

Assessment of some quality parameters of yoghurt produced from fresh brown goat (Hakuya) and cow milks using *Brevibacterium linens* as starter culture

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Abstract

This study investigated the assessment of some quality parameters of yoghurt produced from fresh brown goat milk (Hakuya) and cow milk using *Brevibacterium linens* as starteculture. *Brevibacterium linens* was isolated from three (3) samples of cheese (procured from Jos North, Jos South and homemade cheese). The isolate(s) was tested for the presence of virulence gene. *B. linen* swas inoculated in pasteurized milk to compare its potential as starter culture against commercial starter culture (*L. bulgaricus* and *S. thermophlius*). Physicochemical properties such as pH titratable acidity, total solid, fat, protein, viscosity and microbial activity were determined using standard laboratory procedures. Aroma, mouth- feel and taste were monitored for the sensory quality. The general acceptability of the products was evaluated using twenty (20) trained panelists. The yoghurt produced from *B. linens* as a starter culture was significantly different ($p>0.05$) in term of physical and chemical properties compare to commercial starter culture yoghurt (control). The value for pH, titratable acidity, total solid, viscosity, fat and protein content of yoghurt of yoghurt produced from *B. linens* as a starter culture were in the range: 3.98-4.36%, 18.22-24.01%, 238-280mm², 13.67-14.84, 19.14-23.37% respectively. The yoghurt produced from commercial starter culture were generally accepted by panelist. The result of microbial properties revealed no coliform and mould was detected and small amount of other microbes were present. Inconclusion, *B. linens* can be used as starter culture in yoghurt production. Efforts should intensify toward commercial production of yoghurt and other dairy products using *B. linens* as starter culture.

Key words: Brown goat milk, Cow milk, starter culture, *Brevibacterium linens*, yoghurt.

1.0Introduction

Milk is a complex biological 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.

Nigeria, with a population of more than 170 million is grossly underprovided with essential food components - particularly the milk protein. Reports indicate that cow provides essentially all the fluid milk consumed (Igwegbeet al., 2014); and that milk production has been nose diving or at best has remained constant since 1994 in the country. To ameliorate this problem of low-level protein intake, especially from cheap dairy sources, there is the need for concerted effort to bring about the massive production and utilization of protein based food items from milk of other animal species such as goat, and at competitive costs so that they would be affordable to the general masses.

Goat milk and its products have played an important role in the economic viability in many parts of the world, especially in developing countries. A variety of manufactured products can be produced from goat milk, including fluid products (low fat, fortified, or flavored), fermented products such as cheese, yoghurt or buttermilk, frozen products such as ice cream or frozen yoghurt, butter, and condensed and powdered products (Park, 2011). According to Haenlein and Abdellatif (2004), the world production of goat milk has been relatively minor compared to that of bovine milk (2.1% versus 84.6% of the total milk production, respectively), the worldwide goat population has reached 758 million heads with 55% increase during the last 20 years, and goat milk production has reached 12.2 million tones with 58% increase during the same period.

Producing high quality raw milk is of utmost importance for successful production and marketing of dairy goat products. The products must be safe to consume and free of pathogenic bacteria, antibiotics, insecticides, and herbicides. They should have a good taste with no objectionable flavor or odor, be free of spoilage from bacteria, and contain legal limits of all nutrients (Park, 2011).

Goat milk exhibits beneficial virtues for individuals with certain dietetic problems, thus it is recommended traditional by physicians for infant and others allergic to cow milk. Similarly it has been used in treatment of ulcers (Mereado, 1982, Kumar *et al.*, 2012). Goat milk is a distinctive dairy resource, which is well known as “the king of milk” it is easily digested and has a rich nutrition (Tamime and Robinson, 2000; Agnihotri and Prasad, 1993). Goat milk is more completely and easily absorbed than cow's milk, leaving less undigested residue behind in the

colon to quite literally ferment and cause the uncomfortable symptoms of lactose intolerance (Haenlein, 1992).

Cow milk is the most studied milk since it is the most produced and consumed milk in the world. However, other domesticated mammalian species, such as goats and sheep, have aroused greater interest as an object of study. These species have reached higher production quantities, which meet the nutritional demand of specific populations. On the other hand, goat and sheep milk have their peculiarities varying in the composition of cow's milk. The first major difference is related to seasonality of these species (cow, goat, and sheep), besides the fat content and profile, the protein, the total solids, and the minerals (Park *et al.*, 2007).

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.

Brevibacterium linens is an aerobic halotolerant microorganism that is the major component of the flora of surface-ripened cheeses such as Limburger, Münster, Brick, Tilsiter and Appenzeller (Ratray and Fox, 1999). *Brevibacterium* are irregular rods arranged singly or in pairs. They often orientate at angles to give a V shape. They are 0.6–1.2 µm in diameter by 1.5–6 µm in length. They are strictly aerobic and the colonies are often pigmented with yellow or purple coloration. Their optimum growth temperature is 20 to 35 °C . Both rod and coccoid forms are Gram-positive, but some strains and older cultures decolorize readily. *Brevibacteria* are not acid-fast, produce endospores and are non-motile.

The color of the colonies varies from orange (*B. linens*), through gray-white (*B. epidermis*, *B. casei*) to purple (*B. iodinum*). The orange pigmentation (carotenoids) of the type-strain is often light dependent. The purple coloration of *B. iodinum* results from the production and secretion of purple crystals of a phenazine derivative, called iodinin (Jones & Keddie 1986).

These cheese rind microbial communities can either be inoculated artificially with surface-ripening cultures during the manufacturing process, be present in starting ingredients, or establish themselves through inoculation from the microbial communities of the ripening cellar environment during the ripening process, (Wolfe, et al., 2014, Irlinger et al., 2014, Monnet et al., 2015, Quijada, et al., 2018) Many genera of the bacterial phylum *Actinobacteria*, including – among others - the genus *Brevibacterium*, are important for flavor production during cheese ripening, (Monnet, et al., 2015, Bora, Dodd and Desmaures, (2015), Bockelmann et al., 2005, Rattray & Fox, (1999)). 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 & 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 & 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 investigate the assessment of some quality parameter (pathogenicity test, physicochemical properties, microbial and sensory quality) of yoghurt produced from fresh brown goat (Hakuya) and cow milks using *Brevibacterium linens* as starter culture.

2.0 Materials and Methods

2.1 Source of Milk

Fresh cow and brown goat (Hakuya) milk were 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

Brevibacterium linens were isolated and characterized from cheese. Prior to isolation of *Brevibacterium linens*, cheese was thawed in the dark at 4°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 KH₂PO₄. The pH was adjusted to 6.9 and the medium was sterilized at 121°C for 15 minutes and incubated at 25°C for 48 hours with stirring (150rpm) to oxygenate the medium (Galaup *et al.*, 2005).

Pathogenicity Test of *B. linens* by PCR Amplification of DNA

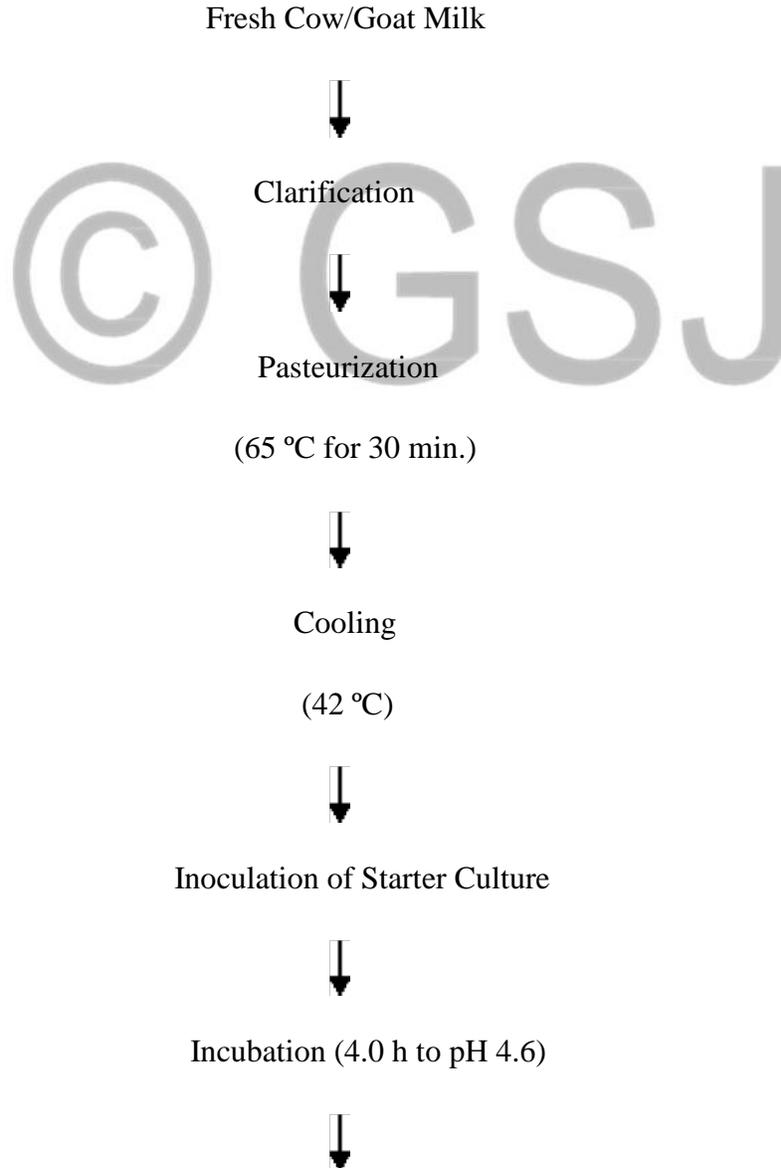
Genomic DNA was extracted using the procedure of (Pitcher *et al.*, 1998). The DNA was amplified in a final volume of 50 μ l. The PCR mix contained 5 μ l of a 10x, 160 mM NH₄-buffer (all products for the PCR are from Bioline, London, UK), 2 μ l of a 50 mM MgCl₂ (final concentration 2 mM), 1 μ l dNTP master mix (final concentration of each dNTP 0.5 mM), 2.5 μ l of each primer (from a 20 mM solution), and 1.25 units *Taq* polymerase. One microlitre of DNA was found to be sufficient for each reaction. Amplification was performed for 25 cycles by denaturing at 94°C for 1 min, annealing at 63°C for 1 min, followed by polymerization at 72°C for 1 min preceded by an initial denaturation step at 95°C for 5 min. The PCR apparatus was the T gradient from Biometra (Göttingen, Germany). Five microlitres of the PCR product were electrophoresed alongside a molecular weight marker on a 1.5% (w/v) agarose gel using a 1x TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). Gels were run for 30 min at 100 V, stained in an ethidium bromide bath and visualized by u.v. transillumination.

2.4 Yoghurt Production

2.4.1 Assessment of the potential of *B.linens* as starter culture against standard culture used in 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 Laboratory at department of Food

Science and Technology, Federal polytechnic, Bauchi. The milk was kept in the refrigerator at 4°C prior to subsequent use. The milk samples were filtered with a clean muslin cloth to remove dirt, debris, and udder tissues. The clarified cow milks were then pasteurized in 65 for 30 min. After which the pasteurized milk samples were cooled to inoculation temperature of 42 °C ± 1 °C and then cooled samples of cow milks were divided into two (2) portions; A, & B. Then, sample A (control) was inoculated with inoculated with (freeze-dried yoghurt starter) consisting of *Lactobacillus bulgaricus* and *Streptococcus* and sample B was inoculated with *B. linens* starter culture the samples were fermented for 4h. The goat milk was inoculated with *Brevibacterium linens*. The yoghurts were homogenized and then packaged in polyethylene terephthalate bottles, chilled in a refrigerator and presented for further analysis.



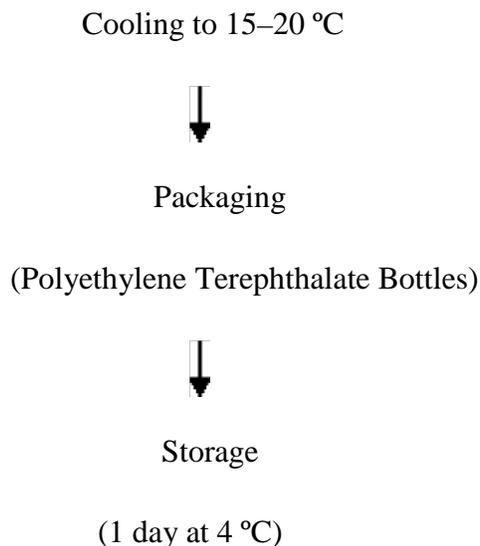


Figure1: Flow Chart for Modifying Method Yoghurt Production (Tamime and Robinson, 1999)

2.5 Chemical 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;

$$\text{Fat \%} \left(\frac{W_1 - W_2}{W_3} \right) = \frac{100(W_1 - W_2)}{W_3} \dots\dots\dots \text{Equation 1}$$

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 °C in forced draft air oven. The Total Solid sample is the weight of the dried sample residue and was calculated as (AOAC, 2005).

$$\% \text{ Total Solids} = \frac{W_2 - W_1}{W_1 - W} \times 100 \dots\dots\dots \text{Equation 2}$$

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 H₂SO₄. Digestion was done initially by slow heating for 45 min. to avoid frothing and then at 80 °C 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 H₂SO₄ solutions till pink color end point appeared. The volume of H₂SO₄ 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:

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

$$\% \text{ Total Protein} = \% \text{ Nitrogen} \times 6.38 \dots\dots\dots \text{Equation 3}$$

2.5.6 Determination 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:

$$\eta = KPt \dots\dots\dots \text{Equation 4}$$

Where η = 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 Sensory Quality Evaluation and Acceptability Test

Acceptance testing method described by Ihekoronye and Ngoddy (1995) was used to investigate the acceptability of the goat milk yoghurt compared with cow milk yoghurt (control) using the optimized processing conditions. Determination of acceptability was done using 20 trained panelists who were familiar with yoghurt and were willing to participate, the panelist were recruited at Federal Polytechnic Bauchi. Briefing regarding the evaluation was given at the beginning of the session. Each panelist was assigned a number for identification purposes and he/she was responsible to evaluate two different samples. Samples were coded using a 3-digit random number and served successively. Panelists were asked to fill out a score sheet for each yoghurt sample they evaluated in term of taste, mouth feel, aroma and overall acceptability. Each sample attribute was rated using a nine-point Hedonic Scale. The nine points on the Hedonic Scale were: dislike extremely = 1, dislike very much = 2, dislike moderately = 3, dislike slightly = 4, neither like nor dislike = 5, like slightly = 6, like moderately = 7, like very much = 8 and like extremely = 9. The average and mean values of scores for each of attributes was computed and analyzed statistically.

2.8 Statistical Analysis

The physico-chemical and microbiological data of yoghurt samples were evaluated using design expert version 8.0 while the sensory analysis of the yoghurt samples was statistically evaluated using paired t-test

3.0 Results and Discussion

PCR Gel Electrophoresis Profile for the Confirmation of Virulence Gene in *Brevibacterium linens*

PCR gel electrophoresis profile for the confirmation of virulence gene in *Brevibacterium linens*



Fig 3: PCR gel electrophoresis profile for the confirmation of virulence gene in *Brevibacterium linens*. Molecular weight ladder (100bp DNA ladder bioline, UK); Lane 1: 100bp ladder, lane 2 : Sample , lane 3; Genomic DNA , lane 4; Negative control

Figure 1: PCR gel electrophoresis profile for the confirmation of virulence gene in *Brevibacterium linens*

The figure 1; showed PCR gel electrophoresis profile for the confirmation of virulence gene in *Brevibacterium linens* using molecular weight ladder of 100bp DNA ladder (bioline, UK). The expected amplicon size generated were; 230, 310 respectively. This was in agreement with the work of (Gelsomino *et al.*, 2014). No gene was recorded been virulence for both the sample and genome DNA. This was in line with work of Alessandra *et al.*, (2016). No genes coding for known toxins were found in the genome of *B. linens* by using Virulence Finder (Kato, *et al.*, 1991), Virulence Factor Database (Chen, *et al.*, 2012), and DBETH (Chakraborty, *et al.*, 2012).

According to the report of Ehirin and Ohin (1993) that, *Brevibacterium linens* have not belonging to human skin flora. *Brevibacterium linens* isolated from clinical materials may be contaminants derived from human skin or from the environment or they may be secondary invaders. The possibility remains, however, that the *Brevibacterium linens* has not been considered as a source of potential pathogen. None of the clinical isolates of *Brevibacterium* spp been studied produced the characteristic pigments of *Brevibacterium linens* and grew at 37°C. At present, however, distinction *B. linens* and *B. epidermidis* cannot be made on the basis of morphology, colonial appearance or biochemical test. The essential difference is one

of habitat: *Brevibacterium linens* isolates are from dairy products and *B. epidermidis* are from human skin Pitcher and Malnick, (2018).

3.2 Physicochemical properties of yoghurt produced from brown goat milk (Hakuya) and cow milk using *Brevibacterium linens* and commercial starter culture

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

Parameter	Goat milk	Cow milk	Control
Fat (%)	4.36 ± 0.04	4.19 ± 0.01	3.98 ± 0.02
Protein (%)	24.01±0.08	20.57±0.04	18.22±0.01
Viscosity (mm ²)	280±0.10	245±1.04	238 ±1.08
Titrate acidity (%)	14.38±1.34	14.84±1.22	13.67±0.11
Total solid (mg/l)	20.42±2.04	19.14±1.08	23.37±2.01
pH	5.64±0.10	5.45±0.08	5.46 ±0.06

Means obtained from triplicate determination (p<0.05)

The result of the physicochemical properties of yoghurt produced from brown goat milk (Hakuya) and cow milk using *Brevibacterium linens* as starter are presented in table 1. The chemical properties showed that the fat content ranged from 3.98-4.36%. There was significant different in fat content (p<0.05) of yoghurt produced from commercial starter (control). Decrease in fat content in yoghurt produced commercial starter culture (*Lactobacillus bulgaricus* and *Streptococcus*) as starter culture was due to hydrolysis of lipid during fermentation Lee and Lucey, (2006). There was increase in protein content in yoghurt produced from brown goat milk using *Brevibacterium linens* as starter culture which ranged from 18.22-24.01. The same observation was also noted in the work of Popoola, (2018) which observed increase in protein, in yoghurt produced from West Africa dwarf goat milk yoghurt. This could be as a result of proteolysis activity of Lactic acid bacteria (LAB) which hydrolyse protein (casein) into peptide and amino acid. Also, the mean composition for viscosity, titrate acidity, total solid and pH values recorded were 238-280mm², 13.67-14.84 %, 19.14-23.37% and 5.45-5.64 respectively.

There was no significant different ($p < 0.05$) in value of pH. 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 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 commercial starter culture due to the activity of Lab produced during fermentation which converts lactose to lactic acid (Lee and Lucey, 2010).

Table 2: Mean sensory score of yoghurt produced from *B. linens* and commercial starter culture

Parameter	Goat milk	Cow milk	Control
Taste	6.57± 0.07	7.49± 0.11	8.09± 0.22
Flavor	6.87±1.02	8.24±0.08	8.18±0.06
Color	8.56±0.04	8.52± 0.04	8.30± 0.01
Texture	8.33±0.08	8.28 ± 0.06	8.14 ±0.04
Overall acceptability	7.64±0.12	8.05 ± 0.09	8.10 ±0.16

Means obtained from triplicate determinations ($p < 0.05$).

The sensory assessment has judged by twenty (20) panelists was presented in table 2. The sensory attribute of the yoghurt is a combination of the flavor, colour (appearance), taste and texture (the mouth feel). The scores for taste, flavor and overall acceptability of yogurt produce from brown goat milk (Hakuya) using *B.linens* as starter culture was significantly ($P < 0.05$) lower than those reported for cow milk yoghurt and control. The variation in taste, flavor and overall acceptability may be attributed to “goaty flavor”. Yoghurt made from goat milk was found to be significantly different ($P > 0.05$) in color (appearance) than cow yoghurt and control (commercial yoghurt), with average scores of 6.57-8.09, 6.87-8.24 for flavor, 8.30-8.56 for color, 8.14-8.33 for texture and 7.64- 8.10 for overall acceptability respectively. The flavor results from chemical compounds in milk and those produced during processing and fermentation of milk. The similarity in flavor between the yoghurt from goat milk and that of the cow is a confirmation that the flavor of yoghurt is always the same irrespective of the milk source. Milk from any animal source is an extremely complicated entity which is comprised of lipids, proteins, carbohydrates, and minerals; and over 400 compounds have been identified in milk products (Lee and Lucey, 2010). The underlying flavor of yoghurt arises principally from

the native volatile components in the milk, influenced by the pasteurization and fermentation processes (Al-Rowaily, 2008). The main flavor compounds found in yoghurt include acetaldehyde, acetoin, diacetylene, acetic acid, propionic and butyric acids (Baglio, 2014). Furthermore, milk of goats produced under sanitary conditions will be free from off-flavour. And, the same factors that adversely affect the flavour of cow's milk also affect goat's milk. However, researchers advise that producers of goat milk must be certain that the buck (male goat) is kept at least 50 m away from the lactating doe (female goat) to prevent the milk from absorbing the buck's odor (Eissa et al., 2010; Ekram and El-Zubeir, 2011). On the other hand, the appearance of the yoghurt is a combination of the color and the visual separation of the whey. It has been reported that the goat is essentially 100 percent efficient in converting carotene into vitamin A, a process that makes goat milk whiter in colour than that of cow. It follows then, that the yoghurt made from the milk is very whitish in colour. The curd of goat milk appeared like small light and friable flakes that dissolved easily upon stirring. Goat milk yoghurt was observed to be more delicate and thinner than the cow milk yoghurt in other words, the yoghurt from the goat's milk was slightly less firm in consistency than that of the cow's milk. These observations are in agreement with those made by other researchers including Janness (1980), Jumah et al. (2001), Maina (2008), Cheng (2010), Eissa et al. (2010), Ekram et al. (2011) and El-Zubeir et al. (2012).

Table 3: Microbial quality of yoghurt produced from *B. linens* and commercial starter culture

Microorganisms	Goat milk	Cow milk	Control
Total plate count (cfu/ml)	2.6 x 10 ⁴	2.8 x 10 ⁴	3.4 x 10 ⁶
Coliform (faecal)	0.00	0.00	0.00
Salmonella count	1.2 x 10 ²	1.0 x 10 ⁴	1.4 x 10 ²
Yeast count	<1.0 x 10 ²	<1.1 x 10 ⁴	<1.2 x 10 ²
Mould count	0.00	0.00	0.00

Means obtained from triplicate determinations (p<0.05)

The mean scores of the microbial counts: total plate count, *coliform*, *salmonella*, and mould and yeast counts, are presented in Table 3. The average total bacteria counts (TBC) of yoghurt produced from *B.linens* ranged from 2.6 x 10⁴ -3.4 x 10⁶ cfu/ml respectively. The TPC consists

dominantly of the lactic acid bacteria (LAB). The smaller numbers of other microorganism recorded: *salmonella*, and yeast, is thought to be as result of the antimicrobial effects of the lactic acid produced by the LAB, causing the pH of the growth environment to decrease to levels quite unfavorable for the growth of those organisms (Pazakova et al., 1997; Lee and Chen, 2004). The *coliforms* were not detected in both samples whereas; *Salmonella* and *Yeast* were present in both samples. This could be as a result of poor hygiene level of milk handlers. According to the FAO (2008) guideline and the specification given by the ICMSF, the milk should contain less than 3.0×10^4 cfu/ml and 5.0×10^4 cfu/ml respectively. Meanwhile, it is extremely important that microbial tests are carried out to ensure that bacterial activity in raw milk is of acceptable level, and that no harmful bacteria remain in the processed products. Furthermore, milk processing of any kind must be done under carefully controlled hygienic conditions. After the incubation period of 4 h, the yoghurt was cooled and stored at below 10°C. This was necessary to slow the multiplication of any contaminating organism.

4.0 Conclusion

The research work revealed that the *B. linens* isolated and screened for the presence of virulence genes such as; *B. linens* (sample) and *B. linens* (Genomic DNA) using specific primers and DNA from *B. linens* revealed the absence of virulence in all the genes. The physico-chemical quality of yoghurt produced from brown goat milk (Hakuya) was significantly difference ($p>0.05$) from yoghurt produced cow milk yoghurt using *B. linens* as starter culture. The yoghurt produced from brown goat milk was recorded higher value in terms of its protein, viscosity, pH and titratable acidity content. The yoghurt produced from commercial starter ingredient (*L. bulgaricus* and *S.thermophilus*) generally accepted by panelists than yoghurt produced *B.linens*. Coliform and mould are not detected in all the production and small counts of other microorganisms were recorded, these was as result of proper hygiene of the environment and handlers. Efforts should be intensified toward commercial production of yoghurt and other dairy products using *B. linens* as the starter ingredient and the awareness of the full usage of *B. linens* as starter culture for yoghurt production at household level should be promoted.

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