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

Partial Purification and Some Properties of Catalase from Red cabbage

Rokayya GhalebAL-Dabbagh and Adnan AL-Mudhafr

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 *- In this work, partial purification of catalase from red cabbage and some properties For the purification, enzyme was extracted from red cabbage with phosphate buffer and centrifuged and activity for crud enzyme was 287.26 unit/ml Then (NH4)2SO4 precipitation was performed to the extracted enzyme and activity was353.26unit/ml*

and was dialyzed and activity was of enzyme after dialyses 552.2unit/ml then were studied The pH optimum function of the effectiveness of the enzyme 7and 30 C optimum temperature and pH was the optimum function of the stability of the enzyme 7 and optimum temperature for the stability of the enzyme was 30 C.

1. Introduction

Cabbage with high nutritional value as it is rich in minerals and vitamins and Monosaccharides and phenolic compounds active number of multiple vital, such as anthocyanins, flavonols, glucosinolates which has positive energy[1][2]. And has value by consumers for its taste and intense red color increases the aesthetic value of the food. For these reasons, it is often consumed vegetables in the form of addition, salad features red cabbage with high life span. And then it can be easily stored and can be accessed in a new form for a long time [2] Catalase enzyme It is one of the first enzymes studied and purification exist in all aerobic respiration objects in cells containing Saitokrom as it has been studied in bacteria, birds, and the liver of animals and human vitality and importance was purified in large quantities and has been studied and purified from plants identified where the effectiveness catalase are yellow apples and Sbang cabbage [3] and has the potential surplus of hydrogen peroxide and thus reduce the dangers caused by the types of interactive oxygen and oxidative stress analysis [4].

2. Materials and Methods

Red 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].

2.1 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.

2.2 Assessment of enzyme activity

Used the method, which made all of Goth, Hadwan, Abei[6][7][8] 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

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

2.2 Determination of the content of protein

Protein was determined by method of green cabbage, absolute method Absloute described by Whitaker and Granium [9] and depends on the frequency of 235-280 Nanwmytr then calculation of protein concentration. The following equation

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

2.3 Enzyme purification

Catalase enzyme was precipitated by ammonium sulfate salt, salt gradient. It was used to satisfy the different ratios And the solubility of the precipitation from the different saturation ratios of the phosphat buffer. The enzymatic efficiency, the protein value for all saturation ratios, and the conservation of the model were estimated at 20 m until use in the manner explained by Segel[10]. Ammonium sulphate is used in precipitation for having high solubility, [11].

2.4 Dialysis

The dialysis was carried out using the dialysis bags of the ammonium sulphate extract with 80% saturation using 24 hours distilled water with distilled water every 6 hours and pH = 5.7 using phosphate precipitator. Then, pH = 7 was adjusted using NaOH and centrifugal 3000 cycles / min For 10 min then enzymatic efficacy and protein were estimated.

2.5 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 [7]

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 [7].

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[7]

3. RESULTS AND DISCUSSION

The results indicated in Table (1) showed that the enzymatic efficacy of the raw extract of the red leaf fraction was 287.26 units / ml and the concentration of the protein was 0.35 mg / ml and the specific efficacy was 82.05 mg / mg. The total effectiveness was 4308.9 units and the number of purification times was 100% The enzyme was then concentrated by ammonium sulphate 80% Enzymatic activity of 353,267 units / ml and the number of purification fold 1,484 times and the enzymatic yield 81.9% then The process of dialysis was estimated at 552.2 units / ml for the enzymatic extract of the red dye. The number of purification times was 3.542

and the enzyme yield was 64.07% The results differed according to the type and source of the enzyme as well as experimental conditions. [12] found that when extracting the catalase enzyme from pea leaves, enzymatic activity showed 1133 units / ml and 100 enzymatic yield and 1 purification times. These results were agreed with [13]was found when extracting the catalase enzyme from lentil seeds. The enzymatic activity of the extract was 274 units, the number of purification times 1 and the enzymatic yield 100. The enzymatic activity after ammonium sulphate deposition was 158 units, the purification times were 2.04 and the enzymatic yield was 61.8%, while [14]When extracting catalase from the leaves of the chard that is effective The quality after the sedimentation with ammonium sulphate was 104 units / mg and the enzymatic yield 38.58 and the number of purification times was 5.1

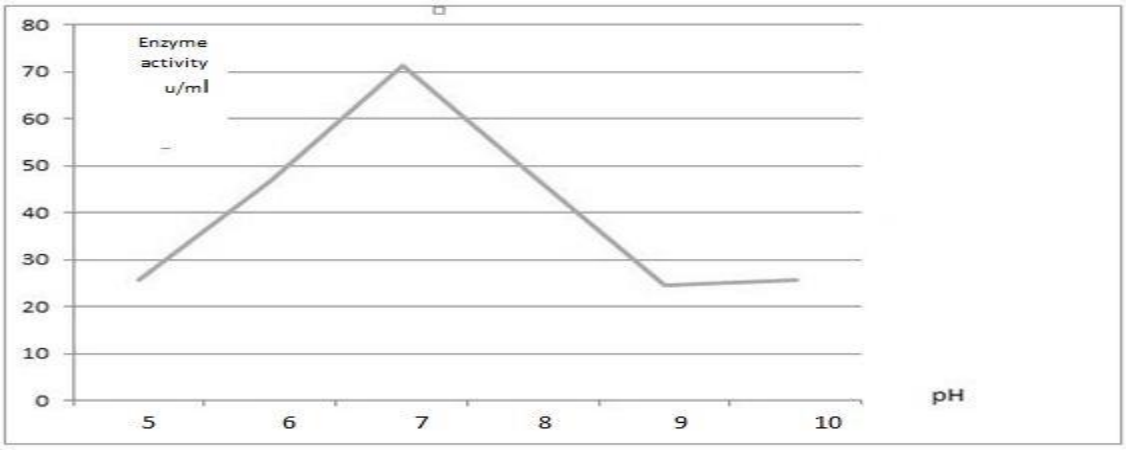
Table (1) shows the stages of purification of catalase enzyme from leaves of the red cabbage

%Yield	Purification fold	Total activity	Specific Activity u/mg	Protein	activity u/ml	Size ml	Purification step
100	1	4308.9	82.05	0.35	287.26	15	Crud
81.9	1.484	3532.6	121.79	0.29	353.26	10	(NH4)2SO4 80%
64.07	3.542	2761	290.63	0.19	552.2	5	*Dialysis*

Determine the optimal enzyme conditions

Measure the pH optimum for the enzyme effectiveness

Optimum pH for function estimated effectiveness of enzyme catalase, extracted from leaves of red cabbage estimated ranging from (5-10) showed the results shown in Figure (1) that the optimal pH for enzyme function was at number 7 as showed higher efficacy of the enzyme shape notes that effective enzymatic grows To increase the pH of the central interaction in extent ranged (6-7) at maximum effectiveness when the number 7 and reached 71.3 units/ml then began to fall, the decline in efficiency Then it began to decline that the drop in efficiency is due to the situation ion effective site substance or both. These results agreed with [15]while he found Nasrabadi[16] appointed pH optimum function 7.5 effectiveness catalase extract from the plant safflower .



Figure(1) The optimum pH of enzyme catalase extracted from red cabbage

Measuring the optimum temperature for the effectiveness of the enzyme

The efficacy of the enzyme was studied at temperatures ranging from 20-60 °C at the optimum pH of the enzyme. The results shown in Fig. 2 showed an increase in the efficiency of the enzyme with high temperature if it reached maximum efficiency at 30 ° C if the activity was 235 units / The enzymatic efficiency of the high temperature increased to 28.39 units / mL at 60 ° C. The increase in the speed of enzymatic reactions due to the collisions between the part of the enzyme and the controlled substance due to increased kinetic energy with high temperature and high heat caused a decrease in efficiency due to Enzyme and composition change. [10]. This result was agreed with the results obtained by Arabaci [17] which found that the optimal temperature of the enzyme catalase extracted from dill plant is 30 °C.

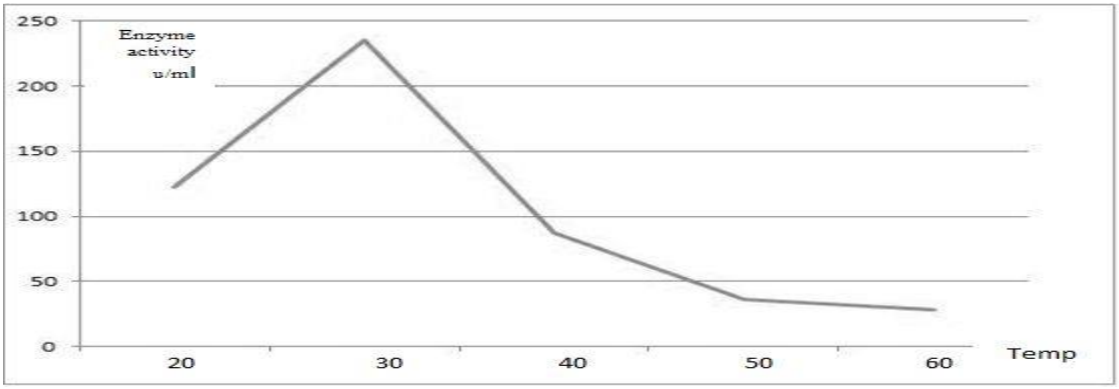


Figure (2): Optimal temperature of the catalase enzyme extract from the red cabbage

Measuring pH optimum stability of the enzyme

The optimum pH for the stability of the enzyme was 7 These results were agreed by Kandukuri [13]who found the optimum pH of the catalase enzyme extracted from lentils 7 and agreed with [15] that the optimum pH of the extracted catalase was 7 and agreed with [17] Found that the optimum pH of the catalase extract from dill was 7 while found in [18] The optimum pH was 7.5 for catalase extract from Malva sylvestris.

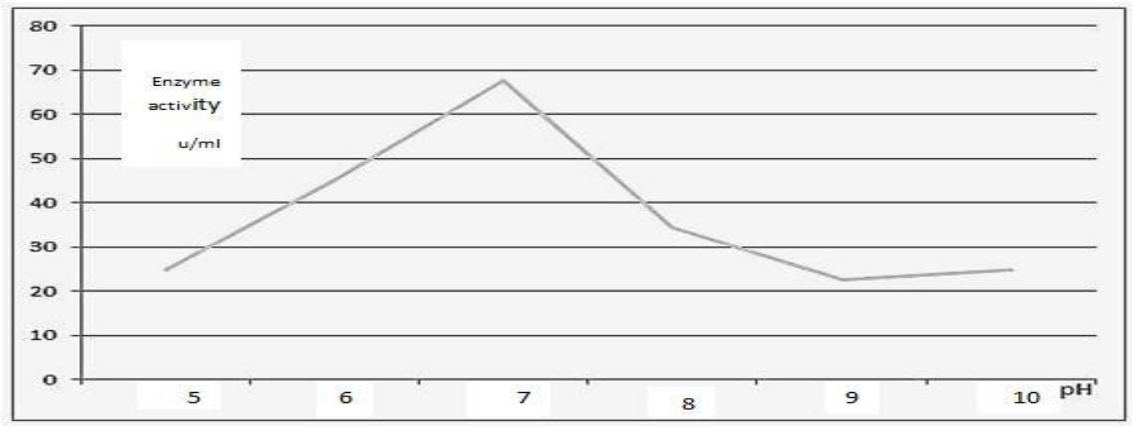


Figure (3) The optimal pH function of the stability of the catalase enzyme extracted from the red cabbage

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 red leaf cells if the enzyme retains enzymatic efficacy of 131.5 units / ml at a temperature of 30 C then it decreased efficiency with high temperature This is due to the nature of the enzyme that is affected by high temperatures and therefore inhibits the action of. These results are consistent with [17] which found that the optimum temperature of the dill extract was 30 and also agreed with [18] Extraction of catalase from the Malva sylvestris if the optimum temperature was found was 30 C while Kandukuri [13] found that when extracting the lentils from the lentils, the optimum temperature was 40 C. This is due to the type of plant source.

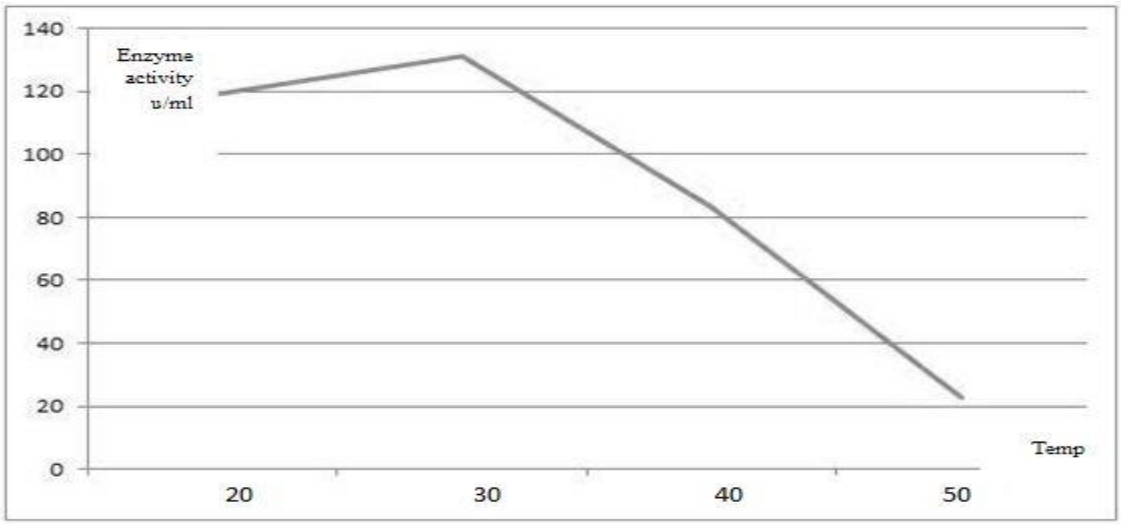


Figure (4): Optimal temperatures for the stability of the catalase extracted from the red Cabbage

References

[1]Volden, J, Borge, G.I.A, Bengtsson, G.B, Hansen, M, Thygesen, I.E, Wicklund, T. (2008). Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (*Brassica oleracea* L. *ssp. capitata f. rubra*). *Food Chem.*, 109: 595–605.

[2]Wiczkowski, W, Szawara-Nowak, D, Topolska, J. (2013). Red cabbage anthocyanins: Profile, isolation, identification, and antioxidant activity. *Food Research Inter.*, 51 (1): 303-309.

[3]Percy, M. E. (1984). Catalase: an old enzyme with a new role?. *Canadian journal of biochemistry and cell biology*, *62*(10), 1006-1014.

[4]Atasever, S., & Erdem, H. (2010). Association of somatic cell count with catalase enzyme activity in bovine bucket milk. *Trends in Animal and Veterinary Sciences*, *1*(1), 24-27

[5]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

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

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

[8]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.

[9]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.

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

[11]Zlatković, D., Jakovljević, D., Zeković, Đ., & Vrvić, M. M. (2003). A glucan from active dry baker's yeast (Saccharomyces cerevisiae): A chemical and enzymatic

investigation of the structure. *Journal of the Serbian Chemical Society*, *68*(11), 805-809

[12]Corpas, Francisco J., et al. (1999). Purification of catalase from pea leaf peroxisomes: identification of five different isoforms. *Free Radical Research*, *31*(sup1), 235-241

[13]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

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

[15]Gholamhoseinian, A., Ghaemi, N., & Rahimi, F. (2006). Partial purification and properties of catalase from Brassia oleracea capitata. *Asian journal of plant sciences*, *5*(5), 827-831.

[16]Tayefi-Nasrabadi, H., Dehghan, G., Daeihassani, B., Movafegi, A., & Samadi, A. (2011). Some biochemical properties of catalase from safflower (Carthamus tinctorius L. cv. M-CC-190). *African Journal of Agricultural Research*, *6*(23), 5221-5226

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

[18]Arabaci, G., & Usluoglu, A. (2013). Catalytic properties and immobilization studies of catalase from Malva sylvestris LJ Chem., 2013, 1-6. *DOI*, *10*(2013), 686185