Biochemical studies on α-amylases from *Euphorbia tirucalli*.

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بسم الله الرحمن الرحيم
﴿ قَالُوا سُبْحَانَكَ لا عِلْمَ لَنَا إلاَّ مَا عَلَّمْتَنَا إنَّكَ
أَنتَ العَلِيمُ الحَكِيمُ ﴾
صدق الله العظيم
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The aim of the work

The aim of this study is purification and characterization of the α-amylases from *Euphorbia tirucalli* with respect to the pH, optimum temperature, thermal stability, the effect of the metals, inhibitors and chelating agents, the ability to degradate different substrates and their kinetics toward these substrates for industrial purposes .

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Abstract

The quantitative determination of α- amylase activity in latex of *E. tirucalii* was found to be 2144 units/ml with a specific activity of 131.7 units mg^{-1} protein , which was a good starting material for preparation of α-amylase. Purification was carried out for the three separated α-amylases namely α–Amylase AI , AII and AIII using columns of DEAE-Sepharose and Sephacryl S-200. Biochemical characterization for α–Amylase AI which has the highest specific activity with respect of molecular weight (40 kDa) , pH optimum (6.0) , temperature optimum (50ºC) , substrate specificity , Michaelis constant , heat stability , effect of different metal cations and different compounds was carried out.

AI is a metaloenzyme; it was activated by Ca^{2+} (2mM) as the most of plant amylases and therefore it is strongly inhibited by the metal chelator EDTA, sodium citrate and sodium oxalate.

Key Words: *E. tirucalii* , α- Amylase , Purification , Characterization , AI , Metaloenzyme.

List of Abbreviations

AI	*Euphorbia tirucalli* amylase 1
AII	*Euphorbia tirucalli* amylase 2
AIII	*Euphorbia tirucalli* amylase 3
Asp	Aspargen
ATP	Adenosine Tri-Phosphate
BSA	bovine serum albumin
cm	Centimeter
CBB	Coomassie Brilliant Blue
ºC	Celsius
DNS	dinitrosalicylic acid
D	Dalton
DEAE	Diethylaminoethyl
DNA	DeoxyriboNucleic Acid
DP	The degree of polymerization term
DTNB	Dithiobis 2-nitrobenzoic acid
EC	Enzyme Commission
EDTA	Ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
Fig	Figure.
g	Gram
Glu	glutamic acid
H- bond	Hydrogen bond
h	Hour
i.d.	Internal Diameter
Km	Mickaelis constant
KD	Kilodalton
LOX	Lipoxygenase
M	Morality
µg	Microgram.
µl	Microliter.
µm	Micromolar
Mg	Miligram
Ml	Milliliter
mM	Millimolar
Min	Minute
nm	Nanometer

M. wt	molecular weight
mmol	Millimole
m	Meter
p-CMB	p-Chloromercuribenzoate
PAGE	Polyacrylamide gel electrophoresis
p-HMB	p-hydroxy mercuric benzoate
pH	Potential of Hydrogen
PMSF	Phenylmethanesulfonyl fluoride
Rf	Relative electrophoretic mobility
rpm	Revolutions Per Minute
Ser	Serine
SDS	Sodium dodecyl sulphate
Spp.	Species(plural)
Sp.	Species(single)
SEM	scanning electron microscopy
S	Substrate
TEMED	Tetramethylethylenediamine
TRI	triose phosphate isomerase
v/v	volume/volume
V0	Void volume
Vmaxs	Maximum volume
Ve	elution volume
w/v	weight/volume
%	Percentage
β-ME	β –mercaptoethanol

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