Journal of Engineering Science and Technology
Special Issue on AASEC2018, February (2019) 1 - 9

ISOLATION AND CHARACTERIZATION OF POLYPHENOL OXIDASES (PPO) ON POTATOES (SOLANUM TUBEROSUM) USING AGE AND ENVIRONMENTAL CONTROL

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Abstract

PPO is an enzyme that plays a role in the browning reaction. This phenomenon impacted on colour, flavour, nutritional properties, and shelf life of food product. The aim of this research was to isolate and characterize PPO from three types of potatoes. Crude PPO was extracted by three types of potatoes namely Desiree, Granola, and Atlantic at the age of 100 days. This is the same environment as soil conditions, climate, and growing conditions. Phosphate buffer (pH=7) was used as solute of PPO and browning reaction of PPO was stopped by adding 2% NaF. Based on experiments towards PPO in all potatoes types, it was found that (i) PPO had optimum activity when catechol was used as a substrate; (ii) the optimum pH and optimum temperature values of PPO were 7 and 35°C; (iii) the most effective inhibitor was EDTA; (iv) PPO from Atlantic had the highest activity; and (v) molecular weight of PPO from Atlantic was about 50 kDa. This research has found optimum conditions of PPO using age and environmental control. Future research is expected to analyse the influence of age and environment on PPO activities.

Keywords: Age control, Characterization, Environmental control, Isolation, Polyphenol oxidases.

1. Introduction

Polyphenol oxidases (PPO) is a group of copper (Cu), containing enzymes that make catalyse process in two different reactions [1]. The first reaction step is enzymatic reaction when monophenol is converted into o-diphenol (monophenol oxidation or monophenolase activity) with hydroxylation ortho position adjacent to an existing hydroxyl group. Then, o-diphenol changed to o-quinone (diphenol oxidase or diphenolase activity or catecholase or oxidase activity). The second step is a non-enzymatic reaction when o-quinone undergoes non-enzymatic oxidative condensation into the complex brown polymer (melanin). PPO can be found in all living organism including plants, animals, and microorganism. In plants, PPO is contained in chloroplasts and it is involved in defence mechanism. This can be seen when the plants got bruises or wounds, they would produce certain phenolic compounds that are oxidized with oxygen to form polymer structures to prevent microbial contamination [2]. However, this compliment can be lost due to aging; wounds; interactions with pests or pathogens; and handling during post-harvest and storage processing [3].

PPO is considered in the food application due to its browning reaction that can decrease food quality. This enzymatic process in the browning reaction has impacts on colour, flavour, nutritional properties, and shelf life of food product [4]. Enzymatic browning on potatoes would decrease selling price and consumer acceptance, especially for processing potato type that is usually made into French fries and potato chips. The potatoes are not a staple food in Indonesia, however many farmers depend on potato plantations such those in Pangalengan, Lembang, Dieng, Wonosobo, and Karo. It is also important to develop an effective maintenance conditions to determine effective methods for preventing enzymatic browning through a study about optimum condition with age and environment control. Activity of PPO has been extensively studied by several researchers. Waleed investigated characterization of PPO from potatoes, apples, apricots and eggplants [2]. Other studies performed characterizations that focused on one kind of the fruits, such as Rambutan [1], Potatoes [5], Apricots [6], and Lemon Balm [7]. Several studies have focused on analysis and treatment of PPO for inhibition of enzymatic browning. Ascorbic acid and ultrasound were used as inhibitor of PPO [8]. The use of ascorbic acid and ultrasound can simultaneously decrease PPO activity more than 12 days. Meanwhile, other researches employed alginate for PPO coating and found that alginate coating on PPO is effective to prevent PPO interacted with oxygen. [9]. Other studies focused on quantification to analysis of reaction rate of PPO and determined reaction rate using fractical browning indicator (FBI) [10]. The FBI method oriented on colour surface change of apple slice using computer vision system (CVS). Chikezie has conducted an analysis of reaction rate of PPO for diocorea rotundata poir [11].

However, the isolation and characterization of PPO from potatoes using age and environment control has not been reported. In general, the chemical composition of potatoes including PPO is significantly different due to three factors that cover types of potato (genetic), age, and environment, such as soil conditions and climate, as well as weather and growing conditions, such as fertilization, pesticides and pests [12]. Therefore, the aim of this research was to isolate and characterize PPO from three types of potatoes using age and environmental control.

2. Methods

2.1. Materials

Three types of potatoes were selected from local source namely Desiree, Granola and Atlantic. The seeds of the potato were controlled on generation 0 (G0). In addition, age and environmental factor such as soil conditions, climate, weather, and growing conditions (e.g., fertilization, pesticides and pests) has been controlled with the same treatment. Collaboration on planting potatoes was made with research centre of plants and vegetable in Lembang. The potatoes were grown in poly bags to minimize pests and harvested in the 100th day. Solvent dissolving PPO was phosphate buffer with pH = 7, and 2% of sodium fluoride (NaF) and was employed used to stop the browning reaction.

2.2. Instrumentation and characterization

Water bath WNB 22 was used to keep the temperature of sample and the browning reaction was monitored by spectrophotometer (UV Jasco V-530, Jasco Corp., Japan). Meanwhile, Multispin centrifuge TC4815D (EITEK) was used to separate precipitated protein.

2.3. Preparation of crude PPO extract

The enzyme of PPO was extracted by 100-gram potatoes that were sliced and cut into small pieces of 2.5×2.5 cm using a stainless knife. Potato pieces were mashed using a saw-milling process by adding 100 mL phosphate buffer pH 7 and filtered by tofu filter. The saw-milling process was the same with previous work [13]. The reaction was stopped by the addition of 50 mL of 2% NaF.

2.4. Specific substrate of PPO

The characteristics of PPO substrate were measured using four kinds of compounds that has similar structures from one to another. The substrates that were used namely catechol, phenol, cyclohexane diol, and resorcinol. 5 ml of enzyme extract (10%) was added to 10 drops of the substrate and calculated the absorbance using a spectrophotometer at wavelength of 420 nm. Incubation of pre-reaction was 5 minutes and incubation of post-reaction was 10 minutes.

2.5. pH and temperature optimum of PPO

The pH optimum of PPO was determined using 0.01 M of catechol as a substrate in sodium phosphate buffer with pH of 5, 7, and 10. Absorbance level was calculated at wavelength of 420 nm using a spectrophotometer. The optimum temperature was determined at 10, 25, 35, and 50°C. 0.01 M of catechol was used as a substrate for enzyme extract (10%). The temperature was monitored using a mercury glass thermometer.

2.6. Inhibitors of PPO

Inhibitor of PPO was determined on three types of inhibitors, i.e., trypsin, pb-nitrate and ethylenediaminetetraacetic acid (EDTA). Aquadest was used as control reaction. 5 mL of enzyme extract (10%), 5 mL of catechol (0.01M) as a substrate, and 5 drops of inhibitor were incubated at 35°C during 5 minutes of mixing process. After the reaction, the mixture was incubated at 35°C for 10 minutes.

2.7. Purification of PPO on atlantic

100 g of Atlantic was homogenized in 100 mL phosphate buffer (pH=7). The homogenate was quickly mixed with 50 mL of NaF (2%) and filtered by a tofu filter. The residue was dissolved twice in phosphate buffer (pH=7) with a ratio of 1:6 and filtered by tofu filter. The filtrate was added to the filtered solution, and it re-filtered twice by a standard filter paper and Whatmann filter paper number one (125 mm). The filtrate was precipitated by saturated ammonium sulphate ($(NH_4)_2SO_4$) with a ratio of 1:6. The precipitated product was separated by a centrifugation at 7000 rpm for 20 minutes.

2.8. Analysis of molecular weight

Polyacrylamide gel electrophoresis (PAGE) was used to check purity of PPO purified with an affinity chromatography. PAGE was performed with 4% of stacking gel and 7.5% of acrylamide stacking gel. Enzyme solution was made with a reaction sample was hydrolysed with 0.2 M of phosphate buffer (pH=7.2).

3. Results and Discussion

3.1. Specific substrate of PPO

PPO was isolated from three types of potatoes, namely Atlantic, Desiree and Granola potatoes. Generally, there are three factors that influence PPO content, i.e., genetic, age, and environmental condition. Age and environmental conditions were controlled, so we get only differences of PPO activities caused by a genetic factor. In the first experiment, substrate specificity of PPO was determined by four different compounds with a similar structure that covers catechol, phenol, cyclohexanediol, and resorcinol with aquadest (used as a comparison). According to spectrophotometric analyses, Atlantic, Desiree and Granola had the highest absorbance when catechol was used as a substrate (see Fig. 1). This is similar with previous studies that used catechol as a substrate for PPO [2, 5, 11, 14-17]. Meanwhile, from three types of potatoes that have been studied, Atlantic has a higher activity than Desiree and Granola. This indicates that PPO content from Atlantic potatoes is highest at the same age and environment.

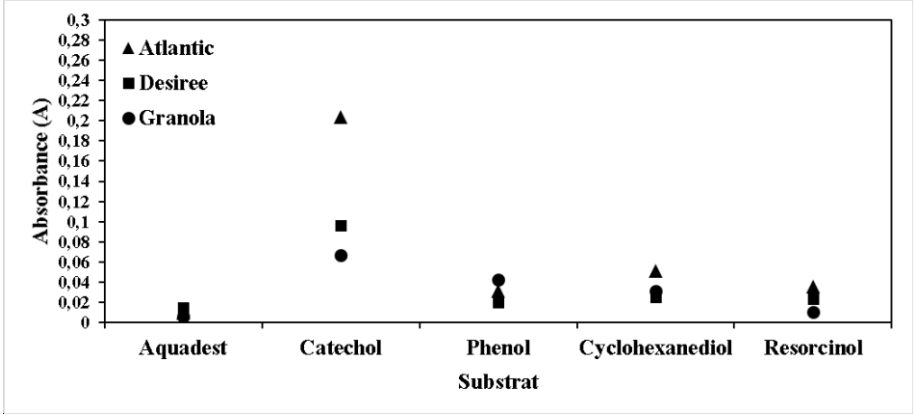

Fig. 1. Absorbance of PPO as a substrate function.

3.2. Determination of optimum pH

PPO absorbance was measured in pH of 5, 6, and 10 using sodium phosphate as a buffer with 10 drops of catechol as a substrate. Optimum absorbance of pH was found at pH of 7 for Atlantic, Desiree and Granola. Figure 2 showed that PPO on potatoes has optimum activity in the neutral pH. This finding is similar to previous research findings that optimum pH of PPO was gained at or close to neutral pH and unsuitable in the acid pH or the basic pH [3, 5, 15]. Atlantic was found to have the highest absorbance at different pH conditions, i.e., pH = 5 (0.15), pH = 7 (0.17), and pH = 10 (0.13). The effects of these results based on pH combination factors are due to the binding of substrate enzyme. The ionization states of the amino acid residues involved in the catalytic activity of the enzyme, ionization substrate, and the variation of protein structure.

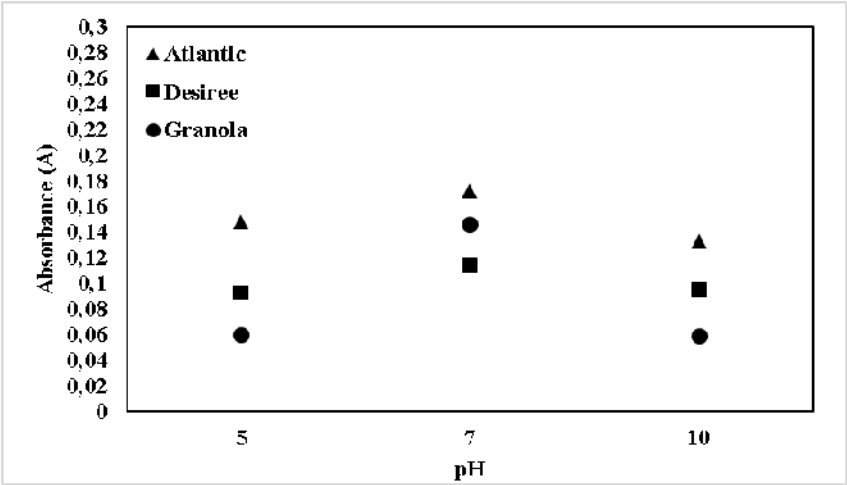

Fig. 2. Optimum pH of PPO absorbance.

3.3. Determination of optimum temperature

Optimum temperature of potatoes PPO were 10, 25, 35, and 50°C. Figure 3 shows that optimum temperature was 35°C for Atlantic, Desiree and Granola potatoes. Above this temperature, the absorbance level rapidly decreased. Generally, Atlantic potatoes had the highest absorbance in different temperature i.e., 10°C (0.09), 25°C (0.19), 35°C (0.27), and 50°C (0.09). Like most chemical reactions, enzyme activity increases as the increasing temperature. However, because enzymes are proteins, a rise in temperature can also cause a denaturation process. If a denaturation process occurs, the active site of the enzyme will be disrupted and its activity will decrease. Previous researches showed that the optimum temperature of PPO was at 35°C [16]. However, it is different from sweet potato and igdir apricot whose optimum temperature was at 30°C [6]. The pulp of apple was at 20°C [3], whereas rambutan peel was at 37°C [1]. However, to determine the most appropriate temperature, the temperatures close to 35°C, informing the need for further tests. The optimum temperature can be more or less than 35°C. The study focused only on the difference in optimum condition in various potato types using age and environmental control.

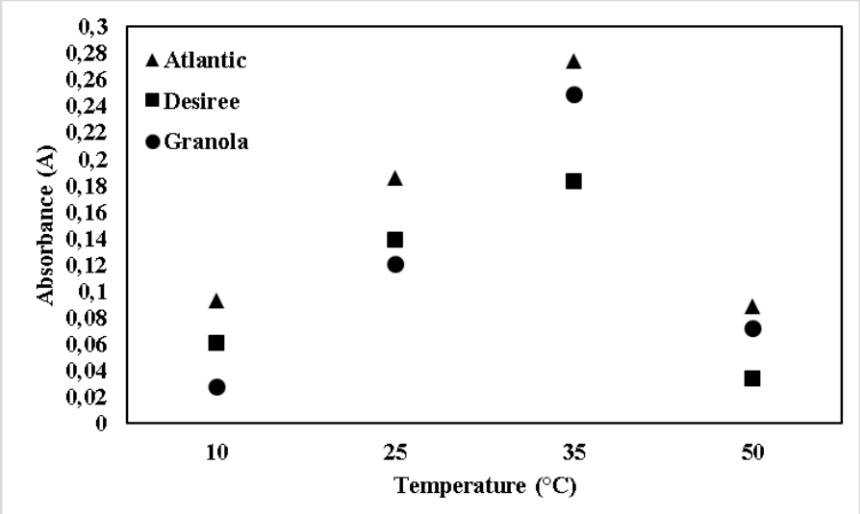

Fig. 3. Optimum temperature of PPO absorbance.

3.4. Determination of PPO inhibitor

Trypsin, Pb-nitrate, and EDTA were set as an inhibitor for PPO potatoes in the same incubation at 35°C. Aquadest was used as a control. Figure 4 shows that PPO was found being able to decrease PPO absorbance with EDTA for all potatoes types and especially for Granola, which had the lowest absorbance. This is possible because the previous absorbance of PPO for Granola was lower than Desiree and Atlantic potatoes. This finding is in accordance with previous researches discovering that EDTA was a good inhibitor to decrease PPO absorbance [18].

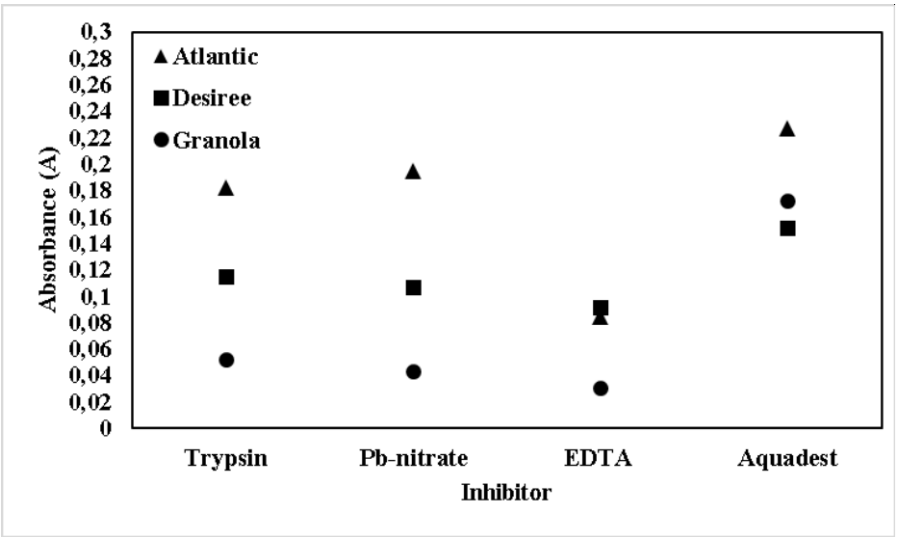

Fig. 4. Optimum Inhibitor of PPO Absorbance.

3.5. Determination of molecular weight

The experimental research found that Atlantic had a greater absorbance than Granola and Desiree potatoes. Further analysis on Atlantic was to analyse the molecular weight of Atlantic with SDS PAGE. Result of SDS PAGE was shown in Fig. 5. The blue band showed the molecular weight of sample and molecular weight of PPO were estimated to be about 50 kDa. This molecular weight was larger than cabbage that is 39 kDa [19]. This is also different from banana with 41 kDa [20] and sunflower seeds with 42 kDa [21]. But, the molecular weight of PPO Atlantic was lower than sago palm that is 53 kDa [22]. This is also lower than mushroom with 58 kDa [23], apple with 65 kDa [24]; and tea leaf with 72 kDa [25].

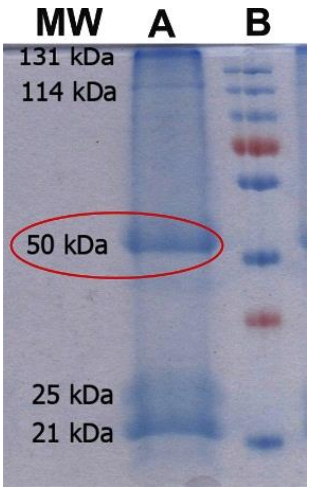

Fig. 5. Electrophoretogram result of the Purified PPO (MW) Molecular Weight, (A) Purified PPO, (B) Protein Marker.

4. Conclusion

This study has successfully isolated and characterized PPO from potatoes as well as studied the optimum conditions of potatoes using the age and environmental control. PPO activity in all potatoes types (i.e., Atlantic, Desiree and Granola) has been revealed. Catechol is the most suitable substrate for PPO whose optimum condition for pH and temperature were 7 and 35°C, respectively. The study has also shown that EDTA can be considered as an effective inhibitor to inhibit PPO activity and has also revealed that Atlantic, Desiree, and Granola potatoes have different PPO activities. This difference is due to the types of potatoes. They were treated the same when all potatoes were harvested at the age of the 100th day and planted in the same environmental condition. The highest PPO activity was found on Atlantic potatoes. According to PAGE, molecular weight of PPO from Atlantic is about 50 kDa. Further researches are expected to analyse the influence of age and environmental conditions (such as fertilization, soil conditions, and pesticide) on PPO activity.

Acknowledgments

The authors gratefully acknowledge financial support from DRPM Kemenristekdikti.

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