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VIABILITY OF LACTIC ACID BACTERIA IN COMMERCIALLY PREPARED AND HOMEMADE BUFFALO CURD DURING REFRIGERATION STORAGE

W. A. S. R. Fernando, T. K. Weerasinghe and R. U. Pathirana

Department of Botany, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka.

INTRODUCTION

Buffalo curd is a popular fermented dairy product and presently people produce them at both cottage and industrial scales. Lactic Acid Bacteria (LAB) are an important group of microorganisms which plays a major role in buffalo curd production. Fermented dairy products contain live microorganisms and have long been considered safe and nutritious foods. These products are also considered to be probiotic because, when consumed in certain quantities, they have beneficial effects on health. A number of health benefits have been proposed including anti-microbial, anti-mutagenic, anti-carcinogenic and hypersensitive properties and reduction in serum cholesterol, alleviation of lactose intolerance and reduction of allergic symptoms (Shah, 2000).

Bacteria that are considered to be probiotic include the *Bifidobacterium* spp. and *Lactobacillus acidophilus* (Shah, 2000). However, they should reach the intestine alive and in a sufficient number (10^6 - 10^7 microorganisms mL^{-1}) for their benefits to be appreciated.

The viability of lactic acid bacteria in fermented cow's and goat milk products such as yoghurt, sour cream, cheese etc. in various storage conditions has been adequately researched. However, the viability of these organisms in Buffalo curd has not been adequately researched (Samona & Robinson,1994). Thus, there is a necessity to analyze the viability of Lactic Acid Bacteria during refrigeration storage, which is the most preferred storage condition, in commercially prepared and homemade buffalo curd, to ensure that its health properties are preserved.

The primary objectives of this study were to investigate the viability of LAB under refrigeration in different curd preparations and to study the changes of physicochemical properties during shelf life. Further, the identification and characterization of the microorganisms in curd were also carried out to ensure the presence of LAB and to decide on the probiotic potential of different curd preparations.

METHODOLOGY

Six different buffalo curd products were selected from local super markets where three of the products A, B and C were commercial curd and the other three products D, E and F were homemade curd. They were stored at refrigeration temperature (4°C) during the study.

Chemical parameters such as pH and titratable acidity (AOAC, 2007) were determined every other day in curd. Three important LAB in curd namely, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and *Streptococcus thermophilus* were isolated and enumerated every other day on selective media, respectively MRS agar (De Man *et al.*, 1960), MRS-NaCl (2%) agar (Dave & Shah, 1996) and *Streptococcus thermophilus* (ST) agar (Dave & Shah, 1996). The colonies were expressed as $CFUg^{-1}$ of Buffalo curd The colonies isolated were identified using identification keys on Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986) and Cowan and Steel's Manual for the Identification of Medical Bacteria (Cowan, 1974). Biochemical tests were carried out to confirm the isolates.

The experiments were in quadruplets and analysis of variance of the data and one sample T test was done using the computer software package MINITAB 14.1 version. A probability level of $P<0.05$ was used to indicate significance.

RESULTS AND DISCUSSION

THE VIABILITY OF LAB IN BUFFALO CURD

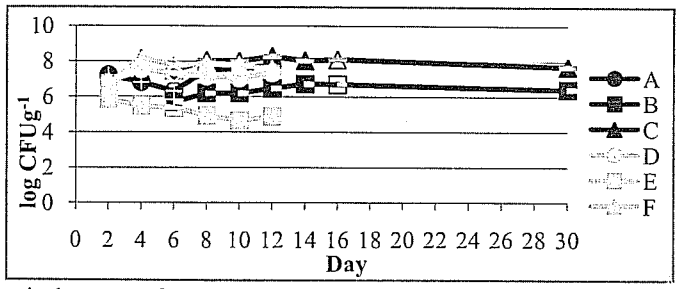


Figure 1. - Survival curves of *Lactobacillus delbrueckii* ssp. *bulgaricus* in different Buffalo curd brands during refrigeration storage - □ (Marker without fill) represents the expiry date of the Buffalo curd.

Different curd products showed different counts of the presence of *Lactobacillus delbrueckii* ssp. *bulgaricus* 5 - 7.5 log $CFUg^{-1}$ initially (Fig.1). It may have depended on the type and the combination of the starter culture used in the production procedure and also the amount of the starter culture. Also during early days of storage, the changes observed among different curd brands may have occurred due to strain variations which change with the starter culture (Dave & Shah, 1997). The brand E showed a less viable count of the *Lactobacillus delbrueckii* ssp. *bulgaricus* compared with the other brands. Also, the number decreased during storage, even after the shelf life, in contrast to other curd brands. It may be due to non availability of necessary conditions to keep the viability of the organism at a constant level and also the higher lactic acid percentage in the curd. In brand A the numbers suddenly decreased as the expiry date approached and again increased afterwards. It may have been due to a suppression of another unknown microorganism and the inhibition of it due to the acidity. Or else, it might have been due to the increasing pH and a positive association with an end product of them after shelf life.

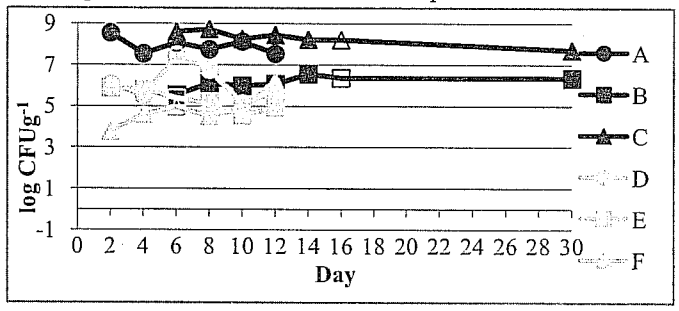


Figure 2. - Survival curves of *Streptococcus thermophilus* in different Buffalo curd brands during refrigeration storage- □ (Marker without fill) represents the expiry date of the Buffalo curd.

According to Fig 2, a higher number of *Streptococcus thermophilus* were present in A and C curds which could be due to the higher amount of them in the starter culture. B had a comparatively lesser number (less than 7 $logCFUg^{-1}$) of *Streptococcus thermophilus* when considering the other commercial Buffalo curd which were above 7 $logCFUg^{-1}$. The viable counts of C and E decreased slightly with the day. It may be due to the increase of the lactic acid in curd and the limited nutrients available in the milk. The counts in B and F increased slightly as the day progressed while the *Streptococcus thermophilus* counts were highly variable in D. In general the counts decreased after the shelf life which may have been due to the post acidification of *Lactobacillus delbrueckii* ssp. *bulgaricus*. According to Dave and Shah (1997), the viable counts of *Streptococcus thermophilus* increases until the 5^{th} day and then decreases in number when they

are in a combination of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and Bifidobacteria in yoghurt. In all buffalo curd brands other than C, the same phenomenon took place.

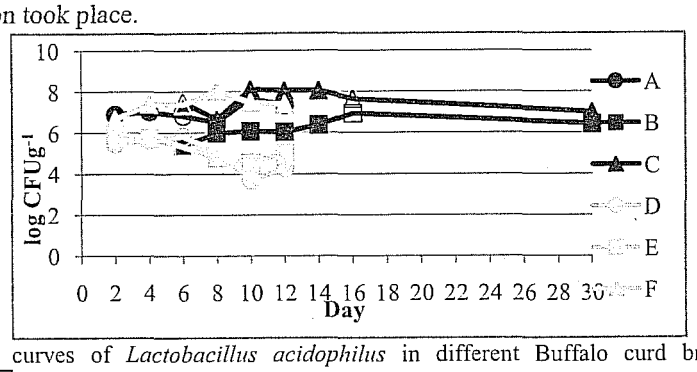


Figure 3. - Survival curves of *Lactobacillus acidophilus* in different Buffalo curd brands during refrigeration storage.- □ (Marker without fill) represents the expiry date of the Buffalo curd.

In Fig 3 , the brands D and E showed a low number of the presence of *Lactobacillus acidophilus* which must be probably due to the low amount of starter organisms from the previous cultures and the low amount of nutrition available in the environment. Decrease in the numbers drastically after the shelf life may be due to the effect of *Lactobacillus delbrueckii* ssp. *bulgaricus* on them with their post acidification (Dave & Shah, 1997). The sudden fall of the number of *Lactobacillus acidophilus* could be due to the higher counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* in D. The sudden increase of *Lactobacillus acidophilus* count after the shelf life in A and F must have been due to a removal of a growth suppressor or a metabolic end product which might have a positive symbiotic effect. The viable counts increased in B and C and they decreased slightly after the expiry date because they were commercially prepared curd with high amount of nutrients. According to Dave and Shah (1997), the viable counts of *Lactobacillus acidophilus* decrease rapidly in number by almost 3 log cycles when they are in a combination of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and Bifidobacteria in yoghurt and the recommended limit of 1 million cells per gram is maintained only for 20-25 days. Such a behavior of the *Lactobacillus acidophilus* viability was not observed in Buffalo curd. It may be due to the nutrition difference in yoghurt and Buffalo curd.

pH AND TITRATABLE ACIDITY

The pH of all curd brands was stable even after the shelf life other than the pH of A, where it decreased as the day progressed (Fig 4). The pH is greatly influenced by the lactic acid production of the lactic acid bacteria and the contribution is mainly by *Lactobacillus delbrueckii* ssp. *bulgaricus*.

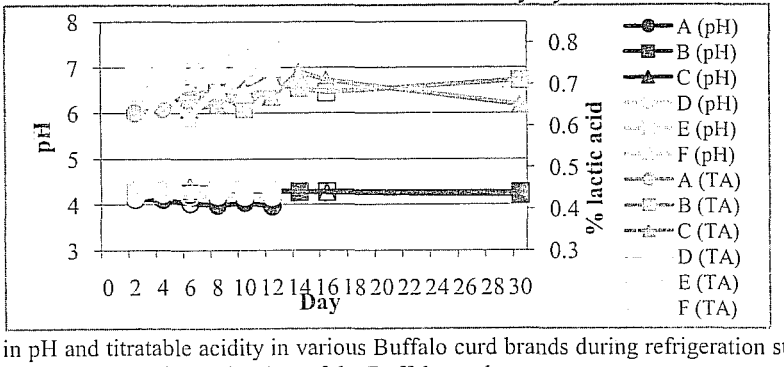
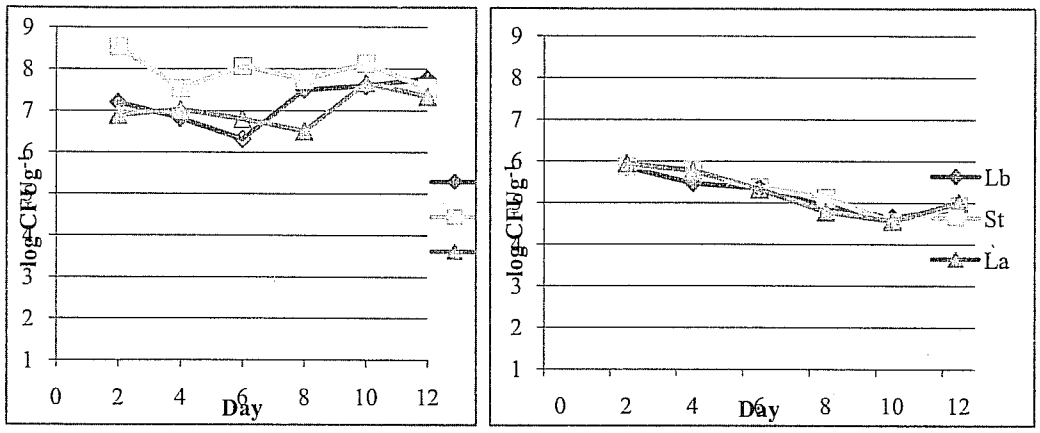


Figure 4. - Changes in pH and titratable acidity in various Buffalo curd brands during refrigeration storage. - □ (Marker without fill) represents the expiry date of the Buffalo curd.

SIGNIFICANT DIFFERENCES BETWEEN COMMERCIAL AND HOMEMADE BUFFALO CURD DURING STORAGE



(a) (b)

Figure 5. - Viability of lactic acid bacteria during refrigeration storage. (a) a representative of commercial curd (A) (b) a representative of homemade curd (E) □ (Marker without fill) represents the expiry date of the curd.

When commercially prepared curds were compared with homemade curd the viable counts were high in commercially prepared curd with a value above the 6 $logCFUg^{-1}$ level. It may be due to the quality and purity of the starter culture added to commercially prepared curd and maintenance of curd in the correct incubation period and temperature and proper sterilization of the buffalo milk. The buffalo milk itself can vary in commercial and home made productions because the buffaloes are treated in different ways like the type of food, quantity of food, their designated area for roaming, milking procedures and equipment. Also, during commercial preparation there is a chance of addition of preservatives, nutrients, flavours, pH maintaining substances and other chemicals like fungicides (Sri Lanka Standard 824, 1988). When a previous culture is used consecutively, there is a chance of the loss of the viability of the organism, for the viability depends on many factors like the acidity and hydrogen peroxide (Dave & Shah, 1998).

According to the results of the ANOVA, commercial and homemade curd were similar in the viable counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* but differed in the viable counts of *Streptococcus thermophilus* and *Lactobacillus acidophilus* before and after the shelf life. The initial starter organism and the amount might have been the reason for the difference in their counts. Commercial curd had a higher number of *Streptococcus thermophilus* and *Lactobacillus acidophilus*. The availability of other infectious, antagonistic microorganisms in homemade curd due to poor sterilization and incubation conditions may have been a reason for the less number of LAB. According to the results, the chemical parameters such as pH and titratable acidity were different in commercial and homemade curd during the shelf life of curd.

PROBIOTIC POTENTIAL OF BUFFALO CURD

Commercial curd demonstrated a significant presence of all three LAB and it proved that the numbers were above 10^6. As a result, commercial curds may be considered as probiotic products. The homemade curd, on the other hand, showed a presence of *Lactobacillus delbrueckii* ssp. *bulgaricus* but not the other two bacteria. So it proved that in homemade curd, the *Lactobacillus delbrueckii* ssp. *bulgaricus* numbers were more than 10^6 and the other two bacteria, were in

numbers less than 10^6. But according to Samona and Robinson (1994), *Lactobacillus acidophilus* is the main probiotic organism while *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* are yogurt bacteria. So homemade curd, cannot be claimed as a probiotic product.

CONCLUSIONS

Viability of the LAB in commercial Buffalo curd during refrigeration storage was higher, compared with homemade Buffalo curd. It was evident especially in the viable counts of *Streptococcus thermophilus* and *Lactobacillus acidophilus* where they were significantly high. The viability of the LAB during the shelf life and after the shelf life did not change significantly. Commercial Buffalo curd could be considered a probiotic product while the homemade Buffalo curd couldn't be considered as a probiotic product, when considering a viable count of 10^6 CFU g^{-1} in the product as the requirement for the probiotic activity.

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