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Production of Kenkey (A Ghanaian Starch-Based Food) Using Starter Cultures

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Abstract: The use of starters in food fermentation had been reported to prevent problems associated with spontaneously fermented indigenous food products such as poor microbial stability, variation in product quality, longer period of fermentation and undesirable sensory attributes. Studies were carried out to investigate the effect of single and combined starter on the fermentation of maize for Kenkey production. The results obtained revealed that the Kenkey sample produced using mixed starters of *L. plantarum* and *C. krusei* was the best in terms of nutritional quality, consumers' acceptability and prolonged shelf life. The result of the nutritional analysis showed the Kenkey sample produced using combined starters of *L. plantarum* and *C. krusei* contained the highest protein content (10.32±0.03), moderate ash content (0.69±0.01), least carbohydrate content (74.89±0.05) and fibre content (2.16±0.02). It also showed that the antinutritional factors were least in the sample produced using combined starters of *L. plantarum* and *C. krusei* with the oxalate, phytate and polyphenol content recording 5.00±0.01, 7.00±0.04 and 0.20±0.01, respectively. However, the elemental investigation depicted the highest calcium, iron and phosphate ions at 130.00±0.05, 5.00± 0.01 and 90.00±0.01, respectively in Kenkey samples produced with combined starters of *L. plantarum* and *C. krusei*.

Key words: Starter cultures, Kenkey, *L. plantarum*, *C. krusei*, spontaneous fermentation

INTRODUCTION

Cereal grains constitute a major source of dietary nutrients all over the world despite the deficiency in some essential amino acid such as methionine, cytosine and phenylalanine (Blandino *et al*., 2003). A large proportion of cereals produced in the world are processed through a simple and economical fermentation technology prior to consumption. This technology had been reported to improve the nutritional quality, sensory attributes coupled with functional properties of food. The application of dry and wet milling techniques in maize processing enhances a relatively large number of intermediary products such as maize grits of different particle sizes, maize meal, maize flour and flaking girts which are useful industrially. In developed countries, >6% of maize produced is used to compound feed for livestock apart from being consumed by humans (Blandino *et al*., 2003) in Africa, the cereal include maize (*Zea may*), sorghum (*Sorghum vulgare*), rice (*Oryza sativa*), pearl millet (*Pennisetum glacicum*) and Finger millet (*Eleusine coracoma*) (Nout, 2009). The cereals serve as substrate for the production of some starchy based fermented food in Africa such as bouza and kishk from barley; busa from rice; ogi and kenky from maize; kunnuzarki from millet; pito, ogi, bogbe kusra, burukutu, injera from sorghum (FAO, 1992).

Indigenously fermented foods prepared from maize are common in many parts of Africa. Some are used as beverages and breakfast or snack fast or snack food while a few is consumed as staples and weaning foods. For example ogi is produced by fermented maize grains which is popular breakfast gruel and a complementary food for young children in several West African countries (Akinrele, 1970; Odunfa, 1985; Adeyemi, 1993). Maize also serves as a substrate in the production of Pozol and Atole in South Western Mexico (Blandino *et al*., 2003), Mawe in Benin Republic (Hounhouigan *et al*., 1993) Banku, Koko and Kenkey in Ghana (Owusu-Ansah *et al*., 1980); Mutwina in Zimbabwe; Rabdi in Indian and Uji in Kenya, Uganda and Tanganyika.

Kenkey an indigenously fermented starchy food is commonly produced in Ghana especially by the Gas in the Greater Accra Region and the Fantis in the Central and Western regions. In Ghana, annual maize production is currently estimated as 1,034,200 metric tons accounting for 3% of the Agricultural Gross Domestic Product

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(AGDP) and the bulk of this quantity is consumed in form of Kenkey which contains nutritionally 9.4 g protein, 4.2 g fat, 73.6 g carbohydrate, 1.9 g fibre per 100 g grain (Nout, 2009). It is consumed as a main meal served with fried or grilled fish and an accompanying sauce or soup. It forms a popular source of food amongst the low-income workers who may eat it as breakfast, lunch or dinner. Kenkey is a heavy meal because of its bulkiness so when taken as breakfast it sustains through dinner thus making life economical for them (Halm *et al*., 2004). The traditionally production of Kenkey is by spontaneous fermentation which involve stages such as soaking, milling, fermentation, gelatinization, mixing, kneading, wrapping in leaves and boiling which are completed in 6 days (Sefa-Dedeh and Plange, 1988; Nche *et al*., 1996; Annan *et al*., 2003). The microorganisms involved in the natural fermentation of maize for Kenkey are responsible for the formation of aroma, sourness, microbial safety and flavor (Akinrele, 1970; Odunfa and Adeyele, 1985; Teniola and Odunfa, 2002).

The activity of yeast and moulds in indigenously fermented African foods and beverages are well documented. Jespersen (2003) reported that during Kenkey production, the occurrence of yeast was very high and contributes immensely to organoleptic and structural quality of the Kenkey dough. All fermented indigenous foods in Africa were produced through spontaneous fermentation process. The product of this process is associated with problems such as short shelf life, increased hygienic risks, undesirable sensory attributes, variations in product quality from one place to the other and relatively long duration of production (Holzapfel, 2002). This research is therefore intended to investigate the selection of starter cultures on the quality status and consumers acceptability of Kenkey.

MATERIALS AND METHODS

Sample collection: Dried white maize grains (*Zea may*) were purchased from Bodija Central market in Ibadan, Oyo State, Nigeria and brought into the laboratory in clean sterile polythene nylons. The defective grains and foreign materials were removed and the remaining were washed with sterile distilled water and used for this study.

Traditional production of Kenkey: Kenkey was produced according to the modified method of Nyarko (1977) 200 g of maize grains was weighed and washed thoroughly with clean water and transferred to 1 L conical flask containing 600 mL of distilled water (Ratio 1:3 w/v) and steeped for 24 h. The steeped water was decanted and the maize grains washed properly and wet milled. The wet milled grains were allowed to ferment for 72 h at 28±2°C. The fermented dough was divided into equal halves and one half of the dough was boiled for 1 h to produce the aflata which was mixed with the other half (unboiled fermented dough) and both were wrapped in plantain leaves for 3 h to obtain Kenkey.

Isolation procedure: Isolation of microorganisms from spontaneously fermented maize grains during traditional production of Kenkey was carried out at 24 h intervals for 72 h using the following media: De Mann Rogosa Sharpe (MRS) agar at pH 5.5 (Supplemented with cycloheximide) for lactic acid bacteria, Mannitol Salt Agar (MSA) for *Staphylococcus* sp., Yeast Extract Agar (YEA) (Supplemented with 2% Streptomycin sulphate) for yeast and fungal mould, Macconkey Agar (MA) for *Coliform or Enterobacter* sp. and Plate Count Agar (PCA) for viable counts. Incubation for yeast species and fungal mould were carried out at 30°C for 5-7 days while other incubations were carried out at 37°C for 48 h.

Purification process: The isolates observed on plates were sub-cultured by repeated streaking until pure colonies were obtained which were maintained on agar slopes in different McCartney bottles and stored in the refrigerator at 4°C.

Identification of microorganisms: Identification of pure microbial isolates was carried out as described (Mulyowidarso *et al*., 1991a, b). Systematic morphological and biochemical tests were investigated as stated by (Cowan and Steel, 1970) to classify the bacterial isolates into genera while identification of bacterial isolates to species was achieved as described by Colins and Lyne (1984).

With reference to Bergey's Manual of Systemic Bacteriology (Bergey *et al*., 1984; Sneath *et al*., 1986). The identities of the yeast isolates were carried out based on morphological, sporulation and biochemical tests reported by Kreger-van Rij (1984). The final identities of the *Lactobacillus* and *Staphylococcus* sp. were confirmed using API 50CH test galleries (API System, SA La Balme les Grotter, France) and API 20EC galleries were used to confirm the identities of species of the Enterobacteriaceae while the fungal isolates (mould) were identified as described by Samson *et al*. (1987) and Pitt and Holdings (1985).

Selection of starter cultures: The selection of starter culture used in the laboratory production of Kenkey was based on parameter such as percentage occurrence of the organisms, ability to produce amylase and antimicrobial substances.

Determination of amylolytic activity of the isolates: This was estimated on the basis of reduction in blue colour intensity resulting from enzymatic hydrolysis of starch (Bajpai and Bajpai, 1989).

Growth at different temperatures by the isolates: Growth of the isolates at different temperatures was monitored using the methods described by Conway (1996).

Production of Antimicrobial substances by the isolates: The antimicrobial substances such as lactic acid, diacetyl and hydrogen peroxide produced by the different isolates were quantitatively estimated using the methods of AOAC (1990) as described by Fadahunsi. Based on the parameter earlier stated, *Lactobacillus plantarum* and *Candida krusei* were selected and used in this study.

Preparation of inocula for fermentation study: The Bacteria and yeast isolates selected for this study or for the fermentation of the maize grains were grown in MRS broth (oxoid) and malt extract broth (oxoid) in different Erlenmeyer flasks, respectively. Incubation was carried out at 30°C and 150 rpm for 24 h. The cells were centrifuged at 4000 rpm for 15 m in washed once and re-suspended in sterile water. This process was repeated before final re-suspension in sterile water. The same treatment was meted to the yeast isolates with exception of medium and period of incubation.

Enumeration of bacterial and yeast cells: About 1 mL of the bacterial suspension was used to inoculate MRS agar in Petri dishes and incubated anaerobically at 30°C for 24-48 h. The colonies formed on plate were counted to obtain the colony forming unit per mL (cfu/mL). Also 1 mL of the yeast isolate suspension was transferred into the haemocytometer and the colonies were counted under x40 objective of the light microscope to obtain its cfu/mL.

Production of Kenkey samples using different starter cultures: Maize grains were fermented to Kenkey according to the traditional method of Nyarko (1977) with some modifications. About 200 g of maize was weighed into three different conical flasks and decontaminated by washing for 15 min at 20°C in filter-sterilized 2.5% sodium hypochlorite solution. The hypochlorite solution was decanted and the maize grains washed thrice with sterile tap water before they were transferred separately into three sterile 1 L Erlenmeyer flasks containing 600 mL of sterile distilled water and steeped for 24 h. The steeped water was decanted and the maize grains wet milled. One flask was inoculated with 2 mL cells suspension of *Lactobacillus plantarum* containing 4.0×108 cfu mL^{-1}, the second flask was inoculated with 2 mL cells suspension of *Candida krusei* containing 3.5×108 cfu mL^{-1} while the third was inoculated with 1 mL cells suspension each of *L. plantarum* and *C. krusei* (mixed cultures).

The wet milled grains in the different conical flasks were allowed to ferment at 28±2°C for 72 h. The fermented dough samples were divided into equal halves, one half boiled for 1 h to produce aflata which was mixed with the other half (unboiled fermented dough) and wrapped in plantain leaves boiled for 3 h to produce Kenkey. Samples were taken aseptically at 24 h intervals from the three different treatments for further investigation.

Physicochemical changes during fermentation of maize to produce Kenkey

pH measurement: The pH was taken at 24 h interval using a Metrohm 620 pH Meter (Metrohm Herisau, Switzerland).

Titratable acidity: This was determined using the method described (Nout *et al*., 1997) by titrating 10 mL of supernatant from the fermenting wet milled maize grains against 0.1 M NaOH using three drops of phenolphthalein as indicator.

Microbiological evaluation of Kenkey samples: This was carried out at 24 h intervals as described earlier for all Kenkey samples.

Nutritional analyses of Kenkey samples: The moisture, ash, crude fibre, crude protein and fat content were determined using the methods described by AOAC (1995) while the mineral contents were quantified using Spectrophotometric Method (AOAC, 1995).

Changes in antinutritional factors

Phytate content measurement: This was determined by employing the Spectrophotometric Method of Maga (1982).

Evaluation of total polyphenols: The Spectrophotometric Method described by Price and Butler (1977) was used.

Sensory evaluation of Kenkey samples: The sensory evaluation panel for the Kenky samples consisted of foreign Ghanaian students and other students in the University of Ibadan who are familiar and had worked with Kenkey earlier. The panelists were instructed to rate the samples for sourness, taste, aroma, appearance and general acceptability. The ratings were presented on a nine-point Hedonic scale ranging from like very much (9 point) to dislike very much (1 point) as described by

Larmond (1977). Each sample were evaluated three times by each panelist. The results obtained were subjected to analysis of variance using one-way ANOVA. Differences between the means were separated using Duncan's multiple range test (Duncan, 1955).

Shelf life of Kenkey samples: This was determined by placing the different fresh samples of Kenkey on a sterile laboratory bench and observed daily for spoilage symptoms and microbial count.

Data analysis: The results or data obtained in this study were analysed using Analysis of Variance (ANOVA) to determine significance differences between the means and these were expressed as mean±Standard Deviation (SD). The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the changes in pH, titratable acidity and microbial count during traditional production of Kenkey. Percentage occurrence of the organisms, the production of amylase by the yeast species isolated during traditional production of Kenkey and the antimicrobial substances of the Lab species isolated during 72 h of fermentation are shown in Table 2-6, respectively. Table 7 shows the result of pH and titrable acidity of the different Kenkey samples. Table 8 shows the microbial analysis of the different Kenkey samples. Table 9 shows the nutritional evaluation of the different Kenkey samples. Table 10 shows the sensory evaluation results of the various Kenkey samples. Table 11 shows the shelf life of the different Kenkey samples.

The result of changes in pH, titratable acidity and microbial count during traditional production of Kenkey showed that the pH of the steeped water and the fermenting maize grains decreased until a final pH of 4.2 was reached. These findings suggest that an acid fermentation occurred naturally during the soaking and fermenting period of wet milled maize grains. Earlier reports by Steinkraus *et al.* (1983), Nout *et al.* (1988) and Mulyowidarso *et al.* (1991a) confirmed this observation.

This occurrence might be due to the increase in the number of yeast cells and Lactic acid bacteria during fermentation. Increase in titratable acidity noted in this study was in conformity with the submission of (Omenu *et al.*, 2007). High titratable acidity in foods had been reported to reduce the incidence of diarrhea in people consuming the products (Mensah *et al.*, 1990). The micro organisms detected in the traditional production of Kenkey included the coliform group, yeast species, species of Lactic acid bacteria and *staphylococcus*. These micro organisms contributed to the ecology of the steeped water and fermentation during the production of Kenkey, Lactic acid bacteria and yeast species increased with fermentation time while coliforms and *Staphylococcus* species were not detected with time as fermentation progressed. Reasons such as inhibitory properties of Lactic acid bacteria and the yeast species may be adduced to be responsible for this observation.

Table 2 shows the percentage occurrence of the organisms. It was observed that *L. plantarum* showed the highest percentage occurrence (Pitt and Holdings, 1985) followed by *C. krusei* (Sneath *et al.*, 1986) and the least occurrence was seen in *Saccharomyces cerevisiae* (07).

Table 3 represents the production of amylase by the isolates during traditional production of Kenkey. The amylolytic ability of yeast was seen to increase throughout the 72 h of fermentation. *Candida krusei* produced the highest of 0.760 mg mL^{-1} at 72 h followed by *Saccharomyces cerevisae* (0.712 mg mL^{-1}) and the least secretion 0.49 mg mL^{-1} was observed in *Rhodotorula gramin*. This result is similar to the earlier reports of Omenu *et al.* (2007) which indicated the amylolytic nature of yeast and suggested that this confers the ability to degrade starch to simple sugars which can be utilized by other co-existing microorganisms in fermenting medium for growth and metabolic activities (Oyewole, 1997). Based on this result, *Candida krusei* was selected as another starter cultures. However, the Lab species isolated did not show any amylolytic activity.

Table 4 shows the production of lactic acid, diacetyl and hydrogen peroxide (antimicrobial substances) by all the Lab species isolated during 72 h of fermentation. The trend observed showed that all the Lactic acid bacteria produced lactic acid throughout the 72 h of investigation. Equally, the quantities produced increased

Table 1: pH, titratable acidity and microbial analysis of traditional produced Kenkey

Duration of fermentation (h)	pH	TTA (Lactic acid %)	TCC (Cfu mL^{-1})	TYC (Cfu mL^{-1})	TLBC (Cfu mL^{-1})	TSTC (Cfu mL^{-1})	TPC (Cfu mL^{-1})
Steep	4.8	0.450	2.6±0.57	5.78±0.57	5.12±0.57	1.52±0.57	6.12±0.57
0	5.3	0.225	1.2±0.57	4.20±0.57	3.89±0.57	0.5±0.57	6.52±0.57
24	4.4	1.351	0.4±0.57	6.70±0.57	6.79±0.57	ND	6.07±0.57
48	4.3	1.126	ND	6.83±0.57	7.07±0.57	ND	7.29±0.57
72	4.2	1.351	ND	7.03±0.57	7.23±0.57	ND	7.50±0.57

Data represent averages of duplicate value; TTA = Total Titrable Acidity; TYC = Total Yeast Count; TCC = Total Coliform Count; TLBC = Total Lactic Acid Bacteria Count; TSTC = Total Staphylococcal Count; TPC = Total Plate Count; ND = Not Detected

Table 2: Percentage occurrence of the isolates

Isolates	Occurrence (%)
S-1	16
S-2	09
48-3	24
72-1	12
O-D	21
O-E	07
72-A	11

Table 3: Amylase production by the isolates

Isolates	Spectrophotometer readings (nm)
S-1	0.0000
S-2	0.0000
48-3	0.0000
72-1	0.0000
O-D	0.7604
O-E	0.7124
72-A	0.0498

Table 4: Quantity of Lactic acid produced by all Lab isolates

Isolates	12 h	24 h	36 h	48 h	60 h	72 h
S-1	0.51	0.56	0.79	0.88	1.22	1.22
S-2	0.79	0.91	1.15	1.28	1.28	1.28
S-4	0.34	0.32	0.32	1.06	1.07	1.13
24-2	0.36	0.38	0.32	0.32	0.49	0.52
24-3	0.39	0.91	0.91	0.91	1.24	1.24
24-4	0.36	0.34	0.38	0.38	0.38	0.38
48-1	0.91	0.79	0.88	0.93	1.01	1.13
48-2	0.79	0.91	1.01	1.01	1.06	1.17
48-3	0.89	0.97	1.13	1.13	1.13	1.31
48-4	0.34	0.43	0.43	0.43	0.43	0.45
48-5	0.29	0.45	0.95	0.96	0.98	1.13
72-1	0.95	0.91	0.72	0.97	0.99	1.13

Table 5: Quantity of diacetyl produced by all Lab isolates

Isolates	12 h	24 h	36 h	48 h	60 h	72 h
S-1	2.28	3.36	6.58	5.78	3.36	4.71
S-2	0.18	2.02	3.89	4.71	4.71	5.01
S-4	3.36	4.71	4.71	4.71	4.71	4.71
24-2	2.55	2.82	5.51	5.51	3.09	4.17
24-3	0.67	4.71	5.51	4.71	4.71	4.71
24-4	0.18	4.71	4.97	6.58	4.71	4.71
48-1	1.21	3.09	3.89	6.05	3.89	4.17
48-2	4.71	4.71	5.51	4.71	4.71	4.71
48-3	0.18	2.82	3.89	4.43	6.05	5.51
48-4	3.36	4.71	4.71	4.71	4.71	4.17
48-5	1.48	3.36	4.17	5.24	4.71	4.71
72-1	2.02	2.55	3.63	4.97	4.71	5.24

Table 6: Quantity of hydrogen peroxide produced by all Lab isolates

Isolates	12 h	24 h	36 h	48 h	60 h	72 h
S-1	0.98	1.11	1.96	0.77	0.65	0.65
S-2	1.31	0.65	0.82	0.52	0.49	0.59
S-4	0.49	0.65	0.49	0.49	0.49	0.49
24-2	1.31	1.37	1.14	0.65	0.46	0.46
24-3	1.71	1.77	1.77	0.65	0.52	0.79
24-4	1.05	0.98	1.71	0.72	0.72	0.65
48-1	1.64	0.98	1.14	1.64	0.46	0.52
48-2	0.65	0.65	0.65	0.49	0.65	0.65
48-3	1.96	0.79	0.65	0.59	0.46	0.52
48-4	0.59	0.65	0.49	0.49	0.62	0.63
48-5	0.72	0.79	0.82	0.79	0.49	0.46
72-1	1.05	0.65	0.72	0.49	0.59	0.59

as fermentation progressed. The highest quantity of lactic acid 1.31 g L^{-1} was produced by isolate 48-3 (*L. plantarum)* followed by isolate S-2 (*L. fermentum*) with the production of 1.28 g L^{-1} and the least (0.38 g L^{-1}) by isolate 24-4. Previously documented results confirmed this occurrence. Fadahunsi reported the production antimicrobial substances by lactic acid bacteria in fermented foods. The highest production of diacetyl (5.51 g L^{-1}) was recorded by *L. plantarum* (48-3) followed by isolate 72-1 which produced 5.24 g L^{-1} and the least quantity (4.17 g L^{-1}) was secreted by isolate *L. fermentum* (S-2). Similarly, the highest quantity of hydrogen peroxide was produced by *L. plantarum* (0.79 g L^{-1}) followed by *L. brevis* (6.65 g L^{-1}) and the least quantity 0.46 g L^{-1} was excreted by *L. fermentum*. The ability of LAB to produce hydrogen peroxide through the oxidation of reduced Nicottin-Amide-adenine Dinucleotide (NADH) by flavin nucleotides which reacts rapidly with oxygen. As LAB lack true catalyst to break down the hydrogen peroxide generated. Hence, it accumulates and becomes inhibitory to some microorganisms (Caplice and Fitzgerald, 1999). None of the Lactic acid bacteria isolated from the traditionally produced Kenkey was amylolytic and based on the ability to produce the highest antimicrobial substances, *Lactobacillus plantarum* was selected as a starter culture. Table 5 shows the result of the pH and titratable acidity of Kenkey produced using different starter cultures. In all the treatment pH decreased throughout the 72 h fermentation period. The highest decrease was recorded when *L. plantarum* and *C. krusei* were used as mixed starters. The pH decreased from 4.6 at 0 h to 3.8 at 72 h. This observation might be due to the larger quantity of acid production resulting from the addition of acid production by the two starters in the fermentation process. The Titratable Acidity (TTA) during the period of fermentation showed an inverse relationship with the pH, as the pH decreased, the TTA increased. The highest increase in TTA was noticeable when *L. plantarum* and *C. krusei* were used as combined starter cultures. The TTA increased from 0.68 at 0 h to 2.7 at 72 h. The importance of high level of TTA in fermented food had been reported (Mensah *et al*., 1990).

Table 8 represents the microbial analyses of Kenkey samples produced using three starter cultures. When *L. plantarum* was used to ferment the maize for 72 h, growth in TYC, TCC, TSTC were not detected but the growth in TLABC increased from 6.72±0.55 in steep waterto 7.35±0.82 after 72 h of fermentation while corresponding growth in TPC increased from 6.01±0.22-7.20±0.88. However, the use of *Candida krusei* revealed that growth increased from 5.47±0.33 in steeped water from to 7.26±0.39 at 72 h while the corresponding growth in TPC increased from 5.22±0.11-7.06±0.23. No growth was detected in TLABC, TCC and TSTC. When

Table 7: pH and titratable acidity of Kenkey produced using different treatments

Treatments	pH					Titratable acidity (actic acid %)			
Starter culture	Steep	0 h	24 h	48 h	72 h	Steep	0 h	24 h	48 h	72 h
L. plantarum	3.8	4.6	4.3	4.1	4.0	0.90	0.68	0.90	2.25	2.70
Candida krusei	4.1	5.3	4.2	4.1	3.9	2.03	0.90	0.90	1.35	2.50
L. plantarum+C. krusei	3.8	4.6	4.1	4.0	3.8	1.35	0.23	0.90	1.80	1.90
Traditionally fermented sample	4.8	5.2	4.4	4.3	4.2	0.45	0.23	1.35	1.13	1.35

Table 8: Microbial analysis of different Kenkey samples

Starter	Fermentation time (h)	TYC (log_{10} cfu/mL)	TLABC (log_{10} cfu/mL)	TCC (log_{10} cfu/mL)	TSTC (log_{10} cfu/mL)	TPC (log_{10}cfu/mL)
L. plantarum	Steep water	ND	6.72±0.55	ND	ND	6.01±0.22
	0	ND	5.32±0.24	ND	ND	6.17±0.13
	24	ND	6.88±0.57	ND	ND	6.56±0.78
	28	ND	7.14±0.35	ND	ND	6.86±0.90
	72	ND	7.35±0.82	ND	ND	7.20±0.88
Candida krusei	Steep water	5.47±0.33	ND	ND	ND	5.22±0.11
	0	4.02±0.57	ND	ND	ND	5.69±0.14
	24	6.49±0.75	ND	ND	ND	6.17±0.53
	48	6.63±0.48	ND	ND	ND	6.44±0.77
	72	7.26±0.39	ND	ND	ND	7.06±0.23
L. plantarum	Steep water	5.42±0.15	6.32±0.19	ND	ND	6.11±0.44
and *C. krusei*	0	4.0±0.63	4.56±0.80	ND	ND	5.70±0.32
	24	5.66±0.78	6.71±0.13	ND	ND	6.62±0.38
	48	6.00±0.50	7.04±0.55	ND	ND	6.72±0.59
	72	6.97±0.58	7.34±0.92	ND	ND	7.18±0.55

ND = Not detected; TYC = Total Yeast Count; TLABC = Total Lactic Acid Bacteria Count; TCC = Total Coliform Count; TSTC = Total Staphylococcal Count; TPC = Total Plate Count

Table 9: Nutritional evaluation of Kenkey samples

Treatments	MC %	Carb %	C. Prt	C. Fat	C. Ash	C. Fibre	Oxa	Phy	PP	Zn^{2+}	Ca^{2+}	Fe^{2+}	Na^{2+}	K^{+}	PO_4^{-}
L. plantarum	71.76	85.21	8.50	1.64	0.75	2.56	6.00	10.00	0.5	0.05	115	4.5	1450.00	38.00	80.00
	±0.03	±0.04	±0.03	±0.03	±0.01	±0.02	±0.01	±0.03	±0.01	±0.05	±0.05	±0.02	±0.01	±0.03	±0.05
C. krusei	71.89	84.63	8.67	1.87	0.82	2.38	5.00	10.00	0.50	0.05	120	4.2	1425.00	32.00	85.00
	±0.02	±0.04	±0.03	±0.03	±0.01	±0.02	±0.02	±0.01	±0.02	±0.05	±0.05	±0.02	±0.01	±0.02	±0.02
L. plantarum	60.82	74.89	10.32	0.62	0.69	2.16	5.00	7.00	0.20	0.05	130.00	5.00	1200.00	38.00	90.00
and C. krusei	±0.03	±0.05	±0.03	±0.03	±0.01	±0.02	±0.01	±0.04	±0.01	±0.01	±0.05	±0.01	±0.01	±0.01	±0.01
Traditionally	69.59	83.71	10.31	0.74	0.63	2.17	5.00	8.00	0.22	0.05	125.00	4.60	1242.00	35.00	75.00
fermented	±0.00	±0.28	±0.00	±0.03	±0.01	±0.28	±0.01	±0.02	±0.01	±0.01	±0.05	±0.02	±0.01	±0.02	±0.03

Values are means±standard error; MC % = Moisture Content %; Carb % = Carbohydrate %; C. Prt = Crude Protein; C. Fat = Crude Fat; C. Ash = Crude Ash; C. Fibre = Crude Fibre; Oxa = Oxalate; Phy = Phytate; Pp = Polyphenols

Table 10: Sensory evaluation of the different Kenkey samples

Sensory parameters	Traditional	L. *plantarum*	*L. plantarum and C. krusei*	*C. krusei*
Sourness	$4.38±0.26^{a}$	$3.63±0.26^{b}$	$4.13±0.30^{ab}$	$2.50±0.19^{c}$
Taste	$3.50±0.33^{bc}$	$4.13±0.23^{ab}$	$4.63±0.26^{a}$	$3.00±0.38^{c}$
Aroma	$3.63±0.53^{ab}$	$3.5±0.330^{ab}$	$4.50±0.27^{a}$	$3.00±0.57^{bc}$
Appearance	$3.50±0.46^{ab}$	$3.6±0.330^{b}$	$3.88±0.44^{a}$	$3.00±0.33^{ab}$
General acceptability	$3.00±0.50^{b}$	$4.0±0.270^{a}$	$4.50±0.33^{a}$	$2.88±0.23^{b}$

Values having the same alphabet superscripts along the rows are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

combined starter cultures of *L. plantarum* and *C. krusei* were employed growth increased from 5.42±0.15 in steep water to 6.97±0.58 at 72 h with corresponding growth was 6.11±0.44-7.18±0.55 in TPC. The growth in TLABC increased from 6.32±0.19-7.34±0.9 after 72 h while the corresponding growth in TPC increased from 6.11±0.32-7.18±0.55. No growth was detected in TLABC, TCC and TSTC. When combined starter cultures of *L. plantarum* and *C. krusei* were employed, growth increased from 5.42±0.15 in steep water to 6.97±0.58 at 72 h ±0.19 and while the corresponding growth was 6.11±0.44-7.18±0.55 in TPC. The growth in TLABC increased from 6.32±0.19-7.34±0.9 after 72 h while the corresponding growth in TPC increased from 6.11±0.32-7.18±0.55.

No growth was seen in TCC and TSTC. The Kenkey sample produced using mixed or combined starter culture, showed that the Lactic acid bacteria count (7.34±0.12) was slightly higher than the yeast count (7.34±0.58) but they co-existed. Reason such as the use of only two starters combined to initiate the fermentation could be adduced for this observation. The yeast and Lactic acid bacteria count increased as the fermentation time proceeded. Such occurrence had been previously reported (Mulyowidarso *et al*., 1991b; Omenu *et al*., 2007). The conspicuous absence of coliform and *Staphylococcus* species in the starter culture produced. Kenky samples might be due to antagonistic properties of both the Lactic acid bacteria and yeast. Black *et al*. (1982) reported that

Table 11: Changes in microbial load of the different Kenkey samples

	Microbial count (cfu g^{-1})						
Kenkey samples	Day 1-6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
L. plantarum	ND	ND	1.6×10^{3}	1.85×10^{3}	1.41×10^{4}	1.30×10^{5}	1.88×10^{6}
C. krusei	ND	1.90×10^{3}	2.50×10^{3}	4.20×10^{3}	3.30×10^{4}	3.80×10^{5}	4.00×10^{6}
L. plantarum+C. krusei	ND	ND	ND	ND	ND	ND	1.25×10^{2}
Traditional sample	ND	ND	2.9×10^{4}	4.22×10^{5}	5.00×10^{5}	3.10×10^{6}	3.80×10^{7}

ND = Not Detected

enteropathogen indication have inferred serious levels of faecal contamination of weaning food in developing countries. High yeast count in both traditional and starter produced Kenkey samples was reported to be responsible for flavor and aroma of maize and fufu (Odunfa, 1985). The significant increase in the growth of *L. plantarum* in the presence of yeast might be due to the availability of growth factors such as vitamins and soluble nitrogen compounds produced by the yeast species (Omenu *et al*., 2007).

Table 9 shows the result of the nutritional evaluation of all the Kenkey samples. The highest moisture content of 71.89±0.02 was recorded in Kenkey sample fermented with the combination of *L. plantarum* and *C. krusei*. High moisture content in fermented foods indicates growth of fermenting. The high moisture content in fermented food had been reported to be potential source of spoilage (44.45). The highest carbohydrate contents (85.21±0.04) and the least (74.84±0.05) were observed in samples produced by *L. plantarum* and the combination of *L. plantarum* and *C. krusei*, respectively. The decrease in carbohydrate content might be attributed to the utilization of sugar produced by amylolytic activity of the yeast. *C. krusei* had been reported and found in previous study to be highly amylolytic (Omenu *et al*., 2007).

The highest crude protein content was detected in the sample produced by the fermentation of *L. plantarum* and *C. krusei*. The relatively high protein content might be linked to combined fermentative abilities of the two starter cultures used because fermentative process improves the nutritional quality of fermented foods (Odunfa, 1985).

The crude fat content observed in sample fermented with mixed starter of *C. krusei* and *L. plantarum* was the highest (10.31) while the least was recorded in the sample fermented with *L. plantarum*. Previous studies had shown that yeast demonstrated lipolytic activity with concomitant increase in the level of fatty acids (Sanni and Ogbonna, 1991). Documented report on *Candida krusei* as a potential odour and flavor enhancing micro organisms are available (Odunfa, 1985). Yeast by the lipolytic disintegration produces precursors of aroma (fatty acids) that may contribute significantly to the final flavours of foods (Roman *et al*., 1987).

The ash content was the least (0.62±0.03) in the sample produced using *C. krusei* and *L. plantarum* while the highest was observed in sample produced by using *C. krusei* (1.87±0.03). The low ash content recorded a low concentrate of phytate and oxalate content with an increase elemented minerals since these compounds are fractional constituent of ash (Berghofer *et al*., 1988). Eka (1980) and Adebowale (1988) reported that low ash content can aid better growth performance and feed utilization efficiency and might also aid high amount of metabolisable energy in diet since ash is a measure of total inorganic matter in food. The result of the crude fibre analysis showed that samples fermented spontaneously and with mixed starters showed lowest content of ash 0.63±0.01 and 0.69±0.01, respectively. Adewusi *et al*. (1991) reported that fibre content in diet decreases the total metabolisable energy through dilution, digestion and intestinal absorption of nutrient. The results of the antinutritional factors monitoring depicted that the least contents of these parameters were observed in the samples fermented using *L. platanrum* and *C. krusei*.

Table 10 shows the results of the sensory evaluation of the different samples of Kenkey. The sample produced using combined starters of *L. plantarum* and *C. krusei* was rated best in terms of taste (4.63±0.26a), aroma (4.50±0.27a), appearance (3.88±0.44a) and general acceptability (4.50±0.33a) while the traditionally produced Kenkey sample scored the highest (4.38±0.26a) in terms of sourness followed by the sample produced by *L. plantarum* and *C. krusei* (4.13±0.30ab). The best samples based on the overall sensory assessment were the sample produced using combined starters of *L. plantarium* and *C. krusei*. This observation could be adduced to reasons such as the better fermentative tendencies of the combined two microorganisms based on higher enzyme and antimicrobial substances production resulting in the best nutritional quality of the sample.

Table 11 shows the result of changes in the microbial load of Kenkey samples for 12 days. In the Kenkey sample produced using *L. plantarum* as a starter microbial count was not detected from days 1-7 but from days 8, the microbial count was detected while in the sample produced by *C. krusei*, no microbial count was seen from days 1-6 but from the 7 days microbes were detected. The

sample produced by combined starters of *L. plantarum* and *C. krusei* did not show any microbe from days 1-11 after which microbes were isolated at day 12th while microbes were isolated in the traditional fermented Kenkey after the 8th day. The presence of microbes is an indication of spoilage. It is therefore inferred that the sample produced using combined starters is most microbiologically stable with the longest shelf life. The use of Lactic acid bacteria as a bio-preservation in food has been documented. Lucke (2000) reported that Lactic acid bacteria possess the ability to lower pH and to produce antimicrobial agents while Omenu *et al.* (2007) reported the production of acid by yeast species.

CONCLUSION

This study shows that the sensory evaluation indicated that the best acceptable Kenkey sample was the one produced using starter cultures of *L. plantarum* and *C. krusei* while the longest shelf life (12 days) was recorded in the sample produced using the same combined starters.

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