Effect of Processing Techniques on Nutritional, Viscosity and Osmolarity of Barnyard Millet Based Diarrheal Replacement Fluids

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Abstract
Barnyard millet is one of the major crops and nutritionally superior to other cereals. It is an excellent source of nutrients and also contains moderate amounts of anti-nutrients such as tannin, phytic acid, and phenolic compounds. Traditional processing techniques enhance the edibility, nutritional quality, and reduce anti-nutritional factors. In the present study, an attempt was made to formulate barnyard millet flour-based diarrheal replacement fluid and analyze the effect of roasting and soaking methods on nutritional composition, viscosity, osmolarity, and sensory characteristics. The results showed there was a significant difference in nutritional, anti-nutritional, viscosity, and osmolarity properties of differently processed barnyard millet flour. Soaking of millet reduced the anti-nutritional factors such as tannin (2.21±0.01-2.96±0.03 mg/100 gm) and phenolic compound (5.2±0.01-5.31±0.01 mg/100 g), similarly, roasting significantly increased the nutrients, and also lowered the viscosity of the replacement fluids (267.31±0.02 -543.90±0.09 cP). The osmolarity of enzyme-treated roasted millet replacement fluids was comparable to the normal osmolarity range of plasma (275-295 mOsm/kg). Roasted barnyard millet-based diarrheal replacement fluids had better sensory properties than raw and soaked millet. The mineral composition, viscosity, and osmolarity of roasted barnyard millet suggested that it has preferable attributes as a diarrheal replacement fluid.

Introduction
Millets are one of the most important cereal crops. It is classified into two categories, major and minor millets. Minor millets are Foxtail (Setaria italica), Pearl (Pennisetum glaucum), Proso (Panicum miliaceum), Barnyard (Echinochola crus galli),...
Finger millet (Eleusine coracana), Brown top millet (Panicum ramosum), Kodo or Ditch millet (Paspalum scrobiculatum), and Teff millet (Eragrostis tef). Barnyard millet is one of the important minor millet. It is a fair source of protein and an excellent source of dietary fiber and it is highly digestible. It is nutritionally superior to other cereals, yet underutilized. It grows in a short duration and under adverse climatic conditions.

In India, barnyard millet is grown in the central part, where it is known as kudiraivali, Sanwa, sawa, samu, etc. In Asian and African countries, millets are used to prepare various traditional foods, beverages, and snack foods. In earlier days, millet was not an important part of the diet among American and European population. Numerous researches on millets have recognized the benefits of millets, leading to their utilization as a breakfast cereal and other products in daily diet.

Barnyard millet is a highly nutritious and versatile food. Barnyard millet contains essential fatty acid such as linoleic acid, palmitic, and oleic acid. Barnyard millet is rich in minerals like iron, calcium, and magnesium. Magnesium and niacin (B3) can help to reduce the cholesterol level. Phosphorus is present in barnyard millet, and it helps to enhance fat metabolism and converting food into energy. They are also considered to be the least allergic grains. Barnyard millet helps to promote the healthy digestive system and prevent constipation. However, millets have certain anti-nutritional factors. Barnyard millet contains low levels of phytic acid. Hence techniques such as soaking, roasting, germination, or fermentation are adopted to reduce the anti-nutrients and also increase the physiochemical accessibility of micronutrients and improve the bioavailability of micronutrients. In the present study, an attempt was made to formulate the diarrheal replacement fluids by using barnyard millet and study the effect of processing techniques units nutritional composition, viscosity, osmolarity and sensory characteristics of diarrheal replacement fluids. Diarrheal replacement fluids should be nutrient-dense as well as have better absorption properties. Cereal based-ORS are better substitutes to WHO-ORS as they are nutritionally balanced and can be prepared at a lesser cost.

**Methodology**

**Selection of the Samples**

The most important cultivated species of millet selected for the present study were Barnyard (Echinochola crus galli) (CO(KV)2, among the cultivated and popular variety of selected minor millet available in the local market at Salem, Tamilnadu, India were procured. The seeds were hand-sorted to remove wrinkled, moldy seeds and foreign material and then stored in polyethylene bags in the refrigerator (4°C±1) until used. The required amount of other food-grade ingredients like sodium chloride, potassium chloride, and sodium bicarbonate, etc was collected from the local analytical laboratory of Salem city and stored.

**Production of Barnyard Millet Flours**

**Soaking of Barnyard Millet**

Barnyard millet without bran was used as a sample to determine water absorption characteristics of millet using different soaking time ranging from 30, 60, 90 and 120 minutes. After soaking, all the soaked millets will be dried, powdered, and packaged. The unsoaked and roasted millet was used as a control, which will be powdered and packed in an airtight container for further analysis.

**Roasting of Barnyard Millet**

Roasting will be done at 70, 80, 90 and 100°C for 4, 5, 7 and 9 minutes. The unroasted millet samples were milled into flour and stored in an airtight container for further preparation.

**Nutritional Composition**

The nutrient analysis of raw and processed millet flour was estimated using standard methods. Proximate compositions such as moisture, ash, energy, carbohydrate were determined by AOAC, 2006 method. The Kjeldahl method, as described in AACC (1986) was used to determine the protein content. The minerals such as sodium, sodium bicarbonate, potassium, were assessed by AACC (1986) and calcium, magnesium, iron, zinc, copper, manganese were estimated by standard analytical methods. Phosphorus content was analyzed by Technician Auto-analyzer method.
Anti-Nutritional Parameters of Raw and Processed Millet Flours
The anti-nutritional parameters, i.e. tannin and total polyphenol compound, were analysed in raw and processed millet flours. Both were described by Sadasivam and Manickem.\textsuperscript{10}

Preparation of Samples
The raw and processed barnyard millet flour was cooked at different concentrations in 5, 10, 15 and 20g / 100 ml of water with sodium chloride, potassium chloride, trisodium citrate dehydrate and alpha amylase enzymes. The temperature was maintained at 90°C for 5-6mins to inactivate the enzyme. After cooling the sample at room temperature, the sample was analyzed for viscosity and Osmolarity.

Viscosity
Viscosity was measured with a micro viscometer (Fungi lab viscolead). The raw and processed millet flour replacement fluids were measured on the same day as prepared with alpha amylase enzymes. Measurements were taken at room temperature and measured twice at eight different speeds: 0.3 to 60 rpm in cP.

Osmolarity
Osmolarity, expressed in mOsm/kg, is measured by freezing point depression with an Osmometer (Rosalina Advanced Instruments; Mumbai, India). The 250 µl pipette was used to transfer the sample into a microtube. Osmolarity was measured on the same day as the replacement fluids as prepared with and without added amylase enzymes.

Organoleptic Evaluation
The formulated fluids were evaluated for organoleptic quality by 9 points hedonic scale scorecard method. The 15 mothers/ caretaker of the child were asked to score the samples for their color, flavor, texture, taste, appearance and overall acceptability based on the child’s facial expression.

Statistical Analysis
The statistical analyses were performed using IBM SPSS Statistics 23 Software package. The analysis was done in triplicates, and results were analyzed by descriptive statistics. The data were subjected to analysis of variance (One-way ANOVA) with Duncan’s Post Hoc test (P<0.05) to determine the significant difference between the means.

Results and Discussion

Nutrient Composition
The nutrient composition of raw and processed barnyard millet flour results was shown in Table-1. The roasted barnyard millet flour protein and Carbohydrate ranges were comparatively high in 70°C roasted barnyard millet flour. Crude fat content was ranged from (2.39±0.01-2.77±0.01g/100g). The result was in line with Aremu; \textit{et al.},\textsuperscript{12} who reported that roasting of groundnut and cranberry bean reduced the crude fat content. The average ash content was 1.28%. There is no significant difference between the moisture content of 70 to 90°C roasted group. The low level of moisture in roasted flours probably was resulted from the high temperature (which eliminated water more quickly), and the intermolecular cross-linking that might occur.\textsuperscript{13} Previous studies reported that drying of high moisture content of the sample was yielded the lower content of samples.\textsuperscript{14}

The average ash content of soaked barnyard millet flour was 0.95%. The lowest ash content was found in 120 mins soaked millet flour. Crude fat and protein were also found to be high in the 60 mins-soaked millet flour group. Studies reported that increased soaking time can drop the protein content of millets. During the soaking periods, the reduction of protein was 5 to 20% at 24 to 72 hrs.\textsuperscript{13} The lowest carbohydrate content was noted in 90mins soaked millet, and there was a significant difference between the groups in CHO, protein and crude fat. The raw barnyard millet had a significant difference in carbohydrates, protein and crude fat.

Sodium and sodium bicarbonate was highest at 70°C roasted barnyard millet flour. Calcium, iron, phosphorus, and zinc were found high in 70°C roasted barnyard millet flour. Manganese content was lowest in 100°C roasted barnyard millet flour. When the grains were roasted, the iron was reduced in grains. The current study was proving that increased the roasting temperature, which decreased the iron content of millet.
Minerals were analyzed in soaked barnyard millet groups. Sodium content was ranged from 2.16-2.57 mg/g. Potassium content was highest in 60 mins soaked millet. Iron and zinc content was lowest in 120 mins soaked millet compared to other millets. Manganese content was highest in 60 mins soaked millet and lowest in 120 mins soaked millet flour. Based on these results was showed 60 mins soaked millet had significantly high in overall mineral composition. In earlier studies was reported that different processing methods affected the mineral content of the barnyard millet.

| Table: 1 Proximate composition of raw and processed barnyard millet flour |
|--------------------------------------------------|
| Proximate analysis | Raw Barnyard millet | 70°C | 80°C | 90°C | 100°C | Soaked Barnyard millet 30 min | 60 min | 90 min | 120 mins |
| Energy (Kcal/100 g) | 271.00±1.00$	ext{a}$ | 287.00±2.00$	ext{d}$ | 273.66±1.52$	ext{b}$ | 253.66±0.57$	ext{c}$ | 221.66±2.08$	ext{a}$ | 307.33±0.57$	ext{b}$ | 315.66±2.08$	ext{b}$ | 285.33±1.52$	ext{c}$ | 241.33±1.52$	ext{a}$ |
| Ash (g/100 g) | 1.75±0.03$	ext{a}$ | 1.15±0.03$	ext{a}$ | 1.21±0.01$	ext{b}$ | 1.25±0.01$	ext{b}$ | 1.54±0.04$	ext{c}$ | 1.15±0.35$	ext{d}$ | 1.07±0.01$	ext{c}$ | 0.98±0.02$	ext{d}$ | 0.85±0.01$	ext{b}$ |
| Moisture (g/100 g) | 3.77±0.02$	ext{a}$ | 3.27±0.00$	ext{b}$ | 3.27±0.00$	ext{b}$ | 3.27±0.00$	ext{b}$ | 2.90±0.07$	ext{b}$ | 9.26±0.02$	ext{b}$ | 9.56±0.01$	ext{b}$ | 9.35±0.01$	ext{b}$ | 9.14±0.01$	ext{b}$ |
| Protein (g/100 g) | 5.62±0.07$	ext{a}$ | 5.45±0.02$	ext{d}$ | 5.35±0.04$	ext{c}$ | 5.17±0.07$	ext{b}$ | 5.05±0.01$	ext{b}$ | 6.09±0.06$	ext{b}$ | 6.61±0.10$	ext{d}$ | 6.33±0.04$	ext{b}$ | 6.07±0.01$	ext{b}$ |
| Crude fat (g/100 g) | 2.97±0.02$	ext{c}$ | 2.77±0.01$	ext{d}$ | 2.70±0.00$	ext{b}$ | 2.42±0.00$	ext{b}$ | 2.39±0.01$	ext{b}$ | 3.43±0.04$	ext{c}$ | 3.57±0.01$	ext{c}$ | 3.25±0.02$	ext{c}$ | 3.07±0.01$	ext{c}$ |
| CHO (g/100 g) | 64.27±0.01$	ext{c}$ | 63.20±0.00$	ext{d}$ | 62.07±0.01$	ext{b}$ | 61.12±0.10$	ext{b}$ | 59.24±0.01$	ext{a}$ | 65.41±0.01$	ext{c}$ | 66.16±0.01$	ext{d}$ | 63.17±0.03$	ext{b}$ | 61.06±0.01$	ext{b}$ |

Each value in the table are represented as Mean ± SD (n=3). Statistically significant at p < 0.05, where a>b>c>d in each column.
The result shown in Figure-1 is the anti-nutrient content of roasted barnyard millet flour. The average content of tannin was 1.9 mg/100 g and ranged between 1.54-2.17 mg/100 g for all groups, and the highest concentration of the tannin was found in 70°C roasted barnyard millet flour. The phenolic compound was ranged between 4.54-5.07 mg/100 g and this indicates lowest levels of phenolic compound present in 100°C roasted millet flour. The 100°C roasted millet flour was the least concentration of anti-nutrients than other variations of the group. In earlier studies, Seifi et al. reported that, during processing, the anti-nutritional parameters are increased in little millets compare to other native millets. The roasting method is used to improve the energy density, and nutrient availability from millet.

The anti-nutrient content of soaked barnyard millet flour was analyzed and represented in Figure-2. Soaked barnyard millet flour has a significant difference between the group in phenolic compounds. Several studies reported that, soaking of legumes in distilled water was an effective way of removing phytic acid from legumes. Because during soaking period legumes may be leaching of phytates ions into the soaking water under the influence of a concentration of gradient, which governs the rate of diffusion.

**Table 3: Anti-nutritional parameters of raw millet flour**

| S. no | Anti nutrient       | Raw barnyard millet flour (mg/100 g) |
|-------|---------------------|---------------------------------------|
| 1     | Tannin              | 1.46±0.01                             |
| 2     | Total phenolic compound | 4.26±0.01                           |
| Concentration     | 5 g | 10 g | 15 g | 20 g |
|-------------------|-----|------|------|------|
|                   | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Raw Barnyard      | 634.33±0.01 | 365.53 ± 1.90 | 823.43±0.01 | 426.67 ± 2.15 | 755.34±0.01 | 456.93 ± 1.56 | 755.45±0.01 | 535.93 ± 1.62 |
| Roasted Barnyard  | 70°C  | 245.67±0.01 | 33.8 ± 2.9 | 267.66±0.02 | 36.4 ± 2.8 | 156.45±0.02 | 38.56 ± 1.06 | 346.48±0.34 | 88.63 ± 0.58 |
| Roasted Barnyard  | 80°C  | 367.43±0.01 | 45.23 ± 0.58 | 299.45±0.05 | 66.73 ± 2.37 | 553.27±0.07 | 57.9 ± 1.45 | 222.40±0.21 | 96.26 ± 2.03 |
| Roasted Barnyard  | 90°C  | 267.31±0.02 | 46.70 ± 2.06 | 367.22±0.01 | 71.80 ± 3.4 | 352.32±0.54 | 77.86 ± 1.55 | 543.90±0.09 | 65.76 ± 9.78 |
| Roasted Barnyard  | 100°C | 221.90±11.05 | 115.60 ± 12.05 | 227.04±0.05 | 82.73 ± 4.34 | 187.45±2.04 | 92.90 ± 6.85 | 193.21±0.56 | 86.30 ± 2.95 |
| Soaked Barnyard   | 30 mins | 456.34±2.12 | 198.27 ± 0.77 | 345.89±0.01 | 191.43 ± 1.82 | 521.09±1.95 | 327.40 ± 2.62 | 434.66±1.78 | 311.90 ± 2.45 |
| Soaked Barnyard   | 60 mins | 421.90±0.03 | 212.70 ± 1.40 | 457.34±3.87 | 223.73 ± 0.65 | 578.34±0.03 | 284.10 ± 0.70 | 452.90±3.10 | 276.53 ± 1.06 |
| Soaked Barnyard   | 90 mins | 321.90±1.23 | 177.80 ± 0.70 | 432.90±3.41 | 233.53 ± 1.09 | 421.90±0.02 | 276.20 ± 1.34 | 378.33±0.02 | 324.00 ± 2.02 |
| Soaked Barnyard   | 120 mins | 345.89±0.03 | 177.90 ± 0.87 | 344.23±1.78 | 188.90 ± 0.70 | 555.34±6.34 | 197.70 ± 1.53 | 235.53±1.09 | 254.13 ± 0.90 |

Mean ± SD values are expressed in centipoise (cP) at 3 rpm.

The anti-nutrient parameter of raw millet flour was presented in Table-3. The tannin and phenolic compounds were the lowest values when compared to processed millet flours. Sudha Rani and Usha Anthony [21] noted that there was a different processing technique for the varieties of finger millet with different seed coat colour has a high impact on the polyphenols content.
Table 5: Osmolarity of raw and processed barnyard millet replacement fluids

| Concentration (g/100 ml) | Raw BM (mOsmol/kg) | Roasted barnyard millet (mOsmol/kg) | Soaked barnyard millet (mOsmol/kg) |
|--------------------------|--------------------|-------------------------------------|-----------------------------------|
| 5g                       | 249.33±1.5        | 247.00±1.00                        | 217.33±0.57                       |
|                          | 236.00±2.64       | 279.33±1.52                        | 289.66±7.57                       |
|                          | 222.00±10.44      | 243.33±2.08                       | 247.66±3.05                       |
| 10g                      | 266.0±1.0         | 248.66±2.08                        | 225.00±3.00                       |
|                          | 243.33±2.08       | 292.66±1.52                        | 274.00±1.00                       |
|                          | 239.00±2.00       | 260.66±2.51                       | 277.00±5.29                       |
| 15g                      | 276.0±1.0         | 278.66±2.08                        | 259.00±4.58                       |
|                          | 277.00±4.58       | 309.66±2.51                       | 254.33±3.05                       |
|                          | 294.00±1.00       | 279.66±4.1                         | 296.00±2.65                       |
| 20g                      | 314.3±3.05        | 286.33±2.08                        | 317.33±6.50                       |
|                          | 294.00±1.00       | 342.33±7.57                       | 302.00±15.77                      |
|                          | 314.33±3.05       | 325.66±4.1                        |                                   |

Each value in the table are represented as Mean ± SD (n=3). Statistically significant at p < 0.05, where a<b<c<d in each column.

Table 6: Organoleptic evaluation of raw and processed barnyard millet replacement fluids

| Parameters            | Raw Barnyard millet Fluid | Roasted barnyard millet Fluid | Soaked barnyard millet Fluid |
|-----------------------|----------------------------|-------------------------------|-------------------------------|
|                       | 70°C                      | 80°C                          | 90°C                          | 100°C                        | 30 mins | 60 mins | 90 mins | 120 mins |
| Appearance            | 3.55±0.60                 | 7.25±0.85                     | 7.95±0.75                     | 6.15±1.03                    | 5.20±1.28 | 5.30±0.86 | 6.05±0.82 | 4.00±0.81 | 3.90±0.30 |
| Flavour               | 4.15±0.82                 | 7.30±0.80                     | 7.90±0.91                     | 6.30±0.92                    | 5.00±1.41 | 5.70±0.86 | 6.00±0.64 | 4.00±0.74 | 4.00±0.85 |
| Colour                | 4.55±0.82                 | 7.50±0.82                     | 7.95±0.82                     | 6.20±1.15                    | 5.05±1.05 | 5.90±0.78 | 6.20±0.41 | 4.36±1.25 | 4.10±0.96 |
| Taste                 | 3.85±1.08                 | 7.25±0.78                     | 7.75±0.09                     | 6.45±1.05                    | 4.40±1.04 | 5.85±1.13 | 6.25±0.63 | 3.94±0.01 | 3.70±0.57 |
| Texture               | 4.20±0.89                 | 7.65±0.87                     | 7.90±0.91                     | 6.20±1.19                    | 4.90±1.11 | 5.70±1.03 | 6.00±0.91 | 4.31±0.74 | 4.25±0.44 |
| Overall acceptability | 4.35±0.74                 | 7.65±0.58                     | 7.94±0.84                     | 6.20±1.19                    | 5.35±1.13 | 5.85±0.67 | 6.05±0.76 | 4.21±0.91 | 4.20±0.61 |

The outcome is expressed in Mean ± SD (n=20). Statistically significant at p < 0.05, where a<b in each column.
During the heat processing, the viscosity was attained to high values to indicate the water-binding capacity of starch in flours. Previous studies stated that a solution with viscosity less than 1,000 cP is free liquid fluid. Amylase enzyme was used to reduce the viscosity in replacement fluids. The raw millet flour fluids had the highest viscosity range when compared to other processed flours fluids. Here, overall the viscosity values were high in initial range after added the enzyme was broke down the starch molecules and expressed a lowest viscosity in both raw and processed flour fluids (Table 4).

Besides the viscosity was decreased in soaked soybean but no difference that can appear in the different soaking periods. Comparative results were observed in roasted soy bean. Similar reduction of viscosity were noted in maize and maize blend flour. In another study reported that, amylose was leach from granules after they gelatinize. Studies revealed that the concentration of starch increase the continuous matrix it contains more amylase and less water and increases the viscosity.

The decrease in viscosity is due to enzymatic breakdown of starch to sugars in different processing. Soaking also leads to the breakdown several components into simpler compounds which alter the texture, flavor, and aroma and taste. Starch, particularly absorbs water on cooking, forming a gelatinous mass, while proteins denature and expose more hydrophilic sites that will take up more water.

Osmolarity
The Osmolarity of raw and processed barnyard millet flour-based replacement fluid is presented in Table-5. The Osmolarity of roasted barnyard millet flour-based replacement fluids has a significant difference between at different concentrations. Osmolarity is the number of dissolved particles per kilogram of solution. The normal plasma osmolarity of solutions to be administered in large animals is approximately 306 mOsm/L; solutions can, therefore, be defined as isotonic (300–312 mOsm/L), hypertonic (>312 mOsm/L), or hypotonic (<300 mOsm/L).

The lowest osmolarity was noted in 5 g of 60 mins soaked barnyard millet flour based replacement fluids (222.00±10.44 mOsmol/kg) and the highest osmolarity was seen in 120 mins soaked barnyard millet flour based replacement fluids (325.66±4.1 mOsmol/kg). There was no significant difference between the osmolarity of raw and processed millet flour based replacement fluids.

**Organoleptic Evaluation**
Organoleptic evaluation results of processed millet flour-based replacement fluids are represented in Table-6. Among the results, mothers have well accepted both roasted and soaked millet flour fluids in all aspects of the sensory profile. As a result, roasted millet flour fluids were more acceptable by mothers when compared to soaked and raw millet flour fluids. In previous study was reported that maize–ORS were successfully rehydrated within a day for an infant who was affected by diarrhoea when compared to glucose–ORS. Chowdury et al.,(1991) reported that mother unanimously agreed that, the rice–ORS had stopped diarrhea more quickly than glucose–ORS.

**Conclusion**
There was a significant difference in nutritional, anti-nutritional, viscosity, and Osmolarity of selected barnyard millet flour-based replacement fluids in response to different processing methods. Among these results, soaked millet was reduced the anti-nutritional factors, and roasted millet significantly reduced the viscosity of replacement fluids. Based on the sensory analysis, the roasted millet replacement fluids were well accepted. A person with moderate to severe diarrhea loses a significant amount of fluids quickly, therefore needs to take in extra fluids to replenish the loss. Therefore, processed millet flour that makes them a good base ingredient an infant food and beverages. The results were concluding that processed barnyard millet was recommended to the diarrheal replacement fluids other than WHO-ORS. Barnyard millet replacement fluid will reduce the dehydration and improve the nutritional status of infants and adults with diarrhea.

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Conflict of Interest
The authors declare that they do not have any conflict of interest.

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