

β – galactosidase

(1)

(1)

(1)

(2)

-

(2) -

(1) -

-

2010/07/04

2010/11/29

5 PH

/ 0.2 +

 β -galactosidase

/ 0.2

%77.14

135.46

187.437

.() Sephacryl S-200

:

Isolation and Purification of β -galactosidase From New Born Goat Brain

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ABSTRACT

β -galactosidase enzyme was isolated from the new born goat brain by nine methods, It was found that the sodium acetate 0.2 Mole/Liter +0.2Mole/Liter NaCl PH5 method have given the highest specific activity of crude enzyme in comparison with the other methods. Also, this enzyme was purified by using four methods, the second one (cold acetone) was the better. As a result the purification fold was about 135.46 times and the yield about 77.14% by using Sephacryl S200 (second step). This enzyme is 187.437 KDa as a molecular weight.

Key words: Enzyme, Enzyme extraction, Enzyme purification,
Lactase, β – galactosidase, Goat Brain

Rat epididymal fluid
 (1997) Leparaoux Mannose
 %6 *achatine achatine*
 Glucose Galactose Mannose Xylose Fructoes Raminose
 N-acetyl hexos amine

(2010) Abed
 %9.9
 %22.3 *A. oryzoa*
.Aspergillus niger %32.5

β – galactosidase (1988) Lim 118
 Ohtsu *Klyveromyces lactis*
Thermus SP 186 ÷ (1998)
 180 ÷ (2004) Hiraiwa
 β-galactosidase

β–galactosidase

Lactose Intolerance
 Lactose Intolerance %70
 Lactose

(Lactic acid)
 (1994 Rings)
 Lactase
 β-galactosidase

()
 Damascus
 20 – 14

(1999) A.O.A.C.
Micro – kjeldahl

6.25

Soxhlet

Petroleum ether
(1987) Vooght Osborn
(1976) Pearson

.100

(pH)

(1985) Egan
.Phillips – 9409
(1) acid

Linolic

(1)

%		%	
167.10	(100/)	61.20	
5.60	pH	26.30	
0.98		4.20	
		7.30	
		1.00	
		100.00	

(1996) Al-Tamimi (1986)Jassim

0.2 %10

%5

/ 0.2 7 /
/ 0.2 3.8 5
/ 0.06 5 / 0.2 +
+ / 0.05 5
(.2) 5 / 0.06

Linoleat Emulsion

0.5 (1998 Liu) 234
0.2 7 PH (/ 0.2)
10 (%95 5)
0.5
4
2 %95 10 2
234 HCl

(Unite of enzyme)
(0.001)

234

(1976) Bradford

(Bovine Serum Albumin, B.S.A.)

β-galactosidase

(2)

()	(/)	(/)	()	()	
6579	19.293	7.93	153	43	
11374	29.150	8.30	242	47	% 0.5 Na ₂ CO ₃
47616	281.500	3.30	992	48	pH 5 %10 NaCl
31602	127.900	5.37	687	46	7 pH / 0.2
25605	90.310	6.30	569	45	5 pH / 0.2
33572	136.250	5.60	763	44	pH 3.8
68352	450.630	3.16	1424	48	+ / 0.2 pH 5 / 0.2 NaCl
18450	78.920	5.40	410	45	pH 5 / 0.06
27730	147.13	4.01	590	47	+ / 0.05 / 0.06

:

(3) (Ultra-filtration)

()

(3)

%		()	(/)	(/)	(/)	()	
100	1.00	67776	442.63	3.19	1412	48	
4.07	0.060	2761	26.64	7.40	197.2	14	%20
31.273	0.927	21196	410.60	3.30	1355	14	%60
100	1.00	66509	445	3.18	1415	47	
68.156	3.04	45330	1355.15	2.23	3022	15	
100	1.00	65274	448.80	3.19	1419	46	
7.643	0.058	3324	34	7.02	239	16	%30
58.73	2.55	38336	1135.50	2.11	2396	16	%60
100	1.00	64584	447.70	3.15	1409	46	

DEAE Cellulose - :
 / 0.25 - - sephadex A 50
 / 0.25HCl - / 0.25
 7 / 0.01 -
 / 1 -
 (1972) Whitaker DEAE Cellulose - Sephadex A 50
 30 500 30
 ()
 () ()
 () 7 PH
 .(58 × 1.6)
 / 5
 5 4 5
 . 280
 () () (Linear Salt Gradient)
 280
 (Fraction collector)
 .(Ultraoc 2070)
 Sephacryl- S-200 Sephadex G- 100
 Pharmacia (Sephacryl S-200)
 ()
 (1)
 Pernosil
 (2010) Abed (1987)

0.2 + / 0.2 (2)
5 /

68352 / 450.63
(2)

(1983) (3)

(1995 Borcelo Munoz ; 2004)

(1997,Schmander) (Salting out)

Lipoxygenase (2004)
(2006) Abed

%70-20 β-galactosidase
Becillus lichenforimis R₅ α-amylase (2004)
/ β-galactosidase 410.6 .%80
% 60-20

/ 442.63
0.927 %60 - 20

% 31.237

1:1 0.5:1 :
2:1 (/) 4:1 3:1 2:1

Attraction Force

(1972) Whitaker

$$F = \frac{Z^+ Z^-}{Dr^2}$$

() :Z⁺Z⁻ ;
 () : D
 () : r
 :F

() D
 30 () D
 () F 80 D

()

Denaturation
Hydrophobic bonds

(3) 3.04 / 1355.15 %68.156

(2004)

2.35 (/) 65.58 %45.64
 Lipoxygenase

(1975) AL-Obaidy

(2006) Abed
β-galactosidase

/ 448.80 / 1135.5 - 34
 %60 - %30

2.55
 %30

... β - galactosidase

/ 239 ()

%60

/ 2396

(..)

Dielectric (Water Miscible) constant

)

(2005 2004 2004 2004

) .

.(1975 Al-Obaidy 1996 1998

2.655 / 1187.60

(1995) Barceló Munoz .%35.67

(2008) (2004) Uhlenbruck Javeri

Lipoxygenase β - galactosidase Amylase

β -galactosidase

β -galactosidase (4)

:

DEAE Cellulose Sephadex-A50

7 / 0.01

280

DEAE Cellulose Sephadex-A50

.14.24 %95.116

β -galactosidase (4)

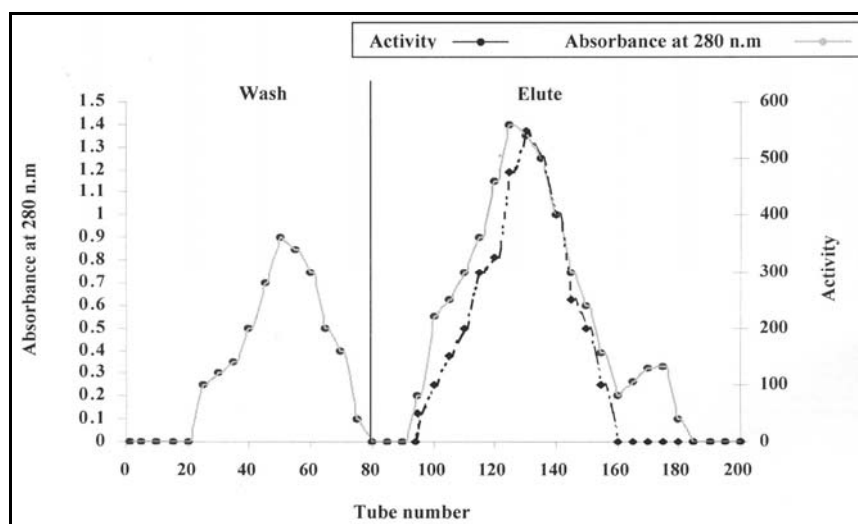
%		()	(/)	(/)	(/)	()	
100	1	67824	321.86	4.39	1413	48	
56.810	6.1477	44640	1978.72	1.41	2790	16	
95.116	14.24	64512	4585	0.67	3072	21	DEAE-Cellulose
98.100	20.26	66540	6523.52	0.51	3327	20	Sphadex G-100
75.128	105.5	50955	33970	0.10	3397	15	Sephacryl S-200
77.140	135.46	52320	43600	0.08	3488	15	Sephacryl S-200

(Peaks)

(1)

Washing

(Elution)

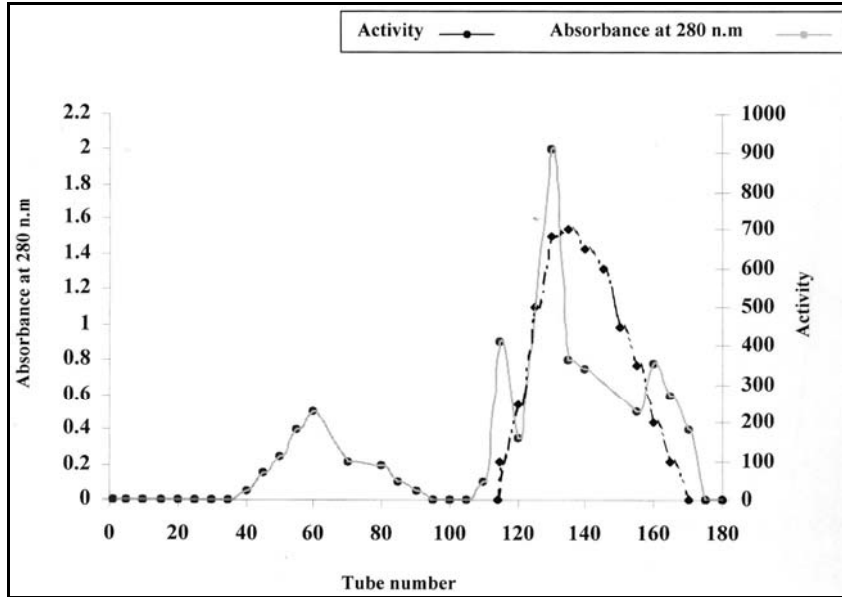


(1)

Sphadex G-100
(2)

%98.10

20.26



Sephadex G-100

(2)

Sephacryl S-200
% 75.128 105.5

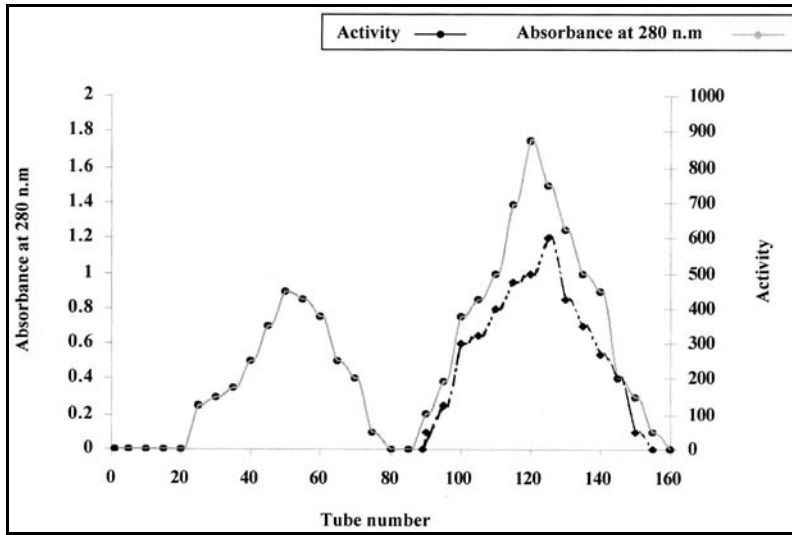
(4)

()

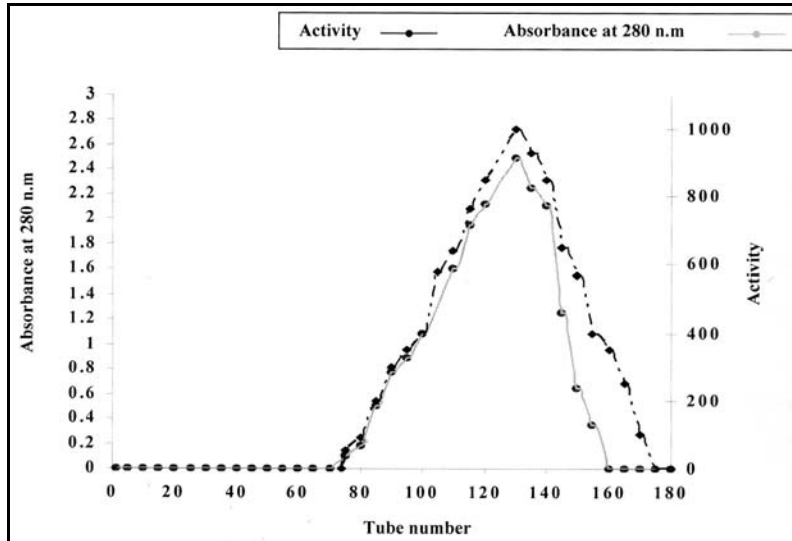
(3)

() Sephacryl S-200
(4)

% 77.14 135.46



Sephacryl S-200 () (3)



Sephacryl S-200 () (4)

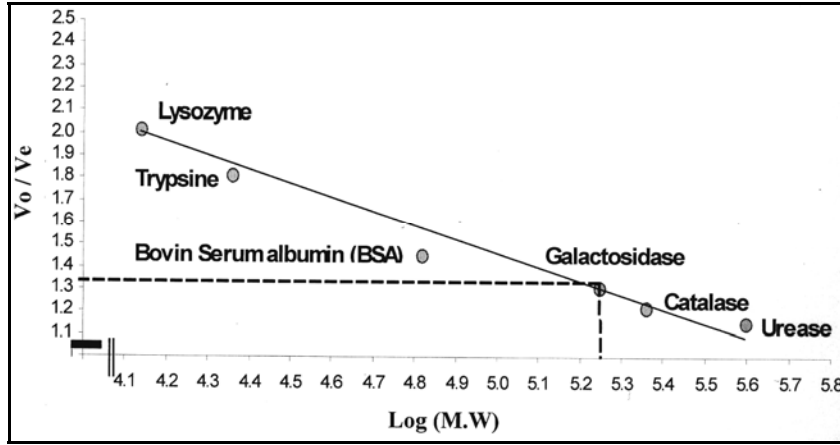
(5) β -galactosidase
 Sepharcyl S-200 β -galactosidase
 187.437

(2010) Abed

185.9
62 β-galactosidase

(2007) Desire
β-galactosidase

(2001) Zoltan *Rhynchophorus palmarum*
Chrysogenum β-galactosidase



(5)

Sephacryl S – 200

β-galactosidase

Elution)

(void volume) (volume
(β-galactosidase Lysozyme Trypsin B.S.A Catalase Urease)
(5)

(void volume) (Elution volume) (5)

β-galactosidase

Log M .W	Ve/Vo		Ve/ ml	
5.60	1.15	398000	63	Urease
5.36	1.22	232000	67	Catalase
4.82	1.45	67000	80	B.S.A
4.36	1.81	22000	100	Trypsin
4.14	2.01	14000	110	Lysozyme
5.25	1.31	unknown	72	β-galactosidase

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 .(2004) .
 .(2004) .
 .112 - 103 :(5) 35
Bacillus .(2004) .
licheniformis R₅
 G6pD .(2005) .
 .(2004) .
 - () .
Lupinus termis L.
 .(1996) .
Aspergillus. sp
Brassica rapa L. .(2004) .
 .(1998) .
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Arachis hypogaea L.
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