Innovations in the production of kenkey, a traditional fermented maize product of Ghana

Nutritional, physical and safety aspects

Pius Fru Nche

Propositions/Stellingen

1. A serious drawback for industrial kenkey manufacture is its high moisture content (up to 60% w/w) which makes it prone to spoilage, especially as the packaging is not adequate to prevent spoilage over long storage periods. (**This thesis**).

2. Acidification of dough from dry-milled maize by accelerated natural fermentation and wet-milling of the aflata portion may be more acceptable than the use of proteolytic enzymes which would raise production costs and arouse consumer fears, as this may be interpreted in the same light as the addition of chemical substances to a product that has always been considered as natural. (**This thesis**).

3. Although a lot of development aid from the West is channelled into African agriculture, it is rather debatable whether improving primary agricultural output alone can begin to tackle the chronic hunger crisis on that continent if a firm post-harvest processing base is not developed. (**This thesis**).

4. Most authors, including Nowak & Steinkraus (1988) tested whole foods for their potential to cause flatus. In the human digestive tract, however, accessible starch is broken down to mono- and disaccharides which are absorbed in the upper gut. *In vitro* models of digestibility and fermentability, therefore, need to take this aspect into account. (**This thesis**).

5. It is reasonable to assume that developing countries will be unable to benefit fully from biotechnology, in terms of economic development and problem solving, unless they are able to utilise results from indigenous biotechnological research. Bridging the gap between research and applications is therefore of vital importance. (R.A. Zilinskas, 1993. *World Journal of Microbiology and Biotechnology* **8**, 145-152).

6. In the industrialised world the concept of sustainability, or in other words minimal resource utilization and environmental impact, has only been applied to a limited extent to agricultural and industrial food production. (Anderson *et al.*, 1994. *Trends in Food Science and Technology* **51**, 134-138).

7.   Foreign aid is a method by which the United States maintains a position of influence and control around the world and sustains a good many countries which would definitely collapse or pass into the communist bloc. (**J.F. Kennedy, 1961**). Will Foreign Aid follow in the food steps of Kennedy and the Communist bloc?

8.   Birth control in an impoverished and uneducated society is simply a non-starter. Those most in need are often victims of the poverty trap which itself does not allow for proper education (**1994 Cairo Conference on World Population**).

9.   In Ghana, like in most West African countries, fermented maize dough is the base material for several types of snacks, staples and beverages. The absence, however, of sustainable agro-industries that can improve the processing and promotion of such indigenous foods has contributed significantly to their perception as "poor peoples' foods".

10.   The distribution of food and wealth in today's "modern" societies guarantees that whilst some people are dieting others are dying of undernourishment and malnutrition.

11.   I have a truly wonderful proof which this margin is too small to contain. (**Pierre de Fermat - 1637**). Bluff or gross understatement?

Propositions belonging to this thesis of P. Fru Nche entitled "Innovations in the production of kenkey, a traditional fermented maize product of Ghana".

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ABSTRACT

Nche P.F. (1995). Innovations in the production of kenkey, a traditional fermented maize product of Ghana: Nutritional, Physical and Safety aspects. *Ph.D. thesis. Agricultural University Wageningen (135 pp, English, Dutch and French summaries)*.

Kenkey is traditionally made from a dough obtained by soaking maize (1-2 days, room temperature), milling and then fermenting naturally for 2-4 days. This thesis was aimed at improving not only the nutritional quality of kenkey, but also the production process. The traditional method for making kenkey was scaled down to a laboratory process and the microbiological, physical and nutritional quality of both maize and maize-cowpea kenkey were investigated. Natural fermentation for 48h or 72h at 30°C was sufficient to obtain properly acidified maize (pH 4.07) or maize-cowpea (pH 4.08) doughs, respectively. Lactic acid bacteria were mainly responsible for acidification. Supplementation of maize (on a replacement basis) with 20% white cowpea resulted in significant increases in protein (by 20.5%) and available lysine (by 74%) contents. This also resulted in significant increases in biogenic amines (total amines < 500 ppm, mainly putrescine and tyramine) compared with maize kenkey (total amines < 60 ppm). Histamine was absent (< 5 ppm). Acceptability tests in Ghana, however, showed that only a 10% cowpea level was comparable with the traditional kenkey in terms of flavour and texture. Process options for producing a dehydrated kenkey meal (kenkey dry-mix) were investigated with the aim of developing a product with a longer shelf-life than traditional kenkey. An *in vitro* method was developed for determining the digestibility and flatulence potential of kenkey. Soaking of grains effected the highest increase in *in vitro* digestibility. *Clostridium perfringens* strain NCTC 8239 produced more gas from the solid residue left over from the *in vitro* digestion of maize-cowpea samples than from the resulting supernatant which contained low molecular weight oligosaccharides, traditionally held responsible for intestinal flatus induction, suggesting that non-starch polysaccharides contribute significantly to the flatulence potential of cowpea-supplemented kenkey.

Keywords: Accelerated fermentation, aflata, biogenic amines, cowpea, digestibility, drum drying, dry milling, flatulence, kenkey, maize, supplementation, texture.

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Contents

I expect to pass this way but once, and any good, therefore, that I can do, or any kindness that I can show to any fellow creature, let me do it now. Let me not defer or neglect it, for I shall not pass this way again

(Etienne de Grellet).

To my family

Chapter 1

General Introduction

More than 60% of the total world food production is provided by cereals which, along with pulses and oil seeds, contribute significantly to the dietary protein, energy, mineral and vitamin requirements of the world population in general and the developing world in particular (Chavan and Kadam, 1989a,b). Whereas over 70% of the total cereal production in the West is fed to animals, the developing world channels almost all of the produced cereals to feeding its large and ever-increasing populations (FAO, 1980; Betschart, 1982). Despite their excellent nutritional quality, foods of animal origin remain a scarcity mainly because high production costs, the need for sophisticated and costly processing technology for storage and distribution and a short shelf-life put them beyond the reach of developing countries (Lay and Fields, 1981; Chavan and Kadam, 1989b).

Of the total world cereal production, maize makes up about 27%, and although other cereals such as rice, sorghum and millet exist in noticeable quantities, maize remains the most important cereal in the developing world, particularly in Africa (Lay and Fields, 1981; Hounhouigan, 1994). Maize has the highest energy value (16.6 kJ/g), compared with pearl millet (16.5kJ/g), brown rice (16.1 kJ/g), sorghum (16.1kJ/g) and wheat (15.7kJ/g) (Chavan and Kadam, 1989a). The total production of maize in Africa in 1991 was estimated at over 33 million tonnes (FAO, 1992).

Cereal processing

The processing technologies employed for maize and other cereals commonly found in the developing world include cooking, sprouting, milling, fermentation and combinations of these. Of these, fermentation is the most commonly practised, particularly in Africa, although the type of raw material, type and conditions of fermentation and sensory qualities of the finished products may vary from culture to culture. Fermentation is especially important to the developing world because it is an inexpensive and simple method of improving the nutritional and organoleptic qualities of otherwise monotonous cereal products (Hesseltine, 1983; Cooke et al., 1987; Chavan and Kadam, 1989a). It does not require expensive equipment or special expertise and can be achieved in a very short period of time (Lay and Fields, 1981). Some of the nutritional advantages of fermentation are said to include the

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decrease in starch and fibre contents, followed by an increase in reducing sugars (Kazanas and Fields, 1981) although it can be expected that the fermenting organisms would use up the available sugars. The process of fermentation itself does not have any significant effect on total protein content, but can result in the qualitative modification of proteins, often resulting in the increase of water-soluble proteins and free essential amino acids. These changes can be effected by endogenous proteases, but have also been attributed to the proteolytic activity of some of the bacteria responsible for cereal fermentations (Kao and Robinson, 1978; Hamad and Fields, 1979; Zamora and Fields, 1979; Umoh and Fields, 1981; Lay and Fields, 1981; Tongnual *et al.*, 1981; Chavan and Kadam, 1988, 1989).

Table 1.1. Some fermented maize products commonly found in Africa.

Product	Type	Microorganism(s)	Country
Agidi	Gruel	LAB[1] & Yeasts	Nigeria
Akasa	Gruel	LAB & Yeasts	Ghana
Banku	Dumpling	LAB	Ghana
Busaa	Beverage	LAB & Yeasts	Kenya
Fufu	Dumpling	LAB & Yeasts	Cameroon
Kaffir beer	Beverage	LAB & Yeast	S. Africa
Kenkey	Dumpling	LAB & Yeast	Ghana
Mahewu	Beverage	LAB	S. Africa
Mawè	Intermediate[2]	LAB & Yeast	Bénin
Ogi	Gruel	LAB	Nigeria
Pito	Beverage	LAB & Yeast	Nigeria
Uji	Gruel	LAB & Yeast	Kenya

[1]LAB = lactic acid bacteria; [2]Fermented dough used for making different products.

Since maize forms the greatest portion of cereals grown in Africa, it is no surprise that most fermented cereal products are either from maize or a mixture of maize and other cereals and in some cases, legumes. Table 1.1 lists some of the many of fermented maize products of Africa, which include staples, gruels and beverages. Most of these fermentations are spontaneous, and involve lactic acid bacteria, yeasts or a mixture of these as the functional microorganisms.

In addition to the enhancement of flavour and nutritional value (Hesseltine, 1983; Halm *et al.*, 1993, Hansen and Hansen, 1994; Hounhouigan, 1994), one of the most important aspects of fermentation in the developing world is the improvement of the microbiological safety of foods. In high temperature and humid regions such as Africa, where cooling facilities are not readily available, fermentation provides a cheap but effective method of food preservation (Hesseltine and Wang, 1979; Umoh and Fields, 1981; Chavan and Kadam, 1989a). Lactic acid fermentations inhibit spoilage and pathogenic microorganisms in a variety of ways. The most important of these is the production of organic acids which lower the pH of foods to levels not favourable to food pathogenic and spoilage bacteria (Nout *et al.*, 1989; Nout and Rombouts, 1992). The depletion of essential nutrients and production of hydrogen peroxide and bacteriocin-like agents by lactic acid bacteria have also been cited as important in limiting the occurrence of spoilage and pathogenic microorganisms in fermented foods (Cooke *et al.*, 1987; Mensah *et al.*, 1991; Mbugua and Njenga, 1992, Nout and Rombouts, 1992; Larsen *et al.*, 1993). Fermentation is also known to reduce the levels of anti-nutritional factors in cereals and legumes, although this is often influenced by the choice of raw materials, fermentation times and the physiological differences in fermenting microorganisms. Lactic acid fermentation can result in rapid decreases in pH, hence the level of enzymatic degradation of antinutrients may depend on pH optima and fermentation times (Chavan and Kadam, 1989a).

Nutritional quality of cereals

Although cereal grains form the bulk of the staple diet of people in the developing world, they are generally inferior in nutritional and sensory quality, compared with foods of animal origin (Chavan and Kadam, 1989a). The protein quantity and quality is low, lacking in essential amino acids such as lysine. Although rich in the sulphur-containing amino acids cysteine and methionine, maize for example, is deficient in lysine and tryptophan, making its protein quality inferior compared with FAO/WHO reference proteins (Newman and Sands, 1984; Plahar and Leung, 1985)

With indications that the ever increasing populations of the developing countries could become increasingly dependent on cereal grains for both energy and protein requirements, there is an urgent need to improve the overall nutritional quality of cereals. Traditionally, cereals are consumed with other plant protein sources such as legumes. Since legumes are deficient in the sulphur-containing amino acids but rich in lysine, the overall protein quality of such mixtures, with

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respect to amino acids, will be better than that from either protein source alone (Chavan and Kadam, 1989b). Various methods have been employed in attempts to improve the protein quantity and quality of cereal foods. These include genetic manipulations, fortification with essential amino acids and supplementation with protein-rich sources such as grain legumes, oil seed meals or their protein concentrates and isolates (Akinrele and Edwards, 1971; Adeniji and Potter, 1978; Plahar and Leung, 1983, 1985; Plahar *et al.*, 1983).

In Ghana, like in most West African countries, fermented maize dough is the base material for several types of snacks, staples and beverages. Fermented maize foods account for more than 95% of the total calories in diets of the coastal peoples of Ghana (Plahar and Leung, 1983). The preparation of fermented maize meal and its nutritional composition have been described by several authors (Christian, 1970; Muller, 1970; Plahar and Leung, 1983; Sefa-Dedeh, 1989).

Kenkey is one of the most popular of the staples of Ghana, prepared exclusively from fermented maize dough. There are several types of kenkey, the most common being Ga and Fanti kenkeys from the Ga and Fanti tribes, respectively. Despite some differences between these kenkeys, processing methods are basically similar, with lactic acid bacteria largely responsible for the fermentation, although other bacteria and yeasts have been implicated (Akinrele, 1970, Wood and Hodge, 1985; Halm *et al.*, 1993).

The low quality and quantity of kenkey protein is a reflection of that of the raw material, maize, which is deficient in lysine. Traditionally, kenkey is eaten with a palm oil and pepper sauce known as **shittoh** or a rich source of protein such as sardine sauce. Fish, however, is expensive and is not often available to especially the poor families.

In addition to its limited protein quality, kenkey is a high moisture containing product (up to 60% w/w) and is, therefore, prone to spoilage (Plahar and Leung, 1985), especially as the packaging (corn husks for Ga and banana leaves for Fanti kenkey) is not adequate to prevent spoilage over long storage periods.

Presently, the production of kenkey is on a micro household scale, operated mainly by women. The whole process, from soaking through milling of maize, fermentation and cooking, can take anything up to a week, and includes one very tedious step during which a gelatinized paste called **aflata**, used as a binder and moisturizer, is prepared by stir-cooking. Usually these producers rely on their intuitive expertise rather than on carefully standardised procedures, and do not always understand the exact nature of the chemical and microbiological changes initiated by the natural fermentation of the maize dough. As a result, kenkey

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producers have very little or no control over the fermentation process, and often end up with products that are highly variable in quality. Such variations occur not only amongst different products, but also within the same product made in the same or different house holds.

Kenkey is a highly popular product amongst the fishermen of the coastal regions, as well as amongst urban populations of workers and students, who for lack of time, tend to prefer ready-to-eat foods. As these populations increase, so will be the demand for a product that is less prone to spoilage and consistent in quality (Hesseltine, 1983; Hollingsworth, 1994). The technologies in place are, however, not adequate to meet this ever-increasing demand for high quality and microbiologically stable kenkey.

Aim and outline of this thesis

The work described here was carried out as part of an E.C. sponsored collaborative project on the evaluation and improvement of traditional fermented cereals and legumes in Ghana. The improvement of the production process and quality of kenkey, are covered in Chapters 2 - 5. Chapters 6-8 and deals with the safety aspects and digestibility of kenkey.

Chapter 2 of this thesis tackles the translation of the traditional kenkey process to a laboratory scale process, and looks at the effects of cowpea supplementation on the fermentation of dough for kenkey production and on the quality of the final product. Chapter 3 discusses the technical feasibilities of reducing production time and increasing output by using a standardized method that can be scaled up in industry. Chapter 4 looks at the biochemical, microbiological and physical changes that occur during the soaking of maize and how these changes could influence the final texture of kenkey. In Chapter 5, the effects of the different process options applied in making kenkey on protein quality, with respect to lysine availability, are discussed. Chapter 6 looks at the influence of the choice of ingredients and fermentation conditions on the formation of biogenic amines and ethyl carbamate in kenkey.

The use of cowpeas increases protein quality but also affects parameters such as digestibility and the level of antinutrients such as flatus-forming oligosaccharides in kenkey. Chapters 7 and 8 discuss the development and use of an *in vitro* method to determine the digestibility and flatulence potential of kenkey.

Chapter 9 is a general discussion of the pertinent issues raised in this thesis, and includes recommendations for possible future work involving the improvement,

not only of the quality of fermented cereal foods, but also the establishment of improved facilities for processing foods in Africa.

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The effect of cowpea-supplementation on the quality of kenkey[1]

Abstract

The effects were evaluated of cowpea-mediated protein enrichment of kenkey on factors relating to kenkey acceptability. Kenkey was prepared at laboratory scale from a 4:1 mixture of maize (*Zea mays*) and red or white cowpea (*Vigna unguiculata*) and compared with an all-maize product in terms of parameters such as the fermentation profile of doughs, colour and fracture profiles. There was no significant difference between the fermentation profiles after 4 days' fermentation at 30°C, with final dough pH values reaching 4.07 and 4.08 for all-maize and maize-cowpea mixtures, respectively. Addition of wholegrain cowpeas resulted in an increase in the crude protein content to 12.99% (w/w) and 13.89% (w/w; dry weight basis) for kenkey supplemented with 20% white and 20% red cowpea, respectively, compared with 10.80% (w/w) for unsupplemented kenkey. Addition of cowpea reduced the whiteness (Hunter L value) of the kenkey by 12% (using white cowpeas) and by 27% (using red cowpeas). The use of dehulled red cowpeas improved the whiteness only slightly. Cowpea-supplemented kenkey was more homogeneous and less prone to fracture than all-maize kenkey. The force required to fracture cowpea-supplemented kenkey was higher than for traditional kenkey, and increased with increasing cowpea concentrations. Kenkey stored for 24 hours required over twice as much force to fracture than freshly prepared kenkey. A group of native Ghanaians familiar with the traditional maize kenkey sampled the new product and concluded that kenkey made from a mixture of wholegrain white cowpea or dehulled red cowpea and maize compared very well with the traditional kenkey.

INTRODUCTION

Kenkey, a dumpling made from fermented maize dough, is a popular staple amongst the peoples of Ghana. There are several types of kenkey (Ga, Fanti), but the processing methods are largely similar. In the traditional kenkey process (Muller, 1970; Muller and Nyarko-Mensah, 1972) maize is cleaned and then

steeped for 1 to 2 days. The steep water is drained and the grain coarsely milled and made into a dough by adding water (1:3 v/w). The dough is then left to ferment naturally in a heaped mass or in covered containers for 2 to 3 days. The fermented dough is then divided into two equal parts, one of which is slurried and cooked to gelatinisation. This gives a sticky paste known as aflata, which is then mixed with the uncooked part to give a kneadable dough. Balls of appropriate size (≈ 500g) are shaped, wrapped (in corn sheaths for Ga kenkey or banana leaves for Fanti kenkey). Salt is also added to dough for Ga kenkey before shaping and wrapping. The wrapped dumplings are immersed in water and boiled in a drum or large iron pot for 2 to 3 hours.

Since maize is the basic raw material, such products are rich in carbohydrate, relatively low in protein and deficient in some essential amino acids, particularly lysine. In a region where animal sources of protein are often in short supply, plant sources such as legumes may be of immense importance. Attempts have been made, with some success, at improving the protein quality and quantity of traditional maize-based fermented products of Ghana (Plahar and Leung, 1983) and Nigeria (Akinrele and Edwards, 1971) by supplementation with soya beans. However, soya beans are rather expensive and not well known in West African households. Being relatively cheaper and readily available, cowpeas can be a suitable alternative to the more expensive soya beans (Akinyele and Fasaye, 1988). In this work, cowpeas were used as a protein supplement to the traditional all-maize Ghanaian kenkey. This improves the protein quantity and certainly its amino acid balance as the methionine-containing maize is complemented by the lysine-containing cowpeas (Bressani, 1985). The rate of fermentation, the colour and textural properties as well as the overall acceptance of cowpea-supplemented kenkey were compared with the conventional all-maize formula.

MATERIALS AND METHODS

Maize (*Zea mays* cv. Obaatanba) and red and white Cowpeas (*Vigna unguiculata* cvs. Benpla and Asontem, respectively) were obtained from the Crops Research Institute, CSIR, Kwadaso, Ghana. Whole grain and dehulled cowpeas were used. Dehulling was done by hand following 48 h of soaking in excess water.

The Laboratory Kenkey Process
In the laboratory process (Fig. 2.1), maize or a 4:1 mixture of maize and cowpea (0.5 - 1.0 kg) was soaked for 48 h in tap water (approx. 3 l).

Clean maize or maize-cowpea mixture

Soak in water for 2 days at ambient temperature

Drain water and coarse mill grain

← Add water (1:3; v/w)

Ferment dough for 4 days at 30°C

Add water (3:1;v/dwt), slurry and cook

Raw dough   AFLATA

Mix (dumpling)

Shape, wrap and boil for 1h
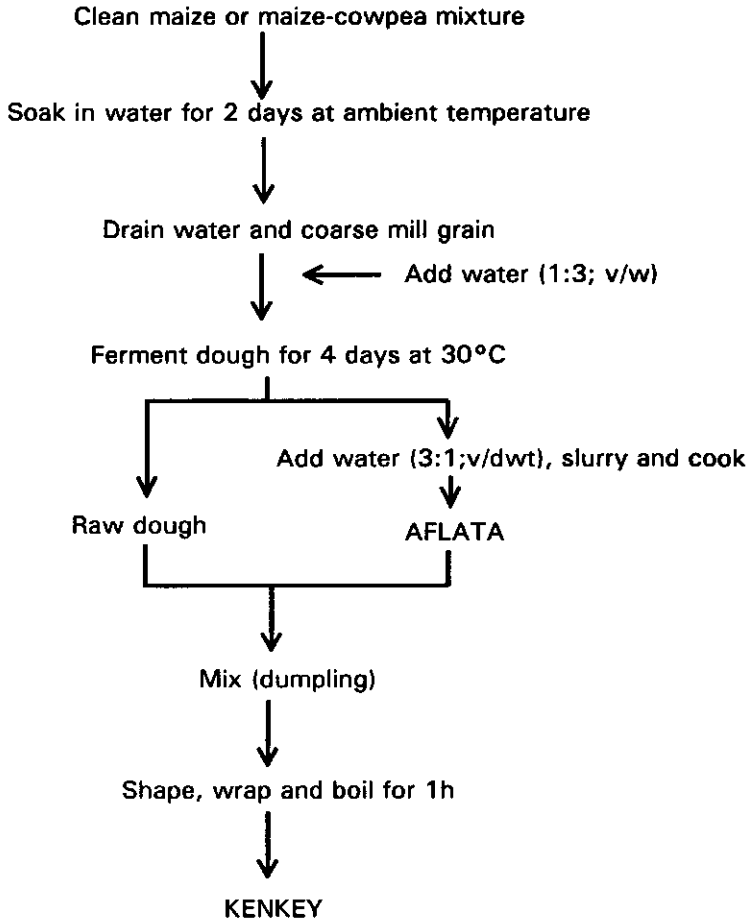
KENKEY

Figure 2.1. Laboratory method for kenkey production

The soak water was drained and the grain coarsely ground in a hammer mill (Fritsch Pulverisette, Type 14.702, Marius Instruments, Utrecht, The Netherlands) with rotor and sieve (4 mm) and made into a dough, M_0, Mw_0 or Mr_0 from maize, maize/white cowpea or maize/red cowpea, respectively, by adding water (1:3 v/w). The dough was then placed in a 1 litre beaker, covered with polyethylene film and allowed to ferment naturally for a standard 4 d at 30°C. The fermented dough, M_4, Mw_4 or Mr_4, was then divided into two equal portions, one of which was slurried with water (3:1 v/w; dry weight basis) and stir-cooked to gelatinisation to give the aflata. The cooked and uncooked portions were mixed, kneaded and dumplings

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were made and wrapped, first in polyethylene film followed by aluminium foil, as direct contact with aluminium foil resulted in darkening of the foil during cooking, imparting a black colour to the outside of the dumpling. The wrapped dumplings were immersed in boiling water to cook. Dumplings were smaller (250 - 300g) than in the traditional household process hence the cooking time (under constant heat in a covered saucepan) was reduced to 1 h. The resulting products were all-maize (M_4C_1), white cowpea-supplemented (Mw_4C_1) and red cowpea-supplemented (Mr_4C_1) kenkeys.

Fermentation profile
During the 4 days of fermentation, the microbial count, pH, and titratable acidity (% lactic acid) were determined according to Nout *et al.* (1987) on a daily basis. Counts were made of the functional groups of micro-organisms in the product, namely lactic acid bacteria (LAB) and yeasts. In addition, Enterobacteriaceae were monitored for public health reasons. Lactic acid bacteria were counted in MRS agar (Merck 10661) pour plates containing 0.1% (w/v) Natamycin ("Delvocid", Gist-brocades, Delft, The Netherlands) overlaid with MRS agar without Delvocid, the yeasts in Oxytetracycline Glucose Yeast Extract agar (Oxoid CM 545) containing 0.01% (w/v) oxytetracycline (Oxoid SR 073A), and the Enterobacteriaceae in Violet Red Bile Glucose agar, VRBG (Merck 10275), with a VRBG overlay.

Characterisation of isolated lactic acid bacteria
Isolates were made daily from appropriate MRS counting plates. Five representative colonies of LAB were isolated and subsequently purified on MRS agar. These were then stored at -80°C in cryotubes containing 15% (w/v) glycerol in MRS broth for later characterisation. Characterisation tests included Gram-stain, morphology (by microscopic observations), catalase reaction, growth at 15°C and/or 45°C, homo/heterofermentation and arginine hydrolysis. Confirmation of isolated lactic acid bacteria was obtained from established descriptions of non-sporing Gram - positive rods (Kandler and Weiss, 1986) based on the utilisation of relevant carbohydrates on the API 50 CHL test strips (API System SA, Montalieu Vercieu, France).

Crude protein content
The crude protein content was determined using a semi-automated version of the micro-Kjeldahl method for nitrogen determination (Roozen and Van Boxtel, 1979). Experiments were done twice and all samples run in duplicates. The values for

nitrogen concentration were converted to crude protein content by multiplying by 5.26 for the maize (Thomas, 1951) and 5.46 for maize-cowpea blends (5.26 x 80% + 6.25 x 20%).

Colour
Colour parameters L, a, and b representing whiteness, redness and yellowness, respectively were measured using a DR LANGE Tricolor LFM3 Colorimeter (Hunterlab, 9529 Lee Highway, Fairfax Virginia), calibrated with a standard white tile (L = 88.20; a = -0.96; b = -1.69). Two measurements were made of the surface of each sample.

Fracture stress
Fracture stress was measured using an Overload Dynamics Instron-type instrument (Marius Instruments, Utrecht, The Netherlands) having two plates between which samples of standard cylindrical dimensions (length 3cm; diameter 2cm) were placed vertically and compressed at constant speed till fracture point and the fracture stress calculated according to Van Vliet (1991). Dehulled cowpeas were used in supplemented kenkeys.

Product evaluation
An untrained 5-member panel of native Ghanaians (3 males and 2 females, aged between 28 and 40 years) familiar with traditional kenkey, was asked to evaluate the control and supplemented products on the basis of their sourness (taste), smell, texture, colour, doneness (degree of cooking) and hardness. This was done by individual structured interview, followed by group discussion. Each panellist was asked to sample the product and comment on paper on how the supplemented kenkey compared with the all-maize traditional kenkey with respect to the above qualities. Each panellist was advised to draw from his or her experience as a traditional kenkey consumer. Individual comments were compared and the group discussion that followed was aimed at reaching a general consensus on the potential acceptability of the new product and recommendations on the possible improvement of the less acceptable qualities.

RESULTS AND DISCUSSION

In the experiments to study lactic acid fermentation in kenkey doughs (Table 2.1), the pH decreased and the titratable acidity increased as the LAB count increased.

Table 2.1. Fermentation profiles of all-maize and cowpea-supplemented maize doughs.

Dough*	pH	TA	LAB (Log_{10} cfu/g)	Yeasts (Log_{10} cfu/g)	Enterobac-teriaceae (Log_{10} cfu/g)
M_0	6.02[a§]	0.49	6.72	4.36	6.53
M_1	4.00	0.97	8.09	<1.70	<1.70
M_2	3.86[b]	1.11	8.01	<1.70	<1.70
M_3	4.05[c]	1.15	7.97	<1.70	<1.70
M_4	4.07[c]	1.12[a]	7.93[a]	<1.70	<1.70
Mw_0	5.87[a]	0.49	6.05	4.79	8.86
Mw_1	4.19	1.31	8.17	<1.70	<1.70
Mw_2	4.11	1.46	8.00	<1.70	<1.70
Mw_3	4.07[c]	1.55	7.94	<1.70	<1.70
Mw_4	4.08[c]	1.57[b]	7.93[a]	<1.70	<1.70
Mr_0	6.65[d]	0.32	7.86	4.61	8.62
Mr_1	4.31	1.32	8.34	<1.70	<1.70
Mr_2	4.11	1.58	8.52	<1.70	<1.70
Mr_3	4.07[c]	1.67	8.13	<1.70	<1.70
Mr_4	4.08[c]	1.73[c]	8.07[a]	<1.70	<1.70

* Sample codes: M = 100% maize; Mw = 80% maize + 20% white cowpea; Mr = 80% maize + 20% red cowpea; subscripts (0, 1, 2, 3, 4) indicate fermentation time (days); § a, b, c, d values in the same column, with the same letter are not significantly different ($p < 0.05$) TA = Titratable acidity, expressed as % lactic acid (w/w).

LAB counts exceeded 10^8/g after only 24h fermentation. Further incubation led to a slight fall in LAB counts. Where yeasts occurred, lower counts ($< 10^5$) than LAB were often noticed. Yeast counts declined sharply with the rapid decrease in pH. The Enterobacteriaceae counts also fell rapidly to below detection level as a result

of acid production by the LAB (Nout *et al.*, 1989). Some authors (Mensah *et al.*, 1991) believe that other substances (e.g. bacteriocins) produced by the dominating LAB may contribute to the disappearance of Enterobacteriaceae.

Table 2.1 confirms the trend observed by previous authors (Chavan *et al.*, 1988) investigating the changes in pH and titratable acidity during the natural fermentation of cereals and legumes. They reported an optimum fermentation period of 2 and 3-4 days at 30°C for cereals and legumes, respectively. The pH of all-maize dough (M_0) fell from 6.02 to 3.86 in just two days, whereas that of supplemented doughs (Mw_0 & Mr_0) required a further day to reach its lowest value of 4.07. After 4 days of incubation, the pH of all three doughs stabilised at 4.08. The higher titratable acidity values obtained for supplemented doughs could be attributed to the high buffering capacity of the medium due to the higher content of proteins and amino acids (Banigo and Muller, 1972; Zamora and Fields, 1979). The addition of 20% cowpeas, therefore, did not hinder proper dough fermentation, though a longer time was required for acidification to stabilise. Two and 3 days of fermentation were sufficient to lower the pH of all-maize and cowpea-supplemented doughs, respectively, to the inhibitory pH range (3.6 - 4.1) for food poisoning bacteria reported by Hamad and Fields (1979). These authors, however, did not specify lactic acid as responsible for lowering the pH to this inhibitory range.

Tests identified *Lactobacillus plantarum, L. confusus, L. brevis* and *Pediococcus pentosaceus* as the main lactic acid bacteria present in the fermenting doughs. In the case of doughs M_0 and Mw_0, *L. confusus* was found to dominate the earlier stages of fermentation (d_0). These were later replaced by *Pediococcus spp* and *L. plantarum* for M_{1-4} and Mw_{1-4} respectively. The pattern was one of acid-sensitive heterofermentors being succeeded by more acid tolerant homofermentors. This was in line with the decrease in pH and the increase in titratable acidity (Table 2.1) as fermentation progressed.

The pattern was less clear for Mr_{0-4}, in which *L. plantarum, L. confusus* and *Pediococcus spp* were present in similar proportions throughout the four days of fermentation.

The addition of 20% red and 20% white cowpea resulted in increases in crude protein contents of 29.2% and 20.5%, respectively, for the supplemented products. The values for crude protein content shown in Table 2.2 are comparable with those reported earlier (Akinyele and Fasaye, 1988) for ogi fortified with cowpeas. With regard to the average essential amino acid contents of maize (Salunkhe *et al.*, 1985) and cowpea (Bressani, 1985), these values represent, respectively, 76.3, 15.6 and 26.8% increases in lysine, methionine and tryptophan

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in maize-red cowpea blends and 65.3, 8.2 and 19.5% increases in lysine, methionine and tryptophan in maize-white cowpea blends.

Table 2.2. Crude protein content of doughs of maize and maize-cowpea blends.

DOUGH[1]	CRUDE PROTEIN CONTENT (%, w/w, dry matter)[2]
M_0	10.41 ± 0.49
M_4	10.80 ± 0.32
Mw_0	12.57 ± 0.21
Mw_4	12.99 ± 0.21
Mr_0	13.51 ± 0.20
Mr_4	13.89 ± 0.24

[1]See Table 2.1 for meaning of sample codes; [2]Mean $\pm$ SD; n $=$ 4

The addition of wholegrain cowpea reduced the whiteness of the product (Table 2.3). This was more pronounced with the addition of red cowpeas where the colour change was noticeable. Dehulling the cowpeas improved the colour visibly even though the Hunter L-values were still very much lower than for all-maize kenkey. Traditional kenkey is usually wrapped in either banana leaves (Fanti) or corn sheaths (Ga), which results in a slight browning of the surface of the product after cooking. This does not seem to be of concern to the consumer, hence a reduction in whiteness by white cowpea is not expected to be a major setback. Red cowpea will, however, require dehulling to prevent the final product becoming brown. Other authors (Plahar and Leung, 1983) used a Hunterlab Model D25D Color Meter, calibrated with a standardised white tile (L $=$ 93.6, a $=$ 0.7 and b $=$ 0.2), and obtained L, a, and b values of 76.94 $\pm$ 0.14, -1.14 $\pm$ 0.06, and 10.55 $\pm$ 0.23, respectively, for traditional fermented maize doughs from Ghana. The differences between these L, a, and b values and those reported here may be due to the use of different standardisation constants. Also, those authors referred to fermented dough and not to the cooked product of fermented doughs used here.

Table 2.3. Colour parameters of all-maize and cowpea-supplemented kenkey (Hunter L, a and b values)

KENKEY[1]	L (mean ± sd)	a	b
M_4C_1	66.89 ± 0.46	0.10 ± 0.74	16.48 ± 1.37
Mw_4C_1	58.86 ± 2.02	3.00 ± 1.25	13.67 ± 2.14
Mw_4C_1[2]	61.09 ± 1.45	0.44 ± 0.08	15.72 ± 0.36
Mr_4C_1	49.70 ± 3.28	6.30 ± 1.13	10.86 ± 1.37
Mr_4C_1[2]	54.03 ± 1.87	8.07 ± 0.25	12.19 ± 0.33

[1] Sample codes in addition to those in Table 2.1; C = cooked, with subscript cooking time (hours); [2] Dehulled cowpeas.

Fracture experiments were conducted on kenkey stored at room temperature for 1, 24, and 72 h. The moisture contents were maintained by storing samples well wrapped up with aluminium foil in a closed bucket. Table 2.4 shows that fracture stress (a_f) increased with increasing cowpea levels. This may be due to the fact that cowpea supplementation results in a more homogeneous product, which is less prone to breakage than the traditional all-maize kenkey. In the traditional kenkey, intact maize hulls form areas of local stress concentrations, which makes the product more prone to fracturing (Luyten, 1988). In addition, starch/protein interactions may contribute to the increased firmness of the product. Fracture stress also increased on storage of the product, the greatest increase occurring in the first 24 h, during which fracture stress (a_f) values doubled. This may be attributable to a number of factors, amongst them setback and retrogradation of starch as the product cools (Bean and Setser, 1992), resulting in a firmer product.

The sharp sour taste of properly fermented kenkey (Plahar and Leung, 1983) was detected in all samples by all panellists. None registered off-smells resulting from the addition of cowpeas. Kenkey made from mixtures of 80% maize and 20% wholegrain white or dehulled red cowpeas were accepted by all the panellists as these compared very well with the traditional product. Three male panellists also

liked the kenkey supplemented with dehulled white cowpea, but the two female panellists thought it was smoother than the traditional product. Kenkey supplemented with wholegrain red cowpeas was rejected by all but one (male) of the panellists on account of its brown colour.

Table 2.4. Effect of cowpea level and storage on hardness of kenkey at constant moisture content.

Kenkey[1]	Cowpea level (%)	Storage time (h)	Moisture content (% w/w)	Stress at fracture $[\times 10^3 \, N \, m^{-2}]$[2]
M_4C_1	0	1	64	2.40 ± 0.10
		24	63	4.50 ± 0.20
		72	63	4.20 ± 0.20
Mw_4C_1	10	1	63	3.40 ± 0.30
		24	63	6.30 ± 0.10
		72	62	6.50 ± 0.10
Mw_4C_1	20	1	67	3.15 ± 0.10
		24	63	6.35 ± 0.20
		72	63	6.95 ± 0.60
Mw_4C_1	30	1	63	4.30 ± 0.20
		24	63	8.30 ± 0.10
		72	63	8.10 ± 0.20
Mw_4C_1	50	1	65	3.20 ± 0.20
		24	65	8.10 ± 0.10
		72	66	7.90 ± 0.10

[1]For abbreviation see Table 2.1 and Table 2.3, with the exception that the level of cowpea substitution ranges from 0-50% as specified above; [2] Mean ± SD; n = 3

Although the results of product evaluation must be interpreted cautiously, they, nevertheless, place additional weight on the argument that traditional kenkey could be enriched with 20% (w/w) cowpeas without necessarily affecting its basic physical and organoleptic properties. Large scale product evaluation is, however, required to establish absolutely the degree of acceptability of enriched kenkey.

Previous reports on cereal-legume mixtures (Bressani, 1985; Bressani and Scrimshaw 1961), and the results reported in this chapter, suggest that cowpea could be used successfully to supplement traditional cereal-based products. This will undoubtedly go some way towards improving the protein requirements of peoples of a region where animal sources of protein are often too expensive to obtain.

Acknowledgements

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Dry-milling and accelerated fermentation of maize for industrial kenkey production[1]

Abstract

The dry-milling of maize and accelerated fermentation of dough for kenkey production were studied as part of a wider investigation into the possibility of industrial production of a dehydrated kenkey flour. Dough containing an enrichment of lactic acid bacteria, was used successfully to achieve, within 24 h incubation at 30°C, the required level of acidification of dry-milled maize flour to obtain kenkey dough. Cabinet- and drum-drying were used to prepare dehydrated kenkey flour and pre-gelatinised aflata, respectively. Drum-drying was an effective method for preparing pre-gelatinised aflata, but it resulted in a 34% reduction in the titratable acidity (TA) of the fermented dough. Cabinet-drying, on the other hand, had a less drastic effect on the TA of fermented dough, suggesting the possible use of a mixture of drum-dried aflata and uncooked cabinet-dried flour for convenient preparation of kenkey at household level. Dry-milled maize flours had pasting and set-back viscosities that were inferior to those of the traditionally prepared doughs and consequently were unsuitable for the production of pre-gelatinised aflatas. Pre-gelatinised aflata from unfermented dry-milled flours resulted in a crumbly and friable kenkey product. It was concluded, therefore, that, although dry-milling of maize and accelerated fermentation of dough could drastically reduce kenkey production time, from about 6 days to within 24h, omission of the soaking step practised traditionally, can result in a product with inferior textural quality.

INTRODUCTION

Kenkey is a popular traditional, fermented maize product, still prepared commercially on an small artisan scale in Ghana. The traditional kenkey process has been described earlier (Muller, 1970; Muller and Nyarko-Mensah, 1972). In this process, maize is cleaned and steeped in excess water for 2 days, followed by milling. The meal obtained is made into a dough by adding water (1:3 v/w) and allowed to ferment naturally. The period for natural dough fermentation and souring

varies from 2 - 4 days, but it has been shown that 2 days at 30°C are sufficient to obtain the desired degree of souring (Nche *et al.*, 1994). The fermented dough is divided into two equal portions, one of which is slurried and stir-cooked to gelatinisation, giving rise to a sticky paste called the aflata, having a glutinous consistency (Muller, 1970). There is some dispute over which intermediate product should be called the aflata, but most reports and most indigenous kenkey consumers agree with the description by Muller (1970), which has also been adopted in this thesis. The aflata is usually mixed in equal proportion with the uncooked portion to produce a dumpling from which balls of appropriate size (≈ 300 - 500 g) are shaped, wrapped in maize sheaths (Ga kenkey) or banana leaves (Fanti kenkey) and cooked for 1 - 3 h, depending on the size, to give kenkey.

In the traditional process, the soaking step not only softens the grains thereby facilitating smooth milling, but also stimulates enzyme activity leading to amylolysis and proteolysis which contribute to the desired physical, chemical and organoleptic properties characteristic to kenkey (Sefa-Dedeh and Plange, 1989).

The technical feasibility of preparing a dehydrated kenkey flour for kenkey manufacture at the household level or in a small scale industry has not been explored to date. The total time taken to prepare kenkey the traditional way, from steeping to cooking, can be as much as 6 days. To adapt such a process to industrial scale would require, amongst other technical adaptations, a reduction in production time. This means, therefore, that the various steps in the whole process have to be looked at and modified, where necessary, to suit an industrial scale process, without significantly compromising the quality of the final product. One way of reducing production time is to skip the soaking step and mill the grains dry. Dough from this can then be fermented rapidly by using a starter dough containing an enrichment of lactic acid bacteria (LAB).

The objectives of this study were to investigate the technological feasibility of producing kenkey from dry-milled maize. This involved an investigation of the effects of dry-milling, compared with soaking-before-milling, on the gelatinisation of maize flour for aflata production and on the subsequent physical characteristics of the final product. Also, the use of a starter dough to accelerate fermentation was investigated.

MATERIALS AND METHODS

Maize (*Zea mays* L cv. obaatanba) was supplied by the Crops Research Institute, Council for Scientific and Industrial Research, CSIR, Kwadaso, Ghana. Grains were

milled using a hammer mill (Fritsch Pulverisette, Type 14.702, Marius Instruments, Utrecht, The Netherlands) with a fine rotor. Rotor speed was set on 2 and grains were milled to pass through a 4 mm sieve.

Microbiological analyses
Sample preparation and enumeration of lactic acid bacteria (LAB), moulds and yeasts and Enterobacteriaceae were as described by Nout *et al.* (1987).

pH and Acidity
A pH meter (Electrofact, Sweden) with a Schott N61 electrode was used to measure pH of fermenting dough. 90ml of distilled water were added to 10 g of sample and mixed in a blender (Stomacher 400 Type BA 7021, Seward Medical, London, U.K.) before recording the pH. Titratable acidity was determined according to Nout *et al.* (1989).

Pasting viscosity
A Brabender Visco-Amylograph (Brabender, Duisburg, Germany) was used to compare the hot and cold paste viscosities of traditionally fermented (TF) and unfermented (TUF) maize doughs as well as dry-milled, unfermented (DUF) and accelerated (starter) fermented (DAF) maize doughs. Samples were tested at 9% (w/w) dry solids basis. The slurry in the Visco-Amylograph bowl was heated from 50°C at a rate of 1.5°C/min to a maximum temperature of 95°C. The temperature was maintained at this maximum for 30 min before cooling at a rate of 1.5°C/min back to 50°C. A 250 cmg cartridge was used and the bowl speed was set at 75 rev/min. Viscosity was recorded in Brabender Units.

Colour determination
Colour parameters L, a and b, representing luminosity (brightness), redness and yellowness, respectively, were measured using a DR Lange Tricolor LFM 3 Colorimeter (Hunterlab, 9529, Lee Highway, Fairfax, Virginia, USA), calibrated with a standard white tile (L = 88.20, a = -0.96, b = -1.69). Two measurements were made of both surfaces of each sample.

Textural measurements
Fracture stress was measured with an Overload Dynamics, Instron-type instrument (Marius Instruments, Utrecht, The Netherlands), with two plates between which samples of standard cylindrical dimensions (length, 3cm; diameter, 2cm) were

placed vertically and compressed at constant speed (50mm/min) until fracture point. Fracture stress was calculated according to Van Vliet (1991).

Accelerated natural fermentation

A LAB-enriched starter dough was prepared by "back-slopping" (Nout *et al.*, 1989). Initially, a previously 24h naturally fermented dough was used to inoculate (at 10% w/w level) fresh dough for a further 24 h fermentation at 30°C. This was repeated until a stable culture, as indicated by a stable pH, TA and microbial count, was obtained. This was then used as a starter to accelerate the fermentation of fresh dry-milled maize dough. Inoculated dough was incubated at 30°C for 24 h to obtain an accelerated fermented dough (Fig. 3.2) that was then used for laboratory preparation of kenkey as described earlier (Nche *et al.*, 1994).

Drying

Two types of drying methods, viz. cabinet- and drum-drying were used. For cabinet-drying, traditionally fermented maize dough was spread on trays and placed in an electrically heated circulating air cabinet drier maintained at 60°C. Drying was done over a period of 3 h during which samples were taken out hourly for moisture, pH and titratable acidity measurements.

For drum-drying, 3 differently treated doughs were used. These were traditionally fermented dough (TF), traditionally soaked but unfermented maize dough (TUF) and dry-milled unfermented maize dough (DUF). During drum-drying, a 20% w/v dough slurry (in tap water) was applied onto the pre-heated (140°C) rotating (1.5 rev/min) drum of the drum drier (N.V. Goudsche Machinefabriek, Waddinxveen, The Netherlands). A film thickness of 0.2 mm was maintained.

Aflata and kenkey processing

Figures 3.1 and 3.2 summarise the schemes used for producing kenkey. Drum-dried dough was reconstituted by adding tap water (1:3 w/v). This gave a gluey paste, the aflata, which was then mixed, in equal proportion, with cabinet-dried, traditionally fermented dough to produce a dumpling from which portions (ca. 300g) were shaped into cylinders (25-30 mm diameter; 10 cm length) and wrapped, first in polyethylene sheaths, and then aluminium foil (Nche *et al.*, 1994).
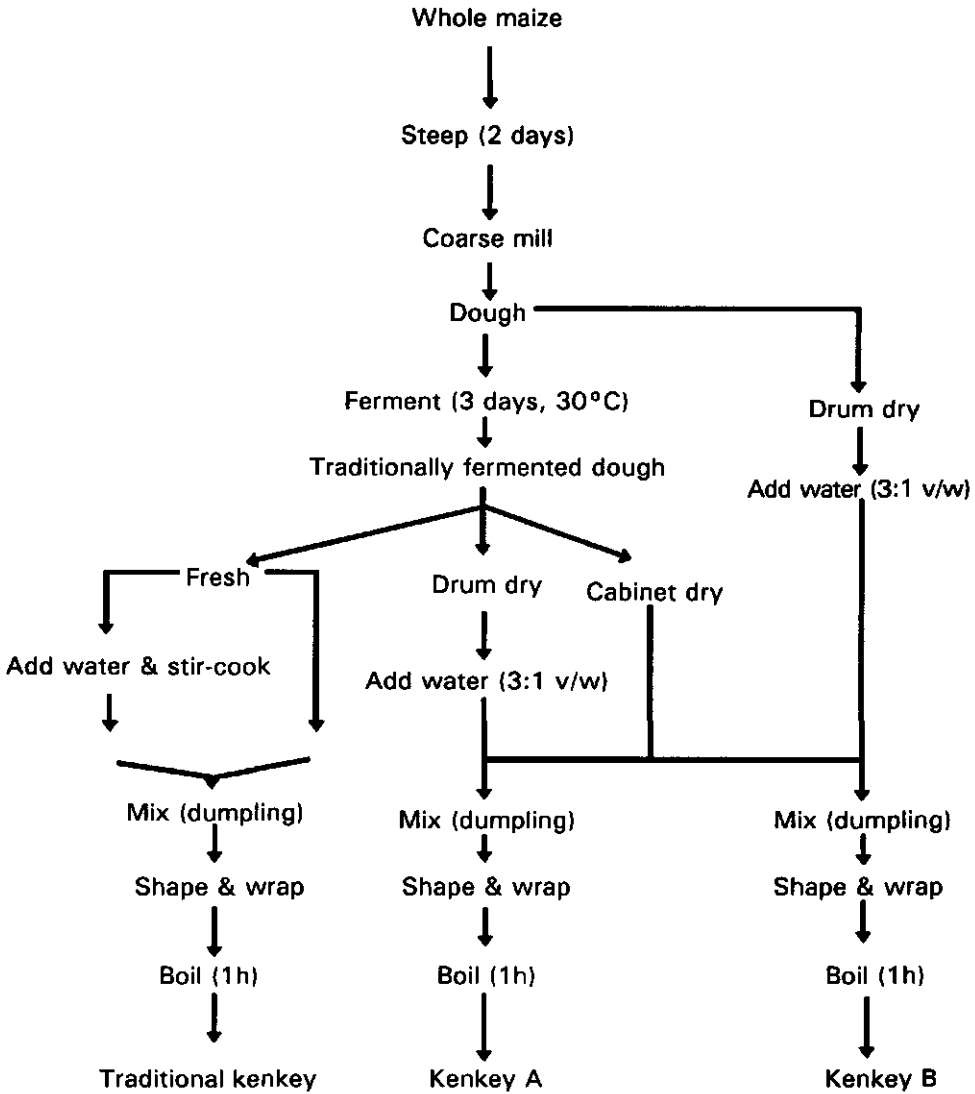
24

Whole maize

↓

Steep (2 days)

↓

Coarse mill

↓

Dough ————————————————————

↓   ↓

Ferment (3 days, 30°C)   Drum dry

↓   ↓

Traditionally fermented dough   Add water (3:1 v/w)

Fresh   Drum dry   Cabinet dry

↓   ↓

Add water & stir-cook   Add water (3:1 v/w)

↓   ↓   ↓

Mix (dumpling)   Mix (dumpling)   Mix (dumpling)

↓   ↓   ↓

Shape & wrap   Shape & wrap   Shape & wrap

↓   ↓   ↓

Boil (1h)   Boil (1h)   Boil (1h)

↓   ↓   ↓

Traditional kenkey   Kenkey A   Kenkey B

Figure 3.1. Production of kenkey from traditionally fermented maize dough

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Whole maize
↓
Dry mill
↓
Add water (80% v/w)
↓

Traditionally
Fermented dough
↓

Drum dry ← Dough
↓

Cabinet dry

Add water (3:1 v/w)

Inoculate with
back-slop (10% w/w)
↓

Mix (dumpling)

Ferment (24 h, 30°C)
↓

Add water and stir-cook
↓

Shape and wrap

Aflata   Raw dough
↓

Boil (1 h)

Mix (dumpling)
↓

Kenkey C

Shape and wrap
↓

Boil (1 h)
↓

Accelerated kenkey, D
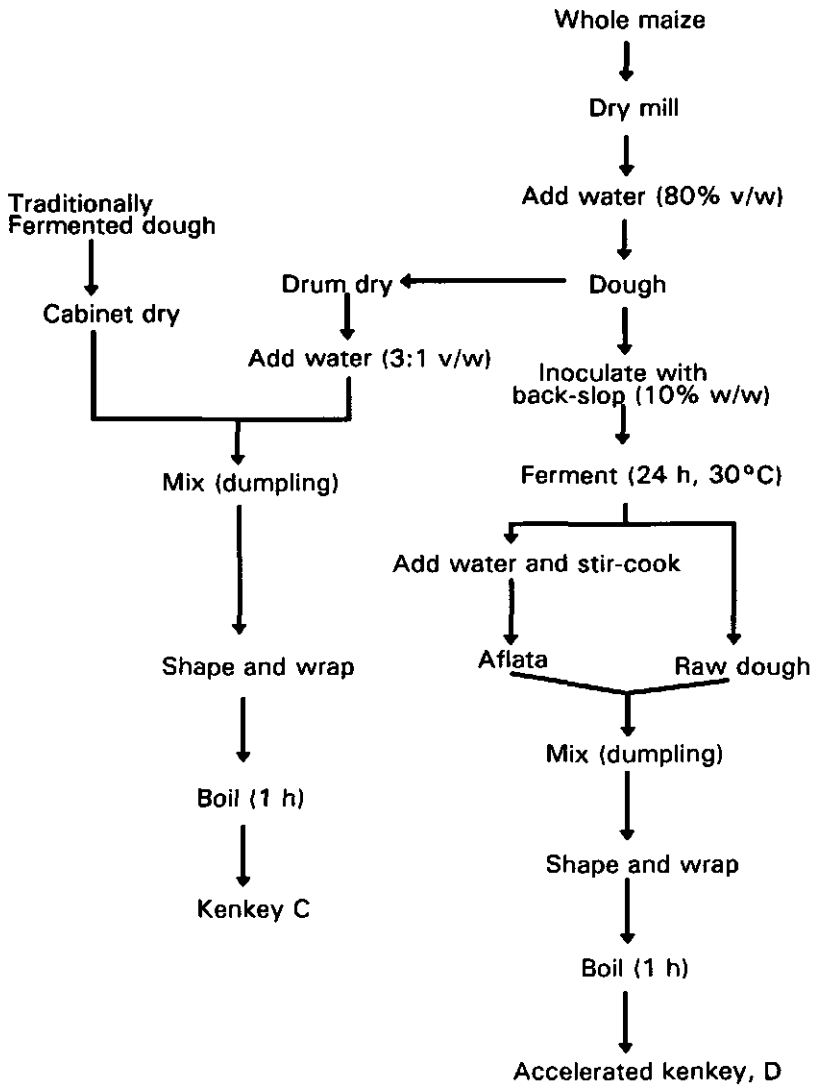
Figure 3.2. Production of kenkey from dry-milled maize flour

These were then boiled for 1h to give kenkey (Fig. 3.1, A). Aflatas from wet and dry-milled, unfermented drum-dried maize doughs were also mixed, in equal proportions, with cabinet-dried traditionally fermented dough to produce kenkey (Fig. 3.1, B and Fig. 3.2, C respectively). Kenkey was also produced from both

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traditionally (Fig. 3.1) and accelerated (Fig. 3.2, D) fermented doughs.

Sensory evaluation
The appearance, texture, smell and taste of all experimental kenkey products were compared with laboratory-made "traditional" kenkey by a 4-member panel consisting of 1 Ghanaian familiar with kenkey and 3 untrained panellists.

Sensory evaluation of samples of kenkey was carried out in Ghana by a 10-member trained taste panel, in triplicate on 3 consecutive days. The samples were judged on the basis of the following attributes and properties: Appearance (usual kenkey colour, acceptable kenkey colour, uniform mix), Texture (breaks easily, crumbliness, softness, hardness, mouldability, stickiness to fingers and palm), Smell (strong, mild, no smell, kenkey), and Taste (pleasant, sour, chewiness, sticks in throat, pleasant after-taste).

RESULTS

The lactic acid bacteria population of naturally fermenting, soaked maize dough increased by about 2 log cycles in 2 days, after which it stabilised (Table 3.1). The mould and yeast counts remained very low ($\log_{10}$ < 2.7 cfu/g) throughout incubation. The Enterobacteriaceae count, on the other hand, fell from an initial high level ($\log_{10}$ 6.1 cfu/g) on day 0 to < $\log_{10}$ 2.7 cfu/g within 24 h.

Table 3.1. Fermentation parameters of wet-milled maize dough.

Time (days)	pH	Titratable acidity(%)[1]	LAB[2]	Yeasts[2]	Enterobacteriaceae[2]
0	5.24	0.37	6.9	< 2.7	6.1
1	3.96	0.83	7.4	< 2.7	< 2.7
2	3.94	0.92	8.8	< 2.7	< 2.7
3	3.95	0.92	8.5	< 2.7	< 2.7

[1]Expressed as lactic acid (w/w); [2]$\log_{10}$ cfu/g

Within the same period of time, the pH of the fermenting dough decreased from an initial 5.24 to 3.96, and thereafter remained constant (Table 3.1). This decrease

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in pH was accompanied by an increase in titratable acidity (Table 3.1) to a final value of 0.92% (expressed as w/w lactic acid, wet basis).

After 3 "back-slopping" cycles at 30°C, the measured parameters of the starter dough remained relatively constant (Fig. 3.3), suggesting the establishment of a stable microflora. This was used successfully to obtain an accelerated acidification of fresh dough. Within 24 h, the pH of the inoculated dough dropped from 5.65 to 3.79, and a titratable acidity value of 1.24% (wet weight basis), was significantly higher than was obtained after 3 days for traditionally fermented dough.
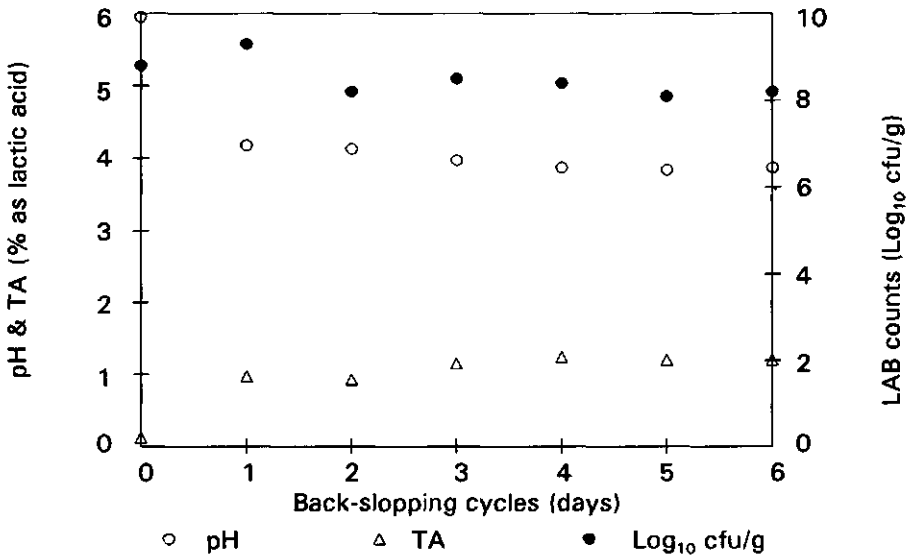
Figure 3.3. pH, titratable acidity and LAB counts during accelerated natural fermentation of dry-milled maize flour

Drying the traditionally fermented maize dough at 60°C for 3 h in a cabinet drier reduced the moisture content from 54 - 10% without significantly affecting its pH (Table 3.2). Only a marginal decrease in titratable acidity from 2.87% to 2.49 % (dry weight basis) was observed. Drum-drying resulted in a significant (p < 0.05) reduction in the TA of freshly fermented dough, however, from 2.87% to 1.90% (w/w as lactic acid).

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Table 3.2. Effect of cabinet drying at 60°C on the pH and titratable acidity of kenkey dough

Drying time(h)	Moisture content(%)[1]	pH	TA(% as lactic acid)[2]
0	54	3.75	2.87
1	35	3.75	2.58
2	20	3.75	2.57
3	10	3.76	2.49

[1]On fresh weight basis.
[2]Titratable acidity, expressed on dry weight basis.
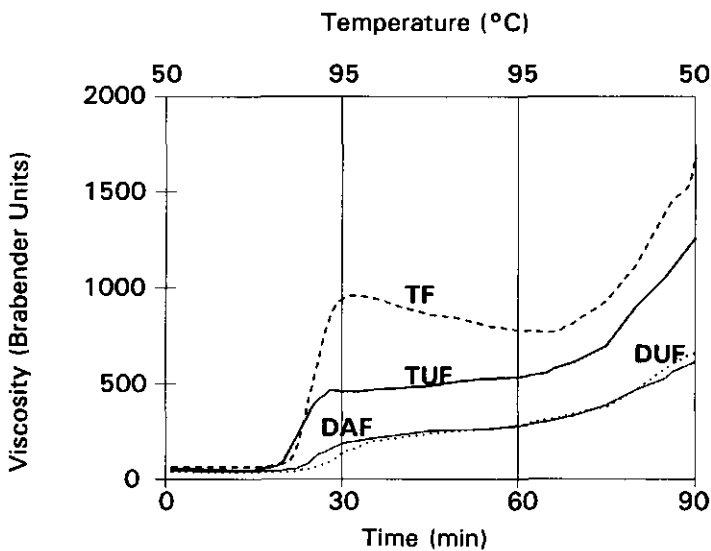
Figure 3.4. Visco-Amylograms of doughs from traditionally-treated and dry-milled maize. **(TF)** Traditional fermented dough; **(TUF)** Traditional unfermented dough; **(DAF)** Dry-milled accelerated (starter) fermented dough; **(DUF)** Dry-milled unfermented dough

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The pasting viscosities of standard slurries of the differently prepared maize doughs are shown in Fig. 3.4. Traditionally fermented maize dough (TF) gave the highest peak viscosity of 950 Brabender Units (B.U.) with the highest set-back value. The corresponding unfermented maize dough (TUF) had lower peak and set-back viscosity values (Fig. 3.4). Both these doughs had significantly higher set-back viscosities than dry-milled accelerated fermented (DAF) and dry-milled unfermented (DUF) doughs.

Table 3.3. Surface colour of dough and kenkey

Sample	L	a	b
Traditional dough	76.08 ± 1.16[1]	-1.51 ± 0.71	14.35 ± 0.25
Traditional kenkey	63.42 ± 0.54	1.47 ± 2.44	13.98 ± 1.40
Accelerated dough	73.54 ± 0.94	-0.81 ± 0.94	14.60 ± 0.43
Accelerated kenkey	63.53 ± 1.55	0.56 ± 1.30	15.05 ± 0.70

[1]Mean ± sd; n = 4.

Table 3.4. Fracture stress of the different kenkey products

Kenkey	Moisture content (%)	Fracture stress (x 10^3 Nm^{-2})
Traditional	63.0	6.43 ± 0.52[1]
Kenkey A	67.0	4.67 ± 0.09
Kenkey B	69.0	4.77 ± 0.26
Kenkey C	68.0	3.67 ± 0.53
Kenkey D	60.6	5.65 ± 0.15

[1]Mean ± SD; n = 4

Table 3.3 shows that there was no significant difference in colour between traditionally made kenkeys and those made by the accelerated process. There was some loss in luminosity of dough when processed into kenkey, however. The

luminosity (L) values for kenkey were comparable with values obtained earlier (Chapter 2) for traditional kenkey.

Table 3.4 shows the values for fracture stress, a textural measure of the hardness of the product. There was no significant difference ($p < 0.05$) in fracture stress values for traditional kenkey and kenkey D. Both these kenkeys had higher, though not significant, fracture stress values than kenkeys A and B. Only Kenkey C had a significantly lower fracture stress value.

Sensory assessment at the laboratory level revealed that, compared with laboratory-made "traditional" kenkey, kenkey products B, C and D were of unsatisfactory texture, taste and smell. On the other hand, kenkey A got promising assessments; this product was selected for sensory evaluation by a local Ghanaian panel.

During sensory evaluation in Ghana, the following samples were compared: (1) Kenkey prepared by reconstituting and cooking of dry kenkey mix (Fig. 3.1, A); (2) Local samples of kenkey bought from the same vendor each day. Both types 1 and 2 were perceived as normal Ga kenkey of commendable quality. However, minor differences were detected. Type 1 scored better with respect to texture (less sticky) and mildness of taste; whereas type 2 had better scores for uniformness and characteristic kenkey smell and taste.

DISCUSSION

The results shown in Table 3.1 and Fig. 3.3 confirm earlier reports on naturally fermenting cereal doughs (Nche *et al.*, 1994; Chavan *et al.*, 1988). As LAB counts increased, more acid was produced, as shown by increasing titratable acidity (TA) and the decline in pH. Within 3 days of fermentation, the pH fell to 3.96, well within the inhibitory range for food pathogenic and spoilage bacteria (Hamad and Fields, 1979). This drop in pH is presumed responsible for the very low levels of Enterobacteriaceae and yeasts (Nout *et al.*, 1989). Other workers attribute this to antibacterial substances such as bacteriocins (Mensah *et al.*, 1991), organic acids, hydrogen peroxide and antibiotic-like substances (Nout *et al.*, 1989; Gibbs, 1987) produced by the dominant LAB. The rate of acidification was even higher when a starter dough was used, with the pH dropping from 5.65 to 3.79 within 24 h. This was an indication that inoculum recycling resulted in a natural selection of LAB which are tolerant to low pH conditions and can bring about rapid acidification of fresh dough when used as an inoculum (Nout *et al.*, 1989). Previous work (Nche *et al.*, 1994) has shown that *Lactobacillus plantarum* and *Pediococcus spp.*

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dominate the latter stages of maize dough fermentation and may, therefore, be responsible for the rapid acidification of inoculated dough.

The decrease in TA following drum-drying could be due to evaporation of some acid at the higher temperature of the drum (140°C), necessary for proper gelatinisation of the starch component of the dough. Since no cooking was required during cabinet-drying, a lower temperature could be used to achieve the required level of dehydration without the loss of acid through evaporation. The fact that dehydration of the fermented maize dough in a cabinet drier did not significantly affect the pH and titratable acidity is interesting as this portion can then be used to prepare dumplings by mixing with drum-dried aflata. This way the diminished acidity of the drum-dried portion can be partly compensated for by the relatively more sour cabinet-dried portion.

Traditionally fermented dough, from soaked maize, gave the highest peak viscosity of 950 B.U. as compared with the lower peak viscosity values (≤ 300 B.U.) of doughs made from dry-milled maize (Fig. 3.4). This finding is contrary to reports on fermented sorghum flour (Adeyemi, 1983), but agrees with the report of Banigo et al. (1974), who found that soaking and wet-milling of maize for ogi manufacture increased the swelling and thickening characteristics of the maize starch component.

Damage of starch granules, as may occur during dry-milling, can result in increased enzymic hydrolysis of starch up to two-fold (Schweizer et al., 1988) or even seven-fold (Wong and Trianedes, 1985) in some instances. Thus fewer intact starch granules undergo swelling and partial solubilisation, especially of amylose. The very low hot and cold paste viscosities of dry-milled flours could, therefore, be the result of mechanical starch damage. Soaking may reduce such damage during milling and thereby result in high viscosities of wet-milled maize doughs. Any inhibition of the release of amylose, which may occur due to the binding of lipids and proteins to starch granules can inhibit gelatinisation and set-back (Eliasson et al., 1981). It has been reported that delipidation resulted in the lowering of the gelatinisation temperatures (T_g) of wheat and maize starches (Vasanthan and Hover, 1992) and in an increase in the pasting viscosity of wheat starch (Eliasson et al., 1981), although a similar treatment increased the T_g of potato and lentil starches (Vasanthan and Hover, 1992). There is, therefore, the possibility that a lack of proper lipolysis and proteolysis, as might be the case with unsoaked maize grains, might lead to the protein and lipid matrix surrounding the starch granules inhibiting the proper swelling of granules for gelatinisation to occur. It would certainly be interesting to investigate the enzyme activities during soaking

and compare this with that in dry-milled grains.

A high set-back viscosity is desired for proper kenkey quality as this determines the cohesive capacity of the aflata, which serves as a binding agent (Sefa-Dedeh and Plange, 1989). If proper gelatinisation is not achieved during the heating phase in the Visco-Amylograph, the set-back viscosity would be low, an indication that any kenkey from such a dough will be crumbly and friable.

During the drum-drying of soaked maize dough, a complete breakdown of the starch granule structure (Bean and Setser, 1992) and hence rapid gelatinisation and solubilisation of released starch occurs. Poor release of amylose from dry-milled flours would, however, result in less gelatinisation and subsequently a lower set-back potential during drum-drying. Rehydration (3:1 v/w) of the drum-dried flour yielded a smooth and gluey aflata, easier to handle than the hot paste obtained from the traditional aflata process. The low degree of set-back in drum-dried, dry-milled unfermented maize flour is reflected in the inferior fracture stress values for kenkey C, produced from this flour (Table 3.4). In fact, kenkey C was a crumbly and friable product that fell apart quite easily. The slightly lower fracture stress values of kenkeys A and B, compared with traditional kenkey, could be accounted for by the higher moisture contents of these kenkeys. Kenkeys A and B were, however, less brittle, reflecting the higher degree of starch gelatinisation and subsequent set-back (Fig. 3.4, see TF and TUF). This is a desirable characteristic of kenkey and can be obtained only in the traditional process if small quantities of kenkey are made at any one time. To obtain such a quality for larger quantities of kenkey would be laborious and time consuming. Bediako-Amoa and Austin (1976) also observed that satisfactory aflata could be produced from pre-gelatinised starch to avoid the strenuous physical work involved in the traditional process.

For logistic reasons, kenkey samples 1 and 2 were made from different batches of maize, and their fermentation was carried out under different conditions (i.e. in the laboratory in The Netherlands, and artisanally in Ghana). These factors will certainly have contributed to the observed difference in smell and taste. For the purpose of the present research, however, the textural behaviour was of major importance, since it could be strongly affected by pre-cooking, dehydration and reconstitution. The positive response on the texture of kenkey 1 (Fig. 3.1, A) is encouraging indeed and indicates that the concept of an intermediate "kenkey dry mix" has good chances of consumer acceptance.

The findings in this Chapter clearly indicate that, although accelerated acidification of dry-milled maize flour can be achieved by using a starter dough,

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proper gelatinisation is necessary during the aflata process in order to obtain the desired physical characteristics of kenkey. The rejection of kenkeys C and D by the laboratory-scale screening panel was a confirmation of the physical evidence showing inferior pasting properties of dough from dry-milled maize, compared with dough from wet-milled maize. Pre-gelatinisation offers an alternative way of aflata production with minimum physical labour, on condition that the treatment of dough prior to gelatinisation is optimised with regard to maximum swelling and set-back.

Acknowledgement

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Soaking of maize determines the quality of aflata for kenkey production[1]

━━━

Abstract

Aflata is a gelatinised maize paste, serving as intermediate in the manufacture of kenkey. The effect of water uptake during soaking of whole or dry-milled maize, extent of starch damage, dough pH, fermentation time and of endogenous and added enzymes on pasting and set-back viscosities of aflata dough were studied. Water uptake by coarsely dry-milled maize (grits) reached 63% in just 1 h, compared with 50% in 3 days for whole grain. High endogenous proteolytic and saccharolytic enzyme activities were recorded in both grits and whole maize when soaked at 4 or 25°C. These were significantly reduced after soaking at 60°C. Soaking of grits at 60°C with a protease, or wet fine-milling of fermented grits resulted in significant ($P < 0.05$) increases in pasting viscosities. Peak viscosities increased with fermentation up to 24 h. Pasting viscosities decreased with increased extent of starch damage caused by repeated milling of dough.

INTRODUCTION

Kenkey, a popular Ghanaian staple is produced from fermented maize dough. During kenkey production (Muller and Nyarko-Mensah, 1972; 1993; Nche et al., 1994a), part of the fermented dough (usually half) is slurried and cooked to gelatinisation to give a thick and sticky paste, called the aflata. This paste is then mixed with the uncooked remainder of the dough and serves as a binding agent (Sefa-Dedeh and Plange, 1989) as well as moisturizer. When kneaded together, the aflata holds the uncooked dough together into a dumpling which can then be shaped, wrapped and boiled to give kenkey.

      The quality of the aflata is very important in determining the desired textural qualities of the final product. The choice of ingredients and the pre-treatment of maize and dough for aflata production are, therefore, crucial to the achievement of these qualities. Local women kenkey producers recognise this fact and are meticulous in their choice of maize, usually favouring maize with a high swelling index during soaking (Sefa-Dedeh & Plange, 1989).

In an attempt to shorten the long and tedious traditional kenkey manufacturing process, dry-milling and accelerated fermentation and the use of pre-gelatinised aflata have been suggested (Bediako-Amoa and Austin, 1976; Nche *et al.*, 1994b). Dry-milling, however, has been shown to cause significant starch damage resulting in a dough of very low pasting and set-back viscosities (Adeyemi and Beckley, 1986). Soaking of maize, on the other hand, is known to limit starch damage during subsequent milling (Akingbala *et al.*, 1987) and is considered crucial to the production of aflata with high pasting and set-back viscosities required for binding uncooked dough in the dumpling prior to cooking (Nche *et al.*, 1994b). As soaking usually takes 24-48 hours, omission or shortening of this step would be desirable if production time is to be reduced for the purpose of industrial-scale kenkey manufacture. However, given the importance of soaking, it is necessary to understand which changes occur during this step and how they affect the optimum properties of aflata dough. These changes could be (bio)chemical (enzymatic, pH, acidity), microbiological (microbial proliferation) or physical (water uptake) and it is the aim of this study to investigate their individual and/or collective roles in determining the behaviour of aflata during kenkey production.

MATERIALS AND METHODS

Maize (*Zea mays* L cv. *obataanba*) was supplied by the Crops Research Institute, CSIR, Kwadaso, Ghana.

Cleaning, disinfection and soaking of whole maize
Before soaking, whole maize kernels were first rinsed with sterile distilled water and then disinfected for 5 min at room temperature in a 1% solution of sodium hypochlorite. Treated kernels were then rinsed with sterile distilled water. Soaking took place at 4 or 25°C for 72 h, during which samples of steep water were analyzed on a daily basis for microbial load. Untreated maize soaked under the same conditions served as a control.

Dry milling
Dry whole maize was coarsely milled in a hammer mill (Fritsch Pulverisette Type 14.702, Marius Instruments, Utrecht, The Netherlands) using a 12 teeth rotor and a 4 mm screen to obtain grits. Rotor speed was set at 2.

Wet milling

Soaked whole grains were also coarsely milled using a 12 teeth rotor and a 4 mm screen. A 24 teeth rotor was then used together with either a 1 mm or 0.5 mm screen for fine-milling fermented dough from coarsely milled grits. Rotor speed was maintained at 2.

Microbiological analysis

Sampling, dilution and enumeration of total aerobic mesophyllic bacteria, lactic acid bacteria, Enterobacteriaceae and fungi were as described earlier (Nout *et al.*, 1987).

Endogenous enzyme activity

Using API-ZYM kit (API Products, Montalieu, Vercieu, France), endogenous enzyme activities in 1:10 homogenates (in distilled water) of soaked and dry maize were evaluated. Soaking was done at $4°C$, $25°C$ and $60°C$. Dry milled maize (DM) was used as a standard and the test samples were either dry maize milled before soaking (DMS) or whole maize soaked before milling (WMS). The levels of enzyme activity were read from the standard API-ZYM colour chart after incubating samples for 4 hours at $37°C$. This was on a scale of 0 - 5 indicating the amount of substrate hydrolysed by the enzymes; where 0, 1, 2, 3, 4, 5 represented 0, 5, 10, 20, 30 and $\geq$ 40 nmoles of degraded substrate, respectively.

Water uptake

Pre-weighed whole kernels were soaked for 12, 24, 48 and 72 h, after which the water was drained and the weight increase measured as water uptake. Samples were taken of dry milled maize grits soaked for 2, 4, 6 and 8 h, weighed and then dried for 48 h in a hot air oven maintained at $80°C$ to determine their moisture contents. Water uptake was determined by subtracting the moisture content of unsoaked maize flour from that of an equivalent amount of soaked grits.

Viscosity of maize dough

A Brabender Visco-Amylograph was used to measure the pasting characteristics of variously treated maize doughs. 9% (w/w dry matter) slurries of dough in distilled water were used and conditions used were as described elsewhere (Nche *et al.* 1994b).

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Treatment with proteolytic enzyme
Some of the dry-milled maize was also soaked at 25 and 60°C in a 1% (v/v) of a heat-stable mixture of acid, neutral and alkaline bacterial proteases (Protease-L 660, Solvay Enzymes, Hannover) solution to determine the effects of proteolytic enzyme activity on the pasting characteristics of the resulting maize dough.

Effect of pH on pasting characteristics
Grits were slurried with distilled water and pH adjusted with 1 N HCl to the desired level. The pasting viscosity of the slurry was then determined using a Brabender Visco-Amylograph as described above.

Fermentation time
Grits were made into a dough by adding water (8:1 v/w), inoculated with recycled starter dough (Nche *et al*. 1994b) and fermented at 30°C for 0, 4, 8, 12 and 24 h. At the end of each fermentation period, the dough was slurried in distilled water and the slurry was adjusted to pH 6.0 with 1 N NaOH to eliminate the pH effect before measuring the pasting characteristics of the dough.

RESULTS AND DISCUSSION

During soaking of whole maize at 4 and 25°C increases of total aerobic counts in soak water were observed after 48 h incubation, reaching levels of $> 10^5$ cfu/ml in non-disinfected samples. On the other hand, 1% (v/v) sodium hypochlorite was very effective at keeping the microbial load of disinfected kernels below 10^4 cfu/ml, and was subsequently used in cases requiring kernel disinfection.

Water uptake at 4 and 25°C by soaking whole kernels reached 42% (w/w, wet basis) after 24 h, and 50% after 72 h of soaking. Predictably, hydration was faster when the maize was coarsely milled before soaking. Water uptake reached 63% in just 1 h of soaking.

A high endogenous enzyme activity was recorded in both dry and soaked maize at 4°C and 25°C (Fig. 4.1). Following soaking at 4°C and 25°C, a-galactosidase and a-mannosidase were stimulated. ß- galactosidase, a- and ß-glucosidase, N-acetyl-ß glucosaminidase, leucine aralymidase and valine aralymidase activities remained unchanged, whilst cystine aralymidase, trypsin and chymotrypsin were suppressed. With the exception of ß-galactosidase and a-mannosidase, all proteolytic and saccharolytic enzyme activities were suppressed following soaking at 60°C.
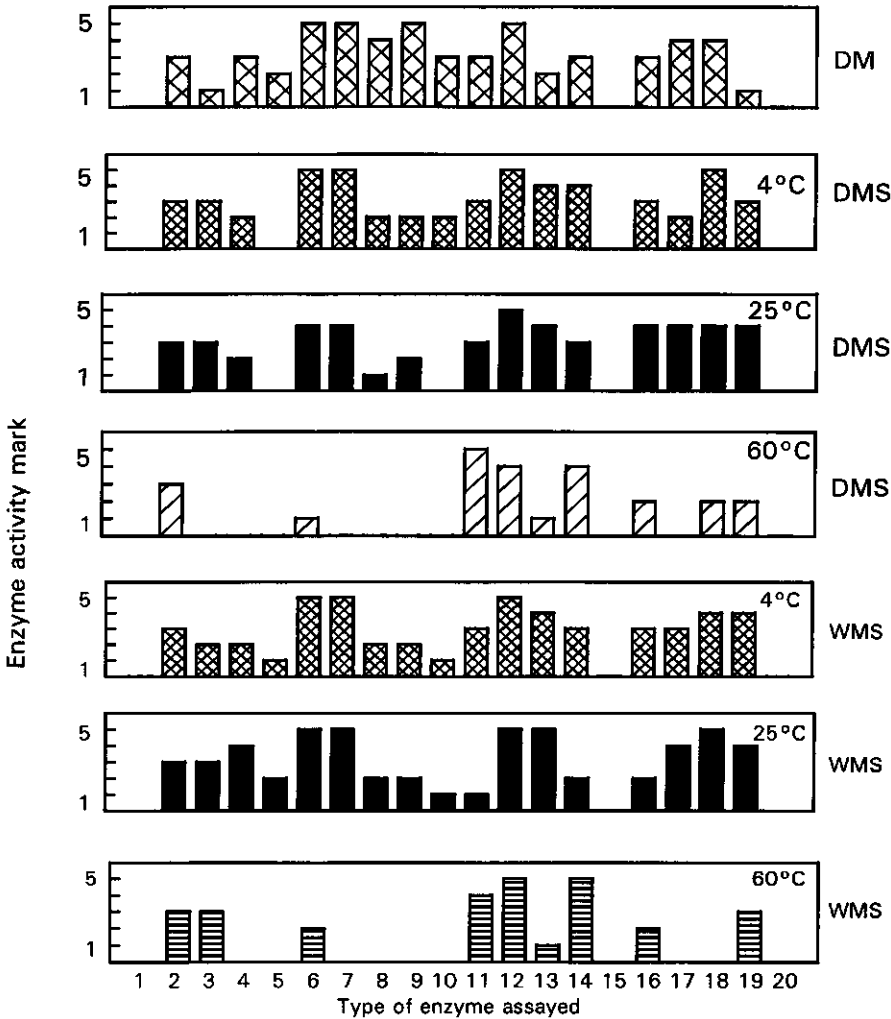
Figure 4.1: Endogenous enzyme profiles of soaked and dry maize as determined with an API-ZYM kit.

DM = dry-milled maize; DMS = dry-milled maize soaked for 2h; WMS = wet-milled maize; (1) control; (2) alkaline phosphatase; (3) esterase(C4); (4) esterase lipase (C8); (5) lipase (C14); (6) leucine aralymidase; (7) valine aralymidase; (8) cystine aralymidase; (9) trypsin; (10) chymotrypsin; (11) acid phosphatase; (12) naphthol-AS-bi-phosphohydrolase; (13) a-galactosidase; (14) ß-galactosidase; (15) ß-glucoronidase; (16) a-glucosidase; (17) ß-glucosidase; (18) N-Acetyl-ß-glucosaminidase; (19) a-mannosidase; (20) a-fucosidase.
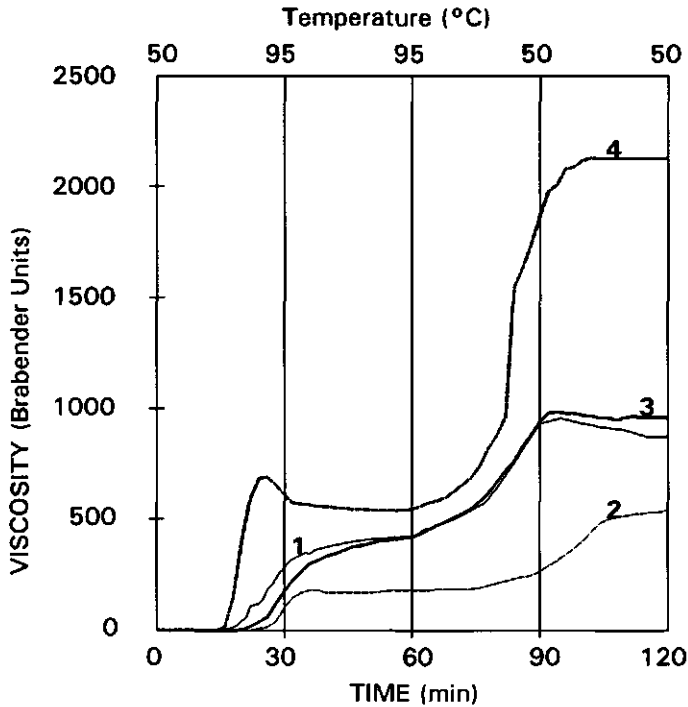
Figure 4.2. The effect of soaking, with and without protease L-660 on the pasting properties of dry-milled maize dough. (1) dry-milled grits + water; (2) dry-milled grits soaked for 2h at 25°C with protease L-660; (3) dry-milled grits soaked for 2h at 60°C; (4) dry-milled grits soaked for 2h at 60°C with Protease L-660.

Dry-milling resulted in a dough with very low pasting and set-back viscosities (Fig. 4.2), confirming earlier reports (Adeyemi and Beckley, 1986; Akingbala *et al.*, 1987) of the detrimental effect of dry-milling of maize on the pasting properties of ogi flour. Hydration alone did not improve the pasting properties of the maize dough since the soaking of grits at 60°C still did not improve on the very poor pasting characteristics. The gelatinisation temperature was 85°C and no peak was obtained during the heating phase, suggesting incomplete swelling of granules and an incomplete gelatinisation (Olkku and Rha, 1978).

When a heat-stable proteolytic enzyme was added to soaking grits at 60°C, it resulted in significantly higher pasting and set-back viscosities (Fig. 4.2). The index of gelatinisation, peak viscosity and set-back viscosity of 610 B.U., 1325

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B.U. and 1260 B.U., respectively, were comparable to those reported for traditional fermented maize dough (Nche et al., 1994b), and ogi from soaked maize (Adeyemi and Beckley, 1986). The gelatinisation temperature was also reduced from 85°C to 73°C. Soaking of grits with protease at room temperature, on the other hand, further reduced the pasting and set-back viscosities of the resulting dough (Fig. 4.2). These observations may be explained in terms of starch damage during dry-milling and the high endogenous enzyme activity in soaked grains. Dry-milling can result in high levels of mechanical starch damage (Akingbala et al., 1987). Adeyemi and Beckley (1986) reported levels of 41.1% damaged starch in dry-milled maize. Such damage renders starch granules susceptible to attack by either endogenous amylolytic enzymes or hydrolytic enzymes of fermenting microorganisms (Akingbala et al., 1987). The result is a dough with very much reduced pasting and set-back viscosities.

How endogenous or added proteolytic enzymes affect the viscosity of either whole or milled maize during soaking may depend on the arrangement of starch granules and the composition of the material embedding the granules in the endosperm. In the endosperm of wheat grains, for example, starch granules are surrounded by a composite of proteins, minerals and enzymes (Knight, 1965; Barlow et al. 1973). If this is true for maize grains, the effect of added or endogenous proteolytic enzymes, during soaking, will be to break down such a matrix embedding starch granules, thus allowing them to swell freely and gelatinise faster and better. Addition of the heat-stable proteolytic enzyme, protease L-660, during soaking of dry-milled maize flour at 25°C, did not affect pasting properties. This could be expected since firstly, 25°C is not the optimum temperature for this enzyme and secondly, because its effect would be counteracted by hydrolysis of damaged starch. The use of other proteases with optimum activities at ambient temperatures had a similar effect (data not included) to that obtained with Protease L-660. At 60°C, however, the activity of protease L-660 is highest and the matrix structure holding granules together (Knight, 1965; Tuschhoff, 1987) may be weakened, thus releasing starch granules. Also, at 60°C, all endogenous and microbial enzyme activity is suppressed, hence no hydrolysis of damaged or freed starch granules will occur. Following such treatment, starch granules will swell freely, when heated, exuding solubilised amylose (Miller et al., 1973) that will significantly increase the pasting and set-back viscosities of the dough. Sulphur-reducing agents have also been used to disrupt protein disulphide bonds and enhance the swelling and gelatinisation of starches (Hamaker and Griffin, 1993).

Soaking of maize and the eventual fermentation of dough results in a

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lowered pH. To investigate how this factor may affect the pasting properties of aflata dough, the doughs from dry grits were adjusted with 1 N HCl to pH 6.0 (initial pH of dry-milled maize), 5.6 (pH of wet-milled maize) and 3.6 (pH of a 3 days' fermented dough), and their pasting and set-back viscosities were measured. Fig. 4.3 shows that lowering the pH of dry-milled flour had no positive effect on the pasting viscosity, which was slightly reduced instead. In all three cases, no peak was obtained during the heating phase, again indicating incomplete gelatinisation and consequently low set-back.
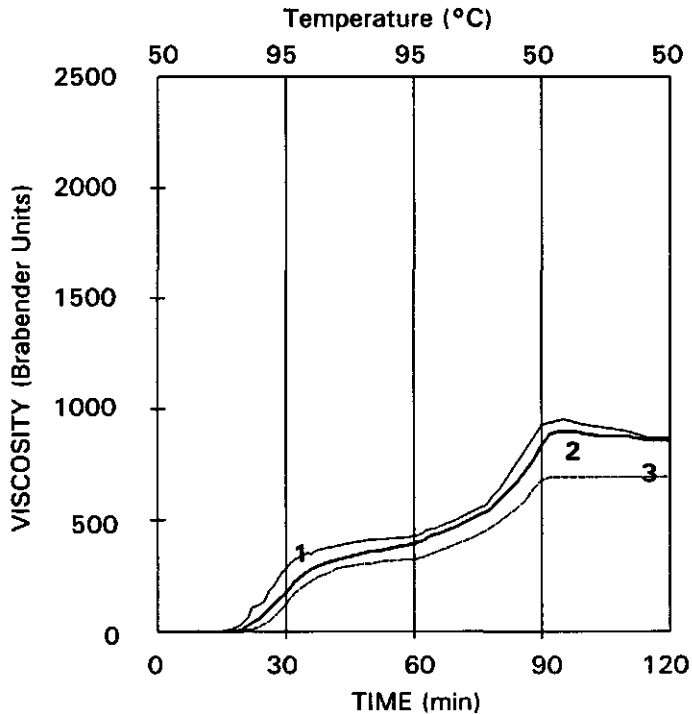
Figure 4.3. The effect of pH on the pasting properties of dough from dry-milled maize. (1) pH 6.0; (2) pH 5.6; (3) pH 3.6

Wet fine-milling (to pass through a 1.0 mm sieve) of fermented dough from grits, however, resulted in a significant increase in pasting viscosity (Fig. 4.4). In this case wet-milling limited mechanical damage of freed starch granules. Also, the lower pH of fermented dough would inhibit endogenous amylases to some extent.
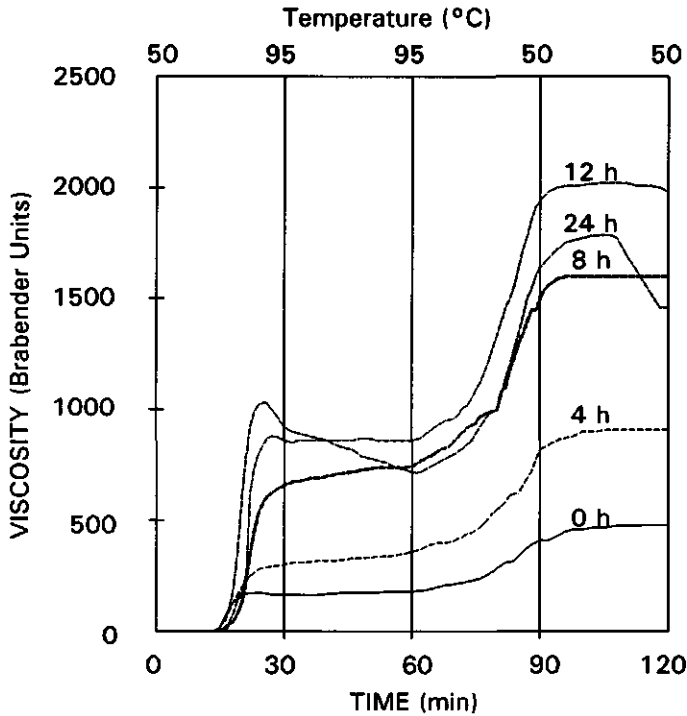
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Figure 4.4. The effect of fermentation time (0, 4, 8, 12, and 24 hours) on the viscosity of dough from dry milled maize.

A limited loss of starch through amylolysis would therefore occur. Only with wet-milling following fermentation could the real effects of fermentation on the viscosity of maize dough be observed. Brabender Visco-Amylograms of doughs fermented for 0, 4, 8, 12 and 24 h and then milled to pass through a 1.0 mm sieve, and adjusted to pH 6.0, show increasing pasting and set-back viscosities (Fig. 4.4). At 0 h, 4h and 8 h, no clear peak viscosities were obtained due to incomplete gelatinisation (Olkku and Rha, 1978), whereas after 12 and 24 h of fermentation, peak viscosities were obtained during the heating phase indicating a higher degree of gelatinisation. Fermentation for 24 h resulted in a lower gelatinisation temperature, a higher peak viscosity, but a reduced set-back viscosity compared with a 12 h fermented dough. These observations are in agreement with earlier reports (Banigo et al., 1974; Adeyemi and Beckley, 1986) that fermentation of maize flour increases the swelling and thickening potential of

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the maize starch component. After 24 h fermentation, the starch component rapidly swells on heating to 95°C. Increasing the temperature of a starch suspension results in a reversible decrease in water mobility (Jaska, 1971) due to absorption by starch granules. At temperatures higher than the gelatinisation temperature, water mobility increases due to a decrease in the microviscosity of starch granules as they break up and exude their solubilised contents. The break up of most, if not all, of the swollen starch granules could mean that it takes longer for significant set-back to occur, hence the reduced set-back viscosity. Despite this, a 12 - 24 h fermentation would give a dough with adequate pasting characteristics provided, of course, that the maize is coarsely dry-milled, fermented and the aflata portion wet-milled before cooking to give aflata.
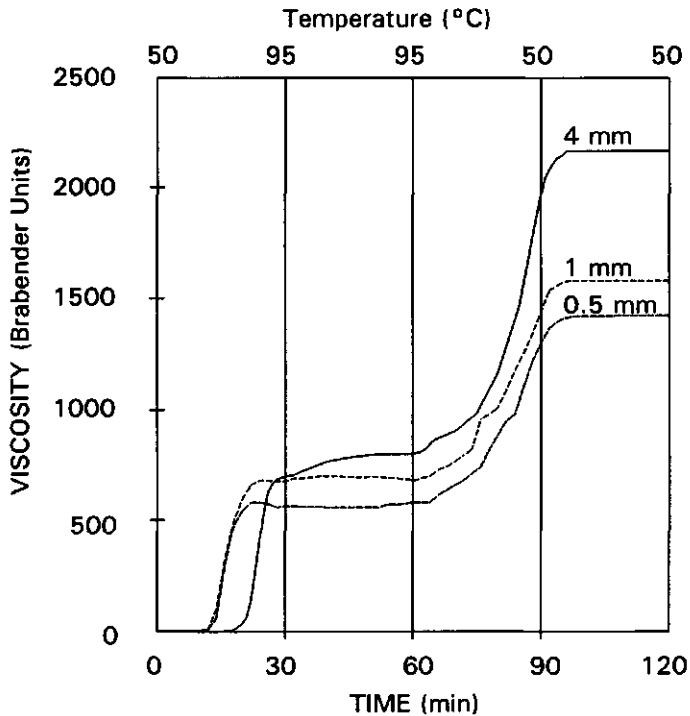

Figure 4.5. The effect of milling levels (to particle sizes of 4mm, 1mm and 0.5mm) on the viscosity of 24 h-fermented dough from wet-milled maize.

Even during the wet-milling of fermented dough or soaked maize, some degree of mechanical starch damage will occur. This is shown in Fig. 4.5, where

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repeated milling of coarsely wet-milled maize, to pass through 1 mm and 0.5 mm sieves, resulted in doughs with lower pasting and set-back viscosities than dough from coarsely wet-milled maize.

Various factors have been listed as being important in the formation and determination of characteristics of starch gels. Amongst these factors are: the type, size and previous treatment of starch granules, as well as paste concentration, amylose/amylopectin ratio, and the temperature and time of cooking (Olkku and Rha, 1978). The results presented here confirm that a complex combination of factors, during soaking and milling of maize and fermentation of the resulting dough contribute to the final pasting characteristics of kenkey dough. Endogenous enzyme activity, hydration and grain softening during soaking combine to limit the degree of mechanical starch damage during milling and thus ensure better hydration and swelling of granules, a high degree of gelatinisation and the eventual set-back necessary for good aflata quality.

Acidification of dough from dry-milled maize by accelerated natural fermentation and wet-milling of the aflata portion may be more acceptable than the use of proteolytic enzymes which would raise production costs and arouse consumer fears, as this may be interpreted in the same light as the addition of chemical substances to a product that has always been considered as natural. Soaking whole maize before milling, however, remains the best option for developing the necessary dough textural characteristics.

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The effects of processing on the availability of lysine in kenkey[1]

Abstract

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/16g 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 and Devi, 1986; Friedman and 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 and 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 also the source of a large proportion of the dietary protein which unfortunately is often seriously deficient in lysine (Clark *et al.*, 1977, Friedman and Finot, 1990). It is, therefore, important that the processing of such foods is carefully controlled in order to maximize lysine availability. This also applies to cereal foods supplemented with legumes in an attempt to improve both protein quality and quantity. In such cases, it is important that the processing methods used do 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 and Fields, 1979a; Umoh and Fields, 1981). The use of rats and mice in growth response experiments is also on the increase (Sherr *et al.*, 1989; Friedman and Finot, 1990; Faldet *et al.*, 1992). Several chemical methods have also been used (Conkerton and 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 analyzed economically (Bakr and Gawish, 1992; Faldet et al., 1992).

The aim of this study 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 laboratory-scale process (Fig. 2.1), 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. 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 as a binder and moisturizer 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 ($\approx$ 300 g) were shaped, wrapped, first in polyethylene sheaths and then aluminium foil, before being cooked in boiling water for 1 h to give kenkey.

In the accelerated process (Chapter 3) 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 preheated to 140°C and set to rotate at 1.5 rev/min. The other portion was dried for 3 h at 60°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 as above before cooking 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).

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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.

Total nitrogen content of samples was determined by a semi-automated version of the micro-Kjeldahl procedure (Roozen and van Boxtel, 1979).

Statistical analyses

All determinations were in duplicate and repeated 3 times. The data were analyzed by an independent t-test (SlideWrite Plus, version 5.0, 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 and 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 5.1 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.

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Table 5.1. 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/16g nitrogen)	Total nitrogen (g/kg sample)	Available lysine (g/16g nitrogen)
None	15.22 ± 0.34	1.30 ± 0.30[2e]	20.49 ± 0.42	2.46 ± 0.11[b]
Soaked	15.48 ± 0.75	1.57 ± 0.00[e]	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[de]
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[de]	20.63 ± 0.45	4.43 ± 0.00[f]

[1]Dry weight basis; [2]Mean ± s.d. (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 ($P < 0.05$)

Table 5.2. 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/16g nitrogen)	Total nitrogen (g/kg sample)	Available lysine (g/16g nitrogen)
Traditional	17.99 ± 1.19	2.36 ± 0.59[2c]	21.78 ± 1.17	4.11 ± 0.30[f]
Cabinet 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]A 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).

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 4.43 g/16g 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 and 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 3.46 g/16 g N for dehydrated fermented maize meal and maize-soy (80:20) flour blends, respectively. Zamora and 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 lysine was, however, reported as unaffected by cooking. This difference in the availability of cystine and methionine was reported (Marletta *et al.*, 1992) to be due to their presence, in different proportions, in various bean protein fractions (albumins, globulins and glutenins) which have been shown to have different digestibilities (Lanfer Marquez and Lajolo, 1981).

The total nitrogen contents of all samples remained largely the same, regardless of the treatment (Tables 1 & 2). Although not determined, it could also be deduced that the total lysine contents of all the samples were not significantly altered. Any changes in the amount of available lysine could, therefore, have been mainly the result of changes in the binding state of total lysine.

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 5.2). Drum drying, on the other hand, significantly ($P < 0.01$) reduced the available lysine contents of the

resulting kenkeys. Adeniji and 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 5.2). 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 5.1) were generally below the range of values of total lysine contents of raw maize and/or cowpeas although Hamad and Fields (1979a) reported even lower values for maize. Calculations from literature values (Hurrell and 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 chapter clearly show that soaking, fermentation and boiling contribute significantly to the protein quality of the final product. Drum drying, however, will induce high loses 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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Investigation of the presence of biogenic amines and ethyl carbamate in kenkey[1]

Abstract

Kenkey is a fermented and cooked maize dough from Ghana. The effect of manufacturing conditions, i.e. fermentation and cooking, and of protein-enrichment by cowpea addition (20% of total weight) on the occurrence of toxic microbial products namely biogenic amines and ethyl carbamate were investigated. The levels of biogenic amines in all-maize kenkey were very low (total amines < 60 ppm), but were significantly increased by addition of red cowpea (total amines < 200 ppm, mainly cadaverine and tyramine), and even more by white cowpea (total amines < 500 ppm, mainly putrescine and tyramine). Histamine was absent (<5 ppm) in all samples. The effects of fermentation and cooking were less pronounced than the influence of cowpea addition. Prolonged cooking of kenkey resulted in lower levels of putrescine, but did not significantly reduce tyramine levels. Ethyl carbamate levels were negligible (<11 ppb) in all treatments.

INTRODUCTION

Biogenic amines and ethyl carbamate are toxic substances which can be formed in foods, mainly by microbial enzymic activity. The biogenic amines of toxicological relevance include histamine, tyramine, ß-phenylethylamine, tryptamine, putrescine and cadaverine (Stratton *et al.*, 1991). The major pathway of formation in foods is by decarboxylation of free amino acids. Various lactic acid bacteria and Enterobacteriaceae possess the required decarboxylase activity (Stratton *et al.*, 1991).

Ethyl carbamate (urethane) has mutagenic and carcinogenic properties (Mirvish, 1986), and can be formed from reaction with ethanol and naturally occurring carbamyl phosphate during the fermentation process. Both compounds mainly result from yeast metabolism; ethyl carbamate can also be formed from reaction with ethanol and urea naturally produced from amino acids like arginine and citrulline (Ough, 1976; Matsudo *et al.*, 1993).

Kenkey is a popular ready-to-eat staple food from Ghana. Basically, it consists of fermented maize dough shaped into balls wrapped in leaves and cooked in water (Muller, 1970; Muller and Nyarko-Mensah, 1972; Nche et al., 1994). The major functional microorganisms in the fermentation are lactic acid bacteria, but yeasts and Enterobactericeae may occur as well in variable quantities. In addition, it is also possible to produce acceptable protein-enriched kenkey (Nche et al. 1994) by substituting 20% of the maize used with cowpea (Vigna unguiculata) which, in Ghana, is more popular than soya beans. The enrichment with cowpea results in increased availability of amino acids from which biogenic amines may also be formed.

The presence of varying levels of biogenic amines in, for example, cheese, sausage, wine, beer, soya sauce and miso (Stratton et al., 1991), and of ethyl carbamate in distilled spirits and wines, and fermented foods e.g. bread, soya sauce, miso and yeast spread (Diachenko et al., 1992) have been reported. In the absence of data concerning kenkey, the present investigation was carried out to evaluate the influence of processing, choice of ingredients and of representative microorganisms on the possible accumulation of biogenic amines and ethyl carbamate in kenkey made with maize or maize-cowpea mixtures.

MATERIALS AND METHODS

Maize (Zea mays cv. obaatanba) and red and white cowpeas (Vigna unguiculata cv. benpla and asontem, respectively) were obtained from the Crops Research Institute, CSIR, Kwadaso, Ghana.

Manufacture of Kenkey

Kenkey was prepared at laboratory-scale according to Nche et al. (1994). In short, 1 kg of maize kernels (or a 4:1 mixture of maize and cowpea) was soaked for 2 days at 4°C or 30°C (regular process) in 3 L of tap water. The soak water was drained and discarded, and the grain coarsely ground in a hammer mill (Fritsch Pulverisette Type 14.702, Marius Instruments, Utrecht, The Netherlands) with rotor and sieve (4 mm aperture) and made into a dough by adding water (1:3 v/w). The dough was placed in a 1 L beaker, covered and allowed to ferment naturally (regular process) for 3 days at 30°C. Alternatively, the dough was inoculated prior to fermentation, as described below. The fermented dough was divided into two equal portions, one of which was slurried with water (3:1 v/w) and stir-cooked to

gelatinisation to give the aflata. The cooked and uncooked portions were mixed, kneaded and dumplings were made and wrapped, first in polyethylene film followed by aluminium foil. The wrapped dumplings were immersed in boiling water to cook for 1 hour (regular process) or 3 hours. Samples for chemical analysis were prepared by grinding 100 g of composite sample from duplicate treatments, followed by frozen storage at -20°C until analysis. Chemical analyses were carried out in duplicate.

Inoculum preparation

Lactic acid bacteria which had been isolated previously from kenkey (Nche *et al.*, 1994) included *Lactobacillus plantarum*, *L. confusus*, *L. brevis* and *Pediococcus pentosaceus*. Pure cultures were grown in MRS broth (Merck Art.10661) at 30°C for 24 hours. At the start of the dough fermentation period, a mixed inoculation of maize dough was achieved by adding 1 ml of each MRS culture per 100 g of dough, followed by thorough mixing. Similarly, 4 unidentified yeast isolates obtained from kenkey dough were pre-grown in Malt Extract Broth (Oxoid CM57) at 30°C for 24 hours. Inoculation of maize dough was as described above.

Determination of biogenic amines

A portion of 15 g dry matter was homogenized with 50 ml 5% trichloroacetic acid at 70°C in a 100 ml glass beaker using a Waring blender. After cooling to room temperature, the mixture was transferred quantitatively into a 100 ml volumetric flask and made up to the mark with 5% trichloroacetic acid. After mixing, a 50 ml portion was transferred into a centrifuge tube and centrifuged at 2500 g for 10 minutes. The supernatant was filtered through filter paper (Schleicher and Schull 595 1/2, no. 311645). A 25 ml aliquot of filtrate was transferred into a 100 ml volumetric flask and diluted to the mark with distilled water. The diluted filtrate was filtered again through a 0.45 μm pore size filter (Millipore filter, type HVLP 04700). Biogenic amines were separated by ion-exchange liquid chromatography (LC) and detected with a fluorescence detector after post-column derivatisation with o-phthalaldehyde adapted from Walters (1984). The detection limit was 1 mg/kg, and data had a coefficient of variation of 10%. The LC conditions were as follows: column: stationary phase Zorbax 300 SCX strong cation exchange resin (Dupont no. 28768); mobile phase (eluent): 70 parts by volume of 0.1 M phosphate buffer, pH 6.1 and 30 parts by volume of methanol; elution rate: 1.0 ml/min. Post-column derivatisation was carried out at 45°C in a 900 x 0.5 mm i.d. Teflon reactor spiral. The reagent for post-column derivatisation was added at

0.8 ml/min and consisted of freshly mixed solutions of A and B. Solution A: 12.5 g boric acid (Merck art. 165) in 475 ml distilled water adjusted with KOH to pH 10.4. Solution B: 300 mg o-phthalaldehyde (Merck art. 11452) in 5 ml ethanol 96% (Merck art. 983) to which 100 μl 2-mercaptoethanol (Merck art. 805740) was added. Detection was carried out with a fluorescence detector (Waters type 420 with lamp 78245) with $\lambda_{excitation}$ = 338 nm and $\lambda_{emission}$ > 420 nm. Samples were extracted in duplicate. Each extract was chromatographed twice. The reported data are means of the 4 determinations.

Detection of ethyl carbamate

Ten grams of sample were homogenized and extracted in duplicate with methylene chloride according to the procedure for bread (Canas *et al.*, 1989). Each concentrated extract was analyzed in duplicate by gas liquid chromatography (GLC) according to Kesselmans *et al.* (1986), applying the following conditions: column: CP wax-52 CB, WCOT fused silica length 50 m, inner diameter 0.32 mm; carrier N_2 (120 kPa), make-up He (115 kPa), detector H_2 (67 kPa), air (102 kPa); NPD-detector in NP-mode (Potassium tablet); injection volume 1.0 μl on-column; temperature programme: 20°C for 20 s, 20-87°C in 40 s, 87-180°C at a rate of 8°C per minute, 180-190°C at a rate of 10°C per minute, 190°C for 20 min. The recoveries for ethyl carbamate (EC) in spiked kenkey samples varied from 45 to 50%. The detection limit of EC in extract by GLC was 10 μg/l, corresponding with 11 μg/kg (11 ppb) in the original sample. Data had a coefficient of variation of 5.5% at 200 ppb level.

RESULTS AND DISCUSSION

Table 6.1 shows the treatments investigated, and the levels of cadaverine, histamine, putrescine, tryptamine and tyramine determined expressed as mg/kg sample dry weight. In addition, the pH of the fermented dough prior to cooking was recorded to serve as an index of fermentation. When soaking and dough incubation were carried out at 4°C (treatment A), no fermentation took place. This was indicated by the almost neutral pH, as well as very low total plate count (data not shown). In doughs, natural fermentation at 30°C resulted in 10^8-10^9 cfu/g of lactic acid bacteria, 10^2-10^3 cfu/g of yeasts, while Enterobacteriaceae could not be detected (data not shown). These levels of microorganisms are in line with those reported earlier for laboratory-made kenkey (Nche *et al.*, 1994)

Table 6.1. Biogenic amines (mg/kg dry matter) in maize- and maize-cowpea-based kenkey processing stages.

	Raw material						
	100% maize		80% maize 20% red CP[1]		80% maize 20% white CP		
Treatment code	A	B	C	D	D′	E	F
Soaking[2] & fermentation							
temperature	4°C	30°C	30°C	30°C		30°C	30°C
Added inoculum	-	-	-	-		LAB[3]	Yeasts[4]
Fermented dough							
pH	6.49	4.01	4.09	4.01		3.75	4.20
Cadaverine	<22	<22	100	<22		78	<22
Histamine	<5	<5	<5	<5		<5	<5
Putrescine	<11	<11	22	377		89	244
Tryptamine	<5	<5	<5	<5		<5	<5
Tyramine	78	11	56	200		200	200
Cooked at 100°C for (***Kenkey***)	1 h	1 h	1 h	1 h	3 h	1 h	1 h
Cadaverine	<25	<25	113	<25	<25	63	<25
Histamine	<5	<5	<5	<5	<5	<5	<5
Putrescine	<13	13	<13	325	<13	63	138
Tryptamine	<5	<5	<5	<5	<5	<5	<5
Tyramine	50	<13	63	163	175	200	175

[1]Cowpea
[2]Whole kernels soaked for 2 days, followed by grinding and 3 days' fermentation of resulting dough.
[3]Multi-strain mixture of lactic acid bacteria isolated from kenkey.
[4]Multi-strain mixture of yeasts isolated from kenkey dough.

When comparing treatments B, C and D, an assessment of the effect of ingredients can be made. Whereas maize alone hardly enabled formation of biogenic amines, addition of 20% red cowpea caused a significant increase of cadaverine and tyramine levels, and addition of 20% white cowpea gave even higher concentrations, mainly of putrescine and tyramine. The general increase can be explained by the supply of precursor amino acids by cowpea. The different effect of red vs white cowpea could be due to different ranges and levels of free amino acids. Also, polyphenols present in red cowpea seed coat may inhibit decarboxylases (Ogun et al., 1989).

Treatments A and B allow an evaluation of the effect of natural fermentation. Although maize was shown not to be a strong precursor for biogenic amines, it was observed that non-fermented maize dough and kenkey (A) had a somewhat higher tyramine content compared with fermented dough and kenkey (B). These limited data suggest that in (A), formation of tyramine can be explained by the combined action of endogenous proteolytic and decarboxylating enzymes (Devi and Prasad, 1992). When actively growing microorganisms are present (B), some of the free amino acids are not converted into biogenic amines. In addition, the low pH in the fermented dough may be inhibitory to decarboxylases causing a lower amine level.

Treatments D, E and F show the difference between natural and inoculum-supplemented fermentations. The inoculation with a mixture of lactic acid bacteria caused a strong reduction of putrescine, but cadaverine slightly increased. Addition of lactic acid bacteria had no significant effect on tyramine levels. Added yeasts had no significant effects on either cadaverine or tyramine, but caused a significant decrease of putrescine levels.

A comparison of dough and kenkey, and of treatments D and D' indicates the effect of cooking. A 1-hour cooking period (compare D,E,F-dough with D,E,F-cooked kenkey) caused a slight (non-significant) decrease of the tyramine level only; extended cooking (D') for 3 hours significantly reduced the putrescine level.

In general, histamine and tryptamine were absent in all samples. Non-enriched whole maize kenkey did not contain significant levels of any of the tested biogenic amines and may be regarded as a safe product with regard to amines. On the other hand, the enrichment of kenkey with 20% cowpea significantly increased the levels of biogenic amines. Not all amines are equally toxic. Histamine and tyramine are especially associated with symptoms of intoxication. Although the other amines are considered to potentiate the toxic effect of histamine and tyramine, few quantitative data about such interactions in vivo are available

(Stratton *et al.*, 1991). Histamine was absent (<5 ppm) in all samples, but tyramine was present in detectable quantities (60-200 ppm). In particular, the tyramine concentrations in cowpea-enriched kenkey could be considered as "moderately high" when compared with similar data for cheese (Stratton *et al.*, 1991). Considering the present lack of knowledge about the toxicity of tyramine in the presence of cadaverine and putrescine, it is not possible to simplify the results by, for example, calculating a weighted total biogenic amines level, or to compensate decreased levels of putrescine with increased cadaverine or tyramine concentrations. Taking into account that kenkey is an everyday staple food, it will nevertheless be important to minimize the level of biogenic amines in cowpea-enriched kenkey as much as possible. The data show that prolonged cooking contributes only marginally to lowering the amine levels. Consequently, it will be of interest to select lactic acid bacteria with the ability to degrade amines (Beutling, 1992), and to investigate their usefulness as starter organisms with a view to obtain low-amine enriched kenkey.

With regard to ethyl carbamate, all samples contained less than 11 ppb (the detection limit). This appears to correspond with the reported absence of ethyl carbamate in a range of fermented foods in which yeasts were not involved as a major population (Diachenko *et al.*, 1992), whereas levels of up to 84 ppb (soya sauce) were found in fermented foods in which alcoholic fermentation had taken place to some extent and an average of approximately 200 ppb was reported for alcoholic beverages. The tolerance level of ethyl carbamate as applied in Canada varies from 30 ppb in wines to 400 ppb in distilled spirits (Diachenko *et al.*, 1992). This implies that ethyl carbamate is not a factor of public health relevance in kenkey or in cowpea-enriched kenkey. Most likely, the absence of ethyl carbamate is due to inadequate levels of ethanol and/or precursors such as citrulline, arginine or urea in the product.

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Gas production by *Clostridium perfringens* as a measure of the fermentability of carbohydrates and processed cereal-legume foods[1]

▓▓

Abstract

A new and improved method was developed for the *in vitro* measurement of gas produced by *Clostridium perfringens*, in an attempt to determine the fermentability of pure carbohydrates, processed legumes and cereal-legume mixtures. The bacterial strain, inoculum and substrate concentrations and type of carbohydrate all proved to be important factors affecting *in vitro* gas production by *Cl. perfringens*. Whilst one strain, NCTC 8239, remained relatively unaffected, strain 83V70-2 tended to be inhibited by glucose concentrations > 1.5%. A 10-fold dilution of the active inoculum reduced the rate of gas production, whilst a 50-fold dilution produced no gas in 24 hours. Strain NCTC 8239 was able to ferment a wide range of carbohydrates, with the exception of xylose. Arabinose, ribose and raffinose were, however, only moderately fermented. Native starch was also poorly fermented but after heating, was fermented with vigorous gas production. Cooked, lactic-fermented cereal-legume composite doughs produced less gas compared to cooked non-fermented doughs. Soaking resulted in decreased fermentability of cowpea and pigeon pea, but the opposite effect was observed with soaked mungbean and soya bean. Fungal fermentation (with *Rhizopus oligosporus*) into tempe also had varying effects on fermentability, depending on the legume. Fungal-fermented cowpea, groundnut and pigeon pea produced more gas than similarly treated mungbean and soya bean. Steaming these products again had contrasting effects, with cowpea and pigeon pea showing reduced fermentability whilst the fermentability of mungbean and soya bean increased.

INTRODUCTION

The large scale consumption of legumes is often hindered by their poor digestibility, a consequence of which is the induction of flatus in both humans and animals (Hellendoorn, 1969; Reddy *et al.*, 1980; Kennedy *et al.*, 1985). Legumes contain

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high levels of indigestible oligosaccharides (α-galactosides) of the raffinose family, unavailable starch and other carbohydrate fractions (Hellendoorn, 1969; Murphy *et al.*, 1972; Fleming, 1981; Kennedy *et al.*, 1985). These carbohydrates escape digestion in the upper gut and end up in the colon where they could be fermented by colon microflora to produce volatile fatty acids (Englyst and Macfarlane 1986, Macfarlane and Englyst 1986, Kikuchi and Sakata 1992) and gas which results in flatus (Richards *et al.*, 1968; Rackis *et al.*, 1970; Speck *et al.*, 1970; Savitri *et al.*, 1986).

Clostridium perfringens is one of the many saccharolytic bacteria (Rockland *et al.*, 1969; Cummings and Macfarlane, 1991) that occur in the colon microflora and contribute to the fermentation of sugars and other carbohydrates reaching the colon. This bacterium has, therefore, been used in *in vitro* methods to determine the fermentability of legumes and legume foods (Rockland *et al.*, 1969; El Faki *et al.*, 1983; Savitri *et al.*, 1986; Nowak and Steinkraus, 1988). Most of the *in vitro* methods reported involve the measurement of gas released from the fermentation of either pure sugars in defined media, or food homogenates inoculated with *Cl. perfringens*. In these methods gas measurement usually involves the use of graduated syringes (El Faki *et al.*, 1983; Savitri *et al.*, 1986; Nowak and Steinkraus, 1988), but inverted (10 x 75 mm) borosilicate glass test tubes (Rockland *et al.*, 1969), head space pressure (Beuvink and Spoelstra, 1992) and horizontal pipettes (Kikuchi and Sakata, 1992) have also been used. These methods vary in accuracy and reproducibility hence the large variability in reported results.

This study was aimed at carrying out comparative determinations of the fermentability of legume food samples as influenced by process conditions viz. soaking, fermentation and cooking. To this effect, the sensitivity and reproducibility of the syringe method was evaluated. A new sensitive U-tube system for measuring gas production was developed, and test conditions were standardized. Using several strains of *Cl. perfringens*, a range of carbohydrates, cereal and legume foods at different processing stages were tested for fermentability in an attempt to establish their flatulence potential after processing.

MATERIALS AND METHODS

Maize (*Zea mays*) and the legumes cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), soya bean (*Glycine max*), mungbean (*Vigna radiata*) and groundnuts (*Arachis hypogaea*) were obtained from Grano-Drente, Meeuwen, The

Netherlands. All processed samples were freeze-dried and milled in a hammer mill (Fritsch pulverisette, Type 14.702, Marius Instruments, Utrecht, The Netherlands) to pass through a 0.5 mm sieve before being tested for fermentability. The test strains were *Clostridium perfringens* 83V70-2, 83.42.1, 84.1.1 and NCTC 8239, obtained from the National Institute of Public Health and Environmental Hygiene (RIVM), Bilthoven, The Netherlands. With the exception of maize starch and ribose (Sigma Chemical Co. St. Louis, USA), all other carbohydrates used were obtained from Merck (E. Merck, Darmstadt, Germany).

Measurement of gas production

I. *Syringe method.*
An active inoculum of *Cl. perfringens* was obtained from a 24 h pre-culture at 37°C in thioglycollate medium U.S.P.(Oxoid CM173). 1 ml of this pre-culture was used to inoculate 20 ml of sugar free thioglycollate medium (per litre: 5.0 g yeast extract, 15.0 g tryptone, 0.5g sodium thioglycollate, 2.5 g NaCl, 0.5 g L-cystine, 0.5 g Agar No. 1, pH 7.1) to which had been added the appropriate concentration of pure carbohydrate or 2% w/w freeze-dried, processed cereal-legume mix to be tested. 5 ml each of this culture suspension were then drawn into pre-weighed graduated 20 ml sterile plastipak syringes (Becton Dickinson, Dun Laoghaire, Co. Dublin, Ireland). After recording the exact weight of culture in the syringe, its hypodermic needle was pushed into a rubber bung to ensure hermetic closure. The syringes were then placed in a 37°C water bath such that the portion containing the culture medium was submerged. Unless otherwise stated, triplicate syringes were used. The volume of gas released was measured by following the movement of the plunger in the syringe. Readings were taken at regular time intervals.

II. *The pressure-free U-tube (PFUT) method*
Figure 7.1 illustrates the experimental device (pressure-free U-tube, PFUT) which was developed to measure periodically the volume of gas produced. A volume of approximately 10 ml of freshly inoculated liquid medium is introduced into a sterile pre-weighed tube (1). The weight of the culture is recorded to enable expression of gas production per unit weight of substrate. With valve (4) opened, the tube is connected to the U-tube system via a rubber bung equipped with a hypodermic needle (2). Because of the connection of the U-tube with the atmosphere, the liquid (7) levels in both arms are at equal height (6). At time = 0, valve (4) is closed and the whole set up is arranged such that the culture tube (1) suspends

in a waterbath (3) maintained at 37°C by a thermostat heater (Haake D1, Karlsruhe, Germany). During incubation, pressure exerted by the gas released forces the liquid (7) into the spiralized arm of the U-tube. The spiral is meant to increase capacity without making the equipment cumbersome. At regular intervals, a 10 ml graduated gastight syringe (5) is connected to valve (4), the valve is opened and the gas produced is drawn up into the syringe until the liquid (7) reaches its original level (6). The valve is closed, the syringe removed and the amount of gas produced is read from the position of the plunger in the syringe.
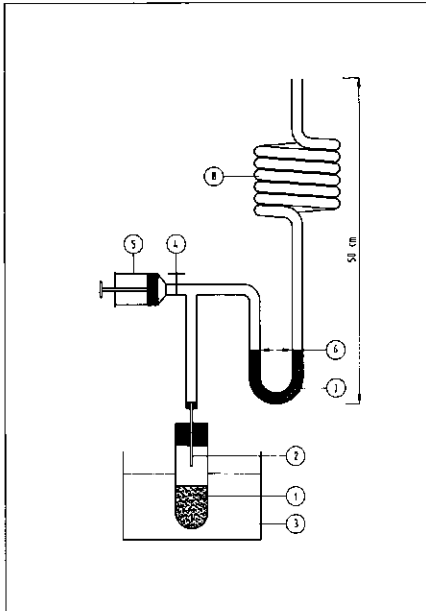

Figure 7.1. Pressure-free U-tube device for gas measurement. (1) culture vessel with known weight of culture medium (about 10 ml); (2) hypodermic needle linking culture vessel with U-tube; (3) thermostat controlled waterbath; (4) Valve; (5) detachable gastight syringe (10 ml Hamilton 1010 Tll); (6) reference mark (7) U-tube liquid (saturated NaCl solution, pH 1); (8) spiralized glass capillary tube (inner diameter = 3 mm; length = 50 cm)

RESULTS AND DISCUSSION

Figure 7.2 shows gas production using the syringe and U-tube methods. The syringe method gave highly variable values due to friction between plunger and syringe, and this is evident in the volume displacements of the syringe plunger which were considerably less than values recorded in the U-tube. A 2 h delay was often required before any movement of the plunger could be recorded. The sensitivity of the U-tube is shown by the early initial recording of gas production and the higher values obtained. Reduced friction in the U-tube resulted in better reproducibility as shown by a lower standard deviation in the recorded values. In addition, the absence of overpressure in the system limits the amount of CO_2 dissolving in the culture medium. Compared to the pressure-free system described by Kikuchi and Sakata (1992), there is no need for graduated pipettes in which to collect the gas. We also found that the larger internal diameter of graduated pipettes resulted in easy collapse of the liquid meniscus.
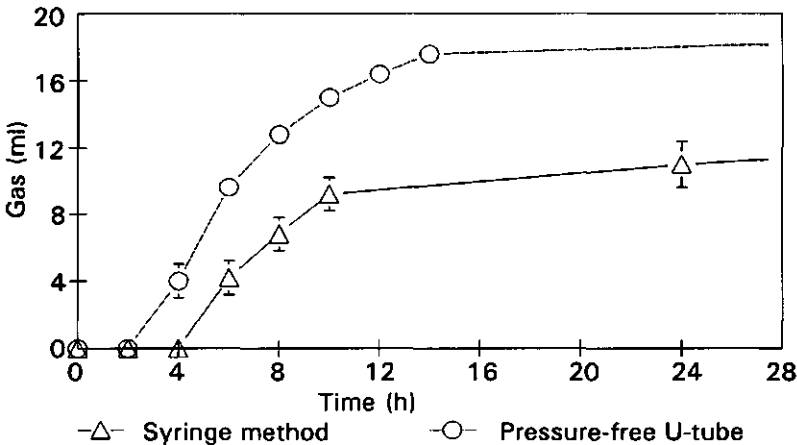

Figure 7.2. Gas production (ml) by *Cl. perfringens* strain NCTC 8239 in 10 ml thioglycollate broth with 2% glucose. (Bars indicate standard deviation).

The effect of substrate concentration on the total gas produced (Table 7.1) was investigated in sugar-free thioglycollate medium to which different glucose concentrations had been added. The two strains of *Cl. perfringens* used showed significant ($p \leq 0.05$) inhibition at glucose concentrations > 1.5%. In order to

standardise experimental conditions and to avoid unwanted inhibitory effects, the level of carbohydrate was limited to 2% in further experiments.

Table 7.1. Effect of glucose concentration on gas production by *Clostridium perfringens*

Glucose (%)	Gas produced (ml)[1]	
	strain 83V70-2	strain NCTC 8239
0.0	0.00 ± 0.00[2]	0.00 ± 0.00
0.5	8.83 ± 0.85	12.98 ± 1.89
1.0	12.00 ± 0.82	15.17 ± 0.62
1.5	13.17 ± 1.17	13.53 ± 0.29
2.0	11.50 ± 1.50	17.58 ± 0.51
2.5	9.35 ± 0.78	12.58 ± 0.31
3.0	6.77 ± 0.92	12.53 ± 0.21

[1]Total gas produced in 10 ml culture after 24 h incubation at 37°C.
[2]Mean ± s.d. (n = 3).

Table 7.2. Effect of inoculum size on growth and gas production by *Cl. perfringens* strain NCTC 8239 in thioglycollate broth with 2% glucose.

Incubation Time (h)	Undiluted inoculum[1]		10-fold dilution	
	Log_{10}cfu/ml	Gas (ml)[2]	Log_{10}cfu/ml	Gas (ml)
0	7.02	0.00 ± 0.00	6.15	0.00 ± 0.00
3	7.88	1.63 ± 0.13	6.25	0.33 ± 0.08
6	8.95	5.75 ± 0.25	8.14	3.70 ± 0.21
24	7.90	17.63 ± 0.63	7.91	21.13 ± 1.47

[1]Pre-cultured in 10% v/v Brain Heart Infusion medium for 24 h at 37°C.
[2]Mean ± s.d. (n = 3).

Table 7.2 shows that the rate and amount of gas produced were very much influenced by the initial cell density of the inoculum. A 10 fold dilution of inoculum resulted initially in a slower rate of gas production, although this had no effect on the total gas produced after 24 h. Further dilution by 50 fold resulted in no gas production within the 24 h of measurement (result not shown). Although not tested here, inoculation at levels exceeding 10^7 cfu/ml could be expected to increase the rate and possibly the amount of gas production. This would, however, require additional preparatory work with centrifugation and re-suspension, whilst avoiding any unwanted contamination. Other authors (Nowak and Steinkraus, 1988) have also reported a good positive correlation between gas production and cell density.

The ability of *Cl. perfringens* strain NCTC 8239 to ferment a wide range of carbohydrates is presented in Table 7.3. With the exception of xylose, all other carbohydrates tested were fermented. The pentoses arabinose and ribose and the trisaccharide raffinose were, however, fermented with only moderate gas production. Native starch was poorly fermented, probably because of its inaccessibility. However, after heating, the then gelatinised starch caused the highest gas production recorded. These results fall in line with previous reports (Nowak and Steinkraus 1988) showing that gas production by *Cl. perfringens* 546 varied with the carbohydrate present in the culture medium. This variation could be related to the ability of the bacteria to adapt to the carbohydrate tested, or in the case of the pentoses, to the different pattern of fermentation products formed. Tests to the effect of induction showed that *Cl. perfringens* pre-cultured on glucose-containing thioglycollate medium produced relatively small amounts of gas on raffinose compared with glucose. However, if pre-cultured on raffinose, gas production by strains 83.42.1 and NCTC 8239 on raffinose was doubled (Table 7.4). Strain 84.1.1, on the other hand, responded differently by producing very little gas on raffinose irrespective of pre-cultivation conditions. An adaptive response to different carbohydrates by *Bacteroides ovatus* was reported by Macfarlane *et al*, 1990. These authors showed that *B. ovatus* was able to synthesize enzymes specific to the carbohydrate contained in the culture medium. The response of the *Cl. perfringens* strains 83.42.1 and NCTC 8239 is probably an indication of their ability to synthesize the enzymes required to degrade the various carbohydrates tested.

Table 7.3. Growth and gas production by *Cl. perfringens* strain NCTC 8239 incubated for 24 h at 37°C on 2% carbohydrate in thioglycollate broth (inoculum pre-cultured in thioglycollate broth with 0.5% glucose)

Carbohydrate	Growth		Gas[1] (ml)	s.d.[2]
	O.D.[3]	Log_{10}cfu/ml		
None	0.64	8.04	0.00	0.00
Arabinose	1.02	ND[4]	3.40	0.20
Fructose	3.39	8.18	20.00	0.30
Galactose	3.20	8.18	16.90	0.10
Glucose	3.67	7.90	22.80	1.70
Maltose	4.25	7.87	23.40	0.40
Mannose	ND	ND	19.20	1.10
Raffinose	1.23	7.88	8.50	0.10
Ribose	1.46	ND	10.00	0.10
Starch (native)	ND	ND	4.10	0.10
Starch (heated[5])	ND	8.63	24.60	1.50
Sucrose	4.16	ND	16.80	1.70
Xylose	ND	ND	0.00	0.00

[1]Total gas measured after 24 h incubation at 37°C; [2]Standard deviation (n = 3); [3]Optical density at 660 nm, measured as diluted samples in the range < 1.0 and multiplied by respective dilution factors; [4]Not determined; [5]Heated for 10 minutes at 90°C.

Growth of the bacteria was determined by both plate counts and spectrophotometric measurements of optical density (O.D.) at 660 nm, using a DU Series 60 spectrophotometer (Beckman Instruments, Mijdrecht, The Netherlands). An interesting observation to note here is that, whereas plate counts of colony forming units, after 24 h incubation at 37°C, were of the same order of magnitude, regardless of the carbohydrate tested (Table 7.3), some carbohydrates e.g. glucose and maltose produced much more biomass (measured as O.D.) compared with raffinose or the sugar-free control. This indicates that a high turnover of cells occurs, and that the difference in O.D. values represents already dead cells which

are, nonetheless, detected spectrophotometrically. High O.D. values also correlate much better with total gas production (Pearson's $r^2 = 0.88$) than do plate counts ($r^2 = 0.09$).

Table 7.4. Effect of pre-culturing conditions on gas production by *Cl. perfringens* in thioglycollate broth containing glucose or raffinose.

Pre-cultured[1] in thioglycollate with:	Gas[2] production (with s.d.[3]) in thioglycollate broth containing:	
	2% glucose	2% raffinose
0.5% glucose		
Strain 83.42.1	13.50 (0.60)	5.30 (0.30)
Strain NCTC 8239	22.80 (1.70)	8.50 (0.10)
Strain 84.1.1	20.60 (0.10)	1.00 (0.10)
2% raffinose		
Strain 83.42.1	17.70 (0.39)	9.10 (0.10)
Strain NCTC 8239	18.80 (0.10)	20.10 (0.10)
Strain 84.1.1	14.17 (0.92)	0.30 (0.10)

[1]24 h at 37°C; [2]Total gas produced after 24 h expressed in ml; [3]standard deviation ($n = 3$);

In addition to pure carbohydrates, several food samples were tested for their fermentability following different process treatments. Table 7.5 shows results obtained with raw and processed cereal-legume mixtures. As can be expected from earlier results with pure carbohydrates (Table 7.3), gas production from food samples reflected the availability of fermentable carbohydrates. Freshly milled raw composite flours containing maize, sorghum and several legumes contain sufficient fermentable carbohydrates such as mono- and disaccharides. After only natural lactic fermentation, the fermentability for *Cl. perfringens* is considerably lower since easily fermentable mono- and disaccharides have already been consumed by lactic acid bacteria. However, the starch fraction is still in native form, not having been heated or degraded and lactic acid bacteria are very poor starch degraders. Cooking the fermented dough, however, results in starch gelatinisation hence increased fermentability of starch (compare Table 7.3) with the exception of

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groundnut mix. With the exception of soya bean mix, less gas was produced from cooked fermented doughs compared with cooked non-fermented flours which still contained higher levels of mono- and disaccharides (data not shown).

Table 7.5. Fermentability of processed cereal-legume mixtures by *Cl. perfringens* strain 83V70-2. (Total gas production in 24 h at 37°C, expressed as ml g^{-1} dry matter).

Processing stage	MSC (30:30:40)[1]	MSG (30:35:35)	MSM (30:30:40)	MSP (30:30:40)	MSS (35:35:30)
Raw	40	55	50	35	30
Fermented[2]	15	10	10	10	15
Fermented & cooked[3]	30	0	35	25	45
Non-fermented & cooked	55	20	60	35	0

[1]Weight ratio on dry matter basis; [2]Accelerated natural lactic fermentation at 30°C for 24 h; [3]Cooked at 100°C for 5 minutes. MSC = maize/sorghum/cowpea; MSG = maize/sorghum/groundnut; MSM = maize/sorghum/mungbean; MSP = maize/sorghum/pigeon pea; MSS = maize/sorghum/soya bean

Table 7.6 summarizes the extent of fermentability of legumes when processed into tempe. Except for groundnuts, raw legumes contain readily fermentable carbohydrates. Soaking, followed by cooking had varying effects on fermentability. In cowpea and pigeon pea, fermentability decreased, possibly due to loss of fermentable carbohydrates by leaching. Soaking and cooking have also been reported to reduce the oligosaccharide content of some legumes, resulting in a decrease in flatulence (Silva and Braga, 1982; Jood *et al.*, 1985). Mungbean and soya bean, on the other hand, showed increased fermentability. This could be attributed to better availability of polysaccharides (i.e. starch in mungbean) or to inactivation of antimicrobial factors such as were described by Nowak and Steinkraus (1988), in soya beans.

Table 7.6. Effect of the tempe manufacturing process on the fermentability of legumes by *Cl. perfringens* strain 83V70-2. (Total gas production in 24 h at 37°C, expressed as ml g^{-1} dry matter).

Processing stage	Cowpea	Groundnut	Mungbean	Pigeon pea	Soya bean
Raw	40	0	20	45	10
Soaked & cooked[1]	15	0	40	0	25
Fermented[2]	40	10	0	110	0
Steamed[3]	20	10	50	30	5

[1]Soaked overnight at 25°C, drained and cooked in fresh water at 100°C for 25 minutes; [2]fermented with *Rhizopus oligosporus* at 30°C for 44 hours; [3]Fresh tempe steamed for 15 minutes.

Fungal fermentation had different effects depending on the legume used. Increases in fermentability were found in cowpea, groundnut and pigeon pea. Such increases might have been caused by improved accessibility of fermentable substrate, due to the enzymes produced by *Rhizopus oligosporus* (Sarrette *et al*, 1992). Fungal fermentation of mungbean and soya bean, on the other hand, resulted in reduced fermentability. Presumably here, any fermentable carbohydrates liberated had been assimilated during the fermentation (as in Table 7.5). In addition, the formation of antimicrobial substances by *R. oligosporus* in soya bean was reported elsewhere (Wang *et al.*, 1969; Nowak and Steinkraus, 1988; Nout and Rombouts, 1990). If present, such antimicrobials might have affected the gas production capacity of *Cl. perfringens*. Steaming resulted in reduced fermentability of fungal-fermented cowpea and pigeon pea. These results contradict those obtained for steamed groundnut, mungbean and soya bean which all showed increased fermentability. A similar observation for soya bean (Nowak and Steinkraus, 1988) has been attributed to the inactivation, by steam, of the factors inhibitory to *Cl. perfringens*, thereby allowing for the bacteria to grow and produce gas.

In conclusion, our results clearly point out potential difficulties that could be encountered when trying to estimate the fermentability of food products by *in vitro* methods. Several important parameters such as the sensitivity of the equipment

used, the type and pre-culture conditions of the bacteria used cannot be overlooked. It must also be made clear that this simple approach which has also been followed by several other authors (Rockland *et al.*, 1969; El Faki *et al.*, 1983; Savitri *et al.*, 1986; Nowak and Steinkraus, 1988; Kikuchi and Sakata, 1992) cannot represent a model of digestion and flatulence in humans. *Cl. perfringens* is, of course, only one of a wide range of different bacteria occurring in the human colon and which ferment carbohydrates reaching the colon. Also, as we have shown, many mono- and disaccharides cause more gas production than indigestible oligosaccharides. In the human digestive tract, accessible starch is broken down to mono- and disaccharides which are absorbed in the upper gut. *In vitro* models of digestibility and fermentability, therefore, need to take this aspect into account. The development of a simple system to this effect will be discussed in Chapter 8.

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An *in vitro* method for determining the digestibility and fermentability of traditional maize and cowpea-supplemented kenkey[1]

Abstract

A method was developed to determine the *in vitro* digestibility and fermentability of cereal and cereal-legume foods. The new method consists of three main parts viz. (1) a digestion step in which food samples are hydrolysed using enzymes similar to those found in the human digestive system; (2) an absorption step in which the yeast *Saccharomyces cerevisiae* is used to selectively remove from the digest simple, easily fermented mono-and disaccharides that would normally be absorbed in the upper gut of humans, but not the flatus-forming oligosaccharides raffinose and stachyose; (3) a fermentation step in which *Clostridium perfringens* is used to ferment residual solids from the digestion step and soluble residues (e.g. oligosaccharides) in the yeast-treated supernatants. This method was used to test the effect of different unit operations of the kenkey process on the digestibility and flatulence potential of kenkey. Soaking was most effective in improving the digestibility of both maize (M) and maize-cowpea (Mw) mixtures. Further processing, which included lactic fermentation and cooking, only slightly improved on the effects of soaking. Overall, M samples were more digestible than Mw samples. *Cl. perfringens* produced gas from yeast-treated supernatants as well as from the undigested residue of Mw samples. No gas was produced from the supernatants of M samples, suggesting that oligosaccharides present in cowpeas were the source of gas in the supernatants of Mw samples. Soaking and fermentation reduced gas production from the supernatants, but not from the residues of Mw samples. Despite the significant amounts of starch in the residue of Mw samples, no correlation between this and gas production was observed, suggesting the presence of other fermentable substrates in the residue. Cooking almost completely inhibited gas production from both the supernatants and residues of fermented samples. It was concluded that fermentation, coupled with cooking, significantly reduced the flatulence potential of both M and Mw kenkey.

INTRODUCTION

A large portion of the diet in developing countries is composed of carbohydrates from either cereals (e.g. maize, sorghum, millet) or tubers (e.g. cassava). These supply much of the required energy but do not meet the protein-calorie requirements, because they are low in protein content and deficient in essential amino acids, e.g. lysine. This means that alternative sources of proteins have to be used to improve the nutritional quality of cereal-based foods. A potential source of proteins is legumes such as cowpeas which are produced in large quantities in developing countries, with more than 70% of the world's production being met by Nigeria, Brazil and Niger (Nnanna and Phillips, 1988). Cowpeas are rich in lysine and are more affordable to the average family in Africa than animal sources of protein. There is, therefore, the possibility that this legume could be used to supplement cereal-based foods, e.g. kenkey in an attempt to improve on protein quality (Nche *et al.*, 1994a). Despite the nutritional potential of cowpeas as a source of significant amounts of protein, its utilization is still limited because of the presence of anti-nutritional factors and indigestible substances (Junek *et al.* 1980). The latter include flatulence-forming oligosaccharides viz. raffinose, stachyose as well as other dietary fibres (Fleming, 1981; Jood *et al.*, 1985), which escape digestion in the human upper gut and end up in the colon where they may be fermented by the intestinal microflora, producing fatty acids and gas, the latter being a source of discomfort to adults, and a cause of diarrhoea in infants.

Despite wide reports of oligosaccharides being the flatus-causing factors in legumes, other factors are thought to be involved (Murphy *et al.*, 1972). Carbohydrates other than the low molecular weight oligosaccharides are increasingly being implicated by virtue of the fact that they escape digestion in the upper gut of humans and other mammals but can be fermented in the colon by resident microflora to produce short chain fatty acids (SCFA) as well as gas (Cummings *et al.* 1989; Macfarlane *et al.*, 1992). Starch and non-starch polysaccharides (NSP) such as hemicelluloses, cellulose and pectins which form part of plant cell walls (Bourquin *et al.*, 1992) represent such poorly digestible substances. A significant proportion of starch escapes digestion in the small intestine of humans (Andrieux *et al.*, 1989) usually because it is resistant to hydrolysis by salivary and pancreatic α-amylases (Ring *et al.*, 1988; Tovar *et al.*, 1992).

Both fungal and lactic acid fermentations have been claimed to reduce the flatulence potential of legumes, either by reducing levels of oligosaccharides (Mital

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and Steinkraus, 1975; Duszkiewicz-Reinhard *et al.*, 1994) or by producing antibacterial substances (Nowak and Steinkraus, 1988; Nout and Rombouts 1990) which prevent the growth of colon bacteria and hence the production of flatus. In order to determine such effects, several *in vitro* methods have been developed to test processed food samples for their ability to induce flatus in human and animal consumers. Such methods have been based largely on the assumption that *a*-galactosides of the raffinose family are the main source of flatus in humans consuming legume-based foods. Hence foods can be tested for the presence of oligosaccharides by extracting them in various solvents followed by analysis by GLC (Hymowitz *et al.*, 1972), GC (Fleming, 1981) or HPLC (Kennedy *et al.*, 1985). Breath hydrogen is also being used as a measure of fermentation in the colon (Fleming *et al.*, 1988). Most common are methods involving the measurement of gas resulting from the incubation of whole foods or extracted non-digestible portions with either pure cultures of saccharolytic bacteria such as *Clostridium perfringens* (Nowak and Steinkraus, 1988; Kikuchi and Sakata, 1992; Nche *et al.*, 1994b) or mixed culture faecal slurries (McBurney *et al.*, 1990; Salvador *et al.*, 1993). Several methods to measure gas production have also been described. These include the use of graduated syringes (El Faki *et al.*, 1983; Nowak and Steinkraus, 1988), headspace pressure of culture vessels (Beuvink and Spoelstra, 1992), horizontal pipettes (Kikuchi and Sakata, 1992) and u-tubes such as the pressure-free u-tube (PFUT) described by Nche *et al.* (1994b). These methods vary in accuracy and substrates used, hence the large variability in reported results.

In most of the methods described in the literature (El Faki *et al.*, 1983; Nowak and Steinkraus, 1988; Nche *et al.*, 1994b) whole food samples from various stages of processing are tested for their potential to cause flatus. This implies that simple easily-fermented sugars as well as oligosaccharides, resistant and available starch and NSPs present in some of these food samples will be fermented to produce gas. In the human intestinal system, however, accessible starch is broken down by digestive enzymes to simple mono- and disaccharides which are absorbed in the upper gut. Only resistant starch, NSPs and oligosaccharides reach the colon where they could be fermented. It has also been shown (Nche *et al.*, 1994b) that more gas is produced from monosaccharides such as glucose than from raffinose or stachyose when these pure sugars are incubated with *Cl. perfringens.* The removal of glucose and other simple sugars in the upper gut of humans, however, means that these sugars do not contribute to the formation of flatus, hence the use of whole foods may result in overestimation of their flatulence potential. It is, therefore, important to take this into account when

designing experiments to test the flatulence potential of cereal, legume or cereal-legume foods. *In vivo* methods would be more representative of the human system but such methods are expensive and often the necessary facilities for such studies are not present in food research laboratories, especially in developing countries. Besides, *in vivo* methods can be laborious and often require several motivated subjects for long periods of time (Granfeldt *et al.*, 1992).

The present work is aimed at developing an *in vitro* approach to obtain a differential view of the direct or indirect digestibility of food constituents. This is attempted by a combination of successive steps of enzymic digestion, biological absorption of low molecular weight degradation products, and fermentation of residual materials using intestinal microorganisms. This method is not meant to be a simulation of the complex human digestive system, but rather a standardized tool to assess the effects of food processing operations on the bio-physiological behaviour of foods. These effects are examined in kenkey, a traditional fermented maize product of Ghana.

MATERIALS AND METHODS

Maize (*Zea mays* L cv. obaatanba) and white cowpeas (*Vigna unguiculata* cv. benpla) were obtained from the Crops Research Institute, CSIR, Kwadaso, Ghana. Baker's yeast, *Saccharomyces cerevisiae*, was obtained from a local bakery. *Clostridium perfringens* NCTC 8239 was obtained from the National Institute of Public Health and Environmental Hygiene (RIVM), Bilthoven, The Netherlands.

Sample preparation
Maize (M) and cowpea-supplemented (Mw) kenkeys were produced as described earlier (Nche *et al.*, 1994b). Samples tested included the untreated maize (M) and 4:1 mixture of maize and white cowpeas (Mw), dough from grains soaked at room temperature for 48 h (M_0 and Mw_0), unfermented maize and maize-cowpea doughs cooked for 1 h (Mw_0C_1), uncooked fermented (4 days) maize and maize-cowpea doughs (M_4 & Mw_4) and fermented maize and maize-cowpea doughs cooked in boiling water for 1 h (M_4C_1 & Mw_4C_1) or for 3 h (M_4C_3 & Mw_4C_3). Except for untreated samples, all soaked grains were first ground to pass through a 4mm screen, in a hammer mill (Fritsch Pulverisette Type 14.702, Marius Instruments, Utrecht, The Netherlands) before further processing. Before testing, all samples were freeze-dried and ground to pass through a 1 mm screen.

In vitro digestibility and fermentability of samples

The method used is composed of three main stages: (1) enzymic digestion in which samples were treated with enzymes similar to those found in the human digestive system; (2) absorption in which the simple sugars resulting from enzymatic digestion were removed by incubating the suspension with the yeast, *Saccharomyces cerevisiae*; and (3) fermentation in which the yeast-treated supernatants and non-digestible residue resulting from stage one were incubated with *Clostridium perfringens* and the amount of gas produced measured.

Enzymic digestion

Two protocols (slow protocol and fast protocol), both of which were adaptations of previously described methods for determining total dietary fibre content of foods were used. The method of Schweizer and Würsch (1979) was adapted and used as the slow protocol to determine *in vitro* digestibility at 37°C. Due to long incubation periods in this method, a preservative, thymol (Merck 8167) was added to prevent unwanted microbial contamination. This method was therefore suitable only for stage one because the presence of an antimicrobial agent would not permit the activity of the yeast and bacteria used in later stages. The fast protocol was an adaptation of AOAC procedure 985.29 (AOAC, 1990) for determining the total dietary fibre content of foods. Here, incubation periods were shorter than in the slow protocol, and the use of higher temperatures removed the risk of microbial contamination in the first stage. Both protocols are described in the sections below.

1.0 *Slow protocol*

1.1   Weigh 5 g of sample (dry matter) accurately (A) into a pre-weighed 250 ml beaker or wide-neck conical flask.

1.2   Add 50 ml distilled water and mix by gentle swirling and adjust pH to 1.5 with 5N HCl. Note volume of acid used.

1.3   Add 100 mg pepsin NF (Merck, 7197, Darmstad, Germany).

1.4   Adjust total weight of suspension to 75 g with distilled water and cover beaker with aluminium foil.

1.5   Incubate in a shaking incubator (120 rpm) at 37°C for 20 h.

1.6   Adjust pH to 6.5 with 3N NaOH. Note volume of alkali used.

1.7   Add 0.5 g of thymol crystals (Merck 8167) as a preservative.

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1.8   Add 200 mg pancreatin FU, USP (Merck 7130) and 100 mg amyloglucosidase (Merck E 21 8432) in 20 ml 0.1 M phosphate buffer (pH 6.5).

1.9   Adjust weight of suspension to 100 g with distilled water and cover beaker as above.

1.10   Incubate in a shaking incubator (120 rpm) at 37°C for 18 h.

1.11   Centrifuge at 4000 rpm for 20 minutes and save supernatant (S1).

1.12   Wash pellet with 20 ml distilled water, centrifuge as above and keep supernatant (S2).

1.13   Dry pellet to constant weight and weigh (R_1).

1.14   Pool supernatants S1 and S2 in a 100 ml volumetric flask.

1.15   Adjust volume to 100 ml with distilled water.

1.16   Transfer 10 ml to a clean dry 100 ml conical flask and weigh.

1.17   Add 4 volumes of ethanol (95%) to precipitate soluble fibres.

1.18   Centrifuge at 4000 rpm for 20 min and discard supernatant.

1.19   Dry pellet to constant weight and calculate total soluble fibre (R_2) in S1 + S2.

Control

A reagent blank without sample and with equal amounts of enzymes, preservative, acid and alkali as used above is passed through the same steps as the sample up to step 1.9 where the dry weight (B) of the final suspension is determined and used to correct for non-sample residue.

Calculations

Apparent *in vitro* digestibility is calculated as follows:

$$Digestibility = \frac{A+B-(R1+R2)}{A} * 100\% \qquad [1]$$

2.0 *Fast Protocol*

2.1   Weigh 5 g of sample (dry matter) accurately (A) into a pre-weighed 250 ml beaker.

2.2   Add 50 ml of 0.1M phosphate buffer (pH 6.0) and re-adjust pH with 0.1N NaOH or 0.1N HCl to 6.0 ± 0.2. Note volume of either acid or alkali used.

2.3 Add 0.5 ml of Termamyl (heat-stable a-amylase, Sigma A-0164) to the suspension.

2.4 Cover the beaker with aluminium foil, place in a boiling water bath and incubate at 95-100°C for 30 minutes with shaking at 5 min intervals.

2.5 Immerse beaker in cold water to cool the suspension to room temperature, and adjust pH to 7.5 ± 0.2 using 3N NaOH solution. Note volume of alkali used.

2.6 Add 25 mg of protease (Sigma P-3910) and incubate the suspension at 60°C for 30 min with regular shaking as above.

2.7 Cool suspension to room temperature and adjust the pH to 4.5 ± 0.1 with 5N HCl. Note volume of acid used.

2.8 Add 1.5 ml of amyloglucosidase (Sigma A-9913) and incubate for 30 minutes at 60°C with regular shaking as above.

2.9 Cool to room temperature and adjust pH to 3.0 ± 0.1 with 5N HCl. Note volume of acid used.

2.10 Centrifuge at 4000 rpm for 15 minutes and save the supernatant (S1).

2.11 Wash pellet with 4 x 10ml changes of distilled water, each wash followed by centrifugation at 4000 rpm for 15 min to give 4 more supernatants (S2 - S5). If necessary, filter supernatants through a Whatman No. 1 filter paper to remove any suspended particles.

2.12 Freeze-dry the final pellet (R_1), weigh and keep for use in the fermentation step.

2.13 Pool the 5 supernatants (S1 - S5) and record the weight of the pool (S6).

2.14 Take out 1g of S6 and precipitate with 4 vols. of 95% ethanol, and determine total weight of water-soluble fibre (R_2).

2.15 If necessary, re-adjust the pH of S6 to about 3 (with 5 N HCl), to limit unwanted microbial growth in the period before the absorption step. Note volume of acid used.

A reagent blank (B) without sample is set up as earlier described and *in vitro* digestibility calculated according to equation [1].

3.0 *Absorption*

This step involves the uptake of degradation products of fast enzymic digestion (protocol 2), mainly monosaccharides from the breakdown of polysaccharides such as starch. Here, the yeast *Saccharomyces cerevisiae* is used to remove the

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absorbable monosaccharides. Fresh Baker's yeast is used because it can be obtained easily in large quantities and also because of its much higher affinity towards monosaccharides compared with oligosaccharides such as raffinose and stachyose. A previous investigation by HPLC (data not included) showed that the major carbohydrate degradation products (glucose, fructose, sucrose) were taken up by the yeast within 3 h, and that fermentation or assimilation of raffinose and stachyose by Baker's yeast was negligible.

Protocol

3.1   Re-adjust S6 ($\approx$ 96 g) with 3 N NaOH to pH 6. Note volume of alkali used.

3.2   Take out 1g and dry (48 h at 80°C) to determine the dry weight (W_1) of S6.

3.3   Mix with 5 g of fresh Baker's yeast.

3.4   Incubate for 3h at 30°C with orbital shaking (120 rpm).

3.5   Centrifuge the suspension (4000 rpm for 15 min) to remove the yeast cells. Save supernatant (S7).

3.6   Wash the pellet with 5 ml of 0.1M phosphate buffer (pH 6.0), centrifuge (4000 rpm) and pool the supernatants with S7. Weigh S7 and determine total dry weight (W_2) as in section 3.2.

3.7   Filter-sterilize S7 for use in the fermentation step

The amount of supernatant taken up by the yeast (W_1 - W_2) is calculated and the apparent absorbability of the original sample calculated as follows:

$$Absorbability = \frac{(W1 - W2)}{A} * 100\% \qquad [2]$$

4.0 Fermentation

Here, *Cl. perfringens* is used to ferment what remains from the first two stages i.e. the residue (R_1), and the yeast-treated supernatant, (S7). Fermentation is carried out in 100 ml serum bottles and gas production measured periodically as described earlier (Nche *et al.*, 1994b).

Protocol

4.1   Prepare both single and double-strength sugar-free thioglycollate medium (per litre: 5.0 g yeast extract, 15.0 g tryptone, 0.5 g sodium thioglycollate, 2.5 g NaCl, 0.5 g L-cystine, 0.5 g Agar No. 1, pH 7.1).

4.2 Dispense 40 ml each of single-strength and 20 ml each of double-strength medium in 100 ml serum bottles and autoclave (15 min, 121°C, 15 psi).

4.3 Add a known amount ($\approx$ 0.2 g) of freeze-dried residue (R_1) from the digestion step to a serum bottle containing single strength medium; or 20 ml of filter-sterilized yeast-treated supernatant (S7) to a serum bottle containing double-strength medium.

4.4 Inoculate with 1 ml of active inoculum (24 h pre-culture of *Cl. perfringens* strain NCTC 8239, in normal thioglycollate medium, U.S.P., containing 0.5% glucose).

4.5 Attach culture bottle via a syringe needle to the gas measuring device (Nche *et al.*, 1994b) and arrange the whole setup such that the culture vessel suspends in a waterbath maintained at 37°C by a thermostat heater.

4.6 Incubate and measure gas production in ml (G) for 24 h.

A control is set up by inoculating 40 ml of single strength sugar-free thioglycollate medium without substrate (r) and measuring the gas (B) produced during 24 h of incubation at 37°C.

Gas production from non-digestible residues per gram of original sample (dry weight basis) is calculated as follows:

$$Gas(ml/g) = \frac{(G-B)*S}{A*r} \qquad [3]$$

Where:

G   = Gas produced (ml) in 24 h from substrate r

B   = Gas produced from blank (ml)

S   = Total amount (g dry weight) of residue (R_1) or supernatant (S_7).

r   = Amount (g dry weight) of residue (R_1) or supernatant (S_7) used in fermentation.

A   = Weight (g) of original sample.

Determination of total (TS) and resistant (RS) starch content of residues

Total starch was determined by a procedure (Edwards, *pers. comm.*) adapted from the method of Englyst *et al.* (1992). 100 mg of freeze-dried sample was placed in screw-capped polypropylene centrifuge tubes containing acetate buffer and boiled for 30 min. After cooling, samples were dispersed in 2 N KOH for 15 min at 0°C,

before hydrolysing with amyloglucosidase in acidic medium. The samples were again boiled for 10 min, cooled and pH adjusted with 4 N KOH. Samples were centrifuged to obtain a clear supernatant, the glucose content of which was determined using a glucose oxidase kit (Merckotest, GOP-PAP, Merck Ltd).

Resistant starch was analyzed according to Faisant *et al.* (1995). 100 mg of sample was incubated with pancreatic amylase for 16 h at 37°C. Ethanol was added to a final concentration of 80%, the samples centrifuged and the soluble fraction removed with the supernatant. The pellet was washed with 80% ethanol and incubated in boiling water for 30 min, after which it was cooled, dispersed in 2 N KOH for 30 min at 0°C, and then proceeded with analysis as in the determination of total starch.

RESULTS AND DISCUSSION

A comparison of the two *in vitro* digestibility protocols as well as the effect of various unit operations on digestibility are presented in Table 8.1. The results show that there were no significant differences in the two protocols. Since the fast protocol involves higher temperatures not representative of the human system, it was hypothesized that the heating could result in higher enzymatic breakdown of samples, and as such, cause an overestimation of *in vitro* digestibility values. The results obtained do not show any such effects and it is possible that the long incubation period in the slow protocol adequately compensates for the increased digestibility due to higher temperatures in the fast protocol. In any case, the results obtained are comparative rather than absolute as either protocols cannot be taken to accurately represent the human system. The fast protocol, however, has the added advantage that the absence of preservatives allows for the incorporation of the biological absorption and fermentation steps.

Table 8.1 also shows that the most significant increase in digestibility is effected by the soaking of the raw grains. Further processing which includes fermentation and cooking for up to 3 h has only limited effect on the *in vitro* digestibilities of the samples tested. Soaking is known to soften grains and lead to absorption of water by the starch moiety of grains which then swells and become more accessible to digestive enzymes (Akingbala *et al.* 1987). Although not determined, further processing such as fermentation and cooking could be expected to increase the speed of digestion, though the total digestibility remained unchanged.

Table 8.1. *In vitro* digestibilities of samples as determined by the slow and fast protocols

| Sample | Slow protocol | Fast protocol |
	%digestibility (mean ± sd)	%digestibility (mean ± sd)[1]
M^2	75.23 ± 0.74	77.83 ± 0.74
M_0^3	82.50 ± 0.04	80.10 ± 1.70
M_4^4	83.40 ± 0.33	80.20 ± 2.20
M_4C_1	83.40 ± 0.79	82.63 ± 0.63
M_4C_3	83.27 ± 0.85	82.23 ± 0.70
Mw^2	71.47 ± 0.46	74.33 ± 0.74
Mw_0^3	78.80 ± 0.71	78.00 ± 0.97
Mw_0C_1	nd	85.66 ± 3.29
*pH-Mw_0C_1	nd	86.29 ± 2.06
Mw_4^4	80.40 ± 0.87	80.80 ± 0.58
Mw_4C_1	80.93 ± 0.79	81.47 ± 0.69
Mw_4C_3	80.70 ± 0.67	80.99 ± 0.53

[1]Mean ± sd (for n = 3); [2]Untreated maize (M) and mixtures of 80% maize and 20% white cowpeas (Mw); [3]Soaked (2 days at room temperature); [4]Four days' fermented dough; C_1 & C_3 = cooked for 1 and 3 hours respectively; *pH adjusted to 4.0 ± 0.1 before cooking.

It was also observed that the digestibilities of all-maize samples were generally higher than maize-cowpea samples. This could be the result of more resistant starch being formed from the legume fraction of the mixtures, given that legume starch is more difficult to digest than cereal starch. The reasons for this difference reportedly could include the relatively high amylose/amylopectin ratio of legume starch, the physical insulation of starch by thick-walled cells and the presence of amylase inhibitors in legumes (Tovar *et al.*, 1992).

Figure 8.1 shows that processing did not significantly affect the absorbability of both maize and maize-cowpea blends, with values ranging from

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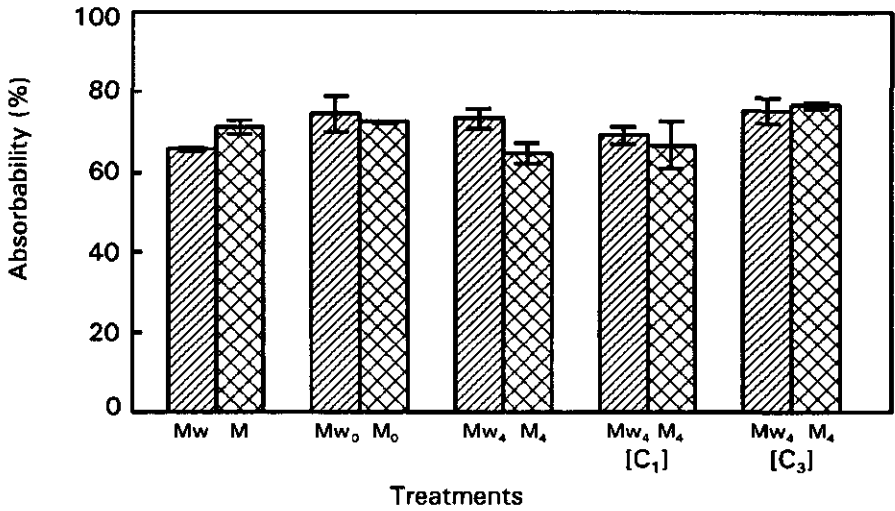
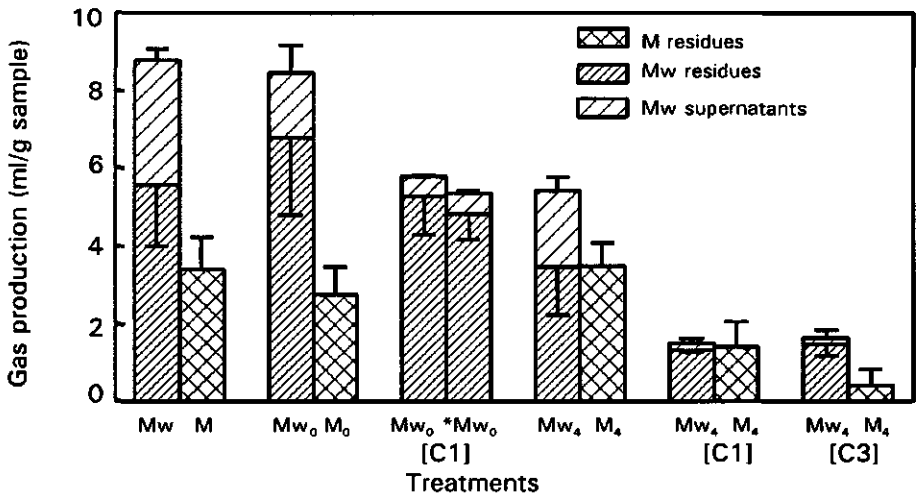Figure 8.1. Apparent absorbability of digested maize and maize-cowpea samples

Figure 8.2. Gas production from maize and maize-cowpea samples
(M) Untreated maize grains; (Mw) untreated maize-cowpea blend; (M_0 & Mw_0) Soaked for 2 days at R.T.; (M_4 & Mw_4) Fermented for 4 days at 30°C, (C1 & C3) Cooked for 1 h and 3 h respectively to kenkey. *pH of dough adjusted to 4.0 ± 0.1.

71% and 66%, respectively in raw maize and maize-cowpea blend to 77% and 75%, respectively in fermented maize and maize-cowpea kenkey cooked for 3 h. There was no significant difference ($p < 0.05$) between the maize and maize-cowpea samples. These values show that less than 85% of the solubilised matter was taken up by the yeast and this could be due to several reasons. First, the yeast did not take up the a-galactosides (raffinose and stachyose) present in the supernatant of Mw samples; secondly, because of the short incubation time, the yeast was not able to assimilate other soluble substrates such as amino acids for biomass formation.

Figure 8.2 shows gas production from the residue and supernatant left over from the enzymic digestion and absorption steps. It can be deduced from these results that, generally, gas production from the residue was higher than from the yeast-treated supernatants. This is not surprising since lactic acid fermentation is known to reduce the levels of flatus-forming oligosaccharides in legumes (Mital and Steinkraus, 1975; Duszkiewicz-Reinhard et al., 1994). Analysis by HPLC (data no included) showed a 64% and 22% reduction of the levels of raffinose and stachyose, respectively, in the supernatants of maize-cowpea blends following 4 days of fermentation. Murphy et al. (1972) reported equal gas production from both alcohol extracts and residues of dry beans, suggesting that substrates other than a-galactosides could also be responsible for inducing flatus in humans. With respect to the residues, gas production from maize-cowpea samples was significantly ($p < 0.05$) higher than from maize samples. The type of fermentation and resulting gas production are known to depend on the chemical composition and botanical source of the substrate (McBurney and Thompson, 1990). It may be the case here as legume starch would be expected to be more resistant to hydrolysis by a-amylase than cereal starch (Tovar et al., 1992) and would therefore contribute more to the residual fraction of maize-cowpea than maize samples following enzymatic digestion. The difference in digestibility between Mw and M samples is supportive of this. The different compositions of legume and cereal NSPs forming the residue could also influence the degree and type of fermentation by Cl. perfringens and hence the amount of gas produced from either sample type within the 24 h of fermentation.

No gas was produced from incubations with M supernatants. The absence of flatulence-forming oligosaccharides in maize would be an obvious reason for this observation but it also suggests that no other soluble fermentable carbohydrates (e.g. starch, oligosaccharides) were present in the supernatants following enzymatic digestion and the absorption steps.

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It can also be observed from Fig. 8.2 that food processing treatments significantly affect gas production from residues and supernatants. Following soaking, an increase in gas production was observed with residues from Mw samples but a significant reduction from Mw supernatants. There was no change in gas production from the residue of M samples. After fermentation, there was reduced gas production from Mw residue, but not for M residue which produced as much gas as untreated and soaked samples. Fermentation also did not effect any further reduction of gas production from Mw supernatants. Cooking of fermented samples further reduced gas production from the residues as well as the supernatants. It was hypothesized that a combination of the acid formed during lactic fermentation and the heat applied during cooking resulted in the hydrolysis of oligosaccharides and any resistant starch that would otherwise add to the undigested fraction. To test this hypothesis, the pH of soaked maize-cowpea (Mw_0) dough was adjusted with a solution of 20% lactic acid and 2% acetic acid to the same level (4.0 ± 0.1) as that of a 4 days' fermented (Mw_4) dough, then digested and the resulting residue and supernatant tested for gas production. The results (cf. pH-Mw_0C_1 in Fig. 8.2) show that, although there was a reduction in gas production from the resulting residue, the amount of gas produced was still higher than from raw fermented (Mw_4) samples. There was no significant difference between Mw_0C_1 samples with or without pH adjustment, suggesting that pH alone may not cause a reduction of the flatus-forming potential of soaked (Mw_0) samples. Lactic acid bacteria may, therefore, play an important part in reducing the flatulence capacity of such foods. Lactic acid bacteria have been reported (Mital and Steinkraus, 1975; Duszkiewicz-Reinhard, 1994) to possess α-galactosidase activity and therefore the ability to utilise raffinose and stachyose, the effect of which would be a reduction in flatus in humans consuming such fermented products.

Table 8.2 shows that significant amounts of starch (TS) were present in the residues resulting from *in vitro* digestion of maize-cowpea (Mw) mixtures. TS and resistant starch (RS) were significantly reduced after soaking, possibly as a result of endogenous α-amylase activity. Further reduction was observed after fermentation, during which some of the starch could have been hydrolysed by microorganisms in the fermenting dough. Significant increases in TS were again observed after cooking. Most of this starch (> 50%) was RS, which could have been formed as a result of retrogradation as the cooked samples cooled.

There appears to be no correlation between starch content of and gas production from the residues. This lack of correlation would suggest that starch is

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Table 8.2. Resistant and total starch contents of and gas production from residues resulting from *in vitro* digestion

SAMPLE	GAS (ml/g residue)	TS[1]	RS[2]	TS-RS	RS/TS (%)
Mw^3	26.75 ± 7.81	7.02	1.96	5.06	27.92
Mw_0	33.92 ± 9.92	2.22	0.89	1.33	40.09
Mw_0C_1	26.36 ± 4.89	23.89	14.26	9.63	59.69
*$pH-Mw_0C_1$	24.15 ± 3.20	9.01	6.79	2.22	75.36
Mw_4	17.42 ± 6.15	1.76	0.65	1.11	36.93
Mw_4C_1	6.7 ± 0.40	14.69	7.38	7.31	50.24

[1]Total starch, [2]Resistant starch, [3]See Table 8.1 for abbreviations.

not the only fermentable substrate in the residue, or that the strain of *Cl. perfringens* used is unable to degrade it. Fleming (1981) reported a negative correlation between the starch contents of legume seed residues fed to rats and gas production and concluded that carbohydrates such as glucans and pentosans induced flatulence when fermented by colonic microflora. Gas production from the residue of cooked fermented samples (M_4C_1 and Mw_4C_1) was significantly lower than in uncooked samples, suggesting that lactic acid fermentation, coupled with cooking, resulted in the modification of the fibre, rendering it less susceptible to fermentation by *Cl. perfringens*. Bourquin *et al.* (1992) noted that the fermentability of dietary fibre was influenced, not only by the source of the fibre, but also by its pre-treatment prior to fermentation. Different sample treatments could influence the rate of fermentation of residues, hence 24 h incubation with *Cl. perfringens* may not be enough for significant fermentation of the residue from fermented and cooked samples. Pre-treatment of material could also cause a shift in the type of by-products resulting from fermentation of the undigested fraction. For example, more SCFA may be produced instead of gas. In order to obtain a clearer idea about the digestibility and fermentability of cereal and cereal-legume foods, gas as well as short-chain fatty acid (SCFA) production during *in vitro* fermentation of the residue would have to be measured.

The results obtained here do give a good indication of the possible causes of flatulence following the consumption of cereal and cereal-legume foods. They confirm that low molecular weight oligosaccharides and undigested starch may not be the only cause of flatus in humans consuming such foods. It must, however, be kept in mind that a simple approach such as this cannot be fully representative of the complex human digestive system. The use of a single organism, for example, does not fully mirror the enormous diversity in colonic microflora. This approach was, however, necessary to keep the already complex 3-part protocol as simple as possible. Fecal preparations may be difficult to standardise, but their use for *in vitro* fermentation of dietary fibres, monitored by their production of gas and short-chain fatty acids, might further increase the representativeness of such a method.

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General Discussion[1]

This study focuses on the processing as well as the nutritional, physical and safety aspects of kenkey. It has laid the foundation for the exploitation of technologies for processing, not only foods for adults, but also infant weaning foods for the tropics from indigenous raw materials such as cereals (e.g. maize) and legumes e.g. cowpea). It is clear from this study that the processing as well as the nutritional value of kenkey can be improved. The implications of these improvements need, however, to be considered before implementation.

Lactic acid fermentation, proved to be an appropriate technology, not only for cereals from the tropics, but for legumes also, especially if these are blended with cereals such as maize. The lowering of pH, due to acidification of maize or maize-cowpea doughs is very important for taste and flavour development and is now widely recognized as crucial from a microbiological safety perspective (Mensah *et al.*, 1991; Larsen *et al.*, 1993). This study also confirmed the positive role of fermentation with respect to the availability of essential amino acids such as lysine.

Improving protein quality and quantity

At first glance the idea of supplementing an adult diet such as kenkey with a legume protein source, seems debatable since kenkey can be eaten with a fish sauce as a side-dish. It could, therefore, be argued that, instead of supplementing maize with cowpea, more of the kenkey could be substituted with the side-dish. This is however not easy for several reasons. First, being a staple, kenkey is always eaten in large quantities, sometimes as breakfast, lunch and dinner. Secondly, the side-dish is often made of fish which is expensive and therefore not readily available to the ordinary folk. A third, though indirect reason for supplementation is that such products can then be extended by converting into infant weaning foods at no extra cost. More often than not infants develop their parents tastes and it would therefore, be easier to introduce as a weaning food, a product that is well known and accepted by parents.

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[1]Parts of this chapter have been presented at "Bioavailability'93" conference of 9-12 May 1993 in Ettlingen, Germany and at the ECSAFoST regional Food Conference of 12-16 September 1994 in Victoria Falls, Zimbabwe.

Cowpea was the logical choice of legume for use in supplementing maize during kenkey production because, like soya bean, it is relatively high in protein content: 24-28% (Akinyele *et al.*, 1986). Also, cowpea is a legume that is commonly found in most West African households (Dovlo *et al.*, 1976). In Ghana and Nigeria, for example, cowpea is already being used widely to make other products such as koose or akara (McWatters, 1983). The locals are therefore familiar with the ways of handling this legume, as well as with its taste. This is in contrast with soya bean which, although widely accepted as a very rich protein source, is more expensive than cowpea and still considered foreign to most West African households.

Table 9.1. Essential amino acids balance in cowpea-supplemented kenkey compared with concentrations in all-maize kenkey and FAO recommendations (Adapted from Bressani and Scrimshaw, 1961).

Amino acid (mg/gN)	100% Maize	Maize:cowpea (80:20)	FAO recommendation
Lysine (mg/gN)	139	207	270
S-amino acids (mg/gN)	195	178	270

Chapters 2 and 5 outline the advantages of supplementing maize with cowpeas. There is a significant increase in the protein content as well as of the level of available lysine. Table 9.1 shows how the essential amino acid balance of maize kenkey could be affected by supplementing (on a 20% replacement level) with whole grain cowpeas. Although there is a drop in the level of S-amino acids, this is not significant as the overall amino acid balance in this mixture is better than that for maize alone. The addition of cowpeas also improves on the texture of the final product. As shown in Chapter 2, addition of cowpea results in a less friable product that requires more force (δ_f) to break. A measurement of the viscosity profile of maize-cowpea dough (Fig. 9.1) shows that cowpea addition gives a dough with higher hot paste and set-back viscosities and hence an aflata with a superior binding capacity compared with the traditional all-maize aflata.
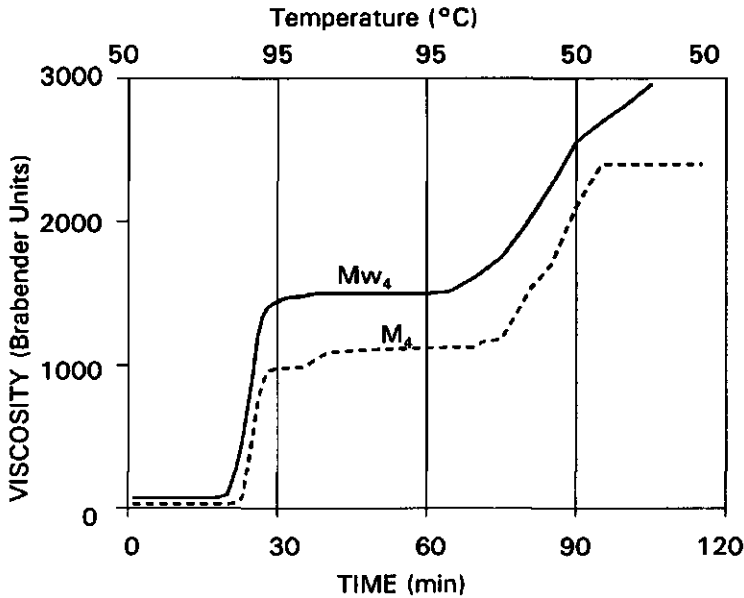
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Figure 9.1. The effect of cowpea on the pasting properties of traditional maize dough. M_4 = traditional kenkey dough; Mw_4 = cowpea-supplemented dough.

A panel of five Ghanaians familiar with kenkey (see Chapter 2) carried out a sensory evaluation of cowpea-supplemented kenkey and could not detect the presence of any beany flavour at 20% cowpea level. A broader sensory evaluation carried out in Ghana, however, resulted in the acceptance of kenkey supplemented at 10% level only. At 20% supplementation the product was not accepted as kenkey. The main points of contention were the unfamiliar flavour and the beany taste imparted by the cowpea. Although the product with 20% cowpea fared poorly with experienced kenkey eaters, indications are that it could be introduced as a different product, possibly as a weaning food for infants, who have not yet developed a strong liking for the particular kenkey flavour and taste. Earlier work (Akinyele and Fasaye, 1988) on ogi, which is widely used as a weaning food in West Africa, indicated that a 30% supplementation of maize with dehulled cowpea obtained a 100% acceptability (80% as very good and 20% as good).

Dry-milling and accelerated fermentation

The traditional kenkey manufacturing process has two major drawbacks; the length of time it takes to make a batch of kenkey, and the short shelf-life of the product resulting from its high moisture content (ca. 60%) and type of wrapping. The process can take up to a week to complete (Sefa-Dedeh Plange, 1989) and is therefore not economically viable if production was to be carried out at an industrial scale. It is therefore logical that this process be made more efficient with respect to processing time. Time-consuming steps are the soaking and fermentation steps, which when combined can take up to 5 days. The use of spontaneous fermentation often results in a dough with variable quality.

Dry-milling and accelerated fermentation were, therefore, seen as possible solutions to the time and quality problems. These alterations were expected to cut down on the process time as well as to allow for a better control of the fermentation process to maintain dough and kenkey quality. Omitting the soaking, however, removed one very crucial step in the development of characteristic kenkey texture. It is reported (Sefa-Dedeh and Plange, 1989) that soaking not only softens the otherwise hard maize grain, but also results in amylolysis and proteolysis which probably contribute to the development of kenkey flavour and texture. A wide range of endogenous enzyme activities was recorded after soaking maize at room temperature. Suppression of these enzymes in whole or dry-milled maize by soaking at 60°C (Chapter 4) resulted in a dough with pasting viscosities inferior to that of dough obtained from traditionally treated maize. A sensory evaluation of kenkey from dry-milled maize showed that it had a texture inferior to that of traditional kenkey. Dry-milling resulted in starch damage, and the resulting dough had an inferior swelling and pasting potential compared with traditionally made dough. Wet-milling, on the other hand, causes less starch damage, while proteolysis and amylolysis during soaking facilitate adequate swelling of starch granules and the subsequent gelatinisation and set-back on cooling. Adeyemi and Beckley (1986) and Akingbala et al. (1987) reported that dry-milling of maize resulted in up to 41% and 32% of starch granules damaged, respectively.

To overcome the problems associated with dry-milling and still obtain a significant reduction of production time, the maize could first of all be cracked before soaking. In this case it takes only 16 hours to achieve the same degree of hydration that occurs when whole grain kernels are soaked for 48 hours. The resulting dough can then be fermented by the accelerated process.

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Accelerated fermentation requires the use of starters. Conventional starters as used in the production of cheeses and yoghurts are pure cultures used either as a single species or a combination of different species of microorganisms. They are used in sufficiently large concentrations to quickly dominate the previously pasteurized substrate and prevent outgrowth of contaminants as well as produce the required taste and flavour components.
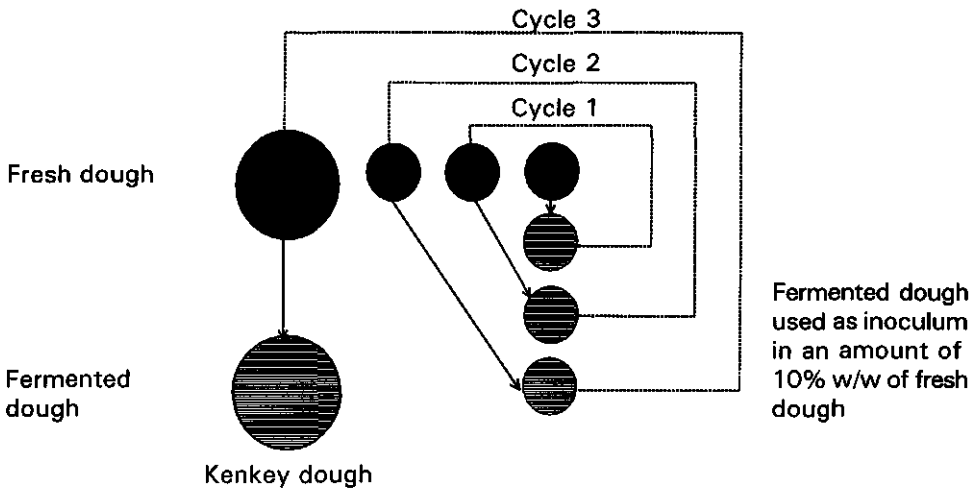

Figure 9.2. Schematic presentation of the back-slopping technique

The use of pure culture starters makes it easier to control fermentations and maintain the quality of the product. Developing and maintaining such pure cultures requires laboratory infrastructure and trained personnel and is too expensive (Nout, 1992) to be suitable for situations such as are usually encountered in developing countries. The "back-slopping" method (Spicher, 1986; Nout et al., 1989) for developing starter doughs (Fig. 9.2), is cheaper and very simple to carry out, and if properly done, results in the same quality of product with respect to flavour and taste. Back-slopping, therefore, provides a useful alternative to pure culture starters.

Although it has not yet been investigated in detail, it is expected that a "back-slop" starter dough would contain the most acid-tolerant LAB, given that a

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natural selection occurs, resulting in the elimination of acid-sensitive (heterofermentative) LAB, which are often present at the start of each spontaneous fermentation. *Lactobacillus plantarum* and *Pediococcus* spp. were shown (Chapter 2) to dominate the later stages of natural fermentations. Hounhouigan (1994) also reported the presence of *L. fermentum* in fermented maize doughs.

Yeasts such as *Candida krusei* and *Saccharomyces cerevisiae* have been reported to be present in fermented maize doughs (Halm *et al.* 1993, Hounhouigan 1994). In this study, it was found that, although yeasts and moulds were present in the early stages of spontaneous fermentation and "back-slopping", they usually disappeared by the end of both processes. Those who report the presence of yeasts, cite them as contributing to the development of flavour components in fermented maize doughs. It could then be argued that the use of "back-slops" which contain mainly LAB, would result in a dough that lacks some of the flavour components of traditionally fermented doughs. Nevertheless, the clean acid aroma and sharp sour taste imparted by the organic acids produced by LAB are notably present and strong enough to meet the consumers' demand for properly fermented kenkey dough. The use of back-slops limits competition from spoilage and pathogenic microbial contaminants during fermentation. This maintains product quality and could prove to be a significant improvement should an industrial-scale process for kenkey production be developed along the lines discussed in this thesis.

Safety of kenkey
The presence of biogenic amines such as putrescine and cadavarine in detectable concentrations in cowpea-supplemented kenkey is an indication of how the introduction of cowpea could influence the safety quality of kenkey. Although the concentration of histamine, most associated with toxigenic effects in humans, was below detection (Chapter 6), tyramine, putrescine and cadavarine were present in concentrations that suggest caution must be exercised when cereal-legume products are processed by spontaneous fermentations, given that such products are staples eaten on a regular basis and in large quantities.
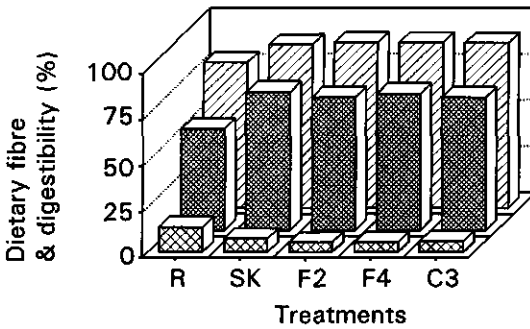
Digestibility and total dietary fibre contents
The choice of ingredients and processing options would influence the digestibility of the final product, kenkey. The effect of three processing steps of kenkey production; soaking, fermentation and cooking on the total dietary fibre content and *in vitro*, total and protein digestibilities of kenkey was investigated.

A. 100% maize
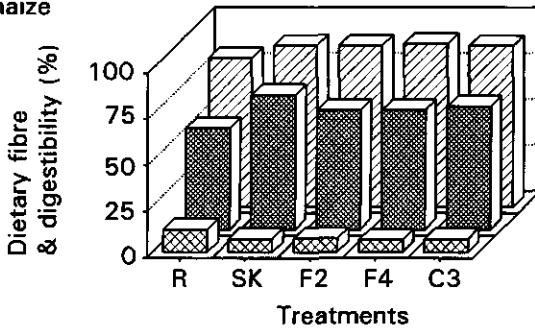
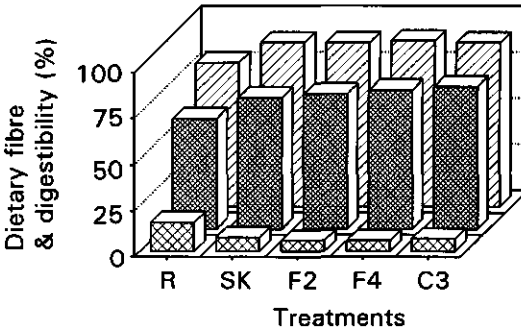B. maize:white cowpea

C. maize:red cowpea

Figure 9.3. Dietary fibre ▨; Protein digestibility ▧; and total digestibility ▨ of maize and maize-cowpea kenkeys. (R) untreated grain, (SK) soaked grains, (F2) 2 days' fermented dough, (F4) 4 days' fermented dough, (C3) kenkey cooked for 3 hours after 4 days' fermentation.

Total dietary fibre was determined by AOAC 985.29 method (AOAC, 1990). *In vitro* total and protein digestibilities were determined on the basis of weight and nitrogen content of residual matter from dietary fibre determination. In general, there were no significant differences ($p > 0.05$) between the dietary fibre contents, the total and protein digestibilities of maize and cowpea-supplemented kenkey. Soaking was found to be most effective in reducing dietary fibre and increasing *in vitro* digestibility. Following soaking, the total dietary fibre content of the raw materials were reduced by 40%, 46% and 51%, whilst total *in vitro* digestibility increased by 9%, 13% and 14% for maize, maize-white cowpea (cv. asontem) and maize-red cowpea (cv. benpla) mixtures, respectively (Fig. 9.3a-c). Increases in protein digestibility of 33%, 36% and 18% were recorded for unfermented maize (Fig.9.3a), maize-white cowpea (Fig. 9.3b) and maize-red cowpea (Fig. 9.3c) doughs, following soaking. Fermentation resulted in further, but non-significant decreases in total dietary fibre. Cooking for up to three hours tended to slightly increase the dietary fibre contents. Fermentation and cooking did not significantly improve on the values for *in vitro* total and protein digestibility obtained after soaking. These results again confirm that soaking is a very important step in the kenkey process.

Antinutritional factors and flatulence

Another important aspect of supplementation with cowpea is the introduction of higher levels of antinutritional factors (ANFs) in the form of protease inhibitors including trypsin inhibitors (TI) and phytic acid (PA). Processing, however, is known to significantly reduce most of these ANFs (Somiari and Balogh, 1993, Duszkiewicz-Reinhard *et al.*, 1994). The traditional kenkey process was found to reduce PA by 49% and TI by 86% from original levels of 12.0 mg/g and 15.4 mg/g (dwt.). The use of *C. krusei, L. plantarum, L. fermentum* and *Pediococcus pentosaceus* as pure culture starters in the fermentation of dough for kenkey production (Table 9.2) did not significantly influence the level of TI, although *L. fermentum* and *P. pentosaceus* initiated significant reductions in PA. Moreover, any significant effects were registered only after cooking and not after fermentation suggesting that cooking and not microbial fermentation was responsible for these reductions.

The introduction of a legume such as cowpea meant that more attention needed to be paid to the presence of flatus-forming components in kenkey. It was necessary to monitor the presence of flatus-forming oligosaccharides such as

Table 9.2. Effect of pure culture starters of yeast and lactic acid bacteria on the antinutritional factor (ANF) content of kenkey

| Microorganism | Total reduction in antinutritional factors | | |
	TI[1]	PA[2]	Oligosaccharides[3]
Control[4]	86	49	43
L. fermentum	71	62	nd[5]
L. plantarum	55	56	nd
P. pentosaceus	63	75	nd
C. krusei	86	49	nd

[1]Trypsin inhibitor (% w/w, dwt. basis); [2]Phytic acid (% w/w, dwt. basis); [3]Raffinose and stachyose; [4]Naturally fermented; [5]Not determined.

raffinose and stachyose, present in cowpeas. The traditional process reduced these oligosaccharides by (43%). A lot of emphasis has been placed on oligosaccharides as the flatus factor in legumes and conventional methods of determining the flatus potential of foods have often involved extractions of these oligosaccharides for measurement by HPLC (Kennedy et al., 1985) or the measurement of gas and short chain fatty acids (SCFA) produced when wholesome foods are incubated with either fecal slurries (Kikuchi and Sakata, 1992, Salvador et al., 1993) or pure cultures of saccharolytic bacteria of colonic origin (Nowak, 1992). However, poorly digestible carbohydrates other than low molecular weight oligosaccharides are known to be fermented in the lower gut, resulting in the formation of SCFAs as well as gas (Bourquin et al., 1992).

Whilst methods involving the use of equipments such as HPLC and GC are complicated and expensive, the simple alternative of incubating whole foods with microorganisms of colonic origin (Nowak, 1992) overlooks one important fact - that not all the carbohydrates present in wholesome foods such as are incubated with colonic microflora, will be present in the portion of the food that escapes digestion in the upper gut. Simple sugars such as glucose and fructose are completely absorbed in the upper gut and are therefore not available for fermentation in the lower bowel. Any procedure that does not take this into

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account would result in an overestimation of the flatulence potential of the tested product. As already shown in Chapter 8 more gas is formed from such sugars than is formed from oligosaccharides such as raffinose. It was therefore necessary to develop a method that takes all the above into consideration, when determining the source of flatus in fermented cereal or cereal-legume foods. The method described in Chapter 8 goes some way to achieving this aim. It also confirms previous reports (Murphy *et al.*, 1972; Salvador *et al.*, 1993) that oligosaccharides from legumes may not be the only cause of flatus in humans. In fact the results presented in Chapter 8 suggest that, in the case of cowpea-supplemented kenkey, indigestible matter appearing as residue (solids) produced more gas than oligosaccharide-containing supernatants treated with yeast to remove simple fermentable sugars such as glucose. Analysis showed that significant amounts of resistant starch (RS) were present in these residues, following *in vitro* digestion. The levels of RS, however, did not correlate well with the amount of gas produced when these residues were incubated with *Cl. perfringens*. This would suggest that other carbon sources were fermented to produce gas. This suggestion cannot, however be conclusive, since other compounds are formed during the anaerobic fermentation of carbohydrates by *Cl. perfringens*. These include SCFAs which were not determined in this study. To obtain a complete mass balance of the original sample as it is passed through *in vitro* digestion, absorption and fermentation, it would be necessary to determine the concentrations of SCFAs produced as well as the amount of residue that is not fermented by the *Cl. perfringens*. In this thesis the method was kept as simple as possible, while placing more emphasis on flatus as a negative aspect of consuming legume-containing fermented foods. SCFAs formed often include acetate, butyrate and propionate each of these having a positive role in body metabolism.

Future Prospects for innovative kenkey processes

Technological options

The results of sensory evaluation of dehydrated kenkey meal (Chapter 3) undoubtedly add more weight to the concept of producing a kenkey "dry-mix". The advantages of having a dehydrated kenkey meal accepted cannot be overemphasised. There are, however, inevitable drawbacks to innovations. The implications of process innovations geared towards the scale-up of the traditional kenkey process to industrial level are presented in Table 9.3 which compares the

traditional process with that discussed in Chapters 3 and 4. It is imperative to consider not only the organoleptic aspects of such innovations, but also how they could affect the affordability of the product for the average kenkey consumer. There is no doubt that the initial investments in setting up an industrial process based on the methods presented in this thesis will be high and would, inevitably in the short-term, trickle down to the consumer. The long-term advantages in removing seasonal variations in price and supply of kenkey due to inadequate storage of and post-harvest losses in raw material must, however, more than offset any short-term costs. In the next 10 - 20 years, producers of products such as kenkey will be faced with a need to meet the demands of an ever increasing urban population, therefore, making the argument for processes that are fast and efficient all the more important.

Although drum-drying was used to investigate the dry-mix concept, other technological concepts are being considered. One of these is extrusion-cooking. The eventual aim is to adopt the system that is most viable in terms of economics of scale as well as product quality and acceptability. Preliminary cost analysis of processes utilizing drum-drying and those utilizing extrusion (based on a single-screw extruder) indicate that drum-drying is economically feasible only at a large throughput of up to 100.000 tons per year. Extrusion on the other hand seems to be more economically attractive both at medium ($\leq$ 50.000 tons per year) and large scale.

It would also be important to investigate the packaging, storage and shelf-life of the dehydrated kenkey dry-mix.

Use of starters

A lot of information can now be found on the microbiology of lactic fermented cereals such as maize (Akingbala et al., 1987, Hounhouigan et al,. 1994, Nout, 1993). It is, therefore, possible to develop single or mixed culture starters for use in the fermentation of cereals (Hounhouigan 1994). This will involve the selection of those microorganisms (LAB and Yeasts) of highly rated functional importance. In the case of cereal and cereal-legume blends, such microorganisms would include yeasts such as C. krusei and S. cerevisiae (for flavour development) and LAB that not only acidify, but can degrade anti-nutritional factors such as PA, TI, oligosaccharides and tannins. Preliminary trials using C. krusei, L. plantarum,

Table 9.3. Comparing the traditional kenkey process with the proposed innovative process

TRADITIONAL	NEW OPTIONS
Long and tedious process (up to 6 days)	Short and cuts down on human labour (≤ 2 days)
Variable product quality	Standardized product quality
Unstable intermediary product (dough)	Stable and convenient intermediary (dry flour mix)
Wet product with short shelf-life	Dehydrated product with a longer shelf-life
Poor and unstable packaging (e.g. Ga kenkey)	Better packaging of dry product possible
Poor hygiene, and prone to contamination & spoilage	Improved hygiene, less prone to contamination and spoilage
Seasonal variations can affect raw material supply	Production less affected by variations in raw material supply
Household scale does not usually meet demand	Large industrial scale to meet demand
Unsophisticated and no special equipment required*	Requires extra sophisticated and expensive machinery
Source of income for home producers*	Could take away income source from small family producers
Affordable to the average kenkey consumer*	Could be more expensive due to extra production costs

*Advantages of the traditional process.

L. fermentum and *P. pentosaceus*, indicate no significant reductions in PA and TI compared with spontaneous fermentation. Bacteriocin- and L-lactate producing lactic acid bacteria would be preferred, especially in the fermentation of foods for weaning purposes, to ensure microbiological safety and avoid the induction of acidosis in infants by the indigestible D-lactic acid which is often produced together with L-lactic acid (Yusof *et al.*, 1993).

Made in Ghana

This study focused on kenkey which is traditionally made in Ghana. It is, however not uncommon to come across similar products throughout West Africa. As discussed in the opening chapter (Table 1.1) kenkey is only one of a wide variety of cereal-based products made by soaking, milling and fermentation. Usually only the final step of cooking determines the unique characteristics of the final product. The rationale here, therefore, is that the methods developed in this study can, with only minor adaptations, be applied to other cereal- and tuber-based products for both adults and infants.

The challenges of developing a truly African food industry

In over 30 years of "independence", Africa has still not come to grips with its ever-mounting food insecurity problems. One only has to look at leaps made by Western food industries in the last decade to see that Africa is a long way from coming anywhere near to developing sustainable food production, processing and preservation systems. Although a lot of development aid from the West is channelled into agriculture, it is rather debatable whether improving primary agricultural output alone can begin to tackle the chronic hunger crisis in Africa, if a firm post-harvest processing base is not developed to limit the huge losses incurred after harvest. Such losses are currently being estimated at between 30-40% (Ngoddy, 1994). Most of the blame, of course, lies with short-sighted governments which, by virtue of their own insecurities, often neglect the fact that the lack of food security is a major root cause of apathy, hunger and disease among its working populations. Whilst Western food industries and institutions cannot determine African government policies with regard to food (in)security in Africa, they can, nevertheless, play an important role in helping set up a firm and sustainable post-harvest food processing base. Funders of major primary agricultural projects and policy makers in Africa must now widen the narrow band

of research to cover research into and application of post-harvest technologies for Africa in particular and the developing world in general. Only then can the governments of these regions begin to replace the spectre of endemic food insecurity and related ailments with strategies for long-term food security for the populations that are increasingly dependent on food aid.

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███████████████████████████ **Summary** ███████████████████████████

Kenkey is a popular traditional product of Ghana made from spontaneously fermented maize (*Zea mays*) dough. The local white maize preferred by traditional kenkey makers, however, is low in protein and is deficient in essential amino acids e.g. lysine. It is, therefore, important to attempt to improve on this aspect of kenkey. Cowpea (*Vigna unguiculata*), a well-known legume in Ghanaian households, is rich in proteins and lysine and was, therefore, considered for use as a supplement protein source for improving the protein quality and quantity of traditional kenkey.

The traditional kenkey process was simulated at a laboratory scale, and the effects of cowpea addition on the fermentation of dough and the quality of the final product were investigated in Chapter 2. Kenkey was made from a 4:1 mixture of maize and red or white cowpea and compared with an all-maize product. After 4 days' natural fermentation at 30°C, final pH values of 4.07 and 4.08 for maize and maize-cowpea doughs, respectively, were obtained. Although 48 h were sufficient for proper acidification of the maize dough, it took a further 24 h before the same pH level could be obtained in maize-cowpea doughs. Due to supplementation with cowpea, the crude protein content increased by 29.2% and 20.5%, respectively, for kenkey supplemented with red and white cowpeas. These represent, respectively, 76.3%, 15.6% and 26.8% increases in total lysine, methionine and tryptophan contents of maize-red cowpea kenkey, and 65.3%, 8.2% and 19.5% increases in lysine, methionine and tryptophan contents, respectively, of maize-white cowpea kenkey.

Cowpea-supplemented kenkey had a more homogeneous structure and was therefore less prone to fracture than the traditional all-maize kenkey (Chapter 2). Hence more force was required to fracture the cowpea-supplemented product and this increased with storage at room temperature. For example, kenkey stored for 24 hours required over twice as much force to fracture as freshly prepared kenkey. Addition of red cowpea reduced the whiteness (Hunter L value) of kenkey by 27% causing it to be rejected by a panel of 5 Ghanaians familiar with kenkey on account of an unfamiliar brown coloration. On the other hand, this panel also concluded that kenkey made from a mixture of wholegrain white cowpea or dehulled red cowpea and maize compared very well with traditional kenkey. A broader sensory evaluation carried out in Ghana itself indicated, however, that 10% but not 20% supplementation was acceptable.

The traditional kenkey process is carried out on a small household scale and is a long (can take up to a week), tedious (especially during aflata production)

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process and often results in a product of variable quality because of varying conditions of fermentation. Also because of its high moisture content (ca. 60%, w/w), kenkey has, in the absence of refrigeration, a rather short shelf-life, ranging from a couple of days for Ga kenkey to about a week for Fanti kenkey. Chapter 3 discusses the technological feasibilities of improving the whole process by reducing production time and increasing output by use of a standardised process that can be scaled up in industry. The aim was also to develop a dehydrated and convenient intermediary product that can be packaged and distributed or stored for longer periods. This can then be made into kenkey simply by rehydrating, wrapping and boiling to taste. Options considered included omission of the soaking step and the use of dry-milled maize and accelerated fermentation. Pre-fermented dough containing an enrichment of lactic acid bacteria was used as a starter in the accelerated fermentation of dough from dry-milled maize flour. Cabinet and drum drying were used to prepare dehydrated kenkey flour and aflata, respectively. Dough from dry-milled maize, however, had pasting and set-back viscosities that were significantly inferior to traditionally prepared dough, and as a result it was not suitable for making good quality aflata for kenkey production. Hence, although dry-milling of maize and accelerated fermentation of the resulting dough could drastically reduce production time from about 6 days to within 24 h, omission of the soaking step practised traditionally, can result in a product with inferior textural quality.

Chapter 4 describes an investigation of the (bio)chemical, microbiological and physical changes that occur during the soaking of maize and how these changes could influence the final flavour and texture of kenkey. During soaking, water uptake by coarsely dry-milled maize (grits) reached 63% (wet weight basis) in just 1 h, compared with 50% in 3 days for whole grain maize. The hypothesis that proteolysis is crucial to the development of the necessary textural characteristics of traditional kenkey was developed on the basis of the hydration experiments with and without microbial activity (at 25 and 4°C) and with and without enzyme activity (at 4, 25 and 60°C). This was tested and confirmed using a heat-stable protease at 60°C. High endogenous proteolytic and saccharolytic enzyme activities were recorded in both grits and whole maize soaked at 4 or 25°C. These enzymes were significantly repressed in grits soaked at 60°C. Although a high degree of hydration of grits occurred at this temperature, there was no significant improvement of the pasting and set-back viscosities. However, when a heat-stable protease was added to the grits during soaking at 60°C, the resulting dough had pasting and set-back viscosities comparable with those of

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traditionally prepared doughs.

The effects of supplementation and the various unit operations of the kenkey process on the availability of lysine were also investigated (Chapter 5). Results indicate that main unit operations such as soaking, fermentation and cooking had significantly positive influence on lysine availability in kenkey. 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. Significant ($p < 0.05$) improvements in lysine availability were also effected by the cooking of fermented doughs, with values of 3.42 or 4.43 g lysine/16g N recorded, respectively, for kenkey made from maize or 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. Kenkey made from a 1:1 mixture of cabinet and drum dried flours, however, had a higher available lysine content than that made from drum dried flour alone, as some of the lysine destroyed during drum drying was compensated for by the cabinet-dried portion.

Chapters 6-8 of this thesis tackle aspects relating to the digestibility and safety of kenkey as influenced by the choice of ingredients and processing conditions.
The traditional kenkey process requires a spontaneous fermentation of maize dough. The quality of this dough and the subsequent kenkey is, therefore, governed essentially by the surface microflora of the grains, the choice of ingredients and the processing conditions used. It is, therefore, essential that such fermented foods be monitored for the presence of any contaminants that may result from processing and microbial activity. Chapter 6 looks at the influence of the above-mentioned factors on the formation of biogenic amines and ethyl carbamate in kenkey. Results obtained indicate that the levels of biogenic amines in all-maize kenkey were very low (total amines < 60 ppm). These were, however, significantly increased by the addition of red cowpea (total amines < 200 ppm, mainly cadavarine and tyramine), and even more by white cowpea (total amines < 500 ppm, mainly putrescine and tyramine). Histamine, which is usually associated with toxic effects in humans, was absent (< 5 ppm) in all samples. The effects of fermentation and cooking were less pronounced than the influence of cowpea addition. Prolonged cooking of kenkey resulted in lower levels of putrescine, but did not significantly reduce tyramine levels. Ethyl carbamate levels were negligible (< 11 ppb) in all treatments.

Cowpeas, like most other legumes, contain antinutritional factors (ANF) as well as the flatus-causing oligosaccharides raffinose and stachyose. Their introduction into kenkey and other cereal-legume foods is, therefore, expected to reduce digestibility and to boost the flatulence potential of these foods. In Chapter 7, the flatulence potential of cereal, cereal-legume foods as well as pure carbohydrates was investigated by monitoring the amount of gas produced when these substrates were incubated with various strains of *Cl. perfringens*. Results obtained indicated that the flatulence potential of foods or pure carbohydrates, as determined by gas production from incubations with *Cl. perfringens*, depend strongly on the choice of food ingredients and processing conditions, the type and concentration of pure carbohydrate used and most importantly the strain of bacteria used.

An *in vitro* method was developed to determine the digestibility of kenkey and to monitor the effects of several important unit operations on its flatulence potential (Chapter 8). The method was designed such that digestible and assimilable carbohydrates were removed, leaving only the indigestible portions of the food sample for testing flatulence potential by fermenting with *Cl. perfringens* strain NCTC 8239. Results show that, although low molecular weight oligosaccharides were fermented to produce gas, more gas was obtained from poorly digestible carbohydrates that make up the solid residue resulting from enzymic digestion.

This thesis has outlined some basic options for improving the quality, processing and preservation of kenkey. It is hoped that this work can form a basis for industrial upgrading of the processing of traditional fermented foods from the tropics, and be extended to cover other cereal and cereal-legume based foods for both adults and weanling infants. It will, therefore, be important to further investigate the nutritional and socio-economic consequences of applying these and similar process options in a bid to develop a sustainable post-harvest food processing system for Africa in particular, and the developing world in general. This must be considered as a very important weapon in the battle against food insecurity in these poorer regions of our world.

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Kenkey , afkomstig uit Ghana, is een traditioneel gefermenteerd maïs-(*Zea mays*)-produkt. De door plaatselijke kenkeyproducenten gebruikte lokale witte maïs is echter relatief arm aan eiwit. Omdat dit eiwit ook nog een laag gehalte aan essentiële aminozuren bijv. lysine heeft, is het vanuit voedingskundig oogpunt van belang dit gehalte te verhogen. De cowpea (*Vigna unguiculata*), een in de Ghanees huishouding veel geconsumeerde erwt, heeft een hoog eiwit- en lysinegehalte. De eiwitverrijking van traditionele kenkey met cowpea is daarom het uitgangspunt voor dit onderzoek geweest.

Het traditionele kenkey bereidingsproces werd op kleine schaal in het laboratorium nagebootst; de invloed van de toevoeging van cowpea op de deegfermentatie en de eigenschappen van de uiteindelijke kenkey zijn beschreven in Hoofdstuk 2. Er werd kenkey gemaakt van een 4:1 mengsel van maïs en rode of witte cowpea, en deze werd vergeleken met maïs-kenkey. Na 4 dagen natuurlijke fermentatie bij 30°C was de pH gedaald tot 4,07 in maïsdeeg, en 4,08 in maïs-cowpea deeg. Deze pH werd in maïsdeeg in 48 uur bereikt; in maïs-cowpea deeg was 24 uur langer fermenteren nodig. Het ruw eiwitgehalte nam met 29,2% toe bij verrijking met rode, en met 20,5% bij witte cowpea. Hiermee gingen toenamen van totaal lysine, methionine en tryptofaangehalten gepaard van respectievelijk 76,3%, 15,6% en 26,8% bij rode cowpea, en 65,3%, 8,2% en 19,5% bij witte cowpea.

Met cowpea verrijkte kenkey had een homogenere structuur, en was minder brokkelig dan maïs kenkey (Hoofdstuk 2). Voor het breken van net cowpea verrijkte kenkey was een grotere kracht nodig dan voor het maïsprodukt; door bewaring bij kamertemperatuur werd het produkt stijver. Na bijvoorbeeld 24 uur was tweemaal zoveel kracht voor breuk nodig als bij vers bereide kenkey. Toevoeging van rode cowpea verminderde de kleur-helderheid (Hunter L-waarde) van kenkey met 27%, en de afwijkende bruine kleur was dan ook de oorzaak voor een panel van vijf Ghanezen om het produkt te verwerpen. Hetzelfde panel vond echter dat kenkeys van mengsels van maïs met volkoren witte, of ontvelde rode cowpea vergelijkbaar waren met traditionele kenkey. Een beoordeling uitgevoerd in Ghana door een consumentenpanel wees echter uit, dat verrijking met 10% cowpea werd geaccepteerd maar 20% niet.

Het traditionele bereidingsproces voor kenkey wordt op kleine huishoudelijke of commerciële schaal uitgevoerd. Het kost veel tijd (maximaal 1 week), lichamelijke inspanning (vooral de bereiding van de zgn. "aflata") en de onbeheerste fermentatie resulteert in een variabele produktkwaliteit. Bovendien heeft kenkey

door het hoge vochtgehalte (ca. 60% m/m) buiten de koelkast één beperkte houdbaarheid, uiteenlopend van enkele dagen (Ga kenkey) tot ongeveer een week (Fanti kenkey). In Hoofdstuk 3 worden de technologische mogelijkheden onderzocht om het proces te versnellen, en tevens te komen tot een gedroogd tussenprodukt dat gemakkelijker zou kunnen worden verpakt, gedistribueerd en bewaard. Hieruit kan eenvoudig kenkey worden bereid door het te mengen met water, te kneden, en in een blad te wikkelen en te koken zoals gebruikelijk. Getracht werd tot procesverkorting te komen door de weekstap over te slaan, en van droog vermalen maïs uit te gaan. De fermentatie kon worden versneld door gebruik te maken van een ophopingscultuur van melkzuurbacteriën in maïsdeeg bereid uit droog gemalen maïs. Gedroogde aflata werd bereid d.m.v. walsdroging; gedroogd kenkey meel werd m.b.v. een kastdroger verkregen. Duidelijk werd dat droog gemalen maïs een inferieure kwaliteit oplevert, veroorzaakt door geringe verstijfseling en gelvorming vergeleken met geweekte maïs. Hoewel het overslaan van de weekstap het proces aanzienlijk zou versnellen, is het geen haalbare keuze omdat het een onacceptabele kenkey textuur geeft.

In Hoofdstuk 4 wordt verder ingegaan op de (bio)chemische, microbiologische en fysische veranderingen in maïs tijdens het weekproces, en hun invloed op aroma en textuur van het eindprodukt. Grof droog gemalen maïs (gries) neemt tijdens weken in 1 uur tot 63% (natgewicht) vocht op, in tegenstelling tot 50% in hele maïskorrels in 3 dagen. Op basis van hydratatie-experimenten met (bij 25°C) en zonder (bij 4°C) microbiële activiteit, en met (bij 4° en 25°C) en zonder (bij 60°C) endogeen enzymactiviteit, werd de hypothese ontwikkeld dat proteolyse van groot belang is voor de ontwikkeling van de textuur van traditionele kenkey. De hypothese werd getoetst en bevestigd door proeven met thermostabiel protease bij 60°C. Zowel bij 4°C en 25°C werden in de hele maïs en in gries tijdens het weken grote endogeen proteolytische en saccharolytische enzymactiviteit aangetoond. Deze activiteiten werden nagenoeg onderdrukt tijdens weken bij 60°C. Hoewel bij deze temperatuur een goede hydratatie van gries optrad, werd het verstijfselings- en gelvormingsgedrag niet verbeterd. Wanneer echter tijdens het weken bij 60°C een thermostabiel protease werd toegevoegd, werd een verstijfseld deeg verkregen met vergelijkbare eigenschappen als het traditionele produkt.

Hoofdstuk 5 betreft de invloed van verrijking en procesbewerkingen op de beschikbaarheid van lysine. Deze werd positief beïnvloed door de voornaamste handelingen in het proces zoals weken, fermenteren en koken. Weken vergrote de lysinebeschikbaarheid met 21% en 22% in respectievelijk, maïs en maïs-cowpea mengsels. Koken van de geweekte grondstof gaf een verdere verbetering van 68%

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en 31% voor respectievelijk, maïs en maïs-cowpea. Het koken van gefermenteerde maïs en maïs-cowpea mengsels resulteerde ook in een significante ($p < 0,05$) verbetering van de lysinebeschikbaarheid en gaf 3,42 g en 4,43 g beschikbaar lysine per 16 g stikstof in resp., kenkey van maïs en maïs-cowpea die vooraf 4 dagen was gefermenteerd en 3 uur was gekookt. Kastdrogen van kenkeymeel had geen significante invloed, maar walsdrogen van gefermenteerde maïs en maïs-cowpea degen leidde tot significante verlaging van de lysinebeschikbaarheid. Door 1:1 mengsels van kastgedroogd meel en walsgedroogde aflata te gebruiken kon het lysineverlies aanvaardbaar blijven.

De hoofdstukken 6-8 van dit proefschrift betreffen de invloed van gebruikte grondstoffen en procesomstandigheden op de veiligheid en verteerbaarheid van kenkey. In het traditionele kenkeyproces wordt van een natuurlijke fermentatie gebruik gemaakt, zodat de kwaliteit van deeg en kenkey vooral bepaald worden door de oppervlakte-microflora van de grondstof, de keuze van de grondstof en de procesomstandigheden. Het is daarom van belang om inzicht te verschaffen in de door procesbewerkingen of door microbiële activiteit mogelijk ontstane verontreinigingen. In Hoofdstuk 6 wordt aandacht besteed aan de vorming van biogene aminen en ethylcarbamaat in kenkey. Biogene aminen komen in maïskenkey slechts in zeer kleine hoeveelheden voor (totaal aminen < 60 ppm). Wordt echter verrijkt met cowpea dan neemt het gehalte biogene aminen aanzienlijk toe tot < 200 ppm (voornamelijk cadaverine en tyramine) bij gebruik van rode cowpea, en < 500 ppm (voornamelijk putrescine en tyramine) bij witte cowpea. Histamine, dat gewoonlijk wordt geassocieerd met vergiftiging bij de mens, werd in geen van de onderzochte kenkey's (< 5 ppm) aangetroffen. De invloed van fermentatie en van koken was minder sterk dan dat van de verrijking met cowpea. Langer koken gaf lagere putrescine concentraties, maar tyramine bleef aanwezig. In alle kenkey's was ethylcarbamaat afwezig (< 11 ppb).

Zoals de meeste peulvruchten bevatten cowpea, antinutritionele factoren (ANF) en flatulentie veroorzakende oligosacchariden zoals raffinose en stachyose. Toevoeging van peulvruchten aan kenkey of andere graanprodukten zal daarom naar verwachting een negatieve invloed hebben op de verteerbaarheid enerzijds, en meer flatus veroorzaken anderzijds. In Hoofdstuk 7 wordt beschreven hoe de flatus capaciteit van levensmiddelen op basis van graan, of graan-peulvruchten mengsels, alsook van zuivere koolhydraten, werd onderzocht aan de hand van gasvolumes die door fermentatie met *Clostridium perfringens* stammen uit deze substraten werden gevormd. De hoeveelheden gevormd gas werden sterk beïnvloed door de gekozen substraten en procesbewerkingen, en tevens door de concentraties zuivere

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koolhydraten en, in hoge mate, door de gebruikte bacteriestammen.

Een *in-vitro* methode werd ontwikkeld (Hoofdstuk 8) voor de bestudering van de verteerbaarheid van kenkey, en van de invloed van procesbewerkingen op de flatus capaciteit van kenkey. In deze methode worden verteerbare en absorbeerbare koolhydraten verwijderd, alvorens de inverteerde resten van het levensmiddel te fermenteren met *Cl. perfringens* stam NCTC 8239. Uit de resultaten blijkt dat niet alleen water-oplosbare oligosacchariden onder gasvorming worden gefermenteerd, maar dat zelfs meer gas wordt gevormd uit inverteerde koolhydraten, die aanwezig zijn in de water-onoplosbare resten overgebleven na enzymatische vertering van het levensmiddel.

Dit proefschrift opent een aantal keuzemogelijkheden voor de verbetering van de kwaliteit, het bereidingsproces en de houdbaarheid van kenkey. Hopelijk zal het bijdragen aan de bereiding op industriële schaal van traditionele gefermenteerde levensmiddelen in de tropen, en zal het bredere toepassing vinden in de produktie van levensmiddelen op basis van granen en graan-peulvruchten mengsels, voor zowel volwassenen als zuigelingen. Het is daarom van belang de voedingskundige en socio-economische implicaties van deze en soortgelijke technologische ontwikkelingen verder te onderzoeken teneinde tot een duurzaam systeem voor levensmiddelentechnologie te komen voor Afrika, en de ontwikkelingslanden in het algemeen. Dit moet beschouwd worden als een zeer belangrijk wapen in de strijd voor voedselzekerheid in deze armere streken van onze wereld.

Résumé

Le Kenkey est un produit traditionnel et populaire du Ghana. Il est fabriqué à partir d'une pâte de maïs (*Zea mays L*) spontanément fermentée. Cependant, le maïs local blanc, préféré par les fabricants traditionnels du kenkey est pauvre en protéine et déficient en acides aminés essentiels tels que la lysine. Par conséquent, il est important d'essayer d'améliorer cet aspect du kenkey. La dolique (*Vigna unguiculata*), une légume bien connue par des ménages Ghanéens, est riche en protéine et lysine. Elle a donc été présentée comme une source supplémentaire de protéines pour améliorer la qualité du kenkey.

Le procédé traditionnel de fabrication du kenkey a été adapté en un procédé utilisable à l'échelle du laboratoire, et les effets de l'addition de la dolique sur la fermentation de la pâte et la qualité du produit final ont été étudiés en Chapitre 2. Le kenkey a été fabriqué à partir d'un mélange à 4:1 de maïs et de la dolique rouge ou blanche, et comparé avec un produit fait uniquement à partir du maïs. Après 4 jours de fermentation naturelle à 30°C, des valeurs finales du pH de 4.07 et 4.08 respectivement pour le maïs et le mélange maïs-dolique ont été obtenues. Quoique 48 heures aient été suffisant pour une bonne acidification de la pâte de maïs, il a fallu 24 heures en plus pour que le même pH soit obtenu avec le mélange maïs-dolique. A cause de l'addition de la dolique, le taux brut de protéine a augmenté de 29,2% et 20,5% pour le kenkey supplémentée respectivement avec la dolique rouge et blanche. Ceci représente respectivement des augmentations de 76,3%, 15,6% et 26,8% du contenu total en lysine, méthionine et tryptophane pour le mélange maïs-dolique rouge et 65,3%, 8,2% et 19,5% d'augmentation en lysine, méthionine et tryptophane respectivement pour le kenkey issu du mélange maïs-dolique blanche.

Le kenkey fait à partir d'un supplément en dolique était plus homogène et par conséquent moins enclin à la fracture que le kenkey fait à partir du maïs uniquement (Chapitre 2). De même, davantage de force était nécessaire pour fracturer le produit issu de l'addition de la dolique et ceci s'est accru avec l'entreposage à température ambiénte. Par exemple le kenkey conservé pendent 24 heures était deux fois plus difficile à fracturer que du kenkey fraîchement préparé. L'addition de la dolique rouge a réduit la blancheur (valeur Hunter L) du kenkey de 27%, ce qui lui a valu d'être rejeté par un panel de 5 Ghanéens connaissant bien le kenkey. Ceci est à mettre au compte d'une couleur brune peu familière. Ce panel a aussi conclu que le kenkey fait avec un mélange de grain entier de dolique blanche ou bien de la dolique rouge décortiquée était bien comparable au kenkey traditionnel. Une enquête d'évaluation plus étendue au Ghana a cependant montré

qu'une supplémentation de 10% était acceptable, mais pas de 20%.

Le procédé traditionnel de fabrication du kenkey est conduit à petit échelle de ménage et est long (peut durer une semaine) et laborieux (spécialement pendant la production d'aflata). Et il résulte souvent en un produit de qualité variable à cause des conditions de fermentation aussi variables. Aussi à cause de sa forte teneur en eau (ca. 60% w/w) le kenkey a en l'absence de réfrigération une très courte durée de conservation variant de deux jours pour le Ga kenkey et près d'une semaine pour le Fanti kenkey.

Le Chapitre 3 examine la faisabilité technologique de l'amélioration de tout le procédé par une réduction du temps de fabrication et une augmentation de la production par l'utilisation d'un procédé standardisé qui peut être utilisé à l'échelle industrielle. Le but était aussi de fabriquer un produit de valeur intermédiaire, déshydraté et qui peut être emballé et conservé plus longtemps. Un tel produit peut être transformé en kenkey par une simple réhydratation suivi d'une mise en paquet et d'une ébullition selon le goût. Les options examinées comprennent l'omission de l'étape de trempage et l'utilisation du maïs sec moulu de même que l'accélération de la fermentation. Une pâte pre-fermentée enrichie en bactéries lactiques a été utilisé pour le démarrage de la fermentation accélérée de la pâte faite à partir de la farine de maïs sec. Le séchage par cabinet et par tambour ont été utilisés pour fabriquer de la farine de kenkey déshydratée et l'aflata respectivement. La pâte issue du maïs sec moulu a cependant montré une viscosité inférieure à celle de la pâte préparée par le procédé traditionnel et par conséquent n'était pas appropriée pour faire de l'aflata de bonne qualité pour la production de kenkey. Quoi-que le séchage et l'écrasage du maïs et l'accélération de la fermentation de la pâte résultante ont puisse réduire le temps de production de 6 jours à 24 heures, l'omission du trempage pratiqué traditionnellement a résulté en une pâte dont la texture était de qualité inférieure.

Le Chapitre 4 décrit une étude les changements (bio)chimiques, microbiologiques et physiques qui ont eu lieu pendant le trempage du maïs et sur l'influence que ces changements peuvent avoir sur le goût et la texture finale du kenkey. Pendant le trempage, l'absorption de l'eau par le maïs sec moulu a atteint un taux de 63% en une heure seulement, comparé avec un taux de 50% en trois jours pour le maïs en grain entier. L'hypothèse que la protéolyse est cruciale au développement des caractéristiques relatives à la texture du kenkey traditionnel a été examinée à travers les expériences avec ou sans activité microbienne (à 25°C et 4°C) et avec ou sans activité enzymatique (à 4, 25 et 60°C). Ceci a été testé et confirmé par l'utilisation à 60°C d'une protéase thermostable. Des fortes

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activités protéolytiques et saccharolytiques endogènes ont été enregistrées tant dans le cas du maïs moulu que dans celui du maïs entier à 4 ou 25°C. Ces enzymes ont été fortement réprimées pour la farine du maïs trempée à 60°C. Quoiqu'un degré élevé d'hydratation ait été obtenu a cette température, il n'y a pas eu une amélioration significative de la viscosité. Toutefois, quand une protéase thermostable a été ajoutée à la farine pendant le trempage à 60°C, la pâte résultante avait une viscosité comparable avec celle des pâtes préparées traditionnellement.

Les effets de la supplémentation et des diverses opérations du procédé de fabrication du kenkey sur la disponibilité de la lysine ont aussi été examinés (Chapitre 5). Les résultats montrent que les principales opérations telles que le trempage, la fermentation et la cuisson ont une influence positive de façon significative sur la disponibilité de la lysine dans le kenkey. Le trempage a fait croître la disponibilité de la lysine de 21% et 22% pour le maïs et le mélange maïs-dolique, respectivement. La cuisson des échantillons trempés a en plus amélioré la disponibilité en lysine de 68% et 31% pour le maïs et le mélange maïs-dolique, respectivement. D'autres améliorations significatives ($p < 0,05$) de la disponibilité en lysine ont aussi été effectuées par la cuisson des pâtes fermentées, avec des valeurs de 3,42 ou 4,43g lysine/16g N enrégistrées, respectivement pour le kenkey fait à partir des pâtes de maïs ou du mélange maïs-dolique fermentées pendant 4 jours et cuites pendant 3 heures. Le séchage par le cabinet n'a eu aucun effet sur la disponibilité en lysine, mais le séchage au tambour des pâtes de maïs et de mélange maïs-dolique a diminué de façon significative la disponibilité en lysine. Le kenkey fait a partir d'un mélange à 1:1 des farines séchées au cabinet et au tambour a, cependant, eu plus de lysine disponible que celui fabriqué à partir de la farine séchée au tambour uniquement, étant donné qu'une partie de la lysine détruite pendant le séchage au tambour avait été compensé par la portion issue du séchage au cabinet.

Les Chapitre 6-8 de la présente thèse affronnent des aspects relatifs à la digestibilité et a la salubrité qui peuvent être influencés par le choix des ingrédients et les conditions de fabrication. Le procédé traditionnel de fabrication du kenkey utilise la fermentation spontanée de la pâte de maïs. La qualité de cette pâte et du produit qui en résulte est donc essentiellement determinée par la microflore à la surface des grains, le choix des ingrédients et les conditions de fabrication utilisés. Il est par conséquent essentiel de controler tels aliments fermentés pour détecter la présence de quelque contamination pouvant provenir de la fabrication et de l'activité microbienne. Le Chapitre 6 examine l'influence des facteurs ci-dessus

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mentionnés sur la formation des amines biogéniques et de la carbamate d'éthyle. Les résultats obtenus montrent que les niveaux des amines biogeniques dans le kenkey issue du maïs uniquement étaient très bas (totale < 60ppm). Ceux-ci ont cependant connus une augmentation significative avec l'addition de la dolique rouge (totale < 200ppm, surtout la cadaverine et la tyramine), et, encore plus avec la dolique blanche (totale < 500ppm, surtout la putrescine et la tyramine). L'Histamine, qui est souvent associée à des effets toxiques chez les humains n'était présent (< 5ppm) dans aucun échantillon. Les effets de la fermentation et de la cuisson étaient moindre par rapport à ceux de l'addition de la dolique. La cuisson prolongée du kenkey a abouti à des niveaux inférieurs de putrescine mais n'a pas réduit de façon significative les niveaux de tyramine. Les niveaux d'éthyle carbamate (< 11ppm) étaient négligeable dans tous les traitement.

La dolique comme la plupart d'autres légumes contient des facteurs anti-nutritionelles (ANF) tels des oligosaccharides raffinose et stachyose qui contribuent à la formation de flatus. Leur introduction dans le kenkey et dans d'autres mélanges alimentaires céréales-légumes devrait par conséquence augmenter le potentiel flatulence de ces aliments. Dans le Chapitre 7, le potentiel de flatulence du céréale, des mélanges alimentaires céréales-légumes ainsi que des glucides pures a fait l'objet de recherches par l'examen des quantités de gaz produites quand ces différents substrats font l'objet d'une incubation avec différentes souches pures de Cl. perfringens. Les résultats obtenus ont indiqué que le potentiel de flatulence des aliments ou des glucides pures tels que déterminés par la production de gaz suite à l'incubation avec Cl. perfringens dépendaient énormément du choix des ingrédients utilisés et surtout de la souche de bactérie.

Une méthode de détermination de la digestibilité du kenkey in vitro a été mise au point pour suivre et évaluer l'effet de plusieurs opérations importantes sur son potentiel de flatulence (Chapitre 8). La méthode a été conçue de telle manière que les glucides digestibles et assimilables ont été éliminées laissant seulement les composantes indigestibles de l'échantillon alimentaire pour tester le potentiel de flatulence avec Cl. perfringens souche NCTC 8239. Les résultats montrent que malgré le fait que les oligosaccharides à faibles poids moléculaires ont été fermentés pour produire du gaz, encore plus de gaz a été obtenu des glucides difficilement digestibles qui constituent le résidu solide résultant de la digestion enzymatique.

Cette thèse a permis d'identifier quelques options fondamentales pour l'amélioration de la qualité, du processus de fabrication et de la préservation de kenkey. Il faut espérer que ce travail formera une base pour une fabrication

industrielle des aliments fermentés traditionnels provenant des tropiques et qu'il sera étendu d'autres aliments basés sur les céréales et sur les mélanges céréales-légumes tant pour les adultes que pour les enfants. Il sera donc important d'examiner plus en profondeur les conséquences nutritionnelles et socio-économiques de l'application de ces options, aussi que d'autres qui leur sont semblables dans les essaies cherchant à développer des systèmes durables de transformation des aliments après récolte en Afrique ou dans les pays en développement en général. Ceci doit être considéré comme une arme très importante dans la bataille contre l'insécurité alimentaire dans les régions qui sont les plus pauvres du monde.

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living so far away from home. Alida, Saskia and Andra, I would like to say thanks on behalf of my daughter Neh. You will always have that special place in all our memories of The Netherlands.

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Pius Fru Nche.   Wageningen, February 1995.

Curriculum vitae

Pius Fru Nche was born on 26-11-1967 in Mankon, in Cameroon. He attended Primary school at Christ the King Catholic School Ntahmbeng, Mankon, from 1971-1978, from where he obtained a First School Leaving Certificate. From September 1978 to June 1986 he attended secondary school at Sacred Heart College Mankon, from where he obtained his General Certificate of Education (GCE), Ordinary and Advanced levels. He then left for the United Kingdom and attended the University of Wales, College of Cardiff, from where he obtained a B.Sc. (Hons) in Microbiology (1986-1989) and a MPhil. in Applied Biology (1989-1991).

It was from Wales that Fru Nche joined the Department of Food Science of the Wageningen Agricultural University, as a Research Assistant, working on an EC-sponsored collaborative project on "The evaluation and improvement of traditional fermented cereals and legumes in Ghana". He was later admitted as a candidate for Ph.D. by WAU, and has been based full time in the Department of Food Science where he carried out all his research.