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ASSESSMENT OF PATHOGENECITY TEST , ORGANOLEPTIC PROPERTIES AND MICROBIAL LOAD OF CHEESE PRODUCED FROM WEST AFRICA DWARF GOAT (WAD) AND COW MILK USING *BREVIBACTERIUM LINENS* AS USING *BREVIBACTRIUM LINENS* AND SODOM APPLE EXTRACT AS COAGULANT

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ABSTRACT

This study investigated the pathogenecity test , Organoleptic properties and Microbial Load of cheese produced from West Africa Dwarf Goat (WAD) and Cow milk using *Brevibactrium linens* and Sodom apple extract as Coagulant. *Brevibacterium linens* were

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

isolated from samples of cheese. Milk samples were filtered and pasteurized at 90 ± 1 °C for 10 min. Sample A (West Africa Dwarf goat's milk) was inoculated with *Brevibacterium linens* as coagulant followed by direct acidification of Sodom apple extract with sample B (Cow's milk). The vats were incubated at 36 °C and gel was pressed, drained, cut, salted and package. The isolate(s) was tested for the presence of virulence gene. *B. linen* was inoculated in pasteurized milk to compare its potential as coagulant against Sodom apple extract. Aroma, mouth- feel and taste were monitored for the sensory quality and microbial activity were determined using standard laboratory procedures 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. The result of microbial properties revealed no coliform and mould was detected and small amount of other microbes were present. 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: West Africa Dwarf (WAD) goat milk, Cow milk, Sodom apple extract, *Brevibacterium linens*, coagulant.

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.

Cheese is a dairy product produced by coagulation of milk using acid or rennet, stirring and heating the curd, draining off whey, pressing the curd. It is further ripened or cured to obtain the final product. The essential ingredients in cheese making are milk and coagulants. Ripening or curing of the curd is one of steps in the development of texture and flavour of cheese (Ozcan & Kurdal, 2012). Cheese can also be made by coagulation of whole milk, skimmed milk, or full cream milk (Bodyfelt, Tobias, & Trout, 1998). The type of coagulant used depends on type of cheese so desired.

Goat cheese was one of the earliest made dairy products that were fermented by allowing raw milk to curdle naturally, draining and pressing the curds. Other techniques used are acid (lemon juice or vinegar) or rennet to coagulate the milk and obtain the curd. Production of cheese from goat milk has a long history. Cheese made from goat milk provides a good source of protein for people in several countries (Seifu, Buys, & Donkin, 2004). It was equally used as a mode of preservation of milk by the nomadic Fulani women of Nigeria. Nowadays, the practice is still in existence and exercised by others who have access to fresh goat milk. Cheese made from goat milk is lower in fat, calories and cholesterol. It also provides more calcium than cream cheese. It is consumed by just a few majority of Nigeria's population due to limited supply of raw goat milk and again the majority are unaware of the nutritional benefits, hence the need to create awareness and meet up with protein demand of the people

One of the key ingredients in cheese making is coagulant and rennin which serves as coagulants from animal origin is the commonest coagulant used (Roseiro *et al*., 2003). To large extent, the yield and quality of cheese is determined by the quality of milk and the type of coagulants used, and several plant coagulant such as *Cynara cardunculus,* sun flower, Moringa extract, pineapple,papaya, *Calotropis procera* (Sodom apple) and so on, have been used to clot milk (Aworth and Muller,1987). In recent development, it has been observed that milk coagulants of plant origin have over-ridden the use animal rennin. The reason being that animal rennin may be limited for diet (vegetarianism), religious reasons (Judaism),or being genetically engineer food, of which the Germans and Dutch for example, forbid the use of recombinant calf rennin (Roseiro *et al*., 2003).

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, 2008). 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. This study therefore investigated the potential of using *Brevibactrium linens* and moringa extract as coagulant in cheese produced from fresh brown goat milk (Hakuya) and cow milk and evaluates nutritional quality of the milk.

Materials and Methods

Source of Milk

Fresh cow and West Africa Dwarf goat (WAD) milks 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.

Source of Sodom apple extract

Sodom apple leaves were plucked from staff quarter in Federal Polytechnic, Bauchi, State Nigeria.

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.*

Isolation of *Brevibacterium linens* from cheese

Brevibactrium 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_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).

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.

Sample preparation

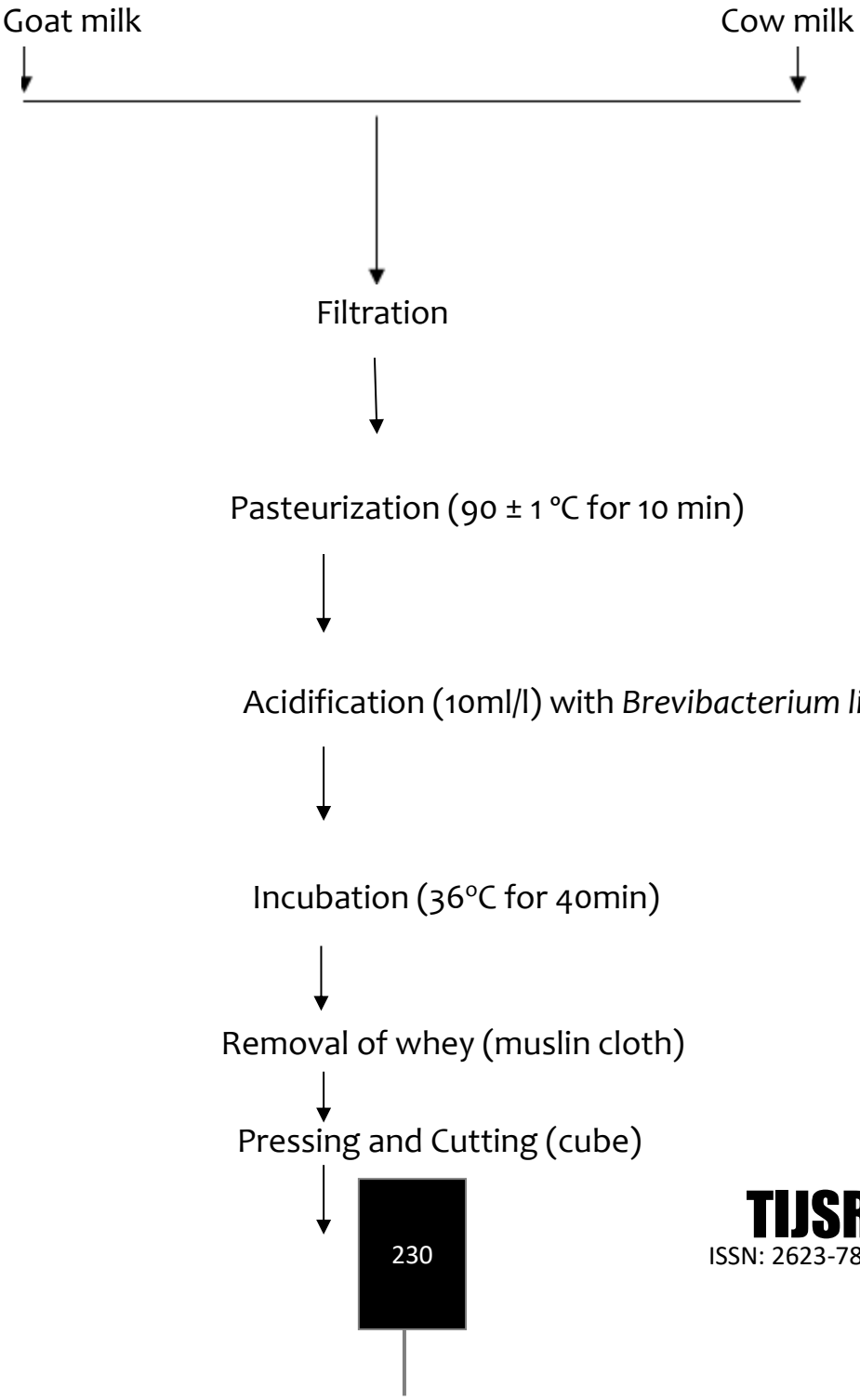
Preparation of extract

Sodom apple leaves were cleaned under run water to remove dirt and other foreign materials. The leaves were drained and squeezed for 5 minutes, filtered and the extract was stored at temperature of 4°C for subsequence use.

Production of cheese

Two different cheese types were made from two samples of fresh milk: CCM (cheese made from cow's milk) and CGM (cheese made from West Africa Dwarf goat's milk). The cheeses were produced using the method described by Adetunji and Babalobi, (2011). 500ml of each sample of milks were filtered, labeled and pasteurized at 90 ± 1 °C for 10 min. Sample A was inoculating with 10ml/l *Brevibacterium linens* and sample B was acidifying with Sodom apple extract. The vats

were incubated at 36 °C until a firm curd was formed (approximately 40 min). The obtained gels were allowed to drain, press, gently cut into cubes, salted in brine (12 g/L NaCl), placed in perforated rectangular containers (approximate capacity of 250 g) and maintained at 10 °C under pressure for 4 h and vacuum packaged. The cheese obtained after storage at 10 °C for 24h was regarded as the final product.



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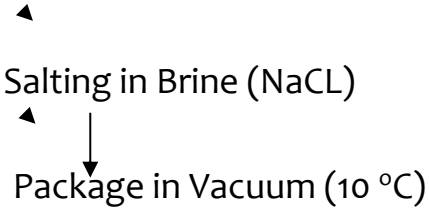

Figure 1: flowchart for the production of goat milk and cow milk.

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 cheese compared with cow milk cheese using the optimized processing conditions. Determination of acceptability was done using 20 trained panelists who were familiar with cheese 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 cheese 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.

Microbiological analysis of goat milk –cheese and cow milk-cheese.

Method of Harrigan and McCane (1976) was employed. Exactly 1.0 g of the cheese was aseptically weighed and carefully introduced into 9 .0 ml of sterile distilled water. This was shaken manually in order to have a homogenous suspension. 1.0 ml of this was taken and introduced into the second tube followed with series of dilutions up to10-2

dilutions. 1ml was taken from 10-2 dilution and pour plated on: (a) Nutrient Agar and incubated at temperature 37 °C for 48 hours; (b) MacConkey Agar was used for the enumeration of total coliform organisms in the sample, the plates were incubated at temperature 35 °C for 48 hours; while (c) Sabouraud Dextrose Agar was used for the enumeration of mould and yeast in the samples. The plates were incubated at temperature 30 oC for 24 hours for yeasts and 3 days for mould.

Microbial counts were calculated as follows. $\frac{DF \times N}{W}$

Where DF = dilution factor.
N = number of colonies.
W = weight of sample used.

Statistical Analysis

The sensory analysis of the cheese samples was statistically evaluated using one way ANOVA and paired t-test

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*

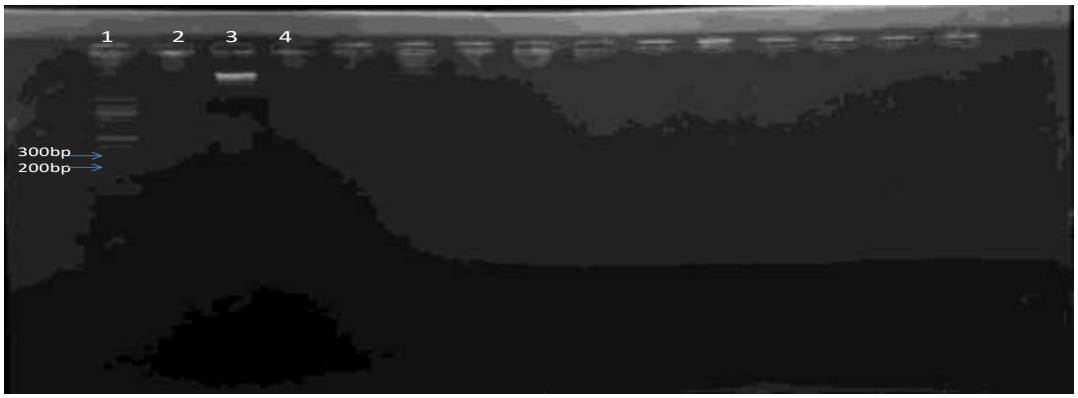

Fig 3: PCR gel electrophoresis profile for the confirmation of virulence gene in *Brevibacterium linens.* Molecular weight ladder (100bp DNA ladder bioline, UK); Lane 1: 100bp ladder, lane 2 *: Sample* , lane 3; *Genomic DNA* , lane 4; *Negative control*

Figure 2: PCR gel electrophoresis profile for the confirmation of virulence gene in Brevibacterium linens

The figure 2; 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).

Sensory attribute of cheese produced from West Africa dwarf goat milk and cow milk

Table 1: Sensory attribute of cheese produced from West Africadwarf goat milk and cow milk

Sample	Appearance (colour)	Aroma	Taste	Texture	Overall acceptability
GC	8.87 ± 0.12	7.22 ± 0.04	7.84 ± 0.01	8.44 ±0.07	7.98 ±0.02

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CC	8.24 ± 0.07	8.09 ±0.14	8.62 ± 0.02	8.27 ± 0.04	8.67 ±0.02

Values are means ± SD of triplicate determination. GC: West Africa dwarf goat milk cheese, CC: cow cheese.

Sensory assessment as judged by 20 taste panelists is presented in Table 1 as means of the scores. The sensory attribute of cheese is a combination of the flavour, colour (appearance), taste and texture (the mouth feel). The cheese made from West Africa dwarf goat milk was found to be significantly different ($P > 0.05$) in colour (appearance) but lower in aroma, taste and overall acceptability ($P < 0.05$) to those made from cow milk, (Table 1), with average scores of 8.87 ± 0.12 – 8.24 ± 0.07 for colour; 8.09 ± 0.14 - 7.22 ± 0.04 for aroma; .8.62 ± 0.02 – 7.84 ± 0.01 for taste, 8.44 ± 0.07-8.27 ± 0.04 for texture and 8.67±0.02- 7.98 ± 0.02 for overall acceptability respectively. The disparity seen in the result of aroma, taste and overall acceptability in cheese produced from cheese produced from West dwarf goat milk, may be attributed to" goaty flavor".

Microbial properties of cheese produced from West Africa dwarf goat and cow milk

Table 2: Microbial properties of cheese produced from Nigeria Africa dwarf goat and cow milk.

Parameters	TBC (cfu/ml)	FCC (cfu/ml)	TFC (cfu/ml)
GC	1.41×10^6	NIL	NIL
CC	1.82×10^4	$<1.0 \times 10^4$	$< 0.01 \times 10^2$

Values are means ± SD of triplicate determination. GC: West Africa dwarf goat cheese, CC: cow cheese. TBC: Total Bacterial Count, FCC: Faecal Coliform Count, TFC: Total Fungi Count, NIL: Not detected.

The microbial (bacterial, faecal coliform and fungi) load of the different milk cheeses are shown in Table 2. The result of total bacterial growth in all the samples of cheese was ranged: 1.14 x 10^6– 1.82x10^4 cfu/ml. There was no significant ($p< 0.05$) different in all the samples of cheese for total bacterial count. The sample of cheese produced from West Africa dwarf goat milk and cow milk revealed no faecal coliform and fungal were detected is thought to be as result of the antimicrobial effects of the *Brevibacterium linens,* 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). It has antimicrobial properties which can reduce the effect of pathogenic *Listeriamono cytogenes* by1-2 log units .This property makes it safer for human consumption (Motta and Brandelli, 2008).
The study thus, reveals all samples were microbiologically safe for human consumption, since the microbial loads did not exceed the acceptable limits of >10^5 recommended by the International Commission of Microbiology Specifications of Foods (Korshina*et al*., 2019). This is an indication that all cheese samples were well processed under good hygienic conditions.

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. This study has proven the potential of *Brevibacterium linens,* as a coagulant, which contributes to the final appearance, flavour, colour and aroma of cheese. Many recent studies have purified and identified *Brevibacterium linens,* for application in food technology, which aims to extend food preservation time, treat pathogen disease and cancer

therapy, and maintain human health. Therefore, *B linens* may become a potential drug candidate for replacing antibiotics in order to treat multiple drugs resistance pathogens in the future. Microbial communities from rinds of surface-ripened cheeses are composed of various bacteria, yeasts and molds, which contribute to the flavor, texture and appearance of the final products.

However, fresh West Africa dwarf goat milk and cow milk produced under good hygienic condition in the manufacture of acceptable cheese of excellent nutritional, microbial and sensory qualities. Goat milk and cow milk are some of the healthiest beverages that are available today, but goat milk is easy to digest than cow milk because of small fat globules and is naturally homogenized. Goat milk is non allergic as compared to cow milk and it can be used in the treatment of certain diseases. Efforts should therefore be intensified toward commercial production of cheese and other dairy products using *Brevibacterium linens,* as a coagulant.

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Competing Interests: The authors declare that they have no competing interests.

Acknowledgement: All authors contributed equally to the conception and design of the study.

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