Kinetics studies of catalase and peroxidase enzymes extracted from garlic cloves (*Allium sativum* L.)

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Abstract

Garlic cloves belongs to the genus Allium and family *liliaceae*, is one of the more commonly used health supplements in the world. It is a medical plant known since a long time for its therapeutic benefits. Many attempts were carried out to elucidate its importance since it contains natural antioxidant enzymes, i.e. catalase (CAT) and peroxidase (POD). These natural enzymes were extracted from garlic cloves and their activities and kinetic characterization were investigated. The results indicated that the activity, protein content and specific activity of catalase and peroxidase were 2.05, 204.4 Units /ml, 4.2, 5.11mg/ml and 0.448, 40Units/mg protein, respectively. The optimum pH and temperature of the enzymes under investigation were 7.0, 5.5 and 40ºC, 50ºC, respectively. The K_m and V_{max} for catalase and peroxidase enzymes were equaled to 0.532, 2.70 ml/100ml and 1.56, 0.60 Units/ml/min, respectively.

Keywords: Garlic cloves; Antioxidant enzymes; Catalase; Peroxidase**.**

Introduction

Garlic cloves (*Allium sativum L.*) are one of the most important vegetables in the world. Garlic cloves had been used an important source for antioxidant enzymes (Catalase and peroxidase) which used in several fields **(Lewis and Elvin-Lewis., 2003)** Catalase (E.C.1.11.16) is a common enzyme found in nearly all living organisms **(Chelikani *et al.*, 2004).** And important cellular antioxidant enzyme that defends against oxidative stress **Neelam (2013**) It efficiently catalyzes the decomposition

of hydrogen peroxide to oxygen and water together with other enzyme systems protects cells against the harmful effects of reactive oxygen species (ROS)such as superoxide anions, hydrogen peroxide and hydroxyl radicals **(Susmitha *et al*., 2013)** . Peroxidase (EC 1.1.11.7) is widely distributed in plants and has ability to applications in many areas including chemical synthesis, diagnostic and food industry **(Singh *et al*., 2017).** Peroxidase from garlic bulbs (cloves) plays a vital role in chemical process as antioxidant factor **(Mamounata *et al*., 2011)**, **(Sfaxi *et al*., 2009)** measured the specific activity of peroxidase and catalase in bulb are respectively about 40, 6.9 U/mg. **(El Ichi *et al*.,2008)** studied anew peroxidase from garlic (Allium sativum) bulb : its use in H_2O_2 biosensing and found that the optimal pH was approx. 5 and the optimal temperature was 30ºC.the K_m (app) values for H_2O_2 obtained for POX_{IA} and POX_{IB} in the presence of o-dianisidine were respectively approx. 3and 0.5mM.this results suggests that POX_{IB} has better affinity for H_2O_2 than POX_{IA}. For POX_{IB}, the K_m (app) values for o-dianisidine and guaiacol were respectively 0.2 and 5.5 mM . The V_{max} values were respectively 0.56 and 31.8 mM . min^{-1}. **(Osuji *et al* .,2014)** extracted an acidic peroxidase from garlic (Allium sativum) and was partially purified threefold by ammonium sulphate precipitation, dialysis and gel filtration chromatography using sephadex G-200. The specific activity of the enzyme increased from4.89 U/mg after ammonium sulphate precipitation to 25.26 U/mg after gel filtration chromatography and they found that the protein content, activity and specific activity of garlic peroxidase were 4.981(mg/ml), 20.39 (U/ml) and 4.09 (U/mg) , respectively. The optimum

temperature and pH of the enzyme were 50°C and 5.0, respectively. The

K_m and V_{max} for H_2O_2 and o-dianisidine were 0.026 mM and 0.8 U/min

and 25 mM and 0.75 U/min, respectively. The application of this enzyme in industrial wastewater treatment especially with hydrogen peroxide. These Vat dyes also exhibited potentials of acting as peroxidase inhibitors at alkaline pH range. **(Marzouki *et al*.,2005)** studied anew thermostable peroxidase from garlic (*Allium sativum*) and they found that the total protein , total activity and specific activity were 497 mg ,50.042 U and 101 U/mg. the optimum temperature ranged from 25 to 40ºC and optimum pH was about 5.0 . The apparent K_m values for guaiacol and H_2O_2 were 9.5 and 2 mM , respectively. These properties permit the use of this enzyme as biosensor to detect H_2O_2 in some food components such as milk or its derivatives. **(Marzouki *et al*.,2010)** studied the kinetic characterization of a basic peroxidase from garlic (*Allium sativum*) and found that the purification of peroxidase obtained from soluble fraction of garlic cloves. Total protein, total activity and specific activity were 96.3mg, 71242 IU and 739.5IU/mg.

The main purpose of this study was to find out if garlic cloves could be used as convenient and rich source of antioxidant enzymes, catalase and peroxidase. The enzyme activities, characterization properties and kinetics parameters of these enzymes also were estimated.

Materials and methods

Enzymes extraction

Garlic cloves were cut into small pieces and homogenized with0.2*M* Tris HCl buffer (pH 7.8) containing 14 m*M* β-mercaptoethanol at a rate of 1:3 (w/v). There for, the extract was filtered through filter paper (Whatman No. 1) and centrifuged at 10000 rpm for 20 min at 4 °C **El-Ichi *et al*., (2008)** for peroxidase enzyme. For catalase enzyme extracted small pieces of garlic cloves homogenized with phosphate buffer (50 mM, pH 7.0) containing 1mM of EDTA. The homogenate filtered through two layers of cheese cloth and the obtained extracted was centrifuged at 7000 rpm for 20 min at 4 °C, **Nur-Hidayah *et al*, (2014)**. The clear supernatants from different extracts of peroxidase and catalase enzymes were kept at 4°C for assays**.**

Catalase and peroxidase enzymes assay

Catalase enzyme (E.C. 1.11.1.6) activity was estimated according to the method described by **Aebi (1984).**

Peroxidase enzyme (E.C.1.11.1.7) activity was determined according to the method described by **(Sfaxi *et al* . 2009)**.

Enzyme protein content

Enzymes protein content for catalase and peroxidase enzymes were determined according to the method described by **Bradford (1976)**, using bovine serum albumin (BSA) as standard.

Kinetics parameters of peroxidase and catalase enzymes extracted from garlic cloves.

Various pH values ranged between (4.0 to 9.0) were tested to determine the optimum pH of catalase and peroxidase activities using acetate buffer (pH 4.0-5.5) , potassium phosphate buffer (pH 6.0-7.5) and Tris Hcl buffer (7.5-9.0) as described by **Osuji *et al.* (2014).** The activity and percent relative activity were calculated as mentioned before.

The effect of different temperatures on catalase and peroxidase activities were tested to determine the optimum temperature by incubating the reaction mixtures of catalase and peroxidase at different temperatures were 30, 35, 40, 45, 50, 55 and 60°C. The experiments were carried out at optimum pH and the standard assay conditions, **(El Ichi *et al.*,2008)**

Different substrate concentrations of H_2O_2 (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 % (v/v) for catalase enzyme and (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4 and 1.5 % (v/v)for peroxidase were utilized to study the effect of substrate concentrations on reaction activity and velocity at optimum pH and temperature as mentioned before.

Results and Discussion

Activity, protein content and specific activity of crude catalase and peroxidase extracted from garlic cloves.

The major aim of this study was to carry out a systematic study of the influence of various parameters i.e. pH, temperature and substrate concentration on the reaction activity of catalase and peroxidase enzymes extracted from garlic cloves.

The activity, protein content and specific activity for catalase and peroxidase extracted from garlic cloves (*Allium sativum*) were carried out and illustrated in Table (1). Data showed that the catalase activity, protein content and specific activity were found to be 2.05 units/ml, 4.2mg/ml and 0.448 units/mg protein, respectively. Whereas, the peroxidase activity, protein content and specific activity were found to be 204.4 units/ml, 5.11 mg/ml and 40 units/mg protein, respectively. The obtained results are lower than that stated by **(Sfaxi *et al.*,2009).** Who found the specific activity of peroxidase and catalase in bulb are respectively about 40, 6.9 U/mg.

Table (1). Activity, protein content and specific activity of crude catalase and crude peroxidase extracted from garlic cloves.

Enzymes extracted	Activity (units/ml)	Protein content (mg/ml)	Specific activity (units /mg protein)
Crude Catalase	2.05	4.2	0.448
Crude Peroxidase	204.4	5.11	40

Factors affecting on the reaction activity of catalase and peroxidase enzymes extracted from garlic cloves.

Effect of pH

The enzyme activities of catalase and peroxidase were demonstrated in Table (2) and Fig (1), the obtained results showed that the maximum reaction activity was 2.00 units /ml/min which recorded at pH 7.0 for catalase. While, the maximum reaction activity found to be 18.38 units /ml/min was found to be at pH 5.5 for peroxidase. These results are higher than with those obtained by **(Osuji *et al* .,2014)** who found that the optimum pH for peroxidase enzyme was 5.0 respectively. and

Table (2): Effect of pH on the reaction activity of catalase and peroxidase enzymes extracted from garlic cloves.

pH values	Catalase enzyme		Peroxidase enzyme	
	Reaction activity (units/ml/min)	Relative activity (%)	Reaction activity (units/ml/min)	Relative activity (%)
4.0	1.16	57.89	13.80	75.08
4.5	1.32	65.91	14.67	79.81
5.0	1.41	70.67	16.49	89.71
5.5	1.60	79.94	18.38	100.00
6.0	1.69	84.46	15.55	84.60

6.5	1.82	91.22	14.67	79.81
7.0	2.00	100.00	14.12	76.82
7.5	1.51	75.68	13.76	74.86
8.0	1.44	71.82	12.84	69.85
8.5	1.23	61.65	12.47	67.84
9.0	1.21	60.50	11.92	64.85

Fig(1): Effect of pH values on the reaction activity of catalase and peroxidase enzymes extracted from garlic cloves.

Effect of temperature

Results presented in Table (3) and Fig (2) showed that the enzymatic reactions were increased with increment of reaction temperature to a certain value in general. Catalase activity showed an optimum reaction temperature at 40°C. On the other hand, the obtained results for peroxidase activity at different temperature values .i.e. 30°C to

60°C had shown in Table (3) and Fig(3).The obtained results indicated that the activities were increased from 30°C till reached its maximum at 50°C beyoud this temperature the reaction activity was decreased. This trend of results were higher than as found by **El Ichi *et al*.,(2008)** who found an optimum activity at 30° C for peroxidase from *Alliun sativum.* and **Belhadj *et al*,(2015)** who found an optimum activity at 40° C for catalase from *Alliun sativum*.

Table (3): Effect of temperature on the reaction activity of catalase and peroxidase enzymes extracted from garlic cloves.

temperature	Catalase enzyme		Peroxidase enzyme	
	Reaction activity (units/ml/min)	Relative activity (%)	Reaction activity (units/ml/min)	Relative activity (%)
30	1.99	87.3	15.38	71.50
35	2.08	91.4	16.07	74.71
40	2.28	100.0	17.14	79.68
45	2.18	95.6	19.29	89.67
50	2.07	90.8	21.51	100.00
55	1.95	85.5	20.36	94.65
60	1.84	80.7	19.29	89.67

Effect of substrate concentration on the reaction activity and reaction velocity of catalase and peroxidase enzymes extracted from garlic cloves.

Substrate concentration is one of the most important factors which effect on the efficiency and velocity of the enzyme reaction. So, the effect of substrate concentration on the reaction velocity of catalase and peroxidase enzymes were evaluated. It was clear that the enzymatic oxidation reaction of H_2O_2 was increased with the increasing of H_2O_2 concentration, then gradually decreased. This reduction is a function of enzyme activity at constant reaction parameters. The reaction activities and reaction velocities of catalase and peroxidase enzymes for various substrate concentrations are plotted in saturation curve , from which maximal activities (V_{max}) and Michealis-Menten constants (K_m) values were calculated.

From this point of view, the obtained results are tabulated in Table (4) and graphically illustrated by Fig. (3,a,b). K_m and V_{max} values were found to be calculated 0.532ml/L and 1.56 units/ml/min for catalase enzyme, respectively. As well as the K_m and V_{max} values of peroxidase

enzyme were recorded in (Table5) and Fig (3,c,d) as 2.70 ml/L and 0.60 units/ml/min, respectively. Lineweaver-Burk plots of experimental data for catalase enzyme was showed in Fig. (3,b). For peroxidase enzyme was showed in Fig. (3,d). The obtained K_m by Lineweaver and Burk plots was equalled to that obtained by experimentally curve. These values for peroxidase enzyme are higher than that reported by

Table (4): Effect of substrate concentration on the reaction activity and reaction velocity of catalase enzyme extracted from garlic cloves.

Substrate concentrations% (v/v)	[1/S]	Reaction activity (U/ml/min)	Reaction velocity (v)	[1/v] $\times 10^{-1}$
0.5	2.0	0.98	1.35	7.41
1.0	1.0	2.04	2.23	4.48
1.5	0.67	2.76	2.85	3.51
2.0	0.50	3.81	3.31	3.02
2.5	0.40	4.37	3.67	2.72
3.0	0.33	4.65	3.95	2.53
3.5	0.28	5.37	4.18	2.39
4.0	0.25	6.43	4.37	2.29
4.5	0.22	6.25	4.53	2.21
5.0	0.20	5.85	4.67	2.14
5.5	0.18	5.60	4.79	2.09
6.0	0.17	5.09	4.89	2.04
6.5	0.15	4.59	4.98	2.01

7.0	0.14	4.47	5.06	1.98
7.5	0.13	4.06	5.14	1.95
8.0	0.12	3.94	5.20	1.92

V_{max} = 1.56 Units/ml/min. K_m = 0.532 ml/100 ml $v = \frac{V_{max} \times [S]}{K_m + [S]}$

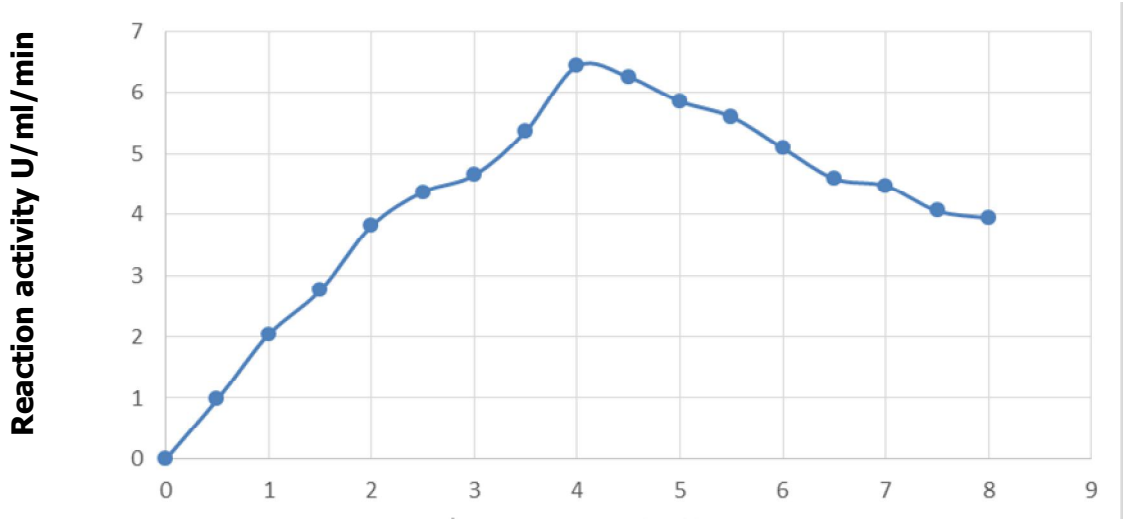


Fig (3,a) :Effect of substrate concentrations on the reaction activity of catalase enzyme extracted from garlic cloves.

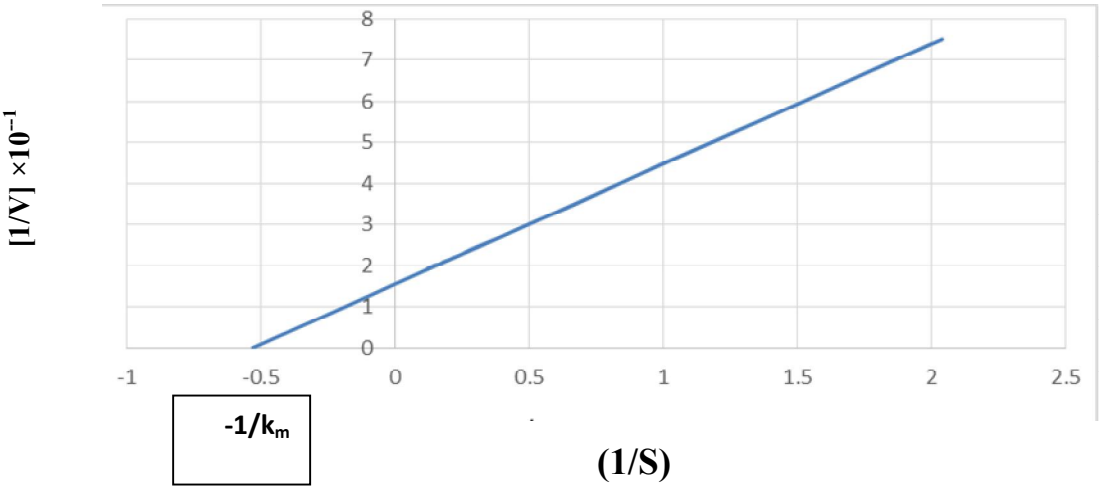


Fig (3, b): **Lineweaver-Burk plots of catalase enzyme**

Table (5): Effect of substrate concentration on the reaction and reaction velocity of peroxidase enzyme extracted from garlic cloves.

Substrate concentration (v/v)%	[1/S]	Reaction activity Units/ml/min	Reaction velocity (v)*	[1/v] $\times 10^{-1}$
0.1	10.0	3.33	3.53	2.83
0.2	5.00	7.00	5.82	1.72
0.3	3.33	8.34	7.42	1.35
0.4	2.50	9.67	8.61	1.16
0.5	2.00	10.68	9.53	1.05
0.6	1.67	11.67	10.26	0.97
0.7	1.42	12.68	10.85	0.92
0.8	1.25	14.02	11.34	0.88
0.9	1.11	15.01	11.75	0.85
1.0	1.00	16.58	12.10	0.83
1.1	0.91	16.02	12.41	0.81
1.2	0.83	15.00	12.67	0.79
1.3	0.77	14.34	12.91	0.77
1.4	0.71	14.03	13.11	0.76

1.5	0.67	13.34	13.30	0.75

V_{max} = 0.60 Units/ml. **K_m = 2.70 ml/100 ml** $v = \frac{V_{max} \times [S]}{K_m + [S]}$

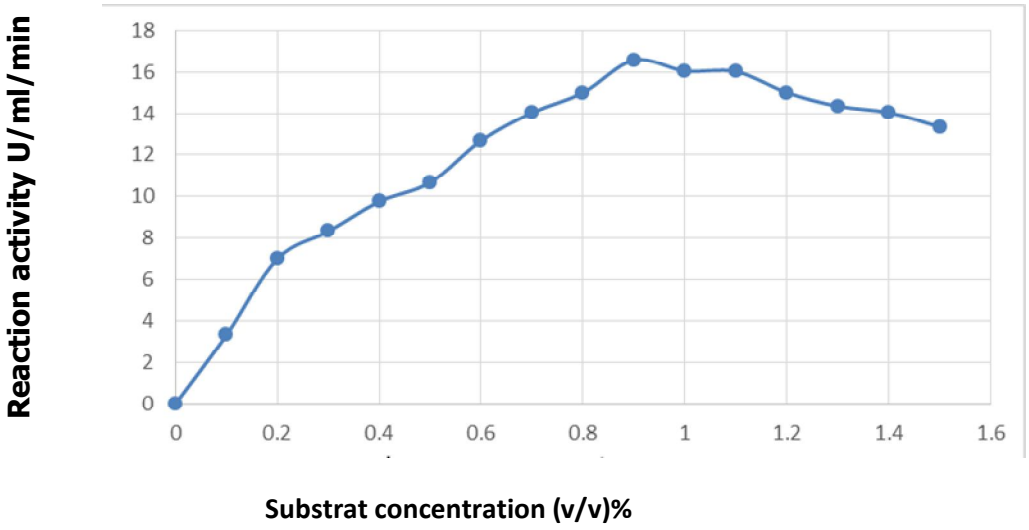
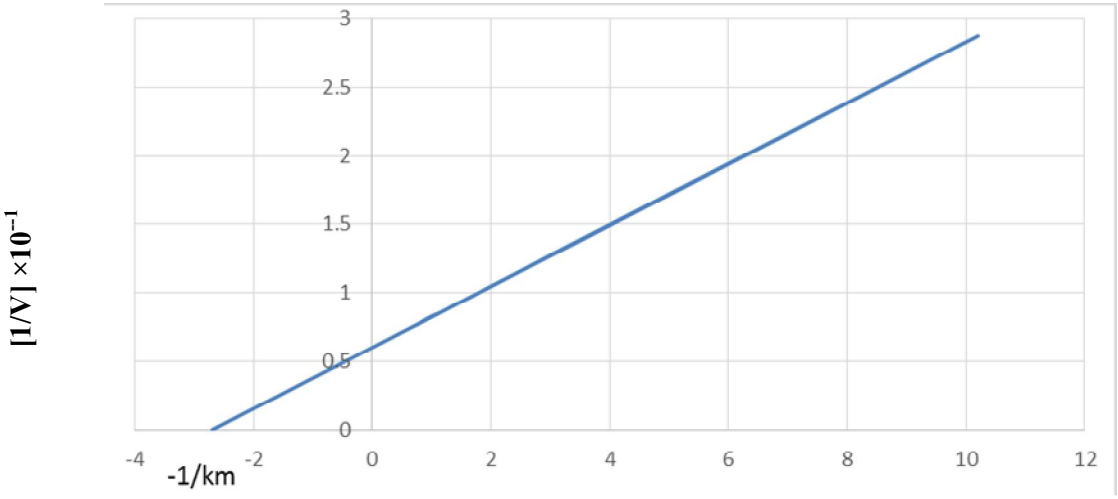


Fig (3,c): Effect of substrate concentration on the reaction activity of peroxidase enzyme extracted from garlic cloves.



1/S

Fig (3, d): Lineweaver-Burk plots of peroxidase enzyme

CONCLUSION

In the present study, we determined antioxidant enzyme activities, CAT and POD, were extracted from garlic cloves and evaluation kinetics parameters of these enzymes in *Allium sativum L.* cloves. Our results showed that cloves have a good antioxidant potential of application in waste water, detoxification and rapid detection of peroxidase in food and beverages. Results , we can conclude that the garlic cloves are a promising source of natural antioxidants and might be used in the treatment of diseases associated with oxidative stress.

References

Aebi, H.E. (1980): Enzymes1:oxidoreductases, trasferasesin: bergmeyer. H, ED. Methods of enzymatic analysis . 57: 273-282.

Belhadj,F.;Messaoud,C.;Benhlel,T.;Demirtas,I.andMarzouki,M.N.(2015): Phytochemical screening , antioxidant and antimicrobial activities of *Allium sativum L.* leaves ,bulbs and roots: Identification of major active compounds . Res, Article, F. Belhadj , IJPRBS, 4(5):46-68.

Bradford ,M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Biochem ., 72: 248-254.

El -Ichi, S.; Abdelghani, A.; Hadji, I.; Helali, S.; Limam, F. and Marzouzi ,M.N.(2008):A new peroxidase from garlic (*Allium sativum*)bulb: its use in H_2O_2 biosensing**.** Biotech, Appl. Biochem., 51, 33-41**.**

Mamounata, D.;Oumou ,H.K.;Nafissetou,G.B;Romaric, H.N.B.; Imael . and Mamoudou ,H.D.(2011): Comparison of

peroxidase activity from *Allium sativum,Ipomoea batatas, Raphanus sativum* and *Sorghum bicolor* grown in Burkina Faso. Afracian J.of Biochem., Res.,5.124-128.

Osuji, A. C.; O.Eze , O. S.; Osayi, E. E. and Chilaka, F .C. (2014). Biobleaching of industrial important dyes with peroxidase partially purified from garlic. The Sci. World.J, Article ID 183163, 8 pages.

Marzouki, S.M.; Almagro, L.; Sabater – Jara , A. B.; Barcrlo, A. R.; and Pedreno,M.A .(2010): Kinetic Characterization of a Basic Peroxidase from Garlic (*Allium sativum* L.) Cloves**.** J. of Food Sci., 75,(9), 740-746.

Marzouki, S.M.; Liman ,F.;Smaali,M.I.;Ulber,R.and Marzouki, M.M(2005): Anew thermostable peroxidase from garlic (*Allium sativum*).Applied Biochem and Biotech.127,201-214.

Neelam, S.(2013): Enhancement of catalase activity under salt stress in germinating seeds .Asian J. of Biocm and pharm., Sci.,3(17),6-8.

Sfaxi,I.H.;Belhadj,F.; Limam , F. and Marzouki,M.N .(2009): Screening of Enzymatic Antioxidant Activities in *Allium sativum* L. Biologia Tunisie Juillet ,(7),1-4.

Singh, S.;Rajani, S.;Ambuj, B. J.; Amarendra, N. M. and Pallavi Sh.(2017): A potential source of peroxidase for wide applications. Inter of food properties, 20, 11, 2658–2664.

Chelikani, P.;Fita ,I. and Loewen ,P.C.(2004): Diversity of structure and properties among catalase. Life sci, 61(2) 192-208.

Susmitha, S.; Shyamala, R.; Gowri, P.;Meenambigai, R.; Ramitha. and Vijayaraghavan, R. (2015): Physiochemical properties of purified Catalase enzyme from Azolla**.** Int. J.Curr. Microbiol .App.Sci .4(8): 836-844

الملخص العربى

دراسات حركيات إنزيمات الكاتلايز والبيروكسيديز المستخلصة من فصوص الثوم

نجوى إبراهيم الخياط أ.د.صلاح مصطفى سعد أ.د.فرحات فودة على فوده د.عبدالله السيد الحضرى

قسم الكيمياء الحيويه – كلية الزراعة– جامعة بنها

تهدف هذه الدراسة الى تقييم اهمية انزيمات المضاده للأكسده حيث انها تعمل على الحد من التاثير الضار للشقوق الحره الناتجه عن عمليات التمثيل الغذائى للمركبات المختلفه داخل جسم الانسان وفى هذه الدراسه قد تم تسليط الضوء على انزيمى (الكاتالايز والبيروكسيديز) ولذلك تم أختيار فصوص الثوم كمصدر طبيعي لانتاج هذه الانزيمات.

وقد اشتملت الدراسه على:

– تقدير النشاط الانزيمى والمحتوى البروتينى لكلا من انزيمى الكتالايز و البيروكسيديز المستخلصه من فصوص الثوم .

– دراسة افضل العوامل التى تؤثر على سرعة التفاعل والنشاط لكلا من انزيمى الكتالايز و البيروكسيديز من تركيز لايونات الايروجين, درجة الحرارة, تركيز مادة التفاعل وايجاد كلا من ثابت ميكاليسوالسرعة القصو ى.

–حيث اوضحت نتائج الدراسه على (فصوص الثوم) ان درجة النشاط والمحتوى البروتينى لانزيمى الكتالايز والبيروكسيديز هى 2,05و204,4 وحدات/ملليلتر2و4 وحدة /ملل و 11و5مللجم/ ملل على التوالى.

كما وجد ان افضل درجة لتركيز ايونات الايدروجين لازمه لنشاط كلا من الكتالايز والبيروكسيديز هى 7 و 5و5 على التوالى . كما وجد ان افضل درجة حرارة لازمة لنشاط كلا من الكتالايز والبيروكسيديز هى 40 و50 درجة مئوية على التوالى.و ان افضل تركيز للمادة المتفاعلة اللازمة لنشاط كلا الكتالايز والبيروكسيديز هو4 ملليلتر /100ملل و 1 ملليللتر/ملل.

كذلك وجد ان قيمة كلا من ثابت ميكاليس والسرعه القصوى لكلا من انزيمى الكتالايز والبيروكسيديز هى 1.88 ملليلتر / 100مل, 6.43 وحدات/ ملليلتر/دقيقه و0.37مل/ 100ملل و 16.58 وحدات/ ملليلتر/دقيقهعلى التو الى .

توصي الدراسة: تبعا للنتائج المتحصل عليها فى هذا البحث يمكن التوصية باستخدام مضادات الأكسدة الطبيعية والموجودة بكثرة فى (فصوص الثوم) وذلك عن طريق تناولها من مصادرها الطبيعية او اضافتها الى المستحضرات الطبية او الغذائية وذلك للحد من مخاطر الشقوق الحرة .كما يمكن استخلاص واستخدام إنزيم الكتالايز البيروكسيديز تحت أفضل الظروف وذلك فى الأغراض الصناعية او الطبية.