Production and Evaluation of Yoghurt Produced from Fresh Cow Milk Using Brevibacterium Linens as Starter Culture

[*1]Lawal, R. A., [1]Musa, H. [2]Adebusoye, M.S. & [1]Haruna, U.S.
[1]Department of Food Science and Technology, Federal Polytechnic, Bauchi, Nigeria
[2]Department of Nutrition and Dietetics, Federal Polytechnic, Bauchi. Nigeria
*Corresponding author: motherofbelievers54@gmail.com

ABSTRACT

Bervibacterium linens are major surface microorganisms that are present in the smear of surface ripened cheeses. This study investigated the production and evaluation of yoghurt from fresh cow milk using *Bervibacterium linens* as starter culture. *Bervibacterium linens* was isolated from sample of cheese (procured from Jos metropolis). The milk samples were clarified to remove foreign materials, pasteurized at 65^{o}C for 30min to destroy pathogenic materials and later cooled to temperature of 42^{o}C and *B. linens* was inoculated in pasteurized milk to compare its potential as starter culture against commo starter culture (*L.bulgaricus* and *S. thermophlius*) , the mixture allowed to ferment for 4 hrs. The semi- solid curds were homogenized; package and cool at 4^{o}C. Physicochemical properties such as pH titratable acidity, total solid, fat, protein, viscosity and microbial activity were determined using standard laboratory procedures. The result of the physicochemical properties were recorded as followed; 3.02 ± 0.01&3.07± 0.02%, 20.12±0.04 & 16.08±0.01%, 201±1.04 mm^2/s& 123 ±1.12mm^2/s, 2.84±0.02% & 1.32 ± 0.04%, 18.64± 0.04mg/l & 22.40 ±0.02mg/l, 4.35 ±0.02 & 4.56 ± 0.02, respectively. Meanwhile, physicochemical properties of yoghurt produced from common starter culture are significant different ($p<0.05$) than yoghurt produced from *B. linens* as starter culture.

Keywords - Production , Evaluation, Yoghurt, Fresh Cow Milk, Brevibacterium Linens, Starter Culture

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1. INTRODUCTION

Milk is a complex biology fluid secreted in the mammary glands of mammals. Its function is to meet the nutritional needs of neonates of the species from which the milk is derived. However, milk and dairy products form a significant part of the human diet. They are rich sources of nutrients such as proteins, fats, vitamins and minerals; ironically, it is because of this that these products are susceptible to rapid microbial growth. In some instances, this microbial growth may be beneficial, while in others it is undesirable. Dairy products are vulnerable to spoilage or contamination with pathogens or microbial toxins; therefore, the microbiology of milk products is of key interest to milk handlers and those in the dairy industry.

An important part of human diet in many regions of the world in ancient times is fermented dairy foods which have been consumed ever since the domestication of animals. Yoghurt is a product made from heat treated milk that may be homogenized prior to the addition of lactic acid bacteria (LAB) cultures containing *Lactobacillus bulgaricus and Streptococcus thermophilus* (Code of Federal Regulations Section 131.203, 2011).

Yoghurt can also be defined as a product of the lactic acid fermentation of milk by addition of a starter culture, which results in a decrease of milk pH to less than or equal to 4.6 (Tamime, 2002). The conversion of lactose to lactic acid has preservative effect on milk; moreover, the low pH of cultured milk inhibits the growth of putrefactive bacteria and other determined organisms, thereby, prolonging the shelf life of the products (Elagamy *et al.,* 1992). An advantage of fermentation of milk of various domesticated animals is the production of products in which their essential nutrients are conserved that otherwise would deteriorate rapidly under the high ambient temperatures. Thus, the process permitted consumption of milk constituents over a period significantly longer than was possible for milk itself (Tammine and Robinson, 2000).

Yoghurt is a semisolid fermented milk product made by the symbiotic activity of a blend of *Streptococcus salivarius subsp. Thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* and can include other lactic acid bacteria. According to the International Dairy Federation definition for fermented milk, it is a milk product fermented by the action of specific microorganisms and resulting in reduction of pH and coagulation. These specific micro-organisms shall be viable, active and abundant (at least 10^7 cfu/g) in the product to the date of minimum durability" (Ouwenhand and Salminen, 1999).

Yoghurt is made from a mix standardized from whole, partially defatted milk, condensed skim milk, cream, and nonfat dry milk. Supplementation of milk solids non -fat (SNF) of the mix with non-fat dry milk is frequently practiced in the industry. The FDA specification calls for a minimum of 8.25% non - fat milk solids. However, the industry uses up to 12% SNF or non-fat milk solids in the yoghurt mix to generate a thick, custard-like consistency in the product. The milk fat levels are standardized to 3.25% for full fat yoghurt. Reduced fat yoghurt is made from mix containing 2.08% milk fat. Low fat yoghurt is manufactured from mix containing 1.11% milk fat. Non-fat yoghurt mix has milk fat level not exceeding 0.5%. These fat levels correspond to the Food and Drug Administration requirement for nutritional labeling of non-fat, reduced fat, and low fat yoghurt (Chandan, 1997).

Brevibacterium linens has long been recognized as an important dairy microorganism because of its ubiquitous presence on the surface of a variety of smear surface-ripened cheese such as Limburger, Munster, Brick, Tilsiter and Appenzeller (Motta and Brandelli, 2006). The growth of *B.linens* on the surface is thought to be an essential prerequisite for the development of the characteristic colour, flavor and aroma of smear surface-ripened cheeses (Ades and Cone, 2009). *Brevibacterium* are of interest to the food industry because they produce amino acids such as glutamic acid which is of use in the production of flavour enhancer such as monosodium glutamate. They also produce important enzymes used in cheese ripening. *Brevibacterium linens* is the type strain and has a growth temperature range of 8–37 °C and an optimum of 21–23 °C (Motta and Brandelli, 2008). *Brevibacterium* have also been isolated from wheat samples (Legan, 2000).*B.linens* produces red or orange or purple-coloured pigment of aromatic carotenoide type which are not common in other bacteria.

This alcalophilic bacterium is able to produce methanethiol fromL-methionine and tolerate a high NaCl concentration up to 15%, *B.linens* produces antimicrobial substances which inhibits the growth many gram positive food poisoning bacteria as well as several yeasts and moulds. *B.linens* synthesizes highly active and multiple proteolytic enzymes during its growth. In acceleration of cheese ripening process, it is possible to improve flavor and eliminate bitterness with the use of enzymes (peptide) from *B.linens* alone or in combination with commercially available enzymes (Motta and Brandelli, 2008). The contribution of *Brevibacterium* towards cheese production has been under investigation for some time, showing that it can break down lipids and proteins (i.e. casein) with the use of extracellular proteases and lipases,(Rattray and Fox, (1999), Ozturkoglu-Budak *et al*., 2016) .

Many *Brevibacterium* isolates also have the ability to modify sulfur-containing amino acids to produce volatile sulfur compounds which are important for flavor development, (Amarita *et al*., 2004, Yvon *et al*., 2000, Bonnarme, Psoni and Spinnler, (2000)). *Brevibacterium* strains are thus often used as surface-ripening cultures in many different cheese types, (Bockelmann *et al*., 2005). Understanding the functional potential of cheese bacteria is essential in the combined effort with cheese producers to shorten ripening times, reduce spoilage, better control cheese aroma, and increase food safety. Therefore, this study aimed to investigate the potential of using *Brevibacterium linens* as starter culture for the production of yoghurt from fresh cow milk.

2.0 MATERIALS AND METHODS

2.1 Source of Milk

Fresh cow milk was purchased from National Veterinary Research Institute (Vom) in division of Animal Health and Production Technology, (AHPT), Jos Plateau State, Nigeria. Milk samples were then kept in an ice box immediately after collection.

2.2 Source of cheese

The cheese was purchased from retail outlet in Jos (North and South). Sample A was purchased from Jos north while sample B from Jos south and sample C was homemade cheese to determine the presence of *B. linens.* A commercial starter culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Freeze- dried yoghurt starter)was purchased from food chemical store in Jos.

2.3 Isolation of *Brevibacterium linens* from cheese

Brevibactrium linens were isolated and characterized from cheese. Prior to isolation of *Brevibacteriumlinens*, cheese was thawed in the dark at 4^{o}C. The smear was collected from cheese, by scraping the surface of the cheese and weighed. The culture was grown in 250ml Erlenmeyer flask containing 50ml of a medium composed of 20g/L D-glucose (Carloerba, London), 5g/L casamino acids (Difco), 1g/L yeast extracts (Biokar), 5g/L NaCl and 1g/L KH_2PO4. The pH was adjusted to 6.9 and the medium was sterilized at 121^{o}C for 15minutes and incubated at 25^{o}C for 48hours with stirring (150rpm) to oxygenate the medium (Galaup *et al.*, 2005).

2.4 Yoghurt Production

Yoghurt was manufactured using the method outlined by Tamime and Robinson (1999) with some modifications (Fig 1). The cow milk was collected from Federal College of Animal Health and Production Technology, VOM. The milk was immediately stored and preserved in cooler containing ice crystals and conveyed down to Food processing Lab. At department of Food Science and Technology, Federal polytechnic, Bauchi. The milk was kept in the refrigerator at 4^{o}C prior to subsequent used. The milk was filtered with a clean muslin cloth to remove dirt, debris, and udder tissues. The clarified cow milk was then pasteurized in 65 for 30 min. After which the pasteurized milk samples were cooled to inoculation temperature of 42 ºC ± 1 ºC and then cooled samples were inoculated with *B. linens* starter culture, the samples were fermented for 4h.The plain yoghurt was then packaged in polyethylene terephthalate bottles, chilled in a refrigerator and presented for further analysis. The same procedure was repeated for control in which common starter culture was used (freeze-dried yoghurt starter) consisting of *Lactobacillus bulgaricus* and *Streptococcus.*

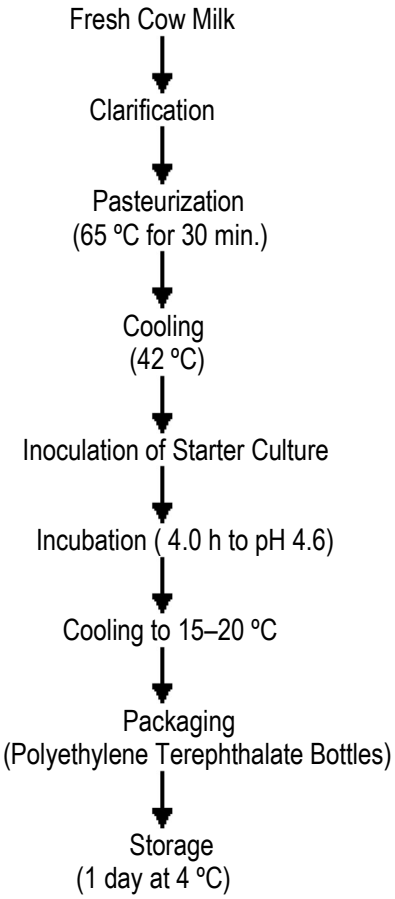

Figure1: Flow Chart for Modifying Method Yoghurt Production (Tamime and Robinson, 1999)

2.5Chemical Analysis of Yoghurt

2.5.1 Fat content determination

The fat content of the yoghurt sample was determined using Acid Digestion Method of Fat determination in Milk (Werner Schmidt Method) as described by Bradley *et al.,* (1992) as follows: In a clean dry Gerber tube, 10 ml of sulphuric acid (density 1.815 gm/ml at 20 °C) was poured, and then 10.94 ml of sample was added in the butyrometer. Amyl alcohol (1-2 ml) was added to the tube. The content is thoroughly mixed till no white particles could be seen. The Gerber tube was centrifuged at 1100 revolutions per minute (rpm) for 4-5 min at 65 °C. The fat column was then read immediately (Bradley *et al.* 1992).

Calculation;

Fat % $\left({}^{W}/_{W}\right) = \frac{100(W_1 - W_2)}{W3}$ .....Equation 1

Where,

W1= Weight in grams of contents in flask before removal of fat.

W2= Weight in grams of contents in flask after removal of fat and

W3= Weight in grams of material taken for the test (10 g)

2.5.2 Total solids determination

The Total Solids was determined as described by AOAC (2005). Ten milliliter of the yoghurt sample was weighed into a dry petri dish of a known weight. The total portion was pre-dried for 25 min. on steam bath and then dried for 3 h at 100 ºC in forced draft air oven. The Total Solid sample is the weight of the dried sample residue and was calculated as (AOAC, 2005).

$$\%\ Total\ Solids\ = \frac{W2 - W1}{W1 - W} \times 100$$ .....Equation 2

Where, W = Weight of the dish
W1= Weight of dish and sample test portion
W2 = Weight of dish and dry sample

2.5.3 Total titrable acidity (TTA) determination

This was determined using the titrimetric method as described by AOAC (2005). One (1) ml of phenolphthalein indicator was introduced into 10ml of the mixed solution. It was then titrated against standard 0.1N sodium hydroxide solution until pink color persisted for about 10 - 15 seconds for complete neutralization. The titration figure was divided by 10 to get the percentage of lactic acid (AOAC, 2005).

2.5.4 pH measurement

The pH of yoghurt was measured with digital pH meter. pH buffers 4 and 7 was used for the calibration of the pH meter. After calibration, 20 ml of yoghurt was taken in a beaker and then electrode is immersed in the milk until constant reading attained (Ong *et al*., 2007).

2.5.5 Protein content determination

Total protein in the yoghurt was determined as described by the international dairy federation method, IDF 20-1 (2001). Three grams of the sample was weighed and poured in digestion tube along with a digestion tablet and 20 ml of concentrated H_2SO_4. Digestion was done initially by slow heating for 45 min. to avoid frothing and then at 80 ºC until appearance of clear or pale green color. The digested sample was allowed to cool for half an hour. Then 100 ml distilled water was added and mixed gradually and transferred to 250 ml volumetric flask, and the digestion flask was rinsed 2 - 3 times with distilled water and the volume made up to 250 ml by adding distilled water.

Ten milliliters of the digested sample and 10 ml of NaOH were distilled in micro Kjeldahl apparatus. The ammonia produced was trapped in 4% boric acid solution containing few drops of methyl red indicator. With the addition of ammonia, boric acid color changed from red to yellow. The distillation was continued for 2 - 3 min. after first appearance of yellow color to catch maximum ammonia. The content was then titrated against 0.1 N H_2SO_4 solutions till pink color end point appeared. The volume of H_2SO_4 used was noted.
Total nitrogen % was calculated with the following formula and the value obtained was multiplied with the factor in the equation to get total protein:

$$\%\ Nittrogen = \frac{\text{Vol. of sulphuric acid used(ml)} \times 250 \times 0.0014}{\text{Vol.used for digestion} \times \text{Vol. of digested sample}} \times 100$$

$$\%\ Total\ Protein = \%\ Nitrogen \times 6.38$$ .....Equation 3

2.5.6Determination of viscosity

The viscosity of the sample was determined using the Ostwald viscometer the sample was allowed to flow through its capillary tube between two etched marks and the time of flow of the liquid was measured (Abbas *et al*., 2010).

Then the viscosity was calculated as follows:

$$\eta = KPt$$ .....Equation 4

Where ŋ = viscosity (mm2/s)
K = constant
T = time (Secs)
P = hydrostatic pressure (mm2)

2.6 Microbial Analyses of Yoghurt

2.6.1 Preparation of serial dilutions

One millimeter of the yoghurt sample was weighted using a micro pipette aseptically into a test tube containing 9 ml sterile distilled water (autoclaved at 121 °C for 15 min). Further serial dilutions were made by mixing one ml of the initial dilution with 9 ml sterile distill water until 1/10 dilution.

2.6.2 Total plate count

The total plate count of raw milk was determined as described by ISO 4833-1:2013 protocol. The colony count method to determine the total spores was followed. One millimeter from the dilution was aseptically transferred into sterile petri-dishes. Then to each plate nutrient milk agar was added. The inoculum was mixed with the medium and allowed to solidify. The plates were then incubated at 37 °C for 24 – 48 h (Buchanan and Gibbons, 2004).

2.6.3 Yeast count

From suitable dilutions of sample, 0.1 ml was aseptically transferred into Sabouraud Dextrose Agar (SDA) containing 0.1g chloramphenicol per one liter to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod and then the plates is incubated at 28 °C for 48 hours (Harrigan and Mc Cance, 1996).

2.6.4 Coliform count

Coliform bacteria will be carried out on violet red bile agar medium and incubated for 24 hours at 37 °C for total coliforms and 44 °C for faecal coliforms according to the standard (ISO 4832); *E. coli* will be streaked onto eosine methylene blue (EMB) agar and then incubated overnight at 37 °C (Seeley and Denmark, 1997).

2.6.5 Enumeration of lactic acid bacteria

Viable bacteria count in the yoghurt sample was enumerated using the pour plate technique. The counts were enumerated on De Man Rogosa Sharpe agar (Oxoid, Australia) and anaerobic incubation at 43 °C for 72 h was used for the differential enumeration of the lactic acid Bacteria (Dave and Shah, 1996).

2.7 Statistical Analysis

The physico-chemical and microbiological data of yoghurt samples were evaluated using design expert version 8.0

3.0 RESULTS AND DISCUSSION

Table 1: Physicochemical Properties of yoghurt produced from *B. linens* and common starter culture

Parameter	BLY	CSCY
Fat (%)	3.02±0.01	3.07±0.02
Protein (%)	20.12±0.04	16.08±0.01
Viscosity (mm^2)	201±1.04	123±1.12
Titratable acidity (%)	2.84±0.02	1.32±0.04
Total solid (mg/l)	18.64±0.04	22.40±0.02
pH	4.35±0.02	4.56±0.02

Means obtained from triplicate determination ($p<0.05$)

Key:
BLY: *Bevibacterium linens* yoghurt.
CSCY: Common starter culture yoghurt

The results of the physicochemical properties of the yoghurts produced from *Bevibacterium linens* and Common starter culture yoghurt are presented in Table 1. The mean composition recorded for fat in yoghurt produced from *Brevibacterium linens* as starter culture and common starter culture yoghurt were 3.02 ± 0.01&3.07± 0.02%. There was significant different in fat content ($p<0.05$). Decrease in fat content in yoghurt produced *Brevibacterium linens* as starter culture was due to hydrolysis of lipid during fermentation Lee and Lucey, (2006). There was increase in protein content in yoghurt produced *Brevibacteriumlinens* as starter culture ranged 20.12±0.04 & 16.08±0.01%. This is in agreement with finding of Thomas and Mills, (2009) who reported increase in protein, could be as a result of proteolytic activity of Lactic acid bacteria (LAB) which hydrolyse protein (casein) into peptide and amino acid. Also, the mean composition for viscosity, titratable acidity, total soild and pH values recorded were 201±1.04 mm^2/s& 123 ±1.12mm^2/s, 2.84±0.02% & 1.32 ± 0.04%, 18.64± 0.04mg/l & 22.40 ±0.02mg/l, 4.35 ±0.02 & 4.56 ± 0.02, respectively. Viscosity of yoghurt is influenced by the composition of the raw milk, incubation temperature and the activity of lactic acid bacteria (LAB) which contributed to higher consistency of the yoghurt produced from *B. Linens* as starter culture (Tamine & Robinson, 1999, Chandan, 2004).

There was increase in titratable acidity of yoghurt produced from *B. linens* as starter culture than common starter culture due to the activity of Lab produced during fermentation which converts lactose to lactic acid (Lee and Lucey, 2010). A pH of less than or equal to 4.6 is an indication of end point of fermentation in yoghurt production (Chandan, 2006).

4.0 CONCLUSION

The physico-chemical quality of yoghurt produced from common starter culture was significantly difference from *B. linens* yoghurt which recoded higher value in terms of its protein, viscosity and titratable acidity content.

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