Chapter 2

Biochemical composition of pulp and peel of culinary banana at various developmental stages and to identify the optimum stage of harvesting

The Chapter 2 has been discussed under two sub-heads as follows:

A) Nutritional composition of culinary banana pulp at different stages of development

2.1 Introduction

Banana plants are the world's biggest herbs, grown in many countries. Culinary bananas, often called as plantains, are mostly evolved from the edible varieties of two species *Musa acuminata* (genome A) and *Musa balbisiana* (genome B).[1] Considering the nutritional aspect, plantains and bananas are the world's 4th leading agricultural crop.[2] Plantains and cooking bananas look almost similar to unripe dessert bananas, but they are larger in size, more fleshy and starchy.[3] Cooking or culinary bananas often named plantains are mostly of the genomic groups of AAB, ABB or BBB is a major staple food in many developing countries.[4] They are considered to be one of the most important sources of energy and starchy staple food for the people of tropical humid regions.[5] According to Doymaz [6] bananas and plantains are rich in nutrients, starch, sugar and vitamins A and C, potassium, calcium, sodium and magnesium. Plantains are nutritionally low protein food material but relatively high in carbohydrates, vitamins and minerals.[7] Plantain and banana cultivation is attractive to farmers due to the low labour requirements compared with cassava, maize, rice and yam.[8] As compared to the dessert banana (also known as commercial banana which has a global distribution), culinary banana is restricted in only a few localities of the world.[9, 10] Ethno botanical studies have revealed that this cultivar of culinary variety possess many medicinal properties along with its pre known health benefits.[11, 12]

The stages of fruit maturity and development have a philosophical influence on the various physical, biochemical, functional and morphological attributes of the fruit, which impinge on the quality of the fruits. When young, fruits develop from the female flowers appear like a slender green fingers, and on attaining maturity, the bracts slowly shed off the fruit which finally turn to be a cluster called "hand" of banana.[13] On further fruit development, it changes its colour from deep green to light green and finally yellow and the pulp turns light yellow from off white. The fruit when young and immature looks firm, taste astringent and feels gummy with latex and slowly turns tender, soft and sweeter when ripe. Therefore, in order to obtain good

quality fruit, harvesting has to be done at proper stage of fruit maturity. Harvesting of immature fruits may lead to poor quality fruit with inconsistent ripening, while on the other hand, delayed harvesting affects the quality of fruit and increase deterioration susceptibility.[14] The nutritional properties of banana and plantains alter significantly with advancement in fruit growth and maturity. The major changes are breakdown of carbohydrates and osmotic transfer form peel to pulp, conversion of starch to sugars, reduction of polyphenols, and synthesis of aromatic compounds etc.[15, 16] Compared to culinary banana, most available reports are on changes in chemical composition of dessert banana cultivars during ripening.[3, 8, 17, 18]

Coming to the point of culinary variety of banana found in Assam and Northeastern region of India, it is called *kachkal* in local language falls under the category of fruit-vegetable, and is one of the commonly consumed vegetable next to potato in the daily diet of local people. It is also used for the preparation of various traditional dishes as it is excellent source of carbohydrates, starch and many other functionally important bioactive compounds. Traditionally, it is used as special diets for babies, elderly and patients with stomach problems, gout and arthritis. Unlike any other fruits or vegetables, the biochemical compositions of culinary banana (*kachkal*) also vary with growth stage and maturity, however, report on its complete nutritional study at various stages of development is hardly available. The nutritional studies will be useful for exploitation of the locally important crop at different stages of growth to identify and quantify the particular compound of interest at particular stage of maturity. Therefore, the present work was undertaken to study the nutritional and biochemical compositions at various developmental stages of culinary banana which can be further helpful in utilization of this underutilized crop in developing value added products

2.2 Materials and methods

2.2.1 Sample collection and preparation

Samples were collected from the experimental plot of Tezpur University, Assam, India. The fingers were harvested at growth stages of 20 days after emergence (DAE) (stage I) of banana inflorescence, 35 DAE (stage II), 50 DAE (stage III), 65 DAE (stage IV), and 80 DAE (stage V) as shown in Fig. 2.1. Samples were washed thoroughly under running water followed

by distilled water and spread out on absorbent tissue papers to remove surface moisture. The pulp and peel samples were separated using a stainless steel knife. The pulp samples were cut into ~6 mm thick slices and dried in a tray drier (IK-112, IKON Instruments, Delhi, India) at 40ºC for 12 h. Dried samples were ground using mechanical grinder (Fritsch, Germany), sieved and stored at ambient temperature (25±2^{o}C) in air tight containers till the time of analyses.

2.2.2 Chemical analysis

2.2.2.1 The proximate composition

The initial moisture, ash, protein, and crude fat contents at various stages of development were determined according to methods described in AOAC.[19] Ash content was determined by ignition in a muffle furnace (Optic Ivymen System, SNOL 8, 2/1100, Utena, Lithuania) at 550°C for 6 h. Nitrogen content was determined using the Kjeldahl apparatus (KelPlus, Pelican Equipment, Chennai, India) and the amount of nitrogen was multiplied by a factor 6.25. Crude fat was determined using the Soxhlet extractor (SocsPlus, Pelican Equipment, Chennai, India) with n-hexane as solvent. Crude fiber was determined following the acid and alkali treatment as described by Maynard[20] and Sadasivam and Manikam.[21] The carbohydrate content was measured by hydrolyzing the polysaccharides (acid hydrolysis) into simple sugars and estimating the resulting monosaccharide by anthrone method.[22]

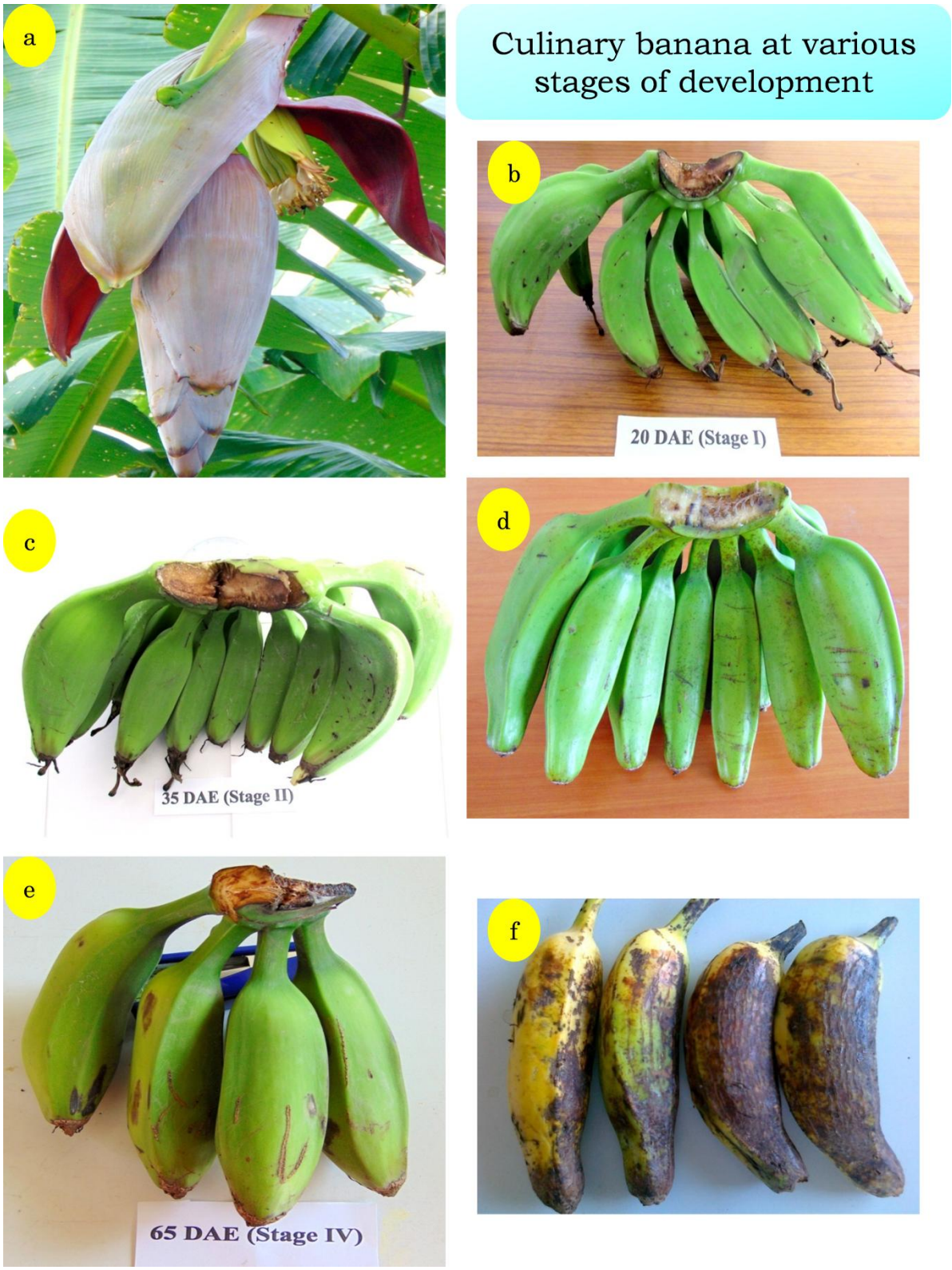

Fig. 2.1 Culinary banana (*kachkal*) at various stages of development **a)** 1 DAE (emergence of inflorescence), **b)** 20 DAE, **c)** 35 DAE, **d)** 50 DAE, **e)** 65 DAE and **f)** 80 DAE

2.2.2.2 Estimation of pH, titratable acidity, ascorbic acid, cellulose and lignin

The pH of samples was measured by blending 10 g of pulp with 40 ml of deionized water[19] and the mixture was shaken at 5 min intervals for 15 min and centrifuged at 3000 rpm for 15 min in refrigerated centrifuge (SIGMA Laborzentrifugen, 3-18 KS, Osterode, Germany). The supernatant was decanted and determined its pH (pH510, Eutech, Ayer Rajah Crescent, Singapore). Titratable acidity and ascorbic acid of samples were determined using the method of Ranganna.[23] Cellulose content was estimated following the method of Sadasivam and Manikam[21] by reacting with acetic/nitric reagent and measured the absorbance at 630 nm spectrophotomerically (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India). Lignin content was estimated by extraction in 0.5N NaOH solution and aliquot samples were adjusted to pH 7.0 and 12.3. The absorbance of aliquots was measured at 245 and 350 nm. The amount of lignin content was calculated by difference between A245 (pH 7.0) and A350 (pH 12.3).[24]

2.2.2.3 Starch and sugars

Following Hodge and Hofreiter[22] starch was extracted with hot perchloric acid and hydrolyzed to glucose which formed green colour with anthrone reagent and the content of starch was measured by reading the absorbance at 630 nm in spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India). Amylose contents were determined with the method of Sadasivam and Manikam[21] where iodine was absorbed within the helical coils of amylose to produce a blue coloured complex which was measured colorimetrically at 590 nm. Soluble sugars were extracted from 1 g of sample in 80% ethanol (hot) and sugar content was quantified by the phenol-sulphuric acid method.[25] The reducing sugar content was estimated by the Nelson-Somogyi method.[26] The amount of non-reducing sugars was determined by subtracting the amount of reducing sugars from the amount of total sugars in the sample.

2.2.2.4 Pectin, tannin, phytic acid estimation

Pectin content was determined by extraction and saponification[23] followed by precipitation as calcium pectate by calcium chloride. After removal of chloride ions, the

precipitate was dried and weighed. Tannin content was determined by Folin-Denis method[27] in which tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a densely blue solution, and the intensity was measured spectrophotometrically (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India). Phytic acid content was estimated by extracting phytate with trichloroacetic acid and precipitating as ferric salt.[28]

2.2.2.5 Total polyphenols and antioxidant activity analysis

Total phenolic content was determined with the Folin-Ciocalteu (F.C.) colorimetric method of Malick and Singh.[29] Phenols were reacted with phosphomolybdate which is an oxidizing agent in F.C. reagent under alkali conditions and result in the formation of a blue coloures complex which was measured at 650 nm colorimetrically. The DPPH radical scavenging activity was measured with the method of Brand-Williams et al.[30] and the assay is based on the ability of antioxidant to scavenge the DPPH cation radical. This method determines the hydrogen donating capacity of molecule and does not produce oxidative chain reactions or react with free radical intermediates. The percentage inhibition of the DPPH radical scavenging activity was calculated colorimetrically at 517 nm using following formula

$$\%\, Scavenging\ activity = \frac{absorbance\ of\ control\ (A_0) - absorbance\ \ of\ sample\ extract\ (A_1)}{absorbance\ \ of\ sample\ extract\ (A_0)}\, x\ 100$$

2.2.2.6 Estimation of carotenoids, vitamin A and thiamine

Carotenoids were extracted and partitioned in organic solvents on the basis of their solubility. The amount of carotenoids present in the sample was measured spectrophotometrically (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India) at 450 nm against concentrations of high purity β-carotene.[21] Vitamin A content was measured by a rapid colorimetric method of Bayfield[31] Thiamine content was estimated by a flurometric method.[21]

2.2.2.7 Minerals estimation

The mineral contents were estimated by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 2100 DV, Bridgeport, USA) following the methods of Naozuka et al.[32] Concentrations were determined in the aqueous solution of acid digest. Powdered samples (1 g) were added to 30 ml concentrated nitric acid and 5 ml concentrated hydrochloric acid. The vessels were immediately closed after addition of oxidants. Samples were digested on a hot plate at 100ºC. At the end of the digestion process, digests were cooled and diluted up to 50 ml with distilled water.

2.2.2.8 Fatty acid analysis

Gas liquid chromatography (GLC) (CP-3800, Varian, USA) was used for analysis of fatty acid profiles. Fatty acids can occur in small amounts in free form, but in general, they are combined in complex molecules through ester or amide bonds. Before GLC analysis non-reactive derivatives of fatty acids methyl esters were prepared.[33] Samples were treated with 0.4N sodium methylate and shaken vigorously at water bath for 2-3 min at 65°C followed by addition of 1 ml carbon disulphide and shaken for 1-2 min and filtered through activated charcoal. The filtrate constituted all methyl esters of fatty acids and were separated by chromatography (Varian) equipped with a flame ionization detector (FID) and electron capture detector (ECD). The column temperature was 190ºC and flow of the nitrogen carrier gas was maintained at 35 ml/min. Peaks were identified by comparison of retention times to those of standard fatty acid esters.

2.2.2.9 Amino acid analysis

Amino acid analysis was done by hydrolyzing with 6 N HCl and measured by ion exchange chromatography using ninhydrin post-column derivatization. Samples equivalent to 5 mg protein were placed in a 20 ml glass ampoule, kept on dry ice to avoid clumps forming and 10 ml 6N HCl was added. Nitrogen gas was flushed to remove oxygen from the ampoule for 1 min and closed with para-film. Samples were kept in oven at 110ºC for 22 h for hydrolysis. After

hydrolysis, samples were removed and allowed to equilibrate at room temperature. The neck of the ampoule was broken and samples transferred to 25 ml volumetric flasks. Volume was made up to 25 ml with distilled water and mixed thoroughly and filtered through nitrogen free Whatman No.1 filter paper. The aliquot (0.5 ml) was evaporated at 45-50ºC and after complete drying, 5 ml deionized water was added and evaporated again. Drying and evaporation of samples were repeated 4 times. Crude dried samples were dissolved with 2.5 ml of sodium citrate loading buffer (pH 2.2). Samples were filtered using a syringe driven filter (0.45 µm) and kept in an auto sampler. Standards (100 pmol) and samples were run in an automated amino acid analyzer (119 CL, Beckman, Palo Alto, California).

2.2.2.10 Estimation of phenolic compounds by HPLC

The determination of major phenolic compounds present in the samples were analyzed using High performance liquid chromatography (Dionex, Ultimate 3000, Germany) equipped with auto sampler UV detection at 320 nm. An acclaimed 120-C18 column (5 µm, 120 Å) with a size of 4.6x250 mm at a constant flow rate of 1.5 ml/min using acidified water (pH adjusted to 2.64 using HCl) as solvent A and mixture of acidified water and acetonitrile in the ratio of 20:80 as solvent B. The linear gradient elution timing was as follows: 100% A (0 min) 90 - 80% (10 min), 70-60% (20 min), 50-40% (30 min) and 30-20% (40 min). The stock solution of each standard (100 ppm) was prepared in 50% methanol-milli-Q water. The retention time (t_R) and DAD (diode-array detector) absorbance spectral matching were used for identification purpose. The data were analyzed using the software Chromeleon ver. 6.80. A slight modification was done on the method of Kumar et al.[34] in order to carry out HPLC study of polyphenols present in culinary banana.

2.2.3 The colour measurement

The colour measurement of samples at different growth stages was analyzed in a Hunter Lab Color Quest (Model Ultrascan Vis-Model, Virginia, USA). The results were expressed in L*, a* and b* systems.

2.2.4 Determination of pulp to peel ratio

Following the method described by Adao and Gloria[35] the pulp-to-peel ratio was determined by weighing the parts of individual sample in an analytical balance and the results were expressed as percent pulp relative to peel weights.

2.2.5 Statistical analysis

Experiments were carried out in 3 replicates. The analysis tool 'Microsoft Excel' was used for statistical analysis. Data were subjected to ANOVA and Fisher's Least Significant Difference (LSD) was used to separate means.

2.3 Results and discussion

2.3.1 Proximate compositions

Moisture content (Table 2.1) decreased gradually from stage I (59.49 g/100g) to stage III (57.02 g/100g) and then increased at stages IV (61.07 g/100g) and V (66.83 g/100g). There was a significant difference in moisture content in all stages except in stages I and II. This might be attributed to respiratory breakdown of starches into sugars and migration of moisture from peel to pulp. Increase in moisture content at stage V might be due to softening tissue texture as ripening progresses.[5] An increase of water content in pulp of two cooking banana hybrids with progress in maturity was attributed to utilization of carbohydrates during breathing and osmotic transfer from peel to pulp.[36] Ash content decreased as plant's maturity progresses and the highest content was recorded at the stage I (7.03 g/100g) which decreased gradually at the fully matured stage (3.05 g/100g). Adeyemi and Oladiji[37] reported that ash content of ripening plantain is affected by developmental stage and unripe plantain contains higher ash compared to ripe ones. Another reason for variation in ash content might be due to differential absorption capacity of minerals at different stages of development. Gradual decrease in protein content as plants matured and decreased from stage I (10.56 g/100g) to stage V (2.01 g/100g). Goswami and Borthakur[38] observed a decline in protein content in culinary banana with maturity and attributed

to protein breakdown and the resulting amino acids being utilized in gluconeogenesis. The samples contained a relatively low amount of fat, which varied from stage I (1.50 g/100g) to stage V (0.63 g/100g) and results are in agreement with Goswami and Borthakur[38] who reported that fat content (0.8-1.2 %) in culinary banana was higher during early developmental stages and gradually decreased with increasing maturity. Fiber content also gradually increased as maturity progresses, indicating there were differences due to stage and the highest amount was recorded at stage IV (1.66 g/100g). Egbebi and Bademosi[39] reported crude fiber content in unripe and ripe plantain (0.7-1.11 %) and increased significantly with progress of maturity. The increase in fiber content at matured stage over tender stage might be due to increase in soluble and insoluble dietary fractions. The carbohydrates content increased from stage I (21.32 g/100g) to III (32.15 g/100g) and decreased at stage V (27.63 g/100g) with significant difference among the stages except in stages III and IV. The variation in carbohydrate contents during growth might be due to degradation of starch for synthesis of sugars.[36]

Table 2.1 Effect of different stages of development on proximate composition of culinary banana (g/100g)

Stage	Moisture content	Ash	Protein	Fat	Crude fiber	Carbohydrate
I	59.49±0.80b[a]	7.03±0.35[d]	10.56±0.86[d]	1.50±0.11[d]	0.61±0.01[a]	21.32±0.05[a]
II	55.84±0.42[a]	6.11±0.73[c]	8.61±0.96[c]	1.27±0.08[c]	0.99±0.08[b]	28.04±0.29[b]
III	57.02±0.5[a]	4.03±0.49[b]	5.56±0.84[c]	0.94±0.05[b]	1.50±0.02[c]	32.15±0.20[d]
IV	61.07±0.77[c]	2.72±0.20[a]	3.99±0.57[b]	0.58±0.06[a]	1.66±0.05[d]	30.93±0.06[c]
V	66.83±1.02[d]	3.05 ±0.92[a]	2.01±0.87[a]	0.63±0.08[a]	0.54±0.87[a]	27.63±0.87[c]

[a]Mean in columns followed by the same letter are not significantly different at p>0.05; values represent mean±SD, n=3

2.3.2 pH, ascorbic acid, cellulose and lignin

The pH (Table 2.2) was the lowest at stage I (5.01) and highest at stage V (5.76). A gradual variation in pH of culinary banana during ripening stages has been reported by Sakyi-Dawson et al.[36] Titratable acidity was the lowest during early developmental stage I (0.16

g/100g) and recorded highest at stage IV (0.32 g/100g). There was no differences between stages I and II and stages III and V; however, stages II and III were different. Acids play an important role in the post-harvest quality of fruits and vegetables, as taste is mainly a balance between sugar and acid contents which is important in evaluation of fruit taste.[40] Sakyi-Dawson et al.[36] reported changes in titratable acidity and pH of cooking banana and indicating a general increase in titratable acidity during plantain ripening. Ascorbic acid content (Table 2.2) varied with stages of development and ranged from 0.74-1.12 mg/100g. Sakyi-Dawson et al.[36] reported that ascorbic acid content in cooking banana varied with maturity with regular decreasing pattern. The present results are in agreement with the report of Segung and Kader[41] in case of fluctuation in ascorbic acid content of horticultural crops with maturity. The lowest amount of ascorbic acid in fully matured tomato is reported by Moneruzzaman et al.[42] Developmental stage affects the cellulose content (Table 2.2) and the highest value was recorded at stage IV (1.06 mg/100g). Komolka et al.[43] reported relatively low amount of cellulose compared to other fruits and vegetables. Lignin content gradually increased with maturity and was the highest at stage IV (1.57 mg/100g). There was no significant difference between stages I and II; however, differences were observed in stages II, III and IV. This might be due to the lignifications of cell wall constituents resulted an increase in other dietary fiber fractions.[44]

Table 2.2 Effect of different stages of development on pH, titratable acidity, ascorbic acid, lignin and cellulose of culinary banana

Stage	pH	Titratable acidity (g/100g)	Ascorbic acid (mg/100g)	Lignin (mg/100g)	Cellulose (mg/100g)
I	5.03±0.15b[a]	0.16±0.01[a]	1.12±0.02[e]	0.56±0.08[a]	0.04±0.01[a]
II	5.01±0.09[a]	0.19±0.02[a]	0.86±0.02[d]	0.68±0.10[a]	0.25±0.01[b]
III	5.12±0.17[a]	0.23±0.03[b]	0.83±0.03[c]	1.25±0.12[b]	0.32±0.02[c]
IV	5.25±0.37[a]	0.32±0.02[c]	0.91±0.11[b]	1.57±0.09[c]	1.06±0.05[d]
V	5.76±0.87[b]	0.25±0.05[b]	0.74±0.90[a]	0.54±0.75[a]	0.28±0.06[b]

[a]Mean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.3.3 Starch, amylose, sugar and pectin contents

Starch, amylose and sugar contents varied with maturity (Table 2.3). Present study revealed that starch is the major storage form of carbohydrates in culinary bananas. The starch content increased from stage I (12.36 g/100g) to stage III (22.66 g/100g) and then declined at stage V (11.21 g/100g). Starch accumulation led to the higher weight in matured plantain.[45] Carbohydrate in the form of starch is the major chemical change which occurs throughout growth and development in cooking bananas and plantains.[36] Amylose content was affected by stages of development and increased from stage I to stage IV (3.77 to 8.81 g/100g) with maturity. An increase in total carbohydrates content might be correlated with active synthesis of starch with growth however, with maturity of cooking banana starch content decreased and total soluble sugars increased significantly. Marriott et al.[8] also reported similar results in ripening banana and plantains. Total soluble sugars content (Table 2.3) increased very marginally from stage I (0.64 g/100g) to stage III (1.35 g/100g) and thereafter sharp increase from stage IV (2.01 g/100g) and in stage V (4.65 g/100 g) and this evinces that starch degrades with more maturity. Ogazi[46] reported that sugars comprise only about 1.30 g/100g of total dry matter in unripe plantain which corroborates the present findings. Reducing and non reducing sugars (Table 2.3) increased with maturity. Non reducing sugars (0.37-3.72 g/100g) were higher than reducing sugars (0.16-1.08 g/100g). Sakyi-Dawson et al.[36] reported a similar trend of non reducing sugar contents in plantain and cooking banana. There was significant difference in pectin content among stages and it increased from stage I (0.92 mg/100g) to stage III (1.37 mg/100g) which thereafter declined at stage V (0.81 mg/100g). The reason for increase in pectin content with the advancement of growth up to stage III might be due to less interaction between the pectin and the other cellular components and as a consequence the pectin was more available for extraction. On the other hand, decrease at stage IV and V might be due to the degradation of pectin under the action of pectic enzymes, such as polygalacturonase (PG), pectin methyl esterase (PME) or pectatelyase (PL). The increase of pectin content up to a certain stage and then decrease was also observed by Lohani et al.[47]

Table 2.3 Effect of different stages of development on starch, amylose, total soluble (TSS), reducing (RS), non reducing (NRS) sugars and pectin content of culinary banana

Stage	Starch (g/100g)	Amylose (g/100g)	TSS (g/100g)	RS (g/100g)	NRS (g/100g)	Pectin (mg/100g)
I	12.36±0.17a [a]	3.77±0.51[a]	0.64±0.03[a]	0.16±0.02[a]	0.37±0.08[a]	0.92±0.15[a]
II	18.47±0.10[b]	5.84±0.48[b]	0.72±0.05[b]	0.29±0.06[b]	0.48±0.04[b]	1.27±0.10[b]
III	22.66±0.61[c]	7.25±0.62[c]	1.35±0.03[c]	0.41±0.01[c]	0.63±0.01[c]	1.37±0.05[b]
IV	20.32±0.99[d]	8.81±0.52[d]	2.01±0.06[d]	0.54±0.04[d]	1.57±0.07[d]	1.26±0.04[b]
V	11.21±0.9[5]	6.65±0.75[b]	4.65±0.97[e]	1.08±0.07[e]	3.72±0.15[e]	0.81±0.13[a]

[a]Mean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.2.4 Tannin and phytic acid contents

The tannin content (Table 2.4) of samples differed significantly with stages of development and the highest amount was recorded at stage I (0.59 mg/100g) which declined with maturity (0.21 mg/100g). The decrease in tannin content with advancement of growth reduces the astringency property. Mendoza et al.[48] reported the tannin content in cooking bananas of Philippians (1.03-5.66 mg tannic acid equivalent/g) which is relatively higher than the present values. As culinary banana attains maturity the astringency property gets reduced which is related to insolubilization and polymerization of polyphenols with other constituents of pulp. The variation might be due to differences in cultivar, growth condition and environmental factors. Fruits and vegetables normally exhibit astringency when it is young and gradually losses this characteristic property with maturity and becomes palatable for exploitation.[48] The stages of development also affect phytic acid content (Table 2.4) and recorded the highest values at stage II (24.15 mg/100g) and lowest at stage V (11.96 mg/100g). The amount of phytic acid was low compared to other starchy foods like cassava (95-135 mg/g).[49]

Table 2.4 Effect of different stages of development on tannin and phytic acid content of culinary banana

Stage	Tannin (mg/100g)	Phytic acid (mg/100g)
I	0.59±0.01[e]	15.50±0.39[b]
II	0.51±0.02[d]	24.15±0.95[e]
III	0.34±0.06[c]	20.05±1.50[d]
IV	0.27±0.03[b]	18.88±0.97[c]
V	0.21±0.06[a]	11.96±1.05[a]

[a]Mean in columns followed by the same letters are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.3.5 Total polyphenols, DPPH radical scavenging activity, total carotenoids, vitamin A and thiamine contents

Wide variations in total polyphenols (91.87-307.99 mg GAE/100g dry matter), DPPH radical scavenging activity (39.64-59.12 % SA), total carotenoids (0.130-0.15 mg/100g), vitamin A (0.029-0.03 mg/100g) and thiamine (0.019-0.03 mg/100g) contents at different stages of development were observed (Table 2.5). Total polyphenols were found higher at stage I compared to the other stages. Bananas have been classified as one of the prominent antioxidant foods by Kanazawa and Sakakibara.[50] The highest DPPH radical scavenging activity was observed in stage I and lowest at stage V and it decreased with maturity. Decreasing trend in scavenging activity of papaya with respect to crop maturity was also observed by Zuhair et al.[51] A gradual increase in total carotenoids, vitamin A from stage I to stage V was observed and recorded the highest at stage V. Increase in thiamine content up to stage IV was observed but declined at stage V. Total carotenoid contents in fruits and vegetables increases during ripening because the chlorophyll undergoes degradation and carotenogenesis takes place resulting in synthesis of greater amount of individual carotenoid compounds at chromoplast rather than the chloroplast.[52]

Table 2.5 Effect of different stages of development on total polyphenols (TPC), DPPH radical scavenging activity (SA), total carotenoids (TC), vitamin A and thiamine contents of culinary banana

Stage	TPC (mg GAE/100g dry matter [a])	DPPH (% SA)	TC (mg/100 g)	Vitamin A (mg/100 g)	Thiamine (mg/100 g)
I	307.99±2.86e [b]	59.12±0.73[d]	0.130±0.07[a]	0.029±0.01[a]	0.002±0.01[a]
II	261.22±2.29[d]	55.60±1.16[c]	0.142±0.05[b]	0.028±0.03[a]	0.019±0.01[b]
III	178.72±2.60[c]	52.66±2.47[c]	0.147±0.01[c]	0.030±0.02[a]	0.027±0.03c
IV	160.96±2.40[b]	46.96±4.20[b]	0.153±0.09[d]	0.033±0.02[b]	0.032±0.03[d]
V	91.87±2.07[a]	39.64±1.75[a]	0.159±0.02[e]	0.038±0.01[c]	0.021±0.07[b]

[a]GAE=gallic acid equivalent; [b]Mean in each column followed by the same letters are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.3.6 Minerals content

Results of mineral contents are presented in Table 2.6. Out of all the minerals Mg was recorded the highest (0.961-1.183 mg/100g) in all stages with significant difference. The highest concentrations of Fe (0.385mg/100g), Cu (0.009mg/100g), and Ca (0.542 mg/100g) were recorded at stage II; however, Zn concentration recorded the highest (0.417 mg/100g) at stage IV. Significant variations of K (0.498-1.273 mg/100 g), Ca (0.241-0.542 mg/100g), Fe (0.173-0.385 mg/100g) and Na (0.115-0.167 mg/100g) were observed at all stages of development. The variation in mineral contents at the growth stages is mainly attributed to preferential absorbance and this might be due to cultivar and/or soil, climate, agricultural practice and the quality of water for irrigation.[53] Most of the minerals are very crucial in many enzymes activities, protecting cells from free radicals attack, regulation of glucose homeostasis etc.[54-55]

2.3.7 Fatty acids composition

Results (Table 2.7) revealed that the major saturated fatty acids are palmitic acid (0.267-1.678 mg/100g) and stearic acid (0.061-0.091 mg/100g). The palmitic acid content decreased from stage I to stage V whereas stearic acid decreased from stage I to stage III and was absent at

subsequent stages. Lauric (0.006-0.092 mg/100g) and myristic (0.008-0.019 mg/100g) acids were recorded in minor quantities during early stages of development, which gradually decreased towards maturity. Among unsaturated fatty acids the most predominant were linoleic (0.329-2.081 mg/100g) and linolenic acids (0.139-1.210 mg/100g). These essential fatty acids were the highest during early developmental stages. The oleic acid (0.128- 0.651 mg/100g) was recorded the highest at stage II and it indicates involvement of lipids to metabolic changes during development. The linoleic acid has nutritional benefits due to its metabolism at tissue levels which produces hormone like compound prostaglandins.[56] The role of α-linolenic acid has been reported in disease prevention.[57] The early rise in linoleic acid appeared to be associated with glycolipid and is a unique component in chloroplast of young plantains. Presence of these unsaturated fatty acids in a reasonable amount enhances nutritional value of this crop.

2.3.8 Amino acids composition

Results (Table 2.8) revealed that all the essential amino acids viz., histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are present in the culinary banana. Amino acids content decreased from stage I to stage V of growth and development (Table 8) of culinary banana. The predominant amino acids are glutamic acid (0.75-3.12 g/100g), aspartic acid (0.05-1.08 g/100g) and alanine (0.19-0.45 g/100g) and declined with maturity. Deka and Harmine[58] also reported the presence of all the essential and non-essential, amino acids in cultivar Borjahaji (AAA) banana. The limiting amino acid methionine (0.017-0.048 g/100g) was recorded in fewer amounts as compared to other amino acids. In plants ethylene plays a key role in fruit ripening and it is synthesized from methionine.[18] There is a marked variation in most of the amino acid contents among all stages except for proline, leucine, tyrosine and phenylalanine. Decrease in amino acids level with advancement in maturity has been previously reported in tomato.[59]

Table 2.6 Effect of different stages of development on mineral contents of culinary banana (mg/100g)

Stage	Na	K	Fe	Cu	Mn	Zn	Mg	Ca
I	0.115±0.87[a]	0.889±0.75[c]	0.302±0.91[a]	0.008±0.01[a]	0.005±0.27[a]	0.350±0.04[b]	0.961±0.65[a]	0.432±3.11[d]
II	0.127±1.34[b]	1.038±0.98[d]	0.385±1.71[e]	0.009±0.31[e]	0.013±0.66[c]	0.389±1.09[d]	1.020±1.71[c]	0.542±4.92[e]
III	0.139±1.28[c]	1.273±0.97[e]	0.210±0.46[b]	0.005±0.03[d]	0.014±0.16[d]	0.353±1.50[c]	1.183±0.37[e]	0.345±6.56[c]
IV	0.167±0.99[e]	0.723±0.96[b]	0.240±1.25[c]	0.004±0.03[c]	0.017±0.32[e]	0.417±1.28[e]	1.109±0.19[d]	0.274±2.03[b]
V	0.164±1.29[d]	0.498±0.98[a]	0.173±1.05[a]	0.002±0.06[b]	0.011±0.89[b]	0.312±1.75[a]	1.024±0.96[b]	0.241±3.67[a]

[a]Mean in each column followed by the same letters are not significantly different at p>0.05; values represent mean±SD, n=3

Table 2.7 Effect of different stages of development on fatty acid profile of culinary banana (mg/100g)

Stage	Lauric acid	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
I	0.013±1.54 [a]	0.019±0.97[b]	1.678±1.52[e]	0.059±1.22[b]	0.091±0.82[c]	0.128±0.51[a]	2.081±0.49[e]	1.210±0.91[d]
II	0.006±1.01[b]	0.008±0.50[a]	0.988±1.84[d]	0.027±0.53[a]	0.061±0.81[a]	0.651±0.11[c]	1.386±0.39[d]	0.670±0.66[c]
III	0.092±1.04[c]	0.013±1.41[c]	0.593±1.32[c]	ND	0.068±1.18[b]	0.524±0.76[b]	0.387±0.05[b]	0.139±0.16[a]
IV	ND [1]	ND	0.422±0.37[b]	ND	ND	ND	0.603±0.58[c]	0.213±0.30[b]
V	ND	ND	0.267±0.75[a]	ND	ND	ND	0.329±0.76[a]	ND

[a]Mean in columns followed by the same letters are not significantly different at p>0.05; values represent mean±SD, n=3;

[1] ND = Not detected.

Table 2.8 Effect of different stages of development on amino acid composition of culinary banana (g/100g protein)

Stage	Tryptophan	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Cystine	Valine	Methionine	IsoleuCine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine
I	0.16± 0.04[b]	1.08± 0.01[d]	0.29± 0.02[e]	0.43± 0.61[d]	3.12± 0.31[e]	0.20 ± 0.03[c]	0.37± 0.55[d]	0.45± 0.06[e]	0.03± 0.41[b]	0.42± 0.07[d]	0.047± 0.01[c]	0.30± 0.51[d]	0.58± 0.99[b]	0.14± 0.03[c]	0.36± 0.05[b]	0.31± 0.05[d]	0.35± 0.08[a]	0.45± 0.90[e]
II	0. 17± 0.22[b]	1.02± 0.09[c]	0.26± 0.04[c]	0.38± 0.70[c]	3.01± 0.06[d]	0.17 ± 0.03[b]	0.33± 0.62[c]	0.41± 0.05[c]	0.04± 0.11[c]	0.38± 0.07[c]	0.048± 0.02[c]	0.27± 0.55[b]	0.62± 0.67[c]	0.13± 0.02[b]	0.41± 0.03[c]	0.24± 0.05[c]	0.47± 0.04[c]	0.40± 0.75[d]
III	0.19± 0.02[c]	0.73± 0.012[b]	0.28± 0.04[d]	0.46± 0.43[e]	1.95± 0.33[c]	0.12 ± 0.04[a]	0.31± 0.46[b]	0.44± 0.06[d]	0.02± 0.15[a]	0.43± 0.04[e]	0.035± 0.03[b]	0.29± 0.95[c]	0.69± 0.58[e]	0.13± 0.02[b]	0.42± 0.04[d]	0.19± 0.03[b]	0.56± 0.05[d]	0.35± 0.55[b]
IV	0.31± 0.65[d]	0.05± 0.02[a]	0.23± 0.02[b]	0.27± 0.25[b]	1.22± 0.95[b]	0.13 ± 0.45[a]	0.30± 0.41[a]	0.32± 0.06[b]	0.03± 0.62[b]	0.34± 0.06[b]	0.021± 0.01[a]	0.23± 0.49[a]	0.65± 0.86[d]	0.09± 0.02[a]	0.43± 0.05[e]	0.12± 0.02[a]	0.63± 0.05[e]	0.38± 0.68[c]
V	0.10± 0.28[a]	N.D	0.15± 0.01[a]	0.20±0. 24[a]	0.75± 0.03[a]	N.D	N.D	0.19± 0.11[a]	N.D	0.16± 0.02[a]	0.017± 0.01[a]	N.D	0.44± 0.52[a]	N.D	0.32± 0.06a	N.D	0.41± 0.08[b]	0.27± 0.75[a]

[a]Mean in columns followed by the same letters are not significantly different at p>0.05; values represent mean±SD, n=3.

2.3.9 Estimation of polyphenols by HPLC

Polyphenols are secondary metabolites of plants, and they are most abundant source of antioxidant present in foods. Generally, they are involved in protection against ultraviolet radiation or aggression by pathogens. In food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability.[60] The various polyphenols present in culinary banana samples at five different stages of growth and development has been evaluated using HPLC and the results are illustrated in Fig. 2.2a to 2.2e. The polyphenols were abundant during early ripening stages which further declines with advancement of growth. The major polyphenols recorded in all the five stages were hydroquinone (27.23-70.22 ppm), catechol (12.15-22.57 ppm), chlorogenic acid (31.52-91.64 ppm), caffeic acid (19.68-47.57 ppm), p-coumeric acid (8.21-71.17 ppm), quinic acid (84.8-228.25ppm) and apigenin (48.76-70.35 ppm). In addition, a small amount of ferulic acid of 21.43 ppm and salicylic acid of 22.36 ppm were observed at stage II and stage V respectively. From the study, it is prudent to say that culinary banana is an excellent source of polyphenols and the statement made by Kanazawa and Sakakibara[50] that banana are considered among one of the antioxidant food has been proved in our study. Among all the polyphenols detected, quinic acid was in major amount, thus, it may help in metabolism of tryptophan as antioxidant. The decrease in polyphenols with respect to growth significantly reduced the bitterness and astringent taste of fruit. The decreasing trend of polyphenols with increase in fruit development has also been reported on different fruits like eggplant, blueberry, maqui etc.[61, 62]

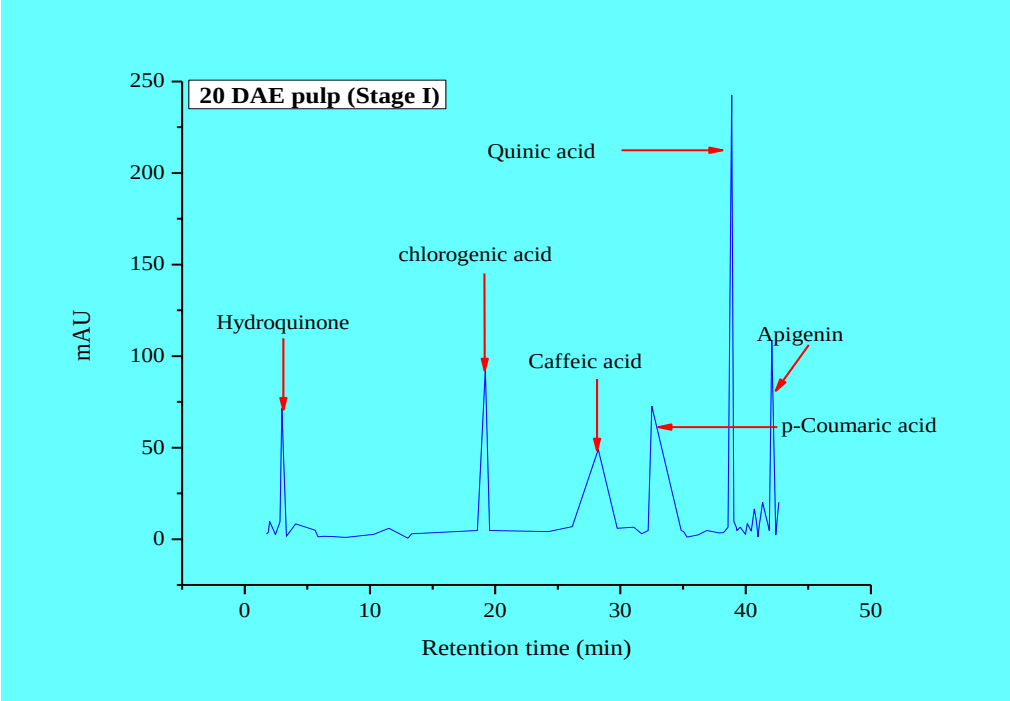

Fig. 2.2a HPLC chromatograms of polyphenols in 20 DAE (stage I) matured culinary banana pulp

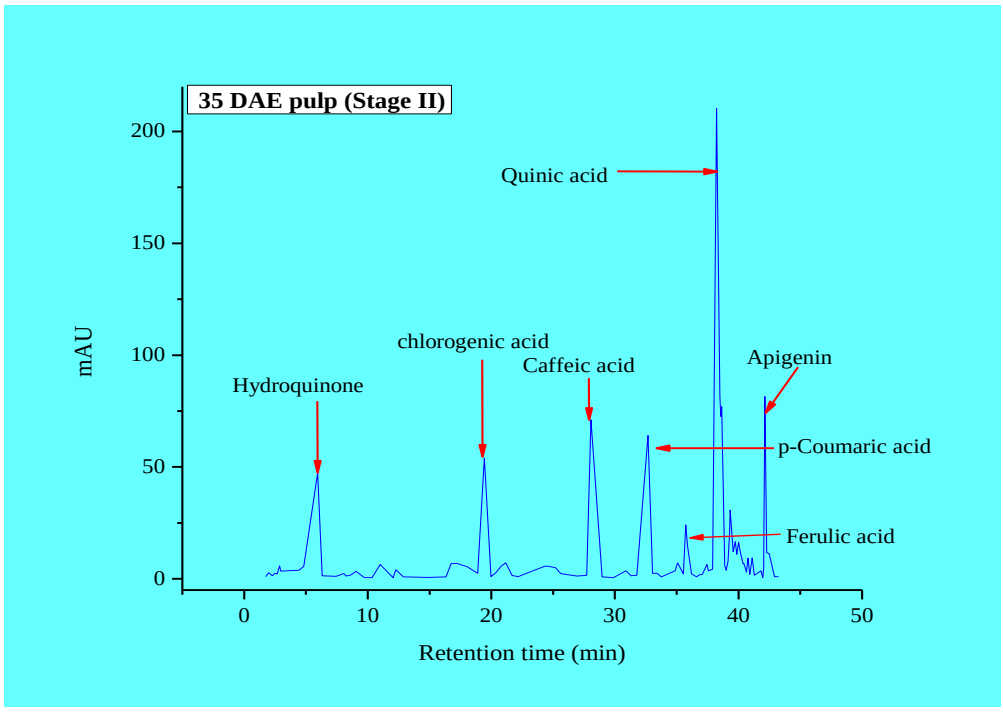

Fig. 2.2b HPLC chromatograms of polyphenols in 35 DAE (stage II) matured culinary banana pulp

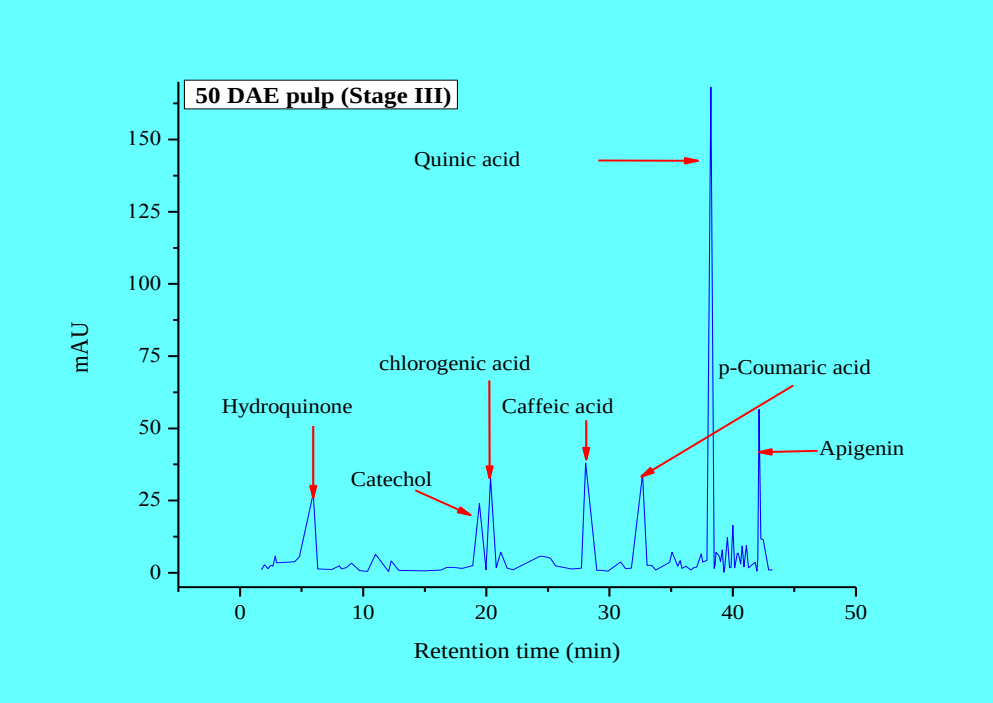

Fig. 2.2c HPLC chromatograms of polyphenols in 50 DAE (stage III) matured culinary banana pulp

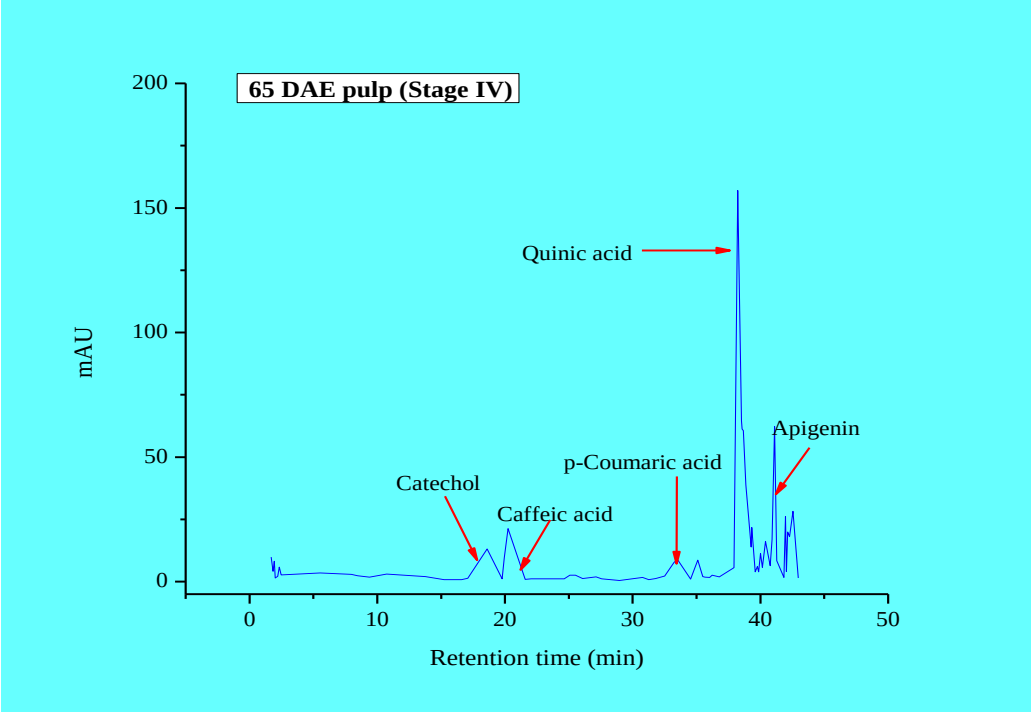

Fig. 2.2d HPLC chromatograms of polyphenols in 65 DAE (stage IV) matured culinary banana pulp

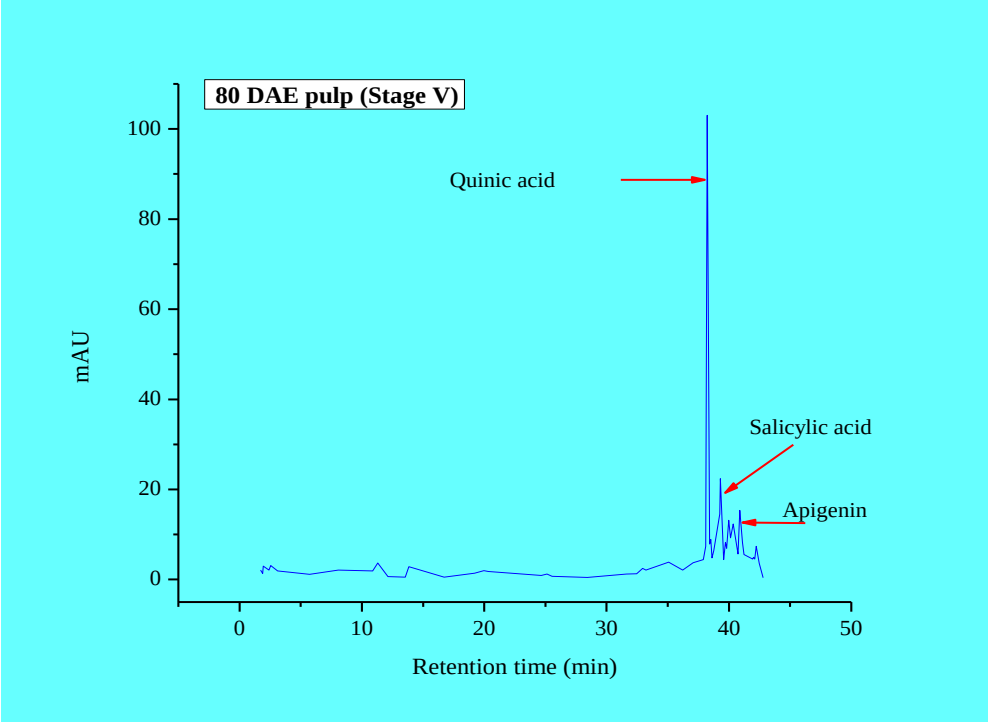

Fig. 2.2e HPLC chromatograms of polyphenols in 80 DAE (stage V) matured culinary banana pulp

2.3.10 Colour measurement

The degree of lightness and yellowness increased with maturity but the degree of redness followed a decreasing trend (Table 2.9). The increase in degree of lightness with maturity might be attributed to reduction in browning potential with growth. Change in colour of the culinary banana is the major physical and chemical changes with the approach of maturation. This could be attributed to degradation of chlorophyll coupled with synthesis of other plant pigments usually carotenoids and anthocyanin. Therefore an increase in yellowness in fully matured culinary banana is associated with increase in total carotenoid content. Several studies have revealed that change in fruit colour is an important indicator to identify stage of crop maturity physically.[63]

Table 2.9 Effect of different stages of development on colour of culinary banana

Stage	L*	a*	b*
I	47.04±1.24a [a]	2.87±0.04[e]	6.49±0.05[a]
II	60.62±1.12[b]	2.19±0.03[d]	7.79±0.12[b]
III	70.31±1.35[d]	2.04±0.03[c]	8.29±0.08[c]
IV	69.06±2.28[c]	1.52±0.02[b]	9.26±0.52[e]
V	72.76±1.97[e]	1.37±0.07[a]	8.77±0.75[d]

[a]Mean in columns followed by the same letters are not significantly different at p>0.05; values represent mean±SD, n=3

2.3.11 Optimum stage of harvesting

Pulp to peel ratio coupled with total soluble sugars are the most important parameters for harvesting. It increases with more maturity/ripeness and indicates differential changes in moisture content of peel and pulp.[35] With increase in pulp to peel ratio or in other words the more is the maturity the more will be the total soluble sugars which is unsuitable for culinary purpose. In the present study, 50 DAE (stage III) is considered to be the optimum stage of harvesting (Table 2.10) because from this stage onwards there was more breakdown of starch and in turn more total soluble sugars were obtained and thereafter sharp increase in total soluble sugars at stage IV and V compared to the earlier stages. Therefore from culinary standpoint, 50 DAE (stage III) is the optimum stage for harvesting of culinary banana.

Table 2.10 Pulp to peel ratio vis-a-vis total soluble sugars of culinary banana

Stage	Pulp to peel ratio (g/100g)	Total soluble sugars (g/100g)
I	1.23±0.75[a]	0.64±0.03[a]
II	1.69±0.94[b]	0.72±0.05[b]
III	2.01±0.95[c]	1.35±0.03[c]
IV	2.11±0.88[c]	2.01±0.06[d]
V	2.15±0.92[c]	4.65±0.97[e]

[a]Means in columns followed by the same letters are not significantly different at p>0.05; values represent mean±SD, n=3

2.4 Conclusions

The present study reveals that the nutritional compositions are affected by various growth stages of culinary banana. Pulp to peel ratio and total soluble sugars suggest that 50 DAE is the optimum stage of harvesting. The culinary banana has potential applications of developing numbers of value added products. For instance, the antioxidant activity makes it an excellent ingredient for developing products like cookies, biscuits, bread etc. Furthermore, increased accumulation of starch renders mature tissue a potential source for commercial starch extraction and also presence of considerable amount of amylose allows for developing products which can be subjected to high temperature.

B) Nutritional composition of culinary banana peel at different stages of development

2.5 Introduction

The peel of banana constitutes to 40% of the total weight of fresh banana and yet it has been underutilized and discarded as waste.[64] This might be due to the ignorance regarding the benefits of commercial application. Like its pulp flour counterpart, banana peel flour can potentially be used in new products with standardized composition for various industrial and domestic uses.[65] Peels are the major by-products of all fruits and vegetables obtained during processing; however some studies show that these are good sources of polyphenols, carotenoids and other bioactive compounds which in turn possess various beneficial effects on human health.[66] But these wastes are either uneconomically utilized or disposed off as they are, thereby causing serious pollution problems. Of particular interest is the finding that banana peel extract contains higher antioxidant compounds than that of the pulp, thus promising a more intense utilization of the peels in food and nutraceuticals. Potential application of banana peel however depends on its chemical composition as well as physicochemical and functional properties.[65]

Banana peel being a key source of many functionally important bioactive compounds are still underutilized and very little scientific effort has been put to identify its functionality in terms of application to food and nutraceuticals. Banana peel can potentially offer new products with standardized composition for various industrial and domestic uses.[67] As reported by Emaga et al.[3, 65] banana peel is a rich source of dietary fibre, protein, crude fat, lipid, pectin, essential amino acids (leucine, valine, phenylalanine and threonine), polyunsaturated fatty acids mainly (linoleic acid and α-linolenic acid) and micronutrients like potassium, phosphorous, calcium, magnesium etc. Numbers of highly valued products like yellow noodles, dietary fiber concentrates, α-amylase, xylose, vinegar, wine etc had been developed using peel of plantains and banana.[64, 68-71]

Keeping in view the importance of banana peel as an potential source of many functionally important biochemical compounds it would be interesting to understand the detailed changes in biochemical composition with respect to ripening and maturation stages. As the fruit precede its maturation stage the composition of both pulp and peel changes significantly.[65] Therefore looking at the backdrop, the present study was undertaken to evaluate the changes

occurs in biochemical composition with advancement in fruit ripening and maturity. This study will not only help in knowing the composition changes but will certainly help in confirming the best stage for obtaining the particular compound of interest when they are at maximum quantity.

2.6 Material and methods

2.6.1 Sample collection and preparation

The fingers of culinary banana were harvested from experimental plot of Tezpur University at five different growth stages viz. 20, 35, 50, 65 and 80 days after emergence (DAE). The samples were thoroughly cleaned, pat dried with towel and pulp and peel was separated using a stainless steel knife. The peels were then washed with the solution of 0.5 % $MgSO_4$ to remove latex from the cut surfaces[72] followed by rising with deionized water and dried at 50^oC for 24 h. The dried culinary banana peel were ground to obtain the flour and passed through 0.25 mm mesh screen, packed in polyethylene bags and stored at 4^oC for further analysis. The flour samples obtained at different stages of maturity was coded as stage I (20 DAE), stage II (35 DAE), stage III (50 DAE), stage IV (65 DAE) and stage V (80 DAE).

2.6.2 Physiochemical analysis

To examine the variation in chemical composition of culinary banana peel at five stages of maturity; initial moisture content, protein, crude fat, ash and crude fiber were determined following the methods describe in AOAC.[19] The carbohydrate and starch content was estimated following anthrone method.[22]. Amylose contents were determined with the method of Sadasivam and Manikam[21]and the sugar content was quantified by the phenol-sulphuric acid method.[25] The reducing sugar content was estimated by the Nelson-Somogyi method.[26] The amount of non reducing sugars was determined by subtracting the amount of reducing sugars from the amount of total sugars in the sample. Cellulose content was estimated following the method of Sadasivam and Manikam[21] while lignin content was determined following the method of Stafford.[24] Determination of pectin content was done following the method explained by Ranganna.[23] Tannin content was determined by Folin-Denis method.[27] of Schanderi. Total

phenolic content was determined with the Folin-Ciocalteu (F.C.) colorimetric method.[29] of Malick and Singh. Total flavonoid content the extracts were determined according to the colorimetric assay following the procedure of Sultana et al.[73] with some modification. Aqueous extract (1 ml) containing 0.01 g/ ml of dry matter was placed in a 10 ml volumetric flask, and then 5 ml of distilled water was added. At zero time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5 min, 0.6 ml of (10% w/v) $AlCl_3$ was added. After another 5 min, 2 ml of 1M solution of NaOH was added. After that, the volume was made up to 10 ml with distilled water. The mixture was shaken vigorously and the absorbance of the pink color of mixture was read at 510 nm using a UV-visible spectrophotometer (Shimadzu UV-1601PC, Tokyo, Japan). A calibration curve was prepared using a standard solution of gallic acid within the range 20-100 μg/ml concentration and the results were expressed as mg gallic acid equivalents (GAE)/100 g of dry matter. The DPPH radical scavenging activity was measured with the method of Brand-Williams et al.[30]

2.6.2.1 Estimation of minerals, fatty acids, polyphenols and amino acids

The concentration of micronutrients present in culinary banana peel undergoing different stages of ripening was examined with the help of inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 2100 DV, Bridgeport, USA) following the methods described at (section 2.2.2.7). Fatty acid profiling was done by Gas liquid chromatography (GLC) (CP-3800, Varian, USA) following the method described at (section 2.2.2.8). Amino acid analysis was studied by following the method described at (section 2.2.2.9). Polyphenols determination by HPLC was performed following the modified method described at (section 2.2.2.10).

2.6.2.2 Fourier transform infrared spectroscopy (FT-IR)

IR spectra of samples were measured using KBr disk (ultra thin pellets) method. The dry sample was ground and blended with KBr in a ratio of sample/KBr 1:4. The blend was pressed to obtain a pellet and introduced in the spectrometer (Nicolet Instruments 410 FTIR equipped with KBr optics and a DTGS detector, Thermo Scientific, USA). Each spectrum was analyzed in the range of resolution from 400- 4000 cm^{-1} with a resolution of 4 cm^{-1} and total of 64 scans were collected.

2.6.2.3 Microstructure Study by SEM

To study the effect of ripening on microstructure of culinary banana peel a small portion of sample was placed in a metal stub using a two-sided adhesive tape and coated with a fine layer of gold using a sputter gold coater. Sample micrographs were observed at a magnification of 3000X at an accelerating voltage of 15 kV under scanning electron microscope (JEOL JSM-6390LV, SEM, Oxford).

2.6.2.4 X-ray Diffraction (XRD)

The powder X-ray diffraction patterns were measured using a Rigaku Miniflex (Japan) instrument at room temperature using CuKα radiation (λ = 0.15418 nm) over the 2θ range of 5 and 50° with a scanning speed of 1.2^{o}/min. The samples were vacuum dried at 60 ^{o}C before the

2.6.3 Statistical Analysis

Experiments were carried out in 3 replicates. The data analysis tool 'Microsoft Excel' was used for statistical analysis. Data were subjected to ANOVA and Fisher's Least Significant Difference (LSD) was used to separate means.

2.7 Results and Discussion

2.7.1 Physicochemical analysis

2.7.1.1 Proximate composition

The proximate composition of culinary banana peel at five different stages of ripening are presented at Table 2.11. Culinary banana peel at younger stage holds more moisture compared to the matured stage. The moisture content at stage I was 62.98% which increased to 65.43% at stage II and from stage III onwards the moisture content appear to be decreasing from 60.65% (stage III) to 57.06 % at stage IV. There was a significant difference in the moisture content in all stages except stage III and IV (58.40%). From the results of decreasing moisture content with

respect to maturity it clearly illustrate that the dry matter content of peel is increasing with advancement of maturity. In case of culinary banana pulp study the moisture content was observed to be increasing with stages of development which is opposite in the peel, the plausible reason behind this may be the moisture from peel is transferring to the pulp as a result decreasing trend in moisture content with maturity is witness in the present study. Furthermore, few authors have also stated that carbohydrates are utilized during breathing and osmotic transfer from the peel to pulp hence water content of pulp is increased which caused variation in osmotic pressure between peel and pulp.[74] The finding of Emaga et al.[65] supports our result as authors have reported the similar finding in case of different varieties of banana peel.

The crude protein content of culinary banana peel ranged from 6.12 to 9.87% in all stages with significant difference among them. Similarly, the dry matter content increase there was increase in protein content with respect to maturity. But the protein content in over ripe peel (stage V) is recorded to be decreased from stage IV. Proteins help in regulation of metabolism in living cells and various metabolic processes persist throughout the fruit developments which necessitate specific proteins in suitable amount at precise time hence they are ubiquitous components of all living tissues. During fruit ripening breakdown and synthesis of protein occurs and amino acids are recycled and during the beginning of ripening the actual concentration of protein increases.[75] Increase in crude protein content with increasing maturity has also been reported by Emaga et al.[65] and Adisa and Okay[76] in case of banana peel. A slight decrease in protein content at stage V may be attributed to the proteins being utilized in the gluconeogenesis Goswami and Borthakur.[38]

The crude fat content in the peel of culinary banana is also recorded to be in increasing trend from stage I (1.96%) to stage V (3.94%) and they varied significantly. As the percentage of dry matter increased with respect to maturity, crude fat content also seems to follow the increasing trend.[77] This may be due to the continuous synthesis of fatty acids during metabolism the lipid content also varies drastically. The increasing trend in crude fat concentration with fruit development has been reported by various authors in case of different fruits which support current finding of Siddika et al.[78] The amount of ash content in peel of culinary banana varied with growth and maturity which increased from stage I (6.24%) to stage IV (9.15%) and decreased slightly at stage V (8.42%). The variation in the ash content did not vary much among the stages of ripening. There was a significant difference among stage I, II and IV but among

stage II, III and V ash content did not vary. Ash content which is generally an inorganic material is directly or indirectly associated with the absorption capacity of mineral salts at different developmental stages. The reported value range of ash content in present study is comparatively less than the values reported (12.8%) by Emaga et al.[65] this may be correlate to the absorption of mineral salt by plant and soil condition of North East India necessary for optimum assimilation of various nutrient ions.

Crude fiber content of culinary banana peel increased as maturity proceeds with the range varied from 15.97% at stage I to 25.65% stage IV which again dropped slightly to 21.23% at over ripe stage V. The values of crude fiber found in the present study is in higher side which suggest that culinary banana peel can be a good source of fiber and can help in treating digestion problem like constipation and improve general health and well being.[55] Apart from this culinary banana peel can also be a potential source for making poultry and cattle feed as its and excellent source of fiber.[79] The increase in fiber content at matured stage over tender stage might be due to increase in soluble and insoluble dietary fractions. Hence culinary banana peel may also be added to the diet which is resistant to enzymatic digestion.[80] Banana peel is an excellent source of carbohydrate with its content varied from 23.30 to 37.07% (stage I to V). The highest amount was recorded at stage III which thereafter noticed decreasing with maturity. This variation might be due to degradation of starch for synthesis of sugar at different developmental stages.[36] The carbohydrate content recorded at present study is comparatively lesser as compared to the report of Anhwange et al.[55] as authors have reported 59% carbohydrates to be present in *Musa sapientunm* peel. This may be because the variety used in the present study is culinary *Musa* ABB.

Table 2.11 Proximate composition at various stages of development of culinary banana peel

Stages	Moisture content (% wb)	Protein (% db)	Crude fat (% db)	Ash (% db)	Crude fiber (% db)	Carbohydrate (% db)
I	62.98±0.52[c]	6.12±0.96[a]	1.96±0.77[a]	6.24±0.11[a]	15.97±0.12[a]	23.30±0.01[a]
II	65.43±0.71[d]	7.86±0.45[c]	2.76±0.53[b]	8.36±0.07[b]	18.44±0.99[b]	27.97±0.05[b]
III	60.65±0.66[b]	9.61±0.25[d]	3.17±0.02[c]	8.86±0.12[b]	22.76±0.11[d]	37.07±0.12[d]
IV	57.06±0.76[a]	9.87±0.44[d]	3.56±0.73[d]	9.44±0.05[c]	25.65±0.19[e]	35.19±0.09[e]
V	58.40±1.43[a]	7.03±0.76[b]	3.94±0.45[e]	8.72±0.07[b]	21.23±0.89[c]	32.43±0.03[c]

[a]Means in columns followed by the same letters are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.7.1.2 Starch, amylose and sugar content

Ripening stages significantly affected starch and amylose content in culinary banana peel (Table 2.12). The starch content varied in the range 4.84 to 15.88% while amylose content was observed in the range 0.89-3.97% in all stages of ripening. Both starch and amylose was in highest amount at stage III and further decreased at stage V with minimum amount of 4.84% starch and 0.89% amylose. Decreasing trend of starch with advancement in maturity has also been reported by Emaga et al.[65] in case of banana peel. The reducing trend in starch content with maturity may correlate to the accumulation of carbohydrate during maturation which causes hydrolysis of starch and sugar storage during maturation. Various enzymes involved in the starch degradation during ripening are amylase, glycosidase, phosphorylase, invertase and sucrose synthase etc which further causes accumulation and formation of soluble sugars.[81] As reported by James et al.[82] amylose consists of 20-30% of native starch present in fruit which supports our finding.

During maturation the sugar contents of culinary banana gradually increased as shown in Table 2.12. Total soluble sugar (TSS) increased from 1.76-4.09%, reducing sugar (0.66-2.09%) and non reducing sugar (1.32-3.63 %) from stage I to stage V. The increase in sugar content evinced the degradation of starch to sugar with maturity. In case of TSS and reducing sugar there was no significant difference observed between stage I and II but thereafter they varied, whereas non reducing sugar varied with respect to maturity. According to the reports of Emaga et al.[65] the

major TSS found in peel of banana are mainly glucose and fructose with little amount of sucrose. In the present study the amount of non reducing sugar studied was higher than the reducing sugar, therefore it can be concluded that culinary banana peel may be a good source of fructose than glucose as fructose is a non reducing sugar. The increasing trend of all sugars with advancement in ripening is in agreement with the report of Adisa and Okey[76]

Table 2.12 Starch, amylose and sugar content (%dry weight basis) at different stages of development of culinary banana peel

Stages	Starch	Amylose	TSS	Reducing sugars	Non reducing sugars
I	6.34 ± 0.9^{b}	1.51 ± 0.01^{b}	1.76 ± 0.02^{a}	0.66 ± 0.97^{ab}	1.32 ± 0.01^{a}
II	8.97 ± 0.54^{c}	2.24 ± 0.09^{c}	2.91 ± 0.09^{a}	0.95 ± 0.06^{a}	2.22 ± 0.13^{b}
III	15.88 ± 0.66^{e}	3.97 ± 0.07^{e}	3.32 ± 0.07^{b}	1.06 ± 0.09^{ab}	2.83 ± 0.18^{c}
IV	10.65 ± 0.05^{d}	2.44 ± 0.54^{d}	3.88 ± 0.01^{c}	1.76 ± 0.45^{c}	3.02 ± 0.32^{c}
V	4.87 ± 0.16^{a}	0.89 ± 0.05^{a}	4.09 ± 0.01^{d}	2.09 ± 0.12^{d}	3.63 ± 0.08^{d}

[a]Means in columns followed by the same letters are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.7.1.3 Cellulose, lignin, hemicelluloses, pectin and tannin content

The culinary banana peel is a rich source of cellulose (Table 2.13) which varied significantly during early ripening stages but towards ripening the cellulose remain almost constant with no significant difference among the stages. The lowest amount of cellulose was recorded at stage I (6.54%) and the highest was at stage IV (16.85%). Cellulose a homopolymer of glucose is most abundant source of polymers and from our results it is confirmed that culinary banana peel can be potentially used in bio polymers. Our results are in line with the values of cellulose content in plantain peels at different ripening stages reported by Emaga et al.[83] Lignin the second most abundant polymer after cellulose is an important constituent of plant provides mechanical support to the cell wall. From the result displayed in Table 2.13 it is seen that lignin varied significantly from 2.87-4.97% among the stages studied. With increase in ripening stages amount of lignin content also gradually increased. This increasing trend of lignin may correlate

to the lignifications of cell wall constituents which result in an increase in other dietary fiber fractions.[44]

The culinary banana peel contains hemicelluloses in the range of 2.98-5.90% from stage I to stage V (Table 2.13). Hemicelluloses are heteropolymer of polysaccharides plays an important biological role in strengthening the cell wall by interacting with cellulose and lignin.[84] It was observed after stage III the hemicelluloses started decreasing and there was no significant difference between stage IV and V. This fluctuation may be because of the cell biosynthesis with respect to ripening and during early stages of fruit development the cell expansion and elongation occurs. Growth of any fruits and vegetables begins with cell wall loosening consist of a cellulose scaffold embedded in a matrix of polysaccharides classified as pectins and hemicelluloses. The growing cell wall is dynamically modified by enzymes that change the structure of pectins and hemicelluloses, thereby altering their interactions with each other and with cellulose. Growth cessation is correlated with reduced expression of genes that promote wall loosening and changes in matrix polysaccharides that lead to a less extensible cell wall.[85] Pectin the heteropolysaccharide found in the primary cell wall is a soluble dietary fiber and they are widely used in the food, pharmaceutical and cosmetic industries.[86] The amount of pectin content in culinary banana peel did not vary much and their amount was limited in the range of 1.23-3.52% in all stages (Table 2.13). The gradual increase in pectin content up to stage IV might be due to less interaction between the pectin and the other cellular components and as a consequence the pectin was more available for extraction. On the other hand, decrease at stage V might be due to the degradation of pectin under the action of pecticenzymes, such as polygalacturonase (PG), pectin methyl esterase (PME) or pectatelyase (PL). The increase of pectin content up to a certain stage and then decrease was also observed by Lohani et al.[47]

An astringent and bitter plant poylphenolic compound that binds and precipitate proteins and other organic compounds including amino acids and alkaloids are known as tannins. The result displayed in Table 2.13 shows that culinary banana peel had this tannin maximum during early developmental stage. It varied from 4.46% (minimum at over ripe stage V) to 6.76% (maximum at stage II). The decrease in tannin content with advancement of growth reduces the astringency property. The astringency property gets reduced as culinary banana attains maturity, the astringency property which is related to insolubilization and polymerization of polyphenols with other constituents of pulp. The tannins content of the peel which act against the availability

of proteins in the rumen decreases with ripening as a consequence of a migration of the polyphenols from the peel towards the pulp and the phenolic oxidative degradation by polyphenol oxidases and peroxidases.[87]

Table 2.13 Cellulose, lignin, hemicelluloses, pectin and tannin content of culinary banana peel (% dry weight basis)

Stages	Cellulose	Lignin	Hemicellulose	Pectin	Tannin
I	6.54±1.06[a]	2.87±0.01[a]	2.98±0.43[a]	1.23±0.65[a]	4.55±0.25[b]
II	9.65±1.32[b]	3.61±0.04[b]	3.47±0.48[b]	1.87±0.43[a]	6.76±0.08[e]
III	15.88±1.22[c]	4.06±0.76[c]	5.90±0.82[d]	2.34±0.87[b]	5.34±0.06[d]
IV	16.85±1.98[c]	4.97±0.08[d]	4.47±0.74[c]	3.43±0.96[c]	5.01±0.95[c]
V	15.43±1.45[c]	3.24±0.77[b]	4.02±0.99[c]	3.52±0.28[c]	4.46±0.18[a]

[a]Means in columns followed by the same letters are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.7.1.4 Total polyphenols, flavonoids and antioxidant activity

Culinary banana peel is an absolute source of polyphenols, the secondary metabolites generally involved in defense against radiation or aggression by pathogens. The polyphenols content in peel was higher when fruit was young and it observed to be decreased with ripening (Table 2.14). They varied significantly in the range of 590.18-1567.84 mg GAE/100g dry matter from stage I to stage V, the highest being observed at stage I and the lowest at stage V. In food polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability[60] and they are important group of antioxidant having ability to absorb free radicals.[88] The plausible explanation behind this variation has been explained by Kiyoshi and Wahachiro[89] that during early ripening stage 60% of polyphenolic compounds have molecular weight above $2x10^5$. With advancement in ripening this 60% polyphenols with higher molecular weight above $2x10^5$ disappears slowly resulting decrease in astringent property. On further ripening only those 40% polyphenols with molecular weight below $2x10^5$ remains and likely the polyphenols content decreased. Our result totally agrees the above statement as we have found similar results. The decreasing trend of polyphenols in banana peel with growth is also reported by Sundaram et al.[90]

Flavonoids, the most potent antioxidative compounds of plant phenolics occurred potentially during early stages of culinary banana peel development (Table 2.14). The maximum amount was recorded at stage I (1137.26 mg catechin/100g dry matter) which gradually decreased with minimum value at over ripe stage V (835.10 mg catechin/100g dry matter). Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups.[91] As maturity progressed biosynthesis of flavonioids occurs which is regulated by coordinated transcriptional control of the enzymes resulting in decrease level of flavonoid with respect to maturity.[92]

Maturity significantly affected the radical scavenging activity of culinary banana peel (Table 2.14). Scavenging activity of peel studied against different concentration of DPPH revealed that compared to pulp peel contains higher percentage scavenging activity. The highest percentage inhibition (up to 97.31%) was showed by peel at early developmental stage I and minimum was noticed at stage V (up to 79.32%). Generally when fruits attain maturity the scavenging activity of fruits found to be decreasing which may be because of fruit ripening is an oxidative phenomena.[93] As reported by Jimenez et al.[94] during ripening the formation of oxygen and accumulation of hydrogen peroxide is more which increase lipid peroxidation and protein oxidation. When fruit attains ripening the active oxygen species (AOS) promote the oxidation which causes the general deterioration of cellular metabolism.[95]

Table 2.14 Total polyphenols, flavonoids and scavenging activity of culinary banana peel

Stages	TPC (mg GAE/100g dry matter	TFC (mg catechin/100g dry matter	DPPH radical scavenging activity (% SA)
I	1567.84 ±2.65^{d}	1137.26±3.44^{e}	97.31±0.99^{e}
II	1184.07±5.98^{e}	1076.28±3.76^{d}	90.54±0.78^{d}
III	1062.56±4.78^{c}	987.87±5.67^{c}	89.72±0.64^{c}
IV	834.61±4.98^{b}	902.65±3.30^{b}	85.25±0.88^{b}
V	590.18±3.65^{a}	835.10±4.59^{a}	79.32±0.09^{a}

^{a}Means in columns followed by the same letters are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.7.1.5 Minerals content

The minerals content of peel presented in Table 2.15 reveals that culinary banana peel is a potential source of micronutrients. Among all the micronutrients present potassium (K) was in most abundant amount ranging from 35025.81-47869.09 mg/kg with significant variation among the stages. The lowest amount was being recorded at over ripe stage V and highest being at stage III. Studies reported by John and Marchal[15] and Emaga et al.[65] also reported that K content in banana peel was highest among other micronutirents. Following K, the second highest amount of micronutrient found was phosphorus (P) which varied significantly in the range of 2831.58 - 4321.88 mg/kg . The highest amount was recorded at stage II which decreased with maturity. Magnesium (Mg) was also recorded in pretty good amount ranged from 1381.23 to 2140.38 mg/kg with significant variation among the stages. The highest amount of Mg was recorded at stage III which slowly decreased with advancement in fruit growth. Insertion of Mg into the porphyrin structure is the first step of chlorophyll biosynthesis[96] as Mg is the central atom of the chlorophyll molecule, and iron and copper, functioning in chlorophyll synthesis. The sodium (Na) content varied significantly from 365.23-653.85 mg/kg in all stages of development. The iron (Fe) content was recorded in between 18.69-30.32 mg/kg and highest was at stage IV. Similarly, zinc (Zn) was in the range of 16.47-22.93 mg/kg with highest value at stage IV. The trace elements like Manganese (Mn) and cupper (Cu) was found in least amounts among all other minerals studied. The maximum amount of Mn (15.96 mg/kg) and Cu (1.14 mg/kg) was at stage IV. K, Mg, Ca, P, Fe, Zn concentrations increased with advancement of fruit development and decreased at over ripe stage. The drop in Mg and Ca content of cell walls at over ripe stage may be because of binding of Ca in the tissue (softening of tissue) just before ripening.[96] The large variation in all micronutrients observed during fruit development is likely to be because of preferential absorbance and this might be due to cultivar and/or soil, climate, agricultural practice and the quality of water for irrigation.[53] Most of the minerals are very crucial in many enzymes activities, protecting cells from free radicals attack, regulation of glucose homeostasis etc.[55] (Anhwange, 2008). From the results of present study culinary banana peel contains higher amount of mineral salts comparing to the fruit pulp. Hence, culinary banana peel could be a good feed material for cattle and poultry.[97]

Table 2.15 Minerals in culinary banana peel (mg/kg)

Stages	Na	Mg	K	Ca	P	Mn	Fe	Cu	Zn
I	365.23±2.46[a]	1761.34±3.05[c]	38469.75±6.78[b]	1581.03±3.97[b]	3768.51±3.45[c]	8.09±0.01[a]	18.69±0.91[a]	2.02±0.01[e]	18.55±0.02[c]
II	512.08±2.68[c]	1964.87±3.76[d]	44032.61±7.88[d]	1727.44±4.82[c]	4321.88±5.64[e]	9.32±0.01[b]	24.39±0.03[b]	1.08±0.03[c]	17.76±0.03[b]
III	603.63±3.55[d]	2140.38±3.98[e]	47869.09±7.21[e]	2068.56±4.56[d]	3892.73±4.87[d]	11.61±0.04[c]	28.02±0.11[d]	0.79±0.01[b]	19.38±0.06[d]
IV	653.85±5.23[e]	1705.54±3.01[b]	43283.75±6.34[b]	2189.67±3.58[e]	3382.89±6.72[b]	15.96±0.21[d]	30.32±0.02[e]	1.14±0.04[d]	22.93±0.01[e]
V	431.90±1.91[b]	1381.23±3.68[a]	35025.81±7.29[a]	1429.30±3.44[a]	2831.58±5.33[a]	8.58±0.07[a]	27.28±0.27[c]	0.07±0.01[a]	16.47±0.02[a]

[a]Means in columns followed by the same letters are not significantly different at $p>0.05$; values represent mean±SD, n=3

2.7.1.6 Fatty acids and amino acid composition

Table 2.16 represents the results of fatty acid compositions of culinary banana peel which varied significantly with its development. Both saturated (lauric, myristic, palmitic, stearic) and unsaturated (oleic, linoleic, linolenic) fatty acids were predominantly occurred during early developmental stages. Lauric acid (3839.64-8134.32 mg/kg) and mysteric acid (1034.63-2463.97 mg/kg) were most abundant during early developmental stage. While palmitic (27349.21-35630.63 mg/kg) and stearic (26334.97-37763.74 mg/kg) first increased with maturity and then gradually decreased with advancement in growth. Oleic (47890.19-78687.25 mg/kg), linoleic (20076.48-30904.58 mg/kg), linolenic (19634.05-27385.26 mg/kg) acids were recorded maximum at stage II of fruit development and gradually decreased with ripening. The unsaturated fatty acids were specially made up of polysaturated fatty acids and they possess great nutritional importance as they cannot be synthesized by animals and have to obtain from plants by diet. The linoleic acid has nutritional benefits due to its metabolism at tissue levels which produces hormone like compound prostaglandins.[56] The role of α-linolenic acid has been reported in disease prevention.[57] It is well known that fatty acid profiles determine the quality of oil in terms of nutritional value thus from our results we can conclude that this locally important agro waste banana peel oil is rich in polyunsaturated essential fatty acids having high nutritional importance.

Amino acids composition of culinary banana peel at different stages of fruit development is presented in Table 2.17. Culinary banana peel may be considered as an outstanding source of amino acids as most of the 18 amino acids studied were present at various developmental stages. Most of the amino acids were observed to be in maximum quantity at stage IV and decreased at over ripe stage. Tryptophan (0.46-1.32g/100 protein), valine (0.03- 0.28 g/100 protein), leucine (0.01-0.27 g/100g protein) phenylalanine (0.02-0.13 g/100g protein) histidine (0.71-1.99 g/100g protein) and argenine (0.06-0.25 g/100g protein) were major essential amino acids present in throughout growth while methionine (0.08- 0.16 g/100 g protein), isoleucine (0.12-0.17 g/100 g protein), threonine (0.02-0.06 g/100 g protein), lysine (0.17- 0.26 g/100 g protein) were recorded in trace amounts. Other nonessential amino acids found were aspartic acid (0.32-0.64 g/100 g protein), threonine (0.68-1.05 g/100 g protein), glutamic acid (0.32- 0.63 g/100 g protein), proline (0.54-1.06 g/100 g protein), glycine (0.21- 0.68 g/100 g protein), alanine (0.07- 0.24

g/100 g protein), cystine (0.01- 0.08 g/100 g protein) which varied with growth and fruit development. The amount of amino acids recorded in culinary banana peel is significantly higher than those in fruit.

2.7.1.6 Polyphenols analysis by HPLC

The major polyphenols found in culinary banana peel at different developmental stage was analyzed and presented in Fig. (2.3a-2.3e). Towards the early development stage most of the polyphenols were found in abundant amount and with advancement in growth the amount of polyphenols gradually decreased and at over ripe stage V most of the polyphenols studied were not present. Analysis of phenolic compounds revealed that hydroquinone, catechol, ferulic acid, quinic acid, salicylic acid, quercetin and apigenin were present throughout the fruit growth while polyphenols like resicerol, chlorogenic acid, p-coumaric acid and naringin were found in trace amount during early stage and slowly disappear with fruit growth. From the present study it can be concluded that culinary banana peel has high antioxidant capacity attributed to high polyphenolic compounds. Polyphenols are considered as significant sources of health promoting bioactives in human diet.[62] The composition of polyphenols which varied with fruit development is contributed to properties such as astringency and colour of fruit. The decreasing trend of polyphenols with increase in fruit development has also been reported on different fruits like eggplant, blueberry, maqui etc.[61, 62]

Table 2.16 Fatty acids in culinary banana peel (mg/kg)

Stages	Lauric Acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
I	8134.32±11.87^{e}	2463.97±7.67^{d}	32689.77±4.57^{c}	29643.33±6.29^{c}	53497.11±8.67^{b}	20786.06±10.29^{b}	22887.19±10.23^{d}
II	7057.08±14.63^{d}	1887.09±9.89^{c}	35630.63±7.86^{e}	32869.58±7.65^{d}	78687.25±3.65^{e}	30904.58±13.44^{e}	27385.26±8.59^{e}
III	4369.13±10.28^{c}	1879.27±10.33^{c}	33543.96±7.92^{d}	37763.74±9.43^{e}	60083.78±7.88^{d}	24638.08±12.58^{d}	23493.63±7.82^{c}
IV	4130.77±15.22^{b}	1308.85±8.48^{b}	30287.17±8.88^{b}	28367.08±8.19^{b}	55345.82±9.39^{c}	22563.14±14.65^{c}	20918.96±5.62^{b}
V	3839.64±9.87^{a}	1034.63±11.43^{a}	27349.21±5.72^{a}	26334.97±4.67^{a}	47890.19±6.78^{a}	20076.48±11.37^{a}	19634.05±6.49^{a}

^{a}Means in columns followed by the same letters are not significantly different at p>0.05; values represent mean±SD, n=3

Table 2.17 Effect of maturation on amino acids content in culinary banana peel (g/100g protein)

Stages	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Tyr	Val	Met	Ile	Leu	Trp	Phe	His	Lys	Arg
I	0.33± 0.01^{b}	0.68± 0.04^{a}	0.20± 0.03^{a}	ND	0.54± 0.12^{a}	0.21± 0.15^{a}	0.07± 0.05^{a}	ND	ND	0.08± 0.01^{b}	ND	ND	0.19± 0.02^{b}	1.32± 0.16^{e}	0.01± 0.00^{a}	1.24± 0.06^{e}	ND	0.06± 0.01^{a}
II	0.43± 0.01^{c}	0.83± 0.01^{c}	0.24± 0.01^{a}	0.32± 0.02^{a}	0.77± 0.05^{b}	0.33± 0.02^{b}	0.13± 0.07^{b}	0.02± 0.01^{a}	0.02± 0.01^{a}	0.24± 0.03^{c}	ND	0.12± 0.02^{c}	0.27± 0.02^{d}	0.98± 0.11^{d}	0.02± 0.01^{a}	1.89± 0.08^{c}	ND	0.18± 0.04^{b}
III	0.64± 0.03^{d}	0.95± 0.04^{d}	0.31± 0.04^{c}	0.51± 0.04^{b}	0.83± 0.04^{c}	0.58± 0.06^{c}	0.24± 0.01^{d}	0.08± 0.01^{b}	0.02± 0.01^{a}	0.28± 0.01^{d}	0.08± 0.01^{a}	0.17± 0.01^{b}	0.20± 0.01^{c}	0.73± 0.05^{c}	0.07± 0.01^{b}	1.99± 0.14^{d}	0.17± 0.01^{a}	0.25± 0.05^{c}
IV	0.32± 0.02^{a}	1.05± 0.05^{e}	0.48± 0.07^{d}	0.63± 0.01^{c}	1.06± 0.09^{d}	0.68± 0.14^{d}	0.20± 0.03^{c}	0.04± 0.02^{a}	0.06± 0.02^{b}	0.03± 0.01^{a}	0.13± 0.01^{b}	0.13± 0.03^{a}	0.01± 0.00^{a}	0.46± 0.02^{b}	0.13± 0.02c	0.74± 0.05^{b}	0.26± 0.02^{c}	0.08± 0.02^{a}
V	ND	0.78± 0.03^{b}	0.26± 0.06^{b}	0.52± 0.01^{b}	0.78± 0.26^{b}	0.32± 0.06^{b}	ND	0.01± 0.00^{a}	ND	ND	0.16± 0.02^{c}	ND	ND	0.18± 0.01^{a}	0.08± 0.01^{b}	0.71± 0.03^{a}	0.21± 0.03^{b}	ND

^{a}Means in columns followed by the same letters are not significantly different at p>0.05; values represent mean±SD, n=3

Asp = aspartic acid; Thr = threonine; Ser = serine; Glu = glutamic acid; Pro = proline; Gly = glycine; Ala = alanine; Cys = cysteine; Tyr = tyrosine; Val = valine; Met = methionine; Ile = isoleucine; Leu = leucine; Trp = tryptophan; Phe = phenylalanine; His = histidine; Lys = lysine; Arg = arginine

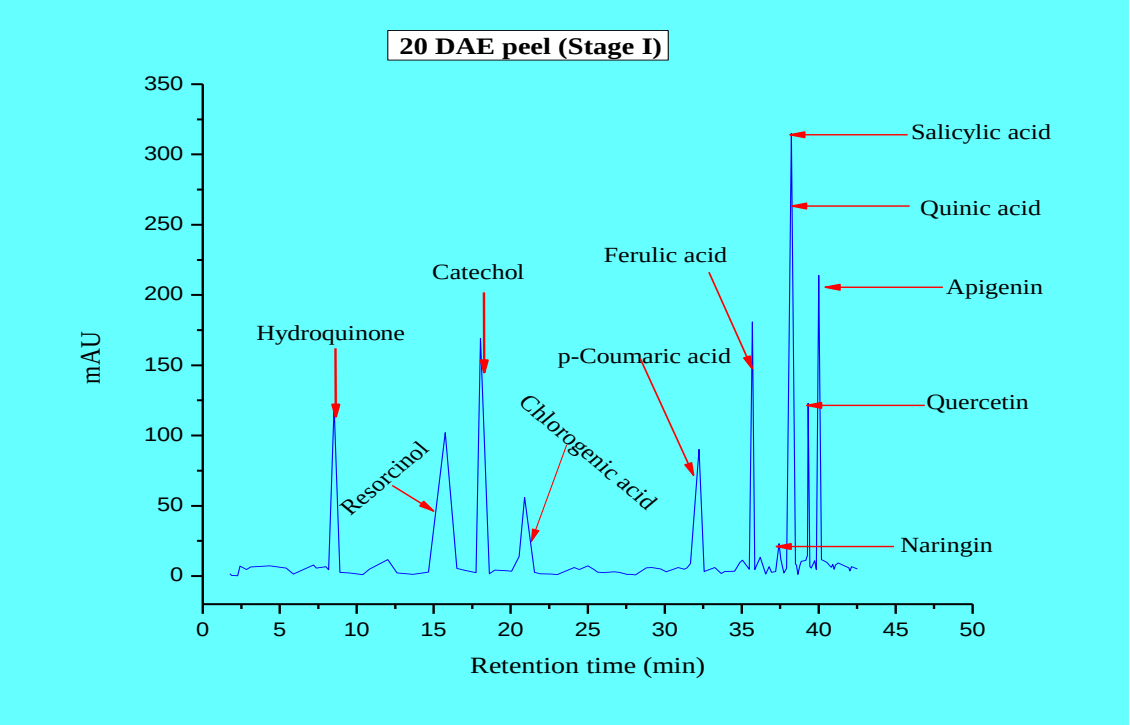

Fig. 2.3a HPLC chromatograms of polyphenols in 20 DAE (stage I) matured culinary banana peel

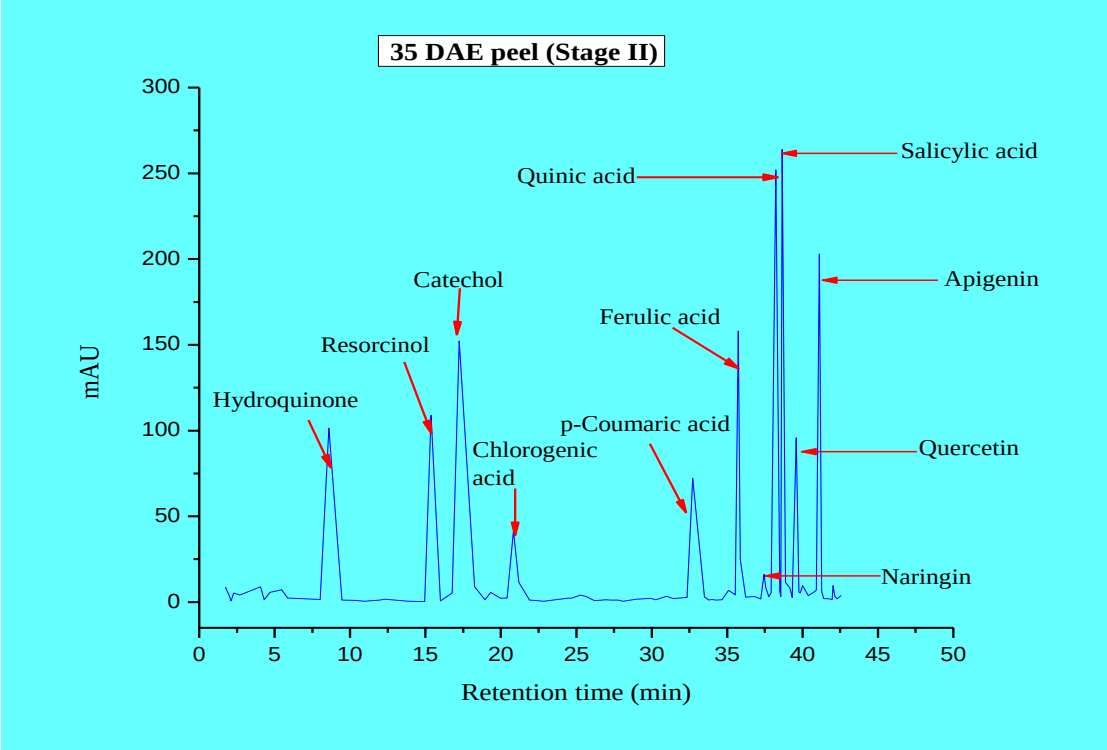

Fig. 2.3b HPLC chromatograms of polyphenols in 35 DAE (stage II) matured culinary banana peel

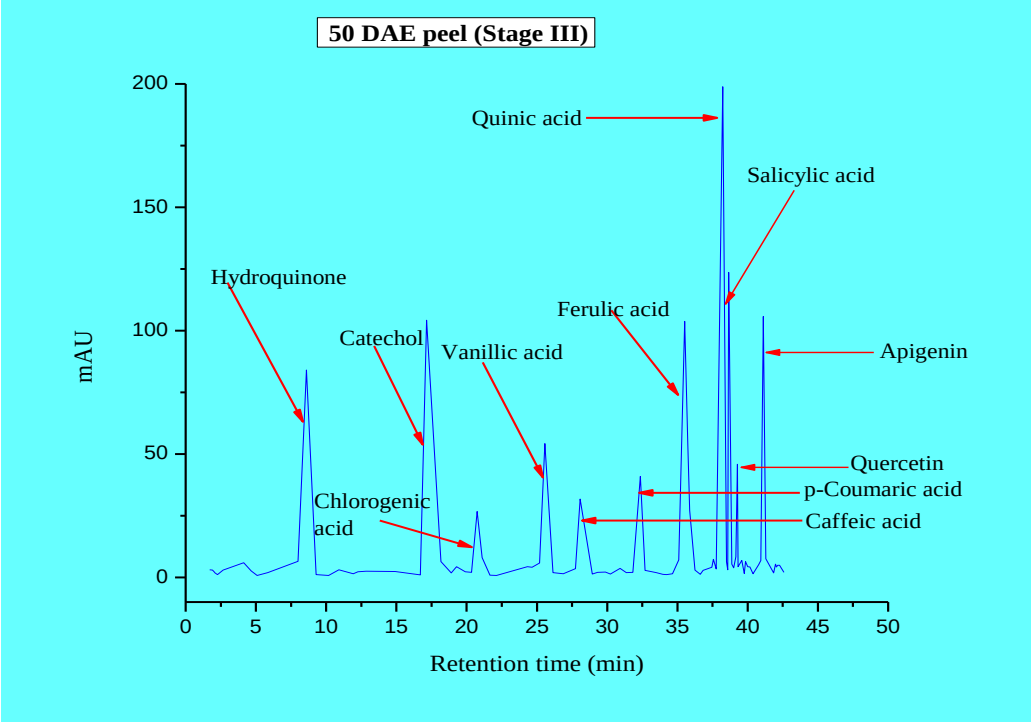

Fig. 2.3c HPLC chromatograms of polyphenols in 50 DAE (stage III) matured culinary banana peel

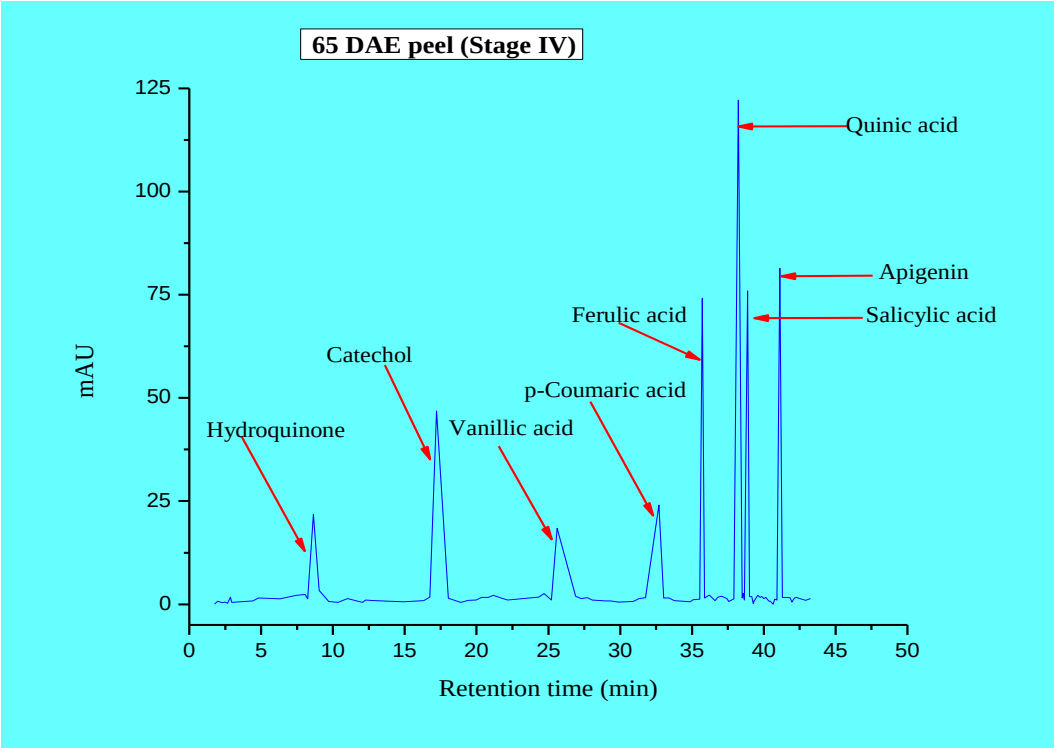

Fig. 2.3d HPLC chromatograms of polyphenols in 65 DAE (stage IV) matured culinary banana peel

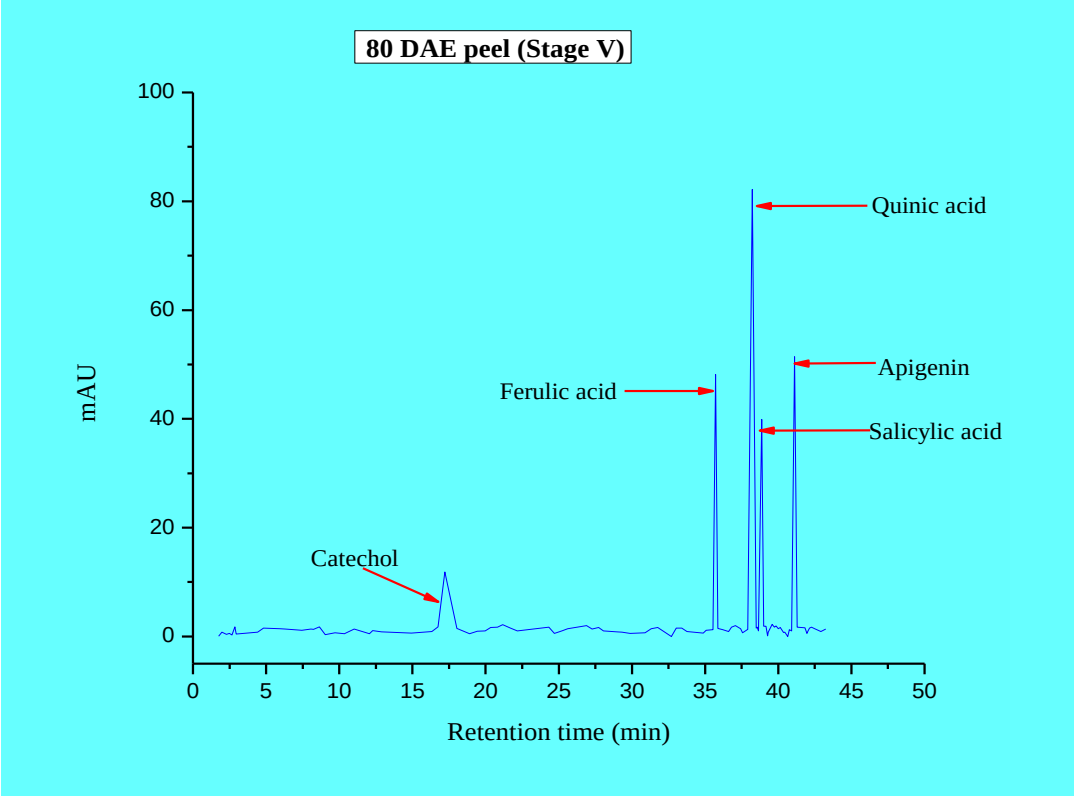

Fig. 2.3e HPLC chromatograms of polyphenols in 80 DAE (stage V) matured culinary banana peel

2.7.1.7 Fourier transform infrared spectroscopy (FT-IR)

The infrared spectra of culinary banana peel were obtained in order to understand the variation in nature of function group at different developmental stages. The spectra presented in Fig. (2.4a-2.4e) can be used to explain the functional groups. The IR-spectra peel at all five stages of development was more or less similar. The characteristic band observed at 3424 cm^{-1} at stage I, II, III and V and at 3433 cm^{-1} at stage IV may be attributed to carboxylic and hydroxyl functional groups and the absorption was caused by stretching of free or H-bonded OH-groups.[98] The sharp peak at At 2923 cm^{-1} is due to -CH stretch for $-CH_2$ and $-CH_3$ bond stretching vibration on OH. Depending upon the various functional groups this -CH stretching may vary. Therefore, this peak may or may not be altered during ripening. The prominent bands observed at 1644 cm^{-1} (stage I), 1635 cm^{-1} (stage II, III, V) and 1626 cm^{-1} (stage IV) represents the bending mode of the absorbed water[99] which might be related to COO- stretching vibration in

a carbohydrate group.[100] Peaks observed at 1412-1447 cm^{-1} was attributable to the bending modes of $-CH_3$.[101] There was a sharp peak at around 1018 cm^{-1} in all for stages studied which may be attributed to C-O-C pyranose ring skeletal vibration stretching indicates presence of xylans associated with hemicelluloses. Further the intense band at this wave number proves higher cellulose content in peel throughout growth. At 589 cm^{-1} (stage I, III), 580 cm^{-1} (stage II), 598 cm^{-1} (stage IV) and 699 cm^{-1} (stage V) are due to the entire anhydroglucose ring stretching vibrations.[102]

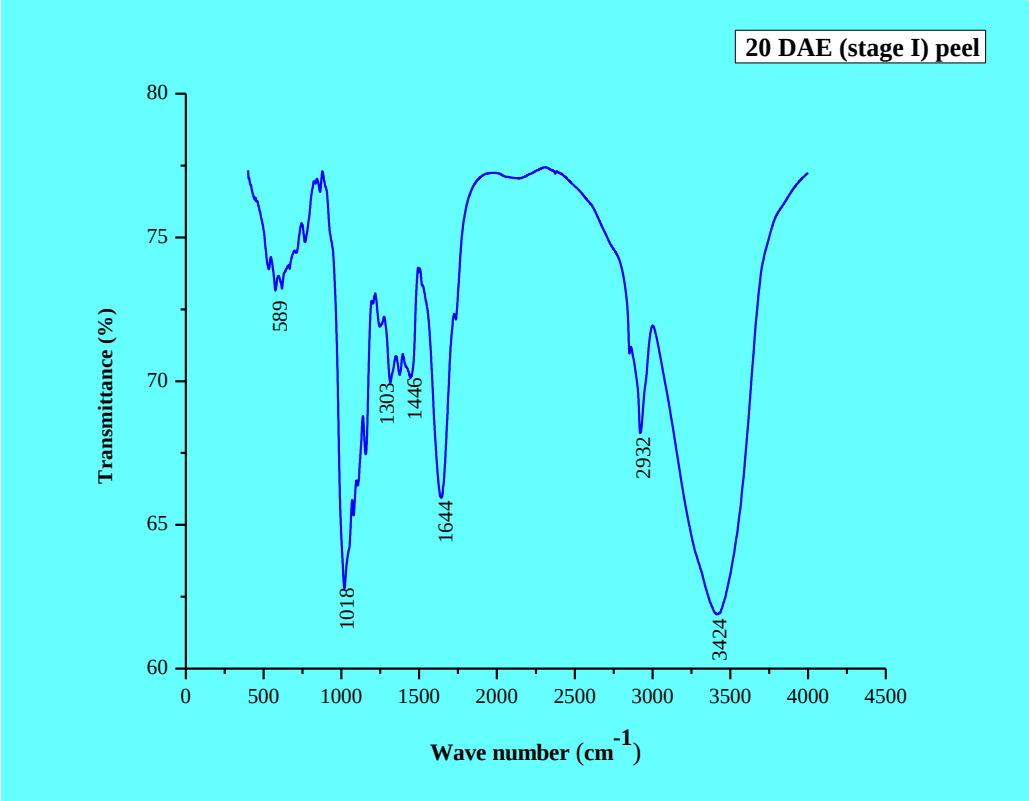

Fig. 2.4a FT-IR spectra of culinary banana peel at 20 DAE (stage I)

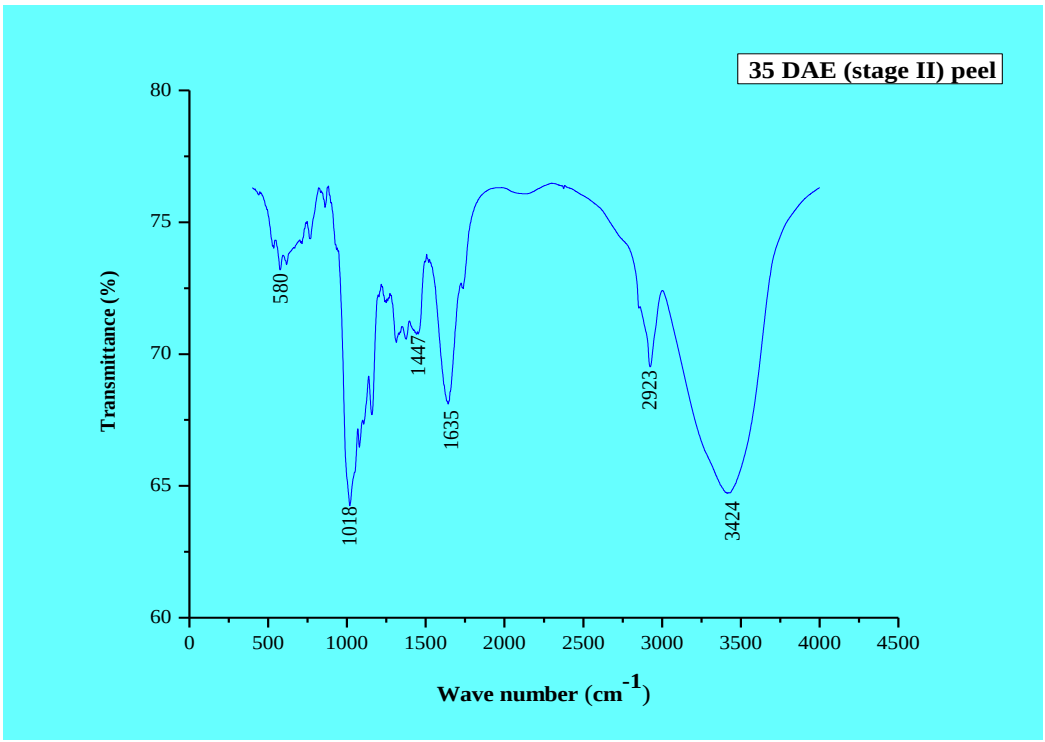

Fig. 2.4b FT-IR spectra of culinary banana peel at 35 DAE (stage II)

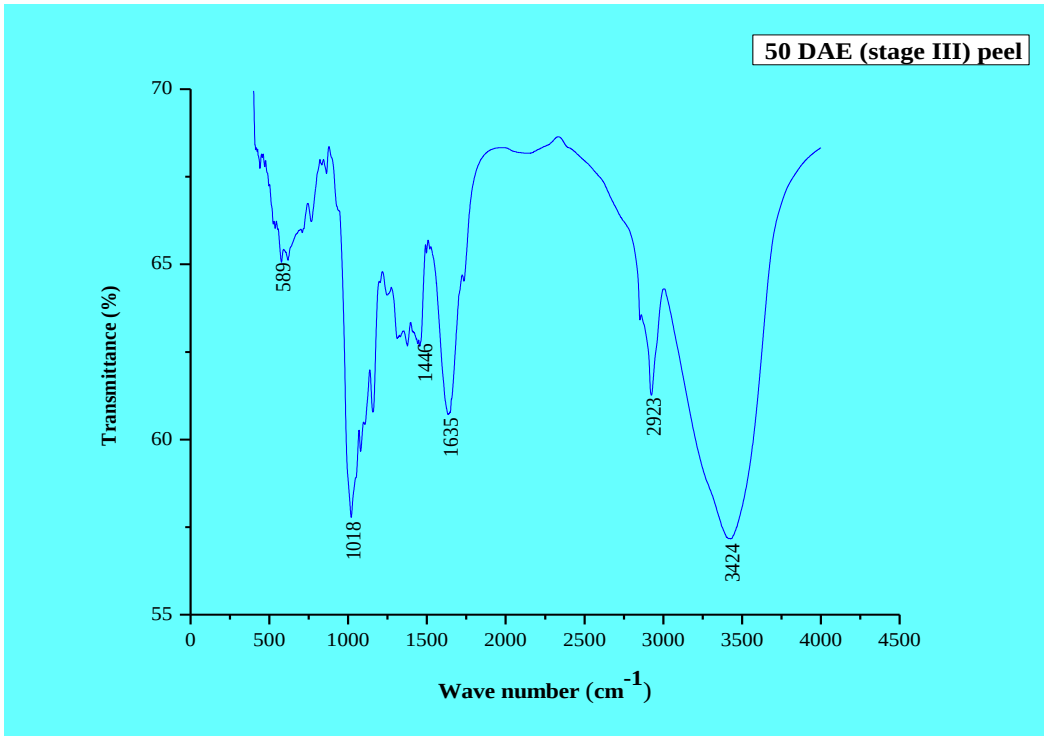

Fig. 2.4c FT-IR spectra of culinary banana peel at 50 DAE (stage III)

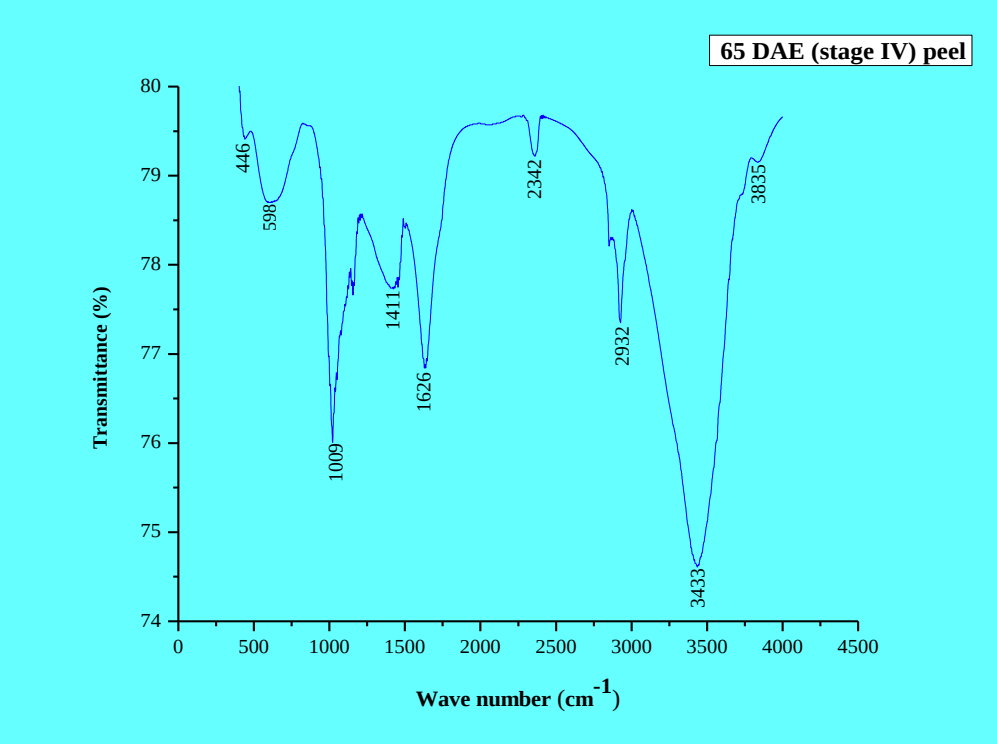

Fig. 2.4d FT-IR spectra of culinary banana peel at 65 DAE (stage IV)

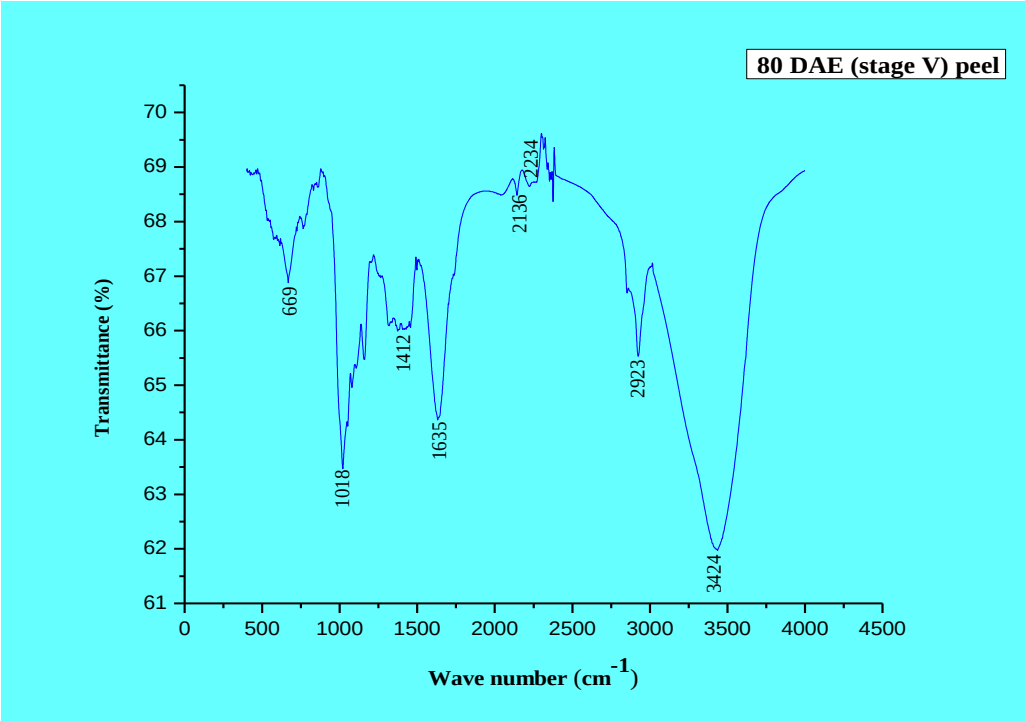

Fig. 2.4e FT-IR spectra of culinary banana peel at 80 DAE (stage V)

2.7.1.8 Scanning electron microscopy

The changes in microstructure of culinary banana peel with respect to growth and development was studied using SEM and presented in Fig (2.5a-2.5e). With the evolution of ripening stage the microstructure of peel drastically changed with irregular shapes and larger intercellular areas. The micrograph of early development stage (Fig. 2.5a) looks hazy with no particular prominent structure and with progress in growth and development at stage II (Fig. 2.5b) some microstructure was observed. From the figures it can be seen that at young stage the biochemical compositions was at limited value and with advancement in growth the components like carbohydrate, starch, fibers, cellulose etc are gradually increasing and hence the difference in microstructure is related to the variation in chemical compositions. At stage III and IV, electron microscope clearly captured the microstructure of starch and carbohydrate (Fig. 2.5c, 2.5d) as they were at dominating side. With further ripening at stage V, the starch is slowly getting degraded and converting to sugars (Fig. 2.5e).

2.7.1.9 X-ray Diffraction Pattern (XRD)

The culinary banana peel at different stages of development was analyzed for its crystalline nature as shown in Fig. (2.6a-2.6e). All the five diffractograms studied varied in their pattern with respect to growth and development but all showed crystalline morphology at each stage. The diffractogram of stage I peel showed five major peaks at 2θ values of 7.95, 12.18, 14.56, and sharp peaks at 16.84 and 21.17 indicates crystalline region. The XRD pattern of peel at stage III and IV particularly showed the presence of starch on it and the diffract grams observed was similar to those of starch. The reflection intensity at 6.90, 7.85, 14.93, 17.01, 23.17, and 30.92 may be attributed to the presence of C-type of starch in culinary banana peel. A 2θ value of 17.01 is the characteristics of B-type starch while peak appeared at 23.17 may be accredited to the characteristics of A-type starch. Thus the starch present in culinary banana peel can be classified as C-type which is the mixture of A and B types.

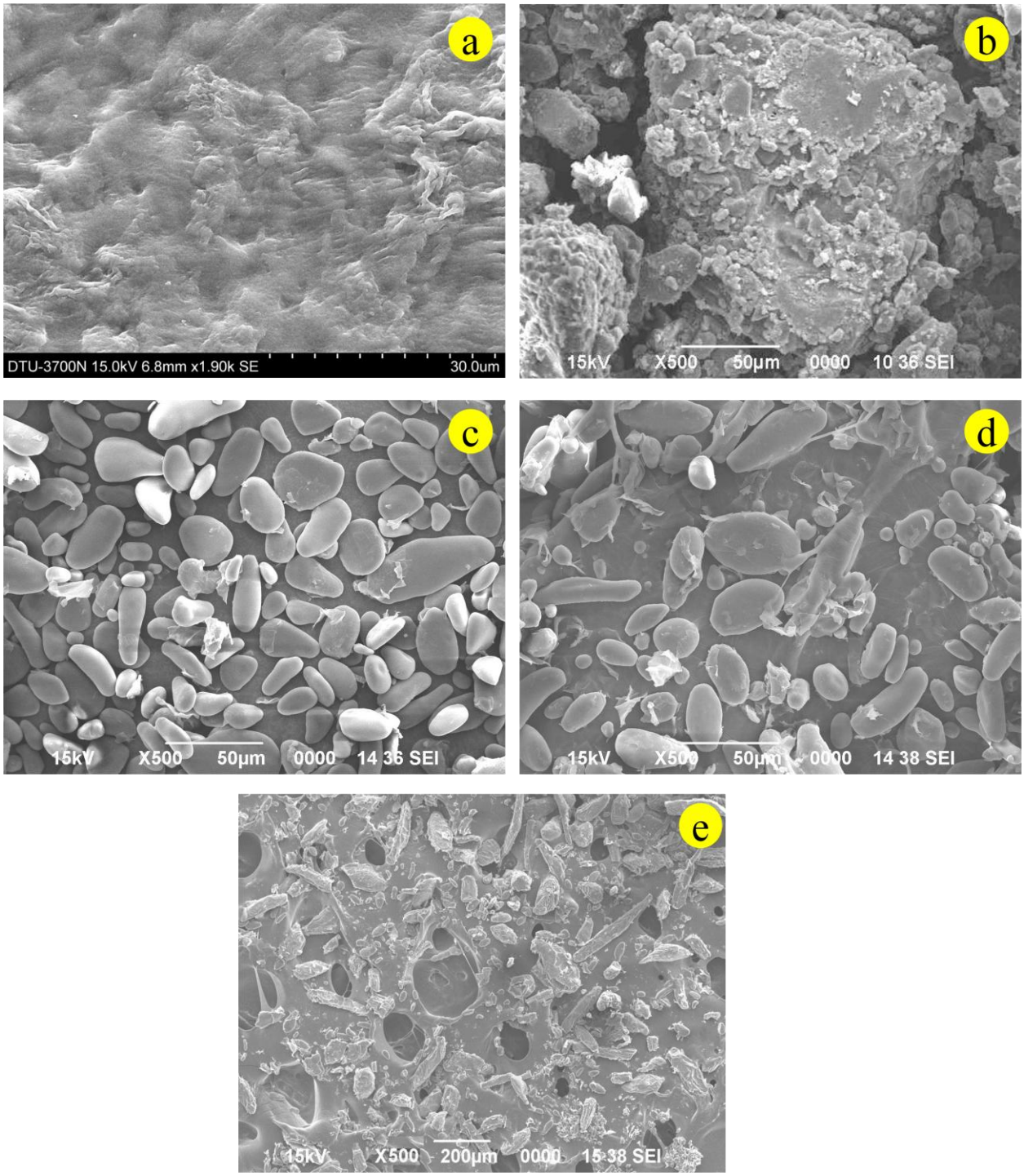

Fig. 2.5 SEM micrographs of culinary banana peel (**2.5a**): 20 DAE (stage I); (**2.5b**): 35 DAE (stage II); (**2.5c**): 50 DAE (stage III); (**2.5d**): 65 DAE (stage IV); (**2.5e**): 80 DAE (stage V)

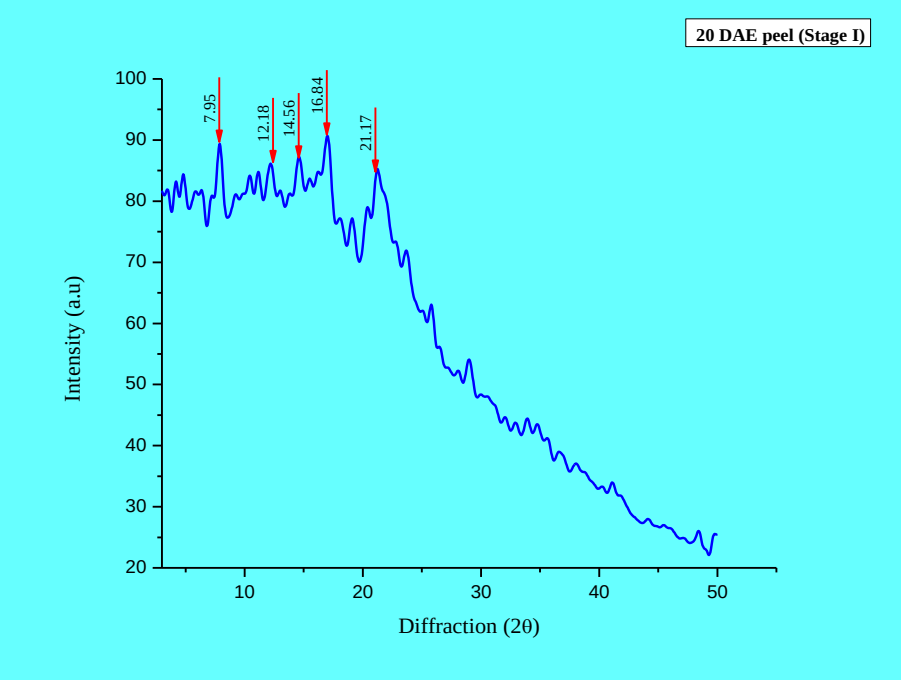

Fig. 2.6a XRD diffraction pattern of culinary banana peel at 20 DAE (stage I)

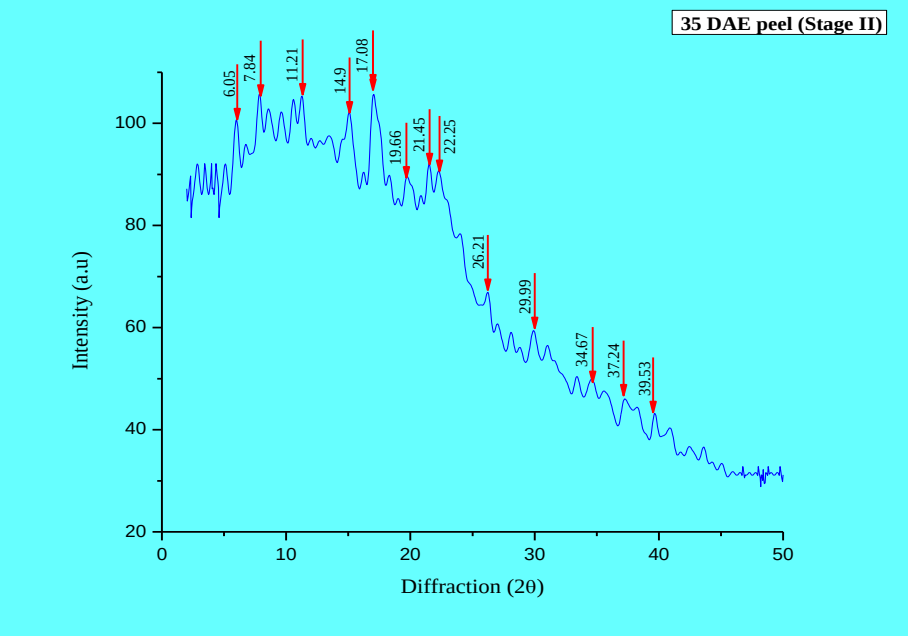

Fig. 2.6b XRD diffraction pattern of culinary banana peel at 35 DAE (stage II)

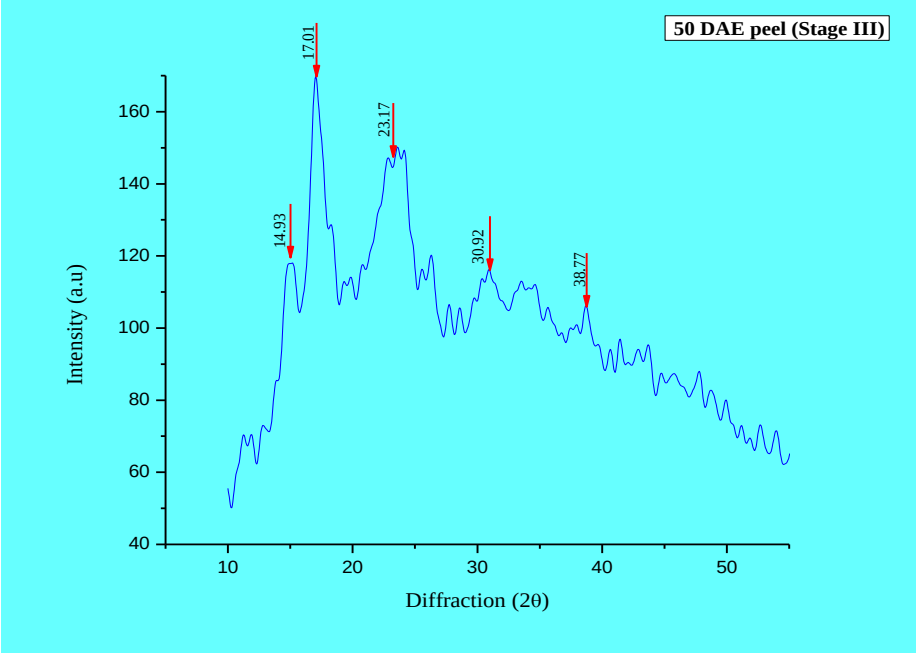

Fig. 2.6c XRD diffraction pattern of culinary banana peel at 50 DAE (stage III)

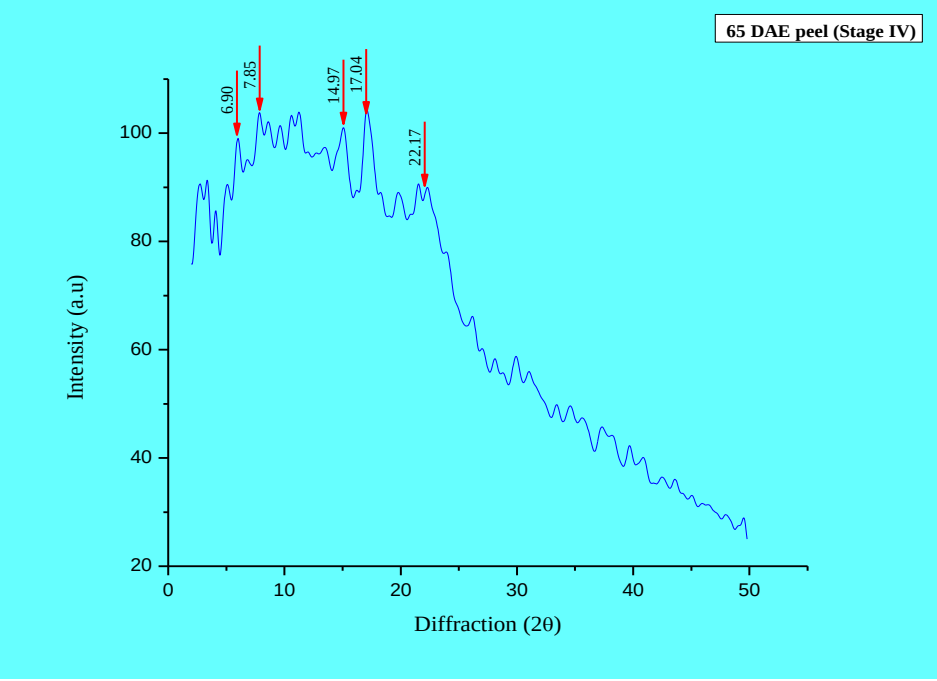

Fig. 2.6d XRD diffraction pattern of culinary banana peel at 65 DAE (stage IV)

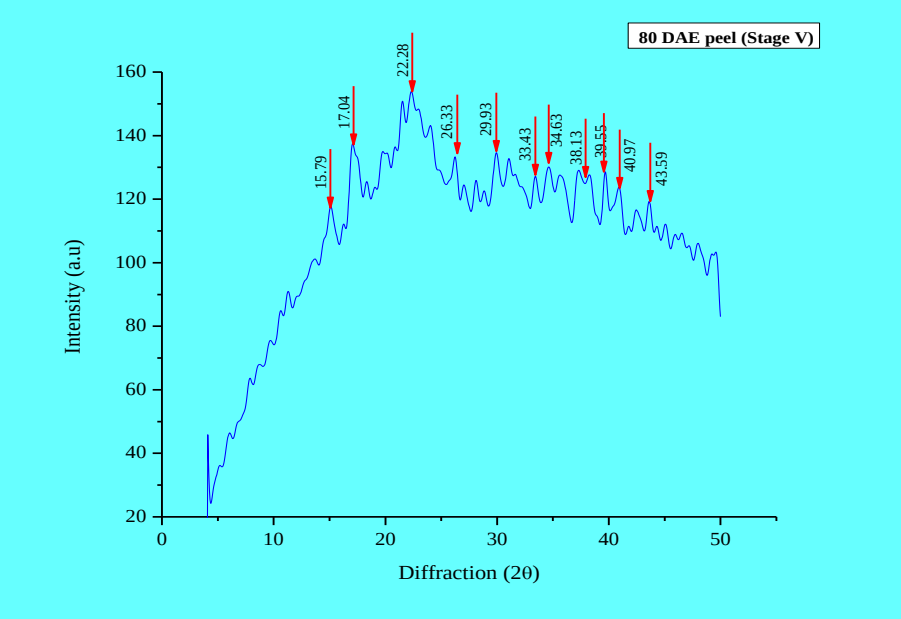

Fig. 2.6e XRD diffraction pattern of culinary banana at 80 DAE (stage V)

2.8 Conclusion

The present study was focused to evaluate the effect of fruit maturation on biochemical and functional compounds in peel at particular stage in order to identify the active compound present in peel. The results obtained indicate that peel of culinary banana is a potential source of many functionally important nutritional and bioactive compounds but are still being underutilized and discarded. The starch present in peel is of C-type as confirmed by x-ray diffractograms. Cellulose may commercially be utilized in the application of reinforcement agent. Phenols, flavonoids and scavenging activity were maximum towards the early development stage. Potassium was the abundant mineral present in all stages followed by phosphorous and magnesium. Fatty acids in peel were also in higher amount compared to pulp, linoleic and linolenic being dominant. Towards the early development stage most of the polyphenols were present in abundant amount. The peel was identified with 18 both essential and nonessential amino acids where tryptophan,valine, leucine, histidine were at dominating side. FT-IR analysis confirmed the presence of various functional groups indicating the complex nature of culinary banana peel. With respect to maturity SEM clearly evinced that microstructure of peel changes drastically and degradation of starch and other compounds occurred at overripe

stage. The crystalline nature of peel at all stages was also confirmed by XRD. Hence the present study favourably justified that peel of underutilized culinary banana has enormous potential for commercial application as a source of nutritional as well as functional compounds that can add high value to this locally important crop.

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