Potential of Using *Brevibactrium linens* and Moringa Extract as Coagulant in Cheese Produced from Fresh Brown Goat Milk (Hakuya) and Cow Milk

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ABSTRACT

This study investigated the potential of using *Brevibacteruim linens* and Moringa extract as coagulant in cheese produced from brown goat (Hakuya) milk and cow milk. *Brevibacterium linens* were isolated from samples of cheese. Milk samples were filtered and pasteurized at 90 ± 1 ºC for 10 min. Sample A (brown goat milk) was inoculated with *Brevibacterium linens* as coagulant followed by direct acidification of Moringa extract with sample B. The vats were incubated at 36 ºC and gel was pressed, drained, cut, salted and package. The samples were analyzed for physicochemical properties and sensory quality using standard laboratory procedures. All the cheeses produced from brown goat's milk using B*. linens* as coagulant was significantly (P > 0.05) different in all the quality parameters compared with cow's milk using moringa extract as coagulant. The mean values for moisture content, fat, protein, ash, pH, TTA and percentage yield were ranged: 48.52-50.44%, 16.13-20.42%, 13.88-14.08%, 1.84-2.01%, 36.65-42.23%, 5.84-5.96% and 0.64-0.68% respectively. The result of the sensory evaluation of cheese produced from brown goat's milk (Hakuya) was generally accepted by panelist. Efforts should therefore be intensified toward commercial production of cheese and other dairy products using *Brevibacterium linens,* as a coagulant.

Keywords: *Brevibactrium linens* , moringa, coagulant, cheese, hakuya

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1. BACKGROUND OF RESEARCH

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

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

Moringa extracts have many health benefits. It contains natural antibiotic, which can be used to give relief from many medical condition like; rheumatism, gout, urinary infection and fungi infection. *Moringa oleifera* extract has been used in the purification of drinking water (Zarkadas and Burron, 2005). The coagulant property of the extract was successfully used in soymilk for the production of *tofu* (Innocent, 2014). The use of this extract will go a long way in substituting the use of rennet which is a bit expensive and does not contribute to the nutritional quality of the cheese. Other coagulants include; alum, and calcium chloride are chemicals in nature and may have side effect because of the quantity that may be used in the coagulation of cheese from milk (AOAC, 2005). *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.

2.0 MATERIALS AND METHODS

2.1 Source of Milk

Fresh cow and brown goat (Hakuya) 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.

2.2 Source of moringa extract

Moringa leaves were plucked from staff quarter in Feredral Polytechnic, Bauchi, State Nigeria.

2.3 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.*

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

2.5 Sample preparation

2.5.1 Preparation of extract

Moringa 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^{o}C for subsequence use.

2.5.2 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 brown 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 ^{o}C for 10 min. Sample A was inoculating with 10ml/l *Brevibacterium linens* and sample B was acidifying with moringa extract. The vats were incubated at 36 ^{o}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 ^{o}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.

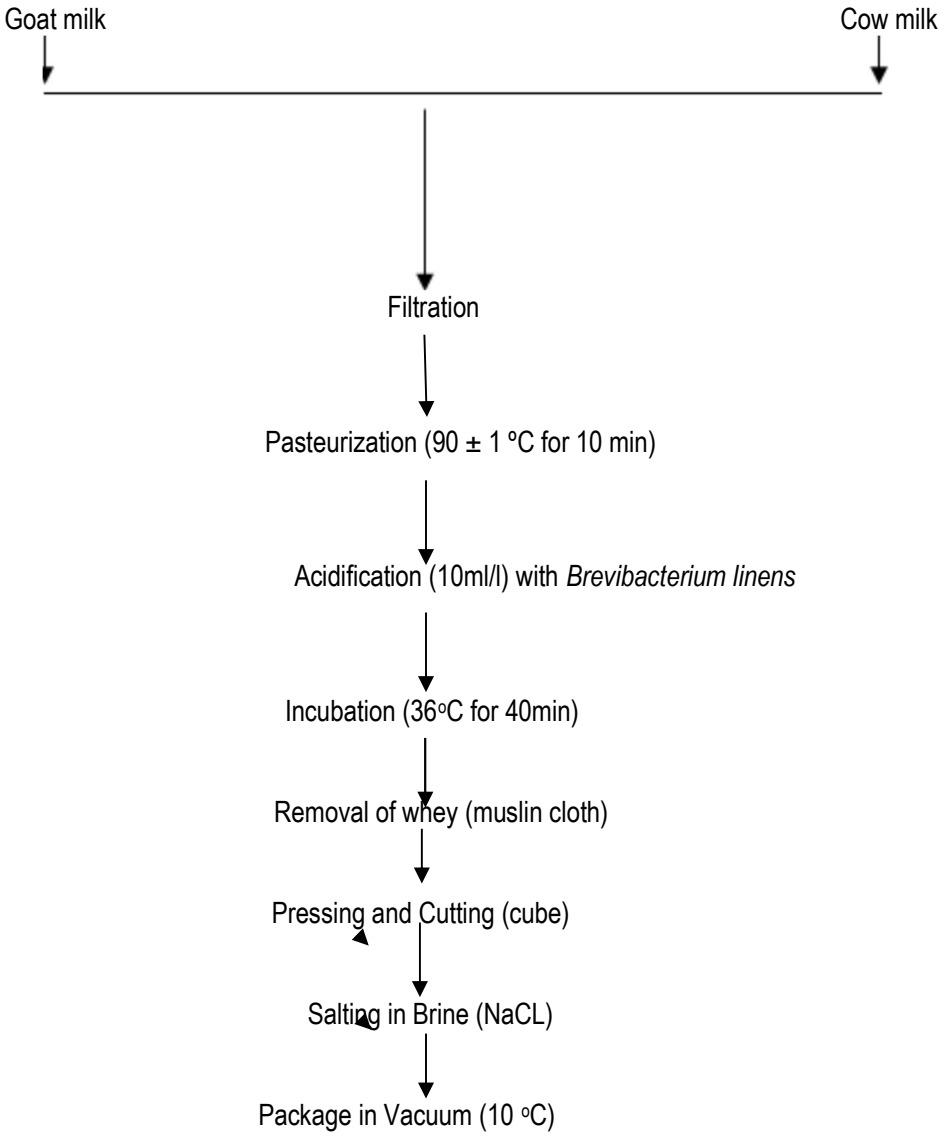

Figure 1: flowchart for the production of goat milk, cow milk and their mixture cheese.

2.6 Determination of proximate composition cheese samples

The moisture, crude protein, crude fat and total ash contents of the cheese samples were determined according to the standard methods of AOAC (2012). The carbohydrate content was determined as shown below:
% Carbohydrate = 100%–(% moisture + %protein + % fat + % ash), (Akume *et al*., 2019).

2.7 Determination of Physical properties of cheese

2.7.1Total Titratable Acidity (TTA)

Total titratable acidity was determined using the AOAC (2005) method. About 10 g of the sample was dissolved in 30 ml of distilled water in a beaker and stirred. The mixture was filtered into 100 ml standard volumetric flask. The filtrate was made up to 100 ml. A 10 ml sample of the filtrate was pipetted into a beaker and 1 drop of phenolphthalein was added. The mixture was titrated against standard 0.01 N Sodium Hydroxide solutions until light pink color was attained. The reading of the burette was recorded.

$$TTA = \frac{N(\text{NaOH}) \times \text{titre value} \times \text{lactic acid value} \times \text{dilution factor} \times 100}{10}$$

Where N = Normality of NaOH (0.01)
Lactic acid value = 0.09
Dilution factor = 10

2.7.2 pH Determination

pH was determined using pH meter (Unicom 9450, Cambridge, UK). About 1.0g of the cheese was dissolved in beaker containing 10 ml of distilled water and stirred. The electrode of the pH meter was dipped into the beaker and readings were obtained from the photo-detector on the pH metre.

2.7.3 Total Soluble Solids

This was determined using the AOAC (2005) method. A clean glass dish was dried in an oven (103-105 ^{o}C) until constant weight was achieved, cooled in a desiccators and weighed. About 2.0 g was dissolved in 50 ml distilled water. About 20 ml of filtered water sample was evaporated on a water bath at temperature 90 ^{o}C followed by oven drying at temperature 103 ^{o}C- 105 ^{o}C for about an hour. The glass was cooled in desiccators, reweighed and the increased weigh recorded.

2.7.4 Determination of Percentage Yield

Percentage yield of cheese was determined by method described by Igyor, Igbian, and Iorbo (2006). The yield of cheese from brown goat's milk and cow's milk were determined by the calculation as follows:

$$\text{Yield of Cheese (\%)} = \frac{W_2 \times 100\%}{W_1}$$

W1 = Weight (g), goat milk, cow milk and cow-goat milk blend.
W2 = Weight (g), cheese produced.

2.8 Sensory evaluation

Sensory evaluation was conducted using a trained panel consisting of twenty members who are familiar with cheese. The Panelists were instructed to evaluate the coded samples for appearance, aroma, taste, texture, and overall acceptability. Each sensory attribute was rated on a 9- point hedonic scale (9 = like extremely and 1 = dislike extremely) (Ekanem and Ojimehkwe, 2017).Cheese samples were served in 3-digit coded white plastics. The order of presentation of samples to the panelists was randomized. Sensory evaluation was carried out under controlled conditions of lighting and ventilation.

2.9. Statistical analyses

The data obtained were subjected to Analysis of Variance (ANOVA), while Duncan Multiple range test was used to separate means where significant differences existed, data analyses was achieved using the Statistical Package for Social Statistics (SPSS) software version 20.0. All analyses were performed in triplicate determination.

3.0 RESULTS AND DISCUSSION

3.1 Physicochemical properties and sensory evaluation of cheese produced from goat and cow's milk using *Brevibacterium linens* and Moringa extract as a coagulant

Table 1: Physicochemical and sensory quality of cheese produced from goat and cow's milk using *Brevibacterium linens* and Moringa extract as a coagulant

Parameters	Sample A	Sample B
Moisture contents (%)	50.44 ± 0.06	48.52 ± 0.02
Protein (%)	20.42 ± 0.02	16.13 ± 0.04
Fat (%)	14.08 ± 0.01	13.88 ± 0.02
Ash (%)	1.84 ± 0.04	2. 01 ± 0.02
Yield (%)	42.23 ± 1.02	36.65 ± 0.12
pH	5.84 ± 1.12	5.96 ± 1.00
Titratible acidity (g/l)	0.64 ± 0.08	0.68 ± 0.04
Taste	8.64 ± 1.04	87.74 ± 1.14
Color	7.88 ± 0.08	7.66 ± 0.04
Flavor	8.32 ± 0.14	7.88 ± 0.02
Texture	8.45 ± 0.06	8.50 ± 0.18
Overall acceptability	8.72 ± 1.14	8.65 ± 1.14

Values represent the means of triplicate analysis result with respective standard deviation. A: cheese from goat's milk using *Brevibacterium linens* as coagulant, B: cheese from cow 's milk using Moringa extract as coagulant.

The results of the physicochemical and sensory quality of the cheese produced from goat and cow milk's are presented in table 1.The moisture content of both cheese samples were ranged from 48.52-50.44% respectively. There was significant different in moisture content of cheese produced from cow's milk using moringa extract as coagulant ($p< 0.05$). This disparity in moisture content of cheese produced from moringa extract as coagulant may be attributed to variation in moisture content of milk samples. Higher moisture content could favour growth and proliferation of microorganisms; thus reducing the shelf-life of cheese (Orhevba and Taiwo, 2016). The protein content of cheese produced from goat's milk using *Brevibacterium linens* as coagulant is slightly higher than cheese produced from cow's milk using Moringa extract as coagulant The disparity seen in the protein content of cheese in this study could probably be due to the denaturization of whey protein during pasteurization and the resulting β-lactoglobulin–κ casein entraps denatured whey proteins, which may lead to some minor differences in amino acid profiles between lactic cheese and soft cheese (Henry *et al*., 2002 cited in Raynal-Ljutovac et al., 2008). This work was in agreement with the contribution of Rattray & Fox, (1999) *Brevibacterium linens* towards cheese production, showing that it can break down lipids and proteins (i.e. casein) with the use of extracellular proteases and lipases,(Ozturkoglu-Budak, et al., 2016).

This study also, in line with report of Henning *et al*., (2006) casein remains in the curd, but caseins are low in sulphur-containing amino acids and the nutritional value of cheese protein is slightly lower than that of total milk protein. Also, progressive breakdown of casein during ripening is reported to increase its digestibility (Henning et al., 2006). Moreover, proteolysis induced by fermentation and ripening increases amounts of bioactive peptides and free amino acids present in the cheese. The fat content of the cheese produced from goat's milk using *Brevibacterium linens* as coagulant was significant (p >0.05) higher than cow's milk using moringa extract as coagulant. The curd contains almost 95 percent of the fat, and during cheese-making the fat is concentrated between 6- and 12-fold, depending on cheese variety (Fox and McSweeney, 2004). Although the content of nutritionally interesting FAs such as CLA can be increased by lipid supplementation of the goat diet, this may be accompanied by a change in cheese flavour (Chilliard and Ferlay, 2004, Chilliard *et al*., 2005 and Chilliard *et al*., 2006a, cited in Raynal-Ljutovac *et al*., 2008).

The ash content of both cheese samples varied from: 1.84-2.01%. The cheese produced from cow milk using moringa extract as coagulant has higher ash content. The physical properties showed that pH ranged from; 5.84-5.96 while titratible acidity ranged from; 0.64-0.68 g/l. There was slight increase in titratible acidity of cheese produced from cow's milk using moringa extract as coagulant. This shows the cheese is slightly acidic but within acceptable level. Sensory quality 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 brown goat's milk was found to be significantly different (P > 0.05) in colour, taste, flavour, texture and overall acceptability.

4.0 CONCLUSION

This study revealed that the cheese produced from goat's milk using *Brevibacteriim linens* as coagulant has high protein content and higher yield compare with work of (Kwaya *et al*., 2018), which reported that the protein contents of cheese produced using moringa seed extract as coagulant are within the range of 29.14 – 30.45% and 16.76-16.69 % respectively .However, the cheese produced from goat milk using *Brevibacteriim linens* as coagulants were generally accepted by panelist. The usage of the *Brevibacteriim linens* should be adopted in dairy industries.

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