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Extraction and purification of *Polyphenol Oxidase* enzyme from tea leaves (*Camellia sinensis*) : A case study of Characterization and inhibitor effect

[1]Yudhistir S.M.F. Jugessur, Researcher

ABSTRACT:
Polyphenol oxidase (PPO) is an enzyme that is known as a catalyste in browning reactions are of significant importance in the fruit and vegetable industry. These reactions proceeding in many foods of plant origin cause deterioration and loss of food quality such as expensive seafood. PPO also is benefic in other instances such as in tea browning , coffee and cocoa industry which as equally quality products. A better knowledge of the factors that influence the action of PPO is imperative in order to control and manipulate its detrimental activity in plant products. Several studies show that polyphenol oxidase is widely found in nature [12 , 13]. The paper describe what are enzymes , their wide uses in industries and food industry and specifically the PPO, which can be extracted fromPolyphenol Oxidase enzyme was extracted from tea leaves (Camellia sinensis). Studies on Enzyme provide the possibility to develop inhibitors that can stop of slow down reactions. An experimental description of anextraction, characterization and purification process is described in this paper.Ion Exchange Chromatography was used.The dialysate was applied to a column (2.5 cm x 30 cm) filled with DEAE-cellulose, balanced with 10 mM phosphate buffer, pH 6.8. In order to remove nonadsorbed fractions the column was washed with 130 mL of the same buffer at the flow rate of 0.5 mL/min. Then, a linear gradient of phosphate buffer concentration from 10 to 200 mM was applied. 4 mL fractions were collected in which the protein level and PPO activity towards catechol as substrate were monitored. A final purification step resulted in a 3.32 fold purification with a recovery of 5.11% was achieved. The optimal pH and temperature for the PPO enzyme activity were found to be 6.02 and
30 °C, respectively. The thermal inactivation studies showed that the enzyme is heat resistant.The enzyme showed the highest activity toward 4-methylcatechol and no activity toward caffeic acid and gallic acid. The most potent inhibitors were sodium metabisulfite and ascorbic acid.

KEYWORDS: Microbiology, Extraction, purification, Enzyme, Polyphenol Oxidase, Camellia Sinensis, Characterization, inhibitor

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I. INTRODUCTION

1. What are Enzymes ?

Enzymes are highly efficient biocatalysts researched for industrial-scale catalysis because of their several distinct advantages that range from their operation in milder reaction conditions, to their exceptional product selectivity, and to their lower environmental and physiological toxicity [1,2,3,4]. Enzymes are complex protein molecules, biocatalysts, which are produced by living cells. They are highly specific both in the reactions that they catalyze and in their choice of reactants, which are known as substrates.An enzyme typically catalyzes a single chemical reaction or a set of closely related reactions. Enzymes can also be defined as soluble, colloidal and organic catalysts that are produced by living cells, but are capable of acting independently of the cells [5,6,7]

2. Mechanism action of enzymes: How enzyme works ?

The mechanism of action is based on a chemical reaction, in which the enzyme binds to the substrate and finally forms an enzyme–substrate complex. This reaction take place in a relatively small area of the enzyme called the active or catalytic site.

a) The Fisher template model (lock and key model)

This is a rigid model of the catalytic site, proposed by Emil Fischer in 1894 . The model explains the interaction between a substrate and an enzyme in terms of a lock and key analogy. In this model, the catalytic site is presumed to be preshaped. The substrate fits as a key fits into a lock.

$E + S \rightarrow ES\ complex \rightarrow E + P$

b) Induced fit model

In contrast to the above method, this model suggests a flexible mode for the catalytic site. To overcome the problems of the lock and key model owing to the rigid catalytic site, Koshland[10] suggested an induced fit model in 1963. The important feature of this procedure is the flexibility of the active site. In the induced fit model, the substrate induces a conformational change in the active site of the enzyme so that the substrate fits into the active site in the most convenient way so as to promote the chemical reaction. This method suggests competitive inhibition, allosteric modulation and inactivation of enzymes on denaturation [8,9,10,11]. Enzymes are considered to lower the activation energy of a system by making it energetically easier for the transition state to form. In the presence of an enzyme catalyst, the formation of the transition state is energetically more favourable (i.e. it requires less energy for the 'kick start'), thereby accelerating the rate at which the reaction will proceed, but not fundamentally changing the energy levels of either the reactant or the product.

3. Rational for doing Research on Polyphenol Oxidase (PPO)

PPO : wound healing ,defense reactions, cancer drug and enhance quality of tea/coffee, to develop inhibitor.

Polyphenol oxidase (PPO) is an enzyme that is known as a catalyste in browning reactions are of significant importance in the fruit and vegetable industry. These reactions proceeding in many foods of plant origin cause deterioration and loss of food quality. A better knowledge of the factors that influence the action of PPO is imperative in order to control and manipulate its detrimental activity in plant products. Several studies show that polyphenol oxidase is widely found in nature [12 , 13]

Note (A): Source of PPO : PPO is typically present in the majority of plant tissues [14 15 16 17 18 19]

Note (B): Adverse effect of PPO: Because of its involvement in adverse browning of plant products, PPO has received much attention from researchers in the field of plant physiology and food science. Enzymatic browning occurs as a result of the oxidation by PPO, of phenolic compounds to quinones and their eventual (nonenzyme-catalyzed) polymerization to melanin pigments [20 21 22 23 24 25]. Similar to vaccine developments, Scientists need to study the virus to develop the vaccines. To develop anti browning agents inhibitors , the enzyme causing the Browning needs to be studied.

II. METHODOLOGY

A Case study of *Polyphenol Oxidase* enzyme from tea leaves (*Camellia sinensis*) Ünal*et al* [48]was described.

Materials and Reagents : In the experiment , Ünal*et al* [48] used (i) tea leaves obtained from Black sea region of Turkey and frozen at -25 °C until used.

(ii) Ünal*et al* [48] purchased Ascorbic acid, catechol, polyvinylpyrolidone (PVPP), pyrogallol, sodium metabisulfite, triton X-100 from Merck (Darmstadt, Germany) . Acetone, ammonium sulphate, L-cysteine, citric acid, gallic acid, caffeic acid 4-methylcatechol, polyethylene glycol(PEG), phenylmethylsulfonyl fluoride (PMSF), cellulose membrane (76x49mm) and DEAE-cellulose were purchased from Sigma-Aldrich (St. Louis, USA) [48] .

4. Enzyme Extraction Method [48]

150 g of frozen tea leaves were homogenized in 225 mL of cold acetone (-25 °C) containing 1.875 g of polyethylene glycol, using a pre-chilled Waring blender (Model HGBTWTS3, Torrington, Connecticut, USA) for 2 min at low speed. The slurry was vacuum filtered through filter paper.The residue was re-extracted with 150 mL of cold acetone. This procedure was repeated until a white powder was obtained. The resultant acetone powder was dried overnight at room temperature and stored at -25 °C [48,49] ,10 g of acetone powder was homogenized for 40 sec in 1 L of 0.1 M phosphate buffer (pH 6.8) containing 10 mM ascorbic acid, 0.1% polyvinylpyrolidene, 0.5% Triton X-100 and1 mM PMSF, using Waring blender. After the homogenate was magnetically stirred for 1 h at 4 °C, it was centrifuged at 10000 x g for 30 min at 4 °C. The resulting supernatant was subjected to ammonium sulphate precipitation. The fraction precipitated between 30-90% saturation was separated by centrifugation at 10000 x g for 30 min at 4 °C. The precipitate was dissolved in a small amount of 10 mM phosphate buffer, pH 6.8, and dialyzed overnight at 4 °C in the same buffer [50]

5. Purification stage [48]

Ion Exchange Chromatography

The dialysate was applied to a column (2.5 cm x 30 cm) filled with DEAE-cellulose, balanced with 10 mM phosphate buffer, pH 6.8. In order to remove nonadsorbed fractions the column was washed with 130 mL of the

same buffer at the flow rate of 0.5 mL/min. Then, a linear gradient of phosphate buffer concentration from 10 to 200 mM was applied. 4 mL fractions were collected in which the protein level and PPO activity towards catechol as substrate were monitored. The fractions which showed PPO activity were combined and were used as enzyme source in the following experiments [51]

Protein Determination using Bradford Method [52]

Protein contents of the enzyme extracts were determined according to Bradford method using bovine serum albumin as a standard [52]

III. DISCUSSION

According to the Food and Agriculture Organization (FAO), handling fruit and vegetables during postharvest treatments causes a 20–40% loss of fruit crops every year [26]. The schematic of enzymatic browning process and inhibition mechanisms of natural extracts [27]. Oxidative browning reactions, proceeding in many foods of plant origin, generally cause deterioration in food quality by changing nutritional and organoleptic properties. These reactions significantly diminish consumer acceptance, storage life and value of the plant products. In addition to its general occurrence in plants, PPO is also found in seafood (crustacean) products, such as shrimp [28 29 30 31] and lobster [32 33 34 35 36]. These highly prized and economically valuable products are extremely vulnerable to deteriorative enzymatic browning, also referred to as melanosis. Owing to its tremendous economic impact to the food industry, inhibition of PPO in seafood products has been widely studied [37 38]

Note (C): Beneficial effect of PPO : The Case for Tea *(Camellia sinensis*)

Browning in some other instances such as in the processing of black tea [39,40]

Coffee [41,42]and cocoa [43,44] .*PPO* is beneficial to some extent as it enhances the quality of the beverages through its forming flavorful products. Polyphenol oxidase (PPO) plays a key role in the oxidation of flavanols to black tea components such as theaflavins and thearubigins. It catalyses the crucial initial reaction during tea fermentation, the oxidation of o-diphenols to their corresponding quinones, which are then spontaneously transformed to more complex fermentation products [45] .Only the tender shoots of the plant are processed. The shoots are a rich source of polyphenols and PPO. The enzyme is found in all parts of the plant, and tea quality is positively correlated with its content in the shoots [46]

Note (D): Beneficial effect of doing research on PPO :

To Develop Inhibitor Effect of PPO Inhibitors to reduce /Prevent Browning effect and food loss (vaccine development synonymous). Enzyme inhibitors are molecules that interact with enzymes (temporary or permanent) in some way and reduce the rate of an enzyme-catalyzed reaction or prevent enzymes to work in a normal manner. The important types of inhibitors are competitive, noncompetitive, and uncompetitive inhibitors. Besides these inhibitor types, a mixed inhibition exists as well. [47]

IV. FINDINGS

6. pH Optima

During the experiment[48] , PPO activity was determined in a pH range of 4.03-5.49 in 200 mM citric acid buffer and 6.02-7.00 in 200 mM phosphate buffer. PPO activity was assayed, using the standard reaction mixture but changing the buffer. PPO activity was calculated in the form of percent residual activity at the optimum pH. The optimum pH value obtained from this assay was used in all the other experiments.

7. Temperature Optima.

During the experiment[48] ,the activity of PPO was determined at temperatures ranging from 20 °C to 80 °C. 0.9 mL of catechol solution in buffer was heated to the appropriate temperature in a water bath. After equilibration of the reaction mixture at the selected temperature, 0.1 mL of the enzyme solution was added and the enzyme activity was measured. PPO activity was calculated in the form of percent residual PPO activity at the optimum temperature.

8. Substrate Specificity [48]

In order to determine Michaelis constant (Km) and maximum velocity (Vm), PPO activities were measured 48 using catechol (25-200 mM), 4-methylcatechol (6.25-100.00 mM), pyrogallol (50-200 mM), gallic acid (50-200 mM) and caffeic acid (0.75-3.00 mM) as substrates. Km and Vm values of the enzyme were calculated from a plot of 1/V vs. 1/S by the method of Lineweaver and Burk.

9. Effects of Inhibitors [48]

The inhibitors examined during that experiment were L-cysteine, ascorbic acid and sodium metabisulfite. The reaction mixture contained 0.8 mL of catechol at a final concentration of 100 mM in 200 mM phosphate buffer (pH 6.02), 0.1 mL inhibitor at a final concentration of 0.01, 0.1 or 1.0 mM and 0.1 mL enzyme solution.

Percentage inhibition was calculated using the following equation:
Inhibition (%) = [(Ao – Ai)/Ao)].100,
where, Ao is the initial PPO activity (without inhibitor)
and Ai is the PPO activity with inhibitor.

Result Analysis

The table shows Inhibitor and their effect on PPO activity [48]
Sodium metabisulfite and ascorbic acid were the most impactful PPO inhibitor.

Effects of inhibitor on tea PPO activity		
Inhibitor	Concentration (mM)	Inhibition (%)
Cysteine	0,01	No inhibition
	0.10	3.3 ± 1.7
	1.00	6.6 ± 2.1
Ascorbic Acid	0.01	12.7 ± 1.5
	0.10	14.5 ± 4.1
	1.00	15.5 ± 0.9
Sodium Metabisulfite	0.01	6.4 ± 0.2
	0.10	7.9 ± 2.0
	1.00	16.4 ± 0.5

Inhibitor and their effect on PPO activity [48]

Kinetic Parameters[48]

Km and Vm values for tea PPO for different substrates are presented in Table on next slide. The affinity of the enzyme varied depending on the substrate used. Tea PPO had a higher affinity for 4-methylcatechol, as evidenced by lower Km value. The criterion for the best substrate is the Vm/Km ratio[54]. Of the substrates tested, the best substrate for tea PPO was 4-methylcatechol. The enzyme showed no activity against caffeic acid and gallic acid.

Results Analysis

The table shows substrate Affinity.
Vm/Km ratio[54] for4-methylcatechol was higher, so higher affinity

Kinetic Parameters of tea PPO			
Substrate	$Km \quad (mM)$	V_m (U/min/mL)	V_m/K_m
Catechol	243.2	28835	118.6
4-Methylcatechol	127.8	54140	423.6
Pyrogallol	3113.4	78988	25.4
Caffeic acid	-	-	-
Gallic acid	-	-	-

Table showing Substrate Affinity

V. CONCLUSION

Studies on Enzymes is useful particularly to be able to control those enzymes as they are being used industrially. The development of inhibitors is essential to stop and slow down enzymes catalytic effects. PPO enzyme has some undesired effect like browning of expensive food but also prove to be useful in Tea industry. The PPO provide the flavour and enhances the quality. The ion exchange chromatography was used but is not the only method. Extraction, Purification and characterization can be performed with various methods. The methods used depends on the properties of the enzyme such as size, mass, polarity , pH of the enzyme. This method produced [48] varied inhibition degree in a dose dependent manner. From the results, it was concluded that

the most potent inhibitors were sodium metabisulfite and ascorbic acid, because a higher degree of inhibition was achieved. Cysteine was the least potent inhibitor.

REFERENCES

[1]. Bommarius, A.S.; Paye, M.F. Stabilizing biocatalysts. Chem. Soc. Rev. 2013, 42, 6534–6565. [PubMed]
[2]. Choi, J.-M.; Han, S.-S.; Kim, H.-S. Industrial applications of enzyme biocatalysis: Current status and future aspects. Biotechnol. Adv.2015, 33, 1443–1454. [PubMed]
[3]. Madhavan, A.; Sindhu, R.; Binod, P.; Sukumaran, R.K.; Pandey, A. Strategies for design of improved biocatalysts for industrial applications. Bioresour. Technol. 2017, 245, 1304–1313. [PubMed]
[4]. Roy, I.; Prasad, S. Converting Enzymes into Tools of Industrial Importance. Recent Pat. Biotechnol. 2017, 12,33–56.
[5]. Berg J M, Tymoczko J L and Stryer L 2002 Biochemistry 5th edn (New York: W H Freeman)
[6]. Agarwal P K 2006 Enzymes: an integrated view of structure, dynamics and function Microb. Cell Fact. 5 2
[7]. Lee H C 2006 Structure and enzymatic functions of human CD38 Mol. Med. 12 317–23
[8]. Lemieux R U and Spohr U 1994 How Emil Fischer was led to the lock and key concept for enzyme specificity Adv. Carbohydr. Chem. Biochem. 50 1–20
[9]. Csermely P, Palotai R and Nussinov R 2010 Induced fit, conformational selection and independent dynamic segments: an extended view of binding events Trends Biochem. Sci. 35 539–46
[10]. Koshland D E 1995 The key–lock theory and the induced fit theory Angew. Chem. Int. Ed. Engl. 33 2375–8
[11]. Boehr D D, Nussinov R and Wright P E 2009 The role of dynamic conformational ensembles in biomolecular recognition Nat. Chem. Biol. 5 789–96.
[12]. Whitaker, J.R. 1994. Principles of Enzymology for the Food Sciences, Second Ed., pp. 271-556, Marcel Dekker, New York.
[13]. Whitaker, J.R. 1996. Polyphenol oxidase. In Food Chemistry, (O.R. Fennema, ed.) pp. 492-494, Marcel Dekker, New York.
[14]. Vamos-Vigyazo , L. 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. Crit. Rev. Food Sci. Nutr. 15, 49-127.
[15]. Zawistowski, J. ,Biliaderis, C.G. and Eskin 1991. Polyphenol oxidase. In Oxidative Enzymes in Foods, (D.S. Robinson and N.A.M. Eskin, 4s.) pp. 217-273, Elsevier Science Publishing, New York
[16]. Sherman , T.D., Vaugh, K.C. and Duke, S.O. 1991. A limited survey of the phylogenetic distribution of polyphenol oxidase. Phytochemistry 30,
[17]. Sherman , T.D., Gardeur T.L. and Lax, A.R. 1995. Implications of the phylogenetic distribution of polyphenol oxidase in plants. In Enzymatic Browning and Its Prevention, (C.Y. Lee and J.R. Whitaker, eds.) pp. 103-1 19, ACS Symposium Series 600; American Chemical Society, Washington, DC
[18]. Fraignier , M., Marques, L., Fleuriet , A. and Macheix , J. 1995. Biochemical and immunochemical characteristics of polyphenol oxidase from different fruits of Prunus. J. Agric. Food Chem. 43, 2375-2380.
[19]. Haruta , M., Murata, M., Kadokura, H. and Homma , S. 1999. Immunological and molecular comparison of polyphenol oxidase in Rosaceae fruit trees. Phytochemistry 50, 1021-1025.
[20]. Mathew A.G. and Parpia , H.A.B. 1971. Food browning as a polyphenol reaction. Adv. Food Res. 29, 75-145.
[21]. Macheix, J. Fleuriet , a an Billot J. 1990. Phenolic compounds in fruit processing. In Fruit Phenolics, (J. Macheix, A. Fleuriet and J. Billot, eds.) pp. 239-312, CRC Press, Boca Raton, Florida
[22]. Sapers, G.M. 1993. Browning of foods: control by sulfites, antioxidants, and other means. Food Technol. 47, 75-84.
[23]. Nicolas JJ, Richard-Forget FC, Goupy PM, Amiot MJ, Aubert SY. Enzymatic browning reactions in apple and apple products. Crit Rev Food Sci Nutr. 1994;34(2):109-57. doi: 10.1080/10408399409527653. PMID: 8011143.
[24]. Sánchez-Ferrer A, Rodríguez-López JN, García-Cánovas F, García-Carmona F. Tyrosinase: a comprehensive review of its mechanism. BiochimBiophys Acta. 1995 Feb 22;1247(1):1-11. doi: 10.1016/0167-4838(94)00204-t. PMID: 7873577.
[25]. Whitaker,J.R. 1995. Polyphenol oxidase. In Food Enzymes Structure and Mechanism, (D.W.S. Wong, ed.) pp. 271-307, Chapman and Hall, New York.
[26]. FAO. The Future of Food and Agriculture–Trends and Challenges; FAO: Rome, Italy, 2017; ISBN 9789251095515.
[27]. Moon, K.M.; Kwon, E.B.; Lee, B.; Kim, C.Y. Recent Trends in Controlling the Enzymatic Browning of Fruit and Vegetable Products. Molecules 2020, 25, 2754.
[28]. Simpson, B.K., Marshall , M.R. and Otwell, W.S. 1987. Phenoloxidase from shrimp (Penaeus setiferus): Purification and some properties. J. Agric. Food Chem. 35, 918-921.
[29]. Simpson, B.K., Marshall , M.R. and Otwell, W.S. 1988. Phenoloxidases from pink and white shrimp: Kinetic and other properties. J . Food Biochemistry 12, 205-217
[30]. Rolle, R.S., Guizani, N., Chen, J .S., Marshall, M.R., Yang, J.S.and Wei, C . I. 199 1 . Purification and characterization of phenoloxidase isoforms from Taiwanese black tiger shrimp (Penaeus monodon). J. Food Biochemistry 15, 17-32.
[31]. Chen J.S., Charest, D.J., Marshall, M.R. and Wei, C.I. 1997. Comparison of two treatment methods on the purification of shrimp polyphenol oxidase. J. Sci. Food Agric. 75, 12-18.
[32]. Ferrer, O.J., Koburger, J.A., Otwell, W.S., Gleeson, R.A., Simpson, B.K. and Marshall, M.R. 1989a. Phenoloxidase from the cuticle of Florida Spiny lobster (Panulirus argus): Mode of activation and characterization. J. Food Sci. 54, 63-67.
[33]. Ferrer, O.J., Koburger, J.A., Simpson, B.K., Gleeson, R.A. and Marhshall, M .R. 1989b. Phenoloxidase levels in Florida Spiny lobster (Panulirus argus): Relationship to season and molting stage. Comp. Biochem. Physiol. 93B, 595-599
[34]. Rolle, R.S., Marshall, M.R., We, C.I. and Chen, J.S. 1990. Phenoloxidase forms of the Florida Spiny lobster: Immunological and spectropolarimetric characterization. Comp. Biochem. Physiol. 97B,
[35]. Chen, J.S., Rolle, R.S., Marshall, M.R. and Wei, C.I. 1991a. Comparison of phenoloxidase activity from Florida Spiny lobster and Western Australian lobster. J. Food Sci. 56, 154-157.
[36]. Ali, M.T., Marshall, M.R., Wei, C.I. and Gleeson, R.A. 1994. Monophenol oxidase activity from the cuticle of Florida Spiny lobster (Panulirus argus). J. Agric. Food Chem. 42, 53-58
[37]. Ferrer, O.J., Otwell, W.S. and Marshall, M.R. 1989c. Effect of bisulfite on lobster shell phenoloxidase. J. Food Sci. 54, 478-480.
[38]. Kim, J., Marshall, M.R. and Wei, C. 2000. Polyphenoloxidase. In Seafood Enzymes Utilization and Influence on Postharvest Seafood Qualiq, (N.F. Haard and B.K. Simpson, eds.) pp. 271-315, Marcel Dekker, New York.
[39]. Eskin , N.A.M. 1990. Biochemistry of food spoilage: Enzymatic browning. In Biochemistry of Foods, (N.A.M. Eskin, ed.) pp. 401-432, Academic Press, New York.
[40]. Ullah , M.R. 1991. Tea. In Food Enzymotogy, (P.F. Fox, ed.) pp. 163-187, Elsevier Science Publishing, New York.
[41]. Amorim, H.W.D. and Silva, D.M. 1968. Relationship between the polyphenol oxidase activity of coffee beans and the quality of the beverage. Nature 219, 381-382.

[42]. Amorim, H.W. and Melo, M. 1991. Significance of enzymes in nonalcoholic coffee beverage. In Food En.ymlogy, (P.F. Fox, ed.) pp. 189-209, Elsevier Science Publishing, New York.
[43]. Lee, P.M., Le, K. and Karim, M.I.A. 1991. Biochemical studies of cocoa bean polyphenol oxidase. J. Sci. Food Agric. 55, 251-260.
[44]. Lopez, A.S. and Dimick, P.S. 1991. Enzymes involved in cocoa curing. In Food Enzymology, (P.F. Fox, ed.) pp. 211-236, Elsevier Science Publishing, New York.
[45]. Finger A. 1994. In-Vitro studies on the effect of polyphenol oxidase and peroxidase on the formation of polyphenolic black tea constituents. J Sci Food Agric, 66:293-305.
[46]. Singh HP, Ravindranath SD. 1994. Occurrence and distribution of PPO activity in floral organs of some standard and local cultivars of tea. J Sci Food Agric, 64:117- 120.
[47]. Introduction to Food Enzymes. Mohammed Kuddus, in Enzymes in Food Biotechnology, 2019
[48]. Ünal, M. &Yabaci, Selin &şener, Aysun. (2011). Extraction, partial purification and characterization of polyphenol oxidase from tea leaf (Camellia sinensis). GIDA. 36. 137-144.
[49]. Coseteng MY, Lee CY. 1987. Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. J Food Sci, 52:985-989
[50]. Serradell MA, Rozenfeld PA, Martinez GA, Civello PM, Chaves AR, Anon MC. 2000. Polyphenoloxidase activity from strawberry fruit (Fragaria x ananassa, Duch., cv Selva): characterization and partial purification. J Sci Food Agric, 80:1421-1427.
[51]. Ding CK, Chachin K, Ueda Y, Imahori Y. 1998. Purification and properties of polyphenol oxidase from loquat fruit. J Agric Food Chem, 46:4144-4149.
[52]. Bradford MM. 1976. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal Biochem, 72:248-254.
[53]. Ünal MÜ, fiener A. 2006. Determination of biochemical properties of polyphenol oxidase from Emir grape (Vitis vinifera L. cv. Emir). J Sci Food Agric, 86:2374-2379.
[54]. Palmer, JK. 1995. Understanding Enzymes. Prentice Hall/Ellis Horwood, Herdfordshire.

Dedication;

I, dedicate this paper to **MrsKritya Jugessur**, my loving caring mother, myfriend , my lover, my baby who passed away on the 30th Jan 2023. After the shock of 15.07.22, her health deteriorated till her death.