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The effects of processing on the availability of lysine in kenkey, a Ghanaian fermented maize food

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The effects of processing steps such as soaking, fermentation, cooking and drying on the availability of lysine in kenkey were investigated. Soaking increased lysine availability by 21% and 22% for maize and maize-cowpea mixtures, respectively. Cooking of soaked samples further improved lysine availability by 68% and 31% for maize and maize-cowpea mixtures, respectively. Further significant improvements in lysine availability were effected by fermentation and cooking and values of 3.42 and 4.43 g/16 g N were recorded, respectively for maize and maize-cowpea doughs fermented for 4 days and cooked for 3 h. Cabinet drying had no significant effect on lysine availability, but drum drying of fermented maize and maize-cowpea doughs significantly lowered lysine availability in the resulting kenkey. A 1:1 mixture of cabinet and drum dried flours gave a product with higher available lysine content than the drum dried flour.

Introduction

Lysine is well established as one of the most important amino acids in animal and human nutrition. It has, however, been reported that in certain circumstances not all the lysine present in a protein is nutritionally available to the animal or human consumer (Björck *et al.*, 1983). This is being attributed to the interaction of the ϵ-amino groups of lysine in heat-processed foods with other food constituents such as sugars, to become nutritionally unavailable (Geervani & Devi, 1986; Friedman & Finot, 1990). Many such interactions have been described and they include the reaction of free amino groups with carbonyl groups of sugars and fatty acids to form Maillard browning products, the formation of cross-linked amino acids such as lysinoalanine and glutamyllysine and the steric blocking of the action of digestive enzymes by newly-formed cross-links, as well as native ones such as disulphide bonds (Otterburn, 1989) which can result in the formation of aggregates that are very poorly susceptible to hydrolysis (Deshpande & Nielsen, 1987). Hence the total lysine content of foods itself is not always an accurate indication of the true nutritional value of the protein with respect to lysine (Hall *et al.*, 1973; Faldet *et al.*, 1992).

Fermented cereal foods such as Ghanaian kenkey are prevalent in developing countries. Although a major energy source, such foods are the only source of a large proportion of the dietary protein which unfortunately is often seriously deficient in lysine (Clark *et al.*, 1977; Friedman & Finot, 1990). It is, therefore, important that the processing of such foods is carefully controlled in order to maximise lysine availability. This also applies to cereal foods supplemented with legumes in an attempt to

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improve both protein quality and quantity. In such cases, it is important that the processing methods used to not undermine the quality improvement process intended with legume supplementation.

Conventional methods used to measure lysine availability in foods are usually either chemical or biological. Biological methods involve the use of microorganisms such as *Pediococcus cerevisiae* (Hamad & Fields, 1979a; Umoh & Fields, 1981). The use of rats and mice in growth response experiments is also on the increase (Sherr *et al.*, 1989; Friedman & Finot, 1990; Faldet *et al.*, 1992). Several chemical methods have also been used (Conkerton & Frampton, 1959; Carpenter, 1960; Hall *et al.*, 1973), and most of these have as a basis, the Sanger reaction (Sanger, 1945) involving free ϵ-amino groups of lysine and fluorodinitrobenzene (FDNB) or its derivatives. Hurrell *et al.* (1979) described a dye-binding method for estimating reactive lysine in foods. Despite some disadvantages, chemical methods involving the use of FDNB and trinitrobenzene sulphonic acid (TNBS) or their derivatives are still being used because they are relatively easy and a large number of samples can be analysed economically (Bakr & Gawish, 1992; Faldet *et al.*, 1992).

The aim of this work was to compare the effects of various treatments of raw materials during kenkey production on the availability of lysine in the final product. Alternative methods used to prepare kenkey were also evaluated for their effects on lysine availability.

Materials and methods

Maize (*Zea mays* L. cv. obantaanba) and cowpea (*Vigna unguiculata* cv. benpla) were obtained from the Crops Research Institute, CSIR, Kwadaso, Ghana.

Preparation of kenkey

Traditional and accelerated kenkeys were prepared in the laboratory as described earlier (Nche *et al.*, 1994a,b). In the traditional process (Figure 1), maize or maize-cowpea mixtures (80:20) were cleaned and soaked for 2 days, after which the soak water was drained and the grains milled and made into a dough that was then placed in sealed plastic containers and allowed to ferment naturally at 30°C for 4 days.

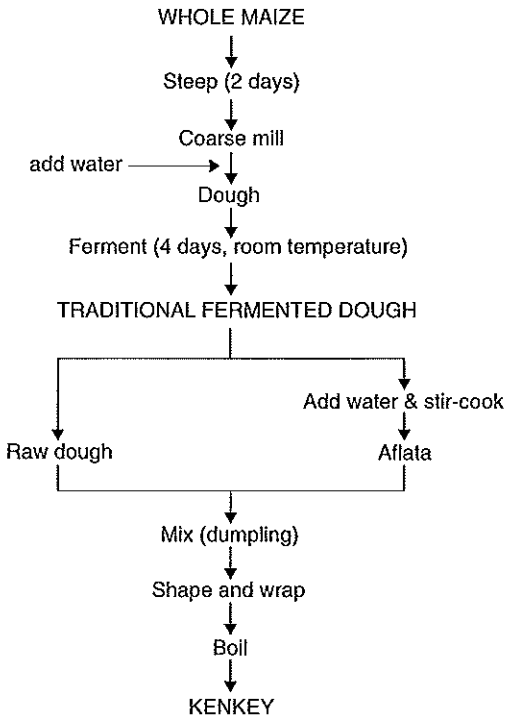

Figure 1. Traditional kenkey process.

Fermented maize or maize-cowpea dough was divided into two parts, one of which was slurried and cooked to gelatinisation to give a gluey paste called the aflata which acts both as a binder and a moisturiser to the uncooked portion. This was then mixed in equal amounts with the uncooked portion, kneaded to give a dumpling from which balls of appropriate size (≈300 g) were shaped, wrapped, first in polyethylene sheaths and then in aluminium foil before being cooked in boiling water for 1 h to give kenkey.

In the accelerated process (Nche *et al.*, 1994b) dry-milled maize was used and dough was fermented for 24 h at 30°C. In this case, fermentation was started by a previously fermented dough called 'back-slop' (Nout *et al.*, 1989). After fermentation, the dough was also divided into two portions, one of which was simultaneously pre-gelatinised and dried on a drum-drier (NV Goudse Machinefabriek, Waddinxveen, The Netherlands) to give a dry flour for convenient aflata production. The drum was pre-heated by steam to 140°C and set to rotate

at 1.5 rev/min. The other portion was dried for 5 h at 45°C in a circulating hot air cabinet drier. A 1:1 mixture of the cabinet and drum dried flours was then re-hydrated to give a dumpling from which balls could be shaped and wrapped in aluminium foil before cooking as above to give kenkey. Samples were stored at –80°C until required for analysis.

Sample preparation and sampling

Wet samples were freeze-dried and all samples were milled to pass through a 0.5 mm sieve using a hammer mill (Fritsch Pulverisette, Type 14.702, Marius Instruments, Utrecht, The Netherlands). 1 g of each sample was accurately weighed and placed in a 100 ml volumetric flask containing 8 ml of acetone. 50 ml of 0.1% agar solution were added and the flask shaken vigorously to ensure adequate mixing. The suspension was then diluted to volume with 0.1% agar solution. The contents of the flask were transferred into a 250 ml beaker. While still stirring with a magnetic stirrer, 0.5 ml of the suspension was pipetted into calibrated 12 ml Kimax tubes with screw caps. Available lysine was determined as described by Hall *et al.* (1973).

Determination of lysine availability

The method described by Hall *et al.* (1973), an improvement of the Carpenter method was chosen for its simplicity and used to investigate the effect of different processing steps on the availability of lysine in kenkey. In this method fluoro-2,4-dinitrobenzene (FDNB) is replaced by 2,4,6-tri-nitrobenzene sulphonic acid (TNBS, Fluka Chemische Fabrik AG, Buchs SG, Switzerland) during the Sanger reaction with free ϵ-amino groups of lysine.

The total nitrogen was determined by a semi-automatic version of the micro-Kjeldahl procedure (Roozen & van Boxtel, 1979).

Statistical analyses

All determinations were in duplicate and repeated 3 times. The data were analysed by an independent *t*-test (SlideWrite Plus, version 5.00, Advanced Graphics Software, Inc., Carlsbad, USA) for significant differences.

Results and discussion

It is important to stress that although most chemical methods including the one used here are useful for a range of animal materials, they suffer significant drawbacks when applied to plant materials. On the one hand, acid hydrolysis steps employed in some of these methods result in some of the available lysine being rendered unavailable by easily reacting with aldose groups of carbohydrates (Hall *et al.*, 1979), on the other hand, reactions between free amino and carbonyl groups during HCl hydrolysis can lead to Maillard browning products imparting unwanted coloration which affects spectrophotometric readings in the later stages of the protocols used. The result could be an overestimation of the available lysine content of the food sample (Friedman & Finot, 1990).

Whilst not ignoring such drawbacks, it must, however, be stressed that this work was aimed more at assessing the comparative effects of processing on the availability of lysine than at absolute quantification of available lysine in kenkey.

The effects of soaking, fermentation time, cooking time, cowpea-supplementation (in traditional kenkey) and cabinet- and drum drying (in accelerated kenkey) on lysine availability were all investigated. Table 1 shows the effects of these treatments on the availability of lysine in raw grains, fermented dough and kenkey from both maize and maize-cowpea blends. Soaking of maize and maize-cowpea blends resulted in significant ($P < 0.05$) increases of 21% and 22%, respectively, in lysine availability. Cooking of unfermented dough (F_0C_1) resulted in increases in lysine availability of 68% and 31%, respectively, for maize and maize-cowpea doughs, compared with soaked samples. Fermentation further increased these values by up to 22% and 12% for 2 days' fermented maize and maize-cowpea doughs (i.e. F_2C_1) respectively, compared with F_0C_1. Prolonged fermentation and cooking effected further increases in lysine availability, with the highest values of 3.42 and 4.3 g/16 g N being obtained, respectively, for maize and maize-cowpea doughs fermented for 4 days and cooked for 3 h. These values represent improvements of 118% and 48%, respectively , on the effects of soaking. These results largely agree with those reported by Hamad & Fields (1979a,b) showing that fermentation of maize and other cereals effected significant improvements in lysine availability. Plahar *et al.* (1983) reported available lysine values of 2.60 and

Table 1. Total nitrogen and available lysine contents of maize and maize cowpea blends during processing into kenkey and kenkey dough

Treatment		*100% Maize*		*Maize:cowpea (80:20)*	
		Total nitrogen (g/kg sample)[1]	*Available lysine (g/16 g nitrogen)*	*Total nitrogen (g/kg sample)*	*Available lysine (g/16 g nitrogen)*
	None	15.22 ± 0.34[2]	1.30 ± 0.30[a]	20.49 ± 0.42	2.46 ± 0.11[b]
	Soaked	15.48 ± 0.75	1.57 ± 0.00[a]	21.30 ± 1.46	2.99 ± 0.19[c]
Fermented					
	F_0C_1[3]	16.19 ± 1.39	2.64 ± 0.17[b]	21.23 ± 0.81	3.91 ± 0.00[e]
	F_1C_1	16.80 ± 0.90	3.25 ± 0.26[d]	ND[4]	ND
	F_2C_1	16.46 ± 0.90	3.21 ± 0.04[d]	22.00 ± 0.50	4.36 ± 0.07[f]
	F_3C_1	16.96 ± 0.38	3.29 ± 0.22[d]	ND	ND
Cooked					
	F_4C_0	18.13 ± 2.13	2.33 ± 0.00[b]	21.45 ± 1.66	3.44 ± 0.40[d,e]
	F_4C_1	17.99 ± 1.19	2.36 ± 0.59[b]	21.78 ± 1.17	4.11 ± 0.30[f]
	F_4C_2	17.49 ± 1.38	3.29 ± 0.04[d]	ND	ND
	F_4C_3	15.36 ± 0.21	3.42 ± 0.17[d,e]	20.63 ± 0.45	4.43 ± 0.00[f]

[1]Dry weight basis; [2]Mean ± SD (n = 6); [3]All samples fermented at 30°C; F_{0-4} = fermented for 0–4 days; C_{0-3} = cooked for 0–3 h; [4]ND = not determined.
[a,b,c,d,e,f]Values with the same letter are not significantly different ($P < 0.05$).

3.46 g/16 g N for dehydrated fermented maize meal and maize-soy (80:20) flour blends, respectively. Zamora & Fields (1979) also found significant improvements in the availability of limiting amino acids such as isoleucine, methionine and tryptophan following the lactic fermentation of cowpeas. In their studies of the availability of sulphur amino acids in six varieties of common beans (*Phaseolus vulgaris*), Marletta *et al.* (1992) reported significant decreases in available cystine in only two varieties, and no changes in the total cystine contents of all six varieties after soaking and cooking. The availability of methionine was, however, reported as unaffected by cooking. This difference in the effects on the availability of cystine and methionine was reported (Marletta *et al.*, 1992) to be due to the presence of different proportions, in various bean proteins (albumins, globulins and glutenins) which have been shown to have different digestibilities (Lanfer Marquez & Lajolo, 1981).

The total nitrogen contents of all samples remained largely the same regardless of the

Table 2. Effects of cabinet and drum drying of maize and maize-cowpea (80:20) doughs on the total nitrogen and available lysine contents of the resulting kenkey

Process	*100% maize*		*Maize:cowpea (80:20)*	
	Total nitrogen (g/kg sample)[1]	*Available lysine (g/16 g nitrogen)*	*Total nitrogen (g/kg sample)*	*Available lysine (g/16 g nitrogen)*
Traditional	17.99 ± 1.19[2]	2.36 ± 0.59[c]	21.78 ± 1.17	4.11 ± 0.30[f]
C abinet dried[3]	17.72 ± 0.87	2.69 ± 0.11[c]	20.93 ± 1.09	4.17 ± 0.23[f]
Drum dried[4]	16.89 ± 1.39	1.59 ± 0.14[a]	21.09 ± 1.08	3.08 ± 0.05[d]
Cabinet + drum dried[5]	15.48 ± 0.60	2.07 ± 0.48[b]	21.00 ± 0.77	3.85 ± 0.05[e]

[1]Dry weight basis; [2]mean ± SD (n = 6); [3]accelerated fermentation plus cabinet drying; [4]accelerated fermentation plus drum drying; [5]1 1:1 mixture of cabinet- and drum dried flours.
[a,b,c,d,e,f]Values with the same letter are not significantly different ($P < 0.01$).

treatment (Tables 1 and 2). Although not determined, it could be deduced from this that the total lysine contents of all the samples were not significantly altered. Any changes in the amounts of available lysine could, therefore, have been mainly the result of changes in the binding state of total lysine during processing.

A 20% supplementation with white cowpea resulted in a 74% increase in the available lysine content of kenkey made by the traditional process (F_4C_1) in comparison with the all-maize product. This increase is in line with increases in total lysine contents of maize-cowpea blends reported earlier (Nche *et al.*, 1994a).

Accelerated lactic fermentation followed by cabinet drying of dough did not affect lysine availability in the resulting kenkey (Table 2). Drum drying, on the other hand significantly ($P < 0.01$) reduced the available lysine contents of the resulting kenkeys. Adeniji & Potter (1978) reported heavy losses of up to 38% in available lysine of ogi following drum drying. We found a 33% and 25% reduction in the amounts of available lysine in drum dried maize and maize-cowpea kenkeys, respectively. Addition of an equal amount of cabinet dried flour to drum dried flour to produce kenkey, did not only contribute to the desired texture of the final product, but also compensated for some of the available lysine lost as a result of drum drying (Table 2). The values obtained for available lysine in such mixtures were only 12% and 6% lower than for traditional maize and maize-cowpea kenkeys, respectively.

Results obtained for lysine availability in the raw materials (Table 1) were generally below the range of values of total lysine contents of raw maize and/or cowpeas although Hamad & Fields (1979a) reported even lower values for maize. Calculations from literature values (Hurrell & Carpenter, 1979; Kent, 1983; Bressani, 1985) give average total lysine contents of 2.5 and 3.6 g/16 g N for maize and 80:20 maize-cowpea blends, respectively. These values are well in line with the crude protein contents of 10.8% and 13% reported by Nche *et al.* (1994a) for maize and a 80:20 maize-cowpea mixture, respectively. The lower values of available lysine for the raw materials, however, could be attributed to the fact that these materials have lower protein digestibilities (Nche *et al.*, 1993) and hence only a fraction of the actual lysine content will be available. Further processing, which includes fermentation and cooking, results in increased protein digestibilities and subsequently increased lysine availability.

The results presented in this paper clearly show that soaking, fermentation and cooking contribute significantly to the protein quality of kenkey. Drum drying will, however, induce high losses in available lysine, but a mixture of cabinet and drum dried flours will, in addition to maintaining the texture of traditional kenkey, limit excessive losses in nutritive value with respect to lysine availability.

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