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Purification and Properties of Catalase from Van Apple (Golden Delicious)

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Abstract: Catalase (CAT:EC1.11.1.6) was purified from Van Apple. The purified enzyme preparation was obtained with a final recovery of enzyme activity of about 11.5% and a specific activity of 29.43 U/mg proteins. The purified catalase has an optimum temperature of activity at 50°C. As regards pH, the enzyme has an optimum activity of pH 5. Vmax and Km values were determined by Lineweaver-burk graphs. Potassium cyanide, citric acid, $MnCl_2$, NaCl, $NaNO_2$ and $CuSO_4$ were used as inhibitor.

Key words: Catalase, characterization, purification, inhibitors

Introduction

Catalase (CAT:EC 1.11.16) is a enzyme to have present in structure of plant cells (Higashi *et al.,* 1974). This enzyme is widely distributed in a variety of life forms, including animals , plants, microbes and usually only absent from strictly anaerobic organisms. Catalase plays an important role in removing toxic hydrogen peroxide in the cell (DeDuve, 1983; Master and Holmes, 1977). Plants have involved in antioxidative enzyme in which catalase are the most efficient enzyme, influencing patterns of fruits (Andrea, 1998). Fruit ripening and senescence may be regarded as oxidative phenomena (Brenan and Frenkel, 1977), especially as at the onset of senescence the activities of other oxygen-detoxifying enzyme such as catalase decrease, superoxide or hydrogen peroxide to accumulate to toxic levels (Bowler *et al.,* 1992). Lipid per oxidation is a important factor in vegetables and fruits (especially apple) (Brenan and Frenkel, 1977). Catalase also removes electrons that can lead to the production of O_2-free-radical (Abassi *et al.,* 1998). Fruit ripening involves a lot of biochemical changes such as cell wall breakdown and membrane alteration, which may favour the infection (Torres *et al.,* 2003). The results suggest that apple fruit is normally submitted to long periods of cold storage (Andrea, 1998). Catalase has been purified from leaves *Zandedeschia aetgiopica* and characterization has been done (Trindade *et al.,* 1998). Catalase seed isozymes showed similarities to those detected in the leaves apple (Korban and Bournival, 1987). Freezing resulted in reductions of 50 to 90 CAT activity compared with the activity measured in crude extracts from tissues (Gong *et al.,* 2000). In addition, catalase from apple, purification, characterization and some properties has not yet been done, and there is not known about the catalase enzyme properties.

The aim of the present study was to characterize the CAT from Van apple and various inhibitors were used.

Materials and Methods

Materials: Van apple fruits used in this study were obtained from local Van region.

Chemicals: Hydrogen peroxide, polyviniylpolypyrolidone, DEAE Cellulose, $(NH_4)_2SO_4$ and other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Extraction and purification: From apple, 10 g was obtained from the equilibration each region of the fruit with a 10-mm-diameter cork borer. After that samples were added to 8 ml 100mM sodium phosphate buffer (pH; 7.0), 0.3 g polyvinylpolypyrolidone (PVPP), and extraction was prepared. The slurry was homogenized with a spatula. After the filtrate was centrifuged at 20.000 g for 30 min and supernatant was collected (Andrea, 1998). Extraction was fractionated with $(NH_4)_2SO_4$, solid $(NH_4)_2SO_4$ was added to the supernatant to obtain 80% saturation. The precipitate obtained was redissolved in extraction buffer containing $(NH_4)_2SO_4$, and the precipitate from 10-90% saturation was dissolved in a minimal volume of the same buffer column with phosphate buffer was washed. The active fractions were applied onto DEAE-Cellulose column (1X5 cm) previously equilibrated with extraction buffer containing $(NH_4)_2SO_4$, and washed with the same buffer to remove unbound proteins. The eluate was used as the CAT enzyme source in the following experiments. CAT active fractions were pooled as purified CAT for characterization.

Determination of protein: Protein amount for CAT was done according to method of Bradford with bovine serum albumin as standard (Bradford, 1976).

Enzyme assay: Catalase (CAT) activity was determined at 25°C according to Aebi (Aebi, 1984). The reaction mixture contained 40 mM H_2O_2 in a 50 mM phosphate buffer pH 7.0, and 0.1 ml pure enzyme in a total volume

Table 1: Partial Purification of CAT From Van Apple

Purification Step	Protein (ig/ml)	Activity (Units/ml)	Specific activity (Units/mg)	Total activity (Units)	Purification (fold)	Yield (%)
Crude Extract	74.6	766	10.27	383000	1.0	100
$(NH_4)_2SO_4$	77.0	883	11.47	176000	2.2	46.0
DEAE-Cellulose	30.0	883	29.43	44150	8.7	11.5

Table 2: Effect of Various Compounds on PPO

Inhibitor	concn(mM)	Relative activity (%)
$CuSO_4$	1	102.6
	5	106.0
	10	102.0
Benzoic acid	1	108.0
	5	131.0
	10	144.0
NaCl	5	99.4
	10	98.6
$MnCl_2$	1	102.6
	5	107.3
	10	101
$NaNO_2$	1	97.9
	5	98.0
	10	104.
KCN	1	118.0
	5	98.5
	10	105.0
Citric acid	1	82.0
	5	74.5
	10	107.0

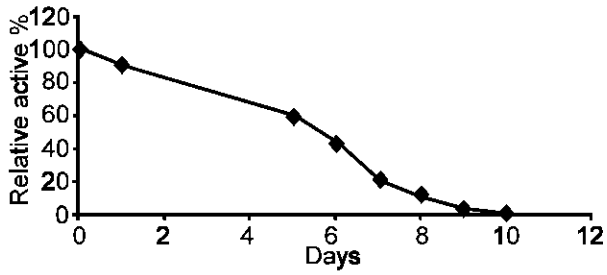

Fig. 1: Effects of time on the activity of van apple catalase

of 3 ml. Catalase (CAT) activity was estimated by decreased in absorbance of H_2O_2 at 240 nm.

Effect of pH: The effect of pH on CAT activity was different pH values (pH 4.0-10.0). After that optimum pH was determined

Effect of temperature: The effect of temperature on CAT activity obtained at different temperature values (20-80 °C). After that optimum temperature was determined.

Kinetic determinations and effect of inhibitors: Kinetic constants as Vmax and Km values were determined by

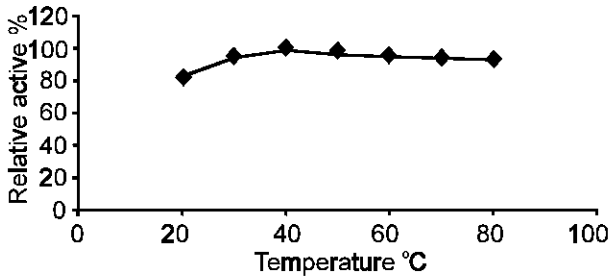

Fig. 2: Effect of temperature on the activity of van apple catalase

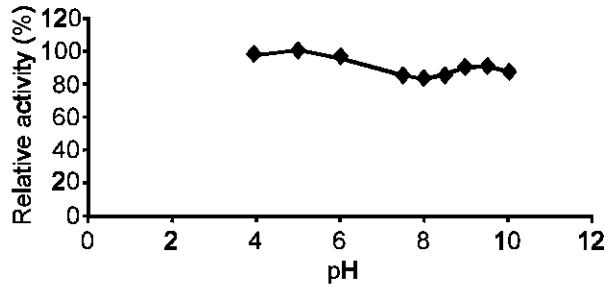

Fig. 3: Effect of pH on the activity of van apple catalase

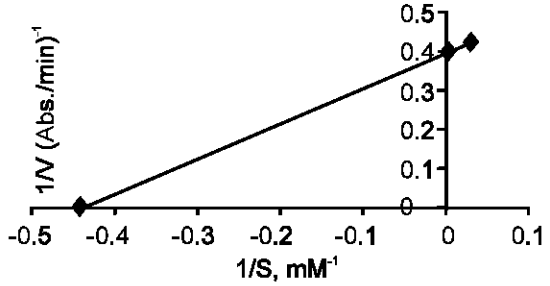

Fig. 4: Lineweaver-burk graph

Lineweaver-burk graphs (Fig. 4). Effect of inhibitors potassium cyanide, citric acid, $MnCl_2$, NaCl, $NaNO_2$ and $CuSO_4$ were used as inhibitor.

Results and Discussion

Determination of optimum pH: The optimum pH for CAT activity was determined at different pH values during incubations at 25°C for 5 min NaPi buffer. The optimal pH for CAT activity was 5 hydrogen peroxide (H_2O_2) as substrate (Fig. 3). Similarly, change in pH and increase in sugar concentrations may provide better conditions,

eig. nutrient availability, for the development of fungi (Torres *et al.*, 2003). The optimum of pH CAT from other studies has been reported (Trindade *et al.*, 1998).

Effect of temperature: The optimum temperature for CAT activity was measured at different temperatures at 20-80°C. The substrate and pure enzyme were incubated for 15 min at various temperatures from 20°C to 80°C, after spectrophotometric measurement for 5 min was carried out at 25°C. The highest activity was observed as 50°C (Fig. 2). The optimum temperature we determined was similar to that given in previous studies (Demir, 2004; Trindade *et al.*, 1998).

Effect of inhibitors: Various inhibitors were used. The inhibitory effect of citric acid, Potassium cyanide, $MnCl_2$, NaCl, $NaNO_2$ $CuSO_4$, and benzoic acid on CAT activity at fixed concentrations was estimated at 25°C and pH 7.0 by using hydrogen peroxide as a substrate at different concentrations (1, 5, 10 mM) (Table 2). These citric acid, Potassium cyanide, $MnCl_2$, NaCl, $NaNO_2$, $CuSO_4$, and benzoic acid were affected to CAT activity. It has recently suggested that the ability of apple extracts to inhibit proliferation of tumor cells in vitro may be due to phenolic/flavonoid antioxidants (Lapidot *et al.*, 2002). These results suggest that $CuSO_4$, $MnCl_2$, NaCl, KCN and benzoic acid at 10 mM able to be used as good.

References

Abassi, N.A., M.M. Kushad and Endress, 1998. Active oxygen-scavengfing enzymes activities in developing apple flowers and fruits. Sientia Horticulturac., 74: 183-194.

Aebi, H., 1984. Catalase. In: L. Packer(Ed), methods in enzymology, Academic pres, Orlando, 105: 121-126.

Andrea, M., 1998. Superoxide dismutase and catalase activities in apple fruit during ripening and post-harvest and with special reference to ethylene. Physiologia Plantarum, 104: 668-672.

Bowler, C., M. Van Montagu and D. Inze, 1992. Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol., 43: 83-116.

Bradford, M.MN., 1976. A rapid and sensitive method for the guantation of microgram quantities of protein utilizing the principle of protein-dye binding anal. Biochem., i72: 248.

Brenan, T.I, and C. Frenkel, 1977. Tarih Involment of hydrogen peroxide in the regulation of senescence in pear. Plant Physiol., 598: 411-416.

DeDuve, C., 1983. Microbodies in the living cells. Sci. Am., 248: 42-52.

Demir, H., 2004. Purification and characterization of polyphenol oxidase from the Ercis apple. Bulletin of Pure and Applied Sciences Chemistry, 42: 1-4.

Gong, Y., P.M. Toivonen, P.A. Wiersma, C. Lu and O.L. Lau, 2000. Effect of freezing on the activity of catalase in apple flesh tiss. J. Agri. Food Chem., 11: 5537-5542.

Higashi, T., F. Kawamata and T. Sakamoto, 1974. Studies on rat liver catalase. VII. Double labeling of catalase by 14C-leucine and 3H-daminolevulinic acid. J. Biochem., 76: 703.

Korban, S.S. and B.L. Bournival, 1987. Catalase, esterase and peroxidase enzymes in seeds and leaves of *Malus domestica*. Borkh. Sientia Horticulturac., 32: 213-219.

Lapidot, T., M.D. Walker and J. Kanner, 2002. Can apple antioxidant inhibit tumor cell proliferation? Generation of H_2O_2 during interaction of phenolic compose with cell culture media. J. Agri. Food Chem., 11: 3156-3160.

Master, C. and R. Holmes and R. Peroxsime, 1977. New aspects of cell physiology and biochemistry. Physiol. Rew., 57: 86-88.

Torres, R., M.C. Valentines, J. Usall and L. Vinas, 2003. Possible involvement of hydrogen peroxide in development of resistance mechanisms in Golden Delicious apple fruit. Postharvest Biology and Technology., 27: 235-242.

Trindade, H., A. Karmali and S.M. Pais, 1998. One- step purification and properties of catalase from *Zandedeschia aetgiopica* .Biochimie., 40: 1759-1764.