PROXIMATE, MINERALS AND BIOACTIVE COMPOUNDS EVALUATION OF PAPAYA POWDERS AS AFFECTED BY ITS DIFFERENT STAGES (GREEN, SEMI RIPE & RIPE)

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Roll No.: 0119/02

Registration No.: 687

Session: January-June/2019

The thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Food Processing and Engineering

Department of Food Processing and Engineering

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JUNE 2021

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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Dedication

DEDICATED TO MY

RESPECTED AND BELOVED

PARENTS AND TEACHERS

Acknowledgements

I am indebted to Almighty Allah who enables me to complete the research work and write up the dissertation successfully for the degree of Master of Science in Food Processing and Engineering under the Department of Food Processing and Engineering, Chattogram Veterinary and Animal Sciences University.

First of all I want to acknowledge National Science and Technology (NST) Fellowship of Ministry of Science and Technology, Bangladesh for giving me the opportunity to take up this fellowship.

I pay heartily gratitude to **Professor Dr. Goutam Buddha Das**, Vice-Chancellor, Chittagong Veterinary and Animal Sciences University (CVASU) for giving special opportunity and providing such research facilities.

At this moment of accomplishment, I am grateful to my supervisor **Mrs. Shireen Akther,** Associate Professor, Department of Food Processing and Engineering, CVASU for her supervision and guidance in successful completion of this work. It was really a great pleasure and amazing experience for me to work under her supervision and it was impossible to complete the dissertation without her constructive supervision.

I feel much pleasure to convey my profound gratitude to my respected teacher **Dr. Jakia Sultana Jothi**, Head, Department of Food Processing and Engineering for her valuable suggestions and inspiration. It is my privilege to acknowledge **Dr. Afroza Sultana,** Associate Professor, Department of Food Processing and Engineering for her moral support and cooperation next ended to me during the course of investigation. I also thank to my Co-supervisor **Mrs. Shamima Ahmed,** Assistant Professor, Department of Food Processing and Engineering for her valuable suggestions and support during the research program.

I sincerely thank to all members of department of Food Processing and Engineering, Poultry Research and Training Center (PRTC) and Department of Physiology, Biochemistry and Pharmacology, for their constant inspiration and kind co-operation in performing the research activities precisely.

I express my deepest sense of gratitude, cordial respect of feelings to my beloved family members and dearest classmates for their immense sacrifice, blessings love and encouragement.

The Author
June 2021

Tables

Abbreviations

AOAC	Association of Official Analytical Chemists
BITC	Benzyl isothiocyanate
DAE	Department of Agriculture Extension
DPPH	2,2-diphenyl-hydrazyl-hydrate
°C	Degree Celsius
E	Extinction Coefficient
GAE	Gallic Acid Equivalent
G	Gram
MG	Milligram
ML	Milliliter
Mmol	Millimole
TAC	Total Anthocyanin Content
TPC	Total phenolic content
TFC	Total Flavonoid Content
PC	Phenolic content
TE	Trolox Equivalent
QE	Quercetin
UV	Ultra Violet
RAE	Retinol Activity Equivalents
ORAC	Oxygen Radical Absorbance Capacity
FRAP	Fluorescence Recovery After Photobleaching

Abstract

Fruit consumption has increased worldwide because of taste, disease prevention and health benefits due to the presence of nutrients such as vitamins, minerals, fiber and other bioactive compounds needed by the human body for a healthy life. Papaya is a rich source of nutrients and widely consumed in Asian countries. The proximate composition, minerals and bioactive compounds of different stages of papaya powders were evaluated using standard methods. The results of moisture, ash, protein, fat, fiber and carbohydrate were ranged from (18.95 to 20.47) %, (3.30 to 6.90) %, (6.50 to 7.05) %, (0.46 to 0.85) %, (4.71 to 8.89) % and (57.91 to 64.01) % for different stages of papaya powders (Green, semi ripe, ripe Papaya) respectively. The highest value of Sodium, Potassium, Calcium, Magnesium and Phosphorus content was found in ripe papaya. The results of Total anthocyanin content (TAC), Total flavonoid content (TFC), Total phenolic content (TPC) and Antioxidant activity were ranged from (0.12 to 0.18) mg/100 g, (27.17 to 51.41) mg QE / 100 g, (4.41 to 5.97) mg GAE / 100 g and (2.53 to 2.86) mg TE / 100 g for different stages of papaya powders (Green, semi ripe, ripe Papaya) respectively. However, it is evident that papaya, which are contained essential nutrients that are useful for human diet.

Keywords: Papaya, Proximate composition, Minerals, Bioactive compounds.

Chapter I: Introduction

Papaya is a member of Caricaceae family. Various parts of the plant, such as the leaves, fruit, seed, flower, and root, are used to make medicine (Veda *et* al., 2007). It is a widely grown perennial, tropical tree and important fruit cultivated throughout the tropical and sub-tropical region of the world. Papaya is short lived fast growing, woody, large, perennial herb, up to 10m in height with self-supporting stems (Martial-Didier *et al.*, 2017). Ripe papaya is consumed as a fruit and also used for processing. At un-ripe stage, the fruit is consumed as cooked vegetable, used as ingredient in papaya salad and cooked dished as well.

The fruit is an excellent source of calcium and other minerals, which are widely used in human diet. Papaya is rich sources of three powerful anti-oxidants: Vitamin C, Vitamin A and Vitamin E and also contain a digestive enzyme called papain that is effectively used for the treatment of trauma, allergies and spot injuries. Papaya seed has contributed to numerous health effect so also papaya skin possess various wound healing properties. They have in content also, thiamin, niacin and riboflavin (Makanjuola *et al.*, 2018). The fruit contains both macro and micronutrients which are Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn.

Papaya fruit is rich in carbohydrate (42.28% starch and 15.5% sugar in pulp), but is deficient in protein and fat (Bari *et al.*, 2006). Papaya has several traditional medicinal applications in human and animals and the fruit is consumed for different digestive conditions, as diuretic and antiseptic. Therefore, this present work is to evaluate the proximate, minerals and bioactive compounds of papaya powders as affected by its different stages.

Objectives of the study

1. To determine the proximate composition of papaya powders as affected by its different stages of maturity.
2. To determine the minerals content of papaya powders as affected by its different stages of maturity.
3. To determine the bioactive compounds of papaya powders as affected by its different stages of maturity.

Chapter II: Review of Literature

2.1 Papaya (*Carica papaya L.)*

The papaya (*Carica papaya L.*) is a tropical fruit that is native to the tropics of South Asia. Bangladesh had produced 492,000 tonnes of papaya in 2014-15. In 2018-19, the production stood at about 9 lakh tonnes, according to the Department of Agricultural Extension (DAE). Currently many genetically modified hybrid varieties are commercially available for cultivation, which are more resistant to diseases (Jiao *et al.*, 2010).

Papaya is normally a single stem plant that can reach up to 10 meters, with spirally arranged leaves confined to the top of the trunk. The plant grows rapidly and starts fruiting within one and half to 3 years. The productive life of the plant is about three and a half year. The flowers appear on the axils of the leaves, maturing into large spherical, pear-shaped fruit whose length can vary from 7 to 20 inches and can reach up to 2.5kg in weight. Papaya fruit has normally greenish yellow, yellow or orange color. The fruit is climacteric and exhibits an increase in respiration and ethylene production during ripening. The fruit ripens rapidly at room temperature. It is ripe when it feels soft and its color changes to amber or orange hue. The shelf life of the ripened fruit is short only 2 to 3 days (Archbold *et al.,* 2003). The two flesh colors (red and yellow) of papaya fruit are controlled by the same single gene, however the yellow color is dominant. The red colour of papaya fruit is due to the accumulation of lycopene, whereas the yellow colour is the result of conversion of lycopene to β-carotene and β-cryptoxanthin (Hirschberg, 2001).

2.2 General characteristics and uses of papaya

Papaya is a deliciously sweet fruit with musky undertones and a distinctive pleasant aroma. It has a soft texture with butter-like consistency (Bari *et al.*, 2006). Its taste and sweetness increase with the ripening process of fruit. However, the overripe fruit quickly starts deteriorating in quality. This is a greatly loved tropical fruit that was sensibly called “The Fruit of Angels” by Christopher Columbus. Papaya plant is also called a "tree of health" and its fruit is termed as a "fruit of long life".

Green papaya

Green papaya is a rich source of papain and chymopapain. Papain, the proteolytic enzyme, is regarded as vegetable pepsin that helps in the digestion of proteins in acid, alkaline or neutral medium. Papain is used like bromelain, a similar enzyme found in pineapple, to treat sports injuries, other causes of trauma and allergies.

Papain is also used in tenderizing meat and other proteins as it has the ability to break down the tough meat fibres and is used since thousands of years. Papain is included as a component in powdered meat tenderizers. Papain is used in chewing gum, in brewing/wine and beer making, textile and tanning industries (Oloyede, 2005). Papain and chymopapain have been shown to help lower inflammation and to improve healing from burns in addition to helping in digestion of proteins.

Figure- 2.2.1: Green Papaya

Semi Ripe Papaya

Semi ripe papayas have blend of sweet buttery consistency and sour taste. They are half green and half yellow. Semi ripe papaya has light orange flesh and slightly black colored seeds at the center. The unripe green fruit can be eaten both as raw and cooked but is usually eaten as cooked in curries, salads and stews.

Semi ripe fruit has a relatively high amount of pectin, which can be used to make jellies. In some parts of Asia, the young leaves of papaya are steamed and eaten like spinach.

The leaves are also made into tea to be used as a preventive agent for malaria, although there is no real scientific evidence for the effectiveness of this treatment.

Figure-2.2.2: Semi ripe Papaya

Ripe Papaya

The ripe fruit is usually eaten raw, without skin or seeds. Ripe papaya flesh has a rich orange color with either yellow or pink hues. The inner cavity contains a wealth of black round seeds, encased in a gelatinous-like substance. The black seeds are edible and have a sharp, spicy taste. The ground seeds are sometimes used as a substitute for black pepper. The stem and bark are also used in rope production. Papaya is also frequently used as a hair conditioner. Papaya is used in making soft drinks, jam, ice cream, and flavoring of crystallized fruit and canned in syrup. Papaya pulp nectar prepared using irradiation and mild heat treatment retained its fresh flavor and nutritional qualities closest to untreated controls and was found to be microbiologically safe with acceptable enzyme levels (Parker et *al*., 2010).

Figure- 2.2.3: Ripe Papaya

2.3 Chemical composition and Nutritional quality of papaya

The nutritional qualities and medicinal value of papaya are closely related. The papaya can be considered as a nutrient dense food, as it provides many more nutrients on per calorie basis as compared to other foods. The chemical composition as well as the mineral and vitamin composition of fresh papaya is given in Tables 2.3.1 and 2.3.2. It contains only small amounts of protein and is almost free from cholesterol and fat. The carbohydrate content of ripe papaya mainly consists of invert sugars, which are easily digestible and absorbed. Ripe fruit can therefore easily boost body's energy.

Table 2.3.1 Proximate Composition of Papaya fruits

Parameters	Range
Energy	39.0 - 41.4 (kcal/100g)
Moisture	86.9 – 89.8 %
Crude protein	0.5 - 0.6 (g/100g)
Total fat	0.1 - 0.14 (g/100g)
Ash	0.5 - 0.7 (g/100g)
Crude fibre	0.4 - 0.8 (g/100g)
Dietary fibre	0.5 - 2.2 (g/100g)
Carbohydrates	7.5 - 10.98 (g/100g)
Total Sugars	7.2 - 9.8 (g/100g)

The values are calculated based on the data reported by Adetuyi *et al.*, (2008), Gouado *et al.*, (2007), Nakamura *et al.*, (2007), Sirichakwal *et al.*, (2005), Wall *et al.*, (2010), Veda *et al.*, (2007).

The whole papaya fruit is an excellent source of dietary fiber and therefore can also help in preventing the constipation. The fiber content of papaya can help in lowering the high blood cholesterol levels. Papaya is rich in vitamins C and A. One serving of papaya can provide about 100% daily requirement for vitamin C and 30% of vitamin A. It also contributes to small quantities of vitamin E, K, thiamine, riboflavin, niacin, pyridoxine and folate. Folic acid is needed for the conversion of homocysteine to cysteine and methionine.

Table 2.3.2 Vitamins and Minerals Composition of Papaya fruits

Parameters	Range
Vitamin A (RAE)*	23 – 55 (μg/100g)
Vitamin E	3.13 – 5.3 (mg/100g)
Thiamine (vitamin B1)	0.04 – 0.05 (mg/100g)
Riboflavin (vitamin B2)	0.05 - 0.07 (mg/100g)
Niacin	0.34 – 44 (mg/100g)
Pyridoxine	0.1 – 0.15 (mg/100g)
Folate	39 – 55 (μg/100g)
Calcium	17 – 24 (mg/100g)
Phosphorous	5 – 9 (mg/100g)
Magnesium	10 – 33 (mg/100g)
Sodium	3 – 24 (mg/100g)
Potassium	90 – 257 (mg/100g)
Vitamin C	57 – 108 (mg/100g)
Manganese	0.01 – 0.03 (mg/100g)
Zinc	0.06 – 0.09 (mg/100g)
Copper	0.06 – 0.14 (mg/100g)
Boron	0.01 – 0.21 (mg/100g)
Selenium	1.2 – 1.5 (μg/100g)

The values are calculated based on the data reported by Adetuyi *et al*., (2008), Gouado *et al*., (2007), Nakamura *et al.*, (2007), Sirichakwal *et al.*, (2005), Wall *et al.*, (2010), Veda *et al.*, (2007).

The increased level of homocysteine in blood is considered a significant risk factor for a heart attack or stroke as it can directly damage the wall of blood vessels (Seo et al., 2010). Papaya could be a candid source to reverse the homocysteine mediated cardiovascular diseases since it has profuse amount of folic acid. The nutrients contained in papaya can also help to prevent the oxidation of cholesterol. Papaya also contains small amounts of calcium, magnesium, potassium, iron, manganese, zinc, copper, boron and selenium. It has low levels of sodium and high levels of potassium and can therefore be helpful for the hypertensive people to balance their overall daily dietary intake of sodium.

2.4 Bioactive compounds of papaya

In addition to its nutritional contents, papaya also contains many bioactive phytochemicals with diverse structure and functional properties which have not yet been fully exploited for their potential health benefits. The phytochemical composition of fresh papaya is given in Table 2.4.1. It contains substantial amounts of carotenoids, flavonoids and polyphenols. Red flesh papaya has been reported to contain significant quantities (4.1 mg/100g flesh) of lycopene. Because of its high phytochemical contents, it shows significant antioxidant activities.

Table 2.4.1 Bioactive compounds of Papaya fruits

Parameters	Range
Total carotenoids	321.2 – 7210 (μg/100g)
Total Polyphenols	51 – 59 (mg GAE/100g)1
Total Antioxidant Activity - ORAC	250 – 350 (μmol TE/100g)2
Total Antioxidant Activity - FRAP	350 – 430 (μmol TE/100g)3
Phytate	1.22- 1.45 (g/100g)
Oxalate (g/100g)	0.45- 57 (g/100g)
Condensed tannins (g/100g)	0.062 -0.087 (g/100g)
Hydrolysable tannins (g/100g)	0.021- 033 (g/100g)

The values are calculated based on the data reported by Adetuyi *et* al., (2008), Gouado *et* al., (2007), Nakamura *et* al., (2007), Sirichakwal *et* al., (2005), Wall *et* al., (2010), Veda *et* al., (2007).

During the growing process, papaya produces some specific compounds (such as benzyl isothiocyanate; BITC and caprine) to protect itself against the attacks of insects, pests and herbivores. However, their levels in mature papaya fruit are low and therefore are considered as safe for humans (Roberts *et* al., 2008). Nakamura *et* al., (2007) reported that papaya seeds represent a rich source of biologically active isothiocyanates and the n-hexane extract of papaya seeds homogenate was highly effective in inhibiting the superoxide generation and apoptosis induction in HL-60 cells, the activities of which are comparable to those of authentic benzyl isothiocyanate. In contrast, the papaya pulp contained an undetectable amount of bezyl-glucosinolate. Papaya is also a good source of carpaine. The extracts of unripe papaya have been reported to contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides and steroids (Ezike *et* al., 2009). The papaya also contains some other anti-nutrient compounds such as phytate, oxalate and tannins. The levels of these anti-nutrients (phytate, oxalate, hydrolysable tannins and condensed tannins) and antioxidants (vitamin C, tocopherols, total phenols, and carotenoids) contents of papaya (*Carica papaya*) can decrease significantly ($P < 0.05$) with increased storage period and temperature (Adetuyi *et* al., 2008). It is also called "cold papaya" as it grows in relatively cooler climates as compared to the popular and widely cultivated *Carica papaya L.* The fruits of mountain papaya are mostly consumed after processing and are also used in the production of jams, beverages, cold drinks and cocktails (Moya-Leon *et* al., 2004).

2.5 Medicinal and health effects of papaya

The overall nutritional and health benefits of papaya are because of the interactions of its nutrients and phytochemicals present in whole fruit, rather than due to a single "active" component. Because of its high antioxidant contents, papaya can prevent cholesterol oxidation and can be used as a preventive treatment against atherosclerosis, strokes, heart attacks and diabetic heart disease. The fresh fruit is commonly used as a carminative, stomachic, diuretic and antiseptic in many parts of the world. Papaya can strengthen the immune system therefore can help in preventing the recurrent colds and flu. After treatment with antibiotics eating papaya or drinking its juice can help to replenish the intestinal microflora. Papaya has also been reported to have significantly high hydroxyl radical and hydrogen peroxide scavenging activity (Murcia *et* al., 2001). The fermented papaya products showed free radical scavenging activity and were effective in providing protection against various pathological disorders including tumors and immunodeficiency many active components from papaya have been isolated and studied. Papain, the main proteolytic enzyme in papaya, is also being studied for relief of cancer therapy side effects, especially in relieving the side effects such as difficulty in swallowing and mouth sores after radiation and chemotherapy as well as boosting up the immune system and helping body to fight against the cancer cells.

Chapter III: Materials and Methods

The research work was conducted at the Department of Food Processing and Engineering, Department of Physiology, Pharmacology and Biochemistry and Poultry Research and Training Center (PRTC) of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

3.1 Study design

Firstly, different stages of papaya (Green, semi ripe, ripe Papaya) fruits was collected from local market. After collection, the selected fruits were washed thoroughly to free from mud, ferns and other extraneous material. Then peeling, cutting & dried on cabinet tray drying (60^0C for 3 days). The dried samples were grounded into powder by using grinder machine and sifted through a 60-mesh screen and then stored in airtight containers for analysis. All analyses were carried out in triplicate and values were expressed as percentages on a dried basis (%DB). The results of minerals were expressed as conversion of mg/dl to mg per 100g.

3.2. Collection of Fruit and Sampling

Different stages of papaya (Green, semi ripe, ripe Papaya) fruits was collected from local market (Zawtala Bazar, Pahartali) in Chattogram, Bangladesh.

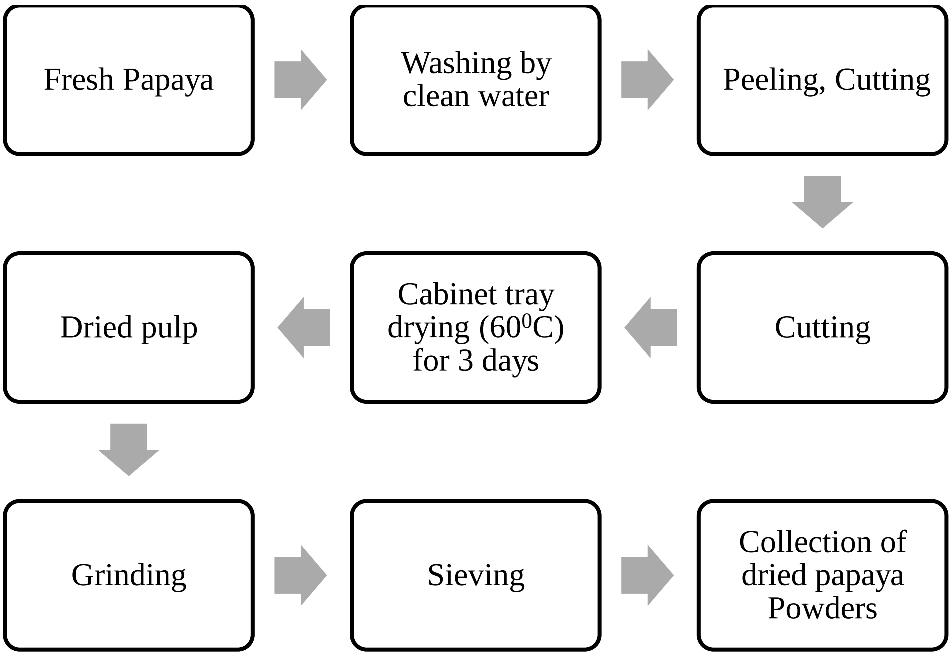

Figure 3.2 Collection of Fruit and Sampling

The freshly collected samples were free from insect's bites and washed with water to eliminate visible dirt and removed the water quickly with a blotting paper. The papaya fruits were peeled by using stainless steel knives. The peeled papaya fruits were cut longitudinally (1/2 slices & 1 cm thickness). After cutting, they were dried in a cabinet dryer at 60°C for 48 hours. The dried samples were ground into powder by using grinder machine and sifted through a 60-mesh screen and then stored in airtight containers for analysis.

3.3 Proximate analysis of different stages of papaya powders (Green, semi ripe, ripe Papaya)

Moisture, protein, fat and ash contents of papaya samples were measured in triplicate according to AOAC methods. The moisture was measured by oven drying at 105^0C to constant weight (AOAC, 2000). The crude protein content was measured by the Kjeldahl procedure (6.25×N). Total lipid was extracted by the AOAC (2000) method using the Soxhlet apparatus. Ash was measured gravimetrically in a muffle furnace by heating at 550^0C to constant weight (AOAC, 2000).

3.3.1 Moisture/Water

At first weight of empty crucibles were dried and 5g of sample was placed on it. Then the crucible was placed in an air oven (thermostatically controlled) and dried at temperature of 105^0C for 24 hrs. After drying, the crucible was removed from the oven and cooled in desiccator. It was then weighed with cover glass. The crucible was again placed in the oven, dried for 30 minutes, took out of the dryer, cooled in desiccator and weighed. Drying, cooling and weighing were repeated until the two consecutive weights were same. From these weights, the percentage of moisture in food samples was calculated as follows:

$$\text{\% Moisture} = \frac{\text{Loss of weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.3.2 Protein

Reagents used: Concentrated H_2SO_4, Digestion mixture (Potassium sulphate 100g + Copper sulphate 10g + Selenium dioxide 2.5g), Boric acid solution, Alkali solution, mixed indicator solution, Standard HCl (0.1N)

For estimation of protein, the steps were followed:

Digestion: 2g sample, 3g digestion mixture and 25 ml H_2SO_4 was taken in a Kjeldahl digestion flask. It was heated for 4 hours in a Kjeldahl digestion and distillation apparatus. The digestion was completed when the color of the substance was pale yellow.

Distillation: After digestion 100ml water, 100 ml 40% NaOH and glass blitz were added to Kjeldahl flask which containing about 10 ml 2% boric acid and 2-3 drops mixed indicator. About 100 ml distillate was collected just before the distillation was stopped. The receiving flask was moved so that the tip of the distilling tube was out the distillate. Some distillate was collected in this way to make sure the condenser tube was free from traces of ammonia.

Titration: The ammonia collected was titrated with 0.1N HCl solution and titer value was recorded.
The calculation of the percent of protein in the sample using protein factor 6.25.

$$\%\ \text{Nitrogen} = \frac{(T_s\text{-}T_b) \times \text{Normality of acid} \times \text{meq. } N_2}{\text{Weight of sample (g)}} \times 100$$

Where, T_s = Titer value of sample (ml), T_B = Titer value of Blank (ml), meq. of N_2 = 0.014, % Protein = % Nitrogen × 6.25

3.3.3 Fat

The fat content of the samples was determined by the standard AOAC method (AOAC, 2000). The dried sample remaining after moisture determination was transferred to a thimble and plugged the top of the thimble with a wad of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet flask. Approximately 75ml or more of anhydrous ether was poured into a flask. The top of the fat extraction tube was attached to the condenser. The sample was extracted for 16hrs or longer on a water bath at 80^0C. At the end of the extraction period, the thimble was removed from the apparatus and distilled off most of the ether by allowing it or collected in Soxhlet tube. The ether was poured off when the tube was nearly full. When the ether reached a small volume, it was poured into a small, dry beaker through a small funnel containing a plug of cotton. The flask was rinsed and filtered thoroughly, using ether. The ether was evaporated on a steam bath at low heat; it was then dried at 100^0C for 1hr, cooled and weighed. The difference in the weights gave the ether soluble material present in the sample.

The presence of fat was expressed as follows:

$$\text{Fat} = \frac{\text{Loss of ether soluble materials}}{\text{Weight of sample}} \times 100$$

3.3.4 Ash

The ash content of the samples was determined by the standard AOAC method (AOAC, 2000). This method performs oxidization of all organic matter by incineration and determines the weight of remaining ash. Briefly, five grams (5 g) of sample was burned and put into muffle furnace with crucible at 550ºC for 8 hrs It was calculated using the following formula:

$$\%\ \text{Ash content} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100$$

3.3.5 Crude fiber determination

Crude fiber was determined according to AOAC method (2005). Principle: Crude fiber is the water insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose and lignin. It is estimated through digestion of fat free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained.

Apparatus: Liebig condenser, Reflux condenser, Gooch crucible

Reagent required:

1. 0.255N Sulphuric acid solution
2. 10.0% Potassium sulphate solution;
3. Asbestos- Gooch grade.

Calculation: The loss in weight represents crude fiber

$$\text{Crude fiber \%} = \frac{\text{Weight of residue with crucible - weight of ash with crucible}}{\text{Weight of sample (moisture and fat free)}} \times 100$$

3.3.6 Determination of total carbohydrates

It was given as the difference between 100 and a sum total of the other proximate components. Hence it was calculated using the formula below:

% CHO = 100% - % (Protein + Fat + Fibre + Ash + Moisture content). (Akther *et* al., 2020).

3.4 Mineral analysis of different stages of papaya powders (Green, semi ripe, ripe Papaya)

3.4.1 Sample preparation

10 ml Nitric and 5 ml $HClO_4$ acid were added to a sample of 1g in a digestion flask. The mixture was digested for 1 hr. The digested mixture was filtered. The filtrate was made up to 100 ml with distilled water. Mineral contents were determined by using a biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox®) was used for biochemical assay (Akther *et* al., 2020).

3.4.2 Determination of Sodium (Na^+)

Principle: Sodium is precipitated as a triple salt with magnesium and uranyl acetate. The excess of uranyl ions is reacted with ferrocyanide in an acidic medium to develop a brownish color. The intensity of the color produced is inversely proportional to the concentration of sodium in the sample

Assay:

Wavelength / filter:	530 nm (Hg 546) /Green
Temperature:	Room Temperature

Light path:	1 cm

Calculations

$$\text{Sodium in} \frac{\text{mmol}}{\text{L}} = \frac{\text{(A) sample}}{\text{(A) Standard}} \times \text{Standard conc. } \left(\frac{\text{mg}}{\text{dl}}\right)$$

3.4.3 Determination of Calcium (Ca)

Principle: Calcium ions form a violet complex with O-Cresol phthalein complex one in an alkaline medium.

Colorimetric method: O-Cresol phthalein complex one, without deproteinization

Assay:

Wavelength / filter:	Hg 578nm (550-590)
Spectrophotometer:	570nm
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm

Calculations

$$\text{Concentration in} \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } \left(\frac{\text{mg}}{\text{dl}}\right)$$

3.4.4 Determination of Potassium (K^+)

Principle: Sodium tetraphenyl boron reacts with potassium to produce a fine turbidity of potassium tetraphenyl boron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample.

Assay:

Wavelength / filter:	630 nm (Hg 623) /Green
Temperature:	Room Temperature
Light path:	1 cm

Calculations

$$\text{Potassium } \frac{mg}{dl} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{mg}{dl})$$

3.4.5 Determination of Magnesium

Principle: The method is based on the specific binding of calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of magnesium in the sample.

Assay

Wavelength / filter:	520 nm, Hg 546 nm 500-550nm (Increase of absorbance)
	628 nm, Hg 623 nm, 570-650 nm (Decrease of absorbance)
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm
Measurement:	Against reagent blank

Calculation

$$\text{Magnesium } \frac{mg}{dl} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{mg}{dl})$$

3.4.6 Determination of Phosphorous

Assay:

Wavelength / filter:	340 nm, Hg 334 nm, Hg 365 nm
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm
Measurement:	Against reagent blank

Calculation

$$\text{Phosphorus concentration } \frac{mg}{dl} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{mg}{dl})$$

3.5. Bioactive compounds of different stages of papaya powders (Green, semi ripe, ripe Papaya)

3.5.1. Preparation of extracts

Preparation of extracts were determined according to a modified method described by Unal *et* al., (2014). Papaya powder samples were transferred into respective beakers added with absolute ethanol, and left to shake on a shaker for 72 hr at room temperature. The solvent was then separated from residue by straining. The filtrate was collected and stored at room temperature while the residue was re-extracted twice, each time with fresh solvent.

Finally, all the filtrates were combined and evaporated under reduced pressure at 60 °C using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4°C until further analysis.

3.5.2 Total anthocyanin content (TAC)

TAC of the Papaya powder samples extracts will be determined calorimetrically following the method described with slight modifications Unal *et* al., (2014). Stock solutions of 15 mg/mL of extracts will be prepared. Extract solution (3 mL) will be pipetted into a cuvette. The intensity of the extract color will be measured at wavelength 520 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu). Ethanol will be used as a blank. TAC will be calculated and expressed as milligrams per 100 g (mg/100 g) using the following equation:

$$TAC = \text{Absorbance of sample} \times DF \times 100 / m \times E$$

Where, DF stands for dilution factor; m means weight of sample used to make stock solution; E refers to extinction coefficient (55.9).

3.5.3Total flavonoid content (TFC)

TFC of the Papaya powder samples extracts will be determined using the aluminum chloride colorimetric method described by Chang et al.,(2002). Stock solution (1 mg/mL) of extracts will be prepared. Quercetin will be dissolved in 80% ethanol to make standard solutions (1.0, 3.0, 5.0, and 7.0 mg/mL) to plot a standard curve. Aliquots of 0.5 mL of diluted extract or standard solution will be mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water in the cuvette. The mixture will be left at room temperature for 30 min. The absorbance will be read at wavelength 415 nm in UV-visible spectrophotometer (UV-2600, Shimadzu). For the blank, 10% aluminum chloride will be substituted with distilled water of the same amount. TFC will be calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

3.5.4 Total phenolic content (TPC)

TPC of the Papaya powder samples extracts will be determined according to the method described with slight modifications (Unal *et* al., 2014). Stock solutions (1 mg/mL) of extracts and standard solutions of Gallic acid **(**1.0, 2.0, 4.0, 6.0, 8.0 mg/mL) will be prepared. Extracts or Gallic acid standard solution (0.3 mL) will be pipetted into a cuvette. Diluted FC reagent (1.5 mL) will be then added and mixed. The mixture will be left for 3 min before adding 1.5 mL of sodium carbonate (75 g/L) solution and left for 60 min. The absorbance was read at wavelength 765 nm using a UV spectrophotometer (UV-2600, Shimadzu). And ethanol will be used as the blank. TPC will be calculated and expressed as milligrams of Gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

3.6. Antioxidant's properties

3.6.1. DPPH assay

Antioxidant capacity of the extracts was determined using DPPH assay as described byAzlim Almey et al.,(2010) with slight modifications . Stock solution (1 mg/mL) of extract was diluted to concentrations of (0.50, 1.00, 1.50, 2.00, 2.50) mg/mL in methanol. Methanolic DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL methanol. The Methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm. Control was prepared by mixing 1 mL of methanol with 2 mL of DPPH solution. Methanol was used as a blank while Trolox was used as a standard. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts was calculated and expressed as milligrams of Trolox equivalents (TE) per gram of extracts (µmol TE/g).

3.7. Statistical tools to be used for data analyses

Statistical analysis Data (Proximate, Minerals and Bioactive compounds) were determined and stored in Microsoft Excel 2013 spread sheet to evaluate statistical analysis. All samples were in three replicates. Descriptive statistics (mean, standard deviation) were done for Proximate, Minerals and Bioactive compounds of different stage of papaya (Green, semi ripe, ripe Papaya). Data were sorted, coded and recorded in IBM SPSS Statistics 16. After that statistical analysis were conducted. Proximate, Minerals and Bioactive compounds data were analyzed by using One-way ANOVA procedures to assess significant level of variation at 95% confidence interval. Post hoc "Tukey" test was conducted to identify the variation within the sample groups. The statistical analysis was conducted for at 5% level of significant ($P < 0.05$) (Akther *et* al., 2020).

Chapter IV: Results

4.1 Proximate Composition of different stages of papaya powders (Green, semi ripe, ripe Papaya)

The result of proximate analysis of different stages of papaya powders (Green, semi ripe, ripe Papaya) are presented in Table 4.1. The results of moisture, ash, protein, fat, fiber and carbohydrate were ranged from (18.95 to 20.47) %, (3.30 to 6.90) %, (6.50 to 7.05) %, (0.46 to 0.85) %, (4.71 to 8.89) % and (57.91 to 64.01) % for different stages of papaya powders (Green, semi ripe, ripe Papaya) respectively.

Table 4.1 Proximate composition of different stages of papaya powders (Green, semi ripe, ripe Papaya)

Parameter	Green	Semi Ripe	Ripe
Moisture (%)	18.95 ± 0.03^{c}	19.06 ± 0.03^{b}	20.47 ± 0.04^{a}
Ash (%)	6.90 ± 0.02^{a}	4.22 ± 0.02^{b}	3.30 ± 0.02^{c}
Protein (%)	6.50 ± 0.01^{c}	6.83 ± 0.03^{b}	7.05 ± 0.01^{a}
Fat (%)	0.85 ± 0.01^{a}	0.64 ± 0.01^{b}	0.46 ± 0.00^{c}
Fiber (%)	8.89 ± 0.04^{a}	6.45 ± 0.02^{b}	4.71 ± 0.02^{c}
CHO (%)	57.91 ± 0.05^{c}	62.80 ± 0.04^{b}	64.01 ± 0.05^{a}

* Significant at $P < 0.05$; Means ± SD and Values followed by different superscript letters denote a significant difference; comparison done across different stage.

4.2 Mineral contents of different stages of papaya powders (Green, semi ripe, ripe Papaya)

The result of Mineral contents of different stages of papaya powders (Green, semi ripe, ripe Papaya) is presented in Table 4.2. The results of Sodium, Potassium, Calcium, Magnesium, Phosphorus were ranged from (11.05 to 12.01) mg/100g, (593.78 to 645.4) mg/dl mg/100g, (21.40 to 23.26) mg/100g, (21.97 to 23.88) mg/100g, (254.77 to 276.92) mg/100g for different stages of papaya powders (Green, semi ripe, ripe Papaya) respectively.

Table 4.2 Mineral contents of different stages of papaya powders (Green, semi ripe, ripe Papaya)

Parameter	Green	Semi Ripe	Ripe
Sodium	11.05 ± 0.01^{c}	11.53 ± 0.04^{b}	12.01 ± 0.01^{a}
Potassium	593.78 ± 0.03^{c}	619.59 ± 0.05^{b}	645.4 ± 0.03^{a}
Calcium	21.40 ± 0.05^{c}	22.33 ± 0.01^{b}	23.26 ± 0.02^{a}
Magnesium	21.97 ± 0.02^{c}	22.93 ± 0.03^{b}	23.88 ± 0.02^{a}
Phosphorus	254.77 ± 0.01^{c}	265.84 ± 0.04^{b}	276.92 ± 0.01^{a}

* Significant at $P < 0.05$; Means ± SD and Values followed by different superscript letters denote a significant difference; comparison done across different stage

Table 4.3 Bioactive compounds of different stages of papaya powders (Green, semi ripe, ripe Papaya)

Bioactive compounds of different stages of papaya powders (Green, semi ripe, ripe Papaya) are presented in Table 4.3. The results of Total anthocyanin content (TAC), Total flavonoid content (TFC), Total phenolic content (TPC) and Antioxidant activity were ranged from (0.12 to 0.18) mg/100 g, (27.17 to 51.41) mg QE / 100 g, (4.41 to 5.97) mg GAE / 100 g and (2.53 to 2.86) mg TE / 100 g for different stages of papaya powders (Green, semi ripe, ripe Papaya) respectively.

Table 4.3 Bioactive compounds of different stages of papaya powders (Green, semi ripe, ripe Papaya)

Parameter	**Green**	**Semi Ripe**	**Ripe**
TAC (mg/100 g)	0.12 ± 0.01^c	0.20 ± 0.01^a	0.18 ± 0.02^b
TFC (mg QE / 100 g)	27.17 ± 0.04^c	31.42 ± 0.03^b	51.41 ± 0.03^a
TPC (mg GAE / 100 g)	5.80 ± 0.05^b	5.97 ± 0.06^a	4.41 ± 0.01^c
Antioxidant Capacity (mg TE / 100 g)	2.86 ± 0.03^a	2.53 ± 0.07^c	2.83 ± 0.03^b

* Significant at P <0.05; Means ± SD and Values followed by different superscript letters denote a significant difference; comparison done across different stage.

Chapter V: Discussions

5.1 Proximate analysis of different stages of papaya powders (Green, semi ripe, ripe Papaya)

The proximate composition of different stages of papaya powders (Green, semi ripe, ripe Papaya) is presented in Table 4.1. The results of moisture content were ranged from 18.95 to 20.47% for different stages of papaya powders. The highest value of moisture content 20.47% was found in ripe papaya powder and the lowest value 18.95 % was found for green papaya powder. The results of moisture content showed that the moisture content of samples was significantly different ($p<0.05$). Hunt *et* al., (1980) and Chukwuka *et* al., (2013) reported that the moisture content of very ripe papaya to be higher than the hard ripe and unripe ones. There was an increase in moisture content from one ripening stage to another (Table 4.1). The moisture contents of ripe papaya were higher than unripe ones reported by Verma & Kaushal, 2014.

The highest value of ash content 6.90% was found in green papaya powder and the lowest value 3.30% was recorded for ripe papaya powder. The results of ash content showed that the ash content of samples was significantly different ($p<0.05$). The results of ash content of different stage of papaya were close agreement with Chukwuka *et* al., (2013) for different stages of ripening of papaya. The ash contents of ripe stage Papaya were lower than reported by Verma & Kaushal, 2014.

The highest value of protein content 7.05% was found in ripe papaya powder and the lowest value 6.50 % was found for green papaya powder. The results of moisture content showed that the moisture content of samples was significantly different ($p<0.05$). The protein contents of ripe stage Papaya were lower than reported by Verma & Kaushal, 2014 and higher than reported by Krishna *et* al., (2008) for ripe and unripe papaya fruits.

The highest value of fat content 0.85% was found in green papaya powder and the lowest value 0.46% was recorded for ripe papaya. The results of fat content showed that the fat content of samples was significantly different ($p<0.05$). The fat contents of ripe stage Papaya were lower than reported by Verma & Kaushal, 2014 and higher than reported by Krishna et al., 2008. For ripe and unripe papaya fruits. The results of fat content of different stages of papaya powders were close agreement with Chukwuka et al., (2013) for different stages of ripening of papaya powders.

The highest value of fiber content 8.89% was found in green papaya powder and the lowest value 4.71% was recorded for ripe papaya powder. The results of fiber content showed that the fiber content of samples was significantly different ($p<0.05$). The fiber contents of different stages of Papaya powders were lower than reported by Verma & Kaushal, 2014; Chukwuka *et* al., (2013) and higher than reported by Krishna *et* al., (2008) for ripe and unripe papaya fruits.

The carbohydrate (CHO) content of different stages of papaya powders (Green, semi ripe, ripe Papaya) varied significantly. It ranged from 57.91 to 64.01%.

The lowest carbohydrate content was found in green papaya powder, whereas highest amount in ripe papaya powder. The carbohydrate content of ripe papaya powder is excellently high showing that it is a good source of energy. The carbohydrate contents of ripe stage papaya powder were higher than reported by Verma & Kaushal, 2014. And higher than reported by Krishna *et* al., (2008) for ripe and unripe papaya fruits.

5.2 Minerals content of different stages of papaya powders (Green, semi ripe, ripe Papaya)

Mineral's content of different stages of papaya powders (Green, semi ripe, ripe Papaya) are presented in Table 4.2. The results of mineral contents were found in all stages of papaya powders in the range as Sodium, Potassium, Calcium, Magnesium, Phosphorus were (11.05 to 12.01) mg/100g, (593.78 to 645.4) mg/dl mg/100g, (21.40 to 23.26) mg/100g, (21.97 to 23.88) mg/100g, (254.77 to 276.92) mg/100g. In case of mineral content, a one-way ANOVA showed that of different stages of papaya powders (Green, semi ripe, ripe Papaya) were significantly different ($p<0.05$). The highest value of Sodium, Potassium, Calcium, Magnesium, Phosphorus content was found in ripe papaya powder. The lowest value of Sodium, Potassium, Calcium, Magnesium, Phosphorus content was found in green papaya powder.

Sodium contents of different stages of Papaya powders were lower than reported by Verma & Kaushal, 2014; Chukwuka *et* al., (2013) and higher than Saeed et al., 2014 and close agreement with Ali *et* al., (2011) for papaya.

Potassium contents of different stages of Papaya powders were lower than reported by Verma & Kaushal, 2014; Chukwuka *et* al., (2013) and higher than Ali *et* al., (2011) and Saeed *et* al., 2014 for papaya.

Calcium contents of different stages of Papaya powders were lower than reported by Verma & Kaushal, 2014; Chukwuka *et* al., (2013) and higher than Ali et *al.,* (2011) for papaya.

Magnesium contents of different stages of Papaya powders were higher than Verma & Kaushal; Chukwuka *et* al., (2013) for papaya and close agreement with Ali *et* al., (2011) for papaya.

Phosphorus contents of different stages of Papaya powders were higher than reported by Ali *et* al., (2011); Chukwuka *et* al., (2013).

5.3 Bioactive compounds of different stages of papaya powders (Green, semi ripe, ripe Papaya)

The Bioactive compounds of different stages of papaya powders (Green, semi ripe, ripe Papaya) are presented in Table 4.3. The results of total anthocyanin content (TAC) were ranged from 0.12-0.20 mg/100g for different stages of papaya powders (Green, semi ripe, ripe Papaya). The highest value of total anthocyanin content 0.20 mg/100g was found in semi ripe papaya powder and the lowest value 0.12 mg/100g was for green papaya powder. The results of anthocyanin content showed that the anthocyanin content of samples was significantly different ($p<0.05$).

The results of total flavonoid content (TFC) were ranged from 27.17-51.41 mg QE/100g for different stages of papaya powders (Green, semi ripe, ripe Papaya. The highest value of total flavonoid content (51.41±0.03 mg QE/100g) was found in ripe papaya powder and the lowest value (27.17±0.04 mg QE/100g) was for green papaya powder. The results of flavonoid content showed that the flavonoid content of samples was significantly different ($p<0.05$). The flavonoid content of the of different stages of papaya powders was higher than reported by Okon *et* al., (2017) for unripe papaya powder and Chukwuka *et* al., (2013) for different stages of ripening of papaya powders.

The results of total phenolic content (TPC) were ranged from 4.41-5.80 mg GAE/100g for different stages of papaya powders (Green, semi ripe, ripe Papaya. The highest value of total phenolic content (5.80±0.05 mg GAE/100g) was found in green papaya powder and the lowest value (4.41±0.01 mg GAE/100g) was for ripe papaya powder. The results of total phenolic content showed that the total phenolic content of samples was significantly different ($p<0.05$). The phenolic content of the different stages of papaya powders was lower than reported by Ali *et* al., (2011) for papaya. The results of phenolic content were close agreement with Okon *et* al., (2017) for unripe papaya and Chukwuka *et* al., (2013) for different stages of ripening of papaya.

The results of antioxidant capacity were ranged from 2.53-2.86 mg TE/100g for different stages of papaya powders (Green, semi ripe, ripe Papaya. The highest value of antioxidant capacity (2.86±0.03 mg TE/100g) was found in green papaya powder and the lowest value (2.53±0.07 mg TE/100g) was for semi-ripe papaya powder. The results of antioxidant capacity showed that the antioxidant capacity of samples was significantly different ($p<0.05$). The antioxidant capacity of the different stages of papaya powders was lower than reported by Ali *et* al., (2011) for papaya.

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Chapter VI: Conclusion

The present study reveals the different stages in nutrient content of Papaya powders. Despite the differences in their nutrient content, the overall nutritive picture of these Papaya appears to be quite sound. They hold out a promise to contribute significantly to the intake of nutrients among our people. This endeavor will certainly improve our micronutrient situation, improving the overall health and ensuring the general well-being of the people. The proximate composition, mineral contents and bioactive compounds of the evaluated different stages of papaya powders (green, semi ripe and ripe) are different. The findings of this study highlight the potential of all stage of papaya, as a valuable source of nutrients such as essential minerals and bioactive compounds. Drying technologies are responsible for reducing the nutritional losses. Based on the above results, it could be concluded that the papaya could be used as a potential source of carbohydrate, energy & nutritive minerals. In addition, it could be further processed into functional food products.

Chapter VII: Recommendations & future perspectives

The worldwide eating of papaya and papaya-based products has importantly increased during recent periods, due to a number of distinct factors. Foremost among these factors is the growing knowledge that papaya and papaya constitutes an important and healthy part of the human diet, mainly owing to the presence of minerals, vitamins and energy value, which play an essential role in human health. Present study is conducted to analysis the proximate composition, minerals and bioactive compounds of different stages of papaya powders. The following recommendation are:

- Effect of different drying methods on proximate composition, minerals and bioactive compounds different stages of papaya powders.
- Correlation between the proximate composition, minerals and bioactive compounds of different stages of papaya powders.
- There should be create awareness about the importance of papaya and papaya-based product being a scope for food as human diet.
- The present studies may be repeated for confirmation of the experimental findings.
- Such types of research should be done for other fruits like mango etc. available in markets especially for off season.
- The findings will be helpful from therapeutic point of view as it has medicinal value.
- Although the sample size was sufficient to perform statistical comparisons between analytical data. Our conclusion should be considered with caution because of the small number of analyzed samples and results would need to be confirmed with another larger study.

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Appendix A: Photo Gallery

Pictures of laboratory activities

Fruits

Washing

Cutting

Peeling

Tray

Drying

Papaya Powder

UV Cell Insert

UV Preparation

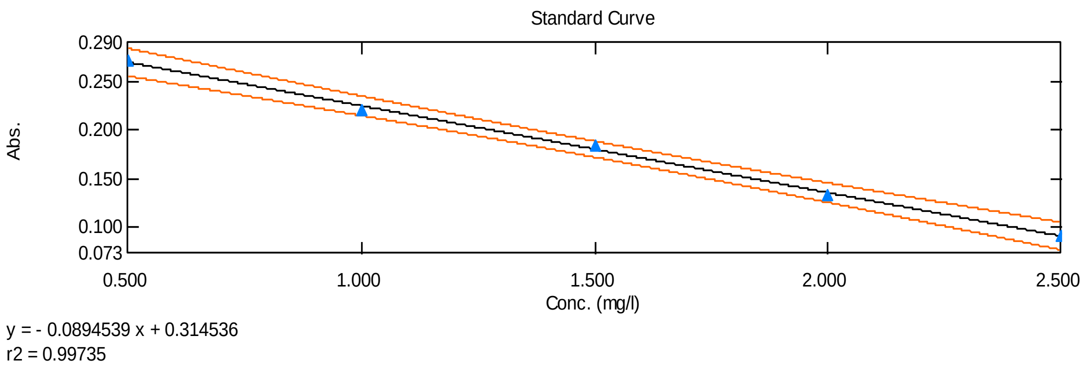
UV Visible

Appendix B: Standard Curve for bioactive compounds

Antioxidant Capacity Standard Table

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt.Factor	Comments
1	Std2	Standard	1.000	0.221	1.000	
2	Std3	Standard	1.500	0.185	1.000	
3	Std4	Standard	2.000	0.133	1.000	
4	Std5	Standard	2.500	0.092	1.000	

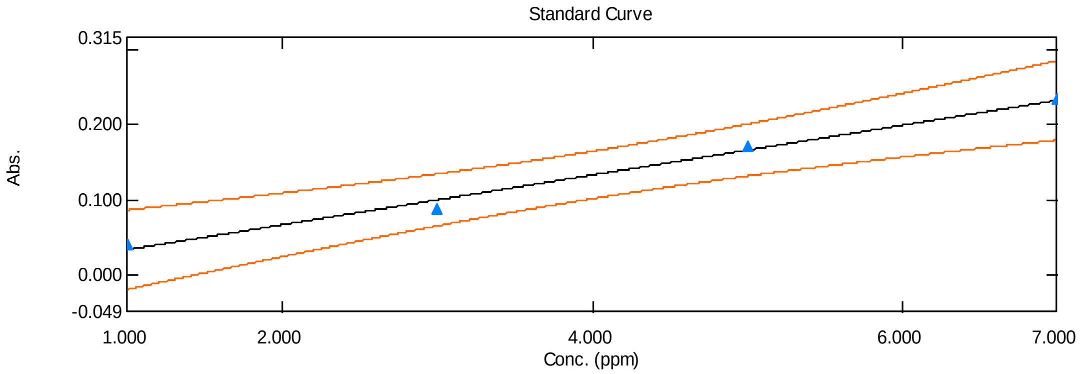
Standard Curve



TFC Standard Table

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt.Factor	Comments
1	Std1	Standard	1.000	0.041	1.000	
2	Std2	Standard	3.000	0.088	1.000	
3	Std3	Standard	5.000	0.171	1.000	
4	Std4	Standard	7.000	0.234	1.000	

Standard Curve

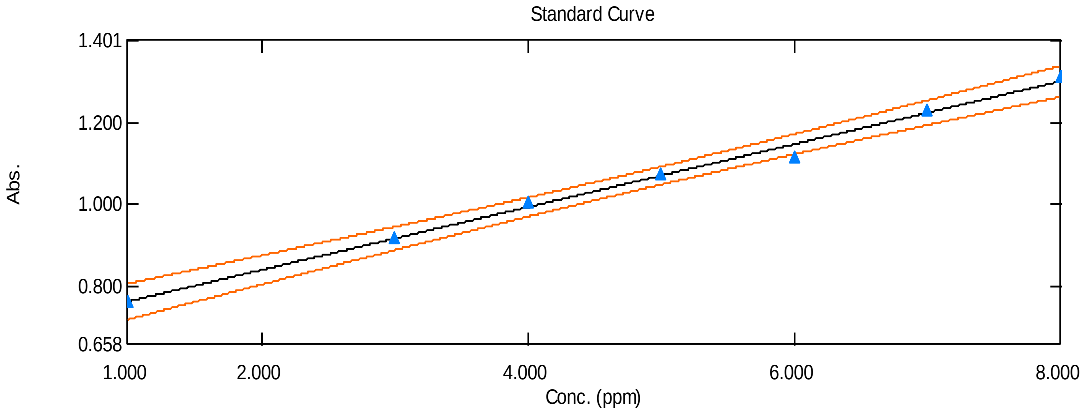


y = 0.0330538 x + 0.00112908
r2 = 0.99052

TPC Standard Table

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt. Factor	Comments
1	Std1	Standard	1.000	0.763	1.000	
2	Std2	Standard	2.000	0.780	1.000	
3	Std3	Standard	3.000	0.920	1.000	
4	Std4	Standard	4.000	1.007	1.000	
5	Std5	Standard	5.000	1.074	1.000	
6	Std6	Standard	6.000	1.115	1.000	
7	Std7	Standard	7.000	1.230	1.000	
8	Std8	Standard	8.000	1.314	1.000	

Standard Curve



y = 0.0768527 x + 0.687090
r2 = 0.99301

Brief Biography

Md. Arif Uddin passed the Secondary School Certificate Examination in 2011 and then Higher Secondary Certificate Examination in 2013. He obtained his B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, he is a candidate for the degree of Master of Science in Food Processing and Engineering under the Department of Food Processing and Engineering, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). His research interests are processing, preservation and development of modified food products, functional food product development and nutritional value analysis etc.