



Full Research Paper

Assessment of Syneresis in yoghurt produced from fresh cow milk using *Brevibacterium linens* as starter culture

¹Lawal ,R. A
¹ Musa, H.
²Adebusoye, M. S
¹Haruna U.S.

¹Department of Food Science
& Technology
²Department of Nutrition and
Dietetics
Federal Polytechnic, Bauchi.
Bauchi State, Nigeria



Corresponding Author's E-mail
motherofbelievers54@gmail.com

Corresponding Author's Phone
1+2348030433283

ABSTRACT

Brevibacterium linens has long been recognized as an important dairy microorganism. This study investigated Assessment of Syneresis in yoghurt produced from fresh cow milk using *Brevibacterium linens* as starter culture. *Brevibacterium linens* was isolated from sample of cheese (procured from Jos metropolis). The milk samples were clarified to remove foreign materials, pasteurized at 65°C for 30min to destroy pathogenic materials and later cooled to temperature of 42°C and *B. linens* was inoculated in pasteurized milk to compare its potential as starter culture against common starter culture (*L.bulgaricus* and *S. thermophilus*), the mixture allowed to ferment for 4 hrs. The semi- solid curds were homogenized; package and cool at 4°C and the same method was repeated for common starter yoghurt. Syneresis was determined by using centrifugal method. The result of syneresis of yoghurt produced *Brevibacteriu linens* as starter was significant ($p<0.05$) lower than that produced from common starter culture. The usage of *B. linens* as starter culture should be encouraged in dairy industries.

Keywords: Syneresis, cow milk, yoghurt, *Brevibacterium linens*, starter culture.

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1. INTRODUCTION

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 microorganisms 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).

All dairy raw materials should be selected for high bacteriological quality. Ingredients containing mastitis milk and rancid milk should be avoided. Also, milk partially fermented by contaminating organisms and milk containing antibiotic and sanitizing chemical residues cannot be used for yogurt production. During milk fermentation, the casein becomes unstable and coagulates to form a firm gel, composed of strands of casein micelles, with whey entrapped within this matrix, which is interlocked via hydrogen bonds, forming a protein matrix. Yoghurt structure is the result of disulphide bonding between k-casein and denatured whey proteins and by aggregation of casein as the pH drops to the isoelectric point of the casein proteins during fermentation (Dave *et al.* 1996). An important aspect of a milk gel is whey separation, which refers to the appearance of a liquid on the surface of milk gel. It is a common defect in fermented milk products such as yoghurt (Lucey *et al.* 1998). Syneresis is defined as the shrinkage of gel, and this occurs concomitantly with expulsion of liquid or whey separation and is related to instability of the gel network resulting in the loss of the ability to entrap all the serum phase (Walstra 1993).

According to Lucey *et al.* (1998), some possible causes of wheying-off in acid gels are very high incubation temperatures, excessive treatment of the mix, low total solids content (protein and/or fat) of the mix, movement or agitation during or just after gel formation and very low acid production (pH > 4.8) (Lucy *et al.* 1999; Dannenberg and Kessler 2009). Monnet *et al.* (2015) reported that factors influencing yoghurt texture and syneresis include total solids content, milk composition (proteins, salts), homogenization, type of culture, acidity resulting from the growth of bacterial cultures and heat pretreatment of milk. 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. *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, 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 flavor 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 (Weimer, et al., 2000). *Brevibacterium* have also been isolated from wheat samples (Legan, 2000).

B. linens produce red or orange or purple-colored pigment of aromatic carotenoide type which is 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 of 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). Therefore, this study was investigated the potential *B. linens* as starter culture against standard culture used in yoghurt production compared the quantity of whey (syneresis) produced.

2. 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; to isolate *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 15minutes and incubated at 25°C for 48hours with stirring (150rpm) to oxygenate the medium (Galaup et al., 2005).

2.4 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 in 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 inoculated with *B. linens* starter culture, 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*.

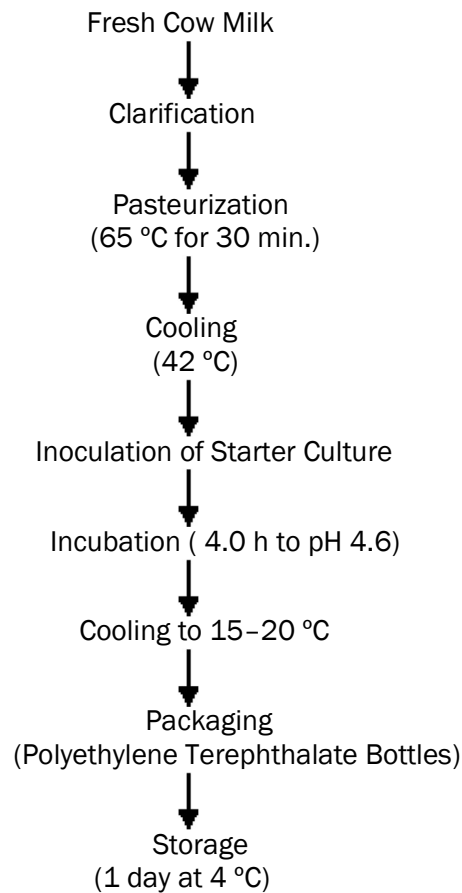


Figure1: Flow Chart for Modifying Method Yoghurt Production (Tamime and Robinson, 1999)



2.5 Determination syneresis by centrifugal method

Whey separation of yoghurt samples were done by using the method as described by Hassan et al., (2015). 25ml of set yoghurt at 5 °C was slowly transferred to 50 ml capacity centrifuge tubes causing minimum disturbance to the coagulum. The centrifuge tubes were balanced by adjusting their weights and centrifuged at 3394 RPM in a Remi centrifuge (Make-Remi, India) for 20 min. The quantity of whey separated at the top of the coagulum inside centrifuge tubes was recorded as milliliters. The weight fraction of the supernatant liquid was used as index of whey syneresis (ml/100 g yoghurt). The higher the volume of whey separated, the higher was the whey separation and vice versa.

2.6 Statistical Analysis

The data obtained from the various experiments during standardization process and storage study of developed product were subjected to One-way analysis of variance (ANOVA) and t-test using SAS 9.3 version under the guidance of statistical,

3. Results and Discussion

What follows are tables presenting results as well as discussion

Table 1: Assessment of syneresis in yoghurt produced from *B. linens* as starter culture against standard culture used in yoghurt production.

Yoghurt (ml)	<i>B. linens</i> (%)	CSCY (%)
Whey separation (mg/100g of yoghurt)		
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 syneresis in yoghurt produced from *B.linens* as starter culture against standard culture used in yoghurt production are shown in Table 1. 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.

4. CONCLUSION

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, this research attempted the assessment of syneresis in yoghurt produced from *B. linens* as starter culture against standard culture used in laboratory. Yoghurt produced from *B. linens* as starter culture produced more whey than those produced from common starter ingredient. However, it is recommended that production of dairy products from *Brevibacterium linens* is to be encouraged. Research in utilization of *Brevibacterium linens* as starter ingredient in dairy production, processing and introduction of new bio-technologies need to be strengthened.



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