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Effect of processing (sprouting and/or fermentation) on sorghum and maize: II. Vitamins and amino acid composition. Biological utilization of maize protein

Matilda Asiedu,[a] Einar Lied,[b] Rune Nilsen[a] & Kjarten Sandnes[b]

[a] *Centre for International Health, University of Bergen, Amauer Hansen Building, N–5021 Bergen, Norway*
[b] *Institute of Nutrition, Directorate of Fisheries, PO Box 1900, Nordnes, N–5024 Bergen, Norway*

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The use of sprouted and or fermented maize and sorghum in improving weaning food nutrient quality was investigated. The cereals were studied for their thiamin, niacin, pyridoxine and amino acid composition and for their protein quality by nitrogen retention in young rats and by protein synthesis *in vitro*. Germination (sprouting) improved the vitamin content whereas fermentation had no substantial effect. The amino acids were slightly improved, but not enough to meet the nutritional needs of infants. Germination and/or fermentation neither improved nor had any detrimental effect on the overall protein quality. In-vitro protein synthesis was not affected by the processing methods.

INTRODUCTION

Weaning foods in developing countries are based on local staples such as cassava, maize, sorghum and millet. These are rich in carbohydrates, mainly starch. Gruels with adequate feeding consistency made from these staples are low in nutrients due to the water volume in relation to total solids. This is a major limitation factor in infant feeding. Sprouting of cereals has been used to reduce the viscosity of cereal based gruels. Studies have been mainly about its effect on dietary bulk (Mosha & Svanberg 1983; Gopaldas *et al.*, 1986). When the viscosity is reduced, total nutrient density is increased. This is because the total solids figure per unit volume is increased at a given consistency (viscosity). The gruel is eaten as the sole source of all nutrients. An appropriate weaning diet should be dense in energy and provide adequate amounts of protein of good quality. It should contain adequate amounts of vitamins and minerals, and the consistency and acceptability should be good. Further, it should be easy to prepare and relatively cheap.

So long as no other foods are eaten, it will be very difficult to meet the protein and other nutrient requirements if only the cereal based gruels are fed to infants. It is, therefore, important that the total nutrient composition is studied. In certain areas, fermentation of the cereals has been employed to achieve the same goal as germination. This paper evaluates some water-soluble vitamins and the amino acid profiles of sprouted and/or fermented sorghum and maize. The protein quality of the unprocessed and processed maize was studied with young rats.

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MATERIALS AND METHODS

The collection of samples and their preparation prior to analysis has been described (Asiedu *et al.*, 1992). Moisture and protein were determined as outlined by the AOAC (1990) and by Crooke & Simpson (1971), respectively.

Microbiological methods

All the vitamins were determined microbiologically. Thiamin was determined, using *Lactobacillus viridescens* (12706 ATCC) after Diebel *et al.* (1957). The vitamin was extracted with 0·05 M H_2SO_4 for 30 min in flowing steam in an autoclave. The extract was incubated with 0·15 M sodium acetate buffer overnight at 37°C. The turbidity of the solutions was measured at 660 nm by a spectrophotometer calibrated against the test organism.

A standard curve was plotted and from this the concentrations in the samples were determined.

The estimation of pyridoxine was carried out by a method described by Atkin *et al.* (1943), using *Saccharomyces carlsbergensis* (9080 ATCC) as the test organism. The analyses were carried out in the dark as pyridoxine is light-sensitive. The vitamin was extracted by autoclaving at 120°C for 4 h with 1 M H_2SO_4. The vitamin content was estimated as for thiamin.

Niacin was determined, using *Lactobacillus plantarum* (8014 ATCC), according to AOAC methods (1980). The vitamin was extracted with 1 M H_2SO_4 for 30 min at 116–120°C and the pH was adjusted to 6·8 with NaOH. This was inoculated with the test organism and incubated at 37°C for 22 h. Turbidity was measured at 575 nm. Concentration was estimated using a standard curve.

Chemical analyses

After hydrolysing the sample with 6 M HCl, the amino acid were separated and analysed with a Waters HPLC system (Millford, MA, USA) (a modification of the Pico-Tag method (Millipore (1987)). Norleucine was used as an internal standard. Tryptophan was determined according to Sachse (1981) and AOAC (1990).

Preparation of porridge

Four porridges were made from the processed maize cereals and freeze-dried.

1. MUG (maize as purchased): 1 part flour to 6 parts water.
2. MUGFD (maize ungerminated fermented dough): 1 part dough to 4 parts water.
3. MG (maize germinated): 1 part flour to 3 parts water.
4. MGFD (maize germinated fermented dough): 1 part dough to 2 parts water.

The porridges containing 0·5% salt (NaCl) were cooked for 15 min.

Table 1. Thiamin, niacin and pyridoxine content in unprocessed and processed maize and sorghum. Values are expressed as mean ± SEM for six determinations

Sample	Thiamin (mg/kg)	Niacin (mg/kg)	Pyridoxine (mg/kg)
Maize			
Mug	2·5 ± 0·20	21·0 ± 0·57	4·1 ± 0·04
MUDFG	2·1 ± 0·08	20·5 ± 0·86	3·8 ± 0·08
MG	3·0 ± 0·04*	34·5 ± 0·29	5·1 ± 0·08
MGFD	3·3 ± 0·08*	34·0 ± 1·14	5·4 ± 0·41
Sorghum			
SUG	7·6 ± 0·08	27·0 ± 0·57	1·7 ± 0·04
SUGFD	7·6 ± 0·53	25·5 ± 0·29	1·7 ± 0·04
SG	9·2 ± 0·37	36·5 ± 1·76	2·1 ± 0·12
SGFD	8·8 ± 0·53	36·0 ± 1·20	2·3 ± 0·08

MUG = maize as purchased.
MUGFD = maize ungerminated fermented dough.
MG = maize germinated.
MGFD = maize germinated fermented dough.
SUG = sorghum as purchased.
SUGFD = sorghum ungerminated fermented dough.
SG = sorghum germinated.
SGFD = sorghum germinated fermented dough.
* $P < 0·05$.

Biological methods

Biological experiments were carried out using Wistar-Møll rats from Møllegard, Denmark, weighing between 60 and 65 g. They were kept in metabolic cages in a room with a temperature of 20 ± 1 °C and maintained on a 12 h cycle of light and dark. Water was available *ad libitum*. There were 5 days acclimatization and a preliminary period and 5 days experimental period. The animals were fed ground freeze-dried porridge (10 g per day). Faeces and urine were collected and nitrogen was determined in them as well as in the diets. $NPU_{operative}$,† balance (%), NDpCal (%)‡ and digestibilty were calculated. In-vitro protein synthesis, using ribosomal extracts from gastrocnemius muscle of the rats, was carried out essentially as described by Omstedt & von der Decken (1972). RNA and DNA were extracted and hydrolysed with perchloric acid at 60 and 90°C, respectively. RNA was determined spectrophotometrically at 260 nm and DNA fluorimetrically at 420 nm (excitation) and 520 nm (emission).

Student's t-tests were used to compare processed samples with the unprocessed ones.

RESULTS AND DISCUSSION

The vitamin compositions of the cereals were within the range of ungerminated whole grains (Brandtzaeg *et al.*, 1981; Passmore & Eastwood 1986; FAO, 1989). The highest content of pyridoxine was observed in maize, whereas sorghum had the highest content of both thiamin and niacin (Table 1). In both cereals, fermentation resulted in either a small decrease or no change in all vitamins. The lactic acid bacteria might have used the vitamins for growth during the fermentation process. The reduction in vitamins during fermentation was previously reported in fermented Ogi (maize porridge) (Akinerle & Bassir, 1967). Sprouting of the cereals led to a considerable increase in the vitamins (Table 1) (Aliya & Geervani, 1981; Brandtzaeg *et al.*, 1981). The recommended dietary intakes for the age groups 0·5–12 months are: 0·3–0·5 mg thiamin; 5·4–9·0 mg niacin; 0·3–0·6 mg pyridoxine (Passmore & Eastwood, 1986). A child in this age group should at least consume porridge with 150 to 250 g total solids of maize gruel to meet the day's requirement of thiamin; this will also meet the needs of pyridoxine. Food is

† NPU = Net Protein Utilization = The proportion of nitrogen that is retained. If a diet is fed as it is consumed then it is termed $NPU_{operative}$.
‡ NDpCal (%) (Net dietary protein calories per cent) = An (estimate of the utilizable protein content of a diet in terms of calories expressed as a percentage of the total metabolizable energy.

Table 2. Amino acid composition (mg/g protein) of maize and sorghum

Amino acid	Sample[a]							
	MUG	MUGFD	MG	MGFD	SUG	SUGFD	SG	SGFD
Asp	61	63	71	72	68	66	82	76
Glu	181	181	199	168	202	198	201	180
Hyp	3	3	3	3	1	1	2	2
Ser	45	46	51	47	44	45	46	44
Gly	33	36	38	36	27	28	28	27
His	23	24	27	24	17	17	17	15
Arg	45	44	49	31	37	38	37	28
Thr	31	40	42	52	31	32	35	37
Ala	67	70	74	68	86	87	81	76
Pro	93	96	104	93	82	80	79	73
Tyr	40	46	48	46	35	40	43	35
Val	44	47	52	50	43	46	47	44
Met	21	23	23	21	15	16	16	15
Ile	31	33	35	35	34	36	38	36
Leu	118	122	128	113	124	126	122	113
Phe	44	46	50	47	45	47	47	44
Lys	23	25	28	21	16	20	25	24
Trp	5	5	8	6	8	9	7	8
Total AA	908	950	1029	933	915	932	953	877
Sum EAA	340	365	392	369	333	349	354	336
E/T_N	2·13	2·28	2·45	2·31	2·08	2·18	2·21	2·10

E/T_N Infants = 2·33

E/T_N = The ratio of essential amino acids to total nitrogen.

[a] See Table 1 for abbreviations.

eaten, first, to meet energy needs, since all bodily metabolic processes require energy and are thus sensitive to energy deprivation. Considering the energy needs of infants in the weaning age group (6–12 months) the average energy requirement is 430 kJ per kg body weight (FAO, 1985). This gives lower and upper limits of energy as 3139 kJ (7·3 kg body weight) and 3870 kJ (9·0 kg body weight) per day, respectively. To obtain this amount of energy one has to consume porridge containing between 172·5 and 212·0 g total solids per day. Within these limits thiamin and pyridoxine requirements could be met, but not that of niacin. Nevertheless, the total solids will have to go up in order to meet the niacin requirement. A child who cannot eat a great volume will need a concentrated diet. Fish which is rich in niacin will be a good supplement.

The amino acid pattern of the cereals is summarized in Table 2. Fermentation increased the lysine value by 25% in sorghum (SUGFD/SUG) and 9% in maize (MUGFD/MUG). A similar increase in fermented maize and millet has been observed (Hamad & Fields, 1979). Germination also increased lysine by 56% in sorghum (SG/SUG) and 21% in maize (MG/MUG). The increase in lysine by germination was in agreement with other workers (Tsai *et al.*, 1975; Dalby *et al.*, 1976; Wu & Wall, 1980). Lysine is a limiting amino acid in both cereals. Tryptophan, however, is the first limiting amino acid in maize. Values between 3 and 4 mg/g have been reported (Steiner-Asiedu, 1989). The combined effect of germination and fermentation resulted in a decrease in arginine contents in both cereals.

Table 3. Dry matter and protein content of the four porridges (± difference between replicates)

Sample[a]	Dry matter (g/kg)	Protein (g/kg dry wt)
MUG	151 ± 0·3	87 ± 2·0
MUGF	167 ± 0·7	88 ± 1·0
MG	222 ± 0·3	89 ± 2·0
MGF	192 ± 1·4	90 ± 3·0

[a]See Table 1 for abbreviations.

Generally, the essential amino acids increased through fermentation and germination. This is reflected in the E/T_N ratio (Table 2). The highest ratio was observed in sprouted grains. Little or no variation existed in the amino acid profiles of the two cereals. Comparing the E/T_N ratios to that of infants (FAO, 1973), all samples are within the reference pattern with very little differences between and within cereals (Table 2). The striking feature, however, is that lysine in both cereals is far below that recommended for infants, and the differences seen between the cereals are very small. Tryptophan in maize is also very much lower than the recommended amount, but increases upon germination. This calls for the incorporation of other foods high in lysine and tryptophan.

The dry matter and protein content of the porridges are presented in Table 3. The protein contents (N × 6·25) were similar. The dry matter content increased through germination. It is very difficult to comment on the dry matter contents since viscosity was not measured. The higher dry matter content would provide a porridge of suitable consistency for spoon feeding. Spoon feeding should be preferred to bottle feeding as it will be easier to keep the spoon and cup clean under the poor hygienic conditions in developing countries. The moisture contents did not have any effect on the biological experiments since all samples were freeze-dried before use.

The E/T^N ratio of maize is well refected in the biological utilization of the proteins. As shown in Table 4, there was relatively no effect of processing on NPU and NDpCal (%), but a considerable increase in digestibility was observed through germination and fermentation. Digestibility values between 70 and 80% have been achieved through germination and fermentation (Svanberg *et al.*, 1992).

Table 4. Effect of processing on the biological utilization of maize protein

Sample[a]	$NPU_{operative}$	BAL (%)	NDPCal (%)	AD
MUG	61·2 ± 2·8	42·7 ± 2·8	5·3 ± 0·2	79·7 ± 1·4
MUGFD	59·2 ± 6·5	39·7 ± 4·1	4·9 ± 0·5	80·9 ± 2·8
MG	58·9 ± 3·1	41·0 ± 3·0	5·2 ± 0·3	82·7 ± 2·2
MGFD	59·8 ± 4·3	41·0 ± 4·3	5·3 ± 0·4	84·8 ± 2·4

[a] See Table 1 for abbreviations.

The values represent the mean (± SD) for six animals in each experimental group.

AD = apparent digestibility.

Table 5. Protein synthesis in rats fed with different types of maize porridge for 10 days. Values are reported as mean (± SD)

Sample[a]	Protein synthesis pmole 1-^{14}C-phenylalanine incorporated/min/g wet wt
MUG	17·5 ± 6·42
MUDFG	19·0 ± 4·72
MG	15·7 ± 2·43
MGFD	17·2 ± 3·68

[a] See Table 1 for abbreviations.

Table 6. Effect of processing on the contents of ribosomal RNA and DNA and the ratio of RNA/DNA in gastrocnemius muscle of rats. Value expressed as mean (± SD) for six animals in each group

Sample[a]	RNA (mg/g wet wt)	DNA (mg/g wet wt)	RNA/DNA
MUG	0·63 ± 0·08	0·64 ± 0·13	1·01 ± 0·18
MUGFD	0·64 ± 0·03	0·63 ± 0·14	1·05 ± 0·20
MG	0·62 ± 0·10	0·70 ± 0·08	0·88 ± 0·13
MGFD	0·63 ± 0·06	0·66 ± 0·12	1·02 ± 0·14

[a] See Table 1 for abbreviations.

The processing methods, germination and/or fermentation did not have any significant effects on in-vitro muscle protein synthesis. The contents of RNA and DNA in the muscle tissues of rats were not affected. The ratio of RNA/DNA remained unchanged (Tables 5 and 6).

The results obtained provide an insight into the chemical, biological and biochemical effects of sprouting and/or fermentation on protein quality. The similarities observed between the cereals' amino acid profiles parallels the results on the balance experiments as well as the protein synthetic activity of the ribosomes.

Sprouting has the potential to reduce viscosity (Mosha & Svanberg, 1983; Gopadals *et al.*, 1986) and fermentation to reduce gastroenteritis (Tomkins, 1983; Mensah *et al.*, 1988; Svanberg & Lorri, 1992), but cannot improve the protein quality of the cereals enough to meet the needs of infants. There is, therefore, the need to include protein-rich foods to complement such diets when used in infant feeding.

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