“Effect of fermentation on physicochemical properties & in vitro starch and protein digestibility of selected cereals”

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Abstract

The effect of natural fermentation on physicochemical parameters (bulk density, water absorption capacity, oil holding capacity, pH and titratable acidity), in vitro starch and protein digestibility of three cereals namely sorghum (Sorghum bicolor), pearl millet (Pennisetum glaucum) and maize (Zea mays) were determined and compared with those of their unfermented counterparts. Cereal flour was fermented for 36 h and samples were withdrawn at 12 h intervals. Results showed that fermentation significantly (p≤0.05) decreased all physicochemical parameters except oil holding capacity and titratable acidity which was significantly (p≤0.05) increased. The pH of the fermented cereal flour decreased with a concomitant increase in the titratable acidity. Percent increase in starch and protein digestibility was highest in sorghum (70 %) and maize (23 %) respectively. Fermentation significantly (p≤0.05) increased in vitro starch and protein digestibility of selected cereal flours.

Key Words: Fermentation, Physicochemical parameters, In vitro starch digestibility, In vitro protein digestibility.

1. INTRODUCTION

Sorghum (Sorghum bicolor), pearl millet (Pennisetum glaucum) and maize (Zea mays) are important cereals of India. It is a major source of protein, carbohydrate and calorie in the diets of large segment of population; however bioavailability is low inherently due to the presence of antinutritional factors such as phytic acid, polyphenols and tannins. These millets contain high amount of starch and its digestibility is greatly influenced by plant type and depends on physicochemical characteristics of the starch and other factors like plant microstructure, composition, processing and storage conditions [1].

Fermentation is one of the processes that decreases the level of antinutrients in food grains and increases the starch digestibility, protein digestibility and nutritive value [2]. The nutritional evaluation of fermented grains has been examined by many workers [3, 4, 5]. Fermentation also leads to an increase in protein content [6], enhancement of carbohydrate accessibility [7], improvement in amino acid balance [8], decrease in antinutritional factors like tannin and phytic acid [9]. Household fermentation technologies have been upgraded to an industrial scale in order to provide value added products that meet urban population demand for traditional products [10]. Commonly consumed millets like Sorghum, Pearl millet and Maize have a high nutrient content but bioavailability is low, inherently due to the presence of antinutritional factors. Functional and nutritional aspects of fermentation have been widely studied in commonly consumed cereals and legumes [11, 12]. There is, however, limited information on the functional properties of fermented sorghum, pearl millet and maize flour and this information is essential for determining potential uses of these products in food formulation.

The objective of the present investigation was to study changes occurring in in vitro starch digestibility, in vitro protein digestibility and physicochemical parameters during natural fermentation of millets.

2. MATERIALS & METHODS

2.1 Procurement of raw materials

Three cereals namely Sorghum (Sorghum bicolor), Maize (Zea mays) and Pearl millet (Pennisetum americanum) were procured from Krishi Sewa Kendra, Allahabad. These varieties include K-65 for maize, Banjara Gold for Pearl millet (Pennisetum americanum) and MFSH-4 for sorghum. These samples were carefully cleaned and freed from foreign materials and the grains were finely ground and stored in polyethylene bags at 4°C. All the chemicals used in analysis were of AR (Analytical Reagent) grade.

2.2 Fermentation

Natural fermentation was carried out by mixing each sample with distilled water (1:2 w/v). Twenty gram selected cereal flours was mixed with 40 mL distilled water
in a 500 ml beaker and were incubated in an incubator (Orbital shaking incubator, REMI/ 396LAG) at 37º C and samples were withdrawn at periods of 0, 12, 24 and 36 h. After the incubation period each sample was mixed with a glass rod and transferred to four aluminum dishes, and dried in a hot air oven drier at 70º C for 3-4 h. Dried samples were finely ground and stored in polyethylene bags at 4ºC for subsequent analysis [13].

2.3 Physicochemical and functional Properties

2.3.1 Bulk Density (BD)

Bulk density was determined according to the method given by Chau and Huang [14] using a graduated cylinder (10 mL), previously weighed, and filled with sample to 10 mL by constant tapping, until there is no further change in volume and the content is weighed. The content was weighed, and from the difference in weight, the bulk density of sample was calculated as grams per milliliter.

2.3.2 Water Absorption Capacity (WAC)

The WAC was determined according to the method of Beuchat, [15]. One g of flour sample was mixed in a vortex with 10 mL of distilled water for 1 min and then centrifuged at 3000-5000 g for 30-45 min. After separation of the content, the volume of supernatant was recorded and used for determination of water absorption; the results are expressed as g/mL of sample.

2.3.3 Oil-Holding Capacity (OHC)

One g of sample was mixed with vegetable oil (1:10). The mixture was stirred for 30 min at room temperature. After samples were centrifuged (2500g, 30 min), the supernatant was transferred to a graduated cylinder of 10 mL, where the volume was measured. The OHC was expressed as milliliters of vegetable oil held per gram of sample [14].

2.3.4 Swelling Capacity (SC)

Swelling capacity was determined according to the method given by Robertson et al., [16]. 100 mg of flour sample was hydrated in a known volume of distilled water (10 mL) in a calibrated cylinder at room temperature. After equilibration (18 h), the bed volume was recorded and swelling capacity expressed as volume occupied by sample per gram of original sample dry weight.

Swelling capacity \% = V₂ - V₁/N x 100

V₁ = volume of flour sample before soaking, V₂ = volume of soaking flour sample, N = grams of flour sample.

2.3.5 pH and Titratable acidity (TA)

The pH of the samples was determined according to the method of AOAC, [17]. The titratable acidity was estimated by titrating against 0.1 N NaOH to phenolphthalein end-point and the acidity was calculated as g lactic acid/100 g [18].

2.3.6 In vitro Starch digestibility (IVSD)

In vitro starch digestibility (IVSD) was determined according to the method of Singh et al., [19]. 50 mg of cereal flour was taken in a test tube and mixed with 1 ml of 0.2 M phosphate buffer (pH 6.9). Pancreatic alpha amylase (0.5 mL) (Sigma, cat. No. 6880, 20 mg enzyme dissolved in 50 mL of the same buffer) was added to the sample and incubated at 37º C for 2 h. After the incubation period 2 ml of 3, 5-DNS reagent (prepared by dissolving 200 mg crystalline phenol, 1 g 3,5-dinitrosalicylic acid and 50 mg sodium sulphite in 1% NaOH solution) was added immediately. The mixture was heated for 5-15 min in a boiling water bath. After heating 1.0 mL of 40% K-Na-Tartarate solution was added in the test tubes and allowed to cool at the room temperature (25 ºC). Thereafter solution was made up to 25 mL with distilled water and filtered prior to measurement of the absorbance at 550 nm. A blank was run simultaneously. A standard curve was prepared using maltose. Values are expressed as mg maltose released per 100 mg of sample.

2.3.7 In vitro Protein digestibility (IVPD)

The in vitro protein digestibility of the samples was determined by enzymatic method [20]. A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 mL of 0.1 M HCl at 37ºC for 2 hours. The reaction was stopped by the addition of 15 mL 10% trichloro-acetic acid (TCA). The mixture was then filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-kjeldahl method. Protein digestibility of the sample was calculated by the following formula:

\[
\text{Protein digestibility (\%) = } \frac{N \text{ in supernatant} - blank N}{N \text{ in sample}} \times 100
\]

3. STATISTICAL ANALYSIS

All the analyses were conducted in triplicate and the mean data ± SD (standard deviation) are reported. Data were subjected to analysis of variance [21]. Significance of mean differences were determined. Significance was accepted at p ≤ 0.05.

4. RESULTS AND DISCUSSION

4.1 Bulk density

Table 1 shows the effect of fermentation on physicochemical properties of selected grains. Bulk density was found highest in sorghum (0.75 g/mL) followed by maize (0.72 g/mL) and pearl millet (0.71 g/mL) respectively. Bulk density values decreased gradually with increase in fermentation periods. The bulk density is a reflection of the load the flour samples can carry, if allowed to rest directly on one another. The density of processed products dictate the characteristics of its container or package product density influences the amount and strength of packaging material, texture or mouth feel [22]. Values obtained from this study were comparable with the values reported by Okaka and Potter [23] for cowpea (0.60 g/mL) and fall in the range for Bambara groundnut (0.6 to 0.75 g/mL) reported by Onimawo et al., [24].

Fermentation of sorghum flour for 24 h decreased the bulk density of the sorghum flour by about 10% [25]. The decrease in bulk density of fermented flour would be an advantage in the preparation of infant foods. Fermentation has been reported as a useful and traditional method for the preparation of low bulk weaning foods [26].

4.2 Water absorption capacity

Water absorption capacity ranged from 0.92 to 1.41 mL/g among all three selected cereal samples and showed decreasing trend with increase in duration of fermentation. Result showed that sequential natural fermentation caused significant (p<0.05) reduction in water absorption capacity in all three selected cereal samples. Udensi and Okoronkwo, [27] also reported that fermentation significantly (p<0.05) decreased the water
Table 1. Effect of natural fermentation on bulk density (BD), oil holding capacity (OHC), water absorption capacity (WAC), swelling capacity (SC), pH and titratable acidity (TA) of unfermented and fermented sorghum, pearl millet and maize flour:

<table>
<thead>
<tr>
<th>Sample</th>
<th>F*</th>
<th>BD (g/ml)</th>
<th>OHC (ml/g)</th>
<th>WAC (ml/g)</th>
<th>SC (%)</th>
<th>pH</th>
<th>TA (g lactic acid/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>0</td>
<td>0.75±0.01a</td>
<td>7.03±0.15b</td>
<td>1.26±0.01c</td>
<td>0.29±0.01b</td>
<td>5.2±0.2</td>
<td>1.06±0.11b</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.69±0.00c</td>
<td>7.46±0.15b</td>
<td>1.19±0.00e</td>
<td>0.25±0.01f</td>
<td>4.16±0.28g</td>
<td>1.8±0.10g</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.66±0.01d</td>
<td>7.3±0.05d</td>
<td>1.19±0.00e</td>
<td>0.23±0.01d</td>
<td>3.86±0.05c</td>
<td>2.1±0.17d</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>0.61±0.01e</td>
<td>8.1±0.1f</td>
<td>1.03±0.04h</td>
<td>0.18±0.02i</td>
<td>3.73±0.05m</td>
<td>2.8±0.11m</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.71±0.01f</td>
<td>6.7±0.17a</td>
<td>1.41±0.01i</td>
<td>0.14±0.01i</td>
<td>5.63±0.15f</td>
<td>1.4±0.1i</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.68±0.00e</td>
<td>7.3±17e</td>
<td>1.39±0.02h</td>
<td>0.15±0.01i</td>
<td>4.63±0.15e</td>
<td>1.83±0.05e</td>
</tr>
<tr>
<td>Pearl millet</td>
<td>24</td>
<td>0.65±0.02g</td>
<td>7.63±0.11m</td>
<td>1.32±0.02i</td>
<td>0.12±0.00g</td>
<td>3.96±0.05ed</td>
<td>2.2±0.2ed</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>0.59±0.00a</td>
<td>8.2±0.1l</td>
<td>1.29±0.01f</td>
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</tr>
<tr>
<td></td>
<td>0</td>
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<td>6.9±0.05h</td>
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<td>0.21±0.01g</td>
<td>5.76±0.15f</td>
<td>0.96±0.11m</td>
</tr>
<tr>
<td>Maize</td>
<td>12</td>
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<td>7.7±0.11f</td>
<td>0.84±0.01b</td>
<td>0.18±0.00h</td>
<td>5.0±0.11f</td>
<td>1.36±0.05t</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.64±0.00g</td>
<td>8.0±0.1l</td>
<td>0.83±0.00g</td>
<td>0.17±0.01i</td>
<td>4.1±0.15f</td>
<td>1.9±0.17de</td>
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<tr>
<td></td>
<td>36</td>
<td>0.60±0.01a</td>
<td>8.5±0.05g</td>
<td>0.77±0.00a</td>
<td>0.10±0.00g</td>
<td>3.5±0.05ab</td>
<td>2.5±0.10a</td>
</tr>
</tbody>
</table>

Values are means ± SD.

F*= Fermentation period

Absorption capacity (WAC) of Mucuna bean. Similar results were also found by Elkalil§a et al., [25] after fermentation of sorghum flour at 37°C for periods of 8, 16, and 24 h. Water binding capacity is a useful indication of flour or isolates whether it can be incorporated into aqueous food formulations especially those involving dough handling [28, 29]. Water absorption capacity gives an indication of the amount of water available for gelatinization. Lower absorption capacity is desirable for making thinner gruels.

4.3 Oil holding capacity

All three selected cereal flour samples contained good oil holding capacity [sorghum (7.03 ml/g), pearl millet (6.7 mL/g) and maize (6.9 mL/g)]. Fermentation significantly (p<0.05) increased oil holding capacity of sorghum, pearl millet and maize by 15.0, 22.0 and 23.0 % respectively after 36 h of fermentation. These results are in agreement with Elkalil§a et al., [25] who reported about 7 % increase in oil holding capacity after 8 h of fermentation of sorghum flour. The higher oil-binding capacity of sorghum flour suggests that this flour would be useful in formulation of foods where an oil holding property is an important consideration.

4.4 Swelling capacity

Swelling capacity decreased with increasing fermentation period. Values of swelling capacity for non-fermented sorghum, pearl millet and maize flour was 0.29, 0.14 and 0.21 % respectively which reduced up to 0.18, 0.10 and 0.10 % respectively after 36 h of fermentation. These results are similar with Adebowale and Maliki, [30] who reported that fermentation was found to reduce the swelling capacity of pigeon pea flour.

4.5 pH and titratable acidity

Changes in pH and titratable acidity (TA) are shown in Table 1. Fermentation was found to cause a gradual reduction in a pH with time. The change in pH from zero to 36 h resulted in a pH drop from 5.2 to 3.73, 5.63 to 3.4 and from 5.76 to 3.5 for Sorghum, pearl millet and Maize, respectively. Similar results were also found by Elyas et al., [31] on two cultivars of pearl millet after 36 h of fermentation. These results also agree with those obtained by Giese, [32] who reported that, as a result of fermentation, acidity increased and pH falls down and this enhanced the keeping quality of millet foods, by inhibiting microbial growth and also contributing to the flavour of processed millet. Usha et al., [33] reported a drop in pH from 6.4 in unfermented finger millet to 5.2 in 24 h and to 4.3 in 48 h of fermentation. This result is in agreement with Murdock and Fields, [34] and Nanson and Fields, [35] who reported that lactic acid fermentation causes a rapid drop in pH of various food grains. Khetarpaul and Chauhan, [36] reported that pH of the fermented dough is lowered due to

Figure 1. Effect of fermentation on in vitro starch digestibility of selected cereals

Figure 2. Effect of fermentation on in vitro protein digestibility of selected cereals
the production of organic acids by the microflora; heterofermentors were reported to convert glucose to equimolar mixture of lactic acid, ethanol and carbon dioxide.

Concomitant with the drop in pH there was a rise in TA of cereal flours throughout the fermentation process. The TA increased from 1.06 to 2.8, 1.4 to 3.06 and 0.96 to 2.5 g lactic acid/100 g for sorghum, pearl millet and maize respectively. This finding was in agreement with the work conducted by Yousif and El-Tinay, [37] and Fadlallah et al., [38]. According to El Hidai, [39] natural sorghum fermentation is mainly lactic acid by Lactobacillus spp. Yeast and acetic acid fermentations occur to a lesser extent during the latter stages of fermentation. This could explain the apparent increase in lactic acid towards the end of fermentation accompanied by lack of changes in pH.

4.6 In vitro starch digestibility (IVSD)

The effect of fermentation on the IVSD is shown in Figure 1; unfermented sorghum, pearl millet and maize flour had the lower IVSD (11, 15 and 19 % respectively) this may be due to the restriction in accessibility of starch caused by endosperm proteins [40]. In our study we found that fermentation causes significant (p<0.05) increase in in vitro starch digestibility of selected cereal flours. Percent increase in starch digestibility was highest of fermented sorghum (70 %), followed by pearl millet (49 %) and maize flour (41 %) respectively. Elkhalfia et al., [7] reported that sorghum flour led to an increase in the IVSD from 34.55 to 56.69% after 28 h fermentation. Hassan and El Tinay, [37] also found an increase in IVSD from 32.3 to 45.2% during fermentation of sorghum cultivar Dabar. This increase may be due to the fact that fermentation led to changes in the endosperm protein fractions [41] and this makes starch more accessible to the digestive enzymes.

4.7 In vitro protein digestibility (IVPD)

The IVPD of unfermented and fermented sorghum, pearl millet and maize flour is shown in Figure 2. Fermentation was found to cause a significant (p<0.05) improvement in IVPD for all three cereal samples. The increase was from 65.0 to 83.0, 68.0 to 84.0 and 63.0 to 81.0 % for sorghum, pearl millet and maize, respectively. Microflora may produce proteolytic enzymes during fermentation which may be responsible for the increased protein digestibility [42]. In addition, the elimination of phytic acid contributes to the improvement in protein digestibility in fermented millet [43]. These results agree with Mohideen et al., [13] who reported that fermentation is found to improve the IVPD of two maize cultivars and this could be attributed to the partial degradation of complex storage proteins into more simple and soluble products. Monawar, [44] reported that the reduction in pH during fermentation plays an important role in enhancing native proteolytic enzymes activity and consequently promotes the breakdown of proteins to smaller polypeptides which are easily digested by enzymes.

5. CONCLUSION

The functional properties, in vitro protein and starch digestibility of sorghum, pearl millet and maize significantly improved after natural fermentation. This suggests possible use of the fermented flour of these cereals as a potential source to improve the nutritional qualities of local staple cereal products. These results suggest the need of further studies about the possibility of using fermented cereal flour or starch isolates from it in food industry.

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