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EMPIRICAL COMPARISON OF CHANGES IN THE PEEL AND PULP OF CAVENDISH BASRAI BANANA RIPENING UNDER VARIOUS STORED STAGES

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Cavendish basrai is considered as the most commonly used banana in Pakistan. This experiment was executed to examine the assortment of basic quality profile of *Cavendish basrai*. Study was carried out on the diversity of four different bioactive characteristics including physical attributes, chemical composition, phenolic constituents and antioxidant potential on *Cavendish basrai* pulp and peel during storage at room temperature from unripe to ripe and ripped to over ripe (R1 to R8) at regular intervals of three days. The continuous increase was observed in pulp to peel ratio, which directly effects on all the bioactive constituents of both pulp and peel. As a result, moisture and sugar contents, TSS, titratable acidity, TDS, conductance, % NaCl and ash contents continuously increased with regular trends from R1 to R8 stages while regular decrease in total organic matter, total dry matter and pH was founded. Chlorophyll a and b, and carotenoids were found to have irregular trends. Phenolic constituents including total phenolic (TPC) and flavonoid contents (TFC), and total antioxidant activity evaluated by three different assays (reducing power, phosphomolybdenum and DPPH assays) increased from R1 to R6 but suddenly decreased from R7 to R8. R5 to R8 considered suitable stages for the utilization of *Cavendish basrai* banana. In comparison to peel, significant fall down observed in bioactive characteristics with decay from R1 to R8.
Keywords: *Cavendish basrai* banana, physical attributes, chemical composition, phenolic and flavonoid, antioxidant

INTRODUCTION

It is observed that diseases are caused by the oxidative stress in the body, which occurs as a consequence of imbalance between formulation and neutralization of reactive free radicals. They are continuously formed and neutralized in body to maintain the internal body environment i.e. redox state. These reactive free radicals are produced due to endogenous factors such as by-products of normal metabolic processes for ATP production (in reducing molecular oxygen) or exogenous factors like air pollution, UV radiation, cigarette smoking, high polyunsaturated fatty acid diet and trace metals in diet (Williams and Jeffrey, 2000). The incidence of diseases due to oxidative stress can be decreased and minimized by antioxidant compounds (*Chu et al*., 2002). They suppress the formation of reactive oxygen species either by chelation of trace elements or by means of enzyme inhibition along with scavenging these species (Haliwell and Guttered, 1999).

Among the most utilizable fruits in the world market, banana is considered as popular fruit. It belongs to the genus *Musa* from the family Musaceae. Banana fruits contain various nutritional constituents and antioxidant compounds including β-carotene, vitamin C and vitamin E (Kanazawa and Sakakibara, 2000). Banana fruit is rich in pyridoxine (vitamin B6) (Leklem, 1999), which relatively provides protection from cancer especially against oesophagus cancer (World Cancer Research Fund, 2007). It also contains phenylpropanoids such as elemicin, eugenol and methyl eugenol but at low concentration (Jordan *et al*., 2001). The peel of banana is also rich in proteins, polyunsaturated fatty acids, essential amino acids and potassium dietary fibers (Emaga *et al*., 2007). Pulp of cooked green bananas have antidiarrheal activity (Rabbani *et al*., 2004) and used traditionally for the treatment of intestinal disorders (Aurora and Sharma, 1990). The composition of nutritional constituents, phenolic contents and antioxidant properties of fruits may be varied by post-harvest conditions and environmental factors during processing and storage period (Robards *et al*., 1999).

Therefore, the main objective of this study was to characterize the physiological matured stage of *Cavendish basrai* banana during ripening for the utilization of antioxidant potential, nutritional constituents and biochemical evaluations at different stages during storage at room temperature without any atmospheric controlled conditions with regular intervals of three days from unripe to ripe, and over ripe to muddy stage.

MATERIALS AND METHODS

***Sample collection*:** *Cavendish basrai* banana bunch was

obtained from the garden located at Department of Chemistry, Federal Urdu University of Arts, Science & Technology, Gulshan-e-Iqbal, Karachi-75300 (24º51.36'N, 67º00.36'E, and an altitude of 26 feet above sea level), Pakistan. Bananas were harvested at the mid of December 2013 to the mid of January 2014 at the stage of inflorescence (fruits above the purple flower bud). Trees of *Cavendish basrai* banana were 2-3 years old and complete description of tree and soil is mentioned in Table 1.

Table 1. Description of *Cavendish basrai* banana tree and soil

Sr.	Parameters	Unit	Numerical value
1	Length of flower on tree	cm	41.23
2	Width of flower on tree	cm	9.34
3	Length of bunch of banana on tree	cm	22.68
4	Width of bunch of banana on tree	cm	25.31
5	Length of leaf on tree	cm	97.80
6	Width of leaf on tree	cm	52.42
7	Height of steam	cm	117.43
8	Width of steam	cm	14.66
9	Difference between two trees	cm	80.02
10	Moisture of soil	%	35.97
11	pH of soil	-	5.63
12	TDS of soil	ppm	558.66
13	Conductance of soil	µS	1050.33

***Storage condition*:** Samples of *Cavendish basrai* banana were uniformly selected for storage and divided equally into different portions randomly. Selected samples were cleaned by washing with tap water and then air dried in order to eliminate any dirt, dust and metallic ions or chemicals on the surface. After cleaning action each portion of samples was stocked up into baskets and sheltered entirely for the prevention from insects at room temperature (25±2ºC) without any atmosphere controlled conditions. Explanation of *Cavendish basrai* banana was observed by 6-members panelist randomly chosen, and asked to rate color (by screening sample carefully) and texture (by hand touching and pressing). There was no visible microbial and transportation damage to the samples. All safety measurements were applied to prevent microbial growth. Analysis was performed on stored samples at various stages (R1-R8) with regular intervals of three days. Condition of samples is described at various stages in Table 2.

***Sample preparation*:** Samples of *Cavendish basrai* banana were peeled off with a clean and sharp stainless steel table knife. Both peel and pulp samples were cut down separately into small pieces and crushed by using mortar and pestle. 10 g of each crushed sample (peel or pulp) was soaked separately into 100 mL selected soaking solvent (water or methanol) and stirred (for 60 min on magnetic stirrer) to prepare the 10% w/v extracts. The extracts were filtered by using Whatman filter paper (Grade 41) and filtrates were used for analysis as test solutions.

Table 2. Description of *Cavendish basrai* banana collected freshly from tree and stored at room temperature at various stages

Sr.	Day	Stage	Description	Figure
1	1st	R1	Mature ripped. Entirely dark green rigid peel and white rigid pulp.	
2	4th	R2	Mature ripped. Medium green rigid peel with rigid white pulp.	
3	7th	R3	Mature ripped. Light green peel with brown patches and decrease in rigidness of white pulp observed.	
4	10th	R4	Mature fully ripped. Light yellowish change along decreases in rigidness of peel observed with increase in softness of pulp.	
5	13th	R5	Mature fully ripped. More yellow than green change along brown spots observed on peel and light yellow pulp becomes soft on pressing.	
6	16th	R6	Mature fully ripped. Entirely yellow with black necks peel with soft light yellow pulp.	
7	19th	R7	Over ripped. Color of peel was converted into blackish shade and yellow pulp becomes watery.	
8	22nd	R8	Over ripped. Fully black peel with watery yellow pulp.	

***Physical attributes*:** Fruit weight of *Cavendish basrai* banana was obtained by weighing the sample on electronic balance (Denver TP-214, Germany). Percentage of peel or pulp was

determined by weighing separately on electronic balance. Length was measured by using measuring tape and diameter of fruit was taken *via* vernier caliper.

Chemical composition

***Moisture contents, total dry matter, ash contents and total organic matter*:** Moisture contents and total dry matter in the samples were determined according to the method of ISI (1984b). Briefly, 5 g sample was weighed in a dried petri dish and kept in hot air oven (Binder E28#05-86486, Germany) at 105±2°C for 2 hours to remove moisture then cooled in a desiccator and weighed again. The process of heating, cooling and weight measuring was repeated to obtain less than 1 mg difference between two successive weighing. Similarly, moisture contents were obtained in the soil.

Ash contents and total organic matter were measured by the method of AOAC (1990). Briefly, 5 g sample was weighed in silica crucible and then dried up in the hot air oven, and finely placed in the muffle furnace (Nabertherm, Germany) by maintaining the temperature about 550°C for 6-8 hours until white ash was achieved. The crucible was cooled, weighed and again placed in the muffle furnace for 1 hour to measure the least weight of total ash.

***pH, titratable acidity (TA), TDS, conductance and % NaCl*:** The pH of the test samples (water extract) and soil were determined by means of pH meter (Jenway 3510, England).

Titratable acidity (TA) of samples was achieved briefly according to the method of AOAC (2000) in which 5 mL of the test sample (water extract) was titrated with 0.1 N solution of NaOH *via* phenolphthalein used as indicator, and acidity reported as mL of 0.1 N NaOH per 100 g or 100 mL as required. TA expressed in g acid per 100 g simply by the factor suitable to the acid as 1 mL of 0.1 N NaOH equals to 0.0060 g acetic acid.

TDS of the test solution (water extract) and soil were obtained by using TDS meter (S 518860, Korea). Conductance was measured by using conductivity meter (Jenway 4510, England).

% NaCl was evaluated simply according to the method of ISI (1984a) in which 5 mL of the test sample (water extract) was neutralized with standard solution of NaOH by using phenolphthalein as indicator. After that 1 mL of 5% aqueous K_2CrO_4 solution was added into mixture and then finely titrated with 0.1 N $AgNO_3$ solution to attain red-brown end point.

***Total soluble solids (TSS) and total sugar contents*:** Total soluble solids (TSS) were determined according to the method of BIS IS (1993). 40-50 g of sample was taken into beaker and 100-150 mL of distilled water was added as suitably, and mixture was allowed to boil with stirring for 5 min. After cooling, the contents were filtered and filtrate was used for analysis. TSS was analyzed simply by placing a drop of filtrate on the refractometer (KRÜSS, Germany), and expressed as sucrose contents.

Total sugar contents were obtained by using the method of AOAC (1990). 1 mL of the test solution (methanol extract) was taken into a test tube, and 1 mL of 5% phenol was added into it. The solution was shaken then 5 mL of 96% H_2SO_4 was added, and finally the test tube was allowed to cool in a water bath (Witeg WIG-32, Germany) at 30°C for 10 min. The absorbance of the mixture was obtained at 490 nm against the prepared blank with the help of spectrophotometer (Jenway 6300, England).

***Total carotenoids, chlorophyll a (C_a) and chlorophyll b (C_b)*:** The amount of total carotenoids, chlorophyll a (C_a) and chlorophyll b (C_b) in the test solution (methanol extract) were measured by using the method of Dere *et al*. (1998). The quantities of these pigments were measured according the formulae as follows:

Total carotenoids = $1000\ A_{470} - 2.860\ C_a - 129.2\ C_b/245$

$C_a = 15.65\ A_{666} - 7.340\ A_{653}$

$C_b = 27.05\ A_{653} - 11.21\ A_{666}$

Where C_a = Chlorophyll a; C_b = Chlorophyll b; A_{666} = Absorbance at 666 nm; A_{653} = Absorbance at 653 nm; A_{470} = Absorbance at 470 nm

Phenolic evaluation

***Total phenolic contents (TPC)*:** Total phenolic contents (TPC) were investigated by using Folin-Ciocalteu reagent according to the method of Lester *et al*. (2012). Briefly, 40 µL of test sample (methanol extract) was mixed with 1.8 mL of pre-diluted (10 times with distilled water) Folin-Ciocalteu reagent and the mixture were kept at room temperature for 5 min. 1.2 mL of (7.5%w/v) sodium carbonate solution was added in the prepared mixture, mixed well and allow to stand for 1 hour at room temperature to complete the reaction. Then absorbance was measured at 765 nm by using spectrophotometer (Jenway 6300, England). Calibration curve was plotted using gallic acid (20 to 100 mg/L, r^2 = 0.997) as standard solution. Results were expressed as gallic acid equivalents per 100 g of sample.

***Total flavonoid contents (TFC)*:** Total flavonoid contents (TFC) were also investigated according to the method explained by Zhishen *et al*. (1999). Briefly, 1 mL of the test sample (methanol extract) was added into 4 mL of distilled water. At zero time, 0.3 mL of (5% w/v) $NaNO_2$ was added in the solution then 0.3 mL of (10 % w/v) $Al(NO_3)_3$ was added after 5 min. The volume of the mixture was made up to 10 mL by the addition of distilled water. Mixture was shaken vigorously then absorbance was noted at 510 nm, and calibration curve was also plotted using a standard solution of catechin (20 to 100 mg/L, r^2 = 0.996). The results were expressed as mg of catechin equivalents (CEQ) /100 g of sample.

Antioxidant evaluation

***Reducing power assay (RPA)*:** Antioxidant capacity by using reducing power assay (RPA) was measured according to the method proposed by Jayanthi and Lalitha (2011). 0.1 mL test sample (methanol extract) was mixed well with 2.5 mL

phosphate buffer (6.6 pH) and 2.5 mL potassium ferricyanide. This mixture was kept on water bath for 20 min at 50°C. After cooling, 2.5 mL of 10% trichloro acetic acid was added into the solution and centrifuged at 3000 rpm for 10 min whenever required. 2.5 mL solution was taken from upper layer and mixed with 2.5 mL distilled water, and after that freshly prepared 0.5 mL ferric chloride solution was added. Absorbance was noted at 700 nm, and calibration curve was plotted using a standard solution of ascorbic acid at various concentrations (20 to 100 mg/L, $r^2 = 0.993$). The results were expressed as mg of ascorbic acid equivalents/100 g of sample.

***Phosphomolybdenum assay (PA)*:** The total antioxidant capacity of sample was also evaluated using phosphomolybdenum assay (PA) by oxidation-reduction mechanism (Prieto *et al*., 1999). 0.1 mL test sample (methanol extract) was mixed well with 4 mL reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate), and obtained mixture was heated on water bath at 95°C for 90 min. After that sample was cooled at room temperature, and absorbance was noted at 695 nm. A calibration curve was acquired using a standard solution of ascorbic acid at various concentrations (20 to 100 mg/L, $r^2 = 0.994$). The results were expressed as mg of ascorbic acid equivalents/100 g of sample.

***DPPH Free radical scavenging assay*:** Antioxidant capacity was also evaluated by radical scavenging mechanism *via* DPPH (2,2-diphenyl-1-picrylhydrazyl) radical according to the method described by De Ancos *et al*. (2002). 0.1 mM methanolic solution of DPPH was used to investigate the % inhibition of samples. 5 µL test sample (methanol extract) was mixed with 95 µL of DPPH solution and this mixture was then mixed on vortex mixer (VM-300, Gemmy Industrial Corp., Taiwan), and finally stored in dark for 30 min. Absorbance was obtained at 515 nm against a blank (methanol without DPPH).

$$\% \text{ Inhibition of DPPH radical} = \frac{(\text{absorbance of control}^* - \text{absorbance of sample})}{(\text{absorbance of control}^*)} \times 100$$

Where, *Control = DPPH solution without sample solution

***Statistical analysis*:** The values are given as standard error of means (Mean±SEM) by using Microsoft Excel. ANOVA as one-way analysis of variance was performed by using statistical program Minitab (Version 16.1.1) software. The significant differences were tested with Tukey's honestly significant difference (HSD) test judged at $p < 0.05$ (95% confidence level).

RESULTS AND DISCUSSION

***Physical attributes*:** Physical parameters including fruit weight, pulp and peel contribution, length and diameter were analyzed with regular intervals of three days from unripe to ripe and ripe to grow moldy stage during storage at room temperature (R1-R8) to evaluate the health promoting stage before spoilage of the *Cavendish basrai* banana and to observe the sustainable changes in banana during storage. The results of physical parameters are revealed in Table 3.

The total fruit weight of the *Cavendish basrai* banana during storage at room temperature did not change significantly from stage R1 to R3, only slightly decrease (7.81%) was observed with the decay of fruit. The decrease in total fruit weight of whole stored banana (both peel and pulp) was due to the decrease in moisture contents and other continuous changes in nutritional contents occurred during storage.

It was also observed that % pulp contribution of stored banana was progressively increased up to 79.00% throughout the experiment with continuous decrease in peel contribution (64.75%) (Table 3), illustrated the indirect correlation between them. It showed that flesh to peel ratio increased in *Cavendish basrai* banana from unripe to moldy stage, which is considered as a coefficient of ripeness. Softness of banana increased, which is possibly due to the conversion of starch contents into free sugary compounds, solubilisation of the pectic substances after breakdown of cell wall in the cohesion of the middle lamella and osmotic transfer of moisture or water from peel to flesh (Smith *et al*., 1989).

Similarly, as total weight, the length of whole banana during storage at room temperature was not changed significantly ($p < 0.05$) throughout the intervals but minor decrease in length was observed during fruit decay. The diameter also showed same trends from R1 to R8 (Table 3). It was due to the loss in firmness and moisture contents as observed.

***Chemical composition*:** It was important to evaluate the chemical composition of Cavendish basrai banana to investigate the complete profile during repining. Fourteen parameters were analyzed to assess the nutritional and

Table 3. Description of physical attributes of *Cavendish basrai* banana under various stored stages at room temperature

Sr.	Parameters	Unit	R1	R2	R3	R4	R5	R6	R7	R8
			Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
1	Fruit weight	g/fruit	33.81 ± 0.30^{A}	33.99 ± 0.57^{A}	34.13 ± 0.06^{A}	32.86 ± 0.25^{B}	32.51 ± 0.31^{BC}	32.08 ± 0.21^{BC}	31.85 ± 0.17^{CD}	31.17 ± 0.05^{D}
2	Flesh contribution	%	45.04 ± 0.64^{G}	51.55 ± 1.68^{F}	58.78 ± 1.09^{E}	60.95 ± 2.42^{E}	67.82 ± 0.54^{D}	71.61 ± 0.20^{C}	75.88 ± 0.16^{B}	80.62 ± 0.11^{A}
3	Peel contribution	%	54.95 ± 0.64^{A}	48.44 ± 1.68^{B}	41.21 ± 1.09^{B}	39.04 ± 2.42^{C}	32.17 ± 0.54^{D}	28.39 ± 0.20^{E}	24.11 ± 0.16^{F}	19.37 ± 0.11^{G}
4	Fruit length	cm	13.85 ± 0.22^{A}	13.61 ± 0.46^{A}	13.15 ± 0.04^{AB}	12.67 ± 0.30^{BC}	12.56 ± 0.67^{BC}	12.18 ± 0.73^{C}	12.11 ± 0.03^{C}	12.18 ± 0.07^{C}
5	Fruit diameter	cm	3.65 ± 0.08^{A}	3.45 ± 0.02^{A}	3.33 ± 0.03^{AB}	3.05 ± 0.24^{BC}	2.72 ± 0.15^{CD}	2.45 ± 0.15^{DE}	2.28 ± 0.06^{E}	2.16 ± 0.03^{E}

SEM = Standard error of the mean of three replicates. (A-G) values in some rows with different subscripts are significant differences, were justified at 95 % confidence level (ANNOVA) by Tukey's HSD ($p < 0.05$).

compositional characteristics in *Cavendish braai* banana pulp and peel during repining under various stored stages at room temperature.

***Moisture contents, total dry matter, ash contents and total organic matter*:** Moisture contents and total dry matter are indirectly correlated to each other. Both parameters are important to scrutinize the maturity level and quality of banana. It was observed that *Cavendish basrai* banana contained extensive amount of moisture in both pulp and peel (Tables 4 and 5) as reported by Wall (2006) during analysis of edible pulp of three different varieties of banana at maturity level (68.52 to 73.84 %). During ripening of banana, regular and significant increase in moisture contents of pulp was observed from R1 to R8 (62.58 to 83.65 %) with the increase in softness and sweetness of pulp (Table 2). It has been reported that increase in the moisture contents of the flesh during ripening was due to carbohydrate breakdown and osmotic transfer of water from the peel to flesh (John and Marchal, 1995). Total dry matter was decreased from R1 to R8 (37.41to 16.34 %), showing indirect connection due to decay of starch contents.

In the peel of banana, the extensive level of moisture contents was observed at unripe conditions but rapidly decline was inspected during ripening from R1 to R8 (85.76 to 64.51 %) with increase in total dry matter (14.23 to 35.48 %). It was due to the development of aromatic changes with continuous drying of peel due to migration of moisture from peel to pulp. Ash contents and total organic matter are also indirectly correlated to each other in both pulp and peel (Tables 4 and 5). The regular increment in ash contents was investigated in pulp during ripening from R1 to R8 (0.52 to 1.06 %). This increment was considered due to the increase in TDS as well as % NaCl in the flesh contribution. The increment of mineral contents in flesh was due to the transfer of minerals from peel to flesh as this ratio increased significantly during analysis. Constant decrease was found in the ash contents of peel from R1 to R8. Total organic matter was found to be decreased in pulp whereas increased in peel during ripening.

***pH, titratable acidity (TA), TDS, conductance and % NaCl*:** pH and acidity are imperative parameters to determine taste and quality of fruits. If pH value of fruit is increased or decreased, it will directly effect on all valuable nutritional

Table 4. Description of chemical composition of *Cavendish basrai* banana pulp under various stored stages at room temperature

Sr.	Parameters	Unit	R1	R2	R3	R4	R5	R6	R7	R8
			Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
1	pH	-	8.23 ± 0.03^{A}	7.63 ± 0.03^{B}	6.74 ± 0.14^{C}	6.27 ± 0.04^{D}	5.71 ± 0.05^{E}	5.24 ± 0.07^{F}	4.92 ± 0.05^{G}	4.83 ± 0.03^{G}
2	Titratable acidity	g/100mL	0.37 ± 0.03^{E}	0.45 ± 0.01^{D}	0.53 ± 0.01^{C}	0.65 ± 0.03^{B}	0.71 ± 0.02^{B}	0.77 ± 0.01^{A}	0.81 ± 0.01^{A}	0.80 ± 0.02^{A}
3	TDS	ppm	875.3 ± 2.081^{H}	1032.3 ± 0.004^{G}	1261.3 ± 0.002^{F}	1647.6 ± 0.004^{E}	1958.3 ± 0.006^{D}	2135.6 ± 0.002^{C}	2334.6 ± 0.012^{B}	2539.6 ± 0.008^{A}
4	Conductance	mS	1.73 ± 0.012^{G}	1.82 ± 0.015^{F}	2.17 ± 0.006^{E}	2.54 ± 0.015^{D}	2.86 ± 0.026^{C}	3.61 ± 0.070^{B}	3.88 ± 0.036^{A}	3.96 ± 0.021^{A}
5	NaCl	%	0.028 ± 0.0006^{F}	0.030 ± 0.0011^{F}	0.034 ± 0.0006^{E}	0.037 ± 0.0006^{D}	0.044 ± 0.0011^{C}	0.051 ± 0.0017^{B}	0.054 ± 0.0006^{A}	0.056 ± 0.0006^{A}
6	Moisture contents	%	62.58 ± 0.39^{G}	65.49 ± 0.32^{F}	68.61 ± 0.51^{E}	71.08 ± 0.82^{D}	75.89 ± 0.54^{C}	78.46 ± 1.28^{B}	80.69 ± 0.42^{B}	83.65 ± 1.38^{A}
7	Total dry matter	%	37.41 ± 0.39^{A}	34.51 ± 0.32^{B}	31.38 ± 0.51^{C}	28.92 ± 0.82^{D}	24.10 ± 0.54^{E}	21.53 ± 1.28^{F}	19.31 ± 0.42^{F}	16.34 ± 1.38^{G}
8	Ash contents	%	0.52 ± 0.005^{F}	0.56 ± 0.010^{F}	0.64 ± 0.006^{E}	0.66 ± 0.023^{E}	0.76 ± 0.115^{D}	0.83 ± 0.032^{C}	0.93 ± 0.025^{B}	1.06 ± 0.030^{A}
9	Total organic matter	%	99.47 ± 0.006^{A}	99.44 ± 0.010^{A}	99.36 ± 0.006^{B}	99.33 ± 0.023^{B}	99.23 ± 0.012^{C}	99.16 ± 0.032^{D}	99.06 ± 0.025^{E}	98.94 ± 0.030^{F}
10	TSS	^{o}Brix	8.63 ± 0.01^{G}	9.08 ± 0.09^{G}	11.29 ± 0.05^{F}	13.33 ± 0.03^{E}	13.88 ± 0.01^{D}	15.01 ± 0.12^{C}	16.56 ± 0.49^{B}	17.64 ± 0.06^{A}
11	Total sugar contents	%	10.39 ± 0.29^{G}	10.71 ± 0.22^{FG}	11.90 ± 0.03^{EF}	12.23 ± 0.58^{E}	14.44 ± 0.38^{D}	16.45 ± 0.59^{C}	19.87 ± 0.39^{B}	21.29 ± 0.87^{A}
12	Total carotenoids	ppm	106.0 ± 1.60^{F}	110.0 ± 0.58^{E}	115.0 ± 1.57^{D}	119.0 ± 0.92^{BC}	122.0 ± 0.97^{AB}	124.0 ± 0.57^{A}	120.0 ± 1.42^{BC}	117.0 ± 1.49^{CD}
13	Chlorophyll a	ppm	0.47 ± 0.024^{F}	0.50 ± 0.024^{EF}	0.51 ± 0.021^{EF}	0.53 ± 0.020^{DE}	0.62 ± 0.020^{BC}	0.70 ± 0.005^{A}	0.67 ± 0.030^{AB}	0.57 ± 0.009^{CD}
14	Chlorophyll b	ppm	0.91 ± 0.033^{E}	0.97 ± 0.041^{DE}	0.99 ± 0.036^{DE}	1.03 ± 0.035^{CD}	1.21 ± 0.035^{B}	1.34 ± 0.011^{A}	1.29 ± 0.051^{AB}	1.10 ± 0.021^{C}

SEM = Standard error of the mean of three replicates. (A-H) values in some rows with different subscripts are significant differences, were justified at 95 % confidence level (ANNOVA) by Tukey's HSD ($p < 0.05$).

Table 5. Description of chemical composition of *Cavendish basrai* banana peel under various stored stages at room temperature

S.No.	Parameters	Unit	R1	R2	R3	R4	R5	R6	R7	R8
			Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
1	pH	-	5.36 ± 0.04^{H}	5.84 ± 0.11^{G}	6.17 ± 0.01^{F}	6.55 ± 0.03^{E}	6.82 ± 0.01^{D}	7.49 ± 0.03^{C}	7.91 ± 0.02^{B}	8.14 ± 0.02^{A}
2	Titratable acidity	g/100 mL	0.93 ± 0.01^{A}	0.87 ± 0.01^{B}	0.80 ± 0.02^{C}	0.71 ± 0.01^{D}	0.66 ± 0.02^{E}	0.55 ± 0.03^{F}	0.53 ± 0.01^{FG}	0.49 ± 0.01^{G}
3	TDS	ppm	1390.6 ± 0.004^{E}	1452.6 ± 0.007^{D}	1672.6 ± 0.009^{B}	1717.3 ± 0.005^{A}	1678.3 ± 0.004^{B}	1565.3 ± 0.004^{C}	1317.3 ± 0.005^{F}	1136.3 ± 0.031^{G}
4	Conductance	mS	3.13 ± 0.015^{D}	3.28 ± 0.015^{BC}	3.34 ± 0.010^{B}	3.54 ± 0.025^{A}	3.267 ± 0.015^{C}	2.96 ± 0.015^{E}	2.20 ± 0.038^{F}	1.86 ± 0.045^{G}
5	NaCl	%	0.075 ± 0.0006^{A}	0.068 ± 0.0006^{B}	0.058 ± 0.0006^{C}	0.053 ± 0.0006^{D}	0.045 ± 0.0007^{E}	0.038 ± 0.0007^{F}	0.035 ± 0.0006^{G}	0.033 ± 0.0006^{H}
6	Moisture contents	%	85.76 ± 0.84^{A}	84.03 ± 0.63^{A}	80.92 ± 0.45^{B}	76.36 ± 0.13^{C}	72.28 ± 0.49^{D}	68.68 ± 0.66^{E}	66.01 ± 0.73^{F}	64.51 ± 1.37^{F}
7	Total dry matter	%	14.23 ± 0.84^{F}	15.96 ± 0.63^{F}	19.07 ± 0.45^{E}	23.63 ± 0.13^{D}	27.71 ± 0.49^{C}	31.31 ± 0.66^{B}	33.99 ± 0.73^{A}	35.48 ± 1.33^{A}
8	Ash contents	%	0.66 ± 0.010^{A}	0.62 ± 0.006^{B}	0.58 ± 0.011^{C}	0.56 ± 0.000^{D}	0.53 ± 0.011^{D}	0.48 ± 0.010^{E}	0.40 ± 0.010^{F}	0.36 ± 0.010^{G}
9	Total organic matter	%	99.34 ± 0.010^{G}	99.37 ± 0.006^{F}	99.41 ± 0.011^{E}	99.44 ± 0.000^{D}	99.46 ± 0.011^{D}	99.52 ± 0.010^{C}	99.60 ± 0.010^{B}	99.64 ± 0.010^{A}
10	TSS	^{o}Brix	12.84 ± 0.02^{A}	12.18 ± 0.02^{B}	11.68 ± 0.00^{C}	9.66 ± 0.01^{D}	8.73 ± 0.01^{E}	6.45 ± 0.04^{F}	5.77 ± 0.23^{G}	5.12 ± 0.02^{H}
11	Total sugar contents	%	13.37 ± 0.58^{A}	12.99 ± 0.20^{A}	12.66 ± 0.43^{A}	10.25 ± 0.30^{B}	8.86 ± 0.35^{C}	7.51 ± 0.11^{D}	7.79 ± 0.47^{D}	7.70 ± 0.15^{D}
12	Total carotenoids	ppm	135.0 ± 0.80^{A}	128.0 ± 0.98^{B}	125.0 ± 1.02^{C}	102.0 ± 1.02^{D}	114.0 ± 0.97^{E}	105.0 ± 0.92^{F}	99.1 ± 0.54^{G}	93.9 ± 0.97^{H}
13	Chlorophyll a	ppm	0.90 ± 0.057^{A}	0.86 ± 0.021^{AB}	0.83 ± 0.005^{B}	0.75 ± 0.017^{C}	0.69 ± 0.008^{CD}	0.62 ± 0.021^{DE}	0.58 ± 0.010^{EF}	0.54 ± 0.008^{F}
14	Chlorophyll b	ppm	1.74 ± 0.097^{A}	1.66 ± 0.036^{AB}	1.60 ± 0.009^{B}	1.47 ± 0.030^{C}	1.34 ± 0.016^{D}	1.22 ± 0.039^{E}	1.14 ± 0.018^{EF}	1.07 ± 0.016^{F}

SEM = Standard error of the mean of three replicates. (A-H) values in some rows with different subscripts are significant differences, were justified at 95 % confidence level (ANNOVA) by Tukey's HSD ($p < 0.05$).

contents and deterioration rate of fruit. That's why pH is necessary to maintain the internal fluids of fruits. pH of the pulp of *Cavendish basrai* banana from R1 to R8 (8.23 to 4.83) continuously decreased during ripening (Table 4), representing pH of the flesh of banana become more acidic during storage. As observed from titratable acidity (TA), which progressively increased during the storage period from R1 to R8 (0.37 to 0.80 g/100 mL), revealed the indirect relationship with pH. Increase in acidity was due to the development of softness in fruit. It has been reported that when the fruits were rigid, these were less acidic and be converted into more acidic as they softened (Kajuna *et al*., 1997). The acidity of banana is due to several organic acids such as tartaric acid, citric acid, oxalic acid and malic acid. The concentration of malic acid increases three to sevenfold during ripening (Barker and Solomos, 1962). Due to increase of these acids, acidity of banana pulp increases and pH decreases. pH of banana peel stored at room temperature showed reverse trend in comparison to pulp. It was significantly increased ($p < 0.05$) with respect to decay of fruit and correspondingly decreased in TA from R1 to R8, showing indirect relationship between pH and titratable acidity again (Table 5). It was due to the loss of moisture contents with decomposition of peel, and decrease in peel contribution established as fruit rotten starts (Table 3).

Total dissolved solids (TDS) and conductance were used to investigate the balance of water or fluids, and the concentration of solids take up to maintain the proper internal density of fruit or ratio of transfer of suspended particles from peel to pulp. TDS was measured to evaluate the total extent of mobile charged ions, counting salts, minerals or metals and some amount of dissolved organic matters suspended in a sample, which determine the impact of fruit quality. In *Cavendish basrai* banana pulp, regular and significant increasing trend was shown in TDS from unripe to over ripe (R1-R8) (875.3 to 2539.6 ppm) (Table 4). It was observed due to increase in softness and moisture of pulp as expressed on pressing or due to continuous increase in pulp weight. In peel of banana, TDS increased from unripe to ripe (R1-R4) (1390.6 to 1717.3 ppm) and then decreased from ripe to over ripe (R5-R8) (1678.3 to 1136.3 ppm) (Table 5). Changes in peel observed due to alteration of texture. Firmness of peel regularly decreased during ripening along color changes from green to yellow (due to loss in chlorophyll and other pigment contents) and after ripening peel color converted into black from yellow (Table 2). Another possible reason was dehydration with loss in weight due to over ripening and due to transfer of dissolved solids from peel to pulp with transportation of moisture contents.

The results of conductance were closely related to TDS and similar development was observed in both parameters. *Cavendish basrai* banana showed the strong capability towards conductance and it also showed conductance of peel was greater than pulp. During ripening of *Cavendish basrai* banana, conductance showed linear with TDS and continuously increased from R1 to R8 (1.73 to 3.96 mS). Conductance is highly dependent on dielectric properties. It was investigated in fruits, the dielectric properties depend upon the temperature, moisture, frequency and soluble solid contents as a quality factor of fruits (Nelson and Bartley, 2002). From the results of moisture, TDS and soluble solids (Tables 4 and 5), it was revealed that the continuous increase found in these parameters. Thus they are responsible for increase in conductance from R1 to R8 in pulp during storage due to the higher dielectric constant. Water is the major constituent of the foodstuff that contributes to the dielectric constant (Ryynanen, 1995). Similarly, as TDS in peel during storage, conductance was also increased from R1 to R4 (3.13 to 3.54 mS) and decreased as fruit over ripping start from R5 to R8 (3.27 to 1.86 mS).

Similarly, conductance, TDS and % NaCl are also directly correlated to each other and each parameter shows comparable increase and decrease during ripening. Increase in % NaCl was monitored in pulp of banana during storage (Table 4). This is due to increase in moisture contents, the average concentration of minerals increases. The concentration of % NaCl was found relatively low between R1 to R8 (0.028 to 0.056%) as reported by Aurore *et al*. (2009), the low concentration of Na in ripe banana is noticed as compared to K. It can be concluded that due to increase in moisture contents solvated species increases and according to their polarities also increases, which leads to increase in conductance and TDS.

***Total soluble solids (TSS) and total sugar contents*:** The total soluble solids (TSS) indicate the maturity index of fruit. A strong direct relation between TSS and total sugar contents was observed as reported previously with continuous increase from unripe to moldy stage (Marriot *et al*., 1981). Significant increase in TSS (8.63 to 17.64 ^{o}Brix) and total sugar contents (10.39 to 21.29%) were observed in the pulp of banana from stages R1 to R8 (Table 4). It exposed the continuous raise in the level of maturity during ripening. Sugar contents were low during ripening but increased with maturity due to conversion of starch contents into sugars such as glucose, sucrose and fructose (Marriot *et al*., 1981; Kulkarni *et al*., 2011). Similar findings have been reported by Bugaud *et al*. (2009) on two different varieties of banana in which TSS level increased from 3 to 20 ^{o}Brix with respect to ripening along the increase in sugar contents.

TSS (12.84 to 5.12 ^{o}Brix) and total sugar contents (13.37 to 7.70%) decreased from R1 to R8 in the peel of banana (Table 5). This decline was due to experiential drying of peel with over ripening. However, it leads to alteration of color and decomposition of phytochemical compounds due to volatilization and reduction of weight.

***Total carotenoids, chlorophyll a (C_a) and chlorophyll b (C_b)*:** Results of chlorophyll a and b, and total carotenoids are shown in Tables 4 and 5. Pigmentation characteristics in the

pulp increased from R1 to R6 during ripening. Increase in carotenoid contents with maturation and ripeness have been reported previously by Lee and Kader (2000). Rapidly fall down in these contents was observed from R7 to R8. As observed from appearance of banana, significant changes occurred in its texture (Table 2). Peel showed the significant loss of pigments from R1 to R8 due to breakdown of chlorophyll reflected by changing in flesh color (Ward and Nussinovitch, 1996).

Phenolic evaluation

***Total phenolic contents (TPC)*:** Phenolic compounds are considerably present in fruits and vegetables with different antioxidant capacity depending on their composition, number of hydroxyl groups and the prevailing conditions where they are embedded (Heo *et al.*, 2007) that's why remarkable attention is paid to fruits as rich resources of phenolic compounds.

The results of phenolic contents (TPC) are shown in Table 6 and correlated with Figs. 1 and 2. In *Cavendish basrai* banana, TPC in pulp varied on different stages during ripening at room temperature. TPC was found to be increased from stages R1 to R6 (65.50 to 86.63 mg/100 g) and reached at maximum level at stage R6but as fruit started to spoil, decline in TPC was observed. 32.25% increase in TPC was observed from R1 to R6 and 4.15 % decline from R7 to R8 after over ripening. Kondo *et al.* (2005) explained phenolic contents and antioxidant activity decreased in banana during storage. It was suggested that the methanolic extract of banana pulp contains greatest amount of phenolic compounds (21 to 263 mg/100 g) (Sulaiman *et al.*, 2011). The pulp of *Cavendish* sub groups contained TPC around 30 to 60 mg/100 g fresh matter (Verde Mendez *et al.*, 2003). Results explained the direct correspondence in correlation with antioxidant assays including DPPH, RPA and PA.

Elevated level of TPC (76.33 mg/100 g) was observed in peel of *Cavendish basrai* banana (Table 6). It has been reported that peel of banana has significantly higher amount of TPC as compared to pulp (Sulaiman *et al.*, 2011). Similarly, TPC was found in higher amount in peel as compared to pulp at R1. Moreover, continuous decline was observed in TPC by the increases of storage period with the maturity of fruit due to rotten of peel as shown from its color and texture (Table 2). Decaying of TPC was found 35.63% in peel, showing significant changes ($p < 0.05$).

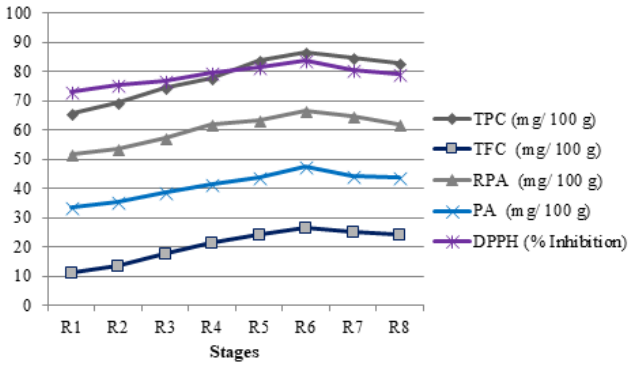

Figure 1. Comparison of antioxidant potential of *Cavendish basrai* banana pulp under various stored stages

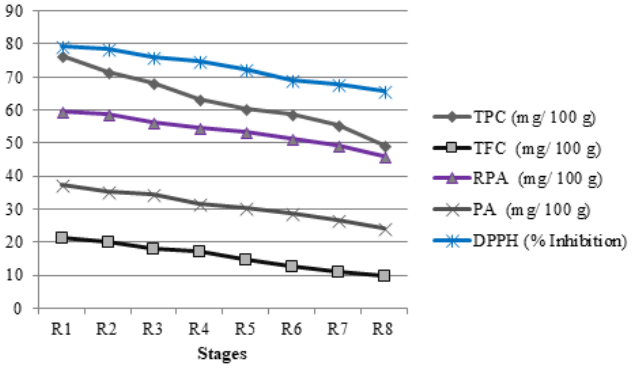

Figure 2. Comparison of antioxidant potential of *Cavendish basrai* banana peel under various stored stages

***Total flavonoid contents (TFC)*:** Flavonoids are extensively distributed in edible plants, involved in photosensitization, energy transfer, control of respiration, photosynthesis, in the

Table 6. Description of antioxidant potential of *Cavendish basrai* banana pulp and peel under various stored stages at room temperature

Sr.	Parameters	Sample	Unit	R1	R2	R3	R4	R5	R6	R7	R8
				Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
1	Total phenolic	Pulp	mg/100 g	65.50±0.36^{G}	69.46±0.35^{F}	74.37±0.25^{E}	77.93±0.55^{D}	83.73±0.32^{C}	86.63±0.30^{A}	84.83±0.11^{B}	83.03±0.41^{C}
	contents (TPC)	Peel	mg/100 g	76.33±0.05^{A}	71.46±0.20^{B}	68.10±0.45^{C}	63.40±0.17^{D}	60.43±0.30^{E}	58.73±0.15^{F}	55.40±0.17^{G}	49.13±0.40^{H}
2	Total flavonoid	Pulp	mg/100 g	11.40±0.17^{G}	13.53±0.20^{F}	17.66±0.11^{E}	21.43±0.20^{D}	24.03±0.21^{C}	26.63±0.05^{A}	25.26±0.15^{B}	24.23±0.12^{C}
	contents (TFC)	Peel	mg/100 g	21.53±0.25^{A}	20.23±0.47^{B}	18.23±0.05^{C}	17.36±0.25^{D}	14.66±0.25^{E}	12.50±0.30^{F}	11.16±0.11^{G}	9.63±0.30^{H}
3	Reducing power	Pulp	mg/100 g	51.50±0.20^{G}	53.6 ±0.34^{F}	57.50±0.17^{E}	61.96±0.30^{D}	63.43±0.35^{C}	66.53±0.35^{A}	64.63±0.23^{B}	61.83±0.11^{D}
	assay (RPA)	Peel	mg/100 g	59.43±0.46^{A}	58.66±1.16^{A}	56.36±0.23^{B}	54.66±0.40^{C}	53.40±0.52^{C}	51.43±0.30^{D}	49.43±0.35^{E}	46.13±0.66^{F}
4	Phosphomolybden	Pulp	mg/100 g	33.63±0.25^{G}	35.33±0.20^{F}	38.50±0.26^{E}	41.66±0.40^{D}	43.66±0.15^{C}	47.70±0.17^{A}	44.46±0.12^{B}	43.60±0.17^{C}
	um assay (PA)	Peel	mg/100 g	37.56±0.28^{A}	35.46±0.37^{B}	34.50±0.30^{C}	31.50±0.26^{D}	30.36±0.21^{E}	28.66±0.20^{F}	26.73±0.55^{G}	24.36±0.21^{H}
5	DPPH assay	Pulp	% Inhibition	73.07±0.35^{G}	75.45±0.53^{F}	76.74±0.22^{E}	79.44±0.26^{D}	81.50±0.05^{B}	83.74±0.33^{A}	80.36±0.18^{C}	79.01±0.31^{D}
		Peel	% Inhibition	79.47±0.26^{A}	78.62±0.18^{B}	75.98±0.28^{C}	74.80±0.13^{D}	72.30±0.15^{E}	69.04±0.23^{F}	67.89±0.23^{G}	65.95±0.08^{H}

SEM = Standard error of the mean of three replicates. (A-H) values in some rows with different subscripts are significant differences, were justified at 95% confidence level (ANNOVA) by Tukey's HSD ($p < 0.05$).

actions of plant growth hormones and growth regulators, morphogenesis and sex determination (Middleton and Chithan, 1993; Harborne and Baxter, 1999). In addition, they also depict the anti-inflammatory, oestrogenic, antimicrobial, antioxidant and antiallergic activities (Middleton and Chithan, 1993; Harborne and Baxter, 1999). Therefore, it is important to investigate the amount of TFC in *Cavendish basrai* banana during ripening with regular intervals.

Results of TFC are given in Table 6 and correlated with Figs. 1 and 2. The flavonoids and their derivatives are the most important and largest group of plant phenolics. *Cavendish basrai* banana pulp contained significant amount of flavonoid contents. The regular increase was found in TFC during maturity from R1 to R6 (11.40 to 26.63 mg/100 g), which was 133.59 % increase. TFC level decreased 9.01 % from R7 to R8 after over ripeness. Similar trend was observed in phenolic contents. Degradation of TFC and TPC after over ripening was due to spoilage of fruit. The correlation in TPC and TFC showed the direct correspondence between them (Figs. 1 and 2). It was also observed the amount of TFC in pulp, has relatively lower as compared to TPC. Our findings are similar to previously reported TFC in the different extracts of banana pulp ranged between 4.70 to 23.7 mg/100 g. In addition, lower amount of TFC was reported as compared to TPC in same cultivar of banana (Alothman *et al.,* 2009).

In the peel of *Cavendish basrai* banana, significant constant decrease was observed in TFC from R1 to R8 (21.53 to 9.63 mg/100 g). It has been investigated that banana contains large quantity of dopamine in both peel and pulp, and its quantity decline with ripening (Kanazawa and Sakakibara, 2000). The concentration of TFC in peel was higher as compared to pulp. Considerable association was observed between TPC and TFC. The % decay of TFC in peel was about 55.27 % relatively higher as compared to decay of TPC during storage, showing flavonoids are more sensitive than phenolic contents.

***Antioxidant evaluation*:** Antioxidant capacity of *Cavendish basrai* banana during storage at room temperature (R1 to R8) with specific time period was evaluated by three different antioxidant assays regarding their measurement theory (Prior and Cao, 1999). The results of antioxidant potential are shown in Table 6 and correlated with Figs. 1 and 2.

Cavendish basrai banana contained the significant quantity of antioxidants in both pulp and peel. Someya *et al.* (2002) has explained that banana is a good source of natural antioxidants. Different stages (R1 to R8) of *Cavendish basrai* banana were used to evaluate the maturity index for utilization of antioxidant potential. Regular trends were observed during analysis of three different antioxidant assays.

In the pulp of *Cavendish basrai* banana, reducing power assay (RPA) was shown the higher antioxidant capacity as compared to phosphomolybdenum assay (PA) during storage at room temperature. Both assays showed the strong linear and significant ($p < 0.05$) correlation. RPA and PA illustrated the similar increasing trend in antioxidant capacity from R1 to R6 and decreasing when fruit decaying started (R7 to R8). Antioxidant potential evaluated by RPA, increased about 29.18 % from unripe R1 (51.50 mg/100 g) to over ripe R6 (66.53 mg/100 g), and decreased 7.06 % as over maturation started from R7 (64.63 mg/100 g) to R8 (61.83 mg/100 g). Similar trend was investigated by PA, 41.83 % increment in antioxidant capacity reached at maturation, and 8.59 % decay found as fruit became muddy. It showed that antioxidant capacity was decreased as decaying of fruit started. Kanazawa and Sakakibara (2000) have explained that bananas are relatively rich in vitamin A (carotene), B (thiamine, riboflavin, B6, niacin) and C (ascorbic acid) at maturity. During storage or processing of fruit products, number of different events occurs, having significant effect on the overall antioxidant activity (Arena *et al.,* 2001). Mostly naturally occurring food antioxidants are unstable, can be substantially lost (Fukumoto and Mazza, 2000).

Antioxidant capacity of *Cavendish basrai* banana peel was relatively higher than pulp at R1 but speedily lost when fruit decaying started. % losses of antioxidant capacity were 22.3% and 35.14% evaluated by RPA and PA, respectively, were showing deteriorative reactions taken place within peel due to degradation of texture. Might be these changes were due to loss of phenolic compounds. Phenolic compounds have diverse antioxidant capacity with respect to their structures, and ripeness process directly affects physiological pathways among antioxidant activity.

Among the three different assays, the DPPH also showed the considerable % inhibition, presenting elevated level of antioxidant potential in banana as observed in RPA and PA. DPPH also illustrated the similar increasing or decreasing manner of antioxidant potential as shown in RPA and PA (Table 6). It was scrutinized that the antioxidant potential in pulp was also retainable during ripening (73.07 to 83.74 % inhibitions) but rapidly decreased as fruit started to spoil (80.36 to 79.01 %inhibitions). It means maximum concentration of antioxidants should not be kept after ripening and fruit should consume as fresh as possible. % Scavenging capacity of *Cavendish basrai* banana peel was shown the regular fell down with respect to storage from R1 to R8 (79.47 to 65.95 % inhibitions) as observed in RPA and PA.

***Conclusion*:** *Cavendish basrai* banana bunches were obtained from the garden located at Department of Chemistry, Federal Urdu University of Arts, Science & Technology, Gulshan-e-Iqbal, Karachi, Pakistan, and the study of changes in nutritional contents was performed during storage at various stages (R1-R8) with regular intervals of three days at room temperature. It was investigated from the physical attributes and chemical composition of *Cavendish basrai* banana that it contained significant amount of nutritional components in the peel and pulp, and also considerable changes taken place in these nutritional components during storage from ripening to over ripening. All parameters were inter-correlated to each

other. It was also observed that ripening reached more rapidly during storage. In our exploration, it was scrutinized that the antioxidant potential of *Cavendish basrai* was retainable during ripening but rapidly decreased in the stored banana at room temperature as fruit rotting started. In order to get maximum concentration of antioxidants, it should not keep after ripening, and consume as fresh as possible.

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