European Journal of Sport Sciences and Public Health 5 (1)

Examination Of Catalase Enzyme In Green Cabbage And Some Characters Of It

Adnan AL-Mudhafr and Rokayya GhalebAL-Dabbagh

Department of food science, Faculty of Agriculture, University of Kufa, _Iraq

Research of master's thesis for the researcher R AL-Dabbagh

A b s t r a c t *- The detection of the enzyme catalase in the leaf of cabbage green enzyme efficiency was estimated, amounting to 268.31 units / ml of crude Alanzymy efficiency and quality at 78.9 unit / mg The total protein concentration of 0.34 mg / ml and a purification of 1 fold and Anzymyt 100% then were studied The pH optimum function of the effectiveness of the enzyme 6 and 35 C optimum temperature and pH was the optimum function of the stability of the enzyme 7.5 and optimum temperature for the stability of the enzyme was 40 C.*

1. Introduction

Featuring green cabbage Brassica oleracea or variants that bold or light slash of white and yellow or white flowers cabbage with four petals in vertical style cabbage leaves vary in terms of softness and hardness and neither did the cabbage is rounded or tapered and these differences depending on the type and category And Genetics[1] execute green leafy vegetables perennial and its leaves with high intensity and can grow in various climatic zones and in most areas of adjustment can be grown all year round and the optimum temperature range for growth and development between (18-35). Have the ability to withstand Frost and cold temperatures up to-8 m and can grow in a variety of soils ranging from sand to clay but prefer fertile[2] contains several of cabbage enzymes, such as enzyme One of antioxidants catalase and oxidative enzymes and redox It stimulates the decomposition of hydrogen peroxide and prevent the formation of free radicals and is used in keeping milk as it works to remove hydrogen peroxide surplus [3] and is used to prevent enzymatic browning with enzyme polyphenols Oksudaiz in dried eggs, fruit juices and wine used in foods to determine the concentration of O2 and H2O2 It consists of the quadrant and with the structure of a molecular weight of 56,000 Dalton[4].

2. Materials and Methods

2.1 Preparation of crude enzyme extract

Green cabbage was purchased from the local market was chosen for the study and classified as the leaves mature and well and free of disease and insect injury, mechanical Then cleaned and washed to remove residual dirt, chemical pesticides found that crude extract was then prepared 50 g of crushed leaves are soft and with a 100 ml solution Dary Fwsf At 0.1 molar potassium pH = 7.8 weight / volume (2:1) extracted and then nominate extracted by cloth of gauze and centrifugal action speed 12000 rpm for 30 min using refrigerated centrifuge with degrees. Temperature of 5 m And the separation of the crude enzymatic extract and neglected precipitation and use the method mentioned by Luhova[5].

The solutions used.

1-phosphate buffer solutionA solution of sodium hydrogen attended the bilateral phosphate solution 60 M by dissolving 71 g NaH2PO4 / one liter of distilled water

Solution B attended the potassium phosphate bilateral hydrogen 60 M by dissolving 27 g / l of K2HPO4 mix Distal. water ratio of 1: 9 (solution 1: solution 2) after adjusting the pH to 7.4 using focus 1 M of HCl and 1 M of NaOH and then Complete the volume to 500 ml

2-Ammonium molybdate solution (32.4 ml Muller) Ammonium molybdate solution and 6.4 g attend dissolving in 500 ml.

3-peroxide solution of hydrogen H2O2 65) ms Mueller) attend 17 ml in 500 ml.

Assessment of enzyme activity

Used the method, which made all of Goth, Hadwan, Abei[5][6][7] took 0.2 ml of extract a lap with 1 ml of the mixture containing 65 molar (H2O2) with Dary phosphates (60 molar) pH = 7.4 to 25 m never It's 4 minutes after stopping the action of the enzyme reaction was stopped by adding 1 ml of the ammonium Mwlbydat to exclude interference resulting from amino acids and proteins. This means that absorption The second part belongs to the hydrogen peroxide remaining not real only interaction was then measured at wavelength of 405 nanometers according the following equation

$$\text{Catalase activity} = (\text{Sample} - \text{Blank1})/(\text{Blank2} - \text{Blank3}) \times 271$$

Determination of the content of protein

Protein was determined by method of green cabbage, absolute method Absloute described by Whitaker and Granium [8] and depends on the frequency of 235-280 Nanwmytr then calculation of protein concentration. The following equation

$$\text{Protein mg/m} = \frac{A235 - A280}{2.51}$$

Determine the optimal enzyme conditions

Measure the pH optimum for the enzyme effectiveness

Preparation phosphate solutions with concentration of (50 mm) and with hydrogen numbers(5,6,7,8,9,10) Mixing 0.1 ml of solution enzymatic with 0.99 ml of each of the solution record in the test tubes of various hydrogen numbers and the lap for one hour at a temperature of 25 ° C Then took 0.2 ml of these solutions (solution Enzymatic +buffer) and added to 1 ml of sodium phosphate H2O2, pH = 7, and incubated at a temperature of 25 ° C and then the measuring device spectrophotometer at the wavelength of 240 nm

Measuring the optimum temperature for the effectiveness of the enzyme

preparation the phosphate concentration of buffer solution (50 m M) and number pH 7 and mixing with 0.1 ml of solution enzymatic With 0.99 ml of each of the buffer solution record in test tubes and incubated for one hour at temperatures (60-20 m). Taken 0.2 ml of solution (enzyme + phosphate buffer) for all pipe and add a 1 ml of 50 m M of sodium phosphate unilateral hydrogen 7 = pH. The lap at a temperature of 25 m to stop the work of the enzyme and then measured to a spectrophotometer at the wavelength of 240 [6]

Measuring pH optimum stability of the enzyme

To determine ph optimum stability of the enzyme was the lap of the solution enzymatic in a water bath at 37 ° C for 30 minutes different extents of hydrogen numbers and that mixing equal volumes of 1: 1 ratio of solution enzymatic with all of the solutions buffer prepared in test tubes and then cooled directly by transferring the tubes to a snowy bath, and then absorbance is measured to a spectrophotometer at the wavelength of 240 nm [6].

Measuring the optimum temperature for the stability of the enzyme

The mixing of 0.1 mL of the solution enzymatic with 0.99 mL of each of the solution phosphate buffer concentration of 50 m M and pH = 7 record in test tubes and incubated at a temperature of 50 ° C and the duration of 60-10 minutes. Taken 0.2 ml of solution record previously (enzyme solution + buffer) and add a 1 ml of 50 m M of mono sodium phosphate hydrogen and 10 m M of hydrogen peroxide pH = 7. And incubated at a temperature of 25 ° C to stop the work of the enzyme and then measured to a spectrophotometer at the wavelength of 240 nm[6]

3. RESULTS AND DISCUSSION

The active enzyme of crude enzyme extract from raw green cabbage 268.31 unit / ml and 0.34 mg / ml protein with specific activity of 78.91 units / mg.

Determine the optimal enzyme conditions

Measure the pH optimum for the enzyme effectiveness

Observed from Fig. (1) That effective enzyme reached its maximum when pH = 6, it reached the effective enzyme 87.01 units / ml thereafter were decreased. In the active enzyme that is so Changing in the enzyme or active site leads to the loss of enzyme efficiency, which affects the change in the pH values in the active aggregates that are Lltayn wall It is found in the enzyme molecule or ion condition of the pillar[9], these results agreed with the [10] extract the enzyme catalase of mint leaves if you find that pH -- like the effectiveness was 6.

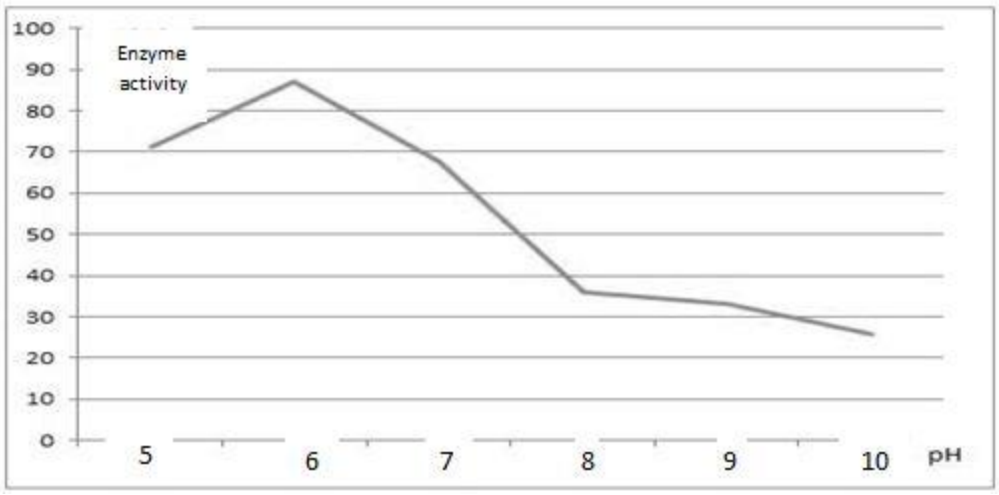

Figure (1) the optimum pH of enzyme catalase extracted from green cabbage

Measuring the optimum temperature for the effectiveness of the enzyme

The enzymatic efficacy was estimated at different temperatures over the range (20-60) ºC. Note from Figure (2) Increase the enzymatic efficiency of the temperature rise to reach a maximum of 35 ° C and then began to decline gradually To reach 28.39 at a temperature of 60 m and two factors involved in this phenomenon, one of the increase of kinetic energy per self Of the enzyme and the sub strait and the other increase the chance of collision between the enzyme and the substance of reaction as a result of increased rate of movement by action of heat[11] When the temperature reaches a certain limit, many hydrogen bonds are broken Which affects the synthesis of the enzyme so that it does not retain its synthesis and loses its catalytic or analytical strength and may occur A breakdown of the synthesis of any enzyme and the loss of its natural properties[8] These results correspond to what he found[12]They found that the optimum heat for the efficiency of the celery extracted from celery was 35 c.

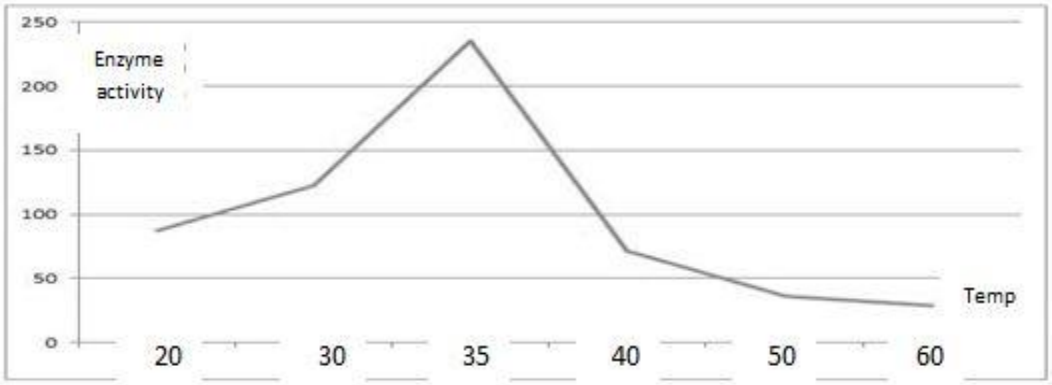

Figure (2): The optimum temperature for the efficiency of the catalase enzyme extracted from the green cabbage

Measuring pH optimum stability of the enzyme

PH affects the speed of enzymatic reactions and this effect is as high as possible at Optimum pH As well as its effect on the ionization of both the enzyme and the controlled substance and enzymatic accompaniments and show the results shown in the figure(3) The optimum pH of the enzyme derived from the green enzyme is 7.5 and these results are consistent with[13] Which found that the optimum pH had reached 7.5 for the catalase extract from the Malva sylvestris.

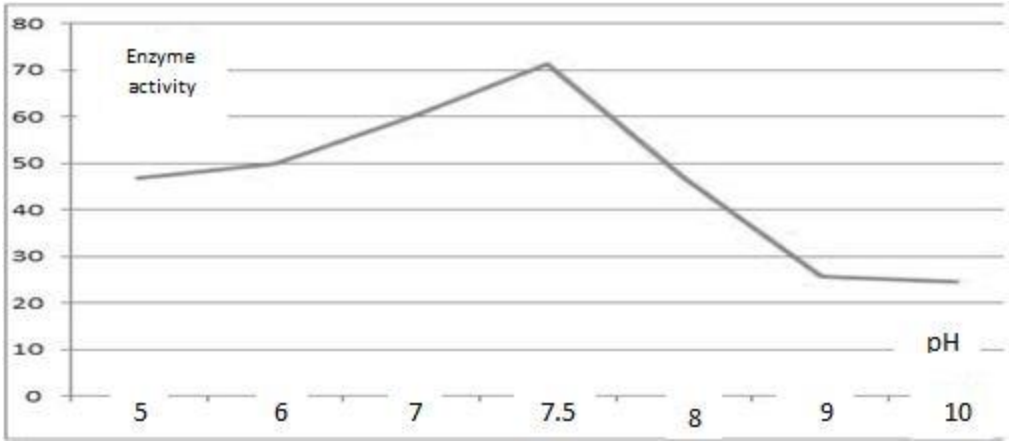

Figure (3) The optimal pH function of the stability of the catalase enzyme extracted from the green cabbage

Measuring the optimum temperature for the stability of the enzyme

The results shown in Figure (4) show the thermal stability of the catalase enzyme extracted from the green leaves of cabbage It was observed that the optimum temperature of the enzyme stability was 40 m. These results were consistent with what[14]Which found the optimal temperature of the stability of the enzyme catalase extracted from the seeds of lentils amounted to 40 C and also agreed with the results found[15] Which found that the optimum heat for the stability of the catalase enzyme extracted from the Chard (*Beta Vulgaris Subspecies Cicla***)** was 40 C.

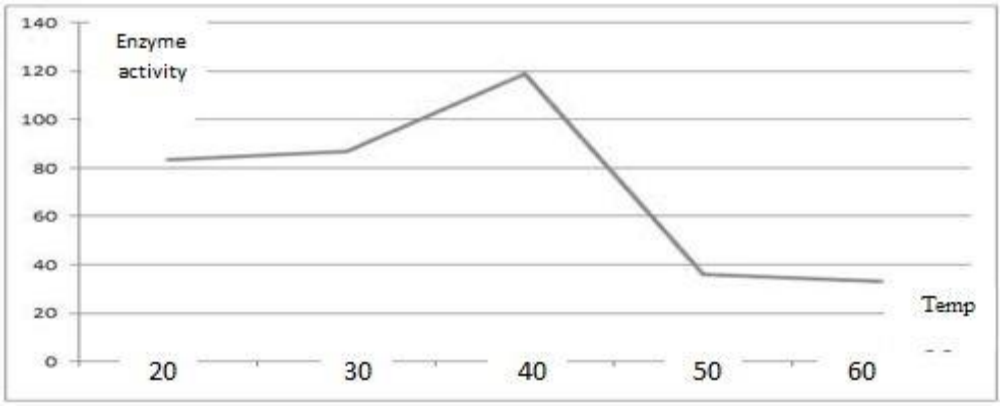

Figure (4): The optimum temperature for the stability of the catalase enzyme extracted from the green cabbage

References

(1) Maggioni, L. (2015). *Domestication of Brassica oleracea L* (Vol. 2015, No. 74).

(2) Salunkhe, D. K., & Kadam, S. S. (1998). Introduction. In Handbook of Vegetable Science and Technology (pp. 19-28). CRC press.

(3) Yuksel, S., Yigit, A. A., Cinar, M., Atmaca, N., & Onaran, Y. (2015). Oxidant and antioxidant status of human breast milk during lactation period. Dairy science & technology, 95(3), 295-302.

(4) Luhova, L., Lebeda, A., Hedererová, D., & Pec, P. (2003). Activities of amine oxidase, peroxidase and catalase in seedlings of Pisum sativum L. under different light conditions. Plant soil and environment, 49(4), 151-157.

(5) Hadwan, M. H. (2016). New method for assessment of serum catalase activity. Indian Journal of Science and Technology, 9(4).

(6) Aebi , H.(1984) .Catalase In Vitro , methods in enzymology .Academic press. Orlando .105 : 121 – 126.

(7) Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica chimica acta*, *196*(2-3), 143-151.

(8) Whitaker, J. R., & Granum, P. E. (1980). An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. Analytical biochemistry, 109(1), 156-159.

(9) Whitaker, J. R. (1972). The glycoside hydrolases. Principles of Enzymology for the Food Science. Marcel Dekker Inc, New York City, NY, USA, 433.

(10) Sondaş, E. (2005). Nane (Menta spicata) ve Pırasa (Allium porrum L.) Bitkilerinden Katalaz Enziminin Kısmı Saflaştırılması, Karekterizasyonu ve Bazı Kinetik Özelliklerinin Araştırılması. Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü.

(11) Segel, I.H. (1976). Biochemical calculations. 2nd edition, John and sons. Inc. New York.

(12) Gungor and Bilgin .(2016). Purification Of Catalase From Celery (Apium Graveolens) Ç.Ü. Fen ve Mühendislik Bilimleri Dergisi Yıl:2016 Cilt:34−1.

(13) Arabaci, G., & Usluoglu, A. (2012). Immobilization of dill (Anethum Graveolens L.) catalase and its properties. Asia-Pacific Journal of Chemical Engineering, 7(S3).

(14) Kandukuri, S. S., Noor, A., Ranjini, S. S., & Vijayalakshmi, M. A. (2012). Purification and characterization of catalase from sprouted black gram (Vigna mungo) seeds. *Journal of Chromatography B*, *889*, 50-54

(15) Dinçler, A., & Aydemir, T. (2001). Purification and characterization of catalase from chard (Beta vulgaris var. cicla). Journal of enzyme inhibition, 16(2), 165−175.