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Effect of fermentation on physico-chemical, textural properties and yoghurt bacteria in mango soy fortified yoghurt

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Mango soy fortified yoghurt (MSFY) samples were prepared by using blends of 78.3% milk containing 3% fat, 14.5% soymilk containing 8.2% total solids and 7.2% mango pulp containing 18% total solids. The physico-chemical, textural properties and yoghurt bacteria counts of MSFY were analyzed at an interval of 30 min during fermentation upto 270 min. The fermentation time had a positive effect on acidity but a negative effect on pH, total solids and sugars in MSFY. Quadratic model were well fitted into different physico-chemical properties of MSFY during fermentation. Textural profile data of MSFY during fermentation were: hardness (46.44 - 154.6 g), cohesiveness (0.4133 - 0.8137), adhesiveness (-2.57 to -59.77 g.s), springiness (1.037 - 1.416) and gumminess (26.22 - 93.80 g). The *Streptococcus thermophilus* counts increased from 1.3×10^8 to 1.75×10^9 cfu/ml and *Lactobacillus bulgaricus* from 0.9×10^8 to 1.17×10^9 cfu/ml. Specific growth rate of *S. thermophilus* is higher than that of *L. bulgaricus* during MSFY fermentation.

Key words: Yoghurt, mango, soymilk, fermentation, texture, physico-chemical properties.

INTRODUCTION

Milk, for thousands of years, has been transformed through microbial fermentation into various food products with high nutritional value (Tamime and Robinson, 1999). Yoghurt is a coagulated milk product obtained by the lactic acid fermentation through the action of *Streptococcus thermophilus and Lactobacillus delbrueckii* spp. *bulgaricus*. The product is accepted by consumers due to its flavor and aroma, mainly attributed to acetaldehyde and texture. Viability and activity of yoghurt bacteria are important commercial considerations so that they survive throughout shelf life, transit through acidic conditions in the stomach as well as enzymes and bile salts in the small intestine.

Fortification of yoghurt with soy solids and mango pulp not only increases its nutrient content including minerals and vitamins but also adds to its health promoting value. Soymilk based yoghurt would offer several distinct nutritional advantages over milk yoghurt to the consumer, that is reduced level of cholesterol, saturated fat and lactose (Lee et al., 1990). Soybean is a good source of niacin, riboflavin, iron, potassium, calcium, magnesium and phosphorus, with several fat and water soluble vitamin B-complex (Zucker and Zucker, 1943). Addition of mango pulp adds a variety for consumer to select as they both add to the nutritional value of the yoghurt. Mango pulp contains a high proportion of sugar, mostly

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monosaccharide that is easily adsorbed by the digestive system.

Bonezar et al. (2002) studied the effects of certain factors on the properties of ewe's milk and observed that the overall properties of yoghurt, such as acidity level, the production of aroma compounds (diacetyl, aceteldehyde) as well as the nutritional value are important traits of the product. These aspects are influenced by the chemical composition of the milk base, processed conditions, the added flavors and the activity of starter culture during the incubation period. There have been many investigations involving optimization of yoghurt texture. These studies have demonstrated that the total solids and fat levels in the milk, heat treatment of the milk prior to inoculation, homogenization, presence of stabilizers (Kumar and Mishra, 2004) and incubation conditions will affect the body of the final product (Schellhaass and Morris, 1985).

Buono et al. (1990) studied the carbohydrate utilization and growth kinetics in the production of yoghurt from soymilk. Utilization of soymilk as an ingredient for human food necessitates a reduction in the concentration of the carbohydrates that can cause flatulence and a reduction in the objectionable beany flavor typically associated with soy products. The fermentation process undergoes several distinct phases based on the changes in pH and acidity and sugar content. The first stage has a rapid decrease of pH and an increase of acidity. These changes are accompanied by a decrease of reducing sugars after the initial lag phase. The next stage shows a gradual drop in pH, a further increase in acidity and a rapid disappearance of reducing sugars. The final stage of fermentation proceeds with slight changes in pH, acidity and reducing sugars.

In the present paper, the effect of fermentation time on physico-chemical, textural and growth kinetics of yoghurt bacteria in Mango soy fortified yoghurt (MSFY) is discussed.

MATERIALS AND METHODS

Pure freeze dried cultures of *S. thermophilus* NCDC 074 and *L. delbrueckii* spp. *bulgaricus* NCDC 009 were procured from National Dairy Research Institute, Karnal, India. These cultures were maintained in 12% skim milk (SM) media and autoclaved at 15 psi for 20 min. Toned milk used for yoghurt preparation was procured from local market and has been standardized to a fat content of 3%. Soymilk was prepared in the laboratory from whole soybeans using the procedure described by Bourne et al. (1976). A canned mango pulp (Kaytis brand) containing 18% total solid was purchased from local market. These were used to prepare experimental samples.

MSFY preparation

The blend of 78.3% milk (3% fat), 14.5% soymilk (8.2% total solids) were heated to 95°C for 5 min, cooled to 45°C and 7.2% mango pulp (18% total solids) was added with high speed stirring. The blends were inoculated with 2% (v/v, *S. thermophilus* and *L.*

bulgaricus in 1:1 ratio) of 24 h old culture. The blend was incubated at 42 ± 2°C in a plastic cup (100 ml). Mango soy fortified yoghurt (MSFY) was prepared by as per method (Kumar and Mishra, 2003a). Samples were kept for 2 h at 8°C before analysis.

Addition of inoculums

Two cultures *S. thermophilus* and *L. bulgaricus* were used for studying the effect of fermentation time on physico-chemical properties and growth kinetics of Mango Soy Fortified Yoghurt (MSFY). The physicochemical properties of MSFY such as pH, acidity, total solids, sugars were analyzed at an interval of 30 min during fermentation up to 270 min. Microbiological and textural properties were also studied for each 30 min after the culture had been allowed to grow for 270 min.

The experiments were carried out in triplicates. All physico-chemical and textural properties were fitted into linear and quadratic equations to see the effect of fermentation time. The effect of fermentation time on microbial growth was described by the exponential phase. The exponential phase may be described by the equation:

$$dx / dt = \mu x \qquad (1)$$

Where:

x = microbial counts (cfu/ml)
t = time (min)
μ = specific growth rate (min^{-1})

Integrating Equation (1) gives:

$$x_t = x_0 \, e^{\mu t} \qquad (2)$$

Where:

x_0 = original microbial counts (cfu/ml)
x_t = microbial counts after the time interval, t (min)
t = Time (min)
e = base of the natural logarithm.

Taking natural logarithms, Equation (2) becomes:

$$\ln x_t = \ln x_0 + \mu t \qquad (3)$$

Thus, a plot of natural logarithm of microbial counts of ST and LB against time should yield a straight line, the slope of which would be the value of specific growth rate. During the exponential phase, nutrients are in excess and the organism is growing at its maximum specific growth rate, μ_{max}, for the prevailing conditions (Stanbury et al., 1995).

Analysis of physicochemical characteristics

Physicochemical properties of MSFY such as pH, titrable acidity (IDF, 1991a) and total solids (IDF, 1991b) were determined. Reducing and non reducing sugars were estimated by Lane and Eynon Method (Ranganna, 1996).

Texture profile analysis

In the recent past, instrumental texture analysis (TPA) has been applied as a useful method to evaluate mechanical properties in a
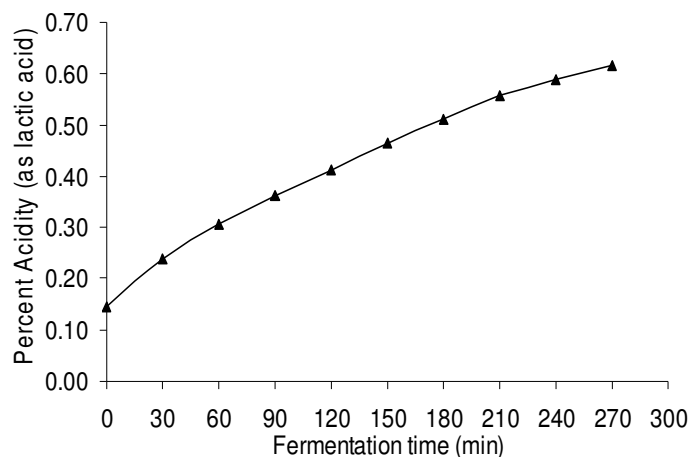
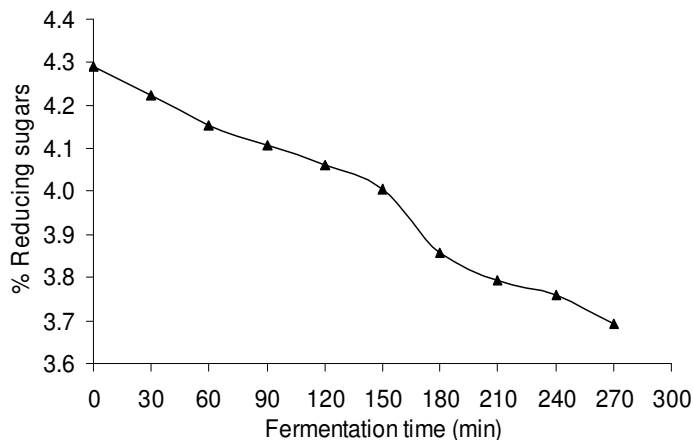**Figure 1a.** Effect of fermentation time on per cent acidity (as lactic acid).

Figure 1b. Effect of fermentation time on pH.

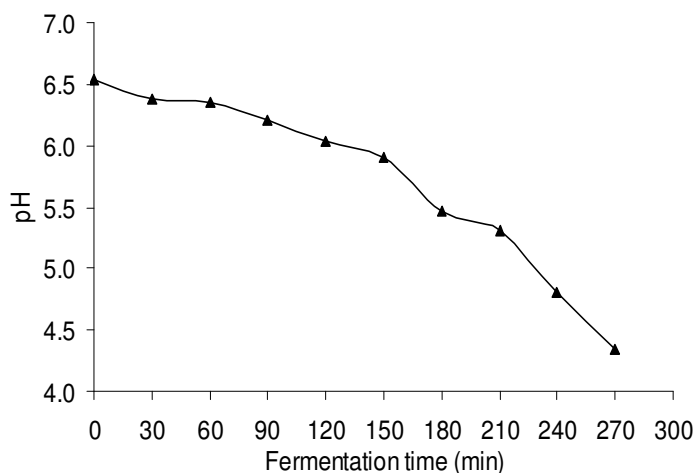**Figure 1c.** Effect of fermentation time on total solids (%).
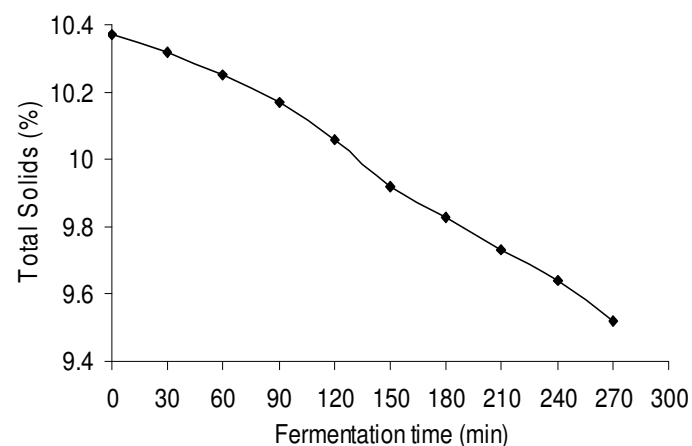

Figure 1d. Effect of fermentation time on reducing sugars (%).

wide range of foods (Pons and Fiszman, 1996). TPA compresses a piece product twice imitating the conditions in the mouth (Szczesniak et al., 1963). Five basic textural parameters may be obtained in only one test (hardness, cohesiveness, springiness, adhesiveness and gumminess), extracted from the resulting force-time curves. Texture profile analysis was carried out by the method described by Kumar and Mishra (2003b). The data obtained from typical texture profile curve was used for the calculation of the textural parameters (Bourne, 1978; Bourne, 1982) as follows:

Hardness, g = maximum force of the first compression

$$\text{Cohesiveness} = \frac{\text{Area under } 2^{nd} \text{ compression (Area 2)}}{\text{Area under } 1^{st} \text{ compression (Area 1)}}$$

Adhesiveness, g.s = negative area in the graph (Area 3).

Springiness = Length 2 / Length 1

Gumminess, g = Hardness × Cohesiveness

Enumeration of *S. thermophilus* and *L. bulgaricus*

International Dairy Federation methods (1988) were used for the enumeration of S. *thermophilus* and *L. bulgaricus*. The *S. thermophilus* agar (M948), *L. delbrueckii* subsp. *bulgaricus* agar (M927) produced from Hi Media Laboratories Limited, Mumbai, were used. The samples were diluted in peptone (1%) in water. The agar plates of *S. thermophilus* were incubated aerobically at 37°C for 48 h while the plates of *L. bulgaricus* were incubated at 37°C for 72 h under anaerobic conditions. After incubation, typical colonies of *S. thermophilus* and *L. bulgaricus* were counted. Plates with 30 - 300 colonies were used in counting and the counts were averaged for three replicates and expressed as c.f.u/ml.

The significance of fermentation time was determined through Single factor analysis (ANOVA) of the data obtained in three experimental runs.

RESULTS AND DISCUSSION

The results of different physico-chemical parameters that is, acidity, pH, total solids, reducing sugars and total sugars are shown in Figures 1a to 1e. The fermentation time

Table 1. Linear and quadratic models of different physico-chemical properties of MSFY.

Parameters	Linear model (y = mx + c)			Quadratic model (y = ax^2 + bx + c)			
	m	**c**	**R^2**	**a**	**b**	**c**	**R^2**
Acidity	0.0017	0.191	0.979	-3 × 10^{-6}	0.0026	0.1569	0.998
pH	-0.0077	6.7781	0.922	-3 × 10^{-5}	-4 × 10^{-5}	6.4699	0.994
Total solids	-0.0033	10.422	0.992	-3 × 10^{-6}	-0.0025	10.393	0.996
Reducing sugars	-0.0023	4.3002	0.985	-1 × 10^{-6}	-0.002	4.2895	0.986
Total sugars	-0.0022	4.939	0.994	-1 × 10^{-6}	-0.0019	4.9272	0.995
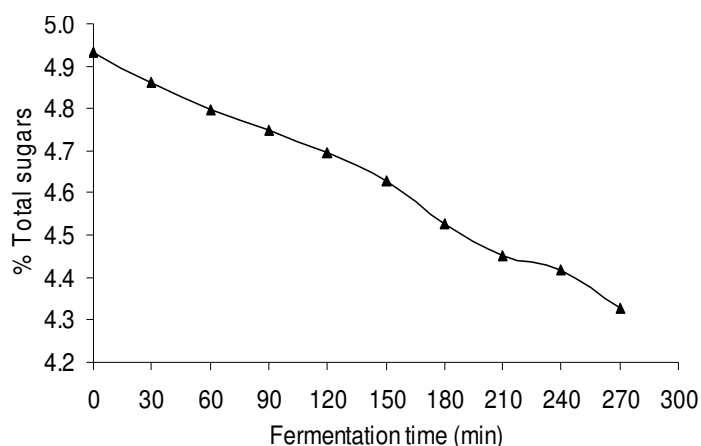
Figure 1e. Effect of fermentation time on total sugars (%) of MSFY.

had a positive effect on acidity but a negative effect on pH, total solids, reducing and total sugars in MSFY (Table 1). The acidity of MSFY as % lactic acid increased from 0.15 at 0 min to 0.62 at 270 min of fermentation and total solids decreased from 10.37 to 9.52. The pH decreased from 6.53 to 4.34 and total sugars from 4.93 to 4.33 at these fermentation times.

Different culture combination of *S. thermophilus* (HST, 197) and *L. bulgaricus* (RTS, Yb) were studied for total acid production in cow milk and buffalo milk from 2 - 10 h of incubation. It was observed that with the increase in incubation time, there was an increase in acid production in the culture combinations of different strains in both types of milk. Earlier reports by Macquot and Hurrel (1970) and Tramer (1973) have indicated that with the increase in fermentation time, there was an increase in acid production in the culture combinations of different strains in both cow and buffalo milk from 10 to 12 h of incubation. In yoghurt, lactose utilization proceeds as long as the external pH allows metabolic activity of the lactic acid bacteria. When lactic acid is produced in yoghurt and pH has reached 4.0, *L. bulgaricus* cease their metabolic activity and lactose is no further utilized. By rerouting metabolism of the lactic acid bacteria towards production of more pH-neutral components, further conversion of lactose is possible (de Vos, 1996).

Goodenough and Kleyn (1976) investigated qualitative and quantitative changes in sugars of yoghurt during its manufacture and reported average lactose concentration of 8.5% in yoghurt mix which dropped to 5.75% with a concomitant increase of 1.20% galactose. Glucose was detected only in trace quantities. Commercial yoghurts had an average of 4.06% lactose, 1.85% galactose, 0.05% glucose.

The dependency of response variables on coefficients associated with linear and quadratic equations and their significance with coefficient of determination (R^2) of physicochemical properties is shown in Table 1. High coefficient of determination (R^2) explained the goodness of fit in the experimental data. The quadratic model was found to be suitable than corresponding linear model for each physico-chemical property. The fermentation time had significant (P < 0.01) effects after analyzing the data for different experimental runs through Single factor analysis (ANOVA) (Table 2) on all physico-chemical characteristics of yoghurt sample during fermentation.

Textural characteristics

Yoghurt rheology is described not only in terms of viscosity, viscosity loss and its recovery, but is also described in terms of how hard, brittle, elastic and cohesive it is. Textural characteristics and rheological properties of coagulated dairy products are affected by their structural characteristics. The structural arrangement of the network determines the textural characteristics of these coagulated products and is affected by factors such as composition and manufacturing processes (Rawson and Marshall, 1997). Force measuring instruments like the TA-XT2 texture analyzer allow food materials to be evaluated in such terms and have been used to assess yoghurt texture (Toba et al., 1990; Kaur et al., 2009).

TPA curves of MSFY were obtained during fermentation at 120,150,180, 210, 240 and 270 min fermentation time. TPA curves at 0, 30, 60, 90 min were not possible as the force required by the probe to mea-sure TPA curves was not attained at these fermentation times. The results of different TPA curves are summarized in Table 3.

Hardness (force necessary to attain a given deformation) is a commonly evaluated parameter when

Table 2. Single factor analysis (ANOVA) for physico-chemical properties and *S. thermophilus* (ST) and *L. bulgaricus* (LB) counts of MSFY.

Physico-chemical and microbiological properties	Source of variation	Sum of squares (SS)	Degree of Freedom (df)	Mean sum of square (MSS)	Calculated F - value	P-value	F-critical
Acidity	Fermentation time	90557.98	1	90557.98	21.95	1.84×10^{-4}	4.41
	Error	74250.2	18	4125.01			
	Total	165808.2	19				
pH	Fermentation time	83550.65	1	83550.65	20.25	2.77×10^{-4}	4.41
	Error	74254.83	18	4125.27			
	Total	157805.5	19				
Total solids	Fermentation time	78148.75	1	78148.75	18.94	4.32×10^{-4}	4.41
	Error	74250.8	18	4125.04			
	Total	152399.6	19				
Reducing sugars	Fermentation time	85812.42	1	85812.42	20.80	2.42×10^{-4}	4.41
	Error	74250.39	18	4125.02			
	Total	160062.8	19				
Total sugars	Fermentation time	84970.82	1	84970.82	20.60	2.54×10^{-4}	4.41
	Error	74250.37	18	4125.02			
	Total	159221.2	19				
ST	Fermentation time	25063.2	1	25063.2	5.24	3.44×10^{-2}	4.41
	Error	86108.27	18	4783.793			
	Total	111171.5	19				
LB	Fermentation time	32213.69	1	32213.69	6.62	1.92×10^{-2}	4.41
	Error	87613.51	18	4867.417			
	Total	119827.2	19				

Table 3. Textural profile data of TPA curves at different fermentation time of MSFY.

Fermentation time (min)	Hardness (g)	Cohesiveness	Adhesiveness (g.s)	Springiness	Gumminess (g)
120	46.44	0.5646	-2.57	1.126	26.22
150	63.6	0.4133	-5.48	1.078	26.28
180	66.9	0.4906	-5.49	1.416	32.82
210	76.6	0.3674	-22.71	1.067	28.14
240	93.1	0.8137	-43.30	1.066	75.76
270	154.6	0.6067	-59.77	1.037	93.80

determining yoghurt texture. Hardness of yoghurt increases with the increase in fermentation time. The hardness of the yoghurt increases with the duration of heating and has been attributed to sulphydral groups (Vijayananda et al., 1989). During the fermentation period of milk, gel is produced to make yoghurt, thus increasing the hardness. In semi solid like products, cohesiveness represents how well the product withstands a second deformation relative to how it behaved under the first deformation. An important characteristic of TPA curve is the negative area between the first and second compression cycle which corresponds to adhesiveness of product. Adhesiveness is the force necessary to remove the material that adheres to the mouth during eating. Springiness is the rate at which the sample returns to its original shape when the deforming force is removed. The textural data of MSFY were hardness (46.44 to 154.6 g), cohesiveness (0.4133 to 0.8137), adhesiveness (-2.57 to -59.77 g.s), springiness (1.037 to 1.416) and gumminess (26.22 to 93.80 g) during fermentation of MSFY. Hardness, gumminess, springiness, adhesiveness and cohesiveness were significantly ($P < 0.01$) affected by

Table 4. Analysis of variance (ANOVA) for textural properties of MSFY.

Properties of MSFY	Source of variation	Sum of Squares (SS)	Degree of freedom (df)	Mean sum of square (MSS)	Calculated F - value	P-value	F-critical
Hardness	Fermentation time	46720.13	1	46720.13	25.03	5.35×10^{-4}	4.96
	Error	18663.72	10	1866.37			
	Total	65383.85	11				
Cohesiveness	Fermentation time	113440.9	1	113440.90	72.03	6.99×10^{-6}	4.96
	Error	15750.13	10	1575.01			
	Total	129191	11				
Adhesiveness	Fermentation time	142859.9	1	142859.9	77.03	5.18×10^{-6}	4.96
	Error	18544.85	10	1854.485			
	Total	161404.8	11				
Springiness	Fermentation time	112754.8	1	112754.8	71.59	7.18×10^{-5}	4.96
	Error	15750.1	10	1575.01			
	Total	128504.9	11				
Gumminess	Fermentation time	72732.75	1	72732.75	40.53	8.18×10^{-5}	4.96
	Error	17945.5	10	1794.55			
	Total	90678.25	11				

fermentation time (Table 4).

Yoghurt bacteria counts

During incubation, as the starter culture grows, a positive interaction is generally observed between *S. thermophilus* and *L. bulgaricus* mixed cultures. The *S. thermophilus* and the *L. bulgaricus* are inoculated simultaneously at 1:1 composition, for instance and remain present throughout the production of yoghurt, as well as in the final product. When both bacteria grow in association, the times for milk coagulation are faster than if either of them is grown separately. The *S. thermophilus* grow and, while they grow, they produce formic acid that, in turn, stimulates the growth of the *L. bulgaricus.* The activity of the latter on casein induces the presence of free amino acids, which, in turn, stimulate the growth of the former (Ginovert et al., 2002).

Production of MSFY involves batch culture fermentation. There were marked changes in starter counts with the fermentation time. Data were plotted as the logarithm of the microbial counts of ST and LB as shown in Figure 2a and 2b. Batch growth curves are prepared based on measurement of viable cell counts (VCC) for *L. bulgaricus* and *S. thermophilus*. As shown in Figure 2, both organisms displayed relatively straight line plots. The *S. thermophilus* and *L. bulgaricus* counts increased from 0 to 270 min of fermentation. The *S. thermophilus* and *L. bulgaricus* counts varied from 1.3×10^8 to 1.75×10^9 cfu / ml and 0.9×10^8 to 1.17×10^9 cfu / ml, respectively.

The fermentation time had a significant effect (P < 0.05) on starter counts (Table 2). F_{cal} value (5.24) is greater than F_{tab} value (4.41) which signifies that fermentation time is significantly affecting the number of Streptococci cells at P < 0.05. The Single factor analysis for *L. bulgaricus* counts (Table 2) also showed the significant effect of fermentation time on *lactobacilli* cells at P < 0.05.

A plot of natural logarithm of microbial counts of ST and LB against time yield a straight line (Figure 2), the slope of which was the specific growth rate (μ_{max}) for each microorganism.
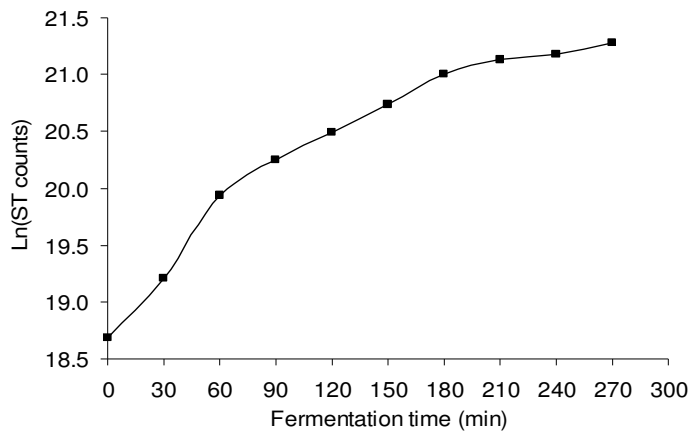
Variations in *S. thermophilus* cell numbers

The equation for the increase in the number of *streptococci* cells in MSFY, 270 min after inoculation:

ln(ST) = 0.0092 × t + 19.145 (R^2 = 0.9012)

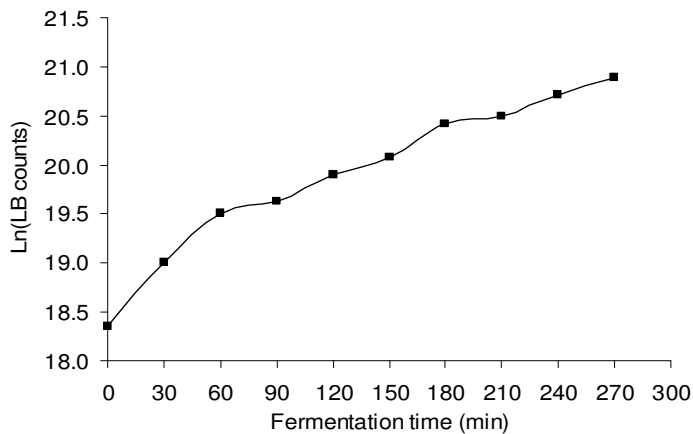Slope = μ_{max} = 0.0092 where μ_{max} is the maximum specific growth rate of *streptococci*.

Variations in *L. bulgaricus* cell numbers

The equation for the increase in the number of *lactobacilli*

Figure 2a. Growth curves of yoghurt bacteria *Streptococcus thermophilus* during fermentation of MSFY.

Figure 2b. Growth curves of yoghurt bacteria *Lactobacillus bulgaricus* during fermentation of MSFY.

cells in MSFY, 270 min after inoculation:

$In(LB) = 0.0085 \times t + 18.745 \ (R^2 = 0.9475)$

Slope = μ_{max} = 0.0085 where μ_{max} is the maximum specific growth rate of *lactobacilli*.

Therefore, μ_{max} for *streptococci* was larger than that for *lactobacilli* which indicated that *S. thermophilus* grows faster than *L. bulgaricus*. This result was in agreement with the earlier findings of Ginovert et al. (2002). They studied the simulation modeling of bacterial growth and noticed a faster increase for both *S. thermophilus* and *L. bulgaricus* in the mixed culture, a consequence of their symbiotic relation.

Conclusion

All the physico-chemical, textural properties and yoghurt bacteria counts were analyzed at an interval of 30 min

after the two cultures that is ST and LB had been allowed to grow for 270 min. The acidity (as % lactic acid) increased whereas pH, total solids and sugars decreased with the increase in fermentation time. The value of acidity of MSFY as % lactic acid ranged from 0.15 at 0 min to 0.62 at 270 min of fermentation. The decrease in pH was from 6.53 to 4.34 and reduction in total sugars was 4.93 to 4.33 while reducing sugars decreased from 4.29 to 3.69 at these fermentation times. The TA-XT2 Texture Analyzer was used to measure the textural characteristics of MSFY. The TPA curves were obtained at 120 - 270 min of fermentation. TPA curves at 0 - 90 min were not possible as the force required by the probe to measure TPA curves was not attained at these fermentation times. The *S. thermophilus* and *L. bulgaricus* counts increased from 0 to 270 min of fermentation. The *S. thermophilus* counts increased from 1.3×10^8 to 1.75×10^9 cfu/ml and *L. bulgaricus* from 0.9×10^8 to 1.17×10^9 cfu /ml.

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