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Yoghurt Production from Commercial Probiotic Starter Cultures and its Chemical Analysis

Prasuna Pakalapati[1], Dr. Santendra Soni[2]
[1]Lecturer, Department of Dairy Technology, Faculty of Science,
Department of Botany at (B.VOC and B.SC courses)
[1,2]Dayalbagh Educational Institute

Abstract
The viability of yoghurt and probiotic bacteria was assessed during production and storage for 35 days. Four commercial starter cultures were used in the yoghurt's preparation. The titratable acidity, pH, and syneresis content portrayed similar patterns of increase and decrease from production to storage of the yoghurt. On the other hand, the percentage of syneresis showed an increase in cultures that contained L. delbrueckii ssp. bulgaricus. The viability of probiotic organisms during production and storage relied on the species and strain of associative yoghurt organisms present. The presence of L. delbrueckii ssp. bulgaricus impacted the viability of L. acidophilus, while thermophilic bacteria showed strong stability in yoghurt developed from cultures containing L. delbrueckii ssp. bulgaricus. The storage temperature of yoghurt affected the survival of the bulgaricus but not L. acidophilus. The variations in titratable acidity, pH, and syneresis were almost indistinguishable at storage temperatures of 4 and 10°C.
Keywords: Viability, yoghurt bacteria, probiotic bacteria,Titratable acidity, syneresis.

Introduction

Consumption of fermented milk products has been linked to numerous health benefits, as stated by Yamamoto et al. (1994). Although Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus microflora present in yoghurt are known to possess nutritious attributes for human well-being (Deeth and Tamime, 1981), there has been a recent emphasis on the production of fermented milk products containing additional Lactobacillus acidophilus. This added emphasis is because of the capacity of these cultures to tolerate bile and acid, enabling them to bind to the intestinal tract. The recommended minimum level for probiotic bacteria present in yoghurt to have a therapeutic effect is 105-106 viable cells per mL or g of product, as indicated by Kurmann and Rasic (1991). Despite the vitality of these beneficial microorganisms, surveys prove that there is poor cell viability of probiotics in market preparations (Shah et al., 1995). Researchers have, therefore, begun to focus on increasing the viability of various products.Numerous factors have been purported to impact the sustainability of probiotics in fermented milk products. The production and preservation of yoghurt have been said to be influenced by acidity, pH, and syneresis (Lankaputhra et al., 1996). Additionally, the viability of probiotic bacteria in yoghurt has been assumed to be altered by other factors, including storage temperature, oxygen concentration, and the concentrations of lactic and acetic acids (Rybka and Kailasapathy, 1995). Although some of these factors have been studied, the impact of all parameters on the viability of probiotic microorganisms has not been examined together.

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The present investigation examined the viability of probiotic bacteria and yoghurt bacteria in yoghurts made from three distinct commercial starter cultures used to produce probiotic yoghurts. The changes in different parameters such as titratable acidity, pH, syneresis and CFU countsof S. thermophilus, L. delbrueckii ssp. Bulgaricus and L. acidophilus were meticulously observed during the production and storage of yoghurt for 35 days, at a temperature of 4°C.

Materials and Methods:

For this investigation, three commercial starter cultures, namely Sl, S2, and S3, were chosen. Sl and S2 were comprised of S. thermophilus (ST), L. delbrueckii ssp. bulgaricus (LB), L. acidophilus (LA), and L. delbrueckii ssp. bulgaricus (LB), S. thermophilus (ST), and L. acidophilus (LA) as the constitutive microflora. It should be noted that among all the commercial culture blends that were studied, the probiotic organisms' strains (LA and BB) remained unchanged, although strains of yoghurt bacteria varied. S3, on the other hand, consisted of a strain of S. thermophilus that produces polysaccharides during fermentation. Out of confidentiality, the supplier's name and the actual nomenclature of the commercial starter cultures were not disclosed. The starter cultures were freeze-dried (DVS) before being procured. Proper precautionary measures as per protocol were implemented concerning the storage and maintenance of the cultures.

Yoghurt Preparation

The yoghurts were produced using a combination of three different commercial cultures. The recommended protocol dictated that starter cultures be added to the milk at specific rates, namely 2 g for Sl and S3 and 1 g for S2 and S3 for every 10 L of yoghurt mix cultures. The incubation temperature was maintained at 43°C for Sl and 40°C for S2 and S3. During the first part of the study, the viability of both yoghurt and probiotic bacteria was evaluated in yoghurt made using all three commercial cultures (Sl, S2, and S3) during the manufacturing process and storage in glass beakers at 4°C. In the second part, the viability of yoghurt bacteria and probiotic bacteria was assessed in yoghurts produced using only two cultures (Sl and S3) during manufacturing and storage in glass beakers at either 4°C or 10°C, or in screw-capped glass bottles at 4°C. A total of six samples were analyzed during each phase of the study.

Sample Preparation

To gauge the quantity of dissolved oxygen, four samples were extracted from each batch of yoghurt and placed in glass beakers for analysis at a heat of 46°C in an aseptic environment while the yoghurt sat in a set form. Following the oxygen assessment, the yoghurt samples were dispensed aseptically into 500 mL sterile glass beakers and uniformly mixed to ensure container consistency. Once mixed appropriately, a sample was aseptically extracted to undergo microbiological analyses, titratable acidity measurement, pH assessment, and syneresis testing, while the quantities of lactic and acetic acids were ascertained.

Analysis

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The pH values of the yoghurt and milk specimens were ascertained at a temperature range of 17 to 20°C by utilizing an Orion 410A pH meter, which was pre-calibrated with fresh pH 4.0 and 7.0 standard buffers. To determine the titratable acidity, a 10 mL proportion of hot distilled water was blended with a sample of yoghurt and titrated with 0.1 N NaOH, using 0.5% phenolphthalein as an indicator. The LC82 Oxygen meter was employed to measure the dissolved oxygen in parts per million (ppm), which was duly calibrated each time before use. Kjeldahl's method was utilized to analyze the protein content of the heat-treated yoghurt mix, where the Kjeldal system and 1002 distillation unit played a significant role. The drying of samples for 2 hours at a temperature of 110°C led to the determination of total solids.

Microbial Analysis

CFU/ml was calculated with One gram of yoghurt specimen blended with 9 mL of peptone water (0.15%) and homogenized with a vortex mixer to create a uniform mixture. Subsequent serial dilutions were produced via the pour plate method for the enumeration of viable quantities. The number of S. thermophilus bacteria was gauged per litre of water using ST agar (pH adjusted to 6.8), with the plates incubated aerobically at 37°C for 24 hours (Dave and Shah, 1996). For the differential enumeration of L. delbrueckii ssp. Bulgaricus, MRS agar was used, pH adjusted to 5.2 and incubated anaerobically at 43°C for 72 hours. MRS-sorbitol agars were used for the selective enumeration of L. acidophilus. The total number of probiotic organisms was gauged using MRS-maltose agar, which was originally designed by Hull and Roberts (1984) for the differential enumeration of L. acidophilus from yoghurt bacteria.

Results and Discussion:

The protein content of the heat-treated yoghurt mixture exhibited a range of 3.55-3.65, while the total solid contents were found to be in the range of 15.99-16.24% (unspecified data). As a result, the variations in composition across the experimental replications were insignificant and therefore the observed differences should not be attributed to compositional factors.

Growth of the probiotic strains evaluated through CFU/ml:

To specify the number of viable probiotic bacteria in yoghurt, the probiotic strains were counted on MRS agar with each of the samples and incubated at 37±1℃ for 3 days . The starter culture count was determined based on the standard method Gahruie (2019). The table explains the viable colony count obtained through the serial dilution method followed by the pour plate technique.

Table- Growth of the probiotic strains evaluated through CFU/ml

Name of the probiotic strain	**D-10^{-1}**	**D-10^{-2}**	**D-10^{-3}**	**D-10^{-4}**	**D-10^{-5}**	**D-10^{-6}**	**D-10^{-7}**	**D-10^{-8}**	**D-10^{-9}**
Lactobacillus delbruekii sub sp. Bulgaricus	876	634	542	360	250	85	25	12	3
Lactobacillus acidophilus	653	243	220	183	62	48	19	8	0
Streptococcus	865	654	492	156	89	64	20	8	0

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thermophilus									

D-Dilution, 10^{-} -Dilution Factor

Eskandari et al (2012) reported a similar observation, which showed the significant effect of viable cell count without any physiochemical changes in the yoghurt samples. The results also agreed with Iqbal et al (2019) showing the best probiotic properties

Titrable acidity and pH:

A. pH of yoghurt samples inoculated with different probiotic strains of bacteria:

Table 4.6-pH of probiotic cultures used for the preparation of yoghurt

Name of the strain	Day 1 (Mean ±SD)	Day 3 (Mean ±SD)	Day 5 (Mean ±SD)	Day 7 (Mean ±SD)
Control	6.3 ± 0.0	4.4 ± 0.1	4.1 ± 0.1	3.6 ± 0.21
Lactobacillus acidophilus	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.0	3.5 ± 0.1
Lactobacillus delbruekii sub sp. bulgaricus	4.6 ± 0.17	4.5 ± 0.15	4.4 ± 0.0	3.3 ± 0.1
Streptococcus thermophilus	4.6 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	3.0 ± 0.0

SD-Standard Deviation

Variable pH was recorded in all different samples of yoghurt which were injected in triplicates with different strains. In general, a slight decrease was noticed in all yoghurt samples till Day 5 and there was a drastic decrease was recorded on Day 7. pH of *Lactobacillus acidophilus* on Day 1, Day 3, Day 5 & Day 7 were 4.6, 4.5, 4.5 & 3.5 respectively. pH of *Lactobacillus delbruekii sub sp. bulgaricus* on Day 1, Day 3, Day 5 & Day 7 were 4.6, 4.5, 4.4 & 3.3 respectively. *Streptococcus thermophilus* pHon Day 1, Day 3, Day 5 & Day 7 were4.6,4.3, 4.2& 3 respectively. Tableexplains that a digital pH meter analysed the samples for pH values in triplicate samples. The standard deviationmean and of pH were calculated from all the days, low or small standard deviation demonstrates how closely data are clustered around the mean.There was a drop in the pH of developed curd samples because of developed acidity. During storage time there was a significant drop in pH up to some extent after which significant changes were noticed. In the last few days, a significant change was observed. Similar results were noted in the study of Soni, (2020).

Statistical Test: Independent sample ANOVA with a single factor has been used along with paired two samples for t-factor has been utilized to access the importance of the Mean Difference At a level of significance (0.05), it appears that there is a notable difference of fortification on pH of developed curd sample for control vs treatment groups between days when stored for days. In the last few days, a significant change was observed. Similar results were noted in the research of Veena, (2017).

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Table -ANOVA calculation of pH obtained at different days in a week

Anova: Two-Factor Without Replication						
SUMMARY	***Count***	***Sum***	***Average***	***Variance***		
Row 1	4	18.4	4.6	1.393333		
Row 2	4	17.11	4.2775	0.270692		
Row 3	4	16.8	4.2	0.366667		
Row 4	4	16.9	4.225	0.189167		
Row 5	4	16.1	4.025	0.495833		
Column 1	5	24.7	4.94	0.578		
Column 2	5	22.11	4.422	0.00742		
Column 3	5	21.5	4.3	0.025		
Column 4	5	17	3.4	0.065		
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.70322	4	0.175805	1.055643	0.419666	3.259167
Columns	6.148615	3	2.049538	12.30671	0.000568	3.490295
Error	1.99846	12	0.166538			
Total	8.850295	19				

Table- Statistical Calculation of t-Test (Day 1 & Day 3) for pH

	6.3	*3.6*
Mean	4.6	3.35
Variance	0	0.07
Observations	4	4
Pearson Correlation	#DIV/0!	
Hypothesized Mean Difference	0	
Df	3	
t Stat	9.449112	
P(T<=t) one-tail	0.001256	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.002512	
t Critical two-tail	3.182446	

Table- Statistical Calculation of t-Test (Day 5 & Day 7) for pH

	6.3	*4.1*
Mean	4.6	4.35
Variance	0	0.016667
Observations	4	4
Pearson Correlation	#DIV/0!	
Hypothesized Mean Difference	0	
Df	3	
t Stat	3.872983	

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P(T<=t) one-tail	0.015233	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.030466	
t Critical two-tail	3.182446	

Along with independent samples single factor ANOVA, the t-test was also calculated to observe a significant change in the pH between the probiotic yoghurt samples preserved between days 1& 3, and days 5 & 7**.**A significant difference has been observed, among the samples that have 0.05 factor was rejected, and 0.005 factor has been accepted. The result determined that the null hypothesis was negligible.

B. Titrable acidity (% Lactic acid) of yoghurt samples injected with probiotic strains of bacteria:

Table -Titrable acidity of probiotic cultures used in the preparation of yoghurt

Name of the strain	**Day 1 (Mean ± SD)**	**Day 3 (Mean ± SD)**	**Day 5 (Mean ± SD)**	**Day 7 (Mean ± SD)**
Control	0.74 ± 0.01	0.74 ± 0.01	0.74 ± 0.01	0.78 ± 0.01
Lactobacillus acidophilus	0.71 ± 0.00	0.73 ± 0.01	0.73 ± 0.01	0.69 ± 0.015
Lactobacillus delbruekii sub sp. bulgaricus	0.70 ± 0.00	0.73 ± 0.01	0.73 ± 0.01	0.68 ± 0.00
Streptococcus thermophilus	0.72 ± 0.01	0.74 ± 0.01	0.74 ± 0.01	0.85 ± 0.01

SD-Standard Deviation

Variable titrable acidity was recorded in all different samples of yoghurt which were injected in triplicates with different strains. The average proportion of the titrable acidity was recorded and calculated. In general, a minor rise was noticed in all yoghurt samples except *Lactobacillus. delbruekii sub sp. bulgaricus*. In *Lactobacillus. delbruekii sub sp. bulgaricus* there was a slight decrease of titrable acidity at Day 7 recorded as 0.68. Titratable acidity of *Lactobacillus acidophilus* on Day 1, Day 3, Day 5 & Day 7 was 0.71, 0.73, 0.73 & 0.69 respectively. The acidity of *Streptococcus thermophilus* on Day1, Day3, Day5 & Day 7 were 0.72, 0.74, 0.74 & 0.85 respectively. similar results were observed by Hamed Mahmoodi Pour (2022). The titrable acidity of prepared yoghurt samples was analysed through the procedure outlined by Rangana (1986). Table explainsthat the probiotic yoghurt samples in the triplicate form of samples were stored for 7 days to check the change in titratable acidity on alternate days. Change in acidity was observed because of increased growth of probiotic bacteria in curd during an incubation time of 7 days. The mean and standard deviationof titrable acidity was evaluated from all the days, low or small standard deviation has shown data are closely grouped around the mean Significant result was found during the analysis and similar was explained by Soni. (2020).

Statistical Analysis: Independent Samples one factor ANOVA has been used to calculate the importance of the Mean Difference in sensory attributes of given samples. At the importance

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level (0.05), it may be concluded that there was a notable variation in fortification on titratable acidity yoghurt sample for control vs treatment groups between days when stored for 7 days.

Table -ANOVA calculation of Titratable acidity obtained at different days in a week

Anova: Two-Factor Without Replication						
SUMMARY	***Count***	***Sum***	***Average***	***Variance***		
Row 1	4	3	0.75	0.0004		
Row 2	4	2.86	0.715	0.000367		
Row 3	4	2.84	0.71	0.0006		
Row 4	4	3.06	0.765	0.0011		
Row 5	4	3.05	0.7625	0.003492		
Column 1	5	3.6	0.72	0.00025		
Column 2	5	3.7	0.74	0.00015		
Column 3	5	3.7	0.74	0.00015		
Column 4	5	3.81	0.762	0.00557		
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.01102	4	0.002755	2.456166	0.102263	3.259167
Columns	0.004415	3	0.001472	1.312036	0.315874	3.490295
Error	0.01346	12	0.001122			
Total	0.028895	19				

Table -Statistical calculation of t-test Factor for titratable acidity:

t-Test: Paired Two Sample for Means		
	0.74	*0.74*
Mean	0.715	0.74
Variance	0.000167	0.0002
Observations	4	4
Pearson Correlation	0.912871	
Hypothesized Mean Difference	0	
Df	3	
t Stat	-8.66025	
P(T<=t) one-tail	0.00162	
t Critical one-tail	5.840909	
P(T<=t) two-tail	0.003239	
t Critical two-tail	7.453319	

Table- Statistical calculation of t-Test Factor (Day 5 & Day 7) for titratable acidity:

t-Test: Paired Two Sample for Means		
	0.74	*0.78*
Mean	0.74	0.7575

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Variance	0.0002	0.007291667
Observations	4	4
Pearson Correlation	0.6900656	
Hypothesized Mean Difference	0	
Df	3	
t Stat	-0.458585	
P(T<=t) one-tail	0.338857	
t Critical one-tail	5.8409093	
P(T<=t) two-tail	0.677714	
t Critical two-tail	7.4533185	

The t-test is also calculated to observe a significant change in the titrable acidity between the probiotic yoghurt samples preserved between days 1 & 3 and, days 5 & 7. A significant difference has been observed, between the samples with 0.05 factor rejected, and 0.005 factor accepted, the results concluded that the null hypothesis was negligible.

C. Syneresis of yoghurt samples inoculated by using probiotic strains:

Table - Syneresis of probiotic cultures used in the preparation of yoghurt:

Name of the strain	**Day 1 (Mean ±SD)**	**Day 3 (Mean ±SD)**	**Day 5 (Mean ±SD)**	**Day 7 (Mean ±SD)**
Control	35.1 ± 0.2	35.1 ± 0.0	35.3 ± 0.3	45.3 ± 0.4
Lactobacillus acidophilus	35.20 ± 0.0	36.3 ± 0.3	36.5 ± 0.3	38.8 ± 0.1
Lactobacillus delbruekii sub sp. bulgaricus	36.7 ± 0.1	37.1 ± 0.1	37.8 ± 0.3	39.1 ± 0.0
Streptococcus thermophilus	39.2 ± 0.1	40.1 ± 0.2	40.6 ± 0.1	47.2 ± 0.1

SD-Standard Deviation

A variable percentage of syneresis was recorded in all different samples of yoghurt which received different treatments in triplicates. The average proportion of the syneresis was calculated. In general, a minorrise was noted in all yoghurt samples till Day 5 and there was a drastic increase was recorded at Day 7. Syneresis of *Lactobacillus acidophilus*onDay 1, Day 3,Day 5 & Day 7 were 35.2, 36.3, 36.5 & 38.8 respectively. pH of *Lactobacillus delbruekii sub sp. bulgaricus*on Day 1, Day 3, Day 5 & Day 7 were 36.7, 37.1, 37.8 & 39.1 respectively. syneresis of *Streptococcus thermophilus*on Day1, Day3, Day5 & Day 7 were 39.2,40.1,40.6 & 47.2 respectively. Table explains syneresis defined as the amount of whey gathered after 2 hours of draining and was expressed as a percentage of whey separated in triplicate form from each bacterial sample. Following the drainage technique suggested by Raju and Pal (2014), syneresis was determined when prepared yoghurt samples were studied for syneresis percentage (%) during the storage of 7 days. The standard deviation and mean of syneresis were calculated from all the days, low or small standard deviation has shown data are clustered tightly about the mean.

Statistical Analysis:

Independent Samples One Factor ANOVA has been used to calculate the importance of

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Mean. The difference in sensory attributes of given samples, At the important level (0.05), it is implied that there existed a considerable variation of fortification on syneresis % of developed yoghurt samples for Control vs Treatment Groups between days when stored for 7 days. In the last few days' significant changeswere observed.

Table - ANOVA calculation of Syneresis% obtained at different days in a week

Anova: Two-Factor Without Replication						
SUMMARY	**Count**	**Sum**	**Average**	**Variance**		
Row 1	4	150.8	37.7	25.68		
Row 2	4	146.8	36.7	2.286667		
Row 3	4	150.7	37.675	1.109167		
Row 4	4	166.5	41.625	9.9025		
Row 5	4	167.1	41.775	13.41583		
Column 1	5	184.3	36.86	3.223		
Column 2	5	188.7	37.74	5.148		
Column 3	5	193.3	38.66	10.043		
Column 4	5	215.6	43.12	15.137		
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	93.127	4	23.28175	6.801397	0.004246	3.259167
Columns	116.1055	3	38.70183	11.30613	0.000823	3.490295
Error	41.077	12	3.423083			
Total	250.3095	19				

Statistical calculation of t-Test Factor for syneresis:

The t-test is also calculated to observe a significant change in the syneresis between the probiotic yoghurt samples preserved between days 1 & 3 days 1 & 3, and days 5 & 7. A significant difference has been observed, between the samples with 0.05 factor was rejected, and 0.005 factor has been accepted. The result concluded the null hypothesis was negligible.

Table - Statistical calculation of t-test factor (Day 1 & Day 3) for syneresis

t-Test: Paired Two Sample for Means		
	35.1	*35.1*
Mean	37.3	38.4
Variance	3.006667	3.96
Observations	4	4
Pearson Correlation	0.944773	
Hypothesized Mean Difference	0	
Df	3	
t Stat	-3.291781	
P(T<=t) one-tail	0.023008	
t Critical one-tail	5.840909	
P(T<=t) two-tail	0.046016	
t Critical two-tail	7.453319	

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Table -Statistical calculation of t-test factor (Day 5 & Day 7) for syneresis

t-Test: Paired Two Sample for Means		
	35.3	*45.3*
Mean	39.5	42.575
Variance	8.686667	18.2025
Observations	4	4
Pearson Correlation	0.842178	
Hypothesized Mean Difference	0	
Df	3	
t Stat	-2.55388	
P(T<=t) one-tail	0.041108	
t Critical one-tail	5.840909	
P(T<=t) two-tail	0.082215	
t Critical two-tail	7.453319	

Conclusion:

The milk samples were pasteurized, homogenized and standardised for the production of yoghurt and to make it economical and affordable to the people. By using the 3 probiotic strains namely *Lactobacillus delbruekii sub sp. bulgaricus,Lactobacillus acidophilus,* and a common strain *Streptococcus thermophilus* were used to check the chemical changes. All of the starter cultures under investigation exhibited variations in the levels of titratable acidity, pH, and syneresis during the production and storage of yoghurt. Notably, a difference existed in the viability of probiotic organisms. While the S. thermophilus and L. delbrueckii ssp. bulgaricus counts increased sharply, the former could not sustain its viability at the highest level while the latter further increased in viability, only to have its viability reduced after two weeks of storage. Multiplication and viability of probiotic bacteria were also subject to the influences of the associated strains and species of yoghurt organisms. For Sl and S2 cultures, the count of L. acidophilus reduced quickly, which could be attributed to the production of hydrogen peroxide by L. delbrueckii ssp. bulgaricus. This finding supported only a slight increase in acetic acid content for products using S3 culture. Additionally, changes in titratable acidity, pH, and syneresis percentage were observed to be related to the fermentation patterns of constitutive microflora in these three cultures. While pH and slight increases in titratable acidity were prominent at 10°C, minimal variations were observed in samples stored in glass bottles.

References:

1. Yamamoto, N., Akino, A. and Takano, T. (1994). Anti-hypersensitive effects of different kinds of fermented milk inspontaneously hypersensitive rats. *Bio-Sci. Biotechnol.Biochem., 58, 776-778.*
2. Deeth H. C. and Tamime, A. Y. (1981). Yoghurt: nutritive andtherapeutic aspects. *J. Food Prot., 44, 78-86.*

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, Journal Volume 10, Iss 12, 2021

3. Kurmann, J. A. and Rasic, J. L. (1991). The health potential ofproducts containing bifidobacteria. In *Therapeutic Propertiesof Fermented Milks* (ed. R. K. Robinson). ElsevierApplied Food Science Series, London, pp. 117-158.
4. Shah, N., Lankaputhra, W. E. V., Britz, M. and Kyle, W. S. A.(1995). Survival of *L. acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigeratedstorage. *Inter. Dairy J., 5, 515.*
5. Lankaputhra, W. E. V., Shah, N. P. and Britz, M. L. (1996a).Evaluation of media for selective enumeration of *Lactobacillusacidophilus* and *Bifidobacterium* spp. *Food Australia,48, 113-118.*
6. Rybka, S. and Kailasapathy, K. (1995). The survival of culturebacteria in fresh and freeze-dried AB yoghurts. *Aust. J.Dairy Technol., 50, 51-57.*
7. Dave, R. I. and Shah, N. P. (1996). Evaluation of media forselective enumeration of *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus acidophilus*and *Bifidobacterium* spp. *J. Dairy Sci., 79,* 1529-l 536
8. Hull, R. R. and Roberts, A. V. (1984). Differential enumerationof *Lactobacillus acidophilus* in yoghurt. *Aust. J. DairyTechnol., 3,* 160-164.
9. Hull, R. R., Roberts, A. V. and Mayes, J. J. (1984). Survival of*Lactobacillus acidophilus* in yoghurt. *Aust. J. DairyTechnol., 39, 164166.*IDF (1984). *Fermented Milks.*
10. Gahruie, H. H., Eskandari, M. H., & Mesbahi, G. (2019). Development of functional yogurt fortified with wheat germ and strawberry as functional ingredients. Progress in Nutrition, 21, 388–398.
11. Eskandari, M. H. Baroutkoub, A.Roushan Zamir, M Beglarian, R. Ghasemkhani, I. and Shekarforoush, S. S,Iranian (2012).Effect of milk supplementation on growth and viability of starter and probiotic bacteria in yogurt during refrigerated storage Journal of Veterinary Research, Shiraz University, Vol. 13, No. 3, Ser. No. 40.
12. Soni, R., Jain, N. K., Shah, V., Soni, J., Suthar, D., & Gohel, P. (2020). Development of probiotic yogurt: Effect of strain combination on nutritional, rheological, organoleptic and probiotic properties. *Journal of Food Science and Technology*, *57*(6), 2038-2050. https://doi.org/10.1007/s13197-020-04238-3
13. Veena, N., Surendra Nath, B., Srinivas, B., &Balasubramanyam, B. V. (2017). Qualityattributes of dahi prepared from milk fortified with omega-3 fatty acids, phytosterolsand polydextrose. Journal of food science and technology, 54(7), 1765-1775.
14. S. Ranganna, (1986) Handbook of Analysis and Quality Control for Fruit and Vegetable Products, Tata McGraw-Hill, 9780074518519.
15. Raju, P. N., & Pal, D. (2012). Effect of dietary fibers on physico-chemical, sensory and textural properties of Misti Dahi. *Journal of Food Science and Technology*, *51*(11), 3124-3133. https://doi.org/10.1007/s13197-012-0849-y
16. Schug, A. R., Bartel, A., Meurer, M., Scholtzek, A. D., Brombach, J., Hensel, V., Fanning, S., Schwarz, S., &Feßler, A. T. (2020). Comparison of two methods for cell count determination in the course of biocide susceptibility testing. *Veterinary Microbiology*, *251*, 108831. https://doi.org/10.1016/j.vetmic.2020.108831
17. Skelte G, Anema and Yuming Li, (2003) Effect of pH on the Association of

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 10, Iss 12, 2021

Denatured Whey Proteins with Casein Micelles in Heated Reconstituted Skim Milk, Journal of Agriculture and Food Chemistry 51: 1640- 1646.

18. Thekkilaveedu, S., Krishnaswami, V., Mohanan, D. P., Alagarsamy, S., Natesan, S., & Kandasamy, R. (2019). Lactic acid-mediated isolation of alpha-, beta- and kappa-casein fractions by isoelectric precipitation coupled with cold extraction from defatted cow milk. *International Journal of Dairy Technology*, *73*(1), 31-39. https://doi.org/10.1111/1471-0307.12646
19. Tulipano, G., Faggi, L., Nardone, A., Cocchi, D., & Caroli, A. M. (2015). Characterisation of the potential of β-lactoglobulin and α-lactalbumin as sources of bioactive peptides affecting incretin function: In silico and in vitro comparative studies. *International Dairy Journal*, *48*, 66-72. https://doi.org/10.1016/j.idairyj.2015.01.008
20. Villhauer, E. B., Brinkman, J. A., Naderi, G. B., Dunning, B. E., Mangold, B. L., Mone, M. D., Russell, M. E., Weldon, S. C., & Hughes, T. E. (2002). 1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(*S*)-pyrrolidinecarbonitrile: a potent, selective, and orally bioavailable Dipeptidyl peptidase IV inhibitor with Antihyperglycemic properties. *Journal of Medicinal Chemistry*, *45*(12), 2362-2365. https://doi.org/10.1021/jm025522z
21. Whitehead, P. (2003), Application Note: Laboratory Monitoring of Total Organic Carbon in Ultrapure Water American Laboratory 35(17), 20-24.
22. Prajapati, J. B. and Dave, R. I. (1994). Associative growth of*L. acidophilus* and S. *thermophilus* in buffalo milk. 24thInter. Dairy Congress, Melbourne, Australia, Sept. 18822,1994.
23. Robinson, R. K. (1987). Survival of *Lactobacillus acidophilus*in fermented products. *SuidAfrikaanseTydskrif VirSuiwelkunde*, 19, 25-27.
24. Rybka, S. and Kailasapathy, K. (1995). The survival of culturebacteria in fresh and freeze-dried AB yoghurts. *Aust. J.Dairy Technol., 50, 51-57.*