Evaluation Of Pathogenicity Test and Organoleptic Properties of Yoghurt Produced From Fresh Brown Goat (Hakuya) and Cow Milk Using Brevibacterium Linens As Starter Culture

Lawal, R. A. & Musa, H.
[1]Department of Food Science and Technology
Federal polytechnic
Bauchi, Nigeria
E-mail: motherofbelievers54@gmail.com

ABSTRACT

This study investigated the evaluation of pathogenicity test and sensory of yoghurt produced from fresh brown goat milk (Hakuya) and cow milk using *Bervibacterium linens* as starter culture. *Bervibacterium 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* was inoculated in pasteurized milk to compare its potential as starter culture against commercial starter culture (*L. bulgaricus and S. thermophlius*). 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 commercial starter culture were generally accepted by panelist. In conclusion, *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.

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

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1. INTRODUCTION

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 (Rattray 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 decolorizereadily. *Brevibacteria* are not acid-fast, produceno 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 isoften light dependent. The purple coloration ofB. iodinum results from the production andsecretion 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 Desmasures, (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

Brevibactrium linens were isolated and characterized from cheese. Prior to isolation of *Brevibacteriumlinens*, 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_2PO4. 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).

2.3.1 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 μl. The PCR mix contained 5 μl of a 10x, 160 mm NH_4-buffer (all products for the PCR are from Bioline, London, UK), 2 μl of a 50 mm $MgCl_2$ (final concentration 2 mm), 1 μl dNTP master mix (final concentration of each dNTP 0·5 mm), 2·5 μl 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 denaturizing 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^{o}C prior to subsequent used. 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 ºC ± 1 ºC 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.

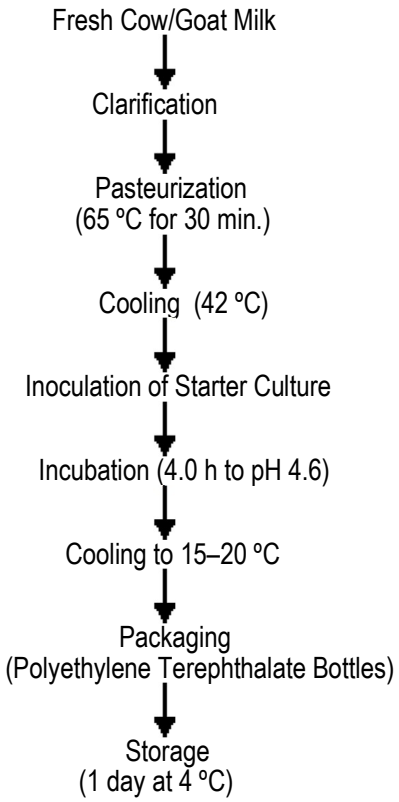

Figure1: Flow Chart for Modifying Method Yoghurt Production (Tamime and Robinson, 1999)

2.5 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 sensory analysis of the yoghurt samples was statistically evaluated using one way ANOVA and 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*

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 andOhin (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 are from human skin Pitcher and Malnick, (2018).

Table 1: 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, diacetyle, 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).

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 yoghurt produced from commercial starter ingredient (*L. bulgaricus and S.thermophilus*) generally accepted by panelists than yoghurt produced *B.linens*.. 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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