Project Completion Report of

Studies on Development of Cereal Based Functional Breakfast Food from the Underutilized Crops of North-East India

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Sponsored by

MINISTRY OF FOOD PROCESSING AND INDUSTRIES

Government of India

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4. Date of commencement: 12.06.2013

5. Planned date of completion:11.09.2015

6. Actual date of completion: 12.06.2015

7. Objectives as stated in the project proposal:

   - ✓ To analyze important physico–chemical and engineering properties of regional underutilized crops of north-east India.
   - ✓ To develop a process technology for production of functional breakfast food from underutilized crops.
   - ✓ To optimize process parameters for functional breakfast product.
   - ✓ To study the change during storage and evaluate the shelf life of prepared functional breakfast cereal product.
   - ✓ Comparative study between conventional breakfast food and developed breakfast food

8. Deviation made from original objectives if any, while implementing the project and reasons thereof: No

9. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:

Material & Methods

Three rice (*Oryza sativa* L) cultivars locally known as Lingkang taker ame (LA), Umling ame (UA), and Pungpo taker ame (PA) were purchased from the farmer of Manigong circle, West Siang district of Arunachal Pradesh, India. All the rice samples were cleaned and grinded in a samples were packed in polyethylene bags and stored in ambient temperature.

Physical properties

Axial Dimensions

The axial dimensions viz., length, breadth and thickness were measured for rice grain with the help of 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 (Mohsenin, 1986).

$$D_g = (LWT)^{1/2} \tag{1}$$

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

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

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 (Mohsenin, 1986).

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

Aspect ratio of rice cultivars were (R_a) calculated using following formula (Mohsenin, 1980).

$$R_a = \frac{W}{L} \tag{4}$$

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 (1997)

$$V = \pi\frac{B^2L^2}{6(2L-B)} \tag{5}$$

$$S = \frac{\pi BL^2}{2L - B} \quad (6)$$

$$B = (WT)^{1/2} \quad (7)$$

Thousand kernel weights of the grains were measured by weighing 100 seeds in an electronic balance to an accuracy of 0.001. It was multiplied with 10 to give mass of 1000 kernels.

Bulk density and true density

Bulk density was calculated using mass/volume relationship. It was determined by method of Gupta & Das (1997). A cylindrical container of 500mL was filled with the grain from a height of 150mm 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 (Selvi, Pinar and Yesiloglu, 2006).

Porosity

Porosity is the percentage of void space in the bulk grain which is not occupied by the grain (Thompson and Issac, 1967). Porosity was calculated using the relationship between the bulk density and true density as shown in the eq. (8)

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \quad (8)$$

Angle of repose

Angle of repose was measured as described by Jain and Bal (1997). 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 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.

Moisture and ash content

The moisture and ash content of rice cultivars and passion fruit pulp and peel were carried out using standard AOAC methods (2000). Total carbohydrate, starch content, crude protein, crude fat and amylase content of rice and fruit was carried out by AOAC (1995).

Mineral Profile

Minerals were quantified by the Atomic absorption spectroscopy (AAS). Sample concentrations were determined in the aqueous solution of acid digest. Grounded rice flour samples (0.5-1 g) were added to 30 mL concentrated nitric acid and later, 5 mL concentrated hydrochloric acid were added. The vessels were immediately closed after addition of oxidants. Samples were digested on a hot plate at 100°C. After digestion process, digests were cooled and diluted up to 50 mL with distilled water. The samples were kept in refrigerated temperature for further analysis. (Naozuka *et al.* 2011)

Pasting properties

Pasting properties of the whole rice flour was measured using a rapid visco analyser (RVA starch master 2 pulverisette instrument). 2g of samples were 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 (Zhou *et al.* 2003).

Color

The colour of the rice cultivars were measured using Hunter Colorimeter (ColorLab Ultrascan Vis). 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.

$$Chroma = [a^{*2} + b^{*2}]^{1/2\theta} \quad (9)$$

$$Hug\ angle = \tan^{-1}(\frac{a^*}{b^*}) \quad (10)$$

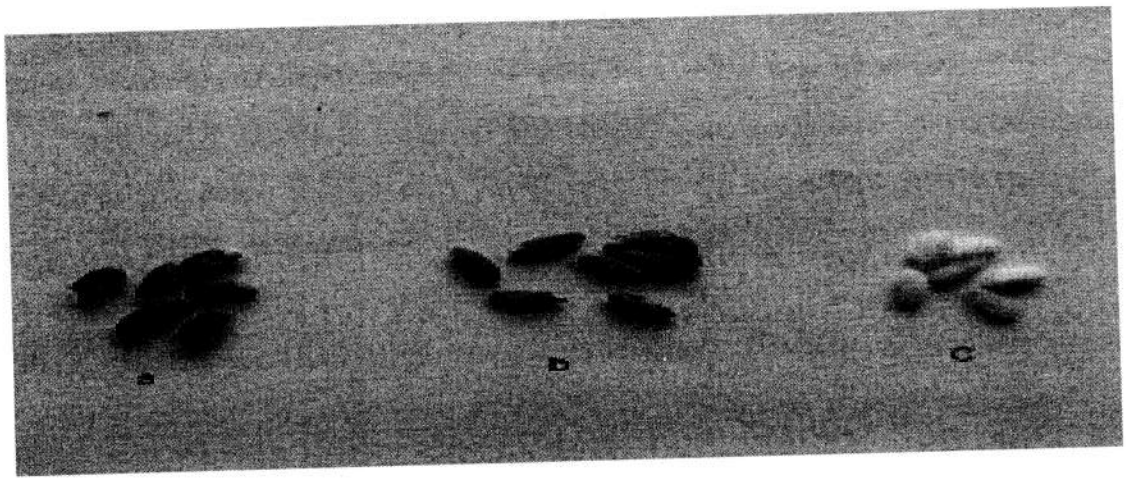
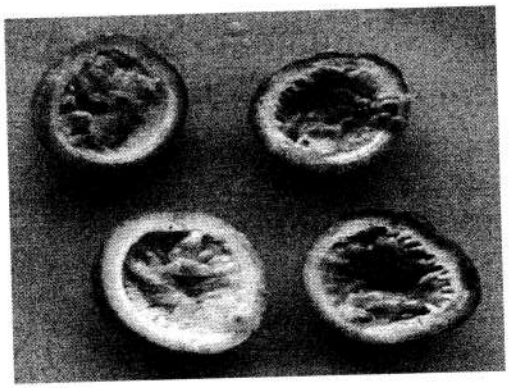
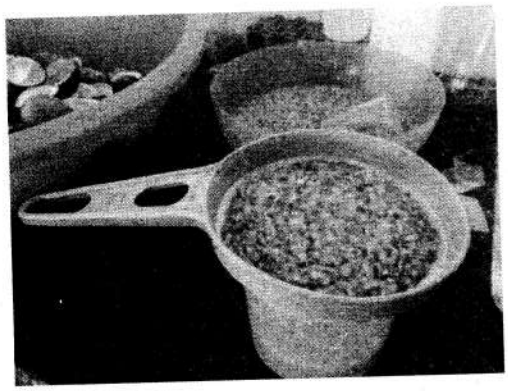


Fig.1 Three rice cultivar a) Umling ame (b) lingkang taker ame (c) Pungpo taker ame



(a)



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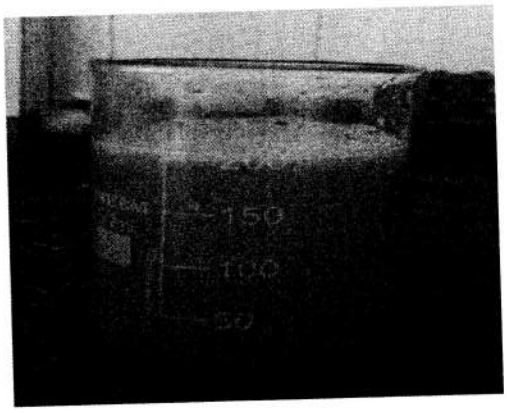

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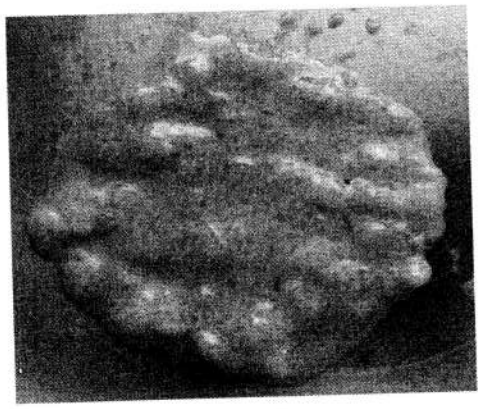
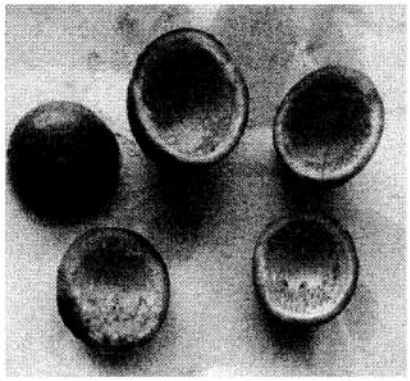


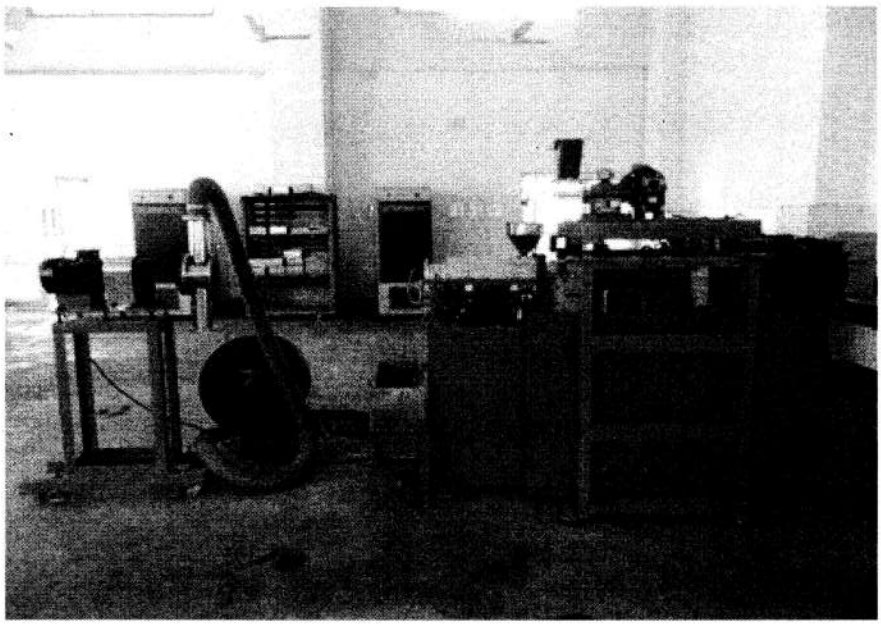
(d)

(e)

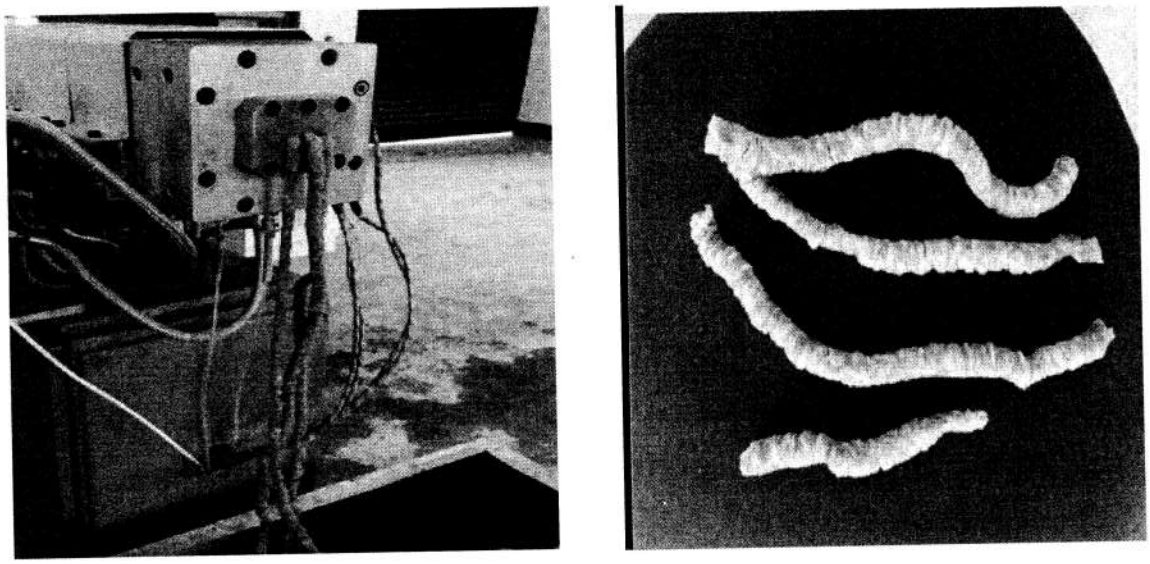


(f)

Fig.2. Passion fruit different forms (a) Ripe passion fruit (b) pulp and seed (c) pulp (d) Pulp mixed with methyl cellulose (e) Dried foam mat powder of (f) dried



(a)



(b) (c)

Fig 2. (a) Twin extruder (b) Product development process and (c) Ready to cook extrudate product.

Cooking characteristics

Optimal Cooking Time

Optimal Cooking Time of three different rice flours was determined using the AACC method 66-50 (AACC, 2000).

Water uptake ratio

Water uptake ratio of different rice samples were measured by the method of Thomas *et al.* (2013). 2 g 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 water uptake ratio was calculated.

Texture Analysis

The fresh cooked rice sample was taken directly to perform texture profile analysis. Parameters like hardness, springiness and cohesiveness of cooked rice were measured using a texture analyzer as the modified method described by Tian *et al.* (2007). The cooked rice sample was put on the sample table at the centre of the probe in a flat form.

Then the cooked rice sample was compressed using a 2.5mm diameter cylindrical probe at a test speed of 0.5 mm/s and a control force of 10 g with a 50kg cell load. The deformation level was 60% of original. This process was repeated for three times for each sample.

Thermal properties

Differential Scanning Calorimeter (DSC)

Thermal analysis was performed with a DSC 60 SHIMADZU instrument. About 5 mg of the sample was used in each experiment. 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. Another empty cup was used after sealing as 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 described by Krueger *et al.* (1987).

Phytochemicals and antioxidant activities

Sample extraction

Sample preparation was done according to the method describe by Atala (2009).Three rice cultivars (10 g each) were powdered in a grinder. The grinded 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.

Total phenolics content

Modified version of the Folin–Ciocalteu assay (Slinkard, 1977) 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 1.58mL 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 UV-VIS spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India). The phenolic content was expressed in mg GAE/100 g.

Total monomeric anthocyanins

The monomeric anthocyanin content of extracted solutions were determined using the pH differential method Giusti (2001) 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.

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical-scavenging activity of rice extracts were evaluated according to Brand-Williams, Cuvelier and Berset (1995) 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.

Metal chelating activity

Metal chelating activity was done as per method described by Dinis (1994) 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.

Reducing power

The method of Oyaizu (1986) was followed. Sample (1 mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_2Fe(CN)_6$]. The mixture was then incubated at 50 °C for 20 min. Trichloroacetic acid (TCA)

was added (2.5 ml of 10% TCA) to the mixture, and centrifuged at 2200 *g* for 5 min. The upper solution (2.5 mL) was mixed with 5.0 mL distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). Absorbance was measured at 700 nm in spectrophotometer and butylated hydroxytoluene (BHT) was used for comparison.

Hydrogen peroxide scavenging capacity

Hydrogen peroxide scavenging activities of the rice cultivars extract were determined according to the method of Ruch (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM) and absorbance of hydrogen peroxide at 230 nm was taken against a blank solution containing the phosphate buffer without hydrogen peroxide. Samples were investigated within the 0–100 µg/mL concentration range, and all samples were run in triplicate.

$$\text{Hydrogen peroxide scavanging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c was the absorbance of the control and A_s was the absorbance of the extract.

Fourier transform infrared (FT-IR) spectra of polyphenols

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} (Kumar, 2014).

Qualitative and quantitative analysis by HPLC

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 upto 50 mL using distilled water and pH was adjusted to 7.0 and 10 mL sample were taken for further analysis.

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.

Statistical Analysis

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

Evaluation of physicochemical properties of passion fruit

Fruits were cut into halves. Pulp and seed were squeezed into to juices .The collected juices were filtered through 3-4-fold muslin cloth and the pulp free juice was collected in clean containers. pH of juice was determined by using pH meter, °Brix reading was taken by refractometer and moisture content of juice were determined method described by AOAC, (2000). Colour of fruit juice and foam mat dried powder were measured by using Colour Measurement Spectrophotometer (Hunter ColorLab Ultrascan Vis). Foaming of pulp by Philips HR1453, 240v 50Hz 175 W Hand Blender, Multiple speeds (3 speed) and turbo function were used to agitate the material in the jar. Whipping time (min) and speed level (1,2 and 3) were noted down.

Ascorbic acid (Vitamin C) determination was described by Thimmaiah in the book standard methods of biochemical analysis). Volumetric method was used for determination of ascorbic acid and Sample (0.5-5g) were taken for preparation of extract. Ascorbic acid was used as standard.

$$Amount\ of\ ascorbic\ acid(\frac{mg}{100g}sample)$$

$$= \frac{0.5\text{mg}}{V_1\ \text{ml}} \times \frac{V_2\text{ml}}{15\text{ml}} \times \frac{100\text{ml}}{\text{wgt of the sample}} \times 100$$

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the fruit extract was measured according to the method of Brand-Williams, Cuvelier, and Berset (1995). Briefly, 100 μL of extracts were added to 1.4 mL DPPH radical methanolic solution (10−4 M). The absorbance was measured at 517 nm for 30 min against. The results were expressed by using the following equation:

$$\text{Radical scavenging activities} = \frac{A_0 - A_s}{A_0} \times 100$$

FT-IR analysis

Fruit pulp sample (0.1-0.5) ml was placed on a multi-bounce ZnSe crystal of ATR-FTIR to identify the functional groups (Vardin et al., 2008). Again, a known amount of foam mat passion fruit powder (2 mg) of properly oven dried and 50 mg desiccated potassium bromide powder were thoroughly mixed in a mortar and pestle before pressing into a thin pellet. The IR- absorption spectra of thin pellet were obtained using a FTIR spectrometer (Nicolet Impact 410, Thermoscientific, United Kingdom) equipped with KBr optics and a DTGS detector. The frequency ranging from 4000 to 400 cm^{-1}.

Experimental Designs for foam mat drying

The independent variables considered were: MC (%), WT (Min), and Temp (^{0}C). Three variable (2 levels) CCRD and a response surface methodology (RSM) were used to understand the inter- actions of MC (%), WT (Min), and Temp (^{0}C).

Table 1. Range of independent variables

Variables	Low level	High level
Temperature range (^{0}C)	40	60
Methyl cellulose (g)	1	3
Whipping time (min)	1	5

Table 2 Experimental table for the passion fruit foam mat drying

Run	WT (min)	Methyle cellulose (%)	Temperature (^{0}C)	Vitamin C	TPC (mg/100g)	Hygroscopicity (%)	Moisture content (%)
1	5	2	50	31.78	223.95	0.018	5.93
2	4.189	2.594	55.94	32.06	184.25	0.009	4.94
3	3	1	50	31.47	238.76	0.005	5.88
4	3	2	50	32.76	256.25	0.068	6.87
5	3	2	40	38.66	319.62	0.007	5.22
6	1.810	1.405	44.05	35.21	240.44	0.002	5.66
7	1.810	2.594	44.05	35.20	243.75	0.080	5.57
8	3	2	60	30.83	143.70	0.083	3.78
9	4.189	1.405	44.05	35.11	273.75	0.005	6.61
10	3	3	50	32.66	206.62	0.059	5.61
11	1.810	2.594	55.94	32.65	182.5	0.054	5.96
12	3	2	50	33.76	248.25	0.066	6.87
13	3	2	50	33.76	258.01	0.066	6.87
14	3	2	50	33.76	246.25	0.066	6.97
15	1.810	1.405	55.94	31.87	182.5	0.076	5.98
16	4.189	2.594	44.05	34.11	230.67	0.071	6.69
17	3	2	50	33.76	206.25	0.090	6.97
18	4.189	1.405	55.94	30.78	196.62	0.011	4.14
19	3	2	50	33.76	246.25	0.066	5.98
20	1	2	50	32.12	224.71	0.048	6.03

Extrusion cooking

The temperature of the extruder barrel was maintained at 70, 80, 90,100 °C throughout the barrel. The screw speed was varied at 200, 300 or 400 rpm while the feed rate was maintained constant at 17 kg/h using a volumetric gravity feeder. The moisture content of the feed at the ranged of 20-27. All extrusion trials were repeated once.

Table 3. Range of independent variables

Variables	Low level	High level
Temperature range (^{0}C)	80	150
Screw speed (rpm)	200	400
Moisture content (%)	22	30
Passion fruit (%)	0	15

Table 4. Experimental design for the extrudate product

Run	Temp (^{O}C)	SS (RPM)	MC(%)	PFP (%)	ER	WAI	TPC	DPPH
1	115	300	25	15	5.87	2.98	87.933	39.53
2	97.5	350	27.5	11.25	6.6	1.29	72.36	98.31
3	132.5	350	22.5	11.25	12.6	1.00	12.0449	8.47
4	132.5	250	27.5	3.75	12.1	3.63	9.775	6.01
5	115	300	20	7.5	9.83	0.23	76.853	33.75
6	132.5	350	22.5	7.75	12.65	1.01	11.764	4.96
7	97.5	350	22.5	3.75	7.9	1.09	97.415	62.12
8	115	300	25	7.5	7.8	2.97	151.6654	57.5
9	132.5	250	22.5	3.75	11.63	1.80	12.808	6.75
10	115	400	25	7.5	10.04	1.50	27.415	18.59
11	97.5	250	22.5	3.75	6.8	2.00	33.764	28.35
12	115	300	25	7.5	9.8	1.95	151.6654	28.14
13	150	300	25	7.5	15.9	2.84	6.905	7.87
14	132.5	250	22.5	11.25	13.13	2.04	10.146	4.37
15	97.5	250	27.5	3.75	7.92	3.21	36.797	57.34
16	115	300	30	7.5	10.9	1.99	134.23	27.18
17	115	300	25	7.5	9.8	1.97	151.6654	57.5
18	115	300	25	7.5	9.8	1.95	151.6654	57.5
19	115	200	25	7.5	12.7	3.70	33.483	14.60
20	97.5	250	27.5	11.25	8	3.12	138.438	70.45
21	115	300	25	0	8.3	2.22	89.887	23.1
22	132.5	250	27.5	11.25	12.48	3.98	12.146	8.10
23	115	300	25	7.5	9.8	1.98	101.95	57.5
24	115	300	25	7.5	9.8	1.97	150.665	57.5
25	97.5	250	22.5	11.25	8.56	1.98	85.012	57.96
26	97.5	350	27.5	3.75	6.5	2.09	69.551	78.43
27	97.5	350	22.5	11.25	8.83	1.11	59.761	76.25
28	132.5	350	27.5	11.25	13.6	2.17	23.483	3.12
29	80	300	25	7.5	6.73	1.09	78.911	85.21
30	132.5	350	27.5	3.75	14	2.18	97.415	8.93

Storage study

Storage study was conducted for the developed breakfast food item made from red rice and passion fruit for 28 days Kulchan (2010). Different packaging material namely Low-density polyethylene (LDPE), High-density polyethylene (HDPE), polypropylene (PP) and Metallized Polyester Film (MP). Experimental RH set at 50 % at 30 degree.

Comparative study between conventional breakfast food and developed breakfast food

RP-HPLC was carried out to compare the phenolic content of products. Sample preparation were same as mentioned above.

10. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:

Physical properties

Axial dimensions, geometric mean diameter, sphericity index (%), moisture content and ash content of three rice cultivars were varied significantly ($p<0.05$). 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 1. Average length (l) of rice grain varied from (UA) 5.37±0.24 mm to (RR_1) 6.80±0.34 mm while the average breadth/width ranged from (UA) 3.47±0.58 to (PA) 3.81±0.02. Therefore, is significantly ($p< 0.05$) longer than the two other grain samples. Equivalent diameters of three samples were varied from (UA) 4.10±0.01 to (PA) 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 (PA) 0.37±0.04 g/cm^3 and (UA) 1.06 ± 0.11 to (PA) 1.56±0.11 g/cm^3 respectively. Bulk density among the three cultivars were differ significantly ($p<0.05$). Angle of repose of different grains varied significantly ranged from (UA) 39.19±0.66° to (RR_1) 43.23± 0.13°. Angle of repose value ranged from WR (41.98^0) to RR_1 (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 (Antequera *et al.* 1994). Porosity of rice samples were varied from (UA) 65.76 to (PA) 75.91 (%).Weight of 1000 grain (g) ranged from (LA) 18.67±0.03g to (PA) 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 (PA) 54.99±2.02. Grain volume (mm^3) of the three different samples was ranged from (UA) 24.99±1.86 to (PA) 35.65 ±1.01. Weight of 1000 grains (g) LA, UA and PA were ranged from 18.67-22.04g.

Table 5 Physical properties of rice cultivars

Properties	**LA**	**UA**	**PA**
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 (slender)	1.54 (slightly round)	1.75 (round)
Equivalent diameter, mm	4.50 ± 0.004^{b}	4.10 ± 0.016^{a}	4.56 ± 0.048^{c}
Sphericity (%)	0.66 ± 0.015^{a}	0.76 ± 0.030^{c}	0.67 ± 0.035^{b}
Bulk density (g/cm^3)	0.372 ± 0.001^{a}	0.371 ± 0.003^{a}	0.378 ± 0.004^{a}
True density (g/cm^3)	1.26 ± 0.057^{b}	1.06 ± 0.115^{a}	1.56 ± 0.115^{c}
Porosity (%)	71.03 ± 0.824^{b}	65.76 ± 0.854^{a}	75.91 ± 0.083^{c}
Angle of repose (deg.)	43.23 ± 0.133^{c}	39.19 ± 0.667^{a}	41.98 ± 0.108^{b}
Weight of 1000 grain (g)	18.67 ± 0.003^{a}	20.96 ± 0.034^{b}	22.04 ± 0.002^{c}
Aspect ratio	1.03 ± 0.018^{b}	0.93 ± 0.010^{a}	1.02 ± 0.020^{b}
Surface area (mm^2)	53.54 ± 2.23^{b}	45.32 ± 4.084^{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}
Colour			
Chroma	7.26 ± 0.05^{a}	8.22 ± 0.104^{b}	9.12 ± 0.067^{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 ± 0.092^{c}	0.35 ± 0.10^{a}
b*	7.50 ± 0.15^{b}	5.98 ± 0.29^{a}	9.06 ± 0.12^{c}
Chroma $[a^{*2}+b^{*2}]^{1/2}$	7.26 ± 0.05^{a}	8.22 ± 0.104^{b}	9.12 ± 0.067^{c}
Hue angle $\tan^{-1}(b^*/a^*)$	64.83 ± 0.08^{b}	54.59 ± 0.53^{a}	87.62 ± 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.

Values expressed as mean ± SD (n=3)

Nutritive quality

The data presented in Table 6 shows that the LA cultivar content significantly ($p<0.05$) high amount of moisture (11.13 %) than UA and PA cultivar. Ash content was found highest in the UA 1.33 %, followed by LA 0.97 % and lowest in the PA 0.93 %. Fat content was significantly ($p<0.05$) high in UA 2.60 % followed by LA (1.80%) and PA (1.77%). Energy values of three rice cultivars (LA, UA and PA) were 16.34, 16.29 and 16.23 kJ respectively. Amylose content of rice cultivars varies from 5.30 -11.86 %

Table 6 Nutritive value of rice cultivars

Parameters	LA	UA	PA
Moisture (%)	11.13 ± 0.02^{a}	11.01 ± 0.005^{b}	11.01 ± 0.005^{b}
Ash (%)	0.97 ± 0.01^{b}	1.33 ± 0.57^{c}	0.93 ± 0.05^{a}
Fat (%)	1.80 ± 0.20^{b}	2.60 ± 0.19^{c}	1.77 ± 0.06^{a}
Protein (%)	1.95 ± 0.02^{c}	0.24 ± 0.03^{b}	0.12 ± 0.02^{a}
carbohydrate (mg)	79.01 ± 0.16^{b}	81.37 ± 0.93^{c}	76.44 ± 0.59^{a}
Energy value(kJ)	16.34 ± 0.01^{c}	16.29 ± 0.01^{b}	16.23 ± 0.02^{a}
Amylose content (%)	6.46 ± 0.025^{b}	11.86 ± 0.04^{c}	5.30 ± 0.04^{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)

Energy in foods can be described by gross energy (GE) i.e. the heat produced by the food and metabolizable energy (ME) as the energy available for body functions or as the adenosine-triphosphate (ATP) available to the human body. Each leads to different energy conversion factors.(Miller,1959 and FAO(2003). According to IRRI (FAO.2005) 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%). Amylose content of these three rice cultivars varied from 5.30 -11.86 % .

Colour values

L*, a*, b* values of three different rice cultivars were analyzed. L* values of the rice flours which indicates whiteness /lightness, varied from 79.83 ±0.05 (PA) to 44.52 ±0.17 (LA). The reason may be because of (PA) has whitish bran than other two grains. The positive a* values for redness ranged from (PA) 0.351 to (UA) 4.192. (UA) have highest a* values, it may be due to more reddish external layers colour than other two rice variety. The yellowness b* value was in the ranged from (UA) 5.98 to (PA) 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 (PA) 87.62 ±0.13.

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 2. Out of eleven minerals, Al (0.97 mg/100g), Cr (0.19mg/100g), Mg (54.25± 0.76 mg/100mg), K (29.00 mg/100g), Zn (0.65 mg/100g), Na (19.54 mg/100g) and Ca (9.55 ± 0.18 mg/100g) concentration were observed highest in (RR_1) Mo, Fe, Mn and Cu concentration were recorded highest in UA. The mean values for most elements were consistent and similar to the result published previously (Shen *et al.* 2013).

Pasting properties

Pasting properties of LA, UA and PA flours are shown in Fig.1. and different viscogram data of rice cultivars were reflected in the Table. 7. Among three rice cultivars UA (90.5± 0.25) showed significantly ($p<0.05$) higher pasting temperature than LA, (83.7°C ± 0.32) and PA (77.7°C ± 0.18) which indicates the minimum temperature needed to cook the rice flour. Previously Huaisan *et al.* (2009) 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 (Miles *et al.*1985; Ding *et al.* 2015). Breakdown viscosity of any mixture can be

depending on degree of mixing, shear stress and temperature (Guha *et al.* 1998). The breakdown viscosity of the flour samples were varied significantly ($p<0.05$) 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 (Adebowale *et al.* 2005; Tester and Morrison, 1990). Break Down value indicates the heat stability of starch at 95^0C. Therefore, low BD value indicates thermal stability (Lee *et al.* 2012; Inglett *et al.* 2015). 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 (Karim *et al.* 2007). It is a measure of gelling ability or retrogradation tendency of the starch. Setback viscosity of three rice cultivars were ranged from PA 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 (Acquaah *et al.* 2015).

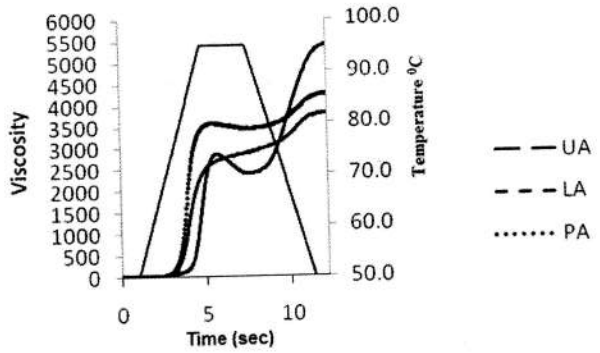


Fig. 1 Viscogram of RVA

Table 7 Viscography parameters of the rice flour suspension

Pasting properties	**LA**	**UA**	**PA**
Pasting temperature(°C)	83.7 ± 0.321^b	90.5 ± 0.251^c	$77.7° \pm 0.185^a$
Peak Viscosity(cP)	1804 ± 0.577^a	1601 ± 0.577^b	2767.6 ± 1.5^c
Hold viscosity (cP)	1762.3 ± 1.5^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

Cooking characteristics

The rice cultivars shows significant ($p<0.05$) different in optimal cooking time (min), ranged from (PA) 21.3- (UA) 31.16 minutes (Table 8). UA shows the highest cooking time (31.16 min) than LA (26.48min) and PA(21.3min). It may be due to the variation in amylose content and size of rice sample. Hogan and Plank (1958) 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 and longer cooking time shows higher internal restructuring of the grain. (Arns *et al.* 2014). 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, Dalgic and Kaya (2007) also reported longer the optimal cooking time higher the water uptake capacity.

Table 8. Cooking characteristics of three different rice grain samples

Properties	**LA**	**UA**	**PA**
Optimal cooking Time (min)	26.48 ± 0.02^{b}	31.16 ± 0.14^{c}	21.3 ± 0.01^{a}
Water uptake ratio (%)	3.066 ± 0.015^{b}	3.65 ± 0.03^{c}	2.666 ± 0.02^{a}

Texture Analysis

Texture Profile Analyser of cooked rice was carried out for three different samples. The data (Table 9) revealed that the cooked UA (3.55±0.84g) rice had higher hardness value than the cooked LA (2.13±0.198g) and PA (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, Ma and Sun (2009) 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 and leads to significant decreases in stickiness of the cooked rice. (Leelayuthsoontorn and Thipayarat, 2006; Katekhong and Charoenrein (2014). 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 PA 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 RR_2 (0.39±0.84) possess higher gel structure than others. Huang, Kennedy, Li, Xu, and Xie, (2007) 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 9. Texture analysis results of three rice cultivars.

Properties	LA	UA	PA
Hardness (g)	2.139± 0.198[a]	3.554± 0.849[b]	3.529± 0.970[b]
Springiness (%)	0.371± 0.103[a]	0.391±0.080[a]	0.389±0.020[a]
Cohesiveness(%)	0.174±0.174[a]	0.231±0.034[c]	0.2166±0.060[b]

Thermal properties

Fig.2 shows the DSC thermograms of three rice cultivars. 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 PA cultivar (67.27, 93.53 and 117.92°C) as shown in the Table 10. 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 (Singh et al.,2000).

Among three samples, highest reaction rate occur in the UA 104.61°C and lowest in PA 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 (Kang *et al.* 2006). Moreover, high amylose starches with longer average chain exhibit higher transition temperatures. For wheat, rice and maize previous researcher (Stevens and Elton 1971) 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.* (2003) also reported the transition temperatures of Basmati cultivars varied between 66.25-74.70°C.

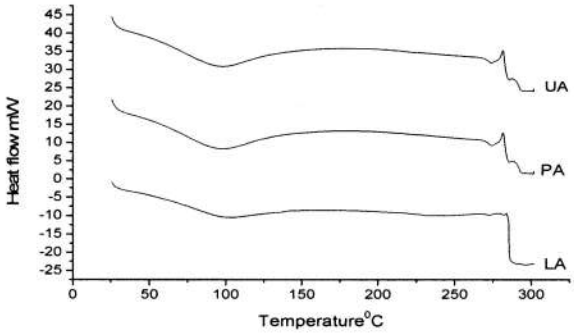


Fig.2. Differential Scanning Calorimetry (DSC) graphs of three different rice cultivars UA, PA and UA.

Phytochemicals and antioxidant activities

Total phenolic content and the anthocyanin content of three cultivars are shown in Table 10. The phenolic compound and anthocyanin content of three rice cultivars varied significantly. The phenolic contents in the white rice (PA) 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.

Table 10 Total Phenolic and anthocynanin content of rice cultivars

Rice cultivars	TPC mg GAE 100 g−1	Anthocynanin content mg cyaniding-3-glucoside Eq/100g
LA	262.3 ±0.2[b]	11.47 ±0.001[b]
UA	349.3 ±0.1[c]	12.79±0.001[c]
PA	142.9 ±0.1[a]	1.34 ±0.001[a]

It was observed from Table 11, 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. 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.

It was observed from the Table 12 that UA (77.53 µg/mL) has the highest iron chelating activity than LA (72.33 µg/mL) and PA (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 colour complex formation.

The correlation coefficients (r^2) of three rice cultivars were UA 0.96, LA 0.85 and PA 0.84 and observed that UA (0.75 mM 100^{-1} g) has the highest reducing power followed by UA (0.64 mM 100^{-1} g) and lowest in PA (0.08 mM 100^{-1} g). The correlation coefficient (r^2) between the reducing power and sample concentration of the three rice cultivars were determined. The reducing power and the sample concentration showed a strong correlation for all the rice viz., LA (r^2) = 0.91, UA (r^2) = 0.84 and PA (r^2) = 0.98.

Among the cultivars the highest H_2O_2 scavenging activity (Table 12) was found in the UA (1.88 µg/ml), followed by LA (1.05 µg/ml) and PA (0.57 µg/ml). This may be attributed to the presence of high amount of phenolic compounds and the correlation coefficient (r^2) of three rice cultivars were UA 0.94, LA 0.98 and 0.92.

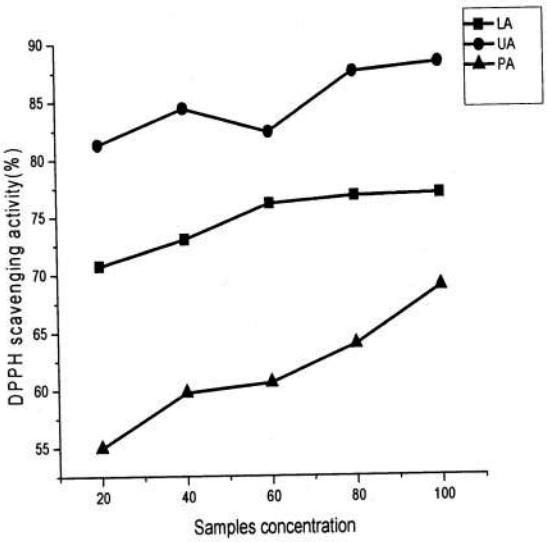


Fig 3. DPPH activity of three rice cultivars LA (Lingkang taker ame: waxy red rice), UA (umling ame non waxy red rice) and PA (Pungpo ame: waxy white rice). Values expressed as mean ± SD (n=3)

Table 11. Anti-oxidant activity of three different rice cultivars with different concentration

Concentration	LA	UA	PA
Reducing Power			
20	0.049 ± 0.01 [a]	0.201±0.02 [a]	0.021±0.01 [b]
40	0.249±0.002 [b]	0.276±0.01 [c]	0.031±0.004 [a]
60	0.410±0.001 [a]	0.358±0.002 [a]	0.057±0.001[a]
80	0.443±0.002 [c]	0.393±0.001 [b]	0.073±0.01[a]
100	0.753±0.02 [c]	0.649±0.03[b]	0.089±0.001[a]
Chelating Activity			
20	37.31±0.01 [b]	63.04 ±0.01[a]	10.23±0.02 [c]
40	45.35± 0.01[a]	64.72±0.02[c]	20.07± 0.01 [b]
80	54.24± 0.02 [a]	67.60±0.08 [b]	41.73±0.01[c]
160	63.17± 0.02 [b]	68.04±0.03 [c]	58.58±0.03[a]
360	72.33± 0.2 [b]	77.53±0.01[c]	58.32±0.2[a]
DPPH Scavenging Activity			
20	70.63±0.13[c]	81.97±0.27[b]	54.97±0.15[a]
40	73.32± 0.10[c]	84.78±0.13[b]	59.62±0.07[a]
60	76.14± 0.04 [c]	82.36 ±0.19[b]	60.15±0.15[a]
80	76.76±0.001[c]	87.55±0.1[b]	63.296±0.3[a]
100	76.97 ±0.15[c]	88.48±0.11[b]	68.87±0.15[a]
Hydrogen peroxide scavenging activity			
20	0.683±0.01[b]	1.385±0.001[c]	0.286±0.01[a]
40	0.742±0.002[b]	1.496±0.003[c]	0.285±0.004[a]
60	0.889±0.003[b]	1.552±0.002[c]	0.4562±0.005[a]
80	0.953±0.003[b]	1.661±0.002[c]	0.514±0.002[a]
100	1.052±0.008[b]	1.883±0.003[b]	0.571±0.002[a]

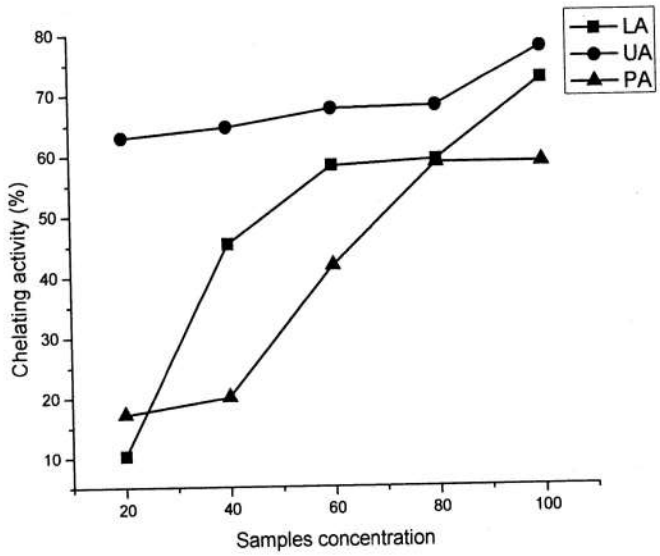


Fig. 4 Chelating activity of three rice cultivars LA (Lingkang taker ame:waxy red rice), UA (umling ame non waxy red rice) and PA (pungpo ame: waxy white rice). Values expressed as mean ± SD (n=3)

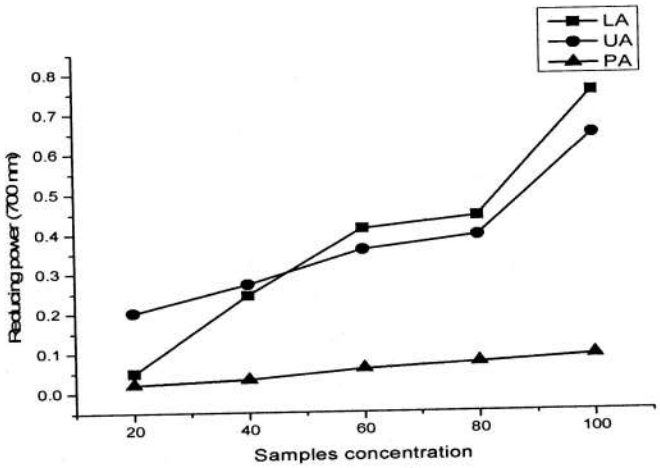


Fig 5. Reducing activity (700nm) of three rice cultivars LA (Lingkang taker ame:waxy red rice), UA(umling ame non waxy red rice) and PA (Pungpo ame: waxy white rice) Values expressed as mean ± SD (n=3)

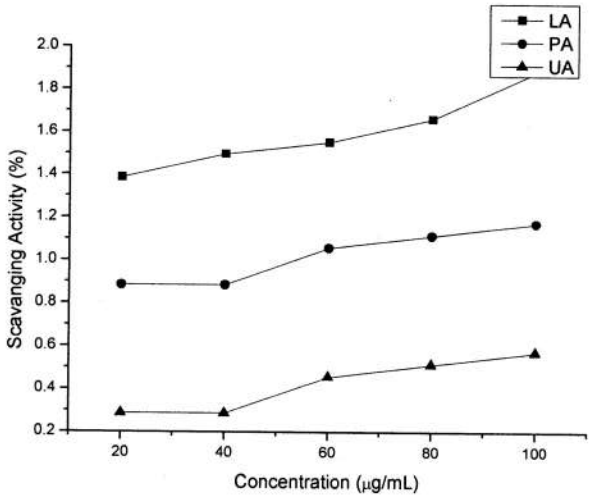


Fig 6. Hydrogen Peroxide Scavenging Capacity of three rice cultivars LA (Lingkang taker ame:waxy red rice), UA (umling ame non waxy red rice) and PA (Pungpo ame: waxy white rice) Values expressed as mean ± SD (n=3)

Fourier transform infrared (FT-IR) spectra analysis of phenolic compounds

The Fourier transform infra red spectrum of three rice cultivars are shown in Fig. (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 PA 1655.63 to 3874.61 cm^{-1}.

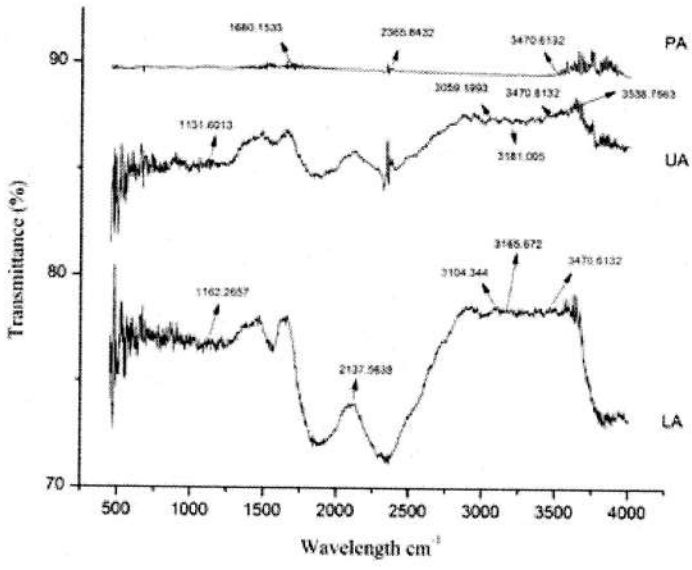


Fig 7. FT-IR chromatograms

HPLC analysis of phenolic compounds

The identification of phenolic compounds by HPLC revealed differences in the, phenolic fraction profile among the rice cultivars Fig. 8 (a), (b) and (c). 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 PA 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 PA (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 PA 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 phenolic compounds react with deep violet colour solution of DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) and convert it to 2, 2-diphenyl-1-picrylhydrazine with decolourisation and measurement of colour reduction allows to estimate the DPPH scavenging activity of the samples. (Brand-Williams, 1995) Similar type of result has been observed by numbers of researchers. (IRRI, 2009 and Hua 2014)

Therefore measurement of colour reduction, allows the estimation of the chelating activity of the sample. (Dinis, 1994).Chelating activity of rice extracts depends upon concentration of extract.

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 colour of the complex is decreased with the sample concentrations (Muller, 2011). The UA cultivar showed significantly ($p<0.05$) the high amount of chelating activity compared to LA and PA rice cultivars.

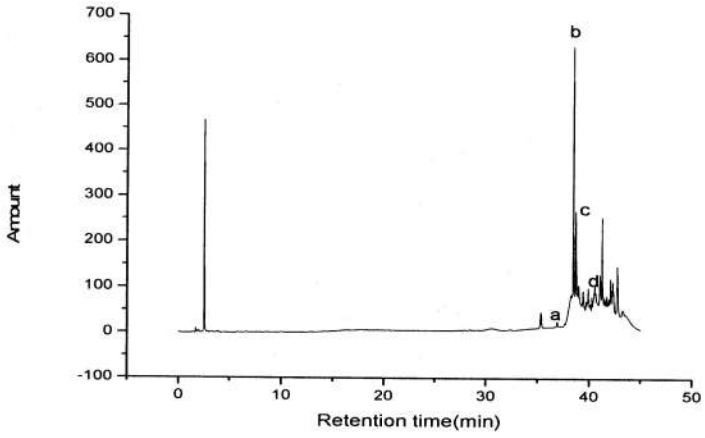
The reducing power assay of the rice samples were determined based on the reduction of ferricyanide complex to the ferrous form in presence of reductants (antioxidants) in the rice samples (Muller,2011). UA cultivars of rice showed significantly higher reducing power with increase of sample concentration than PA and LA cultivars. It may be attributed to the

presence of significantly higher amount of phenolic compounds in UA cultivar than PA and LA cultivars.

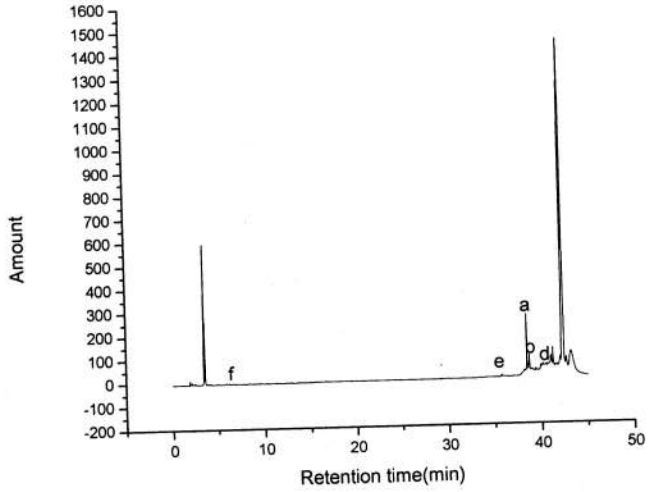
High phenolic contents in the samples result in the high reduction of Fe^{3+} to Fe^{2+} by donating an electron and which can be measured by the formation of pearl's Prussian blue at 700 nm indicates an increase in reductive ability (Khaled-Khodja,2014).

The measurement of H_2O_2 scavenging activity is one of the useful methods for determining the ability of antioxidants to decrease the level of prooxidants (Malar,2014). Phenolic compound in extract act a as electron donor, for conversion of hydrogen peroxide to water may accelerate that increase the scavenging activity of extract. It was also observed that there was strong correlation between hydrogen peroxide scavenging activities of all three samples and concentration and similar results were also observed by Park (Wettasinghe,2014).

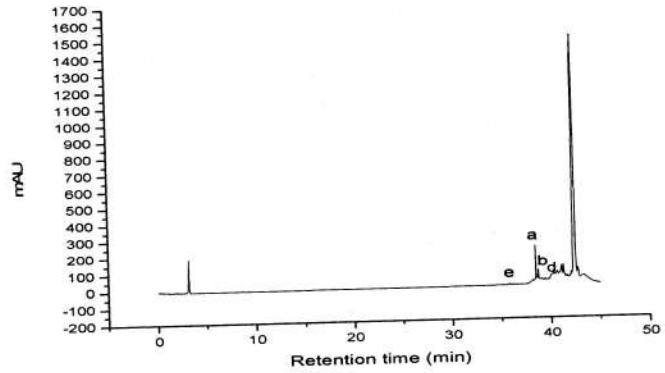
The concentration of total phenolics in the cultivars is associated with the antioxidant activities (Gi, 2003, Itani 2004 and Katalinic 2013) which has potential benefits such as reduction of oxidative stress, cardiovascular problems, blood and lipids related diseases. The rice cultivars used in the present study have various types of phenolic acid content. Salicylic acid, caffeic acid, quinic acid, apigenin, ferulic acid, gallic acid and quercetin were identified in pigmented cultivars whereas the last two acids were not detected in white rice.



(a) LA



(b) UA



(c)

Fig. 8 (a) LA (b) UA and (c) PA HPLC chromatgram

NOTE: a : Quinic acid b : Salicyclic acid c : Quercetin acid d : Apigenin e : Ferulic acid f : Gallic acid

Table 12 Quantification (Mg/L) of Predominant Phenolic Acids Present in three rice cultivars

Active compound	LA	UA	PA
Quinic acid	255.462	611.460	133.927
Salicyclic acid	302.067	44.507	231.943
Quercetin	NA	33.279	NA
Apigenin	7.039	0.421	0.490
Gallic acid	0.983	NA	NA
Caffeic acid	NA	NA	1.835
Ferulic acid	17.218	NA	15.182

Passion fruit Proximate analysis

The moisture and ash content of passion fruit pulp was 82.25 ± 0.01 and 2.60 ± 0.05 %. Brix° and pH were 13.09 ± 0.10 and 3.2± 0.15. Titrable acidity and Ascorbic acid (mg/100g) is 65.191 ± 0.51.

Table 13 Proximate analysis of fruit

Parameter	Passion fruit
Moisture content (%)	82.25 ± 0.01
Ash content (%)	2.60 ± 0.05
Brix °	13.09 ± 0.10
pH	3.2± 0.15
Ascorbic acid (mg/100g)	65.191 ± 0.51
DPPH (%)	90.53

Table 14 Regression coefficients and ANOVA of the second-order polynomial model for the response variables

Parameters	F value	P value	Lack of fit	r^2
Vitamin C (mg/100g)	15.43	<0.0001	4.26	0.9328
TPC (mg/100g)	7.18	0.0024	1.15	0.8661
Hygroscopicity (g/100 g)	6.92	0.0028	4.49	0.8616
Moisture content (%)	12.03	0.0003	0.81	0.9155

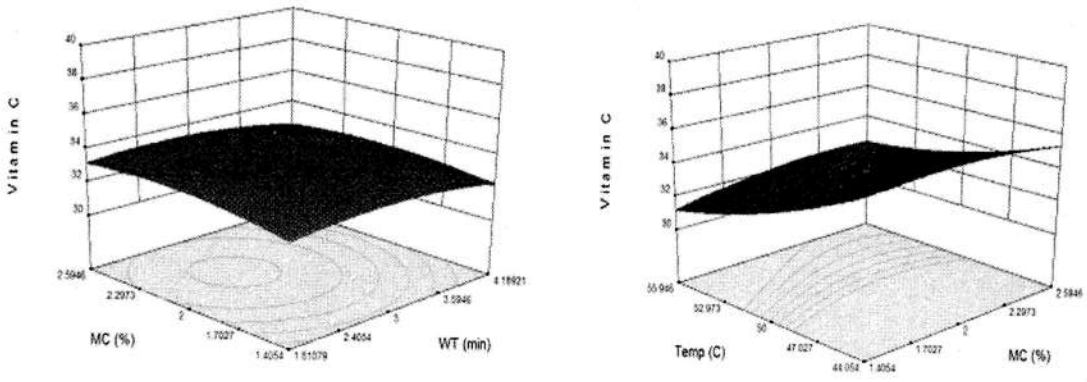
In Table 14 Regression analysis and analysis of variance (ANOVA) for fitting the models were represent and to examine the statistical significance of the model terms. The adequacy of the models were determined using various parameters viz., model analysis, lack-of-fit test, and R^2 (coefficient of determination) analysis (Lee et al. 2000). The significance of the analysis of the judged statistically by computing the F value at 5% significance level shown in the

The second order polynomial equation model was fitted to dependent variables with the experimental data

$$y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta^2_{ii} + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_J$$

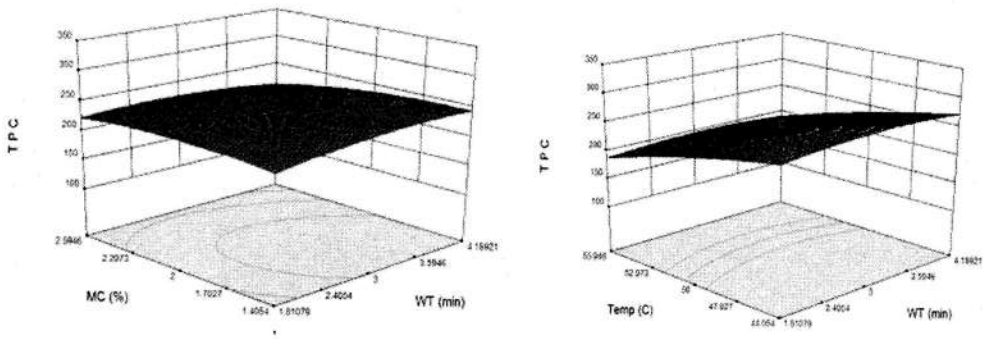
The coefficient of the polynomial equation were β_0 (constant term), β_i (linear effects), β_{ii} (quadratic effects) and β_{ij} (interaction effects) and $X_i's$ represent the coded independent variables.

The effect of Foam density was observed. Fig. 9 It is commonly used to evaluate whipping properties of foam sample. Falade et al., (2003) stated that the more air incorporated during whipping will lower the foam density, the more air present in the foam increases the whip ability



a) b)

In the Fig 9 (a). Effect of the Vitamin C shows no significant effect on the Methyl cellulose (%) and the temperature (°C), whereas the Fig 9 (b) and 1(c) shows slight impact on the methyl cellulose (MC %) temperature (T°C) and whipping time (Min) at 52.4 ^{0}C

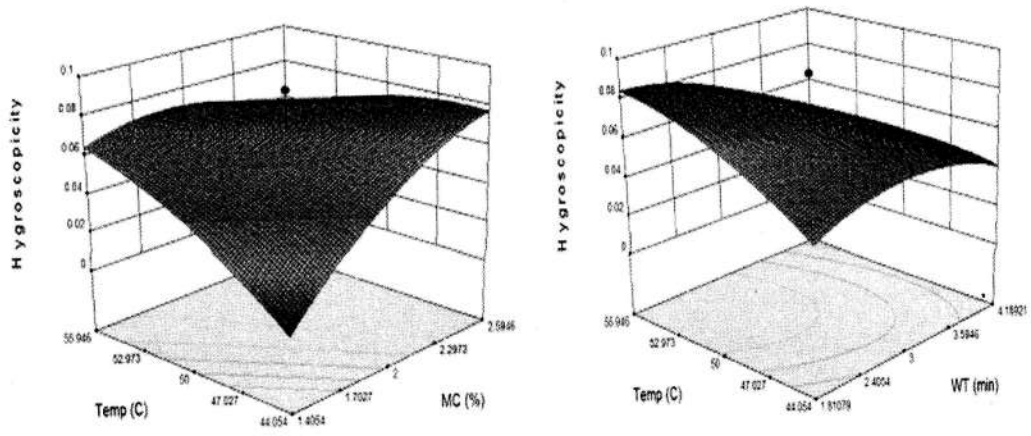


(a) (b)

Fig. 10 Effect of TPC on MC (%), WT (Min) and Temp (^{0}C)

Methyl cellulose (MC %) and whipping time (WT min) Fig 10 (a) shows no effect on total phenolic content (TPC mg/100g) of the foam mat powder but Fig 10(b) shows

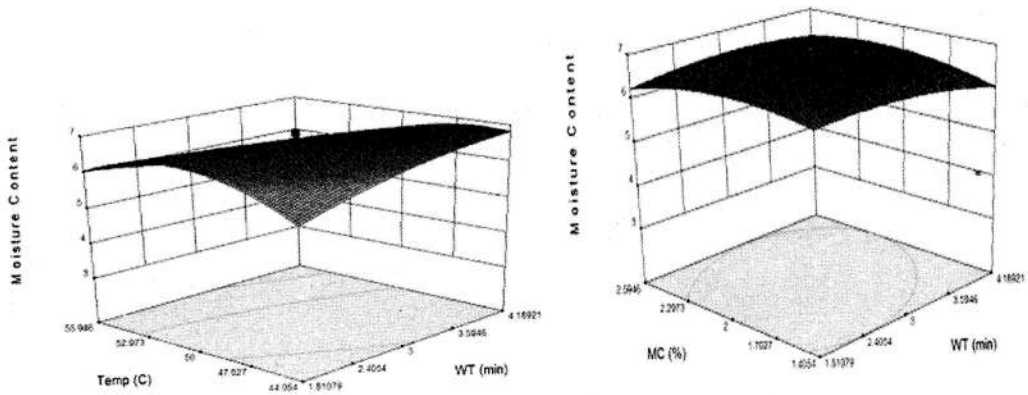
increasing whipping time (min) and methyl cellulose (%) has significant effect on total phenolic content



(a) (b)

Fig 11.Effect of hygroscopicity on MC (%), WT (Min) and Temp (^{0}C)

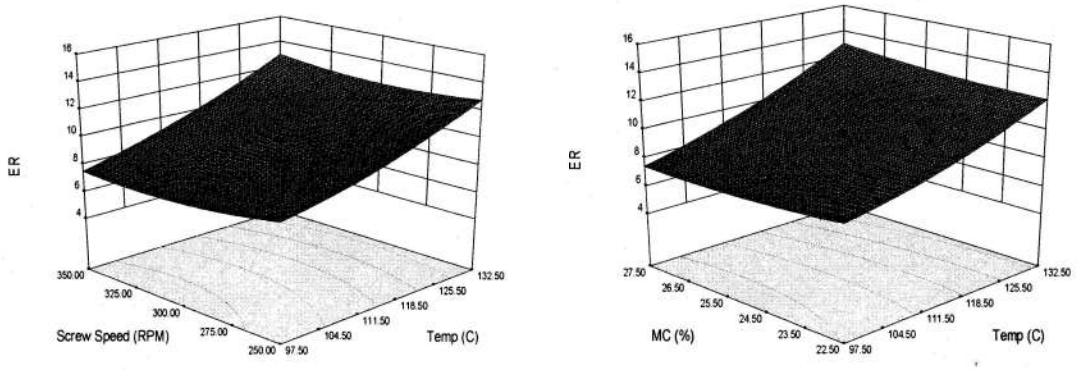
Increase in temperature (T ^{0}C) shows significant effect on the hygroscopicity. As the temperature increase Fig 11(a) Hygroscopicity also shows slight increase graph. However, Fig 11(b) whipping time (min) also shows no significant effect on the hygroscopicity.



(a) (b)

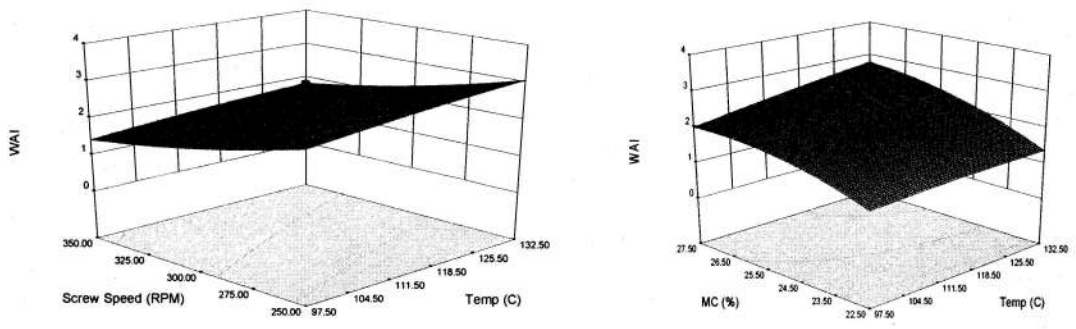
Fig 12. Effect of Moisture content on MC (%), WT (Min) and Temp (^{0}C)

In **Fig** 12. (a) and (b) as the whipping time (min) increases the moisture content also increases. Therefore, it shows slight significant effect on the moisture content. However, Methyl cellulose (%) does not shows any significant effect on the moisture content.



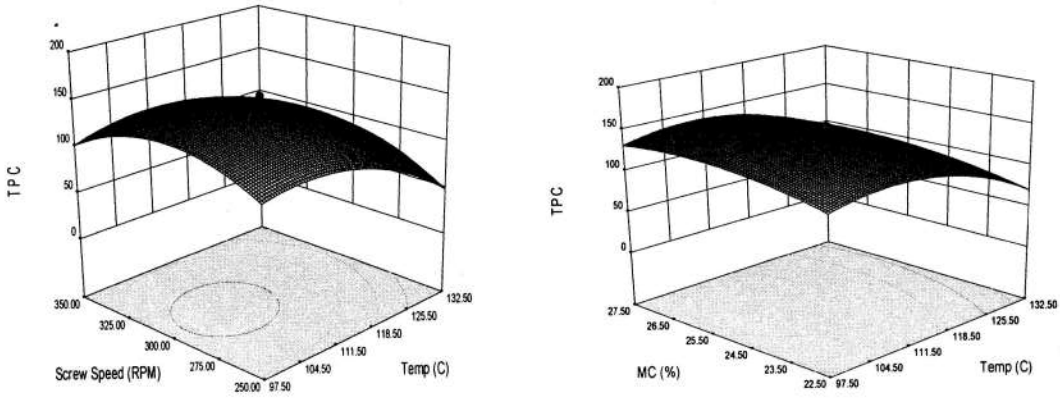
(a) **(b)**

Fig.13 Effect of (a) Screw speed and temperature (b) Moisture content and temperature on expansion ratio



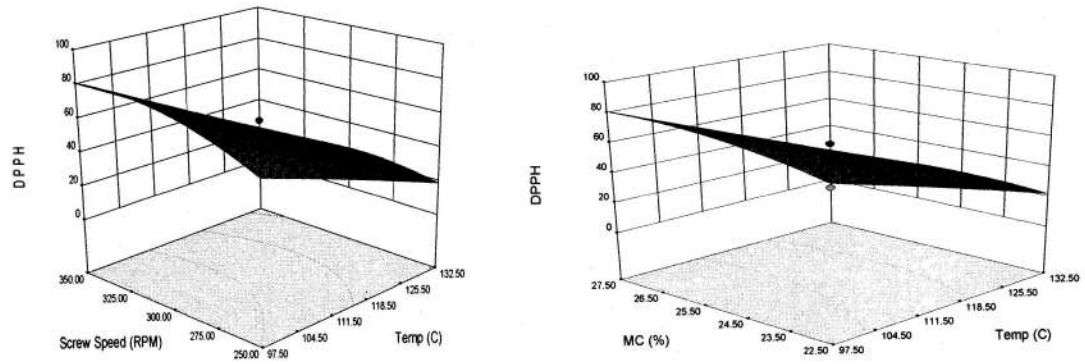
(a) **(b)**

Fig. 14 Effect of (a) screw speed temperature and (b) moisture content and temperature on Water absorption index.



(a) **(b)**

Fig. 15 Effect of (a) screw speed and temperature and (b) Moisture content and temperature on total phenolic content

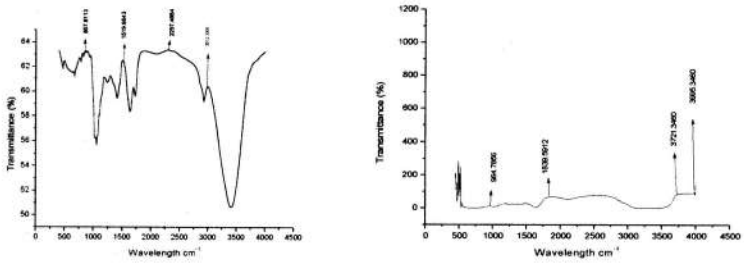


a) **b)**

Fig. 15 Effect of (a) Screw speed and temperature (b) Moisture content and temperature

Table 15. Representing the optimized condition of passion fruit foam mat dried powder

WT (Min)	Methyl Cellulose (%)	Temp (°C)	Vitamin C (mg/100g)	TPC (mg/100g)	Hygroscopicity (%)	Moisture Content (%)	Desirability
1.811	1.505	44.054	35.450	262.506	0.002	5.694	0.737



(a) (b)

Fig. 16 FT-IR spectra of (a) Passion fruit pulp and (b) optimized foam mat powder

Comparison of passion fruit pulp and the foam mat dried powder was illustrated in the Fig. 16. Pulp shows the spectral stretching ranging from 964.7855 to 3995.3460cm^{-1} and foam mate spectral stretching ranging from 857.8113 to 3012.3305 cm^{-1}. Band of C=O stretching was mainly due to the presence of carboxyl (-C=O) group of phenolic compounds. A stretching characteristic peak at approximately at region 3420 cm^{-1} and C–H stretching band at around 2937 cm^{-1} are reported as the hydroxyl group, C=O vibration in the carbonyl group at 1650 cm_1 was hydrogen bonded. (Santhiya, Subramanian, & Natarajan, 2002)

Table 16 Optmized extrudate product parameters

Temp (^{0}C)	**Screw Speed (rpm)**	**Moisture content (%)**	**PF (%)**	**Expansion Ratio**	**WAI**	**TPC (mg Gallic acid equivanlent/100g)**	**DPPH (%)**
98.100	250.000	26.630	11.250	7.931	2.976	133.469	67.146

Storage study of optimized extrudate product with different packaging materials was carried out for 28 days with the interval of 4 days, 50 % RH at 30 °C. Four different packaging materials *viz.*, polypropylene, High-density polyethylene, Low-density polyethylene and metalized polyester were used. In the Fig. 17, It has been found that

Moisture content of Polypropylene (PP) was observed highest i.e. 17.2 % among the four different packaging materials and Lowest moisture content 13.8 % was observed in LDPE.

Comparative study of optimized extrudate product and marketed product by HPLC

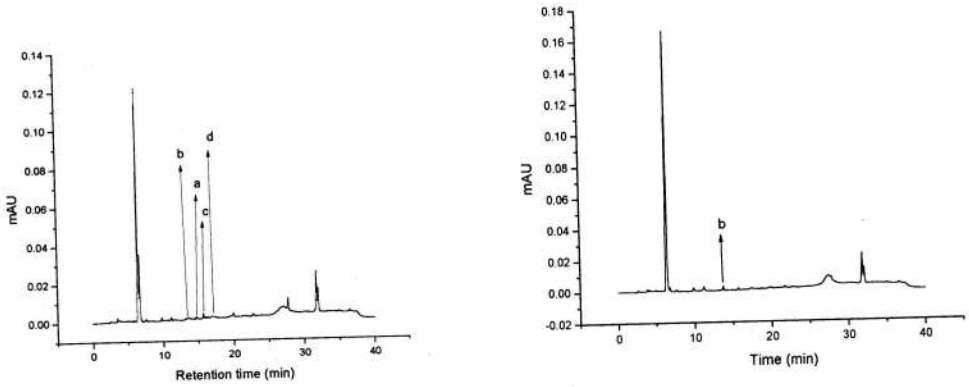


Fig. 18 HPLC chromatogram of (a) optimized extrudate product and (b) market product

In the Fig18.(a) and (b) HPLC chromatogram presence of four phenolic compound have been identified.namely (a) Caffeic acid, (b) (±) Catechin hydrate, (c) Þ- Coumeric and (d) Syringic acid.and only presesnce of one phenolic compound was (b) catechin in the market product only two type of phenolic acid was identified .The optimized extrudate product was prepared from red rice and passion fruit foam mat dried powder this might be the reason of presence and detection of various phenolic compound in the final product compare to marketed one.

Hence the product is rice in the bioactive compounds and in the future can be marketed as the health benefit item.

11. Conclusions summarizing the achievements and indication of scope for future work:

The present investigation was conducted to develop a functional food product from the underutilized crop of north-east India. Lingkang taker ame (LA), Umling ame (UA), and Pungpo taker ame (PA) were the three variety used for development of rice based function breakfast cereal. It was revealed that the pigmented rice variety content more than 12 bioactive compounds. The detected bioactive compounds were β-Carotene, Tocopherol, Tocotrierol, cyanidin-3-glucoside, Peonidin-3-D-glucoside, Caffeic acid, Catechin hydrate, Chlorogenic acid, Coumeric acid, Trans-Ferulic acid, 4-Hydroxybenzoic acid, Syringic acid, Sinapic acid and Vanillic acid. It was also found out that the develop

functional food product also contain. To develop the functional breakfast cereal a twin screw extrusion process was used. The extrusion process was optimized using response surface methodology. The optimize condition were temperature 98.10°C, screw speed of 250 rpm and feed moisture content 26.63%. In the optimized condition the predicted response were 11.250 expansion ratio, 7.931 of water solubility index 2.976, 133.469 mg GAE/100g of total phenolic compounds and 67.146 % of DPPH antioxidant activity. In the final product four types of phenolic acid were detected namely Caffeic acid, (±) Catechin hydrate, Þ- Coumeric and Syringic acid. It confirmed that the developed breakfast cereal had functional properties. The storage life study of the develop product showed that with change of packaging material there was a variation of water adsorption behavior of the product. After 25 day it was observed that the material store in polypropylene was adsorbed highest amount of moisture than other packaging a material

Future scope

1) in-vivo and in-vitro study of develop food product.
2) Degradation behavior of the phenolic compounds
3) Development of functional ingredient from the pigmented rice

12. S&T benefits accrued:

i. List of Research publications

International publication

i. Duyi S, Deka C. S. and Das B. A. (2015) "Phytochemical and antioxidant profile of pigmented and non-pigmented rice cultivars of Arunachal Pradesh, International Journal of Food Properties http://dx.doi.org/10.1080/10942912.2015.1055761.

ii. Duyi S, Deka C. S. and Das B. A. (2015) Evaluation of physical, thermal, pasting and mineral characteristics of pigmented and non-pigmented rice cultivars Journal of Food Processing and Preservation.

National publication

iii. Duyi S, Deka C. S. and Das B. A. (2014) Pulished in 7th international food convention, CFTRI, Mysore. Studies on physicochemical properties of some selected underutilized rice cultivars of arunachal Pradesh.

iv. Duyi S, Deka C. S. and Das B. A. (2014) "Pigmented Rice a Potential Source of Bioactive Compounds: A Review" ETTAE 2014 Conference being held during November 07-09 in NERIST, Nirjuli (Itanagar) Arunachal Pradesh.

v. Duyi S, Deka C. S. and Das B. A. (2014) Effect of different pH and temperature on the stability of anthocyanidin content of red rice, on Innovative Prospects in Food Processing: Integration of Engineering and Biological Sciences 27 -28 th March, 2015, At Tezpur University

vi. Manpower trained on the project

a) Research Scientists or Research Associates

b) No. of Ph.D. produced: **one**

c) Other Technical Personnel trained

vii. Patents taken, if any

vii. Patents taken, if any

13. Financial Position:

No	Financial Position/ Budget Head	Funds Sanctioned		Expenditure	% of Total cost
I	Salaries/ Manpower costs	211200.00	150000.00 (receive without distribution)	302968.00	
II	Equipment	2150000.00		2149288.00	
III	Supplies & Materials	135000.00		176913.00	
IV	Contingencies				
V	Travel	30000.00		45840.00	
VI	Overhead Expenses				
VII	Others, if any				
	Total		2676200.00	2675009.00	**99.95%**

14. Procurement/ Usage of Equipment

S No	Name of Equipment	Make/Model	Cost (FE/ Rs)	Date of Installation	Utilisation Rate (%)	Remarks regarding maintenance/ breakdown
1	Twin Screw Extruder	Yes , Flytech Engineering	1797600	21.07.14	100	Running condition
2	Grinder	Yes, Voltam Furnace industries	161700	21.01.14	100	Running condition
3	Balance	Yes, Mettler-Toledo ME-204	78000	21.01.14	100	Running condition
4	Hot air oven/dryer	Yes, Ovfd-o-OMS (3636D)	111988	21.01.14	100	Running condition
			2149288			

b) Plans for utilizing the equipment facilities in future

Though the project is completed, the equipment are still used by our UG teaching, PG and PhD research activities all the equipment will be under the control of Tezpur University administration.

Name and Signature with Date

Amit Barman Das 12.10.15

(Principal Investigator)

12/10/15

(Co-Investigator)

Statement of Expenditure

30.04.2013 to 31.03.2014 and 01-04-2014 till 31.09.2015

Sr No	Sanctioned Heads	Funds Allocated (indicate sanctioned or revised) (III)		Expenditure Incurred 1st Year (DOS to 31st March next year) (IV)	2nd Year (1st April to 31st March next year) (V)	3rd Year (1st April to 31st March next year) (VI)	4th Year (1st April to project completion) (VII)	Total (IV+V+VI+ VII) (VIII)	Balance, if any (III-VIII)	Remarks
(I)	(II)	1st year	2nd year							
1.	Manpower costs	211200.00	150000.00 (receive without distribution)	142968.00	144000.00	16000	119,432.00	422400.00	-119432.00	Detail in Annexure-I attached
2.	Consumables	135000.00		5060.00	171853.00	--------	93087.00	270000.00	-93087.00	
3.	Travel	30000.00		23666.00	22174.00	--------	14160.00	60000.00	-14160.00	
4.	Contingencies									
5.	Others, if any									
6.	Equipment	2150000.00		351688.00	1797600.00	---------		2149288.00	712.00	
7.	Overhead expenses									
8.	Total		2676200.00	523382.00	2135627.00	16000		2901688.00	-225488.00	

N.B. ₹ 376200.00 were allotted for 2nd year out of which 150000.00 has been received and remaining ₹ 225488.00 need to fulfil the project expenditure.

Amount to be receive (whichever is appropriate): ₹ 225488.00

Amit Boran Das

Name and Signature of Principal Investigator:

Date: 14.03.16

15-03-16

Signature of Competent financial/ audit authority: _____

(with seal) Date: _____

Finance Officer
Tezpur University

UTILISATION CERTIFICATE

Annexure-III

FOR THE FINANCIAL 2015-16 - (01/04/2015-11/09/2015)

1. Title of the Project/ Scheme: Studies on development of cereal based functional breakfast food from the underutilized crops of North-East India

2.	Name of the Institution:	Tezpur University (central)
3.	Principal Investigator:	Amit Baran Das
4.	Department of Science & Technology sanction order No & date sanctioning the project:	SERB/MOFPI/0004/2013 &21/10/2014
5.	Head of account as given in the original sanction order:	Rs. 29, 02,400/-
6.	Amount brought forward from the previous Financial year quoting DST letter no and date in which the authority to carry forward the said amount was given	i. Amount : Rs. 2002818.00 ii. Letter SERB/MOFPI/0004/2013 iii. Date : 30/04/2013
7.	Amount received during the financial year (Please give DST letter/order no and date)	i. Amount: Rs. 150000.00 ii. Letter/Order No SERB/MOFPI/0004/2013 & iii. Date : 21/10/2014
8.	Total amount that was available for expenditure (excluding commitments) during the financial year (Sr. No. 6+7)	Rs. 17191.00
9.	Actual Expenditure (excluding commitments) Incurred during the financial year (upto 30/03/2015)	Rs. 16000 /-
10.	Balance amount available at the end of the financial year:	
11.	Unspent balance refunded, if any (please give details of cheque no etc.):	
12.	Amount to be carried forward to the next financial year (if applicable):	Rs. 1191.00

UTILISATION CERTIFICATE

Certified that out of NIL of grants-in-aid sanctioned during the year 2014-15 in favour of Registrar, Tezpur University, Napaam,Tezpur under this Ministry/ Department letter/ order No NIL and Rs. 17191.00 on account of unspent balance of the previous year, a sum of Rs. 16000 has been utilised for the purpose of implementation of Research Project for which it was sanctioned and that the balance of Rs. 1191.00 remaining unutilized on 11/09/2015 at the end of the year 2015-16.

Signature of PI

Date 11.09.15

Signature of Registrar

Registrar
Tezpur University

Date

Accounts Officer of the Institute

Finance Officer
Tezpur University

11.09.15

Date