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Enzymatic determination of Catechol oxidase and Protease from fruits (orange, apple) and vegetables (carrot, tomato)

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Abstract: *The metabolic changes observed in any physical characters of fruits such as softness or colour change may be a result of changes in the activity of enzymes. The activity of plant polyphenoloxidases can cause enzymatic browning of fruits and vegetables and render food unfit for consumption due to formation of benzoquinone. Proteases degrade polypeptides and facilitate seed germination, leaf senescence, storage and release of amino acids for transport to actively growing regions. Kinetic activity is significant in understanding the process of fruit and vegetable ripening. It is evident from the literature that these enzymes (Catechol oxidase and Protease) have a prominent role in the immunity and defence mechanism.*

The present study has been focused on the enzymatic profiles of Catechol oxidase and Protease in fruits (Orange and Apple) and vegetables (Tomato and Carrot) which can be helpful in isolating and characterizing the enzymes from the above sources. Catechol oxidase and Protease were assayed using catechol and egg albumin as substrates, respectively. The Catechol oxidase activity was determined by measuring the change in colour of solution comprising of substrate and fruit/ vegetable extract due to formation of chromophoric substance, benzoquinone. Protease was assayed by measuring the rate at which the solution of egg albumin got digested by the activity of enzyme present in fruit/ vegetable juice.

An increase in the absorbance due to formation of benzoquinone was observed in tomato, orange and apple juices due to increased activity on enzyme Catechol oxidase implying to more ripening of fruits/ vegetables as compared to carrot juice where the absorbance value was found to decrease indicating less enzyme activity. Protease enzyme activity in tomato juice was found to decrease due to increase in absorbance as lesser amount of egg albumin was digested by the enzyme. However Protease activity was found to increase in orange juice sample. From these results it is evident that, protease has a significant and prominent role in ripening and softening of the fruit (orange) as compared to vegetable (tomato).

Keywords *- apple, benzoquinone, catechol oxidase, carrot, orange, protease, tomato*

I. INTRODUCTION

The metabolic changes that occur in ripening of fruits and vegetables reflect changes in the activity of enzymes within their tissues. Changes observed in any of the physical characters of the fruit, such as softness or color change, may be the result of changes in the activity of one or more of the enzymes. An enzyme may be responsible for degrading a particular component of the plant cell wall, or degradation of a particular pigment leading to a change in color. Some of these naturally occurring enzymes, such as pectinase and cellulase, are used in the food processing industry [1].

1.1 Polyphenol oxidase (Catechol oxidases)

Polyphenol oxidases are a widespread group of enzymes found in plants, fungi, bacteria and animals. In plants, these enzymes are usually found in the chloroplasts, although they can be released from here during ripening or senescence. Polyphenol oxidase (PPO) enzyme is able to catalyze the transformation of an array of aromatic compounds that have two adjacent phenolic groups on them. This includes a number of polyphenols in plants that act as antioxidants. These copper-containing enzymes oxidize the phenolic groups to reactive oxygen molecules known as quinones, which continue reacting with each other and other cellular factors to form brown spots known as melanin. These melanins form barriers and have antimicrobial properties which prevent the spread of infection in plant tissues. Plants, which exhibit comparably high resistance to climatic stress, have been shown to possess relatively higher polyphenol oxidase levels than susceptible varieties. Browning causes the deterioration of fruits and vegetables, resulting in large economic losses. Polyphenol oxidase also occurs in animals and is thought to increase disease resistance in insects and crustaceans. There are two types of PPOs. *viz*., Catechol oxidase (catecholase) can catalyze aromatic groups with two adjacent phenols, a diphenol, to become two quinones. The other is tyrosinase (monophenolase) activity, which involves introducing one atom of oxygen onto a carbon atom of an aromatic group with one phenolic group on it, changing it to a diphenol, with the two phenolic groups being adjacent to each other. Then, the enzyme will mediate catechol oxidase

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