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Isolation of antioxidant enzymes from some wild edible fruits at mature and ripened stage rhizome

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ABSTRACT

The activity of catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), were determined in ten wild edible fruits, viz *Ficus racemosa* Linn., *Elaeagnus conferta* Roxb., *Flacourtia indica* (Burm. f.)Merr., *Glycosmis pentaphylla* (Retz.) DC., *Ziziphus rugosa* Lamk., *Meyna laxiflora* Robyns., *Cordia dichotoma* Linn., *Grewia tiliifolia* Vahl, *Scleichera oleosa* Merr. and *Antidesma ghasembilla* Gaertn. The CAT and SOD activity was increased in mature fruits than ripened fruits, whereas POX activity found to be more in ripened fruits as compare to mature fruits.

Introduction

Fruits have self defense mechanism to protect from oxidative stress by the activation of many antioxidant defense enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POX). The development of oxidative stress in fruit mainly depends upon its cellular antioxidant levels, physical atmosphere of the fruits and its postharvest handling (Niranjana et. al., 2009).

To protect the cells and organ species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radical. These components includes, antioxidant enzymes e.g. superoxide dismutase, catalase, peroxidase, glutathionin peroxidase, glutathionin reductase (Jacob et al., 1995).

Catalase (E.C. 1.11.1.6) is a hydrogen peroxide oxidoreductase, which represents one of the most important oxidoreductase enzymes. Catalases are ubiquitous antioxidant enzymes irrespective of their origin catalyze the same basic reaction, the breakdown of hydrogen peroxide into water and oxygen (Chelikani et al., 2005). Catalases are utilized in cancer and diabetic retinopathy.

Peroxidase catalyses the oxidation of various electron donor substrates. In food, the reaction products of these enzymes may not only affect taste, bitterness, astringency and colour, but when interacting with proteins, these products may hinder digestibility and desirability, thereby reducing the nutritional value of foods.

Enzyme superoxide dismutase is a group of enzymes important for removing biologically generated superoxide anion radical. Superoxide dismutase is a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, and their action helps to protect cells from oxidation of lipids, proteins and DNA (Powers et al., 2008). As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. The superoxide dismutase enzyme mostly is utilized in neurodegenerative diseases.

Material and Method

Mature and ripened Fruits of *Ficus racemosa* Linn, *Elaeagnus conferta* Roxb., *Flacourtia indica* (Burm. f.)Merr., *Glycosmis pentaphylla* (Retz.) DC., *Ziziphus rugosa* Lamk, Meyna *laxiflora* Robyns., *Cordia dichotoma* Linn., *Grewia tiliifolia* Vahl. Scleichera oleosa merr., Antidesma ghasembilla Gaertn. etc were collected from various localities of Kolhapur district. Fresh samples were used for analysis of antioxidant enzymes. The activity of catalase was determined by method of Luck (1974) as described by Sadasivam and Manikam (1992). Peroxidase activity was determined by the method of Maehly (1954) and Superoxide dismutase was determined by following the method described by Giannopolitis and Ries (1977).

Results and Discussion

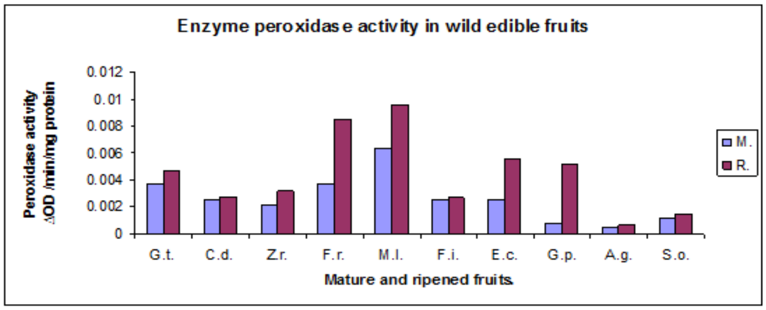
Peroxidase

The changes in the activity of enzyme peroxidase in mature and ripened fruits are shown in Graph 1. It is clear from graph that peroxidase activity is more in ripened fruits as compaire to ripened fruits. The highest peroxidase activity were found in ripened fruits *Meyna laxiflora* (0.006333 $\pm$ 0.004619) and less in *Antidesma ghasembillae* ripened fruits (0.000467$\pm$ 0.000208). In mature fruits, again the highest peroxidase activity were found in *Meyna laxiflora* (0.0096 $\pm$ 0.000265) and less in *Antidesma ghasembillae* (0.000633 $\pm$ 0.000306).

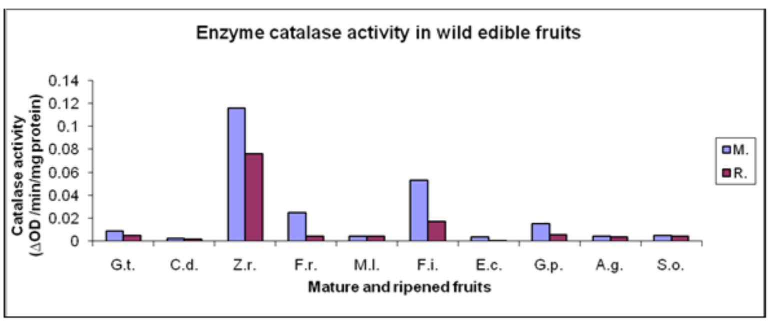
Peroxidases participate in a great number of physiological processes, such as the biosynthesis of lignin and ethylene, defence against pathogens and wounding, auxin metabolism and stress response (Halbrock and Gricebach, 1979; Lagrimini and Rothstein, 1987; O'Neil and Scoot, 1987 and Kim et al., 1999). Peroxidase is recognized to be one of the most heat stable enzymes in plant and its resistance to heat has been reported by a numerous workers (Müftügil, 1985 and Mc Lellan and Robinson, 1987). Ali et al., (2005) reported that peroxidase activity increased when higher temperatures were applied to Phalaenopsis fruits. The activation of peroxidase is correlated to the defense responses of fruit in presence of pathogens (Maksimov et al., 2003).

The activity of antioxidant enzymes is found to be changed in response to drought (Zhang and Kirkham, 1994 and

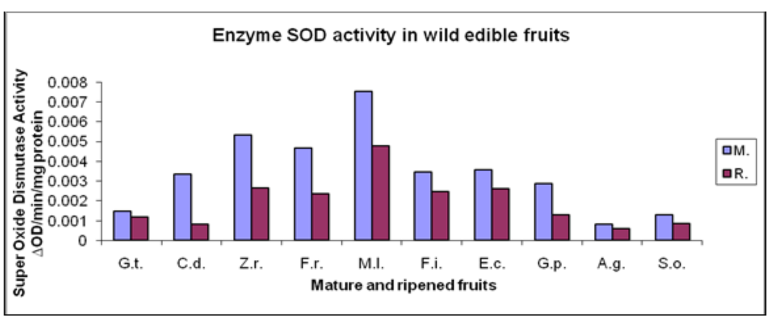
Sairam et al., 1998). Peroxidase constitute the first line of defence against reactive oxygene species (ROS) and changes in its activity and amounts have been identified as an indicator of a redox status change under drought conditions in homoiohydric plants (Schwanz and Polle, 2001). Peroxidase enzymes participate in hormone catabolism, phenol oxidation, polysaccharides and cell wall protein intercrossing, lignin polymerization, fruit ripening, and defense against pathogens. Peroxidase has been reported to be high during early fruit growth period when most metabolic activities are at the maximum (Khali and Selselet-Attou, 2007). During fruit ripening, and particularly during climacterium, peroxidase activity is increased along with the polygalacturonase and cellulase enzymes (Robinson, 1991). Fruits and vegetables may also contain peroxidases which can contribute to or generate browning-like reactions (Vamos-Vigyazo, 1981). Ku et al., (1970) have studied peroxidase activity and enzymic production of ethylene were studied in tomato fruits (*Lycopersicon esculentum* MILL.) at 3 ripening stages. They have reported that the fruit ripens, one isoperoxidase disappears, and 3 new ones are formed and the activity of peroxidase and of ethylene-forming enzyme both increased 3 to 4 times as the fruit ripened. Rogiers et al., (1998) have studied the peroxidase activity during maturation and ripening of fruit of *Amelanchier alnifolia* Nutt. They noticed that activity of enzyme peroxidase remained relatively low and constant from the mature green to the dark red stage of development, then increased towards the end of ripening as fruits turned purple. The similar results were found here in present studied fruits that are peroxidase activity were low in mature fruits, while it is found to be increased in ripened fruits.



Graph 1 enzyme peroxidase activity in wild edible fruits



Graph 2 enzyme catalase activity in wild edible fruits



Graph 3 enzyme SOD activity in wild edible fruits

Catalase

The changes in the activity of enzyme peroxidase in mature and ripened fruits are shown in Graph no. 2. The highest CAT activity shown by mature(0.116 $\pm$ 0.001) and ripened (0.076333 + 0.003786) fruits of *Ziziphus rugosa,* whereas least activity shown by mature *Cordia dichotoma* fruits (0.002667 + 0.000321) and ripened fruits of *Elaeagnus conferta*(0.000667 + 0.000252).

According to Brennan and Frenkel (1977), fruit ripening and senescence may be regarded as oxidative phenomenon and at the onset of senescence the activities of superoxide dismutase and other oxygen detoxyfing enzymes such as catalase decreases allowing superoxide or hydrogen peroxidase to accumulate to toxic levels (Bowler et al., 1992). Various environmental stresses (drought, chilling) have been shown to affect an increase in free radicals and lipid hydroperoxides due to reduced activity of catalase (Hertwig et al., 1992), Fruit ripening has been described as an oxidative phenomenon (Brennan and Frenkel, 1977). It has been reported that the tolerance of plants to conditions causing damage

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may be associated with their higher ability to remove active oxygen species through active oxygen species detoxifying enzymes, SOD, CAT, GR and ascorbate peroxidase, implying that they may play a role protecting fruits from oxidative damage (Sala, 1998). Huang, et al., (2007) studied the activity of enzyme catalase in orange pulp during fruit ripening and maturation of three cultivars ('Red Flesh' navel orange, 'Newhall' navel orange and 'Sanguine' orange,) and noticed the progressive 'Newhall' navel orange and 'Sanguine' orange,) and noticed the progressive decrease in catalase activity with ripening and the most substantial decrease in catalase occurred between young fruit stage and green fruit stage. Similar decline in catalase activity noticed in present work. This decline in catalase activity during development of fruits might be allowing to accumulate H_2O_2 which may be further dehydrogenated by increased levels of enzyme peroxidase during fruit development.

Superoxide dismutase

The changes in the activity of enzyme peroxidase in mature and ripened fruits are shown in Graph no. 3. The highest SOD activity shown by mature(0.007533 ± 0.000252) and ripened (0.004767 + 0.000115) fruits of *Meyna laxiflora,* where as least activity shown by mature(0.0008 + 0.0001) and ripened fruits(0.0006 + 0.000265) of *Antidesma ghasembilla.*

Gratao et al. (2008) worked on the antioxidant enzymes in the mature fruits of tomato. They analysed the four SOD isozymes in leaves, three SOD isozymes in fruits, and two in roots. Niranjana et al. (2009) studied the effect of pre cooling and heat treatment on antioxidant enzymes profile of mango and banana, the highest activity of SOD in mango (T5)was 119.4 units / min/g FW. And lest in T4 is 86 units / min/g FW. Incase of banana (T6) highest SOD activity was (2.84 units / min/g FW) and least in T4 (0.4 units / min/g FW). The results obtained by these authors were higher than present studied fruits.

The superoxide dismutase plays an important role in therapeutic approaches for treatment of various diseases.

Conclusion

Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well being. From our research we find out the levels of antioxidant enzymes, from mature and ripened wild edible fruits. No doubt our body develops its own antioxidant protective system, but antioxidents polynutrients are also present in fruits which keep our body healthy.

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