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Nutritional analysis and enzyme activities from the different stages of papaya flesh (*Carica papaya* L.)

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ABSTRACT

The physico-chemical properties, nutritional parameter changes and enzyme activities in different stages (Green, mature & Ripen) of Bari papaya-1 (*Carica papaya* L.), the most available variety in Rajshahi zone, were studied to obtain a comparative data on their nutritional qualities. The physico-chemical parameters such as pH and moisture content of papaya increased with its advancement of maturity. The ash and TTA content was found to be maximum in mature stage but decrease in ripen stage. The amount of total protein, water-soluble protein, free sugar, reducing sugar and starch increases with advancement of maturity and was found to be maximum in ripen stage. Lipid content was found to be maximum in mature stage but lower in green and ripen stage. The minerals such as calcium, phosphorus, sodium and potassium content of papaya flesh gradually increases with the change of maturity stages and was found to be maximum in ripen stage, whereas the increasing level of iron declines in ripen stage. Ripe papaya is a very rich source of vitamin C as it contained the highest amount (28±0.2 mg/100 gm of flesh). The activities of hydrolytic enzymes in the three stages of papaya flesh were also studied. The activities of protease, amylase and lipase were found to decrease with the advancement of maturity stages while invertase activities were maximum in ripen stage.

KEYWORDS

Papaya (*Carica papaya* L.);
Carbohydrate;
Protein;
Lipid;
Hydrolytic;
Enzymes.

INTRODUCTION

Health and nutritional problem continues to be a worldwide concern. Health profile of a community is greatly influenced by its nutritional status and life style. Bangladesh is one of the poorest countries of the world where eighty percent of the populations live below poverty line[1]. Nutritionists have raised concern on the nutritive value of cooked food because density of the most nutrients content like protein, carbohydrate, vitamins and minerals are lower compared to uncooked food[2]. In Bangladesh different kinds of fruits are available which are rich in nutrients, vitamins and minerals but most of them are expensive and seasonal. Papaya (*Carica Papaya* L.) is one of the cheapest but most nutritious fruit available round the year in Bangladesh. In

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Bangladesh green papaya is the most popular vegetable and ripens papaya is considered as a delicious fruit preferred by all ages of people.

Papaya is an excellent source of vitamin A and vitamin C[3]. Nutritionally, papaya is a good source of calcium iron, potassium, magnesium and sodium. It also contains small amounts of thiamin, riboflavin. Papaya is the only food known to contain papain, the active proteolytic enzyme[4]. It also contains some other enzymes such as lipase, amylase, invertase etc. Analytical data on papaya indicate that it contains 106 volatile components[5]. The value of papaya as a medicinal plant is well known[6]. The United States Food and Drug Administration (FDA) approved the use of chymopapain for treatment of herniated lumbar intervertebral disca in human[6]. Ripe fresh papaya are used in preparation of Jam, soft drinks, ice-cream-flavouring, crystallized fruits and syrup. Papaya can be used as a diuretic (the roots and leaves), anathematic (the fruits). Parts of the plants are also used to combat dyspepsia and other digestive disorders and a liquid portion has been used to reduce to enlarged tonsils. It also eliminates necrotic tissues in chronic wounds, burns and ulcers diphtheria, toothache and fever. Papaya has several industrial uses. Biochemica, the leaves and fruits are complex, producing several proteins and alkaloids with important industrial applications[7]. The bark of papaya plant is also be used for rope making. Papaya is one of the cheapest but most nutritious fruit in our country. Limited works were done in our country on the nutrient contents of papaya at different maturity stages. Therefore, the aim of the present work was to study the nutritional analysis and activities of hydrolytic enzymes of Bari papaya-1 (*Carica papaya* L.), the most available variety in Rajshahi zone at different maturity stages.

MATERIALS AND METHODS

Materials: One of the varieties of papaya (*Carica Papaya* L.), Bari papaya-1 was collected from a local horticulture of Rajshahi and Bangladesh Agriculture research Institute (BARI), Ishwardi, pabna, Bangladesh. The papayas were stored in a deep freeze (-10^{0}C) for further experimental purpose. Glucose, BSA, Dinitrosalicylic acid (DNS) and Sodium tungstate were purchased from Sigma Chemicals Ltd., USA. Chloroform, Ethanol and Trichloro acetic acid (TCA) were purchased from Pharmacia Fine Chemicals Ltd., Sweden. All other chemicals used were commercially available and of high purity.

Preparation of crude enzyme extract

The green papaya (300 gm) was weighted, cut into small pieces and grinded in a pre-cooled mortar with a pestle and homogenized with 50 ml of cold 5 mM phosphate buffer (pH 7.0) by using a homogenizer. The suspension was then filtered through few layers of cheesecloth in a cold room. The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge at 6000 rpm for 15 minutes at 4^{0}C. The clear supernatant was collected and saturated to 100% by adding solid ammonium sulfate with gentle stirring. The precipitate was collected by centrifugation at 6000 rpm for 10 minutes at 4^{0}C. Then the precipitate was dissolved in minimum volume of pre-cooled distilled water, and dialyzed against distilled water for 12 hours and against 5mM phosphate buffer, pH 7.0 for overnight at 4^{0}C. It was again centrifuged at 6000 rpm for 10 minutes to remove any insoluble materials. The clear supernatant thus obtained was designated as "crude enzyme solution".

Measurement of amylase activity

Amylase activity was assayed following the method as described by Jayaraman[8]. 1% of starch solution was used as substrate (1 gm in 100 ml of 0.1 M phosphate buffer, pH 6.7). The amylase activity was measured by estimation the amount of maltose released by it. The amount of maltose released was calculated from the standard curve prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1μg of maltose from starch per minute at 37^{0}C.

Measurement of invertase activity

Invertase activity was assayed following the method of Mahadevan and Sridhar[9]. 2.5% sucrose solution was used as a substrate. The invertase activity was measured by estimating the amount of glucose released. One unit of invertase activity was defined as the amount required for liberating 1μg of glucose and fructose from break down of sucrose per minute at 37^{0}C.

TABLE 1: pH and TTA of papaya flesh

Parameter	Green	Mature	Ripen
PH	5.3	5.5	5.9
TTA ml of 0.1 N NaOH	0.045	0.05	0.04

TABLE 2: Moisture and ash content of papaya flesh

Parameter	Green (gm%)	Mature (gm%)	Ripen (gm%)
Moisture	85.2±0.7	87.3±0.8	90.45±0.6
Ash	0.82±0.05	0.93±0.02	0.81±0.04

Measurement of protease activity

The protease activity was measured following the method of Kunitz[10]. The milk protein casein was used as substrate. The activity was measured by detecting the release of amino acid (Tyrosine). The amount of tyrosine released was calculated from standard curve constructed with tyrosine. One unit of protease activity was defined as the amount required for liberating 1μg of tyrosine per minute at 45^0C.

Measurement of lipase activity

Lipase activity was assayed essentially as described by Sugihara et al.[11]. Olive oil was used as substrate. The lipase activity was measured by estimating the release of fatty acids. One unit of lipase activity is defined as the amount that liberates one μ mole of fatty acid under the specific condition. Specific activity of lipase was expressed as the enzyme unit per mg of protein.

Determination of pH and total titratable acidity (TTA)

The pH of the papaya flesh was determined by preparing a standard buffer solution and total titratable acidity (TTA) was determined by Folin method[12].

Degradation of nutrients

Moisture content was determined by the conventional procedure and ash content was determined following the method of A.O.A.C.[13].

Total protein content of papaya flesh was determined by the method of Micro-Kjeldahl[14] and the water-soluble protein content by the method of Lowry[15]. BSA was used as substrate and protein content was calculated from a standard curve constructed with bovine serum albumin.

Free sugar content was determined calorimetrically by the anthrone method[16]. Glucose was used as standard substrate and sugar content was calculated from a standard curve constructed with glucose.

Reducing sugar content was estimated by DNS (Dinitrosalicylic acid) method[17]. Extraction of sugar from papaya flesh was done following the method of Loomis and Shull[18] and the amount of reducing sugar was calculated from a standard curve constructed with glucose.

The starch content of papaya flesh was determined by the anthrone method[16] and the amounts of starch were also calculated from a standard curve constructed with glucose.

The lipid content of the different stages of papaya flesh was extracted by the solvent extraction process. For this purpose, 1 gm of papaya flesh was first grinded in a glass mortar and the lipid was extracted with petroleum ether (40^0-60^0C): acetone (1:1 v/v) in a Soxhlet apparatus as reported in the literature[19]. The amount of lipid was calculated using electrical balance. Each analysis was performed in triplicates and the averages were taken.

Determination of minerals

Calcium content of papaya flesh was determined by the titrimetric method[20], Phosphorous content was determined by the method of Boltz[21], Iron content was determined spectrophotometrically by thiocyanate method[22], Sodium and potassium content of papaya flesh were also determined by the method of Millner and Whiteside[23].

Vitamin-C content of papaya flesh was determined by the Bessey's titrimetric method[24].

RESULTS AND DISCUSSION

The pH of papaya flesh was in the acidic side of the scale (TABLE 1). The results indicate that the acidity of papaya decreases gradually with the changes of maturity. In TABLE 1 total titratable acidity (TTA) has been expressed as ml of 0.1 N NaOH required per 100 gm papaya flesh. It was found to increase from 0.04 to 0.05.

As demonstrated in TABLE 2, the moisture content in the flesh of different stages of papaya varied between 85.2-90.45%. The moisture content of papaya increases gradually with the advancement of maturity and was found to be highest in ripen stage. Luthfunnesa Bari[25] obtained 94.41±0.1% moisture, which is very

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TABLE 3 : Nutrient content in the different stages of papaya flesh

Parameter	Green (gm%)	Mature (gm%)	Ripen (gm%)
Total protein	0.48±0.04	1.30±0.04	2.20±0.08
Water soluble protein	0.38±0.03	1.1±0.02	2.05±0.01
Free sugar	0.50±0.025	0.68±0.03	0.79±0.015
Reducing sugar	0.43±0.01	0.52±0.04	0.85±0.02
Starch	10.5±0.05	11.11±0.03	12.25±0.06
Lipid	0.3±0.02	0.5±0.03	0.45±0.01

TABLE 4: Calcium, iron, phosphorus, sodium and potassium content in papaya flesh

Parameter	Green (mg%)	Mature (mg%)	Ripen (mg%)
Calcium	100±0.8	120±0.5	140±0.9
Iron	11.68±0.02	15.85±0.02	10.48±0.01
Phosphorus	11.5±0.1	20.2±0.2	30.8±0.15
Sodium	16.4±0.2	26.5±0.5	35.2±0.7
Potassium	80±1.6	90±2.5	130±1.5

TABLE 5 : Vitamin-C (mg/100gm of flesh) content of papaya flesh

Parameter	Green	Mature	Ripen
Vitamin-C	6.50±0.01	7.5±0.5	28±0.2

close to our result. The ash content of different stages of papaya flesh was found to be highest in mature stage.

The amounts of total protein, water soluble protein, free sugar, reducing sugar, starch and lipid present in the flesh of the different stages of papaya are shown in TABLE 3.

As given in TABLE 3, the protein content of papaya is increased with the change of maturity. This finding indicates that ripe papaya contains more protein (2.20±0.08 gm%) than green papaya (0.48±0.04 gm%). Luthfunnesa et al[25] obtained 2.01±0.04 gm% protein in 100 gm of flesh in ripen stage. The amount of free sugar present in the papaya gradually increases with the maturity of the stage. The highest amount of free sugar contain in ripen stage. The reducing sugar content of different stages of papaya has been shown in TABLE 3. The highest amount of reducing sugar is found (0.85±0.02)% in the ripen stage. The present findings indicate that the reducing sugar are increased gradually up to the ripen stage. The starch content in the three stages of papaya flesh is varied from 12.25 to 10.5 gm%. The highest amount of starch (12.25gm%) was found in ripen stage and lowest (10.5gm%) in green stage. This finding indicates that papaya is a good source of carbohydrate. In green stage lipid content was found to be 0.3±0.02% whereas in the mature and ripen stage, it was found to be 0.5±0.03% and 0.45±0.01% respectively. In mature stage, papaya contains highest amount of lipid than that of other stages.

Some minerals such as calcium, iron, phosphorus, sodium and potassium from papaya flesh have been determined. The amount of calcium, iron, phosphorus, sodium and potassium present in different stages of papaya are given in TABLE 4.

In ripen stage, papaya contains the highest amount of calcium (140 mg%). The result indicates that the amount of calcium increases with the advancement of maturity. The iron content in the three stages of papaya flesh is varied from 10.48 to 15.85 mg%. The highest amount of iron present in mature stage of papaya. The present finding indicates that iron content of papaya decreases in ripen stage. The amounts of phosphorus, sodium and potassium in the different stages of papaya also increase gradually with the change of maturity levels. The content of phosphorus, sodium and potassium were ranged from 11.5 to 30.2 mg%, 16.4 to 35.2 mg% and 80 to 130 mg%, respectively. These findings indicate that papaya flesh is a good source of minerals.

The analytical values of vitamin-C of papaya have been presented in TABLE 5. In ripen stage, the amounts of vitamin-C are highest and in the green stage vitamin-C are the lowest. It is evident the vitamin-C content of papaya increases gradually up to the ripen stage. The present finding clearly indicate that ripe papaya is a rich source of Vitamin-C as it contains 28±0.2 mg/100 gm of flesh which is nearer to Luthfunnesa et. al.[25] as they obtained 31.0 ± 2.2 mg of vitamin-C in 100 gm of flesh.

The activities of hydrolytic enzymes in the three stages of papaya flesh are shown in TABLE 6. Proteases hydrolyze peptide bonds in polypeptides into amino acids. The proteolytic activity was measured by detecting the release of amino acid, tyrosine by protease. Activity of protease in the three stages of papaya was determined. The activities were varied between 31.52 to 40.25 unit/ml. In green and ripen stages showed highest (40.25 unit/ml) and lowest (31.52 unit/ml) amount of amylase activities, respectively.

Amylases play a major role in carbohydrate metabolism in several plant tissues (WHO 1985)[26]. Amylase activities in the three stages of papaya ranged from 32.51 to 21.15 unit/ml. The highest amylase activity

TABLE 6: Activities of amylase, invertase, protease and lipase in the different stages of papaya flesh

Enzymes	Activity of enzymes (Units/ml)		
	Green	Mature	Ripen
Protease	40.25±0.01	38.69±0.02	31.52±0.01
Amylase	32.51±0.02	28.25±0.02	21.15±0.03
Invertase	3.32±0.02	4.08±0.01	5.85±0.01
Lipase	11.32±0.02	9.55±0.03	6.94±0.04

was found in green stage (32.51 unit/ml) and lowest in ripen stage (21.15 unit/ml)(TABLE 6).

Invertase, which hydrolyzes sucrose into glucose and fructose, occurs in many plants and microorganisms. The expression and distribution of plant invertases has been especially well documented, because these are considered to play an important role in sugar metabolism[27]. The invertase activities in the three different stages of papaya are shown in TABLE 6. These activities were found to be varied from 3.32 to 5.85 unit/ml. In ripen stage showed the highest invertase activities (5.85 unit/ml) while lowest in green stage (3.32 unit/ml).

Lipases are lipolytic enzymes catalyze the hydrolysis of fats as well as esters of fatty acids with alcohol's[28]. The lipase activities in different stages varied between 6.94 to 11.32 unit/ml. Among the stages, green and ripen showed the highest (11.32 unit/ml) and lowest (6.94 unit/ml) lipase activities, respectively (TABLE 6).

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