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ORIGINAL PAPER

Purification and characterization of polyphenol oxidase from myrtle berries (*Myrtus communis* L.)

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Abstract

Myrtle (*Myrtus communis* L.) belongs to Myrtaceae family in botany and is mostly grown in Mediterranean Basin. Browning reaction because of polyphenol oxidase (PPO) can be observed in the plant that is rich in phenolic substances. This study, it was aimed to determine and purify some biochemical properties, kinetic parameters, the thermal stability of the PPO enzyme of myrtle fruit. In this study, optimum temperature, optimum pH, substrate specificity, the thermal stability of polyphenol oxidase partially purified from white myrtle berries, and the effect of inhibitors on enzyme activity were investigated. Catechol was used as a substrate. The PPO was identified and purified using ammonium sulphate precipitation and ion exchange chromatography. Optimum pH and temperature were found as 6.8 and 30 °C respectively for myrtle PPO while *K*m and *V*max values were calculated as 3.34 mM and 4.1 mL/min. In thermal inactivation studies at 60, 70, and 80 °C reaction rate constants (*k*) were found as 0.0282, 0.526, and 0.1117 /min, thermal half-life times ($t_{1/2}$) were calculated as 24.6, 13.2, and 6.2 min, and decimal reduction time (*D*-value) was calculated as 81.6, 43.8 and 20.6 min, respectively. The activation energy (*Ea*) and *Z*-value were calculated as 67.2 kJ/mol and 33.4 °C, respectively. Similar inactivation ratios from 40 to 100% were observed for both inhibitors of ascorbic acid and disulfide at 0.01–10 mM concentration. PPO from myrtle was successfully purified to 5.5 purification fold with 8% recovery, by using DEAE-Sephacel chromatography.

Keywords *Myrtus Communis* L. · Polyphenol oxidase · Kinetic parameters · Thermal stability · Purification

Introduction

Myrtaceae family comprises approximately 100 genera and 3000 species. *Myrtus*, belonging to this family, is an evergreen shrub and perennial plant. It is usually short, but sometimes it can grow up to 1–5 m. This plant consists mainly of two species, *Myrtus communis* Linn, known as myrtle, and *Myrtus nivellei* Batt, known as Sahara myrtle [1, 2]. *Myrtus communis* L., is one of the main plant species in the vast majority of Mediterranean countries, hot regions of North America, and different regions of Australia. In the wild, it grows mainly on the coasts of Tunisia, Morocco, Turkey, and France. In addition, cultivation efforts continue in Iran, Spain, Italy, and Yugoslavia. In Turkey, Myrtle is grown on the entire coastline, especially in the foothills of the Taurus Mountains at an altitude of 500–600 m above sea level [3, 4]. The plant is also locally known as "hambeles", "myrtle" or "mersin” particularly on the southern coasts of Türkiye [5]. There are two major fruit polymorphisms of myrtle fruits that depend on the color, either dark-blue or white-yellow [6]. The leaves, branches, flowers, and fruit of the myrtle plant have been used as medicinal and/or aromatic plants for a long time to be used in different areas such as pharmaceutics, cosmetics, and liqueur. Moreover, it has a therapeutic and/or protective effect against various diseases due to the bioactive components such as polyphenol they have in these different parts of the myrtle plant [7, 8]. This effect is caused by the plant's antioxidant and anti-inflammatory properties. It is, furthermore, used against liver, kidney, diabetes, lung, skin and eye diseases, infections, diarrhea, rheumatism, bronchitis, and tuberculosis diseases due to its antiseptic and anti-inflammatory effects [1, 9]. Another remarkable feature of the myrtle plant is that it has a pleasant smell and aroma. It is due to the essential oil it contains, which gives the typical pleasant smell and aroma to both

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the fruit flesh and the plant leaves. The main components responsible for the fruit acquiring this characteristic feature are derived from numerous oil components such as monoterpenes: 1,8-cineola, myrtenyl acetate, α-pinene, myrtenol, limonene [10, 11]. Rather than being used as an ornamental plant in landscaping, the myrtle plant is used as a perfume, in cosmetics, in pharmacy, as a seasoning mixture or sauce in ready-made foods such as beverages and confectionery, in ice cream and bakery products, thanks to the essential oils obtained from its leaves [12, 13].

Myrtle berry is susceptible to browning owing to the activity of endogenous enzymes, and so it has a nearly short shelf life [14] The main endogenous enzyme involved in enzymatic browning reactions of fruits and vegetables is polyphenol oxidase PPO (EC 1.14.18.1), which is a copper-containing protein [15] Enzymatic browning is mainly due to the relationship between phenols and polyphenol oxidases in the presence of oxygen when fruits and vegetables are damaged during harvesting and processing operations. For this reason, the PPO enzyme may bring about undesirable changes in the flavor, color, taste, and nutritional value of fruits and vegetables, as well as quality losses in the marketability value, and thus, economic losses. Quality optimization of products requires the control of PPO activity, which is crucial for the food industry [16–18].

Day by day understanding the importance of the PPO enzyme, many researchers have conducted studies to determine the properties of the PPO enzyme in a wide variety of fruits and vegetables. Because of this, PPO has been examined in numerous foods, such as in goldenberry [19], grape [16, 20], blueberry [21, 22], prawn [23], tea leaf [24], cinnamon [15], soursop [25], water yam [26], sweet potato [14]. In the current literature research, no study was found about the purification and characterization of the PPO enzyme from myrtle berries. In this study, it was aimed to determine and purify some biochemical properties, kinetic parameters, the thermal stability of the PPO enzyme of myrtle fruit. It was thought that, results of this study offer some significant data for processing the myrtle berries in engineering point view data for scientific literature.

Materials and methods

Materials

White myrtle (*Myrtus communis* L.) fruits, which were obtained at the same maturity levels in the city center of Mersin province in Turkey and frozen at − 25 °C until analyzed, were used as material. All chemicals used were of analytical grade. In our study, sodium phosphate, sodium acetate, phosphoric acid, and acetic acid were used in the preparation of the extraction medium and buffer solutions. Acetone, triton X-100, ascorbic acid, PVPP (polyvinylpolypyrrolidone), PMSF (phenylmethylsulfonyl fluoride), PEG (polyethylene glycol), sodium disulfide, ammonium sulfate, and catechol used in the purification of the enzyme and determination of its biochemical properties were obtained from merck companies. Toyopearl DEAE-650 M Supelco and cellulose membrane (76 × 49 mm) were purchased from Sigma-Aldrich.

Methods

Extraction and purification of PPO enzyme

Preparation of crude enzyme extract Organic solvents such as acetone and ethanol are frequently used in the precipitation of proteins. Acetone was used in the preparation of crude extracts, as higher efficiency enzyme activity was observed with acetone [18]. Hundred g of fresh frozen myrtle berries were taken and homogenized in a pre-cooled (− 18 °C) 200 mL acetone containing 1 g of polyethylene glycol in a warring blender at − 18 °C for 2 min at high speed**.** The homogenate was filtered through a black band filter paper (640 W- ∅ 125 mm). The filter cake obtained was treated with 100 mL of cooled acetone and the same procedures were applied. The same processes were repeated until the filter cake turned into a white powder. The acetone powder obtained was taken into a large open beaker and after drying at room temperature overnight, it was stored in 50 mL falcon tubes at − 18 °C [27]. 0.5 g of acetone powder was suspended in 37.5 mL of prechilled 0.1 M phosphate buffer, pH 6.8, and then stirred for 1 h at 4 °C. The obtained suspension was centrifuged at 4 °C for 30 min at 7500 × g. The supernatant was used as crude PPO [28].

Partial purification of the enzyme Acetone powder (10 g) was homogenized in 300 mL of 0.1 M phosphate buffer (pH 6.8) containing 0.1% polyvinylpolypyrrolidone, 0.5% Triton X-100, 10 mM ascorbic acid, and 1 mM PMSF. The suspension was magnetically stirred for 3 h at 4 °C. Then, it was centrifuged at 10,000 × g for 45 min at 4 °C. Ammonium sulfate precipitation at 90% saturation was applied to the supernatant. To separate the precipitated parts, it was centrifuged again at 4 °C for 45 min at 10,000 × g. As seen in (Fig. 1a), the solid part obtained was dissolved in a small amount of 0.01 M phosphate buffer, pH 6.8, and placed in cellulose membrane (14,000 Da) and dialyzed overnight against the same buffer at + 4 °C. Semi-permeable membranes used for dialysis are generally used to remove salts and other small molecules from solutions. The enzyme obtained as a result of ammonium sulfate and dialysis processes is now partially purified. Following this, the dialyzed solution was further purified by ion-exchange chromatography [29, 30].

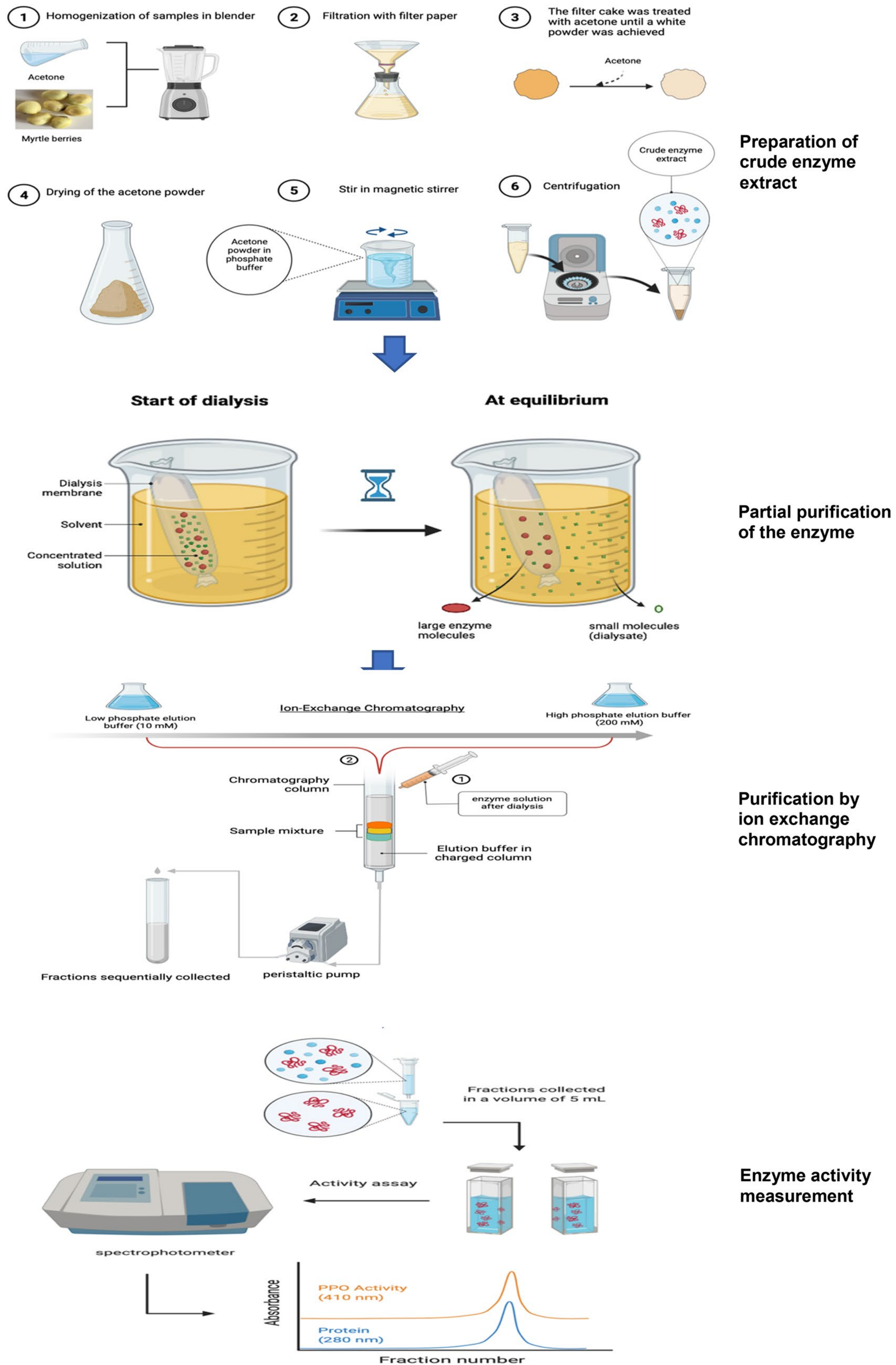

Fig. 1 Purification steps of PPO from myrtle: preparation of crude anzyme extract, partial purification by cellulose membrane, purification by ion exchange chromatography, and assay of enzyme activity

Purification by ion exchange chromatography The purification process using ion exchange chromatography is shown in (Fig. 1b). The ion exchange glass column (2.5 × 30 cm) used for purification was prepared with DEAE-Toyopearl 650-M and balanced with 0.01 M pH 6.8 phosphate buffer. Equilibrium of the resin in the column was achieved by passing through the same buffer approximately 2–3 times. Then, the enzyme solution that we obtained as a result of dialysis was given to the column. Phosphate buffers at pH 6.8, which were gradually prepared at different concentrations (10–200 mM) at 1 mL/min, were used in the washing process. After the fractions in a volume of 5 mL were collected, enzyme activity at 410 nm and protein analysis at 280 nm were performed in the spectrophotometer, as seen in (Fig. 1c). Flow rate is adjusted by a peristaltic pump connected to the end of the column [29, 30].

Assay of enzyme activity

Enzyme activity was determined from the slope of the linear part of the spectrophotometer absorbance-time plot. Before adding enzyme solution for activity measurement, buffer solution (0.1 M and pH 6.8) adjusted to 30 °C, and catechol (25 mM) solution as substrate were kept ready. After mixing 1.45 mL of buffer and 0.5 mL of catechol solutions, 0.1 mL of enzyme solution was added to it and an increase in absorbance was observed at 410 nm. When looking at the effect of the inhibitor on enzyme activity, 1.35 mL buffer, 0.5 mL substrate, 0.1 mL inhibitor, and 0.1 mL enzyme solution were used. 0.001 unit increase in absorbance per minute at 30 °C was defined as 1 unit of PPO activity [31].

Optimum pH

Buffer solutions adjusted at different pH values (pH 4–8) were used to determine the optimum pH value. 0.1 M acetate buffer was used in a pH range 4.0–5.6 to determine PPO activity, while 0.1 M phosphate buffer was used for the pH range 6.0–8.0. 25 mM catechol was used as substrate. The pH value of the PPO enzyme demonstrating the maximum activity was defined to be the optimum pH. The results were shown as a % relative activity-pH graph. After determining the optimum pH value, other studies were carried out at this pH value [28].

Optimum Temperature

To determine the optimum temperature of myrtle PPO, the activity of the enzyme was measured in the temperature range of 20–90 °C. The reaction mixture was incubated in a water bath for 5 min at the appropriate temperatures. After incubation, the enzyme extract was added to the reaction mixture. Then, the PPO activity was calculated in the form of percent residual activity of the optimum temperature at which the highest activity was measured [28].

Determination of kinetic parameters

Michaelis–Menten constant (*K*m) and maximum velocity (*V*max) values of PPO were calculated by using catechol solutions prepared at different concentrations at optimum pH and temperature values. The catechol solution used as a substrate was prepared at concentrations ranging from 2.5 to 50 mM. *K*m and *V*max values of the enzyme were calculated by plotting 1/*V* against 1/*S* according to the Lineweaver–Burk method [28].

Thermal inactivation

To determine the effect of temperature on the enzyme, the enzyme solution was exposed to different temperatures (60, 70, and 80 °C) and times (5, 10, and 15 min). Before adding the enzyme solution to the test tubes used during the experiment, it was ensured to wait for 5 min at the said temperature to reach the relevant temperature. At the end of this period, 0.5 mL of enzyme solution was placed in the tubes and exposed to the relevant temperature for the specified times. At the end of the period, it was immediately cooled in an ice bath and the activity was measured after it was brought to room temperature. To determine k_D (inactivation constant, /min), $t_{1/2}$ (half-life, min), *Ea* (activation energy, kJ/mol), *Z* (temperature change that can cause a tenfold change in reaction rate constant (*k*), °C), and *D* (decimal reduction time, min) values, the residual activity (A_t) in the exposed enzymes were compared with the activity (A_o) of the enzyme exposed to determined optimum temperature [28, 29]

Effects of inhibitors

To determine the effect of inhibitors on enzyme activity, two different inhibitors (ascorbic acid and sodium disulfide) at 0.01, 0.1, 1, and 10 mM concentrations were used. After adding 1.35 mL of buffer, 0.5 mL of the substrate, 0.1 mL of inhibitor, and 0.1 mL of enzyme solution to the test tubes, the activity was measured and kept at 30 °C for 5 min. [28]. The results are given as % inhibition value according to following formula:

$$inhibition(\%) = \left({(Ao - Ai)}/{Ao} \right) * 100 \quad (1)$$

where A_o is enzyme activity determined without using an inhibitor and A_i is enzyme activity determined in the presence of inhibitor.

Determination of protein content

In our study, the total protein content was determined according to the Lowry method using bovine serum albumin (BSA) as a standard. For this purpose, a standard curve was created by using BSA solution prepared at different concentrations (µg/mL) [32, 33].

Statistical analysis

All data were examined in triplicate and standard deviations were obtained using Excel version 16.49 (Microsoft Corporation).

Results and dıscussıon

Extraction and purification

Enzymes are found in nature in a molecular pool mixed with other molecules, either in a structural or functional association. Pure enzymes are needed to determine the structure, function, and some activities. Most isolation protocols require multiple steps to achieve the desired level of purity. These are generally extraction, ammonium sulfate precipitation, and chromatographic methods. The most commonly used chromatographic techniques in enzyme purification are ion exchange and gel filtration chromatography. Ion exchange chromatography, which has an anion exchange feature, was used in our study. In order to purify PPO from myrtle, ammonium sulfate fractionation and ion exchange chromatography using DEAE-Toyopearl 650 M were utilized [17, 18, 29].

Extraction and purification steps of myrtle PPO and total protein, total acticity, spesific activity, purification fold and recovery percents were given in Table 1. While the purification step increased, the total activity and total protein content of the enzyme decreased, but the specific activity increased. PPO from myrtle was successfully purified up to 5.5 purification fold with 8% recovery. Similarly, Peng et al. [26] found that the purification fold of water yam was 4.58. When we look at the specific activity values for myrtle PPO, it was found to be 6 units/mg protein for the crude extract, 24 units/mg protein after dialysis, and 33 units/mg protein after ion-exchange chromatography (DEAE-Toyopearl 650 M). Several reports have shown that there were many purifications folds such as 61.1 and 9.3 for goldnugget loquat and banana pulp PPO, respectively [30], 160 for soursop PPO [25], 8.55 for prawns PPO [23], 14.67 for "Xushu 22" sweet potato skin PPO [14], 4.5 for persimmon fruit PPO, and 5.0 for banana PPO [18], by using DEAE-Sephacel chromatography. These results are consistent with the purification fold we found.

In the ion-exchange chromatography used for purification, 5 mL fractions were collected and protein (absorbance 280 nm) and enzyme activity (absorbance 410 nm) measurements were made in each. The enzyme activity and protein absorbance values of the collected fractions were given in (Fig. 2). As seen in (Fig. 2), the highest activity value was found between the 5th and 8th fractions.

Optimum pH

pH is a critical factor that influences enzyme activity since alteration in the pH of the media may affect the ionization state of the amino acid chains [17]. Due to changing of this ionization state of the amino acids in the active site, the pH can impact PPO's surface charge, its solubility, and enzyme–substrate binding or the affinity with different substrates or inhibitors [17, 18, 26, 34]. The pH value with the highest activity is accepted as 100% and the results are given in (Fig. 3) as % relative activity. As observed, the optimum pH value of PPO obtained from myrtle fruit was 6.8, which was similar to potato PPO using catechol as substrate [35]. It was determined that myrtle PPO did not show any activity between pH 4–4.8 and pH 8.0. As can be seen, a sharp drop in enzyme activity was observed above pH 6.8.

Regarding the optimum pH value of PPO for different fruits, it was determined as 6.5, 7.0, 3.0, 9.0, 8.0, and 7.0 for pear, eggplant, napoleon grape, bean shoots, Indian guava apple, and Anamur banana, respectively [18, 28, 36, 37]. From literatüre, the optimal pH for PPO activity varies from approximately 5.0 to 8.0. The reason why there is an optimum pH value in such a wide range in the literature is that the source from which the PPO enzyme is obtained, the method of extraction, and the location of the enzyme

Table 1 Purification of PPO from myrtle

Purification step	Volume (mL)	Total protein (mg)	Total activity (units)	Specific activity (unit/mg protein)	Purification (fold)	Recovery (%)
Crude extract	196	137.2	823.2	6	1.0	100.0
Ammonium sulfate precipitation and dialysis	50	20	480	24	4.0	58.3
DEAE-Toyopearl 650 M	20	2	66	33	5.5	8.0

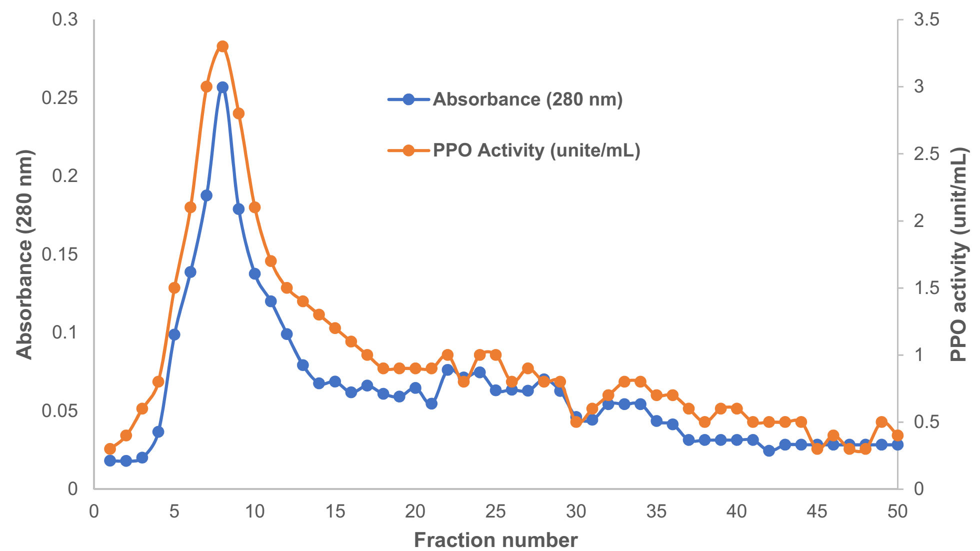

Fig. 2 Elution profile of the myrtle PPO on Toyopearl 650 M

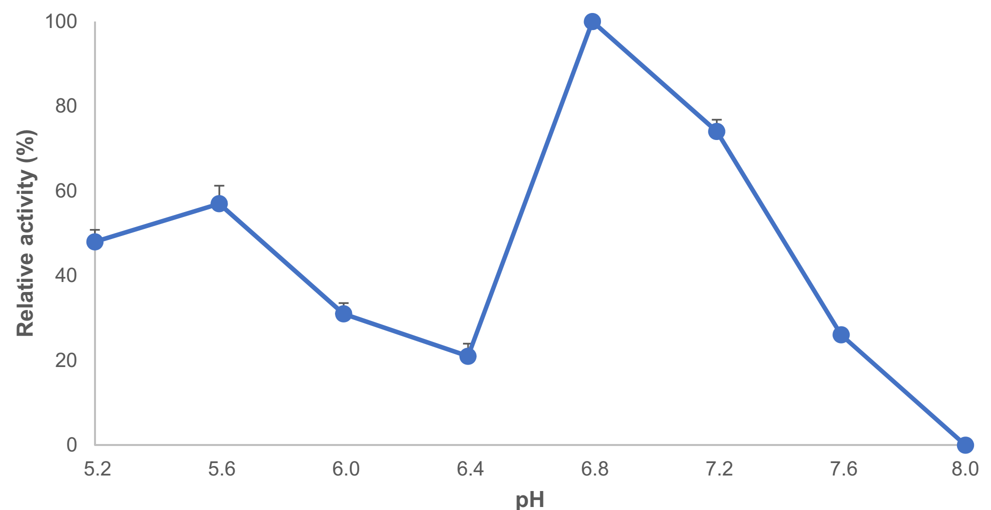

Fig. 3 Effect of pH on myrtle PPO activity

in the cell are different as well as experimental methods used. The type of substrate used in the studies also has a significant effect on the enzyme activity and changes the optimum pH value of the enzyme [38]. For example, in a study in which the PPO enzyme was isolated from borage, it was observed that the optimum pH value of the enzyme was 5.0 for 4-methyl catechol, 5.5 for caffeic acid, and 7.5 for catechol and pyrogallol [39]. Similarly, Bravo and Osorio [19] reported the optimum pH of Cape gooseberry PPO was found to be 5.5 for catechol and 5.0 for chlorogenic acid. The underlying cause of this change is thought to be caused by the difference in the affinity of the relevant substrate to the active center of the enzyme [18]

Optimum Temperature

In our study, activity measurements were made in the temperature range of 20–90 °C to determine the optimum temperature value of PPO obtained from myrtle fruit. The optimum temperature value of myrtle PPO was observed as 30 °C, as seen in (Fig. 4) In similar studies, it was obtained to be 30 °C for banana and snake fruit PPO [28, 40].

As mentioned in the literature, the indicated optimum temperature values were 35 °C for water yam PPO [26], 45 °C for borage PPO [39], 25 °C for soursop PPO [25], 35 °C for blueberry PPO [22] 40 °C for cape gooseberry PPO [19], 45 °C for prawns PPO [23]. These values are consistent with the results we found. Furthermore, the determination of optimum temperature and pH values of the PPO enzyme is a critically important parameter for industrial applications of fruit [41].

Kinetic parameters

The enzyme activity was measured at different increasing catechol concentrations (2.5–50 mM) at optimum pH (6.8)

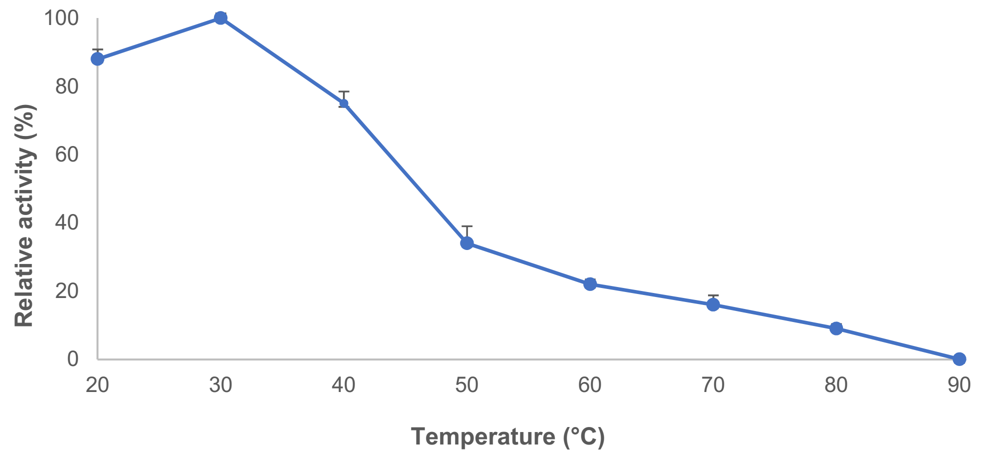

Fig. 4 Effect of temperature on myrtle PPO activity

and temperature (30 °C) to determine the maximum velocity (*V*max) and Michaelis-Mentel constant (*K*m) defined as the substrate concentration that allows maintaining a speed half the maximum speed [38]. By using the reaction velocities at each substrate concentration obtained, *K*m and *V*max values were calculated with the help of the Lineweaver–Burk plot (1/*V* against 1/*S*), as seen in (Fig. 5). For the myrtle PPO, the *K*m and *V*max values were calculated to be 3.34 mM, and 4.21 units/mLmin, respectively. Similarly, Palma-Orozco et al. [25] found that the *K*m value was 3.16 mM for catechol.

The *K*m value is an indicator of the enzyme's tendency to bind to the substrate, and the *V*max/*K*m ratio indicates the best substrate for the enzyme. An enzyme with a low *K*m value has, in other words, a high affinity for its substrate [34]. In this study where catechol was used as a substrate, *V*max/*K*m value for myrtle PPO was found to be 1.26 Units/mLmin. In a study conducted by Siddiq and Dolan [22], the *V*max/*K*m value for blueberry is calculated as 0.17 for catechol and this value is seen to be lower than the myrtle PPO (1.26). In this case, it can be said that myrtle PPO has a higher affinity for catechol than PPO obtained from blueberry. Even though various substrate sources such as 4-methyl catechol, catechin, epicatechin, chlorogenic acid, and pyrogallol are used in the studies carried out to understand which substrate has a higher affinity for PPO, the most preferred among them is catechol [42]. The kinetic parameters *K*m of the truffles PPO were 1.5 mM, 6.1 mM, 8.7 mM, and 3.2 mM, and the kinetic parameters *V*max (EU/mL) were 4331.4, 3458.3, 2873.7, and 2247.2 for catechol, 4-methylcatechol, pyrogallol, and L-tyrosine as substrates, respectively [34].

The effect of inhibitors

The effect of inhibitors on PPO varies according to the type of inhibitor, its concentration, the product from which the enzyme is obtained, and the pH and temperature values of the reaction mixture. A wide variety of inhibitory substances such as ascorbic acid, citric acid, sodium sulfite, L-cysteine, sodium azide, EDTA (ethylenediaminetetracetate) are used to control the activity of the PPO enzyme [18, 21]. In order to demonstrate the effect of inhibitors on PPO obtained from myrtle samples, two different inhibitors (ascorbic acid and sodium disulfide) were used at 0.01, 0.1, 1, and 10 mM concentrations. Inhibition values (%) were given in (Table 2) below. As seen

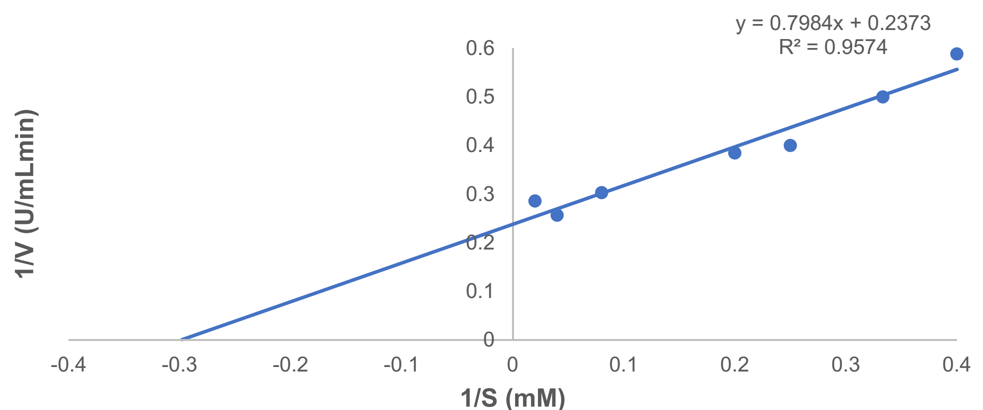

Fig. 5 Lineaweaver-Burk diagram for PPO activity with the catechol substrate

Table 2 Effects of inhibitors on myrtle PPO activity

Inhibitor	Concentration (mM)	Inhibition (%)
Ascorbic acid	0.01	40 ± 1.8
	0.10	43 ± 1.6
	1	69 ± 0.4
	10	100 ± 0.0
Sodium disulfide	0.01	41 ± 0.3
	0.10	49 ± 0.9
	1	69 ± 2.3
	10	100 ± 0.0

in (Table 2), the % inhibition value increased as the concentration of both inhibitors increased. As shown in (Table 2), while both inhibitors were observed at a concentration of 0.01 mM, approximately 40% and 70% at a concentration of 1 mM, while 100% inhibition was observed at a concentration of 10 mM. As argued by Ünal [28], ascorbic acid and sodium metabisulfite were more effective inhibitors against PPO than NaCl and citric acid. Bravo and Osoria stated that the most inhibitory effect was observed in ascorbic acid, sodium sulfite, L-cysteine, and quercetin in their study, in which they examined the effects of various inhibitors against the PPO of cape gooseberry. On the other hand, they stated that citric acid, calcium chloride, tartaric acid, tannic acid, and sodium chloride have relatively lower inhibitory effects [19]. A study carried out by Palma-Orozco et al. [25] showed that the use of ascorbic acid at 3.12 mM concentrations resulted in 77% of inhibition of soursop PPO.

Thermal inactivation

The reaction rate of thermal inactivation of enzymes expressed in first-order reactions is expressed in the following Eq. 2. If the graph of ln (*A/Ao*) against time is drawn at a constant temperature, the graph given in (Fig. 6a) below is obtained. In this graph, the line equations we have obtained for the values (60, 70, and 80 °C) are given. The slope of these line graphs gives us k_D (/min) which means the first-order reaction rate constant for the denatured enzyme. The half-life ($t_{1/2}$) which is calculated with Eq. 3 is another important parameter regarding the characterization of enzyme stability [38].

$$A_t = A_o e^{-kt} \tag{2}$$

$$t_{1/2} = \frac{0.693}{k} \tag{3}$$

$$D = \frac{2.303}{k} \tag{4}$$

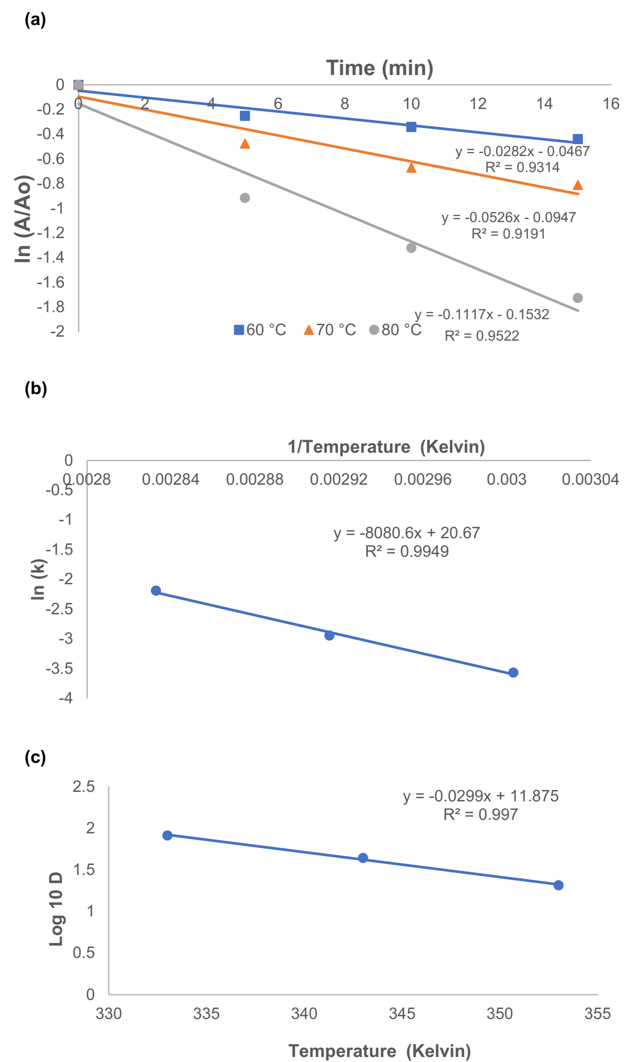

Fig. 6 Determination of the effect of temperature on the enzyme; **a** Logarithmic plot of residual activity of crude extract of myrtle PPO, **b** Arrhenius plots for a crude extract of myrtle PPO, and **c** plots of log D against absolute temperature for a crude extract of myrtle PPO

The reaction rate constants (*k*), the half-life ($t_{1/2}$), and the decimal reduction time (*D*-values) of myrtle PPO for 60, 70, and 80 °C were given in (Table 3). The thermal inactivation rate constants of myrtle PPO were found to be 0.0282, 0.0526, and 0.1117 /min for 60, 70, and 80 °C, respectively, and the half-life times to be 24.6, 13.2,

Table 3 Inactivation parameters of myrtle PPO

Temperature (°C)	*k* (/min)	r^2	$t_{1/2}$ (min)	*D* (min)
60	0.0282	0.9314	24.6	81.6
70	0.0526	0.9191	13.2	43.8
80	0.1117	0.9522	6.2	20.6

and 6.2 min, respectively. The *D* value calculated using the Eq. 4 was found to be 81.6 min at 60 °C, 43.8 min at 70 °C, and 20.6 min at 80 °C. As can be seen from (Table 3), Despite the increasing temperature values, the reaction rate constants (*k*) values increased, while the half-life ($t_{1/2}$) and *D* values decreased.

In the PPO enzyme study of a tropical fruit called *Kalipatti sapota* by Vishwasrao et al. [36], they found the *D* (min) values of PPO in the crude extracts of the fruit as 35.2, 12.5, and 1.8 for 60, 70, and 80 °C, respectively. The reaction rate constants (*k*) values for the PPO enzyme isolated and purified from the cape gooseberry fruit by Bravo et al. were calculated as 0.0122, 0.0399, and 0.0664 /min for 60, 70, and 80 °C, respectively. It is seen that it is lower than the myrtle PPO values at the same temperature. In the same study, half-life times and *D* values were found to be 49.8, 19.0, and 5.4 min, and 165.4, 63.2, and 17.8 min, respectively, for 60, 70, and 80 °C [19]. A study conducted by Ünal [28], was reported the rate constant (*k*) values at 60, 65, 70, and 75 °C for Anamur PPO as 0.0081, 0.0127, 0.0250, and 0.0948 /min, respectively, and it was found to be higher than the myrtle PPO.

The activation energy (*Ea*) for heat inactivation of PPO from myrtle was calculated to be 67.2 kJ/mol by the Arrhenius plot (Fig. 6b). While this value was higher than *Ea* for PPO from soursop (40.97 kJ/mol) [25], it was lower than from Red Fuji apple (139.2 kJ/mol) [43]. If the graph of log *D* values is drawn against the temperature values (60, 70, 80 °C), a graph as in (Fig. 6c) is obtained and the slope of the line gives us the value of 1/*Z*. By using this graph, *Z* value of PPO from myrtle was calculated to be 33.4 °C. The higher the *Z* value, which is an indicator of the enzyme's thermal resistance, indicates that the enzyme is more resistant to temperature. On the other hand, high activation energy (*Ea*) indicates the sensitivity of the enzyme to temperature changes [38].

Şener et al. identified two different isoenzymes for PPO in their study on the biochemical properties of PPO in loquat fruit. While the activation energy value (kj/mol) was 69.4 for isoenzyme A, it was 183.2 for isoenzyme B. They found the *Z* (°C) value as 32.4 and 12.3 for isoenzyme A and B, respectively [30]. It can be said that the activation energy (*Ea*) and *Z* value of myrtle PPO are similar to the results of isoenzyme A and it is resistant to temperature change compared to isoenzyme B. When we look at the activation energy values for different fruits, for example, the *Ea* value for apple PPO is 111.316 kj/mol, the *Ea* value for avocado PPO is 363 kj/mol, and the *Ea* value for pea PPO is 117.43 kj/mol [30]. Our results are within the range of these values.

Conclusion

This study, it was aimed to determine and purify some biochemical properties, kinetic parameters, the thermal stability of the PPO enzyme of myrtle fruit. The purification fold of myrtle PPO was found to be 5.5 by gradual elution using 10–200 mM phosphate buffer in DEAE-Toyopearl 650 resin. The optimum pH and temperature values for PPO activity were found to be 6.8 and 30 °C, respectively. The kinetic parameters *K*m of the myrtle PPO was 3.34 mM, and the kinetic parameters *V*max was 4.21 units/mLmin. According to the thermal inactivation results, the reaction rate constants (*k*) at 60, 70, and 80 °C were found to be 0.0282, 0.0526, and 0.1117 /min, respectively. Half-life ($t_{1/2}$, min) at 60, 70, and 80 °C were 24.6, 13.2, and 6.2, respectively, while *D* values were 81.6, 43.8, and 20.6 min. Activation energy (*Ea*) and *Z* values for myrtle PPO were found to be 67.2 kJ/mol ($r^2 = 0.9949$) and 33.4 °C ($r^2 = 0.9970$), respectively. When the inhibitory effects of ascorbic acid and sodium disulfide inhibitors on myrtle PPO were examined, it was observed that ascorbic acid and sodium disulfide at the same concentration values had approximately the same inhibition percentages on the enzyme. In addition, it is thought that determining the biochemical properties of the PPO enzyme isolated from myrtle fruit, which has the potential to process different food products, will be a guide in terms of processing temperature and other parameters that may affect the quality of the processing of the fruit into different food products.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects.

Informed consent The authors agreed to submit the manuscript to the Journal of Food Measurement and Characterization.

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