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Contents

Executive summary

The production of fermented foods is based on the use of starter cultures. Recently, new starter cultures with an industrially important functionality are being developed. The starter culture can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages. Examples are starter cultures that produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins, or useful enzymes, or that have probiotic properties.

Surveys were conducted in order to collect wild flora found in African cereal based products (Akpan, Gowé, Kenkey, Kishk Sa'eedi) from different rural areas in Benin, Ghana and Egypt. In such places the fermentation process of these products occurs naturally with no addition of industrial starter cultures. Samples during processing were collected and lactic acid bacteria or related cultures were isolated. Out of **280** isolates of lactic acid bacteria from Laban Zeeir and kishk Sa'eedi samples. The isolates were pre identified as 199 *Lactobacillus, 71 Enterococcus, 4 Streptococcus and 6 lactococcus.* 151 isolates, which were able to grow in milk at different temperatures with the absence of off-flavors, were identified using phenotypic methods and the rep-PCR technique. *Lactobacillus* (104 strains) were dominant, followed by genus *Enterococcus* spp (37 strains, and the genus *Lactococcus* (5 strains). Only two of *Streptococci*, two *Leuconostoc* and one *Pediococci* were also isolated and identified from Laban Zeeir. The strains were tested for efficiency of biomass production and separation, acidifying activity, autolytic and aminopeptidase properties, antagonistic activities and slime production. Strains of *Lact. lactis* ssp *lactis*, *E. faecium*, *Lb. fermentum* and *Lb. delbrueckii* ssp *lactis* which showed outstanding performances and are currently investigated in re-engineering Kishk Sa'eedi trials.
In the work of selection culture from Akpan, a total of 88 isolates from MRS were purified and kept at - 80°C in MRS broth containing 30% (v/v) sterilized for further identification. From YEA, a total of 65 yeast isolates were purified and kept in Sabouraud Liquid Medium containing

30% of sterilized glycerol at -80°C. Grouping of these strains is planned for future steps. The identification of isolated from Akpan and Gowe is requested 6 months.

Lactic acid bacteria and yeast isolates from kenkey were selected for potential as starter culture based on investigation of their technological properties including Rate of acidification of steeped dehulled maize grains and dehulled maize dough, amylase secretion and antimicrobial activity towards enteric pathogens. Ten trails of Kenkey were made using dough fermented by different selected cultures. The trials were, 1:control fermentation (without starter culture),2: *L.brevis*, 3: *L.fermentum*, 4: *S. cerevisiea+C. krusei*, 5: *L.fermentum + S. cerevisiae;* 6: *L.fermentum+C. krusei*,7:*L.brevis + C. krusei*, 8: *L.fermentum+S. cerevisiae + C. krusei*, 9:*L.fermentum+L.brevis+ C. krusei;* 10: *L.fermentum+L.brevis+C. krusei + S. cerevisiae*. Trial 9 received the highest score of overall acceptability.

Background

This deliverable report refers to the starter culture for group 1 products include the cereal based products; Akpan and Gowe (Benin), Kenkey (Ghana) and Kishk Sa'eedi (Egypt).

Results

For each product, the summary and detailed reports are given in annexes for Kishk Sa'eedi, Gowe Akpan and Kenkey, respectively. The table and figure numbers refer to each annex respectively.

Annex 1 – detailed report for Kishk Sa'eedi

ABSTRACT (max 300 words)

A total of 280 wild lactic acid bacteria (LAB), belonging to *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Streptcoccus*, *Leuconostoc* and *Pediococcus* isolated from Laban Zeeir and Kishk Sa'eedi were screened and selected according to their production and technological properties. Most strains showed a good separation after centrifugation and some strains were fast acidifying. Aminopeptidase and autolytic activity were generally higher for most lactobacilli compared to other strains. In milk, many strains were able to produce pleasant flavours and some strains produced exopolysaccharides (slime and capsule). Detailed information about the characteristics of each strain is available in the culture collection of Faculty of Agriculture Alexandria University (FAAU). The selected strains are under investigation to be used in second generation of Kishk Sa'eedi.

Introduction

The name "Kishk" refers to a group of popular fermented dairy cereal mix products common to Egypt and the Middle East. The product is made from a combination of wheat with natural local fermented buttermilk, yoghourt or sour milk (Laban Zeeir). On completion of fermentation, the mixture is shaped and sun dried.

The production of fermented foods is based on the use of starter cultures. Recently, new starter cultures with an industrially important functionality are being developed. The starter culture can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages. Examples are starter cultures that produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins, or useful enzymes, or that have probiotic properties.

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The group of lactic acid bacteria (LAB) occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented foods and beverages (Caplice & Fitzgerald, 1999; Ray, 1992; Wood, 1997; Wood & Holzapfel, 1995). They cause rapid acidification of the raw production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance. In this way they enhance shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product.

Traditionally Kishk Sa'eedi is still a very important part of the daily food in Upper Egypt. This product may have a very constant microbial content over time. In general, however, environmental conditions such as temperature, origin of the milk, processing and sanitary

conditions, etc., might have a significant influence on the microbial composition of traditionally made Kishk Sa'eedi.

Materials and methods

Kishk Samples

A total of 99 samples were collected from Mania Governorate in Upper Egypt; the cultures were isolated only from samples having good sensory properties (55 samples); fermented milk (Laban Zeeir) (34), Dough (12), and final product (Kishk) (9).

Enumeration and isolation of lactic acid bacteria

In the case of fermented milk, an enrichment step by holding the samples at 30°C (mesophilic LAB), 42°C (thermophilic LAB) for 24 h was necessary to facilitate the isolation of specific groups of LAB. For Kishk samples, a portion of 10 g were homogenized in 2% (w/v) sodium citrate. In the case of fermented milk samples, 1 ml of samples were diluted in 9 ml sterilized Ringer's solution. Appropriate dilutions were prepared in Ringer's solution and plated on different types of media included M17 agar, MRS agar, Rogosa agar (Oxide), and *Enterococcus faecalis* agar (SF agar, Oxide). MRS and Rogosa agar plates were anaerobically incubated (Generbox anaer, bioMerieux, Vercieu, France) at 37°C while M17 and SF agar plates were aerobically incubated at 37°C. Representative colonies were collected according to their shape and color then they were examined for Gram staining reaction and tested for catalase production. Gram-positive and catalase negative isolates, which were also able to grow in reconstituted skim milk (RSM, 10% w/v), were purified by striking two times to the respective isolation medium.

Identification of isolates

The isolates were identified by morphological and physiological tests (Sharpe, 1979; Garvie, 1986; Hardie 1968; Kandler and Weiss 1986). Gram-positive cocci isolates were tested for growth in M17 broth at 10°C and 45°C; SF medium; in M17 broth containing 6.5% (w/v) NaCl; M17 broth at pH 9.6. Gram-positive rod-shaped isolates were tested for growth in MRS broth at 10°C and 45°C and also for CO_2 production. All isolates were tested for growth in RSM at 37 °C and 45°C. Isolates with the ability to coagulate RSM within 6h without formation of off-flavor were identified. According to the results obtained, isolates were grouped in four major clusters: thermophilic and mesophilic cocci and thermophilic and mesophilic rods. The identification was completed by sugar fermentation patterns obtained with the API 20 and API 50 galleries and CHL medium (bioMerieux, Vercieu, France); results were analyzed by computerized database software provided by the manufacturer. The identity of LAB isolates was confirmed by rep-PCR (Mohammed et al 2009). Bands pattern of isolates were scanned, normalized and compared to a database normalized DNA fingerprints of LAB reference strains by use of GelCompare 5 software (Applied Maths, Kortrijk, Belgium) which was also used for generation of cluster analysis.

Bacterial cultures and media

All strains belonging to lactic acid bacteria (*n* = 280) collected in this study were deposited in the FAAU collection (Faculty of Agriculture, Alexandria University). A total of 140 reference strains were included and their DNA fingerprints were used for identification of isolated strains. Lactic acid bacteria were maintained as frozen stock cultures at – 80°C in 12.5 % w/v reconstituted milk powder containing 15% w/w glycerol (Sigma, St. Louis, MO). Cultures were propagated twice in M17 or MRS medium and incubated at optimum temperatures for 16h before use. Strains of *Lactobacillus* was propagated in MRS broth (Biolife, Milano, Italy; De Man et al., 1960), while *Lactococcus and Enterococcus* strains were maintained in M17 broth (Biolife, Milano, Italy; Terzaghi and Sandine, 1975); for *St. thermophilus* strains a fortified M17 medium (7% saccharose and 0.5% Tween 80) was used.

Selection criteria for starter cultures

The isolates of Kishk were screened for acidification potential, biomass, resistance to drying, flavour potential, antibacterial activity, production of exopolysaccharides and peptidasic activity as described in SOP culture D1.2.3.7.

Results

A total of 280 isolates belongs to lactic acid bacteria were examined for their ability to grow in Reconstitute Skimmed Milk (RSM) at different temperatures with absence of off-flavor. LAB were found at average levels $\log_{10}$ 4.55, and $\log_{10}$ 6.27 CFU/ml or g of fermented milk and Kishk respectively. The highest enterococci count was observed in all Laban Zeeir samples. The domination of this genus in Lban Zeir may be due to the high salt concentration. *E. faecium was* isolated from all Laban Zeeir samples and some of Karish samples. A very complex flora was isolated from Laban Zeeir (*Lc lactis* ssp *lactis, E. feacium, Lb. helveticus, Lb. acidophilus, Lb. delbrueckii* ssp *bulgaricus, Lb. delbrueckii* ssp *lactis, Lb. delbrueckii* ssp *delbrueckii, Lb. paracasei* ssp *paracasei, Lb rhamnosus Lb. plantarum* and *Lb. fermentum*).

Table 1.1. Strains idendified from Laban Zeeir and Kishk Sa'eedi

Source	**Genus**	Number
Laban Zeeir	*Lc lactis* ssp *lactis, E. feacium, Lb. helveticus, Lb. acidophilus, Lb. delbrueckii* ssp *bulgaricus, Lb. delbrueckii* ssp *lactis, Lb. delbrueckii* ssp *delbrueckii, Lb. paracasei* ssp *paracasei, Lb rhamnosus Lb. plantarum* and *Lb. fermentum*	141
Dough		93
Kishk Sa'eedi	*Enterococcus faecium , E. faecalis, E. durans, Lb. brevis, Lb. fermentum*	46

Production effectiveness criteria

In order for the dairy industry to consider any culture as a starter culture, the candidate culture has to fulfill a number of criteria. Economical aspects such as the propagation must be economically feasible with a high yield of biomass; the produced cells should be easily separated by the centrifugation or the microfiltration processes and the starter culture should resist freezing or lyophilization with little practical loss of activity (Buckenhuskes, 1993). The efficiency of isolated strains in the production of biomass was studied. Lactococci strains were generally the highest group among all isolated strains of LAB in biomass production followed by strains belonging to *Lactobacilli*.

With the exception of *Lb. delbrueckii* ssp *bulgaricus* strains, all other LAB cultures showed good biomass separation when collected by centrifugation. A part of strains showing poor pellet formation could be of interest since they were able to produce slime.

The stability of the activity of lyophilized cultures is one of the most critical characters in strain selection. In the present study, the strains are under investigation for stability of the activity of lyophilized.

Technological properties

The fastest acidification rate was obtained by all isolated strains of *St. thermophilus;* one strain, *Lb. helveticus* ; one strain *Lb. delbrueckii* ssp *bulgaricus*; and two strains *Lb. fermentum (*Fig 1.1). It is observed that the majority of the isolated cultures have a medium rate of acidification. Most strains of *Lb. plantarum*, *Lb. rhamnosus* and *Lb. fermentum* showed a slow acidification rate. This result could be expected since their species are grouped with facultatively heterofermentative lactobacilli (Kandler and Weiss, 1986).

In the present study, aminopeptidase (AP) was determined to express the proteolytic activities of isolated cultures (Table 1.2). Aminopeptidase activity was generally higher for most lactobacilli strains when compared to enterococci or lactococci. Although lactic acid bacteria are weakly AP when compared to *Bacillus* and *Pseudomonas* (Law and Kolsted, 1983), they are responsible for the ripening process in many fermented dairy products (Tamine and Deeth, 1980). The proteolytic activities seem to be species and strain dependent. It is reported that peptidases in lactobacilli have a wider range of activities than peptidases in lactococci (Law and Kolstad, 1983). Dako et al. (1995) reported that peptidase activities of lactobacilli are generally higher if compared to lactococci. Results presented here are in agreement with these findings.

The autolytic properties of LAB strains isolated in this study are shown in Fig 2. The results demonstrate that the autolytic activities of most lactobacilli are higher than in lactococci and enterococci. These results are in agreement with findings of Dako et al (1995) who found that *Lactobacillus* autolyse more rapidly than the other LAB which lead to a faster liberation of their intracellular enzymes in the external environment.

In challenge test, strains belonging to the same species were interacted, most strains of *Lc. lactis* ssp *lactis* and *Lc. lactis* ssp *cremoris* were able to inhibit each other (Table 1.3). Higher inhibitory activity was observed among enterococci strains. Lactic acid bacteria are used initially to preserve the raw foods through the fermentation. They produce various antimicrobial substances including; lactic acid, which reduce the pH in the growth medium makes it unsuitable for pathogens and spoilage microorganisms to proliferate; bacteriocins or antimicrobial peptide; reuterin which is a derivative of glycerol with a broad-spectrum antimicrobial activity (De Vuyst and Vandame, 1994). Nisin is one of the most distinguished bacteriocin produced mainly by strains of *Lc lactis* ssp *lactis* (Hurst, 1981) and licensed for use as food additive in over 45 countries (Delves-Broughton et al., 1996).

Slime formation was only obtained by only one strain (results not shown)). The form of slime e.g. exopolysaccharide can be a capsule, closely attached to the cell wall, or loosely attached or excreted slime (Sutherland, 1977).

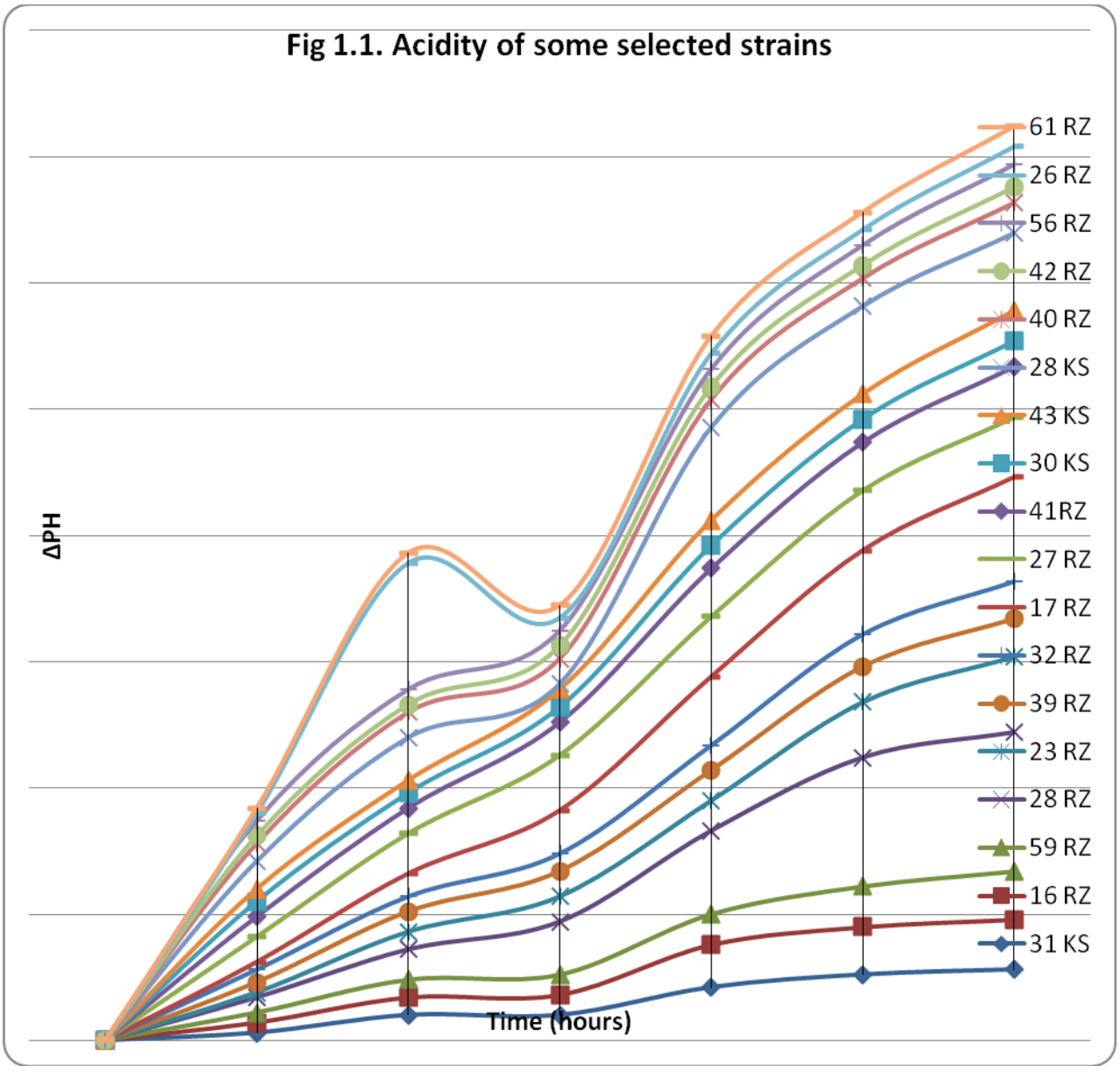
Fig 1.1. Acidity of some selected strains
ΔPH
Time (hours)
61 RZ
26 RZ
56 RZ
42 RZ
40 RZ
28 KS
43 KS
30 KS
41RZ
27 RZ
17 RZ
32 RZ
39 RZ
23 RZ
28 RZ
59 RZ
16 RZ
31 KS

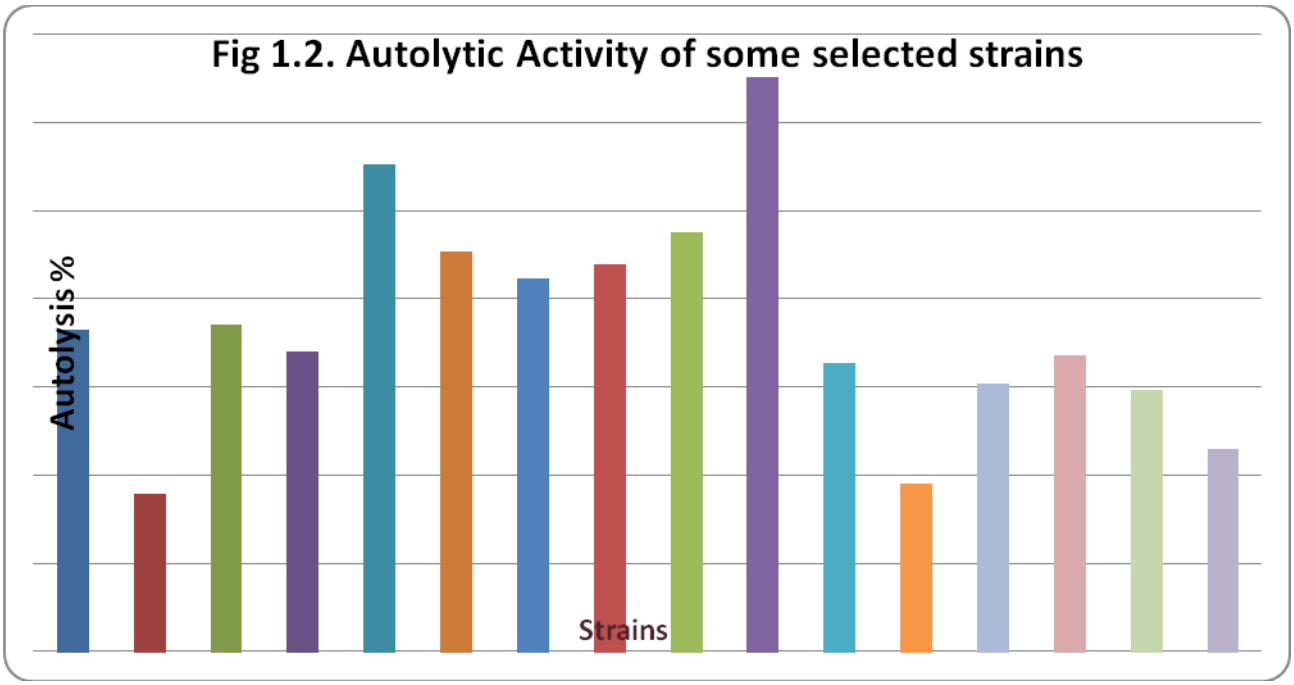

Table 1.2. Aminopeptidase activity of selected strains

Name of sample	Enzyme activity EU
31 KS	0.15
59 RZ	0.70
16 RZ	0.05
39 RZ	0.068
32 RZ	0.047
17 RZ	0.14
27 RZ	0.13
23 RZ	0.03
28 KS	0.020
30 KS	0.08
43 KS	0.01
28 RZ	0.75
56 RZ	0.039
61 RZ	0.027
40 RZ	0.105
41 RZ	0.027
42 RZ	0.058

Table 1.3. Challenge test of some selected strains

Producer Indicator	42 RZ	61 RZ	40 RZ	56 RZ	41 KS	43 KS	26 RZ	38 KS	28 KS	30 KS
42 RZ	-	-	+	+	-	++	+	+	+	+
61 RZ	++	++	-	-	++	++	-	++	+	+
40 RZ	++	-	++	-	++	++	-	++	++	+
56 RZ	+	++	++	+	+	++	++	++	++	++
41 KS	++	+	++	-	++	-	++	++	++	-
43 KS	-	+	+	-	++	-	-	-	++	++
26 RZ	+	-	-	-	++	-	-	+	++	++
38 KS	-	-	-	-	-	-	-	++	-	++
28 KS	++	++	++	++	+	+	++	++	++	+
30 KS	++	-	+	++	++	+	-	++	++	-

Producer Indicator	39RZ	59 RZ	31 RZ	28 RZ	16 RZ	17 RZ	27 RZ	32 RZ	23 RZ
39 RZ	-	++	++	++	++	+	++	++	++
59 RZ	-	++	++	++	++	++	++	++	++
31 RZ	-	++	++	++	++	++	++	++	++
28 RZ	++	++	++	++	++	++	+	++	++
16 RZ	++	++	++	++	++	++	++	+	++
17 RZ	++	+	++	++	++	-	-	-	-
27 RZ	-	++	+	++	+	++	++	++	++
32 RZ	++	++	++	++	+	++	++	++	++
23 RZ	++	++	++	++	++	++	++	++	++

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Annex 2 – detailed report for Gowe

Introduction

Gowe, an indigenous sour beverage is made from a blend of malted and non-malted grains flour that is produced by spontaneous fermentation involving mixed cultures of lactic acid bacteria (LAB) and yeasts. The fermentation can be controlled by using of starters cultures as inoculum enrichment.

Sample collection

Samples at different stages, namely fermentation and cooking were obtained from four producers. Fermentation was monitored and samples were drawn at beginning and at the end. Four samples from each stage were obtained using sterile 500 ml plastic screw-capped glass bottles and transported on ice in cooling boxes to the University of Abomey-calavi.

Microorganisms encountered

Lactic acid bacteria, yeasts and moulds predominated with counts increasing from 4.1 to 5.7 log $cfug^{-1}$ and 3.4 to 5.2 log $cfug^{-1}$ within 24 h of fermentation. After cooking of Gowe, lactic acid bacteria and yeasts and moulds numbers are still present, with mean values of 1.6 and 1.9 log $cfug^{-1}$ respectively.

For each sample 20% of total numbers of colonies were picked at random at a defined area of a plate and purified by successive streaking on the Nutrient Agar. Colonies on MRS were examined on microscopy and tested for Gram-staining and catalase activity. A total of 110 isolates from MRS were purified and kept at - 80°C in MRS broth containing 30% (v/v) of sterilized glycerol for further identification. From YEA, a total of 90 yeast isolates were purified and kept in Sabouraud Liquid Medium containing 30% of sterilized glycerol at -80°C.

Future works (Perspective)

Yeasts isolated will be grouped by internal transcribed spacer-polymerase chain reaction but for lactic acid bacteria the restriction fragment length polymorphism will be also achieved. Identification of yeast or lactic acid bacteria isolated will be done in Benin (UAC). After grouping, the representatives from each group will be selected for sequencing by subcontracting.

In addition, the use of commercial freeze-dried strains of lactic acid bacteria and yeasts of the species encountered in traditional gowé (see below) will be tested.

Other useful information related to previous works on starter culture

Previous works showed that the dominant lactic acid bacteria involved in Gowe production are *Lactobacillus fermentum, Weissella confusa, Lactobacillus mucosae, Pediococcusacidilactici, Pediococcuspentosaceus*and *Weissellakimchii.* The yeasts are *Kluyveromyces marxianus, Pichiaanomala, Candida krusei* and *Candida tropicalis* (Vieira-Dalodé et *al.,* 2007). For the purpose of starter culture testing, some laboratory trials have been performed with the identified

lactic acid bacteria singly or in association with the yeast for a controlled fermentation (Vieira-Dalodé et *al.*, 2008). It was established that Gowe can be obtained by controlling fermentation using *L. fermentum* as inoculum..

Annex 3 – detailed report for Akpan

Summary

It is not possible to conclude on the starter cultures based on the work we did. Currently, a total of 88 isolates from MRS were purified and kept at - 80°C in MRS broth containing 30% (v/v) sterilized for further identification. From YEA, a total of 65 yeast isolates were purified and kept in Sabouraud Liquid Medium containing 30% of sterilized glycerol at -80°C. Grouping of these strains is planned for future steps.

Background

Sorghum or maize beverages in Africa possess similar features in which lactic acid bacteria fermentation plays a key role in safety and acceptability of this product in Benin. Many Beninese cereal fermented foods such as Tchoukoutou, gowé, and Akpan are prepared by the action of diverse of bacteria and yeast. Akpan is a light fermented maize starch in which lactic acid bacteria and yeast are involved. Therefore isolation, characterization and identification of the microorganisms involved in fermentation with a prospective selection of starter culture to obtain a predictable end of product with a desirable quality. These are the works we are doing. Besides, research works attempted to develop starter cultures which presumably can be used for the purpose of fermentation of akpan (Teniola, 2001).

Summary of activities

Enumeration

During process characterization, samples were collected at critical steps (beginning and end of fermentation and after cooking) for microbiological count and then isolation and purification. Counts were carried out as described in the SOPs.

Microorganisms in Ogui and Akpan

Lactic acid bacteria and yeasts and moulds increased from 4. 2 to 5.8 log $cfug^{-1}$ and from 4.3 to 4.9 log $cfug^{-1}$ respectively during 24 h of fermentation. After precooking, lactic acid bacteria and yeasts and moulds numbers were 4.5 and 3.8 log $cfug^{-1}$ respectively.

Isolation and purification

For each sample 20% of total numbers of colonies were picked at random at a defined area of a plate and purified by successive streaking on the Nutrient Agar. Colonies on MRS were examined by microscopy and tested for KOH string test and catalase activity. A total of 88 isolates from MRS were purified and kept at - 80°C in MRS broth containing 30% (v/v) sterilized for further identification. From YEA, a total of 65 yeast isolates were purified and kept in Sabouraud Liquid Medium containing 30% of sterilized glycerol at -80°C.

Future works (Perspective)

Yeasts isolated will be grouped by internal transcribed spacer-polymerase chain reaction but for lactic acid bacteria the restriction fragment length polymorphism will be also achieved. Identification of yeast or lactic acid bacteria isolated will be done in Benin (UAC). After grouping, the representatives from each group will be selected for sequencing. Identification is expected to be done by subcontracting.

In addition, the use of commercial freeze-dried strains of lactic acid bacteria and yeasts of the species encountered in traditional akpan (see below) will be tested.

Table 3.1: Lactic acid bacteria and yeast of ogi

Microorganisms	Starters cultures	Authors
Lactic bacteria	*Lactobacillus fermentum* *Lactobacillus brevis* *Lactobacillus Curvatus* *Lactobacillus buchneri*	Nago et al ., 1998
Yeast	*Candida humicola* *Candida krusei* *Geotrichum spp*	
Lactic bacteria	*Lactobacillus fermentum* *Lactobacillus brevis* *Lactobacillus plantarum*	Omemu A.M.2011
Yeast	*Saccharomyces Cerevisiae* *Rhodotorula Graminis* *C. krusei* *C. tropicalis* *Geotrichum candidum* *Geotrichum fermentum*	
Lactic bacteria	*P.pentosaceus* *L.Brevis* *L.plantarum*	Teniola. O. D. 2001
Yeast	*Saccharomyces Cerevisiae* *Candida valida* *Candida krusei* *Geotrichum Candidum*	

Other useful information related to previous works on starter culture

Table 3.1 presents microorganisms isolated from ogui. Many strains of lactic acid bacteria were isolated during the previous works on Beninese ogi (Table 2.1); there are: *Lactobacillus fermentum, lactobacillus brevis, lactobacillus curvatus, Lactobacillus buchneri* (Nago et al., 1998). These finding are quite similar to those reported by Omemu (2001) for Nigerian ogi. However Teniola (2002; 2001) isolated from Nigerian ogi other bacteria namely *Lactobacillus plantarum* and *Pediococcus pentosaceus*. Many workers reported the presence of yeast such as *Saccharomyces cerevisiae, Candida Krusei* and *Geotrichum ssp* (Nago et al., 1998; Omemu.2001 and Teniola et al., 2001),

It is possible to get combinations from theses isolates for fermenting ogi for akpan

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Annex 4 – detailed report for Kenkey

Summary

To develop a starter culture for white kenkey, the dominant lactic acid bacteria and yeasts responsible for the fermentation of the product were enumerated on MRS and OGYEA respectively and characterized phenotypically. 98 isolates, representing 47.1 2%, were identified as *Lactobacillus fermentum,* 52 isolates representing 25 % as *Lbbrevis,* 30isolates representing 14.42 % as *Lb.plantarum,* 18 isolates representing 8.65 % as *Pediococcuspentosaceus*and 10 isolates representing 4.81 % as *P. acidilactici.* A total of 185 yeast isolates were grouped and identified. *Saccharomyces cerevisiae* was predominant and represented 47.6 % of the yeast isolates, *Candida krusei*29.1 %,*Debaryomyces*spp. 15 %, and *Trichosporon*spp. 8.3 % of the isolates.

The lactic acid bacteria and yeast isolates were selected for potential as starter culture based on investigation of their technological properties which included theirantimicrobial activity towards common enteric pathogens, rate of acidification of dehulled maize grains during steeping and dough fermentation, amylase secretion, protease activity, and production of exo-polysaccharides. Selected cultures were used as single or mixed cultures to produce white kenkey which was assessed by a taste panel.

For amylase secretion, 18.24 % of *Lb. fermentum* isolates produced clear zones of inhibition ranging from 1 mm to 3 mm on Starch Agar, 10.12 % of *Lb. brevis* isolates, 12.78 % of *Lb.plantarum* and 8.28% of *P. pentosaceus* isolates also produced clear zones indicating amylase secretion. None of the *P. acidilactici* isolates demonstrated amylolytic activity. None of the LAB showed any protease activity. 26.4 % of the 108 LAB assayed produced slime (exo-polysaccharide) of >1 mm (and included *Lb. fermentum, P. pentosaceus, Lb. plantarum*), whiles 36.86 % produced slime of <1 mm (*Lb. fermentum, Lb. brevis, P. acidilactici*). Two isolates of *Lb. fermentum*and one isolate of *Lb. plantarum* tested showed antimicrobial activity against *E. coli, Staphylococcus aureus, Salmonella typhimuruim,* and *Vibrio cholera.* A pediococci and *Lb. brevis* 2 showed the least activity against these pathogens

In inoculated fermentations, the use of any of the cultures tested resulted in a more rapid drop in pH due to a faster production of acid than in the uninoculated control sample. The use of LAB resulted in a more rapid drop in pH than use of the yeasts.The isolates which showed the highest rate of acidification at various stages of steeping and dough fermentation were *Lb. fermentum*2 and *Pediococcuspentosaceus.*White kenkey produced using various starter cultures were all acceptable to an untrained 20 member taste panel whoscored between 6.0 and 7.0 on a nine point hedonic scale for all products. The highest score for acceptability of 6.73 which represented like moderately was scored for white kenkey prepared with a mixed culture comprising *Lb. fermentum, Lb. brevis,* and *C. krusei.* The PCA bi-plot showed that kenkey fermented with

starter cultures which included *Lb. brevis* tended to have a good taste and enhanced their acceptability by the taste panel.

Introduction

To develop a starter culture for white kenkey, the dominant lactic acid bacteria and yeasts responsible for the fermentation of the product were enumerated on MRS and OGYEA respectively and characterized. The phenotypic characterization included their pattern of carbohydrate fermentation using API 50 CHL and ID 32C. In summary a total of 208 cultures isolated on MRS (Gm +ve, cat –ve rods, coccoids and cocci) from samples of steep water and fermenting dough grouped and representative isolates identified. 98 isolates, representing 47.1 2%, were identified as *Lactobacillus fermentum*, 52 isolates representing 25 % as *Lbbrevis*, 30isolates representing 14.42 % as *Lbplantarum*, 18 isolates representing 8.65 % as *Pediococcuspentosaceus*and 10 isolates representing 4.81 % as *P. acidilactici.*

For yeasts, a total of 185 isolates on OGYEA from steeping and dough fermentation were grouped and identified. *Saccharomyces cerevisiae* was predominant and represented 47.6 % of the yeast isolates, *Candida krusei*29.1 %,*Debaryomyces*spp. 15 %,and *Trichosporon*spp. 8.3 % of the isolates.

The lactic acid bacteria and yeast isolates were selected for potential as starter culture based on investigation of their technological properties. This includedtheir antimicrobial activity towards common enteric pathogens to assure safety of the re-engineered kenkey, rate of acidification of dehulled maize grains during steeping and dough fermentation, amylase secretion, protease activity, and production of exo-polysaccharides. Selected cultures were used as single or mixed cultures to produce white kenkey which was assessed by a taste panel.

Technological properties of isolates

Amylase activity

Lactic acid bacteria isolates were compared for their ability to secret amylase by growing them on modified MRS agar containing 2% starch. A total 144 isolates comprising *Lb. fermentum*, 43;*Lb.brevis*, 34;*Lb.plantarum*, 22;*P.acidilactici*, 13; and *P. pentosaceu*s, 8 were assayed. Out of these 18.24 % of *Lb. fermentum*isolates produced clear zones of inhibition ranging from 1 mm to 3 mm, 10.12 % of *Lb. brevis*isolates produced clear zones, 12.78% of *Lb.plantarum* and 8.28% of *P .pentosaceus*isolates also produced clear zones indicating amylase secretion. None of the *P. acidilactici*isolates demonstrated amylolytic activity. The technological properties of some of the isolates including amylase production are shown in Table 1.

Proteolytic activity

A total of 108 isolates of lactic acid bacteria isolates were streaked on Plate Count Agar containing 5% casein to assess their ability to secret protease. None of the isolates showed any protease activity (Table 1).

Exo-polysaccharide production

A total 108 lactic acid bacteria were screened for the production of EPS. The isolates produced varied degrees of EPS ranging from 0.5 to 2 mm of a slimy substance. 26.4 % produced slime of >1 mm (*Lb.fermentum, P.pentosaceus, Lb.plantarum*), whiles 36.86 % produced slime of <1 mm (*Lb.fermentum, Lb.brevis, P.acidilactici*) (Table 4.1).

Table 4.1. Amylase, protease and exo-polysaccharides (EPS) secretion by lactic acid bacteria isolated during maize and dough fermentation.

Lactic acid bacteria	Amylase	Protease	EPS
*L. brevis*1	-	-	+
*L. brevis*2	-	-	+
P. pentosaceus	+	-	+
P. acidilatici	-	-	+
*L. fermentum*1	+	-	++
*L. fermentum*2	+	-	++
L. plantarum	+	-	+

-: no inhibition zone, +: 1-2 mm inhibition zone; ++: 3-4 mm inhibition zone; +++:5+ mm inhibition zone

Anti-microbial activity

The inhibitory potential of selected LAB cultures was investigated using the Agar Well Diffusion Method as described by Schillinger and Lücke (1989) and Olsen *et al*. (1995). A volume of 0.1ml of the test culture was transferred into wells in MRS agar plates and left to diffuse into the agar for 4-5 h. The plates were overlaid with 10 ml soft Nutrient Agar containing 0.25 m1 of 10^{-1} dilution of an overnight culture of one of the indicator pathogens: *E. coli,Staphylococcus aureus*, *Salmonellatyphimuruim,* or*Vibrio cholera.* After incubated at 37 °C for 24 h the plates were examined for inhibitory zones around the wells.

The two isolates of *Lb. fermentum*tested showed the strongest antimicrobial activity against the enteric pathogens. Both isolates were able to inhibit all four pathogens and showed strong inhibition against *Staphylococcus aureus*and*Vibro cholera* (Table 4.2). *Lb.plantarum* also sowed activity against all four pathogens but they were weak interactions. None of the remaining isolates showed activity against all four pathogens, the weakest strains being the pediococci and *Lb. brevis* 2.

Table 4.2. Antimicrobial activity of lactic acid bacteria against pathogens indicator strains

Isolates	Indicator strains			
	E. coli	*Staph aureus*	*Salmonella typhimorium*	*Vibrio cholera*
Lb.fermentum 1	++	+++	++	+++
Lb. fermentum 2	+	++	++	++
*Lb. brevis*1	-	+	-	++
Lb. brevis 2	-	-	-	++
Lb. plantarum	+	++	+	+
P. acidilactici	-	+	+	-
P. pentosaceus	-	+	-	-

-: no inhibition, +: 1-2 mm inhibition zone, ++: 3-4 mm inhibition zone, +++:5+ mm inhibition zone

Acid production and rate of acidification of isolates

The rate of acidification of lactic acid bacteria cultures was determined as described by Sawadogo-Lingani et al. (2007). 300 g of dehulled maize grains were steeped in 450 ml of sterile water. The mixture was inoculated with a 16 h culture of a LAB isolate at a concentration of about 10^6 CFU/g and incubated at 37ºC. A control was set up as an uninoculated sample using non-serilized pipe-borne water. During steeping samples of the steep water were taken at 4 h intervals for determination of pH and titratable acidity. The rate of acidification was calculated as ΔpH or ΔTTA according to Ayad*et al.*(2004), being the defence in value recorded between the inoculated and control sample.

For dough fermentation, 200g of the milled steeped grains was mixed with 100ml of sterile water and also inoculation at a concentration of about 10^6 CFU/g.

The changes in pH and titratable acidity in steep water and maize dough during the two stages of fermentation in the production of white kenkey using isolates of *Lb. fermentum, Lb. brevis, C. krusei,* and *S. cerevisiae*are shown in tables 4.3 and 4.4. Two clear patterns emerged; the use of any of the cultures resulted in a more rapid drop in pH due to a faster production of acid than in the uninoculated control sample. Also, as expected, the use of LAB resulted in a more rapid drop in pH than the use of the yeasts

Table 4.3. Changes in pH during steeping and dough fermentation of white kenkeyusing starter cultures

Sample	Fermentation time	Starter culture				
		Control	*Lb. fermentum*	*Lb. brevis*	*C. krusei*	*S. cerevisiae*
Steep water						
	0	6.00	5.96	5.96	6.00	6.02
	24	4.02	3.56	3.58	3.61	3.60
	48	3.54	3.46	3.54	3.56	3.55
Fermenting dough						
	0	6.02	5.99	6.02	6.03	6.03
	4	5.46	5.28	5.32	5.40	5.41
	8	3.72	3.46	3.53	3.50	3.49
	12	3.47	3.38	3.41	3.41	3.44

Table 4.4. Changes in titratable acidity during steeping and dough fermentation of white kenkeyusing starter cultures

Sample	Fermentation time	Starter culture				
		Control	*Lb. fermentum*	*Lb. brevis*	*C. krusei*	*S. cerevisiae*
Steep water						
	0	0.03	0.04	0.04	0.04	0.03
	24	0.26	0.31	0.29	0.28	0.28
	48	0.30	0.38	0.35	0.35	0.34
Fermenting dough						
	0	0.26	0.29	0.27	0.27	0.25
	4	0.28	0.32	0.32	0.30	0.28
	8	0.32	0.38	0.41	0.34	0.33
	12	0.37	0.53	0.47	0.47	0.44

With regards to rate of acidification 7 isolates of LAB were tested and the two isolates of *Lb. fermentum*showed a higher rate of acidification than the other LAB during steeping. In all instances the rate of acidification of the isolates changed at different periods during steeping or dough fermentation. As seen from figure 4.1, the isolates which showed the highest rate of acidification at various stages of steeping and dough fermentation were *Lb. fermentum*2 and *Pediococcuspentosaceus.*

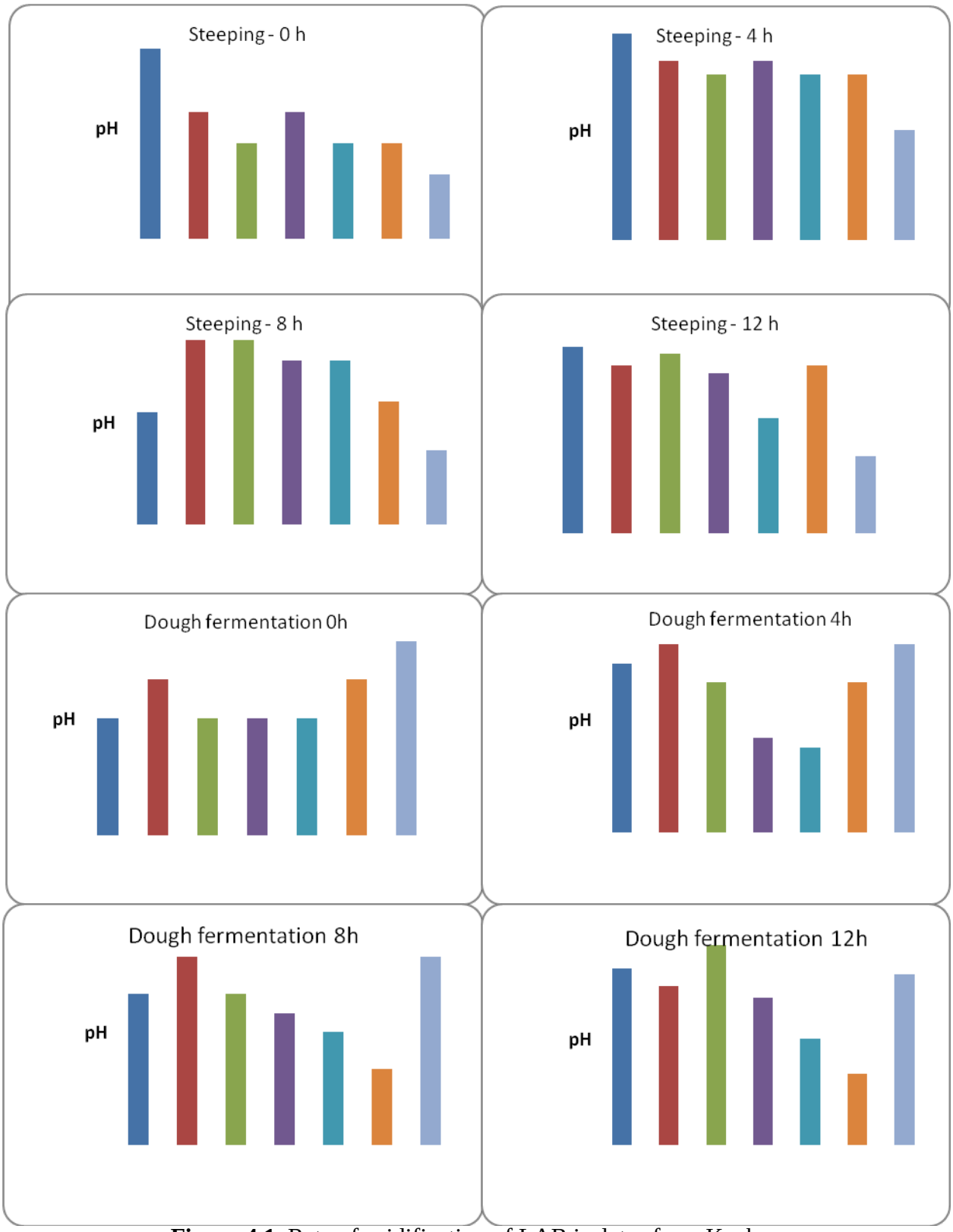
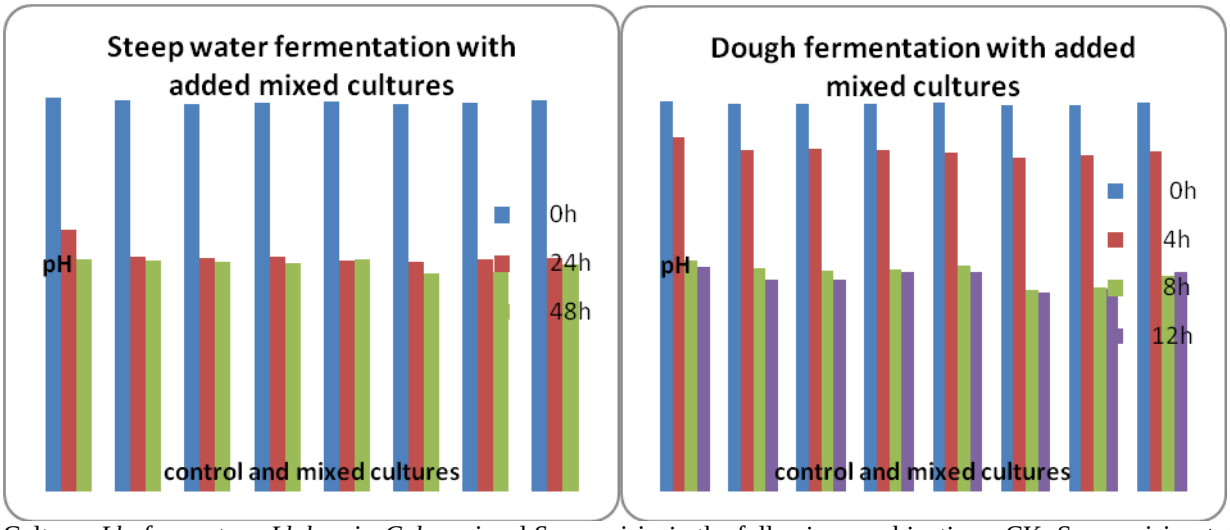


Figure 4.1. Rate of acidification of LAB isolates from Kenkey

Fermentation trials and sensory analysis

Fermentation trials

Dehulled maize grainsweighing 2.5 kg were steeped in 5.0 l of water and inoculated with 10^7CFU/g of LAB and/or 10^6 CFU/g of yeast and left to steep for 24 h. The steeped grains were milled and mixed with 100 ml of water into a dough and allowed ferment for 48 h. The steepwater was sampled at 0, 24, and 48h and fermenting dough at 0, 4, 8 and 12 h for determination of pH, titratable acidity and microbiological analysis. The LAB isolates used were *Lb. fermentum*and *Lb. brevis*, and the yeasts *C. krusei* and *S. cerevisiae.* Inoculations were carried out either as single or mixed cultures.



Cultures:*Lb. fermentum*, *Lb.brevis*, *C. krusei* and *S. cerevisiae*in the following combinations: CK: S. *cerevisiae* + *C. krusei;* FK*: Lb. fermentum* + *C. krusei;* FC*: Lb. fermentum* +S. *cerevisiae*, FCK: *Lb. fermentum* +S. *cerevisiae*+ *C. krusei;* FBKC: *Lb. fermentum*+ *Lb.brevis*+ *C. krusei*+ *S. cerevisiae;* FBK: *Lb.fermentum*, *Lb.brevis*, *C. krusei* and CBK: *S. cerevisiae*+*Lb.brevis*+ *C. krusei*

Figure 4.2. Changes in pH during steeping and dough fermentation following inoculation with mixedstarter cultures

The pH values recorded during the fermentation trials involving mixed starter cultures are shown in figure 4.2.

Sensory evaluation

The dehulled maize dough fermented with different starter cultures were used to prepare white kenkey and assessed by an untrained panel made up of 20 members. The products including a control sample were assessed for colour, odour, texture, taste and overall acceptability using a nine-point hedonic scalewhere 1 = dislike extremely and 9 = like extremely. All the white kenkey produced using the various starter cultures were acceptable to the panel and scored between 6.0 representing like slightly to just less than 7.0 representing like moderately (Table 4.5). The control sample scored 6.3 for overall acceptability. The highest score for overall

acceptability of 6.73 was for white kenkey prepared with a mixed culture comprising *Lb. fermentum, Lb. brevis*, and *C. krusei.* The product with the lowest acceptability score of 6.0 was the mix culture comprising *Lb. fermentum*and the two yeasts *S. cerevisiae* and *C. krusei.* The bi-plot of the principal component analysis of the affective sensory evaluation results is shown in figure 4.3. It showed that starter cultures which included *Lb. brevis* tended to have a good taste which enhanced their acceptability y the taste panel.

Table 4.5. Sensory evaluation of kenkey made using fermented dough by selected culture

Starter culture	Odour	Taste	Texture	Overall acceptability
Control	6.23±0.18	6.40±0.00	6.33±0.11	6.30±0.07
L. brevis	6.63±0.04	6.65±0.42	6.83±0.32	6.58±0.67
L. fermentum	6.65±0.21	6.28±0.60	6.25±0.42	6.25±0.35
C. krusei	6.80±0.35	6.53±0.04	6.73±0.30	6.33±0.46
L fermentum + S. cerevisae(FC)	6.38±0.18	6.50±0.28	6.78±0.04	6.33±0.46
L. fermentum + C. krusei(FK)	6.38±0.18	6.53±0.11	6.63±0.11	6.23 ±0.53
L. brevis + C. krusei(BK)	6.73±0.25	6.43±0.11	6.75±0.14	6.30±0.14
L. ferm + S. cere + C. krusei(FCK)	6.58±0.39	6.28±0.18	6.40±0.07	6.00±0.00
L. ferm + L. brev + C. krusei(FBK)	6.00±0.07	6.65±0.57	7.08±0.46	6.73±0.67
L. ferm + L. brev + C kru + S. cere(FBKC)	6.18±0.18	6.35±0.49	7.09±0.25	6.28±0.18

Mean and standard deviation of scores for 20 panellists on a nine point hedonic scale, 1=dislike extremely, 9=like extremely

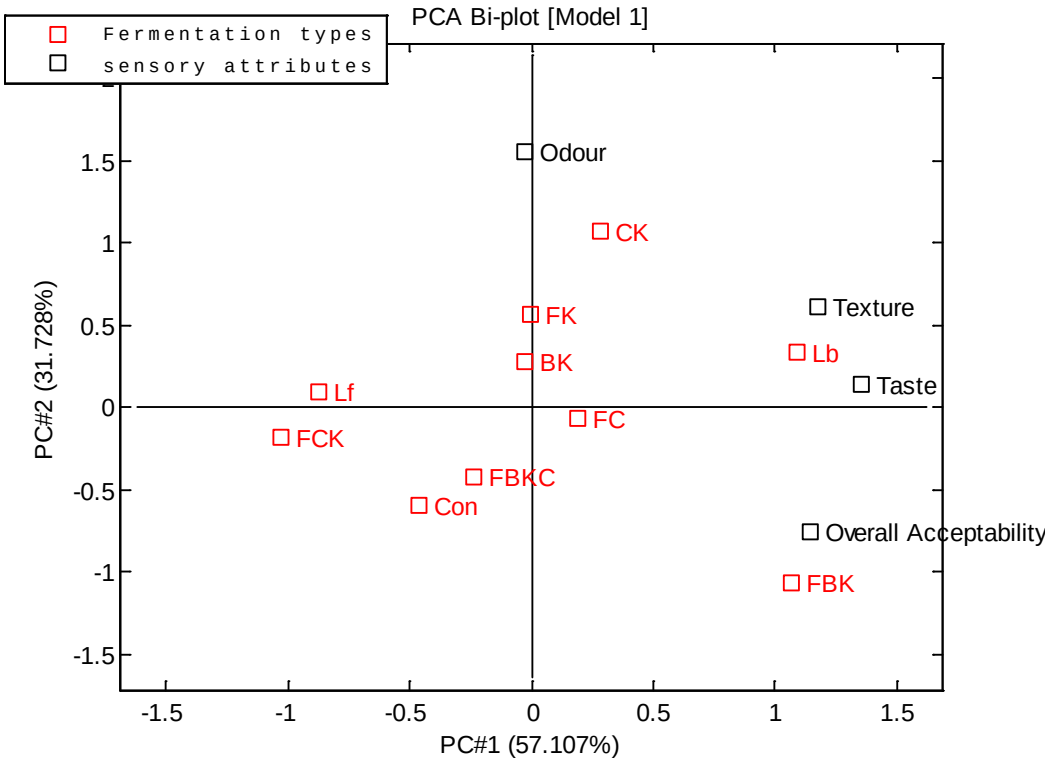


Figure 4.3. Biplot of sensorial analysis of white kenkey prepared with a mixed culture

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