SECTION III
MATERIALS AND METHODS
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3.1. Procurement of Pearl Millet

Two commercially available Pearl millet varieties namely ‘Kalukombu’ (K) and Maharashtra Rabi Bajra (MRB) (Fig 3.1) were procured from the local market of Mysore, India for the study.

- ‘Kalukombu’ (K) is a native variety traditionally grown by farmers in India (Karnataka, Tamilnadu and Maharashtra). This variety is not improved by the modern plant breeding system. It is considered nutritionally very superior by the local people and is used as food crop to make roti, dumpling and chapattis. The seeds of Kalukombu are small and elongated with persisting glumes/husk.

- ‘Maharashtra Rabi Bajra’ (MRB) is a commercially grown hybrid developed by the modern improved plant breeding technique by a commercial seed company. It is basically a winter crop. The seeds are grey/slate coloured, bold and round shaped without persisting glumes/husk (Anon, 2010)

![Kalukombu (K) and Maharashtra Rabi Bajra (MRB)](image)

Figure 3.1. Pictures of the Two Pearl Millet Varieties.
Food Ingredients:

All the ingredients required to prepare food products (Roti, dosa, puttu, cookies, bun, cake, ladoo and burfi) were purchased from local stores.

3.2 Chemical/Enzymes

➢ Termamyl, Amyloglucosidase, Pepsin, Pancreatin, α – amylase (porcine origin) and α, α bipiridyl was procured from Sigma chemicals Co., St Louis, MO, USA.
➢ Bile extract was procured from Loba Chemicals, Mumbai, India.
➢ Butylated hydroxytoulene (BHT) was procured from Qualigens Fine Chemicals, Mumbai, India.
➢ GOD – POD Enzymatic kit was procured from Span Diagnostics, Surat, India.
➢ Dialysis tubing (12,000 cut – off level) and 2,2,Diphenyl – 1 – picrylhydrazyl (DPPH) was procured from Himedia laboratories Pvt. Ltd, Vadhani Ind. Est., LBS marg, Mumbai, India.

All chemicals, reagents and solvents used in the present study were of analytical grade and obtained from reputed companies.

3.3 Processing of Pearl Millet

Milling

The cleaned grains were pulverized using a plate mill to obtain whole flour (WF). A part of the whole flour was further sieved through a 44 mesh sieve (BSS). The ‘+’ fraction was termed as the bran rich fraction (BRF) and the ‘−’ fraction was termed as semi-refined flour (SRF).
Wet Heat Treatment

i. **Boiling**: A batch of pearl millet grains were held in a pan of boiling water (1:1 grain to water, weight to volume basis) for 30 minutes.

ii. **Pressure cooking**: Another batch of pearl millet grains was pressure cooked in water (1:1 grain to water, weight to volume basis) for 10 mins (9.8 x 10^4 Pa).

Dry Heat Treatment

Pearl millet grains were roasted in an open pan for 10 – 15 minutes at 200°C.

Germination:

Pearl millet varieties were soaked in water overnight. The water was drained and the grains were tied in a moist muslin cloth and left to sprout at room temperature for 72 hr (Badau et al 2005).

These treatments are shown in figure 3.2. Each treatment was replicated 2 times. The wet heat treated and germinated grains were laid out on steel trays in thin layers of less than 2 cm. The trays were placed in a hot air oven and dried at 50°C for 24h. The samples were milled to flour using a hammer mill and stored in an air tight polythene bags in a cool and dry place until use.
Pearl millet grains (K and MRB)

Processing

Whole flour (Control)

Semi Refined Flour (sieved through 44 mesh sieve (BSS) (‘-’ fraction)

Bran Rich Fraction (sieved through 44 mesh sieve (BSS) (‘+’ fraction)

Roasted (10 – 15 min)

Boiled for 30 min (1:1 w/v grain to water)

Pressure cooked for 10 min (9.8x10^4)

Germinated (72 hr)

Dried at 50°C for 24hrs

Milled to flour using a hammer mill

Proximate composition – Moisture, Proteins, Fat and Ash

Minerals – Iron, Phosphorus and Calcium

Antinutrient content – Tannin, Oxalate and Phytic acid

Bioaccessible Iron and Calcium

Figure – 3.2. Experimental Design for Determining the Affect of Processing

Treatments on Pearl Millet
The processed Samples were Analysed for the Following –

I. Physicochemical Parameters
   i. Functional properties
   ii. Proximate composition
   iii. Minerals
   iv. Antinutrients
   v. Antioxidant components
   vi. Antioxidant activity
   vii. Carbohydrate profile
   viii. Protein profile – SDS-PAGE Gel Electrophoresis

II. Studies on Isolated Starch from Pearl Millet Varieties
   i. Functional properties
   ii. Proximate composition
   iii. Amylose content
   iv. Starch fractions
   v. X – ray diffraction

III. Nutrient Digestibility
   i. *In vitro* Bioaccessible Iron and Calcium content of processed Pearl millet
   ii. Molar Ratios Influencing the bioaccessible iron and calcium from processed pearl millet.
   iii. *In vitro* protein digestibility of processed pearl millet
   iv. Starch fractions of processed pearl millet
IV. Product Development

i. Preparation of products

ii. *In-vitro* iron and calcium bioaccessibility and nutritionally important starch fractions in breakfast items

iii. Packaging and storage studies

iv. Chemical analysis – Moisture, free fatty acid and peroxide value

v. Sensory analysis

vi. Equilibrium Relative Humidity studies

The Procedures Followed for Analysing the Mentioned Parameters are as follows:

3.4. Functional Properties

**Bulk Density:** Raw and processed pearl millet flour was filled into 10ml measuring cylinder and gently tapped on a cloth. The values were recorded and bulk density was expressed as ml/g.

**Water and Oil Holding Capacity:** Water and oil holding capacities of the raw and processed pearl millet flours were determined by centrifuge method (Sosulki, 1962). Each of the sample (1g) was placed in a 50ml centrifuge tubes, distilled water/oil (30ml) was added to each tube and the contents were mixed well (30sec) using a glass rod. The tubes were allowed to stand for 10 min; additional seven mixings were made with 10 min rest period following each mixing. The suspensions were centrifuged at 2,300rpm for 25min, the supernatant was
decanted, the tubes were drained and dried in the oven at 50°C for 25min cooled in a desiccator and weighed.

**Swelling Power and Solubility:** Swelling power and solubility was determined by centrifuge method (Sosulki, 1962). Each of the sample was placed in a 50ml centrifuge tube, distilled water (30ml) was added, mixed well and heated at 55°C, 65°C, 75°C, 85°C and 95°C respectively in a water bath with intermittent stirring for 30 minutes. Centrifuged at 2000rpm for 20 min and the supernatant was decanted and evaporated on a steam bath to obtain dissolved solids. The sediment flour was weighed to obtain the weights of the swollen flour particles.

3.5. **Proximate Composition**

Standard methods of AOAC (2005) were used to determine Moisture (AOAC – 925.10), fat (AOAC – 2003.05) by soxhlet extraction and ash (AOAC – 923.03) by combustion. Protein (AOAC – 960.52) content (N×6.25) was determined by micro Kjeldahl method.

3.6. **Mineral Content**

Mineral analysis was carried out on samples digested with hydrochloric acid. Total iron (AOAC – 944.02) was analysed by colorimetric method using αα bipyridyyl method (AOAC 2005). Total phosphorus was analysed colorimetrically using Taussky & Shorr method (1953). Total calcium was analysed by Raghuramulu method (1983).

3.7. **Antinutrients**

- **Oxalate** content was determined as described by Baker *et al* (1952). Oxalates were extracted with HCl, precipitated as calcium oxalates from
the deproteinized extracts and were estimated by subsequent titration with potassium permanganate.

- **Phytic Acid** was extracted & determined according to Thompson & Erdman method (Thompson *et al* 1982).

- **Tannins**: Tannin content in the samples was measured using the method described by AOAC (1970).

### 3.8. Antioxidant Components

- **Tannins** as mentioned above

- **Phytic Acid** as mentioned above

- **Flavonoids**: The total flavonoids content of the samples were determined according to Miliauskass *et al* (2004) using rutin as a reference compound.

  One ml of plant extract in methanol (10g/l) was mixed with 1ml if aluminum trichloride in ethanol (20g/L) and diluted with ethanol to 25ml. The mixture was incubated at 20°C and the absorbance was measured at 415 nm. The blank was prepared by diluting 1 ml plant and 1 drop acetic acid to 25 ml with ethanol. The flavonoids in the samples were calculated using the following formula.

\[
X = \frac{(A \times m_{o} \times 10)}{(A_{o} \times m)}
\]

Where:  
\(X\) – Flavonoid content, mg/g plant extract in Rutin Equivalents.  
\(A\) – Absorption of Sample  
\(A_{o}\) – Absorption of standard rutin solution  
\(m\) – Weight of Sample  
\(m_{o}\) – Weight of rutin in the solution
3.9. Antioxidant activity

Raw and processed pearl millet flours (15g) were extracted with 200mL of methanol in a mechanical shaker for 24h, filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The concentrated extract were re-dissolved with methanol to a concentration of 10mg/ml and stored in the refrigerator until analysis. All the analysis was carried out in triplicates.

- **DPPH Radical Scavenging Activity**

  The ability of the methanolic extracts to scavenge free radicals was determined against a very stable free radical DPPH determined spectrometrically (Blois 1958). Aliquot of the sample extract at different concentrations were added to 1mM methanolic solution of DPPH. The mixture was vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

  \[
  \text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
  \]

- **Reducing Power Assay**

  The ability of methanolic extracts to reduce iron (III) to iron (II) was assessed by the method of Yildrim *et al* (2001). The dried extract (125-1000µg) in 1ml of the corresponding solvent was mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of potassium ferricyanide (K₃Fe(CN)₆; 10 g l⁻¹), and then the mixture was incubated at 50°C for 30 min. After incubation, 2.5ml of TCA (100 g l⁻¹) was added and the mixture was centrifuged at 1650 rpm for 10 min. Finally, 2.5ml of the supernatant solution were mixed with 2.5ml of distilled water and
0.5ml of FeCl₃ (1g l⁻¹) and the absorbance was measured at 700 nm. High absorbance indicates high reducing power.

**FRAP (Ferric – Reducing Antioxidant Power)**

The FRAP assay was carried out according to the procedure of Benzine and Strai (1999). The FRAP reagent was prepared by mixing acetate buffer (25 ml, 300mmol/L, Ph 3.6), 10 mmol/L TPTZ solution (2.5mL) in 40 mmol/L HCl and 20 mmol/L FeCl₃ solution (2.5 mL) in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh and warmed to 37°C in a water bath prior to use. One hundred and fifty micro liters of the sample was added to the FRAP reagent (4.5mL). The absorbance of the reaction mixture was than recorded at 593nm after 4 min, the assay was carried out in triplicates. The standard curve was constructed using FeSO₄ solution (0.5 – 10 mg/mL). The results were expressed a µmol Fe (II)/g dry weight of plant material.

**3.10. Carbohydrate Profile**

- **Total Dietary Fiber**: Total dietary fibre (TDF) was measured as the sum soluble and insoluble dietary fibre as described by Asp et al (1983).

- **Amylose**: Total and soluble amylose content of the flour samples were determined according to the method of Sowbhagya et al (1971).

- **Sugars**: Total sugar content was estimated using Michel Dubois et al (1956) method.
3.11. Protein Profile Using Gel Electrophoresis

- **Protein Extraction:** Each of the processed flours (1g) was extracted with tris-HCl buffer (50 mmols, pH-7.2) at 4°C with intermediate mixing. The extracts were separated by centrifuging at 3000 RPM for 15 min. Each step was repeated twice. The extracts were combined and stored at 4°C until analysis. The total soluble protein content was estimated using Lowry’s methods (1983). Each of the extracts (40 to 50 µg) was dissolved in the sample buffer that contained 10% SDS, glycerol, tris buffer (0.5M, pH 6), β- mercaptoethanol, and bromophenol blue. The extracts were boiled for 5 min before being loaded.

- **Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE):** SDS-PAGE was performed using 12.5% separating gels and 5% stacking gels. The gels were 1.5 mm thick and consisted of a 2 cm stacking gel and a 10 cm running gel. Protein extract (40 – 50 µl) was loaded into the sample wells. Electrophoresis was carried out at an initial voltage of 50V till the samples reached the separating gel and later increased to 100V and the electrophoresis was carried out of 2h. Gels were stained with 0.2% Coomassie Brilliant Blue R-250 in 50ml methanol, 12ml acetic acid and 38ml of water and destained in the same solvent. A low \( M_w \) standard was used to estimate protein subunit molecular weights.
3.12. *In-vitro* Bioaccessibility of Iron and Calcium

*In vitro* mineral bioaccessibility was determined by the method of Luten *et al* (1996) (Fig. 3.3.)

- **Gastric Stage:** - The sample (10g) was mixed with 80 ml water in a 250 ml Erlenmeyer flask. The pH was adjusted to 2.0 by adding 6 M HCl. The pH was checked after 15 min and if necessary readjusted to 2.0. Freshly prepared pepsin solution (3 ml) was added and the sample was made up to 100 ml with water. After mixing, the sample was incubated at 37°C in a shaking water bath for 2 h. The gastric digests were stored in ice for 90 min during which the titratable acidity was measured in an aliquot.

- **Titratable Acidity:** - A homogeneous aliquot of pepsin digest (20 ml, 20°C) was taken and 5 ml of freshly prepared pancreatic mixture was added. The pH was adjusted to 7.5 with 0.5 M NaOH. After an equilibrium period time of 30 min the pH was checked and readjusted to pH 7.5 if necessary. Titratable acidity was defined as the amount of 0.5 M NaOH required in order to reach a pH of 7.5.

- **Intestinal Stage:** - Homogenized pepsin digest aliquots (20 ml) were weighed into wide-necked 250 ml Erlenmeyer flasks, which were placed in a water bath at 37°C for 5 min. Segments of dialysis tubing containing 25 ml water and NaHCO₃ were then added immediately, the amount of NaHCO₃, being equivalent in moles to the NaOH used to determine the titratable acidity. The weight of the dialysis bag plus content and clips was determined. The length of the dialysis tubing from clamp to clamp was set at 250 mm. After 30 min the pH was measured and 5 ml of the pancreatic mixture was added to each digest. The digests were incubated in
a shaking water bath for 2h at 37°C. At the end of the incubation period the pH was measured. The Erlenmeyer flasks were closed with parafilm in order to reduce CO₂ losses. The dialysis bags were rinsed with water, carefully dried and weighed. The content of each dialysis bag was transferred into acid-washed containers and analyzed for its iron content using colorimetric method (AOAC 2005) and calcium by Raghuramulu method (1983).
**Gastric Stage**

Sample (15g) + 80ml of demineralized water

Adjust pH to 2 with 6M HCl

Recheck after 15 min and readjust to pH 2 with 6M HCl

Add 3ml of freshly prepared Pepsin solution and make up to 100 ml with demineralized water

Incubate at 37°C for 2hr

Freeze the samples for 90 min

**Titratable Acidity**

20 ml of gastric digest

5ml of Pancreatin mixture

After 30 min check pH and readjust to pH 7.5

Equate the quantity of NaOH required to neutralize with NaHCO₃

**Intestinal Digestion**

20 ml gastric digest (equilibrate to 37°C for 5min in water bath)

Incubate dialysis tube containing 25 ml of water + NaHCO₃

(From titratable acidity) at 37°C for 2hr

Drain the content and note down the volume of the dialysate

Analyse for bioaccessible iron and calcium

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*Figure – 3.3. Flow Chart for In - Vitro Bioaccessible Iron and Calcium Content.*
3.13. *In-vitro* Protein Digestibility

*In-vitro* protein digestibility was estimated by enzymatic method of Akeson and Stahmann (1964) (Fig.3.4). Samples were homogenized and suspended in 15ml of 0.1N HCl containing 1.5mg of pepsin and incubated at 37°C for 3 hrs. The suspension was then neutralized with 0.5N NaOH and treated with 4mg of pancreatin in 7.5ml of phosphate buffer (pH 8.0) containing 0.005M sodium azide. The mixture was gently shaken and incubated at 37°C for 24h. After incubation, the sample was treated with 10 ml of 10% TCA and centrifuged at 3000rpm for 20 min. Protein in the supernatant was estimated by Lowry’s method (1983). %IVPD was calculated using the following formula:-

\[
\text{%IVPD} = \frac{\text{Digested proteins}}{\text{Total proteins}} \times 100
\]

Figure – 3.4. Flow Chart for *In-Vitro* Protein Digestibility

Nutritionally important starch fractions such as total starch and different starch fractions – RDS (rapidly available glucose), RS (resistant starch), and RAG (rapidly available starch) were measured by the methods of Englyst et al (1992) (Fig. 3.5.)

The various starch fractions were measured in the samples after incubation with Invertase (to hydrolyse sucrose), Pancreatin, Amyloglucosidase and guar gum (to standardize the viscosity of the incubation mixture) at 37°C in capped tubes immersed in a shaker water bath. A value for RAG was obtained as the glucose released after 20 min (G_{20}). A second measurement (G_{120}) was obtained as glucose released after the further 100 min incubation. A third measurement (TG – total glucose) was obtained by gelatinization of the starch in the boiling water and treatment with 7M KOH at 0°C, followed by complete enzymatic hydrolysis with Amyloglucosidase. Resistant starch was measured as the starch remained unhydrolyzed after 120 min incubation. Free glucose (FG) was also determined by treating the sample with acetate buffer and placing the tube in water bath at 100°C for 30 min. simultaneous tests were run in a similar manner with glucose standard. A blank tube containing buffer, glass balls and guar gum was also included to correct for the glucose present amyloglucosidase solution. A summary of the analytical strategy in presented in Fig – 3.5. Glucose was determined in all the samples using glucose oxidase – peroxidase diagnostic kit.
Treatment of Data

The values of TS, RDS, SDS and RS were calculated from the values of $G_{20}$, $G_{120}$, FG and TG as follows:

1. $TS = (TG - FG) \times 0.9$
2. $RDS = (G_{20} - FG) \times 0.9$
3. $SDS = (G_{120} - G_{20}) \times 0.9$
4. $RS = TS - (RDS + SDS) \text{ or } (TG - G_{120}) \times 0.9$

The relative rate of starch digestion was calculated as follows –

$$SDI = \frac{RDS}{TS} \times 100$$

$RAG = \text{Free glucose} + \text{glucose from sucrose} + \text{glucose released within 20 min incubation i.e.,} \ RAG = FG + \text{glucose from sucrose} + G_{20}$

$SAG = G_{120} - G_{20}$
Materials and Methods

Sample + guar gum

Add acetate buffer

10 min at 37°C

Add Amyloglucosidase + Pancreatin + Invertase (time zero)

(Incubate with shaking at 37°C)

After 20 min remove 0.5 ml portion in 2 ml 66% Ethanol

After 120 min remove 0.5 ml portion in 2 ml 66% Ethanol

Mix, place in boiling water bath for 30 min

Vortex mix, cool in ice bath

Add 7M KOH

Incubate in ice bath for 30 min

Add 1 ml aliquot in 10 ml 0.5 M Acetic acid

Add Amyloglucosidase

Incubate at 70°C for 30 min

10 min in boiling water bath cool, dilute to 50 ml, centrifuge

Total glucose

Figure – 3.5. Summary of the Analytical Strategy for Measurement of Starch Fractions.
3.15. Isolation of Starch

Starch from the two pearl millet varieties was isolated by steeping in water followed by centrifugation (Tharanathan, 1995) (Fig. 3.6). The crude starch isolate was purified by repeated washings with sodium chloride (0.1 M) – toluene (10:1, v/v) and later by differential sedimentation in water.

Suspend the flour in water overnight

Sieve through BSS (60 and 150)

Repeat till the ‘+’ fraction is free of starch using iodine test

Centrifuge the slurry and wash with water

Set pH to 9.5 using 0.1N NaOH

Wash 3-4 times to remove the alkali

Add water and stir for 5h

Centrifuge and wash till neutral pH

Add NaCl (0.1N): Toluene (1:1) and stir for 3hr

Centrifuge and wash 4 – 5 times

Wash with alcohol

Stir for 3 hr and centrifuge

Add acetone

Stir for 3 hr

Air dry the starch powder

Figure – 3.6. Flow Chart for Isolation of Starch from Pearl Millet.
3.16. X – Ray Diffractometry

The whole flour and isolated starch from pearl millet, were packed in rectangular glass crucibles and exposed to X-ray beam (8 keV) generated by a X-ray diffractometer (MiniFlex –II, Desktop X-ray diffractometer, Japan) equipped with a \( \theta - \theta \) goniometer at 25 mA and 30 kV, with Cu \( \alpha \) filtered radiation. The scanning range for \( 2\theta \) was set to 6–45° to cover all the significant diffraction peaks of sample crystallites with a scan speed of 3°/min.

3.17. Preparation of Products

Following food products were prepared out of two pearl millet varieties

**Breakfast items:**

- Dosa (leavened pancake)
- Roti (unleavened product)
- Puttu (steamed product)

**Traditional sweets:**

- Ladoo
- Burfi

**Baked products:**

- Cookies
- Buns
- Cake
3.18. Acceptability Studies

Acceptability of the products such as dosa, roti, puttu, bun and cake were carried out was done by 30 untrained panelists (Ana et al 2007) who were selected among the research scholars and post graduate students in the department of Food Science and Nutrition. A 9 – point hedonic scale was used for sensory evaluation. All the above food products were presented in small plates labeled with three digit random codes. Panelists were provided with drinking water to rinse their mouth between samples. The samples were presented in random order and panelists were asked to rate their assessment of color, taste, texture and overall acceptability on a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely). A score of 5 or below was considered a limit of acceptability for all sensory attributes tested. Sensory evaluation of buns were carried out according to Indrani et al (2001)

3.19. Packaging and Storage Studies

- The prepared buns were packed in airtight polythene covers and placed in an area cleaned with alcohol. The stored bun and cake was observed for mould growth.
- Two packaging material such as poly ethylene and metallized polypropylene were selected for the study. Control and experimental cookies were packed in the two packaging material and heat sealed and stored for 3 months at room temperature (37°C). Cookies were withdrawn periodically every 15 days for analysis of sensory parameters and chemical analysis
Traditional sweets such as *ladoo* and *burfi* prepared out of pearl millet were stored in air tight stainless steel containers for a period of 1 month. Products were withdrawn periodically every 7 days for analysis of sensory parameters and chemical analysis.

**Chemical analysis:**

Standard method of AOAC (2005) was used to determine Moisture. Peroxide value (PV) and free fatty acid value (FFA) were determined by AOAC (1970a).

**Sensory Analysis**

- **Untrained Panellist:** Sensory evaluation of the products were carried out by 30 panelists (Ana *et al.*, 2007) periodically, selected among the research scholars and post graduate students in the department of Food Science and Nutrition. A 9 – point hedonic scale was used for sensory evaluation. All the food products were presented in small plates labeled with three digit random codes. Panelists were provided with drinking water to rinse their mouth between samples. The samples were presented in random order and panelists were asked to rate their assessment of color, taste, texture and overall acceptability on a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely). A score of 5 or below was considered a limit of acceptability for all sensory attributes tested.

- **Trained Panelist:** The sensory evaluation of the cookies was carried out by a group of 10 trained panel members. Sensory evaluation was carried out under while fluorescent light, with the booth area maintained at temperature 22±20°C and RH 50±5%. A suitable score card was designed using “Free-Choice Profiling”
method. The attributes selected were grouped under modalities such as color, texture, aroma taste etc. A descriptive method, quantitative descriptive analysis (QDA) was adopted to assess the quality of the cookies. Panelists were asked to mark on a scale of 0-15cm to indicate the intensity of each attribute listed on the score card. The scale was anchored as ‘low’ and ‘high’ at 1.25 cm on either end, representing ‘Recognition Threshold’ and ‘Saturation Threshold’ respectively. Samples were presented one at a time in porcelain plates coded with 3-digit random numbers, to the panelists. Along with the samples, warm water and puffed rice were served as palate cleansers.

3.20. Equilibrium Relative Humidity Studies

To study the influence of moisture on cookies prepared out of pearl millet flour, the humidity – moisture content relationship of the product was studied at room temperature by exposing weighed quantities of cookies in Petri dishes to relative humidities (RHs) such as 31%, 64% and 76% using appropriate salt solutions (Rockland 1960). The moisture content of the samples were determined periodically till a constant weight is obtained or showed no signs of mould growth, whichever was earlier.

3.21. Statistical Analysis

Data were analysed by (Analysis of Variance) ANOVA using SPSS 16.0 software. Values were calculated per 100 g of flour. All analysis was carried in 2 replicates. The results were presented as means ± SD of 6 determinations. The means were separated using tukey’s test. Level of significance was set at P ≤ 0.05. Correlation coefficients between mineral bioaccessibility and antinutrient content as well as between % IVPD and tannins and dietary fibre fractions of pearl millet were determined.