Amazonian Journal of Plant Research *©2017 Federal University of Pará*
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http://www.ajpr.online - Amaz. Jour. of Plant Resear. 1:20-23. 2017

Amazonian Journal of Plant Research

Brief communication

Adequacy of the extract aliquot for determining the activity of polyphenoloxidase in sweet potato varieties

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Received: 09 May, 2017. Accepted: 15 July, 2017
First published on the web August, 2017
Doi: 10.26545/b00002x

Abstract

The object of this study was to adjust dosages for measuring polyphenoloxidase activity in sweet potato under refrigerated conditions. The following cultivars were utilized: ESAM 1; ESAM 2; ESAM 3; Mãe de Família; Paraná; and Sr. Antônio. Plants were harvested after 120 of growth. The tubercles were refrigerated for four days at 10 °C and then washed, cut, packed, and stored at 5 °C for 12 days. At initial time (day zero) of conservation, 1 g of tissue sample was macerated in a mortar, under ice bath, containing 6 ml of sodium phosphate buffer [0.2 M; pH 6.0]. The extract was then centrifuged at 7690 x g for 23 minutes at 4 °C. For the PPO activity essay, it was used a mixture of 2.385 µL of phosphate buffer (0.2 M), pH 6.0, and 500 µL of 0.2M catechol as substrate, which remained at 30 °C until temperature stabilization. To this mixture, it was added 10 µL, 15 µL, 20 µL, and 25 µL of the enzyme extract at 425 nm. This was done every 10 seconds in an interval of 2 minutes. It was observed that increasing the volume of extract, increased the activity of polyphenoloxidase independent of the cultivar evaluated. However, the cultivars ESAM 2, ESAM 3, Mãe de família, and Sr. Antônio seemed to slightly lost linearity above 10 µL. Thus, we conclude that the volume of 10 µL is the most appropriate for determining enzymatic activity for all cultivars.

Key-words: *Ipomoea batatas*, Enzymatic Browning, Oxidative Enzyme

Sweet potato cropping assumes a relevant social value in the Northeast region as it contributes for the fixation of the man in the field, creating jobs and increasing income (Santos et al., 2006). Sweet potatoes are a good source of energy and nutrients, contributing to the supply of calories, vitamins and minerals in the human diet (Oliveira et al., 2013).

Harvesting is an stressful event for vegetable products, as it triggers profound transformations, which generates modifications in their metabolism. Post harvesting procedures (handling, packing, transporting, and storing) can also result in mechanical damage of the vegetable, promoting tissue darkening (Chitarra and Chitarra, 2005). The discoloration observed in the surface of some cut fruits and vegetables is the result of ruptures of cell compartments, which allows contact of the oxidases with their substrates. Cutting also induces the synthesis of the enzymes involved in darkening reactions (Rodrigues et al., 2011).

Polyphenoloxidases (PPO, EC 1.14.18.1) promote the formation of dark pigments, often accompanied by undesirable changes in appearance and organoleptic properties of the product, shortening life shelf and decreasing market value (Araujo, 2008).

The intensity of darkening in various tissues may be due to variation in the activity of the polyphenoloxidase (Handche and Boynton, 1986). The promotional action of oxidation reactions and the biodegradation in processed fruits and vegetables

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result in nutritional losses, undesirable changes in aroma, taste, texture and fruits color, which cause economic losses (Mantovani and Clemente, 2010).

The most important factors in the progress rate of the enzymatic darkening caused by PPO are: the active concentration (of active PPO) and concentration of phenolic compounds, the pH, the temperature, and the available oxygen in the tissue (Pineli et al., 2005). The level would depend, among others factors, on the cultivar (Vamos-Vigyazo, 1981). Studies targeting enzymatic activity of plants are essential in biochemistry and plant physiology, as all plant responses involve metabolic processes, which are regulated by various enzymatic complexes.

Darkening is also reported for tuberous roots such as yam (Simões et al., 2016) and table manioc (Freire et al., 2015). However, this enzyme should not be considered only as a villain. It has been observed that plants with high levels of this enzyme may be more resistant to pathogens (Chitarra and Chitarra, 2005). The inhibition and/or control of metabolic changes caused by enzymes is of vital importance for reducing post-harvest losses and maintaining the quality standards of the final product (Alvarenga et al., 2011).

Freire et al. (2015) used 100 µL of the extract volume to quantify polyphenoloxidase activity in table manioc. In order to determine the volume of extract suitable for measuring polyphenoloxidase activity in sweet potato, the establishment of a protocol becomes of fundamental importance.

Thus, the aim of this study is to adjust the aliquots to determine polyphenoloxidiase (PPO) activity in sweet potato cultivars.

The experiment was carried out from June to July of 2015 at the Department of Plant Sciences, UFERSA Mossoro campus. The location is situated at 5 °11'south latitude and 37 °20' west longitude and 18 m altitude. According to Thornthwaite, the climate of the locality is DdAa, which is semiarid, megathermic and with little or no excess of water during the year. According to Koppen, it is BSwh, which is dry and very hot, with a dry season, generally from June to January, and a rainy season, from February to May (Carmo Filho and Oliveira, 1989).

After 120 days of growth, the sweet potato plants (cultivars ESAM 1, ESAM 2, ESAM 3, Mãe de Família, Paraná and Sr. Antônio) were manually collected and transported at room temperature to the Serra Talhada campus of the Universidade Federal Rural de Pernambuco. The potatos were then refrigerated for 4 days at 10 ºC and at 90 ± 5 % of relative humidity, washed in running water, cut with sanitized knives, packed in polystyrene trays sealed with propylene film and held at 5 °C for 12 days in B.O.D.

At the initial time (zero day) of conservation, 1 g of tissue of the samples was extracted and macerated in a mortar under ice bath, containing 6 ml of sodium phosphate buffer [0.2 M; pH 6.0]. Then, the extract was centrifuged at 7690 x g for 23 minutes at 4 °C.

For the PPO activity essay, it was used a mixture of 2.385 µL of phosphate buffer (0.2 M), pH 6.0, and 500 µL of 0.2 M catechol as substrate, which remained at 30 °C until the temperature stabilization. To this mixture, it was added 10 µL, 15 µL, 20 µL and 25 µL of the enzyme extract at 425 nm, were performed every 10 seconds in an interval of 2 minutes. The extraction and the activity of PPO were performed according to Simões et al. (2015).

A regression analyze was carried out for the quantitative average using the program Assistat beta version 7.6, developed by Silva (2013).

It is observed for all cultivars that, increasing the volume of extract also increases polyphenoloxidase enzymatic activity (Fig. 1A; 1B; 1C; 1D; 1E; e 1F). According to Agrios (1997), the concentration of toxic oxidation by-products increases with the activity of polyphenoloxidase enzyme, which confers resistance to infections.

The cultivars ESAM 2, ESAM 3, Mãe de Família; and Sr. Antônio slightly lost linearity above the volume of 10 µL (Fig. 1B; 1C; 1D; 1F), which may indicate external interference such as substrate limitation. An enzymatic extract aliquot of 50 µL was adopted based on the evaluation of PPO activity in yam (*Dioscorea* sp.) by Simões et al. (2016). In manioc, the volume of concentrated enzymatic extract was 0,5 mL (Ramos et al., 2013). PPO activity may also change according to the cultivar. It was not verified any decrease in linearity above 20 µL for the potato cultivars ESAM 1 and Parana (Fig. 1A and 1E, respectively).

After the post-harvest phase, polyphenoloxidase enzyme has the undesirable effect of enzymatic darkening in products of vegetable origin. According to Araújo (2001), most vegetables quickly darkens

after cut or crushed as a result of oxidative reactions of the PPO's enzymes. Besides reduction of nutritional value and the alteration in flavor, darkening also results in great economic losses.

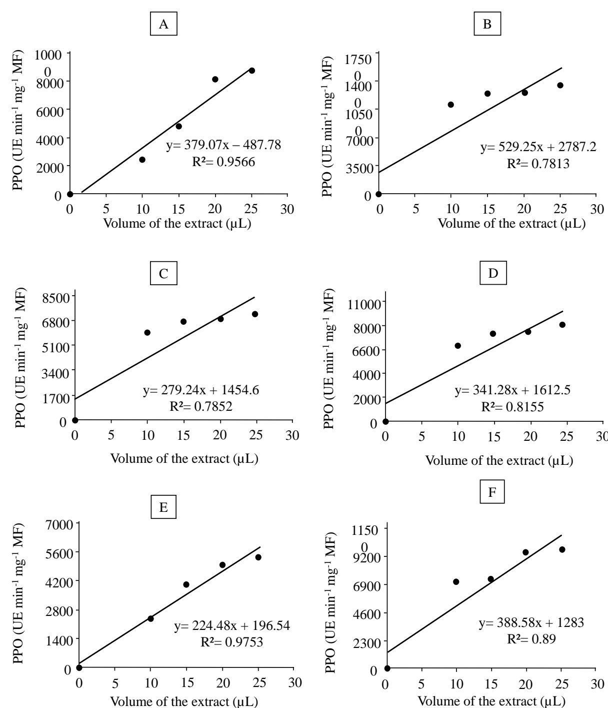

Fig. 1. Activity of the polyphenoloxidase enzyme (PPO) at the initial time (day zero), cultivar ESAM 1 (A), cultivar ESAM 2 (B), cultivar ESAM 3 (C), cultivar Mãe de família (D), cultivar Paraná (E) and cultivar Sr. Antônio (F).

According to Éspin et al. (1995), the agent of tissue darkening (provoked by the action of polyphenoloxidase enzyme) is o-quinone, a product released from tyrosinases active sites. The o-quinones suffer non-enzymatic reactions with many nucleophiles, resulting in the spontaneous appearance of dark brown, red or black pigments, denominated melanin.

According to the obtained results, it was concluded that the volume of 10 µL was the most adequate for the PPO activity assay in the studied cultivars of sweet potato.

Acknowledgements: The authors are thankful to CAPES for providing funds (scholarship); to FACEPE for the use of the equipment; and to UFERSA for making the raw material (sweet potato) available.

Conflict of interest: All authors declare no conflict of interest.

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Cite this article as:

Albuquerque, J.R.T.; Coelho Júnior, L.F.C.; Mélo Neto, D.F.; De Andrade, M.T.; Matias, J.R.; Simões, A.N.; Barros Júnior, A.P. 2017. Adequacy of the extract aliquot for determining the activity of polyphenoloxidase in sweet potato varieties. **Amaz. Jour. of Plant Resear** 1: 20-23.

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