Effect of *Streptococcus thermophillus* and *Lactobacillus bulgaricus* on Acid and Sweet Development of Yoghurt

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ABSTRACT

Yoghurt is a very important fermented dairy product, which is being consumed by the people from ancient times. Micro organisms are mainly used for the fermentation of the milk. Therefore, the characters of the yoghurt may differ according to the type and amount of micro organisms used, composition of milk, period of fermentation and other physical conditions followed in the fermentation unit and the cool storage. There are large numbers of conventional and non conventional types of yoghurts available in the world. For the commercial production of set yoghurt, two bacteria are used namely *Streptococcus thermophillus* and *Lactobacillus bulgaricus*. Bulk culture produced using a freeze dried mixture of the above bacteria is used for the manufacturing of "High Land" yoghurt. Colour separation, setting delay, non setting of yoghurt, differences in acidity and sweetness of produced yoghurt have been identified as major problems encountered in this manufacturing process. Effect of the bacterial count, ratio of the two bacteria and acidity of the bulk culture were tested for six days with the characteristics of the produced yoghurt. There is an effect of acidity of the culture, ratio of *Streptococcus thermophillus* and *Lactobacillus bulgaricus* for the acidity and sweetness of the yoghurt. However, there is no significant effect of them for the colour separation, yoghurt setting and setting delay of yoghurt. Bulk culture could be successfully used up to six days for the yoghurt production while acidity development could be minimized by reducing the fermentation time of the yoghurt.

KEY WORDS: Yoghurt, Culture, "High Land", *Streptococcus, Lactobacillus*, LSD agar, Colony count

1. INTRODUCTION

Yoghurt is a fermented milk product produced by denaturing the milk proteins by lactic acid. Lactose is the sugar substance in milk, which is broken in to lactic acid by *Streptococcus* and *Lactobacillus* bacteria.

There were no records regarding the exact origin of yoghurt. However, belief in its beneficial influence on human health and nutrition has existed in many civilizations for long time. It is likely however, that the place of origin of yoghurt was Middle East, and at present it is being consumed widely throughout the world. With the spreading of refrigeration the consumption of products such as fresh yoghurt, fruit yoghurt, processed yoghurt became popular and many non conventional types of yoghurt are being added to the market (Table 1). Nevertheless, the method of production of yoghurt has in essence, changed little over the years. Although there have been some refinements, especially in relation to the lactic acid bacteria that bring about fermentation, the essential steps in the process are still the same (Tamime and Robinson, 1985)

Variation in milk composition, irregular behaviour of starter organisms, faulty regulation of the incubation temperature, along with number of other process variables, can give rise to an end-product that is deficient in respect of overall quality, resulting high risk of product failure in the market.

There are three types of commercial yoghurt *viz.* plain/natural, fruit, and flavoured. These are manufactured either in set or stirred form (Figure 1 and 2).

The first bacteriological study of yoghurt was made by Grigoroff (1905), and he observed three different micro-organisms present, namely a *Diplostreptococcus*, a rod/coccal-shaped *Lactobacillus* and a rod-shaped *Lactobacillus*.

Table 1. Types of non conventional yoghurts in the world

Pasteurized /ultra high temperature (UHT) /Long - life yoghurt
Lactose hydrolysed yoghurt (LHY)
Drinking yoghurt
Condensed /Concentrated yoghurt
E.g.:- Labneh Anbaris, Chanklish and Kishk
Frozen yoghurt
Carbonated yoghurt
Yoghurt beverages
Dried instant yoghurt
Dietetic/Therapeutic yoghurt
Direct acidification of milk
Soy milk

Table 2. Composition of "High Land" plain yoghurt

Component	Amount (%)
Fat	2.5
Solid Non Fat (SNF)	8.25
Total solids	21.0
Total protein	3.45
Carbohydrate	5.15
Ash	0.75

Genus *Lactococcus*, Genus *Leuconostoc*, Genus *Pediococcus*, Genus *Streptococcus* and Genus *Lactobacillus* were used for yoghurt production as single strain cultures or multi strain cultures for long time. In Sri Lanka, Genus *Streptococcus* and Genus *Lactobacillus* are mainly used as a multi strain culture for the yoghurt production. Other than that "Direct Vat Set " culture is also used. For the production of "High Land" yoghurt in the MILCO Pvt. Ltd., a mixture of *S. thermophillus* and *L. bulgaricus* is used.

Figure 1. Plain set yoghurt production process in the "High Land" milk factory

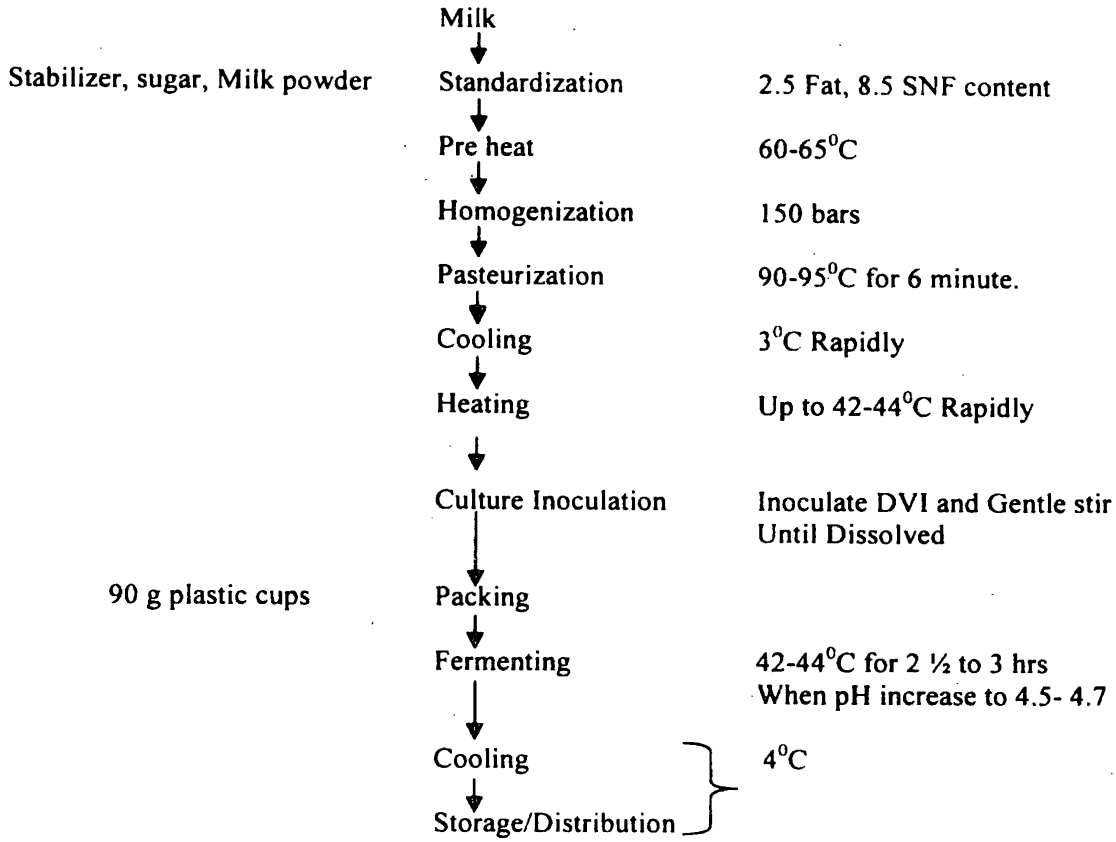
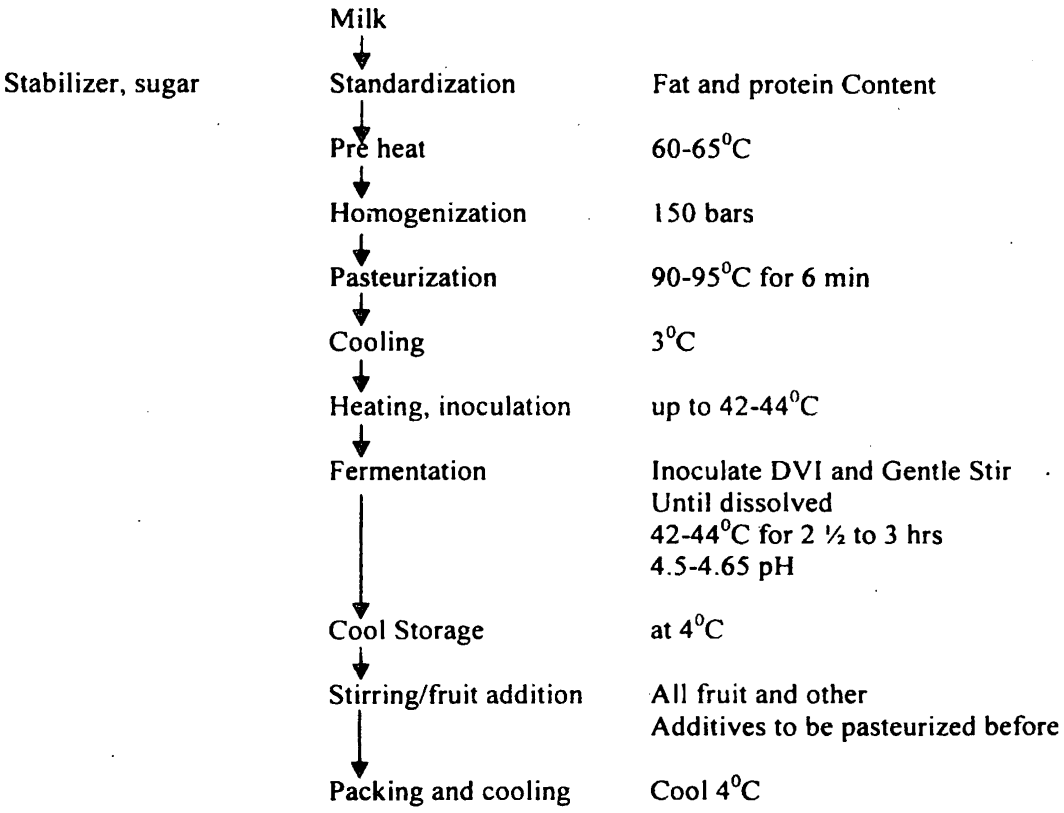


Figure 2. Stirred yoghurt production process in the "High Land" milk factory



1.1 Genus Streptococcus

Cells are spherical or ovoid in shape and 0.5 to 0.2 μm in diameter occurring in pairs or chains, when grown in liquid media. They are sometimes elongated in the axis of the chain to a lanceolate shape. They are non motile, non spore forming, facultative anaerobic, and Gram positive. Some species are encapsulated. They are chemoorganotrophs, requiring nutritionally rich media for growth and some times 5% CO_2. Their metabolism is fermentative, producing mainly lactate but no gas production. It is negative in catalase activity and commonly attacks red blood cells, with either greenish discolouration (α-haemolysis) or complete clearing (β- haemolysis). Growth is usually

restricted to a temperature of 25 – 45 ^{0}C (optimum 37 ^{0}C). Some species are parasites of vertebrates, mainly inhibiting the mouth and upper respiratory tract. Some species are pathogenic for humans and animals. Various antigens associated with Lancified serological groups are characteristic of some of the species and are required for accurate identification (Hoit and Krieg, 2000).

1.2 Genus Lactobacillus

Cells are rod-shaped and usually regular, 0.5-1.2 × 1.0-10.0 μm. They are usually long rods but sometimes almost coccoid, commonly in short chains. Gram positive, non sporing cells are rarely motile by peritrichous flagella. Facultative anaerobes, some times microaerophilic, grow poorly in air but better under reduced oxygen tension and some are anaerobes on isolation. Growth is generally enhanced by 5% CO_2. Colonies on agar media are usually 2-5mm, convex, entire, opaque, and without pigment. Chemoorganotrophs, require rich, complex media and their metabolism is fermentative and saccharoclastic, at least half of the end-product carbon is lactate. Nitrates are not reduced, gelatine is not liquefied, and cells are catalase and cytochrome negative. The major $C_{18:1}$ straight-chain fatty acid is cis-vaccenic. The optimum growth temperature is 30-40 ^{0}C. *Lactobacilli* are widely distributed in the environment, especially in animal and vegetable food products. They generally inhabit the gastrointestinal tract of birds and mammals and the mammalian vagina. They are rarely pathogenic (Hoit and Krieg, 2000).

The growth association between two organisms in a starter culture is known as symbiosis. It has been observed that the acid development is high in the mixed cultures when compared to the single strain cultures.

Consumption of yoghurt in Sri Lanka has been considerably increased during recent years. In a period of a month more than 0.14 million litres of yoghurt is produced by the "High Land" milk factory. It amounts to 2.07 million of 80 ml cups per month. In addition to that, more than 33 thousand of 200 ml drinking yoghurt packets, and more than 8 thousand of 500 ml curd cups are produced per month in the milk factory at Narahenpita alone (Anon 2004 and 2005).

Yoghurt is one of the highly nutritious foods in the world (Table 2). It is a good source of protein, Calcium, Potassium, vitamin B12 and riboflavin. It has a lot of health benefits to the human. It is a milk product that can be consumed by the lactose intolerance people, since it contains very low amount of lactose after fermentation by the bacteria. Acid digestion of milk protein is another important aspect of yoghurt, since it increases the absorption of milk protein to the body. Yoghurt helps to prevent and combat digestive tract infections including gastritis. Recent research findings suggest that the yoghurt consumption has beneficial effects on the immune system, serum cholesterol and in prevention and management of certain cancers.

2. MATERIALS AND METHODS

This study was carried out at the MILCO Pvt. Ltd., No. 45, Nawala road, Narahenpita, Colombo 05, from November, 2004 to July, 2005.

2.1 Materials

Yo-mix (621) thermophilic freeze dried yoghurt culture mixture obtained from Danisco Niebull Gmbh, Bush-Johannsom-strable 1, Niebull-Germany, was used for the inoculation of yoghurt bulk culture.

Commercially available *Lactobacillus Streptococcus* Differential (LSD) media, (Analytical instrument Pvt., 25, Kirula road, Colombo 05, Sri Lanka) was used with filter sterilized 2% 2,3,5, Tryphenyl Tetrasolium Chloride and autoclaved solution of 10 % Antibiotic free Skim milk powder for growing the bacterial strains.

2.2 Methods

2.2.1 Collection of culture samples

Samples were collected using autoclaved sample bottles. Three 500 ml samples were collected at the time of preparation of the bulk culture. Yoghurt were made for six consecutive days from the each sample and tested for its characters. The colony count and acidity values were taken daily from each sample for six days. The procedure was repeated for seven times.

2.2.2. Acidity testing of culture and yoghurt

Ten millilitres distilled water and 1 ml of 0.05% Phenolphthalein were added to 9 ml of culture or yoghurt and titrated with 0.1 N NaOH. When the colour changed into white to pale pink, the titration reading was taken. The reading was divided by 10 to have the percentage of acid (kilograms of acid per 100 kilograms of milk).

2.2.3 Preparation of dilution series

One tenth of the diluted culture was prepared by adding 1 ml of yoghurt culture to 9 ml of 0.1% peptone water and mixed well. Dilution series was made up to 10^6 or in some occasions, up to 10^{14}.

2.2.4 Preparation of *Lactobacillus Streptococcus* differential (LSD) agar

Sixty three point three grams of LSD agar powder was suspended in 1 L of distilled water and boiled to dissolve. Then, the solution was autoclaved at 121^{0}C and 15 lbs/In^2 for 20 minutes after putting in to boiling tubes. Afterwards, 10 % antibiotic free skim milk powder solution was prepared and autoclaved while a 100 ml of milk powder solution and 10 ml of 2 % filter sterilized Tetrazolium Chloride were added to 1 L of LSD agar solution just before testing.

2.2.5. Testing of Colony Count

One millilitre of diluted culture was pipetted to sterilized Petri dish and 10 ml of LSD agar solution was added and was mixed gently by rotating. Petri dishes were incubated in 44 ^{0}C for 48 hours and readings were taken. Two types of colonies were

identified according to the size, shape, colour and margin variation and were counted separately.

2.2.5.1 Identification of *Lactobacillus bulgaricus* Colonies

Shape of this colony is irregular and rhizoidal. The colour of the colony is red while the size is 1.0 mm to 1.5 mm in diameter. The colony is surrounded by a white coloured opaque zone.

2.2.5.2 Identification of *Streptococcus thermophillus* Colonies

These are round or oval shaped colonies. The colour is red and the diameter is 0.2 mm to 0.5 mm. The margin is surrounded by a small clear zone.

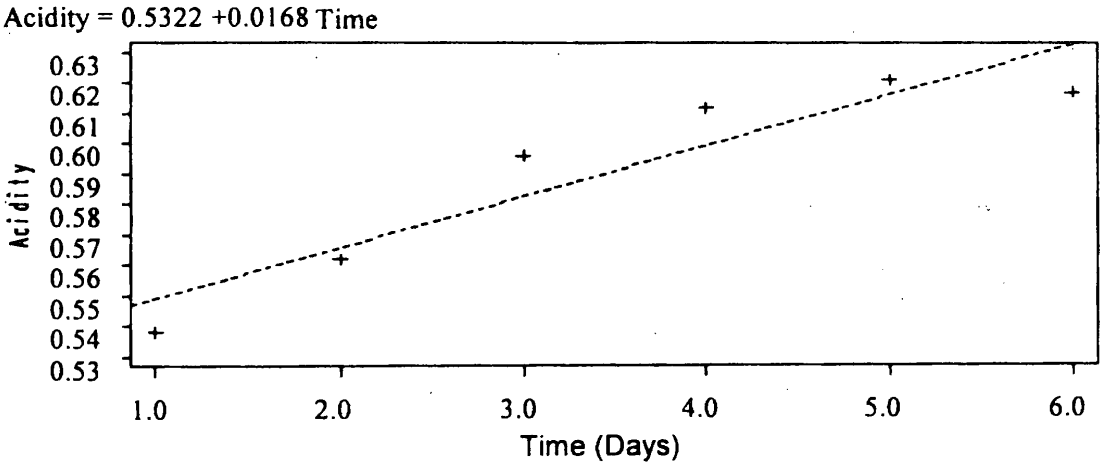
2.2.6 Production of Yoghurt and Testing

Three percent from the culture was added to 90 ml of yoghurt mixture and was kept at 44 ^{0}C in incubator. The time taken for setting and percentage acidity were tested after 3 hours. Six yoghurts were produced for one sample of culture; 3 for acidity testing and 3 for colour separation and sweetness testing. For set yoghurts, sweetness and colour separation were tested. Sweetness testing was done using a series of 1 to 10 % sucrose solution as a control.

Analysis was done by linear regression model of SAS (Statistical Analysis Software) with 95 % confidence interval.

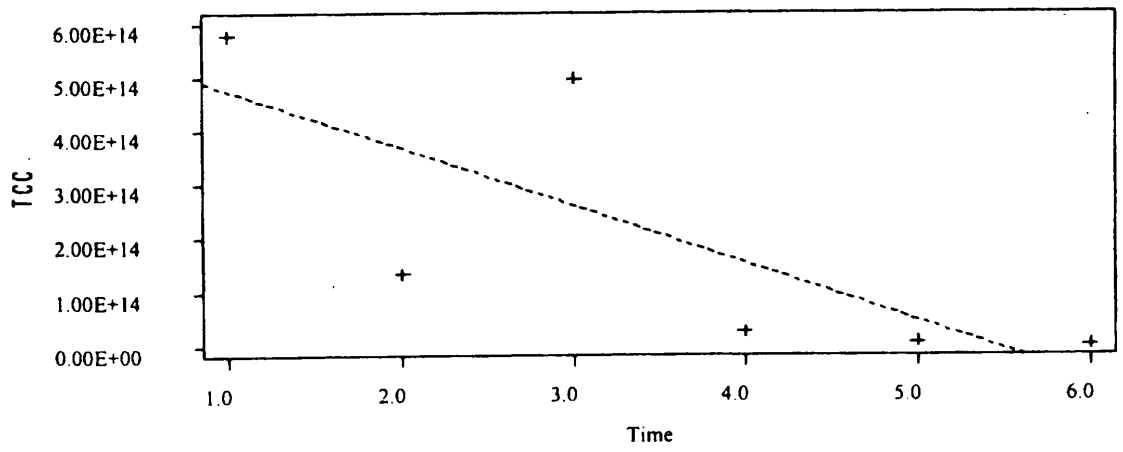
3. RESULTS AND DISCUSSION

Figure 3: - Average Acidity vs. Storage Time of Culture



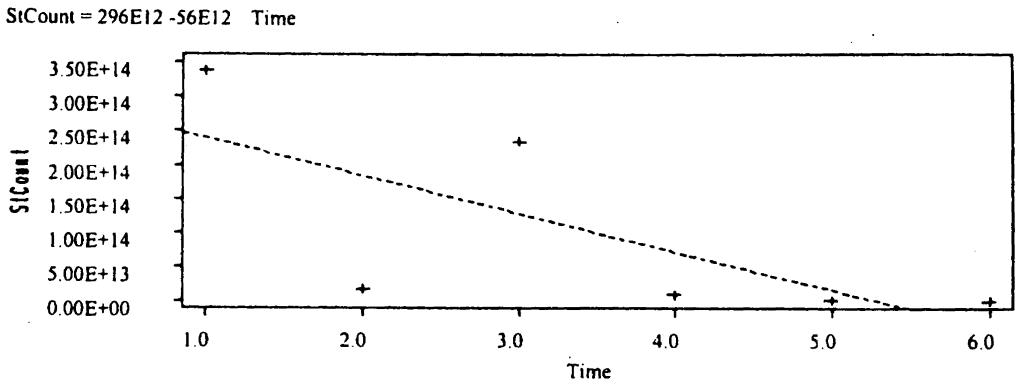
Acidity = Percentage of acidity, Time = Days after incubation,
R-Square = 0.5830, Probability = 0.0656

Figure 4: - Total Colony Count vs. Storage Time of Culture



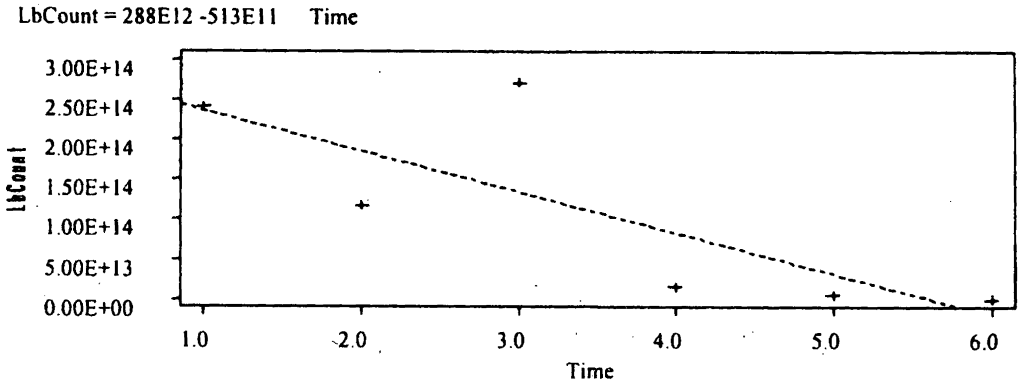
TCC = Total colony count, Time = Days after incubation
R-Square = 0.5830, Probability = 0.0773

Figure 5: - *Streptococcus thermophillus* Count vs. Storage Time of Culture



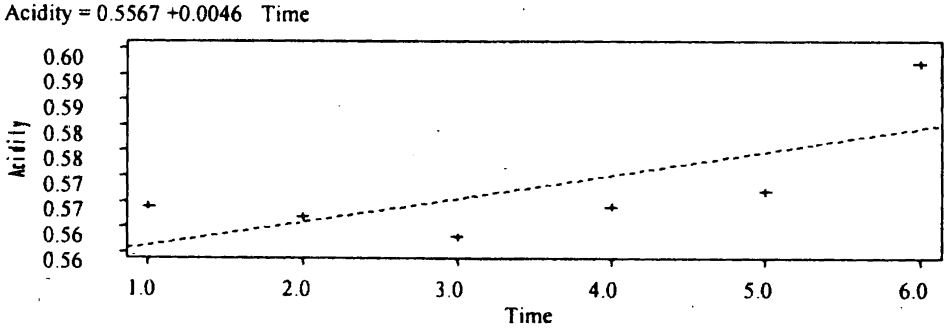
StCount = Streptococcus thermophillus count, Time = Days after incubation
R-Square = 0.5018, Probability = 0.1152

Figure 6: - *Lactobacillus bulgaricus* Count vs. Storage Time of Culture



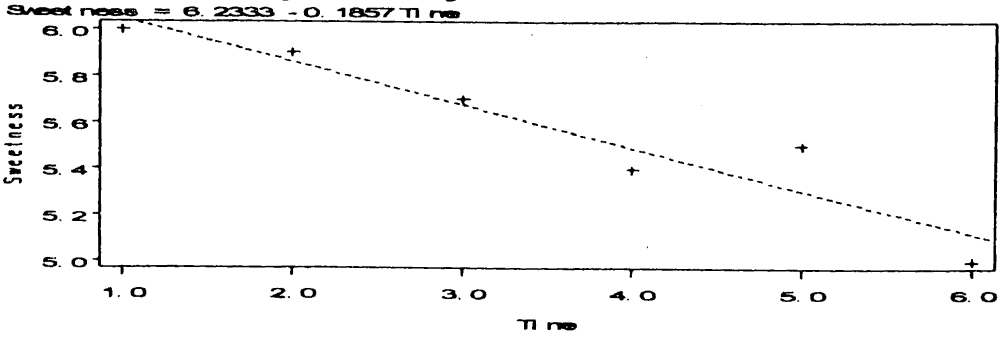
Lbcount = Lactobacillus bulgaricus count, Time = Days after incubation
R-Square = 0.6130, Probability = 0.0656

Figure 7: - Acidity of Yoghurt vs. Storage Time of Culture



Acidity = Percentage of acidity, Time = Days after incubation of culture
R-Square = 0.4972, Probability = 0.1176

Figure 8: - Sweetness of Yoghurt vs. Storage time of Culture



Sweetness = Percentage of Sweetness, Time = Days after incubation of culture
R-Square = 0.9031, Probability = 0.0036

Percentage acidity increased with the time of storage of the culture. Culture setting was observed at the acidity level of 0.5%. Post acidification took place and acidity was increased during the storage period of six days (Figure 3).

Streptococcus thermophillus and *Lactobacillus bulgaricus* colonies were taken together as total colony count (TCC). At the storage of 4 ^{0}C, TCC has declined from the first day up to sixth day. A maximum number of bacterial counts were obtained on the same day of incubation of the culture. The reason for the decline in TCC may be the effects of the increased level of acidity on the growth and multiplication or death of the cells and the reduced multiplication rates in cold storage of culture (Figure 4).

Streptococcus thermophillus count and *Lactobacillus bulgaricus* count reduced from 3.38×10^{14} to 2.85×10^{11} and 2.41×10^{14} to 1.39×10^{11} respectively, within six days storage of culture. The same reason mentioned above could be attributed for the decline in colony count (Figure 5 and 6).

Change of the ratio of *Streptococcus thermophillus* to *Lactobacillus bulgaricus* was not significantly different with the time.

Acidity of yoghurt has not increased linearly, but increased exponentially from the first day of the culture produced up to six days. The rise in culture acidity could be the reason for increased acidity of the produced yoghurt (Figure 7).

Sweetness of yoghurt has significantly decreased with the time. Increase in the acidity of yoghurt may be one of the reasons for this decline in sweetness (Figure 8).

From first day up to six days no non set yoghurt was observed. Setting time was stable at 3 hours. Colour separated yoghurt could not be observed.

The ratio of two bacteria, percentage acidity and TCC of the culture have not significantly affected on the yoghurt setting, setting time and colour separation of yoghurt. Further studies should be carried out to investigate the possible sources of contamination in the manufacturing process which may have an influence on the yoghurt setting, setting delay and colour separation of yoghurt.

4. CONCLUSIONS

When a yoghurt culture is stored at 4 ^{0}C, acidity is increased while the total colony count, *Streptococcus* count and *Lactobacillus* count decreased. The reason for the decrease of bacterial count may be the increase of acidity and cool condition of the storage. The ratio of *Streptococcus* to *Lactobacillus* in culture decreased slightly with time. Though the changes were not significantly different it may affect on the increase of acidity and reduction in the sweetness of yoghurt. Colony count, ratio of two bacteria, acidity of culture and age of the culture up to six days will not affect on the colour separation, setting and setting delay of yoghurt. Yoghurt bulk culture could be successfully used for the production of yoghurt up to 6 days from the day it was produced with the range of the above acidity and storage condition at 4^{0}C.

5. ACKNOWLEDGEMENTS

The authors wish to thank Dr. D.B. Kelaniyangoda, Senior Lecturer, Department of Horticulture and Landscape Gardening and Dr. W.J.S.K. Weerakkody, director, Computer Services Unit, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka., for the valuable assistance given. Sincere thanks are also extended to Mr. Gamini Weerawansa, Research officer, Department of Microbiology, Industrial Technological Institute (ITI), Torinton, Colombo 07.

The support rendered by Mrs. L. Chularathna, Quality Assurance Assistant Manager, all the staff members of the Laboratory, and all the factory workers of MILCO Pvt Ltd., is highly appreciated.

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