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Commercial production of kenkey using *Lactobacillus fermentum* as starter culture for the fermentation of maize

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COMMERCIAL PRODUCTION OF KENKEY USING *LACTOBACILLUS FERMENTUM* AS STARTER CULTURE FOR THE FERMENTATION OF MAIZE

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Abstract

Kenkey is one of the commonly eaten foods in Ghana and therefore there is the need to find ways to make production faster and easier whilst maintaining its natural flavour and quality. A trial test was carried out at a kenkey factory by the Food Research Institute using *Lactobacillus fermentum* (7-11 A) as a starter culture. The dough was allowed to ferment for a maximum of 48 h.

Microbiological analysis coupled with chemical analysis and sensory evaluation carried out suggest that the organoleptic , physical and chemical properties of the kenkey prepared using inoculum does not differ much from traditionally prepared kenkey. pH values and organoleptic assessment showed that with the use of starter culture for inoculum enrichment, maize dough could be fermented for only 24 h instead of the normal fermentation period of 48 - 72 h.

Keywords: *Lactobacillus fermentum*, maize dough, starter culture

INTRODUCTION

Maize is one of the principal cereals cultivated in Ghana and is useful in preparing a variety of traditional foods (FRI, 1986). Interestingly maize in Ghana can enter into all three meals of the day without creating a monotony (Dovlo, 1970) and one of the principal foods is kenkey. Traditional kenkey production involves the general steps shown in Figure 1.

The maize grains are first steeped, milled, mixed into dough with water and left to ferment spontaneously for about 3 days to attain the desired organoleptic quality (Miller and Nyarko-Mensah, 1972). Thereafter, a portion of the dough is half cooked and mixed with the remaining uncooked fermented dough into aflata, a process called "Aflatalization", which leads to the attainment of a characteristic texture (Bediako-Amoa and Austin, 1976; Sefa Dedeh, 1993). This mixture is moulded into balls and wrapped in maize husks and boiled for 3-4 h into Ga kenkey.

From various studies carried out, a variety of microorganisms have been identified as being involved in the process of maize dough fermentation. They include *Lb. plantarum , Cephalosporium spp., Aspergillus spp., Penicillium spp.* and *aerobics-aerobacter* (Akinrele, 1970; Christian, 1970). Yeasts , such as *Candida mycoderma, Saccharomyces cerevisae* and *Rhodotorula* have also been identified.

Studies carried out by Halm et al. (1993) at the Food Research Institute (FRI) identified lactic acid bacteria as the dominant flora with *Lb. fermentum* as more pronounced in the process of fermentation of maize dough. The yeast *Saccharomyces cerevisae* and *Candida krusei* were also found to play a role (Jespersen et al., 1994)

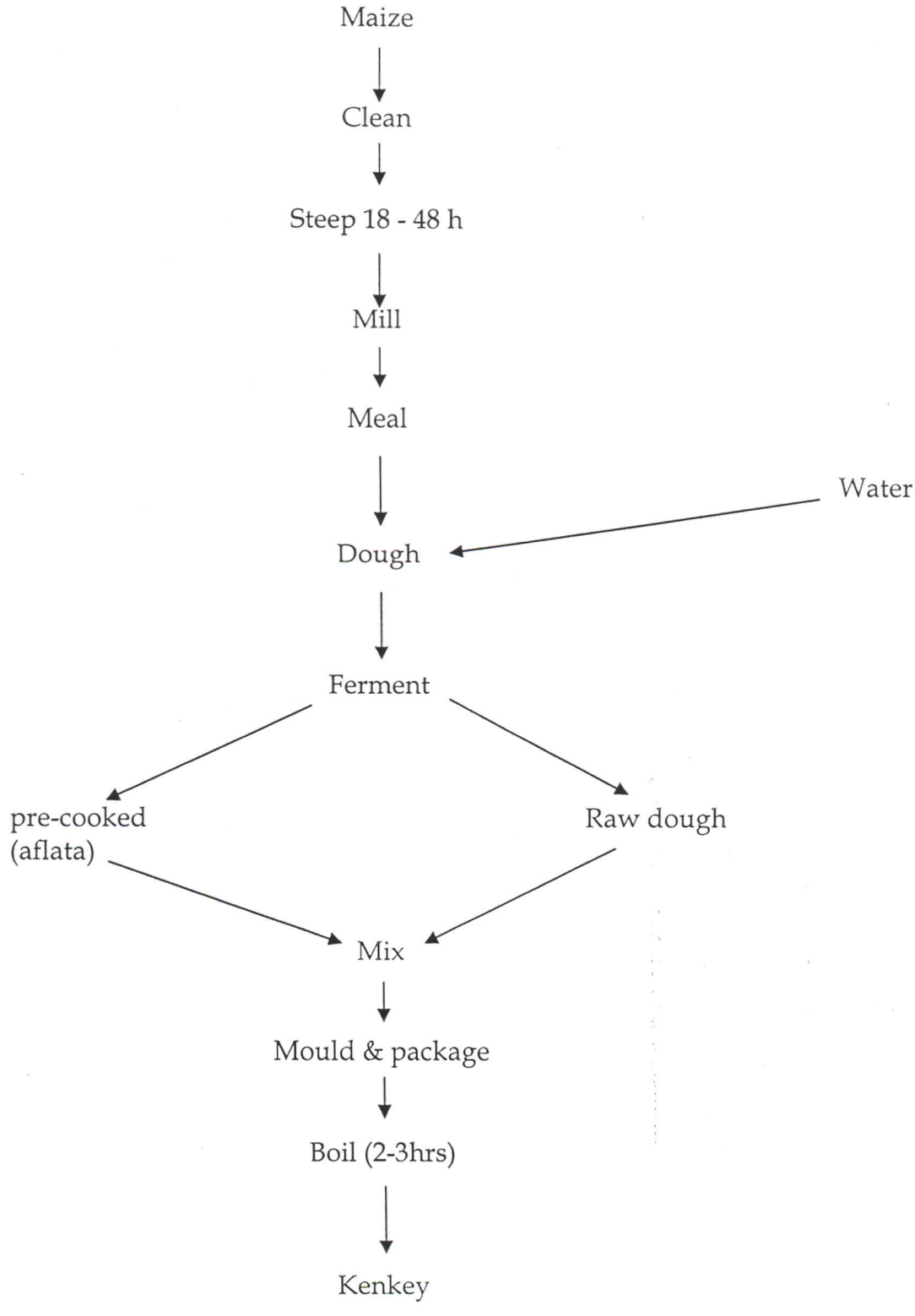

FIGURE 1: Flow diagram of Traditional Kenkey production

1994). This therefore indicates a possibility of controlled fermentation using a starter culture.

The main aroma components in fermented maize dough have been identified as lactic, acetic, butyric and propionic acids (Banigo and Muller, 1972; Plahar and Leung, 1982).

Lactic acid bacteria are reported to be responsible for the fermentation of several traditional African foods such as Gari, Ogi, Sorghum-gruel, agbelima (Amoa-Awua, 1996). They are reported to be responsible for converting sugars to organic acids, reducing pH and depleting carbohydrate sources, thus supressing the growth of spoilage organisms. They also produce antimicrobial compounds like H_2O_2, bacteriocin, diacetyl and secondary reaction products. Furthermore, lactic acid bacteria are used to provide a diversity in food supply by altering the flavour texture and appearance of raw commodities. Lactic acid bacteria also impart a sour aromatic flavour which gives a natural image to the product (Gillliand, 1995; Chassy and Murphy, 1993; Davidson, 1993; Salovaara, 1993).

Lactic acid bacteria have been found to provide microbial safety in Africa's fermented foods (Nout et al., 1987; Mensah et al., 1990; Lacvi & Socinberg, 1991).

Lactobacilli are the largest genera of lactic acid bacteria and are defined as catalase-negative, oxidase-negative non-sporing rods, strictly fermentative and microaerophilic with a complex nutritional requirement (Meisel, 1991). They contribute to the flavour of fermented foods by producing various volatile compounds such as diacetyl and its derivatives and even H_2S (Sharp & Franklin, 1962). They belong to the group of obligate hetero-fermentative lactobacilli (Axelsson, 1993). This group is predominantly found in plants e.g. grains, mashes and fermenting vegetables.

Laboratory studies have been carried out at FRI, using the strains of *Lb. fermentum*, *Saccharomyces cerevisae* and *Candida krusei* isolated from spontaneously fermenting

maize dough as starter culture for controlled fermentation (Krogbeck, 1993; Halm et al. 1996). These studies showed that it is possible to reduce fermentation period of maize from 48 h to 24 h. It also provided a means for controlling the microbial stability and organoleptic quality of the product thus eliminating the quality risks inherent in traditional kenkey production.

The aim of this experiment is to investigate the feasibility of using *Lactobacillus fermentum* (7-11 A) as a starter culture for the industrial production of fermented maize dough during kenkey production.

MATERIALS AND METHODS

Production site

Lactobacillus fermentum 7-11A was used as starter culture ferment maize dough during the commercial production of kenkey at a Kenkey production site at Osu in Accra. The plant owned by a traditional Kenkey producer, Ms Nora Telfar had been upgraded previously with assistance from the Danish International Development Agency (DANIDA) and the Food Research Institute of the Council for Scientific and Industrial Research (CSIR)as part of the collaborative projection 'Capability building in the processing of traditional fermentred foods'.

Maize

Maize used for this project was purchased from the open market through the personnel of the kenkey factory at Osu.

Starter culture

The Lactobacillus strain used in the present studies as starter culture was isolated in a previous study at the Food Research Institute as the dominant *Lactobacillus fermentum* strain responsible for the fermentation of maize. The *Lactobacillus fermentum* strain had been designated as 7 - 11 A.

Production of Inoculum

The starter culture was produced as a batch culture in a 10 litre fermenter (Braun, Biotech International, Germany). About 6 litres of MRS broth was inoculated with 10 mls of MRS broth which had been inoculated with a loopful of a strain of *Lactobacillus fermentum* 7 - 11 A and incubated at 30°C for 48 hrs. The batch culture was run at a temperature of 30°C, a partial pressure of 0% (i.e. anaerobic condition) and stirred at a speed of 50 rpm for about 30 h to obtain a cell concentration of 1.0×10^9 cfu/ml. The cells were harvested by centrifugation at 4000 rpm and washed twice with distilled water after which the cells were resuspended in 100 ml distilled water to form a dense culture.

Inoculation

Out of the dense culture prepared, 400 mls were added to 600 l of water in the steeptank to attain a cell concentration of about 10^8 cfu/g. About 400 kg of maize was added to the water and steeped for 24 h. The steep water was then decanted and the maize wet-milled and made into a dough by adding adequate amount of water. The dough was allowed to undergo fermentation for 2 days.

After 24 and 48 h of fermentation, some of the dough was prepared into kenkey.

Dough fermented spontaneously for 48 h by traditional method was used as a control.

Sampling

The steep water was sampled before and after the addition of inoculum. It was also sampled at the end of the steeping (24 h) The fermented dough samples were taken from within the dough after the surface areas had been removed aseptically.

Microbiological analysis

For all samples taken, 10 g were homogenized in 90 ml sterile diluent (01.% peptone, 0.8% NaCl pH 7.2) using Stomacher (Lab Blender model 4001 Seward Medical , London, England) for 30 s at normal speed. From appropriate tenfold dilutions, poured plate countings were carried out using the following media and incubations, Universal Beer Agar, incubated anaerobically at 30°C in a oxygen-free CO_2 atmosphere for 7 days and Malt Agar containing chloramphenicol and incubated at 25°C for 5-7 days.

From plates of highest sample dilution , all colonies (_10) from a sector of each plate (>75% of the area) were subcultured into their corresponding broth medium and streaked onto the agar substrate until pure cultures were obtained.

Characterisation of Isolates

The isolates were examined and characterised by the following tests: Gram reaction, using 3% KOH, oxidase test (Dry slides; Difco Laboratories, Detroit, Michigan, USA) Catalase Test (H_20_2 3%).

Chemical analysis

pH determination

The pH of steep water was measured directly with a pH meter (Radiometer analytical krogshojves 49, DK-2880, Bagsvaerd, Denmark). The pH of maize dough samples were determined after mixing 5 g of sample with 5 ml of distilled H_2O.

Sensory Evaluation

Ga Kenkey prepared from 24 and 48 h dough fermented with the aid of starter culture were compared to kenkey prepared by traditional method by a taste panel for appearance, odour, taste, sourness, texture and overall acceptability on a scale, ranging from 9 (like extremely) to 1 (dislike extremely).

RESULTS AND DISCUSSION

At only 24 h, the dough fermented with starter culture had attained the final level of pH i.e. 3.7, expected in maize dough fermented spontaneously for 48 h (Table 1). This shows that the production of acid had been increased in the dough inoculated with the starter culture due to the much higher level of lactic acid bacteria. Thus the addition of the starter culture (*Lactobacillus fermentum*) increased the initial load of *L. fermentum* and resulted in an increase in the rate of lowering of pH during the initial stages of fermentation

Examination of the microflora on Universal Beer Agar exhibited the dominance of Gram-positive, catalase-negative and oxidase-negative microorganisms which

TABLE 1: The pH of the steep water and fermented dough samples

	H_2O	Innoculum	Maize	Ohr. Steep H_2O	24hr. steep H_2O	O hr dough	24hr dough	48 hr. dough
Inoculated sample	7.51	7.43	6.13	6.25	4.44	5.99	3.77	3.65
Control				6.15		5.20	3.90	3.81

confirmed the presence of lactic acid bacteria.

In the inoculated sample most of the UBA isolates examined were similar in colony and cell morphology to that of the *L. fermentum* strain used as starter culture. This suggests that the strain of *L. fermentum* used as starter culture multiplied and dominated the microbial population of maize dough during fermentation.

A more remarkable increase in the population of lactic acid bacteria, suspected to be *L. fermentum* , was recorded in the inoculated sample, in comparison with the control(Table 2). This increase in the inoculated sample may be attributed to the introduction of the *L. fermentum* as inoculum enrichment which facilitated the proliferation of this strain at the expense of other microorganisms due to their antimicrobial properties.

The population of yeast in all the test samples was comparable to that of the control

which suggests that the introduction of the starter culture does not affect the microflora of yeast present in fermented dough (Table 3).

TABLE 2:

The population (Log of cfu/g) of Lactic acid bacteria in fermented maize commercially produced with Lactobacillus fermentum 7-11A as starter culture

	Innoculum	Maize	Ohr. Steep H_2O	24hr. steep H_2O	O hr dough	24hr dough	48 hr. dough
Control UBA			6.15		5.20	7.90	8.8
Inoculated sample	6.39	5.00	6.88	8.18	7.20	8.91	8.98

Sensory evaluation

Kenkey produced from all doughs produced with starter culture and fermented for 24 h and 48 h were found acceptable by a taste panel. Analysis of the taste panel scores by Duncan's Multiple Range Test show that there were no significant differences between spontaneously fermented dough kenkey and that of kenkey prepared from dough produced with starter culture and fermented for only
24 h.

TABLE 3:

The population (Log of cfu/g) of yeasts in fermented maize dough commercially produced with *Lactobacillus fermentum* 7-11A as starter culture

	Maize	Ohr. Steep H_2O	24hr. steep H_2O	O hr dough	24hr dough	48 hr. dough
Control	6.17	2.30	6.30	6.17	6.52	6.81
Samplfinoculated with L. fermentum	6.71	2.30	6.36	6.11	6.651	6.60

The results of the work show that *Lb. fermentum* can be used as starter culture to reduce the fermentation period during large scale commercial production of kenkey from 48 to 24 h.

In a previous study, Halm et al. (1996), who used a variety of strains of *Lb. fermentum* as starter culture in the fermentation of maize found 24 h fermented maize produced with starter culture to be suitable for Koko but not kenkey production. However in the present study, the maize used had been freshly harvested rather than been in storage for a period of time as had been used in the previous studies.

Further investigations have to be carried out to establish that the use of single cultures

to ferment maize would fulfill the requirement for food safety and quality since Olsen et al (1995) have demonstrated that each of the various processing stages of maize fermentation during kenkey production has its own microenvironment, with strong antimicrobial activity, which determines the composition of the microflora of the final product.

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