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Examination of Catalase enzyme in fresh leaves and study some chracters of it

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Summary

CATALASEIEC:
1 .1 1.1.6) was eliminated in the lettuce Lactuca sativa ,cabbage Brassia oleracea, spinach Spiacia olearacea, Chard Beta vulgarer leaves , enzyme assay ,protein contains , optimum temperature of .
Activity & stability ,optimum ph of activity & stability .
The result shower The chard Beta bulgaria leaves was biggest activity of database enzyme was (256.897) U/mg , protein contain was (4.797) mg/mi , optimum temperature of activity 50c was(0.256) U/ml , optimum temperature of stability 50C to chelation 30 min , CAT has optimum ph of activity 8 was (0.608) U/ml , optimum ph of stability was (1.455)U/ml

Information
Catalase plays a major role in the protection of tissues from the toxic effect of h202 . CAT also removes electrons that can lead to the production of 02 free- radical (Abassi et al ; 1998) . CAT has been found in all aerobic cells containing cytochrom & CAT enzyme is one of the earliest enzyme had been studied & purified (Percy ; 1984). It is primarily an intra cellular enzyme . The enzyme usually it have been sensitive from extreme temperature degree , the extreme temperature cause changes in primary secondary & active structure of protein & denaturation of it as well as the changes of PH effects in natural of enzyme . The extreme ph decreased activity & * Biology department collage of education / university of dyila

stability of enzyme . Optimum temperature of activity & ph depended on the source of enzyme (Aydemir et al ; 2003) The aim of the present study was to characterize the CAT from the local varieties lettuce Lactuca sativa , cabbage Brassia oleracea , spinach Spiacia olearacea, chard Beta vulgarer leaves & enzyme assays , protein contains , Optimum temperature and ph of activity & stability.
Materials & methods

Chemicals
Hydrogen peroxide , Phosphate buffer (Na2 HPO4 ,K2HPO4) , Bovine serum albumin (BSA) ,Ammonium collide , Acitate buffer , iris buffer.
Enzyme assay CAT activity was determined of fresh leaves at 25c according to A1 - Alwani (Alwani ;2006) The reaction mixture contained (10 mM) h202 in a (60mM) sodium potassium phosphate buffer ph 7.4 & 20 ul crude than addition (34.2M)
ammonium molybdate .CAT activity was estimated by decreased in absorbency of h202 at 504nm .according to equation : -

$$\text{Catalase Activity} = \frac{\text{Sample - Blank 1}}{\text{Blank 2 - Blank3}} \times 271$$

if Blank 1= lml (H2O2 + sodium potassium phosphate buffer) + 1 ml (ammonium Molybdate) + 0.2 sample .

Blank 2= lml (H2O2 + sodium potassium phosphate buffer) + lml (ammonium Molybdate) + 0.2 (sodium potassium phosphate buffer) .

Blank 3| lml (sodium potassium phosphate buffer) + lml (ammonium Molybdate) + 0.2 sodium potassium phosphate duffer) .

Determination of protein Protein amount for CAT was done according to method of Mohamed & abd all (1996 , محمد وعبدالله)with bovine serum albumin as standard
Effect of ph
The effect of ph of CAT activity was investigated in the range of ph 4-5 (0.05M) acetate buffer , ph 6-8 (0.05M)
phosphate buffer , ph 9-10 (0.05M) tries buffer .Then 10 ul enzyme solution was mixed with 0.99 ml buffers at various ph values incubated at 25c for 1 h . Thereafter retained enzyme activity was measured as indieatedin Aebi (Aebi ;1984) after that optimum ph was determined .

Effect of temperature The effect of temperature on CAT activity was investigated by incubating the enzyme solution in (0.05 M) phosphate buffer ph 7.0 for 30 min in the range of 10 -70 C. Then 10 ul enzyme solution was mixed with 0.99 ml phosphate buffer & incubated , Thereafter retained enzyme activity was measured by absorption at 240

nm according to Aebi (Aebi ; 1984) after that optimum temperature was determined .

Optimum ph to stability To CAT stability was determined by incubation of enzyme solution with previously to prepare buffers at 1:1 at 25c for 60 min & measured by absorption at 240 nm .

Optimum Temperature to stability Various temperatures were used to CAT stability was determined by incubation of enzyme solution at 50 C at seven different time (10 - 70 min in bath water , Than 20 ul enzyme solution mixed with 0.99 ml h202 (65 mM) & Sodium phosphate buffer (60 mM) ph 7.0 to stopped the enzyme work & measured by absorption at 240 nm .

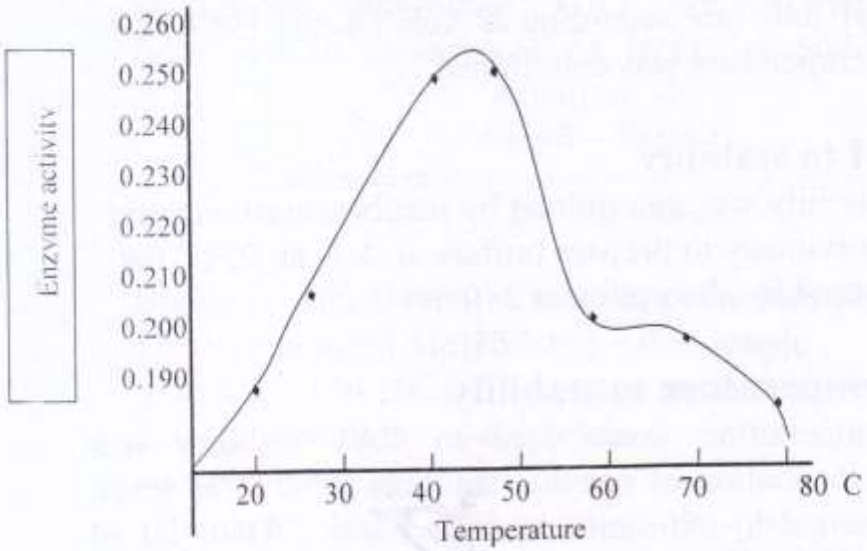
Results & discussion

(Table 1) shown the biggest CAT total activity was determined at chard leaves were 6161.68 units , that affected to contain of protein was measured to be (4.797) mg/ml

Table (1) CAT activity & protein

Crude extract	Specific activity . (Units/mg)	Protein (mg / ml)	Activity (Units/ mg protein)	Total protein (mg/ml)	Total activity (Units)
L.sativa	101.409	1.444	146.435	507.040	732.17
B.oleracea	81.124	1.934	156.897	405.622	784.473
S.olearacea	197.502	4.776	943.270	987.510	4716.350
B.vulgaris	256.897	4.797	1232.336	284.486	6161.684

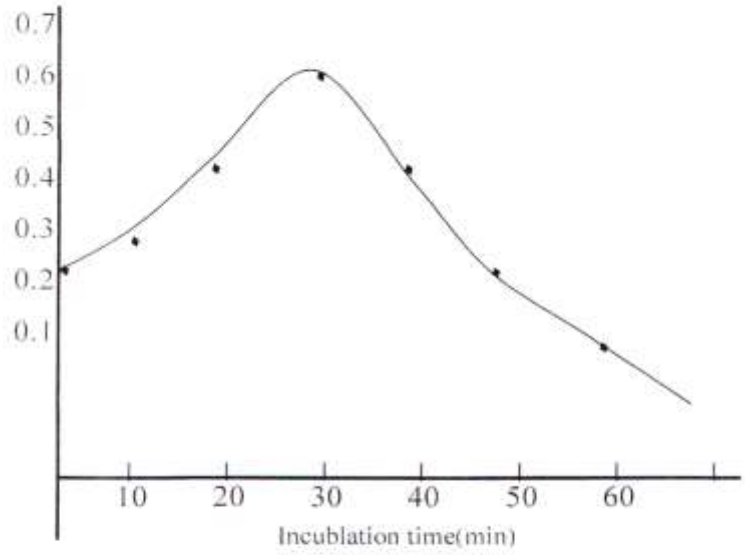
The CAT activity shown in (Fig 1) increasing when the incubating temperature until it was (0.256)U/ml at 50C, Then the activity was decreasing because the increasing temperature increased the speed of reaction due to increasing movement energy to enzyme molecules (Al-Akarky ; 2002)



(Fig 1) effect of Temperature in enzyme activity(CAT)

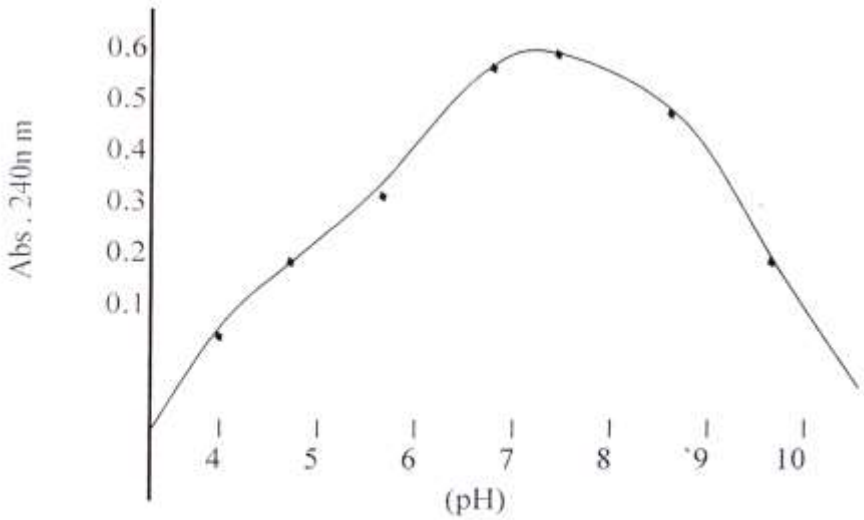
The thermal stability appear in (fig 4) CAT stability in the enzyme solution kept storge there activity when incubating 50C° 30 min was (0.604)U/ml

The optimum temperature we determined was similar to that given in previous studies (Demir ; 2004)



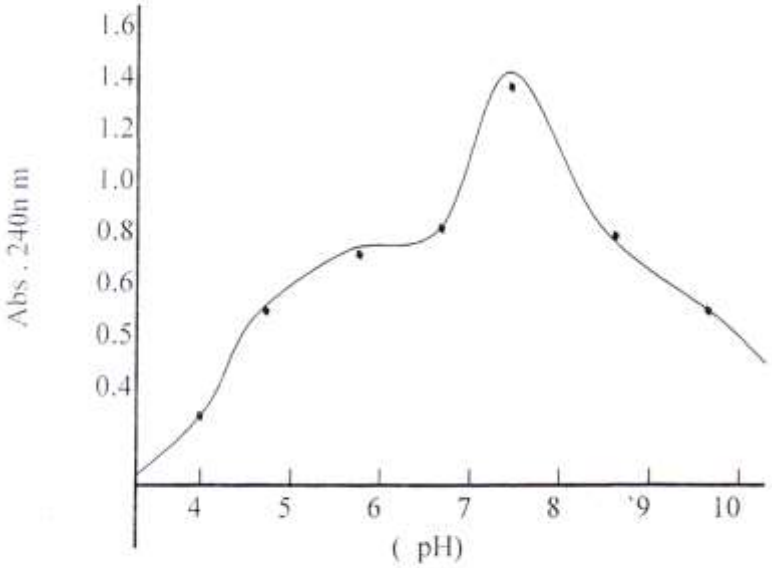
(Fig4) effect of Temperature in stability of enzyme (CAT)

In the study of effect at different pH of CAT activity shown in (fig 3) the biggest activity was (0.608)U/ml at pH 8 ,Then decreasing in crude cause the denaturation of protein molecules , however the same results were arrived
(Yoruk ; 2005 ;Gholamhoseinian ; 2006)



(Fig 2)effect Of pH in to enzyme activity(CAT)

Enzyme stability was expressed as remaining activity .The stability at different pH values was shown (fig 4) when CAT was stored at pH 8 was (1.455)U /ml other studies has been repoted exhibit sharp optimum pH (Torres : 2003)



(Fig 4) effect of PH in stability of enzyme

التحري عن انزيم الكاتليز في الأوراق الخضراء ودراسة بعض خصائصه

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المستخلص

تم التحري عن انزيم الكاتليز CAT في اوراق الخس Lactuca sativa واللهانة Brassia oleracea والسبانخ Spiacia oleracea والسلق Beta vulgaris وتم تقدير الانزيمية له اذ أظهرت أوراق السلق أعلى فعالية بلغت (٢٥٦.٨٩٧) U\mgوتمت دراسة محتوى الاوراق من البروتين الكلي الذي تفوقت اوراق السلق والسبانخ في اعلى محتوى بلغ (٤.٧٧٦ و٤.٧٩٧)ml\mg على التوالي كما تم اختيارلدرجة الحرارة المثلى لفاعلية وثبات الانزيم والدالة الحامضية المثلى لفاعلية وثبات عمل الانزيم في اوراق السلق اظهرت الدراسة النتائج التالية:.

كانت درجة الحرارة المثلى لفاعلية الانزيم C ٥٠ بلغت عندها فاعليته (٠.٢٥٦) U\mlو اظهر الانزيم ثباتا عند حضنهلمدة ٣٠ دقيقة في درجة ٥٠م وصل (٠.٦٠٤)U\ml اما الدالة الحامضية المثلى للفعالية كانت عند (٨) (ph) وبلغت (٠.٦٠٨) U\mlوابدى الانزيم ثباتا عند نفس الدالة الحامضية ووصل الى (١.٤٥٥) U\ml

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