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Estimation of enzymatic activities in under utilized fruits

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ABSTRACT

This study examined the enzymatic activities in five selected fruit pulps viz. Aonla (NA-6 and NA-7), Bael (NB-5 & NB-9), Ber (Karaka and Umran), Jackfruit (NJ-2 & NJ-3) and Kaitha (K-1 and K-2). The present study further indicated that peroxidase, catalase, and cellulase activities in underutilized fruits. Germplasms NA-7, NB-9, Umran, K-2 and NJ-3 have found slightly higher than other respective germplasms.

Key words: *Catalase, Cellulase, Peroxidase, Enzymatic activities and Underutilized fruits.*

Introduction

Underutilized fruits refer to those fruits which may be high in nutritive value but these are not widely grown. These fruits are less consumable due to less palatable or less availability than other fruits (Tripathi *et al.*, 2015). They are nutritionally vital and superior in quality but are rarely used by human. People are generally not aware of its nutritional importance. Several compounds like enzymes as catalases, peroxidases, pectin methylesterase, cellulase, superoxide dismutases, polyphenoloxidase, antioxidant, and some phenolic compounds are present in fruits (Kaur and Kapoor, 1998). Umran showed higher activity of superoxide dismutase, peroxidase and catalase at almost all the stages of storage (Kumar *et al.*, 2011). Oxidative stress were assessed in the liver by estimation of the level of antioxidant markers, i.e. superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), malondialdehyde (MDA) and antihyperlipidemic, better choice to cure the diabetes (Kumar *et al.*, 2013).

Materials and Methods

The experiment was carried out on five underutilized fruits varieties namely Aonla (NA_6 and NA_7), Bael (NB_5 and NB_9), Ber (Karaka and Umran), Jackfruit (NJ_2 and NJ_3) and Kaitha (K_1 and K_2). These varieties are collected from Horticulture Nursery. The samples from each fruit were used to estimate enzymatic activities. Peroxidase activity can be assayed colorimetrically according to method given by Curne and Galston (1959). The enzyme catalyse the oxidation of substrate by removal of hydrogen which combines with H_2O_2.

$$AH_2 + H_2O_2 \longrightarrow 2H_2O + A$$

Taken 200 mg fruit sample and homogenize with 10 ml of phosphate buffer 0.1 M (pH 6.0) centrifuge at 10,000 rpm at 4 ^{0}C for 30 min., collected the super-

natant and store at low temperature. Used the supernatant for enzyme assay. Taken 2.0 ml enzyme extract in a test tube-added 2.0 ml phosphate buffer, 1.0 ml and 0.2 ml H_2O_2 as the test sample. Shaked the mixture well and kept it at 37 ^{0}C on water bath for 10 minutes for the formation of purpurogallin. Measured the activity at 430 nm on spectrophotometer against the blank. Expressed the result as enzyme unit per gram fresh weight. Catalase activity can be assayed colorimetrically according to method given by Sinha (1972). Catalase facilitates the dismutation of H_2O_2 to water and O_2 according to the reaction.

$$H_2O_2 \xrightarrow[\text{Catalase}]{} H_2O + \tfrac{1}{2}O$$

200 mg fruit sample was homogenized with 10 ml of phosphate buffer 0.1 M (pH 7.0) and centrifuged at 10,000 rpm at 40 ^{0}C for 30 minutes. Collected the supernatant and stored at low temperature (4 ^{0}C). Used the supernatant for enzyme assay. Taken 1.25 ml H_2O_2 reagent in a test tube, added 0.5 ml enzyme extract and 3.25 ml phosphate buffer as the test sample. Taken the reaction mixture in conical flask and mixed rapidly at 37 ^{0}C. After 3 minutes withdraw 2.0 ml reaction mixture and added 2.0 ml potassium dichromate acetic acid reagent. Kept on boiling water bath for 10 minutes and recorded O.D. at 570 nm against blank after cooling. Expressed result as enzyme unit per g fresh weight.

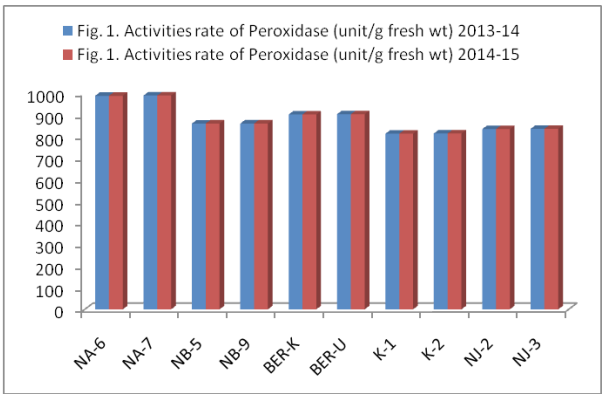
Cellulase enzyme activity was measured by the methods of Mandels *et al.* (1976). Whatman no.1 filter paper strip of 1-6 cm (50 mg approximately) was incubated with 0.5 ml dilute enzyme extract at room temperature for 1 hour. Applied without filter was incubated in order to correct for only reducing sugar present in the enzyme preparation. Tubes were cooled and 3 ml of DNS reagent was added prior to heat in a boiling water bath for 5 minutes. Tubes were cooled and volume was made up to 10 ml with water. The absorbances were taken at 540 nm. The amount of reducing sugar released during reaction estimated by using standard glucose solution and the enzyme was expressed as µ mole glucose produced per minute in enzyme solution.

Results and Discussion

The data pertaining to the peroxidase activities (unit/g fresh wt) in selected underutilized fruit germplasms were given in Table 1 and graphically depicted in figure 1 and this resulted in a slight variation within two germplasm of each fruit pertaining to peroxidase activities. The range of variability in selected two germplasm of five fruits viz. Aonal (NA-6 and NA-7), Bael (NB-5 and NB-9), Ber (Karaka and Umran), Jackfruit (NJ-2 and NJ-3) and Kaitha (K-1 and K-2) were found significant variations in peroxidase activity. The germplasm were recorded from 989.93 to 988.73 unit/g fresh weight (Aonla), 860.38 to 8860.13 unit/g fresh weights (Bael), 903.54 to 902.25 unit/g fresh weight (Ber), 814.24 to 813.18 unit/g fresh weight (Kaitha), 835.63 to 834.54 unit/g fresh weight (Jackfruit). Similar results were observed by Kumar *et al.* (2008) that 1287.0 unit/g fresh weight peroxidase activity in Ber fruit pulp. Kumar *et al.* (2011) reported that higher activity of superoxide dismutase, peroxidase and catalase at almost all the stages of storage. Vijayakumari *et al.* (2012) obsered that peroxidase enzyme activity the fruit extract of *E. officinalis* showed the highest peroxidase activity (924 units/g) compared to other fruit extracts. Very low activity of peroxidase was found in the extract of *F. carica*

Table 1. Peroxidase activity (unit/g fresh wt) in under utilized fruit germplasms

Germplasms	2013-14	2014-15
	Peroxidase activity	Peroxidase activity
NA-6	988.73	988.73
NA-7	989.93	989.95
NB-5	860.13	860.16
NB-9	860.38	860.39
BER-K	902.25	902.26
BER-U	903.54	903.55
K-1	813.18	813.20
K-2	814.24	814.26
NJ-2	834.54	834.57
NJ-3	835.63	835.66
C D at 5%	0.02	0.19



(4.253 units/g). Sirisha *et al.* (2014) also reported that peroxidase activity 875 to 945 units/g fresh wt in five germplasm of jackfruit. The cultivar with short shelf life had higher oxidative stress during storage than one with longer shelf life, increased production of reactive oxygen species induces antioxidative system including enzyme and metabolites.

The data containing to catalase activites (unit/g fresh wt) in selected underutilized fruit germplasms viz. Aonla, Bael, Ber, Jackfruit and Kaitha were given in Table 2 and graphically represented in Figure 2. The results indicate a slight variation within two germplasm of each fruit pertaining to catalase activity. The range of variability in selected two germplasm of five fruit viz. Aonla (NA-6 and NA-7), Bael (NB-5 and NB-9), Ber (Karaka and Umran), Jackfruit (NJ-2 and NJ-3) and Kaitha (K-1 and K-2) were found significant variations in catalase activity. The variations were recorded from 930.23 to 930.15 unit/g fresh weight (Aonla) 985.23 to 985.16 unit/g fresh weight (Bael), 1120.43 to 1120.41 unit/g fresh weight (Ber), 1140.77 to 1040.75 unit/g fresh weight (Kaitha), 910.19 to 910.14 unit/g fresh weight (Jackfruit). Similar findings were reported by Kumar *et al.* (2008) that 1186.21 unit/g fr.wt. catalase activity in Ber fruit pulp. Andrea (2002) reported an increase in catalase activity during storage in apple. Vijayakumari *et al.* (2012) found that catalase level in different plant extracts ranged widely from 3533.333+1533.333 units/g. The maximum catalase activity was exhibited by the fruit extract of *E. officinalis* (3533.333 units/g). The lowest value was observed in the extract of *C. indica* (1533.333 units/g). Fruit extract of *E. officinalis* exhibited the highest activity of super oxide dismutase (2.710 units/g). The minimum activity was showed by the extract of *F. carica* (0.073 units/g). Sirisha *et al.* (2014) also found that catalase activity 975 to 992 units/g fresh wt in five germplasm of jackfruit.

The data regarding to cellulase activities in five selected minor fruits viz. Aonla, Bael, Ber, Jackfruit and Kaitha were given in Table 3 and graphically shown in Figure 3 and resulted in a slight variation

Table 2. Catalase activity (unit/g fresh wt) in under utilized fruit germplasms

Germplasms	2013-14 Catalase activity	2014-15 Catalase activity
NA-6	930.15	930.19
NA-7	930.23	930.21
NB-5	985.16	985.16
NB-9	985.23	985.23
BER-K	1120.41	1120.43
BER-U	1120.43	1120.45
K-1	1040.75	1040.74
K-2	1040.77	1040.78
NJ-2	910.14	910.15
NJ-3	910.19	910.20
C D at 5%	0.11	0.30

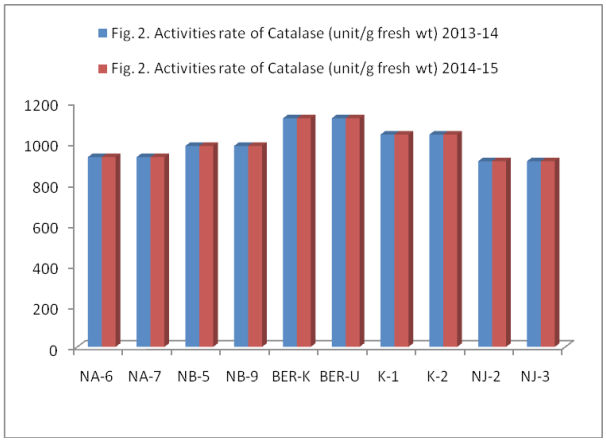
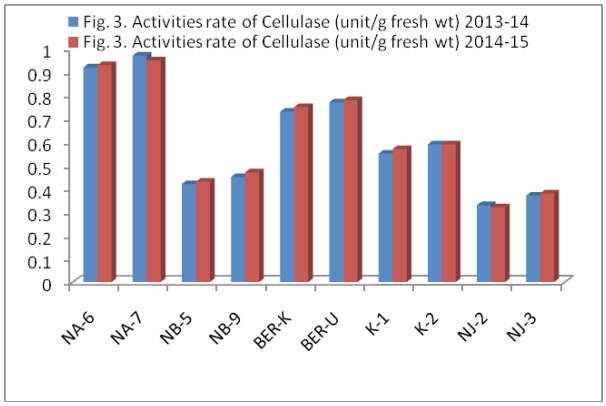


Table 3. Cellulase activity (unit/g fresh wt) in under utilized fruit germplasms

Germplasms	2013-14 Cellulase activity	2014-15 Cellulase activity
NA-6	0.92	0.93
NA-7	0.97	0.95
NB-5	0.42	0.43
NB-9	0.45	0.47
BER-K	0.73	0.75
BER-U	0.77	0.78
K-1	0.55	0.57
K-2	0.59	0.59
NJ-2	0.33	0.32
NJ-3	0.37	0.38
C D at 5%	0.04	0.05



within two germplasm of each fruit pertaining to cellulase activity. The range of variability in selected two germplasm of five fruit viz. Aonal (NA-6 and NA-7), Bael (NB-5 and NB-9), Ber (Karaka and Umran), Jackfruit (NJ-2 and NJ-3) and Kaitha (K-1 and K-2). The variations were recorded from 0.97 to 0.92 unit/g fresh weight (Aonla) 0.45 to 0.42 unit/g fresh weight (Bael), 077 to 0.73 unit/g fresh weight (Ber), 0.59 to 0.55 unit/g fresh weight (Kaitha), 0.37 to 0.33 unit/g fresh weight (Jackfruit). Bhattacharjee (2013) analyzed that cellulase activity (1.41 to 0.98 unit/g fr.wt.) in fresh aonla fruit pulp. Yadav *et al.* (2012) analyzed that the increase in the activity of pectin methylesterase was about 20- and 10-fold, that of polygalacturonase about 8.4- and 5.7-fold, and of cellulase 5.5- and 4.4-fold in ber cultivars viz. Umran and Illaichi, respectivel. Rahman *et al.* (1995) analyzed that Cellulase activity (47.20 to 35.52 unit/g fr.wt) in edible pulp of Jackfruit.

Conclusion

This study examined the enzymatic activities in the selected underutilized fruits, which was carried out at the laboratory of Department of Biochemistry, Narendra Deva University of Agriculture and Technology Narendra Nagar Kumarganj Faizabad (U. P.) India. The results have summarized, peroxidase activity of five minor fruit germplasms viz. NA-7 (Aonla), NB-9 (Bael), Umran (Ber), K-2 (Kaitha) and NJ-3 (Jackfruit) were slightly higher than the other respective fruit germplasms. Catalase activity of five minor fruit germplasms viz. NA-7 (Aonla), NB-9 (Bael), Umran (Ber), K-2 (Kaitha) and NJ-3 (Jackfruit) were slightly higher than the other respective fruit germplasms. Cellulase activity of five minor fruit germplasms viz. NA-7 (Aonla), NB-9 (Bael), Umran (Ber), K-2 (Kaitha) and NJ-3 (Jackfruit) were slightly higher than the other respective fruit germplasms.

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