors), *Springer, New York*, **1975** *103*.
7. Ory.R.L.,Angelo.J.St.and Altschul. A.M. Castor bean lipase action on its endogenous substrate, *J. lipid Research*, **1960,** *1*,208,
8. Ory.R.L.,Yatsu.L.Y.and Kircher,.H.W**.** Association of lipase activity with the spherosoines of Ricinus commun Es, *Arch. Biochem*. (1968),*5*,
9. Ory.R.L. Angelo J.st and Altschul.A.M**.** The acid lipase of castor bean - properties and substrate specificity, *J. Lipid Research 8*,99, Ory.R Acid lipase of castor beans, *Lipids*, **1969,** *4*,177,
10. Huang. A.H.C. and Moreau.R.A. Lipases in the storage tissues of peanut and other oil seeds during germination *Planta*, **1978,** *141*,(i)111,
11. Longenckar.H.E. and Haley.D.E. Nature of Ricinus Lipase and its nature. *J. Amer. Chem. Soc.*, **1935,** *57*, 2019,
12. Kulkarni L.G. and Ramanu musthy.G.V**.** Castor, ICAR, NEW Delhi **1959,**46,
13. Nagraj.G**.** Biochemical Qulaity of oilseed. *J. Oil Seed Research*, **1990** *7*, 47,
14. Ghosh Oil content of castor seeds as affected by climate and other conditions. *Agri. J. India*, **1924,** *19*, 81,

15. Bolley.D.S**.** Composition of castor oil by optical activity. *J. Amer. Oil Chemical Soc*., **1953,** *396*,
16. Lakshminarayan.G., Subha Rao R., Sita Ram Shashtri, Y. and Kale **V.** High pressure spiliting of castor oil, *J. Amer. Oil Chemical. Soc.* **1984** *61*(7) 1204,
17. Gardener.H.K., DaquinE.L. Koltum,S.P.Macourtney Vix H.I.E. and Gastrock.E.A**.** Detoxification and deallergination of castor beans, *J. Amer. Oil Chem. Soc.* **1960** *37*, 142
18. Rao. K.V.S.A., Paulose, M.M. and Lakshminarayan In situ lipolysis of castor oil in homogenous castor seeds, *Biotechnology Letters***,1990,** *12*, 377
19. Longencker.H.E. and Haley.D.E**.** further studies on nature of Ricinus lipase and its action, *J. Am. Chem. Soc*. **1937,** *59*, 2156,
20. Hassanien.F.R. and Mukherjee. K.D. isolation of lipase from germinating oil seeds for biotechnological processes *J. Amer. Oil Chem. Soc.* **1986,***63*(67) 893,
21. Theimer.R.R. and Inge.R. Development and intracellular localization of lipase activity in rapeseed (*Brassica napus* L.) cotyledons, *Planta*, **1978,** *139*, 244,