*Journal of Applied Biosciences (2009), Vol. 13: 714 - 719.*
*ISSN 1997 – 5902: www.biosciences.elewa.org*

# Influence of lactic starters on sensory properties and shelf life of *wara* – a Nigerian (unripened) soft cheese

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[redacted] *Published online on January 5, 2009.*

ABSTRACT
*Objectives*: The effect of lactic starter cultures on the sensory properties and shelf life of *wara* (an unripened soft cheese) was investigated.
*Methodology and results*: Pasteurized fresh milk samples were inoculated with selected lactic acid bacteria (1%) singly or in combination and fermentation was carried out using a modified traditional method. Proximate analysis showed that milk fermented with lactic starters had higher protein contents than that produced by natural fermentation. Samples produced using combined culture of *Lactobacillus bulgaricus* and *L. plantarum* had the highest protein content (29.5%) while the sample produced by natural fermentation had the lowest protein content (26.66%). The moisture content of the samples ranged between 26.64 and 32.09%. According to sensory evaluation report the samples produced with lactic acid bacteria were rated better than those produced by natural fermentation. In addition to this, the product produced with combined starter culture comprising of *L. bulgaricus* and *L. plantarum* was rated best in all the parameters tested. The pH of the *wara* samples ranged between 4.12 and 4.78 while the titratable acidity (TA) was between 0.119 and 0.143% during the storage period (96 h). The cheese produced with lactic acid bacteria had relatively low microbial counts as compared to the sample produced by natural fermentation after 96 h storage. The sample produced by natural fermentation showed signs of spoilage within 72 h of storage whereas samples produced with lactic acid bacteria were still in good condition till the end of storage (96 h).
*Conclusion and application of findings*: It is concluded that the use of combined starter culture of *L. bulgaricus* and *L. plantarum* leads to improvement in nutritional quality, acceptability and shelf life and thus should be used in preparation of *wara*.

Key words: Lactic acid bacteria, biopreservation, milk fermentation, *wara*, shelf life.

Citation: [redacted]

## INTRODUCTION

Milk is one of the key sources of protein and others nutrients to consumers (Anderson & Sobieski, 1978). Milk proteins are ideal in that they are complete and have high essential amino acids composition. Although milk and its various derivatives form a vital human food, it also provides an excellent medium for the growth of many kinds of micro-organisms (Anderson *et al.*, 1978). Examples of fermented milk products are yoghurt (Olubamiwa & Kolapo, 2008), *nono*

(Adesokan *et al*., 2008a), *wara* (Ashaye *et al*., 2006) and cheese (Rodriguez *et al*., 2005; Cetinkaya & Soyutemiz, 2004).

*Wara* is a Nigerian soft white unripened cheese that originated from Fulani cattle rearers in the Northern part of the country (Ogundiwin, 1978). It is commonly produced by Fulani women from unpasteurized cow milk and sold along the major streets of Nigeria. Despite advances in science and technology in Africa, the production of fermented foods is still largely a traditional art associated with poor hygiene, inconsistent quality and short shelf life (Onyekwere *et al*., 1989). The traditional processing method for making *wara* does not take into cognizance quality control measures while unhygienic conditions of milking and processing of cheese make the risk of microbial contamination very high. These contribute not only to the short shelf life of the product but also more importantly to its potential health hazard (Ashaye *et al.,* 2006). As a means of improving the quality and safety of fermented food products the use of lactic starter cultures in fermentation was suggested (Olasupo *et al.,* 1995).

Lactic acid bacteria (LAB) convert milk and its products into other desirable foods (Litopoulou-Tzanetaki *et al*., 1993; Randazzo *et al*., 2002) and are also widely used to improve the flavour, nutritional quality and shelf life of fermented foods (Soomro *et al*., 2002, Ogunbanwo & Okanlawon, 2006; Adesokan *et al*., 2008b). The present work aimed to enhance the nutritional quality and shelf-life of *wara* using lactic acid bacteria.

## MATERIALS AND METHODS

**Milk samples:** Fresh cow milk and *wara* samples for use were collected aspetically from Gaa Apaara, Oyo town, Oyo State, Nigeria. The samples were transported in cooler containing ice packs to the laboratory for immediate analysis.

**Bacteria strains and cultures:** Lactic acid bacteria (LAB) strains were isolated from *wara* samples. One gram of sample was added to 9ml of 0.85% (w/v) NaCl solution and homogenized. Isolation of LAB was selectively made from the dilutions $10^{-5}$ – $10^{-9}$ on MRS (de Mann *et al.,* 1960) agar plates and incubated anaerobically at 30°C for 48 h. Repeated streaking of the isolates was done on fresh MRS agar plates to purify the cultures. LAB isolates were characterized using API 50CH strips and API 50CHL medium (API System, Biomerieux Sa, France), other biochemical tests were performed where necessary. The identity of the isolates was confirmed with reference to Bergey's Manual of Determinative Bacteriology (Kandler & Weiss, 1986). The indicator organisms used for assaying antimicrobial activity of LAB were obtained from the Culture Collection of the Department of Biology, The Polytechnic, Ibadan, Nigeria. The indicator organisms were *Escherichia coli, Staphylococcus aureus, Bacillus cereus, B. subtilis* and *Proteus vulgaris.*

**Antimicrobial activity of lactic acid bacteria:** The method used to detect antimicrobial activity of LAB strains was a well diffusion assay as previously described by Adesokan *et al*. (2008b). The LAB strains with distinct and highest diameter of zones of inhibition were selected for evaluation as starter cultures.

**Preparation of *wara* samples:** *Wara* was prepared using a traditional method described by Ashaye *et al.* (2006) with modifications. The milk was pasteurized at 72°C for 20 s and then cooled to 40°C to enhance the activity of sodom apple proteinase enzyme (applied as described below) and the starter cultures. The pasteurized milk sample was inoculated with the selected lactic starters (1%) singly or in combination, with an uninoculated sample serving as control (Okagbue & Bankole, 1992). Then, sodom apple stem was crushed and the juice extracted (1% w/v) into a little quantity of the warm milk. The mixture was then heated to about 70°C for 20 min. The scum was then removed and *wara* cut into small pieces (50g) and allowed to drain for 2 minutes.

**Physico-chemical analysis:** pH of *wara* samples was measured with a standardized pH meter apparatus (Crison MicropH, 2000). To determine the Titratable Acidity (TA) of *wara*, 1g of the prepared sample was weighed and homogenized in 9ml of distilled water. Two to three drops of phenolphthalein indicator was added to the mixture and this was titrated against 0.1M sodium hydroxide solution (Achi & Akobor, 1999). Proximate parameters such as protein, ash, fat, fibre, carbohydrate and moisture content were determined

using standard procedures as described by AOAC (1990).

Sensory analysis: A 10-member panel of judges who are familiar with the products did sensory analysis of all wara samples produced. The judges evaluated the samples for appearance, taste, texture, colour, aroma and overall acceptability using a 5-point hedonic scale (where 5 = Excellent, 1 = Poor).

Shelf life study: Storage was done according to a modified traditional method described by Ashaye (2006). *Wara* samples were soaked in their whey inside sterile plastic containers and stored at ambient temperature (28±2 °C) for 5 days. During storage, changes in pH and TA were assayed as described above. For microbiological analysis 10 g of *wara* samples was homogenized in 90 ml sterile normal saline in a stomacher. The samples were serially diluted and appropriate dilutions were then plated on MRS agar, nutrient agar and MacConkey agar for enumeration of LAB, bacteria (aerobic and anaerobic) and coliform, respectively. The plates for LAB count were incubated anaerobically at 30 °C for 48 h; while the plates for viable count and coliform count were incubated at 30 °C for 24 h.

## RESULTS

A total of fifty-two strains of lactic acid bacteria (LAB) belonging to *Lactobacillus sp* were isolated from different samples of *wara*. The main species were *Lactobacillus plantarum, L. bulgaricus, L. casei* and *L. fermentum*. The zones of inhibition ranged between 2 and 14mm (Table 1). The strains of *Lactobacillus* with highest zones of inhibition were selected as starter culture for production of *wara.* The proximate analysis of *wara* samples (table 2) revealed that all *wara* produced with lactic starter cultures had increased protein content (P ≤ 0.05) than *wara* produced with spontaneous fermentation (sample D). Sample (C) produced with combined starter culture comprising of *L. bulgaricus* and *L. plantarum* had the highest protein content (29.57%) while *wara* produced with natural fermentation had the lowest (26.66%). Sample D also had the highest moisture content (32.05%) while sample C had the lowest (26.64%).

Table 1: Antagonistic activity of *Lactobacillus* spp. metabolites against some indicator organisms.

| LAB isolates | *Escherichia coli* | *Staphylococuss aureus* | *Bacillus cereus* | *Bacillus subtilis* | *Proteus vulgaris* |
|---|---|---|---|---|---|
| *L. plantarum* | 12[a] | 14 | 13 | 10 | 6 |
| *L. bulgaricus* | 7 | 12 | 11 | 10 | 8 |
| *L. fermentum* | 5 | 9 | 8 | 6 | 2 |
| *L. brevis* | 7 | 10 | 6 | 8 | 5 |
| *L. casei* | 4 | 8 | 5 | 7 | 2 |

LAB = Lactic acid bacteria; [a]Data are diameter of inhibition zones in millimeters

Sensory evaluation (table 3) showed that the use of combined starter cultures (sample C) significantly (P ≤ 0.05) improved the appearance, taste, texture, colour, aroma as well as overall acceptability of the product with sample C being the most generally accepted (4.50) while *wara* produced without LAB was the least accepted (2.50).

The pH of *wara* samples during storage ranged between 4.12 and 4.78 after 96 h of storage (table 4). Moreover, changes in titratable acidity (TA) of the *wara* samples (table 5) were observed during storage showing a significant increase (P ≤ 0.05) ranging from 0.119 and 0.143% at the end of storage.

The viable counts to determine microbiological changes showed that fermented with lactic acid bacteria had relatively low microbial load compared to the fermented by natural fermentation. Also, the samples fermented with starter culture had significantly lower (P ≤ 0.05) coliform count than the sample fermented without lactic acid bacteria. The LAB count of the sample produced lactic starter culture was significantly higher (P ≤ 0.05) than that of the sample produced without starter culture. Also, *wara* sample produced with combined starter culture of *L. bulgaricus* and *L. plantarum* had the highest LAB count (log cfu/g) of 7.96 while the sample produced without LAB had the lowest (7.73) at the end of storage. During storage of *wara*, physical examination showed signs of spoilage within 72 hours of storage in sample produced without

LAB while samples produced with lactic culture were still in good condition until the end of storage (96h).

Table 2: Proximate analysis of *wara* – a Nigerian (unripened) soft cheese produced using various starter cultures.

| Sample | % Protein | % Fat | % Ash | % Fibre | % CHO | % M.C. |
|---|---|---|---|---|---|---|
| A | 27.62±0.02$^{aa}$ | 25.30±0.01$^{ab}$ | 4.75±0.03$^{ac}$ | 3.24±0.02$^{ad}$ | 8.49±0.03$^{ae}$ | 30.570.01$^{af}$ |
| B | 28.72±0.03$^{ba}$ | 25.05±0.04$^{bb}$ | 4.85±0.03$^{bc}$ | 3.67±0.01$^{bd}$ | 11.00±0.02$^{be}$ | 29.040.01$^{bf}$ |
| C | 29.57±0.03$^{ca}$ | 25.18±0.02$^{cb}$ | 4.55±0.01$^{cc}$ | 3.16±0.02$^{cd}$ | 8.49±0.05$^{ce}$ | 26.640.02$^{cf}$ |
| Control | 26.66±0.01$^{da}$ | 25.20±0.02$^{db}$ | 4.63±0.03$^{dc}$ | 3.46±0.02$^{dd}$ | 7.72±0.03$^{de}$ | 32.050.02$^{df}$ |

Values are means (n=3) ± Standard Deviation. Means with different superscripts are significantly different along the rows and columns. **A = *Wara* sample produced with *Lactobacillus bulgaricus*.; B = *wara* produced with *L. plantarum*; C= *wara* produced with a mixture of *L. bulgaricus* and *L. plantarum*; D= *wara* produced by spontaneous fermentation.

Table 3: Sensory evaluation of *wara*- a Nigerian (unripened) soft cheese produced using various starter cultures.

| Sample | Appearance | Taste | Texture | Colour | Aroma | Acceptability |
|---|---|---|---|---|---|---|
| **A | *3.50±0.52$^{aa}$ | 3.75±0.75$^{ab}$ | 3.50±0.51$^{ac}$ | 3.50±0.67$^{ad}$ | 3.58±0.51$^{ae}$ | 3.75±0.45$^{af}$ |
| B | 3.83±0.58$^{ba}$ | 3.67±0.49$^{bb}$ | 3.67±0.78$^{bc}$ | 3.75±0.62$^{bd}$ | 3.75±0.62$^{be}$ | 3.92±0.29$^{bf}$ |
| C | 4.25±0.75$^{ca}$ | 3.75±0.62$^{cb}$ | 3.75±0.75$^{cc}$ | 3.83±0.83$^{cd}$ | 3.75±0.75$^{ce}$ | 4.50±0.52$^{cf}$ |
| Control | 2.42±0.51$^{da}$ | 2.50±0.52$^{db}$ | 2.42±0.51$^{dc}$ | 2.50±0.67$^{dd}$ | 2.83±0.58$^{de}$ | 2.50±0.52$^{df}$ |

*Values are means (n=3) ± Standard Deviation. Means with different superscripts are significantly different along the rows and columns. **Sample codes are as stated in table 2.

## DISCUSSION

The lactic acid bacteria isolated from *wara* samples belong to the genus *Lactobacillus* species. Members of this genus have been reported in milk and fermented milk products (Okagbua & Bankole, 1992; Oyewole, 1997; Sayadogo *et al.,* 2004; Guessas & Khal, 2004). All the strains of *Lactobacillus sp.* demonstrated antimicrobial activity against the indicator organisms employed in the agar diffusion assay. Lactic acid bacteria have been reported to exhibit antagonistic activity against many pathogenic microorganisms (Harris *et al*., 1989; Rodriguez *et al.,* 1997; Savagodo *et al*., 2004; Belfiore *et al.,* 2007).

The *wara* samples produced with lactic acid cultures had a better protein content than the one produced by natural fermentation. Furthermore, the *wara* produced with combined starter culture comprising of *L. bulgaricus* and *L plantarum* had higher protein content than all other samples. Lactic acid bacteria are known to enhance nutritional quality of fermented food products (Caplice *et al*., 1999; Onilude *et al*., 2002; Adesokan *et al*., 2008a).

The sensory properties of the *wara* produced with lactic acid bacteria were superior to that of *wara* produced by spontaneous fermentation. This could be due to flavouring compounds such as diacetyl and acetoin produced by the lactic starter cultures (Okagbue & Bankole, 1992; Bassit *et al.,* 1993; Boumerdassi *et al.*, 1996, Boumerdassi *et al.*, 1997).

During the five day storage of *wara* samples, a decrease in pH was observed with a corresponding increase in TA. This might be as a result of fermentation of the product during storage. The *wara* samples produced with starter cultures had lower microbial counts than *wara* produced by natural fermentation, possibly as a result of antimicrobial metabolites such as lactic acid, diacetyl, hydrogen peroxide and bacteriocin produced by the lactic starter cultures (Harris *et al*., 1989; Lewus *et al*., 1991; Bassit *et al.*, 1993; Einarsson & Lauzon, 1995; Zalan *et al*., 2005; Veljovic *et al.*, 2007). Furthermore, the shelf life of *wara* produced with lactic cultures was 96 hours while the one produced by natural fermentation was 72 hours. This agreed with the findings of Ogunbanwo *et al.* (2004) who extended the shelf life of *fufu* using bacteriocinogenic *Lactobacillus spp*.

Table 4: Changes in pH during storage of *wara*- a Nigerian (unripened) soft cheese produced using various starter cultures.

| Sample | 0 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|
| A** | *5.96±0.02$^{aa}$ | 5.74±0.04$^{ab}$ | 4.92±0.02$^{ac}$ | 4.25±0.02$^{ad}$ | 4.12±0.007$^{ae}$ |
| B | 5.94±0.04$^{ba}$ | 5.83±0.06$^{bb}$ | 4.96±0.01$^{bc}$ | 4.28±0.03$^{bd}$ | 4.16±0.02$^{be}$ |
| C | 5.45±0.66$^{ca}$ | 5.89±0.02$^{cb}$ | 5.00±0.007$^{cc}$ | 4.36±0.02$^{cd}$ | 4.21±0.01$^{ce}$ |
| Control | 6.07±0.007$^{da}$ | 5.25±0.02$^{db}$ | 5.12±0.01$^{dc}$ | 4.97±0.01$^{dd}$ | 4.78±0.01$^{de}$ |

*Values are means (n=3) ± Standard Deviation. Means with different superscripts are significantly different along the rows and columns. **Sample codes are as stated in table 2.

Table 5: Changes in titratable acidity (mg/100g) during storage of *wara*- a Nigerian (unripened) soft cheese - produced using various starter cultures.

| Sample | 0 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|
| A | 0.028±0.001$^{aa}$ | 0.047±0.0003$^{ab}$ | 0.089+0.0002$^{ac}$ | 0.133±0.0002$^{ad}$ | 0.133±0.0003$^{ae}$ |
| B | 0.026±0.0002$^{ba}$ | 0.044±0.0002$^{bb}$ | 0.090±0.0007$^{bc}$ | 0.116±0.0002$^{bd}$ | 0.128±0.0002$^{be}$ |
| C | 0.034±0.004$^{ca}$ | 0.053±0.0002$^{cb}$ | 0.085±0.0002$^{cc}$ | 0.109±0.0007$^{cd}$ | 0.119±0.0002$^{ce}$ |
| Control | 0.062±0.0001$^{da}$ | 0.083±0.0002$^{db}$ | 0.098±0.0001$^{dc}$ | 0.130±0.0001$^{dd}$ | 0.143±0.0002$^{de}$ |

*Values are means (n=3) ± Standard Deviation. Means with different superscripts are significantly different along the rows and columns. **Sample codes are as stated in table 2.

The findings of this study demonstrate that the use of lactic acid starter cultures improves the nutritional quality, acceptability and shelf life of *wara*. Use of such bacterial isolates should therefore be encouraged, more so in the communities that produce and consume *wara* more often, so that they can harness the benefits of using lactic acid starter cultures.

ACKNOWLEDGEMENTS: The authors wish to thank Avanrenren E.R and Clement J.B of the Department of Biology, The Polytechnic, Ibadan for technical assistance.

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