Research Article [Araştırma Makalesi]

Characterization of polyphenol oxidase during three ripening stages of an eggplant (*Solanum melongena* L.) fruit: a local type in northeast Anatolia

[Patlıcan (*Solanum melongena* L.) meyvesinin polifenol oksidazının üç olgunlaşma safhası boyunca karakterizasyonu: Doğu Anadolu'da yerel bir tip]

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ABSTRACT

Objective: A relatively new local type of eggplant, *Solanum melongena* L. 'Kadife' is widely consumed in Turkey because of its economic availability and good nutritional qualities. However, the high polyphenol content of eggplant renders it susceptible to unattractive oxidative browning catalyzed by polyphenol oxides (PPOs). Therefore, the main aim of this study was to characterize PPO in this eggplant cultivar at three stages of its development.

Methods: In this study, we determined substrate specificity, optimum pH and temperature, and optimum substrate concentration of the partial purified eggplant fruits PPO during ripening.

Results: *L*-DOPA was proved to be the preferred PPO substrate in all three stages of ripening. Optimum activity was observed at pH 7.0 for PPO in extracts of ripening and overly-ripe eggplant, while activity in extracts of immature eggplant exhibited a broad pH optimum between, pH 5.0 and 7.0. In general, at all ripening stages, PPO was most active at 30°C and was most sensitive to inhibition by sodium metabisulphite and ascorbic acid. The metal ions (Hg^{2+}, Mn^{2+}, Fe^{3+} and Co^{2+}) mostly inhibited PPO activities.

Conclusion: These data regarding the properties of PPO should enhance understanding of the browning reaction in eggplant and lead to the development of techniques for controlling this undesirable process.

Key Words: Browning, eggplant, polyphenol oxidase, ripening, *Solanum melongena*

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Kısmen yeni bir yerel tip patlıcan çeşidi *Solanum melongena* L. 'Kadife', ekonomik uygunluğu ve iyi besin kalitesinden dolayı Türkiye'de yaygın olarak tüketilir. Bununla birlikte, patlıcanın yüksek polifenol içeriği, bu meyveyi polifenol oksidaz (PFO) tarafından katalizlenen istenmeyen oksidatif esmerleşmeye karşı duyarlı kılar. Dolayısıyla, bu çalışmanın temel amacı, gelişiminin üç safhasında bu patlıcan kültivarındaki PFO'yu karakterize etmektir.

Metod: Bu çalışmada, olgunlaşma boyunca patlıcan meyvelerinden elde edilen kısmi saflaştırılmış PFO'nun substrat özgünlüğünü, optimum pH ve sıcaklığını, ve optimum substrat konsantrasyonunu belirledik.

Bulgular: Olgunlaşmanın her üç safhasında, *L*-DOPA'nın tercih edilen PFO substratı olduğu ortaya konuldu. Olgunlaşmamış patlıcan özütlerinde optimum pH 5.0 ile 7.0 arasında geniş bir aktivite gösterirken olgun ve aşırı olgun patlıcan özütlerindeki PFO aktivitesi için optimum pH'nın 7.0 olduğu gözlendi. Genel olarak, tüm olgunlaşma safhalarında, PFO, 30°C'de en aktiftir ve inhibitör olarak sodyum metabisülfit ve askorbik aside oldukça duyarlıdır. Metal iyonları (Hg^{2+}, Mn^{2+}, Fe^{3+} ve Co^{2+}) çoğunlukla PPO aktivitesini inhibe ettiler.

Sonuç: PFO'nun özelliklerine ilişkin bu veriler, patlıcandaki esmerleşme reaksiyonunun anlaşılmasını arttırmalı ve istenmeyen bu sürecin kontrolü için teknikler geliştirmeye öncülük etmelidir.

Anahtar Kelimeler: Esmerleşme, patlıcan, polifenol oksidaz, olgunlaşma, *Solanum melongena*

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Eggplant (*Solanum melongena* L.) or aubergine is one of the most important vegetable crops, grown on over 1.7 million ha world-wide [1]. On a world-wide basis, the fruit of most commercial eggplant varieties are purple, but it is highly variable for fruit color, as well as fruit shape and size [2]. Since eggplant is an important source of essential nutrients and polyphenolics, identification of eggplant varieties with a high nutrient content would be particularly beneficial, especially to low-income consumers. *In vitro* studies have shown that eggplant extracts suppress the development of blood vessels required for tumor growth and metastasis [3], inhibit protein-activated receptor 2 inflammation that has been implicated in atherosclerosis [4]. Nevertheless, one drawback of increasing the concentration of the polyphenolic antioxidants in eggplant is that their oxidation causes browning of the fruit flesh after exposure to the air, which may in turn reduce the apparent quality of the eggplant in consumers' eyes.

Despite the high antioxidant capacity of eggplant attributable to its high content of phenolic compounds, the strong tendency of eggplant fruit to undergo browning remains a major problem during storage and processing. Preserving the quality and nutritional value of fruits and vegetables between harvest and consumption is widely agreed to be very important. Eggplant quality and shelf-life are markedly reduced by development of skin and tissue browning [5]. The unattractive appearance and concomitant off-flavor development associated with browning adversely affects consumer acceptability and palatability [6]. Furthermore, the brown pigments may change the biochemical and nutritional characteristics of eggplant. In general, browning of foods is caused by the enzyme-catalyzed oxidation of naturally-occurring phenolic compounds by polyphenol oxidase (PPO).

PPO is a bifunctional copper protein complex that catalyzes the cresolase reaction (the *o*-hydroxylation of monophenols to ortho-diphenols) and the catecholase reaction (the oxidation of orthodiphenols to ortho-quinones) [7]. Quinones are highly reactive molecules capable of polymerization, leading to the formation of brown pigments [8]. Especially, in eggplant the problem is intensified because of its high phenolic content of substances [7].

The activity and properties of PPO have been extensively studied in broccoli, apples, avocado, yacon roots and many other plants [8-11]. However, in most of these studies, the stage of development of the plant was not specified. The literature also contains several reports of PPO in eggplant. However, in general these did not involve investigation of PPO across a range of developmental stages. Therefore, in order to acquire new data regarding PPOs at different developmental stages of eggplant fruit, we studied the activity level and properties of PPO in extracts of *S. melongena* fruit during ripening. To achieve these aims, we collected fruit samples of a local eggplant, which has been named as "Kadife" for years by the local growers in northeast Anatolia (Turkey), at three different stages of maturation. The plant, with its light purple and white striped fruit is widely cultivated and consumed in Trabzon and elsewhere in the region compared to other species. Specifically, our aim was to determine PPO-specific activity from eggplants at time of flowering and at intervals of 2 weeks thereafter. In addition, we studied the effects of pH and temperature, as well as the effects of a variety of inhibitors and metal ions, on PPO activity.

Materials and Methods

Plant material

The seeds belonging to the eggplant (*Solanum melongena*) were obtained from local growers, but they have not certificated yet. They were grown within and near the campus of Karadeniz Technical University's (Trabzon) several open fields during summer. Standard cultivation techniques were used for eggplant in the field. Eight plants were separated in each field and the fruits were harvested at three different stages of maturation. The flowers were considered to be in full bloom on June 24, 2010 and the fruit was sampled after 17th, 30th and 43rd days of full bloom. The fruit was divided into three distinct maturity classes: stage 1 (immature, 17 days), stage 2 (ripening, 30 days) and stage 3 (overly-ripe, 43 days) (Table 1). The fruits for each stage were treated with liquid nitrogen and stored at -80ºC and freeze-dried.

Reagents and chemicals

All chemicals and reagents were analytical grade and purchased from the Merck A.G. (Darmstadt, Germany) and from the Sigma Chemical Company (St. Louis, USA).

Enzyme preparation

Crude enzyme fractions were prepared as described previously elsewhere [12]. Briefly, eggplant fruits (10 g each) were placed in a dewar flask under liquid nitrogen and homogenized at 4°C for 2 min using a blender and 10 mL of ice-cold 50 mM phosphate buffer (pH 7.0) containing 2 mM ethylenediaminetetraacetic acid (EDTA), 1 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1% (w/v) polyvinylpolypyrolidone (PVPP) and 6% (w/v) Triton X-114. The homogenate was filtered before centrifugation in a Sigma 2-16 K centrifuge at 15,000 rpm at 4°C for 20 min. Then, the enzyme extract was precipitated by using an equal volume of cold acetone and the precipitate was dissolved in phosphate buffer (pH 7.0). This solution was utilized as the enzyme sample. Small volumes was separated into eppendorf tubes and stored at -20°C. A new sample was used for each measurement which was repeated and averaged. Thus, losing the stability of the enzyme was prevented over the time.

Protein determination

The soluble protein content of the enzyme preparations (*n*=3) was determined according to the method of Lowry et al. [13].

Enzyme assay

PPO activity was determined in the presence of 3-methyl-2-benzothiazolinone hydrazone (MBTH) by measuring the increase in absorbance at 494 nm for 4-methylcatechol (4-MC) and 500 nm for all other substrates at pH 7.0 (50 mM potassium phosphate buffer) and room temperature. Enzyme activity was assayed using a 1 mL sample cuvette containing 100 μL of substrates (stock 100 mM), an equal volume of MBTH (stock 10 mM) and 20 μL of dimethylformamide (DMF). Subsequently, 50 μL of eggplant extract was added to initiate the reaction. One unit of PPO activity was defined as 1 μM of product formed per mL of reaction medium. Specific activity was defined as units of enzyme activity per milligram of protein [9,12].

Electrophoresis of PPO

Native-PAGE (8%, w/v) electrophoresis was performed as described by Laemmli [14]. Then, the gel was stained for 30 min in 24 mM *L*-3,4-dihydroxyphenylalanine (*L*-DOPA) in water to reveal the location of the brown-staining PPO bands.

Characterization of PPO

Substrate specificity

Catechol, 4-MC and *L*-DOPA as diphenolic substrates, and *p*-hydroxyphenyl propionic acid (PHPPA) and tyrosine as monophenolic substrates were used to monitor PPO activity. The rate of the reaction was measured in terms of the increase in absorbance at the absorption maxima of the corresponding quinone products for each substrate [9].

Effects of pH and temperature on PPO activity

The effect of pH on PPO activity was determined using 100 mM of *L*-DOPA as substrate and the following buffers (50 mM): glicine-HCl, pH 3.0; sodium acetate, pH 4.0 and 5.0; potassium phosphate, pH 6.0 and 7.0 and Tris-HCl, pH 8.0 and 9.0.

The optimum temperature for PPO activity was determined by measuring enzyme activity at various temperatures over 0°C to 80°C.

Enzyme kinetics

PPO activity was monitored by using 0.5-50 mM *L*-DOPA at pH 7.0. The kinetic data was analyzed as 1/specific activity (1/V) against 1/substrate concentration (1/[S]). The Michaelis-Menten constant (Km) and maximum velocity (V_{max}) parameters were obtained from Lineweaver-Burk plots [15].

Effect of various inhibitors on PPO activity

Inhibition of PPO by sodium azide (1-60 mM), sodium metabisulfite (0.01-5 mM), ascorbic acid (0.01-5 mM) and thiourea (0.01-5 mM) was determined at 500 nm using *L*-DOPA as substrate. The concentration of inhibitor causing 50% reduction of PPO activity (IC_{50}) was determined graphically by plotting the percentage of control activity (no inhibitor) against inhibitor concentration.

Effect of metal ions on PPO activity

The effect of 1 mM of the following metal ions on PPO activity was determined: K^+, Ni^{2+}, Zn^{2+}, Hg^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}, Ca^{2+}, Al^{3+} and Fe^{3+}. The percentages of remaining activities were expressed by comparison with the standard assay mixture with no metal ion added [16,17].

Results and Discussion

During the eggplant fruit ripening and over-ripening, rapid enzymatic browning and pronounced changes were observed in the color of the mesocarp throughout the three stages of ripening. The variations in the color of the skin, pulp and state of maturity during fruit maturation of the eggplant are summarized in Table 1. As indicated in the table, the color of the skin of the fruit varied from whitish to purple, fruit hardness from rather hard to rather soft, and the length and diameter of the fruit from 6.32 to 21.73 cm and 1.7 to 3.51 cm, respectively, between 17 and 43 days.

Table 1. Variation in the colour of the skin and pulp of eggplant (*S. melongena*) fruit at different stages of ripening

Harvest date	DAFB*	Fruit ripeness	Fruit and pulp colour	Fruit length (cm)	Fruit diameter (cm)
11 July 2010	17	Immature	Skin whitish (75%) at background and slightly purple with longitudinal sparsely distributed purple stripes (25%), fruit rather hard	6.32±0.19 a	1.74±0.03 a
24 July 2010	30	Ripe	Skin whitish (47%) at background, sometimes divided into frequent open purple stripes longitudinally sparsely distributed (53%), fruit table soft	13.65±0.83 b	2.46±0.16 b
06 August 2010	43	Over-ripe	Skin whitish (30%) entangled with longitudinally elongating purple stripes at background (70%), fruit table soft or a little bit over it	21.73±0.80 c	3.51±0.18 c

*DAFB: Days after full bloom

Table 2. Substrate specificity of PPO from eggplant (*S. melongena*) fruit throughout ripening and over ripening at three stages

Substrates (100 mM)	Relative Activity (%)					
	Enzyme Extraction Buffer					
	pH 7.0			pH 5.0		
	Days After Full Bloom (DAFB)					
	17	30	43	17	30	43
Monophenols						
L-Tyrosine	27	38	85	15	12	71
PHPPA	None	None	8	None	15	None
Diphenols						
4-Methylcatechol	17	None	None	100	100	100
Catechol	36	None	None	52	44	53
L-DOPA	100	100	100	47	50	50

A good correlation was obtained between fruit length and diameter (r=0.956, *P*<0.05). A rapid increase in the fruit length and diameter, approximately 3.44 and 2.02-fold, respectively, took place in accordance with the above characteristics between early immature fruit and the late stage of maturity as the fruit progressed to texture softening.

In this study, we aimed to determine the optimum harvest time with regard to consumer preference and exportation considerations. It can be concluded that harvest time or season, or even the state of maturity, may have a considerable effect on the distribution of PPO isoforms. However, to the best of our knowledge, there is no information in the literature concerning PPO during eggplant fruit's rapid ripening and enzymatic browning through stages in different genotypes.

The PPO of the cultivar in this study showed activity with various substrates, such as tyrosine and PHPPA, as monophenolic substrates, and 4-MC, catechol and *L*-DOPA as diphenolic substrates. The enzyme extractions were performed at pH 5.0 and pH 7.0, separately and observed activities are shown in Table 2. It has been known that types and relative concentrations of natural phenols vary widely in different plant sources. The substrate specificity of PPO, therefore similarly depends on the species and the cultivar. Compounds used in this study as substrates were also used as substrates for other plant PPOs [11,18]. In further studies, enzyme extracts prepared at pH 7.0 and *L*-DOPA substrate were used because they supported the highest specific activity at all stages of development. This result is consistent with a previous report from another fruit, potato [19]. Searching literature revealed little information in terms of using *L*-DOPA as the substrate, and no definitive reports during ripening and over-ripening.

In this study, using *L*-DOPA as a substrate, native polyacrylamide gel electrophoresis revealed the presence of at least two prominent PPO isoforms in eggplant fruits at each of the three stages of development (Figure 1). In general, variations in the PPO isoenzyme pattern ranged from two to four bands that have also been observed in other fruits, such as papaya [20] and medlar [12]. There are few published studies of PPO isoenzymes in eggplant fruits, and none for the eggplant fruit examined in the present study. However, cultivar variations and differentiation in genotype affecting the distribution of PPO isoforms have been reported. The development of the ripening stages and the harvesting time also affect the pattern of PPO isoforms [12].

The activity of PPO in extracts of eggplant at stages 1, 2 and 3 was measured at different pH values ranging from 3.0 to 9.0 using *L*-DOPA as substrate. Although the pH optimum of PPO activity in enzyme extracts was 7.0 in the fruits that reached ripe and overly-ripe maturity, there
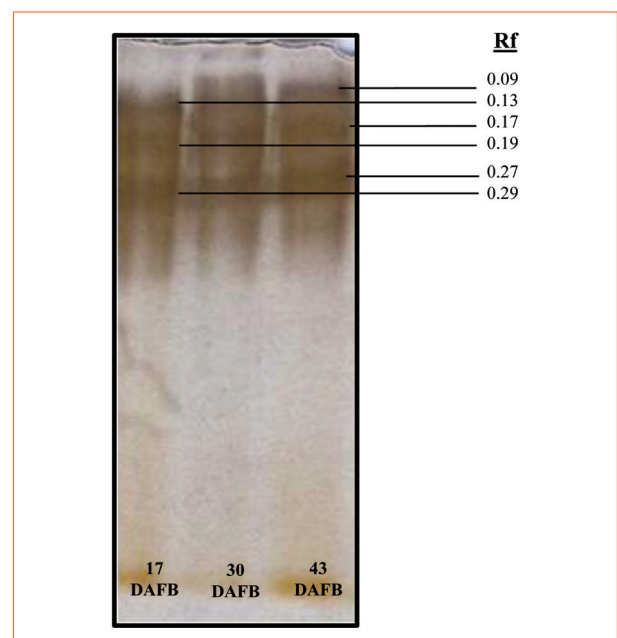

Figure 1. Native polyacrylamide gel electrophoresis from eggplant (*S. melongena*) PPO stained by *L*-DOPA at three stages of ripening. DAFB: Days after full bloom.

was a broad pH optimum between pH 5.0 and 8.0 for the immature stage (Figure 2a). pH alters the charge of amino acid side chains or the ionization of the substrates, and therefore is the determining factor in the expression of enzymatic activity. Maximum PPO activity has been obtained at or near neutral pH values in most plants [12,21]. In this study, PPO from eggplant fruits demonstrated marked decreases in activity below pH 5.0 and a slight decrease above pH 8.0 (Figure 2a). pH 7.0 was chosen for analyzing PPO activity at each stage of the eggplant fruit. The optimum pH of PPO varies with the source of the enzyme and also depends on the phenolic substrate chosen for the assay. A range of values of maximum PPO activity has been reported in the literature of between pH 4.0 and 8.0 for enzymes from several sources with various substrates [11]. For example, pH optimum values of 7.0 for marula [22] and the Amasya apple [23] and 6.0-9.0 for sweet basil [24] have been reported.

The temperature dependence of PPO activity in each of the three extracts of the eggplant fruit measured at pH 7.0 using L-DOPA between 0°C and 80°C (Figure 2b). PPO activity was almost highest between 10°C and 30°C for all stages. As a different, the temperature dependence of PPO activity in the extracts of fruit at immature maturity was much lower at temperatures greater than 30°C. However, even at 80°C, the enzyme still exhibited activity through ripe to over-ripe maturity. Despite many investigations of the optimum temperatures related to species- and substrate-dependent PPO [11 25], there is little information available on the effect of developmental stage on PPO activity [12]. Optimal temperatures for PPO activity have been reported by other authors [6,20,23,26]. While the values of optimum temperatures related to plant sources range from 18°C to 40°C, there are some exceptional differences in the literature. Fruits of a cherry laurel cultivar (*Laurocerasus officinalis* 'Oxygemmis') exhibited a temperature optimum at 40°C; however, the PPO activity of that fruit decreased to less than 50% with temperature increases to 80°C [27]. Apart from the cherry laurel fruit, the activities of PPO in mulberry [28] and medlar [12] fruits were also reported at 75°C and 70°C, respectively.

Table 3. Kinetic constants for the oxidation of L-DOPA by the eggplant (*S. melongena*) PPO throught ripening and over ripening at three stages

Stage of maturity	K_m (mM)	V_{max} (U/mg protein)	V_{max}/K_m
17 DAFB*	33.3	142.8	4.29
30 DAFB	86.0	1000	11.6
43 DAFB	25.4	200.0	7.87

*DAFB: Days after full bloom

K_m and V_{max} values for all maturation stages were calculated from Lineweaver-Burk plot by estimating activities toward a series of L-DOPA concentrations (0.5-50 mM) at constant protein concentration and optimum conditions. Whereas the lowest K_m values for PPO were found only at the beginning of the immature and at the overly-ripe stages of maturity, the highest V_{max} value was seen in particular at the ripe stage of maturity (Table 3). The K_m value depends on the structure of the enzyme, pH, temperature and ionic strength [21]. Table 3 shows, using L-DOPA as the diphenolic substrate, that the enzyme kinetic constants, K_m and V_{max}, depended on the stage of the plant. K_m and V_{max} values were in the range of 25.4-86 mM and 142.8-1000 U/mg protein, respectively. In terms of K_m values, these results were consistent with previous reports [12,23]. On the other hand, values obtained for the present eggplant fruit's PPO for each stage are lower than the 682.5 mM value reported for cabbage [29] and higher than the 2 mM reported for lettuce [21] and 1.62 mM for *Ocimum basilicum* [24]. Because of the high antioxidant capacity and phenolic compounds in eggplant fruits [30], the reduction in K_m and V_{max} values at the third stage of maturation is a natural outcome. In terms of maturation, the expression level of each isoenzyme can be variable. Since we did not perform exact purification, kinetic characteristics can be variable too, depending on at least one of the PPO isoenzymes of the present study, which was not dominant at any stage of maturation.

Enzymatic browning can be prevented by using inhibitors. We chose to study, thiourea, sodium metabisulfite,
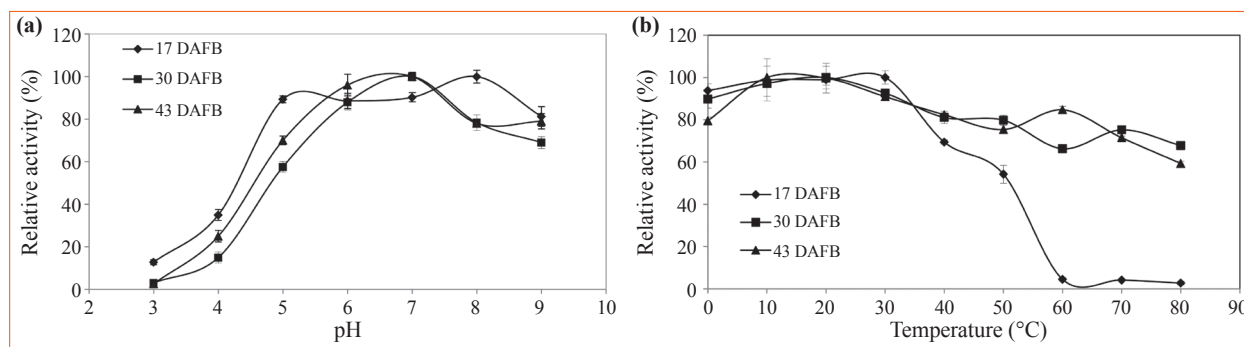

Figure 2. Optimum pH **(a)** and temperature **(b)** profiles in eggplant (*S. melongena*) fruit PPO at three stages maturation. The buffers were used for pH activity glycine-HCl (pH 3.0), acetate (pH 4.0 and 5.0), phosphate (pH 6.0 and 7.0) and Tris-HCl (pH 8.0 and 9.0). DAFB: Days after full bloom.

ascorbic acid and sodium azide because other investigators have demonstrated that these compounds inhibit PPO activity to one extent or another, depending on the stage of the plant representing the source of PPO. In the presence of L-DOPA as substrate, each inhibitor was tested over a wide range of concentrations sufficient to reduce PPO activity by 50% or more. Table 4a summarizes the IC_{50} values for each inhibitor when tested against PPO prepared from eggplant at the three stages of development. Results show that the most effective inhibitors of PPO activity were sodium metabisulfite and ascorbic acid. These findings are consistent with earlier reports, such as loquat [26], mamey [31] and mulberry [32]. Thiourea and sodium azide were ineffective inhibitors at all stages of eggplant fruit maturation. In addition, PPO activity at the overly-ripe stage of maturity was more sensitive to inhibition by sodium metabisulfite and ascorbic acid compared to PPO in extracts of plants, particularly at the immature and ripe stages of maturity, respectively. The mechanism of inhibition by ascorbate may involve reduction of quinonoid compounds produced by the diphenolases, and chelation of the copper at the active site and reduction of Cu^{2+} to Cu^+ [10]. Inhibition assays indicate that thiol compounds, such as sodium metabisulfite, are one of the potent inhibitors the PPO enzyme [26,33].

Since previous studies showed that various metal ions either inhibited or stimulated PPO activity, the fruit extracts of the present eggplant we studied at the three stages of fruit maturity were assayed for PPO activity at pH 7.0 using L-DOPA in the presence of a 1 mM final concentration of each of the following metal chlorides: K^+, Ca^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, Fe^{3+}. The inhibitory effect of the chosen metals varied greatly and was strongly stage-dependent (Table 4b). For example, Hg^{2+}, Mn^{2+}, Fe^{3+} and Co^{2+} were the most potent inhibitors of PPO activity. However, while Fe^{3+} inhibited 100% of PPO activity in extracts of fruits at the initial (immature) stage of maturity (17 days), it inhibited PPO activity at a level of only 32% in overly-ripe fruit at 43 days' maturity. None of the metal ions caused significant stimulation of PPO activity of the fruits throughout maturation. In terms of Hg^{2+}, similar results have been reported for medlar PPO [12]. Since metal ions may have different coordination numbers and geometries in their coordination structures, and potentials as Lewis acids, they may behave differently toward proteins as ligands. These differences may also result in metal binding to different sites, and therefore, disturb the enzyme structure in different ways.

This study quantified eggplant PPO activity during the ripening and over-ripening periods. We conclude that PPOs isolated from the present local eggplant fruits (Kadife) exhibit an activity which is stage-dependent. The enzyme is a catecholase, active toward diphenols, and has the greatest substrate specificity towards L-DOPA for all three stages. Harvest time, season, fruit maturity and genotype differences during ripening result in con-

Table 4. Inhibition of eggplant (*S. melongena*) fruits' enzyme by some general PPO inhibitors **(a)** and effects of various metal ions[a] **(b)** during ripening and over ripening at three stages (DAFB: Days after full bloom)

(a)

Substrate	IC_{50} (mM)		
	17 DAFB	30 DAFB	43 DAFB
Sodium azide	142	450	100
Sodium metabisulfite	0.21	1.0	0.03
Ascorbic acid	0.24	0.1	0.04
Thiourea	95	42.5	32.4

(b)

Metal ions	Relative activity (%)		
	17 DAFB	30 DAFB	43 DAFB
None	100±1.0	100±1.0	100±1.0
K^+	90±1.0	103±1.0	102±1.0
Ca^{2+}	98±1.3	102±2.1	113±1.7
Co^{2+}	30±2.1	38±2.3	103±1.5
Cu^{2+}	86±1.1	88±1.6	86±0.5
Hg^{2+}	18±1.1	91±1.1	57±1.0
Mn^{2+}	25±2.2	67±1.2	92±2.4
Ni^{2+}	107±1.6	101±2.6	112±2.0
Zn^{2+}	91±2.6	102±1.9	95±1.6
Al^{3+}	107±1.0	106±1.4	111±1.1
Fe^{3+}	0±1.2	11±3.6	68±1.1

[a]Values, means±standard deviation of triplicate determinations (*n*=3).

siderable effects in the distribution of PPO isoforms and their activity and characterization in eggplant fruit. Eggplant fruit has a high antioxidant capacity, attributed to high phenolic compound content [30]. The oxidation of phenolics, a drawback of increasing the concentration of these antioxidants in eggplant, triggers browning of the fruit. The reduction of the apparent quality therefore affects consumption and exportation of eggplant fruits. It is extremely important to take into account the stage of maturity of the fruit in order to optimize the health benefits of consuming eggplants in terms of enzymatic browning, storage and industrial processing. Moreover, acknowledge of the properties of eggplant PPOs will help producers and consumers control the browning reaction. Consequently, in the light of our experimental results, as a potent local type, the present eggplant fruit (Kadife) should be consumed at stage 3 (ripe maturity following 30 days of maturation after 6 weeks of full bloom).

Conflict of Interest

There are no conflicts of interest among the authors.

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