Effect of Direct Vat Set Culture and Bulk Culture on Shelf Life of the Fermented Butter Milk Based Mango Drink

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ABSTRACT

Increasing acidity during storage, unacceptable growth of yeast, mould and coliform organisms and reduction of organoleptic properties are major problems related with most cultured products as well as for Mango Delight drink. These chemical, physical and microbiological parameters affect on products quality which determines the shelf life. General objective of this study was to test the ability of direct vat set culture to improve the shelf life of the Mango Delight drink. Bulk cultured Mango delight samples and direct vat set cultured Mango delight samples were tested for chemical, physical and microbiological parameters over the storage time while keeping the products at 4°C storage temperature and 10°C storage temperature. Direct vat set cultured product had significantly low acidity development than the bulk cultured product. There was no significant difference of yeast and mould count, coliform and total soluble solids between both products. At 10°C storage temperature both direct vat set cultured product and bulk cultured product showed significant difference between acidity development and yeast and mould growth with time than at 4°C storage temperature. Direct vat set cultured Mango delight had long shelf life due to acceptable level of acidic taste. Even in high storage temperature direct vat set cultured product had low acidity development rate than Bulk cultured product. Increasing storage temperature resulted poor quality product with in short period of time. Both Bulk cultured and Direct vat set cultured products were required to keep under proper storage condition (<5°C temperature) to obtain expected shelf life.

KEY WORDS: Bulk culture, Coliform, Colony count , Direct vat set culture, Mango Delight, Mould , Starter culture, Yeast

INTRODUCTION

The product, fermented buttermilk based Mango drink (commercial name: "Mango Delight") is manufactured basically from buttermilk and Mango pulp (Figure 1). Buttermilk is the aqueous phase of the milk, which remains when cream is churned in to butter. Buttermilk has gained popularity among community due to its low caloric value. Fresh Mango pulp is a good source of vitamin A, vitamin C, and soluble fibers. Soluble fibers have been shown to be effective in reducing the risk of cardiovascular diseases and diabetes by reducing total blood cholesterol and regulation blood sugar levels (Anon, 1998).

The use of culture to produce fermented milk products had been adopted long before anything was known about bacteriology. With the time scientists began to investigate the question of souring from various points of view. Conn in U.S.A, Storch in Denmark demonstrated the main course of the reaction almost simultaneously but quite independently and by Weighman in Germany in 1890 (Anon, 1978). These workers laid the foundation for the preparation and use of cultures in the dairy industry. Starter culture is the term generally applied to the organisms used to ferment the culture product. Correct selection, preservation, handling and propagation of the starter culture helps to maintain the quality of the end product. Starter culture of the Mango Delight (MD) consist two species, *i.e. Streptococcus thermophillus* and *Lactobacillus bulgaricus* they derive their energy by fermentation of lactose, which is the only sugar found in milk. Lactic acid is resulted by the action of Lactose dehydrogenase on Pyruvate. Lactic acid gives the distinctive and characteristic yoghurt taste. Also it enhances the aromatic flavor of the product.

Traditional method of manufacturing cultured milk product is the use of bulk starter culture. Freeze dried bulk culture obtained by the manufacturer is inoculated to the bulk starter medium under aseptic conditions (Table 1). Small proportion (for MD 1.5% - 2%) of prepaid bulk starter culture is used for inoculation of the actual product. The use of bulk starter is becoming increasingly uncommon amongst commercial producers.

Table 1 - Physical / Chemical specifications for production of bulk starter

Culture medium:	
Reconstituted skim milk with 9% of dry matter heated at 95 ± 3°C for 30 min	
Fermentation:	
Inoculation amount: 1 pouch / 500 l	
Inoculation and incubation temperature: 42°C	
pH after 6 h	<=4.75
Typical value for bulk starter activity:	
pH after 2 h / 42°C	< =5.40

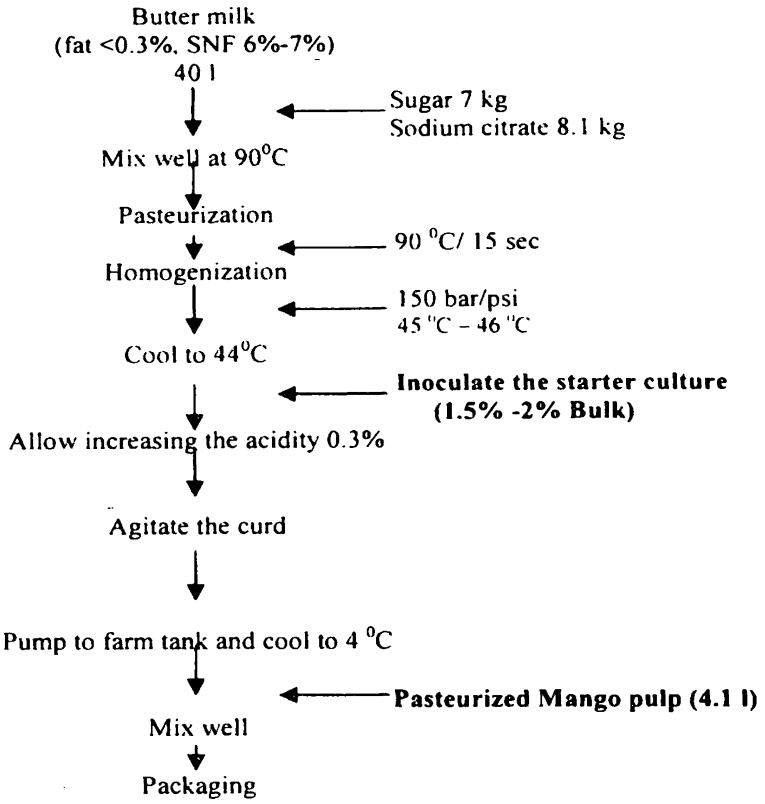

Figure 1 - Process flow of "MANGO DELIGHT" drink (Fermented milk base)

A technique often referred to as direct vat set (DVS) culture inoculation is becoming more popular. DVS culture involves inoculating the production mix directly with a very large number of freeze-dried starter organisms. There high concentration allows for their ability to be inoculated directly to the production mix. DVS cultures are available in different pack sizes (50U, 200U, 500U, 1000U) and recommended dosage should be used during manufacturing (Table 2).

DVS culture contain many advantages over bulk culture, such as less batch to batch variation by maintaining the correct ratio (1:1) of starter organisms, more predictable performance, better pH control at production and post acidification, improved quality, easy to use, high flexibility, less risk of contamination and phage attack and no cost for bulk starter preparation (Anon, 2000).

Table 2 - Recommended dosage of freeze-dried DVS Culture in units to liters:

DVS inoculation (percentage)	Amount of milk to be inoculated		
500U/2500 l	1000 l 200U	5000 l 1000U	10000 l 3000U

Moulds (*Aspergillus, Penicillium etc.*) are aerobic and can grow over a wide range of acidity and temperature. Moulds are undesirable in most dairy products because they injure flavor and produce a musty odor. Yeasts (*Saccharomyces, Torulae etc.*) are microorganisms that can ferment solution of sugar in to alcohol and carbon dioxide gas, also responsible for yeasty odor (Lampert, 1970). The group of coliform bacteria includes the genera *Escherichia* and *Aerobacter*. Yeast, mould and coliform are wide spread in air and can cause contaminations during the manufacturing process.

Viable cultured organisms present in end product are responsible for the further acidity development during storage time. This is the major problem related with MD, which reduce the shelf life. Increasing acidity provide better medium for yeast, mould and coliform growth and other putrefactive organisms. To minimize the action of mesophilic cultured organisms MD should store under refrigerated condition (<5°C). Increasing storage temperature at commercial level may reduce the shelf life.

Analysis of end-product is an essential feature of quality control because it's important to protect the consumer from the purchasing of poor quality product, and assist in the smooth operation of a plant by identifying variations in product quality at a early stage, which enable any necessary corrective action be taken before the on set of serious problems (Tamime and Robinson, 1978).

MATERIALS AND METHODS

The study was carried out at the MILCO Pvt. Ltd, No 45, Nawala road, Narahenpita, Colombo 05 from February to August 2006.

1 *Sample Collection*

Bulk cultured samples and DVS cultured samples were collected from each batch separately. Chemical, microbiological and sensory analyses were done for each sample daily over the storage time. Experiment was repeated 5 times while storing at 4^0C temperature. Same experiment was done while keeping samples at 10 ^{0}C storage temperature.

1.1Testing Acidity

One milliliter of phenolphthalein was added to 9ml of sample and titrated with 0.1N NaOH. When the colour changed in to 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 100kgs of milk).

1.2 pH Testing

pH was tested using calibrated pH meter.

1.3 Testing Total Soluble Solids (TSS)

TSS was tested using hand refractometer (refractometer gives percentage of sugar in the product).

1.4 Testing of Yeast and Mould Colony Count.

One tenth of the diluted sample was prepared by adding 1ml of MD sample to 9ml of sterilized 0.1% peptone water in MaCartney bottle and mix well.

One milliliter of diluted sample was pipetted to sterilized Petri dish aseptically. Ten milliliters of melted Potato dextrose agar (PDA) and 1ml of Tartaric acid were mixed and pour in to inoculated dish and was mixed gently by rotating.(Tartaric acid was used to adjust the pH to 3.5 of the medium) Petri dishes were incubated in 25^{0}C for 5 days. Two types of colonies were identified according to the size, shape, colour and marginal variation. Numbers of colonies were counted separately by colony counter.

1.4.1 Identification of Yeast colonies.

These are white or cream coloured round or oval shaped colonies found on surface of the agar medium.

1.4.2 Identification of Mould colonies.

These are white coloured irregular shaped colonies with cottony or wooly appearance. Found on surface of agar medium.

1.5 Testing for Coliform bacteria.

Five milliliters of MacConkey's broth was distributed in to a test tube with inverted fermentation tubes (Durham tubes). One milliliter of 10^{-1} diluted MD sample was inoculated to the liquid broth aseptically. Test tubes were incubated at 30^{0}C for 18-24 hours.

Gas production indicates the positive test for colifom.

1.6 Sensory Analysis

Taste and aroma were tested daily.

2 Data Analysis

The data were analyzed by two sample T-test model of Minitab with 95% confidence interval.

RESULTS AND DISCUSSION

1 Comparison of Two Cultured Products at 4^0C Storage Temperature.

Acidity was increased gradually with storage time. DVS cultured product had significantly low acidity development with the storage time than Bulk-cultured product (Figure 2). Gradual reduction of pH with the storage time was observed. DVS cultured product had significantly high pH level than Bulk cultured product (Figure 3). Acidity increase can be attributed to the conversion of lactose to lactic acid by the cultured organisms.

There was no significant difference between TSS of DVS cultured product and Bulk cultured product. TSS decreased with storage time (Figure 6). That was due to the reduction of lactose.

There was no significant difference between the yeast count of the DVS and Bulk cultured products. With in first 6 days product was in satisfactory level to consume (<100 Yeast count). Beyond the 7th day the yeast count increased rapidly (Figure 4). Mould count of the DVS cultured product and Bulk-cultured product had no significant difference. With in first 7 days the product was in satisfactory level to consume (<100 Mould count). Beyond 8th day the mould count increased rapidly (Figure 5). Yeast and mould contaminations were common during the manufacturing process of MD. Growth of these organisms favored the acidity development of the MD in storage.

Taste and aroma of the both product, were reduced gradually with the storage time. Bulk cultured product was acceptable with in first 10 days. DVS cultured product was acceptable with in first 12 days. DVS cultured product had acceptable taste due to low acidity (Table 3).

Coliform contaminations showed no relationship with storage time in both products (Table 4).

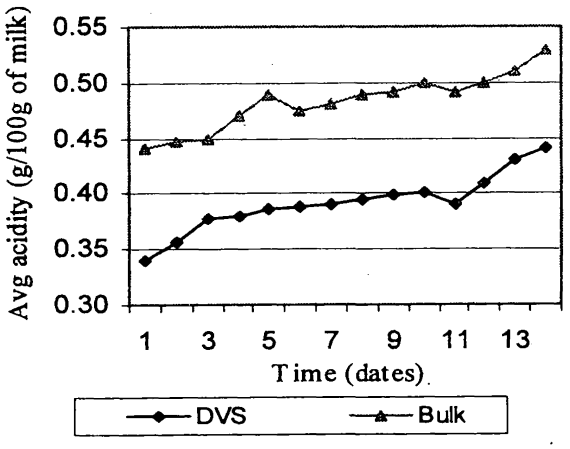

Figure 2 - Average acidity vs. storage time:
Probability =0.0000(<0.05-significant)

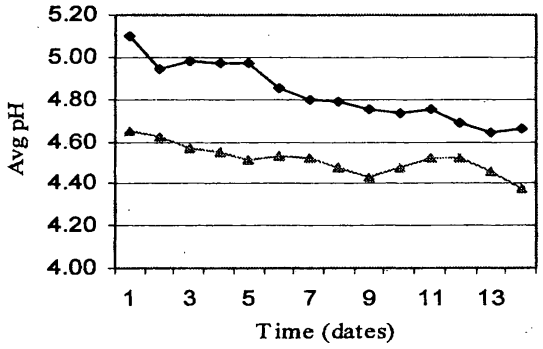

Figure 3 - Average pH vs. storage time:
Probability =0.0000(<0.05-significant)

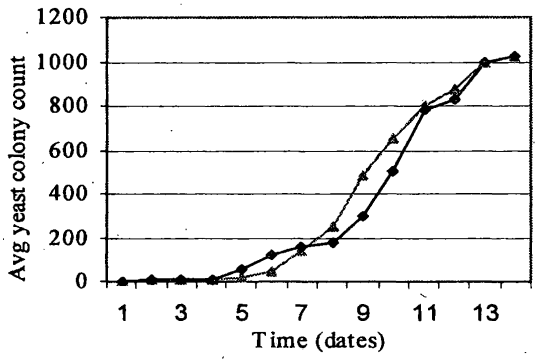

Figure 4 - Average Yeast colony counts vs. storage time:
Probability =0.88(>0.05-Not significant)

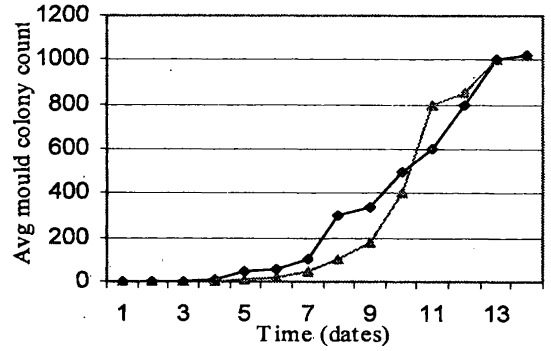

Figure 5 -Average Mould colony counts vs. storage time:
Probability=0.87(>0.05–Not significant)

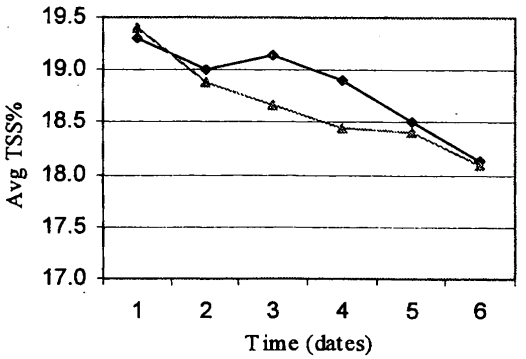

Figure 6 - Average TSS% vs. storage time:
Probability =0.50(>0.05–Not significant)

Table 3 - *Sensory analysis with storage time (Taste and aroma):*

Time (dates)	Bulk (4^0C)	DVS (4^0C)	Bulk (10^0C)	DVS (10^0C)
1	Excellent	Excellent	Excellent	Excellent
2	Excellent	Excellent	Good	Excellent
3	Good	Excellent	Good	Good
4	Good	Good	Good	Good
5	Good	Good	Fair	Fair
6	Fair	Good	Fair	Fair
7	Fair	Good	Fair	Fair
8	Fair	Fair	Unacceptable	Fair
9	Fair	Fair	Unacceptable	Fair
10	Fair	Fair	Unacceptable	Unaccept
11	Unacceptable	Fair	Unacceptable	Unaccept
12	Unacceptable	Fair	Unacceptable	Unaccept
13	Unacceptable	Unacceptable	Unacceptable	Unaccept
14	Unacceptable	Unacceptable	Unacceptable	Unaccept

Table 4 - *Average results of Coliform with time:*

Dates	Bulk (4^0C)	DVS (4^0C)	Bulk (10^0C)	DVS (10^0C)
1	--	+	--	+
2	--	--	--	--
3	+	--	+	+
4	+	+	--	+
5	--	+	+	--
6	--	--	+	--
7	+	--	--	--
8	--	--	--	+
9	--	+	--	+
10	+	--	+	--
11	+	--	+	--
12	--	+	--	+
13	+	--	+	+
14	+	+	+	+

+ Coliform present - Coliform absent

2 Comparison of Storage Temperature Difference.

There was significantly high acidity development in Bulk cultured MD and DVS cultured MD at 10^0C storage temperature than 4^0C storage temperature (Figure 7). Rate of acidity development of Bulk cultured MD was higher than DVS cultured MD at 10^0C temperature. At 10^0C temperature pH decreased rapidly in both products than the pH at 4^0C storage temperature (Figure8). These were due to the higher activity of the cultured organisms at the high temperature.

There was no significant difference between TSS at 10^0C and 4^0C storage temperatures in both products. But at 10^0C temperature reduction of TSS was higher than at 4^0C temperature (Figure11). This was due to the higher rate of lactose reduction at 10^0C.

Both DVS and Bulk cultured products had significantly high amount of yeast and mould count at 10^0C storage temperature than at 4^0C storage temperature. Both products were satisfactory to consume with in first 4days at 10^0C storage temperature. Beyond 5^{th} day yeast and mould counts were rapidly increased (Figure 9 and 10).

At 10°C storage temperature taste and aroma of both products were decreased rapidly than at 4°C storage temperature. Bulk cultured product was acceptable with in first 7 days. DVS cultured product was acceptable within first 9 days (Table3). Both products were unacceptable beyond those days due to high acidity and unfavorable odor.

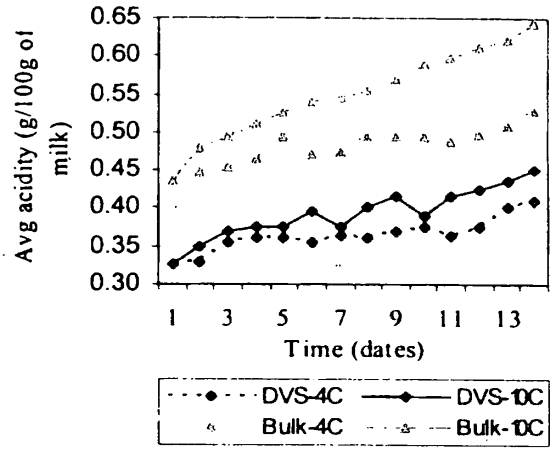

Figure 7 - Average acidity at two storage temperatures vs. time:
DVS Prob =0.018 (<0.05- Significant)
Bulk Prob =0.0008(<0.05- Significant)

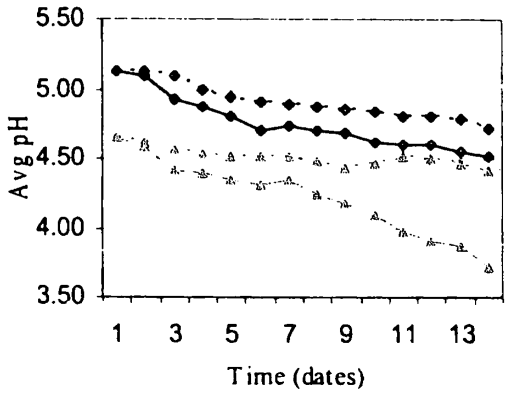

Figure 8 - Average pH at two storage temperatures vs. time:
DVS Prob =0.018 (<0.05- Significant)
Bulk Prob =0.0015(<0.05 - Significant)

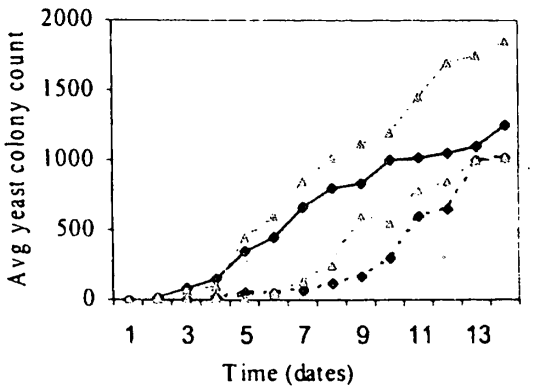

Figure 9 - Average Yeast counts at two storage temperatures vs. time:
DVS prob =0.040 (< 0.05 – Significant)
Bulk Prob =0.029 (< 0.05- Significant)

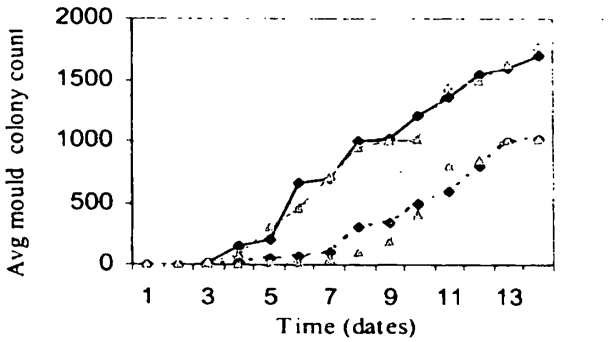

Figure 10 - Average Mould counts at two storage temperature vs. time.
DVS prob =0.032 (<0.05- Significant)
Bulk prob =0.035 (<0.05- Significant)

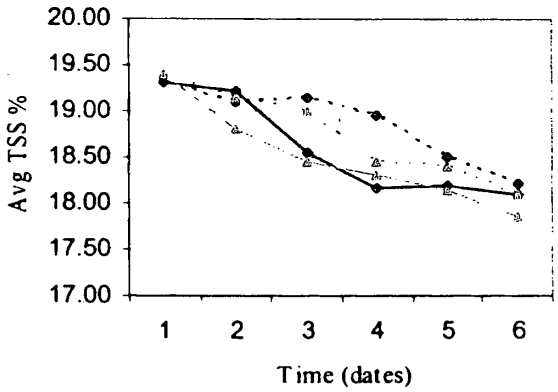

Figure 11 - Average TSS% at two storage temperatures vs. time:
DVS Prob=0.34(>0.05- Notsignificant)
Bulk Prob=0.42(>0.05-Not significant)

CONCLUSION

Direct vat set culture extended the shelf life of the Mango Delight. That was due to slow acidity development and acceptable taste over the storage time. DVS culture did not show improvement of microbiological parameters than Bulk culture.

Storage temperature was the main factor determines the shelf life of the product. Fluctuation (increasing) of storage temperature resulted poor quality product within short period of time. DVS cultured product was acceptable than Bulk cultured product even in high storage temperature conditions. DVS cultured product can be recommended for commercial level.

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