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Investigation of the effects of pesticides on 'Jonagold' apple (*Malus x domestica*) polyphenol oxidase enzyme activity

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Abstract: In this study, the effects of a number of commonly used pesticides on the activity of the polyphenol oxidase (PPO) enzyme from pesticide-free and pesticide-applied 'Jonagold' apples were comparatively evaluated. Substrates 4-methylcatechol, pyrocatechol, pyrogallol and L-tyrosine were used to determine the substrate specificity of the PPO enzyme obtained from apple. According to the results, on the contrary, PPO enzyme from 'Jonagold' apple did not show any activity against L-tyrosine substrate which is a monophenolic substrate, the enzyme had high affinity against 4-methylcatechol, pyrocatechol and pyrogallol which are di and tri phenolic substrates. K_m values of PPO enzyme obtained from pesticide-free apples against 4-methylcatechol, pyrocatechol, and pyrogallol substrates were determined as followed 0.27 mM, 2.27 mM, and 0.37 mM, respectively. V_{max} values were found as 0.133 mM/min, 0.081 mM/min, and 0.051 mM/min. Optimum pH values were found to be 4.5 for 4-methylcatechol, 7.0 for pyrocatechol, and 7.5 for pyrogallol. Optimum temperature values were determined as 40 °C for 4-methylcatechol, 10 °C for pyrocatechol, and 50 °C for pyrogallol. K_m values for PPO enzyme activity obtained from pesticide-treated apples against 4-methylcatechol, pyrocatechol, and pyrogallol substrates were 0.98 mM, 3.94 mM, and 0.37 mM, V_{max} values were 0.08 mM/min, 0.02 mM/min, and 0.034 mM/min. Optimum pH values were found to be 7.0 for 4-methylcatechol and pyrocatechol and 7.5 for pyrogallol. Optimum temperature values were determined as 50 °C for 4-methylcatechol, 30 °C for pyrocatechol, and 40 °C for pyrogallol. Overall, the results showed that the PPO enzyme from pesticide-free apples had higher activity than pesticide-treated apples. The effects of metals and storage stability on PPO enzyme activity were also investigated. The results reveal that pesticide use affects PPO enzyme activity. The obtained data bring to light new pesticide functional features of great interest in the medicinal, agro-chemical and environmental circles.

Key words: Apple, kinetic characterization, purification, inhibition

1. Introduction

Pesticides are usually applied simultaneously or one after another for crop protection, and such pesticide application can impair the activities of various enzymes in the fruit structure, as it usually stays which significantly affects the value of the fruit remains in the fruit for a long time. According to the UN's Food and Agricultural Organization, pesticides means any substance or mixture of substances intended to prevent, destroy or control any pest and vectors of human or animal diseases, unwanted species, including insecticides, fungicides, herbicides, and disinfectants. Substances that may be administered to animals for the control of plants or animals or insects, spiders, or other pests on or on their bodies, which cause harm or otherwise interfere with the production, processing, storage, transportation, or marketing of food, agricultural products, wood and wood products, or animal feed. Otherwise, pesticides are compounds recommended for use as plant growth organizers, desiccants, defoliants, or compounds to thin fruit or prevent mutational fruit shedding, and are compounds used in crops before or after harvest to protect crops from damage during transport and storage (FAOSTAT, 2002). The use of pesticides is inevitable in growing agricultural products. Nevertheless, the unconscious increase in pesticide use in the last years has significantly affected both environmental pollution and human health. Food safety is a growing area of concern worldwide due to its direct impact on human health (Darko and Akoto, 2008). In recent years, the presence of harmful pesticide residues (insecticide, fungicide, etc.) has caused great concern in foods (Karalliedde et al., 2003). Despite their ability to protect crops and human health,

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their overutilization and misuse, their instability, and their ability to be transported over long distances ultimately cause widespread environmental pollution (Singha et al., 2008). In addition, since very little pesticide is usually involved directly in the pesticide's mechanism of action, many of these chemicals end up as deposits and "waste" in the environment where they are active (Kaushik et al., 2009). These residues can affect both target and nontarget organisms (Sinan et al., 2007). Some part of these chemicals has structural similarities with natural phenolic substrates for PPO. This has inspired scientists to invent biosensors that use immobilized enzymes to identify pesticide residues in environmental and food samples (Anh et al., 2002a, 2004b). A recent study (Rung and Schwack, 2005) showed that the parathion, aminoparathion, an organophosphorus insecticide can behave as a substrate for PPOs in forming red colored polymerized compounds. Polyphenol oxidases (PPO; EC 1.14.18.1 and EC 1.10.3.1), also known as tyrosinases or catechol oxidases, are metalloproteins containing copper atoms and protected by three histidine rings with amino acids in the active center. These enzymes, which are common in nature, are found in animals, plants, and microorganisms. They catalyze two different reactions; hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and oxidation of o-diphenols to *o*-quinones (diphenolase activity). Many researchers reported that PPO activity causes browning reactions in fruits and vegetables. Also, lots of researchers reported that the main reason for browning reactions, especially in apples, is due to PPO activity (Nicolas, et al., 1994). Therefore, a lot of work has been done to inhibit PPO activity. In one of these studies, it was determined that as a result of the characterization of the PPO enzyme purified from Jonagored apple, there was high activity against 4-methylcatechol and L-dopa substrates (Rocha et. al., 2001).

PPO enzyme was purified and characterized from Yomra apple cultivar grown in Turkey and sodium metabisulfite was found to be the most suitable inhibitor that inhibits Yomra PPO (Can et.al., 2014). PPO enzyme was purified from Amasya apple cultivar and inhibitor studies were carried out after characterization. A study showed that the most effective inhibitor was L-cysteine (Oktay et.al., 1995). Gianfreda and Rao studied the interaction of soil enzymes with pesticides (2004) and it was observed that pesticides increased enzymatic activity. 'Jonagold' (*Malus x domestic)* apple cultivar which belongs to *Rosaceae* family was used in our study. 'Jonagored' is a mutant of 'Jonagold' which is a controlled cross of 'Jonathan' with 'Golden Delicious' (Trillot, 1993). There is a lack of studies that have been done on 'Jonagold' apple PPO so that our study was carried out in a specific area in the 'Jonagold' apple growing area in Atmaca village of Prizren which is a city of Kosovo.

When the literature was examined, the absence of any study examining the effects of 'Jonagold' apple PPO and pesticides on this enzyme encouraged us to do this study. For this purpose, in our study, pesticide applied and untreated apples were grown in a special area in the 'Jonagold' apple growing area in Atmaca village of Prizren province of Kosovo. The data obtained show the effect of pesticides on Jonagold PPO. These results contain important information for the use of Jonagold apple PPO as a bioactive agent in biosensors to be designed for pesticide determination in the future. The obtained data bring to light new pesticide functional features of great interest in the medicinal, agro-chemical, and environmental circles.

2. Materials and methods

2.1. Plant materials (apples) and reagents

'Jonagold' apples used in this study were harvested from Atmaca (42°14'39.7"N 20°40'57.6"E) village of Prizren district of the Republic of Kosovo and stored at 4 °C. Polyvinyl pyrrolidone was purchased from company VWR. Dipotassium hydrogen phosphate, potassium dihydrogen phosphate and L-Ascorbic Acid were purchased from Lachner. Trizma base, Pyrogallol, Catechol, L-Tyrosine, and 4-methylcatechol were purchased from Acros, Germany. All other chemicals used were purchased from Fluka and Sigma Aldrich. All chemicals were analytical grade.

2.2. Pesticide application

In recent years, pesticides have been sprayed on fruits and vegetables to provide protection against external factors. Like other vegetables and fruits, pesticide spraying is done in 'Jonagold' apple. Certain apple trees (approximately 50 trees) were not sprayed so that they would not be affected by pesticide spraying on the 'Jonagold' apple plantation. These trees have been grown in a completely natural environment. Then the apples were collected and transported to the laboratory environment. In laboratory, PPO has been purified from 'Jonagold' apples. Based on this, the aim of the study is to compare the PPO activity (AWP = apple with pesticide) in pesticide-sprayed apples and the PPO activity in nonpesticide-sprayed apples (AFP = Pesticide Free Apple). While comparing, the difference between kinetic values such as optimum pH, temperature, Michealis-Menten constant (Km), maximum velocity (Vmax), resistance to metals, and storage stability were determined experimentally.

'Jonagold' apple trees that were previously detected in the apple growing area, Atmaca village of Prizren were marked and pesticides were applied to these trees, while the others were not.

In our study, there were about 400 trees in an area of one ha. Pesticide application was not applied to 1225

m^2 of one-ha area. This area was marked as the AFP area and there were about 50 'Jonagold' apple trees in this area. The apples in this AFP area were picked specifically from the extreme region. The reason is that, as a result of pesticide spraying in the AWP region, AFP apples were inadvertently exposed to pesticide spraying in the border area. Therefore, apples were especially selected and collected from the extreme area.

Pesticide application was made in two steps:

• In the first step, Acrobat (2 kg/ha), Kumulus (3 kg/ha), and Deltamethrin (0.4 l/ha) were used twice 2021, May.

• In the second step, Armethyl (2.5 kg/ha) and Grande (0.5 l/ha) were used for once 2021, June.

Apples were collected in middle of July (2021). After the apples were harvested, they were stored under the conditions described in section 2.1.

2.3. Enzyme extraction

30 g of 'Jonagold' apples were chopped and mixed with 30 mL of 0.1 M phosphate buffer (pH 7.0) containing 1% triton X-100, 1.5% polyvinyl pyrrolidone, and 5 mM ascorbic acid. It was completely homogenized using a blender for 5 min. The homogenate was then filtered and centrifuged at 5000 rpm for 20 min. Enzyme assays were carried out with the aid of a Thermo Scientific Genesys UV-VIS Spectrophotometer. Centrifugation was done until no precipitate formed in the extraction tubes. The supernatant obtained after centrifugation was used as the crude enzyme extract (Oktay et al., 1995).

2.4. Effect of pH

Optimal pH for 'Jonagold' apples PPO activity was determined using 10 mM catechol, 4-methylcatechol, and pyrogallol as substrate in buffers of pH values ranging from 3.0 to 9.0. The buffer systems used were citrate buffer for pH 3.0–5.0; phosphate buffer for pH 6.0–7.5; Tris-HCl buffer for 7.5–9.0. Observing absorbance increases at 420 nm for 60 s with the spectrophotometric method was performed for enzyme activity determinations.

2.5. Effect of temperature

PPO enzyme activity was measured at temperature values ranging from 0 °C to 80 °C to determine the optimum temperature of 'Jonagold' apple PPO using 10 mM catechol, 4-methylcatechol, and pyrogallol as substrate. The enzyme was incubated in phosphate buffer for 1 min at the appropriate temperature. After the incubation, the increase in absorbance was observed at 420 nm for 60 s with the addition of the substrate solution.

2.6. Substrate specificity

A substrate specificity study was carried out to determine which substrates the enzyme can transform and against which it has a higher affinity. The activity of the enzyme against four different substrates was determined. For this purpose, 4-methylcatechol, pyrocatechol, pyrogallol, and L-tyrosine were used as substrates. The Km value was calculated for each substrate and the substrates used were compared. Vmax and Km were calculated according to the Lineweaver and Burk method (Wong et al. 1971).

2.7. Enzyme kinetics

Substrate solutions ranging from 0.05 mM to 50 mM were used in kinetic studies. These different substrate solutions were used to determine the enzyme's Vmax and Km. Enzyme activity was monitored spectrophotometrically at 420 nm for 60 s. After the measurements were taken, the initial velocities were calculated using the absorbance-time graph. Km and Vmax constants were obtained after the necessary calculations were made in the Michaelis-Menten (substrate concentration [S] versus velocity (V)) and Lineweaver-Burk method (1/[S] versus 1/V) (Lineweaver and Burk, 1934).

2.8. Effect of metals

In order to measure and examine the effect of metals on PPO enzyme activity obtained from 'Jonagold' apples, metal solutions containing different concentrations of metal were prepared. Studies were carried out under standard conditions. The tested concentrations of metals were 0.5 mM, 1 mM, and 5 mM. The enzyme solution was incubated with metals in pH 7.0 phosphate buffers at 4 °C for 30 min. The reaction was initiated by adding 4-methylcatechol in constant concentration to the reaction medium. In the study, Al^{+3}, Ba^{+2}, Ca^{+2}, Co^{+2} Cu^{+}, Fe^{+3}, Mg^{+2}, Ni^{+2}, K^{+}, Na^{+} and Zn^{+2} metals were used. As a control, the reactions were performed under the same conditions in a metal-free environment and the results were compared (Cerrahoğlu and Arabacı, 2016).

2.9. Storage stability of the enzyme

The storage stability study of the PPO enzyme was carried out at 3 different temperatures, –20 °C, 4 °C, and room temperature (average 25 °C). In order to find the storage stability of the enzyme at room temperature, the decrease in its activity at room temperature was recorded by measuring the change in absorbance at 420 nm for 60 s. Here, 10 mM 4-methylcatechol was used as the substrate. In order to see the stability of the enzyme in storage at –20 °C, the enzyme was stored at –20 °C and recorded by measuring the absorbance value at 420 nm for 60 s once a week. Again, 10 mM 4-methylcatechol was used as the substrate. In order to measure the stability at 4 °C, activity was measured in the same way, but the measurement frequency was set to every 8–12 h (Cerrahoğlu and Arabacı, 2016).

3. Results

In this study, PPO enzyme was extracted and characterized from 'Jonagold' apple cultivar (*Malus x domestica*). In the

apple growing area, the trees were marked by dividing them in half. Some of the apple trees were pesticide-free, while others were affected by pesticide chemicals as described in section 2.3. They all grew up in their natural habitat. In order to optimize the pH and temperature values of PPO enzyme activity obtained from free and pesticide-treated apples, enzyme activity was determined at different pH and temperatures, and the results are shown in Figures 1 and 2. As shown in Figure 1 at the optimum pH values for AFP and AWP PPO enzyme, we can see that the optimum pH range is between 4.5 and 8.0.

When both PPO enzyme activities for each substrate are compared, it is seen that the optimum pH of 4-methylcatechol in AFP PPO enzyme is in the acidic region with pH 4.5, whereas it is in the neutral region with pH 7.0 in AWP PPO enzyme. Pyrocatechol has an optimum pH value similar to pH 7.0 in both enzyme activities. While pyrogallol has an optimum pH value with pH 7.5 in AFP PPO enzyme, it shifts its optimum pH value to the basic region with a pH of 8.0 in AWP PPO enzyme. In general, we can say that pesticide application affects the optimum pH of the substrate in enzyme activity.

In the present study, the optimum temperature of the enzyme activity from both sources was also determined. For this purpose, the enzyme solution was incubated for 1 min in the buffer at the desired temperature, and then its interaction with the substrate was measured in spectrophotometry. When the optimum temperature of PPO enzyme from the AWP and AFP in the presence of different substrates is compared, it is seen that the PPO enzyme from the AWP is active higher temperature than the one in the AFP in Figure 2. Figure 2 shows that the optimum temperature values have been changed from 10 to 60 °C for both PPO enzymes. When using only the pyrocatechol substrate, the optimum temperature with the highest PPO enzyme activity from AFP is 10 °C. By comparison, it can be seen that the PPO enzyme from pesticide-treated apples has activity at a higher temperature than the PPO enzyme from AFP.

Considering the kinetic activity and substrate specificity of the PPO enzyme obtained from two different sources, four different substrates were used and the results are presented in Table 1. These substrates were 4-methylcatechol, pyrocatechol, pyrogallol, and L-tyrosine. Among these substrates, both PPO enzymes did not have any activity with L-tyrosine as a monophenol substrate. The PPO enzyme from AWP and AFP showed very good activity with diphenolic (pyrocatechol and 4-methylcatechol) and triphenolic (pyrogallol) substrates (Table 1). When the kinetic values in Table 1 are examined, it is seen that the PPO enzyme from AFP has the best affinity for 4-methylcatechol with a Km value of 0.27 mM and Vmax/Km value of 0.492 (U/mM.min). Figure 3 shows that the Lineweaver–Burk plot for 4–methylcatechol as a substrate for AWP and AFP PPO enzyme. PPO enzyme from both sources has a similar affinity to pyrogallol with a Km value of 0.37 mM.

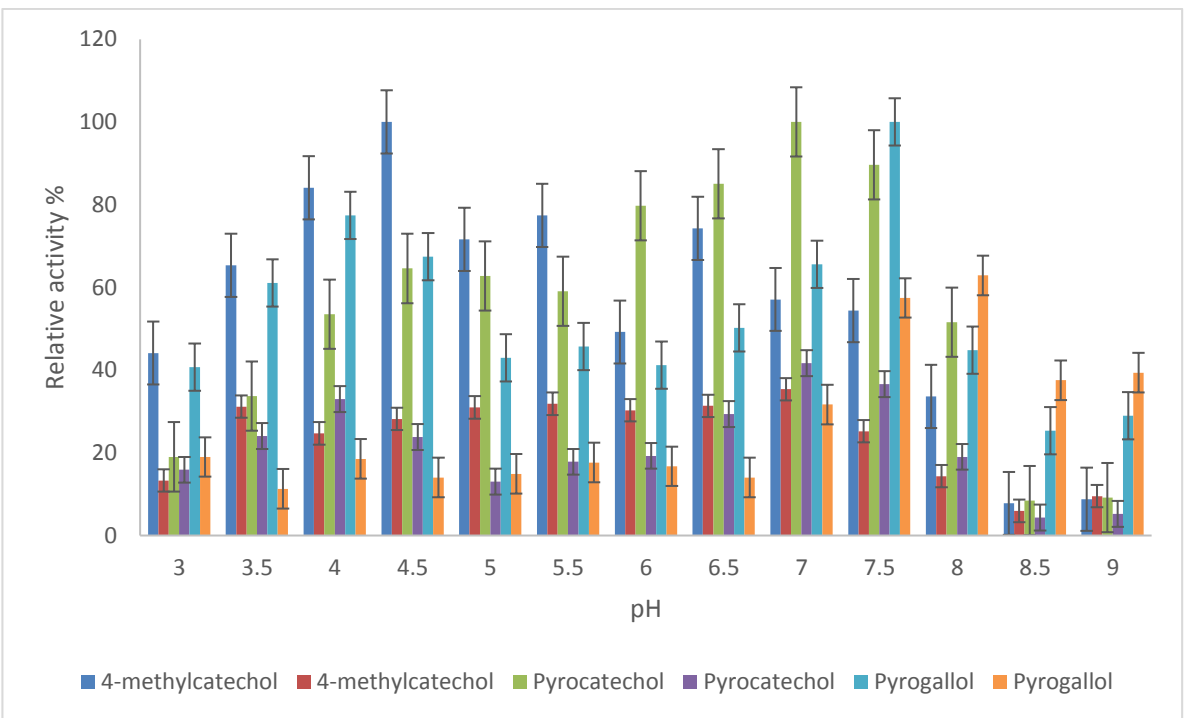

Figure 1. Comparison of optimum pH values of PPO enzyme activity from AWP and AFP in the presence of different substrates.

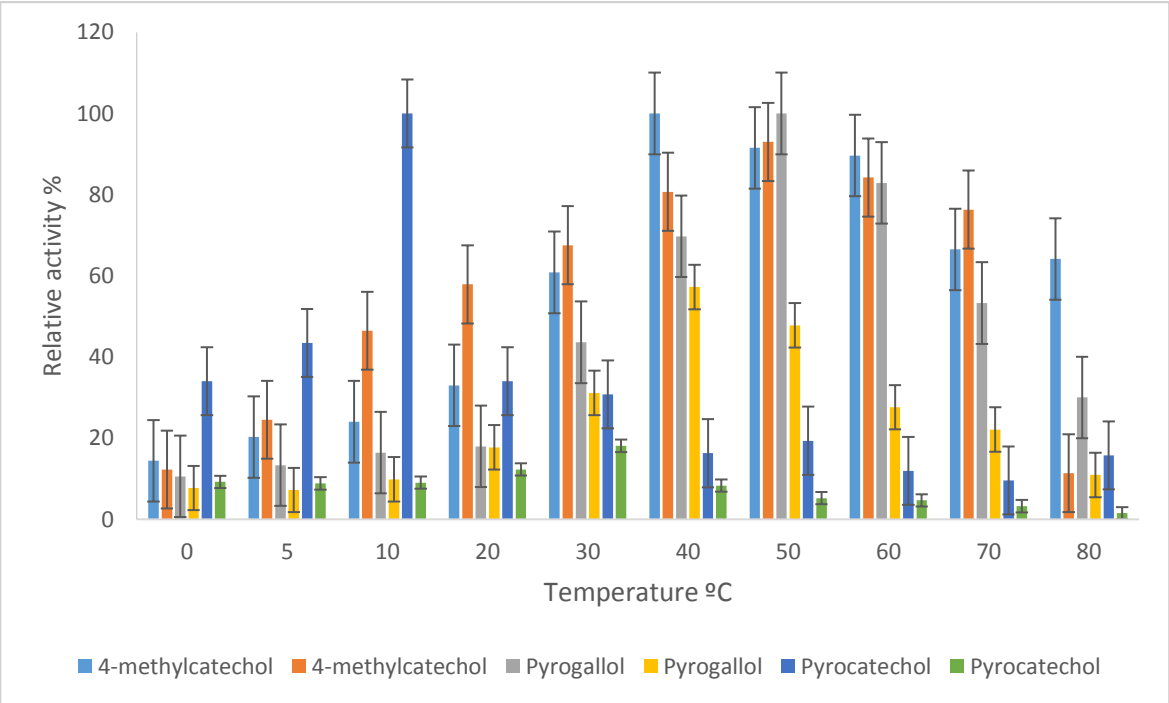

Figure 2. Comparison of optimum temperature values of PPO enzyme activity from AWP and AFP in the presence of different substrates.

Table 1. Optimum temperature, optimum pH, and kinetic parameters of PPO enzyme from AWP and AFP.

	Optimum pH		Optimum temperature (°C)		Km (mM)		Vmax (U/min.)		Vmax/Km (U/mM.min.)	
	AWP	AFP	AWP	AFP	AWP	AFP	AWP	AFP	AWP	AFP
4-methyl catechol	7.0 ± 0.2	4.5 ± 0.1	50 ± 4	40 ± 3	0.98 ± 0.01	0.27± 0.01	0.08 ± 0.002	0.133 ± 0.003	0.081 ± 0.003	0.492 ± 0.002
Pyrocatechol	7.0 ± 0.2	7.0 ± 0.2	30 ± 3	10 ± 4	3.94 ± 0.02	2.27 ± 0.03	0.02 ± 0.001	0.081 ± 0.002	0.005 ± 0.001	0.035 ± 0.001
Pyrogallol	7.5 ± 0.1	7.5 ± 0.2	40 ± 4	50 ± 5	0.37 ± 0.02	0.37 ± 0.01	0.034 ± 0.002	0.051 ± 0.001	0.091 ± 0.002	0.137 ± 0.003
L-tyrosine	-	-	-	-	-	-	-	-	-	-

The metal effects on PPO enzyme activity obtained from AWP and AFP were also investigated with a constant 5 mM concentration of 4-methylcatechol as substrate. The results are presented in Table 1 and Figure 4. When the values of the metal solutions in Table 2 on the PPO enzyme activity are examined, it is seen that the metals tested at different concentrations, except for the Cu^{+2} atom, have an inhibitory effect on the PPO enzyme activity from both sources. Cu metal had an activator effect on the PPO enzyme activity form AWP and AFP. In general, it can be concluded that metals tested at different concentrations (0.5 to 5 mM) did not have much effect on PPO enzyme activity from AWP. However, it is seen that metals are effective on PPO enzyme activity in AFP. The storage stability of the PPO enzyme in AWP and AFP was investigated in order to determine how the activity of the PPO enzyme would change when stored under changing temperature conditions. As a result of this, the enzyme was stored at room temperature (25 °C), +4 °C' and –20 °C , and enzyme activity was determined at certain time intervals (Figure 5). In the presence of 4-methylcatechol substrate, the activity of PPO enzyme in AFP and AWP apples at room temperature decreased to 50% after 150 h and had an activity of 15% after 400 h. In the storage stability at +4 °C, it was found that there was a 50% decrease in the relative activity of the enzyme after 100 h substrate (4-methylcatechol) and that the enzyme had 10% activity at the end of 405 h. However, as seen in Figure 5, the PPO enzyme activity is higher in AFP. In the storage stability at –20 °C, in the presence of 4-methylcatechol substrate, PPO activity in AWP and AFP decreases regularly in the first 100 h. However, after 150 h, there is a 50% decrease in

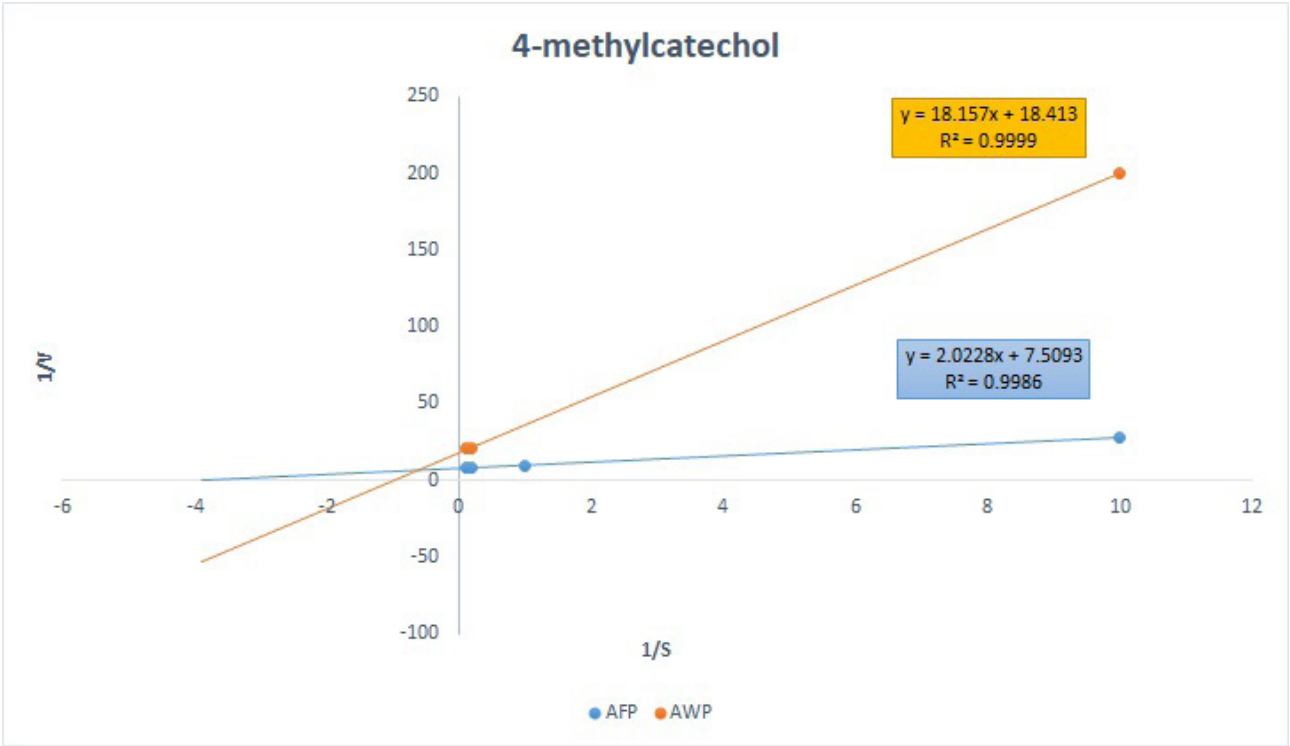

Figure 3. Lineweaver-Burk plot for 4-metyl catechol substrate of PPO enzyme from AWP and AFP.

the relative activity of the PPO enzyme in AWP, whereas there is a 30% decrease in the relative activity of the PPO enzyme in AFP. By the end of 400 h, the PPO enzyme in AWP was found to have an activity of 1%. It was found that the PPO enzyme in AFP had an activity of 30% at the end of 405 h.

4. Discussion

Pesticides that are used to protect fruits and vegetables against harmful external factors affect enzymatic activity. When the results of substrate specificity of the PPO enzyme purified from 'Jonagold' apple are examined (Table 1), it can be seen that it did not show any activity against the monophenolic substrate L-tyrosine. However, the PPO enzyme showed high activity with diphenolic (4-methylcatechol and pyrocatechol) and triphenolic (pyrogallol) substrates. It showed the highest activity with 4-methylcatechol and pyrogallol substrate. However, when evaluated over Vmax values, it is seen that the PPO enzyme showed high activity with the 4-methylcatechol substrate (Figure 3). Based on the specific substrate actions pesticides are classified into three different types: tyrosinases, cathecol oxidases, and laccases. Tyrosinases, have both cresolase and catecholase activities (Sánchez-Ferrer et. al., 1995). Catechol oxidases, also known as o-Diphenol oxidases, catalyze the oxidation of *o*-diphenols to *o*-quinones. Also, laccases have the ability to oxidize various aromatic compounds by a radical-catalyzed reaction mechanism (Fronk et. al., 2015). It has been determined that the PPO enzyme purified from 'Jonagold' apple has Laccase character due to its interaction with diphenolic (4-methylcatechol and pyrocatechol) and triphenolic (pyrogallol) substrates. This study, was found to be in agreement with the studies of Palma-Orozco et al. (2014) that were made on the PPO enzyme purified from mango fruit. Optimum pH studies for the 'Jonagold' PPO enzyme showed that the PPO enzyme had the best activity in the acidic and neutral regions. The enzyme showed high activity with the 4-metylcatechol substrate in the pH range of 4.5 to 7.0, while in the neutral region it showed high activity only with the pyrocatechol and pyrogallol substrates (Figure 1).

In the study of Rocha et al. in 2001, it was experimentally proven that the interaction of the PPO enzyme purified from Jonagored apple with the substrates was only with diphenols and triphenols. In addition, it was determined that the PPO enzyme showed high activity with the 4-methylcatechol substrate in the pH range of 4.5 to 7.0. The substrate specificity and optimum pH results of the PPO enzyme purified from 'Jonagold' apple were found to be similar to the previous studies. Interesting results were obtained when comparing the substrate specificity of the PPO enzyme purified from AWP and AFP apples. When the substrate specificity of the PPO enzyme is evaluated,

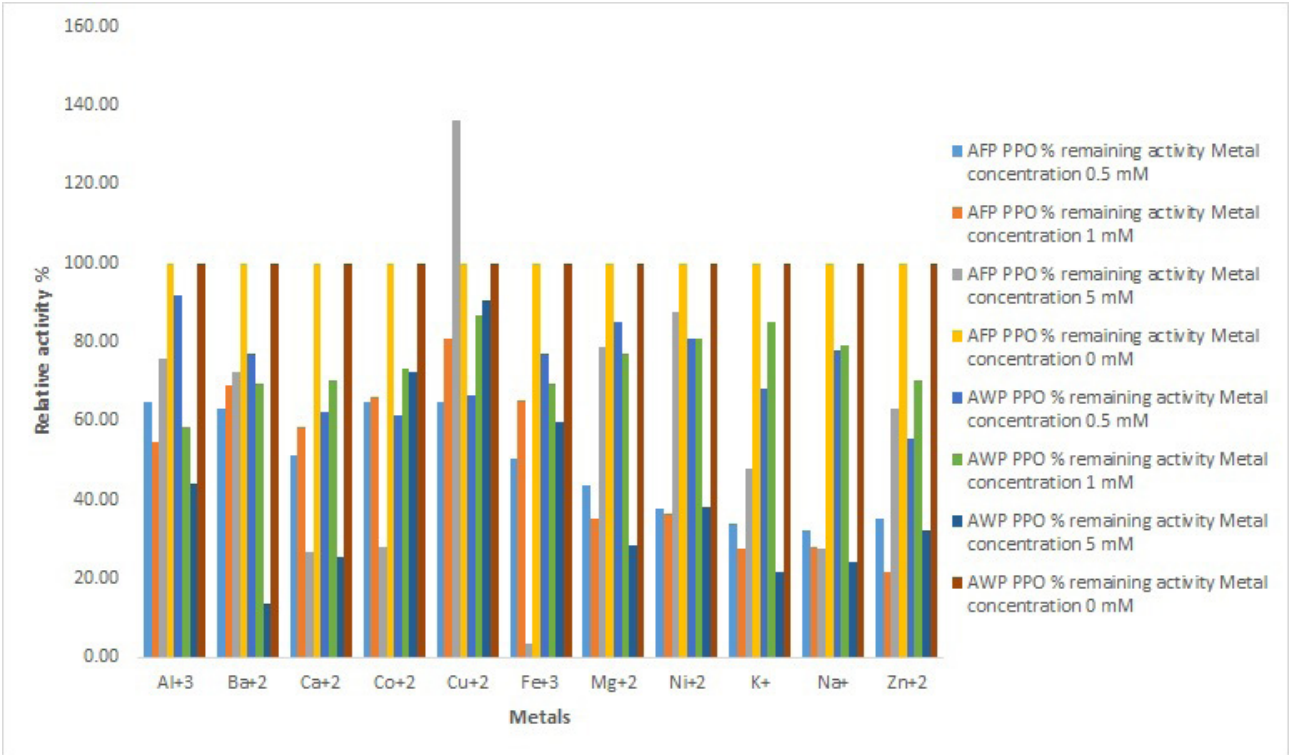

Figure 4. Comparative graph showing the effect of metals on the PPO enzyme activity from AFP and AWP.

Table 2. Effect of metals on the PPO enzyme activity from AWP and AFP.

	AFP				**AWP**			
	PPO % remaining activity				**PPO % remaining activity**			
	Metal concentration				**Metal concentration**			
Metals	0.5 mM	1 mM	5 mM	0 mM	0.5 mM	1 mM	5 mM	0 mM
Al^{3+}	64.60674	54.77528	75.8427	100	91.70732	58.53659	43.90244	100
Ba^{2+}	63.20225	68.82022	72.33146	100	77.07317	69.26829	13.65854	100
Ca^{2+}	51.26404	58.28652	26.68539	100	62.43902	70.2439	25.36585	100
Co^{2+}	64.60674	66.01124	28.08989	100	61.46341	73.17073	72.19512	100
Cu^{2+}	64.60674	80.75843	139.0449	100	66.34146	86.82927	90.73171	100
Fe^{3+}	50.5618	65.30899	3.511236	100	77.07317	69.26829	59.5122	100
Mg^{2+}	43.53933	35.11236	78.65169	100	84.87805	77.07317	28.29268	100
Ni^{2+}	37.92135	36.51685	87.7809	100	80.97561	80.97561	38.04878	100
K^{+}	33.70787	27.38764	47.75281	100	68.29268	84.87805	21.46341	100
Na^{+}	32.30337	28.08989	27.38764	100	78.04878	79.02439	24.39024	100
Zn^{2+}	35.11236	21.76966	63.20225	100	55.60976	70.2439	32.19512	100

especially with 4-methylcatechol, it is seen that the AWP PPO activity is lower than the PPO activity in AFP (Table 1). Considering the Vmax values, it was determined that the Vmax value of AFP PPO was approximately 2.5 times higher than the Vmax value of AWP PPO. Deltamethrin was used when spraying pesticides, as described in section 2.2.

In the study of Fattouch et al. (2010), the effect of a range of commonly used pesticides on enzyme activity has been evaluated using the purified quince (*Cydonia*

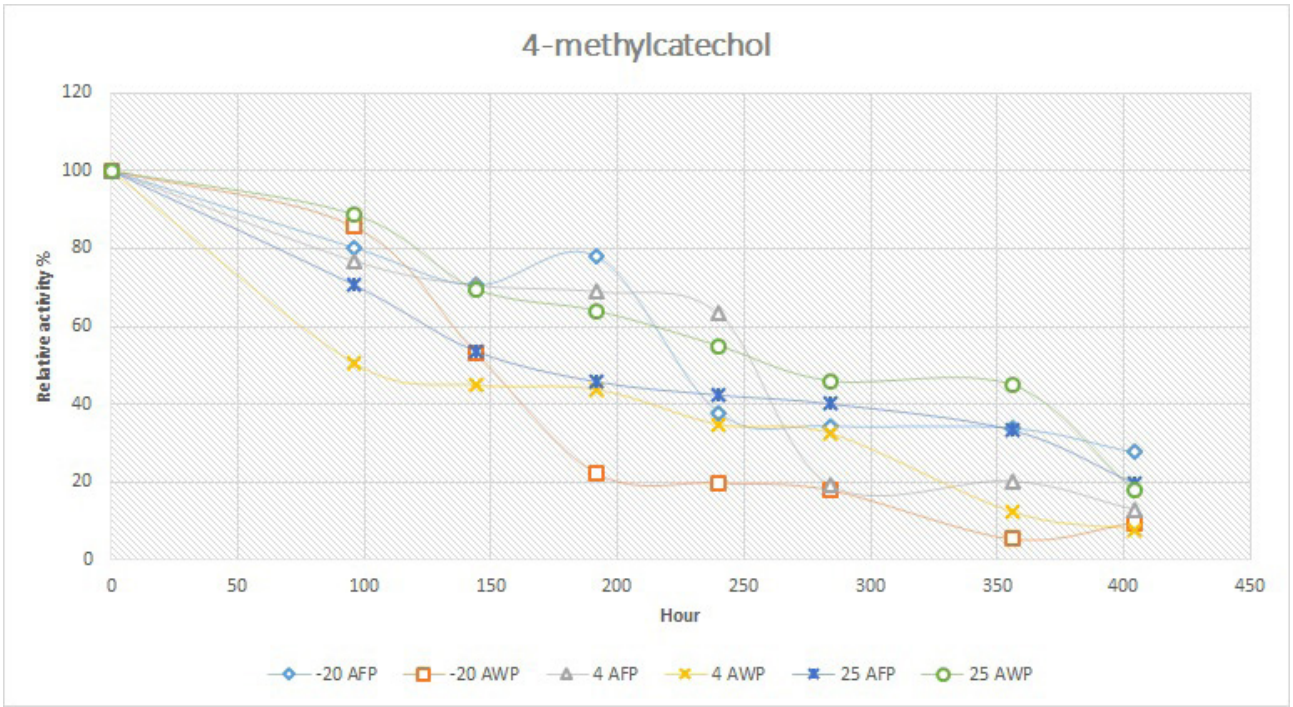

Figure 5. Time- varying comparative graph of the activity of PPO enzyme obtained from AWP and AFP in the presence of 4-methylcatechol substrate at different temperatures.

oblonga) PPO. Purified Deltamethrine was used as a pesticide and it was found that PPO inhibited the enzyme by 33%. According to our study, it was determined that it inhibited the PPO enzyme purified from AWP apples by 80%. As described in section 2.2, deltamethrin was not used alone besides, Acrobat and Kumulus were also used as a mixture. In the first step, the mixture in the pesticide spray significantly inhibited the 'Jonagold' PPO enzyme. The reason is that the molecules in the mixture that is used in the pesticide spraying in the first stage have organic properties and bind to the region outside the active center of the PPO enzyme. The fact that the Km values of the pyrogallol substrate are the same but the Vmax values are different (Blat, 2010) reveals that the PPO in AWP has a noncompetitive inhibition effect. The effect of deltamethrin pesticide on PPO enzyme purified from quince by Fattouch et al. in 2010 was recorded as competitive. In our results, it was determined that the results obtained with the pyrogallol substrate were close and similar results were obtained.

When the optimum temperature values of the PPO enzyme purified from AWP and AFP 'Jonagold' apples are examined, it is seen that the PPO enzyme activity in AFP is higher than the PPO enzyme activity in AWP. It has been established that the PPO enzyme in temperature-dependent AWP is nonresistance. In the kinetic study of Oktay et al. (1995), on the PPO activity of Amasya apple, the optimum temperature value was found to be high which is similar to our study. Therefore, it is seen that this study is similar to the studies that were done.

This study also investigated the effect of metals on the PPO enzyme purified from AWP and AFP 'Jonagold' apples. Therefore, the enzyme activity was measured for 60 s against time with metal solutions prepared at 0.5 mM, 1 mM, and 5 mM ratios. 4-methylcatechol at a concentration of 5 mM was used as a substrate. In Table 2, it is seen that metal solutions used at low concentrations did not inhibit AWP PPO enzyme activity too much. However, AFP PPO enzyme activity was found to be inhibited more than AWP PPO. Therefore, AWP PPO enzyme activity was found to be resistant to low metal concentrations. It has been determined that the pesticides that were used do not affect the enzyme activity in large amount and even provide resistance against external factors. When looked at Table 2 and Graph 4, it was determined that Cu ions increased the enzyme activity. Five mM Cu^{+2} ions concentration increased AFP PPO enzyme activity by 139%. Also, it was founded that Fe^{+3} ions, which inhibit AFP PPO enzyme activity the most, inhibited the enzyme by 96.5% when they were present in the reaction medium at 5 mM level. Afterwards, it was determined that Ca^{+2}, Na^{+}, and Co^{+2} ions inhibited the AFP PPO enzyme by 75%. Whereas, Ba+2 ions, which inhibit AWP PPO enzyme activity the most, were found to inhibit the enzyme by 86.5% when they were present in the reaction medium at 5 mM level. Then, it was determined that Ca^{+2}, Na^{+}, K^{+}, and Mg^{+2} ions

inhibited the AWP PPO enzyme by 75%. When the effect of Na^+ and K^+ ions was examined, it was determined that when used at low concentrations, AWP did not inhibit the PPO enzyme very much, whereas AFP inhibited the PPO enzyme by almost 60% to 70%. These results show that the AWP PPO enzyme is resistant to external factors. Pesticides have been found to be effective in the study. Since K^+ and Na^+ ions play an important role in the cell structure of living organisms and various other biochemical factors, it is thought that they have no effect on the enzyme. Other tested metals have the properties to easily interact with ligands such as imidazole, thiol and carbonyl, which contain atoms such as N, S, and O, which are found in the basic structures of biological systems. Accordingly, it is thought that the tested metals may interact with the ligands in the enzyme, causing changes in the conformation of the enzyme and inhibiting the enzyme at different rates. In some studies, there are also findings that the effects of metal solutions increase the activity on PPO enzymes. According to the study conducted by Gedikli et al. (2010), the effect of denim dyes containing metal ions (Mn, Pb, Ni, Zn, Co, Cd, and Cu) on laccase enzyme was examined. Palma-Orozco et al. (2014) tested metal ions at 0.1 mM, 1 mM, and 10 mM concentrations on the PPO enzyme that they purified from mango fruit varieties. Their results determined that the effect of K^+ ions on the PPO enzyme was low. However, they determined that the Na^+ ions used in 0.1 mM concentration inhibited the PPO enzyme by 10%. Cu^{+2} ions, on the other hand, inhibited PPO enzyme at the rate of 87% at 0.1 mM concentration, 67% at 2.5 mM concentration, 56% at 5.0 mM concentration, and 49% at 10 mM concentration. According to the studies, AFP and AWP 'Jonagold' PPO enzymes are inhibited at high metal concentrations, but maintain their activity at 1 mM and lower metal concentrations. Since metal ions in high concentrations are known to be toxic to living things, it is thought that metals inhibit the enzyme by interacting with various regions of enzymes at high concentrations.

The storage stability of the 'Jonagold' PPO enzyme was investigated in order to determine how the activity would change when stored under changing temperature conditions. Therefore, the enzyme was stored at room temperature, +4 °C and –20 °C, and enzyme activity was determined at certain time intervals. The study found that, the activity of PPO enzyme purified from AFP and AWP 'Jonagold' apples decreased by 70% at the end of 400 h. Also, AWP PPO lost its activity at the end of 400 h. It has been found that pesticides significantly reduce the shelf life of apples. In addition, it was determined that the polyphenol oxidase enzyme in the plant *Prunus dulcis* lost its activity in 16 h at room temperature and in 13 days (312 h) at –20 °C (Güngör and Badem, 2008). According to the results obtained, 'Jonagold' PPO enzyme was determined to be quite stable at –20 °C.

5. Conclusion

It is important to control the PPO enzyme activity since more than 50% of losses occur in the fruit and vegetable sector as a result of the browning reaction with the PPO enzyme. This study demonstrates the behavior of the Jonagold PPO in the presence of a range of pesticides used worldwide in human activities. Additionally, the study shows that pesticides protect apples from harmful external factors, while at the same time inhibit the PPO activity in the product. We have determined that inhibiting PPO activity extends the shelf life of the product and will greatly benefit the industry. Also, in this study's results, it was found that pesticides bind to the active site of the PPO enzyme in apples, reducing the enzyme activity and making it more resistant to metals. Therefore, it can be said that pesticides will prevent the browning reaction in apples, extend the spoilage of the products and reduce the losses due to rot in the fruit and vegetable sector. Our findings are likely to have general utility in predicting environmental toxicants, like pesticides, which could bind and modulate enzymatic systems. It also presented good data for examining the utility of Jonagold PPO enzyme as a bioactive agent in the development of biosensors for pesticide determination.

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References

Anh TM, Dzyadevych SV, Soldatkin AP, Duc CN, Jaffrezic-Renault N et al. (2002). Development of tyrosinase biosensor based on pH sensitive field-effect transistors for phenols determination in water solutions. Talanta 56 (4): 627–634. doi: https://doi.org/10.1016/S0039-9140(01)00611-7

Anh TM, Dzyadevych SV, Van MC, Jaffrezic-Renault N, Duc CN et al. (2004). Conductometric tyrosinase biosensor for the detection of diuron, atrazine and its main metabolites. Talanta 63 (2): 365–370. https://doi.org/10.1016/j.talanta.2003.11.008

Blat Y (2010). Non-Competitive Inhibition by Active Site Binders. Chemical Biology & Drug Design 75 (6): 535-540. https://doi.org/10.1111/j.1747-0285.2010.00972.x

Can Z, Dincer B, Sahin H, Baltas H, Yildiz O et al. (2014). Polyphenol oxidase activity and antioxidant properties of Yomra apple (*Malus communis L.*) from Turkey, Journal of Enzym Inhibition and Medicinal Chemistry 29 (6): 829-835. https://doi.org/10.3109/14756366.2013.858144

Cerrahoğlu E, Arabacı G (2016). Eflatun çiçekli ballıbaba (*Lamium purpureum*) polifenol oksidaz enziminin karakterizasyonu ve inhibisyonu. Sakarya Üniversitesi, Fen Bilimleri Dergisi 20 (2): 245-250. (in Turkish with an abstract in English). 10.16984/saufenbilder.80440

Darko G, Akoto O (2008). Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. Food Chemical Toxicology 46 (12): 3703-3706. https://doi.org/10.1016/j.fct.2008.09.049

Fattouch S, Raboudi-Fattouch F, Ponce JVG, Forment JV, Lukovic D et al. (2010). Concentration dependent effects of commonly used pesticides on activation versus of the quince (*Cydonia Oblonga*) polyphenol oxidase. Food and Chemical Toxicology 48 (3): 957–963. https://doi.org/10.1016/j.fct.2010.01.006

Food and Agriculture Organization of the United Nations (2002). FAOSTAT. URL: https://www.fao.org/3/bt472e/bt472e.pdf

Fronk P, Hartmann H, Bauer M, Solem E, Jaenicke E et al. (2015). Polyphenoloxidase from Riesling and Dornfelder wine grapes (*Vitis vinifera*) is a tyrosinase. Food Chemistry 183: 49–57. https://doi.org/10.1016/j.foodchem.2015.03.016

Gedikli Ş, Aytar P, Çabuk A, Ünal A, Kolankaya N (2010). Lakkaz Enzimi İle Kot Boyarmaddesinin Dekolorizasyonu, Anadolu University Journal of Science and Technology Life Sciences and Biotechnology 1 (1): 59-70. (in Turkish with an abstract in English) https://hdl.handle.net/11421/1518

Gianfreda L, Rao MA (2004). Potential of extracellular enzymes in remediation of polluted soils: a review. Enzyme and Microbial Technology 35 (4): 339-354. https://doi.org/10.1016/j.enzmictec.2004.05.006

Güngör K, Badem Ç (2008). (*Prunus dulcis*) bitkisinden polifenol oksidaz enziminin saflaştırılması ve karakterizasyonu. Sakarya Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. (in Turkish with an abstract in English) https://hdl.handle.net/20.500.12619/81188

Karalliedde LD, Edwardsb P, Marrsc TC (2003). Variables influencing the toxic response to organophosphates in humans. Food Chemical Toxicology 41 (1): 1–13. https://doi.org/10.1016/s0278-6915(02)00232-6

Kaushik G, Satya S, Naik SN (2009). Food processing a tool to pesticide residue dissipation–a review. Food Research International 42 (1): 26–40. https://doi.org/10.1016/j.foodres.2008.09.009

Lineweaver H, Burk D (1934). The determination of enzyme dissociation constants, Journal of the American Chemical Society 56: 658-661. https://doi.org/10.1021/ja01318a036

Nicolas JJ, Richard-Forget FC, Goupy PM, Amiot MJ, Aubert SY (1994). Enzymatic browning reactions in apple and apple products. Critical Reviews in Food Science Nutrition 34 (2): 109-157. https://doi.org/10.1080/10408399409527653

Oktay M, Küfrevioglu I, Kocaçaliskan I, Sakiroglu H (1995). Polyphenoloxidase from Amasya apple. Journal of Food Science 60 (3): 494–496. https://doi.org/10.1111/j.1365-2621.1995.tb09810.x

Palma-Orozco G, Marrufo-Hernandez NA, Sampedro JG, Najera H (2014). Purification and partial biochemical characterization of polyphenol oxidase from Mango (*Mangifera indica* cv. Manila). Journal of Agricultural and Food Chemistry 62 (40): 9832-9840. https://doi.org/10.1021/jf5029784

Rocha AMCN, Morais AMMB (2001). Characterization of polyphenoloxidase (PPO) extracted from 'Jonagored' apple. Food Control 12 (2): 85–90. https://doi.org/10.1016/S0956-7135(00)00026-8

Rung B, Schwack W (2005). Aminoparathion: a highly reactive metabolite of parathion. 1. Reactions with polyphenols and polyphenol oxidase. Journal of Agricultural and Food Chemistry 53 (23): 9140–9145. https://doi.org/10.1021/jf051520m

Sánchez-Ferrer A, Rodríguez-López JN, García-Cánovas F, García-Carmona F (1995). Tyrosinase: A comprehensive review of its mechanism. Biochimica et Biophysica Acta. 1247 (1): 1–11. https://doi.org/10.1016/0167-4838(94)00204-T

Sinan S, Gencer N, Turan Y, Arslan O (2007). In vitro inhibition of the carbonic anhydrase from saanen goat (*Capra hircus*) with pesticides. Pesticide Biochemistry Physiology 88 (3): 307–311. https://doi.org/10.1016/j.pestbp.2007.01.006

Singha PB, Singha V, Nayak PK (2008). Pesticide residues and reproductive dysfunction in different vertebrates from north India. Food Chemical Toxicology 46 (7): 2533–2539. https://doi.org/10.1016/j.fct.2008.04.009

Trillot M, Masseron A, Trovel C (1993). In*: Pomme les variétés* Paris: Centre Technique Interprofessionnel des Fruits et Légumes. CTIFL (pp. 97-99).

Whitaker JR, Lee CY (1995) Recent advances in chemistry of enzymatic browning. Enzymatic browning and its prevention. American Chemical Society Symposium Series 600: 2–7. https://doi.org/10.1021/bk-1995-0600.ch001

Wong TC, Lun BS, Whitaker JR (1971). Isolation and characterization of polyphenoloxidase isoenzymes of clingstone peach. Plant Physiology 48 (1): 19–23. https://doi.org/10.1104/pp.48.1.19