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EXPERIENCES IN THE APPLICATION OF HACCP TO TRADITIONAL FOOD PROCESSING AT A SEMI-COMMERCIAL KENKEY PRODUCTION PLANT IN ACCRA, GHANA

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Abstract

Hazard Analysis Critical Control Point was implemented at a traditional semi-commercial plant for processing maize into Kenkey. The quality system was assessed for its effectiveness over a two months period, involving evaluation of the resident microbiota in the air and on the surface of processing equipment on three separate occasions, monitoring of Kenkey production on two occasions and laboratory analysis of raw materials, intermediary and final product on two separate occasions as a means of verification. Air sampling revealed a population of aerobic mesophiles ranging between 23 and 45 cfu/min; and yeasts and moulds between 12 and 16 cfu/min. The population of aerobic mesophiles on the surface of the steeping tank between batch productions was between 3.1×10^5 and 1.3×10^6 cfu/g; count on MRS between 4.6×10^5 and 5.7×10^5 cfu/g; and yeasts and moulds between 7.7×10^3 and 3.1×10^4 cfu/g. The fermentation trough also recorded aerobic mesophilic population ranging between 4.1×10^4 and 6.1×10^5 cfu/g; MRS counts between 2.1×10^3 and 5.0×10^6 cfu/g; and yeasts and moulds counts between 2.6×10^3 and 8.4×10^3 cfu/g. The aerobic mesophiles found at the plant included *Bacillus* spp., *Staphylococcus* spp. and *Micrococcus* spp. The lactic acid bacteria population was composed of *Lactobacillus fermentum* and pediococci; and the moulds *Aspergillus* spp., *Mucor* spp., *Penicillium* spp. and *Fusarium* spp. Very little variation was observed between the monitoring results on the two separate occasions and they all fell within the stipulated critical limit in each instance. Laboratory analysis showed the presence of coliforms and *E. coli* in the steep-water on the first occasion of sampling but could not be detected after 24 h of dough fermentation. Of the pathogens tested for on the second occasion of sampling, *E. Coli, Staphylococcus aureus, Enterococcus, Salmonella,* and *Vibro cholera* were not detected in any of the fermented maize dough and Kenkey samples analysed. *Bacillus cereus* was found in the fermented dough but was not detected after

cooking into Kenkey. The level of total aflatoxin in the maize samples were 17.0 and 13.7 µg/kg and in the Kenkey samples 17.2 and 14.7 µg/kg. Levels of aflatoxins in Kenkey reported at the plant before implementation of HACCP ranged from 64.1 to 196 ug/kg.

1. Introduction

Kenkey which is a cooked stiff porridge prepared from fermented maize dough, is a major staple food consumed in Ghana. In recent years it has received extensive scientific research at the Food Research Institute of the Council for Scientific and Industrial Research in Ghana, in collaboration with the Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Denmark (Halm et al., 1993, 1996, 2004; Halm and Olsen 1996; Jespersen et al., 1994; Kpodo et al., 1995, 2000; Kpodo 2003; Olsen et al., 1995; Hayford, 1998; Hayford and Jespersen, 1999; Hayford and Jakobsen, 1999; Hayford et al., 1999; Annan et al., 2003a; 2003b; 2003c).

Initial studies by Halm et al. (1993) found the microbial population at the advanced stage of maize dough fermentation to consist of lactic acid bacteria and yeasts dominated by an obligately heterofermentative lactobacilli whose pattern of carbohydrate fermentation was closely related to *Lactobacillus fermentum* and *Lactobacillus reuteri.* RAPD profiling by Hayford et al. (1999) confirmed the dominant lactobacilli as *Lactobacillus fermentum.* The study by Hayford et al. (1999) also showed the presence of several strains of *Lactobacillus fermentum* based on DNA specific-probe hybridization. *Candida krusei* and *Saccharomyces cerevisiae* were identified by Jespersen et al. (1994) as the dominant yeasts in Kenkey fermentation based on phenotypic methods. These results were confirmed by Hayford and Jakobsen (1999) and Hayford and Jespersen (1999) using whole cell DNA, PCR and REA

profiling. Hayford and Jespersen (1999) also showed the presence of four different *MAL* genotypes within the *Saccharomyces cerevisiae* group.

Annan et al. (2003a) identified a total of 76 aroma compounds in fermented maize dough by GC-MS analysis including 21 carbonyls, 19 alcohols, 17 esters, 12 acids, a furan, 2 phenolic compounds, an alkane and four unidentifed compounds. GC-sniffing technique showed 46 compounds as contributing to the aroma of fermented dough.

In 1996, Kpodo et al. had identified mycotoxin contamination of maize as a major hazard in kenkey production, and over an 18 months sampling period analysed twelve samples of fermented maize dough from two kenkey production sites in Accra. They reported aflatoxin levels ranging from 0.7 to 313 µg/kg, with seven of the samples containing more than 100 µg/kg aflatoxin.

Different strategies were devised by the CSIR-Food Research Institute to manage mycotoxin contamination of maize and maize products in Ghana. (i) The relevant agencies were made aware of the problem and there are programmes now which educate farmers on procedures for handling and storing maize efficiently, in order to minimise contamination with moulds and mycotoxin production. (ii) An HACCP system was also developed for the processing of maize into fermented maize meal and Kenkey to assure the microbiological and mycotoxicological safety of the products (Amoa-Awua et al. 1998). (iii) In collaboration with the Institute of Hygiene and Toxicology, Karlsrule, Germany and other institutions, microbial species capable of degrading aflatoxins were isolated. (iv) Finally, starter cultures have also been developed for use in Kenkey production based on their antimicrobial and probiotic properties amognst other factors (Halm et al, 1996; Hayford 1998; Annan 2003b, 2003c).

In testing the effectiveness of the HACCP system developed, it was implemented at a traditional semi-commercial Kenkey production plant in Accra after the plant had been upgraded. The Kenkey plant, which is owned by a traditional processor, consists of a small processing hall and a detached maize storage room as well as a milling room. It processes about five tons of maize per week into fermented maize meal and Kenkey, and these are retailed directly to the general public. This work was carried out to assess the effectiveness and impact of the HACCP system at the plant by evaluating the resident microbiota on the three separate occasions and carrying out laboratory analysis of raw materials, intermediary and final products on two occasions over a two months period.

2. Materials and methods

2.1. Determination of the microbiota in the air and on the surface of the steeping tank and fermentation toughs

The microbiota in the air and on the surface of the steeping tank and four fermentation troughs were sampled on three separate occasions between batch productions within a period of two months.

2.1.1. Sampling of air

Plate Count Agar (PCA Difco 0479-17-3, Difco Laboratories, Detroit, USA) and Malt Extract Agar (Merck 5398, Merck, Darmstadt, Germany) plates containing 100 mg chloramphenicol (Sigma C-0378, Sigma Chemical Co, St Louis. MO, USA) and 50 mg chlorotetracycline (Sigma C-4881) were exposed in the processing hall for 5 min. The lids were replaced and the plates sent immediately to the laboratory for incubation at 30°C for 3 - 5 d.

2.1.2. *Swabs*

A sterile swab was dipped into sterile peptone water containing 0.1 % peptone (Difco 0118-17, Becton Dickinson & Co, Sparks, USA) and 0.85 % NaCl with pH adjusted to 7.2 and used to swab an area of 5 cm x 5 cm of the inner surface of either the steeping tank or a fermentation trough and plunged into 10 ml of sterile peptone water in a test tube. Swabs were taken from four different areas of the inner surface of the steeping tank and from four different fermentation troughs. Swabs were placed in an ice chest and taken immediately to the laboratory for analysis.

2.2. *Production of Kenkey*

On two separate occasions within a period of two months, Kenkey being produced at the plant was monitored and also sampled for laboratory analysis. On each occasion, about 1.8 tons of maize were washed and soaked in water in the steeping tank for 24 h. The steep-water was drained off and the maize grains milled in a plate mill (Premiere-Lister, U.K.). The meal was kneaded in lots of about 10 kg of dough with 3 l of water to make a dough of about 50 – 55% moisture. The dough was packed in lots of about 50 kg per fermentation trough and fermented for 48 h. After fermentation, one-third of the fermented dough was partially cooked and mixed with the remaining uncooked dough into Aflata. The mixture was moulded into balls, wrapped in maize husks and cooked for 3 h into kenkey.

2.2.1 *Monitoring of Kenkey production*

The monitoring procedure used was according to the HACCP Plan of Amoa-Awua (1998) for Kenkey production shown in Table 1. This involved a visual examination of maize kernels, water, steep-water, milled maize and maize husks. Maize kernels were assessed for their

extent of dryness by their shininess and crispiness. Steep-water and water for mixing dough were visually examined for clarity and odour and pH by use of a pH strip. The milled maize meal was visually examined for the presence of metal pieces, whilst fermenting maize dough was examined for pH, odour and mould growth on the surface. Maize husks were visually examined for discolouration and mould growth.

2.2.2 Verification of HACCP

Maize kernels, 0-h and 24-h steep-water, fresh dough, 24-h and 48-h fermented dough and Kenkey were sampled on the two occasions that monitoring was carried out and analysed in the laboratory for pH, titratable acidity, aerobic mesophilic count, yeasts and moulds count, enumeration/detection of coliforms, *E. coli*, *Staphylococcus aureus, Bacillus cereus, Enterococci, Salmonella* and *Vibrio cholera*.

2.3 Microbiological analysis

The four swab samples from the steeping tank were pooled together and shaken to dislodge the microorganisms from the swabs. The four samples from the different fermentation troughs were also pooled together. Each pooled swab sample was homogenised in a Stomacher (Lab Blender, Model 4001, Seward Medical, London, England) for 30 s at normal speed. For maize dough samples, 10 g were homogenized in 90 ml sterile diluent containing 0.1 % peptone, 0.8% NaCl, pH 7.2 using Stomacher (Lab Blender, Model 4001) for 30 s at normal speed. From appropriate tenfold dilutions, aerobic mesophiles were enumerated on Plate Count Agar (PCA Difco 0479-17-3) incubated at 30° C for 3 d. Lactic acid bacteria were enumerated on deMan, Rogosa and Sharpe Medium (MRS, Merck 10660) incubated anaerobically at 30° C using Anaerocult A (Merck) for 5 d. Yeasts and moulds were

enumerated on Malt Extract Agar (Merck 5398) added per 1000 ml, 100 mg chloramphenicol (Chloramphenicol Selective Supplement Oxoid) and 50 mg chlortetracycline (Sigma Chemical Co.) incubated at 25° C for 7 d.

2.3.1 Isolation, characterization and identification of bacteria

Colonies totalling about 20 from a segment of the highest dilution or suitable plate were subcultured in the corresponding broth medium and streaked onto the agar substrate until pure cultures were obtained. PCA isolates were characterised by cell morphology, Gram reaction and catalase catalase test

2.3.2. Identification of lactic acid bacteria

Isolates on MRS were examined by Gram reaction, catalase production, oxidase test, aerobic and anaerobic growth, colony and cell morphology. Gram-positive catalase-negative regular rods, coccoid and cocci were examined by gas production in MRS broth (Merck 10661) with Durham tube and also in MRS broth in which glucose was replaced with gluconate as sole carbon source; growth at 10 °C and 45 °C, at pH 4.4 and 9.6, in 6.5 and 18% (w/v) NaCl; and Hugh and Leifson's test (Hugh and Leifson, 1953). The species of isolates were identified by determining their pattern of carbohydrate fermentation and comparing them to known carbohydrate fermentation profiles (Kandler and Weiss 1986). Modified MRS-broth was prepared from 10 g peptone (Oxoid L37, Oxoid, Basingstoke, Hampshire, England), 5 g yeast extract (Difco,212750, Becton Dickenson and Co, Sparks, MD, USA), 1ml polyoxyethylene sorbitan mono-oleate (Tween 80) (Merck Art 822187, Merck-Scuhardt, Schuhardt, Hohenbrunn, Munich, Germany), 2 g K_2HPO_4 (Merck 4873.025, Merck, Darmstadt, Germany), 5 g sodium acetate trihydrate (Sigma S-8625, Sigma Chemical Co, St Louis, MO, USA) 2 g triammonium citrate (Aldrich 37,469.5, Aldrich Chemical Co Inc., Milwauke, WIS,

USA), 0.2 g magnesium sulphate heptahydrate (Sigma M.1880), 0.05 g manganese sulphate monohydrate (Merck 1.05963, Merck, Darmstadt, Germany), 1000 ml distilled water, 0.2 % chlorophenol red, pH 6.2. A 10 % solution of 17 different carbohydrates all obtained from Merck (amygdalin, arabinose, cellubiose, fructose, galactose, glucose, gluconate, lactose, maltose, mannitol, melezitose, melibiose, raffinose, salicin, sorbitol, sucrose and trehalose) was each filtered sterile and 4.5 ml of the modified MRS broth added to 0.5 ml of each carbohydrate. Each broth containing a different carbohydrate was inoculated with the isolate and incubated at 30 °C for 7 d. Fermentation of the carbohydrate was indicated by a change in colouration from red to yellow as a result of acid production. By comparing the carbohydrate fermentation profile of each isolate to known profiles (Kandler and Weiss, 1986) the species of the isolate was identified.

2.3.3. Identification of yeasts

Yeasts were examined by colony and cell morphology. Their pattern of fermentation and assimilation of glucose, sucrose, raffinose galactose, maltose, lactose, trehalose, cellobiose, xylose and meliobiose were determined according to Kreger-Van Rij (1984). .

2.3.4 Identification of moulds

Moulds were identified by morphological characteristics according to Samson et al. (1994). Isolates were inoculated at three points on Czapek Yeast Extract Agar (CYA) and Malt Extract Agar (Merck 5398) incubated in the dark at 25 °C for 7 d. Czapek Yeast Extract Agar contained g/l distilled water; 3.0, $NaNO_3$ (Merck 11,8598), 1.0, K_2HPO_4 (Sigma no P5504), 0.5, KCl, 0.5, $MgSO_4.7H_2O$ (Merck 11,5573), 0.01, $FeSO_4.7H_2O$ (Merck 11,4006), 5.0, yeast extract (Oxoid L21, Oxoid Ltd., Basingstoke, Hampshire, England), 30.0, sucrose (Sigma no S9378) and 20.0, agar (Merck 1.01614), pH 6.5. The colony diameter was measured after 5 d

and colony characteristics observed. Morphological examination was carried out by staining and wetting a piece of the mould on a microscope slide with lactophenol (Merck), squashing the fruit bodies and teasing the mycelium to determine the general form of growth.

2.3.5. Detection of indicator organisms and pathogens

Coliform bacteria was detected according to NMKL 44 (1995). Enterococci were enumerated according to NMKL 68 (1992). Coagualse positive Staphylococci were enumerated according to NMKL 66 (1992). *Bacillus cereus* was determined according to NMK 67 (1996) using *Bacillus cereus* selective agar (Oxoid CM617). *Salmonella* was determined according to NMKL 71 (1999). *Vibrio cholera* was determined according to NMKL

2.4. Chemical Analysis

2.4.1. pH determination and titratable acidity

Samples of dough weighing 10 g, were mixed with 90 ml distilled water and pH determined with a pH meter (Crison pH meter, Crison Instrument, S.A. France). Titratable acidity was determined by the titration of 80 ml of filtrate obtained from 10 g of dough dissolved in 200 ml distilled water, against 0.1N NaOH with 1% phenolphthalein. 1ml of 0.1N NaOH was taken as equivalent to 9.008 x 10 g lactic acid.

2.4.2. Determination of aflatoxins

Aflatoxins were extracted based on the procedure of Pons (1979) as described by Kpodo et al. (1994; 2000). Finely ground maize kernels, fermented dough or Kenkey were extracted with methanol followed by precipitation of colour pigments using zinc acetate then extraction into dichloromethane, with further clean-up by column chromatography using cellulose and silica

gel. Aflatoxins were eluted with dichloromethane:acetone (80:20 v/v), which was evaporated off, and the residue quantitatively transferred into 10ml of HPLC-grade dichloromethane. Five millilitres were evaporated to dryness under a stream of nitrogen, and the final residue dissolved in 0.1-1.0 ml of HPLC mobile phase and used for HPLC analysis.

Aflatoxin standards were obtained from Sigma Chemical Co. Ltd (St Louis, MO, USA). Standard stock and HPLC working solutions were prepared by evaporating and dissolving in a mobile phase consisting of methanol, acetonitrile and water (10:30:60 v/v/v) to give concentrations of 0.1μg/ml for aflatoxins B_1 and G_1 and 0.03μg/ml for B_2 and G_2. Reagents used for HPLC separations were of HPLC grade (Merck Chemicals, Darmstadt, Germany). All other chemicals and reagents were of analytical grade. Distilled and deionized water were used throughout. All mobile phase solutions were filtered through a 0.45 μm Millipore HV disc filter. They were degassed prior to use througha Millipore filtration unit (Millipore Corp. Bedford, MA, USA)

HPLC analysis for aflatoxins was by reversed-phase liquid chromatography with post-column iodine derivatization. Separation of aflatoxins was carried out on a Spherisorb S5 ODS-1 column of dimension 25 x 4.6mm packed with 5μm particles (Phase Separations Inc., Norwalk, CT, USA) maintained at 35°C. The flow rate of the HPLC mobile phase was 1.2 ml/min and post-column iodine derivatization of aflatoxins B_1 and G_1 was achieved using saturated iodine solution according to the procedure of Shepherd and Gilbert (1984). Iodine was pumped at a flow rate of 0.4 ml/min using an Eldex precision metering pump (Eldex Laboratories Inc., San Carlos, CA, USA). The derivatization tube consisted of stainless steel tubing (5m x 0.3mm) maintained at 75°C. The excitation and emission wavelengths used

were 360 and 440 nm respectively. The aflatoxins were identified by their retention times, and peaks areas were used to determine their concentrations in the samples by reference to standard curves obtained by chromatographing pure aflatoxin standard solutions under identical conditions.

3. Results and discussion

3.1. Upgrading of the traditional Kenkey plant

Kenkey was produced at the traditional processing plant by steeping maize in large plastic drums in the courtyard for 2 d. After steeping, the grains were drained and milled in a plate mill in a room detached from the small processing hall. The milled maize was kneaded with water into a dough in wooden containers, and left in the processing hall to ferment for at least 48 h. Part of the fermented dough was cooked in the processing hall on an open fire using firewood, and kneaded with the rest of the fermented dough into Aflata. The mixed dough was kneaded into balls, wrapped with maize husks, and cooked into Kenkey on the open fire in the processing. In upgrading the traditional plant, the small processing hall was renovated, the walls painted, and the open windows covered with a nylon mesh to keep away flies and other flying insects. A large soaking tank was constructed in the processing hall using cement blocks lined with white porcelain tiles. This replaced the metal drums used to steep maize kernels in the courtyard. Large aluminium containers for fermenting maize dough were constructed and placed on metal shelves erected in the processing hall. Two large gas stoves were constructed to replace the traditional tripod firewood stoves.

3.2. *Implementation of HACCP*

HACCP was implemented at the plant based on the system described by Amoa-Awua et al (1998). The staff were given adequate training before implementation of HACCP. After six months the system was audited and corrective actions taken.

3.3. *The resident microbiota at the Kenkey production plant*

The micropopulation on the surface of the steeping tank, fermentation trough and in the air at the Kenkey production plant sampled on three separate occasions over a two months period are presented in Table 2. The micropopulation of aerobic mesophiles in the air ranged from 23 to 45 cfu/min, on the surface of the steeping tank from 10^5 to 10^6 cfu/ml, and in the fermentation trough from 10^4 to 10^5 cfu/ml. The population of the aerobic mesophiles in all instances were dominated by Gram-positive, catalase-positive rods which bore phase bright spores and were assumed to be *Bacillus* spp. The population of aerobic mesophiles also contained glucose fermenting Gram-positive aerobic cocci which were catalase positive and occurred in pairs or clusters, were non-motile and non-sporing and were suspected to belonged to the genus *Staphylocccus* or *Micrococcus*.

The resident microbial population enumerated on MRS incubated anaerobically were found at levels of 10^5 cfu/g on the surface of the steeping tank and 10^3 to 10^6 cfu/g on the surface of the fermentation trough (Table 2). The cultures were mostly Gram-positive, catalase-negative, oxidase-negative regular fermentative rods, which produced gas from glucose and were considered to be lactic acid bacteria. The most frequently occurring rods fermented arabinose, fructose, galactose, glucose, gluconate, lactose, maltose, melibiose, raffinose, sucrose and

trehalose and were tentatively identified as *Lactobacillus fermentum*. *Lactobacillus fermentum* had previously been isolated from the plant by Halm et al. (1993) as the dominant lactic acid bacteria responsible for the fermentation of maize dough. The micropopulation on MRS also contained Gram-positive, catalase negative cocci that did not produce gas from glucose and occurred in pairs and tetrads, and were suspected to be pediococci. Halm et al. (1993) had isolated *Pediococcus pentosaceus* and *Pediococcus acidilactici* in the flora of fermented maize dough produced at the Kenkey plant.

The population of yeasts and moulds in the air at the plant were between 12 and 16 cfu/min, on the surface of the steeping tank between 7.7 x 10^3 and 3.1 x 10^4 cfu/g and on the fermentation troughs between 2.6 x 10^3 and 8.4 x 10^3 cfu/g. Cultures isolated on Malt Extract Agar (Merck 5398) from the steeping tank and fermentation troughs, consisted of yeasts and moulds based on their cell and colony morphologies. The yeasts were similar in colony and cell characteristics to dominating yeasts isolated from fermenting maize dough at the plant in a previous study (Jespersen et al., 1994) and were tentatively identified as *Saccharomyces cereviseae* and *Candida krusei* based on their patterns of carbohydrate fermentation and utilisation (results not shown).

Various types of moulds were isolated from the air and the surfaces of the steeping tank and fermentation troughs. One type of mould attained a size of 4.5 cm on MEA after 5 d at 25 °C and had black conidiophores with a radiate black conidial head, floccose smooth-walled conidiophore stipes, globose to subglobose veside and conidia, septate metulae and an unbranched tall sporulating structure. These moulds were tentatively identified as *Aspergillus niger*. A second type of mould which was whitish to cream in colour, also attained a diameter of 4.5 cm, and had branched conidiophores with slender mono and poly phialides, floccose

aerial mycellium, one celled micro-conidia produced in false heads in the aerial mycelium, three to five slightly sickle shaped macro-conidia and was tentatively identified as *Fusarium subglutinans*.

A third type of mould which was tentatively identified as *Mucor racemosus,* was fast growing forming a dense mycelium mat which covered the whole MEA plate in 7 d at 25 °C. It had white hyphae with both short and tall branched smooth-walled ellipsoidal to subglobose sporangiophores, the short branches sometimes recurved with encrusted walls. The sporangia hyaline became brownish to grey with age and the collumella was ovoboid or ellipsoidal, slightly pyriform with truncated base. Another type of mould isolated, grew very rapidly on MEA appearing as a dense mat when sporulating and covered the agar plate within 7 d. The colonies which were cottony white, became dark grey with age. Smooth-walled globose, ovoid or irregular shaped unbranched sporangiophores rose directly from stolons without rhizoids and the sporangia were globose or subglobose, whilst the columella was ovoid or globose. This type of mould also produced asexual spores and had a single septa which separated the mycelium from the sporangium, and was tentatively identified as *Rhizopus oryzae*. The last type of mould isolated, was tentatively identified as *Penicilium citrinum.* It attained a size of only 1.5 cm after 5 d growth on CYA and had dense felt conidiophores which appeared leathery and blue green, with a pale yellow colony reverse. The conidiophores were smooth walled with divergent metulae, and the phialides were flask shaped. The conidia, which were produced in columns, were globose to subglobose, smooth walled or finely rough hyaline to greenish.

The population of the different types of moulds found in the air and on the surfaces of the steeping tank and the fermentation trough are presented in Table 3. Isolation of various

moulds in the air sample and from the surfaces of the two equipment examined, was not surprising. This is because in Ghanaian households, fermented maize dough is usually seen covered with moulds which are scrapped off before the dough is used. Jespersen et al (1994) isolated *Penicillium, Aspergillus* and *Fusarium* species from raw maize, but during the maize dough fermentation, the initial high counts of 10^5 cfu/g were reduced to less than 10^2 cfu/g within 24 h.

3.4 Monitoring of the processing of maize into Kenkey

The results of monitoring carried out during the production of Kenkey on the two separate occasions are presented in Table 4. Visual assessment of maize used for Kenkey production on both occasions, indicated that the maize supplied was of satisfactory quality since it did not contain discoloured grains and was very crispy and shiny. This suggested that on both occasions, the maize was fairly well inspected before it was purchased.

The washed maize contained many pieces of broken cobs and chaff, and had therefore not been adequately dry cleaned and sorted. The use of such inadequately cleaned maize is expected to have an adverse effect on the organoleptic but not the microbiological quality of the product. Pipe borne water used for steeping maize on both occasions was clear, odourless and had almost a neutral pH. It was therefore suitable for the production of good quality Kenkey.

Steeping was carried out successfully on both occasions, since there was a drop in pH to levels indicating rapid growth of lactic acid bacteria and conditions not favourable for the

proliferation of spoilage microorganisms. This was confirmed by the absence of offensive odour in the 24-h steep-water.

The grinding plates of the mill used to mill the steeped maize were in good condition and no metal pieces broken off the grinding plates were found in any of the milled products. In both dough fermentations, the final pHs attained were below 4 indicating successful fermentations. The doughs had no offensive smell to indicate proliferation of spoilage microorganisms. The Kenkey on both occasions were cooked by boiling for 3 h.

3.5. Verification of the quality of maize, intermediary products and Kenkey

Results of laboratory analysis of samples taken on the two separate occasions during Kenkey production are presented in Table 5. The verification results confirmed that Kenkey had been produced on both occasions within the critical limits for the production of microbiologically safe Kenkey. Steeping was assessed in the first batch production only and by the end of steeping, coliforms including *E. coli* were detected in the steep-water indicating faecal contamination. The likely source of contamination could have been the maize or water used, or from the hands of the processors. Maize dough analysed on both occasions, showed that the final pH attained in each case was close to the optimal value of 3.7. At that low pH value, spoilage organisms and pathogens were not expected to survive the fermentation, and indeed the coliforms including *E. coli*, found in the steep-water in the first batch, were not detected in 10 g of sample at the end of fermentation. In the second batch production, coliforms were detected at 24 h of fermentation but could not be detected in 10 g of dough by the end of fermentation. *E. coli* was not detected in this dough at all.

The lowering of pH during maize dough fermentation, correlated with the production of lactic and acetic acids as expected. The percentage of titratable acid produced in both fermentations were between 1.44 and 1.5%. These were within the range of 1.4-2% stipulated as the critical limit for maize dough fermentation during kenkey production (Halm et al., 1996; Amoa-Awua et al., 1998). In both dough fermentations there was an increase in the population of aerobic mesophiles enumerated on Plate Count Agar (PCA Difco 0479-17-3) from a level of 10^7 to 10^8 cfu/g. In the second batch, a more extensive analysis was carried out to determine the presence of some indicator organisms and pathogens in the fermented dough. Of the pathogens investigated, only *Bacillus cereus* was found present and had a population of 100 cfu/g. No counts in cfu/g were obtained for *E. coli, Staphylococcus aureus* and enterococci, and neither *Salmonella* nor *Vibrio cholera* were found in 25 g of sample.

Three hours of cooking Kenkey on both occasions, was sufficient to render the product microbiologically sterile, and no growths were obtained on Plate Count Agar (PCA Difco 0479-17-3) and Malt Extract Agar (Merck 5398). *Bacillus cereus* which was found in the second fermented dough was not detected in the Kenkey produced. It could be concluded that three hours of cooking was sufficient to destroy even the spores of the *Bacillus* species. *E. coli, Staphylococcus aureus,* enterococci and *Salmonella* were not found in any of the Kenkey samples. The Kenkey produced on the two occasions were therefore microbiologically safe ,and any threat of food intoxication would be the result of poor finished product handling during retailing.

The main hazard in Kenkey making is the presence of aflatoxin if contaminated maize is used (Kpodo et al. 1995). On the first occasion of sampling, the total aflatoxin content in the maize kernels was 17.0 µg/kg and on the second occasion 13.7 µg/kg. The Codex

Alimentarius standard for aflatoxins in maize is set at 15 ppb (FAO, 1995) and this was exceeded slightly on the first but not on the second occasion. The total aflatoxin content of Kenkey samples collected on the two occasions were 17.2 and 14.7 µg/kg. Earlier studies carried out at this same plant before implementation of HACCP, reported aflatoxin levels in fermented maize meal ranging from 17.9 to 313 ug/kg and in kenkey samples from 64.1 to 196 ug/kg. This study was carried by Kpodo et al (1995) over an 18 months period. Therefore, with the implementation of HACCP at the plant which necessitated the changing of the maize supplier, the problem of aflatoxin contamination of Kenkey is being better managed by discriminate purchase of maize, better storage and handling of maize at the plant, and adherence to the principles of HACCP. However, it is necessary for inspection of maize during purchasing to be more stringent to ensure compliance to the critical limits set for aflatoxins at 10 ppb in the HACCP Plan (Amoa-Awua et al. 1998).

3.6. Impact of the quality system at the Kenkey Production Plant

The monitoring and verification results showed that processing operations were fairly standardised at the traditional semi-commercial Kenkey production plant, at least during the period of evaluation. Little variations were observed in the monitoring and verification results on the two separate occasions. A major factor which affected the organoleptic quality of the Kenkey produced, was the absence of a suitable equipment for cleaning and sorting maize kernels. The maize kernels were cleaned manually using sieves of two different sizes, but because of the relatively large volumes of maize handled, the traditional processors were unable to do this effectively. An unexpected consequence of the upgrading of the plant and implementation of HACCP, was the temptation of the traditional processors to increase the volume of maize which was processed in a batch. This was because the clientele of the plant

increased several fold. The plant owner rather than adhere strictly to the parameters which had been laid down, tended to increase the output of the plant to take advantage of the additional clientele to the disadvantage of plant hygiene and the HACCP system, since this led to spillage of maize dough and other materials due to space limitations. This issue was discussed at great lengths with the plant owner but not much progress was made. One could argue that HACCP had been 'imposed' on the traditional plant owner as part of a project rather than the plant owner haven't realised the need to have such a quality system in place at the plant. This emphasises the need for unwavering commitment to HACCP by management of companies implementing the system, and this was the main obstacle to the success of implementing HACCP at the level of traditional food processing in Ghana.

With the experiences acquired, the CSIR-Food Research Institute build its own Kenkey pilot plant as a model for transferring technology. The plant is installed in a small processing hall measuring 12.8 m by 7.6 m. The objective of the design is to permit an orderly flow of material through the plant, give enough elbow room for processing and cleaning and to prevent/minimise cross contamination of processing materials. The limiting factor in designing the plant layout was the space which was available, since the plant was installed in a hall which had already been put up.

The processing hall is divided into a main hall, a milling room, a fermentation room and a cooking section. This layout reduces noise pollution by the plate mill due to its seclusion in a milling room. The partitioning of the processing hall, also minimises the possibility of cross contamination of materials during processing. The plant consists of a weighing scale, a maize cleaning and sorting machine, a washing trough, a rinsing trough, four steeping tanks, a plate mill, 20 small plastic fermentation containers, a walk-in cabinet dryer and four gas stoves.

Apart from Kenkey, dehydrated fermented maize meal is also produced at the plant by drying fermented maize meal at 60 °C in the walk-in dryer. The product obtained is shelf stable for more than six months as compared to the wet fermented maize meal sold in the local markets which have a shelf life of less than a week.

4. Conclusion

This work has demonstrated the possibility of integrating a quality system into traditional food processing in the African context. HACCP proved an effective system which could address some of lapses experienced in traditional food processing which lacks the sophistication of a modern food processing factory. It could be implemented at minimal cost depending on simple techniques and equipment such as visual inspections, pH strips, thermometers and timing of unit operations such as cooking. Implementation of HACCP drastically reduced the levels of aflatoxins which had been recorded at the traditional plant, but for effective maintenance of the system, the commitment of the plant owner and traditional processors to the system is crucial.

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Table 1. HACCP Control Plan for the processing of maize into Kenkey (Amoa-Awua et al., 1998)

PROCESS STEP CCP	HAZARD	CONTROL MEASURE	CRITICAL LIMITS	MONITORING PROCEDURE	CORRECTIVE ACTION
MAIZE CCP	▪ Mycotoxins: Aflatoxins Citrinin fumonisins ▪ Foreign materials	▪ Purchase good quality material	▪ ppm aflatoxins ▪ 13.0% moisture	▪ Visual inspection	▪ Reject raw material and inform supplier
STEEPING CCP	▪ Spoilage and pathogenic micro-organisms	▪ Clean water ▪ .pH control to desired level ▪ keep hands off	▪ Transparent, clear, odourless and colourless ▪ pH 4.2+ 0.1 ▪ Keep hands off.	▪ Visual inspection ▪ Use of pH strips	▪ Boil water ▪ Educate ▪ Steep longer
DOUGH FERMENTATION CCP	▪ Mycotoxins ▪ Spoilage and pathogenic micro-organisms	▪ Adherence to fermentation time ▪ Cover dough	▪ Titratable acidity: Lactic: -1.4-2% Acetic:0.18-023% ▪ Volatile/Non volatile acid ratio about 0.16 ▪ pH not > 3.9	▪ Visual inspection ▪ Use of pH strips	▪ If fermentation is slow backslop with old dough
PACKAGING CCP	▪ Mycotoxins in maize husks	▪ Selection of non-mouldy maize husks ▪ Good Hygiene	▪ free from moulds ▪ >.....ppm aflatoxins	▪ Visual inspection	▪ Reject
COOKING CCP	▪ Residual mycotoxins in kenkey; ▪ Aflatoxins ▪ Citrinin	▪ Adequate cooking	▪ not less than 3 hrs cooking ▪ > 10 ppb of aflatoxins	▪ Recording of time	▪ Heat longer
KENKEY Final product			▪ >100 cfu/g of foreign bacteria (non LAB) ▪ >100 cfu/g of moulds ▪ >10 ppb of aflatoxins		
CLEANING CCP	▪ Contamination with spoilage and pathogenic micro-organisms	▪ Good housekeeping and personal hygiene ▪ Maintenance of equipment	▪ Clean premises, processing equipment and vessels and processing staff	▪ Visual inspection	▪ . Clean premise, hall equipment and vessels ▪ . Clean factory coats ▪ . Wash hands ▪ . Use gloves

Table 2. Microbial population in the air and on processing facilities at the Kenkey production plant

Date	Sampling area	Count on microbiological media		
		Plate Count Agar	MRS Agar	Malt Agar
06/01/03	Sampling of air*	23 cfu/min	No plating	12 cfu/min
	Steeping tank	8.0×10^5 cfu/g	5.1×10^5 cfu/g	7.7×10^3 cfu/g
	Fermentation trough	1.0×10^5 cfu/g	1.1×10^5 cfu/g	2.6×10^3 cfu/g
01/02/03	Sampling of air	45 cfu/min	No plating	12 cfu/min
	Steeping tank	3.1×10^5 cfu/g	4.6×10^5 cfu/g	1.9×10^4 cfu/g
	Fermentation trough	4.1×10^4 cfu/g	2.1×10^3 cfu/g	$8.4.1 \times 10^3$ cfu/g
28/02/03	Sampling of air	45 cfu/min	No plating	16 cfu/min
	Steeping tank	1.3×10^6 cfu/g	5.7×10^5 cfu/g	3.1×10^4 cfu/g
	Fermentation trough	6.1×10^5 cfu/g	5.0×10^6 cfu/g	3.0×10^3 cfu/g

* Exposure of Plate Count Agar plate for 5 min.

Table 3. Population of different species of moulds isolated at the Semi-commercial Kenkey Production Plant

Date of sampling	Sampling area	Species of mould isolated	Count in cfu/g
03/05/02	Sampling of air*	*Aspergillus niger*	3 cfu
		Mucor spp.	1
		Penicillium spp.	2
	Steeping tank	*Aspergillus niger*	2.0×10^3
		Aspergillus paraciticus	1.0×10^3
		Fusarium spp	2.0×10^3
		Rhizopus spp	2.0×10^3
	Fermentation trough	*Mucor spp*	1.0×10^3
		Penicillium citrinum	1.0×10^3
09/05/02	Sampling of air	*Aspergillus niger*	4
		Mucor spp	1
		Penicillium spp.	3
	Steeping tank	*Aspergillus niger*	3.0×10^3
		Fusarium spp	2.0×10^3
		Mucor spp	2.0×10^3
	Fermentation trough	*Mucor* spp	10
		Penicillium citrinum	10
		Rhizopus spp	20
15/05/02	Sampling of air	*Aspergillus niger*	7
		Aspergillus paraciticus	5
		Mucor spp	2
		Penicillium spp.	2
	Steeping tank	*Aspergillus niger*	2.4×10^3
		Fusarium spp	4.0×10^2
		Mucor spp	5.0×10^2
		Penicillium citrinum	7.0×10^2
	Fermentation trough	*Fusarium spp*	40
		Mucor spp	20
		Penicillium citrinum	60

* Exposure of Plate Count Agar plate for 5 min

Table 4. Results of monitoring Critical Control Points at the Semi-commercial Kenkey Production Plant in Accra: Visual characteristics and approximate pH of maize and intermediary products during Kenkey production.

Monitoring criterion	First batch	Second batch
Maize		
Discoloured grains	Nil	Nil
Extend of dryness	Very crispy	Very crispy
Steep water		
0 h		
pH	7	7
Clarity	Clear	Clear
Odour	Odourless	Odourless
24 h		
pH	4	4
Dough fermentation		
0 h		
Surface of dough	No visible mould growth	No visible mould growth
pH	5	5
24 h		
Surface of dough	No visible mould growth	No visible mould growth
pH	4	4
48 h		
Surface of dough	No visible mould growth	No visible mould growth
pH	>4	>4

Table 5. Results of verification of HACCP at the Semi-commercial Kenkey Production Plant in Accra: Chemical and microbiological analysis of maize, Kenkey and intermediary products during processing

Test	First batch	Second batch
Maize		
Aflatoxin	17.0 µg/kg	12.7 µg/kg
Steep water		
0 h		
pH	7.48	nd
Aerobic mesophilic bacteria (cfu/ml)	5.9×10^2	nd
Coliforms	Not detected	nd
24 h		
pH	3.96	nd
Aerobic mesophillic bacteria (cfu/ml)	1.2×10^8	nd
Coliforms	Present	nd
E. coli	Present	nd
Dough Fermentation		
0 h		
pH	4.71	4.54
Aerobic mesophilic bacteria (cfu/g)	nd	1.1×10^7
Aflatoxin	22.6µg/kg	nd
24 h		
pH	3.93	3.97
Titratable acidity (%)	1.44	1.46
Aerobic mesophilic bacteria (cfu/g)	5.6×10^7	1.7×10^6
Yeasts and moulds (cfu/g)	3.4×10^5	5.2×10^6
Coliforms	Not detected	Present
E. coli	Not detected	Not detected
Aflatoxin	30.3 µg/kg	nd
48 h		
pH	3.73	3.75
Titratable acidity (%)	1.41	1.47
Aerobic mesophilic bacteria (cfu/g)	1.4×10^8	2.6×10^8
Yeasts ans mould (cfu/g)	9.4 x 104	
Coliforms	Not detected	Not detected
E. coli	nd	0 cfu/g
Staphylococcus aureus	nd	0 cfu/g
Enterococcus	nd	0 cfu/g
Bacillus cereus	nd	100 cfu/g
Salmonella	nd	not found in 25 g
Vibro cholera	nd	not found in 25 g
Aflatoxin	36.4 ug/kg	nd
Kenkey		
pH	3.73	3.70
Aerobic mesophiles	No growth*	No growth
Yeast and moulds	No growth	No growth
Coliforms	Not detected	Not detected
E. coli	nd	0 cfu/g
Staphylococcus aureus	nd	0 cfu/g
Enterococcus	nd	0 cfu/g
Bacillus cereus	nd	0 cfu/g
Salmonella	nd	not found in 25 g
Vibro cholera	nd	not found in 25 g
Aflatoxin	20.3 ug/kg	14.5 ug/kg

nd = not determined,
*no growth on appropriate 10^{-2} agar plate