Kinetic 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 and 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 Units/ml, 204.4 Units/ml), (4.2 mg/ml, 5.11mg/ml) and (0.448 Units/mg protein, 40 Units/mg protein), respectively. The optimum pH and temperature of these enzymes under investigation were 7.0, 5.5 and 40ºC, 50ºC, respectively. The K_m and V_{max} for catalase and peroxidase enzymes were equalled to (1.88 ml/100ml, 0.37ml/100ml) and (6.43 Units/ml/min, 16.58Units/ml/min) , respectively.

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

Introduction

Garlic cloves had been used an important natural 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 and important cellular antioxidant enzyme that defends against oxidative stress, **Chelikani *et al.* (2004) and 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)**.

Marzouki *et al.* (2005) studied a new thermostable peroxidase from garlic (*Allium sativum*) and they found that the total protein , total activity and specific activity were 497 mg, 50042 U and 101 U/mg, the optimum temperature was 40ºC and optimum pH was 5.0 . The apparent K_m values for guaiacol and H_2O_2 were 9.5 and 2.0 mM, respectively.

El Ichi *et al.* (2008) studied a new peroxidase from garlic (*Allium sativum*) bulb and used in H_2O_2 biosensing. They found that the optimal pH was 5.0 and the optimal temperature was 30ºC.The K_m (app) values for H_2O_2 obtained for POX_{IA} and POX_{IB} were 3.0 and 0.5mM, respectively. The K_m (app) values for O-dianisidine and guaiacol were 0.2 and 5.5 mM, and V_{max} values were 0.56 and 31.8 mM/min, respectively.

Sfaxi *et al.* (2009) measured the specific activity of peroxidase and catalase in bulb were 40 and 6.9U/mg, respectively.

Marzouki *et al.* (2010) studied the kinetic characterization of a basic peroxidase from garlic (*Allium sativum*) and found that the total protein, total activity and specific activity were 96.3mg, 71242 IU and 739.5IU/mg.

Osuji *et al.* (2014) extracted an acidic peroxidase from garlic (*Allium sativum*) and was partially purified. They found that the specific activity of the enzyme increased from 4.89 U/mg after ammonium sulphate precipitation to 25.26 U/mg after gel filtration chromatography. Also, the protein content, activity and specific activity of garlic peroxidase were 4.981mg/ml, 20.39 U/ml and 4.09 U/mg, respectively. The optimum temperature and pH of the enzyme extracted were 50ºC 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 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). Therefore, the extracted was filtered through two layers of cheesecloth and centrifuged at 10000rpm 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 cheesecloth and the obtained extracted was centrifuged at 7000 rpm for 20 min at 4°C, **Nurhidayah *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 as mentioned before, which reported by **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 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, 4.2 mg/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 in agreement of these reported **Sfaxi *et al*. (2009)** for peroxidase while, the specific activity of catalase is lowest.

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	40.0

Factors affecting on the reaction activity of catalase and peroxidase enzymes 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.

Effect of pH

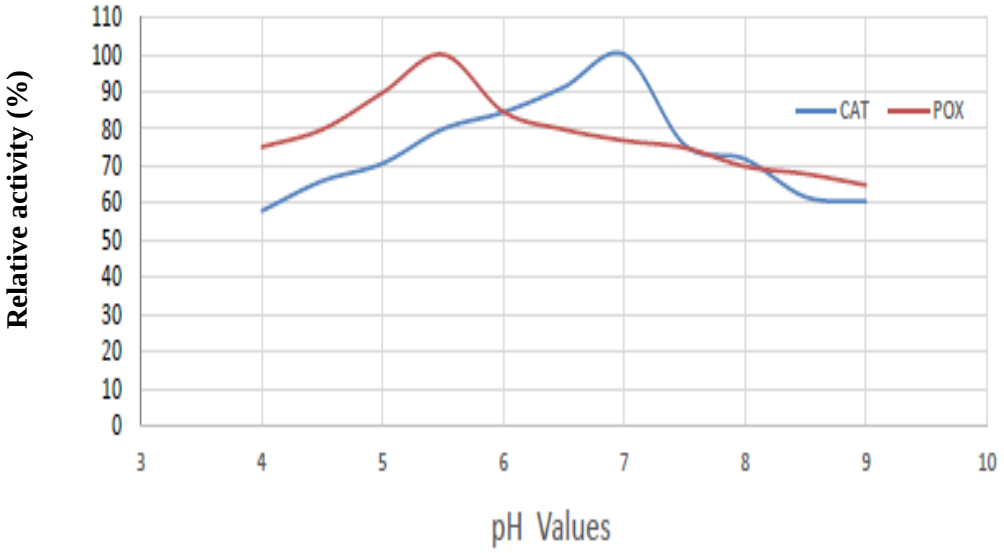
The enzyme activities of catalase and peroxidase were demonstrated in Table (2) and Fig (1), the obtained results showed that the maximum reaction activity was 2.00 units /ml/min which recorded at pH 7.0 for catalase. While, the maximum reaction activity found to be 18.38 units /ml/min was found to be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

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 *Allium sativum.* While, the results of catalase were similar to results reported by **Belhadj *et al*. (2015)** who found an optimum activity at 40° C for catalase from *Allium sativum.*

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	58.00	13.80	75.08
4.5	1.32	66.00	14.67	79.81
5.0	1.41	70.50	16.49	89.71
5.5	1.60	80.00	18.38	100.00
6.0	1.69	84.50	15.55	84.60
6.5	1.82	91.00	14.67	79.81
7.0	2.00	100.00	14.12	76.82
7.5	1.51	75.50	13.76	74.86
8.0	1.44	72.00	12.84	69.85
8.5	1.23	61.50	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.

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	18.36	85.35

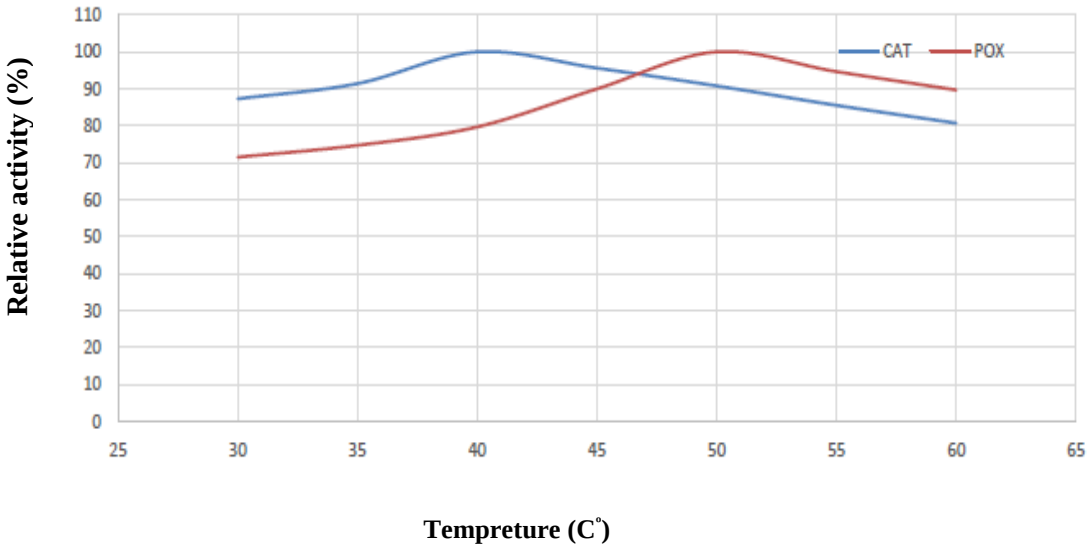


Fig (2). Effect of temperature on the reaction activity of catalase and peroxidase enzymes extracted from garlic cloves.

Effect of substrate concentration on the reaction activity and 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 calculated and found to be 1.88 ml/100ml and 6.43 units/ml/min for catalase enzyme, respectively. As well as, these values of peroxidase enzyme were recorded in (Table 5) and Fig (3,c,d) as 0.37 ml/100ml and 16.58 units/ml/min, respectively. Lineweaver-Burk plots of experimental data for catalase and peroxidase enzymes are illustrated in Fig. (3,b ,d). The obtained K_m and $_{Vmax}$ by Lineweaver and Burk plots were equalled to that obtained by experimentally curve. These values for peroxidase enzyme are higher than that reported by **Osuji *et al.* (2014)**.

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

Substrate concentrations(%)	[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

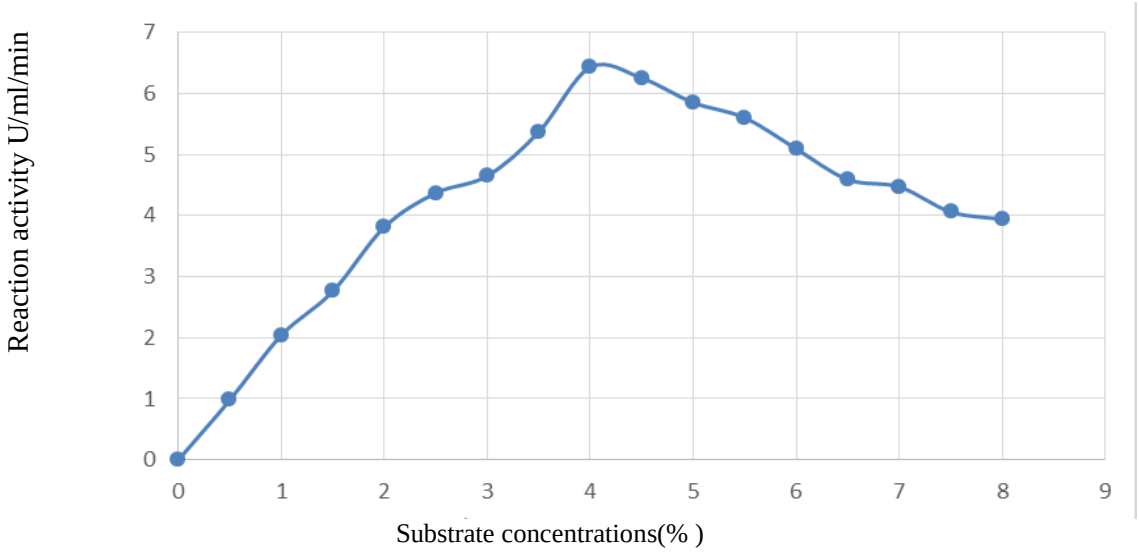


Fig (3, a). Effect of substrate concentrations on the reaction activity of catalase enzyme extracted from garlic cloves.

V_{max} = 6.43Units/ml/min, K_m = 1.88 ml/100 ml, $$\mathbf{v = \frac{V_{max} \times [S]}{K_m + [S]}}$$

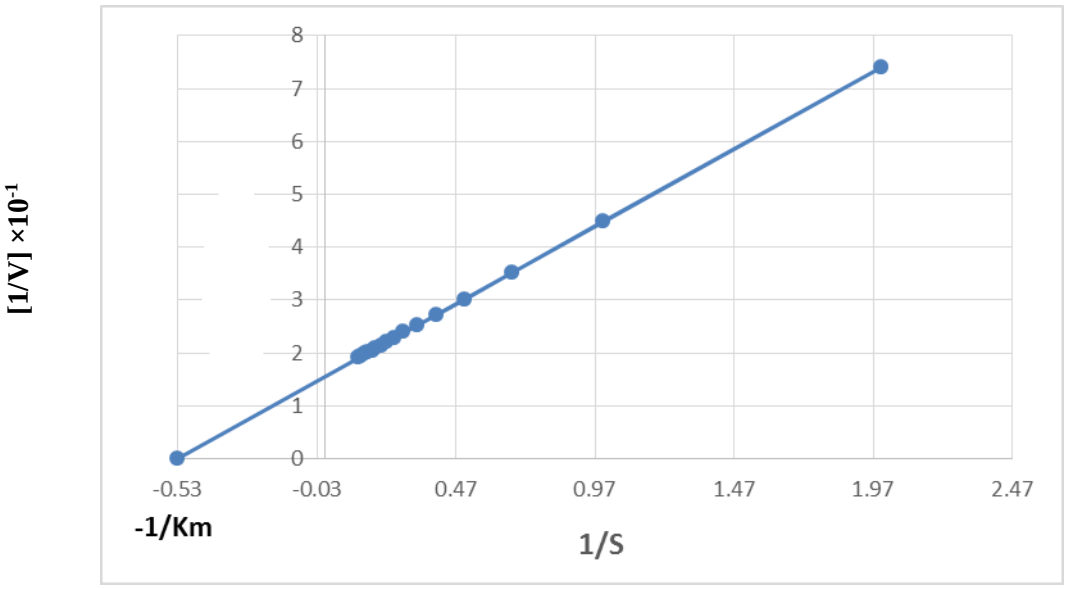


Fig (3, b). Lineweaver-Burk plots of catalase enzyme

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

Substrate concentration (%)	[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	9.76	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	16.05	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

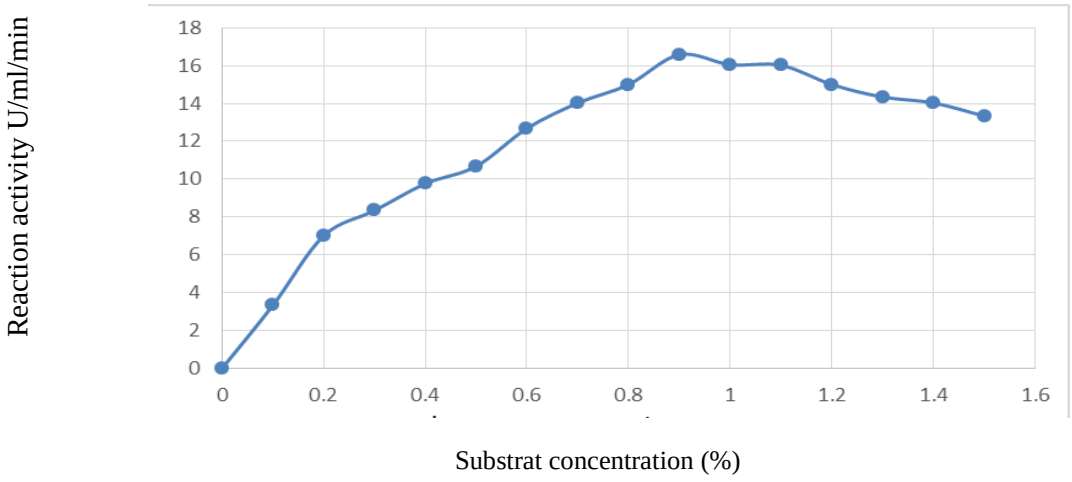


Fig 3, c. Effect of substrate concentration on the reaction activity of peroxidase enzyme extracted from garlic cloves.

V_{max} = 16.58 Units/ml, K_m = 0.37 ml/100 ml, $$\mathbf{v = \frac{V_{max} \times [S]}{K_m + [S]}}$$

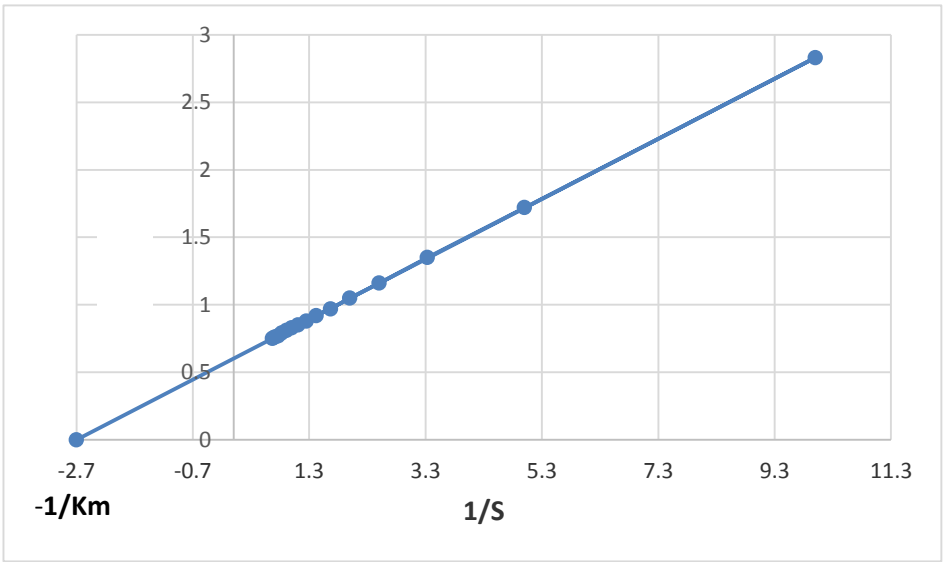


Fig (3, d). Lineweaver-Burk plots of peroxidase enzyme

Conclusion

In the present study, antioxidant enzyme activities, CAT and POD, extracted from garlic cloves and kinetics parameters of these enzymes were evaluated. The achieved results showed that cloves have a good antioxidant potential of application in wastewater treatment, detoxification and rapid detection of peroxidase in food and beverages. Also, from the obtained results, can be concluded that the garlic cloves are a promising source of natural antioxidants, catalase and peroxidase and might be used in the treatment of diseases associated with oxidative stress.

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دراسات حركيات إنزيمات الكاتاليز والبيروكسيديز المستخلصة من فصوص الثوم

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قسم الكيمياء الحيويه – كلية الزراعة– جامعة بنها

تهدف هذه الدراسة الى امكانيه استخلاص وانتاج مصادر طبيعيه وبديليه للانزيمات الطبيعيه والمضاده للاكسده وهى انزيمات الكاتاليز والبيروكسيديز نظرا لاهميتها فى المجالات المتنوعه من اهمها التخليق الكيماوى ومصانع الاغذيه والمعالجه البيولوجيه لمياه الصرف الصحى وأجهزه الاستشعار البيولوجى ومجال التكنولوجيا الحيويه والمجالات الطبيه ودورها فى صناعه كثير من الادويه والمستحضرات الطبيه متعدده الاستخدمات . لذلك تم اختيار نبات الثوم (فصوص الثوم) كمصدر طبيعى لاستخلاص هذه الانزيمات المضاده للاكسده .

كذلك تقدير المحتوى البروتينى ومعدل النشاط الانزيمى للانزيمات المستخلصه تحت الدراسه كما تم دراسه خواص وحركيات هذه الانزيمات وذلك بدراسه افضل الظروف التى تؤثر عل معدل النشاط الانزيمى من درجه حراره ودرجه حموضه وكذلك تاثير تركيزات من الماده المتفاعله على معدل سرعه التفاعل وحساب ثوابت الانزيمات من كلا من ثابت ميكالس منتن بالطرق المختلفه والسرعه القصوى لمعدل النشاط الانزيمى بغرض تحديد ومعرفه الظروف المثلى لاستخدمات مثل هذه الانزيمات فى مجالاتها المختلفه.

حيث اوضحت النتائج ان درجة النشاط للانزيمات المستخلصه من فصوص الثوم وكذلك المحتوى البروتينى لهما هى على التوالى2,05 ، 204,4وحدات/ملليلتر 4,2 ،5,11ملجم/ ملل.

كما أظهرت النتائج ان أعلى معدل لنشاط انزيم الكاتاليز عند درجه حموضه 7 بينما كانت لانزيم البيروكسيديز 5,5. بينما اعطت الانزيمات المستخلصه من فصوص الثوم أعلى معدل نشاط انزيمى على درجه حراره 40 درجه مئويه لانزيم الكاتاليز ودرجه حراره 50 درجه مئويه لانزيم البيروكسيديز وبذلك انه يتحمل درجات الحراره المرتفعه وتعتبر هذه النقطه هامه من الناحيه الصناعيه.

كذلك وجد ان قيم كل من ثابت ميكاليس منتن والسرعه القصوى لانزيمى الكاتاليز والبيروكسيديز هي 1,88ملليلتر / 100مل, 6,43 وحدات/ ملليلتر/دقيقه 0,37 مل/ 100ملل ، 16,58 وحدات/ ملليلتر/دقيقه على التوالى .

توصي هذه الدراسة الى امكانيه استخدام أنزيمات الكاتاليز والبيروكسيديز المستخلصه من فصوص الثوم كمصدر طبيعى لمضادات الاكسده الطبيعيه وكذلك استخدامها فى بعض المجالات الصناعيه .