



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 (Tammime 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, 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 carotenoid type which are not common in other bacteria. This alcalophilic bacterium is able to produce methanethiol from L-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**

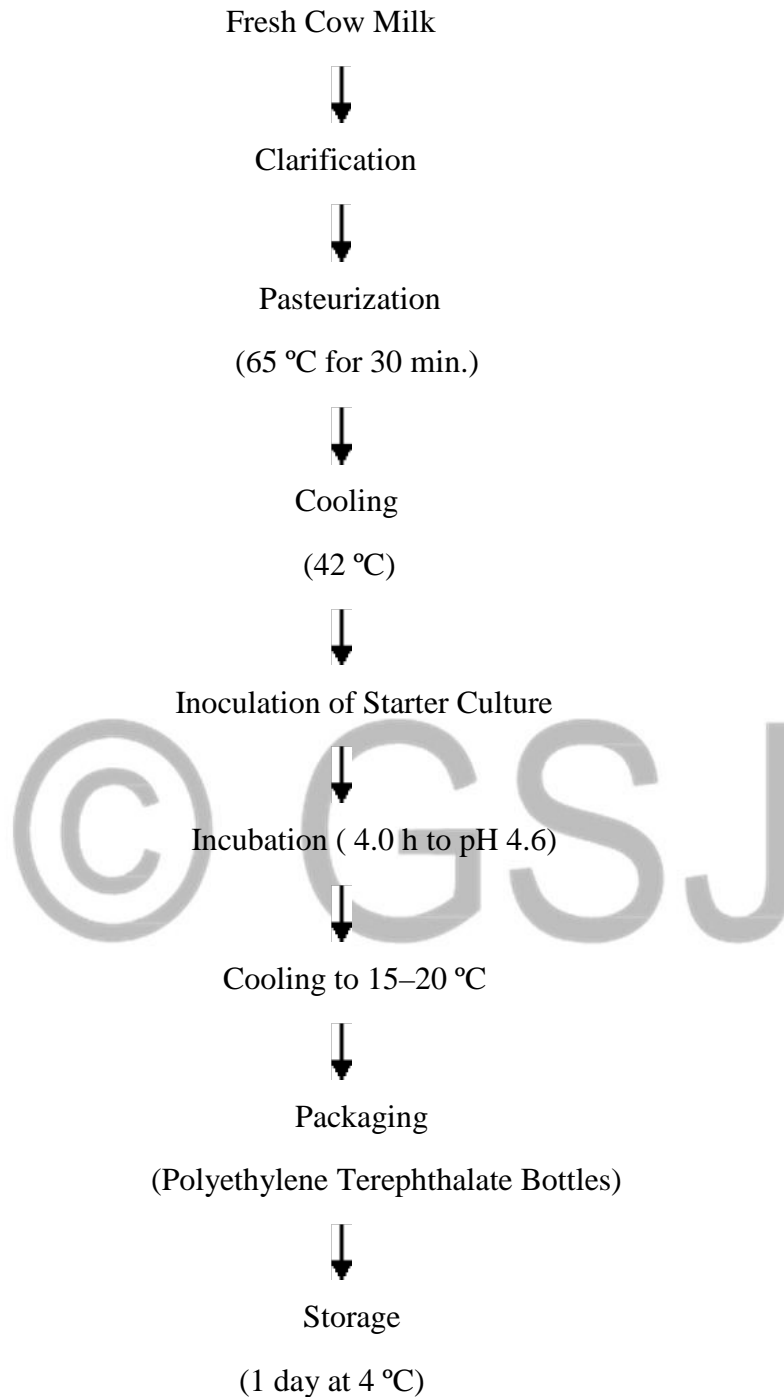
*Brevibacterium linens* were isolated and characterized from cheese. Prior to isolation of *Brevibacterium linens*, cheese was thawed in the dark at 4°C. The smear was collected from cheese, by scraping the surface of the cheese and weighed. The culture was grown in 250ml Erlenmeyer flask containing 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 KH<sub>2</sub>PO<sub>4</sub>. The pH was adjusted to 6.9 and the medium was sterilized at 121°C for 15minutes and incubated at 25°C for 48hours with stirring (150rpm) to oxygenate the medium (Galaup *et al.*, 2005).

## **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 Lab. At department of Food Science and Technology, Federal polytechnic, Bauchi. The milk was kept in the refrigerator at 4°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 °C ± 1 °C and then cooled samples were divided into six (6) portions; A,B,C,D& E. Then inoculated with *B. linens* starter culture (5ml, 10ml, 15ml, 20ml, 25ml& 30ml) 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)**

## 2.5 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;

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

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 H<sub>2</sub>SO<sub>4</sub>. 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 H<sub>2</sub>SO<sub>4</sub> solutions till pink color end point appeared. The volume of H<sub>2</sub>SO<sub>4</sub> 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  $\eta$  = viscosity (mm<sup>2</sup>/s)

K = constant

T = time (Secs)

P = hydrostatic pressure (mm<sup>2</sup>)

**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

**Table 1: Assessment of the potential of *B.linens* as starter culture against standard culture used in yoghurt production**

Culture (ml)	<i>B. linens</i> (%)	CSCY (%)
5.0	6.0	0.0
10.0	8.0	1.0
15.0	10.0	1.2
20.0	12.0	1.4
25.0	14.0	1.8
30.0	16.0	2.0

All data are means of triplicate determination Standard deviation (P <0.05).

**KEY:**

*B.linens* Y: Yoghurt produced from *Brevibacterium linens* as starter culture.

CSCY:Yoghurt produced from common starter culture (*L. bulgaricus* and *S.thermophilus*).

The results of the assessment of the potential of *B.linens* as starter culture against standard culture used in yoghurt production are shown in Table 1. The starter culture is the most important factor for determination of the overall quality of yoghurt, defining its qualitative and nutritional characteristics and also determining the type of fermentation process and the final fermentation metabolites. Regularly, for the yoghurt production, there are used symbiotic cultures consisting mainly of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria, in specific growth conditions. This study investigates the potential *B.linens* as starter culture against standard culture used in yoghurt production compared the quantity of whey (syneresis) produced.

Serum release, known as syneresis, is considered as one of the most important parameters indicating the quality of yoghurt during storage. Table 1, shows the changes in the syneresis rates of yogurts produced from *B.linens* as starter culture and common starter ingredient (*L. bulgaricus* and *S.thermophilus*). Here, the syneresis rate was expressed as milliliters of serum phase released per gram of sample per unit of time. The decrease in the syneresis rate was observed in yoghurt produced from common starter culture. Therefore, the proportion of the serum separation was significantly less in yoghurt produced from common starter culture ( $P < 0.05$ ). In the case of yogurts produced *B.linens* as a starter culture, resulted in increase in the syneresis rate ( $P > 0.05$ ). This result was in agreements with the finding of Lee and Lucey, (2016). Lee and Lucey (2006) investigated the structural breakdown of the original (intact) yogurt gels that were prepared *in situ* in a rheometer, as well as, the rheological properties of stirred yogurts made from these gels.

**Table 2: 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 <sup>2</sup> )	201±1.04	123±1.12
Titrateable 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: *Brevibacterium linens* yoghurt.

CSCY: Common starter culture yoghurt

The results of the physicochemical properties of the yoghurts produced from *Brevibacterium linens* and Common starter culture yoghurt are presented in Table 2. The mean composition recorded for fat in yoghurt produced from *Brevibacterium linens* as starter culture and common starter culture yoghurt were  $3.02 \pm 0.01$  &  $3.07 \pm 0.02$ %. 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 *Brevibacterium linens* as starter culture ranged  $20.12 \pm 0.04$  &  $16.08 \pm 0.01$ %. This is in agreement with finding of Thomas and Mills, (1981), 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 solid and pH values recorded were  $201 \pm 1.04 \text{ mm}^2/\text{s}$  &  $123 \pm 1.12 \text{ mm}^2/\text{s}$ ,  $2.84 \pm 0.02\%$  &  $1.32 \pm 0.04\%$ ,  $18.64 \pm 0.04 \text{ mg/l}$  &  $22.40 \pm 0.02 \text{ mg/l}$ ,  $4.35 \pm 0.02$  &  $4.56 \pm 0.02$ , 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).

**Table 3: Organoleptic properties of Yoghurt produced from *B. linens* and common Starter culture**

Parameter	BLY	CSCY
Taste	5.06± 0.01	7.00 0.02
Flavor	6.30±0.02	7.14±0.01
Color	6.50± 0.04	7.50± 0.01
Texture	6.20 ± 0.01	7.66 ±0.04
Overall acceptability	6.01 ± 0.02	7.50 ±0.02

Means obtained from triplicate determinations (p<0.05)

Key:

BLY: *Bevibacteriumlinens* yoghurt

CSCY: Common starter culture yoghurt

The sensory assessment has judged by twenty (20) panelists was presented in table 3. The sensory attribute of the yoghurt is a combination of the flavor, colour (appearance), taste and texture (the mouth feel). The scores for flavour, texture, colour, taste, and overall acceptability of yogurt produce from *B.linens* as starter culture was significantly (P < 0.05) lower than those produced from common starter culture. The low organoleptic properties of *B.linens* yogurt was attributed to many factors such as; high quantity of whey and fat hydrolysis during fermentation Gran *et al.*, (1990). These made it to play a key role in cheese ripening and development of color pigment Arif *et al.*; (2006). This observation was in agreements with finding of Igwegbe *et al.*, (2015). The appearance of the yoghurt is a combination of the colour and visual separation of the whey.

**Table 4: Microbiological quality of yoghurt produced from *B. linens* and common starter culture**

Microorganisms	B.LY	CSCY
Total plate count (cfu/ml)	$4.8 \times 10^4$	$4.2 \times 10^6$
Coliform (faecal)	0.00	0.00
Salmonella count	$2.1 \times 10^4$	$1.0 \times 10^2$

Yeast count	$2.4 \times 10^4$	$1.4 \times 10^2$
Mould count	0.00	0.00
Lactic acid bac (LAB)	$1.8 \times 10^4$	$1.1 \times 10^2$

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

Key:

BLY: *Brevibacterium linens* yoghurt

CSCY: Common starter culture yoghurt.

The mean scores of the microbial counts: total plate count, *coliform*, *salmonella*, and mould and yeast counts, are presented in Table 7. The average total bacteria counts (TBC) of yoghurt produced from *B. linens* ranged from  $4.8 \times 10^4$  and  $4.2 \times 10^6$  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  $5.0 \times 10^4$  cfu/ml and  $3.0 \times 10^4$  cfu/ml respectively.

#### 4.0 Conclusion

The results from this study showed that yoghurt produced from *B. linens* as starter culture produced more whey than those produced from common starter ingredient. 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. The yoghurt produced from common starter ingredient (*L. bulgaricus* and *S. thermophilus*) generally accepted by panelists than yoghurt produced *B. linens*.

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