# Distribution of Enzymes (Rhodanese, 3-Mercaptopyruvate Sulphurtransferase, Arginase And Thiaminase) in Some Commonly Consumed Plant Tubers in Nigeria.

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**Abstract**

Four different enzymes (Rhodanese, 3-mercaptopyruvate sulphurtransferase (3-MST) , Arginase and thiaminase) activities were detected in crude plant extracts of nine randomly selected plant tubers which includes sweet potato (*Ipomoea batatas*), irish potato (*Solanum tuberosum*), white yam, yellow yam, bitter yam (*Diascorea bulbifera*), sweet yam (*Diascorea esculentu*), water yam (*Diascorea alata*), cocoyam and cassava (*Manihot esculentu*) . In each case, the enzymes exhibited high activities. The *p* value activity of 3-MST varies significantly in the various plant tuber. White yam showed the highest activity with a mean of 0.2 which varies significantly when compared to, red cocoyam with a mean of 0.005. Arginase was also seen to vary significantly in the different plant samples with Irish potato having the highest mean value of activity while cassava showed the lowest mean value. Thiaminase activity varied significantly in the plant tubers. Cassava stem had the highest mean value of thiaminase activity while bitter yam peel showed the lowest mean value of activity. These studies confirm the activities and nutritional values of these enzymes in the commonly consumed plant tubers.

**Keywords:** Plant Tubers, Arginase, Rhodanese, 3-Mercaptopyruvate Sulphurtransferase, Thiaminase, Activity.

## Introduction

Nigeria is the highest producer of yam, producing 73% of the world total, most of which is locally consumed. Other tubers like cassava, coco-yam and potatoes make up a large proportion in our diet (FAO, 1990). These plants are equally necessary to maintain the balance in the eco-system. Tubers (both stem and root tubers) are various types of modified plant structures that are enlarged to store nutrients (Kyte and Klein, 1996; Hammerson, 2004). In the present time human activities have reduced the amount of plants on the planet. Although many current researches confirm that plant extracts contain some chemical compounds which are biologically active within the human body. For centuries humans have used plants and plant extracts to treat various disease conditions and more recently to produce new drugs. Still most of the plants carry a large number of unidentified compounds which can be useful for making new drugs and for the treatment of disease.

Some of the best sources for enzymes are fresh fruits, vegetables, and sprouted grains. Just as the body needs enzymes to function, so do plants need enzymes for growth and reproduction. Foods are such rich sources of enzymes that some enzyme supplements are actually derived from food sources; these include pineapple (the source of the enzyme bromelain), and papaya (the source of papain), as well as kiwi and figs. Rhodanese has been confirmed to exist in plants but its function in plants is poorly understood (**Hatzfeld and Saito, 1999**). The enzyme has been proposed to have a role in cyanide detoxification, but no correlation between cyanide accumulation by plants and rhodanese activity has been observed (King and Cho, 1990). Arginine is the most important single metabolite for nitrogen storage in plant seeds. Plant arginase is housekeeping enzymes found in many if not all plant species (Witte and Medina-Escobar, 2001; Brownfield et al., 2008; Cao et al., 2010).

The potato tuber for example, represents an important reservoir of nitrogen and an approach was made to the characterization of Glutamine Synthatase (GS) in this tuber, particularly at the stages of sprouting and of new tuber formation (Pereira *et al*., 1996). Tubers represent an important reservoir of nitrogen both in the form of proteins and of a free amino acid pool which consists mainly of glutamine and asparagine (Burton, 1989). In this paper we describe the activities of four different metabolic enzymes in some plant tubers and discuss their possible functions.

## Materials

Trizma base, Trizma-HCl, ethylenediamine tetraacetic acid (EDTA), Reactive Blue 2- crosslinked agarose (suspension), thiamine, aniline and p - dimethylaminobenzaldelyde (Ehrlich reagent) were purchased from Sigma Chemical Company, St. Louis, Mo, USA. Sodium chloride, orthophosphoric acid and manganese chloride tetrahydrate were purchased from BDH Chemicals Limited, Poole, England. All reagents used were of

analytical grade and were either obtained from Sigma Chemical Company, St. Louis U.S.A or BDH Chemicals Limited, Poole, England. Microfield SM23A-Sphetophotometer

**Methods**

**Preparation of Crude Extract**.

The plant tubers were washed with saline water to remove dirty particles and were cut in to small bits. Twenty gram of each plant tubers were homogenised in three volumes of 0.02 M phosphate buffer, pH 7.2. This was followed by filtration using cheese cloth. The filtrate was then centrifuged at 5000 rpm (IEC, DPR 6000) for 30 min. The supernant was used for the final analysis.

**Enzyme and Protein Assays**

**Protein Assays:** Protein concentration was determined by Bradford method by extrapolated each protein concentration from standard curve using bovine serum albumin (BSA) as standard (1976).

Mercaptopyruvate sulphurtransferase activity was measured according to the modified method of Taniguchi and Kimura (1974). The reaction mixture in a final volume of 1 ml contained 0.38 M of Tris-HCl buffer, pH 7.8, 0.5 M potassium cyanide, 0.3 M mercaptoethanol and 30 µl of enzyme solution. Absorbance of each sample was measured at 460 nm using spectrophotometer (SM 23A-sphetophotometer). The unit of enzyme activity was defined as micromoles mercaptocyanate formed per minute at 37°C and pH 7.8.

Rhodanese activity was assayed by the method of Agboola and Okonji (2004). The reaction mixture contained 50 mM sodium thiosulphate, 50 mM potassium cyanide, 0.25 mM borate buffer, pH 9.4 and 10 µl of enzyme solution in a final volume of 1.0 ml. The reaction was carried out for 1 min at 37°C and stopped by adding 0.5ml 15% formaldehyde and 1.5 ml of Sorbo reagent (which is made up of ferric nitrate solution containing 0.025 g $Fe(NO_3)$ 3,9 $H_2O$ in 0.74 ml water and 0.26 ml concentrated nitric acid). Absorbance was measured at 460 nm. The unit of enzyme activity was defined as micromoles thiocyanate formed per minute at 37°C and pH 9.2.

Arginase activity was determined according to the method of Kaysen and Strecker (1973). The reaction mixture contained, in final concentration, 1.0 mM Tris-HCl buffer, pH 9.5 containing 1.0 mM $MnCl_{2,}$ 0.1 M arginine and 50 µl of the enzyme preparation was added in a final volume of 1.0 ml. The mixture was incubated for 10 minutes at 37 °C. The reaction was terminated by the addition of 2.5 ml Erhlich's reagent. The optical density reading was taken after 20 minutes at 450 nm. The urea produced was estimated from the urea curved prepared by varying the concentration of urea between 0.1 µmol and 1.0 µmol and a graph of optical density against urea concentration was plotted. The unit of activity of arginase is defined as the amount of enzyme that will produce one µmol of urea per min at 37$^o$C.

Thiaminase Assay**:** The enzyme sample was incubated at 37°C in 0.1 M Tris-HCl buffer, pH 8.0, with $10^{-5}$ M thiamine and $4\times10^{-3}$ M aniline and incubated for 30 min. The remaining thiamine was oxidized with the addition of 1.0 ml of 200 g/l NaOH and assayed fluorometrically (Nishimune *et al*., 2000). Absorbance was read at 411nm immediately. One unit of enzyme activity is that amount of enzyme which catalyses the formation of one micromole of heteropyrithiamine in 30 min.

**Statistical analysis**

The results are presented as means ± SD. Data were analyzed by one-way ANOVA by using SAS/PC soft ware to examine whether there was any statistical difference among groups. If the difference evaluated with the ANOVA was significant, Duncan multiple range test was used for paired comparisons. A $p$ value less than 0.05 was considered statistically significant.

**Results and Discussion**

The present study showed the activities of four different metabolic and cyanide detoxifying enzymes (arginase, rhodanese, 3-MST and thiaminase) in higher plants. The results given in Figure 1 shows the protein concentrations in the different plants. White yam contained the highest mean value of protein while different parts of cassava showed the least mean protein concentration value (Figure 1). Protein concentration of plants has always been used as index for food quality of such plant and its classification into the three classes of food. Although, the plant tubers are predominantly carbohydrate plants, the protein concentration reported in this work is in good agreement with the low protein and high carbohydrate nature of the tubers. (Anosike and Ugochukwu, 1981). Cyanide is highly toxic to the cell, especially as a potent inhibitor of metalloenzymes. In plants, at least two proteins could act in its detoxification, sulphurtransferases (rhodanese and 3-mercaptopyruvate sulphurtransferase). Sulphurtransferases would catalyze the formation of the less toxic thiocyanate; in mammals this compound is mainly excreted in the urine (Nagahara *et al*. 1999; Ressler and Tatake 2001; Meyer *et al*., 2003). Mercaptopyruvate sulphurtransferase (MST, EC 2.8.1.2) and rhodanese (Rhodanese, EC 2.8.1.1) are

evolutionarily related enzymes that catalyze the transfer of sulfur ions from mercaptopyruvate and thiosulphate, respectively, to cyanide ions (Nagahara *et al*., 1999). The activity of 3-MST in our study varies significantly in the various plant tuber samples. Water yam showed the highest specific activity with a mean value (*p*) of 0.2 and cocoyam had the least mean value of 0.005 (Figure 2). The presence of rhodanese was first reported in higher plant tissues other than the leaves by Anosike and Ugochukwu (1981) and the enzyme was hypothesized to be distributed in plants by Shirai and Kurihara (1991). Rhodanese activity has been established in the leaves, peels and in the flesh of *Manihot esculenta* (Anosike and Ugochukwu, 1981). Rhodanese specific activity at 95% confidence interval at *p* value less than 0.05 was considered to be statistically significant, but the *p* value of 0.194 was obtained for rhodanese which is less than 0.05, this shows that the rhodanese specific activity does not vary significantly in the plant tubers. Though white yam peel had the highest specific activity of rhodanese; cassava plant also showed very high rhodanese activity while cocoyam showed the least mean value (Figure 3).

Rhodanese activity in higher plants is not as high as that in animals (Sorbo, 1951; Nagahara *et al*., 1996). Cyanogenic plants showed higher rhodanese activities than non-cyanogenic plants. This may indicate that the main function of rhodanese is cyanide detoxication. But its general distribution may also suggest other function as seen in its distribution in the other plant tubers. It has been suggested that rhodanese plays a role in the modulation of energy production enzymes (Volini and Ogata, 1991). It is also possible that rhodanese activity in plants is due to the action of other enzyme(s) (Kakes and Hakvoort, 1992). No relation between cyanogenesis and rhodanese activity was found. A rhodanese purified from tapioca leaves showed properties similar to that of bovine rhodanese (Boey, *et al*., 1976), but rhodanese activity in plants is so low that the very existence of a rhodanese enzyme has been controversial (Lieberei and Selmar, 1990; Kakes and Hakvoort, 1992).

Arginase, which plays an important role in regulating the metabolism of L-arginine in mammalian cells, the enzyme has also been reported to be involved in stress responses in higher plants (Zhang *et al*., 2010). In higher plants, which also utilize arginine for the production of polyamines and nitric oxide, the potential role of arginase as a control point for arginine homeostasis has not been investigated. The specific activity of arginase was highest in irish potato while cassava had the lowest mean activity (Figure 4). In view of the well-established roles of polyamines, nitric oxide and proline in plant tolerance to chilling stress; arginase may play an important role in fruit chilling tolerance by regulating the metabolism of arginine. Most of the studies on plant arginase have focused on its role in mobilizing arginine during early seedling germination. Storage proteins are mobilized to provide amino acids for protein synthesis in the dividing cells. Arginase activity increases sharply during germination in several species including soybean (Matsuburu and Suzuki, 1984; King and Cho, 1990; Jenkinson *et al*., 1996), arabidopsis and loblolly pines (Zonia *et al*., 1995; Ging and Gilford, 1997). In higher plants urea and ornithine formed as products of arginase reaction are used in the assimilation of nitrogen into amino acids and the synthesis of polyamines through urease and ornithine decarboxylase respectively (Dawnum *et al*., 1983, Jenkinson *et al.*, 1996). The enzyme has been isolated and characterized from cotyledons as well as axis of soybean (Dabir *et al.,* 2010).

Thiaminases are enzymes found in a few plants and they function by cleaving the thiamine molecule and render it biologically inactive. Highest levels of enzyme activity were found in vigorously growing plant material (McCleary and Chick, 1977). The presence of thiaminase in very high activity in the various cassava parts (peel, stem and the flesh) is in agreement with the toxic nature of the plant. Cassava stem was found to have the highest specific activity of thiaminase while bitter yam peal showed the least mean activity (Figure 5). Reports have shown that the highest thiaminase activity in plants is found in the Rhizomes, but all areas of the plant contain some thiaminase and show seasonal variability (McCleary and Chick, 1977). Potato *(Solanum tuberosum)* was also found to possess high activity of thiaminase, and research has shown that many parts of potato are poisonous. The potato poisoning follows eating tubers that have decayed, sprouted or become green through being exposed to sunlight. The green leaves and shoots as well as the berries of the plant itself are also been reported to be poisonous. Other tubers like Fronds of the fern nardoo (*Marsilea drummondii*) contain a thiaminase enzyme at very high levels of activity.

In conclusion, consumption of cassava over time has been linked to ataxia (a neurological disorder affecting ability to walk), goitre, and konzo (FAO, 1990; Ademolekun, 1994) in some East African countries (Ademolekun, 1994). It can also cause severe calcific pancreatitis in humans, leading to chronic pancreatitis. The presence of these enzymes (3-MST, rhodanese, arginase and thiaminase) in these plant tubers, indicate the presence of a powerful detoxification mechanism of cyanide in these tubers. These enzymes may have evolved due to the exposure of these tubers through ages to high level of cyanogeic substances in the environment. It is apparent that theses enzymes may perform primary biological function as well as detoxification in these tubers.

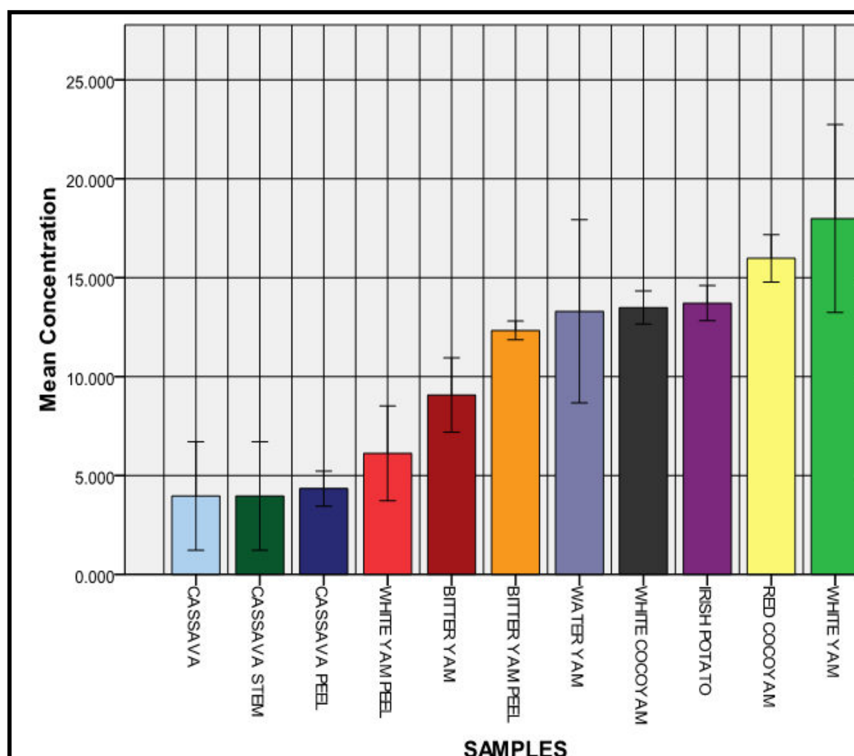


**Figure 1: Mean Protein Concentration of protein (µg in 10g tuber) the Various Plant Tubers.**

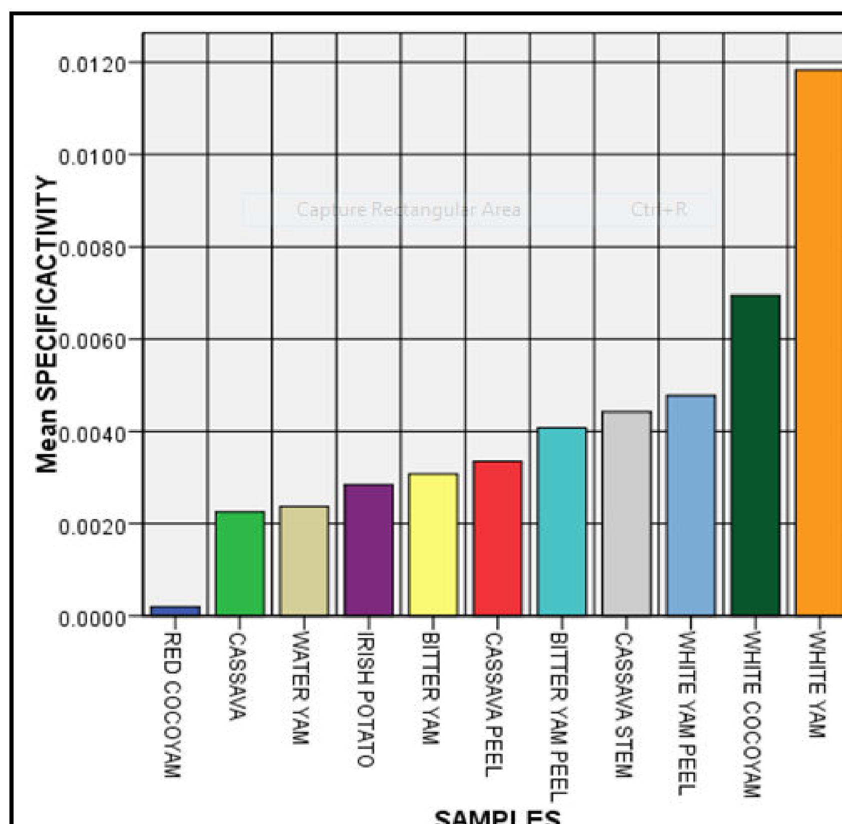


**Figure 2: *p* value of mean Specific Activity of 3-Mercaptopyruvaye Sulphurtransferase**

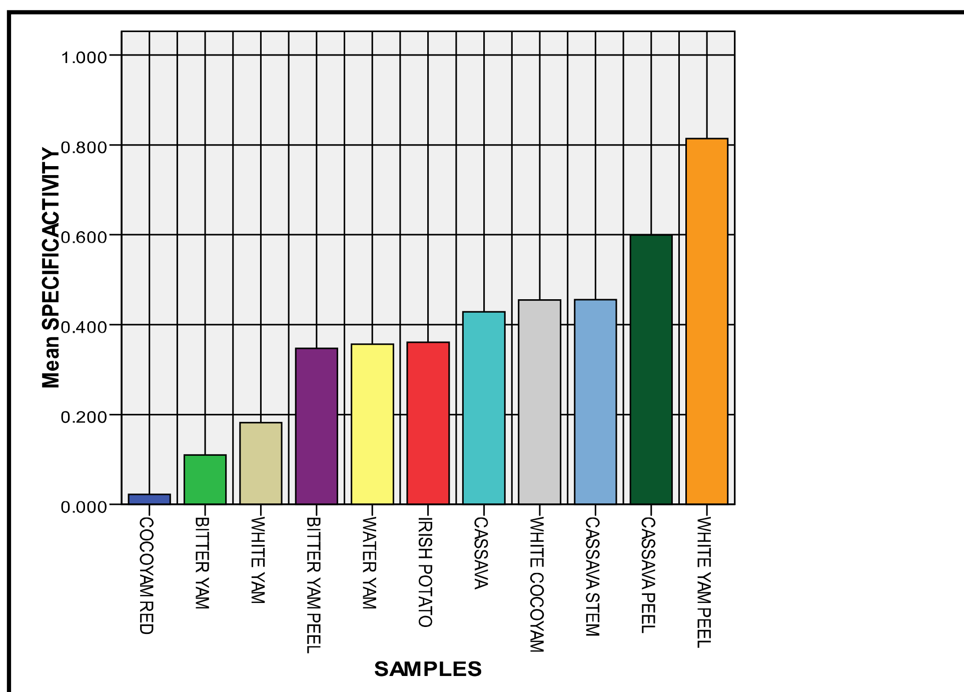


**Figure 3: *p* value mean Specific Activity of Rhodanese**

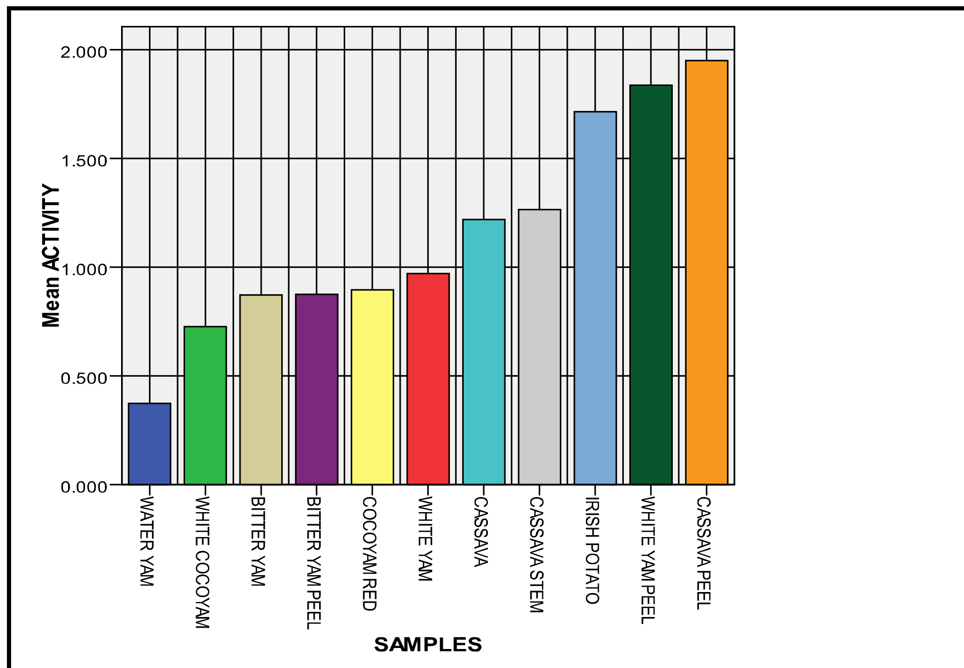


**Figure 4: *p* value mean specific activity of Arginase**

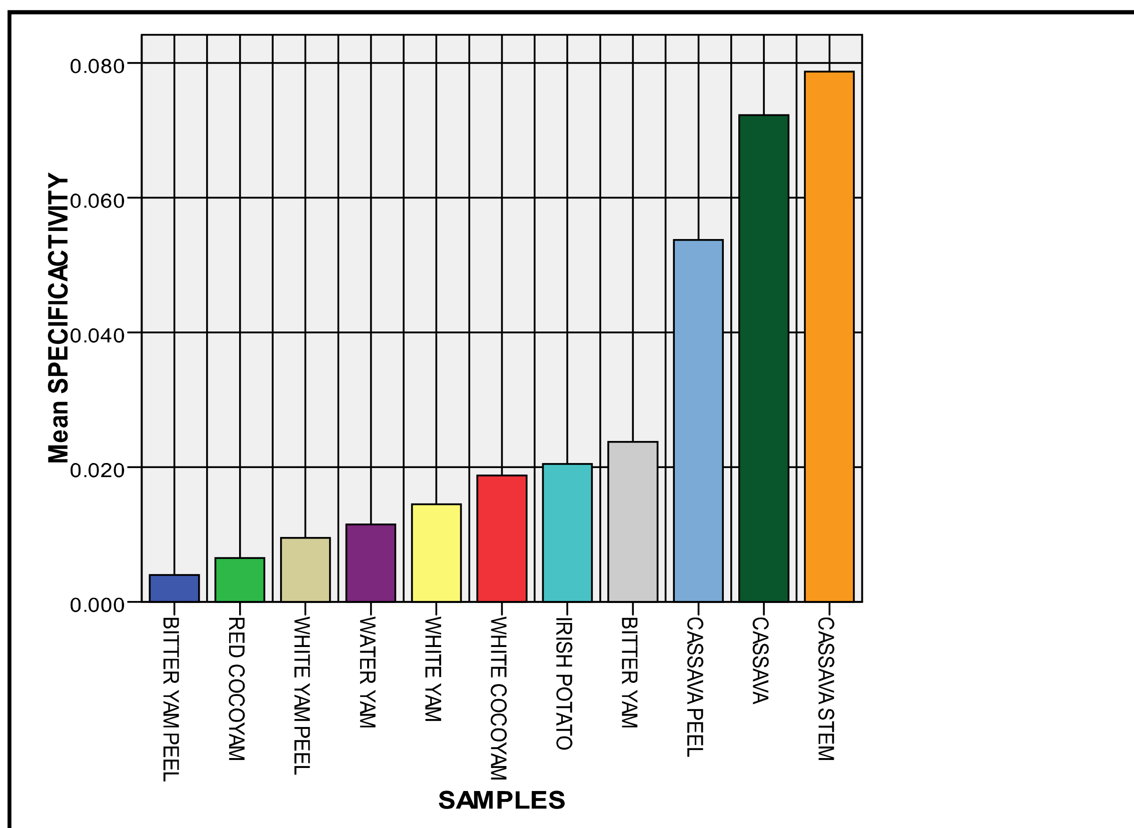


**Figure 5:** ***p*** **value mean Specific Activity of Thiaminase**

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