Chapter 3

Physicochemical analysis of pigmented and nonpigmented rice and phytochemical analysis of purple passion fruit

The Chapter 3 has been discussed under two sub-heads as follows:

A) Physicochemical analysis of pigmented and nonpigmented rice cultivars

3.1 Introduction

Rice (*Oryza sativa* L.) is considered as major staple food worldwide. The Ayurvedic Treatise (Indian Materia Medica) records showed that some Indian rice has medicinal properties.[17] Northeastern region of India namely, Assam, Arunachal Pradesh and Manipur grow pigmented rice cultivars. In Arunachal Pradesh, red rice is grown abundantly in some part of the state but it is still considered as underutilized rice cultivars. The categories of rice on the basis of amylose content are high amylose, intermediate amylose, low amylose, and waxy rice types.[10]

Physical properties of different grains are important parameters to determine the quality and conditions for processing and safe storage.[48] Amin et al.[3] also stated that the parameters like bulk density, kernel density and porosity reveal important information to design grain hopper. During heating, starch-rich cereal flour undergo starch gelatinization by melting starch crystallites and produce viscous mass. These thermal properties should be known for novel food development such as tortillas, beverage, pudding and gluten-free snacks.

Pigmented rice are good source of antioxidant compounds *viz.*, flavonoid, anthocyanin, phytic acid, proanthocyanidin, tocopherols, tocotrienols, γ-oryzanol, and phenolic compounds respectively.[12]

Therefore, the present study was conducted to investigate physicochemical and phytochemical analysis of two pigmented (Lingkang ame:LA and Umling ame:UA), and one non-pigmented rice (Pungpo ame:WR) rice cultivars of Arunachal Pradesh, India. Also, investigate about the pasting and thermal properties of flour of three rice was carried out.

Samyor et al. (2016). Phytochemical and antioxidant profile ofpigmented and non-pigmented rice cultivars of Arunachal Pradesh, India, International Journal of Food Properties, 19, 1104–1114.

Samyor et al. (2016). Evaluation of physical, thermal, pasting characteristics and mineral profile of pigmented and non -pigmented rice cultivars. Journal of Food Processing and Preservation, 40, 174–182.

3.2 Materials and methods

3.2.1 Materials

Two red rice (*Oryza sativa* L.) cultivars locally known as Lingkang ame (LA: long grain) and Umling ame (UA: short grain) and one white rice known as Pungpo ame (WR: short grain) were purchased from the farmers of Yingkiong, Upper Siang district and Manigong circle, West Siang district of Arunachal Pradesh, India. All rice samples were cleaned and ground, and the samples were packed in polyethylene bags and stored in ambient temperature.

3.2.2 Proximate compositions

The moisture, ash, total carbohydrate, crude protein, crude fat, and amylose contents were analyzed using standard methods.[5]

3.2.3 Physical properties of rice

3.2.3.1 Moisture and ash content

The moisture and as content (%) of samples were carried out by using standard methods.[5]

3.2.3.2 Axial dimensions

The axial dimensions, *viz.*, length, breadth and thickness, were measured for rice grain with the help of a vernier calliper with an accuracy of 0.01 mm. The geometric mean diameter of grain (*Dg*) and equivalent diameter (*Dp*) of all the three rice grain were calculated using the following expression.[42]

$$D_g = (LWT)^{\frac{1}{3}}$$

Where, *L* is the length, *W* is the width and *T* is the thickness of grain in millimeters.

$$D_P = \left[L \frac{(W+T)^2}{4} \right]^{1/3}$$

The sphericity (Sp) defined as the ratio of the surface area of sphere having the same volume as that of the grain to the surface area of the grain. The sphericity of three rice cultivars was calculated using the following formula.[42]

$$Sp = \frac{(LDT)^{1/3}}{L}$$

Aspect ratio of rice cultivars were (R_a) calculated using following formula [41]

$$\mathrm{R_a} = \frac{\mathrm{W}}{\mathrm{L}}$$

Where W and L are width and length of rice grain, respectively;

Grain volume (V) and surface area (S) was also calculated according to Jain and Bal [30]

$$V = \pi \frac{B^2 L^2}{6(2L-B)}$$

$$S = \frac{\pi B L^2}{(2L-B)}$$

$$B = (WT)^{1/2}$$

To evaluate thousand kernel weights, the grains were measured by counting 100 seeds and then weighing in an electronic balance to an accuracy of 0.001. It was multiplied with 10 to give mass of 1000 kernels.

3.2.3.3 Bulk and true densities

Bulk density was calculated using mass/volume relationship. It was determined by method of Gupta and Das[20] were a cylindrical container of 500 mL in volume was filled with the grain from a height of 150 mm at a constant rate and then weighing the contents. The true density was define as the ratio between the mass of grain and the true volume of the grain, was determined using the kerosene displacement method.[48]

3.2.3.4 Porosity

Porosity is the percentage of void space in the bulk grain which is not occupied by the grain.[58] Porosity was calculated using the relationship between the bulk density and true density as shown in the eq.

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_t} \times 100$$

Where ρ_t is true density and ρ_b is bulk density of the grain (g/ cm^3).

3.2.3.5 Angle of repose

Angle of repose was measured as described by Jain and Bal.[30] A Plywood box measuring 300mm×300mm×300mm with a removable front panel was filled with grains at the desired moisture content and the front panel was quickly removed. Grains were allowed to flow to their natural slope. The angle of repose was calculated from measurements of grain free surface depths at the end of the box and midway along the sloped surface and horizontal distance from the end of the box to its midpoint.

3.2.3.6 Color

The color of the rice cultivars were measured according to Saikia et al.[55] Hunter Colorimeter (Color Lab Ultra scan Vis) was used to measure the color of the rice cultivars. The *L*, a* and b** values were recorded as the mean of three replicates. Hue angle and chroma of all the three rice cultivars were also determined using the following equations.

$$Chroma = [a^{*2} + b^{*2}]^{1/2}$$

$$Hue\ angle = \tan^{-1}(b^*/a^*)$$

3.2.4 Mineral profile

The mineral profiles of the rice cultivars were determined by AOAC.[5]

3.2.5 Pasting properties

Pasting properties of the whole rice flour samples were measured using a rapid visco analyzer (RVA starch master 2 pulverisette instrument). 2 g of sample was taken for viscosity profiles. Rice flour suspensions were prepared using 25 ml distilled water. The sample holding temperature was initially at 50˚C to 95˚C in 3:45 min, a second holding phase was at 95˚C for 2:40 min, a cooling phase from 95˚C to 50˚C in 4 min and a final holding phase at 50˚C for 1 min. The pasting point (PP), corresponding gelatinization temperature (GT), peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), breakdown (BD), and total setback (SBt) were recorded. HPV is the minimum

viscosity at 95˚C and CPV is the final viscosity at 50˚C. BD = PV–HPV and SBt = CPV–HPV.[61]

3.2.6 Cooking characteristics

3.2.6.1 Optimal cooking time

Optimal cooking time of three different rice flours was determined using the AACC approved method.[5]

3.2.6.2 Water uptake ratio

Water uptake ratio of different rice sample was described by the method of Thomas et al.[57] 2 grams of rice samples were added into 20 ml of distilled water and cooked in a boiling water bath for a minimum cooking time. The remaining water contents in the samples were drained out. The adhering superficial water present on cooked rice was further removed by pressing the samples between filter papers. Cooked samples were weighed and the water uptake ratio was calculated.

3.2.7 Texture profile analysis (TPA)

The fresh cooked rice sample was taken directly to perform texture profile analysis. Hardness of cooked rice were measured using a texture analyzer as the modified method described by Tian et al.[59] Briefly, the cooked rice sample was put on the sample table at the centre of the probe in a flat form. A load cell of 50 kg was used. Then the cooked rice sample was compressed using a 2.5 mm diameter cylindrical probe at a test speed of 0.5 mm/s and a control force of 10 g. The deformation level was 60% of original. This process was repeated for three times for each sample.

3.2.8 Differential scanning calorimeter (DSC) analysis

Thermal analysis was performed with a DSC 60 SHIMADZU instrument. About 5 mg of the sample was used in each experiment with measured amounts of water, hermetically sealed, and kept it for equilibrium (~1hr) prior to analysis. Heating was carried out from 30 to 350˚C at the rate of 10˚C/min. The sample was placed in the silver cup, covered with the silver lid, and sealed very carefully with the sealer supplied by the manufacturers. Another empty cup was used after sealing as before, making this the reference (air). The DSC instrument's software was used to calculate the onset (T_o), endset

(T_p), conclusion temperature (T_c) and gelatinization temperature range (R) was calculated as $2\times(T_p - T_o)$ as described by Krueger et al.[34]

3.2.9 X-Ray diffraction (XRD) analysis of rice cultivars

The X-ray diffraction analysis was carried out for three rice flour samples to obtain the X-ray diffraction (XRD) pattern using an X-ray diffractometer (D8 Focus, Bruker AXS,Germany) with acceleration potential of 35 kV with 25 mA current. The scans adjusted for Bragg angle were in the range of 7 to 80˚ on a 2θ scale with a step-size of 0.05. Software used was XRD Commander and Diffrac. EVA. The total area under the curve and the area under each prominent peak was determined using OriginPro 8.0 software and percentage crystallinity formula was given below

% Crystallinity = (Area under peaks/total area)× 100

3.2.10 Fourier transform infrared (FT-IR) spectra of different compounds

Fourier transform infrared (FT-IR) spectra were used for detecting functional groups present in the three different rice cultivars from the state of Arunachal Pradesh. The rice grain was ground into flour and mixed with KBr (spectroscopic grade) powder. Mixer was pressed into pellets for FT-IR measurement in the frequency ranging from 4000 to 400 cm^{-1} and spectra of the materials were obtained at a resolution of 8 cm^{-1}.[35]

3.2.11 Phytochemicals and antioxidant activities

3.2.11.1 Sample extraction

Sample preparation was done according to the method describe by Atala et al.[6] Three rice cultivars (10 g each) were powdered in a grinder. The ground sample was extracted with 100 mL of extraction solvent (75:25 v/v, acetone: water). Extracts were shaken in a water bath at 25˚C for 90 min, and centrifuged in refrigerated condition (SIGMA Laborzentrifugen, 3-18 KS, Osterode, Germany) at 950 g for 15 min. Supernatant was stored at -20˚C for further analysis.

3.2.11.2 Total phenolics content

Modified version of the Folin-Ciocalteu assay described by Slinkard and Singleton [46] was used to determine the total phenolic content in the extracts from the three different rice

cultivars. Gallic acid was used for preparation of standard curve at various concentrations. Independently extract (20 µL each), gallic acid, and blank were prepared and mixed with 1.58 mL distilled water. Folin-Ciocalteu reagent (100 µL) was added to 300 µL of sodium carbonate within 8 min. The samples were vortexed immediately and incubated for 30 min at 40˚C. The absorbance was measured at 765 nm in ultraviolet-visible (UV-VIS) spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India). The phenolic content was expressed in mg GAE/100 g.

3.2.11.3 Total monomeric anthocyanins

The monomeric anthocyanin content of extracted solutions were determined using the pH differential method [21] Absorbance was measured at 515 and 700 nm. Anthocyanin was calculated as cyanidin-3-glucoside using a molar extinction coefficient of 26,900 and a molecular weight of 449.2.

$$A=(A_{515}-A_{700})pH1-(A_{515}-A_{700})pH4.5$$

$$\text{Anthocyanin content (mg/L)}=\frac{(A\times MW\times DF)}{\varepsilon\times L}\times 1000$$

Where, DF was dilution factor, MW cyanidin-3-glucoside molecular weight (449.2) and ε molar absorptivity (26,900). All measurements were done in duplicates.

3.2.11.4 DPPH scavenging activity

DPPH radical-scavenging activity of rice extracts were evaluated according to Brand-Williams [11] method. Briefly, extracts (100 µL) were taken and added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). After 30 min of incubation period, absorbance reading was taken at 517 nm using a spectrophotometer (Chemito, Spectrascan UV 2600, double beam UV-VIS Spectrophotometer Thermo Scientific). The percentage of radical-scavenging activity was calculated using the formula:

$$\text{Radical scavanging activity (\%)}=\frac{A_o-A_s}{A_o}\times 100$$

Where, A_o is absorbance of control blank, and A_s is absorbance of sample extract.

3.2.11.5 Metal chelating activity

Metal chelating activity was done as per method described by Dinis.[16] Ferric chloride (50 µL of 2 mM) was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) and 0.2 ml of 5 mM ferrozine solution was added. The mixture was vigorously shaken and kept at room temperature for 10 min. The absorbance reading was taken at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as $[(A_o- A_s)/A_s] \times 100$, where A_0 was the absorbance of the control and A_s was the absorbance of the extract. EDTA was used as standard.

3.2.12 Rverse phase-High-performance liquid chromatography (RP-HPLC) analysis of phenolic compounds

3.2.12.1 Sample preparation

Sample (50 g) was mixed with 0.5 g ascorbic acid and added with 100 ml of 80% methanol followed by filtration (Whatman no.2). The excess amount of the methanol and water was evaporated. Sample was washed in a separating funnel with hexane to remove carotenoid and other nonpolar compounds. Volume was made up to 50 mL using distilled water and pH was adjusted to 7.0 and 10 mL sample were taken for further analysis.

3.2.12.2 Detection

A HPLC system (Ultimate 3000 Liquid Chromatography Systems) with an ultimate 3000 variable wavelength UV detector at 215 nm and Ultimate 3000 pump were used for analysis. The column was Acclaim 120 C18 column (5 µm, 120Å) with a size of 4.6×250 mm. The HPLC analysis was performed with 20 µl of sample injected into the column. The solvent system was eluent A-acidified water pH adjusted to 2.64 with the dil. hydrochloric acid and eluent B–acidified water: acetonitrile (20:80). A constant flow rate of 1.5 mL/min with a gradient run was maintained. The quantification of polyphenolic compounds was quantified using the calibration curves of their respective standards. The software chameleon ver. 6.80 was used for analyzing data.

3.2.13 Statistical analysis

Experiments were carried out in triplicates and presented as mean ± standard deviation of mean using SPSS version 16. The data were statistically analyzed by Duncan's multiple range tests at 5% significance level. The Origin 8.5 (Origin Lab Corporation, Northampton, USA) software was used for statistical analysis.

3.3 Results and discussion

3.3.1 Nutritive quality

The data presented in Table 3.1 shows that the LA cultivar content significantly ($p \leq 0.05$) high amount of moisture (11.13 %) than UA and WR cultivar. Ash content was found highest in the UA 1.33 %, followed by LA 0.97 % and lowest in the WR 0.93 %. Fat content was significantly ($p \leq 0.05$) high in UA 2.60 % followed by LA (1.80%) and WR (1.77%). Very low amylose content (%) was observed in three rice were LA (6.46±0.026), UA (11.86±0.04) and WR 5.30±0.04 respectively. According to IRRI, rice varieties were classified into five groups as per their amylose content: waxy (1–2%), very low (2–9%), low (10–20%), intermediate (20–25%), and high (25–33%).

Table 3.1 Proximate composition of rice cultivars

Parameters	LA	UA	WR
Moisture (% w.b)	11.13 ± 0.02^{a}	11.01 ± 0.005^{b}	11.01 ± 0.005^{b}
Ash (% d.b)	0.97 ± 0.01^{b}	1.33 ± 0.57^{c}	0.93 ± 0.05^{a}
Fat (% d.b)	1.80 ± 0.20^{b}	2.60 ± 0.19^{c}	1.77 ± 0.06^{a}
Protein (% d.b)	1.95 ± 0.02^{c}	0.24 ± 0.03^{b}	0.12 ± 0.02^{a}
Carbohydrate (% d.b)	79.01 ± 0.16^{b}	81.37 ± 0.93^{c}	76.44 ± 0.59^{a}

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

Values expressed as mean ± SD (n=3)

3.3.2 Physical properties

Axial dimensions (mm), geometric mean diameter (mm), sphericity index (%), moisture content (%) and ash content (%) of three rice cultivars were varied significantly ($p \leq 0.05$). Moisture and ash content ranged from 11.00 to 11.50 % (db) and 0.93 to 1.33% (db) respectively. Physical properties of three rice cultivars are presented in Table 3.2. Average

length (l) of rice grain varied from (UA) 5.37±0.24 mm to (LA) 6.80±0.34 mm while the average breadth/width ranged from (UA) 3.47±0.58 to (WR) 3.81±0.02. Therefore, it is significantly ($p \leq 0.05$) longer than the two other grain samples. Equivalent diameters of three samples were varied from (UA) 4.10±0.01 to (WR) 4.56±0.04. Sphericity (%) of grains ranges from (LA) 0.66±0.01 to (UA) 0.76±0.03 %. The bulk and true density of three different rice cultivars were in the ranged of (UA) 0.37±0.01 to (WR) 0.37±0.04 g/cm^3 and (UA) 1.06 ± 0.11 to (WR) 1.56±0.11 g/cm^3 respectively. Bulk density among the three cultivars were differ significantly ($p \leq 0.05$). Angle of repose of different grains varied significantly ranged from (UA) 39.19±0.66˚ to (LA) 43.23±0.13˚. Angle of repose value ranged from WR (41.98˚) to LA (43.23˚) shows a good flowability and handling properties. The result was supported by previous literature which confirmed that a material with an angle of repose between 40˚ and 45˚ are free-flowing and powders with repose angles above 50˚ are very cohesive and could cause handling problems.[4] Porosity of rice samples were varied from (UA) 65.76 to (WR) 75.91 (%).Weight of 1000 grain (g) ranged from (LA) 18.67±0.03g to (WR) 22.04±0.02g. Aspect ratio of three samples were varied between (UA) 0.93±0.01 to (LA) 1.03±0.01. Surface areas (mm^2) were ranged from (UA) 45.32±4.08 to (WR) 54.99±2.02. Grain volume (mm^3) of the three different samples was ranged from (UA) 24.99±1.86 to (WR) 35.65 ±1.01. Weight of 1000 grains (g) LA, UA and WR were ranged from 18.67-22.04g. The bulk and true density of three different rice cultivars (LA, UA and WR) showed no significant differences between the cultivars at the 0.05% probability. Thousand grain weights (g), angle of repose and porosity of LA, UA and WR were varied significantly. It was revealed that all the rice variety was a good source mineral as a micronutrient.

L, a*, b** values of three different rice cultivars were shown in the Table.3.2 L* values of the rice flours which indicates whiteness /lightness, varied from 79.83 ±0.05 (WR) to 44.52 ±0.17 (LA). The reason may be because of (WR) has whitish bran than other two grains. The positive a* values for redness ranged from (WR) 0.351 to (UA) 4.192. (UA) have highest a* values, it may be due to more reddish external layers color than other two rice variety. The yellowness b* value was in the ranged from (UA) 5.98 to (WR) 9.06. Chroma of three different samples were ranged from (LA) 7.26±0.05 to 9.12±0.06 and hue angle varied from (UA) 54.59±0.53 to (WR) 87.62 ±0.13. *L*, a*, b** color parameters varied from sample to sample. UA cultivar exhibited higher degree of

redness and yellowness than the LA and WR. Mineral profiles of the rice cultivars were analyzed.

Table 3.2 Physical properties of raw rice cultivars

Properties	**LA**	**UA**	**WR**
Length (mm)	6.60±0.34^{b}	5.37±0.24^{a}	6.73±0.30^{c}
Breadth (mm)	3.73±0.11^{b}	3.47±0.58^{a}	3.83±0.02^{c}
L/B	1.76±0.01	1.54±0.02	1.75±0.01
Equivalent diameter (mm)	4.50±0.04^{b}	4.10±0.01^{a}	4.56±0.04^{c}
Sphericity (%)	0.66±0.01^{a}	0.76±0.03^{c}	0.67±0.03^{b}
Bulk density (g/cm^3)	0.372±0.01^{a}	0.371±0.03^{a}	0.378±0.04^{a}
True density (g/cm^3)	1.26±0.05^{b}	1.06±0.11^{a}	1.56±0.11^{c}
Porosity (%)	71.03±0.82^{b}	65.76±0.85^{a}	75.91±0.08^{c}
Angle of repose (deg.)	43.23±0.13^{c}	39.19±0.66^{a}	41.98±0.10^{b}
Weight of 1000 grain (g)	18.67±0.03^{a}	20.96±0.03^{b}	22.04±0.02^{c}
Aspect ratio	1.03±0.01^{b}	0.93±0.01^{a}	1.02±0.02^{b}
Surface area (mm^2)	53.54±2.23^{b}	45.32±4.08^{a}	54.99±2.02^{c}
Grain volume (mm^3)	34.12±1.27^{b}	24.99±1.86^{a}	35.65±1.01^{c}
Color			
Chroma	7.26±0.05^{a}	8.22±0.10^{b}	9.12±0.06^{c}
*L**	44.52±0.17^{a}	59.25±0.17^{b}	79.83±0.05^{c}
*a**	3.51±0.02^{b}	4.19±0.09^{c}	0.35±0.10^{a}
*b**	7.50 ±0.15^{b}	5.98±0.29^{a}	9.06±0.12^{c}
Chroma [a*^{2} +b*^{2}]$^{1/2}$	8.28±0.05^{a}	7.30±0.10^{b}	9.06±0.06^{c}
Hue angle tan^{-1}(b*/a*)	64.85±0.08^{b}	54.84±0.53^{a}	87.78±0.13^{c}

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

3.3.3 Mineral profile

The concentration of elements such as Al, Ca, Cu, Cr, Fe, K, Mg, Mn, Mo, Na and Zn found in rice samples are shown in Table 3.3. Out of eleven minerals, Al (0.97 mg/100g), Cr (0.19mg/100g), Mg (54.25mg/100mg), K (29.00 mg/100g), Zn (0.65 mg/100g), Na (19.54 mg/100g) and Ca (9.55 mg/100g) concentration were observed highest in (LA) Mo, Fe, Mn and Cu concentration were recorded highest in UA (Table 3.3). The mean values for most elements were consistent and similar to the result published previously.[49]

Table 3.3 Mineral profiles of three rice cultivars determined by atomic absorption spectroscopy (AAS)

Mineral (mg/100g)	LA	UA	WR
Al	0.97	0.57	0.74
Cr	0.19	0.16	0.16
Mg	54.25	52.39	45.91
Mo	0.09	0.04	0.02
Ca	9.55	5.74	8.60
Fe	1.45	1.72	0.25
K	29.00	21.73	19.48
Zn	0.65	0.46	0.47
Cu	0.27	0.36	0.27
Na	19.54	16.60	13.72
Mn	0.25	0.33	0.24

3.3.4 Pasting properties

Pasting properties of LA, UA and WR flours are shown in Fig 3.1 and different viscogram data of rice cultivars were reflected in the Table 3.4. Among three rice cultivars UA (90.5± 0.25) showed significantly ($p \leq 0.05$) higher pasting temperature than LA, (83.7˚C ± 0.32) and WR (77.7˚C ± 0.18) which indicates the minimum temperature needed to cook the rice flour. Previously, Huaisan et al.[25] reported that in the rice starch, pasting temperature (PT) was ranged from 79.1˚C to 79.5˚C. Peak viscosity (PV) is the maximum viscosity attained by gelatinized mixture during heating in water i.e. water holding capacity of the mixture. PV was observed highest in WR (2767.6± 1.5 cP) followed by LA (1804±0.57cP) and UA (1601±0.57cP). It may be because of the higher damage caused to starch during dry grinding process. Final viscosity shows the ability of starch to form viscous paste. Final viscosity of samples was varied significantly, ranged from (LA) 2709 to (UA) 3477 cP. Variation in final viscosity might be due to the variation in amylose molecules and its amount.[40] Breakdown of any mixture can be depending on temperature, degree of mixing and shear stress. The breakdown viscosity of the flour samples were varied significantly ($p \leq 0.05$) from (UA) 17 to (LA) 43. Higher the breakdown in viscosity, lower the ability of starch sample to withstand heating and shear stress during cooking.[2] Break down value indicates the heat stability of starch at 95˚C. Therefore, low BD value indicates thermal stability.[37] In UA, break down (cP) results shows the lowest value i.e 17 cp. Therefore, it can be concluded that UA can be an ideal sample to withstand the heating and shear stress

during cooking. Setback viscosity results in rearrangement of amylose molecules that have been leached out from the swollen starch granules during cooling.[32] It is a measure of gelling ability or retrogradation tendency of the starch. Setback viscosity of three rice cultivars were ranged from (WR) 591 to (UA) 1876 cp. The paste properties of the mixture can provide information about the organoleptic and functional properties of rice and thus influence the type of formulations rice flour can be used in the future.[1] Guha et al.[19] reported that the viscosity of a completely gelatinized starch slurry decreases during heating. Inglett et al.[27] also stated in their study that the pasting curves of the quinoa, oat products and their composites shows dissimilar patterns. Initially, pasting viscosity shows high (~223 RVU) and sharp viscosity around (~20 RVU min $^{-1}$) at initial 11-min heating period at ~90˚C followed by a rapid decrease in viscosity (69 RVU min^{-1}) to ~20 RVU during continued heating to 95˚C at 15 min. According to Tester and Morrison [56] pasting enhance the changes that occur after gelatinization due to further heating and leads to further swelling of granules, leaching of molecular components from the granules and finally, disruption of granules especially with the application of shear forces. Ding et al.[15] also stated that the change of swelling power of ozone-treated waxy rice starch was due to data of molecular size distribution, which further explained the change of pasting property.

Table 3. 4 Viscography parameters of three rice flour

Pasting properties	**LA**	**UA**	**WR**
Pasting temperature(°C)	83.70 ± 0.32^{b}	90.5± 0.25^{c}	77.7± 0.18^{a}
Peak Viscosity(cP)	1804±0.57^{b}	1601±0.57^{a}	2767.6± 1.5^{c}
Hold viscosity (cP)	1762.30± 1.50^{b}	1583.3±0.57^{a}	2743.3±0.57^{c}
Final Viscosity (cP)	2709± 1.54^{a}	3476.6± 1.52^{c}	3361±1.73^{b}
Break Down (cP)	43± 1.52^{c}	17±1.00^{a}	26±1.00^{b}
Set Back (cP)	905±0.57^{b}	1876±0.57^{c}	591±0.57^{a}

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

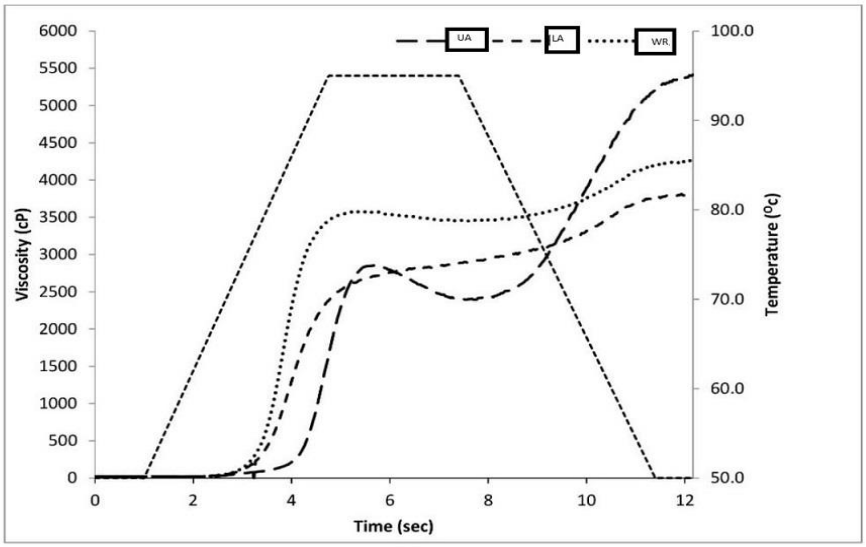


Fig.3.1 Viscosity profile of three different rice cultivars

3.3.5 Cooking characteristics

The rice cultivars shows significant ($p \leq 0.05$) different in optimal cooking time (min), ranged from (WR) 21.3- (UA) 31.16 minutes (Table.3.5). UA shows the highest cooking time (31.16) min than LA (26.48min) and WR (21.3min). It may be due to the variation in amylose content and size of rice sample. Hogan and Plank [24] suggested that the hydration characteristics of rice influenced by variety and size of grain. Water uptake properties of rice grain directly relate with cooking time of the grain. Arns et al.[8] reported that the longer cooking time shows higher internal restructuring of the grain with increasing time of heat–moisture treatment, requires longer time and a greater amount of water for their cooking. Highest water uptake properties was found in UA (3.65±0.01) and hence, optimal cooking time (31.16±0.01 mins) was also highest in UA. Sozer et al.[52] also reported longer the optimal cooking time higher the water uptake capacity.

Table 3.5 Cooking characteristics of three rice grain samples

Properties	**LA**	**UA**	**WR**
Optimal cooking time (min)	26.48±0.02[b]	31.16±0.14[c]	21.3±0.01[a]
Water uptake ratio (%)	3.06±0.01[b]	3.65±0.03[c]	2.66±0.02[a]

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

3.3.6 Texture profile analysis (TPA)

Texture profile analyser (TPA) of cooked rice was carried out for three different samples. The data (Table 3.6) revealed that the cooked UA (3.55±0.84g) rice had higher hardness value than the cooked LA (2.13±0.198g) and WR (3.52 ± 0.97g). It may be attributed to the presence of higher amount of amylose in UA variety than other two varieties. In a previous study, Yu et al.[60] stated that the hardness was positively correlated with the amylose contained in rice grain. Rice with higher amylose content was liable to leach more into the cooking water and formed a coating on rice grain, which increase the hardness.[39] In a study conducted by Katekhong and Charoenrein [33] stated that the increasing ageing duration and the number of freeze-thaw cycles, leads to the significant ($P \leq 0.05$) decreases in stickiness of the cooked rice. According to the Chrastil [13] and Chrastil and Zarins [14] stated about the possible factors contributing to the harder texture of cooked rice. During rice grain ageing number of disulphide bonds and the high molecular weight peptide subunit of the storage protein oryzenin increases. Apparently, disulphide linkage might retard water uptake by granules. Texture profile such as hardness (g), springiness (length/length) and cohesiveness of cooked rice had significant variation.

Amylose, on the other hand, is easier to retrograde and increased the hardness of the cooked rice in a short period. Springiness (length/length) is a measure of how much the gel structure is broken down by initial compression. Springiness value of three rice samples ranged from (LA) 0.37±0.10 to (WR) 0.38 ±0.02 (length/length) and cohesiveness from (LA) 0.17±0.17 to (UA) 0.23±0.03 were found to be non-significant. These changes may be due to the variation in amylose and amylopectin in rice cultivars, responsible for variation in gel network formed in rice during temperature cooking.

Although, there was less difference in springiness in three rice cultivars but UA (0.39±0.84) possess higher gel structure than others. Huang et al.[26] also discussed that a high springiness appears as a gel structure is broken into few large pieces during the first TPA compression, whereas low springiness results from a gel breaking into many small pieces.

Table 3.6 Texture profile analysis (TPA) of cooked rice

Properties	**LA**	**UA**	**WR**
Hardness (g)	2.13± 0.19[a]	3.55± 0.84[b]	3.52± 0.97[b]

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range tests.

3.3.7 Differential scanning calorimetry (DSC) analysis

Fig.3.2 showed the differential scanning calorimetry (DSC) thermograms of three rice cultivars. The thermal properties (melting point) of cooked rice were studied using DSC and summarized in Table 3.7. For endothermic enthalpy, heating was carried out from 30 to 350˚C at the rate of 10˚C/min. Among the three rice flour the onset (T_o), peak (T_p) and endset (T_c) value were highest in the UA (77.12, 104.61 and 140˚C) and lowest was found in the WR cultivar (67.27, 93.53 and 117.92˚C) as shown in the Table 3.7. The reason of high onset temperature in the UA may be because of bran layer losses during hulling which contained bran oil. Previously the authors have reported that defatting marginally increased the melting points.[50] Among three samples, highest reaction rate occur in the (UA) 104.61˚C and lowest in (WR) 93.53˚C. Differences in the range of T_o, T_p and T_c in three rice cultivars may be attributed by differences in amylose content, starch structure, amylose to amylopectin ratio, the degrees of heterogeneity of crystallites within granules, and the content of amylose-lipid complex.[31] Moreover, high amylose starches with longer average chain exhibit higher transition temperatures. For wheat, rice and maize previous researcher [53] was reported the onset (T_o), peak (T_p) and conclusion (T_c) temperatures as 54, 69 and 86˚C (wheat) 66, 82 and 100˚C (rice) and 67, 78 and 95˚C (maize) respectively. Sodhi et al. [51] also reported the transition temperatures of Basmati cultivars varied between 66.25-74.70˚C. In thermal properties onset (T_o), peak (T_p) and endset (T_c) value of UA cultivar were shown higher than WR and LA. The above results could provide important

information for the utilization of rice cultivars and to develop new food product. In thermal properties onset (T_o), peak (T_p) and endset (T_c) value of UA cultivar were shown higher than WR and LA.

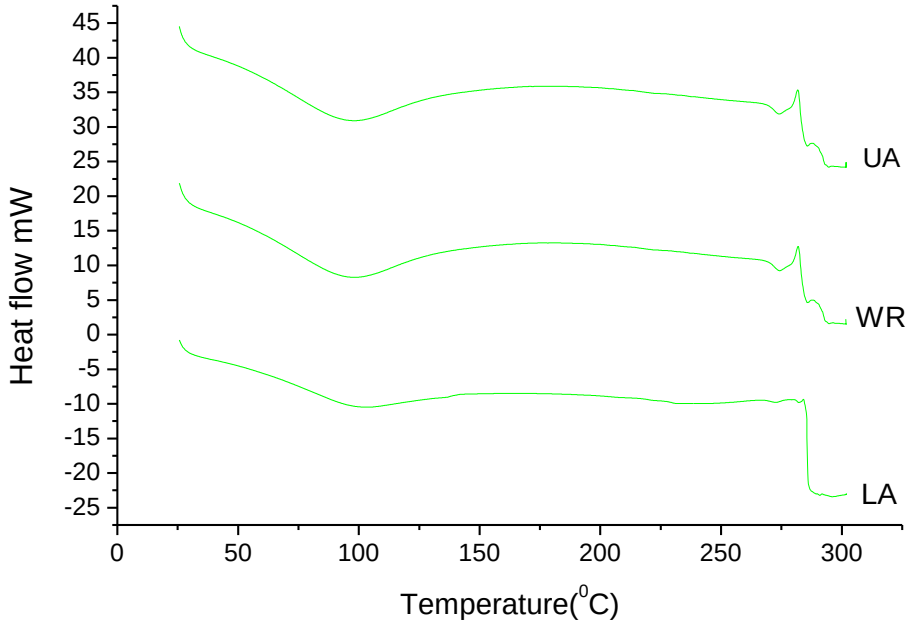
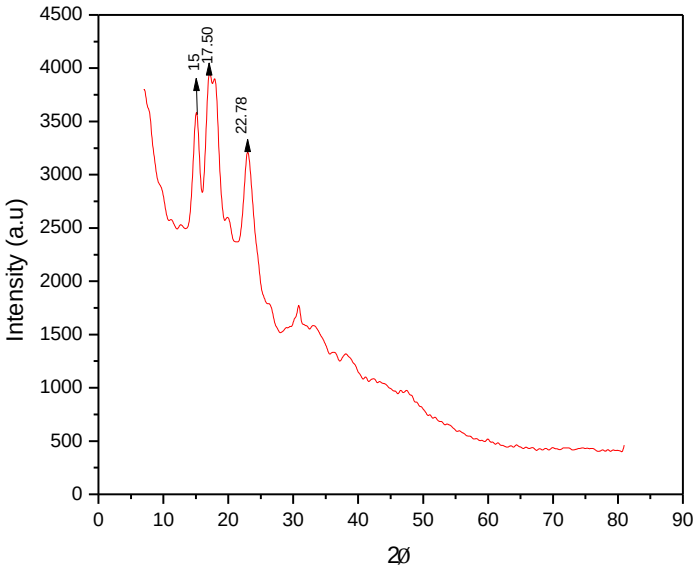


Fig 3.2. Differential scanning calorimetry (DSC) graphs of three rice cultivars **Table 3.7** DSC thermograms of rice flours

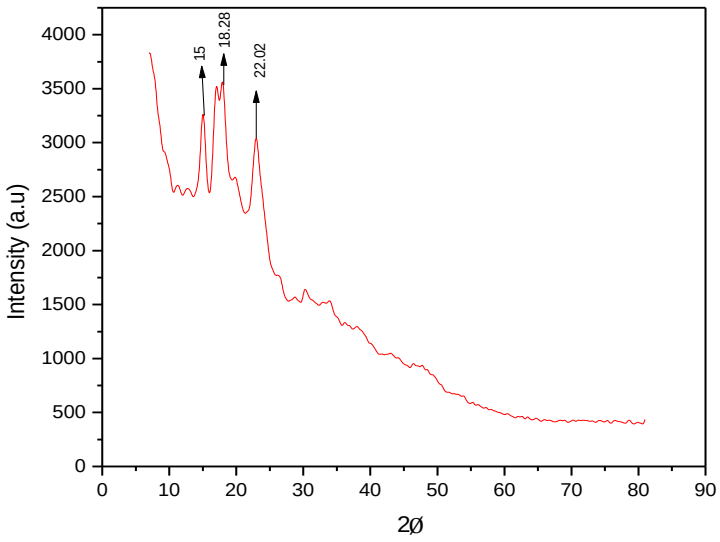
Types	**Gelatinization temperature (˚C)**			
	(To)	***(Tp)***	***(Tc)***	**R**
LA	68.51± 0.02[b]	97.70±0.20[b]	123.91± .23[b]	58.38±0.17[c]
UA	77.12± 0.02[c]	104.61±0.33[c]	141.66±1.52[c]	54.8± 0.16[b]
WR	67.27±0.015[a]	93.53± 0.60[a]	117.92± .03[a]	52.71±0.21[a]

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range tests.T_o: onset temperature; T_p: peak temperature; T_c: endset temperature.

3.3.8 X-Ray Diffraction (XRD) analysis of rice flour

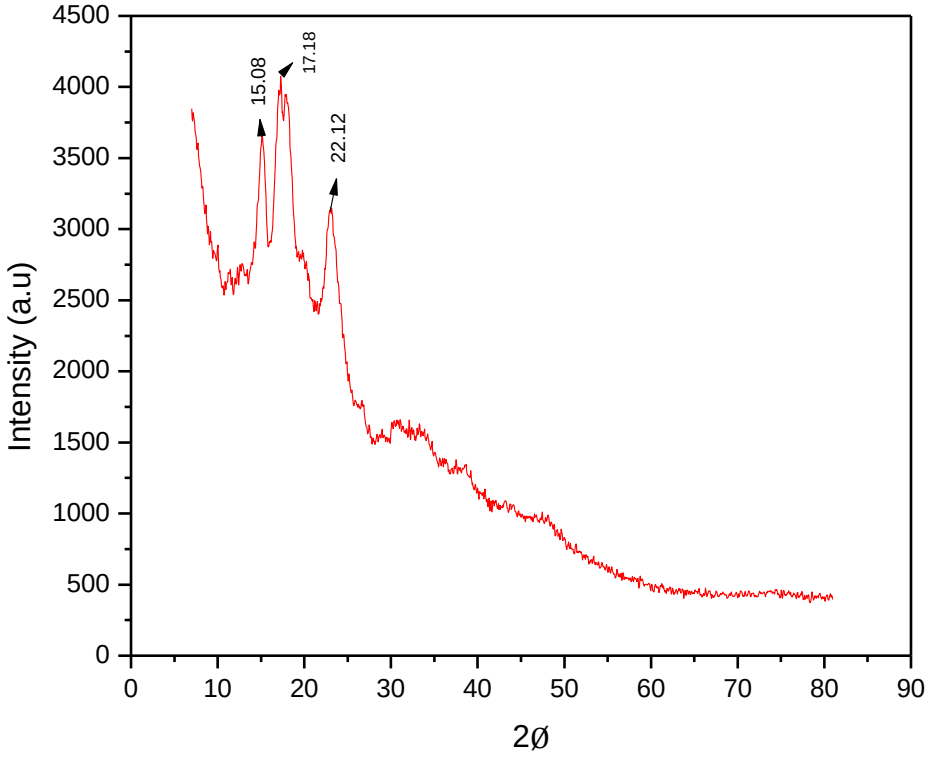


(LA)



(UA)

Contd



(WR)

Fig. 3.3. X-ray diffraction of three rice flour a) LA b) UA and c) WR

In the Fig. 3.3., the rice flour of three sample revaled the presence of A type pattern in the granular level. The angles obtained were in the ranged of 15.02-23 θ which usually present in the cereal crop. The % crsytallinity of the LA, UA and WR were 61.16 ,70.39 and 59.78 %.

3.3.9 Phytochemicals and antioxidant activities

3.3.9.1 Total phenolic content

Total phenolic content and the anthocyanin content of three cultivars are shown in Table 3.8. The phenolic compound and anthocyanin content of three rice cultivars varied significantly. The phenolic contents in the white rice (WR) and the two red types rice (LA) and (UA) ranged from 142-349.30 mg GAE/100 g and anthocyanin content ranged from 1.34-12.79 mg cyanidin-3-glucoside Eq/100g respectively.

3.3.9.2 Chelating activity

It was observed from the Table 3.8 that UA (77.53 µg/mL) has the highest iron chelating activity than LA (72.33 µg/mL) and WR (58.92 µg/mL). It might be due to the presence of higher amount of phenolic compounds which reacted with iron and disrupted the red color complex formation. Therefore, measurement of color reduction, allows the estimation of the chelating activity of the sample.[9] Chelating activity of rice extracts depends upon concentration of extract. From the Fig. 3.3(b) it was also observed that there was strong correlation between iron chelating activity and concentration of UA and LA sample. In general ferrozine can quantitatively form complexes with Fe^{2+} but for presence of phenolic compounds which act as chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased with the sample concentrations.[43] The UA cultivar showed significantly ($p \leq 0.05$) the high amount of chelating activity compared to LA and WR rice cultivars.

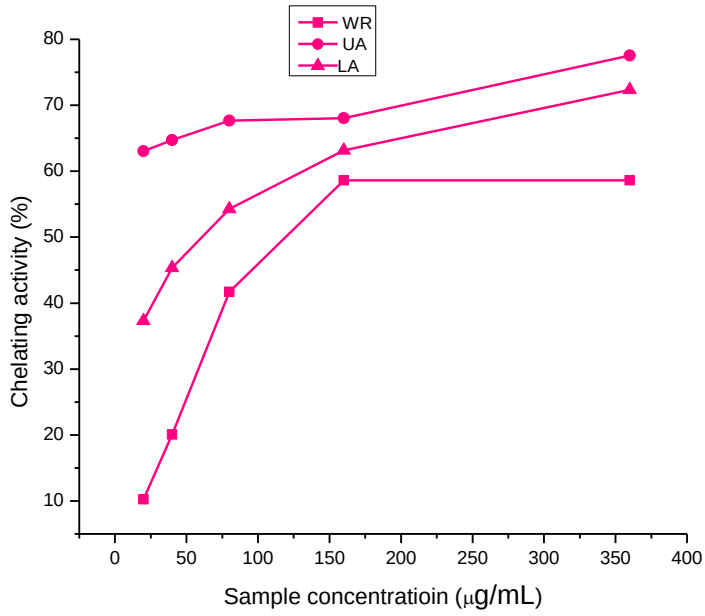
3.3.9.3 DPPH scavenging activities

It was observed from Table 3.8, the DPPH scavenging activity was highest in UA (88.48 %) followed by LA (76.97%) and lowest in PA (68.87 %). It may be attributed to the presence of higher amount of phenolic compounds. Fig 3.3. Shows that DPPH scavenging activities of all the three samples were strongly dependent (R^2 value of LA 0.86, UA 0.71 and PA 0.93) on the concentration of the sample. It could be due to marked effect of phenolic compounds of pigmented rice on DPPH scavenging activity. The phenolic compounds react with deep violet color solution of DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) and convert it to 2, 2-diphenyl-1-picrylhydrazine with decolonization and measurement of color reduction allows to estimate the DPPH scavenging activity of the samples.[11] Similar type of result has been observed by numbers of researchers.[29, 23]

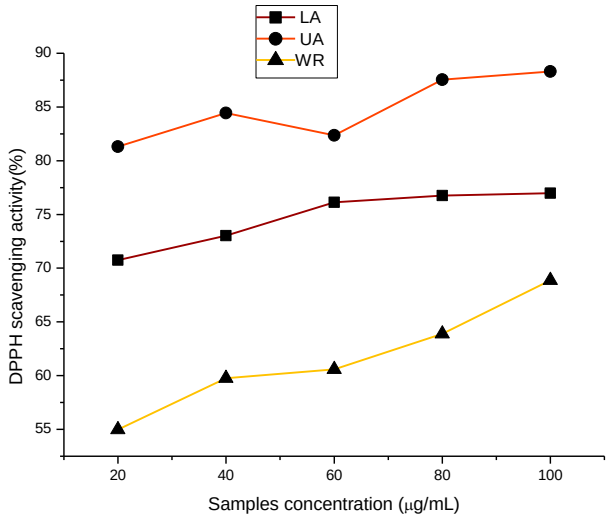
Table 3.8 Antioxidant activity of three rice cultivars

Concentration (µg/mL)	**LA**	**UA**	**WR**
Metal chelating activity			
20	37.31±0.01^{b}	63.04 ±0.01c	10.23±0.02^{a}
40	45.35± 0.01^{b}	64.72±0.02^{c}	20.07± 0.01^{a}
80	54.24± 0.02^{b}	67.60±0.08^{c}	41.73±0.01^{a}
160	63.17± 0.02^{b}	68.04±0.03^{c}	58.58±0.03^{a}
360	72.33± 0.02^{b}	77.53±0.01^{c}	58.92±0.2^{a}
DPPH scavenging activity			
20	70.63±0.13^{b}	81.97±0.27^{c}	54.97±0.15^{a}
40	73.32± 0.10^{b}	84.78±0.13^{c}	59.62±0.07^{a}
60	76.14± 0.04 ^{b}	82.36 ±0.19^{c}	60.15±0.15^{a}
80	76.76±0.001^{b}	87.55±0.1^{c}	63.296±0.3^{a}
100	76.97 ±0.15^{b}	88.48±0.11^{c}	68.87±0.15^{a}

Values expressed as mean ± SD (n=3). Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.



(a)



(b)

Fig.3.4 Scavenging capacity of three (LA, UA and WR) rice cultivars (a) Chelating activity and (c) DPPH activity

3.3.9.4 Fourier transform infrared (FT-IR) spectra analysis

The Fourier transform infra-red spectrum of three rice cultivars are shown in Fig.3.4 (a, b and c). It was observed from the figures that the broad band of LA varied from 1455.51 to 3643.40 cm^{-1}, UA 1650.63 to 3751.16 cm^{-1} and WR 1655.63 to 3874.61 cm^{-1}. Intense characteristic peak at approximately in the region of 3600 to 3700 cm^{-1} and 3200–3500 cm^{-1} have been seen in all the three rice cultivars. Broad band at 3610–3640 cm^{-1} and 3200–3500 cm^{-1} is attributed to –OH stretching vibrations, which represents possibly the presence of phenolic OH.[45] On the other hand, a weak C–H stretching band at around 2891 to 2937 cm^{-1} was observed in the rice cultivars, which represents the asymmetrical and symmetrical stretching vibration of hydroxyl group, respectively. The weak C–H stretching band in FT-IR spectrum was the plausible reason for the presence of phenolic acid in the rice cultivars.[7, 38] The presence of carbonyl (C=O) linkages were confirmed from the peaks at 1076 and 1162 cm^{-1}. The FT-IR band of C=O stretching was mainly due to the presence of carboxyl (-C=O) group of phenolic compounds of the pigmented rice cultivars. The bands approximately in the regions of 3400, 2930 and 1650 cm^{-1} are characteristic of a carbohydrate ring.[54]

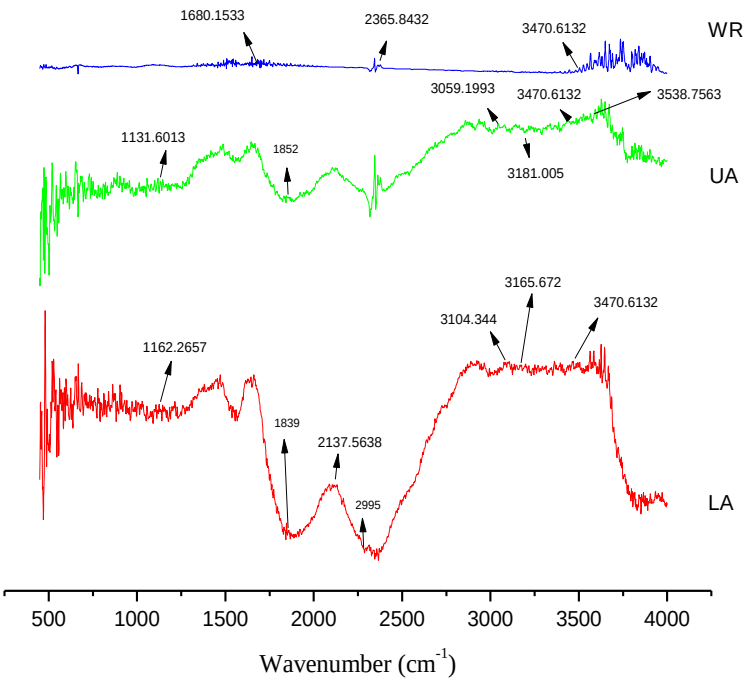


Fig. 3.5 Typical FTIR spectra of (a) LA (b) UA and (c)

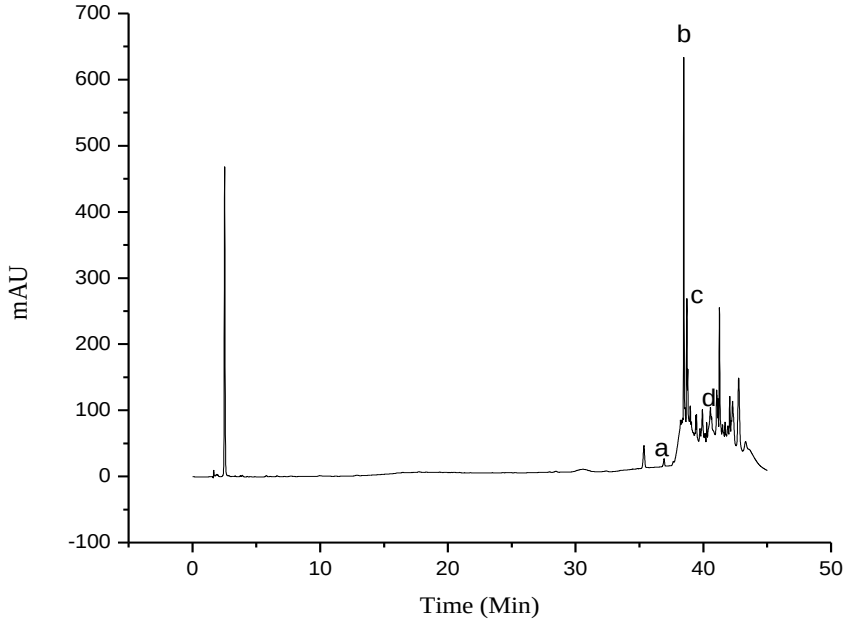
3.3.9.5 Reverse Phase-High-performance liquid chromatography (RP-HPLC) analysis

The identification of phenolic compounds by RP-HPLC revealed differences in the, phenolic fraction profile among the rice cultivars (Fig. 3.5a, b and c). The distribution of phenolic acids in all samples is illustrated in Table 4. The main phenolic acids identified in all three cultivars were salicylic acid, apigenin and quinic acid. The salicylic acid was present at 302.06 ±0.03, 44.50 ± 0.01 and 231.94±0.02 mg/L level in UA, LA and WR respectively. The highest amount apigenin was detected in UA (7.03±0.01 mg/L) followed by LA (0.42±0.02 mg/L) and WR (0.49±0.01 mg/L). Quinic acid was detected in all the three rice cultivars in high amount. It was found that UA, LA and WR had 255.46±0.01, 611.46 ± 0.01 and 133.92±0.02 mg/L of quinic acid respectively. Quercetin was only detected in LA (33.27±0.01 mg/L) cultivar and there was no detectable amount of ferulic, gallic and caffeic acid in LA. UA rice cultivar contained detectable amount of gallic acid (0.98±0.04mg/L) and ferulic acid (17.21±0.02 mg/L) whereas white rice cultivars contained only ferulic acid (15.18±0.01 mg/L) and very less amount of caffeic acid (1.83±0.02 mg/L). The concentration of total phenolics in the cultivars is associated with the antioxidant activities [28,36] which has potential benefits such as reduction of oxidative stress, cardiovascular problems, blood and lipids related diseases.[36] The rice cultivars used in the present study have various types of phenolic acid content. Salicylic, caffeic, quinic, apigenin, ferulic, gallic and quercetin acid were identified in pigmented cultivars whereas the last two acids were not detected in white rice.

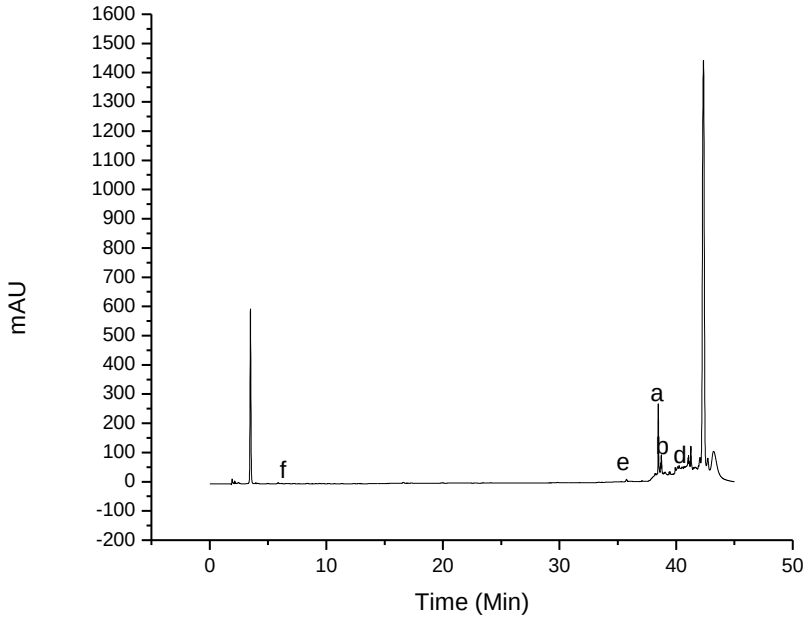
Table 3.9 Quantification of predominant phenolic acids present in three rice cultivars

Phenolic acid (mg/L)	LA	UA	WR
Quinic acid (a)	611.46 ± 0.01^{c}	255.46 ± 0.01^{b}	133.92 ± 0.02^{a}
Salicylic acid (b)	44.50 ± 0.01^{a}	302.06 ± 0.03^{c}	231.94 ± 0.02^{b}
Quercetin (c)	33.27±0.01	ND	ND
Apigenin (d)	0.42 ± 0.02^{a}	7.03 ± 0.01^{b}	0.49 ± 0.01^{a}
Gallic acid (f)	ND	0.98±0.04	ND
Caffeic acid (g)	ND	ND	1.83±0.02
Ferulic acid (e)	ND	17.21±0.02	15.18±0.01

Note: N.D (not detected)



(a)



(b)

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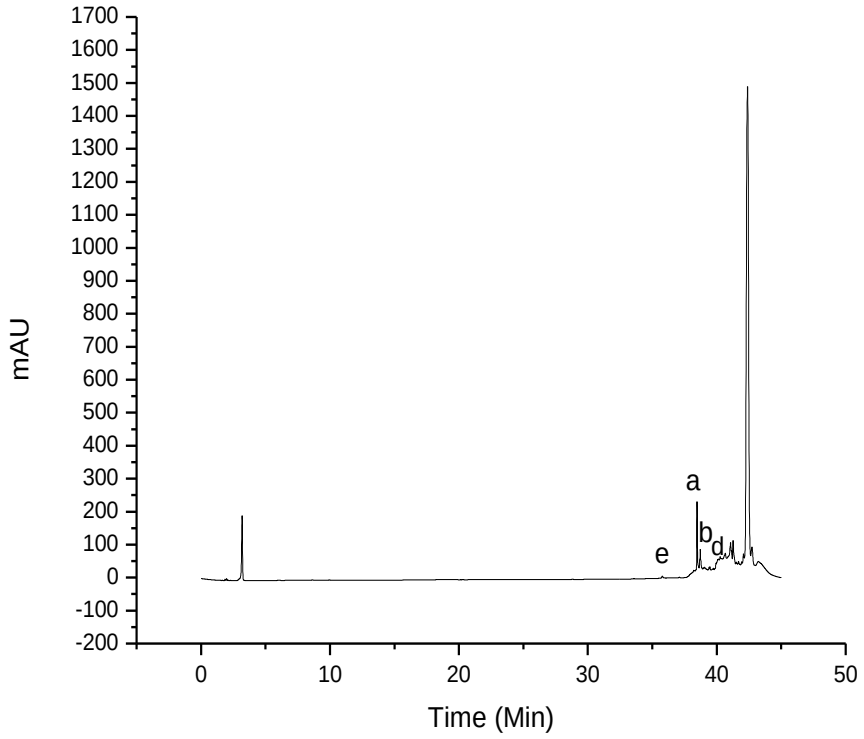

(c)

Fig. 3.6 RP-HPLC chromatograms of (a) LA (b) UA and (c) WR

3.4 Conclusion

The results of the proximate composition of the pigmented rice cultivars differed significantly over the non-pigmented rice. Physical and thermal attributes varied with difference in cultivars. Moisture and ash content of different rice cultivars varied significantly ($P \leq 0.05$) from 11 to 11.50% (d.b.) and from 0.93 to 1.33% (d.b.). The bulk and true density of the three different rice cultivars (LA, UA and WR) showed no significant differences between the cultivars at the 0.05% probability. Thousand grain weights (g), angle of repose and porosity of LA, UA and WR varied significantly. *L*, *a** and *b** color parameters varied from sample to sample. UA cultivar exhibited higher degree of redness and yellowness than LA and WR. Mineral profiles of the rice cultivars were analyzed. It was revealed that all of the rice varieties were a good source of mineral as a micronutrient. Texture profile such as hardness (g), springiness (length/length) and cohesiveness of cooked rice had significant variation. In thermal properties, T_o, T_p and T_c values of UA cultivar were shown to be higher than WR and LA. In addition, pigmented rice cultivars evinced substantial amount of phenolic compounds (UA-349.3 ± 0.02 and LA-262.30 ± 0.01 mg GAE/100 g) compared to white rice (142.90 ± 0.01 mg GAE/100 g) and revealed high antioxidant activity. The pigmented cultivars also showed a higher amount of anthocyanin content in UA (12.79 ± 0.001) and LA (11.47 ± 0.001 mg/100 g) compared to WR (1.34 ± 0.001) further reinforced its potential for high value addition. FT-IR and RP-HPLC analysis of the rice cultivars deciphered the presence of seven phenolic compounds viz., quinic, salicylic, quercetin, apigenin, ferulic, gallic, and caffic acid which are paramount for functional foods. It is prudent to summarize that these pigmented untapped rice cultivars of Arunachal Pradesh, India have enormous potential in the field of the pharmaceutical industry vis-a-vis its health benefits. The above results could provide important information for the utilization of rice cultivars and to develop new food products. The results of the present study clearly suggest that the pigmented rice cultivars of Arunachal Pradesh, India are rich in phytochemicals. These novel cultivars can undoubtedly be used in preparation of functional foods and therefore it elucidates that these cultivars has enormous potentials as nutraceuticals and can be exploited industrially

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(B) Physicochemical and phytochemical analysis of purple passion fruit

3.5 Introduction

The genus *Passiflora* L. (family Passifloraceae) is predominant in Brazil.[10] Passion fruit (*Passiflora edulis*) is an exotic fruit i.e. restricted fruits usage in limited or particular geographic areas[14,1] is oval shape and size about 6-12 cm belongs to Passifloraceae family. Among the different species, two well-known edible passion fruit species are purple (*Passiflora edulis* Sims) and yellow (*Passiflora edulis* f. *flavicarpa* Deg.). Also, possibilities of natural hybrid between purple and another related species.[2] Yellow passion fruit mostly grown in lowland tropical conditions and purple type are found more in the higher altitudes in the tropics. The purple passion fruit is originally from southern Brazil through Paraguay to northern Argentina and yellow one perhaps from Amazon region of Brazil.[9] Purple fruit is well known for its nutritional benefits and medicinal properties and its rind have the anti-hypertensive effect and vasodilatory effect on human body.[15,16] Therefore, the present chapter deals with the physiochemical and phytochemical analysis of purple passion fruit (*Passiflora edulis* Sims).

3.6 Material and methods

3.6.1 Raw material

Fully ripe purple passion fruit was purchased from the local market of village Sinchung, West Kameng District, Arunachal Pradesh, in the month of July-September. The fruit pulp with seed was squeezed by using muslin cloth. The pH of fruit pulp was measured by pH meter and the total soluble solids (TSS) content was recorded with the help of hand refractometer in the laboratory condition (0-32˚ Brix). The pulp was collected was store at -20˚C for further analysis.

Samyor et al. (2017). Effect of foam mat drying on physicochemical and phytochemical properties of passion fruit powder. International Journal of Food Properties (Under Review)

3.6.2 The color measurement

The color measurement of pulp was measured by using color measurement spectrophotometer (Ultra Scan VIS, Hunter Lab, a41-1013-504, Reston, VA) (method described in section 3.2.3.7)

3.6.3 Phytochemical analysis

3.6.3.1 Ascorbic acid (vitamin C) determination

Ascorbic acid (vitamin C) was determined according to Sadasivam and Theymoli[17]. The volumetric method was used for determination of ascorbic acid in passion fruit. Sample (1g) was weighed and taken for preparation of extract and ascorbic acid solution was used as standard.

$$\text{Ascorbic acid} = \frac{0.5}{V_1} \times \frac{V_2}{15} \times \frac{100}{s} \times 100$$

Where, V_1 is the mL of solution taken for estimation, V_2 is the volume made up, S is the weight of sample.

3.6.3.2 Total phenolic content

Determination of total phenolic content of passion fruit pulp was carried out by Folin–Ciocalteu assay[18] (method described in section 3.2.4.2).

3.6.3.3 DPPH scavenging activity

DPPH radical scavenging activity of the fruit pulp was measured according to the method of Brand-Williams et al.[4] (method described in section 3.2.4.4).

3.6.3.4 Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis

Fruit pulp sample (0.5 mL) was placed over on a multi-bounce Zn Se crystal of ATR-FTIR to identify the functional groups.[23] The IR- absorption spectra of pulp were obtained using a FT-IR spectrometer (Nicolet Impact 410, Thermoscientific, USA) equipped with KBr optics and a DTGS detector and the frequency ranged from 4000 to 400 cm^{-1}.

3.6.3.5 Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of phenolic acids

3.6.3.5.1 Sample preparation

For phenolic acid analysis, 50 ml of fruit pulp and 0.5 g ascorbic acid were mixed together. In the mixture 100 ml of 80% methanol was added and filtrated through WhatmanNo.2. (method was described previously in the section 3.2.4.7)

3.6.3.6 Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of (±) α-tocopherol, D-α-tocotrienol and β-carotene

3.6.3.6.1 Sample preparation

The (±)-α-tocopherol, D-α-tocotrienol and β- carotene were estimated by the method described by Aguilar-Garcia et al.[3] with slight modification. Passion fruit pulp (50 ml) was extracted twice with 6mL of methanol. Then, the extract was centrifuged for 10 min at 825g. The supernatant was collected and evaporated to 4 mL and volume made up to 5.0mL with methanol in a volumetric flask. This solution was filtered with Whatman ™ No. 1 and then, filtered through GD/X sterile 0.45 µm CA filter media of 25 mm before being subjected to HPLC analysis.

3.6.3.6.2 Detection

For detection of (±)-α-tocopherol, D- α- tocotrienol and β- carotene, the same RP-HPLC as used for phenolic, was used with UV Detector at 292 and 325nm. The C18, 5.0 µm (4.6 mm x 250mm) column was used to separate the compounds. The mobile phase was a mixture of methanol and acetonitrile (20:80 v/v) at a flow rate of 0.8 mL/min with isocratic mode. The software empower 2 was used for analyzing data.

3.6.4 Statistical analysis

Microsoft office excel 2013 was used for average and standard deviation calculation. The Origin 8.5 (Origin Lab Corporation, Northampton, USA) software was used for graphs.

3.7 Results and discussion

3.7.1 Moisture content (%), pH and total soluble solid (˚Brix)

Moisture content (%), pH and total soluble solid (˚Brix) value of pulp were 82.25 ± 0.01%, 3.91 ± 0.2 and 16.85 ± 0.4 respectively. Soluble solids referred to the sugars and acids combined with minute amount of dissolved vitamins, proteins, pigments, phenolics, and minerals.[21, 22,6,11,12] TSS is a great indicator to study quality parameters to indicate sweetness of post harvested horticultural crops in laboratories for research and development purpose, also in food industry to determine marketing standards. In the Table 3.10, the color measurement and phytochemical composition were presented. *L** value represent whiteness/lightness of the pulp sample which was 36.68±2.02, the lower value of *a** value is indicates redness part 3.79± 0.61 and *b** value observed was 19.82 ±1 which indicates the yellowness. Chroma and hue were 0.19±0.01and 0.19±0.01.The term chroma (C_{ab}), or saturation index represent the quantitative attribute of colorfulness of sample which is proportional to its intensity whereas Hue (h_{ab}) is a qualitative indicator.[5]

Table 3.10 Color measurement and phytochemical compositions of pulp

Parameters	**Passion fruit pulp**
*L**	36.68±2.02
*a**	3.79± 0.61
*b**	19.82 ±1.44
Chroma $(a^{*2}+b^{*2})^{1/2}$	20.17±0.02
Hue angle $\tan^{-1}(b^*/a^*)$	79.15±0.01
Vitamin C(mg/100g)	39.85.19± 0.01
Total phenolic content(mg GAE/100g)	206.29 ±0.10
DPPH scavenging activity (%)	70.53±0.03

3.7.2 Phytochemical analysis

3.7.2.1 Vitamin C (mg/100g)

Vitamin C content of purple passion fruit pulp was 39.85.19 ± 0.01 (mg/100g). Valente et al.[24] reported that in purple passion fruit from Colombia ascorbic acid content was 36.3

mg/100g. Ramaiya et al.[16] stated that *passiflora edulis* (purple) fruit collected from Malaysia showed ascorbic acid of 0.32 ± 0.72 g/kg i.e. 32mg/100g. Therefore, from the study ascorbic acid content can be differ from plant origins.

3.7.2.2 Total phenolic content (mg GAE/100g)

Total phenolic content can be calculated by using Folin–Ciocalteu reagent (FCR) method were the reduction of the reagent by phenolic compounds present in the sample can be observed. Blue complex obtained can be measured at 765nm against gallic acid as a standard. In the passion fruit pulp total phenolic content (mg GAE/100g) determined was 206.29 ±0.10 (mg GAE/100g).

3.7.2.3 DPPH scavenging activity (%)

DPPH is a stable free radical which is widely used in research laboratory to analyses the ability of exotic fruit different solvents extracts to act as free radical scavengers or hydrogen donors, which predicts the antioxidant activity of a sample. In passion fruit pulp, DPPH scavenging activity (%) at 517 nm obtained was 70.53 %.

3.7.2.4 ATR-FT-IR analysis

ATR-FT-IR analysis of pulp has been illustrated in Fig.3.7 and spectral stretching ranging from 625 to 3312 cm^{-1}. The absorption bands appeared were 625, 1039.54, 1270, 1375, 1658, 2122 and 3312 cm^{-1}. A very sharp band observed in 1658 cm^{-1}. Among all bands, 3312 cm^{-1} was the broadest band. Spectral peak at 1057 cm^{-1} is sucrose. The medium peak ranged from 1250 to 1020 cm^{-1} denotes C–N stretch bond indicating aliphatic amines.[13] A stretching characteristic bands at 3420 cm^{-1} and 2937 cm^{-1} were due to the O–H stretching band.[20]

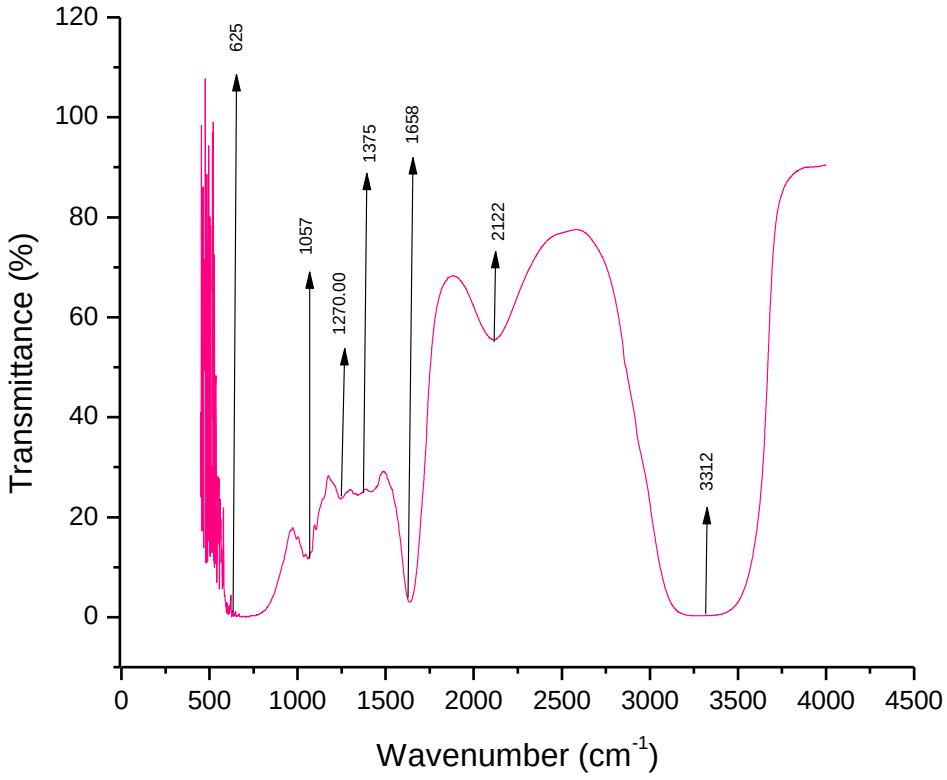

Fig.3.7. ATR-FT-IR spectra of pulp

3.7.2.5 RP-HPLC analysis

The vitamins and phenolic acids from pulp were identified and quantified by RP-HPLC (Table 3.11). The β-carotene, (±)-α-tocopherol and D-α-tocotrienol were detected at the retention times of 3, 3.5 and 3.8 min, respectively. The β-carotene, (±)-α-tocopherol and D-α-tocotrienol content in pulp were 11.79, 171.10 and 27.19 mg/100g. Cavalcante et al.[8] stated that compounds like carotene and vitamin accumulation in passion fruit are contributed by various internal as well as external factors *viz.*, maturity stage, cultivation system etc. Six phenolic acids were prominently observed in the raw pulp extract (Table 3.11). The pulp sample reveal highest content of vanillic acid 873.75 mg/100g.

Table 3.11 Quantification of vitamins and phenolic acids of passion fruit pulp

Phytochemicals (mg/100g)	**Retention time (min)**	**Passion fruit pulp**
Vitamin		
1. β-carotene	3.00	11.79
2. (±)-α-tocopherol	3.50	171.10
3. D-α-tocotrienol	3.80	27.19
Phenolic acid		
1. Caffeic acid	14.66	ND
2. (±) Catechin hydrate	13.00	ND
3. Chlorogenic acid	13.80	789.00
4. *p*- Coumeric acid	17.25	268.75
5. Transferulic acid	14.36	766.26
6. 4-Hydroxybenzoic acid	18.03	ND
7. Syringic acid	15.06	643.46
8. Sinapic acid	17.80	630.00
9. Vanillic acid	14.96	873.75

Note: ND (not detected)

3.8 Conclusion

In the present chapter physiochemical and phytochemical analysis of purple passion fruit (*Passiflora edulis* Sims) content were revealed. The pH and total soluble solids (˚Brix) of fruit pulpmeasured by pH meter and hand refractometer were 3.91 ± 0.2 and 16.85 ± 0.4. Color measurement showed that *b** which indicates yellowness were higher than *a** (redness). Phytochemical analysis *viz.*, DPPH scavenging activity (%), total phenolic content (mg GAE/100g) and vitamin C (mg/100g) presented good value of antioxidant property. The vitamins and phenolic acids from pulp were identified and quantified by RP-HPLC respectively. Some phenolic acid were found in good amount. These data can be useful for further in-depth study in the field of passion fruit utilization in food industries.

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