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Biocatalysis of tyrosinase using catechin as substrate in selected organic solvent media

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Abstract

The enzymatic activity of mushroom tyrosinase was investigated using catechin as substrate in selected organic solvent media. The results showed that optimal tyrosinase activity was obtained at pH 6.2, 6.6, 6.0 and 6.2 in the organic solvent media of heptane, toluene, dichloromethane, and dichloroethane, respectively, and at a temperature between 25°C and 27.5°C. In addition, the kinetic studies showed that the K_m values were 5.38, 1.03, 2.52 and 4.03 mM, for the tyrosinase-catechin biocatalysis in the reaction media of heptane, toluene, dichloromethane, and dichloroethane, respectively, while the corresponding V_{max} values were 1.22×10^{-3}, 0.33×10^{-3}, 1.47×10^{-3} and 1.20×10^{-3} δA per μg protein per second, respectively. The use of acetone as co-solvent for the tyrosinase-catechin biocatalysis showed that acetone concentrations ranging from 5% to 30% (v/v) in the heptane reaction medium produced a decrease of 4.3% to 96.7% in tyrosinase activity. The results also indicated that the presence of 12.5% acetone in the reaction medium of dichloromethane, and 22.0% in those of toluene and dichloroethane produced a maximal increase of 42.6%, 92.1% and 71.8%, respectively, in tyrosinase activity. However, the overall findings indicated that additional increases in acetone concentration resulted in an inhibition of tyrosinase activity.

Keywords: Tyrosinase; Biocatalysis; Organic solvents; Catechin

1. Introduction

Polyphenol oxidase (PPO) is a term used to describe a large number of related enzymes, including catecholase, diphenol oxidase, phenolase, polyphenol oxidase and tyrosinase [1]. PPO catalyzes the hydroxylation of monophenols to produce diphenols and their subsequent oxidation to *o*-quinones, which upon further reaction lead to the production of brown pigments [2]. The presence of PPO is responsible for the occurrence of the enzymatic browning reaction in injured or cut fruits and vegetables upon contact with oxygen. The rate of the enzymatic browning reaction depends on the degree of PPO activity present in the food, the nature and content of phenolic compounds, the pH and temperature, as well as the presence of oxygen reducing substances and metal ions [3].

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For many years, the biocatalysis of PPO has been investigated using a wide range of aqueous media [4–6]. However, PPO is limited in its use as a practical catalyst in aqueous media due to the polymerizarion of *o*-quinones which inactivate the enzyme [7]. Recently, the use of organic solvents as potential reaction media for PPO activity has been reported, particularly, for the production of natural stable pigments of selected color intensity, due to limited *o*-quinone polymerization [8].

The selection of the appropriate organic solvent as a reaction medium for enzyme biocatalysis depends on several factors such as solvent hydrophobicity, density, viscosity, surface tension, toxicity, flammability, waste disposal, and cost [9]. Solvent hydrophobicity is represented as the logarithm of the partition coefficient (log P), where P is defined as the partitioning of a given solvent between water and 1-octanol in a two-phase system [10]. Solvents with a log P value lower than 2 were reported not to be suitable for PPO biocatalysis, as they strongly distorted the essential water layer required to maintain the enzyme in its native catalytically active conformation. Solvents with a log P value between 2 and 2.5 showed higher enzymatic conversions since they did not interfere with the essential water coat surrounding the enzyme molecule in its active state [8,11,12].

This work is part of on-going research aimed at the optimization of tyrosinase biocatalysis in organic solvent media. The specific objective of this work was to study the effect of enzymatic protein concentration, substrate concentration, pH, temperature, and acetone as co-solvent on the activity of the commercially purified mushroom tyrosinase, using catechin as substrate, in selected organic solvent media including heptane, toluene, dichloromethane and dichloroethane.

2. Materials and methods

2.1. Preparation of enzyme suspension

Commercially purified tyrosinase, possessing an activity of 3000–3400 units/mg solid, was purchased from Sigma (St. Louis, MO) and used throughout this study. The tyrosinase suspension was prepared in citrate–phosphate buffer solution (0.1 M) (ACP Chemicals, Montréal, Qc) at a pH ranging from 4.0 to 7.0.

2.2. Preparation of substrate solution

A stock solution (400 mM) of catechin, obtained from Sigma, was prepared in methanol and homogenized using a Branson water-bath sonicator (Branson, Danbury, CT).

2.3. Enzyme assay

The tyrosinase assay was carried out using selected organic solvent media (Fisher Scientific, Pittsburgh, PA) of different polarity including heptane (log $P = 4.0$), toluene (log $P = 2.5$), dichloromethane (log $P = 2.0$), and dichloroethane (log $P = 2.0$). All organic solvents used in this study were dried overnight with anhydrous sodium sulfate prior to the assays. The enzymatic reaction was initiated by the addition of the tyrosinase suspension (1.1 μl), prepared in citrate–phosphate buffer solution (0.1 M, pH 6.0), to the organic solvent reaction mixture containing 5 μl of catechin stock solution (400 mM) so that the final assay volume was 200 μl. The reaction mixture was incubated at 25°C with vigorous agitation between 5 s to 2 min. The enzymatic reaction was subsequently stopped by the addition of 200 μl acetone. A blank, containing all the components except the tyrosinase suspension, was performed at the same time. The color intensity of the pigments resulting from the tyrosinase-catalyzed end products in the reaction media of heptane, toluene, dichloromethane and dichloroethane was measured spectrophotometrically (Beckman DU-650) at 376, 379, 375 and 375 nm, respectively. One unit of enzyme activity was defined as the amount of enzyme, which produced an increase of 0.001 in absorbance per minute at a defined wavelength, temperature and pH. The specific activity of tyrosinase was expressed as the change in the absorbency unit at a defined wavelength, per μg enzymatic protein per second.

2.4. Effect of protein concentration on tyrosinase activity

Protein concentrations ranging from 10.4 to 36.7, 39.8 to 70.5, 18.2 to 64.4 and 22.5 to 61.3 μg were suspended in 1.1 μl phosphate–citrate buffer solution (0.1 M, pH 6.0) and used in the enzymatic assays (200 μl) with the reaction media of heptane, toluene, dichloromethane and dichloroethane, respectively; the enzymatic assays contained 5 μl of catechin stock solution (400 mM).

2.5. Effect of pH on tyrosinase activity

The effect of pH on the tyrosinase biocatalysis in the heptane, toluene, dichloromethane and dichloroethane reaction media was determined by preparing the tyrosinase-buffer suspension in phosphate–citrate buffer solutions (0.1 M) at a pH ranging from 4.6 to 7.0.

2.6. Effect of temperature on tyrosinase activity

The effect of temperature on tyrosinase activity was determined by incubating the enzymatic reaction at a wide temperature range of 20°C to 30°C, 15°C to 30°C, 15°C to 40°C and 10°C to 40°C for the reaction media of heptane, toluene, dichloromethane and dichloroethane, respectively.

2.7. Effect of substrate concentration on tyrosinase activity

The effect of substrate concentration on tyrosinase activity, in the four selected reaction media, was assayed under the same experimental conditions described previously. Catechin was used as substrate at different concentrations ranging from 2 to 20, 2 to 12, 0.5 to 20 and 2 to 14 mM for the reaction media of heptane, toluene, dichloromethane and dichloroethane, respectively.

2.8. Effect of acetone on tyrosinase activity

The effect of acetone on the tyrosinase biocatalysis reaction was studied by using different concentrations of acetone as co-solvent in the enzymatic reaction mixture. The concentration of acetone used in the reaction media varied from 0% to 90% (v/v) for heptane, 0% to 60% (v/v) for toluene, 0% to 30% (v/v) for dichloromethane and 0% to 35% (v/v) for dichloroethane. The concentration of acetone as a co-solvent in the reaction medium was expressed as its percentage ratio (in μl) to the total assay volume (200 μl) [13].

3. Results and discussion

Commercially purified tyrosinase may contain contaminating oxidizing enzymatic activities, particularly laccase [14]. In order to determine the absence or presence of such contaminating activity, the commercially purified tyrosinase (Sigma) was examined using two specific substrates for laccase activity: 3,5-dimethoxy-4-hydroxybenzaldehyde azine "syringaldazine" and 2,6-dimethyoxyphenol [15]. The experimental results indicated that the commercially purified tyrosinase, used throughout this study, contained negligible laccase activity (0.03%–0.4%). Kermasha et al. [14] as well as Tse et al. [13] also indicated similar results on the purity of the commercially purified tyrosinase (Sigma).

3.1. Scanning of tyrosinase-catalyzed end-products

Fig. 1 shows the spectrophotometric scanning of the colored compounds resulting from the enzymatically-oxidized end products of catechin in the selected organic reaction media. The results show that the tyrosinase-catechin biocatalysis gave distinct absorbency peaks with maximum absorption at 376, 379, 375 and 375 nm for the colored compounds of the enzymatically produced *o*-quinones in the reaction media of heptane, toluene, dichloromethane and dichloroethane, respectively. In addition, the results show that in the absence of the enzyme in the reaction mixture, there was no indication of these peaks. These findings suggest that the bioconversion of catechin into the corresponding *o*-quinones in the selected organic media required the presence of tyrosinase. According to the results obtained by Good-

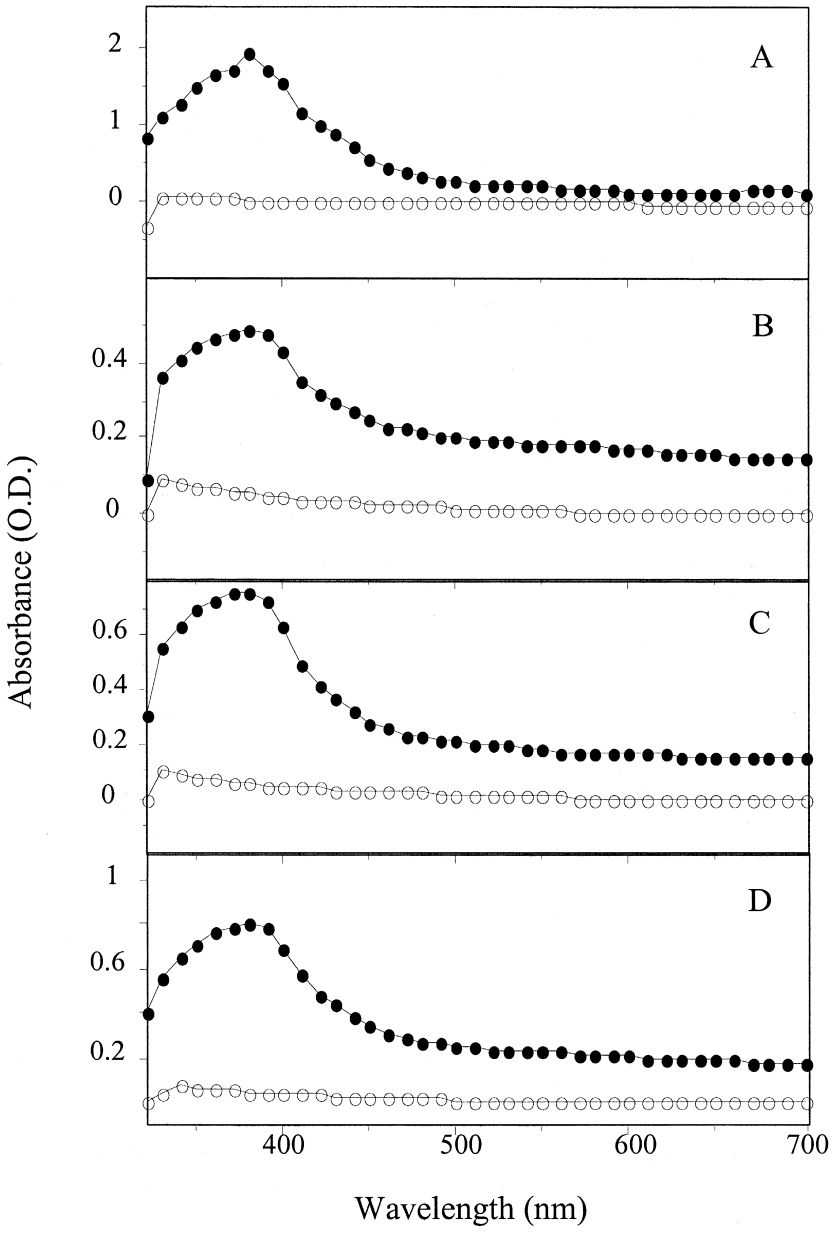

Fig. 1. Scanning profiles of catechin (○—○) and the corresponding pigments of the tyrosinase-catalyzed end products (●—●) in the selected organic solvent media of heptane (A), toluene (B), dichloromethane (C) and dichloroethane (D).

enough et al. [16] and Tse et al. [13], the PPO oxidation of catechin and vanillin in chloroform medium yielded colored compounds which gave distinct peaks of maximum absorption at 372 and 332 nm, respectively, whereas that of catechin in aqueous media showed optimal absorption at 390 nm.

3.2. Effect of enzymatic protein concentration on tyrosinase activity

The effect of enzymatic protein concentration on tyrosinase activity in the selected organic solvent media using catechin as substrate was investigated.

The results (not shown) indicated that the optimal amount of enzymatic protein for tyrosinase biocatalysis was 31.4, 60.0, 45.8 and 53.1 μg per 200 μl reaction media of heptane, toluene, dichloromethane and dichloroethane, respectively. The initial addition of increasing amounts of enzymatic protein produced a concomitant increase in the specific activity of tyrosinase in all selected organic media; however, after maximal tyrosinase activity was obtained, further increases in enzymatic protein resulted in a decrease in the specific activity. This phenomenon may be due to the limited solubility of the enzymatic protein in the selected organic solvents thereby resulting in the aggregation of enzyme molecules and a subsequent reduction of enzyme and substrate interaction [17,18].

3.3. Effect of pH on tyrosinase activity

The effect of pH on tyrosinase activity (Fig. 2) was studied over a wide pH range. Fig. 2 shows that the optimal pH for mushroom tyrosinase biocatalysis using catechin as substrate was 6.2 in the reaction media of heptane and dichloroethane, and 6.6 and 6.0 in those of toluene and dichloromethane, respectively. Kermasha et al. [19] also reported that the optimal pH for mushroom tyrosinase activity was 6.0 using chlorogenic acid as substrate in aqueous buffer solutions. Guyot et al. [20] investigated the PPO biocatalysis of catechin in aqueous solutions ranging from pH 3.0 to 7.0 and showed that reaction media with low pH values gave almost colorless solutions, whereas those with higher pH values yielded yellow solutions due to the presence of higher concentrations of pigments, reaching a maximum at pH 6.0; these results suggest that the color of the pigments resulting from the enzymatically oxidized catechin could be due to the coupling of *o*-quinones, whose nature and relative abundance is influenced by the pH of the incubation medium.

The overall results, obtained in this study, also show that there were small shifts in pH ranging from

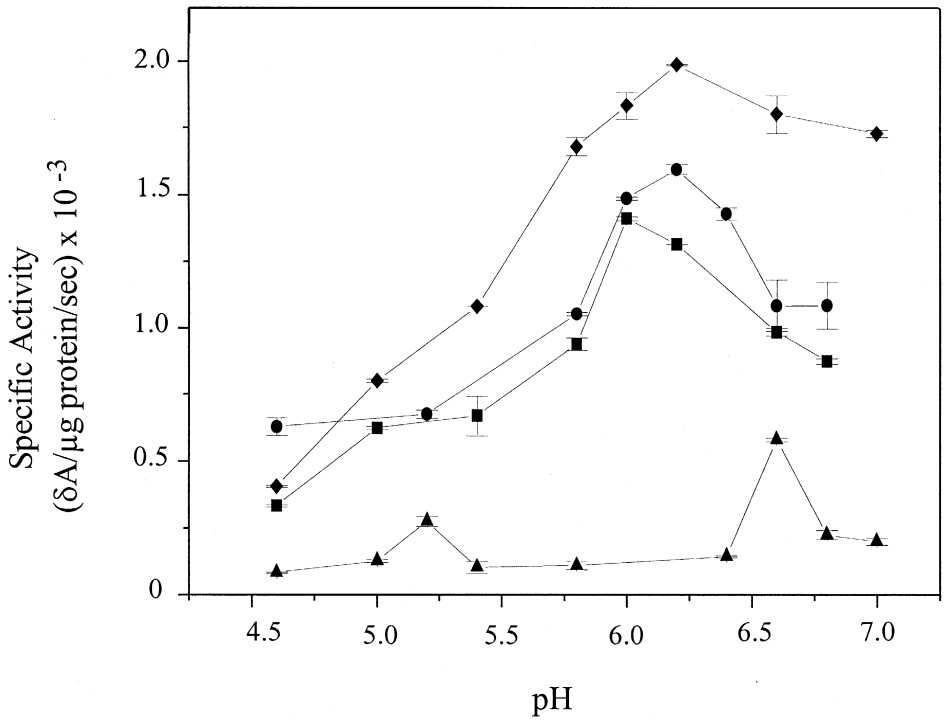

Fig. 2. The effect of pH on tyrosinase activity in the reaction media of heptane (●—●), toluene (▲—▲), dichloromethane (■—■) and dichloroethane (◆—◆), using catechin as substrate, where the specific activity was defined as the change in absorbance at $\lambda = 376$ nm for heptane, $\lambda = 379$ nm for toluene, and $\lambda = 375$ nm for dichloromethane and dichloroethane per μg protein per second.

6.0 to 6.6 for maximal tyrosinase activity in the different selected organic solvent media. Similar findings were also obtained by Khamessan et al. [21], who reported that the optimum pH for the hydrolytic activity of chlorophyllase was slightly affected by the type of organic solvent used as the reaction medium. The same author suggested that the slight shifts in the pH values from 7.0 to 8.0 for optimal chlorophyllase activity may be due to the effect of the organic solvents used on the hydrogen ion dissociation of the enzyme. Zaks and Klibanov [11] also reported that enzymes maintain a 'pH memory' in organic solvent media, so that their catalytic activity in organic reaction media is characteristic to that exhibited at their optimal pH in aqueous media; these findings may be explained by the fact that the enzyme's ionogenic groups maintain the corresponding ionization states acquired at the particular pH of the aqueous solution in which it is suspended before being added to the organic solvent medium.

3.4. Effect of temperature on tyrosinase activity

The results (not shown) indicated that the optimal temperature for mushroom tyrosinase oxidative activity using catechin as substrate was 25°C in the reaction media of heptane, toluene and dichloromethane, and 27.5°C in that of dichloroethane. Estrada et al. [8] also reported that the optimal temperature for the enzymatic oxidation of phenol in the chloroform medium was 30°C. The influence of temperature on maximal enzymatic activity in organic solvents having log P values ranging from 1.9 to 2.5 was studied by Estrada et al. [22], who suggested that the optimal temperature for biocatalysis may be influenced by the intrinsic characteristics of the organic solvents such as boiling point, partition coefficient, interfacial tension at the aqueous and organic solvent interface, as well as the solubility of substrates and/or products.

The Arrhenius plot (Fig. 3) shows that the activation energy E_a was 88.36, 42.78, 12.24, and 34.31

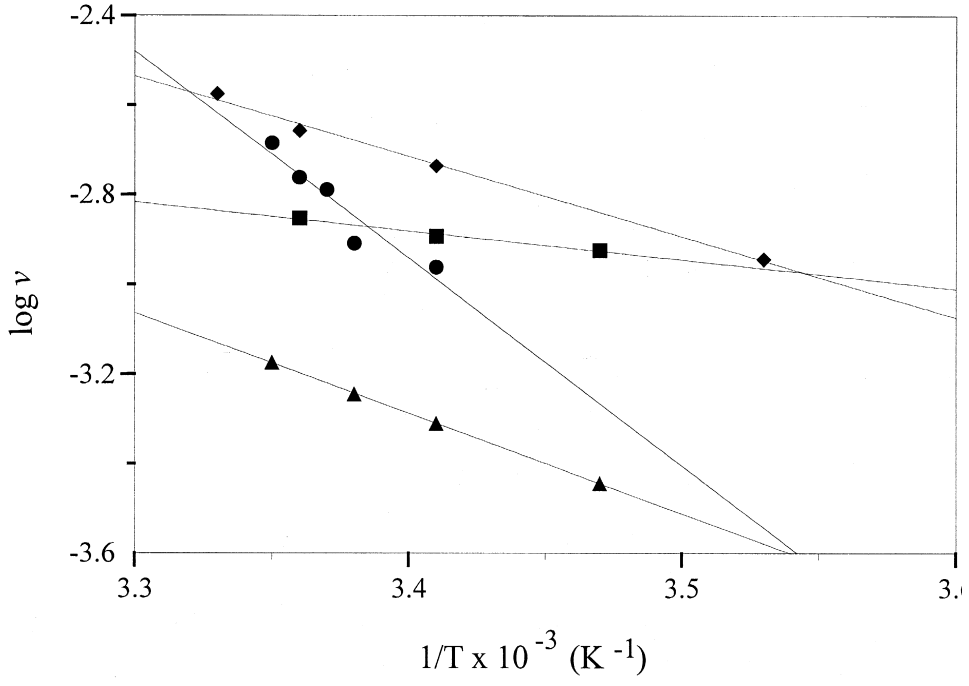

Fig. 3. The Arrhenius plots for tyrosinase biocatalysis in the reaction media of heptane (●—●), toluene (▲—▲), dichloromethane (■—■) and dichloroethane (◆—◆), using catechin as substrate, where the specific activity was defined as the change in absorbance at $\lambda = 376$ nm for heptane, $\lambda = 379$ nm for toluene, and $\lambda = 375$ nm for dichloromethane and dichloroethane per μg protein per second.

kJ/mol for the tyrosinase biocatalysis in the reaction media of heptane, toluene, dichloromethane and dichloroethane, respectively, while the corresponding temperature coefficient (Q_{10}) was 1.04, 1.86, 1.18 and 1.17. These results suggest that the rate of tyrosinase biocatalysis in the heptane reaction medium was more temperature dependent as indicated by its relatively high E_a value of 88.36 kJ/mol; however, the tyrosinase oxidative activity in the reaction media of toluene, dichloroethane and especially dichloromethane was less influenced by changes in temperature in the range of 10°C to 25°C. The trend of these results is in agreement with those reported by Estrada et al. [22] who indicated that the PPO biocatalysis of the immobilized mushroom enzymatic extract showed E_a values of 33.53, 29.17 and 32.40 kJ/mol in the reaction media of toluene, dichloromethane and dichloroethane, respectively, using *p*-cresol as substrate. The results obtained by Tse et al. [13] also demonstrated that the E_a values for the tyrosinase oxidative activity in the chloroform medium, using catechin and vanillin as substrates, were 18.01 and 22.12 kJ/mol, respectively, while the respective Q_{10} values were 1.91 and 1.88.

3.5. Kinetic parameters of tyrosinase biocatalysis

Table 1 shows that tyrosinase possessed a higher affinity for catechin in the toluene and dichloromethane reaction media than that obtained in the reaction media of heptane and dichloroethane, as indicated by the respective K_m values of 1.03 and 2.52 in comparison to those of 5.38 and 4.03 mM. The catalytic efficiency, expressed as the V_{max}/K_m ratio [23], was also used as an indication of the ability of tyrosinase to convert catechin into the corresponding end products in the selected organic solvent media. Table 1 shows that the catalytic efficiency of tyrosinase in the dichloromethane reaction medium was approximately twice as high as that obtained in the reaction media of heptane, toluene and dichloroethane. The overall findings show that maximal tyrosinase activity was demonstrated in the dichloromethane medium, followed by those of toluene and dichloroethane; however, the lowest tyrosinase-catechin biocatalysis was observed in the heptane reaction medium. These results are in agreement with those reported by Estrada et al. [8] who stated that reaction media consisting of organic solvents with log P values between 2.0 and 2.5 showed higher PPO biocatalysis due to a higher conversion of substrate whereas those composed of organic solvents with log P values higher than 2.5 such as octanol (2.9), hexane (3.5) and heptane (4.0) showed very low PPO activity; the same authors suggested that this low enzymatic activity could be explained by the effect of solvent polarity on the conformation of the enzyme as well as the solubility of substrates and/or products.

3.6. Effect of acetone on tyrosinase activity

The effect of acetone as co-solvent on the tyrosinase-catechin oxidative reaction in selected organic solvent media (Fig. 4) was investigated. The results show that the addition of 5% to 30% (v/v) acetone

Table 1
Kinetic parameters of tyrosinase biocatalysis in selected organic solvent media

Reaction medium	Without inhibitor			With acetone as inhibitor				
	V_{max}^{a} (10^{-4})	K_m (mM)	Catalytic efficiency[b]	Acetone[c] (%)	V_{maxapp}^{a} (10^{-4})	K_{mapp} (mM)	Catalytic efficiency[b]	Type of inhibition
Heptane	12.2	5.38	2.3	15.0	11.9	12.7	0.94	Competitive
Toluene	3.3	1.03	3.2	40.0	4.85	0.84	5.70	Mixed noncompetitive
Dichloromethane	14.7	2.52	5.8	20.0	5.24	4.24	1.24	Mixed noncompetitive
Dichloroethane	12.0	4.03	3.0	30.0	4.59	4.63	0.99	Mixed noncompetitive

[a] The maximal enzymatic reaction rate was defined as the change in absorbance unit at a specific wavelength per μg enzymatic protein per second.
[b] The catalytic efficiency was defined as the ratio of V_{max} or V_{maxapp} to K_m or K_{mapp}, respectively.
[c] Acetone concentration was expressed as the percentage of volume of acetone to the total reaction volume.

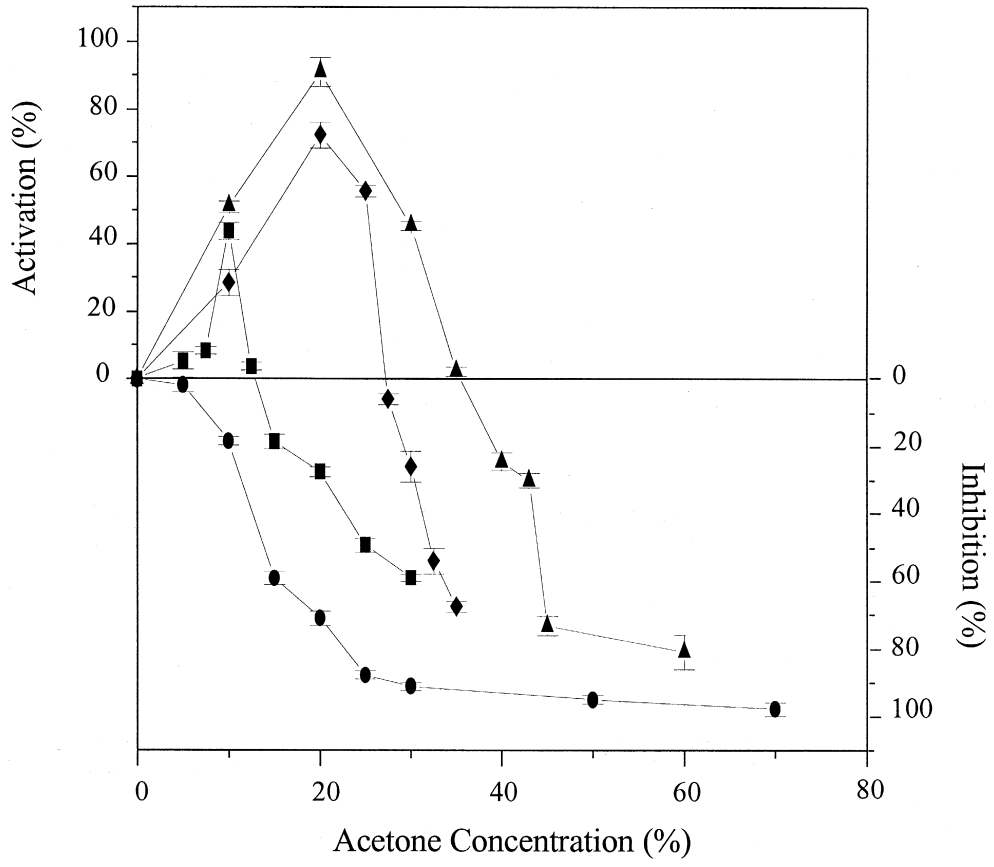

Fig. 4. The effect of the presence of acetone as co-solvent on tyrosinase biocatalysis in the reaction media of heptane (●—●), toluene (▲—▲), dichloromethane (■—■) and dichloroethane (♦—♦), using catechin as substrate. The percentage of activation or inhibition of tyrosinase activity was defined as the increase or decrease in enzymatic activity, respectively, relative to the initial value (without activator or inhibitor).

produced a 4.3% to 96.7% decrease in tyrosinase activity in the heptane reaction medium. In contrast, the results (Fig. 4) also indicate that the presence of 12.5%, 22.0% and 22.0% acetone in the reaction media of dichloromethane, dichloroethane and toluene, respectively, produce a maximal increase of 42.6%, 71.8% and 92.1%, respectively, in tyrosinase activity; however, the overall findings indicate that additional increases in acetone concentration still resulted in a subsequent inhibition of tyrosinase activity, due to the denaturing effect of acetone in removing the essential layer of water from the enzyme [24].

The overall results show that lower concentrations of acetone were required to inhibit tyrosinase biocatalysis in the more hydrophobic reaction medium of heptane (log $P = 4.0$) whereas higher concentrations were needed in the less hydrophobic reaction media of toluene (log $P = 2.5$), dichloromethane (log $P = 2.0$) and dichloroethane (log $P = 2.0$). These findings suggest that the addition of acetone to the more hydrophilic reaction media of toluene, dichloromethane and dichloroethane produced an initial increase in tyrosinase activity due to the enhanced solubility of substrate and end products thereby allowing more substrate interaction and less end-product inhibition. These findings also suggest that the observed enzyme activation may be due to the "lubricating effect" of acetone on protein structure, thereby giving the protein molecule greater

flexibility necessary for enzymatic catalysis by reducing the intermolecular protein–protein contacts [24]. These results are in agreement with those reported by Almarsson and Klibanov [25] who indicated that the addition of a denaturing solvent, such as formanide and dimethyl sulfoxide, could actually dramatically increase enzymatic activity in anhydrous reaction media due to enhanced conformational flexibility as a result of weakening intermolecular protein0–protein contacts. The same author reported that the rate of transesterification, catalyzed by the protease subtilisin Carlsberg in anhydrous acetonitrile medium, was increased by more than 50% with the addition of 10% to 50% of formamide as co-solvent [25].

The results (Table 1) also show that the addition of acetone to the heptane reaction medium showed a competitive inhibitory effect on tyrosinase activity as indicated by the K_{mapp} value of 12.7 mM. This finding suggests that in the heptane reaction medium, acetone interacted with the active site of the enzyme in a similar fashion as the catechin substrate thereby disrupting the partitioning of catechin; subsequently higher concentrations of substrate were required to obtain the V_{max} of 11.9. The results (Table 1) also show that the addition of acetone to the toluene, dichloromethane and dichloroethane reaction media produced a mixed noncompetitive inhibitory effect on tyrosinase biocatalysis. These findings suggest that the inhibitory effect of acetone on tyrosinase activity in the more hydrophilic (log $P = 2.0$ to 2.5) reaction media of toluene, dichloromethane and dichloroethane is similar in that acetone competes with catechin to bind to the enzyme and distorts its conformation sufficiently to prevent proper positioning of its catalytic center, thereby rendering the enzymatic complex nonproductive upon interaction with the substrates; consequently, there is less bioconversion of catechin into the corresponding end products due to both enzyme inactivation as well as lower substrate concentrations in the presence of acetone [26]. The overall results also suggest that the differences in the inhibitory effects of acetone on tyrosinase activity in the heptane reaction medium in comparison to that obtained in the reaction media of toluene, dichloromethane and dichloroethane, may be due to the effect of solvent polarity on the conformation of enzyme. Tse et al. [13] reported that Fourier Transform Infrared (FT-IR) spectroscopic analyses revealed that the native mushroom tyrosinase is predominately of α-helical conformation while in the chloroform medium the enzyme was mainly composed of β-pleat structure.

4. Conclusion

The results demonstrate that mushroom tyrosinase showed high enzymatic activity in the dichloromethane reaction medium in comparison to that obtained in the toluene, dichloroethane and heptane media, using catechin as substrate. The kinetics analysis also confirmed that the results obtained in this study are in agreement with the current general theories of the effect of organic solvent reaction media on the biocatalysis of PPO.

Acknowledgements

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References

[1] C.Y. Lee, J.R. Whitaker, Enzymatic Browning and its Prevention, American Chemical Society, Washington, D.C., 1995, pp. 11–16.
[2] J. Oszmianski, C.Y. Lee, J. Agric. Food Chem. 38 (1990) 1202–1204.
[3] F. Shahidi, M. Naczk, Food Phenolics Sources: Chemistry, Effects and Applications, Technomic Publishing Company, Lancaster, PA, 1995, pp. 210–313.
[4] F.C. Richard-Forget, M. Rouet-Mayer, P.M. Goupy, J. Philippon, J. Nicolas, J. Agric. Food Chem. 40 (1992) 2114–2122.
[5] C.M. Robert, F.R. Cadet, C.C. Rouch, M. Pabion, F. Richard-Forget, J. Agric. Food Chem. 43 (1995) 1143–1150.
[6] F. Gauillard, F. Richard-Forget, J. Sci. Food Agric. 74 (1997) 49–56.
[7] R.Z. Kazandjian, A.M. Klibanov, J. Am. Chem. Soc. 107 (1985) 5448–5450.
[8] P. Estrada, R. Sanchez-Muniz, C. Acebal, R. Arche, M.P. Castillon, Biotechnol. Appl. Biochem. 14 (1991) 12–20.
[9] S.J. Dordick, Enzyme. Microb. Technol. 11 (1989) 194–211.
[10] R.F. Rekker, H.M. de Kort, Eur. J. Med. Chem. 14 (1979) 479–488.

[11] A. Zaks, A.M. Klibanov, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 3192–3196.
[12] C. Lanne, S. Boeren, K. Vos, C. Veerger, Biotech. Bioeng. 30 (1987) 80–87.
[13] M. Tse, S. Kermasha, A. Ismail, J. Mol. Catal. B: Enzym. 2 (1997) 199–213.
[14] S. Kermasha, M. Goetghebeur, A. Monfette, M. Metche, B. Rovel, Phytochemistry 34 (1993) 349–353.
[15] D. Dubourdieu, C. Grassin, C. Deruche, P. Ribereau-Gayon, Connaiss. Vigne Vin 18 (1984) 237–252.
[16] P.W. Goodenough, S. Kessell, A.G.H. Lea, T. Loeffler, Phytochemistry 22 (1983) 359–363.
[17] R.Z. Kazandjian, J.S. Dordick, A.M. Klibanov, Biotech. Bioeng. 28 (1986) 417–421.
[18] S. Kamat, E.J. Beckman, A.J. Russe, Crit. Rev. Biotechnol. 15 (1995) 41–71.
[19] S. Kermasha, M. Goetghebeur, A. Monfette, M. Metche, B. Rovel, J. Agric. Food Chem. 41 (1993) 526–531.
[20] S. Guyot, V. Cheynier, J. Souquet, M. Moutounet, J. Agric. Food Chem. 43 (1995) 2458–2562.
[21] A. Khamessan, S. Kermasha, P. Marsot, Process Biochem. 30 (1995) 159–168.
[22] P. Estrada, W. Baroto, M.P. Castillon, C. Acebal, R. Arche, J. Chem. Tech. Biotechnol. 56 (1993) 59–65.
[23] S.G. Burton, J.R. Duncan, P.T. Keye, P.D. Rose, Biotech. Bioeng. 42 (1993) 938–944.
[24] P.L. Poole, J.L. Finney, Int. J. Biol. Macromol. 5 (1983) 308–310.
[25] Ö. Almarsson, A.M. Klibanov, Biotech. Bioeng. 49 (1996) 87–92.
[26] I.H. Segel, Rapid equilibrium partial and mixed-type inhibition, Enzyme Kinetics Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems, Wiley, New York, NY, 1993, pp. 161–226.