STUDY ON CHEMICAL BREAKDOWN AND INCREASE IN NUTRITIVE VALUE OF BARNYARD MILLET (*ECHINOCHLOA SPP*) BY FERMENTATION IN EARTHEN POT

Abstract

Barnyard millet (*Echinochloa spp*)has lot of health promoting factors. So the aim of this study was to evaluate the fermentation process related to its endogenous microorganism and effect of *Lacto bacillus* and yeast (*Saccharomyces*) which can enhance bioavailability of nutrients. Hence the study was carried out using Earthen pot for fermentation of Barnyard millet flour, it is aimed to assess the microbial changes and simultaneously biochemical changes. The microbial changes was observed in primary (usingraw millet) and secondary (cooked millet) fermented Barnyard millet flour. A comparative Study using *Lacto bacillus* and yeast and its biochemical changes were carried out. Increase in total titrable acid in fermented millet is the result of increase in reducing sugar and protein. Quantifiable amount Vitamin B2, B5 and B9 were detected.Ca and Fe was also reported. Fatty acid of C16 and C18 were detected.

Keywords: Barnyard millet, Endogenous microorganism, Fermentation, Nutritive value

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I. INTRODUCTION

Millets has large quantities of nutrients. They include macro and micronutrients which helps the human body to function correctly. Hence they can help withstand malnutrition (1). Compared to other cereals, millet is considered as rich source of energy containing complex carbohydrates, resistant starch and allows slow release of sugar there by preventing blood sugar spike. Of the total fibres, the soluble fibres are around 3.4 to 6.5 percent. It has a low fat content from 1.1 to 5.0 percent and are rich in B vitamins especially niacin, pyridoxine and folic acid. Millets offer good amount of calcium, iron, potassium, magnesium and zinc. Millets contain about 8.0 percent protein and 4.0 percent fat. Prolamines and glutelins form the major portion of their proteins and the fats from millet contain a higher portion of unsaturated fatty acids and supply essential fatty acids. Millets are especially rich in calcium. In traditional fermentation processes, microorganism play an important in enhancing the flavor, aroma, texture of food, and other desirable qualities associated with digestibility and edibility and contributes toward the preservation of food by the production of organic compounds (2). By- products produced by Lactic acid bacteria being less toxic or carcinogenic than current antimicrobial agents, have shown to be more effective and flexible in several applications. Most inhibitory substances produced by microorganisms are safe and effective natural inhibitors of pathogenic and food spoilage bacteria in various foods (3).Extensive studies has been carried out in Traditional fermented foods preparation with respect to the changes in microbiological, enzymological and biochemical aspect (4, 5 and 6).In finger millet major biochemical changes takes place due to fermentation from 5 - 15 hrs indicating a window period of 10 hrs. During primary fermentation, hydrolysis of starch occurs irrespective of the grain type and was reported in Fox tail and Pearl millet fermentation had significant reducing sugar content probably due to easily digestible starch type (7).The microorganisms may be indigenous to the food, or may be added as a starter culture after pre-treating or cooking the product (8).Lactic acid bacteria (LAB) increases the acidity subsequently decreasing the pH of the substrate and there by inhibiting many pathogens (9). A number of lactic acid bacteria (LAB) are used as probiotics which when administered in adequate amounts, confer a health benefit on the host (10).Millets can be consumed as breakfast cereal in the form of porridge, soups, stews and bread (11). Volatile compounds in cooked millet impart flavour perception and acceptability of the product by the consumers. Volatile compounds vary depending upon the variety and its preparation (12). A vast number of compounds have been identified such as fatty acids phenolic compounds and alkaloids (13). So the study has been carried out on the nutritive value in fermented millet using earthen pot.

II. MATERIALS AND METHODS

MILLET- Barnyard Millet - Kuthiraivalli- *Echinochloa spp*

1. **Collection of Sample:** Dehusked Barnyard millet grain was brought from organic store, Chennai. All samples were segregated, cleaned and stored in air tight containers till further use.

2. **Preparation of Samples.** Millets used for study was steeped in water and the impurities were removed. Shade dried millet grains was grinded in a mixer sieved and then stored in air tight container in the laboratory refrigerator for use in experiments.

III. EVALUATION OF MICROBIAL PROFILE

1. **Isolation and Morphological Study of Bacteria:** Nutrient agar were used. 1 ml of serially diluted sample solution from the desired test tube was added to each petridish and poured with the respective media as pour plate technique. These plates were incubated at 37^{o} C for 24 hours. The number of bacteria colonies were assessed in the first and third days of incubation, and the number of Colony forming Unit (CFU) per ml of sample was determined.

2. **Identification of Bacteria:** The isolated bacteria were identified up to generic level by performing the biochemical tests (14,15).

   - **Gram Staining:** Individual colony was smeared on slide and was flooded crystal violet and allowed to stand for a one minute, and then the slide was washed with water and then flooded with Gram's iodine solute and left for one minute. The slide was drained and decolorized with 95% ethanol rinsed with tap water. This slide was counterstained for a minute with safranin and washed. The slide was blot dried and examined under oil immersion.

   - **Mannitol Motility Test:** Manitol test medium is suspend 28.0 g of powder in 1 litre of distilled or deionized water boil it by continuously shaking it so as it dissolves completely. Dispense in test tube and autoclave it at 121°C for 15 minutes. Cool it and inoculate the tubes with a pure culture by stabbing the centre of the column of medium to greater than half the depth. Incubate tubes for 24-48 hours at 35 ± 2°C in an aerobic condition.

     Bacterial motility can be observed directly from examination of the tubes following incubation. Growth extends away from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of non-motile organisms only occurs along the stab line but no further

   - **Catalase Test:** A clean glass slide was placed in the petridish saline suspension of the organism was made on the slide. Immediately drop of 3% H_2O_2 was added using dropper.

   - **Oxidase Test:** Oxidase disc was placed on a clean glass slide which is placed in the petridish. The dish was moistened with sterile water. The colony to be tested is picked up using a tooth pick and smeared over moist area.

   - **Indole Production Test:** 5 ml of sterile peptone broth was inoculated with the test culture and incubated at 37^{o}C for 48 hours. Following incubation with 0.2 ml of Kovac's reagent which is composed of p-dimethylaminobenzaldehyde, when it produces a cherry red colour shows the presence of indole.

   - **Methyl Red Test:** 5 ml sterile glucose broth was inoculated with the test culture and incubated at 37^{o}C for 48 hours. Following incubation,5-6 drops of methylred solution was added.

- **Voges-Proskauer Test:** 5 ml of sterile glucose broth was inoculated with the test culture and incubated at 37^{o}c for 48 hours. Following incubation 1 ml of 40% potassium hydroxide and 3 ml of 5% solution of alpha naphthol in absolute ethanol was added.

- **Citrate Utilization Test:** Streak the slant with a inoculum in Simmons citrate medium and incubated at 37^{o}C for 24 hours. Positive reaction shows change from green to blue colour along the slant.

- **Triple Sugar Iron (Tsi) Agar Test:** To the TSI slants agar medium incorporate phenol red which is the acid base indicator to detect carbohydrate fermentation. The medium was inoculated with the test culture by first stabbing the butt down to the bottom and then streaking the surface of the slant and incubated at 37^{o}C for 24 hours. Use a loosely fitting closure to permit access of air.

- **Urease Test:** For the detection of presence of urease, the test culture is inoculated in urea broth medium containing phenol red and incubated for 24 hours at 37^{o}C . The pH shift is detected by the change in colour of phenol red from light orange at pH 6.8 to magenta (pink) at pH 8.1.

- **Carbohydrate Fermentation Test:** In fermentation, substrate such as carbohydrates and alcohols undergo anaerobic dissimilation and produce an organic acid that may be accompanied by gases such as hydrogen or carbon-dioxide. The broth was inoculated with the test culture in aerobic and anaerobic condition and incubated at 37^{o}C for 18-24 hrs. The appearance of any yellow colour is indicative of a positive reaction.

- **Oxidative Fermentation Test:** Duplicate tubes of oxidative –fermentative medium are inoculated by stabbing with the test organism; one of the two tubes is covered with liquid paraffin to a depth of 5-10 mm and both are incubated at 37^{o}C for 48 hours or longer. This overlay of liquid paraffin prevents the diffusion of oxygen into the medium and creates an anaerobic condition in the tube. After 24 hours change in the pH was observed. Acidic pH is observed on the surface of the tube, due to oxidation of sugar. With prolonged incubation (more than 48 hours), when acid is found throughout the tube, including the lower layer. The acid produced changes the pH indicator, bromthymol blue, from green to yellow. No colour change or reaction occurs in the paraffin covered tube.

3. **Isolation and Identification of Fungi:** Potato dextrose agar (PDA) media was prepared for the isolation of fungi. Antibiotic (Streptomycin) was added to the media before solidification to inhibit the bacterial growth. Pour plate technique was followed to which 1 ml of millet sample is added to the petridish and poured the potato dextrose agar medium.

   Identification of Fungi: Identification of culturable molds was done using their macroscopic appearance and their microscopic appearance. The fungi were identified with the help of standard text-books and mono graphs (16,17).

- **Macroscopic Appearance:** Morphological characters of the culture like the colour, shape, pigmentation, reverse pigmentation were studied by using the hand lens.

- **Morphological Appearance:** Morphological characters was studied under light microscope. The characters of conidia bearing structure, shape, size, septate or non septate, colour and ornamentation were observed.

- **Slide Preparation:** Lactophenol and lactophenol with cotton blue [for hyaline molds] were used for examination. The preparation of lacto phenol with cotton blue is as follows:

  Phenol - 10 g
  Lactic acid - 10 g
  Glycerol - 10 g
  Distilled Water - 10 g

  Lacto phenol is prepared by warming phenol with water and then adding lactic acid and glycerol. To prepare lacto phenol with cotton blue, 0.05 g of cotton blue stain is added to 100 ml of lacto phenol.

- **Presentation of Data:**

  $$\text{Percentage contribution} = \frac{\text{Total no. of colonies for an individual species}}{\text{Total no. of colonies recorded for all species}} \times 100$$

IV. COMPOSITION OF SKIM MILK MEDIA

52.15% lactose, 38.71% protein (31.18% casein, 7.53% whey protein), 1.08% fat, and 8.06% ash.

V. FERMENTATION TREATMENTS IN EARTHEN POTS:

Formulation of Millet Beverage taken for biochemical test:

- **Water-based fermented millet**
- **Water-based fermented millet** with inoculum-;***Lactobacillus sps***
- **Water-based fermented millet** with sugar as substrate**,** inoculum- ***Lactobacillus sps***
- **Water-based fermented millet** with milk as substrate and inoculum-***Lactobacillus sps*.**
- **Water-based fermented millet** with milk, sugar as substrate and inoculum**-;** ***Lactobacillus sps***
- **Water-based fermented millet** with inoculum-;Yeast (*Saccharomyces cerevisiae*)
- **Water-based fermented millet** with sugar substrate and- inoculum- Yeast (*Saccharomyces cerevisiae*)
- **Water-based fermented millet** with milk substrate and inoculum**-;** Yeast (*Saccharomyces cerevisiae*)

- **Water-based fermented millet** with sugar, milk substrate and inoculum-; Yeast (*Saccharomyces cerevisiae*) .

1. **Water-Based Fermented Millet:** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water in earthen pot. In secondary fermentation the soaked, the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked. Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 -12hrs .This fermented millet is taken for biochemical analysis

2. **Water-Based Fermented Millet with inoculum-*Lactobacillus sps:*** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water in earthen pot. During secondary fermentation, the soaked the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked, cooled and then inoculated with bacterial culture i.e *Lactobacillus sps.* Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis.

3. **Water-Based Fermented Millet with sugar as substrate and inoculum- *Lactobacillus sps*:** To prepare water-based fermented millet –primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water in earthen pot. During secondary fermentation the soaked, the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked**.** 5% sugar (sucrose) was added as substrate to cooked millet. cooled and then inoculated with bacterial culture i.e *Lactobacillus sps.* Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis

4. **Water-Based Fermented Millet with milk as substrate and inoculum-*Lactobacillus sps*:** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water and milk (1:1) in earthen pot. During secondary fermentation the soaked, the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked**.** Milk was added as substrate to cooked millet. cooled and then inoculated with bacterial culture i.e *Lactobacillus sps.* Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis

5. **Water-Based Fermented Millet with milk and sugar as substrate and inoculum-; *Lactobacillus sps:*** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water and milk (1:1) in earthen pot. During secondary fermentation the soaked, the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked**.** Milk and 5% sugar (sucrose) was added as substrate to cooked millet. cooled and then inoculated with bacterial culture i.e *Lactobacillus sps.* Cover the earthen pot with sterile muslin

cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis

6. **Water-Based Fermented Millet Inoculum with inoculum *Yeast* (*Saccharomyces cerevisiae*):** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water in earthen pot. During secondary fermentation, the soaked the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked, cooled and then inoculated with fungi -yeast (*Saccharomyces cerevisiae*) . Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis

7. **Water-Based Fermented Millet with sugar as substrate and inoculum- Yeast (*Saccharomyces cerevisiae*):** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet- *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water in earthen pot. During secondary fermentation the soaked, the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked**.** 5% sugar (sucrose) was added as substrate to cooked millet, cooled and then inoculated with inoculum fungi culture –yeast (*Saccharomyces cerevisiae*) . Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis

8. **Water-Based Fermented Millet with milk as substrate and inoculum-Yeast (*Saccharomyces cerevisiae*):** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water and milk (1:1) in earthen pot During secondary fermentation the soaked, the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked**.** Milk was added as substrate to cooked millet, cooled and then inoculated with inoculum fungi culture –yeast (*Saccharomyces cerevisiae*) . Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis

9. **Water-Based Fermented Millet with sugar and milk as substrate and inoculum-Y*east* (*Saccharomyces cerevisiae*):** To prepare water-based fermented millet ,–primary and secondary fermentation is done. In primary fermentation take 20 gm of Barnyard millet *i.e* dehusked millet is steeped in water for 8 hours in 250 ml of distilled water and milk (1:1) in earthen pot. During secondary fermentation the soaked, the primary fermented millet is cooked in the earthen pot at 90–95 °C for 20 min till it is completely cooked**.** 5% sugar (sucrose) and milk was added as substrate to cooked millet, cooled and then inoculated with inoculum fungi culture –yeast (*Saccharomyces cerevisiae*) . Cover the earthen pot with sterile muslin cloth and leave it for fermentation for 8 hrs. This fermented millet is taken for biochemical analysis

VI. MILLET –BIOCHEMICAL TEST

1. Carbohydrate:

- **Reagents:**
  - Standard for carbohydrate is prepared using D- Glucose (0.1mg/ml)
  - Anthrone reagent 33%

- **Estimation of Reducing Sugars:** Cooked fermented Barnyard Millet (CF-BM) broth was taken to estimate the reducing sugar. One of the test tube is considered as blank with 1ml of distilled water and to another test tube 0.1 ml of millet broth is made to 1ml with distilled water. To each test tube add 4 ml of anthrone reagent and mix it thoroughly. Keep it in boiling water for 8 min. Cool it rapidly and green colour formed was measured at 620 nm using colorimeter(18). For quantitative analysis, reducing sugars was calculated from the regression equation of the standard plot **$y = 2.325x - 0.0005$; $R^2 = 0.9709$** and is expressed as gm of equivalent of D-glucose per gram of dry weight.

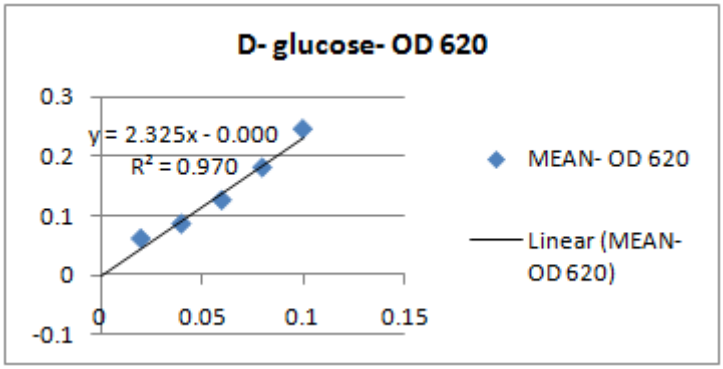


Figure 1

2. Protein (Modified Lowry's Method) (48)

- **Reagents:**
  - **Standard for protein:** Bovine Serum Albumin (BSA) is taken as standard (1mg/ml)
  - **Alkaline reagent:** 0.3 N NaOH with 2.9% Na2CO3.
  - **FolinCiocalteau reagent-** This reagent has to be diluted with two times its volume with distilled water.

- **Estimation of Protein:** Cooked fermented Barnyard Millet (CF-BM) broth was taken to estimate the reducing sugar. One of the test tube is considered as blank with 1ml of distilled water and to another test tube 0.1 ml of millet broth is made to 1ml with distilled water. To these test tubes add 2.5 ml of alkaline reagent and 0.75 ml Folin's reagent was add. Blue colour formed was measured at 640 nm using colorimeter. For quantitative analysis, protein was calculated from the regression equation of the

standard plot **of BSA is y = 0.0009x + 0.008, R^2 = 0.9908** and is expressed as gm of equivalent of BSA per gram of dry weight.

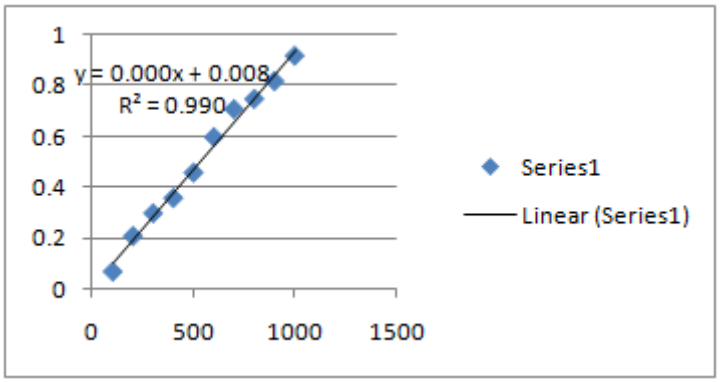


Figure 2

3. Quantitative Estimation of Acidty in Fermented Millet

- **Total Titrable Acid and PH Determination:**

Reagents:

- 0.1 M NaOH.
- 0.5 % Phenolphthalein indicator

The pH and titrable acid of cooked fermented Barnyard millet (CF-BM) during secondary fermentation was taken after 12 hrs of fermentation. The titrable acid of 10 ml of cooked fermented Barnyard millet CF-BM is pipetted out in 100 ml conical flasks and to this 1ml of 0.5% phenolphthalein indicator is added. This was titrated with 0.1 M NaOH until consistent pink colour appeared. Readings of titre values were obtained in triplicates. The acidity was expressed as based on the conversion of 1ml of 0.1M NaOH was taken as equivalent to 9.008 $x10^{-3}$ g (90.08 mg) of lactic acids. The titratable acidity was then calculated as stated in A.O.A.C (1980) (20,21)

- **Total Titrable Acid and PH Determination at different Incubation Time:** The quantity of lactic acid and volatile acid was taken for quantification using cooked fermented Barnyard millet (CF-BM) and with inoculum (*Lactobacillus* and yeast-*Saccharomyces cerevisiae*,) with and without substrate was incubated for 24hrs, 48hrs and 72hrs. The determination of titrable acid was carried out by taking out sample for analysis at interval of 24hrs for a period of 72 hrs. The titrable acid of 10 ml of cooked fermented Barnyard millet CF-BM is pipetted out in 100 ml conical flasks and to this 01ml of 0.5 % phenolphthalein indicator is added. This was titrated with 0.1 M NaOH until consistent pink colour appeared. Readings of titre values were obtained in triplicates. The acidity was expressed as based on the conversion of 1ml of 0.1M NaOH was taken as equivalent to 9.008 $x10^{-3}$ g (90.08 mg) of lactic acids. The titratable acidity was then calculated as stated in A.O.A.C (1980) (20,21)

$$\% \text{ acidity} = \frac{ml\ NaOH\ X\ MNaOH\ X\ M.E}{volume\ of\ sample} X\ 100$$

Where; ml NaOH = volume of NaOH used

Lactic Acid and Volatile Acid:

$$\text{Total acidity of lactic acid} = \frac{ml\ of\ alkali\ X\ Molarity\ of\ alkali\ X\ 7.5}{Weight\ of\ sample\ in\ grams}$$

$$\text{Volatile acidity} = \frac{ml\ of\ alkali\ X\ Molarity\ of\ alkali\ X\ 6.0}{Weight\ of\ sample\ in\ grams}$$

- **Estimation of Diacetyl:** Diacetyl production was determined at 24hrs, 48hrs and 72hrs by transferring 25ml cooked fermented Barnyard millet CF-BM into 100 ml flasks. Add 7.5 ml of Hydroxylamine solution (1 M) was added to this Bromothymol blue as indicator is added. This was titrated with 0.1 M concentration of HCl until consistent greenish yellow appears**.**

The equivalence factor of HCl to diacetyl is 21.52 mg. The concentration of diacetyl produced was calculated using the A.O.A.C. (1980). Where Ak = % of diacetyl, b- s = volume of HCl used, E = equivalence factor (21.52/mg), W = volume of broth and 100 = constant (22)

$$\text{AK} = \frac{(b-s)(100E)}{W}$$

VII. CALCIUM, IRON, ZINC-ICPMS PROCEDURE

1. **Closed-System Microwave Mineralization:** Place a portion of the sample weighing 1.0 g in digestive vessel, add 5 mL of deionized water, then 5 mL of nitric acid to the digestion vessel and close To ensure that there has been no contamination, each series of analyses should include a blank test (a matrix-free test performed with the same amounts of reagents that undergoes digestion at the same time as the samples). Each series of analyses should contain a reference material containing a known amount of the elements of quantify. This reference material should undergo digestion under the same conditions as the sample under examination.

2. **Digestion:** Install the digestion flask on the rotor, and then apply the appropriate digestion programmed. Oven programming (power/time, for example) should be performed according to manufacturer's recommendations. For microwave digestion times are 45 minutes. A gradual increase between selected phases is recommended so as to avoid pressure spikes inside the vessel. To reduce the temperature and pressure inside the digestion vessel, a cooling phase is included at the end of the program. The final state of digestion of the sample depends on the digestion temperature. In general, the higher the temperature, the less residual carbon is left in the solution and the better the quality of the mineral deposit. The digestion solution should be limpid, without any suspended

particles, and its volume should be practically the same as before digestion. After digestion, open the vessels, then rinse the covers and walls with deionized water, take up in polypropylene flasks, and dilute to 25 mL with deionized water.

VIII. VITAMINS-LC MS MS PROCEDURE

1. Instrument: LC/MS/MS

- Agilent: LC: (G1312B) HPLC-1260 Binary pump.
- MS MS:G6430A Triple Quad MS,
- EclipsPlus C18 column 100 X 4.6, 3.5 um

2. Instruments Conditions for LC/MS/MS:

- **HPLC Conditions:** The LC-program for the Vitamins compounds. Eclipse Plus C18 column 100 X 4.6, 3.5 um was used with methanol and 0.1 % Formic acid in gradient. The flow rate was 0.400 ml/min. The column was maintained at 40˚C. The injection volume was 25 micro litre and run time 12 min.

- **Sample Preparation:** Weigh 10 gm of sample in a screw capped conical flask and dissolve with 10-15 ml of water and then cover the flask with aluminum foil. Add 10 ml of 0.1N hydrochloric acid and then shake it. The sample was heated for half an hour over a boiling water bath and stirred frequently. The sample was next cooled in a water bath and then adjust pH to 4- 4.5 with 2.5M sodium acetate solution. The extract was then transferred into a 100 ml volumetric flask and then made up to the mark with water. After shaking well, the extract was filter through 0.22 µm membrane filter. Inject 20µl of the filtrate into LC MS MS and compare against known concentration of mixture of standards.

IX. RESULTS AND DISCUSSION

1. Microbial Studies and Fermentation

Table 1: Microbiological analysis - Raw Barnyard millet- Primary fermentation and Cooked and fermented Barnyard millet- Secondary fermentation

Bacterial species isolated from primary and secondary fermented millet					
S. NO	Species	Raw Barnyard millet- Primary fermentation		Cooked and fermented Barnyard millet-Secondary fermentation	
		Colony Forming Unit (CFU)	Per cent contribution	Colony Forming Unit (CFU)	Percent contribution
1	*Bacillusalvei*	Nil-	Nil	2.33×10^{9}	6.4
2	*Bacillus sp*	4.66×10^{9}	21.1	Nil	Nil -
3	Micrococcus *lylae*	7×10^{9}	31.48	9.33×10^{9}	25.9

4	*Micrococcus kristinae*	10.33×10^{9}	46.9	Nil	Nil
Fungal species isolated from primary and secondary fermented millet					
S. NO	Species	Raw Barnyard millet-Primary fermentation		Cooked and fermented Barnyard millet- Secondary fermentation	
		Colony Forming Unit (CFU)	Percent contribution	Colony Forming Unit (CFU)	Percent contribution
1	*Aspergillus flavus*	0.33×10^{2}	0.26	0.33×10^{2}	0.24
2	*Penicillium citrinum*	$126x10^{2}$	99.7	$133x\ 10^{2}$	99.5
3	*Saccharomyces cerevesis*	Nil	Nil	0.33	0.24

Microbial fermentation was carried out in earthen pot using Barnyard Millet.

- **Primary Fermentation in Bacteria:** In primary fermentation of raw Barnyard millet was soaked with distilled water in earthen pot for 8hrs. The total number of bacterial Colony Forming Units (CFU) were 66 colonies in primary fermentation(uncooked millet). Of these *Micrococcus kristinae* 31 colonies was reported more number of colonies followed by *Micrococcus lylae* 21 colonies and *Bacillus sps* 14 colonies.

- **Secondary Fermentation in Bacteria:** In secondary fermentation Barnyard millet was cooked and fermented in earthen pot for 8hrs. The total number colony forming unit of bacteria is 112 colonies . Among these *Micrococcus lylae* 28 colonies followed by *Bacillus alvei* 7 colonies. Similar the percentage contribution of bacterial species (67.4%) and followed by *Micrococcus lylae* (25.9%) and *Bacillus alvei* (6.4%).

- **Primary Fermentation in Fungi:** In primary fermentation of raw Barnyard millet was soaked with distilled water in earthen pot for 8hrs. The total number of fungal colony forming units is 379 colonies. Among these colonies 378 colonies of *Penicillium citrinum* had maximum number of colonies and least number of colonies in *Aspergillus flavus* of 1colony. Similar to these isolated fungi species, the percentage contribution fungal isolate in primary fermentation is *Penicillium citrinum* (99.7%) and the *Aspergillus flavus* (0.24%).

- **Secondary Fermentation in Fungi:** In secondary fermentation of cooked fermented Barnyard millet in earthen pot for 8hrs, the total number of fungal colony forming unit is 401. Among these *Penicillium citrinum* maximum of 399 colonies, whereas *Aspergillus flavus* and *Saccharomyces cerevesiae* reported 1 colony only. The percentage contribution of fungal isolates in secondary fermentation is maximum in *Penicillium citrinum* (99.5%) followed by *Aspergillus flavus* and *Saccharomyces cerevesiae* (0.24%)

From the five the best starchy based food substrate *Saccharomyces cerevisiae, Candida sp. Aspergillus niger, Aspergillus flavus* and *Penicillium sp* and bacteria were *Lactobacillus plantarum, Lactobacillus casie, Lactobacillus fermentum, Lactobacillus lactis, Klebisella pnemoniae, Escherichia coli, lavobacterium sp., Proteus vulgaris* (23) were isolated.

In millet and defatted Soybean blends, decrease in pH from 9.2-9.3 to a range of 6.6-6.8 was recorded from day 1 (0 hrs) to day 4 (72 hrs) *Bacillus cereus, Bacillus subtillis, Enterococcus* sp., *Micrococcus* sp. and *Lactobacillus* sp were isolated during fermentation. In fermentation of flour some microorganism such as *Pseudomonas* sp., *Proteus* sp., and coliforms (*Enterobacter* sp. and *Klebsiella* sp.) may not have played any role in the process of fermentation (24).

Biochemical Test on Formulated Barnyard Millet

- **UNSOAKED** = Raw millet
- **SOAKED**= Steeped in water for 8 hrs –Primary fermentation
- **CF-BM**= Cooked and fermented- Barnyard millet for 8 hrs- Secondary fermentation
- **LB** = CF-BM with *Lactobacillus sps*
- **LB+S-**= CF-BM with *Lactobacillus sps* with substrate sugar
- **LB+M**= CF-BM with *Lactobacillus sps* with substrate milk
- **LB+S+M**= CF-BM with *Lactobacillus sps* with both substrate sugar and milk
- **Y =** CF-BM with Yeast (*Saccharomyces cerevisiae*)
- ***Y*+S-**= CF-BM with Yeast (*Saccharomyces cerevisiae*) with substrate sugar
- **Y+M**= CF-BM with Yeast (*Saccharomyces cerevisiae*) with substrate milk
- **Y+S+M**= CF-BM with Yeast (*Saccharomyces cerevisiae*) with both substrate sugar and milk

Reducing sugar in Barnyard millet grains:

Table 2: Reducing sugar in Barnyard millet grains

s.no	Barnyard Millet	gm of Reducing sugar/gm of millet
1	Unsoaked	0.004
2	Soaked	0.016
3	CF-BM	0.004
4	LB	0.017
5	LB+S	0.012
6	LB +M	0.010
7	LB +S+M	0.006
8	Y	0.004
9	Y+S	0.009
10	Y+M	0.004
11	Y+S+M	0.008

It is evident from the Table 2 that due to fermentation the polysaccharides get reduced to reducing sugar. The concentration of reducing sugar in unsoaked Barnyard millet is less, but the concentration of reducing sugar in soaked gram of millet is 0.004gm/g of Barnyard Millet there is a sharp decrease in the reducing sugar of carbohydrate in cook and fermented Barnyard millet of 0.004 gm / g of Barnyard millet. The change in carbohydrate value with fermentation was reported in fermented pumpkin seeds and fermented cowpea respectively (25, 26).

The effect of two substrate milk and sugar and the combined effect of both substrate on the carbohydrate content due to fermentation was studied using *Lactobacillus* bacteria and yeast separately cooked and fermented Barnyard millet (CF-BM) when inoculated with *Lactobacillus* bacteria only, showed increase in reducing sugar of 0.017 gm but there is a study decrease in carbohydrate value when added substrate of sugar 0.012 gm, milk 0.010 gm of reducing sugar separately and the level of carbohydrate value 0.006 gm showed sharp decrease with the combined effect of both the substrate with sugar and milk to CF-BM with Lactobacillus. Lactic acid bacteria lack hydrolytic enzymes which is necessary for fermentation of polysaccharides like starch or dextrins, but can ferment sucrose, maltose, galactose (27).

Cooked fermented Barnyard millet (CF-BM) when inoculated with yeast and supplemented with non-reducing sugar sucrose, increase in reducing sugar was noticed, which also was noticed in pearl millet (28).

Table: 3 Protein content in Barnyard Millet Grains

	Sample	**Protein gm/gm of millet**
1	UNSOAKED	5.83
2	SOAKED	11.39
3	CF-BM	30.83
4	LB	16.94
5	LB+S	12.78
6	LB +M	29.44
7	LB +S+M	11.39
8	Y	14.17
9	Y+S	19.72
10	Y+M	14.17
11	Y+S+M	11.39

It is evident from the about Table 3 that the concentration of protein per gram of Barnyard millet (BM) in unsoaked is less 5.83 gm/g of millet, but the protein concentration gradually increases from soaked Barnyard millet and cooked fermented Barnyard millet (CF –BM) of 11.39 to 30.83 gm / g of millet respectively. The increase in protein content after fermentation than in raw Barnyard millet was also reported in finger millet (29).

This may be due to utilization of carbohydrate content by the action of enzyme produced by fermentation (30).The effect of two substrate milk and sugar and the

combined effect of both substrate on the protein content due to fermentation was studied using *Lactobacillus* bacteria and yeast separately

Cooked and fermented millet (CF-BM) when inoculated with *Lactobacillus sps* bacteria, in presence substrate milk showed increase in protein of 29.44 gm /gm of millet, but there is a steady decrease in protein value with only *Lactobacillus sps*, with sugar and with both milk and sugar to cooked and fermented millet (CF-BM). Increase in protein content was also observed in fermented of pearl millet (31).

Cooked and fermented Barnyard millet (CF-BM) when inoculated with yeast and supplemented with sucrose there was a slight increase in protein of 19.72 gm, but with only yeast and substrate milk showed the same in the value of 14.7 gm of protein, but there was decrease in protein value 11.39 gm with of both the substrate of milk and sucrose was used in yeast inoculated CF-BM. Increase in protein content in fermented finger millet was reported when inoculated with yeast (29).

2. Acidity of Fermented Millet

- **Titrable Acid, Diacety and pH of Barnyard Millet Produced on Fermentation:**

Table 4: Titrable acid, Diacety and pH of Barnyard millet produced on fermentation

S. No	Sample	Total Acidity of Lactic Acid	Volatile Acid	% Total Titrable Acid	Diacety	pH
1	UNSOAKED	0.019	0.015	45.0	0	6
2	SOAKED	0.0152	0.012	36.0	0	6
3	CF-BM	0.38	0.3	90.1	0	6
4	CF-BM +LB	0.0228	0.018	54.0	0	5
5	CF-BM+LB+S	0.076	0.06	18.0	0	5
6	CF-BM +LB+M	0.019	0.015	45.0	0	5
7	CF-BM +LB +S+M	0.0228	0.018	54.0	0	5
8	CF-BM +Y	0.228	0.18	54.0	0	5
9	CF-BM +Y+S	0.0304	0.024	72.1	0	5
10	Y+M	0.0228	0.018	54.0	0	5
11	Y+S+M	0.0228	0.018	54.0	0	5

To determine the acid production of soaked , cooked and fermented with inoculum *Lactobacillus sps*, and Yeast (*Saccharomyces cerevisiae*); substrate of milk, sucrose and combined effect of milk and sucrose with the inoculum lactic acid , volatile acid and percent titrable acid was measured using 0.1 N NaOH it showed different level of acidity**.**

The result showed that during fermentation there was much decrease in pH (Table 4) i.e from pH6 to pH 5, but shows corresponding increase in the lactic acid ,

volatile acid and percent titrable acid in unsoaked millet 0.019, 0.015 and 45 percent, is less as compared with soaked millet pH 5.6 0.015, 0.012, 36 percent, and cooked and fermented Barnyard millet CF-BM pH 6 has maximum acidity of 0.038, 0.3 and 90.1 percent, respectively.

In CF-BM the pH6, has dropped to pH 5 when inoculated with *Lactobacillus sps* bacteria having no substrate , but subsequently showed increase lactic acid , volatile acid and percent titrable acid 0.023, 0.018 and 54 percent, and with substrate milk 0.19, 0.015 and 45 percent, but with sucrose as substrate and substrate milk and sucrose at pH 5 decrease in acidity was noted as 0.023, 0.018 and 54 percent, with only sucrose, 0.076, 0.076 and 18 per cent respectively.

There was decrease in pH from 8.50 at 0 hr to pH 7.60 for pearl millet and pH 7.90 for finger millet, while total titrable acid (TTA) increased from 0.0038 to 0.18 g/L during germination. The decrease in pH and increase in total titrable acid (TTA) might be due to degradation of some complex organic molecules such as lipids, phytin, and protein to simpler compounds (30)

Lactobacillus generate energy only during breakdown of carbohydrate but *Lactobacillus* do not ferment polysaccharides that starch or dextrin because they lack hydrolytic enzyme. Lactose are absent in plant (27). In homo fermentative species of *Lactobacillus* convert sugars in milk mostly into lactic acid, whereas the hetero fermentative species convert lactose into lactic acid, acetic acid, ethanol and CO_2. Production of lactic acid by *Lactobacillus* is strain dependent (31).

Lactose present in milk is utilised by *Lactobacillus* and have a role in fermentation of milk to produce acid which is important as preservative agents and generating flavour of the products.Exopolysaccharides are also produced which are essential as texture formation and has several health promoting properties and is widely used in developing new fermented products (32).

Cooked and fermented Barnyard millet (CF-BM) when inoculated with yeast and supplemented with sucrose pH 6 was recorded increase in lactic acid , volatile acid and percent titrable acid of 0.75, 0.59 and 1.78 was noted. With only yeast and substrate milk and with both substrate of milk and sucrose supplemented along with the inoculum yeast showed almost the same value but the pH 5 was noted 0.023, 0.18 and 54 percent. But with sucrose was used in yeast inoculated cooked and fermented (CF-BM) had pH 5 with decrease in acidity of 0.030, 0.024 and 72.1 percent.

Glucose is the important nutrient which stimulates a variety of growth-related events in the yeast *Saccharomyces cerevisiae* (33). Diacety in cooked and fermented (CF-BM) was negative at pH 6-5 since gram negative was not observed during fermentation. It is reported that the optimum pH for diacetyl production is pH 4.5–5.5 (34) and also the test carried out using Diacetyl compound showed lactic acid bacteria was uneffected even at the concentration of 100 µg/ml and 350 µg/ml at pH 5 to 7, gram-positive non-lactic acid bacteria, were inhibited by 300 µg/ml at pH 7.0 and in yeasts and gram-negative bacteria that grew at pH 5.5 were inhibited by 200 µg/ml (35).

- **Quantity of Lactic Acid, Volatile Acid, Total Titrable Acid, Diacetyl produced on fermentation at different incubation times**

Table 5: Quantity of Lactic Acid, Volatile Acid, Total Titrable Acid on fermentation at different incubation times

Sample	Hours	Total Acidity Of Lactic Acid	Volatile Acid	% Total Titrable Acid
Cooked And Fermented	24 HRS	0.038	0.03	90.1
	48 HRS	0.064	0.051	153.1
	72 HRS	0.083	0.066	198.2
Lacto Bacillus	24 HRS	0.023	0.018	54.0
	48 HRS	0.045	0.036	108.1
	72 HRS	0.075	0.06	180.2
Milk+ Lacto Bacillus	24 HRS	0.019	0.015	45.0
	48 HRS	0.049	0.039	117.1
	72 HRS	0.079	0.063	189.2
Milk+Sugar+Lacto Bacillus	24 HRS	0.023	0.018	54.0
	48 HRS	0.049	0.039	117.1
	72 HRS	0.071	0.057	171.2
Yeast	24 HRS	0.023	0.018	54.0
	48 HRS	0.056	0.045	135.1
	72 HRS	0.083	0.066	198.2
Milk+ Yeast	24 HRS	0.023	0.018	54.0
	48 HRS	0.034	0.027	81.1
	72 HRS	0.056	0.045	135.1
Milk+Sugar+Yeast	24 HRS	0.023	0.018	54.0
	48 HRS	0.045	0.036	108.1
	72 HRS	0.079	0.063	189.2

It is evident from Table 5 that the Cooked and fermented Barnyard millet (CF-BM) was incubated i.e. fermented for 24hrs, 48 hrs and 72 hrs showed increase in lactic acid from 0.038 to 0.083 ,volatile acid 0.03 to 0.066 and percent of total titrable acid (TTA) 0.09 to 0.198. Cooked and fermented Barnyard millet (CF-BM) when inoculated with Lactobacillus and incubated for 24hours, 48 hours and 72 hours there was increase in lactic acid from 0.023 to 0.075 ,volatile acid 0.18 to 0.06 and percent of total titrable acid(TTA) 54 to 180.2.

Cooked and fermented Barnyard millet (CF-BM) when inoculated with *Lactobacillus* and supplemented with substrate milk and incubated for 24hrs, 48 hrs and 72 hrs there was increase in lactic acid from 0.019 to 0.079 ,volatile acid 0.015 to 0.063 and percent of total titrable acid (TTA) 45 to 189.2. Cooked and fermented Barnyard millet (CF-BM) when inoculated with *Lactobacillus sps* and supplemented with substrate milk and sugar and incubated for 24hrs, 48 hrs and 72 hrs there was

increase in lactic acid from 0.023 to 0.071 ,volatile acid 0.018 to 0.057 and percent of total titrable acid (TTA) 54 to 171.2.

Cooked and fermented Barnyard millet (CF-BM) when inoculated with yeast and incubated for 24hrs, 48 hrs and 72 hrs there was increase in lactic acid from 0.023 to 0.083 ,volatile acid 0.018 to 0.066 and percent of total titrable acid(TTA) 54 to 198.2.

Cooked and fermented Barnyard millet (CF-BYM) when inoculated with yeast and supplemented with substrate milk and incubated for 24hrs, 48 hrs and 72 hrs there was increase in lactic acid from 0.023 to 0.056 ,volatile acid 0.018 to 0.045 and percent of total titrable acid (TTA) 54 to 135.1.

Cooked and fermented Barnyard millet (CF-BM) when inoculated with yeast and supplemented with substrate milk and sugar and incubated for 24hours, 48 hours and 72 hours there was increase in lactic acid from 0.023 to 0.079 ,volatile acid 0.018 to 0.063 and percent of total titrable acid (TTA) 54 to 189.2. Fermentation treatments were found effective with increase in incubation time the nutritional value and decrease in anti-nutritional of finger millet (*Eleusine coracana L*.) (29).

Eighteen volatile compounds were identified in Barnyard millet which included cyclohexasiloxane, dodecamethyl (7.22%), cycloheptasiloxane, tetradecamethyl (5.82%), benzene,1,3-bis-1,1-dimethylethyl (5.65%), dodecane (5.52%), eicosane (4.61%) and cyclooctasiloxane, hexadecamethyl (4.55%). The notable amount present in the form of decanal, heptanes, 2,4-dimethyl, benzene, 1-ethyl-3-methyl and mesitylene etc.**(36).**

Volatile compound that heptanal was produced in higher amounts (2-heptaone and 2-nonaone touched 25.56 and 10.67 µg /l respectively) by *L. delbrueckii subsp.bulgaricus* during storage period was reported in fermented milk **(37).**These compounds were reported in dairy products, including milk, fermented milk and cheese (38).

- **Vitamin B and minerals in cooked fermented Barnyard millet:**

  - **Vitamin B level:** Water Soluble vitamins the millets are rich sources of B-complex vitamins (except Vitamin B 12). In cooked and fermented (CF-BM) the total vitamin B2 - Riboflavin content present is 0.0653 mg/100 ml, vitamin B5 Pantothenic acid is 0.3176 mg/100ml and vitamin B9- Folic acid is 0.013 mg/100ml of extract. The other vitamin like -. Vitamin B1- Thiamin, Vitamin B3-Niacin, Vitamin B6, B7- Pyridoxine, Biotin and Vitamin B12- Cyanocobalamin was found Below the Limit of Quantification (BLQ) (Table 6). The presence of water Soluble Vitamins in other millet, Bajara, Sorghum, , Ragi, Kodo and , Foxtail showed the presence Vitamin B1, B2, B3 , B5 , B6, B7, B9 , Proso millet reported the presence of vitamin B2, B3, B5, B6, but in Barnyard millet showed the presence vitamin B2, B3 and B5 only (39).

Table 6: Vitamin B level

S.No	Vitamin	Generic Name	Units	MG/100ML
1	Vitamin B1	Thiamin	mg/100ml	BLQ (LOQ 0.01)
2	Vitamin B2	Riboflavin	mg/100ml	0.0653
3	Vitamin B3	Niacin	mg/100ml	BLQ (LOQ 0.01)
4	Vitamin B5	Pantothenic acid	mg/100ml	0.3176
5	Vitamin B6	Pyridoxine	mg/100ml	BLQ (LOQ 0.01)
6	Vitamin B7	Biotin	mg/100ml	BLQ (LOQ 0.01)
7	Vitamin B9	Folic acid	mg/100ml	0.0103
8	Vitamin B12	Cyanocobalamin	mg/100ml	BLQ (LOQ 0.01)

Note:
- BLQ- Below the Limit of Qualification
- LOQ- Limit of Quantification

- **Mineral:** This study evaluated the extent of variability of micronutrients (Fe, Zn) and macronutrients (Ca,). In Barnyard millet has shown presence calcium content (Ca) is nearly 39.6 mg/l, Iron (Fe) 2.4 mg/l and Zinc (Zn) 0.396 mg/l (Table 7).

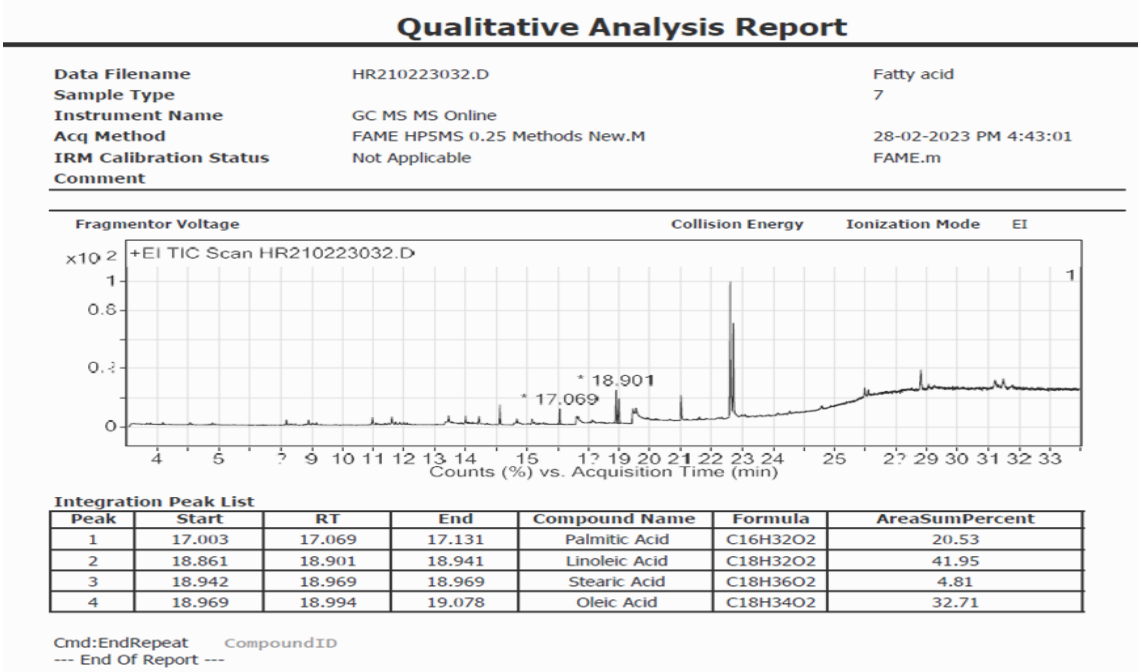
In Finger Millet or *Ragi* (*Eleusine coracana*) calcium content is nearly 350 mg for 100g whereas in wheat and rice it is even below 50mg. Pearl Millet or B*ajra* (*Pennisetum glaucum*) contains magnesium, copper, zinc. and is rich in calcium and unsaturated fats which benefits our health. Sorghum or *Jowar* (*Sorghum vulgare*) good amount of calcium with small amounts of iron and sodium. Jowar helps to maintain heart, body weight and arthritis (40). Mineral content of millets such as pearl millet, finger millet, foxtail millet, little millet, proso millet and Kodo millet namely calcium content of the above mentioned millet was reported as 10-46, 240-410,10-30,12-30, 20-33 and 10-31 mg/100g; Zinc content 2.95-3.1, 2-2.3, 2.14-9,3.5-11, 1.4-2.4,0.7-1.5 mg/100g and iron content 7.49-8, 3.9-7.5, 3.2-19, 13-20, 4-5.2and 0.7-3.6 mg/100g (41).

Table 7: Evaluation of Minerals in fermented in cooked fermented Barnyard millet (CF-BM)

S.NO	Mineral	Units	Unit- mg/l
1	Calcium as Ca	mg/l	39.6
2	Iron as Fe	mg/l	2.4
3	Zinc as Zn	mg/l	0.396

mg/l - milligrams per litre

3. Fatty Acids of Barnyard Milett



Peak	Start	RT	End	Compound Name	Formula	AreaSumPercent
1	17.003	17.069	17.131	Palmitic Acid	C16H32O2	20.53
2	18.861	18.901	18.941	Linoleic Acid	C18H32O2	41.95
3	18.942	18.969	18.969	Stearic Acid	C18H36O2	4.81
4	18.969	18.994	19.078	Oleic Acid	C18H34O2	32.71

Figure 3: GC-MS of Fattyacids

Table 8: Fatty Acid Profile of Barnyard Milett

Compound Name	Formula	Peak	Start	RT	End	Area Sum Percent
Palmitic Acid (C16:0)	C16H32O2	1	17.003	17.069	17.131	20.53
Linoleic Acid (C18:2)	C18H32O2	2	18.861	18.901	18.941	41.95
Stearic Acid (C18:0)	C18H36O2	3	19.942	18.969	18.969	4.81
Oleic Acid (C18:1)	C18H34O2	4	18.969	18.994	19.078	32.71

Fatty Acid Composition in Barnyard Millet: The analysis of fatty acid composition is carried out by GC /MS. The fatty acid is converted into fatty acid methyl ester by acid – catalysed esterification and it showed the calibration curve having four major peaks. The first peak *i.e* the fatty acid has the lowest boiling point which convert liquid to gaseous phase and has the retention time of 17.069 is palmitic acid whereas the other three peaks has retention time of 18.901 is linoleic acid, 18.969 is stearic acid and 18.994 is oleic acid. The percent area of the peak with respect to retention time has maximum of 41.95 is linoleic acid followed by 32.71 oleic acid, 20.53 palmitic acid and 4.81 stearic acid (Table 8). Foxtail millet bran oil is rich in linoleic acid (66.5%) and oleic acid (13.0%) of saturated fatty acids- palmitic acid (6.4%) and stearic acid (6.3%) (42). several fatty acid were detected in pearl millet, the major fatty acid was linoleic acid (47.5%)

(43). Linoleic acid (38-40%), oleic acid (27-37%), palmitic acid (16-22%) and linolenic acid (1-4%) are the major fatty acids found in small millets. The presence of unsaturated fatty acids account for more than 85% of the total fatty acid content in millets (44,45). Barnyard millet oil could be a good source of natural oil rich in linoleic acid and tocopherols (46).

X. CONCLUSION

Fermentation of Barnyard millet was carried out in earthen pot. Biochemical changes accompanied by fermentation of Barnyard millet by endogenous microflora. Increase in total titrable acid (TTA) *i.e* organic acid with decrease in pH was observed due to fermentation. Starch being the major source of energy is utilized by the microflora and results in increased reducing sugars due hydrolysis. Fermentation resulted in increased protein content. Vitamin B2, B5 and B9 were present in quantifiable amount and rich in Ca and Fe is reported in Barnyard millet. Vitamins and minerals helps for normal functioning of human body. C16 , C18 fatty acid has been observed , which would decreases the risk of heart disease and stroke. Remarkable rise in nutritive value was observed when cooked and fermented in earthen pot.

Conflict of Interest: "The authors declare that there is no conflict of interest"

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