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Activity of polyphenoloxidase and peroxidase in non-dormant potato tubers treated with sprout suppressors

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Abstract

One of the major problems affecting potatoes quality on stored is the susceptibility to enzymatic browning, which occurs mainly after wounds during the harvest, at postharvest handling and by aging, leading to the development of dark color compounds. Enzymatic browning is associated with the action of polyphenoloxidases (PPO) and peroxidases (POD), which use phenolic compounds and oxygen as substrates. The products of these enzymes cause undesirable changes of color and flavor of processed potato products. The present study aimed to evaluate some kinetic properties based to optimum pH and reaction temperature, and to determine changes on activity of potato tubers PPO and POD treated with sprouting suppressors compounds menthol and eugenol. For each treatment, samples were collected in six different periods, before application (day 0) and 10, 20, 30, 40, and 50 days after the treatments to determine the influence of sprout inhibitors on the activity of PPO and POD. Changes in pH and temperature affected drastically the activity of both enzymes. The use of eugenol and menthol as sprouting suppressors decreased enzymatic activity on the treated tubers compared to control with an inverse relationship between enzymes activity and content of phenolic compounds.

Keywords: *Solanum tuberosum*; essential oils; oxidative enzymes; postharvest storage.

Practical Application: Sprouting suppressors such eugenol and menthol may contribute to the potato production chain for processing by reducing enzymatic browning.

1 Introduction

Potato (*Solanum tuberosum*) is one of the most consumed staple foods in the world due to its nutritional composition, gastronomic and processing versatility. The quality of potatoes is determined by the combination of various nutritional, physical-chemical characteristics that are heavily influenced by field and storage conditions.

Because of the current demand for fresh and processing potato markets, concerns with quality become a priority for farmers and Brazilian processing industries. Dormancy keeps the tubers from sprouting, but appropriate storage conditions should keep tubers edible and marketable, avoiding cold sweetening, deterioration and early sprouting (Nourian et al., 2003). One of the main problems is the significant susceptibility to enzymatic browning, which occurs mainly after wounds to the tissues during the harvesting, shipping and handling practices for further processing, which results in the appearance of dark color compounds (Mdluli, 2005).

Many studies have shown that PPO and POD activities increase in response to biotic and abiotic stresses. In general, enzymatic browning is related to the action of the PPO and POD isozymes, which use phenolic compounds as substrates and cause undesirable changes in the color and flavor of fruits, vegetables and tubers (Valderrama et al., 2001).

Polyphenoloxidase enzymes catalyze the oxidation of phenolic compounds to quinones, with further polymerization to brown or black pigments known as melanin. In potatoes, the PPO action results in enzymatic browning causing major losses during the processing into flakes, chips, and frozen French fries. The PPO is generally encoded by a multigenic family, which are expressed differently in parts of the plant (Thygesen et al., 1995; Aniszewski et al., 2008; Saeidian, 2012), its level is dependent on the species, cultivar, maturity and conditions of cultivation and storage conditions (Vamos-Vigyazo, 1981; Vitti et al., 2011; Wang et al., 2015). The enzyme has great heterogeneity regarding substrate, sensitivity to inhibitors, optimum pH, latency, thermal inactivation, number of isoforms and molecular mass (Mayer & Harel, 1979).

Peroxidases enzymes belong to a multigenic family, which catalyze the oxidation of phenylpropanoid metabolites at the expense of hydrogen peroxide. PODs are involved of lignin formation, a phenolic heteropolymer in the cell wall, providing rigidity, strength, and resistance to chemical, physical and biological attacks in plants (Pandey et al., 2017). The multigenic forms of isoperoxidases differ in molecular weight, isoelectric point, pH and optimum temperature, thermal stability and inhibitors sensitivity (Veitch, 2004). Peroxidases are important enzymes from the point of view of their industrial applications

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because of their ability to catalyze the oxidation of a wide range of phenolic pollutants present in the rejects.

This work was undertaken to report some kinetic properties of polyphenoloxidase and peroxidase enzymes based to optimum pH and reaction temperature, and their activities on potato tubers treated with the sprouting suppressors compounds eugenol and menthol.

2 Materials and methods

2.1 Plant material

Potato plants of Asterix were grown from May to August at micro-region of Alto Paranaíba (19° 35'S, 46° 56' W, 1000 m a.s.l.) and after the death of the vines, the tubers were harvested. Tubers weighting between 150-200 g were cured for ten days at 14 °C and 90% relative humidity for 7 days. Afterward, the temperature was lowered at a rate of 1 °C per day down to 8 °C and relative humidity 90 ± 5%. Tubers were stored for three months and when the natural dormancy was broken, at early sprouting stage (sprouts with less 5 mm in length), tubers were treated with eugenol and menthol at 50% solution in 95% ethanol. A total of 35 tubers were placed in 65 L sealed containers containing a petri dish with 2 mL of solution, with a final concentration of 100 mg/kg of tubers. The essential oils were vaporized, according to the method described by Vaughn & Spencer (1991) for a period of two hours with the help of a hot plate, returning to the cold storage afterward. Control tubers were treated with the vapor of ethanol for the same period. The tubers were removed from the buckets and stored at 8 °C and relative humidity of 90 ± 5%, in the dark. For each treatment, samples were collected in six different periods, before application (day 0) and 10, 20, 30, 40, and 50 days after the treatments.

2.2 Extraction and assays of peroxidase (POD) and polyphenoloxidase (PPO)

Samples containing 0.5 g of potato pulp was removed and stored in liquid nitrogen until analysis. The material was homogenized with the help of a polytron with 15 mL of extraction buffer (0.1 M phosphate buffer, pH 6.5). This homogenate was filtered in four layers of gauze and centrifuged at 17000 *g* for 30 min at 4 °C (Lagrimini et al., 1997) with modifications. The supernatant was used for the enzyme assays.

To determine the enzymatic activity of POD, an aliquot of 100 µL of the enzyme extract was added to the reaction medium containing 0.5 mL of guaiacol (1.68%), 1.5 mL of 0.1 M phosphate buffer (pH 7.0) and 0.5 mL H_2O_2 (1.8%) and the volume completed to 3 mL with distilled water. Blanks were constituted by all components of the reaction medium, except the enzymatic extract, which was replaced with water (Lagrimini et al., 1997).

To determine the enzymatic activity of PPO, a 100 µL aliquot of the enzyme extract was added to the reaction medium containing 1.5 mL of 0.1 M phosphate buffer (pH 7.0), added with 0.5 mL of catechol (120 mM), completing the volume to 3.0 mL with distilled water. The blank assays had all the components for the reaction, except the enzymatic extract, which was replaced with water (Kavrayan & Aydemir, 2001).

The enzymatic activity was analyzed in a spectrophotometer, observing the variation in absorbance units at wavelengths of 470 nm for POD and 420 nm for PPO at 30 °C. The results were expressed in AU min^{-1} mg $protein^{-1}$.

The same extract used to determine the enzymatic activity was used for protein quantification by the method of Bradford (1976), using bovine serum albumin (BSA) as standard.

2.3 Optimum pH for POD and PPO activity

Buffer solutions for the enzyme assays were used at 0.1 M concentration containing citrate buffer (pH 4.0 to 5.5), phosphate buffer (pH 6.0 to 7.5) and Tris-HCl buffer (pH 8.0 to 9.0). The pH range that provide highest activity was considered as 100%, and the relative activity of the others was calculated based on this one.

2.4 Optimum temperature for POD and PPO activity

The enzymatic activities were performed in reaction buffer (phosphate buffer pH 7.0 for both enzymes) were preincubated for 10 min at temperatures ranging from 10 to 60 °C, at intervals of 10 °C. The temperature that provided the highest activity was considered as 100%, and the relative activity of the others was calculated based on this one.

2.5 Total phenolic compounds

The content of total phenolic was determined by the method of Fu et al. (2010) with modifications. After extraction, an aliquot of 0.2 mL was taken and mixed with 1.0 mL of the Folin-Ciocaltieu reagent 1:10 and 0.8 mL of 7.5% $NaCO_3$, followed by stirring. After 30 min of rest in the dark, the spectrophotometer reading at 760 nm was performed, using gallic acid as standard. The results were expressed in mg gallic acid $100g^{-1}$ FW (fresh weight).

2.6 Statistical analysis

The experimental design was performed in a randomized complete block design, with menthol and eugenol in the plots and six observation periods in the subplots. The experiment was composed of four replicates per treatment and the experimental unit consisted of five potato tubers. The averages of the relative activity of POD and PPO were submitted to the standard error of the average (n=4). The peroxidase (POD) activity, polyphenoloxidase (PPO) activity and total phenolic contents data were compared by analysis of variance (ANOVA) by the Tukey test at 5% probability, using the System of Statistical Analysis and Genetics of the UFV (Sistema de Análises Estatísticas e Genéticas, 2008). SigmaPlot software was used for graph design.

3 Results and discussion

Among the most important factors that determine the browning caused by the enzymatic reaction of vegetables are pH and temperature. The catalytic action of an enzyme is usually achieved within a narrow pH range and each reaction has an optimum value, in which its activity is maximal. The optimum

pH range for the PPO of potato tubers of cv. Asterix was achieved at 6.5-7.5, using catechol as substrate and phosphate buffer in the enzyme reaction, showing an average activity value of 0.992 AU min^{-1} mg $protein^{-1}$. Below and above from optimum pH range there were reductions in PPO enzymatic activity (Figure 1). Likewise, the optimum pH for POD was 7.0, using guaiacol as substrate and the phosphate buffer in the enzyme reaction, with an average activity value of 0.502 AU min^{-1} mg $protein^{-1}$. Under pH values below 6.5 and above 7.0, the activity of POD dropped rapidly reaching lower values, with no activity between pH 8.5 and 9.0 (Figure 1).

Usually, maximum enzyme activity for most vegetables are obtained near neutral pH, but optimum values of pH for peroxidases and polyphenoloxidases activities described in the literature vary according to the source and purity of the enzymes, presence of isoforms, substrates and buffer solutions (Vamos-Vigyazo & Haard, 1981).

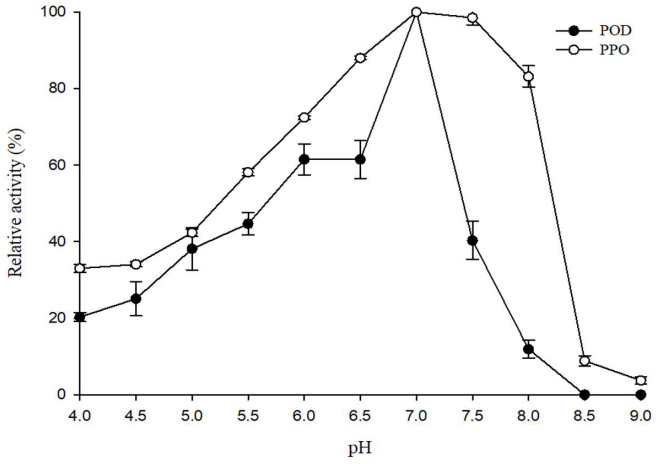

Figure 1. Effect of pHs on the activity of peroxidase (POD) and polyphenoloxidase (PPO) in Asterix potato tubers. The vertical bars represent the standard error of the average (n=4).

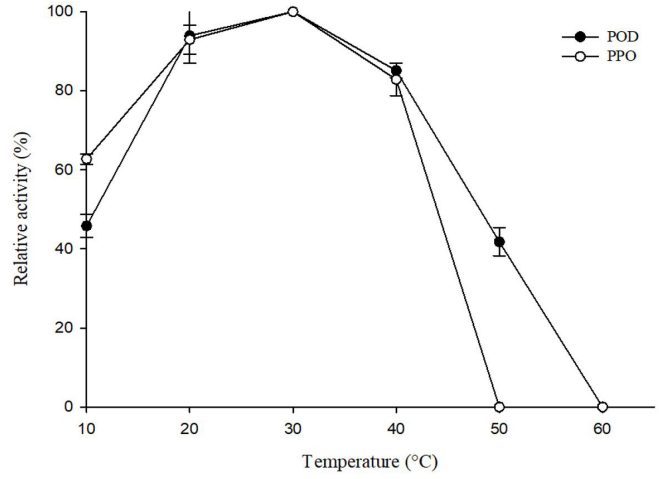

Figure 2. Influence of reaction medium temperature on the activity of peroxidase (POD) and polyphenoloxidase (PPO) in Asterix potato tubers, evaluated at pH 7.0. The vertical bars represent the standard error of the average (n=4).

In this study, both enzymes evaluated showed higher enzymatic activities near neutral pH, however there are variations related to the optimal pH of POD activity. In other species including sweet potato pulp showed optimal pH values between 3.5 and 5.5 (Castillo et al., 2002), red beet taproots between 5 to 6, while in radish, the pH ranged from 4 to 5 (Rudrappa et al., 2007). Optimum pH values for PPO extracted from tubers using catechol as substrate was 4.6 for taro rhizomes and 6.8 for potato cv. Romano (Kiattisak et al., 1999), and 7.2-7.8 in yacon tubers (Araújo & Figueiredo, 2018).

POD and PPO enzymatic activities were affected similarly by the changes in temperature. Both evaluated enzymes showed optimum temperature ranging from 30 to 35 °C. At temperature between 50 and 60 °C the activities of both enzymes were reduced drastically (Figure 2).

The temperature effect on the stability of an enzyme depends on several factors including pH, ionic strength of the medium, and the presence or absence of binders such as substrates which protect the enzymes from heat denaturation (Sellés-Marchart et al., 2006). On the other hand, thermal treatment, generally between 70-90 °C, inactivates PPO, but the use of intense heat treatments can produce changes in color, texture and formation of off flavors (Vamos-Vigyazo & Haard, 1981; Martinez & Whitaker, 1995).

The results obtained in the study are according to the literature, agreeing with the results found in strawberry, where the POD activity was highest at 35 °C (Martínez et al., 2001) and in arracacha tubers had its maximum activity at 30 °C (Menolli et al., 2008). In yacon tubers, the optimum temperature for PPO activity was 30 °C (Neves & Silva, 2007) and in sweet potato at 30 °C (Manohan & Wai, 2012).

Among the enzymes evaluated, the POD was more resistant to heat treatment. Usually, the peroxidases of most plant tissues and present an optimum activity in the range of pH 5.0 to 6.0, being considered by some authors the most heat-resistant enzyme among those present in fruits and vegetables (Lee et al., 1984; Rodrigo et al., 1996), thus being used as an index of effectiveness of the bleaching of products by the food processing industry.

Regardless the treatment, there was an increase in the enzymatic activity during the storage time, with higher values in the control tubers and smaller activities in the tubers treated with eugenol or menthol sprouting suppressors up to 40 days of storage (Figure 3). But in the end of the storage period of 50 days, POD activity was similar in all treatments, a fact that can be explained by the loss of effectiveness by sprouting suppressants.

Similar results were previously reported by Afify et al. (2012), which observed lower enzymatic activity, disease incidence and percentage of germination after the application of the essential oils from Caraway and Clove and its main component carvone and eugenol. In addition, they noticed that POD activity increased during the aging of the tubers and the length of storage resulting in lipid peroxidation over time. These results corroborate the

increase of POD activity at the end of storage in the present study (Figure 3).

According to Abbasi et al. (2015) the lower activity in tubers treated with essential oils may be related to a lower rate of growing verified in the sprouts over time, since POD activity was present in all treatments, but with the maximum activity in the control tubers. The same authors determined that the application of peppermint oil as sprouting suppressor resulted in moderate enzymatic activity at the end of the storage period, similarly to the results obtained with the cultivar and storage conditions of this study.

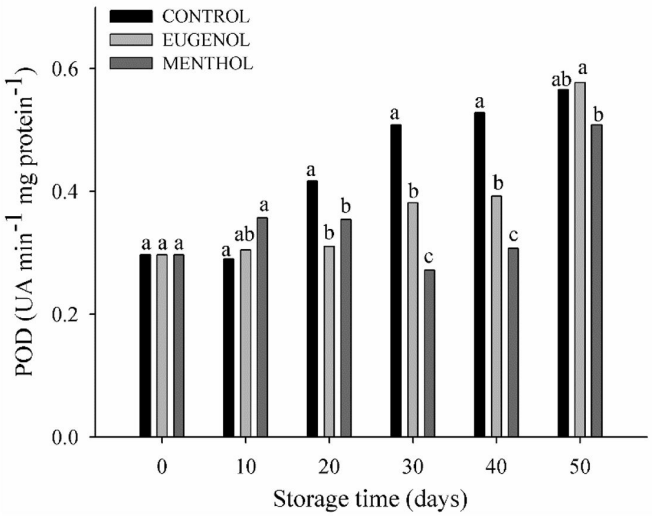

Figure 3. Peroxidase activity (AU min^{-1} mg protein^{-1}) in non-dormant potato tubers cv. Asterix treated with sprouting suppressants (eugenol and menthol) during storage. Means followed by the same letter, do not differ by the Tukey test at 5% probability.

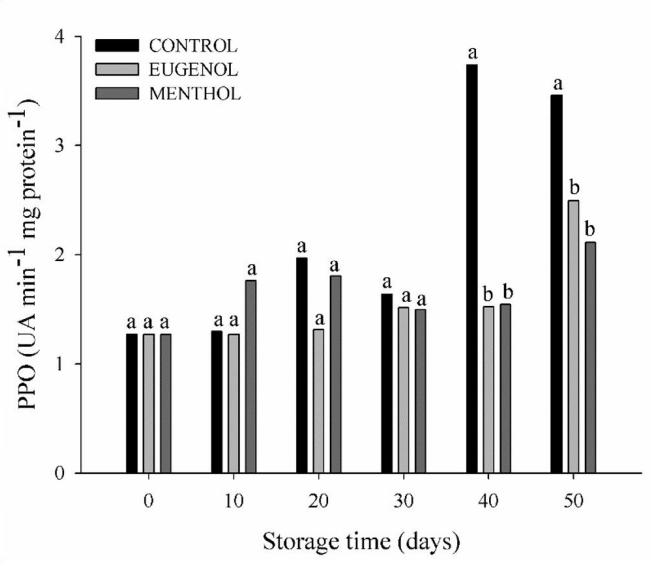

Figure 4. Polyphenoloxidase activity (AU min^{-1} mg protein^{-1}) in potato tubers cv. Asterix treated with sprout suppressants (eugenol and menthol), during storage. Means followed by the same letter, do not differ by the Tukey test at 5% probability.

The increase in PPO activity in vegetables during postharvest storage can be associated with the consumption of free phenolic compounds used as a substrate in the presence of oxygen. In the present study, PPO activity remained stable in all treatments during the first 30 days of storage (Figure 4), followed by a higher enzymatic activity on control tuber, compared to potatoes treated with eugenol and menthol (Figure 4).

The increased PPO activity in control tubers may be attributed to the faster sprouting growth rate during storage compared to the eugenol and menthol treated tubers. Likewise, Abbasi et al. (2015) observed that the polyphenoloxidase activity in potatoes presented a constant increase with the progression of storage in most treatments, however, the increase was significant in the control tubers in comparison with CIPC, clove oils, hot water and spearmint treatments.

Nourian et al. (2003) also found small changes in PPO and POD activity, especially with short storage time at temperatures of 4 and 8 °C. As mechanical damages are among factors that trigger the activity of these two enzymes in stored potatoes, the small changes associated with the activity of these enzymes could mean the lack of wounding in the tubers.

Delaplace et al. (2008) justified that the application of essential oil, in addition of preventing sprouting, has suppressive activity in protecting the tuber against the incidence of pathogens due to its antiviral and antifungal characteristics. Because of that, the essential oils alleviate the stress conditions, which results in lower POD and PPO activities. This fact was also observed in the experiment under discussion.

In addition to pH and temperature, the concentration of phenolic compounds is extremely important for the development of enzymatic darkening reactions. The phenolics are substrates for the enzyme, are usually fisically separated within the cells. With wounding or any treatment that damages the cell integrity, the enzyme and the substrate come in contact allowing the reaction to occur rapidly (Escribano et al., 1997).

Phenolic compounds are secondary metabolites that confer significant functional attributes on plants involved in quality characteristics, including taste and appearance (Abbasi et al., 2015). The POD catalyzes browning by oxidizing free phenolic or quinones compounds using hydrogen peroxide as the electron acceptor (Wongsheree et al., 2009), and PPO increases the browning in conjunction with POD enzyme or by acting independently.

Regarding the total phenolic compounds content, there was an increase over time during storage in all treatments (Figure 5). At the beginning of storage, the treatments had an estimated average total phenolic compound content of 5.72 mg gallic acid 100g^{-1} FW. Eugenol and menthol treated tubers presented increased contents beginning 20 days, keeping somewhat constant up to the end of storage (Figure 5). The changes on phenolics during the storage seemed to be associated with the aging of the tubers and without any effect by the essential oils treatments. Similarly, Abbasi et al. (2015) noted an initial increase followed by a gradual decline until the end of storage of the total phenolic compounds.

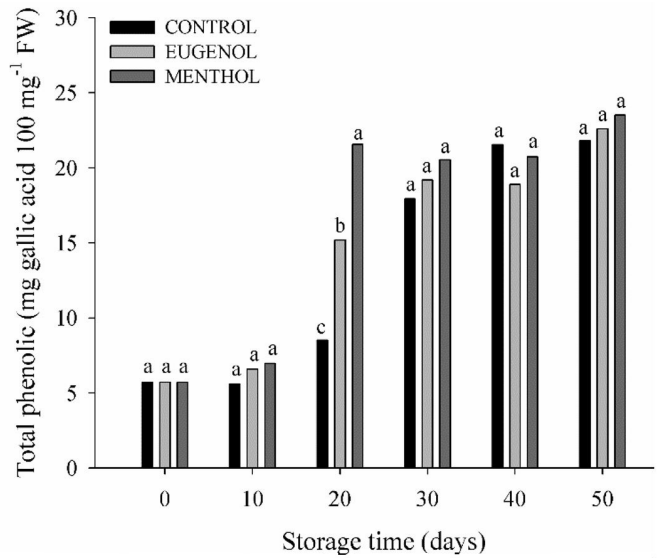

Figure 5. Total phenolic contents (mg gallic acid 100g^{-1} FW) in potato tubers 'Asterix' treated with sprouting suppressants (eugenol and menthol) during storage. Means followed by the same letter, do not differ by the Tukey test at 5% probability.

4 Conclusion

The optimum pH and reaction temperature for both enzymes of potato cv. Asterix were defined, contributing to the thermal inactivation of these enzymes on industrial processing. The sprouting suppressors reduced enzymatic activity of POD and PPO, which can result in less browning during potato processing.

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