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Characterization of the dominant microorganisms responsible for the fermentation of dehulled maize grains into *nsiho* in Ghana

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***Nsiho* (white *kenkey*) is a type of *kenkey*, a sour stiff dumpling, produced from fermented maize meal in Ghana. The dominant microorganisms responsible for the fermentation of *nsiho* were characterized by analysing samples from four traditional production sites at Anum in the Eastern Region of Ghana. During 48 h of steeping dehulled maize grains, the pH values decreased from 6.05 to 5.93 to 3.59 to 3.55, whilst titratable acidity increased from 0.02 to 0.03 to 0.27 to 0.32%. In the subsequent 12 h dough fermentation, the pH decreased from 6.02 to 5.80 to 3.52 to 3.46, whilst titratable acidity increased from 0.25 to 0.27 to 0.35 to 0.38%. The lactic acid bacteria population increased by 2 to 5 log units to concentrations of 10^7 to 10^8 CFU/ml during steeping and by 2 to 3 log units from 10^5 to 10^6 CFU/g to 10^8 to 10^9 CFU/g during dough fermentation. Yeasts counts increased by 3 to 4 log units during steeping and by 2 to 4 log units during dough fermentation. The most frequently isolated lactic acid bacteria responsible for *nsiho* fermentation were identified as *Lactobacillus fermentum* (47.1%), *Lactobacillus brevis* (25%), *Lactobacillus plantarum* (14.42%), *Pediococcus pentosaceus* (8.65%) and *Pediococcus acidilactici,* (4.8%). The dominant yeasts species were *Saccharyomyces cerevisiae* (47.6%), *Candida krusei* (29.1%), *Debaryomyces* spp., (15%) and *Trichosporon* spp., (8.3%). This is the first study to report on the micororganisms involved in *nsiho* fermentation.**

Key words: *Nsiho*, dehulled maize, *kenkey*, lactic acid bacteria, indigenous African fermented foods.

INTRODUCTION

Traditional processing of maize into various fermented food products plays an important role in the food supply system of Ghana, and contributes to curtailment of post-harvest losses and national food delivery (Sefa –Dedeh,

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Abbreviations: OGYEA, Oxytetracycline-glucose yeast extract agar; **SPS**, salt peptone solution.

1993). Maize grains, by a variety of indigenous processes, are transformed into an intermediate or finished product with a stable shelf-life, improved digestibility and nutritive quality as well as desirable organoleptic properties. Processing of the grains may also include improvement of the protein quality of the product by fortification with legumes such as cowpeas and soybeans (Plahar and Leung, 1982; Nche et al., 1996; Obiri-Danso et al., 1997; Plahar et al., 1997; Sefa-Dedeh et al., 2000). One of the most common traditional products made from maize in Ghana is *kenkey*. Two main types of *kenkey* are known. *Ga kenkey* and *Fanti kenkey* have been the subject of much scientific investigation. A less common type of *kenkey* is produced from dehulled maize grains and is called *nsiho* or white *kenkey*. Production of *nsiho* involves dehulling or degerming maize grains which are then steeped in water for 48 h and milled into a meal. The meal is kneaded with water into a dough and left to ferment spontaneously for 12 to 24 h. Some producers however do not carry out dough fermentation. The dough is now pre-cooked, moulded into balls, wrapped in corn husks and steamed for 1 to 2 h. Some producers do not pre-cook the dough whilst others pre-cook part of the dough and mix it with the remaining dough before moulding and steaming into nsiho.

Production and vending of *nsiho* as well as the other types of *kenkey* is an important socio-economic activity in Ghana. This informal industry serves as a means of livelihood for numerous traditional food processors and their families. Presently, there is a pressing need to improve the informal traditional food processing sector as a whole in the advent of fast foods which could outcompete the indigenous foods. It is in this regard that *kenkey* and a lot of other indigenous African fermented foods have become subjects of intense scientific studies. Such investigations provide a basis for injection of suitable scientific and technological know-how to upgrade the traditional operations and the quality of the indigenous foods. With regards to *nsiho*, no detailed studies of its fermentation have been reported and there is very little information in the literature on the product. This work was carried out to characterize the dominant microorganisms involved in *nsiho* fermentation which could eventually lead to the development of a starter culture for its controlled fermentation during industrial production.

MATERIALS AND METHODS

Brief field study and sample collection

The brief study was carried out in three towns in the Asuogyaman District in the Eastern Region of Ghana, Anum, Senchi and Atimpoku. These towns are noted for the production of *nsiho* which is mainly produced in this part of the country. *Nsiho* producers were briefly interviewed to obtain information on their production practices and collect samples for analysis. Samples were only collected from Anum where a two stage fermentation is carried out during *nsiho* production, that is, during steeping of dehulled maize grains and fermentation of dehulled maize dough. At Senchi and Atimpoku which are next to each other, fermentation is limited to steeping as no dough fermentation is carried out. Samples were aseptically collected from four production sites at Anum on three separate occasions for laboratory analysis. The samples were taken from various stages of production. They were dehulled maize grains, steep water at 0, 24 and 48 h, dough at 0, 4, 8 and 12 h of fermentation and the final product. The samples were transported in an ice chest to the Food Research Institute, CSIR, in Accra for analysis.

Chemical analysis

The pH of steep water was determined directly using a pH meter (Radiometer pHM 92. Radiometer Analytical A/S, Bagsvaerd, Denmark) after calibration using standard buffers. The pH of fermenting dough was determined after blending with distilled water in a ratio of 1:1. Titratable acidity was determined as described by Amoa-Awua et al. (1996). 80 ml of filtrate obtained from 10 g of dough dissolved in 200 ml distilled water was titrated against 0.1 N NaOH with 1% phenolphthalein. 1 ml of 0.1 N NaOH was taken as equivalent to 9.008 x 10 *g* lactic acid.

Microbiological analysis

Enumeration of microorganisms

For all solid samples, 10 g were added to 90.0 ml sterile salt peptone solution (SPS) containing 0.1% peptone and 0.8% NaCl, with pH adjusted to 7.2 and homogenized in a stomacher (Lad Blender, Model 4001, Seward Medical), for 30 s at normal speed. From appropriate ten-fold dilutions 1 ml aliquots of each dilution were plated on the appropriate media for enumeration and isolation of microorganisms. Aerobic mesophiles were enumerated by pour plate method on plate count agar (Oxoid CM325; Oxoid Ltd., Basingstoke, Hampshire, UK). Plates were incubated at 30°C for 72 h in accordance with the NMKL., No. 86 (2006). Lactic acid bacteria were enumerated by pour plate on deMan, Rogosa and Sharpe Agar (MRS, Oxoid CM361), pH 6.2, containing 0.1% cycloheximide to inhibit yeast growth and incubated anaerobically in an anaerobic jar with anaerocult A at 30°C for five days. Yeasts and moulds were enumerated by pour plate on oxytetracycline-glucose yeast extract agar (OGYEA), (Oxoid CM545) containing OGYEA supplement with pH adjusted to 7.0 and incubated at 25°C for 3 to 5 d in accordance with ISO No 21527-1 (2008).

Isolation and identification of lactic acid bacteria

About 20 colonies of LAB were selected from a segment of the highest dilution or suitable MRS plate and purified by plating repeatedly. The colonies were tested for Gram catalase and oxidase reaction and observed under phase contrast microscope. The colonies were tested for their ability to grow at different temperatures by inoculating them into MRS broth and incubating at either 10°C or 45°C for 72 to 96 h to observe growth as visual turbidity in the broth. Isolates were tested for growth at different pH in MRS broth (Oxoid CM359) with pH adjusted to 4.4 or 9.6 incubation at 30°C for 72 and observing for growth as visual turbidity in the broth. Isolates were tested for salt tolerance in MRS broth (Oxoid CM359) containing 6.5 and 18% (w/v) NaCl incubated

at 30°C for 5 days and observing for growth as visual turbidity. Isolates were tested for gas production from glucose in MRS basal medium to which glucose had been added. The medium was composed of peptone 10 g, yeast extract 5 g, tween 80 1 ml, di-potassium hydrogen phosphate 2 g, sodium acetate 5 g, tri-ammonium citrate 2 g, $MgSO_4.7H_2O$ 0.2 g, $MnSO_4.4H_2O$ 0.05 g, 1 L distilled water, pH 6.5, but without glucose or meat extract. The basal medium was dispensed in 5 ml amounts into test tubes containing inverted Durham tubes and sterilized by autoclaving at 121°C for 15 min. Glucose was prepared as 10% solution and sterilized by filtration and added aseptically to the basal medium to give a final concentration of 2%. The inoculated tubes were examined for production of gas after 3 d incubation at 30°C. Isolates were tentatively identified by determining their pattern of carbohydrate fermentation using the API 50 CHL kit (BioMérieux, Marcy-l'Etoile, France) and comparing them to the API database.

Isolation and identification of yeasts

All colonies totaling 15 from a segment of the highest dilution or suitable OGYEA plate were selected and purified by successive sub-culturing in Malt Extract Broth (Oxoid CM57) and streaked repeatedly on OGYEA until pure colonies were obtained. The colonies were identified by carbohydrate fermentation and utilization patterns using ID 32 C kit (BioMérieux, Marcy-l'Etoile, France).

RESULTS

Nsiho production at Senchi, Atimpoku and Anum

The brief field study confirmed *nsiho* or *white-kenkey* to be the most common type of *kenkey* produced in the Asuogyaman District in the Eastern Region of Ghana. It also showed that Senchi, Atimpoku and Anum were the most important towns in the production of *nsiho*. A previous extensive survey involving the current authors had shown *Ga-* and *Fanti-kenkey* to be the most common types of *kenkey* in Ghana, with *nsiho* being less common and confined to a few parts of the country (Obodai et al., 2014). All the *nsiho* producers interviewed in the present work were women and most of them had little formal education. They were engaged in *nsiho* production or vending as a family business in home-based operations with skills acquired within the family. A production unit usually involved 3 or 4 women who produced between 5 and 10 kg of *nsiho* per batch. The producers did not have any equipment of their own and used large utensils including plastic drums for their manual operations. For the mechanized operations, that is, dehulling and milling of maize grains, they used customer service mills available in the neighbourhood. *Nsiho* production was the main source of employment for the families concerned and it was considered a profitable business. Two different methods (variations) were observed for the production of *nsiho* as shown in Figure 1. Maize grains are cleaned by winnowing and sorting to remove, chaff, dust, stones and other debris. The cleaned maize is dehulled in a mill and steeped in water for 48 h. The steeped grains are then milled in a plate mill into a meal. At Senchi and Atimpoku, the meal is pre-cooked for about 30 to 60 min into a thick gelatinous paste, *ohu,* which is then moulded into balls and wrapped in clean maize husks. The balls are packed into a pot lined with sticks and maize husks and containing a small amount of water. The balls are then steamed for 1 to 2 h into *nsiho*. At Anum, however, the meal is kneaded with a little water into stiff dough and fermented spontaneously for 6 to 12 h. Two-thirds of the dough is pre-cooked for about 30 to 60 min into *ohu* and mixed with the remaining uncooked dough. The mixture is then moulded into balls and wrapped in clean maize husks. The balls are packed into a perforated pan and placed over a pot of boiling water and steamed for 1 to 2 h.

Changes in pH and titratable acidity during steeping and dough fermentation

The results of pH and titratable acidity of steep water and fermenting dough from the four production sites at Anum are shown in Table 1. The pH values during 48 h of steeping decreased from 6.05 to 5.93 at the start of steeping to 3.59 to 3.55 by the end of steeping. During dough fermentation, the pH decreased from 6.02 to 5.80 for the freshly prepared dough to 3.52 to 3.46 at the end of the fermentation. The most pronounced drop in pH occurred within the first 24 h of steeping and in the dough between 4 and 8 h of fermentation. Percentage titratable acidity increased during steeping from 0.02 to 0.03 to 0.27 to 0.32% after 48 h of steeping. Similar results were observed for dough fermentation with titratable acidity changing from 0.25 to 0.27 to 0.35 to 0.38% at the end of the process. The drop in pH values and the corresponding increases in percentage titratable acidity during both steeping and dough fermentation indicate the occurrence of lactic acid fermentation, as has been reported during the production of *Ga* and *Fanti kenkey* from whole maize grains (Halm et al., 1993, 1996, 2004; Obiri-Danso et al., 1997; Amoa-Awua et al., 1998; 2006).

Changes in microbial population during steeping and dough fermentation

The population of aerobic mesophiles recorded during steeping and dough fermentation at the four production sites at Anum are shown in Table 2. The values represent mean counts for samples taken on three separate occasions. The counts were at concentrations of 10^4 to 10^6 CFU/ml at the start of steeping. These counts increased by 2 to 4 log units within the first 24 h to 10^8 CFU/ml at all four production sites. Between 24 and 48 h of steeping the concentration of aerobic mesophiles

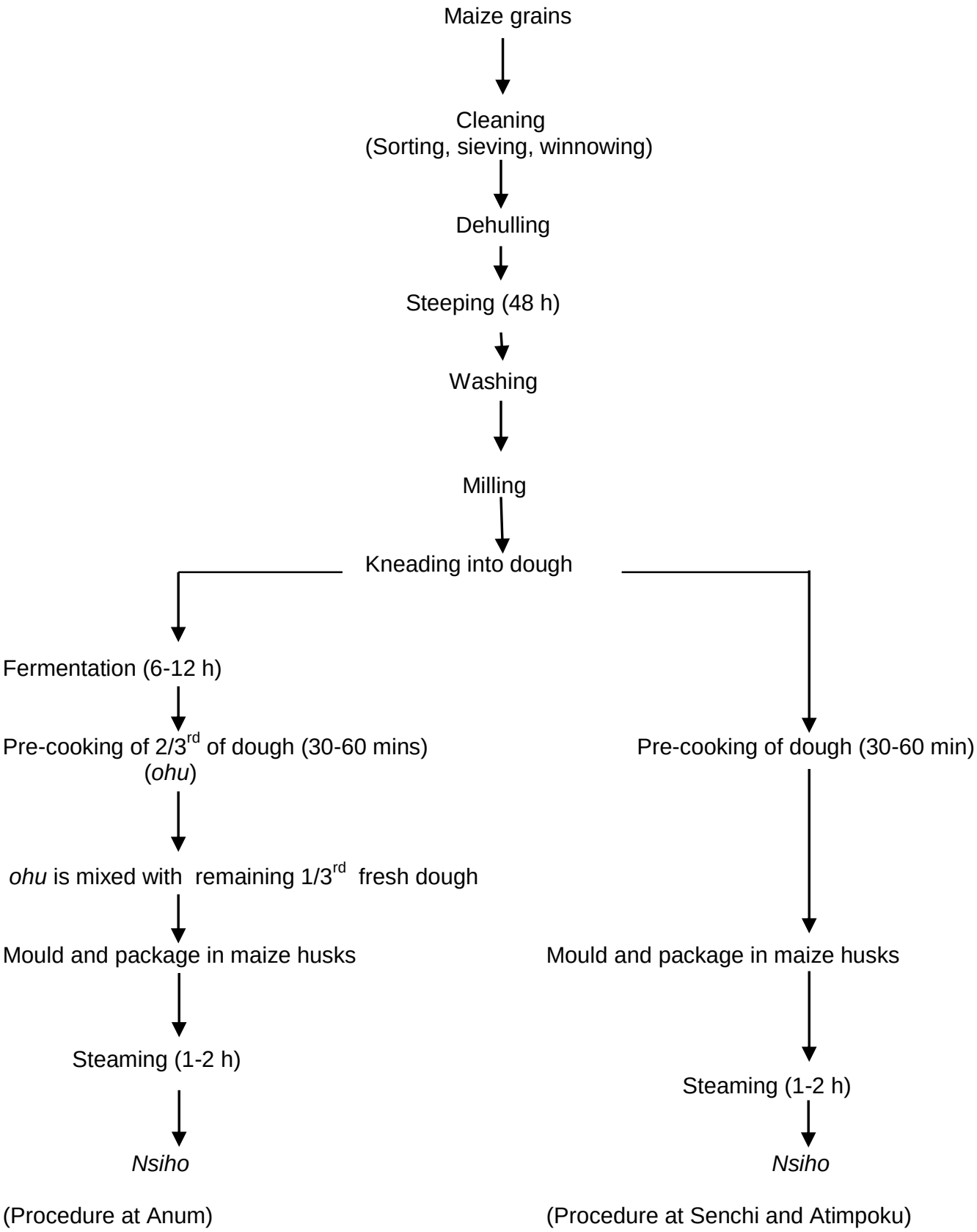

Figure 1. Flow diagram of the production of *Nsiho* (white-*kenkey*).

Table 1. Changes in pH and percentage titratable acidity during the fermentation of dehulled maize grains into *nsiho* (white *kenkey*) at Anum.

Sample	Production site 1	Production site 2	Production site 3	Production site 4
pH				
Steep water (h)				
0	5.98 ± 0.05	6.00 ± 0.03	6.05 ± 0.03	5.93 ± 0.01
24	4.01 ± 0.03	4.00 ± 0.01	3.81 ± 0.10	3.90 ± 0.01
48	3.59 ± 0.04	3.59 ± 0.03	3.57 ± 0.03	3.55 ± 0.02
Fermenting dough (h)				
0	5.98 ± 0.02	6.02 ± 0.01	5.98 ± 0.01	5.80 ± 0.02
4	5.44 ± 0.01	5.51 ± 0.01	5.51 ± 0.06	5.46 ± 0.01
8	3.55 ± 0.01	3.58 ± 0.03	3.76 ± 0.03	3.57 ± 0.04
12	3.51 ± 0.02	3.52 ± 0.04	3.49 ± 0.01	3.46 ± 0.01
Percentage titratable acidity				
Steep water (h)				
0	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
24	0.25 ± 0.01	0.25 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
48	0.27 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.32 ± 0.01
Dough fermentation (h)				
0	0.27 ± 0.03	0.25 ± 0.02	0.26 ± 0.01	0.27 ± 0.04
4	0.28 ± 0.01	0.26 ± 0.03	0.28 ± 0.01	0.28 ± 0.03
8	0.31 ± 0.03	0.29 ± 0.03	0.31 ± 0.02	0.32 ± 0.05
12	0.36 ± 0.01	0.35 ± 0.04	0.35 ± 0.01	0.38 ± 0.01

Table 2. Changes in the population of aerobic mesophiles in CFU/ml or g during the fermentation of dehulled maize grains into *nsiho* (white *kenkey*) at Anum.

Sample	Production site 1	Production site 2	Production site 3	Production site 4
Steep water (h)				
0	$(3.4 \pm 1.6)\ 10^5$	$(4.5 \pm 0.3)\ 10^4$	$(1.1 \pm 0.2)\ 10^6$	$(2.0 \pm 1.2)\ 10^6$
24	$(5.5 \pm 2.4)\ 10^8$	$(4.6 \pm 1.7)\ 10^8$	$(1.0 \pm 0.2)\ 10^8$	$(3.0 \pm 0.4)\ 10^8$
48	$(6.1 \pm 1.3)\ 10^8$	$(4.8 \pm 1.6)\ 10^8$	$(3.9 \pm 0.9)\ 10^8$	$(9.1 \pm 0.4)\ 10^8$
Fermenting dough (h)				
0	$(1.7 \pm 0.6)\ 10^6$	$(3.0 \pm 1.4)\ 10^6$	$(5.1 \pm 0.4)\ 10^7$	$(2.2 \pm 1.5)\ 10^6$
4	$(7.6 \pm 0.8)\ 10^6$	$(7.6 \pm 0.8)\ 10^6$	$(1.1 \pm 0.2)\ 10^8$	$(2.2 \pm 0.6)\ 10^7$
8	$(2.1 \pm 0.6)\ 10^7$	$(7.8 \pm 0.7)\ 10^7$	$(8.6 \pm 0.4)\ 10^8$	$(4.8 \pm 0.6)\ 10^8$
12	$(4.9 \pm 0.9)\ 10^8$	$(5.6 \pm 0.5)\ 10^8$	$(1.0 \pm 0.5)\ 10^9$	$(8.7 \pm 1.0)\ 10^8$

remained at 10^8 CFU/ml at all sites. For dough fermentation the aerobic mesophilic counts increased steadily over the 12 h of fermentation by 2 log units from 10^6 to 10^8 CFU/g at production sites 1, 2 and 4, and from 10^7 to 10^9 CFU/ml at production site 3 (Table 2). The aerobic mesophiles consisted of Gram positive catalase-negative rods and cocci, Gram positive catalase positive cocci and Gram negative bacteria. These isolates were not characterized any further because it was assumed that they played no role in *nsiho* fermentation as reported for *kenkey* by Halm et al. (1993) and Olsen et al. (1995). Lactic acid bacteria were enumerated as Gram positive catalase negative rods, coccoids and cocci on MRS. They were present at the start of steeping at a mean concentration of 10^5 CFU/ml at production site 1, 10^4 CFU/ml at production sites 2 and 3, and at 10^5 CFU/ml at

Table 3. Changes in the population of lactic acid bacteria in CFU/ml or g during the fermentation of dehulled maize grains into *nsiho* (white *kenkey*) at Anum.

Sample	Production site 1	Production site 2	Production site 3	Production site 4
Steep water (h)				
0	$(1.1 \pm 0.1)\ 10^5$	$(5.1 \pm 0.6)\ 10^4$	$(7.2 \pm 0.4)\ 10^4$	$(1.7 \pm 0.8)\ 10^3$
24	$(5.4 \pm 1.0)\ 10^8$	$(2.2 \pm 0.4)\ 10^8$	$(2.8 \pm 1.3)\ 10^8$	$(7.7 \pm 0.7)\ 10^6$
48	$(1.8 \pm 0.4)\ 10^7$	$(4.1 \pm 0.8)\ 10^7$	$(8.8 \pm 0.9)\ 10^7$	$(3.2 \pm 0.9)\ 10^8$
Fermenting dough (h)				
0	$(1.0 \pm 0.1)\ 10^5$	$(2.3 \pm 1.3)\ 10^6$	$(2.8 \pm 1.2)\ 10^6$	$(2.1 \pm 1.1)\ 10^6$
4	$(6.7 \pm 0.6)\ 10^6$	$(3.1 \pm 0.9)\ 10^6$	$(3.2 \pm 2.0)\ 10^7$	$(2.5 \pm 1.3)\ 10^7$
8	$(3.0 \pm 0.6)\ 10^7$	$(1.6 \pm 0.1)\ 10^7$	$(2.1 \pm 1.5)\ 10^9$	$(5.0 \pm 0.9)\ 10^7$
12	$(6.3 \pm 1.2)\ 10^8$	$(5.1 \pm 1.5)\ 10^8$	$(2.4 \pm 0.7)\ 10^9$	$(2.6 \pm 2.1)\ 10^8$

Table 4. Changes in yeast population in CFU/ml or g during the fermentation of dehulled maize grains into *nsiho* (white *kenkey*).

Sample	Production site 1	Production site 2	Production site 3	Production site 4
Steep water (h)				
0	$(4.1 \pm 0.5)10^2$	$(2.8 \pm 0.6)\ 10^2$	$(1.6 \pm 0.8)\ 10^2$	$(7.5 \pm 0.6)\ 10^2$
24	$(4.9 \pm 0.3)\ 10^4$	$(6.9 \pm 0.4)\ 10^4$	$(1.9 \pm 1.1)\ 10^5$	$(9.7 \pm 0.8)\ 10^4$
48	$(1.8 \pm 0.5)\ 10^5$	$(9.8 \pm 0.1)\ 10^4$	$(9.1 \pm 0.4)\ 10^5$	$(1.9 \pm 0.9)\ 10^6$
Fermenting dough (h)				
0	$(5.7 \pm 0.8)\ 10^3$	$(5.9 \pm 0.1)\ 10^4$	$(7.8 \pm 1.1)\ 10^4$	$(9.1 \pm 0.7)\ 10^3$
4	$(2.5 \pm 0.6)\ 10^5$	$(4.9 \pm 0.5)\ 10^5$	$(1.8 \pm 0.1)\ 10^5$	$(5.9 \pm 0.5)\ 10^4$
8	$(6.0 \pm 0.3)\ 10^5$	$(1.0 \pm 0.1)\ 10^6$	$(1.0 \pm 0.4)\ 10^6$	$(4.0 \pm 0.6)\ 10^5$
12	$(1.8 \pm 0.7)\ 10^7$	$(7.8 \pm 0.6)\ 10^7$	$(2.7 \pm 0.6)\ 10^6$	$(7.8 \pm 0.5)\ 10^6$

production site 4 (Table 3). These are also mean values for samples taken on three separate occasions. At 24 h, the concentrations were at 10^8 CFU/ml at production sites 1, 2 and 3 but at 10^6 CFU/ml at production site 4. By the end of steeping at 48 h a 1 log unit drop in concentration was recorded at production sites 1, 2 and 3 to 10^7 CFU/ml, whilst a 2 log unit increase occurred at production site 4 to 10^8 CFU/ml. In the 12 h dough fermentation, LAB counts increased by 2 log units from 10^6 to 0^8 CFU/g at production sites 2 and 4 and by 3 log units at production site 1 (10^5 to 10^8 CFU/g) and production site 3 (10^6 to 10^9 CFU/g).

Yeasts counts at the start of steeping at all production sites were at concentrations of 10^2 CFU/ml representing mean values for sampling on the three separate occasions (Table 4). Within 24 h, the counts increased by 2 log units at production sites 1, 2 and 4, and by 3 log units at production site 3. At 48 h no further increase was recorded at production sites 2 and 3, whilst an increase by 1 log unit was recorded at production site 1 and by 2 log units at production site 4. During 12 h dough fermentation yeast count increased by 4 log units from 10^3 and 10^4 CFU/g at production sites 1 and 2, respectively. At production site 3 the yeast population increased from 10^4 to 10^6 CFU/g and at production site 4 from 10^3 to 10^6 CFU/g respectively.

Tentative identification of lactic acid bacteria and yeasts

Only lactic acid bacteria and yeasts were tentatively identified in the present work because they have consistently been shown to be responsible for the fermentation of *kenkey* (Halm et al., 1993; Hayford and Jespersen, 1999; Hayford and Jakobsen, 1999; Hayford et al., 1999). A total number of 208 LAB were isolated from steep water and fermenting dough samples. They were isolated as Gram positive catalase negative rods, cocobacilli or cocci on MRS. They were assumed to be lactic acid bacteria belonging to the genera *Lactobacillus* and *Lactococci*. After grouping based on biochemical characterization, the most frequently occurring species was found to account for 47.1% of the total number of

LAB isolates. They were heterofermentative based on their ability to produce CO_2 from glucose. They also grew at pH 4.4 and 9.6 and at 45°C, but not at 10°C nor in 18% NaCl. They mostly fermented galactose, D-glucose, D-fructose, D-mannose, ribose, melibiose, saccharose, gluconate, maltose, D-raffinose, 5-ketogluconate, D-xylose, lactose, cellobiose, esculin, trehalose, β-gentobiose, salin, amygdalin, l-arabinose, galactose and mannitol in the API 50 CHL galleries. Based on this pattern of carbohydrate fermentation they were tentatively identified as *Lactobacillus fermentum.*

The second most frequently occurring species accounted for 25% of the isolates. They were very short rods or cocobacilli and grew at pH 4.4 and 9.6 and at 45°C but not at 10°C and 18% NaCl. They were able to ferment L-arabionose, ribose, D-xylose, galactose, D-glucose, D-frucrose, amygdaline, maltose, melibiose, saccharose, gluconate and 2 keto-gluconate but did not utilize glycerol, erythritol, sorbose, or rhamnose. They were identified as *Lacobacillus brevis.*

The third most dominant species which accounted for 14.42% of the LAB isolates were rods which were identified as *Lactobaccillus plantarum.* This was because they grew at pH 4.4 and 9.6, but not in 6.5 and 18% NaCl. They were also able to ferment arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, D-turanose, mannitol, esculin, salicin, sorbitol, maltose, lactose, cellobiose and gluconate.

Other LAB species which were cocci and appeared as tetrads were identified based on their carbohydrate fermentation profiles as *Pediococcus pentosaceus* (8.65%) and *Pediococcus acidilactici (*4.81%). *P. pentosaceus* isolates mainly fermented L-arabinose, ribose, galactose, D-xylose, D- fructose, D-glucose, D-manose, salicin, cellobiose, esculin, lactose, mellibiose, saccharose and β-gentobiose. *P. acidilactici* isolates mainly fermented ribose, D-xylose, L-xylose, D- fructose, D-glucose, D-manose but not mellibiose and sacchrarose.

A total of 185 yeasts were isolated from steep water and fermenting dough samples from the four production sites. The isolates were characterized by colony and cell morphology as well as by their pattern of carbohydrate fermentation and utilization in ID 32C galleries. The most frequently occurring species accounted for 47.6% of all the yeast isolates. They utilized galactose, glucose, sucrose, raffinose, maltose, DL-lactate, trehalose, α-metyl-D-glucoside, melibiose but could not assimilate lactose. They were identified as *Saccharomyces cerevisiae*. The second dominant yeast which made up 29.1% of the yeasts, utilized glucose, N-acetyl-glucosamide and DL-lactate out of the 32 carbohydrate tested. They were identified as *Candida krusei.* The third species constituted 15% of all the total yeast isolates and utilized D-melizitose, D-melibiose D-glucose. They were identified as *Debaryomyces* spp. and *Trichosporon* spp., was the least frequently isolated yeast (8.3%). It utilized only D-melibiose and D-glucose. *Debaryomyces* spp. and *Trichosporon* spp occurred mainly at the initial stages of steeping, whilst the others occurred at all the various stages of processing.

DISCUSSION

Souring of *nsiho*

In this work samples were only collected for analysis from *nsiho* production sites at Anum. In the process at Anum fermentation occurs at two different stages, during steeping and during dough fermentation. At Senchi and Atimpoku fermentation occurs only during steeping since no dough fermentation is carried out. Therefore, by studying the process at Anum information was obtained on both steeping and dough fermentation. The current study has shown that a steady increase in titratable acidity with a corresponding decrease in pH occurred during steeping of dehulled maize grains and *nsiho* dough fermentation. This was expected since previous studies have shown that other types of kenkey, notably *Ga*- and *Fanti-kenkey*, undergo lactic acid fermentation (Halm et al., 1993; Obiri-Danso et al., 1997; Hayford and Jakobsen, 1999). LAB counts in the present work increased during both steeping and dough fermentation and was responsible for the reduction in pH and increase in titratable acidity. Homofermentative lactic acid bacteria metabolize glucose to lactic acid by the Embden Meyerhof pathway. Heterofermentative lactic acid bacteria on the other hand metabolize glucose through the phosphoketolase pathways. This yields lactic acid and acetic acid if the bifidus pathway is used or lactic acid, acetic acid, ethanol and CO_2 through the 6P-gluconate pathway (Kandler, 1983). Several authors have reported a decrease in pH and an increase in titratable acidity during steeping of whole maize grains and fermentation of maize dough in *kenkey* production. According to Plahar and Leug (1982) the main carboxylic acids produced in maize dough fermentation are D+L-lactic acid and acetic acid in concentrations of 0.8 to 1.4% and 0.1 to 0.16%, respectively. Other acids produced are propionic and butyric acids with values of 30 and 40 mg/kg (Plahar and Leung, 1982; Halm et al., 1993; 1996; 2004). The present work has shown that dehulling or degerming maize grains does not change the trends in acidification or souring of *kenkey* during production.

Role of lactic acid bacteria in *nsiho* fermentation

Wide variations, 2 to 5 log unit increases, were recorded in the lactic acid bacteria population during steeping at

the four different production sites at Anum. This could be attributed to wide variations in the lactic acid bacteria population at the start of steeping, that is, mean counts of 10^5, 10^4, 10^4, 10^3 CFU/ml at the different production sites. This initial LAB population was dependent on the conditions at the different production sites. The important factors included the population of LAB on the dehulled grains, on utensils and containers used, in the steeping tanks, etc. These are the sources of LAB for the spontaneous fermentation of the grains during steeping. By the end of steeping, there were very little differences in the mean LAB population at the different production sites; 10^7 and 10^8 CFU/ml. In steeping of whole maize grains in *kenkey* production Halm et al. (1993) recorded a LAB population of 1.7×10^8 CFU/ml at the end of steeping from the initial concentration of 8.2×10^6 CFU/ml. Increases in LAB population during 12 h of dough fermentation were by 2 and 3 log units at the different production sites. The LAB population as well as titratable acidity at the start of dough fermentation was lower than at the end of steeping which was the first fermentation stage. This could be attributed to loss of cells and acids in the steep water which was decanted off and also addition of water (dilution) to the milled meal to form the dough. This will also explain the changes in pH at these stages. The LAB counts at the end of 12 h dough fermentation were between 10^8 and 10^9 CFU/g. In whole maize dough fermentation Halm et al. (1993) reported LAB population of 10^9 CFU/g at the end of fermentation. The dominant lactic acid bacteria identified in the present work to be responsible for *nsiho* fermentation was *L. fermentum* which accounted for nearly half of the lactic acid bacteria population. This result is in agreement with the work of Halm et al. (1993) who found fermentation of whole maize meal in *Ga*- and *Fanti- kenkey* production to be dominated by a group of obligately heterofermentative lactobacilli consistent with *L. fermentum* and *Lactobacillis reuteri* in their patterns of carbohydrate fermentation. Hayford et al. (1999) later confirmed the dominant species to be *L. fermentum* using molecular characterization. It is therefore not surprising that *L. fermentum* has been found in the present work to be responsible for the fermentation of *nsiho*, a different type of *kenkey*. This study therefore shows that polishing of maize grains by removal of the testa and germ has little effect on the composition of the LAB which ferments the cereal. In Benin, Hounhouigan et al. (1993) also reported *L. fermentum* to be the dominant lactic acid bacteria responsible for the fermentation of maize into *mawe* which involves fermentation of partially dehulled maize grains.

In this study, *L. plantarum* and *L. brevis* were also isolated in high numbers during steeping and dough fermentation. The presence of *L. plantarum* in maize dough fermentation has been reported. Nche et al. (1996) identified *L. plantarum, L. brevis, Lactobacillus confuses* and *Pediococcus* species as the main lactic acid bacteria present in fermenting maize and maize cowpea dough. Olasupo et al. (1997) in their studies on selected African fermented foods obtained 48 lactobacillus isolates from *kenkey* which they identified as *L. plantarum, L. fermentum, L. brevis, Lactobacillus delbruckii* and *L. acidophilus*. Olsen et al. (1995) found *L. plantarum* at the initial stage of *kenkey* fermentation where it dominated the heterofermentative lactic acid bacteria present. In whole maize kenkey production, Olsen et al. (1995) showed that about half of all *L. plantarum* and practically all *L. fermentum* isolates inhibited all other Gram positive and Gram negative bacteria and explained the elimination of these organisms during the initial stages of *kenkey* production.

The presence of *P. pentosaceus* and *P. acidilactici* which were identified in the lactic acid bacteria composition in *nisho* in the current work have also been reported in kenkey by Halm et al. (1993). Their presence can be linked to production of propionic acid which both Plahar and Leung (1982) and Halm et al. (1993) have reported to be one of the main organic acids present in *kenkey*. These organisms may also ferment lactic acid and do so as a primary end-product of CHO catabolism.

Role of yeasts in *nsiho* fermentation

The dominant yeasts identified in the current work to be involved in the *nsiho* fermentation were *S. cerevisiae* and *C. krusei*. The other yeasts species which occurred only at the initial stages of steeping were *Debaryomyces* and *Trichosporon* species. In whole maize *kenkey* production, Hayford and Jesperson (1999) and Hayford and Jakobsen (1999) confirmed the dominant yeast species during steeping and dough fermentation to be *S. cerevisiae* and *C. krusei* by molecular methods. Obiri-Danso et al. (1997) had previously reported *S. cerevisiae* and *C. krusei* as the yeasts species involved in *kenkey* fermentation. Jespersen et al. (1994) isolated *S. cerevisiae* and *C. krusei* as the dominant yeast in maize dough fermentation and suggested that since yeast are known to produce a wide range of aromatic compounds including organic acids, esters, aldehydes, alcohols, lactones and terpenes, they are likely to influence the organoleptic and structural quality of fermented maize dough. Jespersen et al. (1994) also identified *Debaryomyces* and *Trichosporon* species in the yeast population during *kenkey* production. The present work has shown that *L. fermentum* and *S. cerevisiae* are the predominant microbial species responsible for the fermentation of dehulled maize grains into *nsiho*. They accounted for nearly half of the lactic acid bacteria and yeast populations. Thus, the same predominant organisms responsible for the fermentation of whole maize grains into *Ga*- and Fante-*kenkey* are also respon-

sible for fermentation of dehulled maize grains into *nsiho*. In 1996, Halm et al. successfully developed and tested a mixed starter culture containing a strain each of *L. fermentum* and *S. cerevisiae* for the production of *Ga* and *Fanti kenkey*.

Conclusion

The fermentation of dehulled maize grains in *nsiho* production is similar in character to fermentation of whole maize grains in *Ga* and *Fanti-kenkey* production. This is with respect to the microbiological and biochemical changes which take place during fermentation. In *nsiho* production, lactic acid fermentation occurs during both steeping of maize grains and dough fermentation. This results in a sour product with a low pH and high percentage titratable acidity. The lactic acid population is dominated by *L. fermentum* and also includes *P. pentosaceus*, *P. acidilactici, L. plantarum* and *L. brevis*. Yeasts are also involved in these fermentations with *S. cerevisiae* and *C. krusei* being dominant.

Conflict of interests

The authors have not declared any conflict of interests.

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