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STUDY OF ACTIVITY OF CATALASE ENZYME IN VARIOUS FRUITS

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ABSTRACT

Hydrogen peroxide (H_2O_2) plays an important role in fruit growth and its preservation. But too much hydrogen peroxide (H_2O_2) can be harmful to fruit cells. According to previous studies, the fruits contain an enzyme called catalase which converts the extra H_2O_2 into water and oxygen (O_2). Therefore, an experiment conducted to find the presence or absence of catalase enzyme in various fruits as well as the activity of catalase was compared among the different fruits; including Apple, banana, pineapple, strawberry, watermelon, dragon fruit, kiwi and papaya. When an additional hydrogen peroxide (H_2O_2) (6% concentration) was added to the test tube containing the fruit sample, O_2 bubbles were found in the test tube, which showed that the catalase enzyme in the fruit pieces decomposed the excess hydrogen peroxide (H_2O_2) in the test tube, resulting in the release of water (H_2O) and oxygen (O_2). The According to the present study, the highest amount of catalase enzyme was found in a banana while the lowest was found in watermelon.

Keywords: *Enzyme, Catalase, Hydrogen peroxide (H_2O_2), Oxygen, Water, Fruits*

INTRODUCTION

Enzyme: The notion of an enzyme as biocatalysts was originally presented in 1833 with the discovery of the conversion of starch into sugars catalyzed by diastase [1]. Enzymes are biological catalysts (also known as biocatalysts) that speed up biochemical reactions in living organisms. They can also be extracted from the cell and then used to catalyze a wide range of commercially important processes. The word 'enzyme' was first used by the German physiologist Wilhelm Kuhner in 1878, when he was describing the ability of yeast to produce alcohol from sugars, and it is derived from the Greek words en (meaning 'within') and zyme (meaning 'yeast') [2]. The name, classification, chemical composition and properties of the originator vary according to their function. The condition selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect the rate at which enzymatic reactions proceed – temperature, pH, enzyme concentration, substrate concentration and the presence of any inhibitors or activators [3].

Catalase is a common enzyme found in almost all organisms exposed to oxygen. Catalase was spotted as an unknown enzyme when Louis Jacques Thenard discovered Hydrogen Peroxide (H_2O_2) in 1818. In 1900, Oscar Loew first named it 'catalase'. Catalase was crystallized from the beef liver in 1937 by James B Sumner and Alexander Dounce [4]. Catalase enzyme is an oxidoreductase enzyme as it plays a crucial role in quenching the reactive oxygen species (ROS), i.e. hydrogen peroxide, often produced as a by-product of aerobic respiration. The spectrophotometric method can measure the breakdown of hydrogen peroxide (H_2O_2) by catalase [5]. Hence it acts as an antioxidant and protects the cell against oxidative stress [6]. The enzyme is found in a wide range of aerobic and anaerobic organisms. Catalase has one of the highest turnover numbers as one molecule of enzyme hydrolyzing over a million molecules of the substrate i.e. hydrogen peroxide per second. New applications for catalases are constantly emerging [7, 8] with characterization of catalase [9] and well-defined reaction mechanisms and novel roles of catalase [10]. The basic mechanism of the working of this enzyme involves the breakdown and subsequent breakdown of the reactive oxygen i.e. hydrogen peroxide (H_2O_2) into oxygen (O_2) and water (H_2O) thus relieving the oxidative stress caused by this substrate as depicted in the following reaction [11].

Catalase

$2H_2O_2$ $2H_2O + O_2$ 

(Substrate) (Enzyme) (Water) (Oxygen)

The decomposition of hydrogen peroxide by catalase is regarded as involving two reactions, namely, the catalytic decomposition of hydrogen peroxide, which is a maximum at the optimum pH 6.8 to 7.0, and the "induced inactivation" of catalase by the "nascent" oxygen produced by the hydrogen peroxide and still adhering to the catalase surface. This differs from the more generally accepted view, namely that the induced inactivation is due to the H_2O_2 itself [12]. The process of catalase can also be measured by a catalase meter. It contains flotation time data which is expressed in ten seconds [13].

Catalases are among the enzymes with the highest turnover rates known: under optimal conditions each subunit can dismutate 2×10^5 mole of hydrogen peroxide per second [14]. The molecular weights of the catalases range between 230 and 250 kDa [15]. Catalase activity is stable at a broad pH range between 5.0 and 10.5, and at temperatures between 10˚C and 30˚C [16]. Catalase is a tetramer of four polypeptide chains, each over 500 amino acid long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide (H_2O_2) [17, 18, 19]. Catalase is usually located in a cellular, bipolar environment organelle called the peroxisome [20]. As this enzyme is found in mainly all organisms (aerobic and anaerobic), it has been exploited in many applications including food processing, textile, paper, pharmaceutical industry and also in the field of bioremediation as one of the upcoming areas of its application [21, 22, 1, 23].

Depending on the physical and biochemical properties of catalases, these encompass four different types: mono-functional heme catalases (classical catalase), catalase-peroxidases (atypical catalase), non-heme catalases (pseudo catalases) and minor catalases [24]. Mono-functional heme catalase belongs to the original class of catalases found ubiquitously in animals, plants and microorganisms [25]. The heme group is responsible for catalase enzymatic activity and is located between the internal walls of the beta-barrel and several helices [26, 27]. The absence of these older taxonomic groups of catalases in organisms suggests that they arose later in evolution [19]. Catalase-peroxidase is a less prevalent class and belongs to the second group of catalases [28]. These are found only in aerobic bacteria and their molecular weight ranges from 120-340 kDa [29]. A distinctive feature in most catalase-peroxidase structures is the presence of a covalent adduct in which tyrosine is attached at its ortho position with methionine on one side and tryptophan is linked on the other side [30]. The third class is the non-heme catalases and which is a minor bacterial protein family with manganese in the active site rather than a heme molecule and is also called pseudo catalase or manganese catalase [31]. The fourth minor class of catalases includes several heme-containing proteins and these exhibit a very low level of catalytic activity [32]. They include bi-functional enzymes, such as chloroperoxidases, bromoperoxidases [33] and catalase-phenol oxidases [34].

Catalase is an antioxidant enzyme present in all aerobic organisms. It is known to catalyze Hydrogen production in the cellular environment of higher plants. Multiple molecular forms of catalase isozymes indicate its versatile role within the plant system. Catalase deficiency in plants develops anomalies such as chlorosis and head sterility and sensitivity to normal photorespiratory conditions. The molecular phylogeny of plant catalase proteins also reveals the structure and functional links among a wide range of plant species [35].

Fruit information used during the experiment:

Apple (*Malus domestic L.*) belongs to the Rosaceae family. The following nutrition is provided by the USDA for medium size (182g) apple (3" in diameter): Energy 95kcl, Fat 0.3g, Sodium 1.8mg, Carbohydrates 25g, Fiber 4.4g, Sugars 18.9g, Protein 0.5g and water 56g [36].

Banana (*Musa acuminate L.*) belongs to the Musaceae family. The following nutrition is provided by the USDA from one medium banana (118g): Energy 105kcl, Fat 0.4g, Sodium 1.2mg, Carbohydrates 27g, Fiber 3.07g, Sugar 14.4g, Protein 1.3g and Water88.4g [37].

Pineapple (*Ananas comosus L.*) belongs to the Bromeliads family. The following nutrition is provided by the USDA from 1 cup fresh pineapple chunks (165g): Energy 82.5kcl, Fat 0.2g, Sodium 1.7mg, Carbohydrates 22g, Fiber 2.3g, Sugars 16.3g, Protein 0.9g and Vitamin-c 79g [38].

Strawberry (*Fragaria x. ananassa L.*) belongs to the Rosaceae family. The following nutrition is provided by the USDA from 1 cup (152g) of strawberry halves: Energy 49kcl, Fat 0.5g, Sodium 1.5mg, Carbohydrates 11.7g, Fiber 3g, Sugars 7.4g, Protein 1g and Water 138g [39].

Watermelon (*Citrullus lanatus L.*) belongs to the Cucurbitaceae family. The following nutrition is provided by the USDA from 1 cup (154g) of raw, balled watermelon: Energy 46kcl,

Fat 0.2g, Sodium 1.5mg, Carbohydrates 11.6g, Fiber 0.6g, Sugars 9.5g, Protein 0.9g, Vitamin-c 12.5mg and Water 141g [40].

Dragon fruit (*Hylocereus undatus L.*) belongs to the Cactaceae family. The following nutrition is provided by the USDA from one 6-ounce (170g) serving of cubed dragon fruit: Energy 102kcl, Fat 0g, Sodium 0g, Carbohydrates 0.22g, Fiber 5g, Sugars 13g, Protein 2g and Vitamin-c 4.25mg [41].

Kiwi (*Actinidia deliciosa L.*) belongs to the Actinidiaceae family. The following nutrition is provided by the USDA from one green kiwi (69g): Energy 42kcl, Fat 0.4g, Sodium 2mg, Carbohydrates 10.1g, Fiber 2.1g, Sugars 6.2g, Protein 0.8g and Water 57.3g [42].

Papaya (*Carica papaya L.*) belongs to the Caricaceae family. The following nutrition is provided by the USDA from 1 cup (145g) of raw papaya sliced into one-inch cubes: Energy 62kcl, Fat 0.4g, Sodium 11.6mg, Carbohydrates 16g, Fiber 2.5g, Sugars 11g, Protein 0.7g and Water 128g [43].

MATERIALS AND METHODS

Equipment for the experiment consisted of test tubes, a test tube stand, a pipette, a stopwatch, a beaker, a weighing scale, a solution of hydrogen peroxide (H_2O_2) (6% concentration) and 8 different fruits. Apple, Banana, Pineapple, Strawberry, Watermelon, Dragon fruit, Kiwi and Papaya were taken as 8 different fruits.

Pieces of different fruits weighing 2g each were taken, including apple, banana, pineapple, strawberry, watermelon, dragon fruit, kiwi and papaya. For each fruit cleaned, separate test-tube was used. 2 ml of 6% hydrogen peroxide (H_2O_2) was added to each test tube (figure 1). The test tube was then observed with the help of a stopwatch for a fixed period of 3 minutes and the result was noted in terms of number of oxygen bubbles evolved. The experiment was repeated thrice.

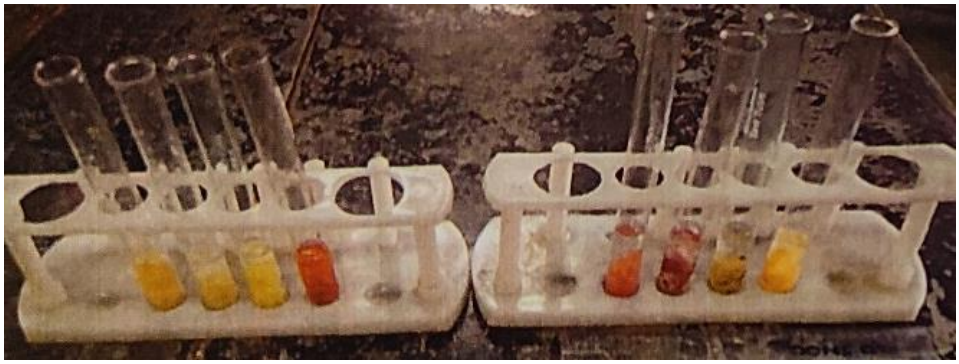

Figure 1: Experiment to study catalase activity of 8 different fruits including apple, pineapple, banana, strawberry, watermelon, dragon fruit, kiwi and banana

RESULT AND DISCUSSION:

The presence of catalase enzymes was observed in various fruits taken during the experiment. Catalase enzyme decomposes excess hydrogen peroxide (H_2O_2) and releases atoms of water (H_2O) and oxygen (O_2) [44]. When an additional 2ml of hydrogen peroxide (H_2O_2) (6% concentration) was added to the test tube containing the fruit sample, O_2 bubbles were found in the test tube. The O_2 bubbles produced in the test tube showed that the catalase enzyme in the fruit pieces decomposed the excess hydrogen peroxide (H_2O_2), resulting in the release of water (H_2O) and oxygen (O_2). The amount of O2 bubbles found in each test tube was different, indicating that the amount of catalase enzyme was different in each fruit. Just as this experiment studied the amount of catalase enzyme in different fruits, Solanki *et al.* also compared the amount of catalase in different species of *Ficus* in the polluted area and the non-polluted area [45]. Experiments were conducted to study the presence of citrate enzymes present in mango fruit [46].

In the present study, the maximum activity of catalase was observed in banana, followed by papaya, strawberry, dragon fruit, apple, kiwi, pineapple and watermelon, respectively (Figure 2). Relatively low catalase enzyme was reported in watermelon, kiwi and pineapple. Earlier, however, Papaya was reported to contain catalase enzyme less than that of banana [47]. Udoh *et al.* (1938) studied the activity of the catalase enzyme present in the blood of a diabetic patient [48]. The study was conducted on a phytoalexin called Danielon obtained from papaya

[49]. The important contribution of catalase enzyme was studied in damaged and aging seeds [50].

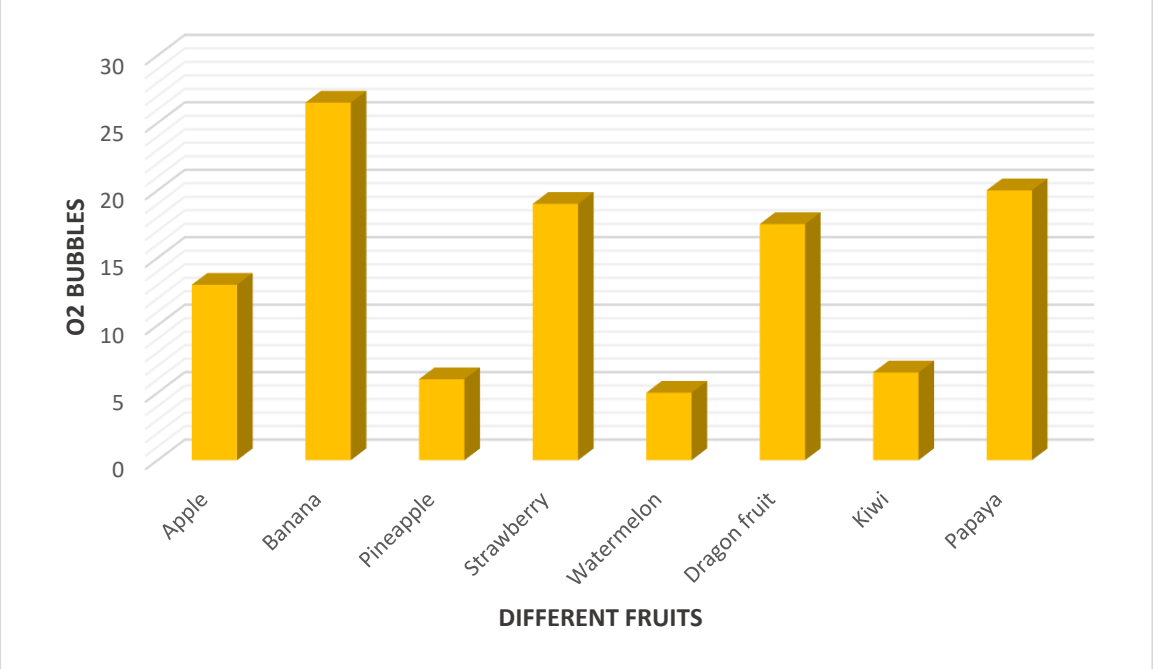

Figure 2: Effect of catalase enzyme in different fruits showing result in mean when experiment was repeated thrice

CONCLUSION

Every fruit contains some amount of hydrogen peroxide in it. Though normal amount of hydrogen peroxide (H_2O_2) is not harmful to the consumers, large amount of it can cause harmful effects. Apart from the self-generated excess hydrogen peroxide (H_2O_2), external means like preservatives etc. can also increase the hydrogen peroxide (H_2O_2) content of fruits. Catalase enzyme is a substance that helps in decomposing this harmful hydrogen peroxide (H_2O_2) into harmless components like water (H_2O) and oxygen (O_2). This experiment shows that all the fruits contain at least some amount of catalase enzyme, that vary in each fruit. This experiment showed that among those fruits, banana contains the highest amount of catalase enzyme while watermelon contains the least amount.

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