

Status of biochemical content in papaya (*Carica papaya* L.) after post-harvest pathogenesis by fungi

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Abstract

Post-harvest fungi depleted the pectin, sugar, ash, phosphorous, calcium and ascorbic acid content of papaya fruit.

Keywords: Fruits, fungal diseases, post-harvest, biochemical content, papaya

INTRODUCTION

Papaya (*Carica papaya* L.) is an important and popular fruit crop. Papaya fruits are also produced in many countries on a small scale for local consumption only. This fruit is rapidly becoming internationally an important fruit, both as fresh and processed products but papaya fruit are very susceptible to diseases caused by many microorganisms especially fungi, as papaya fruit is high in moisture and nutrients [1]. The papaya fruits is a fleshy, juicy usually green but turning yellow when ripe. The fruit consist largely of water, sugar, vitamin A and C, protein and ash. It is one of the most nutritious and cheapest fruits grown and consumed in all over parts of the world. The fruit can be fleshy eaten or cooked. It can also be used in the preparation of jellies, juice and jams. It has a pleasant sweat taste and flavour and has a great application in the preparation of the fruit salad and deserts. It has a mild laxative action and the seeds are used medicinally against worms and ulcer [2].

In India, the papaya fruit production has improved the diet of the local people, whose diet generally consisted of starch staples lacking essential vitamins and minerals. There has been a great increase in the demand for the papaya fruits over the years and this may be due to their increased consumption pattern in the topic [3].

Nutritional value of the fruits mainly depends on their quality and quantity of sugars, vitamins and other essential substances. Fruits are considered as the best sources of sugars, amino acids, organic acids, vitamins and other nutrients. During pathogenesis various fungi and bacteria not only blemish, disfigure or cause rot to a number of fruit but the post-infectional biochemical changes reduce their food and market values considerably [4 and 5]. Considering the fact biochemical content of papaya under the influence of post-harvest fungi was studied.

MATERIALS AND METHODS

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Freshly harvested mature and apparently healthy papaya fruits of three varieties viz. Taiwan, Washington and Local were collected from the markets. Hundred gram pulps from each variety was collected in aseptic container. 01 ml of spore suspension of *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium equiseti*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Penicillium digitatum* and *Rhizopus stolonifer* were inoculated separately in pulp of all variety. Pulp without inoculation was served as control. After ten days of incubation pulp was collected in separate sterilized containers and biochemical changes were estimated by standard biochemical methods.

Estimation of pectin

The pectin was estimated by the standard method given in (A.O.A.C., 1970) [6] 5 gm of fruit pulp was taken into beaker to it 30 ml of 0.01N HCL was added and boil it up to 30 min. after cooling filter with Whatman's filter paper no.54 wash distilled water and collect the filtrate into conical flask and labeled them as 1. The residue was taken into beaker 10 ml of 0.05N HCL was added and boil it up to 20 min. and again filters with the Whatman's filter paper no.54, the filtrate was collected into another conical flask and numbered them as 2. The residue was again taken into beaker and 10 ml of 0.03 N HCL was added boil it for 10 min. filter the same with Whatman's filter paper No.54, wash with hot water and labeled the filtrate as no.3. Residues keep aside.

The filtrate was collected into conical flasks and make the volume up to 50 ml. Pipette out 10 ml of aliquot 25 ml of water was added and neutralize the acid with 1 N NaOH using Phenolphthalein as indicator. Then an excess of 1 ml of 1 N NaOH was added with continuous stirring and leave it overnight. 5 ml of 1N acetic acid was added and after 5 min, again 25 ml of 1N CaCl₂ solution was added with stirring. Leave it for 1 hrs. Boil for 2-3 min. After cooling, filter through a pre-weighed Whatman's filter paper No.1. The precipitate was washed with almost boiling water until the filtrate is free from chloride. Test the filtrate with AgNO₃ for chloride. Dry the filter paper at 100°C overnight.

Estimation of total sugar

The sugar content in the fruit pulp was estimated by the procedure recommended by Oser (1979) [7] as follows. The non reducing sugars are hydrolyzed by acid. Acid hydrolysis converts

them in reducing monosaccharide. The reducing sugars are then estimated and recorded as total soluble sugars.

100-500 mg fruit pulp was taken into 125 ml conical flask about 15 ml distilled water was added and heat on a hot plate. Allow solution to boil for 1-2 minutes to gelatinize starch. Include one conical flask containing only water which will serve as the enzyme blank. Cool to room temperature. Pipette 10 ml buffer solution and exactly 10 ml 0.5%. A diastase enzyme solution was taken into the conical flask.

This amount of enzyme is sufficient to hydrolyse disaccharides and starch. Stopper the conical flask and incubate for 44 hours at 38°C. Swirl the flask occasionally to stir mixture. Filter solution through Whatman's no.1 paper into a suitable volumetric flask. Conical flask and filter paper was washed several times with distilled water. 2 ml 10 % neutral lead acetate was added to volumetric flask. Bring to volume with distilled water and mix well. Decant about 30 ml into a 50 ml centrifuge tube, centrifuge for 5 minutes. Decant into a 50 ml conical flask containing 100 mg powdered potassium oxalate. Refrigerate for 4-5 hours or overnight. Filter through Whatman's no.42 paper into a small conical flask without washing. Analyse aliquot directly for reducing power by Shaeffer Somogyi method (3:1b) after acid hydrolysis.

Estimation of reducing sugar

The sugar content in the fruit pulp was estimated by the procedure recommended by Oser (1979) [7] as follows.

The majority of methods for the determination of glucose are based upon the ability of glucose in hot alkaline solution to reduce certain metallic ions of which the cupric and ferric cyanide ions are most commonly used. The following method was used for estimating water soluble reducing sugars.

500 mg of fruit pulp was taken in 50 ml distilled water and boiled it, then filtered it and the filtrate is diluted up to 100 ml. Three Folin-Wu tubes were taken and added following manner. (1) Blank tube – D. W. – 2 ml (2) 2 ml glucose 'C' solution. (3) 2 ml filtrate. In each tube 3 ml alkaline solution of copper was added. Then tube was boiled in boiling water bath for 8 minutes. Cooled the tubes under tap water and add 2 ml of phosphomolybdic acid solution which give blue colour. Then this solution was diluted upto 25 ml distilled water and optical densities determined at 420 nm and calculate the amount of reducing sugar present in fruit pulps.

Estimation of non reducing sugar

The percentage of non-reducing sugars was calculated by subtracting the value of the percentage of reducing sugars from that of total sugars.

Estimation of ash

The residue after incineration of sample at 550 – 600°C is known as ash. For this purpose the sample is subjected to a high temperature up to 600°C and then the ash content is determined. During ignition to such a high temperature all organic compounds decompose and pass off in the form of gases, while the material elements remain in the form of ash. For this the procedure is followed by A.O.A.C. (1970) [6] and Mungikar (1999) [8].

2 gm of fruit pulp was placed in a previously weighed crucible and it was subjected for heating on hot plate till the sample

was sufficiently turned black about 30 minutes. Then it was placed in muffle furnace, pre-heated to 600°C for 2 hours with automatic control. Crucible were transferred directly to desiccators, cooled and weighed immediately. Weight of ash was obtained per 2 gm of sample and further calculated the ash content.

Estimation of calcium

An aliquot (25 ml) of the acid solution ash portion was diluted to about 150 ml with distilled water. Few drops of methyl red are added and the mixture is neutralized with ammonia (NH₃) solution till the pink colour changes to yellow. The solution was heated to boiling and the 10 ml ammonium oxalate solution was added. The mixture was allowed to boil for a few minutes. Glacial acetic acid was then added till distinctly pink colour reappeared. The mixture was then kept aside for 12 to 24 hours at room temperature. When the precipitate at calcium oxalate settles down, it was filter through Whatman's filter paper No.42. The precipitate was washed several times with distilled water, to make it free from acid. It was then transferred in a small beaker by piercing a hole in the filter paper and by pouring over it about 15 ml 2N H₂SO₄. This is heated to above 40°C and titrated against 0.01N KMNO₄ solution until the first drop which gives the solution a pink colouration persisting for at least 30 second.

The amount of calcium was calculated using an equation. 1ml of KMNO₄=0.2004 mg of Ca. The percent Ca on DM basis was then calculated on the basis of the amount of sample used for ashing, the volume to which acid solution of ash is diluted and the volume of the aliquot taken for the precipitation of calcium. The procedure of estimation of calcium is recommended by A.O.A.C. (1970) [6].

Estimation of phosphorus

The estimation of phosphorus is carried out by the method given by Fiske and Subba Rao (1925) [9] and recommended by Oser (1979) [7].

0.5 ml of acid soluble portion of ash was taken in a test tube. Diluted it to a volume of 10 ml with distilled water. Simultaneously taken a blank containing only 10 ml distilled water. 1 ml molybdate solution was added to each test tube and mix, and then 0.4 ml ANSA reagent was added and again mixes. Allowed to stand for 5 minutes and noted/observed the optical density (O.D.) at 660 nm using colorimeter by setting it to a zero with the blank.

The O.D. of standard phosphorus solution was established by preparing a standard graph containing 0 to 1 ml standard phosphorus solutions in series of test tubes. Determine the amount of phosphorus in an aliquot with the help of standard graph and calculated the phosphorus content in the fruit pulp considering its amount taken for ashing, volume of the acid soluble ash and amount of aliquot used for the reaction.

Estimation of ascorbic acid

Vitamin C content was estimated by standard titration method. 5 ml of standard solution of standard Ascorbic acid (100mg/ml) was pipette out into a conical flask, then 10ml of 0.4 % oxalic acid was taken and it was titrated with dye solution. After that 2gm sample was extracted in 0.4% oxalic acid and volume was made up to 100ml by 0.4% oxalic acid. From that solution 5ml of sample was pipette

out into conical flask and titrated with dye solution. End point was pink colour. Finally amount of ascorbic acid in mg / 100ml pulp was estimated by using following formula.

Amount of Ascorbic acid mg / 100ml pulp = $0.5\text{mg} / V1 \text{ ml} \times V2\text{ml} / 5\text{ml} \times 100\text{ml} / \text{wt. of sample} \times 100$.

Where, V1 ml = volume of Standard Ascorbic acid.
V2ml = volume of sample's Ascorbic acid.

RESULTS AND DISCUSSION

It was observed that all fungi deteriorate pectin contents of papaya fruits in all tested varieties. It is also observed from the results that in Taiwan variety maximum loss of pectin contents was observed by *Fusarium moniliforme*. *Curvularia lunata*, *Fusarium moniliforme* and *Rhizopus stolonifer* showed maximum degradation of pectin of Washington variety while *Curvularia lunata* drastically degraded the pectin contents of Local variety (Table 1).

It is clear from table 2 that all fungi reduced the total sugar in all varieties. It was also observed from the result that maximum of total sugar of Taiwan variety was reduced due to *Aspergillus niger*. While in case of Washington maximum loss of total sugar observed due to *Alternaria alternata*, and *Aspergillus niger*. *Aspergillus niger* reduced maximum total sugar in local variety.

It was found that all fungi reduced the reducing sugar in all varieties of papaya fruits. It is also found that in Taiwan, Local and Washington varieties showed maximum depletion of reducing sugar due to *Aspergillus niger* (Table 3).

It was found that all fungi reduced the non-reducing sugar in all varieties fruits. It is observed from the results that in Taiwan variety non reducing sugar was found more decrease due to *Aspergillus niger* and *Curvularia lunata* while in case of Washington maximum loss of non reducing sugar was caused by *Rhizopus stolonifer*. It was also found that *Alternaria alternata* reduce more non reducing sugar in Local variety (Table 4).

It is clear from table 5 that all fungi reduced the ash contents in all varieties. It was found that *Alternaria alternata* and *Penicillium digitatum* deplete maximum ash contents in Taiwan variety while *Fusarium moniliforme* and *Rhizopus stolonifer* were caused maximum loss of ash contents in Washington variety. It was also reported that in Local variety *Alternaria alternata* was responsible for maximum depletion of ash contents.

Table 6 show that all fungi reduced the calcium contents in all varieties of papaya fruits. It was observed that *Alternaria alternata* reduced maximum calcium contents in Taiwan variety. Maximum loss of calcium content in Washington and Local variety was caused due to *Penicillium digitatum*.

It is observed from table 7 that all fungi reduced the phosphorus contents in all varieties. It is clear from the table that *Penicillium digitatum* was responsible for maximum depletion of phosphorus content in Taiwan and Washington variety. It was also found that maximum loss in Local variety was caused due to *Fusarium moniliforme*.

It was found that all fungi reduced the ascorbic acid contents in all varieties of fruits. It was found that *Alternaria alternata* deteriorate maximum ascorbic acid in Taiwan variety while and *Rhizopus stolonifer* and *Penicillium digitatum* deplete maximum ascorbic acid content in Washington and Local variety (Table 8).

Table 1. Changes in pectin (gm/100ml) contents of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	1.3	1.5	1.4
<i>Aspergillus flavus</i>	1.2	1.3	1.8
<i>Aspergillus niger</i>	1.5	1.4	1.6
<i>Colletotrichum gloeosporioides</i>	1.2	1.4	1.7
<i>Curvularia lunata</i>	1.6	1.2	1.2
<i>Fusarium equiseti</i>	1.4	1.5	1.4
<i>Fusarium moniliforme</i>	0.80	1.2	1.6
<i>Fusarium oxysporum</i>	1.6	1.3	1.3
<i>Penicillium digitatum</i>	1.0	1.3	1.5
<i>Rhizopus stolonifer</i>	1.4	1.2	1.8
Control	1.8	1.7	1.9

Table 2. Changes in total sugar (gm/100 gm pulp) of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	3.80	3.40	3.80
<i>Aspergillus flavus</i>	4.20	3.90	3.40
<i>Aspergillus niger</i>	3.60	3.40	3.20
<i>Colletotrichum gloeosporioides</i>	4.50	4.70	4.80
<i>Curvularia lunata</i>	4.20	5.20	5.20
<i>Fusarium equiseti</i>	5.80	4.00	3.40
<i>Fusarium moniliforme</i>	5.00	4.60	4.70
<i>Fusarium oxysporum</i>	4.90	4.20	4.90
<i>Penicillium digitatum</i>	5.90	3.80	3.80
<i>Rhizopus stolonifer</i>	5.80	4.20	3.50
Control	6.7	6.40	5.80

Table 3. Changes in reducing sugars (gm/100 gm pulp) of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	3.00	2.60	3.00
<i>Aspergillus flavus</i>	3.20	3.00	2.20
<i>Aspergillus niger</i>	2.00	1.20	1.00
<i>Colletotrichum gloeosporioides</i>	2.60	2.30	2.80
<i>Curvularia lunata</i>	2.80	2.50	2.80
<i>Fusarium equiseti</i>	2.70	1.70	2.50
<i>Fusarium moniliforme</i>	3.00	2.10	2.20
<i>Fusarium oxysporum</i>	2.40	2.80	2.30
<i>Penicillium digitatum</i>	3.20	2.40	2.50
<i>Rhizopus stolonifer</i>	3.70	3.80	2.90
Control	4.20	4.50	3.40

Table 4. Changes non-reducing sugars (gm/100 gm pulp) of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	0.80	0.80	0.80
<i>Aspergillus flavus</i>	1.00	0.90	1.20
<i>Aspergillus niger</i>	1.60	2.20	2.00
<i>Colletotrichum gloeosporioides</i>	1.90	2.40	2.00
<i>Curvularia lunata</i>	1.00	2.70	2.40
<i>Fusarium equiseti</i>	3.10	2.30	1.20
<i>Fusarium moniliforme</i>	2.00	2.50	2.40
<i>Fusarium oxysporum</i>	2.50	1.40	2.10
<i>Penicillium digitatum</i>	2.70	1.40	1.30
<i>Rhizopus stolonifer</i>	2.40	0.40	1.00
Control	2.50	1.90	2.40

Table 5. Changes in ash (mg/100ml) contents of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	230	340	220
<i>Aspergillus flavus</i>	260	280	222
<i>Aspergillus niger</i>	295	320	310
<i>Colletotrichum gloeosporioides</i>	280	340	340
<i>Curvularia lunata</i>	322	310	310
<i>Fusarium equiseti</i>	310	255	308
<i>Fusarium moniliforme</i>	228	225	310
<i>Fusarium oxysporum</i>	235	340	280
<i>Penicillium digitatum</i>	230	335	276
<i>Rhizopus stolonifer</i>	236	225	330
Control	390	360	400

Table 6. Changes in calcium contents (mg/100ml) of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	10.2	13.3	10.3
<i>Aspergillus flavus</i>	10.4	12.5	16.4
<i>Aspergillus niger</i>	10.5	13.2	15.4
<i>Colletotrichum gloeosporioides</i>	11.5	10.8	16.9
<i>Curvularia lunata</i>	10.5	10.1	16.6
<i>Fusarium equiseti</i>	11.2	07.2	15.5
<i>Fusarium moniliforme</i>	12.5	08.8	08.9
<i>Fusarium oxysporum</i>	13.0	07.3	06.7
<i>Penicillium digitatum</i>	15.0	06.5	06.5
<i>Rhizopus stolonifer</i>	13.2	10.5	16.2
Control	16.0	15.2	18.6

Table 7. Change in phosphorus contents (mg/100ml) of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	10.5	10.2	07.3
<i>Aspergillus flavus</i>	09.9	10.5	10.2
<i>Aspergillus niger</i>	09.1	09.5	09.9
<i>Colletotrichum gloeosporioides</i>	08.4	07.5	09.2
<i>Curvularia lunata</i>	09.2	08.6	08.9
<i>Fusarium equiseti</i>	09.5	09.2	07.4
<i>Fusarium moniliforme</i>	09.1	09.3	03.5

<i>Fusarium oxysporum</i>	10.2	10.3	10.5
<i>Penicillium digitatum</i>	06.9	05.7	09.9
<i>Rhizopus stolonifer</i>	10.5	09.2	04.1
Control	13.0	10.5	14.2

Table 8. Change in ascorbic acid (mg/100ml) of papaya fruits due to post-harvest fungi

Fungi	Varieties of papaya		
	Taiwan	Washington	Local
<i>Alternaria alternata</i>	42.8	48.9	43.1
<i>Aspergillus flavus</i>	57.1	57.8	47.3
<i>Aspergillus niger</i>	52.3	56.1	53.3
<i>Colletotrichum gloeosporioides</i>	56.5	53.4	48.4
<i>Curvularia lunata</i>	46.6	54.4	55.8
<i>Fusarium equiseti</i>	64.7	62.2	42.3
<i>Fusarium moniliforme</i>	56.3	60.4	54.3
<i>Fusarium oxysporum</i>	61.2	42.6	52.8
<i>Penicillium digitatum</i>	65.5	45.3	41.3
<i>Rhizopus stolonifer</i>	59.3	33.7	52.6
Control	62.6	68.3	56.6

Chaudhary *et al.* (1980) [10] reported that *Pestalotia anonicola*, *Stachybotrys* sp. and *Trichoderma viride* were decrease the total sugar and increase the reducing sugar. Similarly *Cladosporium oxysporum* and *Drechslera rostrata* loquat and capegoose-berry, respectively utilized their total sugar contents within ten days (Singh, 1980) [11]. Singh and Sinha (1982) [12] found that *Aspergillus flavus* and *A. parasiticus* cause depletion in total, reducing and non reducing sugars of *Citrus sinensis* fruits similar results were observed by Singh and Sinha (1983) [13] in guava fruits. They found that decrease in total, reducing and non reducing sugars of guava fruit was observed due to *Aspergillus flavus* and *A. parasiticus*. Bilgrami *et al.* (1983) [14] revealed that there was sharp decline in the level of total, reducing and non reducing sugars of dry fruit during *Aspergillus flavus* infestation. Madhukar and Reddy (1991) [15] revealed the *Pestalotiopsis versicolor* and *Rhizoctonia solani* decrease the reducing sugar content in guava fruit. Verma *et al.* (1991) [16] revealed the total, reducing and non reducing sugars were greatly reduced by *Aspergillus niger*, *A. fumigatus* and *A. luchuensis* in bael fruits. Recently Sawant and Gawai (2011a) [17] found that *Rhizopus stolonifer*, *Aspergillus flavus*, *Penicillium digitatum*, *Curvularia lunata* and *Fusarium moniliforme* were responsible for decrease in total sugar and increase in reducing sugar content of papaya fruit. Sawant and Gawai (2011b) [18] also reported that *Aspergillus niger*, *Fusarium roseum*, *Rhizopus stolonifer* and *Gleospodium musarum* were decreases the total sugar and increases the reducing sugar content of banana fruits.

Ghosh *et al.* (1966) [19] reported the vitamin C was totally absent after 8 days in mango fruit tissues infected with *Colletotrichum gloeosporioides*. Srivastava and Tandon (1966) [20] observed the vitamin C content was depleted due *Botryodiplodia* in Langra and Dashehari varieties of mango fruits. Tandon (1970) [21] found that ascorbic acid of mango pulp was decreased due to *A.niger*. Vitamin C content of mango fruit was depleted by *Phomopsis mangiferae* and *Phoma exigua* [22]. Similarly Arya (1993) [5] reported the mango fruit infected with *Botryodiplodia theobromae* showed decrease in vitamin C content. Similar results have been reported in guava [23], apple [10], anola [24], banana [25], Jujube [26], citrus [27], Musambi [12].

Verma *et al.* (1991) [16] reported *Aspergillus niger*, *A. fumigatus* and *A. luchuensis* were slightly decrease the ash content in bael fruits. Recently Sawant and Gawai (2011a) [17] found that ash content of papaya fruit was depleted by *Rhizopus stolonifer*,

Aspergillus flavus, *Penicillium digitatum*, *Curvularia lunata* and *Fusarium moniliforme* Sawant and Gawai (2011b) [18] also reported that *Aspergillus niger*, *Fusarium roseum*, *Rhizopus stolonifer* and *Gleospodium musarum* were responsible loss in ash content of banana fruits.

CONCLUSION

It is concluded that post- harvest fungi are responsible for reduction in biochemical content of papaya fruit which revealed that the fungi might have utilized it as a substrate.

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