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Effect of fermentation on protein fractions and in vitro protein digestibility of maize

Nabila E. Yousif *, Abdullahi H. El Tinay

Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Shambat, Sudan

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Abstract

Changes in pH, titratable acidity, total soluble solids and protein of maize during natural fermentation at 37°C up to 36 h were monitored. The pH of the fermenting material decreased sharply with concomitant increase in the titratable acidity. Total soluble solids increased with progressive fermentation time. The crude protein and non-protein nitrogen increased during the first stages of fermentation. The in vitro protein digestibility markedly increased as a result of fermentation. The globulin and albumin fraction increased significantly ($P \leqslant 0.05$) during the first 16 h of fermentation. The zein fraction was the major protein fraction, which decreased during the first 16 h of fermentation but increased sharply with progressive fermentation. G_1-glutelin increased during the first 16 h but fluctuated thereafter. The G_2-glutelin, which is the minor fraction and G_3-glutelin the second most abundant fraction, together with insoluble protein, fluctuated during the fermentation process. The results indicated that natural fermentation of corn significantly improves in vitro protein digestibility.

1. Introduction

After wheat and rice, maize ranks as the most important cereal grain in the world, and provides over one-half of the total calories and the total protein for peoples in the developing countries (Hanson, 1974). Maize is the principal source of food for millions of people, particularly in Latin America and Africa. It is an excellent source of carbohydrate, but its protein quality is relatively poor because it is deficient in the essential amino acids lysine and tryptophan (Paulis, 1982).

Maize grain proteins can be separated into six fractions according to Landry and Moureaux (1970), namely albumin, globulin, zein, G_1-glutelin, G_2-glutelin and G_3-glutelin. The zein fraction was considered nutritionally undesirable because it was shown to be low in lysine content and lacking in tryptophan. The albumin, globulin and glutelin fractions on the other hand have relatively higher levels of lysine and tryptophan. Another important feature of the zein fraction is its higher content of leucine, an amino acid implicated in isoleucine deficiency (Patterson, Brown, Linkswiter & Harper, 1980).

Maize can be fortified with lysine and tryptophan but enrichment raises the price and puts this product out of the reach of the poor (May-Gi Lay & Fields, 1981). Fermentation is an inexpensive and simple method for improving the nutritive value of maize. Cameron and Hofvander (1971) stated that it is usual to ferment maize in African countries. Wang and Fields (1978) reported that germination and fermentation are feasible methods to improve the protein quality of maize. Hamad and Fields (1979) also reported a significant increase in available lysine in fermented maize.

The objective of the present investigation was to study changes occurring in proteins of maize using the Landry and Moureaux technique (1970) and the in vitro protein digestibility during natural fermentation of maize.

2. Materials and methods

2.1. Materials

Grain maize was purchased from Khartoum North local market. The sample was carefully cleaned and freed from foreign materials and the grains were ground using a pestle and mortar. Natural maize fermentation was carried out by mixing corn flour with distilled water (1:2 w/v). This mixture was incubated at 37°C for periods of 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 h. Samples were withdrawn and transferred to aluminium dishes and dried in a hot air oven-drier at 70°C for 3 – 4 h. Dried samples were ground and stored at 4°C for analysis. All samples were analyzed for titratable acidity,

* Corresponding author.

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total soluble solids, crude protein, non-protein nitrogen, in vitro protein digestibility and protein solubility.

2.2. Determination of pH and titratable acidity

The pH of the fermented dough was monitored initially and every 4 h using a glass electrode pH meter. Titratable acidity, expressed as lactic acid, was determined according to the established AOAC method (Association of Official Analytical Chemists [AOAC], 1975).

2.3. Determination of crude protein and non-protein nitrogen

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

Non-protein nitrogen was determined according to Gheysuddin (1970).

2.4. Determination of in vitro protein digestibility

This was carried out according to Saunders, Connor, Booth, Bickoff and Kohler (1970), 0.2 g of the sample was placed in a 50 ml centrifuge tube, 15 ml of 0.1 N HC1 containing 1.5 mg pepsin, were added and the tube was incubated at 37°C for 3 h. The suspension was then neutralized with 0.5 N NaOH (ca. 3.3 ml), then treated with 4 mg of pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH = 8.0), containing 0.005 M sodium azide; the mixture was then gently shaken and incubated at 37°C for 24 h. After incubation the sample was treated with 10 ml, 10% trichloroacetic acid, and centrifuged at 50,000 *g* for 20 min at room temperature. Nitrogen in the supernatant was estimated using the Kjeldahl method. Digestibility was calculated using the formula:

$$\text{protein digestibility}\% = \frac{\text{nitrogen in supernatant}}{\text{nitrogen in sample}} \times 100$$

2.5. Determination of total soluble solids

Total soluble solids were determined at 20°C using an Abbe refractometer according to the method of Joslyn (1970).

2.6. Protein fractionation

The nitrogen from the defatted meal was extracted stepwise by a series of solvents according to the Landry and Moureaux (1970) procedure. Thus, triplicate 3.5 g samples were kept in suspension with 35 ml of extractant by magnetic stirring in 50 ml centrifuge tubes.

2.6.1. Step 1

To obtain the first fraction, 0.5 M NaC1 was added to the sample powder and the mixture was stirred three times, 60, 30 and 30 min at 4°C.

2.6.2. Step 2

The residue was extracted with the same volume of distilled water twice for 15 mm at 4°C.

2.6.3. Step 3

To obtain the third fraction, the residual material was stirred with 60% ethanol twice for 30 min at 20°C and then at 60°C for 30 min, followed by extraction with 55% isopropanol (Pr-OH) at 20°C three times (60, 30 and 15 min with stirring).

2.6.4. Step 4

To obtain the fourth fraction, the residue was extracted with 60% ethanol plus 0.6% 2-mercaptoethanol (2-ME) and stirred twice for 30 min (20°C), then extracted with 55% Pr-OH containing 2-ME (0.6%) at 20°C twice for 30 min.

2.6.5. Step 5

To obtain the fifth fraction, borate buffer, pH 10 (0.0 125 M $Na_2B_4O_7$. $12H_20$ and 0.02 M NaOH) with 0.6% 2-ME and 0.5 M NaC1 was used with stirring for 60, 30 and 30 min (20°C).

2.6.6. Step 6

To obtain the sixth fraction, borate buffer, pH 10 with 0.6% 2-ME and 0.5% sodium dodecyl sulphate (SDS) was used with stirring for 60, 30 and 15 min (20°C). Fractions I and II contained the albumin and globulin, the free amino acids and small peptide fragments. Fraction III contained the prolamin zein. Fraction IV contained the zein-like protein (G_1-glutelin). Fraction V contained the glutelin like (G_2-glutelin) protein. Fraction VI contained the true glutelin (G_3-glutelin). The solid material was isolated from extractants by centrifugation at 30,000 *g* for 15 min. For each solvent. supernatants were combined to give the total extract. The nitrogen content of each of these six fractions was determined by the micro-Kjeldahl method. The residue left after extraction was also analyzed for nitrogen content.

2.7. Statistical analysis

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

3. Results and discussion

Changes in pH, titratable acidity (TA), total soluble solids (TSS), crude protein and non-protein nitrogen (NPN) are shown in Table 1. The pH of the fermented dough dropped from 4.5 to 3.7 during the first 20 h. The TA increased from 29.3 to 65 mg/100 g expressed as lactic acid. According to Ramirez (1988) the traditional pozol fermentation, a Mexican fermented maize food, is mainly done by lactic acid bacteria and nitrogen-fixing bacteria followed by *Candida* sp., *Goetrichum candidum* and *Monilia sitophila* in the latter stages. This could explain the apparent increase in lactic acid towards the end of fermentation accompanied by lack of changes in pH. The TSS of the fermented dough ranged from 1.5 to 4.1%. El Tinay, El Mahdi and El Soubki (1985) reported a TSS increase from 5.1 to 8.2% during sorghum fermentation. Padhye and Salunkhe (1979), who studied the fermentation of idli prepared from rice and black gram, also reported an increase of TSS. The protein content of the fermented dough ranged from 8.5 to 11.2%. It increased during the first period of fermentation, reaching its maximum at 12 h; however, after 12 h the protein content started to decrease. Azoulay (1978) reported 15–30% increase in protein content as a result of maize fermentation, with *Candida tropicalis*. Non-protein nitrogen increased significantly ($P \leqslant 0.05$) during the first stages of fermentation and then remained constant during the latter stages of the fermentation period. Increase in NPN was reported by El Tinay, Abdel Gadir and El Hidai (1979).

Table 1
Changes in pH, titratable acidity, total soluble solids, crude protein and non-protein nitrogen during the natural fermentation of maize[ab]

Fermentation period (h)	pH	TA (mg/100 g)	TSS (%)	Protein (%)	Non-protein nitrogen (%)
0	4.5 (0.00)a	29.3 (1.53)g	1.5 (0.00)g	9.5 (0.12)d	0.13 (0.00)c
4	4.3 (0.00)b	33.7 (1.15)f	2.6 (0.00)f	9.5 (0.00)d	0.13 (0.00)c
8	4.3 (0.00)b	35.0 (0.00)e	3.1 (0.00)d	10.3 (0.00)b	0.14 (0.01)b
12	4.1 (0.06)c	38.0 (0.00)d	3.1 (0.00)d	11.2 (0.10)a	0.15 (0.00)a
16	3.9 (0.06)d	50.0 (0.00)c	3.5 (0.00)c	9.7 (0.38)c	0.15 (0.00)a
20	3.7 (0.06)f	59.3 (0.58)b	3.6 (0.00)b	9.8 (0.10)c	0.15 (0.00)a
24	3.7 (0.00)f	60.0 (0.00)b	3.6 (0.00)b	9.3 (0.31)ef	0.15 (0.01)a
28	3.7 (0.00)f	65.0 (0.00)a	3.6 (0.00)b	9.2 (0.10)f	0.15 (0.00)a
32	3.7 (0.06)f	65.0 (0.00)a	4.1 (0.00)a	9.4 (0.83)de	0.14 (0.00)b
36	3.8 (0.00)e	65.0 (O.00)a	3.0 (0.00)e	8.5 (0.00)g	0.15 (0.00)a

[a] Values are means ± S.D.
[b] Means not sharing a common letter in a column are significantly different at $P \leqslant 0.05$.

3.1. Changes in protein fraction as a result of fermentation

Table 2 shows variation in protein fractions during natural fermentation of corn. The globulin plus albumin fraction increased significantly during the first stages of

Table 2
Changes in protein fractions and in vitro protein digestibility during natural fermentation of maize[a,b]

Fermentation period (h)	pH	I + II Globulin + albumin (%)	III Zein (%)	IV G_1-glutelin (%)	V G_2-glutelin (%)	VI G_3-glutelin (%)	Insoluble protein (%)	Total protein recovered (%)	IVPD (%)
0	4.5 (0.00)a	18.5 (0.23)g	29.1 (0.67)cd	21.4 (0.35)bc	6.6 (3.50)abcd	21.1 (3.70)abc	2.2 (0.20)cd	98.9	74.9 (0.00)i
4	4.3 (0.00)b	19.9 (1.85)e	28.7 (1.20)cde	23.9 (6.35)abc	3.0 (0.60)f	23.9 (1.20)ab	1.8 (0.00)cd	101.2	79.0 (0.00)h
8	4.3 (0.00)b	21.1 (0.00)c	24.5 (0.00)gh	23.7 (0.75)abc	4.5 (0.00)bcdef	23.0 (0.55)abc	1.5 (0.00)d	98.3	80.9 (0.00)f
12	4.1 (0.06)c	20.3 (0.00)d	26.7 (1.90)efgh	21.4 (1.35)bc	5.7 (0.82)abcdef	21.9 (0.00)abcd	2.1 (0.38)cd	98.1	83.3 (0.00)e
16	3.9 (0.06)d	22.6 (1.20)a	20.5 (0.00)i	27.4 (0.00)a	7.5 (3.84)a	15.3 (3.02)f	4.1 (0.00)ab	97.4	84.1 (0.10)d
20	3.7 (0.06)f	22.0 (0.00)b	29.5 (2.40)bc	21.5 (1.97)bc	7.4 (1.84)ab	20.0 (0.35)cde	1.7 (0.32)cd	102.1	85.0 (0.00)c
24	3.7 (0.00)f	17.6 (0.00)h	28.5 (0.69)cdef	23.9 (1.22)abc	6.9 (1.67)abc	15.0 (1.87)f	4.5 (1.25)a	96.9	93.5 (0.50)a
28	3.7 (0.06)f	17.4 (0.42)h	31.0 (0.00)b	25.4 (0.98)ab	7.3 (0.97)ab	16.2 (0.65)ef	3.4 (0.38)b	100.7	88.1 (0.15)b
32	3.7 (0.06)f	19.1 (0.00)f	27.6 (0.64)cdefg	20.8 (1.22)c	6.2 (0.00)abcde	24.4 (0.35)e	2.4 (0.15)c	100.5	79.1 (0.10)g
36	3.8 (0.00)e	20.3 (0.40)d	34.3 (1.35)a	24.6 (0.90)abc	3.9 (0.76)def	17.8 (0.00)ef	1.5 (0.10)d	102.4	64.8 (0.10)j

[a] Values are means ± (S.D.).
[b] Means not sharing a common letter in a column are significantly different at $P \leqslant 0.05$.

fermentation reaching its maximum at 16 h, but it started to decrease after 20 h. The albumin plus globulin fraction is characterized by higher levels of lysine according to Patterson et al. (1980). Umoh and Fields (1981) reported an increase in tryptophan and lysine content in Agidi, a Nigerian fermented maize food. Hamad and Fields (1979) also reported a significant increase in available lysine in fermented maize. Thus the nutritional value of maize would be expected to increase as a result of fermentation. The zein fraction decreased significantly ($P \leqslant 0.05$) during the initial 16 h but it increased thereafter. The G_1-glutelin increased during the first stages, reaching its maximum at 16 h, and then fluctuated. The G_2-glutelin, G_3-glutelin fraction and insoluble protein fluctuated during fermentation. Abdel Moneium, El Tinay and Abdalla (1996) fractionated germinated maize proteins and found that the albumin plus globulin fraction increased significantly during germination, accompanied by a decrease in the prolamin zein fraction. This would seem to agree with our results and with earlier results of Wang and Fields (1978), who stated that both germination and fermentation are feasible methods to improve the protein quality of maize.

3.2. Changes in IVPD of corn dough during fermentation

The IVPD of naturally fermented corn dough is shown in Table 2. The IVPD increased from 74.9 for the control to 93.5 for 24 h dough. It gradually decreased after 24 h. The results indicate that fermentation of maize improves protein digestibility. Dirar (1992). reported that the fermentation process, that African women employ, increases maize protein digestibility by more than 86%.

4. Conclusions

Results showed that natural fermentation of maize increased total soluble solids and non-protein nitrogen and slightly increased protein content. Sixteen hour-fermented dough had higher levels of the albumin plus globulin fraction, indicating that natural fermentation of maize results in improvement in the nutritional value of the grain. Also, maize protein digestibility is elevated.

References

Abdel Moneium, Z. S., El Tinay, A. H., & Abdalla, A. H. (1996). Effect of germination on protein fractions of corn cultivars. *Food Chemistry, 57*(3), 381–384.

Association of Official Analytical Chemists (1975). *Official methods of analysis* (12th ed.). Washington DC: Association of Official Agricultural Chemists.

Azoulay, E. E. Y. (1978). Protein enrichment of maize, cassava and other starch products by direct fermentation. United-States-Patent. Patent, USA; Protein-Products.

Cameron, M., & Hofvander, Y. (1971). *Manual of feeding infants and young children*. New York: Protein Advisory Group of the UN (p. 73).

Dirar, H. A. (1992). Traditional fermentation technologies and food policy in Africa. *Appropriate Technology, 19*(3), 21–23.

Duncan, D. B. (1955). Multiple range and multiple F- test. *Biometrics, 11*, 1–42.

El Tinay, A. H., Abdel Gadir, A. M., & El Hidai, M. (1979). Sorghum fermented kisra bread. 1. Nutritive value of kisra. *Journal of Science of Food and Agriculture, 30*, 859–863.

El Tinay, A. H., El Mahdi, Z. M., & El Soubki, A. (1985). Supplementation of fermented sorghum kisra bread with legume protein. *Journal of Food Technology, 20*, 679–687.

Hamad, A. M., & Fields, M. L. (1979). Evaluation of the protein quality and available lysine of fermented cereals. *Journal of Food Science, 44*, 456–459.

Hanson, H. (1974). Role of maize in world food needs to 1980. In *Symposium Proceedings: Worldwide Maize Improvement in the 70s and Role for CIMMYT*, pp. 1–10.

Joslyn, A. M. (1970). *Methods in food analysis* (2nd ed.). New York: Academic Press.

Landry, J., & Moureaux, T. (1970). Heterogeneity of the glutelins of the grain corn: selective extraction and composition in amino acids of the three isolated fractions. *Bull. Soc. Chem. Biol., 52*, 1021–1037.

May-Gi Lay, M., & Fields, M. L. (1981). Nutritive value of germinated corn and corn fermented after germination. *Journal of Food Science, 46*, 1069–1073.

Padhye, V. W., & Salunkhe, D. K. (1979). Biochemical studies on black gram (*Phaseolus mungo* L.) 111. Fermentation of black gram and rice blend and its influence on the in vitro protein digestibilities of proteins. *Journal of Food Biochemistry, 2*(4), 327–347.

Patterson, J. I., Brown, R. R., Linkswiter, H., & Harper, A. E. (1980). Extraction of tryptophan- niacin metabolites by young men: effect of tryptophan, leucine and vitamin B_6 intakes. *American Journal of Clinical Nutrition, 33*, 2157–2167.

Paulis, J. W. (1982). Recent development in corn protein research. *Journal of Agricultural and Food Chemistry, 30*, 14–20.

Ramirez, J. F. (1988). Biochemical studies on a Mexican fermented corn food — pozol. *Dissertation Abstracts-International, B, 48*(9), 2511.

Saunders, R. M., Connor, M. A., Booth, A. N., Bickoff, E. N., & Kohier, C. O. (1973). Measurement of digestibility of alfalfa protein concentrate by in vitro and in vivo methods. *Journal of Nutrition, 103*, 530–535.

Gheysuddin, S. (1970). *Studies of sunflower seeds proteins and polyphenols*. Ph. D. thesis. Texas A & M University.

Snedecor, G. W., & Cochran, W. G. (1987). *Statistical methods* (17th ed.). Ames IA: The Iowa State University Press (pp. 221–222).

Umoh, V., & Fields, M. L. (1981). Fermentation of corn for Nigerian Agidi. *Journal of Food Science, 46*, 903–905.

Wang, Y. D., & Fields, M. L. (1978). Germination of corn and sorghum in the home to improve nutritive value. *Journal of Food Science, 44*, 456–459.