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Full Length Research Paper

Nigerian indigenous yoghurt (kindirmo) production using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* mutants as starter culture

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The production of Nigerian indigenous yoghurt (kindirmo) using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* mutants as starter culture was investigated. The results of milk fermentations using *L. bulgaricus* and *S. thermophilus* mutant isolates when compared with their wild-type strains (control) indicated that the pH values of "kindirmo" produced at different incubation periods (h) and the mean percentage titratable acidity were not significantly different ($p > 0.05$) when compared with the control. The pH values of "kindirmo" produced using *L. bulgaricus* mutant isolates at different incubation periods (h) and the mean percentage titratable acidity were not significantly different ($p > 0.05$) when compared with the control. Also, these physicochemical properties of color [yellow (9/12) and white (3/12)], consistency [(thick and uniform (2/12), thick (6/12), not so thick (1/12), very thick (1/12), very thick and uniform (1/12)] and presence of water [trace amount of water (4/12), water present (4/12), water moderately present (2/12), water in large amount present (1/12)] were observed. The pH values of "kindirmo" produced by *S. thermophilus* mutant isolates at different incubation periods (h) and the mean percentage titratable acidity were not significantly different ($p > 0.05$) when compared with the control. Also, these physicochemical properties of color [yellow (5/6) and white (1/6)], consistency [(thick and uniform (1/6), thick (3/6), not so thick (2/6)] and presence of water [(water present (3/6), trace amount of water present (2/6), water moderately present (1/6)] were observed. It should be noted that, the "kindirmo" produced in this work was not from a mixed culture of *L. bulgaricus* and *S. thermophilus* mutant isolates when compared with the normal procedures known. It is possible that if these mutant isolates are to be used in dairy industry as a mixed culture, it will give better and high quality yoghurt.

Key words: Starter culture, titratable acidity, consistency, physicochemical properties, kindirmo.

INTRODUCTION

Lactic acid bacteria are used in the production of fermented milk products, beverages, vegetable foods, sourdough, cheese and sausage. In northern Nigeria, the skimmed (defatted) milk is known as "nono" while the full fat or partially skimmed milk is referred to as "kindirmo" (Hayakawa, 1994). According to Kroger et al. (1992), modern yoghurt is defined by the regulation of many governments to be made from and to contain *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* but with no hard and fast rules and theoretically, any combination of organisms could be utilized to make a fermented milk product. The ultimate taste is palatability. According to Adams and Moss (1995), both *L. bulgaricus*

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and *S. thermophilus* lack an alcohol dehydrogenase which could reduce acetaldehyde to ethanol.

L. bulgaricus belong to the genus Lactobacillae which are gram positive, non-spore forming rods, catalase negative, microaerophilic with homofermentative and some have heterofermentative metabolism (Bock and Madigan, 1991; Prescott et al., 1999; Tserovska et al., 2002). *Lactobacilli* form the normal flora of the human body in the gut and vagina. They are non-pathogenic and toxigenic. They retain viability during storage (Macfarlane and Cummings, 1999). They inhibit the growth of other microorganisms and form the basis of their ability to improve the keeping quality and safety of many food products. *Lactobacillus delbruieki* sub-specie *bulgaricus* is used in preparation of yoghurt, *Lactobacillus acidophilus* in preparation of a*cidophilus* milk and other species are used in the production of vegetable foods, beverages, sourdough, cheese and sausage (Brock and Madigan, 1991; Fashakin and Umokiwedi, 1993; Adams and Moss, 1995; Prescott et al., 1999). This species is closely related to *Lactobacillus lactis* being morphologically indistinguishable, producing the same amount of D (-) lactic acid in milk. The only significant difference is that *L. bulgaricus* ferment few sugars than *L. lactis*, which might be a mutant or variant of the former, produces no gas from glucose or gluconate, does not ferment ribose and produces D (L) lactic acid (Buchanan and Gibbons, 1974).

S. thermophilus and other cocci belong to the genus lactococci with quite different habitats, whose activities are of considerable practical importance to humans. Some are pathogenic to humans and animals (Brock and Madigan, 1991). *Streptococcus* spp form the dairy starter culture and have a complex proteolytic system which enables them grow in milk by degrading casein into small peptides and free amino acids. This leads to the development of texture and flavor of various dairy products (Rao et al., 1998). This specie is easily recognizes by its high temperature limit of growth, thermal tolerance, inability to ferment maltose and inability to grow in media containing 2.0% NaCl. Its optimum temperature is between 40 to 45°C. Growth occurs at 50°C but not at 35°C. Final pH range in glucose broth is 4.0 to 5.0. Acid is produced from glucose, fructose, lactose and sucrose. No acids are produced from trehalose, maltose, inulin, glycerol, mannitol, sorbitol or salicin and are rarely from rafinose, xylose or arabinose (Buchanan and Gibbons, 1974). The aim of this study was to compare the "Kindirmo" produced by lactic acid bacterial mutant isolates and their wild-type so as to enhance indigenous yoghurt (kindirmo) production.

MATERIALS AND METHODS

'Kindirmo' starter culture (wild-type strain) was purchased from Fulani women hawking "kindirmo" in the Federal University of Technology, Yola, mutagenesis and mutants were selected as described by Sudi et al. (2008) and fresh milk was obtained from Fulani women at Bajabure, Girei Local Government Area of Adamawa State, Nigeria. Measurement of pH and percentage titratable acidity was done according to standard methods. Physicochemical properties of "kindirmo" were determined as described by Sudi (2006).

RESULTS

The results of milk fermentations using *L. bulgaricus* and *S. thermophilus* mutant isolates compared with their wild-type strains (control) are shown in Tables 1 and 2, respectively. The pH values of "kindirmo" produced by *L. bulgaricus* and *S. thermophilus* mutants at different incubation periods (h) and the mean percentage titratable acidity were not significantly different ($p > 0.05$) when compared with the control. These physicochemical properties of color [yellow (9/12) and white (3/12)], consistency [thick and uniform (2/12), thick (6/12), not so thick (1/12) and very thick (1/12), very thick and uniform (1/12)] and presence of water [trace amount of water (4/12), water present (4/12), water moderately present (2/12), water in large amount present (1/12)] were observed.

The pH values of "kindirmo" produced by *S. thermophilus* mutant isolates at different incubation periods (h) and the mean percentage titratable acidity were not significantly different ($p > 0.05$) when compared with the control. These physicochemical properties of color [(yellow 5/6 and white 1/6)], consistency [(thick and uniform 1/6, thick 3/6, not so thick 2/6)] and presence of water [(water present 3/6, trace amount of water present 2/6, water moderately present 1/6)] were also observed.

DISCUSSION

The technology of yoghurt production by Fulani is as old as dairying itself (Adams and Moss, 1995). Sometimes there are complains about the taste of "kindirmo", its sourness (not always but rampant during hot periods) due to acidity, thus, it has low pH. Sometimes the fermentation process may be slow, probably due to drop in temperature or other contaminants (Adams and Moss, 1995; Abdullahi et al., 2001; Igyor, 2005) and are referred to as "lazy-milk". There were attempts to address some of these problems enumerated earlier like fermentation of "lazy-milk" using yeast extract supplementation (Igyor, 2005) which is still at the experimental stage.

The result of the fermented milk from *L. bulgaricus* and *S. thermophilus* mutants respectively showed no significant difference in pH and mean percentage titratable acidity when compared with the control, but the result of the physicochemical properties (color, consistency and presence of water) would be good for dairy industry. This

Table 1. Titratable acidity, pH and physicochemical properties of fermented milk ("Kindirmo") produced by *L. bulgaricus* mutants (Lbm) and their wild-type (LbWc).

Isolate	pH at incubation period (h)				Mean% titratable acidity	Physicochemical properties		
	4	8	16	24		Color	Consistency	Water
LbWc	6.36±0.19	5.90±0.24	5.36±0.07	4.95±0.29	0.315	Yellowish	thick	+
Lbm 101	6.27±0.19	5.97±0.22	5.15±0.15	4.77±0.28	0.243	Yellowish	thick	+
Lbm 102	6.29±0.17	5.85±0.10	5.18±0.10	4.63±0.30	0.234	Yellowish	TU	+
Lbm 104	6.32±0.16	5.79±0.27	5.19±0.16	4.86±0.29	0.243	Yellowish	thick	+
Lbm 113	6.33±0.19	6.01±0.08	5.33±0.06	4.74±0.04	0.216	Yellowish	TU	+
Lbm 119	6.27±0.20	5.45±0.44	5.07±0.44	4.79±0.38	0.297	Yellowish	VTU	+
Lbm 121	6.35±0.01	5.82±0.13	5.24±0.33	4.66±0.61	0.243	Whitish	NT	+++
Lbm 122	6.33±0.07	5.84±0.34	5.10±0.40	4.68±0.57	0.207	Yellowish	VT	+
Lbm 131	6.29±0.08	5.69±0.36	4.97±0.47	4.51±0.35	0.243	Yellowish	NTU	+
Lbm 132	6.40±0.11	8.2±0.39	5.7±0.07	4. 88±0.29	0.252	Yellowish	thick	++
Lbm 133	6.32±0.14	5.76±0.33	5.24±0.31	4.79±0.42	0.261	Whitish	thick	++
Lbm 134	6.28±0.15	5.81±0.28	5.18±0.03	4.65±0.49	0.243	Whitish	thick	+

±, Trace amount of water present; +, water present; ++, water moderately present; +++, water in large amount present; U, thick and uniform; VTU, very thick and uniform; NT, not so thick; VT, very thick; NTU, not so thick and uniform. Values are mean of three determinations ± S.D.

Table 2. Titratable acidity, ph and physicochemical properties of fermented milk ("kindirmo") produced by *streptococcus thermophilus* mutants (Stm) and their wild-type (Stwc).

Isolate	pH at incubation period (h)				Mean% titratable acidity	Physicochemical properties		
	4	8	16	24		Color	Consistency	Water
StWc	6.26 ± 0.12	5.70 ± 0.29	5.04 ± 0.40	4.57 ± 0.37	0.216	Yellowish	thick	+
Lbm 104	6.34 ± 0.04	5.81 ± 0.28	5.02 ± 0.23	4.46 ± 0.38	0.225	Whitish	thick	+
Lbm 109	6.28 ± 0.12	5.89 ± 0.14	5.14 ± 0.37	4.70 ± 0.031	0.225	Yellowish	thick	+
Lbm 140	6.42 ± 0.35	5.98 ± 0.08	5.10 ± 0.42	4.78 ± 0.29	0.225	Yellowish	NT	+
Lbm 142	6.35 ± 0.10	5.84 ± 0.021	5.29 ± 0.18	4.74 ± 0.33	0.252	Yellowish	thick	++
Lbm 143	6.30 ± 0.07	5.90 ± 0.06	5.20 ± 0.26	4.54 ± 0.52	0.243	Yellowish	NT	++
Lbm 144	6.35 ± 0.13	5.80 ± 0.16	5.13 ± 0.06	4.72 ± 0.21	0.243	Yellowish	TU	+

±, Trace amount of water present; +, water present; ++, water moderately present; TU, thick and uniform; = not so thick. Values are mean of three determinations ± S.D.

might be because the creamy texture of the "kindirmo" produced with low water content could be indicative of complete milk precipitation (coagulation is induced by *S.thermophilus* and *L. bulgaricus*) (Bautista et al.,1966) and suitable fermentation period of 24 h incubation. This was supported by Abu-Foul et al. (2007) that yoghurt from cottonseed with thin consistency and light green color was rated poor by sensory panelist.

The use of *L.bulgaricus* LB207 as a starter culture is still recommended in this work because of its high antioxidant activity as reported by Kim et al. (2005) that, antioxidant activity of *L. bulgaricus* LB207 is "due to its strong hydroxy radical scavenging activity and reducing power". It was also reported by Soomro and Masud (2008), that "the strains of *L. delbrueckii* ssp. *bulgaricus* Lb5, Lb7 and *S. thermophilus* St4 and St7, produced more acidity after 6 h of incubation in skim milk media".

It should be noted that, the "kindirmo" produced in this work was not from a mixed culture of *L. bulgaricus* and *S. thermophilus* mutant isolates when compared with the normal procedures known (Adams and Moss, 1995; Abdullahi et al., 2001; Buchanan and Gibbons, 1974; Hayakawa, 1994). Observation by Bautista et al. (1966) showed that *L. bulgaricus* gradually accumulates glycine and histidine thereby, increasing rapid production of acid by *S. thermophilus*. It is possible that if these mutant isolates are to be used in dairy industry as a mixed culture, it will give better and high quality yoghurt to take care of the

teaming human population.

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