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A COMPARATIVE STUDY OF THE POLYPHENOL OXIDASE ENZYME ASSAY AND POLYPHENOL PROFILE OF THREE TYPES OF TOMATO

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ABSTRACT

The objective of this study was to make a comparative study of the polyphenol composition, protein and enzyme activity of poly phenol oxidase present in three cultivars of Tomato (*Solanum lycopersicum*). Gel diffusion assay gave a preliminary insight into the PPO enzyme activities of the three types of tomato. This was later confirmed by the spectrophotometric enzyme assay. Among the three varieties of tomato, cherry tomatoes proved to be the best having the highest activity and polyphenol profile.This research has established the fact that the cherry tomatoes are comparatively better in terms of PPO enzyme activity and the polyphenol composition.

Keywords: Polyphenol, Protein, Enzyme, Gel diffusion

INTRODUCTION

Tomato (*Solanum lycopersicum*) constitutes an integral part of the human diet and is one of the most consumed agricultural crops worldwide, cultivated in fields, greenhouses, or small home gardens. Classified as a functional food, it is one of the most widely consumed fresh and processed vegetables for its nutritional and bioactive antioxidants such as vitamin A, C, and E. It has not only the nutritional antioxidants, but also a great quantity of non-nutritional antioxidants such as carotenoids, flavonoids and phenolic compounds, etc. **[1, 2]** Phenolic compounds

are one of the main groups of dietary phytochemicals found in fruits, vegetables and grains. They are found in plant tissues, and frequently serve as pigments in plants to attract pollinators, or as plants chemical defence mechanism against infections caused by microorganisms and injuries by insects **[3, 4]**. A significant role of phenolics that has been under active research in recent years is their possible beneficial health effects for humans. Phenolic compounds have been recognized for their antioxidant activity which has been linked to slow down the ageing process and lowered risks of many prevalent chronic diseases such as cancer and coronary heart disease **[5]**.

Polyphenol oxidase (PPO) is ubiquitous enzyme found in almost all living organisms, including animals, plants and microorganisms **[6]**. Copper present as a prosthetic group in the enzyme utilizes molecular oxygen which is considered as a co-substrate. It catalyzes two important reactions: the first a cresolase activity, adding a hydroxyl group to a monophenol at ortho position to convert it into an *o*-diphenolic compound. The second a catecholase activity, converting the diphenolic compound into quinone which is polymerized into red, brown or black pigments **[7]**. In living tissues, enzyme and phenolic compounds are separated from each other. During post harvesting processes due to cell damaging, PPO and its substrates come in contact with each other leading to browning, which lowers the nutritive value of food and alters the protein functions as well **[8]**. Browning negatively affects the commercial value of many agricultural productions, including apple, banana, cucumber, grape, mango, pear, peach, apricot, eggplant, lettuce, potato and cereals **[9]**. PPOs have been largely investigated in relation to the commercial importance of browning **[10]**. Several studies report a positive correlation between PPO expression and resistance/tolerance to biotic stresses. For instance, potato varieties displaying enhanced PPO activity also exhibited higher tolerance to soft rot disease caused by the bacteria *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *brasiliensis* and *Dickeya* spp. **[11-13]**. In a few cases, it is associated with the formation of flavour compounds positively influencing the food processing quality, for instance in the production of black tea, coffee and cocoa **[14]**. Based on substrate specificity and mechanism of action, polyphenol oxidases are classified into three types: tyrosinase, catechol oxidase and laccase **[15, 16]**. The purpose of this research work is to compare the enzyme activities in three varieties of tomato and also their polyphenol composition.

MATERIALS AND METHODS

Sample Collection and Preparation of Tomato extract:

Fresh tomatoes were bought from the local vegetable market and were washed, wiped dry. Tomatoes were cut into pieces, deseeded and oven dried in separate trays at 50°C for 48hrs. The dried pieces were powdered and 10 g of each of the powder was soaked in 100 ml of distilled water and ethanol which were kept for shaking in orbital shaker at room temperature for 48hrs. The extracts of the peel and pulp were filtered and the extraction was repeated for two more times to make sure the complete extraction of the phytochemicals. The resulting ethanolic extracts were pooled together and left to air dry for the complete evaporation of ethanol. The aqueous extracts were placed in the water bath for the evaporation of water. The samples were dried to get a constant weight and then reconstituted in their respective solvents. These solutions were then used as aqueous and ethanolic extracts for all the investigations.

PPO Enzyme assay by Catechol Agarose Gel Diffusion method:

Agarose (1.5% (w/v) in phosphate buffer pH 7.0 was heated in microwave oven and 50 mM catechol was added **[17]**. 25 ml of this buffered solution was poured into petri dish, cooled to room temperature and stored at 4° C in dark conditions. Three 5 mm diameter wells were bored with a cork borer in the catechol agarose plate and 100µl PPO enzyme extracts of the three tomatoes were loaded into the wells. The petri plates were covered and then incubated at 35^0C in an incubator for 12 hours. Diameters of the darkly stained zones were measured.

Protein Estimation:

Protein estimation was carried out by Lowry's method. Unknown protein concentration in samples, was determined using a standard calibration curve of Bovine serum albumin (BSA). The standard calibration curve has absorbance at 660nm in y axis and the known BSA concentration (µg/mL) in x-axis. By plotting the absorbance (y) of the sample in the slope equation (y= mx+c) as deduced by BSA calibration curve, we can get the concentration estimate of protein in sample (x). Estimation of Protein in the three tomato extract samples was carried out using Lowry's Method.

Enzyme Activity

PPO activity was determined by measuring the absorbance at 420 nm using a spectrophotometer (Jasco). To determine the concentration of enzyme sample corresponding to the highest enzyme activity, the activity was determined in 3 mL of reaction mixture which consisted of 2.0 mL substrate (0.02 M catechol) and different concentrations (0.1-0.3 mL) of the

enzyme preparation. To this mixture 3.0 mL phosphate buffer (pH 6.8) was added. The blank was 3.0 mL 0.1 M phosphate buffer (pH 6.8). PPO activity was then calculated from the linear portion of the curve. An enzyme sample of 0.2 mL showed the highest activity using catechol as a substrate. Enzyme assays were carried out at room temperature and results were the averages of at least three assays. One enzyme unit was considered to represent the amount of enzyme that produced an increase of 0.001 absorbance in one minute at 420 nm.

Total Flavonoid content:

10 ml of tomato extract of was added to 4 ml of distilled water and allowed to stand for 5 minutes. Different aliquots of (0.5 ml –3.0 ml) of Working Standard Quercetin (100µg/ml Methanol**)** were taken in the test tubes and the volume made up to 4 ml with distilled water. Blank was without sample.0.3 ml of 5% Sodium Nitrate and 0.3 ml of 10 % Aluminum chloride was added in all tubes. After shaking well the solutions were allowed to stand for 10 minutes at 37^0C. 2 ml of 1 M Sodium Hydroxide was added and finally the volume was made up to 10 ml with distilled water. Test tubes were shaken well and test sample filtered using filter paper and the filtrate was collected. The absorbance of the reaction mixture was read at 510 nm against a blank colorimeter. The total Flavonoid content in the ethanolic and aqueous extracts were expressed as mg of Quercetin equivalent by using the standard curve.

Total phenolics content:

The total phenolics content of tomato was estimated using Folin-Ciocalteau reagent. Different volumes of (0.1 ml - 0.5ml) tomato extracts and Gallic acid were taken in test tubes. The volume was made up to 0.5 ml with distilled water. Control was treated without sample. 0.5 ml of Folin's Ciocalteau Reagent was added in all the tubes followed by 5 ml of 7% Sodium Carbonate. The tubes were incubated for 30 minutes in dark place. The blue colour was read at 640 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10–50 µg/mL. The total phenolics content in the tomato extracts were denoted as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

RESULTS AND DISCUSSIONS

Catechol Agarose Gel Diffusion method for PPO Enzyme assay:

This method can be used as identification for PPO enzyme activity **[18]**. After 12hrs of incubation of the PPO enzyme extracts from three varieties of tomatoes in the catechol agarose plates, dark zones were seen as in **Figure 1**. These were an indication of PPO enzyme in the tomato extracts oxidizing catechol. The diameters

of the dark zones were measured and found that cherry tomato extract had a slightly larger diameter compared to the other two varieties of tomatoes.

Diffusion method

The PPO enzyme activity seen in **Figure 2** is in consonance with that which we see in **Figure 1**. The cherry tomato shows a higher activity followed by Bangalore tomato and then the Desi tomato. The protein concentration was determined by Lowry's method and it was found to be that protein concentration was higher in cherry tomato followed by Desi tomato and least in Bangalore Tomato **[19]**.

It has been reported that the individual phenolics and flavonoids content strongly depend on the type of the solvent as well as on the different concentrations of the tomato extracts used [20]. The polyphenol composition is taken in two solvents, aqueous and ethanolic[21]. As seen in Fig 3 the aqueous solvents show lower level of polyphenols while the ethanolic extracts show higher concentration **[22]**. The flavonoids exceed the level of phenols in both the extracts **[23, 24, 25]**. Cherry tomatoes have the greater concentration of polyphenols in both the aqueous and ethanolic extracts when compared to the other two varieties of tomatoes.

Figure 1: PPO enzyme assay of Desi, Bangalore and cherry tomatoes by Catechol Agarose Gel

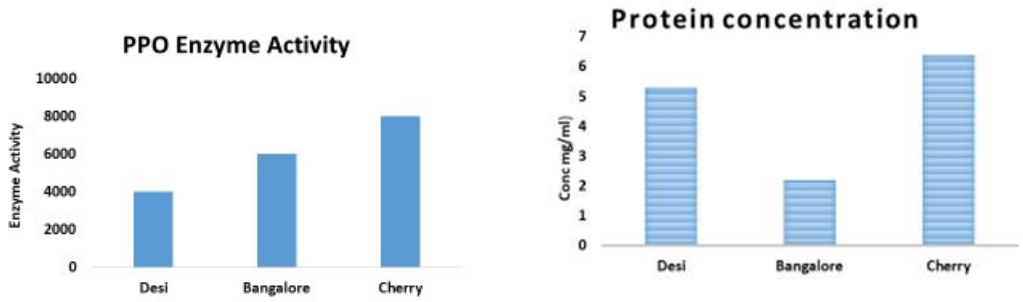

Figure 2: PPO enzyme activity and protein concentration of Desi, Bangalore and cherry tomatoes

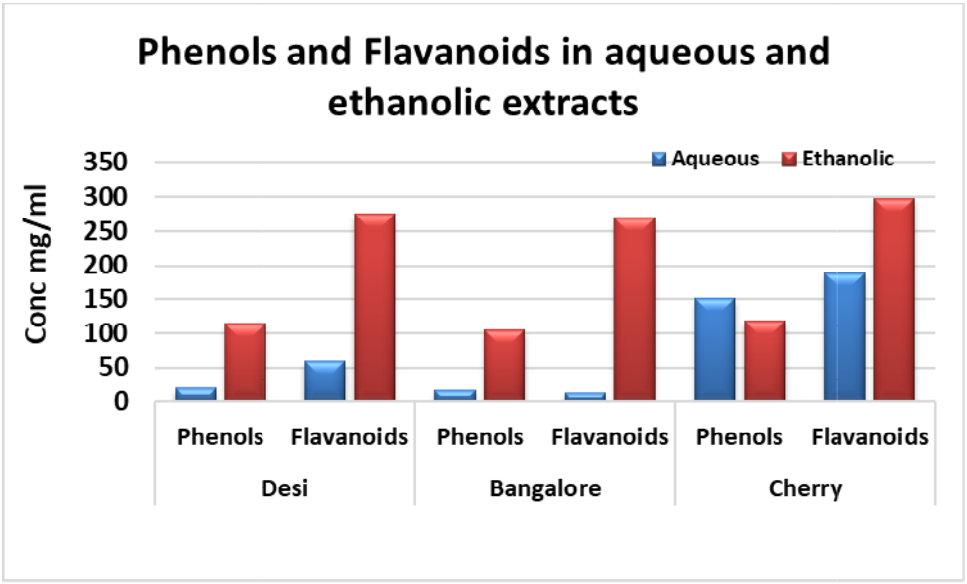

Figure 3: Phenols and Flavonoids in aqueous and ethanolic extracts of Desi, Bangalore and cherry tomatoes

CONCLUSION

This research has established the fact that the cherry tomatoes are comparatively better in terms of PPO enzyme activity and the polyphenol composition. Tomato is a promising source of bioactive compounds. These tomatoes can be used to harness their potential in terms of the extra effects that they have in terms of being antioxidants which can also be antimicrobial to treat diverse diseases ranging from life style diseases to microbial illnesses. We conclude that the tomatoes possess pharmacological properties which can not only be used in the management of diseases but also as nutritional supplements.

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