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Effect of fermentation on the in vitro protein digestibility of pearl millet

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Abstract

Two pearl millet genotypes, Madelkawya and Population 1/Shambat, were used in this study. Investigation showed that the dry matter, ash, crude fibre, crude fat, crude protein and starch contents for Madelkawya cultivar were 92.5, 2.1, 2.8, 7.8, 13.6 and 63.2%, respectively, and for Population 1/Shambat were 92.7, 2.4, 3.2, 6.7, 12.5 and 64.9%, respectively. The two cultivars were fermented for 14 h and the pH, crude protein and in vitro protein digestibilities were determined at 2 h intervals. The results indicated that there was a gradual reduction in pH with increase in time, a marginal change in the protein content and a highly significant ($P \leqslant 0.05$) improvement in the in vitro protein digestibility for both cultivars.

Keywords: Millet; Protein digestibility; Fermentation

1. Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br] is the most widely grown product of millet species, which are grown for their edible starchy seeds. Pearl millet probably originated in the African savannah zone, having been grown in Africa and Asia since prehistoric times. Pearl millet is sown on about 15 million hectares in Africa and 12 million hectares in Asia (Riley, Gupta, Seetharama, & Mushonga, 1993).

Pearl millet is consumed as a staple food by large sections of the population in India and Africa and, recently, attempts have been made to combine high grain yield with good nutritional qualities. The nutritional properties of pearl millet have received more attention than those of the other common millets, because it is the largest-seeded, most widely grown type (Hoseney, Rew, & Clark, 1987).

Millet is usually ground for feeding to animals other than poultry and it is very important in human diets, particularly those of the poorest people of the semi-arid tropics.

The protein quality of pearl millet is low in the levels of lysine and tryptophan; hence, there is growing emphasis on the improvement of protein quantity and quality in cereal crops. Characteristics often used to define protein quality of a feed or food are its amino acid composition and its protein digestibility (Hahn, Faubion, Ring, Doherty, & Roonew, 1981).

Fermentation is one of the processes that decrease the levels of anti-nutrients in food grains and increase the protein availability, in vitro protein digestibility and nutritive value. Chavan and Kadam (1989a,b) reported that germination and fermentation enhance the nutritional value of sorghum and millets by causing significant changes in chemical composition and elimination of anti-nutritional factors.

The objective of the present investigation was to study changes occurring in crude protein and in vitro protein digestibility during fermentation of pearl millet.

2. Materials and methods

2.1. Materials

2.1.1. Seeds

Two pearl millet genotypes were obtained from the Department of Agronomy, Faculty of Agriculture, University of Khartoum, Sudan. They were selected, based on their protein quality [relative abundance of albumin plus globulin fractions: 31.1% for Madelkawya and 35.1% for Population 1/Shambat; Ali (2001)] and cultivated in Shambat Demonstration Farm to obtain

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sufficient material for analysis. The grains were harvested in the season of 1999.

2.1.2. Preparation of samples

The grains were cleaned from dust, foreign materials and broken seeds, then milled into fine powder and passed through 0.4-mm mesh.

2.2. Methods

2.2.1. Dough fermentation

Fermented dough was prepared according to the traditional domestic method, as described by El. Hidai (1978). Five hundred grams of pearl millet flour were mixed with 1 l of distilled water. One hundred and twenty-five grams of previously fermented dough, called Khumara, were added to the mixture to act as a starter. Fermentation was carried out at room temperature (30 + 2 °C). A portion of the fermented sample was sampled at 2 h intervals starting from zero until the end of fermentation (14 h). The pH of the dough was measured by means of a pH meter (Krich-Digital pH meter-type 644/1). The withdrawn samples were dried in an air oven for 24–30 h at 70 °C. Dried samples were ground and stored at 4 °C for analysis. All samples were analyzed for crude protein and in vitro protein digestibility.

2.2.2. Determination of moisture, ash, crude fibre and crude fat

The moisture, ash, crude fibre and crude fat were determined according to AOAC (1984).

2.2.3. Determination of crude protein

Crude protein was determined by the micro-Kjeldahl method of the AOAC (1975).

2.2.4. Determination of starch

Starch was determined by the method of dispersal in calcium chloride, followed by iodine spectrophotometry (Kerr, 1950).

2.2.5. Determination of in vitro protein digestibility

This was carried out according to the Saunder, Connor, Booth, Bickoff, and Kohler (1973) method. Of the sample, 0.2 g was placed into a 50-ml centrifuge tube; 15 ml of 0.1 N HCl containing 1.5 mg pepsin, were added and the tube was incubated at 37 °C for 3 h. The suspension was then neutralized with 3.3 ml of 0.5 M NaOH, then treated with 4 mg of pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH 8.0), containing 0.005 M soldium azide; the mixture was then gently shaken and incubated at 37 °C for 24 h. After incubation, the sample was treated with 10 ml of 10% trichloroacetic acid and centrifuged at 5000 $\times g$ for 20 min at room temperature. Nitrogen in the supernatant was estimated using the micro-Kjeldahl method.

Digestibility was calculated using the formula:

$$\text{Protein digestibility}\% = \frac{\text{Nitrogen in supernatant}}{\text{Nitrogen in the sample}} \times 100$$

2.2.6. Statistical analysis

Each determination consisted of three separate samples, which were analyzed and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1987) and by Duncan's multiple range test with probability $P \leqslant 0.05$ (Duncan, 1955).

3. Results and discussion

3.1. Proximate composition and starch content

The proximate composition and starch content of the two pearl millet genotypes are shown in Table 1. The dry matter was 92.5% for Madelkawya and 92.7% for Population 1/Shambat. The results are higher than the range of 88.3–90.9% reported by Abdalla, El Tinay, Mohamed, and Abdalla (1998) . Ash content was 2.1% for Madelkawya and 2.4% for Population 1/Shambat. These results are within the range of 1.5–3.9% reported by Burton, Wallace, and Rachie (1972). The crude fibre contents of Madelkawya and Population 1/Shambat cultivars were 2.8 and 3.2%, respectively. These values are within the range 2.6–4.0% reported by Abdalla et al. (1998). The fat content was 6.7% for Population 1/Shambat and 7.8% for Madelkawya. The results are higher than the range 4.1–6.4% reported by Saxena, Sharma, Sehgal, and Bakhshi (1992), but the fat content of Population 1/Shambat cultivar is within the range 3.4–7.4% reported by Hadimani, Ali, and Malleshi (1995) and 2.7–7.1% reported by Abdalla et al. (1998).

Table 1
Proximate composition and starch content of two pearl millet cultivars

Cultivar	Dry matter (%)	Ash (%)	Crude fibre (%)	Crude fat (%)	Crude protein (%)	Starch (%)
Madelkawya (Breeder seeds 1999)	92.5 (±0.12)a	2.1 (±0.14)	2.8 (±0.02)b	7.8 (±0.09)a	13.6 (±0.05)a	63.2 (±0.00)b
Population I/ Shambat (Breeder seeds 1999)	92.7 (±0.06)a	2.4 (±0.03)a	3.2 (±0.02)a	6.7 (±0.15)b	12.5 (±0.10)b	64.9 (±0.00)a

Values are means (±S.D.). Means not sharing a common letter in a column are significantly different at $P \leqslant 0.05$ as assessed by Duncan's multiple range test.

Crude proteins of the two pearl millet cultivars investigated were 12.5% for Population 1/Shambat and 13.6% for Madelkawya. Values obtained were within the range 8–19% reported by Sullivan, Douglass, Andrews, Bond, Hemcock, Bramel-cox et al. (1990). The starch content was found to be 63.2% for Madelkawya and 64.9% for Pupulation 1/Shambat. These results were within the range of 58.5–70.0% reported by Abdalla et al. (1998) for 10 pearl millet genotypes.

3.2. In vitro protein digestibility

Data presented in Table 2 show the mean value of the in vitro protein digestibilities (IVPD) for the two pearl millet cultivars. The IVPD was 68.1% for Madelkawya and 75.9% for Population 1/Shambat. These results are higher than the value of Khetarpaul and Chauhan (1991) who reported 51%; also the results are higher than those reported by Elyas (1999) who reported IVPD of 60.5 and 61.9% for the two pearl millet cultivars.

3.3. Changes in crude protein of pearl millet dough during fermentation

The protein contents of fermented pearl millet dough for Madelkawya and Population 1/Shambat cultivars are shown in Table 3. Fermentation was found to cause a marginal change in the protein content for the two cultivars. Fermentation did not alter the protein content of the two genotypes in the first 2 h. It increased at 4, 6 and 8 h, then it decreased at 10, 12 and 14 h for the Madelkawya cultivar. Fermentation decreased the protein content of Population 1/Shambat cultivar at 4 and 6 h, then it increased at 10 and 12 h but decreased at the end of fermentation. These results indicate that fermentation does not seem to be a viable method for raising the protein content in pearl millet. Khetarpaul and Chauhan (1989) and Abdalla et al. (1998) reported a marginal and insignificant change in the protein content of pearl millet flour during fermentation.

3.4. Changes in IVPD of pearl millet dough during fermentation

The IVPD of fermented pearl millet dough is shown in Table 3. The IVPD increased from 69.0 to 77.5%, after 14 h, for Madelkawya fermented dough and from 76.9 to 86.8%, after 14 h, for Population 1/Shambat fermented dough. The results indicate that fermentation causes a highly significant ($P \leqslant 0.05$) improvement in IVPD and an increase in protein availability for both pearl millet cultivars. Khetarpaul and Chauhan (1990)

Table 2
Protein digestibility of pearl millet cultivars

Cultivar	pH of dough	Protein content (%)	Protein digestibility (%)
Madelkawya (Breeder seeds 1999)	6.3 (±0.00)a	13.6 (±0.05)a	68.1 (±2.88)b
Population I/Shambat (Breeder seeds 1999)	6.3 (±0.00)a	12.5 (±0.10)b	75.9 (±0.00)a

Values are means (±S.D.). Means not sharing a common letter in a column are significantly different at $P \leqslant 0.05$ as assessed by Duncan's multiple range test.

Table 3
Protein content and digestibility of fermented pearl millet cultivars Madelkawya (1999) and Population 1/Shambat (1999)

Fermentation period (h)	pH	Protein content (%)	Protein digestibility (%)
Madelkawya			
0	5.5 (±0.00)a	13.6 (±0.05)bc	69.0 (±0.00)ef
2	5.4 (±0.00)b	13.6 (±0.05)bc	73.7 (±0.00)abcd
4	5.3 (±0.00)c	14.0 (±0.00)a	73.2 (±2.75)bcde
6	5.0 (±0.00)d	14.0 (±0.10)a	72.6 (±2.73)bcdef
8	4.8 (±0.00)e	14.0 (±0.06)a	74.6 (±2.75)abc
10	4.5 (±0.00)f	13.7 (±0.06)b	75.7 (±2.79)ab
12	4.3 (±0.00)g	13.7 (±0.06)b	73.2 (±0.00)bcde
14	4.2 (±0.00)h	13.5 (±0.05)c	77.5 (±2.86)a
Population 1/Shambat			
0	5.5 (±0.00)a	12.4 (±0.05)c	76.9 (±3.24)ef
2	5.4 (±0.00)b	12.4 (±0.05)c	78.8 (±3.10)def
4	5.2 (±0.00)c	12.1 (±0.05)de	82.4 (±0.00)abcde
6	5.0 (±0.00)d	12.00 (±0.00)e	85.6 (±3.16)abc
8	4.7 (±0.00)e	12.4 (±0.09)c	84.3 (±3.11)abcd
10	4.5 (±0.00)f	12.5 (±0.05)b	87.7 (±3.10)a
12	4.3 (±0.00)g	12.7 (±0.05)a	85.6 (±3.02)abc
14	4.2 (±0.00)h	12.2 (±0.05)d	86.8 (±3.07)ab

Values are means (±S.D.). Means not sharing a common letter in a column are significantly different at $P \leqslant 0.05$ as assessed by Duncan's multiple range

observed an improved IVPD of pearl millet when subjected to germination and even better when further fermented.

4. Conclusions

Fermentation caused marginal changes in the protein contents of the two pearl millet cultivars. The cultivar Population 1/Shambat being richer in the albumin plus globulin fraction, and hence of superior protein quality compared to cultivar Madelkawya, also had the higher protein digestibility, which improved markedly due to fermentation. Fermentation could be utilized to improve the IVPD and hence protein quality and protein availability of pearl millet.

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