I J R B A T, Issue (Special-17), June 2021: 529-534 e-ISSN 2347 – 517X
A Double-Blind Peer Reviewed & Refereed Journal Original Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY

BIOCHEMICAL ANALYSIS OF *PITHECELLOBIUM DULCE* (ROXB.) BTH. FRUIT DURING ITS SUCCESSIVE STAGES OF DEVELOPMENT

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ABSTRACT:
The *Pithecellobium dulce* (Roxb.) Bth. is an underutilized fruit plant. To encourage use of the underutilized fruits and come upon the need of nutritional fruits the following work has been carried out for their nutritional analysis. For that morphological and physic-chemical parameters such as Length, Diameter, Volume, Moisture, Ash, pH and total acidity of fruits has been Measured. The Biochemical changes in carbohydrates, proteins and phenols have been observed during the development of the fruit. pH is High at ripened stage while total acidity is high at pre-ripened stage. Moisture is high at the young stage while ash content is high at ripened stage. Chlorophyll a and b and total chlorophyll is high at maturity while carotene and anthocyanin is high at ripened stage. Reducing sugar is high at ripened stage, non-reducing sugar high at young stage and total sugar and starch is high in ripened stage. Proteins and phenols are high at ripened stage. Amylase activity is high at maturity and Invertase activity is high at premature and Catalase activity is high at ripened stage. Also the histological observations are made during the successive stages of development.

Keywords: *Pithecellobium dulce*, underutilized fruits, developmental changes.

INTRODUCTION:

Pithecellobium dulce (Roxb.) Bth. is a tree that reaches a height of about 10 to 15 m (33 to 49 ft). Its trunk is spiny and its leaves are bipinnate. Each pinna has a single pair of ovate- oblong leaflets that are about 2 to 4 cm (0.79 to 1.57 inch) long. The flowers are greenish-white, fragrant and sessile and reach about 12 cm (4.7 in) in length, though appear shorter due to coiling. The flowers produce a pod, which turns pink when ripe and opens to expose an edible pulp. The pulp contains black shiny seeds that are circular and flat. The seed is dispersed via birds that feed on the sweet pulp. The tree is drought resistant and can survive in dry lands from sea level to an elevation of 1,500 m (4,900 ft), making it suitable for cultivation as a street tree. The seed pods contain a sweet and sour pulp that which is eaten raw in Mexico as an accompaniment to various meat dishes and used as a base for drinks with sugar and water.

MATERIAL AND METHODS

The fruits were collected with sequential development stages from the M. J. Commerce College, M. K. B. University campus, Bhavnagar and subjected for their nutritional analysis. (Figure - 1)

The physic-chemical analysis in Length, Diameter and Volume were carried out by the method of Mazumdar and Majumder, 2003. Moisture Content of fresh fruits was observed by following the method of Berwal et al., 2004. The ash content of fruits was measured by the method of Berwal et al., 2004. For recording the pH and total acidity of fruits method of Pandya and Raman Rao, 2010 has been followed. The pigment analysis was done by

the method of Pandya and Mehta, (2016) and Devi (2002). For their 5 gm tissues of leaves and fruit wall were homogenized in 80% acetone and centrifuged. The absorbance was taken at different wavelengths for chlorophyll a, chlorophyll b, total chlorophyll, carotene and anthocyanin. Following the method of Hedge and Hofreiter (1962) total sugars were estimated by extracting 1gm sample in 5 ml 2.5 N HCl for 3 hrs in boiling water bath. The supernatant was neutralized, cooled and after adding anthrone reagent sugars were estimated spectro-photometrically. Reducing and non-reducing sugars were estimated spectro-photometrically extracting 1gm sample in 10 ml 80% ethanol and the supernatant was evaporated in boiling water bath. The residue was dissolve in 5ml distilled water and was estimated spectro-photometrically by using DNS (Dinitrosalicylic acid) reagent (Miller, 1972). Proteins were estimated by extracting 1gm sample in 5 ml phosphate buffer pH 7.2. The supernatant was estimated using Folin-Ciocalteu reagent (FCR) method described by of Lowry et al (1951). The amount of total phenolic contents were estimated by extracting 1gm sample in 10ml 80% ethanol and the extract was evaporated in boiling water bath. The residue was dissolved in 5ml distilled water and by using Folin-Ciocalteu reagent (FCR) under alkaline condition (20% Na_2co_3) phenols were estimated spectro-photometrically (Pandya and Raman Rao, 2010). The anatomical work was carried out the methods described by Johansen, 1940.

RESULTS:

Morphological measurements

The morphological measurements of aril of *Pithecellobium dulce* fruit have been measured at the time of collection. Aril of the fruit 17.11cm length and 15.02mm diameter the weight of the aril is 2.56 gm.

Physico-chemical changes:

The pH of the fruit pulp increases from 3.43 at young stage to 4.86 at ripened stage. In contrast, the Total acidity of the fruit decreases with increasing maturity, measuring highest with 0.90 % at premature stage, but with the onset of ripening the acidity declines to 0.73 %, whereas the Moisture content of the fruit which is initially high with 83.01 % in the young fruit decreases marginally to 70.31 % in the ripened stage. Also, the Ash content of the fruit is increases from 0.30 % in young fruit to 0.53 % in the ripened fruit.

Pigments:

With the advancement of growth and ripening, a visual change occurs in its color from white to light pink. The quantitative analysis of pigments reveals that the amount of chlorophyll 'a' increased by one-fold from the young stage to the pre-ripened stage, but thereafter it remained more or less unchanged. Also, at the mature stage the amount of Total chlorophylls is recorded to be as high as 1.13 mg/100 gm, but subsequently it showed a declining trend. The quantity of Carotenoids in the presently worked out *Pithecellobium dulce* fruit is very little amount. In contrast, Anthocyanins got accumulated to higher levels from 1.31 mg/100 gm at the young stage to 3.80 mg/100gm at the ripened stage.

Carbohydrates:

Total sugar content exhibits an initial decrease in its amount from 14.30 mg/gm at young stage to 12.04 mg/gm at the premature stage, but eventually it increases to the tune of 19.09 mg/gm during ripening. Consistency in the quantity of reducing sugars of presently

worked out fruit at its sequential stages was also observed to initial decrease in the premature stage and thereafter increased until ripening. In contrast, non-reducing sugars observed inconsistency in its amount during the successive growth stages with 4.06 mg/gm at young stage, decreased to 2.09 mg/gm at the premature stage, increased once again to 3.57 mg/gm in the mature stage, remained consistent in the pre-ripened stage and finally decreased to 2.98 mg/gm in the ripened stage. In contrast, the amount of starch was found to get decreased by more than one-fold from 39.80 mg/gm at young stage to 20.28 mg/gm at the mature stage, but thereafter the accumulation of starch was observed with 50.61 mg/gm and 60.77 mg/gm in the pre-ripened and ripened stages respectively.

Proteins and Phenols:

In contrast with increasing maturity the amount of both, phenols and proteins from 1.23 mg/gm and 1.87 mg/gm in the young stage to 4.73 mg/gm and 12.59 mg/gm at the ripened stage. The phenolic compounds amplified by three-fold, while that of proteins exhibited rise by eleven-fold.

Enzymes:

The specific activity of enzyme amylase is found to get increased from young stage (0.032 mg maltose released/min/mg protein) to mature stage (0.058 mg maltose released/min/mg protein), but subsequently it declines to 0.044 and 0.022 mg maltose released/min/mg protein during pre-ripened and ripened stages respectively. In contrast, a consistent and gradual increase in the specific activity of enzyme invertase was found with 0.010 mg maltose released/min/mg protein at young stage to 0.035 mg maltose released/min/mg protein at premature stage. Invertase, however, inconsistent in its activity with its decrease to 0.013 & 0.017 mg maltose released/min/mg protein in the mature and pre-ripened stages and a minor increment 0.019 mg maltose released/min/mg protein with the onset of ripening. While Catalase activity it shows gradual increase from young to ripened stage.

Histological changes in relation to growth and ripening of *Pithecellobium dulce* fruit:

The edible portion in fruit is mesocarp only according to the difference in the cell size and arrangement of cells. The mesocarp can be having very deep splits. The cells of mesocarp are 16-24 layered made up of thin walled parenchyma cells, which are smaller in size than the inner mesocarpic cells. The middle layers of the mesocarp possesses cells that are polygonal in shape and possesses scanty cytoplasm and/ or are found vacuolated, shows both anticline and periclinal divisions. Some cells of mesocarp are observed to enlarge in their size and become vacuolated then their adjoining mesocarpic cells. The arrangement of cells in the mesocarp remains well organized until the mature stage, but in the pre-ripened and ripened fruit stages disruption of cell walls are observed. The cells of the inner mesocarp possess more intercellular space than that of outer mesocarpic cells. Histo-chemical tests of the presently studied fruit of Goras aamli reveal that during its young stage and with the advancement in the growth and ripening the cells of mesocarp accumulate large quantities of Tannin. However, insoluble polysaccharides did not show much variation at all the successive stages of growth and ripening of *Pithecellobium dulce* fruits. (Plate 1).

DISCUSSION:

The present results are near about accordance with the finding of Pandya and Mehta (2017), Patel and Rao (2009, 2011 and 2014). The pH is high at ripened stage while acidity is high at pre-ripened stage. Moisture content is highest at young stage and lowest at ripened stage while ash content shows opposite results from Moisture. Chlorophyll pigments accumulation riches high towards maturity and decrease again during ripening while Carotene and Anthocyanin increases during the course of ripening. Reducing sugar, total sugar and starch expressed high at ripening while non-reducing sugar is high at young stage. During the course of ripening the protein and phenols are increasing during the course of ripening. Amylase activity is high at maturity and Invertase activity is high at premature and Catalase activity is high at ripened stage.

CONCLUSION:

From the present results concluded that the *Pithecellobium dulce* fruits is a rich source of sugar and protein. Anthocyanin is also accumulating during course of ripening. Due to the presence good amount of nutritional components *Pithecellobium dulce* can be incorporate for manufacture the commercial products.

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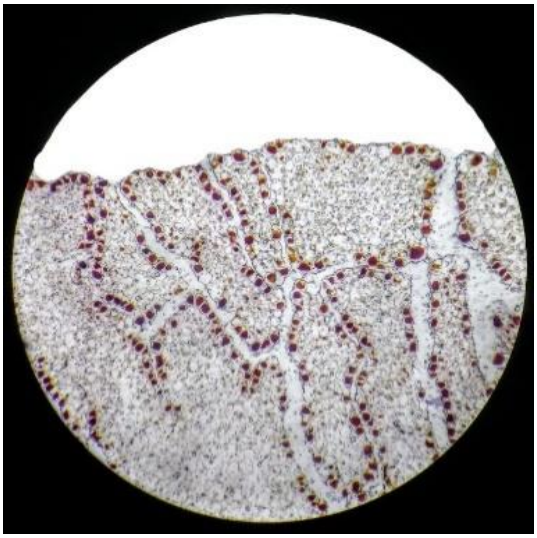
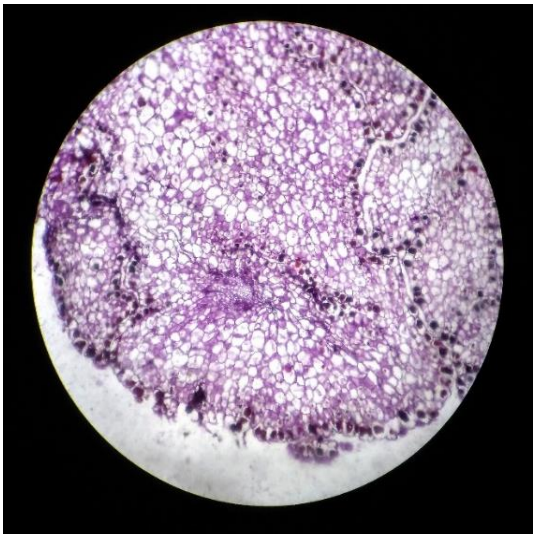
Table – 1 Physico and Biochemical Analysis of *Pithecellobium dulce* fruits

Parameters	Stages of Fruit				
	Young	**Pre-mature**	**Mature**	**Pre-ripened**	**Ripened**
pH	3.43± 0.001^{d}	3.87± 0.002^{b}	4.26± 0.004^{c}	4.55± 0.006^{a}	4.86± 0.003^{b}
Total acidity %	0.46± 0.003^{c}	0.62± 0.001d	0.76± 0.007^{e}	0.90± 0.002^{b}	0.73± 0.002^{c}
Moisture content %	83.01± 0.004^{c}	79.55± 0.005^{c}	77.83± 0.003^{a}	74.02± 0.004^{c}	70.31± 0.001^{e}
Ash content %	0.30± 0.003^{c}	0.34± 0.002^{e}	0.42± 0.003^{b}	0.49± 0.001^{d}	0.53± 0.002^{c}
Chlorophyll 'a' mg/100 gm	0.32± 0.004^{a}	0.65± 0.003^{c}	0.67± 0.002^{a}	0.59± 0.003^{a}	0.38± 0.003^{a}
Chlorophyll 'b' mg/100 gm	0.27± 0.002^{d}	0.43± 0.001^{a}	0.46± 0.005^{d}	0.43± 0.002^{d}	0.41± 0.001^{e}
Total Chlorophyll mg/100 gm	0.59± 0.004^{a}	1.07± 0.004^{c}	1.13± 0.001^{d}	1.02± 0.003^{c}	0.79± 0.006^{a}
Carotenoids mg/100 gm	0.23± 0.002^{e}	0.27± 0.004^{a}	0.26± 0.002^{c}	0.29± 0.005^{d}	0.32± 0.003^{a}
Anthocyanins mg/100 gm	1.31± 0.003^{a}	1.57± 0.002^{d}	1.92± 0.002^{c}	2.45± 0.003^{b}	3.80± 0.004^{a}
Reducing Sugar mg/gm	10.23± 0.002^{a}	09.37± 0.003^{b}	11.31± 0.005^{c}	13.07± 0.003^{a}	16.03± 0.006^{c}
Non-Redu. Sugar mg/gm	4.06± 0.001^{c}	2.09± 0.004^{b}	3.57± 0.003^{a}	3.32± 0.004^{d}	2.98± 0.003^{b}
Total Sugar mg/gm	14.30± 0.003^{d}	12.04± 0.004^{b}	14.77± 0.002^{c}	16.37± 0.001^{d}	19.09± 0.005^{a}
Starch mg/gm	39.80± 0.005^{c}	30.22± 0.004^{a}	20.28± 0.002^{d}	50.61± 0.003^{d}	60.77± 0.006^{b}
Phenols mg/gm	1.23± 0.002^{a}	1.77± 0.004^{f}	2.15± 0.003^{b}	2.99± 0.003^{b}	4.73± 0.004^{a}
Proteins mg/gm	1.87± 0.001^{c}	2.55± 0.004^{d}	4.78± 0.003^{b}	9.74± 0.005^{a}	12.59± 0.003^{b}
Amylase (mg altose/min/ mg protein)	0.032 ± 0.002^{c}	0.040 ±0.003^{b}	0.058 ± 0.010^{a}	0.044 ± 0.001^{a}	0.022 ± 0.001^{a}
Invertase (mg glucose/hr/ mg protein)	0.010± 0.006^{c}	0.035 ± 0.008^{d}	0.013 ± 0.001^{a}	0.017 ± 0.001^{a}	0.019 ± 0.002^{a}
Catalase (units/min/mg protein)	0.012 ± 0.001^{b}	0.013 ± 0.0006^{b}	0.015 ± 0.004^{c}	0.017 ± 0.001^{a}	0.022 ± 0.002^{b}

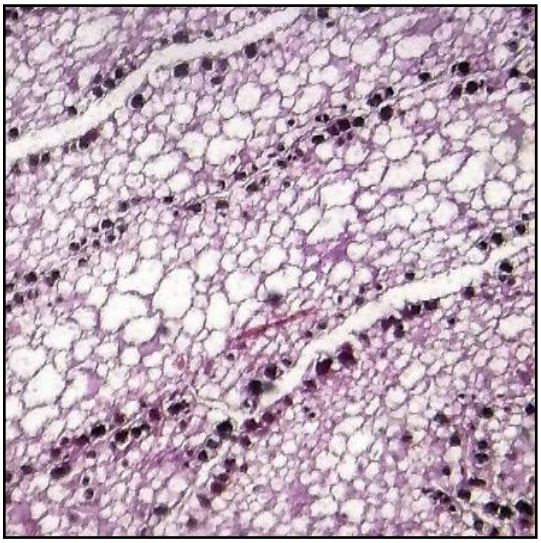
Values of means followed by different letters are statistically significant according to Duncan's multiple range test (DMRT) at 5% level (n=3).



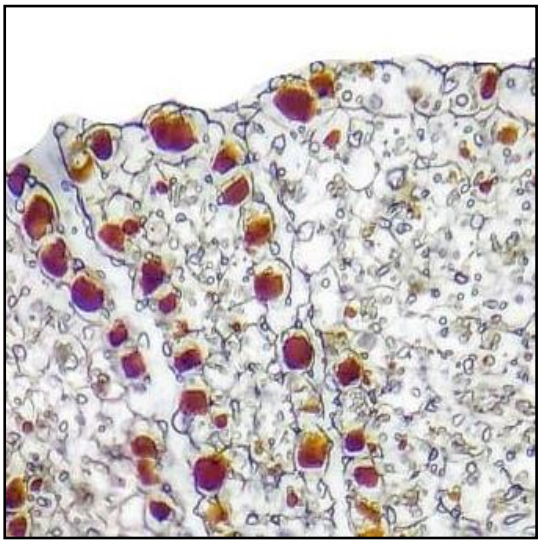
Pithecellobium Dulce Fruit Mesocarp Stained By Safranin with Tannin



Pithecellobium Dulce with Vascular Bundles That Is Transversally Cut



Pithecellobium Dulce Fruit Stained by Periodic Acid and Schiff Reagent Exhibits the Insoluble Poly Saccharides



Pithecellobium Dulce Fruit Mesocarp Outer Layer Cells with Tannin Content That is Indigestive

PLATE 1 ***Pithec ellobium dulce*** **FRUIT HISTOLOGY**