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Study of Biochemical and Phytochemical Variations in *Carica papaya*, Linn. During Storage

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ABSTRACT: Papaya is a tropical fruit and is usually harvested prior to the onset of climacteric rise. The moisture content of Papaya is always higher than 70% throughout the season. The moisture content increases significantly with season. The phytochemical analysis of Papaya leaves contain carbohydrates, saponins, terpenoides, alkaloids, phenols, glycosides, cardiac glycosides and flavonoids. Temperature has a major role in the post harvested biochemical changes and the activity of polyphenol oxidase in Papaya. Flesh color development in nonstored fruits did not change significantly during the first six days of storage, and then rapidly increases. Unripe fruit stored for 5 days at above 27 ºC exhibits faster ripening rates. The optimal temperature for storing Papayas are found to be between 21.5 to 25 ºC. Biochemical changes in Papaya fruits at room temperature revealed that the level of phenolics and reducing sugars are gradually increases . But there is a gradual decrease in the protein content of the fruits. As the PPO quality deteriorating enzyme, the nutritional quality was assayed by estimating the activity of PPO. The enzyme was responsible for the browning of Papaya fruits. Post harvest storage of Papaya is a major problem for many farmers. High moisture content of papaya fruit may affect the contamination of disease spreading microbes and insect vectors. They may cause spoilage of soft tissues of Papaya. High temperature storage may cause the nutrient quality of fruit. Many enzymes and proteins degraded in extreme temperature condition.

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INTRODUCTION

Papaya is belongs to the family Caricaceae and is botanically known as *Carica papaya*, Linn. It is a short lived perennial plant growing to 30ft high and is distributed over the whole tropical area. Many scientific investigation have been conducted to evaluate the biological activities of various parts of papaya including fruits, shoots, leaves, rinds, seeds, roots and latex. Flowers are fragrant developing from leaf axils. Papaya fruits are smooth skinned with fleshy pulp enclosing seeds. Young green Papaya contain latex. The latex consists of a chemical substance known as papain. The latex is either sun dried or oven dried and solid in powdered form to be used in beer industries. Yellow and orange fruits varying amount of antioxidants such as vitamin C as well as carotenoids and bioflavonoids, two classes of phytochemicals that scientists are studying extensively for their health promoting potential. Papaya seed extracts might contain effects in toxicity induced kidney failure.(Uppal.D.S and Uqma.S.C,1983)

Papaya has nutritional benefits. It is rich in anti-oxidants, B vitamins, folate and pantothenic acid, minerals, potassium, magnesium and fiber. Papaya stored at controlled conditions of temperature and humidity. Optimum storage conditions was obtained is at 22.5 to 25.6 ºC. The relative humidity is about 70% to 85%. Such a condition of fruit can stored at 5 days. Long term storage of Papaya has been done with the help of chemicals, growth regulators and controlled conditions of humidity and atmospheric temperature.

High carbondioxide concentration may affect the rate of respiration of fruits. It may lead to carbondioxide toxicity in plants (Brito-A,2007). Papaya artificially stored in CO_2 controlled freezers for increasing the shell life. This method is most efficient than they stored in normal ice freezers. .Chilling storage is widely used short term preservation methods to increase the storage time of fruits. It may affect the normal metabolic activities. The metabolic activities continue post harvest and thus make most fruits highly perishable commodities.

MATERIALS AND METHODS

Fresh mature unripe papaya was collected. Then stored for five consecutive days at room temperature. Papaya samples from the storage conditions were analyzed for reducing sugar, protein, polyphenol oxidase enzyme.

Analytical Method

Determination of moisture content

The papaya fruit was chopped into small slices. The tissue pieces were kept in dried previously weighed petridish with a lid and weighed. The dish was placed without lid into the oven at 103º for 2 hours. The oven was opened and the petridish was closed immediately with a lid. The dish was cooled in a desiccator and weighed. The amount of moisture was calculated from the differences in weight.

Biochemical Method

Extraction and estimation of total soluble sugars

The total soluble sugars were estimated using DNS method.

DNS method

The DNS method of sugar estimation is very simple sensitive and adaptive during handling of a large number of samples at a time. The alkaline solution of Dinitro Salicyclic acid is reduced to a brown coloured product Nitro amino Salicilicacid.

1g of papaya tissue was weighed and extracts the sugars with 10ml of distilled water. Filtered the homogenate and centrifuge at 10,000 rpm for 10 minutes. From this 0.2g sample was pipetted out into a clean dry test tube. Makeup the volume to 2ml with distilled water and 3ml DNS reagent was added. Heat the contents in boiling water bath for 5 minutes. When the contents of tubes were still warm add 1ml of 40% Rochelle salt solution. Cool and read the absorbency at 560nm.

Extraction and estimation of total soluble protein Lowrys Method

Total soluble protein was extracted in 0.1M Phosphate buffer with pH7.The buffer was prepared using 0.1M Na_2HPO_4 and 0.1M NaH_2PO_4.1g of fresh papaya was taken and grinds it well in 15ml of 0.1M Phosphate buffer. Filter the homogenate and squeeze gently through cheese cloath. Centrifuge the filtrate at 10,000rpm for 10 minutes. The supernatant was collected and used for estimation. From this, 0.02ml sample was pipetted out in a test tube and made upto 1ml using 0.1M Phosphate buffer pH 7. To this 5ml alkaline copper sulphate reagent was added. The reagent was prepared by mixing 50ml of alkaline Na_2CO_3 and 1ml of $CuSO_4$. Reaction mixture was mixed thoroughly and undisturbed for 10 minutes.

Then 0.5ml of Folins Reagent was added and mixed well and kept for 30 minutes. Absorbency was read at 660nm using spectrophotometer. Amount of total soluble protein was estimated using a standard graph.

Extraction and assay of polyphenol oxidase

1g of fresh papaya was taken and homogenized in 10ml of chilled 0.1M Citrate phosphate buffer pH7. Filter the homogenate through a cheese cloath and centrifuge at 10,000 rpm for 20 minutes. Collect the supernatant and make up to 20ml.

Polyphenol oxidase was assayed according to the method of Okay *et al* (1995). PPO activity was determined spectrophotometrically by recording the increase in absorbance at 420nm for 10 minutes. Total volume of reaction mixture was 3ml. 2.5 ml of substrate so;ution was taken in the cuvette. Reaction was initiated by the addition of 0.5ml of enzyme and the absorbance was read at 420nm immediately.3ml substrate solution was taken as blank.

Preliminary phytochemical analysis were carried out according to the method of Harbone(1973).

RESULT AND DISCUSSION

The outer wall of ripen papaya is soft that is a major problem to maintain the shelf life to avoid degradation and microbial attacks. Softening of fruit occur due to the presence of gaseous phytohormone,ethylene. The ethylene response of ripening papaya fruit was determined with 5 days of storage at room temperature conditions. Temperature also has a major role in ripening of Papaya. But extreme temperature may degrade the protein content in Papaya. Papaya fruit has excellent flavor because of the presence of phytochemicals. It is associated with essential volatile oils and aromatic acids. It also consists of a wide range of aromatic phenolic compounds that gives more aromas to the ripened papaya which increases the economic value of fruit.

Moisture content

Ripened Papaya was collected, sliced out and stored in five consecutive days in room temperature. The moisture content was calculated periodically. On the first day of storage, highest moisture level was found to be 23g. It was highest value got under observation. Then reduces the moisture content. In the final day of storage the moisture level was found to be 9.7 g.

Days	Fresh weight in grams	Dry weight in grams	Moisture content in grams
1	32.8	9.8	23
2	25.3	5.7	19.6

3	18.2	3.9	14.3
4	14.6	2.6	12
5	11.1	1.4	9.7

Variations in reducing sugar

Ripened Papaya was collected directly from the tree and sliced into small pieces and stored in five consecutive days at room temperature. The amount of reducing sugar estimated by DNS method. It was found that the sugar content of the fruit exhibits progressive level of increase during storage. There is rapid variation occur after third day of storage.

At first day of storage the amount of reducing sugar was very little. But its level increases during the consecutive days and reaches the maximum at the fifth day. At the first day, the amount of sugar was 1.26mg/g in 1g of tissue. On the fifth day, sugar level increases to 8.71mg/g in 1g of tissue. Increase in sugar content makes them more sweet and delicious.

When storing Papaya at room temperature reducing sugar content will increases. In low temperature storage may increases the sugar content in fruits because, rate of metabolic activity is lesser in cold storage. So retain the high concentration of reducing sugars in long periods, than storing in room temperature. But there are limits for storing Papaya at room temperature because of microbial condaminations.

High sugar level in Papaya make them commercially valuable. The ripening period can control by regulating storage temperatures. But it reduces the quality of Papaya and nutrient contents. So room temperature storage is better than stored in refrigerator.

Table showing variations reducing sugars in Papaya for 5 days of storage.

Days	Reducing sugar mg/g
1	8.71
2	9.85
3	1.04
4	1.08
5	1.26

Variations in Protein content

Protein content of Papaya during five days of storage at room temperature was estimated by Lowrys method (Lowry *et al*., 1951). It was found that protein content of Papaya decreases gradually. At first day the level of protein was 14.8mg/g in 1g of tissue. The fifth day of storage, the level of protein gradually decreases to 5.2mg/g in 1g of tissue.

The amount of protein content in Papaya was decreases during room temperature due to climatic as well as biochemical factors. At the first day of storage, the protein content was high. After third day, there is a gradual decrease in protein content was observed. It may be due to the denaturation of aminoacids present in Papaya. When continuing the denaturation may affect the quality of Papaya. Fruit may produce foul smell lead to spoilage.

Now various techniques have been adapted to overcome the denaturation of protein content in Papaya during storage. Storage of Papaya in low temperature conditions or high carbon dioxide conditions may prevent the early denaturation of Papaya.

Table showing the variation in protein in Papaya for 5days of storage

Days	Protein mg/g
1	14.8
2	13.7
3	12.8
4	9.6
5	5.2

Variations in Polyphenol Oxidase (PPO)

The ripened Papaya was kept in room temperature for continuous five days of storage. Polyphenol oxidase is major enzyme responsible for the browing of fruit tissues. The extraction and estimation of polyphenol oxidase was done by Okay *et al* (1995) method. The activity and assay of PPO was determined spectrophotometrically

During first day of storage, the amount of PPO was maximum. But the level gradually decreased during storage. At first day the amount of PPO activity was 0.31mg/g in 1g of Papaya tissue. At the fifth day, PPO activity was 0.04 mg/g in 1g of tissue.

Table showing the variations of PPO activity in Papaya for 5days of storage

Days	Polyphenol oxidase mg/g
1	0.31
2	0.26
3	0.12
4	0.09
5	0.04

In addition to the complex mixture of phenolic compounds, Papaya contain Polyphenol oxidase activity. PPO is a copper containing enzyme. It is widely distributed in plant tissues and has been related to enzymatic browning of fruits including apple, pear, plum and Papaya. The main step in enzymatic browning is the oxidation of phenolic compounds to corresponding quinines by PPO in presence of oxygen.

Phytochemical analysis of Papaya

Sl.No	Phytochemicals	Test performed	Ethanol extract	Methanol extract
1	Alkaloids	Mayors Test	+	-
2	Carbohydrates	Benedicts Test	+	-
3	Saponins	Chloroform and H2SO4 Test	+	+
4	Glycosides	Solkowiski Test	+	+
5	Cardiac Glycosides	Keller-Killani Test	+	+
6	Protein and aminoacids	Millions Test	+	+
7	Flavonoids	Shinoda Test and Alkaline reagent Test	+	+
8	Phenolic compounds	Shinoda Test	+	+
9	Tannins	Neutral FeCl3	-	-
10	Terpenoids	Solkowiski Test	+	+

SUMMARY AND CONCLUSION

Papaya is a tropical fruit and is usually harvested prior to the onset of climacteric rise. The moisture content of Papaya is always higher than 70% throughout the season. The moisture content increases significantly with season. Late season fruits have the highest moisture content while early season fruits had the lowest content. Moisture content also increases during ripening under normal room temperature conditions especially with early season fruits. Such highly moisture Papaya fruits were reported in India (Zaman *et al*., 2006).

The phytochemical analysis of Papaya leaves contain carbohydrates, saponins, terpenoides, alkaloids, phenols, glycosides, cardiac glycosides and flavonoids. Tannin was absent in Papaya leaves. The presence of saponins supports the fact that papaya leaves has a cytotoxic effect such as permealization of the intestine as saponins are cytotoxic (Schneider, G and Wolfing, 2004). Saponins also gives a bitter taste in the leaves. Alkaloids were also present. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic properties. The cardiac glycosides has the ability to increase heart beat and at the same time steady excess the heart beat without strain in the organ(Nagar P.K., 1964) .Papaya leaves are highly aromatic in nature. Fruit has also pleasant smell. Because Papaya plant produce many phenolic compounds. They may include volatile oils and gummy substances. They are also refered to as secondary metabolites. They have actually no direct significance in the normal

metabolic activities of the plant. But have defensive role. Flavonoids are derivatives of phenolic compounds that are also present in Papaya leaves. Due to all this, no any microorganism can easily affected papaya plant.

Studies revealed that Papaya leaves are also acidic in nature(Njoku,P.C and Akumefula M.I., 2007)

Temperature has a major role in the post harvested biochemical changes and the activity of polyphenol oxidase in Papaya. Flesh color development in non-stored fruits did not change significantly during the first six days of storage, and then rapidly increases.

Unripe fruit stored for 5 days at above 27 ºC exhibits faster ripening rates. Problem of weight loss and other abnormalities and contamination has been observed when storing Papaya over 30ºC at room conditions(Ahmed, J., Shivhare U.S, 2002).So maintain of temperature is one of the main problem. The optimal temperature for storing Papayas are found to be between 21.5 to 25 ºC. Ethylene did not ripen immature Papayas completely in terms of skin and flesh color development. Ethylene reduced the coefficient of variation for skin color, softening rate and flesh color development in treated fruits. Ethylene increased the rate of skin degreening and hastened the rate of carotenoids development and softening in the outer mesocarp while having little effect on the inner mesocarp.

Biochemical changes in Papaya fruits at room temperature revealed that the level of phenolics and reducing sugars are gradually increases .But there is a gradual decrease in the protein content of the fruits. As the PPO quality deteriorating enzyme, the nutritional quality was assayed by estimating the activity of PPO. The enzyme was responsible for the browning of Papaya fruits. Post harvest storage of Papaya is a major problem for many farmers. Because it is not an easy way to maintain the proper nutrient quality of Papaya. High moisture content of papaya fruit may affect the contamination of disease spreading microbes and insect vectors. They may cause spoilage of soft tissues of Papaya. High temperature storage may cause the nutrient quality of fruit. Many enzymes and proteins degraded in extreme temperature condition.

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