EFFECIENCY OF IMMOBILIZED POLYPHENOL OXIDASE ON SOME TEXTILE DYES DEGRADATION USING BATCH OPERATION SYSTEM BY PACKED BED BIOREACTOR

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ABSTRACT

In the current study, three types of common plants, namely Tomato (*Solanum lycopersicum*), Cucumber (*Cucumis sativus*) and Orange (*Citrus sinensis*) were obtained and screened for their polyphenol oxidase (PPO) activity, Among the three plants, *Solanum lycopersicum* was chosen with maximum enzymatic activity, it had the highest productivity of the enzyme (23733 U/mg protein).The PPO from *Solanum lycopersicum* was purified using two steps: concentration by sucrose and gel filtration by using Sephacryl S-200. The results showed an increase in the final purification folds by 2.4 times with an enzyme yield of 32.6%. The immobilization studies showed that PPO was more stable when immobilized on chitosan by covalent linkage with immobilization ratio of 62%, in comparison with agar-agar by entrapment method (36%). The removal efficiency of crude and partial purified PPO was studied with textile dyes, including yellow, red, black and blue dyes at optimum conditions: pH 5, temperature 40^{o}C after 3 hrs. Maximum removal efficiency of dyes observed with crude PPO were 53.9, 81.4, 86.5 and 79.6% respectively. However, purified PPO displayed removal efficiency reached 60.3, 84.3, 84.6 and 77.5% respectively. The potential of immobilized PPO on chitosan was evaluated by decolorization of black textile dye in packed bed bioreactor in batch operation. The results indicated that immobilized PPO in batch operation has the ability to remove 99% of the dye after 2 hrs, and the results showed a positive relationship between the degradation rate and incubation time in batch operation.

Keyword: Enzymes, Tomato Plants, Bioreactor, Industrial Dyes

مجلة العلوم الزراعية العراقية -2019 :50(3):943-950 حسين

كفاءة انزيم البولي فينول اوكسيديز المقيد في تفكيك بعض صبغات الانسجة باستخدام نظام الوجبه في المفاعل الحيوي

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مدرس

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المستخلص

تضمنت الدراسه الحاليه غربله ثلاثة نباتات في قابليه انتاجها لانزيم polyphenol oxidase شملت الطماطة (*Solanum lycopersicum*) والخيار (*Cucumis sativus*) والبرتقال (*Citrus sinensis*) انتخب نبات الطماطه باحتواءه على اعلى فعاليه انزيميه اذ بلغت 23733 وحدة/ ملغم بروتين. نقي انزيم polyphenol oxidase المنتج من النبات المنتخب بخطوتين تضمنت الاولى التركيز بالسكروز اما الثانيه فكانت بالترشيح الهلامي باستخدام السيفاكريل s–200، بينت النتائج زيادة في عدد مرات التنقيه في الخطوه الاخيره اذ بلغت 2.4 والحصيله الانزيمية 32.6 %. قيد الانزيم بطريقتين حيث كان اكثر ثبات عند تقييده بماده الكيتوسان بوساطه الربط التساهمي اذا وصلت نسبه التقييد الى 62% مقارنه بتقييده بوساطه الحجز بماده الاكار اكار اذا وصلتالى 36%. درست كفاءه الانزيم الخام والمنقى في ازاله صبغات معامل الانسجه المتمثله بالصبغه الصفراء ، الحمراء، السوداء والزرقاء عند الظروف المثلى المتمثله بداله حموضه 5 ودرجه حرارة 40 م^{o} بعد ثلاث ساعات. وصلت اعلى كفاءه لازله الصبغات الاربعه باستخدام الانزيم الخام الى 53.9 و81.4 و 86.5 و79.6% على التوالي ، كذلك وصلت اعلى كفاءه لازاله الصبغات باستخدم الانزيم المنقى الى 60.3 و84.3 و84.6 و77.5 % على التوالي. درست كفاءه الانزيم المقيد بماده الكيتوسان في ازاله الصبغه السوداء بنظام الوجبه في المفاعل الحيوي ، اذا بلغت 99% بعد ساعتين. بينت الدراسه الحاليه هناك علاقه طرديه بين معدل ازاله الصبغه وفترة الحضانه في كفاءه ازاله الصبغه بنظام الوجية .

كلمات مفتاحية: انزيم ، نبات الطماطة، مفاعل حيوي، صبغات صناعية

***Received:12/7/2018, Accepted:1/11/2018**

INTRODUCTION

In line with the evolution of the petroleum industry, the area of oil-polluted soil has increased continuously. Thus, it becomes a natural question of treating the oil-polluted soil to retrieval level in a short time (30). The bioremediation technology could be beneficial because of its pollution-free, low cost and highly efficient characteristics. In recent years, this technology has developed quickly and different microorganisms with special biochemical and physiological functions have been screened, which promote the bioremediation technology for further application and development (12). The crude oil filtrated into soil through varying ways and interacted with soil enzymes (2). On the one hand, the enzymes from oil-degrading plants could be an indicator for assaying the amount and physiochemical property of crude oil. On the other hand, the enzymes had the great ability to degrade the petroleum. The activities of the enzymes indicated the intensity and tendency of various biochemical reactions, though the amounts of the enzymes were quite small. In a sense, the enzyme, a living molecular unified with the environmental factors, could degrade the oil to simple chemical compounds. It is necessary to give greater attention to investigating the relationship between environmental pollution and the enzymes from oil-degrading plants and the suitable parameters of enzyme activity. Polyphenol oxidase (PPO) is a monophenol, dihydroxy-L-phenylalanine oxygen oxidoreductase, (E.C.1.14.18.1), which occur in many plants together with phenolics. It's an enzyme that catalyzes the oxidation of mono-, di- and poly-hydric phenols to o-quinones. Different names have been associated with this enzyme including catecholase, cresolase, tyrosinase, phenolase and diphenolase (21). The enzyme is widely distributed on the phylogenic scale and is responsible for the harmful effect of enzymatic browning reactions in foods and other materials. It is known that browning reactions, initiated by the enzyme, occur generally post-harvest when the tissues are exposed to stress conditions, deterioration, or during handling, storage and processing (29). PPO has been widely studied and characterized in a variety of tissues, such as tomato (26), sweet potato (20), potato and taro (10), eggplant (7), palmito (20), apple (22), pears (13), grapes (24), peppermint (20), oregano (10) and artichoke (1). The aim of this study is the extraction, purification, immobilization and decolorization of some textile dyes by polyphenol oxidase from plant sources.

MATERIALS AND METHODS

Plants

The plants used throughout this study were locally available in the market. *Solanum lycopersicum* (Tomato), *Cucumis sativus* (Cucumber) and *Citrus sinensis* (Orange) were used as a source of material to screen for polyphenol oxidase activity.

Extraction and recovery of PPO

One hundred gm of each plant were homogenized, with the mixing performed by using a blender for 10 mins at room temperature. The slurry was filtrated through gauze to remove any cell debris from the preparation, then centrifuged at 8000 rpm for 10 mins. The clear supernatant obtained represented the crude extract, and was assayed for PPO activity.

PPO activity

Polyphenol oxidase enzyme activity was estimated according to the method described by Zian and Pekyardimici (33) using ortho-catechol as a substrate. The oxidation of ortho-catechol was detected by measuring the absorbance increase at 420 nm using a VIS-spectrophotometer. The protein concentration was measured according to the method described by Bradford (4).

Purification of PPO

Purification of the enzyme was determined by two methods: concentration by dialysis and gel filtration.

Enzyme concentration by dialysis

The crude enzyme solution was concentrated by using sucrose, then the enzyme activity and the protein concentration was measured.

Gel filtration chromatography

The column was prepared and packed according to the instructions of the manufacturing company (Pharmacia-Sweden). The concentrated enzyme by dialysis was passed through sephacryle S-200 column with the dimensions 21×1.6 cm. The elution was done by using 0.2M of sodium phosphate

buffer solution, pH 7.0 with flow rate of 20 ml / h, 3ml for each fraction. The protein was estimated in each fraction at wave length 280 nm, then enzyme activity was measured in these fractions. Effective fractions were collected and then its volume was measured as well as its activity and protein concentration were estimated.

Immobilization of PPO enzyme

Immobilization of PPO by entrapment methods

Entrapment in agar-agar

2ml of purified PPO was mixed with 5ml of sterile agar-agar solution (2%) at 40^{o}C. It was shaken for a few minutes and poured into a sterile petri dish and allowed to solidify. After solidification, cubes of 1×1×1cm were cut and washed with distilled water. Next, the cubes of agar-bounded enzyme were stored in 0.1 M of sodium acetate buffer pH 5 at 4^{o}C, and then the immobilized enzyme activity was assayed (28).

Immobilization of PPO by covalent methods

Covalent linkage by chitosan

1gm of chitosan was added to 10ml of 2% gluteraldehyde solution, stirred gently and mixed for 2 hrs at 4 ^{o}C, followed by an overnight incubation. The gluteraldehyde bounded chitosan was washed extensively with water to remove the unbounded gluteraldehyde, then mixed with 10ml of purified enzyme solution at 4^{o}C overnight for enzyme immobilization (5). The resulting chitosan-gluteraldehyde-PPO conjugates were separated and washed, then immobilized enzyme activity was determined.

Decolorization (%) = Initial Absorbance – Final Absorbance / Initial Absorbance x 100

Determination of the immobilized PPO activity

Immobilized PPO activity was estimated according to the method described by (33) by using 30 mg of immobilized enzyme instead of 0.1 ml of enzyme. The oxidation of ortho-catechol was detected by measuring the absorbance increase at 420 nm using a VIS-spectrophotometer.

Application of PPO enzyme

Dye decolorization of textile black dye in packed bed-reactor by an immobilized crude PPO

Batch operation

Cylindrical packed bed-reactor (420 ml) was prepared with working volume 385 ml and then was loaded with the immobilized PPO 734 U/mg. The dye (textile black) was obtained from the Al-diwaniyah textile factory, which had a concentration of 30 mg/l. To determine the decolorization rate of the dye by chitosan immobilized PPO, the samples from the outlet of the reactor were taken at different incubation times (0, 1, 2, 3 and 24hrs). The percentage of removal efficiency for the dye was calculated by the difference in absorbance at λ max according to Zhang *et al* (32).

Decolorization (%) = Initial Absorbance – Final Absorbance / Initial Absorbance x 100

RESULTS AND DISCUSSION

Extraction and recovery of PPO enzyme

In this study, three types of commonly used plants, namely Tomato (*Solanum lycopersicum*), Cucumber (*Cucumis sativus*) and Orange (*Citrus sinensis*) were obtained and screened for their PPO activity. It was observed from the results that *Solanum lycopersicum* gave the highest enzymatic activity. PPO specific activity reached 23733 U/mg protein, whereas the PPO specific activity of *Cucumis sativus* and *Citrus sinensis* were 1688 and 0 U/mg protein respectively (Fig.1). Rodríguez *etal* (25) were extracted PPO enzyme from tomato roots. Also Shanti with coworkers (26) were extracted PPO from the peel and pulp of tomato.

Purification of PPO

Polyphenol oxidase enzyme was extracted from *Solanum lycopersicum* by homogenization in the blender at room temperature. The slurry was filtrated through gauze to remove any cell debris that remained from the preparation and then it was placed in the cooling centrifuge at 8000rpm for 10mins. The crude PPO was purified from the unwanted proteins and other components through concentration by sucrose, and gel filtration using Sephacryl –S200, as follows:

Enzyme concentration by sucrose

The crude extract produced was initially subjected to concentration by sucrose. The results showed that 75.8% of PPO enzyme was yielded with purification fold 1.3 as shows in

Table 1. Sucrose was formerly widely used for the concentration of proteins, as an inexpensive way of precipitating and concentrating a protein extract with the sucrose as a nucleophilic effector (activator) on the enzyme. Bajaj and Singh (3) described the method for the concentration of the enzymes by reducing the volume of the active solution by dialyzing against a concentrated solution of sucrose, through withdrawal of water molecules from the enzymatic solution. The other methods of concentration of the enzyme by precipitation techniques, including ammonium sulfate, ethanol, acetone, dialysing against 96% of ethyl alcohol or acetone as the outside solution (provided that the active substance (enzyme) is not inactivated by them). Using this procedure, the volume of the active substance is rapidly reduced (14).

Gel filtration chromatography

The solution of concentrated enzyme was passed through a gel filtration column Sephacryl - S200 with the dimensions 21×1.6 cm, and equilibrium with 0.2M of phosphate buffer pH 7. Results showed one peak of protein in the eluted fractions from the column with a single peak of PPO, then the active fractions were collected (Fig. 2). The result indicated the presence of one peak of protein that is compatible with the peak of PPO activity in fractions 12-20. At this step, the specific activity was 48900 U/mg protein with a purification fold 2.4 and yield of 32.6% (Table 1). (16) purified the enzyme by using acetone precipitation and gel filtration chromatography with 35% yield and 1.24 purification fold. PPO was extracted and purified from *Portulaca oleracea* by using a Sephadex G-100 column. The specific activity of the purified PPO was determined as 11421 U/mg, with a purification fold of 23.5 and a yield of 31.3% (15).

Immobilization of PPO enzyme

Immobilization of PPO by entrapment and covalent methods

Crude PPO was immobilized by entrapment in agar-agar (Fig. 3) and by covalent linkage in gluteraldehyde activated chitosan (Fig. 4). The results showed that the immobilization ratio of the chitosan linkage PPO reached 62% and higher than that of agar-agar at 36% (Fig. 5). Agar is resistant to acidic hydrolysis, it's a naturally occurring heterogeneous colloidal polysaccharide complex of agarose and agaropectin having alternating α-(1 → 3) and β-(1 → 4) linkages. Agarose is an alternating copolymer of 3-linked~-D galactopyranose and 4-linked 3,6-anhydro-α- galactopyranose units. The structure of agaropectin is not fully known but it is a sulphonated polysaccharide in which galactose as well as uronic acid is partly esterified with sulphuric acid. However, agar has the ability to form gel in salt-free conditions due to the ionic nature of the polymer (agaropectin). But the decrease in the immobilization ratio of PPO entrapped in agar-agar was due to the weakness of cohesion of agar-agar for entrapped enzyme, the easy dissolution, disintegration and high solubility of the gel during the immobilization of the enzyme (33). Therefore, the immobilized PPO covalent linkage by chitosan was used for further studies. The immobilization of enzyme to chitosan carrier is commonly carried out via multipoint covalent attachment at high ionic strength, because it has been postulated that, in a first step, a salt-induced association between the protein and the support surface takes place (31).

Application of PPO

Dyes decolorization: The various textile dyes (textile yellow, textile red, textile black and textile blue) degradation capability of PPO (crude and purified) were studied at dyes concentration of 30 mg/l, in pH 5.0 at 40^{o}C after 3 hrs, as can be seen in Table 2 and Fig. 6 and 7. Absorbance of each dyes was recorded at suitable wave length for each one. Results showed that the value of each absorbance was decreased as the incubation time increases and stabilized after 3 hrs and even after 24 hrs, compared with the absorbance of the control, which did not change during the 24hrs. These results indicated that PPO have the ability to degrade different dyes. Results in Table 2 show significant differences in rate of degradation which is due to each dye having a different structure that effects the degradation capability of PPO enzyme. Textile black exhibited higher degradation capacity with purified PPO and showed maximum removal extent of 84.6% after 3 hrs with initial purified PPO. This was followed by textile red dye, textile blue and textile yellow with removal

efficiency of 84.3, 77.5 and 60.3% respectively (Table 2). Whereas crude PPO has approximately the same efficiency to degrade dyes compared with purified enzyme, researchers observed that crude PPO is more stable and cheaper compared to purified PPO. Nevertheless, using crude PPO would reduce the cost of the enzymatic-based decolorization process in an industrial scale. These encouraging results suppose a step forward towards the industrial application of PPO. These dyes were not decolorized to the same extent; that may be due to the difference of the redox potentials and the suitability of their steric structure with the active site of the enzyme (27). The extent of decolorization activity depends on the chemical structure of the dye and the source of the enzyme (8). It has been reported that the chemical structures of dyes largely influence their decolorization rates with PPO and that its decolorization efficiency was limited to several azo dye structures (6). This variation in the ability of the PPO for dyes degradation is due to the complexity of the dye structure which influence the degradation rate by PPO. (23) noted that heterocyclic dyes were resistant to enzymatic oxidation.

Decolorization of textile black dye in packed bed-reactor by an immobilized crude PPO

Batch operation

The decolorization capability of immobilized PPO on chitosan was studied with black dye at concentration of (30 mg/l) in packed bed-reactor with pH 5.0 at room temperature. The bioreactor was operated as a batch system for 3 hrs (Fig. 8). Absorbance of dye was recorded at suitable wave length 530 nm. The results in Fig. 8 showed that the decolorization rate increased as the incubation time increases and stabilized after approximately 2hrs. These results indicated that immobilized PPO in batch operation has the ability to remove 99% of dye after 2 hrs. Also, the results in Fig. 8 showed the positive relationship between the degradation rate with incubation time in batch operation. In the batch system operation, the black textile dye decolorized appeared by decreasing the absorbance at 530 nm which indicated degradation of dyes (azo conjugated structure) into low molecular weight aromatic compounds. The decreasing at 530 nm suggests that there were changes in the aromatic group in the black dye decolorized sample (17). Couto (8) have observed an oxidation of phenolic dyes having electron donating methyl or methoxy substituents by PPO. Although a large number of structurally related dyes can be successfully oxidized by PPO, decolorization occurs at different rates and to different extents and many dyes are not degraded at all (18). Further, there is a correlation between redox potential of the dyes and decolorization rates (33). However, complementary studies are required to study a relationship between the redox potential of the azo dyes and the decolorization efficiency of *Solanum lycopersicum* PPO, besides further information on the mechanisms involved in the degradation of this dye. This study on application of *Solanum lycopersicum* PPO in the degradation of dyes and the focus on the structural requirement of a dye to be biodegradable is important in order to optimize potential bioremediation systems for industrial textile process water treatment.

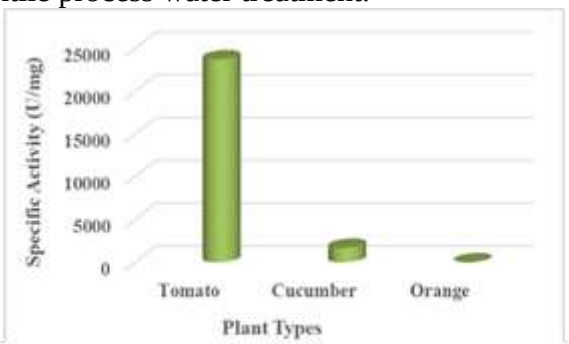

Figure 1. Extraction of Polyphenol Oxidase from Different Plant Types

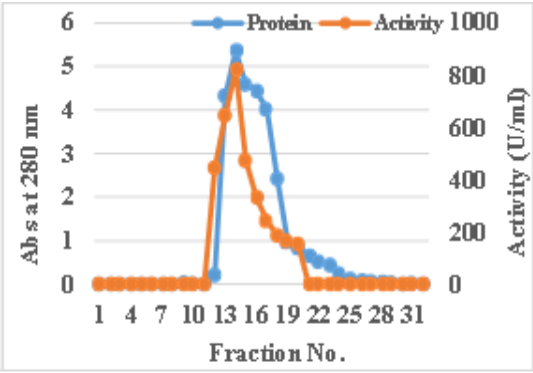

Figure 2. Gel Filtration Chromatography for Polyphenol Oxidase Purification from *Solanum lycopersicum* by using Sephacryl S-200 Column (21 x 1.6) cm Equilibrated and Eluted with Phosphate Buffer (0.2 M, pH 7), in Flow Rate 20ml/hr. 3ml for Each Fraction.

Figure 3. Immobilized PPO from *Solanum lycopersicum* in Agar-agar

Figure 4. Immobilized PPO from *Solanum lycopersicum* on Gluteraldehyde Activated Chitosan

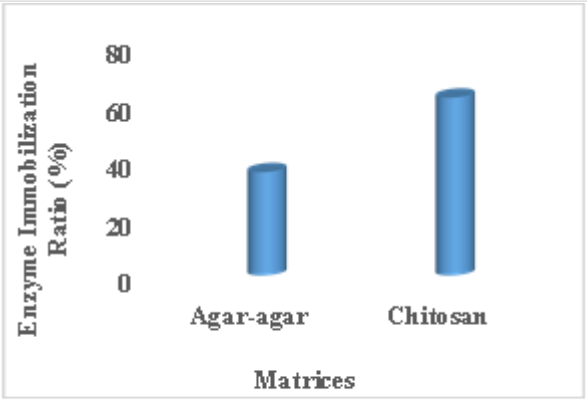

Figure 5. Enzyme Immobilization % of *Solanum lycopersicum* PPO by using Chitosan and Agar-

Figure 6. Dye Decolorization by Crude PPO Extracted from *Solanum lycopersicum* in pH 5.0 and 40 °C at a Concentration of 30 mg/ml after 3 hrs.

Figure 7. Dye Decolorization by Purified PPO Extracted from *Solanum lycopersicum* in pH 5.0 and 40 °C at a Concentration of 30 mg/ml after 3 hrs.

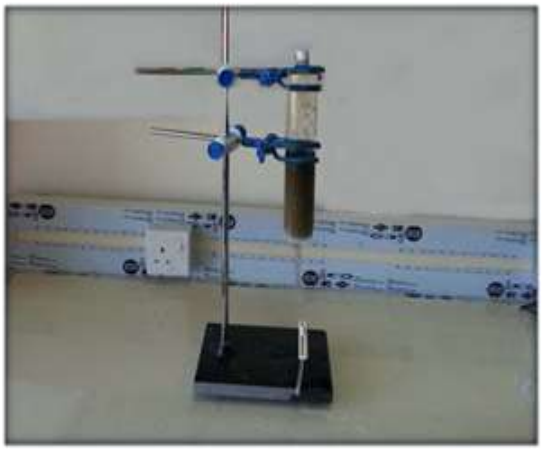

Figure 8. The Decolorization Rate of Textile Black Dye in Immobilized Crude PPO using Batch System in Packed Bed-Reactor at pH 5.0 at 40 °C

Table 1. Purification Steps of PPO Enzyme from *Solanum lycopersicum*

Sample	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specific Activity (U/mg)	Total Activity (U)	Purification Fold	Yield (%)
Crude	50	810	0.04	20250	40500	1	100
Sucrose	20	1534	0.06	25567	30680	1.3	75.8
Gel Filtration (Sephacryl S-200)	27	489	0.01	48900	12203	2.4	32.6

Table 2. Degradation % by PPO Enzyme (Crude and Purified) from *Salanum lycopersicum* at 40 °C in pH 5.0 after 3 hr

Dyes	Dyes Degradation (%)	
	Crude PPO	Purified PPO
Textile Yellow	53.9	60.3
Textile Red	81.4	84.3
Textile Blue	79.6	77.5
Textile Black	86.5	84.6

REFERENCES

1. Aydemir, T. 2004. Partial purification and characterization of polyphenol oxidase from artichoke (*Cynara scolymus* L.) heads. Food Chemistry Journal. 87: 59- 67

2. Azaizeh, H., P.M.L. Castro and P. Kidd. 2011. Biodegradation of organic xenobiotic pollutants in the rhizosphere. Org. Xenobiotics and Plants Journal. 8: 191-215

3. Bajaj, B. K. and N. P. Singh. 2010. Production of xylanase from an alkali tolerant *Streptomyces* sp. 7b under solid-state fermentation, its purification, and characterization. Journal of Applied Biochemistry and Biotechnology. 162: 180-188

4. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Analytical Biochemistry Journal. 72: 248-254

5. Carrara, C. R. and A. C. Rubiolo. 1994. Immobilization of α-galactosidase on chitosan. Journal of Biotechnology. 10: 220-224

6. Chivukula, M. and V. Renganathan. 1995. Phenolic azo dye oxidation by laccase from *Pyricularia oryzae.* Journal of Applied Environmental Microbiology. 61:4374-4377

7. Concellon, A., M. Anon and A. Chaves. 2004. Characterization and changes in polyphenol oxidase from eggplant fruit (*Solanun melongena* L.) during storage at low temperature. Food Chemistry Journal. 88: 17-24

8. Couto, S.R. 2007. Decoloration of industrial azo dyes by crude laccase from *Trametes hirsute*. Journal of Hazardous Materials. 148: 768–770

9. Dogan, S., O. Arslan and F. Ozen. 2005a. Polyphenol oxidase activity of oregano at different stages. Food Chemistry Journal. 91: 341-345

10. Duangmal, K. and R. K. Owusu Apenten. 1999. Acomparative study of polyphenol oxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum* var. *Romano*). Food Chemistry Journal. 64: 351- 359

11. Edalli, V.A. and C.M. Kamanavalli, 2013. Application of immobilized mushroom polyphenol oxidase for the removal of phenol and 4-chlorophenol. International Journal of Biochemistry-Photon 108: 144-150

12. Gauillard, F. and F. Richard-Forget. 1997. Polyphenol oxidases from Williams pear (*Pyrus communis* L., cv. *Williams*): activation, purification and some properties. Science of Food and Agriculture Journal. 74. 49-56

13. Geethanjali, S. and A. Subash. 2012. Comparative study on precipitation techniques for protease isolation and purification from *Labeorohita Viscera*. Journal of Aquatic Food Product Technology. 22:2

14. Gul Guven, R., K. Guven, F. Matpan Bekler, O. Acer, H. Alkan, and M. Dogru, 2017. Food Science Technolology. Campinas Journal. 37(3): 356-362

15. Guray, M. Z. 2009. Partial Purification and Characterization of Polyphenol Oxidase from Thermophilic *Bacillus* sp. A Thesis Submitted to the Graduate School of Engineering and Sciences of İzmir Institute of Technology in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

16. Kalme, S.D., G.K. Parshetti, S. U. Jadhav and S.P. Govindwar, 2007. Biodegradation of benzidine based dye Direct Blue-6 by *Pseudomonas desmolyticum* NCIM 2112. Journal of Bioresource Technology. 98: 1405-1410

17. Kandelbauer, A., O. Maute, R.W. Kessler, A. Erlacher and G.M. Gubitz, 2004. Study of dye decolorization in an immobilized laccase enzyme-reactor using online spectroscopy. Journal of Biotechnology and Bioengineering. 87:552–63

18. Kavrayan, D. and T. Aydemir. 2001. Partial purification and characterization of polyphenol oxidase from peppermint (*Mentha piperita*). Food Chemistry Journal. 74: 147-154

19. Lourenço, E. J., V. A. Neves and M. A. Da Silva. 1992. Polyphenol oxidase from sweet potato: Purification and properties.

Journal of Agricultural and Food Chemistry. 40: 2369-2373

20. Mayer, A.M. 2006. Polyphenol oxidases in plants and fungi: going places? Areview. Phytochemistry Journal. 67 (21): 2318-31

21. Nicolas, J. J., F. C. Richard-Forget, P. M. Goupy, M. J. Amiot and S.Y. Aubert. 1994. Enzymatic browning reactions in apple and apple products. Critical Review of Food Science and Nutrition Journal. 34: 109-157

22. Ollikka, P. K., V. M. Alhonmaki, Leppanen, T. Glumoff, T. Raijola. and I. Suominen. 1993. Decolorization of azo triphenyl methane, heterocyclic and polymeric dyes by lignin peroxidase isozymes from *Phanerochate crysosporium*. Journal of Applied Environmental and Microbiology. 59:4010-4016

23. Rapeanu, G., A. Van Loey, C. Smout and M. Hendrickx. 2006. Biochemical characterization and process stability of polyphenol oxidase extracted from Victoria grape (*Vitis vinifera* ssp. *Sativa*). Food Chemistry Journal. 94: 253-261

24. Rodríguez, Y., E. P. Ernestina Solórzano, A. R. Meneses and F. Fernández, 2001. Peroxidase and polyphenol oxidase activities in tomato roots inoculated with *Glomus clarum* or *Glomus fasciculatum*. Journal of Cultivos Tropicales. 22: 1.11-16

25. Shanti, R., M. Anannya, M. Parijatham, Kanchana and Sr. Sandra horta 2014. Extraction, purification and characterization of polyphenol oxidase from peel and pulp of tomato. International Journal of Current Research Acadimic Review. 2(3):74-82

26. Tavares, A. P.M., R.O. Cristóvão, J.M. Loureiro, R.A.R. Boaventura and E.A. Macedo 2008. Optimization of reactive textile dyes degradation by laccase–mediator system. Journal of Chemical Technology and Biotechnology. 83: 1609–1615

27. Thakur, V., P. Kumar, A. Verma and D. Chand. 2015. Decolorization of dye by alginate immobilized laccase from *Cercospora* SPF-6: Using compact 5 stage plug flow reactor. International Journal of Current Microbiology and Applied Sciences. 4 (1): 183-200

28. Tomás-Barberán, F. A. and J. C. Espín. 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. The Science of Food and Agriculture Journal. 81:853-876

29. Turgay, O.C, E.E. Erdogan and A. Karaca. 2010. Effect of humic deposit (leonardite) on degradation of semi-volatile and heavy hydrocarbons and soil quality in crude-oil-contaminated soil. Environmental Monitoring and Assessment Journal. 170:45-58

30. Wheatley, J.B. and D.E. Schmidt. 1999. Salt-induced immobilization of affinity ligands onto epoxide-activated supports. Journal of Chromatography A. 849:1–12

31. Zhang, Z., Y. Shan and J. Wang. 2007. Investigation on the rapid degradation of congo red catalyzed by activated carbon powder under microwave irradiation. Journal of Hazardous Materials. 147 (1-2): 325- 333

32. Zian, E. and S. Pekyardimici. 2004. Purification, Characterization of pear *Pyrus communis* Polyphenol Oxidase. Turkish Chemistry Journal. 28:547-557

33. Zille, A., P. Ramalho, T. Tzanov, R. Millward, V. Aires and M.H. Cardoso. 2004. Predicting dye biodegradation from redox potentials. Journal of Biotechnology Progress. 20:1588–92.