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Biochemical characterization with kinetic studies of melanogenic enzyme tyrosinase from white button mushroom, *Agaricus bisporus*

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In *Agaricus bisporus*, color is a key determinant for marketability and consumer acceptability. However, postharvest browning has become a major concern, affecting the overall economics of the mushroom industry. In button mushrooms, the tyrosinase enzyme (E.C.1.14.18.1) is responsible for the browning reactions by catalyzing the conversion of monophenols and diphenols into quinones which polymerize to form melanin. Thus, the present study focused on the purification and characterization of tyrosinase from *A. bisporus.* This enzyme was purified with a final yield of 19.71% and 32.05 purification fold. The study of enzymatic activity over a temperature (5-45°C) and pH range (3-10) showed that the optimum temperature was 35°C with pH 7. The kinetic studies revealed that K_m values were different for catechol (0.71 mM) and L-dopa (0.87 mM), which indicated a higher affinity of the enzyme for catechol. Inhibition studies showed that cinnamic acid is a non-competitive inhibitor while salicylic acid is a competitive inhibitor of tyrosinase. The molecular weight of the enzyme was found to be 43 kDa and different amide regions were reflected by the FTIR spectra of the enzyme. This study may provide valuable insights into the structure, biochemical properties, and inhibition of tyrosinase enzyme for controlling mushroom browning.

Keywords: Enzyme Inhibitors, FTIR, Ion exchange chromatography, SDS-PAGE, Substrate specificity

The white button mushroom, *Agaricus bisporus*, is highly popular with consumers due to its nutritional and medicinal properties[1]. Their economic value is rising globally day by day in the form of potential nutraceuticals and dietary supplements, which act as a source of antimicrobial and antioxidant compounds[2,3]. However, the shelf life of this mushroom is quite less which results in postharvest deterioration, thus quality preservation is still a major drawback[4]. The major factors contributing to quality loss are browning, veil opening, texture changes and weight loss[5]. Browning is an undesirable enzymatic reaction that leads to a less attractive appearance and reduction in nutritional quality[6]. It is a major impediment in the marketing of fresh mushrooms as colour is perceived as an indicator of freshness by the consumers[7]. Browning is a complex process that gets initiated by the disruption of cell membrane integrity leading to close contact between enzymes and the phenolic compounds[8]. Tyrosinase (E.C.1.14.18.1) is one of the key enzymes involved in browning reactions that oxidizes phenolic compounds by two different types of reactions[9,10]. One of the reactions involves monophenolase activity (hydroxylation of monophenols) and the second reaction involves diphenolase or catecholase activity, oxidizing ortho-diphenols to ortho-quinones[11]. Therefore, the present study was conducted to extract, purify and characterize the tyrosinase enzyme from *A. bisporus* which may be helpful to disseminate important information about the tyrosinase enzyme for futuristic practical applications.

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Materials and Methods

Materials

The chemicals used (Analytical grade) in this study were purchased from Sigma-Aldrich. Mushroom fruit bodies were freshly harvested from Dr. HS Garcha Mushroom Research Laboratory, Punjab Agricultural University, Ludhiana, Punjab, India.

Methods

Enzyme extraction and purification

The crude enzyme was extracted from the fruit bodies of *A. bisporus* in 50 mM phosphate buffer (pH 7.0). Ammonium sulfate precipitation of the supernatant was carried out to obtain the solution with 60-90% saturation. For desalting, the crude extract was subjected to dialysis and further purified by gel

filtration and ion exchange chromatography. The enzyme was eluted from the column using a phosphate buffer saline gradient of 25, 50, 100 and 150 mM with a flow rate of 1 mL/min. The collected salt eluent enzyme fractions were screened for maximum activity. Tyrosinase active fractions were pooled and concentrated by lyophilisation[12].

Characterization of the purified enzyme

Assay of enzyme activity

Tyrosinase activity was estimated using catechol (40 mM) as substrate[13]. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001 per min.

Optimum temperature

To determine the optimum temperature for the purified enzyme, the substrate solution (Catechol 40 mM) was equilibrated at a temperature range of 10-40°C for half an hour and the activity was estimated using the temperature equilibrated substrate solutions[14].

Optimum pH

To determine the effect of pH, enzyme activity was estimated at a range of pH (3-10)[15]. To obtain the pH range of 3-10, citrate (3.0-3.5), sodium acetate (4.0-5.8), phosphate (6.0-8.0) and tris buffer (8.5-10) were used.

Thermal stability

Thermal stability was investigated by incubating aliquots of the enzyme at a temperature range (25-70°C) for 60 min, then allowed to cool at room temperature (25°C). These pre-incubated enzyme aliquots were used for the estimation of enzyme activity[14].

Substrate specificity

To determine the substrate specificity, different substrate solutions of the same concentration (40 mM) were used to estimate the monophenolase (L-tyrosine and Phenol), diphenolase (catechol, L-dopa) and triphenolase activity (pyrogallol) of the purified enzyme at a wavelength of 420 nm[16].

Kinetic properties of the purified enzyme

The kinetic properties of the enzyme were studied using 1-10 mM concentrations for two substrates *i.e*., catechol and L-dopa at pH 7.0. After measuring the activity of the enzyme at different concentrations of both the substrates, values of Michaelis constant (K_m) and maximum velocity (V_{max}) were determined using the method given by Lineweaver and Burk (1934)[17].

Effect of inhibitors on the enzyme activity

The inhibitory effect of salicylic acid and cinnamic acid (0.5-2 mM) was determined using catechol as a substrate. Inhibition patterns were studied from double reciprocal plots of reaction velocity versus initial substrate concentration. The inhibition constant (K_I) was calculated by plots of the apparent $1/V_{max}$ or K_m/V_{max} *vs* the concentration of the inhibitor[18].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS- PAGE)

To determine the molecular weight of the enzyme, SDS-PAGE electrophoresis (12%), was performed for 4 h at 150 V[19].

Fourier transfer infrared spectroscopy (FTIR) of the enzyme

FTIR analysis of the purified tyrosinase was carried out by loading the lyophilized enzyme sample on the ATR crystal (4000-400 cm^{-1})[20].

Results

Purification of the tyrosinase enzyme from *Agaricus bisporus*

The purification steps involved the estimation of enzyme activity and total proteins for calculating the specific activity, yield and multiple purification fold of the enzyme (Table 1). The specific activity of the crude extract during the initial extraction phase was 2.31 U/mg, volume activity 2592 U/mL along with 100% yield and purification fold 1 (Table 1). Ammonium sulfate precipitation for the crude protein extraction was carried out in three steps that are from 0-30%, 30-60% and 60-90%, the precipitate fraction obtained with 60-90% ammonium sulfate precipitation was found to be having highest volume activity of 1989 U/mL with

Table 1 — Purification summary of tyrosinase from the fruit bodies of button mushroom, *A. bisporus*

Fractions	Volume (mL)	Total Protein (mg)	Total activity (U/ml)	Specific activity (U/mg)	Purification fold (x)	Yield (%)
Crude Extract	400	1120	2592	2.31	1	100
Ammonium Sulphate precipitate	90	315	1989	6.31	2.73	76.73
Dialysis	48	145	1640	11.26	4.87	63.27
Sephadex G-100	28	52	1303	25.05	10 84	50.27
DEAE-Cellulose	16	6.9	511	74.05	32.05	19.71

315 mg total protein content. An increase in the specific activity was recorded from 2.31 to 6.31 U/mg with a yield of 76.73% and 2.73 purification fold through ammonium sulfate precipitation. Later on, the enzyme extract was desalted through dialysis to obtain concentrated extract (48 mL) with specific activity as 11. 26 U/mg and 145 U/mg total protein content. The yield after dialysis decreased from 76.73 to 63.27% with an increase in the purification fold from 2.73 to 4.87.

The partially purified extract was subjected to molecular filtration through a Sephadex G-100 column (30 × 2 cm). The elution profile of Sephadex G-100 gel filtration showed the major peaks of the tyrosinase activity in the active fractions. The pooled active fractions (28 mL) showed 25.05 U/mg specific activity with 52 mg total protein content and 50.27% yield. The pooled active factions from Sephadex G-100 were loaded onto the DEAE-cellulose column (30 × 1 cm) to obtain pure enzyme and the fractions (3 mL) were collected at a flow rate of 1 mL/min. From the elution profile, the active fractions with major peaks were eluted using the 0-150 mM NaCl gradient (Fig. 1). The fractions with maximum activity were pooled which showed 74.05 U/mg specific activity of the pure enzyme, 6.9 mg total protein and 19.71% yield.

Characterization of tyrosinase enzyme from *Agaricus bisporus*

Temperature optima for the enzyme

An increase in the enzyme activity was recorded with the increasing temperature and maximum activity (468 µmole/min/mL) was observed at 35°C (Fig. 2). The enzyme activity at 5°C was 44.87% while at 40°C it was 91.36% of the activity at 35°C. The activity over the temperature range (5-45°C) showed that the optimum temperature for the maximum enzyme activity was 35°C (Fig. 2).

Effect of pH on the enzyme activity

An increase in the activity was recorded with the increase in the pH from the acidic to neutral range. However, with progression towards alkalinity, it followed a decreasing trend. At pH 3, the enzyme activity was low (13.57%; 63 µmole/min/mL) and a sudden increase in activity was observed by increasing the pH from pH 5.8 (212 µmole/min/mL) to pH 6.2 (395 µmole/min/mL). Later on, the activity increased moderately with maximum activity at pH 7.0 (464 µmole/min/mL). A significant decline was observed from pH 7.0 to 8.8 and at pH 9.0, no activity was recorded (Fig. 3). From this study, it was observed that the enzyme was active over a pH range of 6.4 to 7.2 with maximum activity at pH 7.

Thermal stability

The purified enzyme extract was subjected to thermal shock between 25 and 70°C for 30 min and residual activity was measured using catechol solution (40 mM) as substrate prepared in phosphate buffer (pH 7.0). The enzyme was found to be stable at a temperature range of 25-40°C. The maximum residual activity (287 µmole/min/mL; 100%) was observed at 35°C and 75.75% activity was retained by the enzyme at 45°C, while this activity decreased to 38.68% with an increase in temperature (50°C). A rapid decline in the activity was observed at 65°C (6.6%) and the enzyme was found to be inactive at 70°C (Fig. 4).

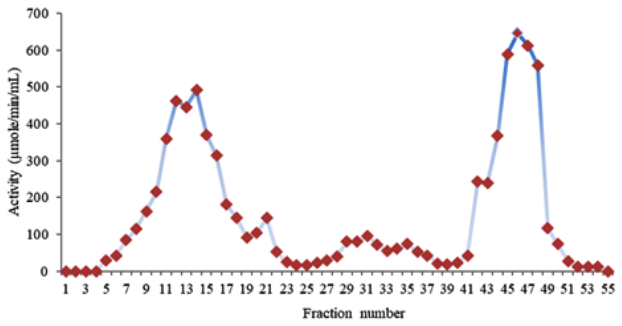

Fig. 1 — DEAE-Cellulose elution profile of tyrosinase from *A. bisporus*

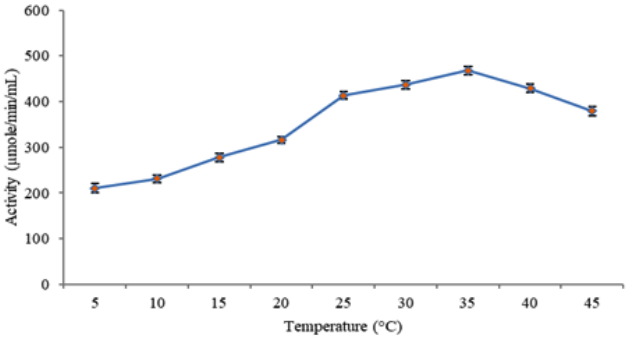

Fig. 2 — Effect of temperature on the activity of purified tyrosinase enzyme

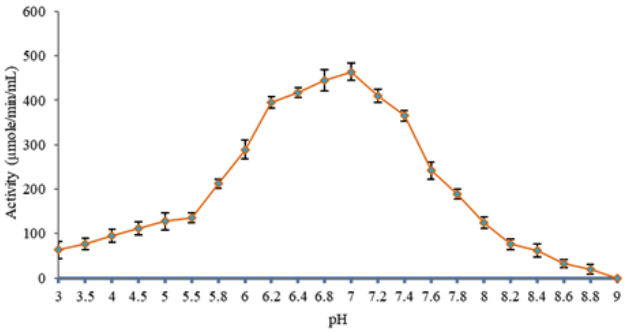

Fig. 3 — Effect of pH on the activity of the purified tyrosinase enzyme

Substrate specificity

In the study of substrate specificity, different substrates were used to estimate monophenolase (L-tyrosine and Phenol), diphenolase (catechol, L-dopa) and triphenolase activity (pyrogallol) of the purified enzyme (Fig. 5). The results indicated that maximum enzyme activity of 276 µmole/min/mL (100%) for catechol as substrate, while activity for L-dopa pyrogallol and was only 36.96% and 33.33%, respectively. The monophenolic substrates exhibited very low activity which was found to be only 4.71% (phenol) and 3.26% (tyrosine) of the activity for catechol as substrate.

Kinetic studies of the purified enzyme

For kinetic studies, the activity of the tyrosinase was estimated at a varied concentration of catechol and L-dopa as substrates and K_m and V_{max} were calculated by plotting 1/[V] against 1/[S]. In the case of catechol, the K_m was found to be 0.71 mM with V_{max} 2518 µmole/mL/min (Fig. 6A) while for L-Dopa, the K_m value was found to be 0.87 mM with V_{max} 1714 µmole/mL/min. The K_m values for both the substrates were different. The lower value of K_m indicated higher affinity of tyrosinase for catechol as compared to L-dopa (Fig. 6B).

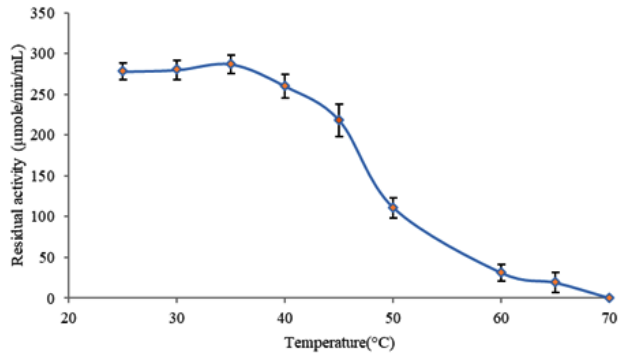

Fig. 4 — Thermal stability of the tyrosinase enzyme purified from *Agaricus bisporus*

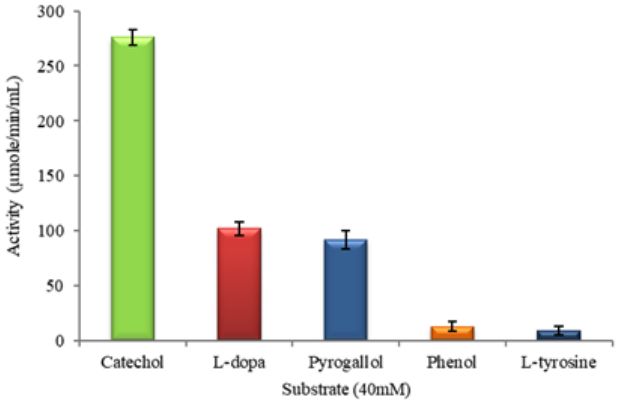

Fig. 5 — Effect of different substrates on the activity of purified tyrosinase enzyme

Effect of inhibitors on the enzyme activity

The inhibitory modes of salicylic acid and cinnamic acid on the purified enzyme were determined from Lineweaver–Burk double reciprocal plots using catechol as substrate. The plot of 1/[V] *vs.* 1/[S] with cinnamic acid as an inhibitor showed straight lines with different slopes having intersections at the X axis. By increasing the inhibitory concentration, V_{max} decreased, however, K_m remained unchanged. This indicated that the mode of inhibition by cinnamic acid was non-competitive and the site of binding was other than the active site of the enzyme (Fig. 7A). The inhibition constant value K_I was found to be 1.91 mM (Fig. 7B) which revealed that cinnamic acid can bind to both free enzyme and enzyme-substrate complex.

In the presence of salicylic acid, the kinetics of the catecholase activity of the purified enzyme was investigated from double-reciprocal Lineweaver–Burk plots. The plots of 1/[V] *vs* 1/[S] showed several straight lines with different values of the slope, intersecting with one another on the Y-axis (Fig. 8A). The value of V_{max} remained almost

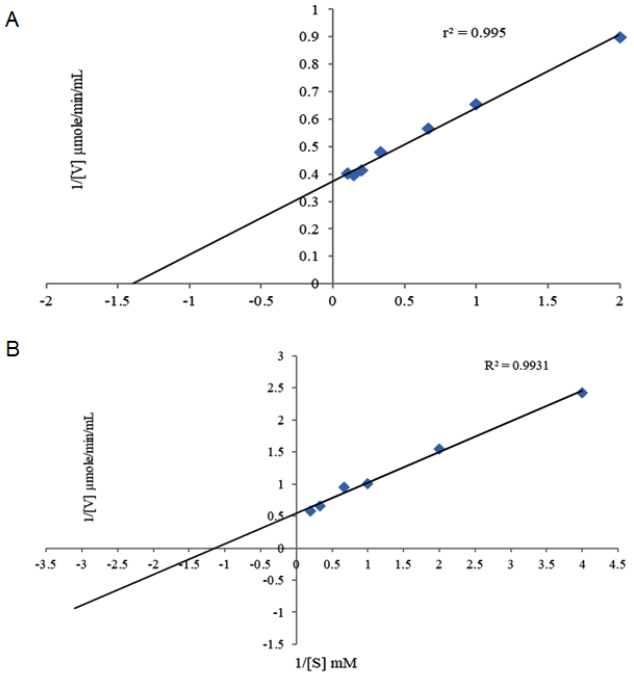

Fig. 6 — The Lineweaver Burk plot of tyrosinase enzyme using substrates (A) catechol; and (B) L-dopa

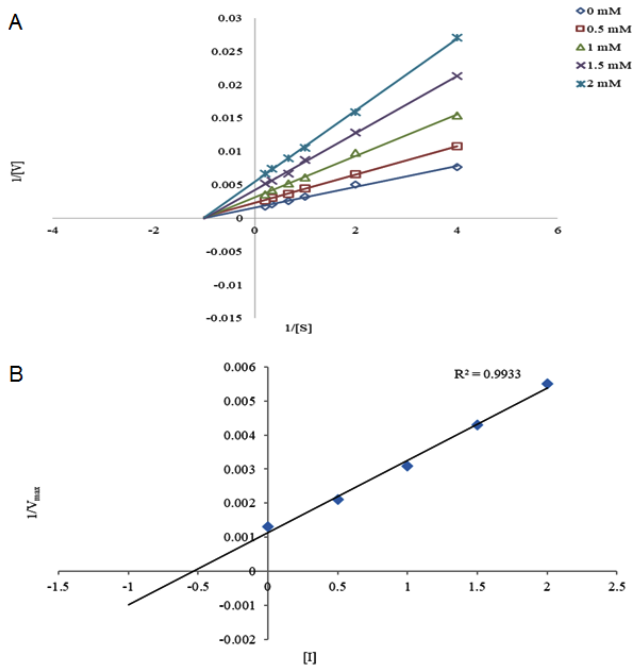

Fig. 7 — (A) Lineweaver–Burk plot with cinnamic acid (0-2 mM) as an inhibitor for tyrosinase; and (B) $1/V_{max}$ *vs* [I] plot to determine inhibition constant (K_I)

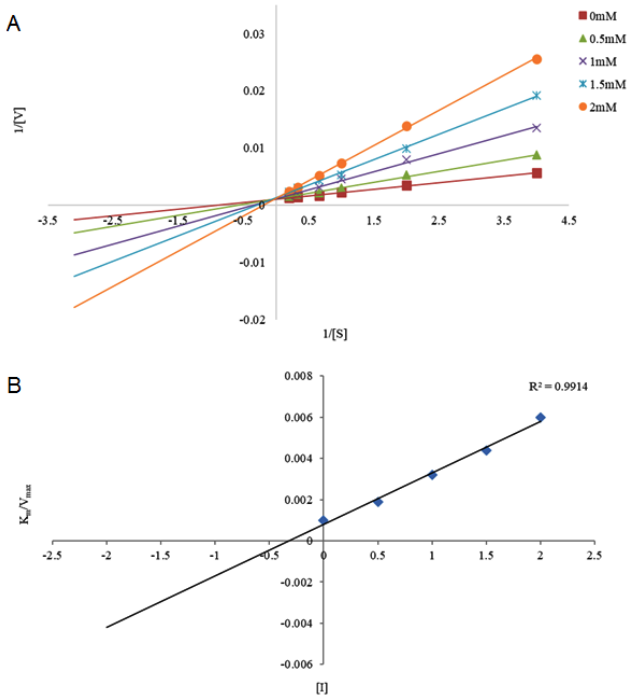

Fig. 8 — (A) Lineweaver–Burk plot with salicylic acid (0-2 mM) as an inhibitor for tyrosinase; and (B) K_m /V_{max} *vs* [I] plot to determine inhibition constant (K_I)

same whereas, K_m increased with increase in the concentration of salicylic acid. Our findings revealed that salicylic acid is a competitive inhibitor of tyrosinase which binds to the enzyme in a free state only. The inhibition constant (K_I) was found to be 3.1 mM (Fig. 8B).

Molecular weight determination of the enzyme by SDS-PAGE

In the crude fractions, eight bands with different molecular weights 64, 43, 34, 27.8, 26, 22, 16, 11 kDa) were observed. However, in the Sephadex G-100 and DEAE cellulose eluted fractions, a single distinctive band appeared with a molecular weight of 43 kDa (Fig. 9).

Fourier transform infrared spectroscopy (FTIR) study of the purified enzyme

FTIR spectra of the enzyme reflected the amide I and amide II bands, particularly the former which absorbed around 1620 to 1690 cm^{-1} wave number (Fig. 10). The spectra defined reflectance at 3343. 5 cm^{-1} for an in-plane stretch of N-H in amide A region. The FTIR spectra of the tyrosinase showed a peak at 1643.7 cm^{-1} which defined the amide I region. This peak was due to the stretching vibration of the C=O group. This might be due to the involvement of C=O in the hydrogen bonding that occurs between different elements of the secondary structure of the

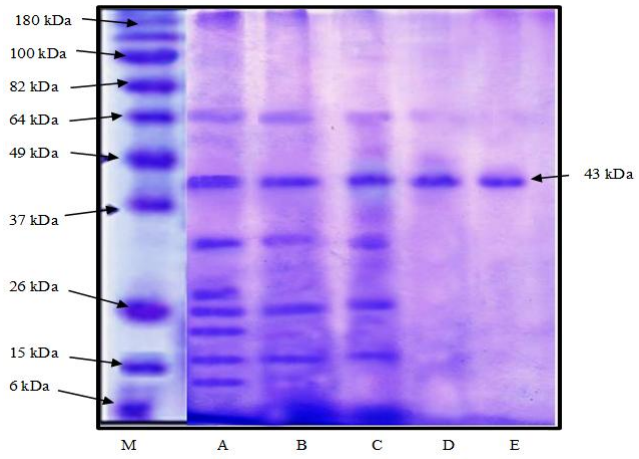

Fig. 9 — Electrophoretic profile of purified tyrosinase enzyme. M: Marker, A: Crude extract, B: Precipitated fraction, C: Dialyzed fraction, D: Sephadex, E: DEAE cellulose fraction

protein. The spectra showed smaller peaks in the range 1550.49-1492.63 cm^{-1} which could be due to the C-N stretching in amide II of the peptide linkage. A peak at 1288.22 cm^{-1} was also recorded indicating a C-N stretch of the amide III of the peptide linkage. A peak at 1066.44 cm^{-1} defined the carbonyl (C-O) stretching, while peaks at 649.89 cm^{-1} to 613.25 cm^{-1} indicated O-C=N bending of the amide IV in peptide linkage.

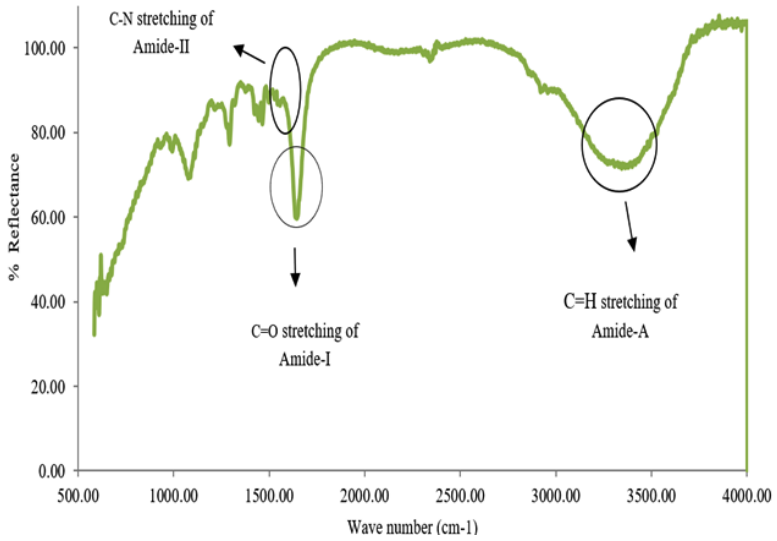

Fig. 10 — FTIR spectra of the tyrosinase enzyme purified from *Agaricus bisporus*

Discussion

Tyrosinase, a type III copper containing enzyme, plays a pivotal role in mushroom browning[21] and inhibition of this enzyme during post-harvest storage is of great concern to improve the shelf life of mushrooms. In this study, the tyrosinase enzyme from *Agaricus bisporus* was studied in depth for its biochemical and structural properties. Tyrosinase was purified through gel filtration and ion-exchange chromatography with a final yield of 19.71%. Previous purification studies have reported the % yield of tyrosinase enzyme which varied from 2.67% in tea leaf (*Camellia sinensis*), 9.8% in *Pholiota nameko* to 17.9% in northern mauxia shrimp (*Acetes chinensis*)[22-24]. In this study, tyrosinase showed maximum enzyme activity at a temperature of 35°C. It has been previously reported that temperature optima for tyrosinase enzyme range from 20-45°C depending on the source[25]. In enzyme catalysis, pH is an important factor, if the pH of the solution is near the optimum level, the rate of catalysis will be significantly higher as compared to the pH farther away from the optima[26]. From this study, it was observed that the enzyme was active over a pH range of 6.4 to 7.2 with optimum pH of 7. However, activity was quite low at acidic and alkaline pH. The reduction in activity at acidic and alkaline pH could be due to the denaturation of the structural subunits, and the change in the surface charges of the enzyme which in turn causes the reduction in binding of the substrate with the active sites, therefore, reducing the overall enzymatic activity[27].

Tyrosinase enzyme has been reported to be thermostable in the moderate temperature range[28]. In our study, the purified tyrosinase was found to be stable at a temperature range of 25-40°C, with a slight reduction in activity on increasing the temperature up to 45°C, while no activity was observed at 70°C. Similarly, in *Pleurotus djamor*, tyrosinase activity was found to be quite stable at a temperature range of 30–40°C, however, the enzyme was completely inactive at 70°C[29]. A sudden decrease in the enzyme activity at higher temperatures can be attributed to the dissociation of structural subunits and unfolding of the tertiary structure thereby, shielding the active sites of the enzyme[30]. Tyrosinase enzyme revealed varied specificity towards different substrates with higher affinity for diphenolic substrate catechol. Tyrosinase can accept mono, di and triphenols as substrates, which might be due to the structural flexibility of this enzyme that makes the binding of different phenolic substrates to the active site possible[31,32]. The higher affinity for catecholic compounds than phenolic compounds can be attributed to the binding of catecholic compounds to dicopper center at the active site with higher affinity by forming two to four coordination bonds[33].

The K_m and V_{max} of the enzyme were found to be substrate dependent. In the case of catechol, the K_m was found to be 0.71 mM with V_{max} 2518 µmole/mL/min while for L-Dopa, the K_m value was found to be 0.87 mM with V_{max} 1714 µmole/mL/min. Similarly, in a previous study the value of K_m and V_{max} of tyrosinase from the peel of African bush mango (*Irvingia gabonensis*) was 17.84 mM, 0. 42 U/min using L-dopa as substrate while the K_m was 14.34 mM with V_{max} 0.46 U/min using catechol as substrate[34]. Inhibition of

enzymatic browning is a foremost strategy for enhancing the quality of fresh mushrooms. Thus, the use of tyrosinase inhibitors as anti-browning agents is an attractive approach to control browning[35]. In this study, the mode of inhibition by cinnamic acid and salicylic acid was found to be non-competitive and competitive, respectively. Phenolic compounds such as salicylic acid possess structures similar to tyrosinase substrates. This similarity resulted in competitive inhibition that might be due to the binding of salicylic acid to the active site of the enzyme[36]. Inhibition by cinnamic acid on diphenolase activity of tyrosinase enzyme was found to be non-competitive which can bind to the free enzyme and enzyme-substrate complex rather than binding to the active site[37]. The tyrosinase purified in our study showed a molecular weight of 43 kDa as estimated by SDS-PAGE. The previous studies have reported that the molecular weight of tyrosinase varies among the species. For example, the molecular wight of tyrosinase was observed to be 35 kDa from *Bacillus aryabhattai*[28], 66 kDa from *Armillaria ostoyae*[38], and 90 kDa from *Pleurotus djamor*[29].

FTIR spectra of tyrosinase reflected the secondary structural components such as the amide I, and amide II bands and in-plane stretch of N-H in the amide A region. It has been reported that the most sensitive spectral region to the protein secondary structural components is the amide I band (1700−1600 cm^{-1}), which was due to the C=O stretch vibrations of the peptide linkages (approximately 80%). The frequencies of the amide I band components were found to be correlated closely to each secondary structural element of the proteins. The amide II band, in contrast, derives mainly from in-plane N-H bending (40−60% of the potential energy) and the C-N stretching vibrations (18−40%)[39]. Similarly, in FTIR spectra of the crude protein extract of *A. bisporus*, a wide peak was around 3500 cm^{-1} referred to the stretching vibration of primary amines and hydroxyl groups (–OH), which were related to the proteins. A sharp peak at 1637.84 cm^{-1} indicated the amide I, which was due to the vibrations of C=O stretch bonded to the protein while the wave number at 1450.23 cm^{-1} corresponded to the C=O, NH, and NH_2[40].

Conclusion

The major enzyme (tyrosinase) responsible for melanogenic reactions in *Agaricus bisporus* was isolated and purified by precipitation, gel filtration, and ion exchange chromatography with a final yield of 19.71% and 32.05 purification fold. The purified enzyme showed different specificity towards different substrates with the highest affinity for catechol and was found to be found stable at a temperature range of 25-40°C. The lower K_m values indicated a higher affinity of the enzyme for catechol. The enzyme was found to be active at neutral and near neutral range with optimum pH 7. The activity over the temperature range (5-45°C) showed that the optimum temperature for enzyme activity was 35°C. The purified enzyme fractions showed a single distinctive band with a molecular weight of 43 kDa. The inhibition studies showed that cinnamic acid was a non-competitive while salicylic acid was a competitive inhibitor of purified tyrosinase which could be used to curb browning in button mushrooms.

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Conflict of interest

All authors declare no conflict of interest.

References

1 Mwangi RW, Macharia JM, Wagara IN & Bence RL, The antioxidant potential of different edible and medicinal mushrooms. *Biomed Pharmacother*, 147 (2022) 112621.

2 Shaffique S, Kang SM, Kim AY, Imran M, Khan MA & Lee IJ, Current knowledge of medicinal mushrooms related to anti-oxidant properties. *Sustainability*, 13 (2021) 7948.

3 Maiety P, Sen IK, Chakraborty I, Mondal S, Bar H, Bhanja SK, Mandal S & Maity GN, Biologically active polysaccharide from edible mushrooms: A review. *Int J Biol Macromol*, 172 (2021) 408.

4 Wang T, Yun JM, Zhang Y, Bi Y, Zhao FY & Niu YX, Effects of ozone fumigation combined with nano-film packaging on the postharvest storage quality and antioxidant capacity of button mushrooms (*Agaricus bisporus*). *Postharvest Biol Technol*, 176 (2021) 111501.

5 Zalewska M, Marcinkowska-Lesiak M, Onopiuk A, Stelmasiak A & Półtorak A, Modified atmosphere packaging for extending the shelf life of fresh *Agaricus bisporus*. *J Food Process Preserv*, 42 (2018) 13839.

6 Qian X, Hou Q, Liu J, Huang Q, Jin Z, Zhou Q, Jiang T & Zheng X, Inhibition of browning and shelf life extension of button mushroom (*Agaricus bisporus*) by ergothioneine treatment. *Sci Hortic*, 288 (2021) 110385.

7 Gholami R, Ahmadi E & Farris S, Shelf life extension of white button mushrooms (*Agaricus bisporus*) by low temperatures conditioning, modified atmosphere, and nanocomposite packaging material. *Food Packag Shelf Life*, 14 (2017) 88.

8 Shekari A, Hassani RA & Aghdam MS, Exogenous application of GABA retards cap browning in *Agaricus bisporus* and its possible mechanism. *Postharvest Biol Technol*, 174 (2021) 111434.

9 Cabezudo I, Ramallo A, Alonso VL & Furlan RLE, Effect directed synthesis of a new tyrosinase inhibitor with anti-browning activity. *Food Chem*, 341 (2021) 128232.

10 Peng Z, Wang G, Zeng QH, Li Y, Liu H, Wang JJ & Zhao Y, A systematic review of synthetic tyrosinase inhibitors and their structure-activity relationship. *Crit Rev Food Sci Nutr*, 62 (2022) 4053.

11 Zhou X, Iqbal A, Li J, Liu C, Murtaza A, Xu X, Pan S & Hu W, Changes in browning degree and reducibility of polyphenols during autoxidation and enzymatic oxidation. *Antioxidants*, 10 (2021)1809.

12 Zhou XR, Xiao YJ, Meng XH & Liu BJ, Full inhibition of Whangkeumbae pear polyphenol oxidase enzymatic browning reaction by l-cysteine. *Food Chem*, 266 (2018) 1.

13 Wu J, Gao J, Chen H, Liu X, Cheng W, Ma X & Tong P, Purification and characterization of polyphenol oxidase from *Agaricus bisporus*. *Int J Food Prop*, 16 (2013) 1483.

14 Marrufo-Hernandez NA, Palma-Orozco G, Beltran HI & Najera H, Purification, partial biochemical characterization and inactivation of polyphenol oxidase from Mexican Golden Delicious apple (*Malus domestica*). *J Food Biochem*, 41 (2017) 12356.

15 Kebeish R, El-sayed A, Fahmy H & Abdel-Ghany A, Molecular cloning, biochemical characterization, and antitumor properties of a novel L-asparaginase from *Synechococcus elongates* PCC6803. *Biochem (Mosc)*, 81 (2016) 1173.

16 Derardja AE, Pretzler M, Kampatsikas I, Barkat M & Rompel A, Purification and characterization of latent polyphenol oxidase from apricot (*Prunus armeniaca* L.). *J Agric Food Chem*, 65 (2017) 8203.

17 Lineweaver H & Burk D, The determination of enzyme dissociation constants. *J Am Chem Soc*, 56 (1934) 658.

18 Chen CQ, Li ZC, Pan ZZ, Zhu YJ, Yan RR, Wang Q, Yan JH & Chen QX, Inactivation kinetics of polyphenol oxidase from pupae of blowfly (*Sarcophaga bullata*) in the dimethyl sulfoxide solution. *Appl Biochem Biotechnol*, 160 (2010) 2166.

19 Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227 (1970) 680.

20 Kumari V & Sangal A, Antimicrobial study of *Arjuna Terminalia* loaded PLGA nanoparticle. *Indian J Biochem Biophys*, 57 (2020) 291.

21 Mahdavi A, Mohammadsadeghi N, Mohammadi F, Saadati F & Nikfard S, Evaluation of inhibitory effects of some novel phenolic derivatives on the mushroom tyrosinase activity: Insights from spectroscopic analyses, molecular docking and *in vitro* assays. *Food Chem*, 387 (2022) 132938.

22 Öztürk C, Aksoy M & Küfrevioğlu Öİ, Purification of tea leaf (*Camellia sinensis*) polyphenol oxidase by using affinity chromatography and investigation of its kinetic properties. *J Food Meas Charact*, 14 (2020) 31.

23 Kawamura-Konishi Y, Tsuji M, Hatana S, Asanuma M, Kakuta D, Kawano T, Mukouyama EB, Goto H & Suzuki H, Purification characterization and molecular cloning of tyrosinase from *Pholiota nameko*. *Biosci Biotechnol Biochem*, 71 (2007) 1752.

24 Zhang J, Zhou G, Fei L, Chen L, Sun L, Lyu F & Ding Y, Study on purification and characterization of polyphenol oxidase from *Acetes chinensis*. *Molecules*, 26 (2021) 7545.

25 Rasmussen CB, Enghild JJ & Scavenius C, Identification of polyphenol oxidases in potato tuber (*Solanum tuberosum*) and purification and characterization of the major polyphenol oxidases. *Food Chem*, 365 (2021) 130454.

26 Nannipieri P, Trasar-Cepeda C & Dick RP, Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biol Fertil Soils*, 54 (2018) 11.

27 Maamoun HS, Rabie GH, Shaker I, Alaidaroos BA & El-Sayed ASA, Biochemical properties of tyrosinase from *Aspergillus terreus* and *Penicillium copticola*; undecanoic acid from *Aspergillus flavus*, an endophyte of *Moringa oleifera*, is a novel potent tyrosinase inhibitor. *Molecules*, 26 (2021) 1309.

28 Wang F, Xu Z, Wang C, Guo Z, Yuan Z, Kang H, Li J, Lu F & Liu Y, Biochemical characterization of a tyrosinase from *Bacillus aryabhattai* and its application. *Int J Biol Macromol*, 176 (2021) 37.

29 Sharma J, Sharma D, Sharma A & Bansal S, Thermo stable tyrosinase purified from *Pleurotus djamor* grown in biomimetic calcium carbonate: A biological strategy to industrial waste remediation. *Environ Technol Innovat*, 21 (2021) 101294.

30 El-sayed ASA & Shad G, *Aspergillus flavipes* is a novel efficient biocontrol agent of *Phytophthora parasitica*. *Biol Control*, 140 (2020) 104072.

31 Panis F, Krachler RF, Krachler R & Rompel A, Expression, purification, and characterization of a well-adapted tyrosinase from peatlands identified by partial community analysis. *Environ Sci Technol*, 55 (2021) 11445.

32 McLarin MA & Leung IKH, Substrate specificity of polyphenol oxidase. *Crit Rev Biochem Mol Biol*, 55 (2020) 274.

33 Matoba Y, Oda K, Muraki Y & Masuda T, The basicity of an active-site water molecule discriminates between tyrosinase and catechol oxidase activity. *Int J Biol Macromol*, 183 (2021) 1861.

34 Adeseko CJ, Sanni DM, Salawu SO, Kade IJ, Bamidele SO & Lawal OT, Purification and biochemical characterization of polyphenol oxidase of African bush mango (*Irvingia gabonensis*) fruit peel. *Biocatal Agric Biotechnol*, 36 (2021) 102119.

35 Zeng H, Sun D, Chu S, Zhang J, Hu G & Yang R, Inhibitory effects of four anthraquinones on tyrosinase activity: Insight from spectroscopic analysis and molecular docking. *Int J Biol Macromol*, 160 (2020) 153.

36 Liao T, Zhou L, Liu J, Zou L, Dai T & Liu W, Inhibitory mechanism of salicylic acid on polyphenol oxidase: A cooperation between acidification and binding effects. *Food Chem*, 348 (2021) 129100.

37 Yu Q, Fan L & Duan Z, Five individual polyphenols as tyrosinase inhibitors: inhibitory activity, synergistic effect, action mechanism, and molecular docking. *Food Chem*, 297 (2019) 124910.

38 Li T, Zhang N, Yan S, Jiang S & Yin H, A novel tyrosinase from *Armillaria ostoyae* with comparable monophenolase and diphenolase activities suffers substrate inhibition. *Appl Environ Microbiol*, 87 (2021) 00275.

39 Bunaciu AA, Aboul-Enein HY & Hoang VD, Raman spectroscopy for protein analysis. *Appl Spectrosc Rev*, 50 (2015) 377.

40 Eskandari-Nojehdehi M, Jafarizadeh-Malmiri H & Rahbar-Shahrouzi J, Optimization of processing parameters in green synthesis of gold nanoparticles using microwave and edible mushroom (*Agaricus bisporus*) extract and evaluation of their antibacterial activity. *Nanotechnol Rev*, 5 (2016) 537.