PREPARATION, EVALUATION AND USE OF BULK FREEZE-DRIED STARTER CULTURES IN "DAHI" AND YOGHURT MANUFACTURE

THESIS
SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE
KARNAL (HARYANA)
IN
PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

IN

DAIRY MICROBIOLOGY

By

S. VENKATA NAGA VIJAYENDRA
M. Sc. (MICROBIOLOGY)

DIVISION OF DAIRY MICROBIOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL – 132001 (HARYANA), INDIA

1994

REGN. NO. 90 – P – DM – 143

PREPARATION, EVALUATION AND USE OF BULK FREEZE-DRIED STARTER CULTURES IN "DAHI" AND YOGHURT MANUFACTURE

THESIS

SUBMITTED TO THE

NATIONAL DAIRY RESEARCH INSTITUTE

KARNAL (HARYANA)

IN

PARTIAL FULFILMENT OF THE REQUIREMENT

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

DAIRY MICROBIOLOGY

By

S. VENKATA NAGA VIJAYENDRA

M. Sc. (MICROBIOLOGY)

DIVISION OF DAIRY MICROBIOLOGY

NATIONAL DAIRY RESEARCH INSTITUTE

(I. C. A. R.)

KARNAL - 132 001 (HARYANA), INDIA

1994

REGN. NO. 90-P-DM-143

DEDICATED

TO

LORD SRI HANUMAN

&

MY BELOVED PARENTS

SRI S. VIVEKANANDA & SMT.S. VASUNDHARA DEVI

PREPARATION, EVALUATION AND USE OF BULK FREEZE-DRIED STARTER CULTURES IN DAHI AND YOGHURT MANUFACTURE

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL (HARYANA)
(DEEMED UNIVERSITY)

IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY
IN
DAIRY MICROBIOLOGY

BY
S. VENKATA NAGA VIJAYENDRA
M.SC.(MICROBIOLOGY)

APPROVED BY

( )
EXTERNAL EXAMINER

(DR. R.C. GUPTA)
MAJOR ADVISOR & CHAIRMAN (GUIDE)

MEMBERS OF ADVISORY COMMITTEE

1. Dr. S. Neelakantan,
   Principal Scientist & Ex-Head,
   Dairy Microbiology Division, NDRI

2. Dr. D.N. Prasad,
   Principal Scientist,
   Dairy Microbiology Dvn., NDRI

3. Dr. M.P. Tiwari,
   Principal Scientist,
   Dairy Microbiology Dvn., NDRI

4. Prof. R.D. Verma,
   Principal Scientist,
   Dairy Engineering Dvn., NDRI

5. Dr. S.R. Anand,
   Principal Scientist,
   Animal Biochemistry Dvn., NDRI

6. Dr. B.D. Tiwari,
   Senior Scientist,
   Dairy Technology Dvn., NDRI

Dr. R.C. Gupta
M.Sc(Dairy Microbiology), Ph.D (Dairying)
Senior Scientist

Division of Dairy Microbiology
National Dairy Research Institute
(Indian Council of Agricultural Research)
Karnal-132 001

CERTIFICATE

This is to certify that the thesis entitled, "Preparation, evaluation and use of bulk freeze-dried starter cultures in dahi and yoghurt manufacture" submitted by Mr. S. Venkata Naga Vijayendra in partial fulfilment of the requirement for the award of the DOCTOR OF PHILOSOPHY in Dairy Microbiology of the National Dairy Research Institute (Deemed University), Karnal, India is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

(R.C. GUPTA)
Major Advisor & Chairman (Guide)

Date: 14th Nov. 94

ACKNOWLEDGEMENTS

I wish to express my deepest sense of gratitude to Dr. R.C. Gupta, Senior Scientist, Dairy Microbiology Division for his valuable guidance, constructive criticism, meticulous attention, sustained encouragement and pertinent suggestions in planning and execution of this investigation successfully.

Grateful acknowledgements are due to Dr. O.S. Tomer, Director; Dr. S.C. Sarma, former Director, National Dairy Research Institute; Dr. R.N. Sinha, Head for providing necessary facilities to carry out the present investigation.

Sincere appreciation is extended to the members of my Advisory Committee- Dr. S. Neelakantan, Principal Scientist & former Head; Dr. D.N. Prasad, Principal Scientist & former Head; Dr. M.P. Tiwari, Principal Scientist & Incharge, NCDC Lab of Dairy Microbiology Division; Prof. R.D. Verma, Principal Scientist, Dairy Engineering Division; Dr. S.R. Anand, Principal Scientist (Retd.), Animal Biochemistry Division and Dr. B.D. Tiwari, Senior Scientist, Dairy Technology Division, for their valuable suggestions during the period of this study.

Financial assistance received from the Institute in the form of NDRI Senior Research Fellowship, without which this endeavour would not have been possible, is gratefully acknowledged.

My sincere thanks are due to Mr. Madan Lal, Technical Officer; Mr. I.T. Zariawala, Technical Officer; Mr. Roop Chand, T-I-3; Mr. Vinod Kumar and all other members of Dairy Microbiology Division for their cooperation during the course of this study.

My special thanks are due to Dr. S.V.N. Rao, Senior Scientist, Dairy Extension Division; Dr. D.L. N. Rao, Senior Scientist, Central Soil Salinity Research Institute, Karnal; Dr. T. Chandra Sekhar, Scientist and Dr(Mrs) Sangeeta Nair, Scientist, Dairy Cattle Physiology Division; Mr. K.S.N. Prasad, Scientist, Dairy Cattle Nutrition Division; Dr. G.K. Goyal, Senior Scientist, Dairy Technology Division and Mr. A.K. Sharma, Computer Centre, for their valuable suggestions, timely help and encouragement for the completion of this study.

I also wish to express my special thanks to Prof. S.R. Tatini, Department of Food Science & Nutrition, University of Minnesota, Minnesota (USA) for kindly providing N-acetyl-D-glucosamine & Dr. N. Subrahmanyam, Senior Scientist, Central Food Technological Research Institute, Mysore and M/s.Rolla Tainers, Faridabad, for providing the packaging material free of cost.

Sincere thanks are due to Dr. K. Jaya Raja Rao, Scientist, Dairy Technology Division and Mr. K. Nageswara Rao (D.M) for their endless effort and help rendered during the study and in the preparation of this thesis.

My special thanks are also due to Dr. J.R.K. Prasad, Dr. M.M. Prasad, Dr. B. Sreenivas, Mr. Rajeev Prashar, Mr. Rajesh Bajaj and Mr. C. Ganesh Kumar who had been a good company and constant source of encouragement throughout this investigation. I feel fortunate to have the company of my friends to name a few, B.L.V. Ramana, G. Sambasiva Rao, M.S. Rao, R.S. Rao, S. Rama Rao and all seniors for their cooperation and inspiration during my stay at NDRI.

I am grateful to all the Telugu families of Karnal for their immense affection and good company during my stay at Karnal.

I am indebted to my affectionate grand mother, parents, brothers, sister-in-law, Kirti Sai and my childhood friends, B.V. Jayaram and K. Gowri Shanker, whose everlasting love and inspiration made my endeavour a success.

[S.V.N. VIJAYENDRA]

ABBREVIATIONS

ANOVA	Analysis of variance
@	At the rate of
C	Centigrade
CD	Critical difference
CF	Correction factor
CFF	Cell free filtrate
cfu	Colony forming unit
cm	centimeter
CoA	Co-enzyme A
DF	Degrees of freedom
DMSO	Dimethyl sulfoxide
DVI	Direct to vat inoculation
EDTA	Ethylene diamine tetra acetic acid
eg	for example
FD culture	Freeze-dried culture
Fig.	Figure
g	Gram
Hg	Mercury
hrs	hours
ISI	Bureau of Indian Standards (formerly Indian Standards Institution)
kg	Kilogram
LA	Lactic acid
LAB	Lactic acid bacteria
M	Molar
mcg	Microgram
mg	Milligram
min.	Minute(s)
ml	Millilitre
mm	Millimetre
MSS	Mean sum of square
nm	nanometer
NDRI	National Dairy Research Institute
No	Number
OD	Optical density
%	Percent(age)
PA	Proteolytic activity
PEG	Polyethylene glycol
rpm	Revolutions per minute
psi	Pounds per square inch
RSM	Reconstituted skim milk
SS	Sum of square
TA	Titratable acidity
TMS	Total milk solids
VP	Voges-Proskaur
wt	Weight
w/v	Weight by volume
µ	Micron

C O N T E N T S

Sl.No.	Title	Page No.
1.	INTRODUCTION	1
2.0	REVIEW OF LITERATURE	2-31
2.1	Starter cultures of dahi and yoghurt	2
2.2	Use of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in dahi, yoghurt and other fermented dairy products	3
2.3	Associative growth of mixed starter cultures of dahi and yoghurt	4
2.3.1	Effect on titratable acidity	5
2.3.2	Effect on volatile acidity	6
2.3.3	Effect on flavour	7
2.3.4	Effect on proteolytic activity	9
2.4	Preparation of bulk freeze-dried starter cultures of dahi and yoghurt	10
2.4.1	Growth media	10
2.4.2	Growth conditions	13
2.4.2.1	Effect of pH	13
2.4.2.2	Effect of neutralizer	14
2.4.2.3	Effect of temperature	14
2.4.3	Rate of inoculum	15
2.4.4	Harvesting of cell biomass	16
2.4.5	Influence of cryoprotective agents	17
2.4.6	Freeze-drying of bulk starter cultures	20
2.4.7	Packaging of bulk freeze-dried starter cultures	21
2.5	Stability of bulk freeze-dried starter cultures during storage	21
2.5.1	Effect of storage temperature	21
2.5.2	Effect of sealing atmosphere	22
2.6	Use of bulk freeze-dried starter cultures of dahi and yoghurt	23
2.6.1	Preparation of dahi	24
2.6.2	Preparation of yoghurt	24
2.7	Nutritional and therapeutic value of dahi and yoghurt	25
2.7.1	Effect on body weight gain	25
2.7.2	Effect on serum cholesterol level	26

2.7.3	Antibacterial activity	28
2.8	Objectives of the present study	31
3.0	MATERIALS AND METHODS	32-61
3.1	Collection of raw materials	32
3.2	Collection of bacterial cultures	33
3.3	Maintenance of the bacterial cultures	33
3.4	Purity of the cultures	34
3.5	Composition of bacteriological media	34
3.6	Biochemical methods for the evaluation of starter cultures, dahi and yoghurt	37
3.6.1	Titratable acidity	37
3.6.2	Culture activity	37
3.6.3	Volatile acidity	38
3.6.4	Flavour producing capacity (Voges-Proskauer test)	38
3.6.5	Diacetyl content	38
3.6.6	Acetaldehyde content	40
3.6.7	Proteolytic activity	40
3.6.8	Residual moisture content	40
3.7	Chemical analysis	43
3.7.1	Determination of fat content	43
3.7.2	Cholesterol content	43
3.8	Performance of formulated cultures of dahi and yoghurt	46
3.8.1	Selection of dahi, yoghurt and therapeutic cultures	46
3.8.2	Performance of selected dahi and yoghurt cultures with and without therapeutic cultures	47
3.9	Preparation of bulk freeze-dried starter cultures	49
3.9.1	Selection of formulated growth media	51
3.9.2	Optimization of growth conditions	52
3.9.2.1	pH of the medium	52
3.9.2.2	Neutralization of cultures	52
3.9.2.3	Incubation temperature	52
3.9.2.4	Inoculum rate	53
3.9.3	Production of cell biomass	53
3.9.4	Harvesting of cell biomass	53
3.9.5	Cryoprotective agents	54
3.9.6	Freeze-drying of bulk starter cultures of dahi and yoghurt	54

3.9.7	Packaging of bulk freeze-dried starter cultures	55
3.10	Stability of bulk freeze-dried starter cultures of dahi and yoghurt during storage	55
3.11	Predicting the shelf life of bulk freeze-dried starter cultures	56
3.12	Use of bulk freeze-dried starter cultures of dahi and yoghurt preparation	56
3.12.1	Determination of inoculum size	56
3.12.2	Activity and biochemical performance of bulk freeze-dried cultures of dahi and yoghurt	57
3.12.3	Preparation of dahi	57
3.12.4	Preparation of yoghurt	57
3.12.5	Lactic count and biochemical quality of dahi and yoghurt	58
3.12.6	Sensory evaluation of dahi and yoghurt	58
3.13	Nutritional and therapeutic values of dahi and yoghurt	58
3.13.1	Body weight and serum cholesterol level of albino rats	59
3.13.2	Determination of antibacterial activity of dahi and yoghurt	60
4.0	RESULTS AND DISCUSSION	62-145
4.1	Formulation and selection of dahi and yoghurt cultures	63
4.1.1	Selection of formulated culture combinations	63
4.1.2	Viability and biochemical performance of selected cultures of dahi and yoghurt in buffalo skim milk	66
4.2	Preparation of bulk freeze-dried starter cultures of dahi and yoghurt	82
4.2.1	Selection of growth media	82
4.2.2	Optimization of growth conditions	89
4.2.2.1	Effect of pH on the growth medium	89
4.2.2.2	Effect of neutralization of growth medium	92
4.2.2.3	Effect of incubation temperature	94
4.2.2.4	Effect of inoculum size	97
4.2.3	Influence of cryoprotective agents	100
4.3.	Stability of bulk freeze-dried dahi and yoghurt cultures during storage	104

4.3.1	Influence of storage temperature and sealing atmosphere on the stability of freeze-dried cultures	104
4.3.1.1	Bulk freeze-dried dahi culture D	104
4.3.1.2	Bulk freeze-dried dahi culture DAB	104
4.3.1.3	Bulk freeze-dried yoghurt culture Y	111
4.3.1.4	Bulk freeze-dried yoghurt culture YAB	115
4.3.2	Prediction of shelf life of bulk freeze-dried cultures of dahi and yoghurt	119
4.4	Use of bulk freeze-dried cultures of dahi and yoghurt	122
4.4.1	Determination of inoculum size	122
4.4.2	Culture activity and biochemical performance of bulk freeze-dried cultures of dahi and yoghurt in cow and buffalo milk	124
4.4.3	Analysis of dahi and yoghurt	126
4.4.3.1	Lactic count and biochemical quality	126
4.4.3.2	Viability of *Bif. bifidum* and *Lb. acidophilus*	133
4.4.3.3	Sensory evaluation of dahi and yoghurt	135
4.5	Evaluation of nutritional and therapeutic values of dahi and yoghurt	137
4.5.1	Effect on body weight gain of male albino rats	137
4.5.2	Effect on serum cholesterol level of male albino rats	140
4.5.3	Evaluation of antibacterial activity	143
5.0	SUMMARY AND CONCLUSION	146-150
	BIBLIOGRAPHY	i-xxx
	APPENDICES	I-XI

LIST OF TABLES

Table No.	Title	Page No
Table-1	Changes produced in titratable acidity, proteolytic activity, Voges-proskauer reaction and body and texture of buffalo skim milk curd, inoculated with various dahi culture combinations added @ 1% after 16 hrs of incubation at 30°C.	64
Table-2	Changes produced in titratable acidity, proteolytic activity and body and texture of buffalo skim milk curd, inoculated with yoghurt cultures and combinations of acidophilus and bifidobacteria @ 1% after 16 hrs of incubation at 37°C.	65
Table-3	Viable cell counts of selected cultures of dahi and yoghurt in buffalo skim milk at different periods of incubation.	68
Table-4	Titratable acidity of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation.	72
Table-5	Volatile acidity of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation.	74
Table-6	Diacetyl/acetaldehyde content of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation.	77
Table-7	Tyrosine content of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation	80
Table-8	Suitability of various formulated media for the growth of dahi cultures.	83
Table-9	Suitability of various formulated media for the growth of yoghurt cultures.	84
Table-10	Viable cell counts and titratable acidity of dahi cultures in certain formulated media at different periods of incubation.	86
Table-11	Viable cell counts and titratable acidity of yoghurt cultures in certain formulated media at different periods of incubation.	87

Table-12	Influence of pH of the selected growth medium (skim milk + yeast autolysate + glucose) on viable cell count and titratable acidity of dahi cultures at different periods of incubation.	90
Table-13	Influence of pH of the selected growth medium (skim milk + tryptone + glucose) on viable cell count and titratable acidity of yoghurt cultures at different periods of incubation at 37°C.	91
Table-14	Influence of neutralization of selected growth media to maintain initial pH on viable cell count of cultures of dahi and yoghurt at different periods of incubation.	93
Table-15	Influence of incubation temperature on viable cell count and % titratable acidity of dahi cultures in selected growth medium (skim milk + yeast autolysate + glucose) at different periods of incubation.	95
Table-16	Influence of incubation temperature on viable cell count and % titratable acidity of yoghurt cultures in selected growth medium (skim milk + tryptone + glucose) at different periods of incubation.	96
Table-17	Influence of inoculum level of dahi cultures on viable cell count and % titratable acidity in selected growth medium (skim milk + yeast autolysate + glucose) at different periods of incubation.	98
Table-18	Influence of inoculum level of yoghurt cultures on viable cell count and % titratable acidity in selected growth medium (skim milk + tryptone + glucose) at different periods of incubation at 37°C.	99
Table-19	Influence of formulated cryoprotective agents on viable cell count and biochemical performance of bulk freeze-dried dahi cultures grown in selected growth medium (skim milk + yeast autolysate + glucose) under standardized growth conditions.	101
Table-20	Influence of formulated cryoprotective agents on viable cell count and biochemical performance of bulk freeze-dried yoghurt cultures grown in selected growth medium (skim milk + tryptone + glucose) under standardized growth conditions.	102
Table-21	Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried dahi culture D during storage.	105

Table-22	Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried dahi culture D during storage.	106
Table-23	Influence of sealing atmosphere and temperature on diacetyl content and proteolytic activity of bulk freeze-dried dahi culture D during storage.	107
Table-24	Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried dahi culture DAB during storage.	108
Table-25	Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried dahi culture DAB during storage.	109
Table-26	Influence of sealing atmosphere and temperature on diacetyl content and proteolytic activity of bulk freeze-dried dahi culture DAB during storage.	110
Table-27	Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried yoghurt culture Y during storage.	112
Table-28	Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried yoghurt culture Y during storage.	113
Table-29	Influence of sealing atmosphere and temperature on acetal dehyde content and proteolytic activity of bulk freeze-dried yoghurt culture Y during storage.	114
Table-30	Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried yoghurt culture YAB during storage.	116
Table-31	Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried yoghurt culture YAB during storage.	117
Table-32	Influence of sealing atmosphere and temperature on acetaldehyde content and proteolytic activity of bulk freeze-dried yoghurt culture YAB during storage.	118
Table-33	Influence of inoculum size of bulk freeze-dried dahi and yoghurt cultures on titratable acidity and body and texture of standardized buffalo milk (4.0% fat) curd.	123

Table-34	Culture activity and biochemical performance of 0.01% (w/v) bulk freeze-dried dahi culture D in skimmed and standardized milk (4.0% fat) of cow and buffalo after 14 hrs incubation at 30°C.	125
Table-35	Culture activity and biochemical performance of 0.01% (w/v) bulk freeze-dried dahi culture DAB in skimmed and standardized milk (4.0% fat) of cow and buffalo after 14 hrs of incubation at 37°C.	127
Table-36	Culture activity and biochemical performance of 0.01% (w/v) bulk freeze-dried yoghurt culture Y in skimmed and standardized milk (4.0% fat) of cow and buffalo after 12 hrs incubation at 37°C.	128
Table-37	Culture activity and biochemical performance of 0.01% (w/v) bulk freeze-dried yoghurt culture YAB in skimmed and standardized milk (4.0% fat) of cow and buffalo after 12 hrs incubation at 37°C.	129
Table-38	Comparison of lactic count and biochemical properties of dahi prepared with liquid and bulk freeze-dried dahi cultures using standardized (4.0% fat) buffalo milk.	131
Table-39	Comparison of lactic count and biochemical properties of yoghurt prepared with liquid and bulk freeze-dried yoghurt cultures using standardized (4.0% fat) buffalo milk at 37°C.	132
Table-40	Viable cell counts of *Bif. bifidum* I and *Lb. acidophilus* R in dahi and yoghurt prepared with liquid and bulk freeze-dried cultures of dahi (DAB) and yoghurt (YAB).	134
Table-41	Sensory evaluation of dahi and yoghurt prepared with liquid and bulk freeze-dried cultures of dahi and yoghurt.	136
Table-42	Chemical analysis of various products fed to male albino rats.	138
Table-43	Effect of feeding dahi and yoghurt prepared with bulk freeze-dried cultures of dahi and yoghurt on serum cholesterol level of male albino rats.	142
Table-44	Antibacterial activity of dahi and yoghurt prepared with bulk freeze-dried cultures of dahi and yoghurt.	144

LIST OF FIGURES

Figure No.	Title	Page No.
Fig.1	Standard curve for the estimation of diacetyl concentration	39
Fig.2	Standard curve for the estimation of acetaldehyde concentration	41
Fig.3	Standard curve for the estimation of tyrosine concentration	42
Fig.4	Standard curve for the estimation of cholesterol concentration	45
Fig.5	Viable cell counts of dahi cultures D and DAB in buffalo skim milk	69
Fig.6	Viable cell counts of yoghurt cultures Y and YAB in buffalo skim milk	70
Fig.7	Titratable acidity production by dahi and yoghurt cultures in buffalo skim milk	73
Fig.8	Volatile acidity production by dahi and yoghurt cultures in buffalo skim milk	75
Fig.9	Diacetyl production by dahi cultures D and DAB in buffalo skim milk	78
Fig.10	Acetaldehyde production by yoghurt cultures Y and YAB in buffalo skim milk	79
Fig.11	Proteolytic activity of dahi and yoghurt cultures in buffalo skim milk	81
Fig.12	Effect of dahi and yoghurt prepared by using different culture combinations on mean body weight gain of albino rats	139
Fig.13	Effect of dahi and yoghurt prepared by using different culture combinations on serum cholesterol level of albino rats	141

LIST OF PLATES

1. Microscopic examination of *Bif. bifidum*

2. Working with Edwards Vacuum Freeze-drier (Model 30.P.2T)

3. Laminated aluminium foil pouches packed with bulk freeze-dried cultures of dahi and yoghurt

4. Microscopic examination of dahi prepared with bulk freeze-dried dahi culture DAB

5. Microscopic examination of yoghurt prepared with yoghurt culture YAB

6. Antibacterial activity of dahi and yoghurt samples prepared with bulk freeze-dried cultures against *Salmonella typhimurium* A KIGM

INTRODUCTION

1. INTRODUCTION

The commercialisation of fermented dairy products has not yet been possible in the country due to the non-availability of Direct-Vat-Inoculation starter cultures. The present preparation of cultures in the factories is time consuming and laborious. The bulk freeze-dried starters provide, in terms of convenience, reliability and the ideal inoculum for direct-vat-inoculation. The production of an active, contaminant-free bulk starter is the cornerstone for the successful manufacture of fermented dairy products. Once these are made available to industry there will be vast scope to set up industries for fermented dairy products manufacture. Popularity of the fermented dairy prdoucts among the masses will soon follow.

It is widely accepted that using selected strains of desirable lactic acid bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* along with conventional starters, is essential for good health. These are capable of controlling the intestinal disorders, reduce the carcinogenic activity and help in controlling serum cholesterol level (Kim, 1988; Gilliland, 1989; Laroia and Martin, 1990). According to Tamura (1983) bifidobacteria produce vitamins B_1, B_6, B_{12}, folic acid and also digestive enzymes (human casein phosphatase and lysozyme). These strains also help to maintain a proper balance of the resident flora. Japan and some European countries have already developed fermented dairy products having these cultures (Puhan, 1990). There is a greater need to incorporate such cultures to the conventional starters to enrich the products both from nutrition and therapeutic points of view.

There is a good scope for the manufacture of bulk freeze-dried mixed strains starter cultures which could be made available to industry for the manufacture of high quality dahi and yoghurt.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Starter Cultures of Dahi and Yoghurt

Laxminarayana *et al.* (1952) reported the presence of *Streptococcus lactis*, *Str. thermophilus*, *Str. faecalis*, *Str. dextranicum*, *Lactobacillus bulgaricus*, *Lb. casei* and *Lb. brevis* in market and home made samples of dahi. It has been found that 78.9% of dahi flora was of streptococci and 17.5% was of lactobacilli (Sheikh *et al.*, 1970). Indian standard (ISI 1973) specifies two types of dahi starter, viz., (i) mesophilic starters, *Str. lactis* ssp. *lactis*, *Str. lactis* ssp. *lactis* biovar. *diacetylactis* and *Str. lactis* ssp. *cremoris* either single or in combination with or without *Leuconostoc* spp. for sweet dahi preparation and (ii) thermophilic starters like *Lb. bulgaricus*, *Lb. casei*, *Lb. acidophilus* and *Str. salivarius* ssp. *thermophilus* along with the above mentioned mesophilic starters for sour dahi preparation. The ratio of the individual cultures in the mixed lactic cultures, however, was not mentioned in the standard. Baisya and Bose (1974) and Dave *et. al.* (1992) prepared dahi using *Str. thermophilus*. The use of this culture and *Str. lactis* ssp. *lactis* biovar. *diacetylactis* in 1:1 ratio was advocated by Baisya and Bose (1975) and Garg and Jain (1980). Use of mutant strain of *Str. lactis* ssp. *lactis* biovar. *diacetylactis* to improve the quality of dahi was reported by Bhatt (1976). There is a report of using *Str. lactis* ssp. *lactis* alone for the manufacture of sweetened dahi (Panday, 1985). LF-40, a mixed culture (consisting of *Lc. lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc* ssp) was also used for dahi preparation (Ghosh and Rajorhia, 1990).

Ashton (1963) pointed out that conventional yoghurt contains equal number of *Lb. bulgaricus* and *Str. thermophilus* strains. Yoghurt preparation using single cultures of either of the above cultures was reported (Marshall and Mabbit, 1980) whereas several

reports indicated the use of both of the above cultures (Rakesh, 1979; Bakshi, 1982; Labropoulos *et al.*, 1982; Lee *et al.*, 1988; Robinson, 1988; Mehanna and Mehanna, 1989; Ratnakar, 1989; Mohammed and Younis, 1990 and Kebary and Kamaly, 1991).

Certain strains of slime producing *Str. thermophilus* and *Lb. bulgaricus* cultures which can improve the consistency, viscosity of the coagulum were also used in yoghurt preparation (Tamime, 1977; Tamime *et al.*, 1984 and Schellaass and Morris, 1985).

2.2 Use of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in Dahi, Yoghurt and Other Fermented Dairy Products

The selected intestinal bacteria, which are capable of growth and acid production in milk, are being used in the manufacture of various fermented milks because of their potentially beneficial role in human health. It is believed that fermented milk products of the third generation which are prepared with selected intestinal cultures have a future and will be in widespread use by 2000 AD (Kurmann, 1986).

The potentially beneficial role of indigenous bifidobacteria and lactobacilli in the human gut have been stressed (Sandine *et al.*, 1972; Speck, 1976 and Goldin and Gorbach, 1984). To enhance the therapeutic values and dietary qualities of the fermented milk products, the use of suitable strains of *Bif. bifidum* and *Lb. acidophilus*, which are having greater potential to synthesize antibiotic substances, vitamins, essential amino acids and to establish the normal flora in the intestines, has been advocated (Kurmann, 1988; Driessen and de Boer, 1989 and Gilliland, 1989).

A variety of fermented milk products containing bifidobacteria and/or acidophilus bacteria along with either mesophilic or thermophilic starters have been developed in the past few years.

The strains of bifidobacteria and acidophilus have been used along with the mesophilic lactic starters to prepare the progurt and acidophilus butter milk products (Kurmann and Rasic, 1988). Recently, an example of fermented milk drink, fermented by lactic mesophilic bacteria (*Lactococcus lactis*, *Lc. cremoris*, *Lc. lactis* ssp. *diacetylactis*) to which a mixed culture of *Lb. acidophilus* and bifidobacteria (in a ratio of 1:3) has been added, was cited by Klupsch (1989).

As early as in 1969, successful attempts were made to replace *Lb. bulgaricus* of yoghurt starter with *Lb. acidophilus* and *Lb. bifidus* (Mulhens and Stamer, 1969). Prior to this, Siegenthaler *et al.* (1960) prepared 'Acoyoghurt' using *Lb. acidophilus* along with yoghurt flora. Gandhi (1979) replaced the *Lb. bulgaricus* of yoghurt with *Lb. acidophilus*. In order to improve the nutritive and therapeutic value of yoghurt, supplementation of *Bif. bifidum* and *Lb. acidophilus* to yoghurt cultures has been tried (Gupta and Tiwari, 1985 and Tamime and Robinson, 1988).

Recently, Mahdi *et al.* (1990) reported the use of concentrated deep frozen *Bif. bifidum* B6-12, *Lb. acidophilus* along with *Str. thermophilus* in the preparation of *Labneh*. Daly (1991) reviewed about the fermented milks, named 'AB Yoghurt' containing *Lb. acidophilus* and *Bif. bifidum* along with yoghurt cultures, 'Cultura AB' made by using *Lb. acidophilus* and *Bif. bifidum* and Mil Mil having *Bif. bifidum* along with yoghurt cultures.

2.3 Associative Growth of Mixed Starter Cultures of Dahi and Yoghurt

Numerous instances of associative inter-relationships among micro-organisms are found in nature. In particular, lactic acid bacteria have the ability to enter into associative relationships with other microbes. Yet the associative and especially symbiotic relationships of mesophilic LAB in mixed populations

are very little explored and the chemical nature of associative action is not known. Likewise the reports dealing with the effect of associative growth between dahi and yoghurt cultures with bifidobacteria and acidophilus are very scanty.

Czulak and Hammond (1954) found increased starter activity of *Str. lactis* ssp. *lactis*, *Str. lactis* ssp. *cremoris* and *Str. lactis* ssp. *lactis* biovar. *diacetylactis* when grown together. Domination of *Str. lactis* ssp. *lactis* biovar. *diacetylactis* cells with five subcultures in mixed cultures was observed (Henning *et al.*, 1964 and Vedamuthu *et al.*, 1966). Mixed cultures of *Str. lactis* and *Str. lactis* ssp. *cremoris* or *Lb. delbrueckii* ssp. *bulgaricus* and *Str. salivarius* ssp. *thermophilus* were better in bacterial counts than each culture of these strains (Kim, 1990). *Lb bulgaricus* is able to produce large quantities of amino acids (histidine, glycine, valine) that stimulated the growth and acid production of *Str. thermophilus* (Pette and Lolkema, 1950a and Shankar and Davies, 1977). On the other hand, Galesloot *et al.* (1968), Veringa *et al.* (1968), Pulusani *et al.* (1979) and Pulusani and Rao (1984) noted that *Str. thermophilus* stimulated the growth of *Lb. bulgaricus* by producing formic acid.

2.3.1 *Effect on Titratable Acidity*

Dahiya and Speck (1966) reported that *Str. lactis* and strains of *Lactobacillus* isolate produced acid at a faster rate in a mixed culture than when either strain was grown individually. Mixed cultures of *Str. lactis* ssp. *lactis* and *Str. lactis* ssp. *cremoris* are better in acid development than single cultures (Kim, 1990). Maximum acid development was noted in case of mixed mesophilic cultures *Str. lactis* ssp. *cremoris*, *Str. lactis* ssp. *lactis* and *Str. lactis* ssp. *lactis* biovar. *diacetylactis* than when these cultures were grown individually in buffalo skim milk (Sunita, 1986). Stimulation of acid production by paired cultures of *Str. lactis* ssp. *lactis* and *Lb. acidophilus* was also documented (Kothari, 1970)

Relatively high acid production by mixed cultures of *Str. thermophilus* and *Lb. bulgaricus* due to increased growth of streptococci was put forward (Moon and Reinbold, 1976). Several reports indicating more acid production by mixed yoghurt cultures are available (Accolas *et al.*, 1977; Khanna and Singh, 1979; Suzuki *et al.*, 1980; EL-Soda *et al.*, 1986; Lee *et al.*, 1988; Robinson and Tamime, 1989; Ghosh and Rajorhia, 1990; Kim, 1990 and Rajagopal and Sandine, 1990). Accolas *et al.* (1977). estimated 0.36-0.72% and 0.31-0.63% lactic acid (LA) production by *Str. thermophilus* and *Lb. bulgaricus*, respectively, whereas the acidity developed by the mixed culture was 0.7-1.1% LA after 6 hrs of incubation at 42°C. Rajagopal and Sandine (1990) had obtained a pH of 6.05 and 5.89 with *Str. salivarius* ssp. *thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus* respectively and still lower pH (4.21) was recorded by mixed cultures.

No significant difference in the acid development by yoghurt cultures alone and in association with *Lb. acidophilus* after 12 hrs of incubation at 37°C in skim milk was observed by Sharma and Singh (1982) whereas Yu and Nakanishi (1975) reported decrease in acidity from 2.75% to 1.62% LA with mixed yoghurt cultures when grown in association with *Lb. acidophilus* for 72 hrs at 37°C. Incorporation of *Bifidobacterium* into yoghurt starter had resulted in slight reduction of acidity (Kisza *et al.*, 1978). Acid production by mixed cultures of *Bif. breue* or *Bif. infantis* with *Lb. casei* or *Lb. acidophilus* grown in skim milk was greater than the sum of acidity developed by these cultures, when grown individually (Cheng and Nagasawa, 1983).

2.3.2 *Effect on Volatile Acidity*

Hammer and Bailey (1919) found that butter cultures contained organisms which commonly did not curdle milk but which in combination with *Str. lactis* ssp. *lactis* gave high volatile acidity. Similarly, Boekhout and Ott de Vries (1920) found that symbiosis of the two types of butter culture organisms not only yielded flavour but also considerable volatile fatty acid which

was acetic. The quantity of the acid was considerably greater than with cultures of single species.

An increased volatile acidity development (3.6 ml of 0.1N NaOH/50g curd) by mixed cultures of yoghurt compared to that of the single cultures (0.6 ml by *Str. thermophilus* and 2.0ml by *Lb. bulgaricus*) was found (Singh *et al.*, 1982). Contrary to this, however, no significant difference in the volatile acidity in mixed cultures of either yoghurt or yoghurt cultures added with *Lb. acidophilus* was reported (Yu and Nakanishi, 1975 and Sharma and Singh, 1982).

2.3.3 *Effect on Flavour*

The flavour components of major importance in fermented dairy products are diacetyl and acetaldehyde. Diacetyl in dairy products is formed by the fermentation of milk citrate by aroma bacteria and the characteristic butter like flavour is attributed to this compound (Marshall, 1987).

In mixed species mesophilic starter the balance between acid producers and citrate fermenters influences the accummulation of diacetyl and acetoin during the growth period (Jonsson and Pettersson, 1977) and higher levels of diacetyl are produced at 22°C than at 30°C (Pack *et al.*, 1968). The strains of *Str. lactis* ssp. *lactis*, *Str. lactis* ssp. *cremoris*, *Str. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc citrovorum* have produced diacetyl in the range of 0.1 to 3.24 ppm whereas the mixed cultures of these species have produced as high as 5.45 ppm diacetyl (Lindsay and Day, 1965).

Slightly less diacetyl development (3.1 to 3.4 ppm) by commercial freeze-dried cultures of mixed mesophilic cultures (*Str. lactis* ssp. *lactis*, *Str. lactis* ssp. *cremoris* and *Str. lactis* ssp. *lactis* biovar. *diacetylactis*) was observed when compared with diacetyl production by *Str. lactis* ssp. *lactis* biovar. *diacetylactis* (3.7 ppm). Nevertheless, this value is

relatively very high compared to that produced (0.3 ppm) by the combined culture of *Str. lactis* ssp. *lactis* and *Str. lactis* ssp. cremoris in cultured milk (Kneifel *et al.*, 1992a).

The amount of diacetyl + acetyl methyl carbinol (AMC) produced by *Str. salivarius* ssp. *thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus* was 5.6 and 3.0 mcg/g curd, respectively. However, only 2.3 mcg diacetyl + AMC per gram curd was developed by mixed culture of these two species during *misti dahi* preparation (Ghosh and Rajorhia, 1990).

Pette and Lolkema (1950b) showed acetaldehyde as the chief flavouring compound in yoghurt. Acetaldehyde production was greatly reduced by single strains of *Str. thermophilus* or *Lb. bulgaricus* when compared to that produced by combined cultures (Hamden *et al.*, 1971; Khanna and Singh, 1979; Singh *et al.*, 1982 and El-Soda *et al.*, 1986). According to Hamdan *et al.* (1971) *Str. thermophilus* 7233 and *Lb. bulgaricus* 9311 produced 3 and 9 ppm of acetaldehyde respectively whereas the mixed cultures of these starters (1:1 ratio) produced as high as 21 ppm of acetaldehyde. Similarly El-Soda *et al.* (1986) obtained 58.8 mcg and 20.25 mcg from *Lb. bulgaricus* and *Str. thermophilus*, respectively.

Sharma and Singh (1982) had shown no appreciable difference in the acetaldehyde content with the yoghurt starters added with *Lb. acidophilus*. The combination of two local yoghurt isolates (*Str. thermophilus* and *Lb. bulgaricus*) with *Bif. bifidum* and *Lb. acidophilus* yielded higher acetaldehyde than the combination of both the yoghurt cultures (Gupta and Tiwari, 1988). Incubation with mixed starter culture (*Bifidobacterium* along with yoghurt cultures) stimulated the production of acetaldehyde and diacetyl (Yuguchi *et al.*, 1989). This is contradicting the earlier observation of Kisza *et al.* (1978) who reported production of less acetaldehyde and diacetyl by mixed yoghurt culture along with *Bifidobacterium* than by the mixed yoghurt culture alone.

2.3.4 *Effect on Proteolytic Activity*

Lactic acid bacteria are nutritionally fastidious organisms needing an exogenous supply of pre-formed amino acids to initiate growth. Proteolytic activity of the lactic streptococci is subtle and manifestations of these proteolysis is relatively obscure. Presumably, the proteinase system of these micro-organisms is needed to obtain certain nitrogenous constitutents from milk proteins. The ability of lactic cultures to grow properly in milk may, therefore, be directly related to their proteinase activity. LAB have varying degrees of proteolytic activity which is generally strain rather than species specific. Searles *et al.* (1970), Khanna and Singh (1979), El-Soda *et al.* (1986) and Rajagopal and Sandine (1990) observed that lactobacilli possessed greater proteolytic activity than streptococci. The range of tyrosine produced per ml of curd was 25-144.6 mcg by lactobacilli and 2.4-34 mcg by streptococci. Contrary to this, Kneifel *et al.* (1992b) stated that the proteolytic activities of the mesophilic cultures are comparable to those of yoghurt and yoghurt related products.

A reduction in the proteolytic activity of combined culture, compared to its component strains has been reported (Mattick and Hirsch, 1949 and Barridge *et al.*, 1952). Similarly, decrease in the proteolysis by mixed culture (*Lc. lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris* and *Lc. lactis* ssp. *lactis* biovar. *diacetylactis*) compared to that exhibited by one of the component culture *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* was observed by Sunita (1986) and Kneifel *et al.* (1992a).

There can be an increase in the proteolytic activity with the increase in incubation period in yoghurt made from cow/goat milk (Rasic and Kurmann, 1978). El-Soda *et al.* (1986) and Rajagopal and Sandine (1990) have observed increased proteolytic activity by mixed cultures of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. salivarius* ssp. *thermophilus* (34-70 mcg tyrosine/ml) whereas

the individual cultures gave 25-49 mcg and 18-34 mcg tyrosine, respectively.

Addition of *Lb. acidophilus* R to the yoghurt culture reduced the proteolytic activity (0.26 mg tyrosine/g curd) when compared to the proteolytic activity of mixed yoghurt culture (0.32 mg tyrosine/g curd) when grown in skim milk for 18 hrs at 37°C (Sharma, 1982). The rate of proteolysis was comparatively less in acidophilus yoghurt than that of plain yoghurt (Sharma and Prasad, 1986).

Lack of information with regard to the associative growth pattern between therapeutic cultures and that of dahi and yoghurt emphasizing the need to study on this aspect to gather necessary information on the quality of dahi and yoghurt when therapeutic cultures are incorporated in the conventional cultures of respective products.

2.4 Preparation of Bulk Freeze-dried Starter Cultures of Dahi and Yoghurt

In preparing culture concentrate, it is desirable to obtain large number of cells possible that will actively grow and produce the desired metabolic products when placed in milk. A number of factors such as growth medium, growth conditions (pH, temperature and period of incubation) method of harvesting etc. may affect the quality of the starter.

2.4.1 *Growth Media*

The choice of a growth medium is guided by costs and efficiency in cell production. The medium must be adequate in maintaining the balance among the strains in mixed starter cultures. Loss of viability can occur unless appropriate medium is used for growth (Champagne *et al.*, 1991b). Thus, the development of suitable growth medium has received much attention.

Various types of growth media mainly based on milk and/or whey have been used for the production of dairy starter cultures (Stadhouders *et al.*, 1969; Richardson, 1978; Peterkova, 1980; Ram, 1984; Kegal and Wallace, 1989; Rajagopal *et al.*, 1990; Champagne *et al.*, 1991a and Gandhi and Shahani, 1994).

The utilization of steamed skim milk or skim milk supplemented with 1% yeast extract (Stadhouders *et al.*, 1969) or skim milk with 0.1% yeast extract (Ram, 1984) has been reported. Sterilized reconstituted skim milk (RSM) with 9.1% solids was used to produce mixed species lactic starters by Bettersson (1975).

Kornacki *et al.* (1974) obtained good growth of lactic streptococci (upto 10×10^{11} ml) using papain digested whey medium containing yeast extract (0.5%) along with 0.012% each of $MnSO_4$, $MgSO_4$ and $ZnSO_4$ and suggested that addition of glucose (0.5%) or crude β-D- galactosidase will reduce the duration of the multiplication phase.

Porubcan and Sellars (1975) patented a milk-based medium containing ascorbic acid, monosodium glutamate (MSG) aspartate and cryoprotective agents inositol, sorbitol, mannitol, glucose, sucrose etc., for the production of freeze-dried starter cultures. According to Jonas *et al.* (1977) pH controlled phage inhibitory whey based medium is most economical for the production of bulk lactic cultures.

A whey-based medium containing 10-20% whey, 0.5-2.0% maize extract, 1.2% sodium citrate, 0.016-0.164% $MnSO_4$ was patented (Lagoda *et al.*, 1978). Lagoda *et al.* (1981) proposed a whey medium with 1% skim milk, 0.008% pepsin with which they obtained 32-56% increased biomass of mesophilic lactic streptococci.

Priidak *et al.* (1982) found increased yield of lactic streptococci biomass from 3.5 to 5.2 kg/1000 litres when 0.5-1.0%

of acid hydrolysed casein is added to the base medium. Wright and Richardson (1982) reported 36% of increase in cell mass and 38% of activity increase of bulk lactic cultures using a phosphated whey medium having 5.2% whey solids, 0.71% yeast autolysate and 0.43% casein hydrolysate.

Enrichment of 8% reconstituted whey medium with 1% tryptone and 0.5% yeast extract increased the activity of the BD type cheese culture by forty times (Toyoda *et al.*, 1987). Christopherson and Zottola (1989) had obtained higher cell number and increased developed acidity (0.88%) with *Lc. lactis* ssp. *lactis* C2, *Lc. lactis* ssp. *cremoris* K4 and *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC3 in ultrafiltered skim milk than with cultures grown in RSM (TA 0.72%).

Willrett and Comotto (1989) got a patent for a medium with 45-50% non-fermentable carbohydrate, 20-35% milk protein, 2-10% growth stimulant, 5% phosphate or citrate salts, <2% salt and <1% stabilizer for the production of cheese starters in bulk quantities. Another patented medium contains 38.45% dried whey, 16.4% each of ammonium and magnesium phosphates, sodium citrate, 5.6% yeast extract and 6.7% xanthan gum (Kegal and Wallace, 1989).

A medium for the production of bacterial concentrate of yoghurt symbiont culture, having a composition of 2.5% spray dried skim milk, 2.5% spray-dried sweet whey, 0.5% yeast extract, 0.5% lactose, 0.25% sucrose, 5.85 mg calcium chloride, 0.12 mg magnesium sulphate per litre, 0.2% dipotassium hydrogen phosphate and 0.002% bacterial proteinase was developed (Kapoor, 1976). The cultures grown in this medium had longer shelf life. Kondratenko and Kondareva (1978) developed a simple medium consisting of 500 ml of skim milk, 500 ml of water, 3% of yeast extract and 2% of sodium acetate, suitable for the production of bacterial concentrates of *Str. thermophilus* and *Lb. bulgaricus*. Champagne *et al.* (1991a) revealed that papain hydrolyzed whey proved better growth medium than untreated whey and enabled increased biomass

production of *Lb. delbrueckii* ssp. *bulgaricus*.

A formulation of whey medium supplemented with maize extract, vitamin B_{12} complex, trypsin, buffering salts and growth promoting substances which is useful for the production of *Lb. acidophilus* cell biomass was developed (Lagoda and Bannikova, 1978). Pyatkova and Lukonikova (1979) noticed stimulatory effect of RSM with 18-32% total solids on *Lb. acidophilus*. Gandhi and Shahani (1994) obtained higher number of *Lb. acidophilus* cells in case of cheese whey supplemented with 0.5% yeast extract as compared to MRS medium at all the stages of preparation of freeze-dried acidophilus powder.

Wright and Klaenhammer (1981) suggested that media supplemented with phosphates, may not be appropriate for the preparation of *Lb. acidophilus* cultures aimed for freeze-drying due to the morphological changes.

Bily (1987) claimed increased acid production by *Lb. acidophilus* in skim milk medium containing 4.5% lecithin. Marshall *et al.* (1982) have successfully grown *Bif. bifidum* in 2x ultrafiltered skim milk and 5x cheddar cheese whey. The addition of cysteine (0.05%) plus pyruvic acid (0.05%) and ascorbic acid (0.2%) to 12% RSM permitted the growth of *Bif. bifidum* (Collins and Hall, 1984). Misra and Kuila (1991) obtained optimal growth (7.51 log Cfu/g) of *Bif. bifidum* isolates, after incubating at 37°C for 48 hrs in cow milk digested with 20% pepsin.

2.4.2 *Growth Conditions*

2.4.2.1 Effect of pH

The populations of several lactic acid bacteria can be increased by controlling the pH of the growth medium at a favourable level. Freeze-drying of acid cultures is detrimental to survival, hence, it is necessary to control the pH at two

levels, i.e., during cell growth and in the suspension medium (Champagne *et al.*, 1991b).

In producing culture concentrates, it is economically important to obtain the largest yield of cells possible that possess maximum biological activity. The population of lactic streptococci can be greatly increased by controlling the pH of the growth medium at a favourable level (Berger, 1968; Cogan *et al.*, 1971 and Lloyed and Pont, 1973).

Growth of *Str. lactis* ssp. *cremoris* under pH control (6.0) not only resulted in higher biomass yields but also increased survival during freezing (Peebles *et al.*, 1969). Maintaining of pH 6.5 was suggested for mass production of mesophilic streptococci (Speckman, 1975). The cells of *Str. salivarius* ssp *thermophilus* had lower viability losses when grown at pH 6.0 rather than at pH 6.5 and the same was generally found to be the case with *Lb. delbrueckii* ssp. *bulgaricus* (Bozoglu *et al.*, 1987).

2.4.2.2 Effect of neutralizer

The type of neutralizer used for pH control influences the maximum obtainable population of the dairy cultures in the media (Peebles *et al.*, 1969; Lloyed and Pont, 1973 and Mitchell and Gilliland, 1983). When a conventional mixed lactococci culture (with pH 4.8) was neutralized, the survival to freeze-drying was higher when $Ca(OH)_2$ rather than NaOH, was used for neutralization (Stadhouders *et al.*, 1969).

Fernandes *et al.* (1986) observed marked increase in the growth of *Lb. acidophilus* in MRS broth, when neutralized with $Ca(OH)_2$. Gilliland *et al.*(1970) found no significant difference either with NaOH or NH_4OH whereas Peebles *et al.* (1969), Blaine (1972), Mitchell and Gilliland (1983)obtained relatively higher yields with NH_4OH and Na_2CO_3 than with NaOH, KOH or $Ca(OH)_2$.

Several other publications showed two-fold increase in the cell population in the medium neutralized with NH_4OH rather than

with NaOH (Lloyed and Pont, 1973; Efstathiou *et al.*, 1975 and Ram, 1984). Recently Hall and Antonvcci (1988) observed five fold increase in the activity of cheese starters when grown in skim milk medium neutralized by NH_4OH.

2.4.2.3 Effect of temperature

The temperature chosen for biomass production is a function of the optimum growth temperature of a specific strain and the temperature at which it will be used at the dairy plant (Gilliland, 1976).

Pont and Halloway (1968) found maximum growth rate and population density with an optimum temperature of 30°C for *Str. lactis* ssp. *lactis* C10. Contrary to this, Efstathiou *et al.* (1975) reported that incubation temperature of 25°C was favourable not only for obtaining maximum population but also for proper species balance between *Str. lactis* ssp. *cremoris* and *Str. lactis* ssp. *lactis* biovar. *diacetylactis*. Similarly Pettersson (1975) also suggested the incubation at 25°C for optimal yield of mixed species starter consisting of *Str. cremoris Str. lactis* and *Str. diacetylactis*.

Although the activity of mixed strain culture is optimum at 45°C, it is recommended that in order to maintain and/or achieve a ratio of 1:1 between *Str. thermophilus* and *Lb. bulgaricus*, propagation of these organisms together at 42°C was advocated (Kurmann, 1967 and Tamime, 1977).

2.4.3 *Rate of Inoculum*

Ayunts *et al.* (1979) studied the effect of age and amount of inoculum and duration of cultivation on yield of biomass of lactic acid bacteria. Maximum yield of bacterial concentration was obtained using 1% inoculum and incubating for 14-16 hrs.

Belova *et al.* (1977) used 3-4% inoculum for bacterial population of *Str. lactis*, *Str. lactis* biovar. *diacetylactis* and *Str. paracitrovorus*.

2.4.4 *Harvesting of Cell Biomass*

In order to obtain an acceptable number of cells, they must be harvested from the growth medium and concentrated in a suspending medium suitable for freeze-drying.

The cells of *Lb. helveticus* and *Str. thermophilus* (Gehriger, 1968) and lactic streptococci (Shurda, 1980) harvested from late logarithmic or early stationary phase were found to give greatest viability after freeze-drying. Similar view was expressed by Woodward (1980).

The initial bacterial load affects the survival rate. Kilara *et al.* (1976) observed higher survival levels of *Str. lactis* ssp. *lactis*, *Str. thermophilus*, and *Lb. bulgaricus*, when the highest initial cell densities were freeze-dried. Bozoglu *et al.* (1987) showed that the protective effect of high initial bacterial load is a function of the interactions between micro-organisms through decreasing the exposed area of each cell to the environment and therefore, preventing the possible damage. They further suggested that an initial biomass that would be too concentrated (10^{12} cells/ml) would, however, be harmfull because of an imbalanced osmotic pressure,

Stadhouders *et al.* (1969) established a procedure to harvest cells of *Str. lactis* C10 from skim milk, by neutralizing with 33% NaOH and addition of 1% sodium citrate prior to centrifugation.

The survival rate of the preserved starter culture depends on the method of harvesting. Continuous neutralization of the growth medium at around pH 5.8-6.0 to produce a culture with high cell culture concentrate has been reported (Barach and Kamara, 1986). Laboratory centrifuges enable 99% of *Str. lactis* ssp.

lactis C10 to be concentrated in 5% of the original volume (Stadhouders *et al.*, 1969).

Sharples and Cepa centrifuges are adequate for collecting cells but require a discontinuous operation in order to recuperate the cell deposits. Stadhouders *et al.* (1969) observed that use of sharples centrifuge for the collection of cell biomass was inferior because it gave intermittent harvesting and removal of bacterial sludge was difficult. Contrarily, Blaine (1972) reported 99.9% recovery of cells from the medium using sharples centrifuge.

The stress caused by centrifugation increases sensitivity to freezing and drying due to physical damage (Tamime, 1990). Osborne (1977) developed a diffusion culture technique to remove lactate and achieved 10^{11} cells/ml in a concentrated culture.

In recent times, ultrafiltration is being used to concentrate starter cultures (Boyaval *et al.*, 1987; Ferras *et al.*, 1986 and Prigent *et al.*, 1988). Champagne *et al.* (1991b) observed breaking of the *Str. lactis* ssp. *cremoris* chains to form diplococci following ultrafiltration resulting in an increase of cell count.

2.4.5 *Influence of Cryoprotective Agents*

A good suspending medium should provide cryoprotection to the cells during freezing, get easily dried and provide a good matrix to allow stability and ease of rehydration. It was shown that dairy starters could be freeze-dried directly in their growth medium with some success (El-Sadek *et al.*, 1975 and Nagmoush *et al.*, 1978). Better recovery is generally achieved when cryoprotective agents are used.

Skim milk was used to protect LAB during freeze-drying because it is easy to dry (Sinha *et al.*, 1974; El-Sadek *et al.*, 1975 and Kilara *et al.*, 1976). Since drying damages cell wall

proteins and makes them more easily extractable, it was thought that proteins contained in milk provide a protective coat for the cells.

Stadhouders *et al.* (1969) and Pettersson (1975) found that 7% lactose was a useful cryoprotectant for lactic acid bacteria whereas Kilara *et al.*(1976) and Nikolova (1978) achieved 90-100% of survival with yoghurt cultures when 10% lactose solution used as a cryoprotective agent. Sucrose, which inhibit free radical formation was used successfully to get higher survival rate (Nikolova, 1978; Grudzinskaya, 1982; Lagoda *et al.*, 1982; Sidorova *et al.*, 1983 and Alaeddinoglu *et al.*, 1989).

Damjanovic and Radulovic (1967) found best survival (99%) of *Bif. bifidum* when a suspending medium containing 8% sucrose, 1.5% gelatin in 5% skim milk was used. By using the same suspending medium Radulovic (1971) achieved 90-100% survival of *Lb. acidophilus*.

Many reports on using monosodium glutamate for lactic cultures, to achieve 60-95% survival on freeze-drying are available (Speckman, 1975; Pettersson, 1975; Kilara *et al.*, 1976; Misra, 1978; Garcia Pina *et al.*, 1979; Ozlap and Ozlap, 1978; Lagoda *et al.*, 1982; Font de Valdez *et al.*, 1983a and Kim *et al.*, 1987).

Pectin (Kilara *et al.*, 1976 and Prajapati and Dave, 1994) and dextran (Font de Valdez *et al.*, 1983a) increased the survival of most lactobacilli and lactococci during freeze-drying process and storage. The addition of ascorbic acid, thiourea and ammonium chloride (0.5% each) to skim milk with 10% T.S. was proved better for mesophilic streptococci and for *Str. thermophilus* and *Lb. bifidus* (Sinha *et al.*, 1970, 1972 and 1974). However, ascorbic acid was proved to be toxic to cells (Heckly and Quay, 1983).

Morichi (1972) obtained 40-66% viability of *Str. lactis* ssp. *cremoris*, *Str. lactis* ssp. *lactis*, *Str. lactis* ssp. *lactis* biovar.

diacetylactis, *Lb. bulgaricus* and *Lb. acidophilus* using *L-glutamic acid/L-* arginine/acetylglycine/DL malic acid as cryoprotective agent. Reconstituted skim milk (10% T.M.S.) containing either yeast extract or 0.03% vitamin E was proved to be more effective in preserving *Str. lactis* ssp. *lactis* activity during storage (Nagmoush *et al.*, 1978).

Polyethylene glycol (PEG) was found to accelerate the drying and thereby reduces the drying period from 45 to 30 min to have a residual moisture level of 1.1-1.7% and addition of PEG to milk did increase survival of freeze-drying by 10-20% with lactococci (Font de Valdez *et al.*, 1985). Aspartic acid and cysteine were successfully used as cryoprotectants (Morichi, 1970 and Font de Valdez *et al.*, 1983a).

Misra (1978) found 95.6% survival of *Lb. bulgaricus* with a suspending medium containing 0.5% whey powder and 0.5% malt extract. These cultures have retained better activity following freeze-drying when 0.01% Tween 80 or 1% sheep's serum was added to 10% skim milk prior to freeze-drying (Nagmoush *et al.*, 1978).

More than 80% survival of yoghurt cultures and *Str. lactis* ssp. *cremoris* was obtained with adonitol (Font de Valdez *et al.*, 1983b) and similar view was expressed by Alaeddinoglu *et al.*(1989) who made an attempt of using 1 M adonitol in whey medium or in skim milk.

Very recently, Belova *et al.* (1993) found effective protection of *Str. salivarius* ssp. *thermophilus*, *Lb. helveticus* and *Lb. lactis* with a suspending medium having sucrose, magnesium sulphate, ascorbic acid, sodium acetate, apilac and calcium hydroxide.

Dulcitol, mannitol, minositol and sorbitol were found to provide little or no protection, to lactic acid bacteria when added to milk (Font de Valdez *et al.*, 1983b). Because of its potential human toxicity dimethyl sulfoxide (DMSO) cannot be used

as a suspending medium (Simatos *et al.*,1974). Best results were obtained with glycerol (0.75M) in whey medium (Alaeddinoglu *et al.*, 1989). However, glycerol remains liquid at normal storage temperature (4^{o}C) and was not found suitable for freeze-drying (Simatos *et al.*, 1974). The addition of glycogen was not very effective for lactic cultures (Font de Valdez *et al.*, 1983a). The L-cysteine, DL-threonine gave 40% of survival for both mesophilic and thermophilic lactic cultures (Morichi, 1972). Asparagine was not an effective protectant (Morichi, 1970 and Font de Valdez *et al.*, 1983a). Peptone, yeast extract, protein hydrolysate and gelatin increased survival of most strains when compared to water but are generally less effective than milk or sucrose (Nikolova, 1978). Bovine serum albumin was not a good cryoprotectant than milk (Font de Valdez *et al.*, 1983a).

2.4.6 *Freeze-drying of Bulk Starter Cultures*

The lactic acid bacteria were freeze-dried by Rogers (1914) for the first time. Later on, it came to be widely used in the preservation of lactic cultures. Morichi (1974), Speckman *et al.* (1974) and Pettersson (1975) reported that lactic cultures gave 50% of survival during freeze-drying. Advances in lyophilization have made it possible to produce freeze-dried starter cultures of various lactic cultures to be used for direct vat inoculation (Bannikova and Lagoda, 1970; Kornacki *et al.*, 1977; Lagoda and Bannikova, 1978b and Ram, 1984).

Bulk freeze-dried starter cultures of single species cultures of dahi and yoghurt have been prepared (Dalai, 1983). But the study was not giving the required data. Successful freeze-drying of bifidobacteria has been proclaimed (Obayashi *et al.*, 1961; Damjanovic and Radulovic, 1967; Ervol'der and Gudkov, 1982; Collins and Hall, 1984 and Ishibashi *et al.*, 1985). Freeze-drying of *Lb. acidophilus* has been successfully carried (Kujawa *et al.*, 1987). No reports are available regarding the freeze-drying of mixed cultures of either dahi or yoghurt in combination

with therapeutic cultures. Hence, the present study has a great practical significance for dairy industry.

2.4.7 *Packaging of Bulk Freeze-dried Starter Cultures*

The material for the packaging of cultures has special significance in so far as viability and activity of bacteria in the concentrate are concerned. Moisture as well as the oxygen has been found to be lethal to the bacteria in dried preparation of cultures.

Common practice of dispensing freeze-dried cultures is in glass ampoules. Endeavours have also been made to disburse the freeze-dried cultures in glass vials sealed with rubber stoppers along with nylon or aluminium sachets etc. (Ram, 1984).

Tsvetkov and Nikolova (1974) manifested that survival rate of yoghurt cultures was higher in foil pouches than in nylon pouches. Laminated aluminium foil was proved superior to metalized polyester for packaging of freeze-dried *Lb. bulgaricus* in bulk quantities (Dalai, 1983). Shah *et al.* (1987) advocated the use of polyethylene laminated aluminium foil pouches as a suitable packaging material for dried dairy products containing live lactobacilli.

2.5 Stability of Bulk Freeze-dried Starter Cultures During Storage

A variety of factors, such as temperature and storage atmosphere besides residual moisture and relative humidity, affect the viability of dried lactic cultures during storage period.

2.5.1 *Effect of Storage Temperature*

The storage temperature is the most important aspect which influences the activity and viability of freeze-dried starter

cultures. Working with mesophilic and thermophilic LAB, Ciblis (1966) reported 1-5% survival at 5°C after four years of storage. Blaine (1972) observed more than 80% loss in the activity of lyophilized *Str. lactis* ssp. *lactis* after 7 days of storage at 22°C. No significant loss of viability during 3 months of storage either at -22° or 20°C of dried LAB was shown (Speckman *et al.*, 1974). Freeze-dried concentrates of commercial mixed strain lactococci were active and stable at ambient temperatures (25°C) for several months in vacuum sealed and stored in amber containers filled with argon (Yang and Sandine, 1979).

The increase in the temperature of storage had decreased the survival rate of freeze-dried cells (Dalai, 1983). Ram (1984) revealed that the freeze-dried cheese starter concentrates stored at -20°C were viable upto 4 months. Seventy three percent viability of *Lactobacillus* cultures when stored at -20°C for a period of 2 years was indicated (Barbour and Priest, 1986). Longer shelf life of *Str. lactis* ssp *lactis* C2 at 22°C rather than at 37°C has been established (Sinha, 1989).

2.5.2 *Effect of Sealing Atmosphere*

Survival of dried cells is affected by the atmosphere of storage. Hawrylewiez *et al.* (1962) reported lower survival rates *in vacuo* as compared to nitrogen. Sinha *et al.* (1974) obtained 93.7% survival rate for *Lb. bifidus* with vacuum sealed and 92.1% with air packed freeze-dried cultures, immediately after 2 months storage at 30°C. This observation was contradictory to that reported by Obayashi *et al* (1961).

Bozoglu *et al.* (1987) obtained 5.2 log cfu/g of freeze-dried *Str. thermophilus* cells with nitrogen packaging whereas the count of cells packed with air, after 75 days of storage at 4°C was 4.8 log cfu/g. The useful shelf life of freeze-dried *Str. lactis* ssp. *lactis* C2 based on the survival of 1000 cells per ampoule when packed under vacuum was maximum (112 months) whereas the cells packed under carbondioxide, nitrogen, air and oxygen resulted in

lower expected shelf life which was in the decreasing order from 93 months to 6 months (Sinha, 1989). This is in accordance with the result of Ram (1984) who obtained 30% of viability with vacuum sealed CH-9 cheese starter after 6 months storage at $-20^{o}C$ whereas the same culture, sealed under air gave only 10% of the survival.

2.6 Use of Bulk Freeze-dried Starter Cultures of Dahi and Yoghurt

Although extensive developments in the production of starter concentrates have been made during the last two decades, little information has been published on the actual performance of starter concentrates in the preparation of fermented dairy products. Even though the reports regarding application of bulk freeze-dried starters in the preparation of a variety of cheeses are available publications on the direct inoculation of milk with freeze-dried starters for the production of dahi and yoghurt are very scanty.

Speckman (1975) prepared good quality of yoghurt by direct inoculation of milk with lyophilized *Lb. bulgaricus* and *Str. thermophilus* in equal quantities and achieved a pH of 4.4 to 4.7, as with conventional yoghurt cultures. Under limited laboratory trials consistent results were achieved with the use of bulk freeze-dried dahi and yoghurt cultures (Dalai, 1983). A freeze-dried conventional yoghurt culture named 'Jointec-R' was developed and used for direct-vat inoculation (Rottigni, 1987). 'Yo-Flex'- DVS yoghurt culture, having defined single strains of yoghurt cultures was developed by Chr. Hansen's Laboratory (Spork, 1990). The freeze-dried starter culture having a viable count of 10^{9}-$10^{10}/cm^{3}$ of bifidobacteria for the preparation of bifidus milk under both domestic and industrial conditions are made available (Khamagaeva and Stolyarova, 1993).

2.6.1 *Preparation of Dahi*

The cow or buffalo milk, either whole or skim, was simmered for a long time (Sinha, 1984) or boiled for 10 min (Garg, 1988) cooled to body temperature and transferred to earthenware pots or pans. In households, a small amount of previous day's dahi or butter milk is being added and incubated at room temperature for 16-20 hrs. Ray (1970) studied microbiology and preparation of sweetened dahi or misti dahi prepared with 1-3% of culture consisting of *Str. lactis* ssp. *lactis* or *Str. lactis* ssp. *lactis* biovar. *diacetylactis*. Recently, Ghosh and Rajorhia (1990) published the details of misti dahi preparation. Dahi was also prepared using reconstituted whole and skim milk (Panday, 1985).

2.6.2 *Preparation of Yoghurt*

Yoghurt is an exotic counterpart of dahi. Many reviews on yoghurt preparation have been published (Humphreys and Plunkett, 1969; Robinson and Tamime, 1975; Tamime and Robinson, 1985; Shukla *et al.*, 1987 and Vedamuthu, 1991). The various types of yoghurts differ according to their chemical composition, method of preparation, flavour and nature of post-incubation processing.

The manufacturing process of yoghurt was described by Tamime and Robinson (1985). Milk was standardised to 0.5-3.0% fat and 14-16% total solids. After adding sugar and stabilizers the yoghurt milk was heated to 50-60^{o}C to homogenize at a pressure 100-200 kg/cm^{2}. The homogenized milk was heated to 85^{o}C for 30 min and cooled to incubation temperature, either 42^{o} or 37^{o}C and was inoculated with yoghurt starter culture @ 1-2%. Then it was packed and incubated at 42^{o} or 37^{o}C for 4 or 10 hours, respectively. This fermented product was cooled to <10^{o}C and stored at refrigeration temperature.

2.7 Nutritional and Therapeutic Values of Dahi and Yoghurt

Sages Charaka and Sushruta, the great exponents of Ayurvedic system of medicine in India, have described the virtues of dahi and stated that this increases apetite, vitality, cures diarrhoea, nasal catarh, dysentry, intermittent fever and other diseases (Bishagranta, 1907 and Kaviratna, 1922). The yoghurt, besides its nutritive value (Tomar, 1988) is believed to be effective both in prevention and treatment of various illnesses in man as well as in animals, viz., gastrointestinal disorders, heart diseases and tumour development (Deeth and Tamime, 1981).

Recently many reviews have been published revealing the nutritional and healthful aspects of cultured and culture containing dairy products (Shahani and Chandan, 1979; IDF, [illegible] 1991; Gilliland, 1989; Driessen and de Boer, 1989; Hughes and Hoover, 1991).

2.7.1 *Effect on Body Weight Gain*

Experiments have been performed to examine the effect of feeding cultured dairy products on growth performance of animals.

As early as in 1950 Khambatta performed an experiment to know the effect of dahi on growth rate of rats. In this, the rats were reared on mineralised milk and dahi prepared from raw and boiled milk. The results of total growth and body weight varied widely but in no case did dahi groups show superior growth to milk group. It was also suggested that the lowering in the growth promoting efficiency of dahi was due to the hydrolysis of casein and subsequent metabolization of some of the essential amino acids by the lactic acid bacteria. Similarly, the rats given milk grew slightly faster than those given dahi or acidophilus milk and there was no difference in growth rates of rats given acidophilus milk or dahi (Chawla, 1982).

Greater weight gains and feed efficiency ratio in rats were obtained when fed with yoghurt than that of the rats given unfermented milk (Hargrove and Alford, 1978). Similar observations in rats were made by McDonough *et al.* (1982) Broussalian and Westhoff (1983), Hitchins *et al.* (1983) and Grunewald and Mitchell (1983) and in pigs by Pollman (1984). Grunewald (1985) found more gain in weight of mice fed with acidophilus milk than fed with milk. Similar growth rates were observed in rats fed with drinking water or 10% liquid skim milk or 10% milk fermented with *Lb. acidophilus* along with water. The differing results were attributed to specific strain of *Lb. acidophilus* used, the host animal employed, the number of bacteria fed etc.

McDonough *et al.* (1985) opined that the stimulation of growth with yoghurt feeding might be due to the increasing absorption and utilization of one or more minerals. According to Mantere-Alhonen (1986), the efficiency of probiotics like lactic and propionic acid bacteria, may be due to the occurrence of useful metabolites (lactic and propionic acids), improved feed efficiency, increased resistance to diseases, the elimination of unfavourable drug effects and/or the inhibition of the growth of harmful micro-organisms in the intestinal tracts.

2.7.2 *Effect on Serum Cholesterol Level*

A splendid correlation exists between dietary saturated fat or cholesterol intake and serum cholesterol level. The level of serum cholesterol is a major factor of coronary heart diseases. Mann and Spoerry (1974) observed that inspite of consuming large quantities of saturated fat and cholesterol through fermented milk, the Masai tribesmen of Africa had fairly low level of cholesterol in their blood. They suggested that there exists an anticholesterolemic milk factor for the hypocholestermic effect of cultured milk.

According to Astrup (1977), the serum cholesterol levels in young and middle aged men are reduced within a few weeks by daily consumption of yoghurt. Mann (1977) and Nair and Mann (1977) described that the effective agent in yoghurt may be inhibiting the hydroxymethyl glutaryl CoA reductase, the regulatory enzyme in cholesterol synthesis.

The hypocholesterolemic effect theory of fermented milks was also supported by Hepner *et al.* (1979) who found 5-10% reduction in cholesterol level in human volunteers after supplementation of diet with yoghurt for one week. This report is in contrast to the earlier work published by Gold and Samuel (1965) and Payens *et al.* (1976) who have found no significant difference in serum cholesterol levels of human volunteers fed either yoghurt, milk or dairy product.

Kansal and Chawla (1984) reported depression in the cholesterol level from 58 mg to 39 mg/100 ml plasma in rats fed with dahi for a period of 90 days. The increased serum cholesterol level in rats fed by the supplemented butter oil was lowered down by the addition of yoghurt (Kaul, 1984) or butter milk (Metwally *et al.*, 1988 and Abd-El Gawad *et al.*, 1988).

The experiments of Grunewald (1982), Kansal and Chawla (1984) and Ishida and Kubo (1985) had shown that the fermented milks gave a lower serum cholesterol concentration in rats fed with fermented milk containing *Lb. acidophilus* than did the water-fed or milk-fed rats. In contrast to the aforesaid results, Grunewald (1985) did not observe significant difference in serum cholesterol among mice fed with fermented milk and it was thought that the differing results may be due to (i) the specific strains of *Lb. acidophilus* used (ii) host animal employed (iii)number of bacteria fed (iv) feeding procedure and (v) storage of cultured product.

Gorbach *et al.* (1988) have observed reduction of cholesterol level in 35 healthy volunteers treated for two weeks with a

fermented product containing *Lactobacillus* GG strain. Similarly, significant reduction in serum cholesterol level by feeding acidophilus milk, 200 g/head/day to human volunteers of aged 50-60 years, for a period of 30 days was reported (Khedkar *et al.*, 1993). Prior to this, Danielson *et al.* (1989) found reduction in cholesterol level in boars by *Lb. acidophilus* 16 strain.

Comparatively, limited information is available on this aspect, with regard to bifidobacteria; Homma (1988) reported the lowering of serum cholesterol level in rats fed orally with bifidobacteria. In contrary, in the same year Kim ascribed the hypocholesterolemic effect to lactobacilli but not to bifidobacteria.

2.7.3 *Antibacterial Activity*

The importance of the antibacterial activity of cultured milks and culture containing dairy products in human health has been reviewed (Shahani and Chandan, 1979; Driessen and de Boer, 1989; Fuller, 1989; Gilliland, 1989 and IDF, 1991).

The antibacterial activity in lactic acid bacteria has been ascribed to hydrogen peroxide (Hirsch and Wheater, 1951; Gilliland and Speck, 1969) diacetyl (Jay, 1982) or polypeptides like bacteriocins (Klaenhammer, 1988 and Daeschel, 1989) and antibiotics (Reddy and Ranganathan, 1983; Daeschel, 1989 and Spelhaug and Harlander, 1989).

Marshall (1987) reviewed the antimicrobial compounds produced by lactic cultures, those are lactocidin/acidophilin/acidolin by *Lb. acidophilus*, diplococcin by *Str. lactis* ssp. *cremoris*, nisin by *Str lactis* ssp. *lactis*, bulgarican by *Lb. delbrueckii* spp. *bulgaricus*. Anti-microbial compounds have been isolated from *Str. lactis* ssp. *lactis* biovar. *diacetylactis* by Branen *et al.* (1975) and from *Str. thermophilus* by Pulusani *et al.* (1979). Nisin is effective against numerous Gram positive bacteria (Klaenhammer, 1988) and is being

successfully used in the preservation of dairy and other food products (Gupta and Prasad, 1989). Piard *et al.* (1989) had characterized a bacteriocin called lactocin 481 from *Str. lactis* ssp. *lactis* CNRZ 481. The antibacterial substances bifidin from *Bif. bifidum* and bifilong from *Bif. longum* were purified by Anand *et al.* (1985) and Kang *et al.* (1989) respectively.

Gandhi and Nambudripad (1975) reported the antimicrobial properties of market dahi samples against *Escherichia coli*, *Aerobacter aerogenes*, *Bacillus subtilis* and *Staphylococcus aureus*. According to the observations dahi samples showed maximum inhibitory activity against *B. subtilis* and against *E. coli*. Frank and Marth (1977) reported the inhibition of enteropathogenic *E. coli* by homofermentative LAB like *Str. lactis* and *Str. cremoris*.

Pulusani *et al.* (1979) found strong inhibition by *Str. thermophilus* of *B. subtilis*, *E. coli* and *Salmonella typhimurium*. Singh *et al.* (1979) proved the antibacterial activity of pure and mixed strains of *Str. thermophilus* and *Lb. bulgaricus* against *E.coli*, *Pseudomonas fragi* and *Micrococcus flavus*. Reddy (1980) reported the antibacterial activity of *Str. lactis* ssp. *diacetylactis* strains DRC1, DRC2, against *Staph. aureus* S6 and *P. fragi*.

Ratnakar (1989) had proved weak inhibitory activity of market dahi samples against *E.coli*, *B.subtilis* and *Staph.aureus* and no inhibition was observed against *Sal.typhi*. The dahi samples prepared using *Lc. lactis* ssp. *lactis* C10 had shown a weak antibacterial activity against *B.subtilis* and *Sal.typhi* whereas dahi prepared with mixed cultures showed no inhibitory action against the test organisms.

In vitro studies showed that *Lb. bulgaricus* and *Str. thermophilus* do not produce any antibiosis against *E.coli* (Bianchi-Salvadori *et al.*, 1988).

The yoghurt samples prepared by using *Str. salivarius* ssp. *thermophilus* H, *Lb. delbrueckii* ssp. *bulgaricus* W and *Lb. acidophilus* R showed mild inhibitory effect against *Staph.aureus* and no inhibition was noted against *E.coli*, *B.subtilis* and *Sal.typhi* (Ratnakar, 1989).

As early as in 1965, Vakil and Shahani showed high antibacterial activity of a strain of *Lb. acidophilus* against bacterial pathogens belonging to genera *Salmonella*, *Shigella*, *Klebsiella*, *Pseudomonas* and *Staphylococcus*. Similar observations were reported (Hamdan and Mikolajcik, 1973; Mikolajcik and Hamdan, 1975; Babel, 1977; Gilliland and Speck, 1977; Sandine, 1979; and Keating, 1985). The production of hydrogen peroxide, which is inhibitory to many pathogens, by *Lb. acidophilus* was observed (Wheather *et al.*, 1952; Collins and Aramaki, 1980; Martin and Gilliland, 1980). Acidophilus yoghurt, containing *Lb. acidophilus* and *Str. thermophilus* suppressed *Staph.aureus* more effectively than standard yoghurt (Attaie *et al.*, 1987). Ferreira and Del (1987) had observed that bacteriocin produced by *Lb. acidophilus* was inhibitory towards *Staph.aureus*, *E.coli* and *Salmonella* spp. Very strong antibacterial activity by *Lb. acidophilus* R (Rao and Gandhi, 1988) than *Lb. acidophilus* 1899 was found against *Staph.aureus* and weak against *Sal.typhi* (Ratnakar, 1989). Khedkar *et al.* (1990) proved strong inhibitory activity of cell free culture filtrates of *Lb. acidophilus* BV3 against *Ps.aeruginosa* and *Sal.typhosa*.

Lin *et al.* (1986) examined 91 cultures of lactic acid bacteria for their antibacterial activity based on the formation of an inhibitory zone against *E.coli*, *Staph.aureus*, *Clostridium sporogenes*, *Ps.fluorescens*, *Sal.typhimurium* or *B.subtilis*. The highest antimicrobial activity was obtained with *Lb. acidophilus*, *Lb. bulgaricus* and *Str.lactis* cultures.

Bifidobacterium bifidum was found to exhibit maximum antagonistic action against several pathogenic micro-organisms tested. Maximum inhibitory action was against *M.flavus* followed

by *Staph.aureus*, *B.cereus*, *E.coli*, *Ps.fluorescens*, *Sal.typhosa*, *Sh.dysenteriae* in decreasing order (Anand *et al.*, 1984; Tojo *et al.*, 1987 and Tomåda *et al.*, 1988).

Rao (1993) reported very strong antibacterial activity of yoghurt-ice cream supplemented with *Lb.acidophilus* and *Bif.bifidum* against *E.coli*, *Staph.aureus* and *Sal.typhi* than that of the yoghurt-ice cream prepared with yoghurt cultures alone.

2.8 *Objectives of the Present Study*

Keeping in view of the necessity of the concentrated cultures of dahi and yoghurt, the present investigation was carried out with the following objectives:

- To develop improved, mixed starter cultures for dahi and yoghurt and to enhance the therapeutic value of these products by incorporating *Lb. acidophilus* and *Bif. bifidum* in both dahi and yoghurt cultures.
- To make available the direct vat inoculation freeze-dried starter cultures for the commercial production of dahi and yoghurt of uniform quality.
- To predict the shelf life of bulk freeze-dried starter cultures of dahi and yoghurt.
- To manufacture dahi and yoghurt of uniform quality.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was carried out in four phases. In the first phase, the effect of associative growth in mixed cultures of dahi and yoghurt with the therapeutic cultures (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) was studied to select the best combination of cultures for the preparation of dahi and yoghurt. In the second phase, selection of suitable growth medium, and optimization of growth conditions for bulk production of selected dahi and yoghurt cultures and freeze-drying of these cultures were carried out. In the third phase, the bulk freeze-dried cultures of dahi and yoghurt were evaluated for viability and biochemical activity during storage period. In the fourth phase, dahi and yoghurt were prepared using bulk freeze-dried cultures and the products were evaluated for sensory properties and therapeutic values viz., effect on the growth and serum cholesterol level of experimental animals and antibiotic properties against selected pathogenic cultures.

3.1 Collection of Raw Materials

In this investigation, samples of buffalo milk, cheese whey and spray-dried skim milk powder were collected from the Experimental Dairy of National Dairy Research Institute (NDRI), Karnal. The laminated aluminium foil (12 microns polyethylene + 12 microns aluminium foil + 150 gauge polypropylene) used for the packaging of freeze-dried cultures of dahi and yoghurt was collected from Rollatainers Limited, Faridabad and Dr. N. Subrahmanyam, Senior Scientist, Central Food Technological Research Institute, Mysore. All the chemicals used in the present study were of analytical grade and of S.D. fine, Qualigens, B.D.H. and Merck make. The ingredients used in the bacteriological media were procured from Hi-Media Laboratories (P) Limited, Bombay.

3.2 Collection of Bacterial Cultures

The following lactic and non-lactic cultures were obtained from the National Collection of Dairy Cultures, Dairy Microbiology Division, N.D.R.I., Karnal.

a) **Lactic Cultures:**

S.No.	Culture	Strain No.	Code
1.	*Lactococcus lactis* ssp. *cremoris*	C1	LC C1
	Lactococcus lactis ssp. *cremoris*	H	LC H
2.	*Lc. lactis* ssp. *lactis*	C10	LL C10
	Lc. lactis ssp. *lactis*	ML8	LL ML8
3.	*Lc. lactis* ssp. *lactis* biovar.*diacetylactis*	DRC1	LD DRC1
	Lc. lactis ssp. *lactis* biovar.*diacetylactis*	DRC2	LD DRC2
4.	*Streptococcus salivarius* ssp. *thermophilus*	HST	ST HST
	Streptococcus salivarius ssp. *thermophilus*	I	ST I
5.	*Bifidobacterium bifidum*	Isolate	BB I
6.	*Lactobacillus acidophilus*	R	LA R
	Lactobacillus acidophilus	111	LA 111
7.	*Lb. delbrueckii* ssp. *bulgaricus*	RTS	LB RTS
	Lb. delbrueckii ssp. *bulgaricus*	LBW	LB W

b) **Non-lactic Cultures:**

Escherichia coli	K-12
Salmonella typhimurium	A-KIGM
Shigella dysenteriae	KIGM

3.3 Maintenance of the Bacterial Cultures

All the lactic cultures except *Bif. bifidum* were maintained in chalk litmus milk in duplicates and sub-cultured after every 30 days. The lactic cultures (under 3.2a, at 1-3) were incubated

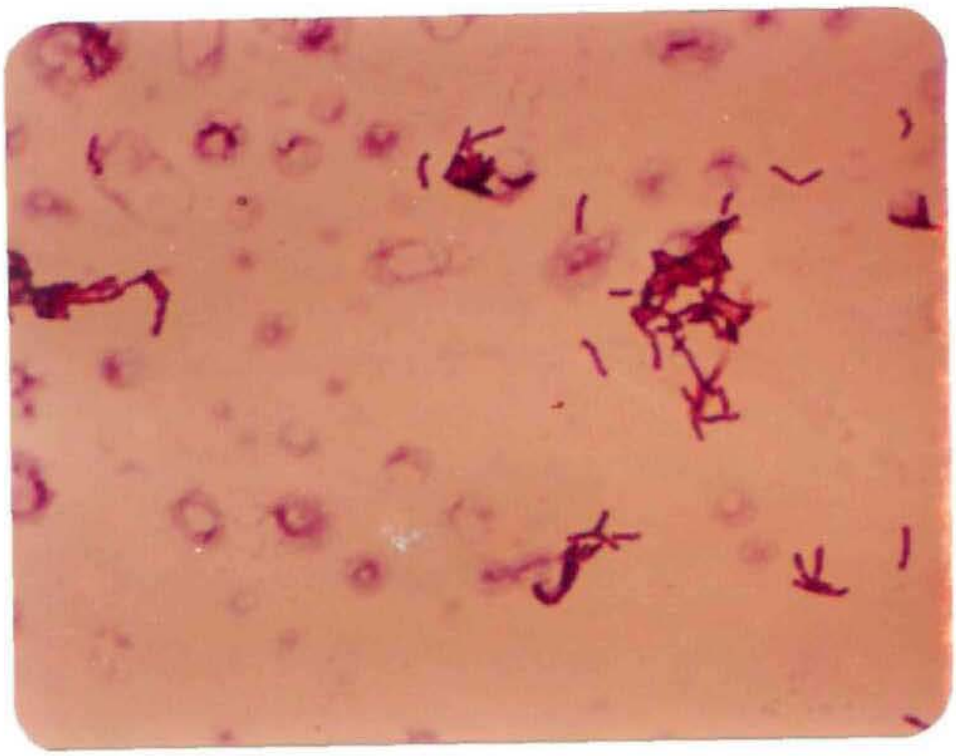


Plate.1. Microscopic examination of *Bif. bifidum*

at 30°C whereas the lactic cultures (under 3.2.a at 5-7) were incubated at 37°C for 16 hrs.

Bif.bifidum Isolate was maintained in deMann Rogosa and Sharpe (MRS) broth supplemented with 0.1% N-acetyl-D-glucosamine and 0.05% cysteine hydrochloride (both were separately sterilized by Seitz filter). The culture was stored in refrigerator and sub-cultured after every 7 days. This culture was anaerobically propagated in yeast dextrose milk for routine use.

The non-lactic cultures were maintained on nutrient agar slants and sub-cultured after every 30 days.

3.4 Purity of the Cultures

The purity of the bacterial cultures was examined by Gram staining technique.

3.5 Composition of Bacteriological Media

Owing to the fastidious growth requirements of various cultures, different agar/broth media were used for maintenance and plating purposes. All the media and buffer were sterilized by autoclaving at 15 psi for 15 min and were subjected to sterility checking by incubating at 30°C for 4 days.

i) Brain Heart Infusion (BHI) broth:

Composition:

Calf brain infusion		20.00%
Beef heart infusion		25.00%
Proteose peptone		1.00%
Na_2HPO_4	0.25%	
NaCl		0.50%
Glucose		0.20%
pH 7.4		

ii) de, Man Rogosa and Sharpe (MRS) broth:

Composition:	
Peptone	1.0%
Meat extract	1.0%
Yeast extract	0.5%
K_2HPO_4	0.2%
Ammonium citrate	0.2%
Glucose	2.0%
Sodium acetate	0.5%
Tween 80	0.1%
Salt A	0.5ml
pH 6.8	

Salt A:

Composition:

$MgSO_4 . 7H_2O$	11.5%
$MnSO_4 . H_2O$	2.4%
Distilled water	100ml

iii) de, Man Rogosa and Sharpe (MRS) broth supplement

Filter sterilized solutions of N-acetyl-D-glucosamine and cysteine hydrochloride were added to MRS medium at the rate of 0.1 and 0.05%, respectively when used for the maintenance of *Bif. bifi*

iv) LA agar (Klupsch, 1984)

This medium has been designed principally for the enumeration of *Bif. bifidum* and *Lb. acidophilus* in mixed starter cultures and fermented milks.

Composition:

Peptone	1.0%
Meat extract	0.5%
Yeast autolysate	0.5%
Glucose	1.0%
lactose	1.0%
Sodium acetate	0.5%

Sodium citrate	0.3%
Tween 80	0.1%
$MgSO_4 . 7 H_2O$ (1% solution)	2.0ml
$MnSO_4 . 4 H_2O$ (1% solution)	0.5ml
Agar	2.0%
Sheep blood (defibrinated)	5.0ml
before sterilization 7.2	
pH	
after sterilization 6.8	

vi) Tryptone Glucose Vegetable (TGV) agar:

Composition:

Tryptone	1.0%
Meat extract	1.0%
Yeast extract	0.5%
Glucose	2.0%
Tomato juice	4.0ml
K_2HPO_4	0.2%
Tween 80	0.1%
Agar	1.5%
pH 7.0	

vi) Yeast Dextrose Agar (YDA)
Composition:

Peptone	0.5%
Yeast extract	0.5%
Glucose	1.0%
Sodium acetate	1.0%
Agar	1.5%
pH 6.9	

vii) Yeast Dextrose Milk (YDM)
Composition:

Yeast extract	0.1%
Dextrose	1.0%
Skim milk	100 ml

viii) Phosphate buffer

Composition

Na_2HPO_4	0.60%
KH_2PO_4	0.45%
Tween 80	0.05%
Agar	0.10%
pH 7.0	

Filter sterilized solution of L-cysteine hydrochloride was added @ 0.05% to the buffer before it was used for making serial dilutions of cultures containing *Bif. bifidum*.

3.6 Biochemical Methods for the Evaluation of Starter Cultures, Dahi and Yoghurt

3.6.1 *Titratable Acidity*

Titratable acidity of the samples was determined according to the procedure described in ISI (1981) and the results were reported as % titratable acidity (expressed as lactic acid).

3.6.2 *Culture Activity*

activity of starter cultures was determined according to the procedure described by Horrall and Elliker (1950).

The reconstituted milk was prepared at the rate of 10% of the non-fat dry milk solids in distilled water and was heated in flasks at 15psi pressure for 10 min. Respective 10 ml quantities of sterile milk were pipetted aseptically into sterile screw cap tubes and adjusted to 37.8 °C in a water bath. Each tube was inoculated with 0.3 ml of the starter culture to be tested and incubated for 3.5 hrs. Then the entire contents of the tube, together with 5 ml of distilled water used to rinse the tube, were titrated with 0.1 N NaOH to a faint pink colour using phenolphthalein as an indicator. The results were recorded as percent lactic acid. This figure was termed the activity factor

of the culture. The cultures with an activity factor of above 0.35 were graded active.

3.6.3 *Volatile Acidity*

Volatile acidity was determined as per the method of Hempenius and Liska (1968).

A 50g sample was weighed into a test tube containing antifoam A and 3 ml of 1N sulfuric acid was added. The test tube was lowered into a one litre round bottom flask. The steam was introduced into the flask through the side arm of the flask. The first 100 ml fraction of the distillate was collected and titrated against 0.01N NaOH using 0.1% phenolphthalein indicator. The volatile acidity was expressed as ml of 0.01N NaOH/50g of sample.

3.6.4 *Flavour Producing Capacity(Voges-Proskauer Test)*

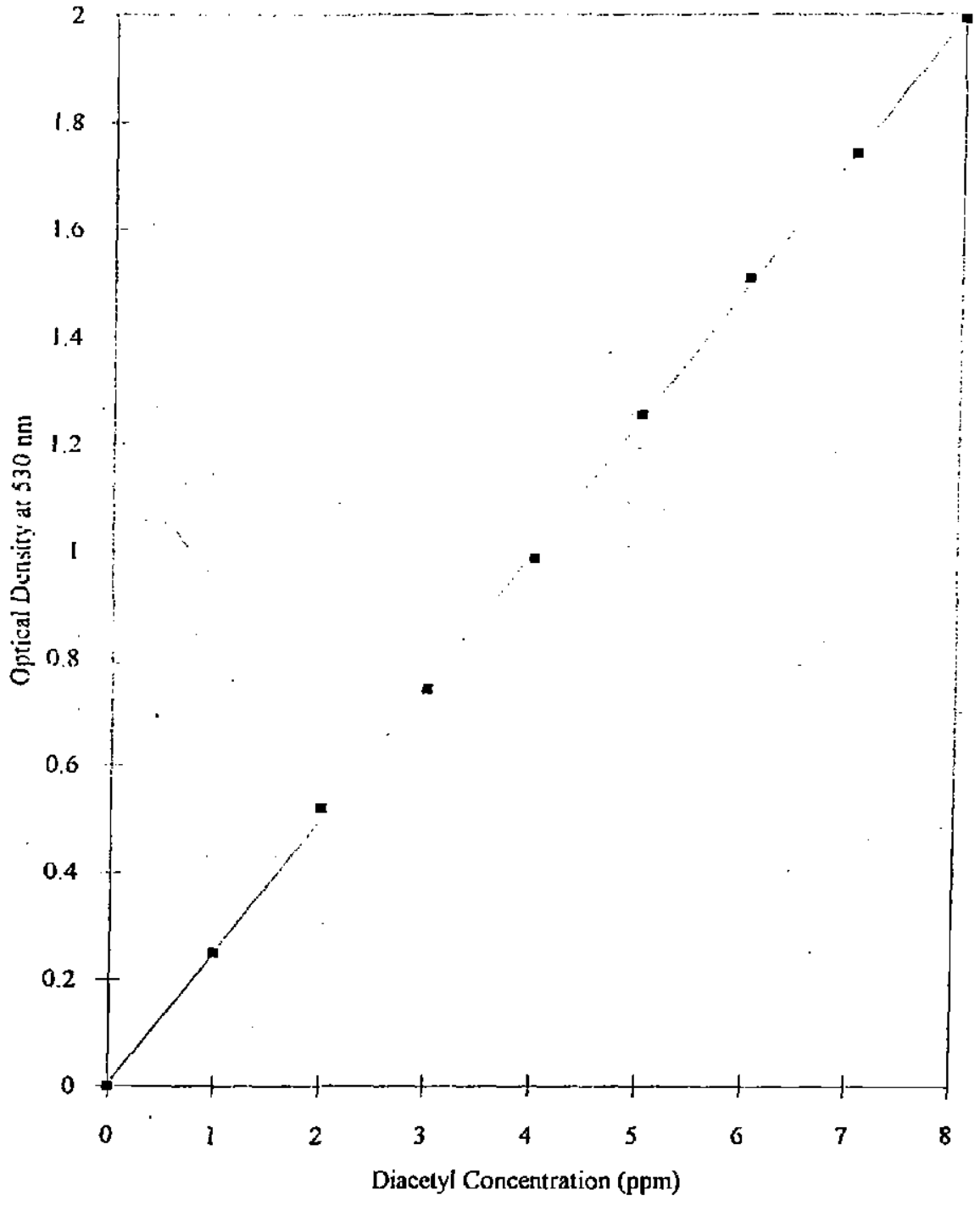
The flavour producing capacity of the sample was determined by modified O'Meara's test as described by Hammer (1935).

A 2.5 ml sample was transferred into a test tube and 2.5 ml of strong aqueous sodium hydroxide (40% w/v) solution was added to it. A pinch of creatine powder was added and the contents were mixed thoroughly and allowed to stand for 10 min at 37^{o}C. A red colour development at the surface indicates the flavour development by the cultures.

3.6.5 *Diacetyl Content*

Diacetyl content of the samples was estimated by the method of Pack *et al.* (1964) and the diacetyl content (ppm) was determined by reading with the standard curve (Fig.1) of diacetyl.

Fig. 1 Standard curve for the estimation of diacetyl concentration.



3.6.6 *Acetaldehyde Content*

Acetaldehyde content of the samples was estimated by the method of Lindsay and Day (1965) and the acetaldehyde content (ppm) was determined by reading with the standard curve (Fig.2) of acetaldehyde.

3.6.7 *Proteolytic Activity*

Proteolytic activity of the samples was determined by the method of Hull (1947) and the tyrosine content (mg/g curd) was determined by reading with the standard curve (Fig.3) of tyrosine.

3.6.8 *Residual Moisture Content*

The residual moisture content of the bulk freeze-dried starter cultures was determined by the method of Nei *et. al.* (1966)

The weighing bottles with stoppers tilted open were placed in the vacuum oven at 60°C for 2 hrs. These were placed directly in a desiccator containing freshly scraped P_2O_5 and allowed to cool. After the temperature equilibrium attained the bottles were removed and weighed quickly. The bottles were now filled with 1.0g of freeze-dried culture powder and after replacing the stoppers, the bottles were weighed to get exact weight. With the stoppers tilted, the bottles were transferred to vacuum oven. The vacuum pump was turned on and allowed to run continuously for 3hrs in a vacuum of 10^{-5} mm Hg. After admitting atmospheric air slowly into the oven, the oven was opened and stoppers were replaced instantly and the bottles were transferred to the desiccator for cooling. Then the bottles were weighed. The residual moisture (expressed in %) was calculated using the following formula:

Fig. 2 Standard curve for the estimation of acetaldehyde concentration.

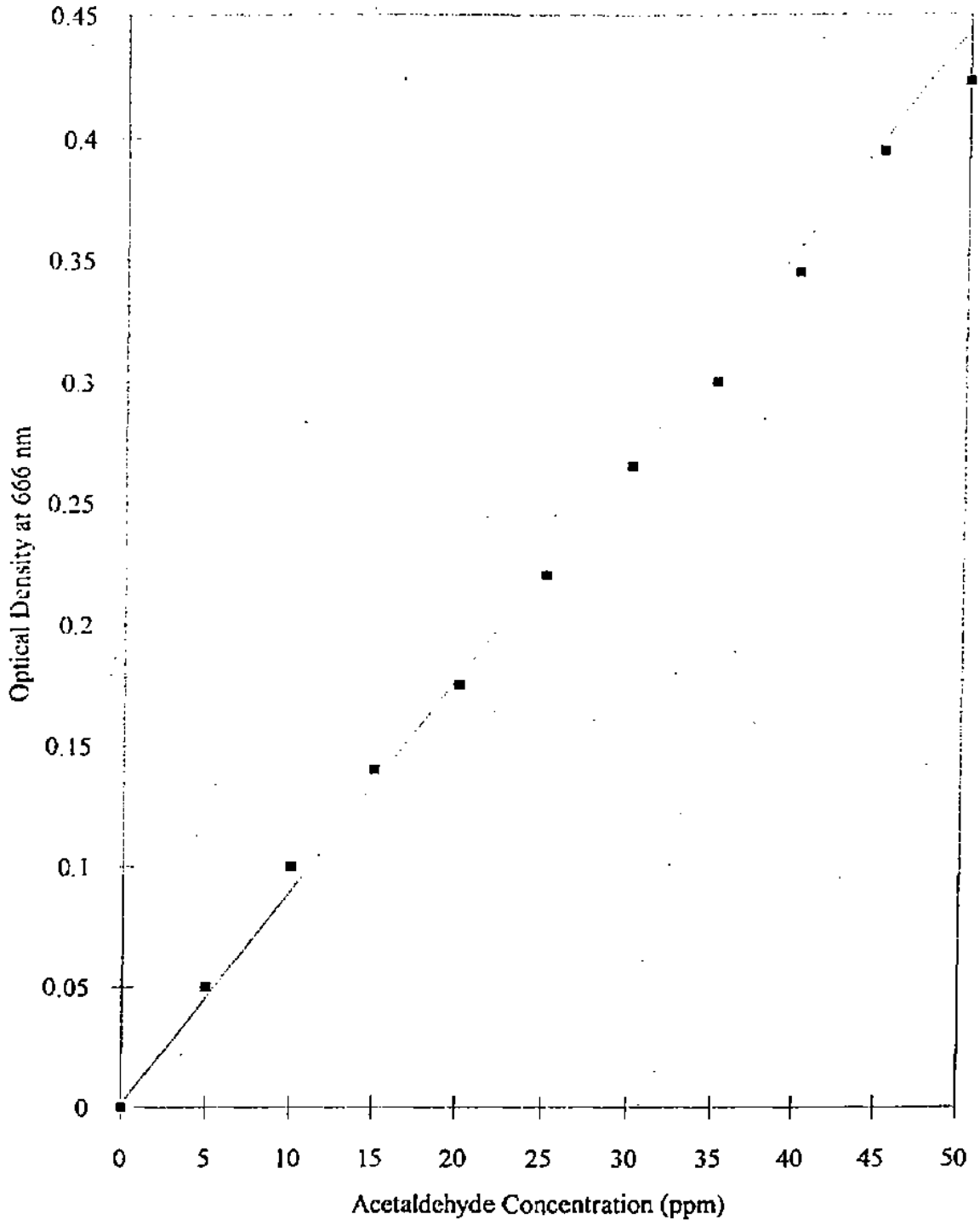
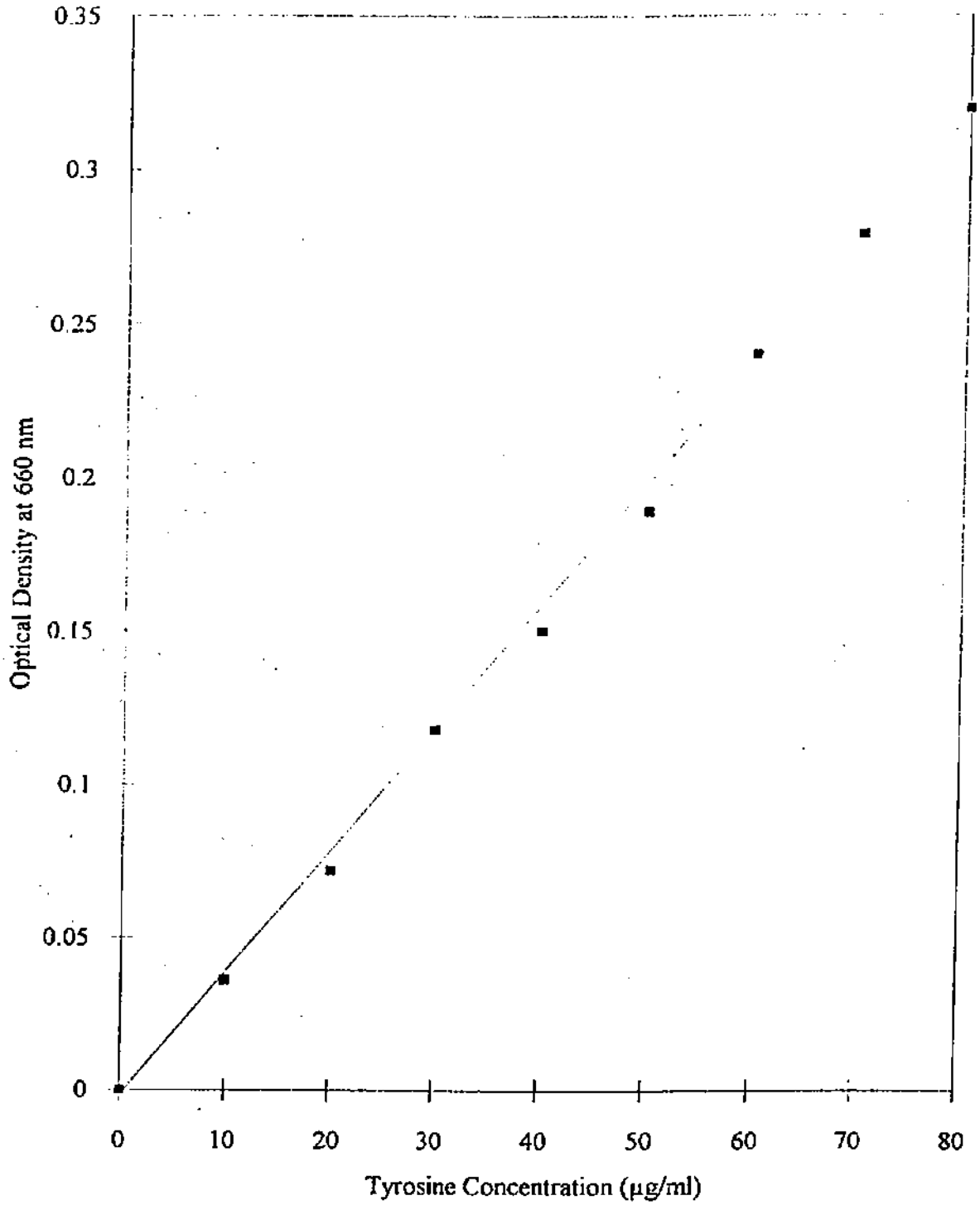


Fig. 3 Standard curve for the estimation of Tyrosine concentration.



$$\frac{A-B}{B} \times 100$$

where A = initial weight

B = weight after drying.

3.7 Chemical Analysis

3.7.1 *Determination of Fat Content:*

a) Buffalo milk

Fat content was determined by Gerber method specified in IS:1479 (ISI, 1977). The fat content was expressed in percentage.

b) Dahi and yoghurt

Ten grams of dahi and yoghurt samples were taken to determine the fat content by Mojonnier method as specified in IS:4079 (ISI, 1967). The results were expressed in percentage.

3.7.2 *Cholesterol Content:*

The total cholesterol content of serum samples was estimated according to the method of Zlatkis *et al.*(1953) with some modifications. Instead of using formula given in the original procedure a standard curve for cholesterol content was prepared.

Reagents:

i) Glacial acetic acid (aldehyde free)

ii) Conc. H_2SO_4

iii) Ferric chloride solution: It was prepared freshly by

dissolving 0.05g of $FeCl_3 . 6H_2O$ in 100 ml of glacial acetic acid.

iv) Cholesterol

a) Procedure:

One hundred microliters of test serum were taken in 10 ml of screw cap tube and 5 ml of ferric chloride solution was added to it and was left at 37°C for 15 min. It was centrifuged at 3000 rpm for 15 min. and the supernatant was decanted into another screw cap tube. To this, 3 ml of conc. H_2SO_4 was added and after capping the contents were mixed thoroughly by reversing the tube twice. After 30 min, the absorbance of the pink colour developed was red at 560nm using Spectrocol (Model CI: 23, Elico, India) spectrophotometer and the concentration of total cholesterol content was determined by using the standard curve of cholesterol.

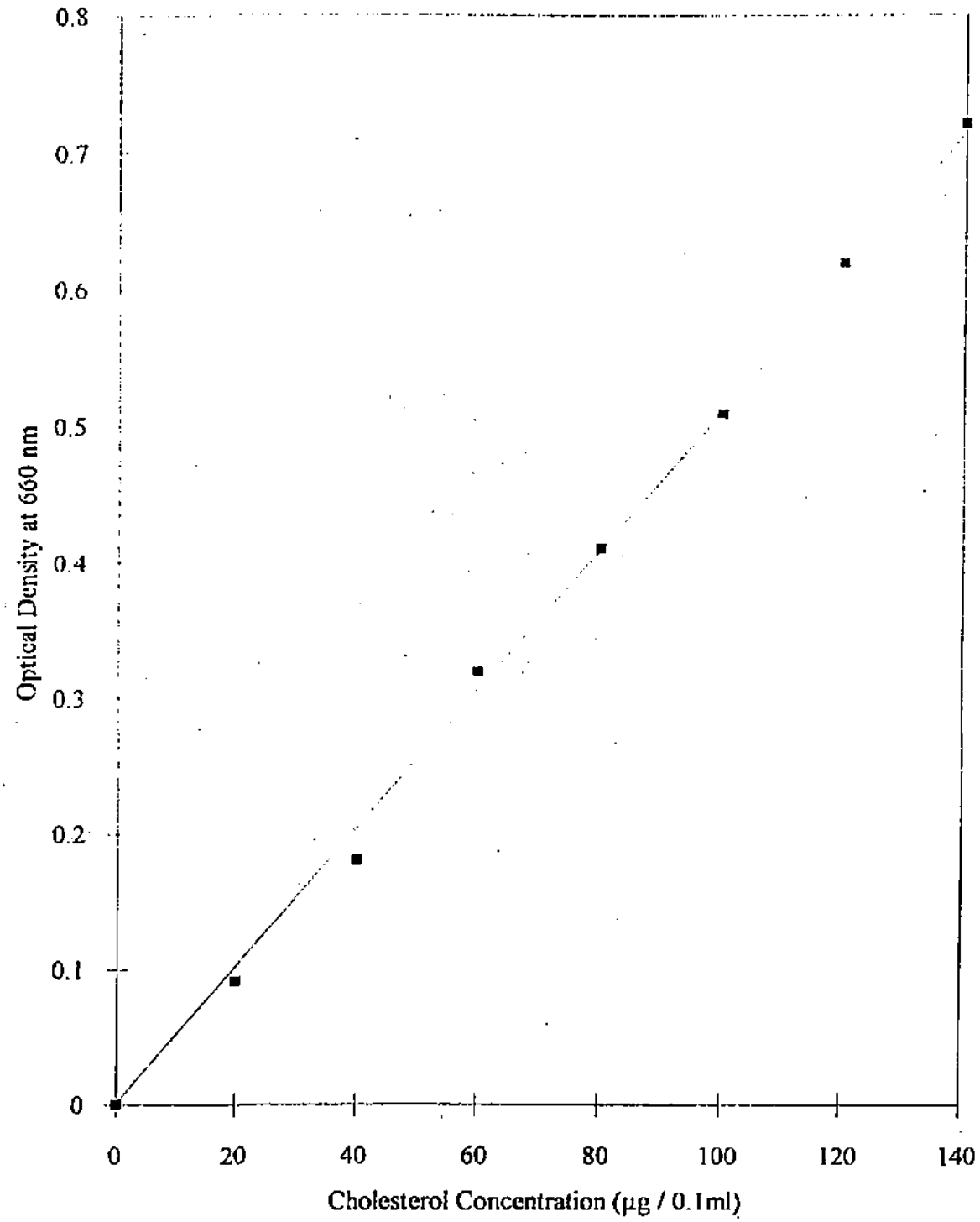
b) Extraction of cholesterol from milk, dahi and yoghurt:

Ten grams of sample was saponified with 5.0 ml of freshly prepared methanolic KOH (20% w/v) for 55 min. at 65°C. The cholesterol was extracted thrice with 5.0 ml of petroleum ether. The pooled extract was evaporated to dryness under a stream of air on boiling water bath. The cholesterol crystals were then dissolved in 10.0 ml of petroleum ether. It was evaporated to dryness and the residue was then dissolved 0.5 ml of 0.05% ferric chloride solution. One hundred microliters of this was subjected to colour development as per the procedure described earlier (a).

c) Standard Curve:

A stock solution of cholesterol was prepared by dissolving 20 mg of cholesterol crystals in 10 ml of glacial acetic acid by keeping it in boiling water bath, thus getting a conc. of 2000 ug per ml. A series of dilutions were then prepared from the stock solution to have solutions with cholesterol conc. ranging from 20

Fig. 4 Standard curve for the estimation of cholesterol concentration.



to 160 ug/0.1 ml. The cholesterol solutions were treated as given in the procedure under (a) for developing the colour. The standard curve (Fig.4) was drawn by plotting optical density values against the cholesterol concentrations.

3.8 Performance of formulated cultures of dahi and yoghurt

3.8.1 *Selection of Dahi, Yoghurt and Therapeutic Cultures:*

A) Dahi Cultures:

To study the biochemical performance of mixed mesophilic lactococci, eight possible combinations with one strain each of *Lc. lactis* ssp.*lactis*, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* and *Lc. lactis* ssp. *cremoris* in 1:1:1 ratio were prepared and subcultured thrice successively. These combinations were selected based on the observations of a preliminary study conducted with all possible combinations.

Freshly prepared buffalo skim milk was distributed in 200 ml quantities in conical flasks of 500 ml capacity and the flasks were plugged with cotton and sterilized at 15 psi for 15 min. After cooling to 30°C, the milk samples were inoculated individually with active cultures of above combinations at the rate of 1% and incubated at 30°C till the milk samples were curdled (16 hrs). The curd samples were subjected to biochemical analysis, i.e., titratable acidity, Voges-Proskauer test and proteolytic activity(Section 3.6) and the body and texture of the curds were examined to select the best combination.

B) Yoghurt Cultures:

In a way similar to dahi cultures, four possible combinations with one strain each of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. salivarius* ssp. *thermophilus* were screened to select the best combination. The titratable acidity production, proteolytic activity (Section 3.6) and the observations of body

and texture were recorded, after the sterilized buffalo skim milk samples inoculated with 1% of active cultures were set for 16 hrs at 37°C.

C) Therapeutic Cultures:

The cultures of *Bif. bifidum* and *Lb. acidophilus* were mixed in 3:1 ratio and sub-cultured successively for three times. These are inoculated individually into the sterilized buffalo skim milk at the rate of 1% and incubated at 37°C till the milk was curdled (16 hrs) and analysed for titratable acidity and proteolytic activity (Section 3.6) and the body and texture of the curds were observed in order to select the best combination.

3.8.2 *Performance of Selected Dahi and Yoghurt Cultures with and without Therapeutic Cultures*

On the basis of performance of mixed culture combinations, the promising mixed cultures of dahi and yoghurt were selected for detailed study to evaluate the behaviour of these cultures, when grown together with *Bif. bifidum* and *Lb. acidophilus* in terms of biochemical activity and viable cell count. The study was carried out with the following selected combinations.

Formulated cultures	Ratio	Incubation temperature (°C)
1. LD DRC1+LC C1+LL C10	1:1:1	30
2. LD DRC1+LC C1+ LL C10+BB I+LA R	1:1:1:3:1	30 & 37
3. ST HST+LB RTS	1:1	37
4. ST HST+LB RTS+BBI+LAR	1:1:3:1	37

The formulated culture combination (i) LD DRC1, LC C1 and LL C10 was henceforth referred as Dahi Culture D. The dahi culture combination containing LA R and BB I was referred as Dahi Culture DAB. Likewise, the culture combination consisting of ST

HST and LB RTS was designated as **Yoghurt Culture Y** and with the addition of LAR and BBI to this combination, it was called as **Yoghurt Culture YAB.**

Freshly prepared buffalo skim milk was distributed in 200 ml quantities in conical flasks of 500 ml capacity and the flasks were plugged with cotton and sterilized at 15 psi for 15 min. After cooling, the milk samples were inoculated with the above active cultures, individually. A set of milk samples inoculated with combinations 1 and 2 were incubated at 30°C. The milk samples inoculated with combinations 2, 3 and 4 were incubated at 37°C.

At periodic intervals, viz., 6, 12 and 18 hrs of incubation at respective temperatures, the samples were withdrawn aseptically and the biochemical activity in terms of titratable acidity, volatile acidity, estimation of diacetyl (for dahi cultures) acetaldehyde (for yoghurt cultures) and proteolytic activity of all the cultures was determined (Section 3.6).

The viable cell count of the samples withdrawn at periodic intervals was enumerated. For this, serial dilutions of the samples were prepared in phosphate buffer (Section 3.5) and appropriate dilutions of formulated dahi cultures were spread onto YD agar and LA agar plates, whereas the formulated yoghurt cultures were spread onto TGV agar and LA agar plates. The YDA and TGV agar plates were incubated aerobically at 30° and 37°C, respectively and the LA agar plates were incubated at 37°C under anaerobic conditions. The colony count was made after 48 hrs of incubation and the results were expressed in terms of log colony forming units per gram.

Bif. bifidum appeared as elevated, chocolate brown coloured colonies and *Lb. acidophilus* as flat, greyish and larger in colony size.

3.9 Preparation of Bulk Freeze-dried Starter Cultures

Different steps involved in the freeze-drying of dahi cultures (D and DAB) and yoghurt cultures (Y and YAB) and their storage pattern are depicted in Appendix-I.

3.9.1 *Selection of Formulated Growth Media*

In a preliminary study different buffalo skim milk and cheese whey based media were prepared as given below and tried for their suitability for the growth of dahi and yoghurt cultures. The pH of all the media was adjusted to 7.0 and were sterilized at 15 psi for 10 min.

A) **Skim milk-based Media:**

1. Skim milk media for dahi cultures:

Medium No.1		Medium No.2	
Skim milk	- 5%	Skim milk	- 5%
Sodium citrate	- 1%	Yeast autolysate	- 1%
$MgSO_4.7H_2O$	- 0.02%	Glucose	- 1%

Medium No.3		Medium No.4	
Skim milk	- 1000 ml	Skim milk	- 50%
Yeast extract	- 0.1%	Tryptone	- 1%
		Glucose	- 0.1%

Medium No.5
(This medium was also tried for the growth of yoghurt cultures)

Skim milk (trypsin digested)*	- 100 ml
Sodium acetate	- 0.5%
Yeast extract	- 0.5%
Glucose	- 1.0%

*Trypsin Digestion

Five grams of non-fat dried milk and 0.1g of Tween 80 were added to 100 ml of distilled water and pH was adjusted to 8.5 and then 0.5g of trypsin was added and incubated at 37°C for 30 min.

2. Skim milk media for yoghurt cultures:

Medium No.1

Skim milk	- 75%
Sodium acetate	- 3%
Yeast extract	- 4.5%

Medium No.2

Skim milk	- 9%
Cysteine	- 0.075%
Ascorbic acid	- 0.150%
Yeast extract	- 1.5%
Tween 80	- 0.15%

Medium No.3

Skim milk	- 5%
Yeast extract	- 0.5%
Lactose	- 0.5%
Sucrose	- 0.25%
$MgSO_4$ 7 H_2O	- 0.013%
K_2H PO_4	- 0.2%

Medium No.4

Skim milk	- 50%
Tryptone	- 1.0%
Glucose	- 0.1%

Medium No.5

Skim milk	- 20 ml
Distilled water	- 130 ml
Yeast autolysate	- 1.0%
Glucose	- 1.0%

B) **Cheese Whey-based Media:**

Treatment of Whey

Freshly collected cheese whey was boiled for 15-20 min, cooled and filtered through four-folded muslin cloth. The heated and cooled whey was stored in refrigerator for settling of the precipitated proteins. After 24 hrs, it was decanted and the clarified whey was filtered through Whatman filter paper No.4 and the filtrated whey was used in the preparation of media.

1. Whey Media for Dahi Cultures:

Medium No.1

Cheese whey	- 1000 ml
Skim milk	- 10 ml
Yeast extract	- 5 g

Medium No.2

Cheese whey	- 20%
Tryptone	- 1%
Yeast extract	- 0.05%

Medium No.3

Cheese whey	- 1000 ml
Yeast extract	- 5 g

Medium No.4

Cheese whey	- 10%
Sodium citrate	- 2%

$MnSO_4$	- 120 mg	$MnSO_4.H_2O$	- 0.17%
$MgSO_4$	- 120 mg	Cysteine	- 0.5%
$ZnSO_4$	- 120 mg	Yeast extract	- 0.5%
		Glucose	- 0.5%

2. Whey Media for Yoghurt Cultures:

Medium No.1		Medium No.2	
Cheese whey	- 1000 ml	Cheese whey	- 1000 ml
Yeast extract	- 10 g	Tryptone	- 10 g
Tween 80	- 1 g	Glucose	- 10 g

Medium No.3

Cheese whey	- 10%
Yeast extract	- 0.5%
Glucose	- 0.5%
Sodium citrate	- 2.0%
$MnSO_4.H_2O$	- 0.17%
Tween 80	- 0.1%
Cysteine	- 0.5%

The media tried were distributed in 100ml quantities in 250 ml capacity Erlenmeyer's flasks and sterilized at 15 psi for 10 min. Sterilized media were inoculated with the selected cultures of dahi and yoghurt, individually at the rate of 1%. The flasks inoculated with dahi cultures were incubated both at 30°C and 37°C whereas the yoghurt cultures were incubated at 37°C. All the flasks were removed after 18 hrs and the intensity of growth was measured by recording absorbence at 600 nm. In case of milk-based media, the curdled milk was chilled and treated with 0.2% EDTA and 10 M NaOH to facilitate reading in spectrophotometer.

The selected media were inoculated with 1% active culture and the titratable acidity, and viable cell count of the cultures were determined after 14, 16 and 18 hrs of incubation at respective temperatures (Sections 3.6 and 3.8.2).

3.9.2 *Optimization of Growth Conditions*

Skim milk medium with yeast autolysate and glucose (SYG) for dahi cultures (D and DAB)and skim milk medium with tryptone and

glucose (ST6) for yoghurt cultures (Y and YAB) were finally selected for the preparation of starter concentrate and was used to optimize the growth conditions for dahi and yoghurt cultures.

The cultures of the following experiments were removed after 14, 16 and 18 hrs of incubation and analysed for viable cell counts and titratable acidity (Sections 3.6. and 3.8.).

3.9.2.1 pH of the medium

The selected growth media for dahi and yoghurt cultures were prepared and pH of the media was adjusted to 6.0, 6.5 and 7.0. The adjustment of pH of the sterilized medium was made with the help of sterile 20% NH_4OH solution. The media with differing pH values were inoculated with respective cultures of dahi and yoghurt @ 1% and incubated at respective temperatures.

3.9.2.2 Neutralization of cultures

Dahi and yoghurt cultures were inoculated @ 1% into the respective selected media and incubated at 30°C for dahi culture D and 37°C for cultures DAB, Y and YAB. At the end of 10, 12, 14, 16 and 18 hrs of incubation, the cultures were removed and neutralized to the initial pH of the respective media with 20% solution of NH_4OH. Viability of the cultures in the flasks with and without (control) neutralizer was determined (Section 3.8.2).

3.9.2.3 Incubation temperature

The media with selected pH were prepared and inoculated with respective dahi and yoghurt cultures @ 1%. The media inoculated with dahi cultures were incubated at 25°, 30° and 37°C whereas the media inoculated with yoghurt cultures were incubated at 37° and 42°C to study the suitability of the temperature.

3.9.2.4 Inoculum Rate

The selected media for dahi and yoghurt cultures were inoculated individually with respective cultures @ 0.5%, 1.0% and 1.5% and the media inoculated with dahi culture D was incubated at 30°C and the media inoculated with cultures DAB, Y and YAB were incubated at 37°C.

3.9.3 *Production of Cell Biomass*

The selected media were prepared in 10 litre quantities and were inoculated individually with dahi (D and DAB) and yoghurt cultures (Y and YAB) @ 1% and incubated at 30° and 37°C for D and DAB, Y and YAB, respectively. The cultures were removed between 16-18 hrs and the curd was broken and 30% sterilized solution of sodium citrate was added @ 1%. The cultures were shaken well and left undisturbed for 10 min. The pH of the contents was brought to 6.8-7.0 by using 20% NH_4OH solution. After 15 min., the contents were transferred to chemically sterilized glass aspirator bottles of 10 litres capacity.

3.9.4 *Harvesting of Cell Biomass*

Aspirator bottle containing the shaken milk culture was kept at a higher level on the cemented platform and its outlet was connected to the bowl of the sharples super centrifuge (Sharples Centrifuges Ltd, Camberley, UK). The culture was allowed to enter the bowl after the speed of the centrifuge had attained 15,000-20,000 rpm. Inflow of the culture into the centrifuge was regulated in such a way that approximately 6 litres of medium was centrifuged in one hour.

After completion of the centrifugation, bowl containing the cell biomass was dismantled. It was brought to the inoculating chamber aseptically and the cell mass sticking to the walls of the bowl was scraped off, using sterile plastic knife. The remnant of cell mass adhering to the wall was dislodged by

rinsing with normal saline (250 ml)and the whole content was centrifuged (Kubota Centrifuge, Japan) at 3000 rpm at 4°C to collect the cells.

3.9.5 *Cryoprotective Agents*

To select the best combination of cryoprotective agents, the cell biomass was suspended in three formulated combinations of different cryoprotective agents. The formulations tried were:(i) 5% buffalo skim milk + 1.5% gelatin + 8% sucrose (ii) 10% buffalo skim milk + 1.5% gelatin + 7% sucrose + 1M monosodium glutamate (iii) 10% buffalo skim milk + 7% sucrose + 1M monosodium glutamate. The cell biomass obtained (Section 3.9.4.) was resuspended in all the formulated suspending media in a volume of 2% of the growth medium. Clumps of cells in the suspension were broken with the help of Omini mixer (Sorvall Inc., USA) at low speed. A portion of the cell suspension in different formulations was distributed in 10 ml quantities in sterilized petri dishes and subjected to freeze-drying.

For evaluating the cryoprotective effect of different combinations, dahi and yoghurt cultures were analysed before and after freeze-drying. The viable cell counts (Section 3.8.2), titratable acidity, culture activity, diacetyl/acetaldehyde content and proteolytic activity were determined (Section 3.6).

3.9.6 *Freeze-drying of Bulk Starter Cultures of Dahi and Yoghurt*

Cell biomass of dahi and yoghurt cultures (D, DAB, Y and YAB) after suspending in selected suspending medium was freeze-dried separately using vacuum freeze-drier model 30P.2.T, Edward, U.K.(Plate 2).

In different trials the chilled cell suspension prepared (Section 3.9.3) was distributed in rectangular stainless steel trays (53 x 23 x 2 cm^3) in 125 ml quantities and were placed in the chambers (at -15°C) of the freeze-drier with lids of the

plates partially displaced. The temperature of the chamber was allowed to lower to -20°C whereas the condenser maintained at -40°C. The vacuum pump was switched on and the pressure was maintained at 0.01 mm Hg. After completing the primary drying, the refrigerator of the chamber was switched off and the temperature was allowed to rise upto 10°C slowly by switching on the heater and care was taken to prevent frothing. The instrument was switched off after observing complete dryness in the starter concentrate and the trays were removed from the chamber for packing purpose.

3.9.7 *Packaging of Bulk Freeze-dried Starter Cultures*

Twenty five grams quantity of freeze-dried starter cultures of dahi (D and DAB) and yoghurt (Y and YAB) were packed in sterilized laminated aluminium foil pouches (Plate.3), under aseptic conditions. The sealing of the pouches was done under vacuum (Quick 2000 vacuum sealer) and in the atmosphere of air.

3.10 Stability of Bulk Freeze-dried Starter Cultures of Dahi and Yoghurt during Storage:

The sealed pouches of dahi and yoghurt cultures were stored at 4° and -20°C for a period of three months. The cultures stored at 4°C were analysed once in a fortnight whereas the cultures stored at -20°C were subjected to analysis after every one month.

The microscopic examination of the cultures was performed by Gram staining (Section 3.4) and the viability of cultures was determined (Section 3.8.2). The titratable acidity, activity test, diacetyl/acetaldehyde content, proteolytic activity and the residual moisture content were determined (Section 3.6).

Plate.2. Working with Edwards Vacuum Freeze-drier (Model 30.P.2T)

Plate.3. Laminated aluminium foil pouches packed with bulk freeze-dried cultures of dahi and yoghurt

3.11 Predicting the Shelf life of Bulk Freeze-dried Starter Cultures

Storage studies data (Section3.10) of freeze-dried cultures with regard to viable cell count and the biochemical properties of the cultures were subjected to multiple regression analysis given by Senedcor and Cochran (1967). The data were fitted to the following models in order to find out the best model.

1. $Y = A + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + B_5X_5 + B_6X_6$

2. $Y = AX_1^{B1}X_2^{B2}X_3^{B3}X_4^{B4}X_5^{B5}X_6^{B6}$

The best suited model was chosen based on maximum R^2 values (>0.85).

3.12 Use of Bulk Freeze-Dried Starter Cultures in Dahi and Yoghurt Preparation

Dahi (plain) and yoghurt (sweetened) were prepared in laboratory by direct inoculation of the milk with respective freeze-dried cultures. The products prepared using liquid cultures served as controls. All the experimental and control samples were analysed for biochemical properties (Section 3.12.5) and sensory evaluation (3.12.6).

3.12.1 *Determination of Inoculum Size*

For the standardization trials, the bulk freeze-dried starter cultures of dahi and yoghurt were inoculated into the heat treated (90°C for 15') and cooled (30°C) buffalo milk (4% fat) at levels varying from 0.001 to 0.10% (w/v) and 1.0% (v/v) for liquid cultures (control) and incubated at 30°C for culture D and at 37°C for cultures DAB, Y and YAB till the milk has set. The curds were examined for body and texture and the titratable acidity (Section 3.6.1) was estimated in order to determine the

required inoculum size for the preparation of dahi and yoghurt using freeze-dried starter cultures.

3.12.2 *Activity and Biochemical Performance of Bulk Freeze-Dried Cultures of Dahi and Yoghurt*

The activity and biochemical performance of bulk freeze-dried cultures of dahi and yoghurt were evaluated as per the following procedure.

Skimmed cow milk, standardized cow milk, skimmed buffalo milk (4.0% fat) and standardized buffalo milk (4.0% fat), were distributed in 100 ml quantities in conical flasks of 250 ml capacity and sterlized at 15 psi for 15 min. After cooling all the milk samples were inoculated separately with 0.01 % (w/v) of freeze-dried cultures of dahi and yoghurt (D, DAB, Y and YAB) and incubated at 30°C for D and 37°C for rest of the cultures till the curd has set. The curd samples were subjected to activity test, titratable acidity, volatile acidity, diacetyl/acetaldehyde content and proteolytic activity (Section 3.6).

3.12.3 *Preparation of Dahi*

Each lot of two litres of standardized milk (4% fat) was heated at 90°C for 15min. After cooling to room temperature, one lot of milk was inoculated with 1% of respective liquid cultures and another with freeze-dried cultures of dahi @ 0.01%. The samples were incubated at 30°C for culture D and at 37°C for culture DAB till the milk has set and transferred to refrigerator to cool the product to 5^{o}C.

3.12.4 *Preparation of Yoghurt*

The solids-not-fat content of standardized buffalo milk (4.0% fat) was increased to 13-14% by adding spray-dried skim milk powder. To this, cane sugar was added @ 8% and subjected to homogenization at a pressure of 100 kg/cm^2. The milk samples were

heat treated at 90°C for 15 min and cooled to room temperature. Then the yoghurt milk mix was inoculated with liquid cultures @ 1.0% or freeze-dried yoghurt cultures @ 0.01% and distributed in 100 ml plastic cups having lids. Samples were incubated at 37°C and cooled to 5°C after the curd has set.

3.12.5 *Lactic Count and Biochemical Quality of Dahi and Yoghurt*

All the samples of dahi and yoghurt were subjected to biochemical analysis i.e., titratable acidity, volatile acidity, diacetyl/acetaldehyde content and proteolytic activity (Section 3.6). The viable counts of *Bif. bifidum* I and *Lb. acidophilus* R and lactic counts of dahi and yoghurt were determined (Section 3.8.2).

3.12.6 *Sensory Evaluation of Dahi and Yoghurt*

The appearance, body and texture and flavour of dahi and yoghurt samples were evaluated by subjecting it to sensory evaluation. The score card used for sensory evaluation is given in Appendix II. The scores obtained were subjected to statistical analysis using 2 way analysis of variance (ANOVA) method. The products were rated using the following scale.

Excellent : >18-20; Very Good : >16-18, Good : >14-16, Fair : >10-14, Poor : <10.

3.13 Nutritional and Therapeutic Values of Dahi and Yoghurt

Dahi and yoghurt (with and without *Bif. bifidum* I and *Lb. acidophilus* R) prepared by using the freeze-dried starter cultures were evaluated for their influence on weight gain and serum cholesterol level in experimental animals and antibacterial activity *in vitro* against pathogenic bacteria.

3.13.1 *Body Weight Gain and Serum Cholesterol Level of Albino rats*

The study was carried out on male albino rats (wistar). Thirty six healthy rats, weighing between 110-160 g were collected from the stock colony of Small Animal House of NDRI and housed individually in anodized aluminium cages and had free access to feed and water.

The fresh products of dahi and yoghurt, were prepared (Section 3.12.3 and 3.12.4) to feed the animals. The standardized buffalo milk, dahi and yoghurt used for feeding were analysed for titratable acidity, fat and cholesterol content (Sections 3.6.1, 3.7.1 and 3.7.2), respectively.

The experimental animals were divided into six groups with six rats in each group. On day 'zero', blood samples from the rats were collected by eye puncture and the cholesterol content was estimated. All the animals of six groups were fed in the following way upto a period of 30 days.

Group	Products fed (per day basis)
C	Rat chew *ad lib.*
B	Buffalo milk (4% fat) 25g+Rat chew *adlib.*
D	Dahi prepared with culture D 25g+Rat chew *ad lib.*
DAB	Dahi prepared with culture DAB 25g+Rat chew *ad lib.*
Y	Yoghurt prepared with culture Y 25g+Rat chew *ad lib.*
YAB	Yoghurt prepared with culture YAB 25g+Rat chew *ad lib.*

Control and experimental rats were weighed at weekly intervals and blood samples were collected at intervals of 15 days. The serum was separated from the clotted blood by centrifugation at 3000 rpm and stored at $4^{o}C$ until the assay. The serum cholesterol content was estimated (Section 3.7.2).

The data obtained were subjected to analysis of variance given by Senedcor and Cochran (1967).

3.13.2 *Determination of Antibacterial Activity of Dahi and Yoghurt*

A) Test organisms:

The following organisms were used as test organisms to observe the antibacterial activity of dahi and yoghurt (with and without *Bif. bifidum* I and *Lb. acidophilus* R) samples prepared using bulk freeze-dried starter cultures.

Escherichia coli	K-12
Salmonella typhimurium	A-KIGM
Shigella dysenteriae	KIGM

B) Seeding of agar plates:

Active cultures of all the test organisms were inoculated into BHI broth individually at the rate of 1% level and incubated at 37°C for 16-18 hrs. The cells were collected aseptically by centrifugation at 3000 rpm for 30 min. The bacterial cells thus collected were washed twice with normal saline and the cell concentration was adjusted to 50% transmittance at a wavelength of 430 nm with normal saline using spectrophotometer (Spectrocol, Model CL:23, Elico make). BHI agar was melted and cooled to 45±2°C and 20 ml of media was added to sterilized flat bottom petri dishes on a level platform and allowed to solidify. The petri dishes were then transferred to a refrigerator for one hour to facilitate the boring of wells into the poured medium. The plates were moved to an incubator at 37°C and kept for one hour to make the plates dry after spreading 0.5 ml of active cell suspension of test organisms individually on the agar surface uniformly.

C) Preparation of cell-free filtrates

Dahi and yoghurt (with and without *Bif. bifidum* and *Lb. acidophilus*) were individually transferred to 6" x 1" test tubes. They were centrifuged at 3000 rpm for 20 min and the clear supernatant layer was transferred to other sterile tubes. The supernatant material was then passed through seitz filter and the cell free material was kept in the refrigerator until use.

The control filtrate was prepared by adding lactic acid in the sterilized buffalo milk (fat 4.0%) to achieve 2% lactic acid. This was then centrifuged for 20 min. at 3000 rpm and the pH of the supernatant was adjusted to the pH of the cell-free filtrates.

D) Modified agar cup assay method

With the help of a sterile glass borer, five wells (6 mm diameter) were made on the surface of the medium in each plate taking aseptic precautions. One hundred microlitres of cell-free filtrates of all the four cultured products were dispensed into different wells, whereas the fifth well was filled with 0.1 ml of control filtrate.

The plates were kept undisturbed for 1 hr in order to ensure uniform diffusion of the filtrates into the medium and incubated without inverting at 37^{o}C for 24 hrs and the diameter of inhibitory zones was measured with the help of Vernier calipers and expressed in terms of millimeters inclusive of the well diameter.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

Keeping in view the non-availability of good culture combinations for the preparation of dahi and yoghurt in our country, the formulation of starter cultures to ensure good quality products assumes great significance. The strains of *Bif. bifidium* and *Lb. acidophilus* are well known for their therapeutic values, and the addition of these organisms to starter culture combinations greatly helps in improving the therapeutic value of the products. In the absence of information on the associative growth pattern of the therapeutic organisms and the lactic cultures used in the preparation of dahi and yoghurt, the present study plays an important role in revealing the nature of associative growth between these cultures.

Another aim of this study was to prepare the promising mixed cultures, in a ready-to-use form, for the commercial manufacture of dahi and yoghurt. This will ensure uniform quality of the products, besides eliminating the tedious process of culture propagation. The present study, the first of its kind in India has great relevance to the Indian dairy industry.

In this chapter the results on the formulation of culture combinations, their growth pattern in association with therapeutic cultures; selection of growth medium and optimization of growth conditions, i.e. pH, neutralizer, temperature, rate of inoculum; freeze-drying of starter concentrate and its stability during storage, the preparation of dahi and yoghurt and their therapeutic values, viz. effect on body weight gain, serum cholesterol level in rats and *in vitro* antibacterial activity are presented and discussed.

4.1. Formulation and Selection of Dahi and Yoghurt Cultures

4.1.1 *Selection of Formulated Culture Combinations*

The performance of different laboratory formulated cultures prepared with various single strain lactic cultures of dahi, yoghurt and therapeutic cultures (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) is presented in Tables 1 and 2. The parameters considered were body and texture, titratable acidity and proteolytic activity for all the combinations and the Voges-Proskauer reaction for flavour development by formulated dahi culture.

It is evident from Table 1 that among the various culture combinations, maximum acid development (0.92% lactic acid) was noted with the combination No.3 followed by the combination No.1 with 0.89% acidity. Lowest acid development was recorded with the combination No.2 with 0.75% titratable acidity in buffalo skim milk after 16 hrs of incubation at 30°C.

The body and texture of the curd samples prepared with different combinations varied from firm to hard and some appeared to be glossy while others had dull appearance.

The proteolytic activity ranged from 0.25 mg of tyrosine released per g curd in combination No.4 to 0.43 mg in combination No. 3. With regard to flavour development, none of the combinations except No.3 showed a significant positive result with Voges-Proskauer reaction.

Therefore, the combination of *Lc. lactis* ssp. *Lactis* biovar. *diacetylactis* DRC1+ *Lc. lactis ssp. cremoris* C1 + *Lc. lactis* ssp. *lactis* C10 was adjudged as the best combination for dahi preparation.

Various cultures of yoghurt were evaluated similarly in skimmed buffalo milk after 16 hrs of incubation at 37°C (Table-

Table-1: Changes produced in Titratable Acidity, Proteolytic Activity, Voges-Proskauer reaction and Body & Texture of buffalo skim milk curd, inoculated with various dahi culture combinations added @ 1%, after 16 hrs of incubation at 30°C

Combination No.	Formulated culture (in 1:1:1 ratio)	% Titratable acidity (expressed as lactic acid)	Proteolytic activity (mg tyrosine/g)	Voges - proskauer reaction	Body & texture of the curd
I	*Lactococcus lactis* ssp. *lactis* C10 *Lc. lactis* ssp. *cremoris* H *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC1	0.89	0.32	+	Semi glossy and semi firm
II	*Lc. lactis* ssp. *lactis* ML8 *Lc. lactis* ssp. *cremoris* C1 *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC1	0.75	0.28	++	Semi glossy and firm
III	*Lc. lactis* ssp. *lactis* C10 *Lc. lactis* ssp. *cremoris* C1 *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC1	0.92	0.43	+++	Glossy and firm
IV	*Lc. lactis* ssp. *lactis* ML8 *Lc. lactis* ssp. *cremoris* H *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC1	0.78	0.25	++	Glossy and semi firm
V	*Lc. lactis* ssp. *lactis* C10 *Lc. lactis* ssp. *cremoris* H *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC2	0.80	0.26	++	Dull and semi firm
VI	*Lc. lactis* ssp. *lactis* ML8 *Lc. lactis* ssp. *cremoris* C1 *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC2	0.76	0.30	++	Glossy and hard
VII	*Lc. lactis* ssp. *lactis* C10 *Lc. lactis* ssp. *cremoris* C1 *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC2	0.86	0.36	++	Semi glossy and semi firm
VIII	*Lc. lactis* ssp. *lactis* ML8 *Lc. lactis* ssp. *cremoris* H *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC2	0.78	0.29	+	Dull and semi firm

Values are averages of five trials in duplicate

Table-2: Changes produced in Titratable Acidity, Proteolytic Activity and Body & Texture of buffalo skim milk curd inoculated with yoghurt cultures and combinations of acidophilus and bifidobacteria @ 1% after 16 hrs of incubation at 37°C

Combination No.	Formulated culture (in 1:1:1 ratio)*	% Titratable acidity (expressed as lactic acid)	Proteolytic activity (mg tyrosine/g)	Body & texture of of the curd
I	*Streptococcus salivarius* ssp. *thermophilus* HST *Lactobacillus delbrueckii* ssp. *bulgaricus* LBM	1.52	0.26	Dull and semi firm
II	*Str. salivarius* ssp. *thermophilus* HST *Lb. delbrueckii* ssp. *bulgaricus* R15	1.64	0.40	Glossy and firm
III	*Str. salivarius* ssp. *thermophilus* I *Lb. delbrueckii* ssp. *bulgaricus* R15	1.55	0.24	Semi glossy and semi firm
IV	*Str. salivarius* ssp. *thermophilus* I *Lb. delbrueckii* ssp. *bulgaricus* LBM	1.58	0.28	Dull and firm
A	*Lb. acidophilus* R *Bifidobacterium bifidum* I	1.52	0.24	Glossy and firm
B	*Lb. acidophilus* 111 *Bif. bifidum* I	1.40	0.18	Semi glossy and semi firm

The values are averages of five trials in duplicate.

* *Lb. acidophilus* and *Bif. bifidum* are in 1:3 ratio.

2). It is interesting to note that yoghurt culture combination II produced maximum acidity of 1.64% lactic acid, which is comparable to the results of Jain (1979) and Sharma (1982). Maximum proteolytic activity and good body and texture were also recorded with this combination. Hence, yoghurt culture combination II was considered the best.

The therapeutic culture combinations were formulated using the strains of *Lb. acidophilus* and *Bif. bifidum*. The growth rate of the latter culture is slow in milk, so to attain sufficiently large populations in the product the ratio of the two cultures in the inoculum was kept at 1:3 as advocated by Klupsch (1989). Out of the two combinations formulated with therapeutic cultures, the combination of *Lb. acidophilus* R + *Bif. bifidum* I produced more titratable acidity (1.52% lactic acid) and showed maximum proteolytic activity compared with the other combination. The body and texture of the former combination was more firm and glossy than that of the latter. Therefore, the same was selected for further studies along with the selected dahi and yoghurt cultures.

4.1.2 *Viability and Biochemical Performance of Selected Cultures of Dahi and Yoghurt in Buffalo Skim Milk*

Results on the associative growth between the therapeutic culture and that of dahi or yoghurt cultures, with regard to viable cell counts, titratable acidity, volatile acidity, diacetyl or acetaldehyde content and proteolytic activity in buffalo skim milk are depicted in Tables 3 to 7 and Figures 5 to 11. Variation was noted in the biochemical activity of dahi and yoghurt cultures when grown together with both *Bif. bifidum* I and *Lb. acidophilus* R.

a) Viable Cell Count

Viable cell counts increased upto 18 hrs of incubation but

with further incubation the count decreased considerably (Table-3, Fig. 5 and 6).

The lactic count of dahi culture combination DAB after 24 hrs was comparatively less (7.450 log cfu/g) when incubated at 37°C than when grown at 30°C (7.799 log cfu/g) which has indicated the necessity of optimum temperature for getting maximum viable cell counts. The counts of *Bif. bifidum* I and *Lb. acidophilus* R of dahi culture DAB was maximum at an incubation temperature of 37°C compared to the incubation at 30°C.

A difference of nearly one log cell count of lactic culture counts (*Lc. lactis* ssp. *lactis* C10 + *Lc. lactis* ssp. *cremoris* C1 + *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC1) was observed between dahi cultures D and DAB, after 24 hrs of incubation at 30°C. This can be attributed to the presence of *Lb. acidophilus*, a comparatively high acid producing culture in the latter combination, which might have affected the growth of lactic cultures. This effect was more prominent when grown at 37°C where the growth rate of *Lb. acidophilus* was optimum. The viable cell counts of both *Lb. acidophilus* and *Bif. bifidum* were high when incubated at 37°C than at 30°C which indicated the need for incubation at 37°C to achieve higher counts of these cultures and ensure adequate therapeutic values.

The observations pertaining to yoghurt culture combinations (Table 3 and Fig.6) indicated a very slight decrease in viable cell count of lactic cultures (Str. *salivarius* ssp. *thermophilus* HST + *Lb. delbrueckii* ssp. *bulgaricus* RTS) in yoghurt culture YAB than in Y after 18 hrs of incubation at 37 °C. The decreasing effect was augmented on further incubation.

The viable cell counts of therapeutic cultures especially of *Bif. bifidum* I were comparatively less in YAB (7.079 log cfu/g) than in dahi culture DAB (7.505 log cfu/g), after 24 hrs at 37°C which may be due to the presence of high acid producing cultures

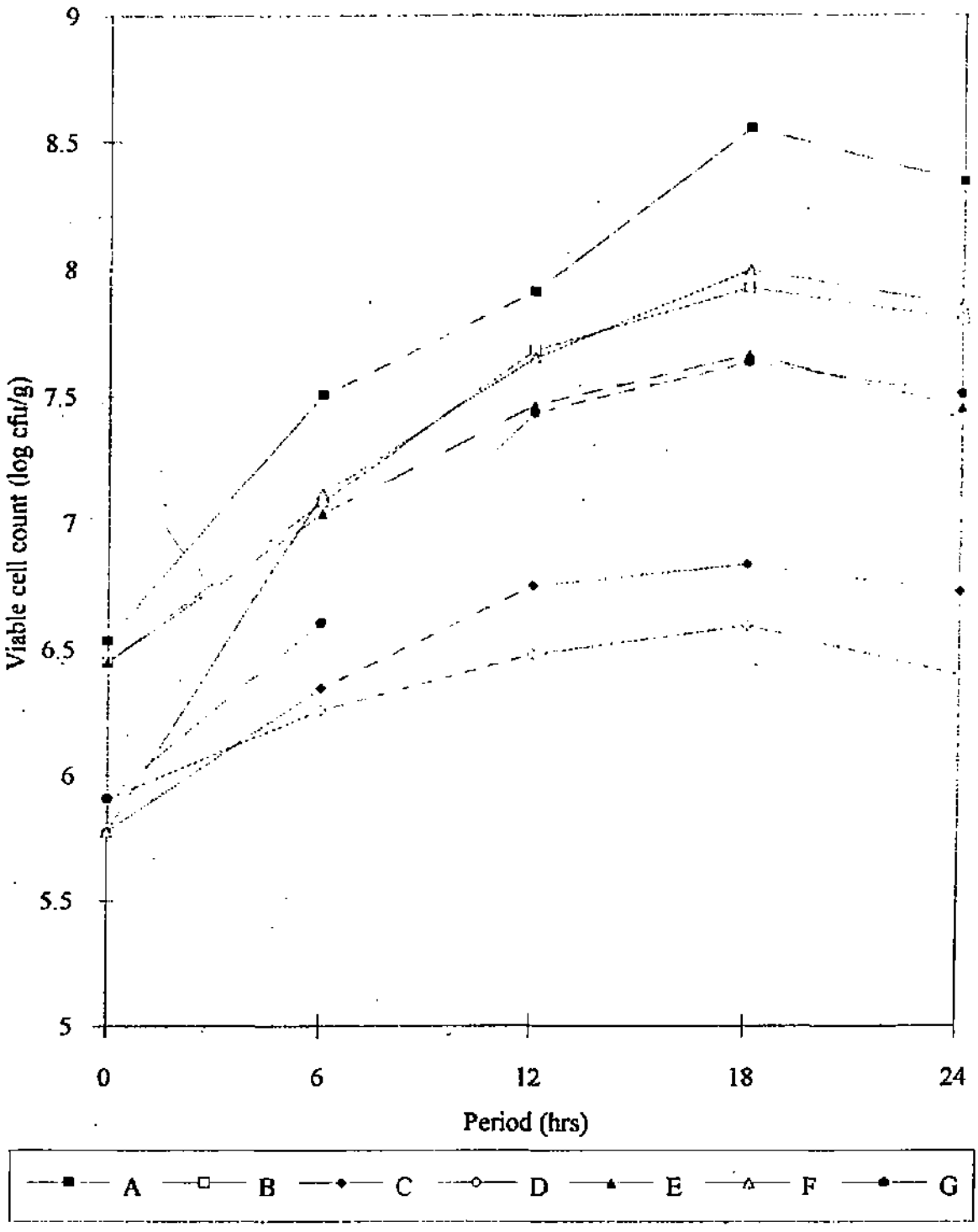
Table-3: Viable cell counts of selected cultures of dahi and yoghurt in buffalo skim milk at different periods of incubation

Sl. No.	Culture combinations	Growth temperature (°C)	Cultures	Incubation period (hrs)				
				0	6	12	18	24
				Viable cell count (log cfu/g)				
1.	Dahi culture D	30	Lactic cultures	6.553	7.505	7.913	8.556	8.342
2.	Dahi culture DAB	30	Lactic cultures	6.447	7.079	7.681	7.926	7.799
			*Lb. acidophilus**	5.778	6.342	6.748	6.832	6.724
			*Bif. bifidus**	5.903	6.255	6.477	6.591	6.397
3.	Dahi culture DAB	37	Lactic cultures	6.447	7.033	7.462	7.664	7.450
			Lb. acidophilus	5.778	7.113	7.653	7.944	7.857
			Bif. bifidus	5.903	6.602	7.431	7.633	7.505
4.	Yoghurt culture Y	37	Lactic cultures	6.692	7.690	7.973	8.643	8.447
5.	Yoghurt culture YAB	37	Yoghurt cultures	6.662	7.447	7.724	8.623	8.380
			Lb. acidophilus	5.812	7.146	7.505	7.799	7.681
			Bif. bifidus	5.924	6.078	6.779	7.255	7.079

The values are averages of five trials in duplicate.
*The colony counts were taken from the same LA agar plate

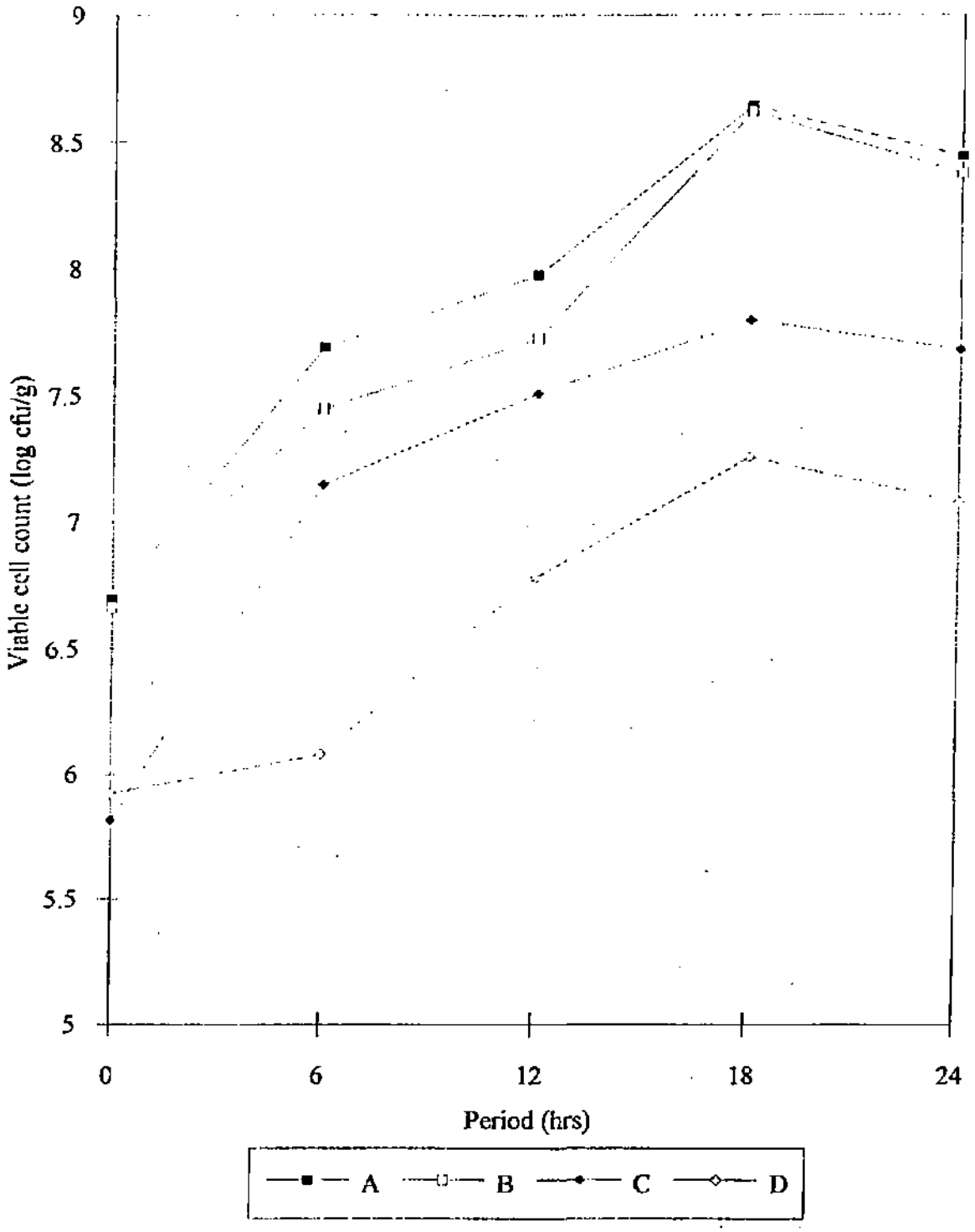
A	Lactic count of combination D
B	Lactic count of combination DAB grown at 30° C
C	*Lb. acidophilus* count of combination DAB grown at 30° C
D	*Bif. bifidum* count of combination DAB grown at 30° C
E	Lactic count of combination DAB grown at 37° C
F	*Lb. acidophilus* count of combination DAB grown at 37° C
G	*Bif. bifidum* count of combination DAB grown at 37° C

Fig. 5 Viable cell counts of dahi cultures D and DAB in buffalo skim milk.



A	Lactic count of combination Y
B	Lactic count of combination YAB
C	*Lb. acidophilus* count of combination YAB
D	*Bif. bifidum* count of combination YAB

Fig. 6 Viable cell counts of yoghurt cultures Y and YAB in buffalo skim milk.



of yoghurt whose acidity might have hampered the growth of these cultures.

b) **Titratable Acidity**

The titratable acidity of buffalo skim milk curds prepared with different cultures of dahi and yoghurt is presented in Table 4 and Fig.7. The titratable acidity produced after 18 hrs by dahi culture DAB at 30°C was slightly more than that produced by dahi culture D and less than the acidity produced by the same culture, when incubated at 37°C. This can be explained by the presence of *Lb. acidophilus* in the former combination. The optimum incubation temperature of 37°C for *Lb. acidophilus* might have led to more acid production by this particular culture combination. The rate of acid development by yoghurt culture Y decreased significantly with the addition of *Lb. acidophilus* and *Bif. bifidum*. The present observation is in agreement with Yu and Nakanishi (1975) who also recorded a decrease in titratable acidity on the addition of *Lb. acidophilus* to yoghurt culture.

c) **Volatile Acidity**

The pleasing flavour of dahi and yoghurt can be attributed to the optimum level of volatile acid production by the lactic acid bacteria. Variations have been observed with regard to volatile acid production between dahi culture combinations D and DAB (Table-5 and Fig.8). The addition of therapeutic cultures with dahi culture D drastically decreased the volatile acid production from 36.0 ml of 0.01 N NaOH to 15.8 ml after 18 hrs of incubation at 30°C. No significant difference in volatile acid production by dahi culture DAB was noted, irrespective of incubation temperature.

No significant change in the volatile acidity development was observed upon the addition of therapeutic cultures to yoghurt culture combination Y. Observations of Yu and Nakanishi (1975)

Table-4: Titratable acidity of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation

Sl. No.	Formulated culture	Incubation temperature (°C)	Incubation period (hrs)		
			6	12	18
			% Titratable acidity (expressed as lactic acid)		
1.	Dahi culture D	30	0.56	0.72	0.93
2.	Dahi culture DAB	30	0.48	0.64	0.99
3.	Dahi culture DAB	37	0.66	0.82	1.18
4.	Yoghurt culture Y	37	0.91	1.42	1.68
5.	Yoghurt culture YAB	37	0.85	1.12	1.44

Values are averages of five trials in duplicate

A	Dahi Culture D at 30° C
B	Dahi culture DAB at 30° C
C	Dahi culture DAB at 37° C
D	Yoghurt culture Y at 37° C
E	Yoghurt culture YAB at 37° C

Fig. 7 Titratable acidity production by dahi and yoghurt cultures in buffalo skim milk.

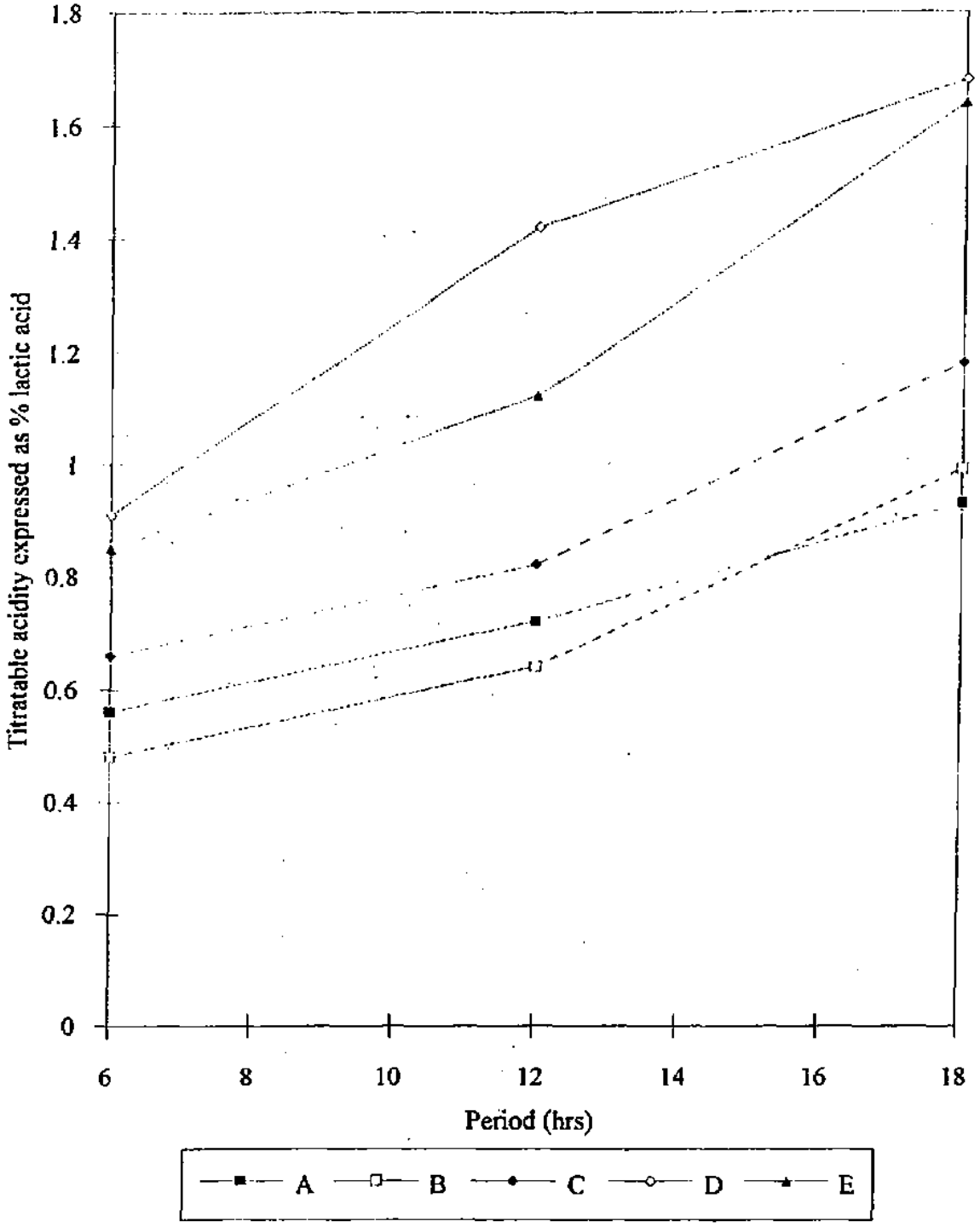


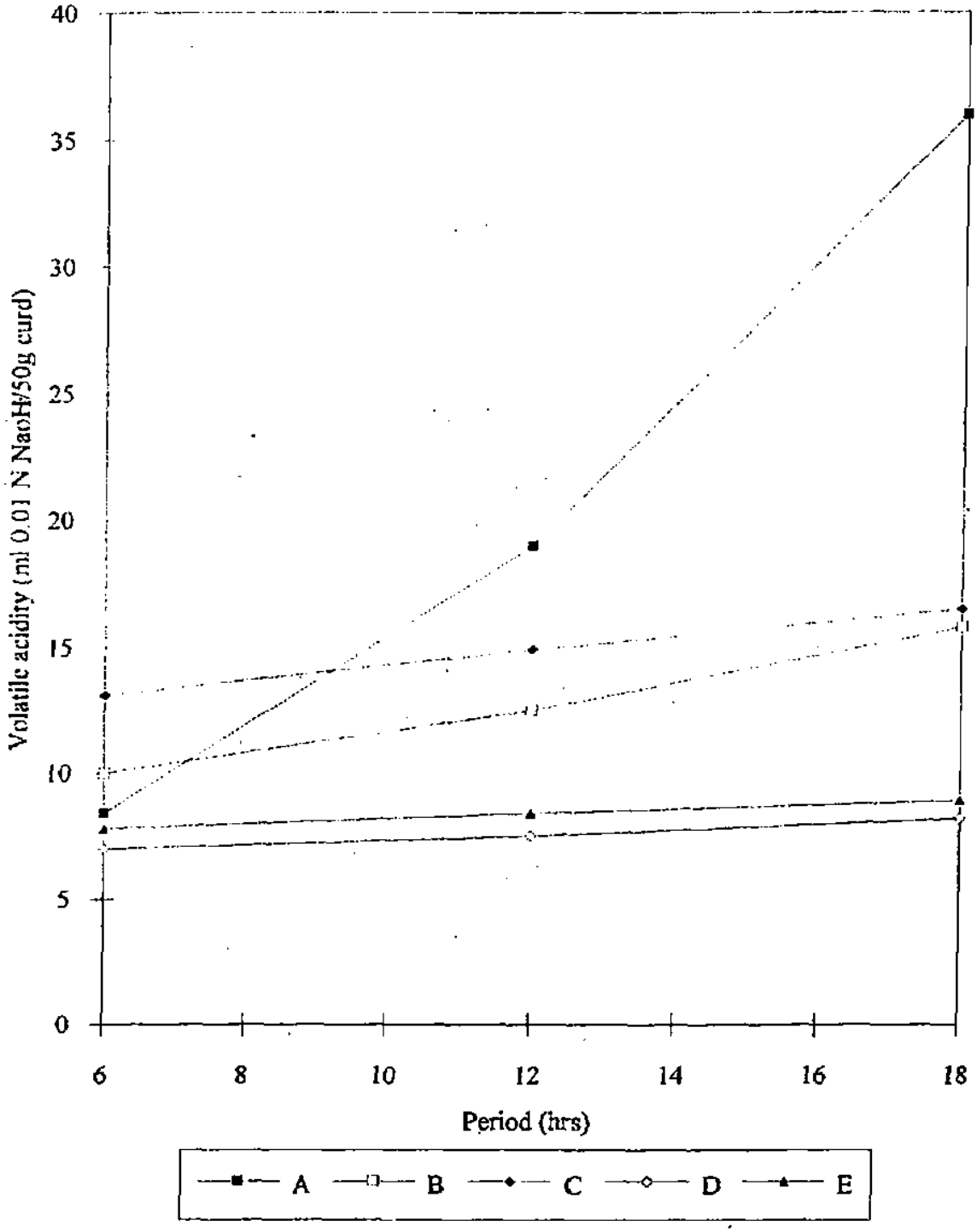
Table-5: Volatile acidity of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation

Sl. No.	Formulated culture	Incubation temperature (°C)	Incubation period (hrs)		
			6	12	18
			Volatile acidity (expressed as ml of 0.01N NaoH used/50g curd)		
1.	Dahi culture D	30	8.4	19.0	36.0
2.	Dahi culture DAB	30	10.0	12.5	15.8
3.	Dahi culture DAB	37	13.1	14.9	16.5
4.	Yoghurt culture Y	37	7.0	7.5	8.2
5.	Yoghurt culture YAB	37	7.8	8.4	8.9

Values are averages of five trials in duplicate

A	Dahi Culture D at 30° C
B	Dahi culture DAB at 30° C
C	Dahi culture DAB at 37° C
D	Yoghurt culture Y at 37° C
E	Yoghurt culture YAB at 37° C

Fig. 8 Volatile acidity production by dahi and yoghurt cultures in buffalo skim milk.



and Sharma and Singh (1982) with mixed yoghurt cultures when grown along with *Lb. acidophilus* supported the present findings.

d) Flavour Production

The diacetyl content of buffalo skim milk curd samples prepared by dahi cultures D and DAB is presented in Table 6 and Fig.9. From the results, it is evident that the amount of diacetyl liberated by dahi culture D was reduced considerably from 4.05 ppm to 2.80 ppm with the addition of therapeutic cultures. With dahi culture DAB, no significant change in diacetyl content was observed with the change in incubation temperature from 30° to 37°C. The decrease in diacetyl production might be due to the difference in lactococci count as can be observed in Table 3.

The acetaldehyde content of buffalo skim milk curd prepared by yoghurt cultures Y and YAB is presented in Table 6 and Fig.10. Addition of *Lb. acidophilus* and *Bif. bifidum* to yoghurt cultures increased the acetaldehyde content from 28.4 ppm to 34.6 after 18hrs of incubation at 37°C. These findings are in agreement with the report of Gupta and Tiwari (1988). Similarly, Yuguchi *et al.* (1989) also found stimulation of production of acetaldehyde by yoghurt cultures supplemented with *Bif. bifidum* 15696.

e) Proteolytic Activity

There was a reduction in the tyrosine content liberated by dahi culture combination D with the addition of therapeutic cultures especially when incubated at 37°C (Table-7 and Fig.11). This may be due to the decrease in the viable count of lactococci as evidenced in Table 3. The effect was less intensive when dahi culture DAB was incubated at 30°C than at 37°C because of the high cell count of lactococci in this combination particularly in the former.

Table-6: Diacetyl/Acetaldehyde content of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation

Sl. No.	Formulated culture	Incubation temperature (°C)	Incubation period (hrs)		
			6	12	18
			Diacetyl content (ppm)		
1.	Dahi culture D	30	3.75	3.90	4.05
2.	Dahi culture DAB	30	2.30	2.62	2.88
3.	Dahi culture DAB	37	2.50	2.55	2.68
			Acetaldehyde content (ppm)		
4.	Yoghurt culture Y	37	24.80	26.20	28.40
5.	Yoghurt culture YAB	37	28.30	31.80	34.60

Values are averages of five trials in duplicate

Fig. 9 Diacetyl production by dahi cultures D and DAB in buffalo skim milk.

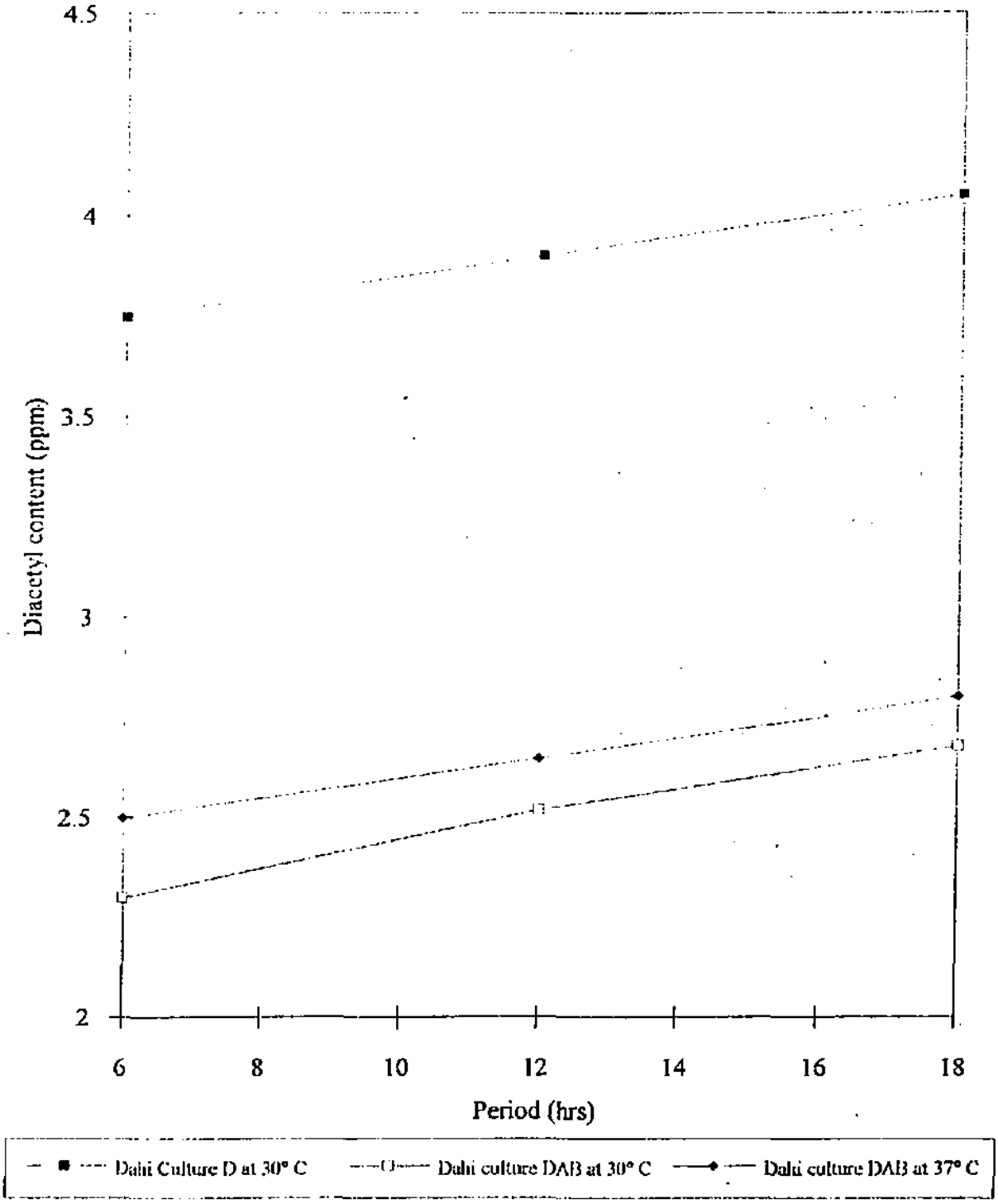


Fig. 10 Acetaldehyde production by yoghurt cultures Y and YAB in buffalo skim milk.

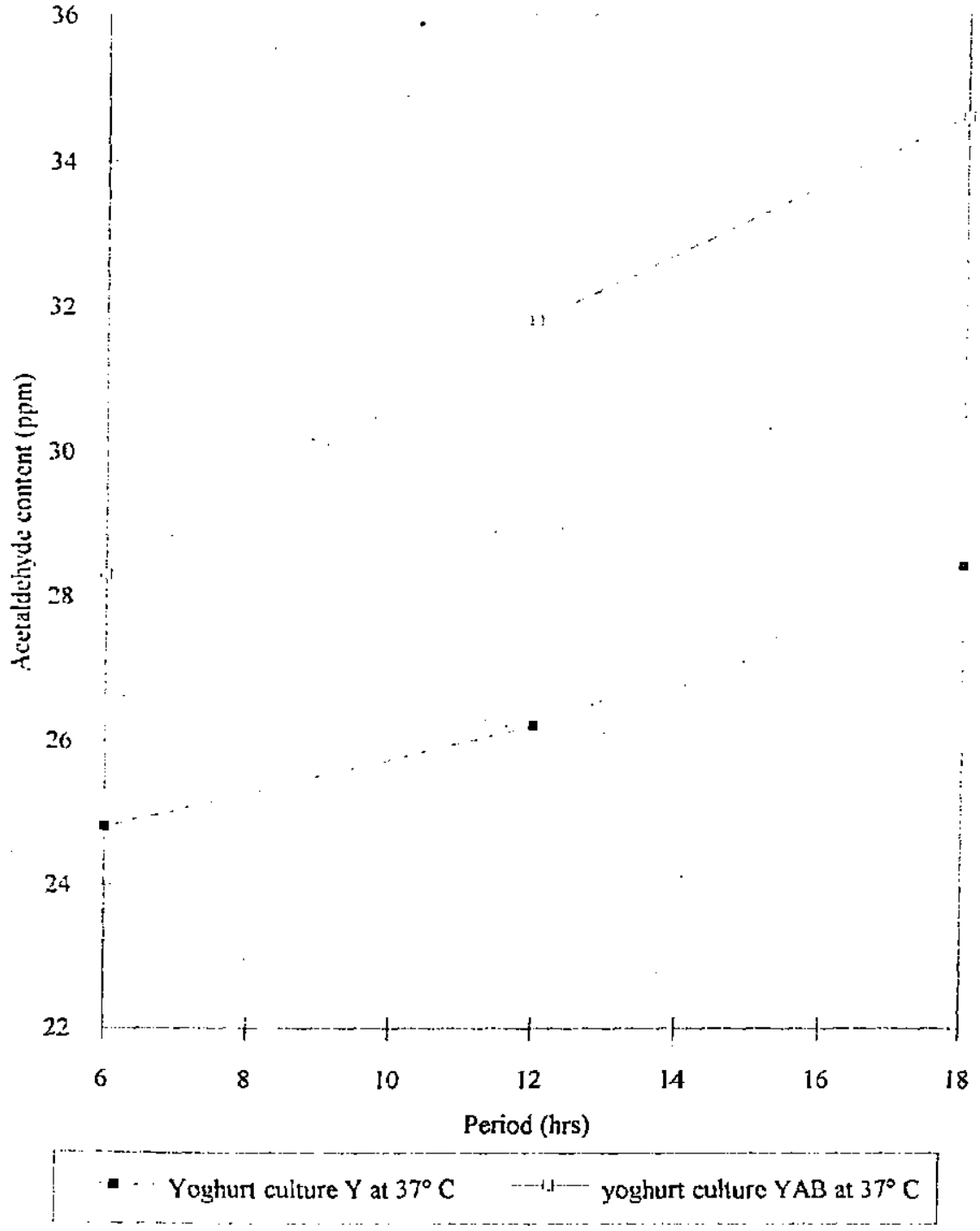


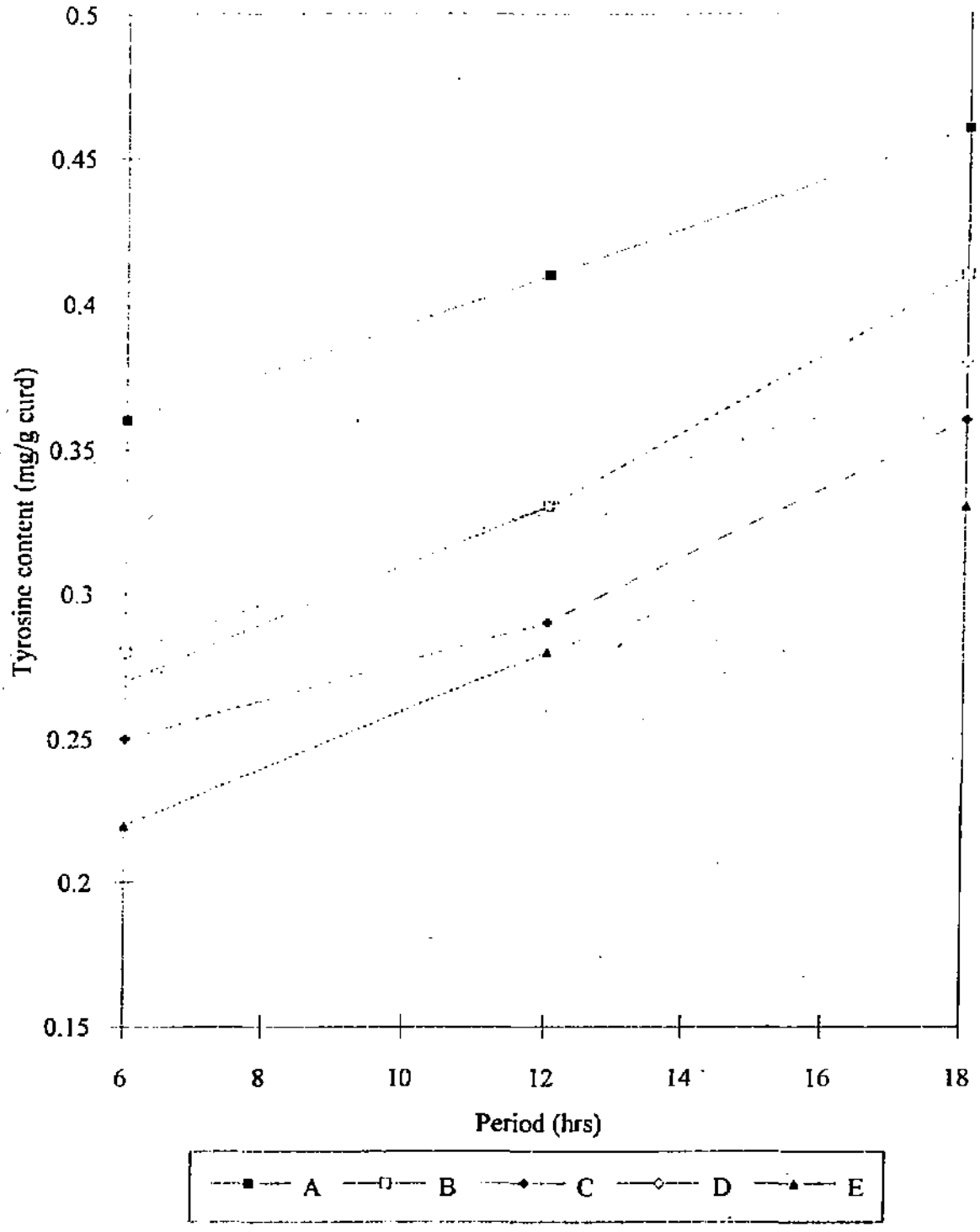
Table-7: Tyrosine content of buffalo skim milk curd inoculated with selected cultures of dahi and yoghurt at different periods of incubation

Sl. No.	Formulated culture	Incubation temperature (°C)	Incubation period (hrs)		
			6	12	18
			Proteolytic activity (mg tyrosine/g curd)		
1.	Dahi culture D	30	0.36	0.41	0.46
2.	Dahi culture DAB	30	0.28	0.33	0.41
3.	Dahi culture DAB	37	0.25	0.29	0.36
4.	Yoghurt culture Y	37	0.27	0.33	0.38
5.	Yoghurt culture YAB	37	0.22	0.28	0.33

Values are averages of five trials in duplicate

A	Dahi Culture D at 30° C
B	Dahi culture DAB at 30° C
C	Dahi culture DAB at 37° C
D	Yoghurt culture Y at 37° C
E	Yoghurt culture YAB at 37° C

Fig. 11 Proteolytic activity of dahi and yoghurt cultures in buffalo skim milk.



With the addition of therapeutic cultures in yoghurt culture combinations, no significant decrease in proteolytic activity was noticed and the present observation is in agreement with that of Sharma and Singh (1982) who have reported similar results with mixed yoghurt culture combination containing *Lb. acidophilus*.

4.2 Preparation of Bulk Freeze-dried Starter Cultures of Dahi and Yoghurt

4.2.1 *Selection of Growth Media*

A bacterial growth medium has to adequately support the growth by supplying all the essential nutrients required. When the medium has to be used commercially, its ingredients have to be cheap and its method of preparation should be convenient. Bacteria, in general, have flexible growth requirements. However, lactic acid bacteria are fastidious in this respect because of their weak biosynthetic machinery. Keeping this in view, a preliminary trial was undertaken to apprise the suitability of various buffalo skim milk and cheese whey based media for dahi and yoghurt cultures. Results with regard to the growth of formulated cultures of dahi and yoghurt in these media are presented in Tables-8 and 9, respectively.

Relatively better growth of dahi culture combination D and yoghurt culture combination Y, was observed in buffalo skim milk based media than in cheese whey based media. The cheese whey based media were equally good for dahi and yoghurt cultures added with the therapeutic cultures (*Lb. acidophilus* R + *Bif. bifidum* I) when grown at 37°C. However, the buffalo skim milk medium containing yeast autolysate and glucose was found best for dahi culture combinations D and DAB at 37°C as well as 30°C. Similarly, buffalo skim milk medium with tryptone and glucose was found to be good for both the yoghurt cultures Y and YAB.

The study was further continued using certain formulated media to choose the best medium with respect to each culture for

Table-8: Suitability of various formulated media for the growth of dahi cultures

Sl. No.	Formulated media	Dahi culture D 30°C	Dahi culture D 37°C	Dahi culture DAB 30°C	Dahi culture DAB 37°C
		Culture growth (as % transmittance at 600 nm after 18 hrs)			
A.	Skim milk based media:				
1.	Skim milk - 5% + Sodium citrate - 1% + $MgSO_4.7H_2O$ - 0.02%	98	99	98	98
2.	Skim milk - 5% + Yeast autolysate - 1% + Glucose - 1%	35	60	55	30
3.	Skim milk - 1000ml + Yeast extract - 0.01%	95	98	93	97
4.	Skim milk - 50% + Tryptone - 1% + Glucose - 0.1%	60	90	91	65
5.	Trypsin digested skim milk - 100 ml + Sodium acetate - 0.5% + Yeast extract - 0.5% + Glucose - 1.0%	90	92	98	96
B.	Cheese whey based media:				
1.	Cheese whey - 1000 ml + Skim milk - 10 ml + Yeast extract - 0.5%	90	95	93	65
2.	Cheese whey - 20% + Tryptone - 1% + Yeast extract - 0.05%	88	89	94	58
3.	Cheese whey - 1000 ml + Yeast extract - 0.5% + $MnSO_4.H_2O$-120 mg + $MgSO_4.7H_2O$ - 120 mg + $ZnSO_4$ - 120 mg	65	93	95	60
4.	Cheese whey - 10% + Sodium citrate - 2% + $MnSO_4.H_2O$ - 0.17% + Cysteine - 0.5% + Yeast extract - 0.5% + Glucose - 0.5%	96	95	98	96

Values are averages of six observations

Table-9: Suitability of various formulated media for the growth of yoghurt cultures

Sl. No.	Formulated media	Yoghurt culture Y 37°C	Yoghurt culture YAB 37°C
		Culture growth (as % transmittance at 600 nm after 18 hrs)	
A.	Skim milk based media		
1.	Skim milk - 75% + Sodium acetate - 3% + Yeast extract - 4.5%	55	94
2.	Skim milk - 9% + Cysteine - 0.075% + Ascorbic acid - 0.150% + Yeast extract - 1.5% + Tween 80 - 0.15%	95	55
3.	Skim milk - 5% + Yeast extract - 0.5% + Lactose - 0.5% + Sucrose - 0.25% + $MgSO_4$. 7 H_2O - 0.13% + K_2HPO_4 - 0.2%	92	65
4.	Trypsin digested skim milk - 100 ml + Sodium acetate - 0.5% + Yeast extract - 0.5% + Glucose - 1.0%	65	60
5.	Skim milk - 50% + Tryptone - 1.0% + Glucose - 0.1%	39	28
6.	Skim milk - 20 ml + Distilled water - 130 ml + Yeast autolysate - 1.0% + Glucose - 1.0%	98	96
B.	Cheese whey based media:		
1.	Cheese whey - 1000 ml + Yeast extract - 1% + Tween 80 - 0.1%	92	94
2.	Cheese whey - 1000 ml + Tryptone - 1% + Glucose - 1%	90	95
3.	Cheese whey - 10% + Yeast extract - 0.5% + glucose - 0.5% + Sodium citrate - 2.0% + $MnSO_4$. H_2O - 0.17% + Tween 80 - 0.1% + Cysteine - 0.5%	95	65

Values are averages of six observations

the preparation of culture concentrates for freeze-drying purpose. The results are tabulated in Tables 10 and 11 for dahi and yoghurt cultures, respectively.

Skim milk based media supported good growth when compared to whey based media (Table-10). Maximum viable cell count of dahi cultures in different media was attained after 18 hrs of incubation at respective temperatures. Further incubation for another two hours resulted in decrease of viable cell count in all the media excepting the whey medium containing tryptone and yeast extract, where slight increase in the viable cell count of dahi culture DAB was observed. The maximum viable cell count observed in skim milk supplemented with yeast autolysate (1.0%) and glucose (0.1%) was 9.087 and 9.155 log colony forming units per gram, respectively for dahi cultures D and DAB after 18 hrs of incubation.

The analyses of variance (ANOVA) are depicted in Appendices III and IV. The ANOVA revealed that the differences in the viable cell counts were significant ($P \leq 0.01$).

Skim milk (50%) supplemented with tryptone (1.0%) and glucose (0.1%) was the best medium for yoghurt cultures Y and YAB, which gave the maximum cell yield of 9.096 and 9.176 log cfu per gram, respectively after 16 hrs of incubation at 37°C (Table-11).

The results obtained with respect to viable cell counts of yoghurt cultures Y and YAB were subjected to least square analysis and the analyses of variance are presented in Appendices V and VI. The differences among the cell counts were found to be significant at 1% level.

The lowering trend in acid production by all cultures of dahi and yoghurt in whey media compared to that in skim milk media at different intervals of incubation was recorded (Tables 10 and 11). Maximum titratable acidity in skim milk medium

Table-10: Viable cell counts and Titratable acidity of dahi cultures in certain formulated media at different periods of incubation

Sl. No.	Formulted media	Incubation Period (hrs)							
		14	16	18	20	14	16	18	20
		Viable cell count (log cfu/g)				% Titratable acidity (expressed as lactic acid)			
		Dahi culture D (30°C)							
1.	Skim milk + Yeast autolysate + Glucose	8.763	8.991	9.087	9.049	0.73	0.82	0.89	0.92
2.	Trypsin digested skim milk + Sodium acetate + Glucose + Yeast extract	8.760	8.903	8.990	8.944	0.60	0.67	0.72	0.76
3.	Cheese whey + Yeast extract + $MnSO_4.H_2O$ + $MgSO_4.7H_2O$ + $ZnSO_4$	8.792	8.929	8.988	8.977	0.64	0.71	0.77	0.81
		Dahi culture DAB (37°C)							
1.	Skim milk + Yeast autolysate + Glucose	8.838	9.008	9.155	9.133	0.84	0.98	1.20	1.23
2.	Trypsin digested skim milk + Sodium acetate + Glucose + Yeast extract	8.857	8.925	9.002	8.982	0.80	0.88	0.96	0.98
3.	Cheese whey + Skim milk + Yeast extract	8.740	8.913	8.970	8.959	0.73	0.84	0.87	0.91
4.	Cheese whey + Tryptone + Yeast extract	8.812	8.980	8.994	8.962	0.75	0.87	0.91	0.93
5.	Cheese whey + Yeast extract + $MgSO_4.7H_2O$ + $MnSO_4.H_2O$ + $ZnSO_4$	8.707	8.942	8.938	8.892	0.74	0.84	0.89	0.94

Values are averages of six observations

Table-11: Viable cell counts and Titratable acidity of yoghurt cultures in certain formulated media at different periods of incubation

Sl. No.	Formulated media	Incubation Period (hrs)					
		14	16	18	14	16	18
		Viable cell count (log cfu/g)			% Titratable acidity (expressed as lactic acid)		
		Yoghurt culture Y					
1.	Skim milk + Sodium acetate + Yeast extract	8.771	8.891	8.840	1.28	1.36	1.43
2.	Trypsin digested skim milk + Sodium acetate + Yeast extract + Glucose	8.868	8.960	8.934	1.32	1.41	1.49
3.	Skim milk + Tryptone + Glucose	8.990	9.096	9.040	1.44	1.52	1.64
		Yoghurt culture YAB					
1.	Skim milk + Cysteine + Ascorbic acid + Yeast extract + Tween 80	8.732	8.831	8.968	1.18	1.22	1.28
2.	Trypsin digested skim milk + Sodium acetate + yeast extract + glucose	8.944	8.969	8.919	1.12	1.24	1.28
3.	Skim milk + Tryptone + Glucose	9.000	9.176	9.141	1.28	1.32	1.48
4.	Cheese whey + Yeast extract + Glucose + Sodium citrate + $MnSO_4$. H_2O + Tween 80 + Cysteine	8.826	8.866	8.813	0.98	1.12	1.20

Values are averages of six observations.

supplemented with yeast autolysate and glucose was noted with dahi cultures D and DAB. The difference in acidity between these cultures can be attributed to the presence of *Lb. acidophilus* which is generally a high acid producer in the latter combination.

Titratable acidity of 1.64 and 1.48 (% L.A) was observed with yoghurt culture combinations Y and YAB, respectively in skim milk medium supplemented with tryptone and glucose, after 16 hrs of incubation. The lower acidity production by the latter combination may be due to the associative growth effect of *Lb. acidophilus* R and *Bif. bifidum* I with the yoghurt cultures as evidenced by Singh *et al.* (1982) and Kisza *et al.* (1978) who observed the ability of *Lb. acidophilus* and *Bif. bifidum* to reduce the acid development by yoghurt cultures.

Out of several media tested, skim milk supplemented with yeast autolysate and glucose was selected for the growth of dahi cultures D and DAB whereas skim milk medium containing tryptone and glucose was chosen for the growth of yoghurt cultures Y and YAB as these media were found to support maximum cell growth and titratable acidity development.

Christopherson and Zottola (1989) suggested ultra-filtered skim milk medium for *Lc. lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris* and *Lc. lactis* ssp. *lactis* biovar. *diacetylatis*. Prior to that Petterson (1975) recommended the use of sterilized reconstituted skim milk medium for the growth of mixed species mesophilic lactic cultures. Kapoor (1976) formulated a medium for the growth of yoghurt symbiont cultures, however, it did not receive much attention due to its complexity. Later a simplified medium was proposed for the production of bacterial concentrate of yoghurt cultures (Kondratenko and Kondareva, 1978) using diluted skim milk containing yeast extract and sodium acetate. In the present study, the objective was to formulate a simple medium which is convenient for common usage. A high cell count of yoghurt cultures was achieved by using a simple medium prepared

using skim milk fortified with tryptone and glucose as can be seen in Table 11. The use of skim milk medium has another advantage of minimizing the lag phase of cultures as proposed by Speckman *et al.* (1974) and the problem associated with whey media centrifugation, employed for the purpose of clarification, which is time-consuming and costly affair, is also eliminated.

4.2.2 *Optimization of Growth Conditions*

In order to maximize the cell population and activity in the starter concentrate for freeze-drying, experiments were conducted to determine the optimum conditions with respect to pH of the medium, neutralization of the acidity developed, temperature of incubation and rate of inoculation for all the combinations of dahi and yoghurt cultures in respective selected formulated medium.

4.2.2.1 Effect of pH of the growth medium

All the combinations of dahi and yoghurt cultures grew well in the pH range of 6.0 to 7.0 (Table-12). However, maximum viable cell count and titratable acidity with dahi cultures D and DAB were observed at pH 6.5 rather than at pH 6.0 or 7.0. In both the cases significantly ($P \leq 0.01$) high viable cell counts were achieved nearly after 18 hrs of incubation at their optimum growth temperatures 30° and 37°C, respectively (Appendices III and IV).

It may be inferred from the results (Table-13) that a pH of 6.0 to get a high cell count, was favoured by yoghurt culture Y, whereas pH 6.5 was proved to be good to achieve maximum viable cell count of yoghurt culture YAB, after 16 hrs of incubation at 37°C. The counts were highly significant at 1% level (Appendices V and VI).

The findings are in accordance with that of the earlier reports of Cogan *et al.* (1971) and Speckman (1975) who have

Table-12: Influence of pH of the selected growth medium (skim milk + yeast autolysate + glucose) on viable cell count and titratable acidity of dahi cultures at different periods of incubation

Sl. No.	Formulated culture	Inoculum level (%)	Growth medium pH	Incubation Period (hrs)							
				0	14	16	18	0	14	16	18
				Viable cell count (log cfu/g)				% Titratable acidity (expressed as lactic acid)			
1.	Dahi culture D (30 °C)	1.0	6.0	6.301	7.255	8.568	8.687	0.14	0.65	0.78	0.83
			6.5	6.477	8.646	9.278	9.246	0.16	0.76	0.88	0.96
			7.0	6.447	8.322	8.964	8.812	0.15	0.70	0.82	0.92
2.	Dahi culture DAB (37 °C)	1.0	6.0	6.590	7.813	8.947	8.806	0.15	0.72	0.88	0.94
			6.5	6.698	8.906	9.285	9.222	0.16	0.79	0.98	1.26
			7.0	6.770	8.598	8.963	8.903	0.16	0.74	0.90	1.12

Values are averages of six observations

Table-13: Influence of pH of the selected growth medium (skim milk + tryptone + glucose) on viable cell count and titratable acidity of yoghurt cultures at different periods of incubation at 37°C

Sl. No.	Formulated culture	Inoculum level (%)	Growth medium pH	Incubation Period (hrs)							
				0	14	16	18	0	14	16	18
				Viable cell count (log cfu/g)				% Titratable acidity (expressed as lactic acid)			
1.	Yoghurt culture Y	1.0	6.0	6.514	8.547	9.462	9.481	0.16	1.34	1.56	1.62
			6.5	6.612	8.610	9.102	9.084	0.17	1.22	1.48	1.56
			7.0	6.716	8.779	8.954	8.886	0.17	1.27	1.44	1.58
2.	Yoghurt culture YAB	1.0	6.0	6.241	8.554	8.864	8.921	0.14	1.07	1.12	1.18
			6.5	6.232	8.870	9.291	9.155	0.15	1.22	1.38	1.46
			7.0	6.257	8.802	9.141	9.112	0.15	1.28	1.34	1.40

Values are averages of six observations

suggested a pH of 6.3-6.5 for mass production of mesophilic lactococci and that of Bozoglu *et al.* (1989) who had observed less viability losses when yoghurt cultures were grown at pH 6.0 rather than at pH 6.5.

4.2.2.2. Effect of neutralization of growth medium

Maintenance of optimum pH throughout the incubation period is very essential for obtaining maximum cell yields. This is especially true with lactic cultures which produce high acidity which leads to cell losses during the growth period. Neutralization of the medium is required to avoid such cell losses. However, the neutralizer used should not have adverse effect on the bacterial growth. The results are presented in Table-14.

There was a marked increase in the cell yield of all the four cultures tested, with the neutrailization of the growth medium, to maintain the initial pH, after 10, 12, 14, 16 and 18 hrs of incubation when compared to respective control samples, where the acidity of the growth medium was not neutrailzed to initial pH. Rapid death of cells was observed in the control samples.

Five fold increase in the cell count was recorded with dahi cultures (D and DAB) whereas, it was only twofold in yoghurt cultures (Y and YAB). The differences in the viable cell counts of all the four cultures of neutralized and un-neutralized growth media were found to be significant at 1% level (Appendix VII).

The results are in confirmation with the reports of Hall and Antonvcci (1988) who have observed fivefold increase in NH_4OH neutrailized cheese starter cultures and with that of Lloyed and Pont (1973), Efstathiou *et al.* (1975) and Ram (1984) who have reported twofold increase in cell population in the medium neutralized with ammonium hydroxide vis-a-vis sodium hydroxide.

Table-14: Influence of neutralization of selected growth media to maintain initial pH on viable cell count of cultures of dahi and yoghurt at different periods of incubation

Sl. No.	Formulated culture	Growth medium composition	Growth medium pH	Incubation temperature (°C)	Percentage of inoculum	Incubation Period (hrs)						
						0	14	16	18	14	16	18
						Control medium				Neutralised medium		
						(Viable cell count : log cfu/g)						
1.	Dahi culture D	Skim milk 5% + Yeast autolysate 1%	6.5	30	1.0	6.696	8.693	9.100	9.042	9.677	9.698	9.708
2.	Dahi culture DAB	+ Glucose 1%	6.5	37	1.0	6.968	8.982	9.260	9.164	9.253	9.650	9.686
3.	Yoghurt culture Y	Skim milk 50% + Tryptone 1%	6.0	37	1.0	6.579	9.128	9.412	9.218	9.552	9.764	9.882
4.	Yoghurt culture YAB	+ Glucose 0.1%	6.5	37	1.0	6.568	8.579	8.858	8.615	8.864	9.454	9.502

Values are averages of six observations

4.2.2.3 Effect of incubation temperature

In order to obtain maximum cell density and activity of the culture combinations of dahi and yoghurt in starter concentrates, trials were conducted to optimize the temperature of incubation for each of the combination. For this purpose, the inoculated medium was incubated at 25°, 30° and 37°C for dahi cultures and at 37° and 42°C for yoghurt cultures. The observations made are recorded in Tables 15 and 16 for dahi and yoghurt cultures, respectively.

With regard to dahi culture D based on viable cell counts and titratable acidity, incubation at 30°C was found to be suitable rather than at 25° or at 37°C. The incubation period was shortened by 2 hrs with the increase in incubation temperature from 25°C to 30°C. Incubation at 37°C was found unsuitable for this combination as the cell yield was less than what was obtained either at 25°C or at 30°C and a drastic decrease in the cell count was observed after 16 hrs of incubation at 37°C.

Dahi culture DAB grew better at 37°C than at the rest of the temperatures tested. The titratable acidity was also found to be more at this temperature of incubation. The viable cell count as well as titratable acidity showed a decreasing trend with the reduction in incubation temperature which may be due to the deviation from the optimum temperature of *Lb. acidophilus* and *Bif. bifidum*, where the activity of these two cultures is at minimum. The ANOVA (Appendices III and IV) for the data obtained has indicated that the viable cell counts achieved differ significantly at 1% level.

In the case of yoghurt culture Y, maximum cell count (9.477 log cfu) was achieved after 16 hrs of incubation at 37°C. Though a log count of 8.732 was obtained with a short incubation period of 5 hrs at 42°C, upon further incubation the viable cell count decreased drastically making it unsuitable for the preparation of starter concentrate. The maximum titratable acidity was also

Table-15: Influence of incubation temperature on viable cell count and % titratable acidity of dahi cultures in selected growth medium (skim milk + yeast autolysate + glucose) at different periods of incubation

Sl. No.	Formulated culture*	Growth medium pH	Incubation temperature (°C)	Incubation Period (hrs) 0	14	16	18	0	14	16	18
				Viable cell count (log cfu/g)				% Titratable acidity (expressed as lactic acid)			
1.	Dahi culture D	6.5	25	6.344	8.348	8.902	8.702	0.15	0.68	0.80	0.86
			30	6.505	8.608	8.991	9.063	0.16	0.72	0.86	0.94
			37	6.462	8.579	8.748	8.653	0.15	0.70	0.82	0.89
2.	Dahi culture DAB	6.5	25	6.623	8.334	8.518	8.630	0.16	0.81	0.92	0.98
			30	6.653	8.440	8.681	8.814	0.17	0.76	0.95	1.04
			37	6.662	8.568	8.984	9.062	0.17	0.79	0.96	1.15

Values are averages of six observations
*Inoculum level: 1.0%

Table-16: Influence of incubation temperature on viable cell count and % titratable acidity of yoghurt cultures in selected growth medium (skim milk + tryptone + glucose) at different periods of incubation

Sl. No.	Formulated culture*	Growth medium pH	Incubation temperature (°C)	Incubation Period (hrs)									
				0	5	14	16	18	0	5	14	16	18
				Viable cell count (log cfu/g)					% Titratable acidity (expressed as % lactic acid)				
1.	Yoghurt culture Y	6.0	37	6.616	7.913	8.730	8.933	8.845	0.16	0.88	1.32	1.54	1.68
			42	6.623	8.132	8.079	7.986	7.914	0.16	1.29	1.32	1.36	1.38
2.	Yoghurt culture YAB	6.5	37	6.301	7.653	8.866	9.101	9.062	0.15	0.92	1.23	1.33	1.44
			42	6.342	7.981	7.912	7.862	7.813	0.15	1.08	1.12	1.16	1.19

Values are averages of six observations.
*Inoculum level: 1.0%

recorded at 37°C. Similar observation was made with yoghurt culture YAB. Thus, the incubation temperature of 37°C was found to be equally suitable for both the combinations of yoghurt cultures. When the data was subjected to statistical analysis by paired t-test, the differences in the cell counts obtained at two temperatures were found to be significant for both the combinations with a t-value of 36.24 for yoghurt culture Y and 327.85 for yoghurt culture YAB.

The observations made with dahi cultures in this study are contradicting the reports of Efstathiou *et al.* (1975) who have suggested the incubation at 25°C for proper species balance and optimal yield of mixed mesophilic starters. This may be due to the differences in strains used, pH and composition of the growth medium and initial cell count. However, incubation at 30°C was found to be more suitable for the growth of *Lc. lactis* ssp. *lactis* C10 and *Lc. lactis* ssp. *cremoris* H as observed by Pont and Halloway (1968), Blaine (1972) and Ram (1984).

4.2.2.4 Effect of inoculum size

It is clear that viable cell counts and titratable acidity enhanced as the rate of inoculum was increased from 0.5 to 1.5% irrespective of the culture (Table-17 and 18). With the increase in the inoculum rate the time taken to attain maximum cell count decreased. This effect was observed with all the cultures of dahi and yoghurt.

After 18 hrs of incubation, maximum viable cell count of 9.099 and 9.149 (log cfu/g) and titratable acidity of 0.92 and 1.18 (% lactic acid) were observed with dahi cultures D and DAB, respectively when added at the rate of 1.0% to the growth medium and incubated at respective growth temperatures. Similarly, maximum log count of 9.219 and 9.236 colony forming units and titratable acidity of 1.64 and 1.46 (% LA) were noted after 16hrs with yoghurt cultures Y and YAB, respectively, when added at the rate of 1.0% and incubated at 37°C. Interestingly, in all the

Table-17: Influence of inoculum level of dahi cultures on viable cell count and % titratable acidity in selected growth medium (skim milk + yeast autolysate + glucose) at different periods of incubation

Sl. No.	Formulated culture	Growth medium pH	Inoculum level (%)	Incubation Period (hrs)							
				0	14	16	18	0	14	16	18
				Viable cell count (log cfu/g)				% Titratable acidity (expressed as lactic acid)			
1.	Dahi culture D (30°C)	6.5	0.5	6.431	7.792	7.902	7.991	0.16	0.54	0.69	0.78
			1.0	6.602	8.763	9.007	9.099	0.18	0.68	0.88	0.92
			1.5	6.980	8.724	8.697	8.644	0.18	0.66	0.84	0.96
2.	Dahi culture DAB (37°C)	6.5	0.5	6.778	7.732	7.954	7.784	0.17	0.76	0.84	0.94
			1.0	6.897	8.820	9.088	9.149	0.18	0.81	0.96	1.18
			1.5	7.104	8.881	8.988	9.064	0.20	0.82	0.93	1.08

Value are averages of six observations.

Table-18: Influence of inoculum level of yoghurt cultures on viable cell count and % titratable acidity in selected growth medium (skim milk + tryptone + glucose) at different periods of incubation at 37°C

Sl. No.	Formulated culture	Growth medium pH	Inoculum level (%)	Incubation Period (hrs)							
				0	14	16	18	0	14	16	18
				Viable cell count (log cfu/g)				% Titratable acidity (expressed as lactic acid)			
1.	Yoghurt culture Y	6.0	0.5	6.462	8.532	8.768	8.737	0.15	0.92	1.22	1.34
			1.0	6.653	8.797	9.219	9.160	0.15	1.28	1.50	1.64
			1.5	6.857	8.968	8.866	8.712	0.16	1.26	1.38	1.42
2.	Yoghurt culture YAB	6.5	0.5	6.477	8.668	8.960	9.080	0.15	0.89	1.12	1.20
			1.0	6.698	8.908	9.236	9.149	0.15	1.16	1.32	1.46
			1.5	7.126	9.061	8.928	8.876	0.16	1.22	1.28	1.34

Values are averages of six observations.

cases reduction in incubation period to get maximum population, was observed with the increase in the rate of inoculum.

The observations made with regard to viable cell count were found to be highly significant at 1% level (Appendices III to VI) with all the combinations dahi and yoghurt cultures.

The results obtained are in agreement with those of Ayunts *et al.* (1979) who had obtained maximum yield of bacterial concentrate of lactic acid bacteria using 1.0% inoculum after incubating for 14-16 hrs. The population changes with higher rate of inoculum were too rapid, and after 16 hrs of inoculation, significant decline in the cell number was started making it unsuitable for the preparation of concentrates, whereas lower level of inoculum (0.5%) needed longer incubation period to get a good number of viable cells.

4.2.3. *Influence of Cryoprotective Agents*

Use of cryoprotective agents is recommended to protect micro-organisms during freeze-drying process. Three formulations viz. 1)5% buffalo skim milk + 1.5% gelatin + 8% sucrose; 2)10% buffalo skim milk + 1.5% gelatin + 7% sucrose + 1M monosodium glutamate and 3)10% buffalo skim milk + 7% sucrose + 1M monosodium glutamate were tried in this study and their effect on culture viability was studied and the results are presented in Tables 19 and 20 for dahi and yoghurt cultures, respectively.

A perusal of the results with formulation 1 indicated a survival rate of 80.00% for dahi culture D, 90.73% for dahi culture DAB, 78.40% for yoghurt culture Y and 84.55% for yoghurt culture YAB. Addition of monosodium glutamate to the same suspending medium increased the survival rate of all the four combinations giving 87.50, 96.20, 88.46 and 89.51% survival for the respective cultures studied. The differences observed in the viability were found to be highly significant at one percent level as shown in ANOVA tables (Appendices III to VI).

Table-19: Influence of formulated cryoprotective agents on viable cell count and biochemical performance of bulk freeze-dried dahi cultures grown in selected growth medium (Skim milk + Yeast autolysate + Glucose) under standardized growth conditions

Sl. No.	Tests performed	Formulated cryoprotective agents*	Dahi cultures			
			D		DAB	
			Before F.D**	After F.D.	Before F.D.	After F.D.
1.	Viable cell count (log cfu/g)	Formulation 1	11.544	11.462	11.158	11.120
		Formulation 2	11.602	11.540	11.198	11.181
		Formulation 3	11.397	11.301	11.112	11.070
2.	Culture activity factor (Horrall & Elliker test)	Formulation 1	0.52	0.50	0.56	0.54
		Formulation 2	0.57	0.50	0.56	0.54
		Formulation 3	0.48	0.44	0.53	0.49
3.	Diacetyl content (ppm)	Formulation 1	4.25	4.05	3.14	2.92
		Formulation 2	4.35	4.18	3.08	2.98
		Formulation 3	4.20	4.00	3.12	2.86
4.	Proteolytic activity (mg tyrosine/g curd)	Formulation 1	0.42	0.39	0.39	0.34
		Formulation 2	0.45	0.43	0.40	0.38
		Formulation 3	0.44	0.40	0.41	0.35
5.	Residual moisture content (%)	Formulation 1	-	1.86	-	1.90
		Formulation 2	-	1.22	-	1.36
		Formulation 3	-	1.94	-	2.00

Values are averages of six observations, *Formulation 1: 5% buffalo skim milk + 1.5% gelatin + 8% sucrose
Formulation 2: 10% buffalo skim milk + 1.5% gelatin + 7% sucrose + 1M MSG
**F.D: Freeze-drying
Formulation 3: 10% buffalo skim milk + 7% sucrose + 1M MSG

Table-20: Influence of formulated cryoprotective agents on viable cell count and biochemical performance of bulk freeze-dried yoghurt cultures grown in selected growth medium (Skim milk + Tryptone + Glucose) under standardized growth conditions

Sl. No.	Tests performed	Formulated cryoprotective agents*	Yoghurt cultures			
			Y		YAB	
			Before F.D**	After F.D.	Before F.D.	After F.D.
1.	Viable cell count (log cfu/g)	Formulation 1	11.079	10.991	11.076	11.007
		Formulation 2	11.113	11.060	11.155	11.102
		Formulation 3	11.096	10.989	11.133	11.096
2.	Culture activity factor (Horrall & Elliker test) (acidity expressed as % lactic acid)	Formulation 1	0.50	0.46	0.45	0.43
		Formulation 2	0.56	0.53	0.48	0.46
		Formulation 3	0.48	0.44	0.44	0.41
3.	Acetaldehyde content (ppm)	Formulation 1	28.00	26.20	29.00	26.60
		Formulation 2	30.00	28.80	32.10	29.40
		Formulation 3	27.40	25.80	28.70	25.20
4.	Proteolytic activity (mg tyrosine/g curd)	Formulation 1	0.37	0.34	0.35	0.29
		Formulation 2	0.48	0.45	0.42	0.38
		Formulation 3	0.39	0.35	0.32	0.26
5.	Residual moisture content (%)	Formulation 1	--	1.62	--	1.78
		Formulation 2	--	1.29	--	1.42
		Formulation 3	--	1.96	--	1.94

Values are averages of six observations, *Formulation 1: 5% buffalo skim milk + 1.5% gelatin + 8% sucrose
Formulation 2: 10% buffalo skim milk + 1.5% gelatin + 7% sucrose + 1M MSG
**F.D: Freeze-drying
Formulation 3: 10% buffalo skim milk + 7% sucrose + 1M MSG

The culture activity of all the combinations that were freeze-dried in formulation 2 was found to be satisfactory. The residual moisture content in freeze-dried cultures of all the combinatioins was at minimum with formulation 2. The biochemical activity in terms of flavour production (diacetyl/acetaldehyde) and proteolytic activity of all the cultures was also superior with the suspending medium formulated with skim milk, gelatin, sucrose and monosodium glutamate.

Skim milk has been in use since long as a cryoprotective agent and Rogers (1914) was the first to use it for lactic cultures. Use of skim milk gave only 60% viability with certain lactococci (Yano *et al*, 1960). Addition of other cryoprotective agents to milk can improve the protective effect of skim milk as observed in the present study. The activity and biochemical performance of all the cultures freeze-dried using a suspending medium (formulation 2) was maximum as the survival rates of the respective combinations of cultures were also high. This is in agreement with Pettersson's (1975) observation who also noted a relationship between activity of concentrates and viability of cells.

Addition of sucrose to the suspending media has an advantage of inhibiting free radical formulation and thus helps to improve the survival rate (Nikolova, 1978; Lagoda *et al.*, 1982; and Alaeddinoglu *et al.*, 1989). Use of monosodium glutamate to get 60-95% survival of lactic cultures during freeze-drying was reported by Speckman (1975), Kilara *et al.* (1976), Lagoda *et al.* (1982) and Kim *et al.* (1987).

The present findings are also comparable with the reports of Damjanovic and Radulovic (1967) and Radulovic (1971) who have achieved 90-100% survival of *Bif. bifidum* and *Lb. acidophilus* when a suspending medium containing sucrose, gelatin and skim milk was used. The minor differences in the survival rate of different combinations of dahi and yoghurt can be attributed to

the species and strain variation of cultures or to their associative growth in mixed cultures.

4.3. Stability of Bulk Freeze-dried Formulated Dahi and Yoghurt Cultures during Storage

4.3.1 *Influence of Storage Temperature and Sealing Atmosphere on the Stability of Freeze-dried Cultures*

Freeze-dried dahi and yoghurt cultures, after sealing under air and vacuum in laminated aluminium foil pouches, were stored at 4° and -20°C for a period of 90 days, and their activity was monitored for viability, culture activity, acidity development, proteolytic activity, flavour production and residual moisture content and the observations are recorded in Tables 21 to 32.

4.3.1.1. Bulk freeze-dried dahi culture D

Variation in survival rate was observed with culture D as shown in Table-21. Both sealing atmosphere and temperature of storage were proved to be deleterious and the effect was severe in cultures stored for longer periods.

The survival rate after 90 days of storage at 4°C for air and vacuum sealed cultures was 70.4 and 83.6%, respectively. For samples stored at -20°C it was comparatively higher, 83.6 and 94.7% for air and vaccum sealing, respectively.

During storage no significant change in biochemical activity and residual moisture content of dahi culture D was observed with both air and vacuum sealed cultures, irrespective of storage temperature (Table 21 to 23).

4.3.1.2 Bulk freeze-dried dahi culture DAB

Results presented in Table-24, show that both sealing environment and storage temperature have detrimental effect on

Table-21: Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried dahi culture D during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)
0#	11.531	1.20	11.531	1.20	11.531	1.20	11.531	1.20
15	11.502 (94.1)	1.21	--	--	11.529 (99.4)	1.20	--	--
30	11.490 (90.9)	1.22	11.516 (96.4)	1.20	11.524 (98.6)	1.21	11.528 (99.1)	1.20
45	11.462 (85.2)	1.23	--	--	11.512 (99.9)	1.21	--	--
60	11.426 (78.4)	1.24	11.500 (92.9)	1.20	11.504 (93.85)	1.22	11.520 (97.4)	1.20
75	11.408 (75.2)	1.24	--	--	11.460 (84.8)	1.22	--	--
90	11.379 (70.4)	1.25	11.483 (89.4)	1.21	11.454 (83.6)	1.22	11.508 (94.7)	1.21

Values are averages of three trials in duplicate
#Immediately freeze-drying
Values in parentheses indicate percent survival

Table-22: Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried dahi culture D during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)
0*	0.52	0.93	0.52	0.93	0.52	0.93	0.52	0.93
15	0.52	0.93	--	--	0.52	0.93	--	--
30	0.51	0.92	0.52	0.93	0.52	0.93	0.52	0.93
45	0.51	0.92	--	--	0.51	0.92	--	--
60	0.51	0.91	0.52	0.93	0.51	0.92	0.52	0.93
75	0.50	0.90	--	--	0.51	0.91	--	--
90	0.50	0.90	0.51	0.92	0.50	0.91	0.51	0.92

Values are averages of three trials in duplicate

*Immediately after freez-drying

Table 23: Influence of sealing atmosphere and temperature on diacetyl content and proteolytic activity of bulk freeze-dried dahi culture D during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)
0*	4.10	0.48	4.10	0.48	4.10	0.48	4.10	0.48
15	4.08	0.48	—	--	4.09	0.48	--	--
30	4.07	0.46	4.10	0.47	4.09	0.47	4.10	0.48
45	4.07	0.46	—	--	4.08	0.47	--	--
60	4.06	0.45	4.08	0.47	4.08	0.46	4.10	0.47
75	4.06	0.44	--	--	4.06	0.46	--	--
90	4.04	0.44	4.08	0.46	4.06	0.45	4.09	0.47

Values are averages of three trials in duplicate
*Immediately after freeze-drying

Table-24: Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried *dahi* culture DAB during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)
0*	11.146	1.32	11.146	1.32	11.146	1.32	11.146	1.32
15	11.124 (95.0)	1.33	--	--	11.144 (99.5)	1.32	--	--
30	11.101 (90.0)	1.34	11.137 (97.8)	1.33	11.138 (98.1)	1.33	11.141 (98.8)	1.32
45	11.094 (88.6)	1.35	--	--	11.132 (96.8)	1.33	--	--
60	11.077 (85.2)	1.37	11.122 (94.6)	1.34	11.129 (96.0)	1.34	11.136 (97.6)	1.33
75	11.052 (80.5)	1.37	--	--	11.121 (94.3)	1.34	--	--
90	11.038 (77.9)	1.38	11.102 (90.2)	1.35	11.118 (93.7)	1.35	11.124 (95.0)	1.34

Values are averages of three trials in duplicate.
Immediately after freeze-drying
Values in parentheses indicate percent survival

Table-25: Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried dahi culture DAB during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)
0*	0.46	1.20	0.46	1.20	0.46	1.20	0.46	1.20
15	0.45	1.18	--	--	0.46	1.20	--	--
30	0.45	1.16	0.46	1.20	0.45	1.20	0.46	1.20
45	0.43	1.15	--	--	0.45	1.19	--	--
60	0.43	1.14	0.45	1.18	0.44	1.19	0.46	1.20
75	0.42	1.13	--	--	0.44	1.19	--	--
90	0.42	1.12	0.45	1.18	0.43	1.18	0.45	1.19

Values are averages of three trials in duplicate
*Immediate after freeze-drying

Table 26: Influence of sealing atmosphere and temperature on diacetyl content and proteolytic activity of bulk freeze-dried dahi culture DAB during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)	Diacetyl content (ppm)	Proteolytic activity (mg tyrosine/g)
0*	2.90	0.36	2.90	0.36	2.90	0.36	2.90	0.36
15	2.89	0.35	--	--	2.89	0.36	--	--
30	2.87	0.35	2.88	0.36	2.89	0.34	2.90	0.36
45	2.86	0.33	--	--	2.88	0.34	--	--
60	2.86	0.33	2.88	0.35	2.87	0.34	2.90	0.35
75	2.85	0.33	--	--	2.87	0.33	--	--
90	2.84	0.32	2.87	0.35	2.87	0.33	2.89	0.35

Values are averages of three trials in duplicate
*Immediately after freeze-drying

the cultures and the deterioration increased with the increase in storage period.

The viable cell counts in the culture sealed under air was found to be 11.038 (log cfu/g) after 90 days storage at 4°C resulting in 22.1% cell death. Compratively higher cell count (11.118 log cfu/g) was observed with vacuum sealed samples when stored at 4°C for a period of 90 days. In this case 93.7 percent viability of cells was achieved.

Storage at -20°C has resulted in higher cell counts with both air and vacuum sealed samples with 11.102 and 11.124 (log cfu/g), respectively at the end of storage period resulting in as high as 90.2 to 95.0 percent viability. On the basis of observations, it can be inferred that the cell death was rapid in samples sealed under air as compared to vacuum, which is due to the deleterious effect of oxygen on the cultures and this effect is more pronounced in samples stored for longer periods. The trend observed with regard to biochmeical activity and residual moisture content for dahi culture DAB (Table 24 to 26) was also similar to that of dahi culture D at all the conditions of storage.

4.3.1.3 Bulk freeze-dried yoghurt culture Y

Bulk freeze-dried starter concentrate of yoghurt culture Y, after sealing under air and vacuum in laminated foil pouches has showed variation in viability and biochemical activity as depicted in Tables 27 to 29.

The survival rate, with this culture, after 3 months storage at 4°C was found to be 70.6 and 83.0 percent for air and vacuum sealed, respectively whereas the percentage viability recorded for air and vacuum sealed samples was 83.0 and 91.6 when stored at -20°C for a period of 90 days which are significantly higher than when stored at 4°C.

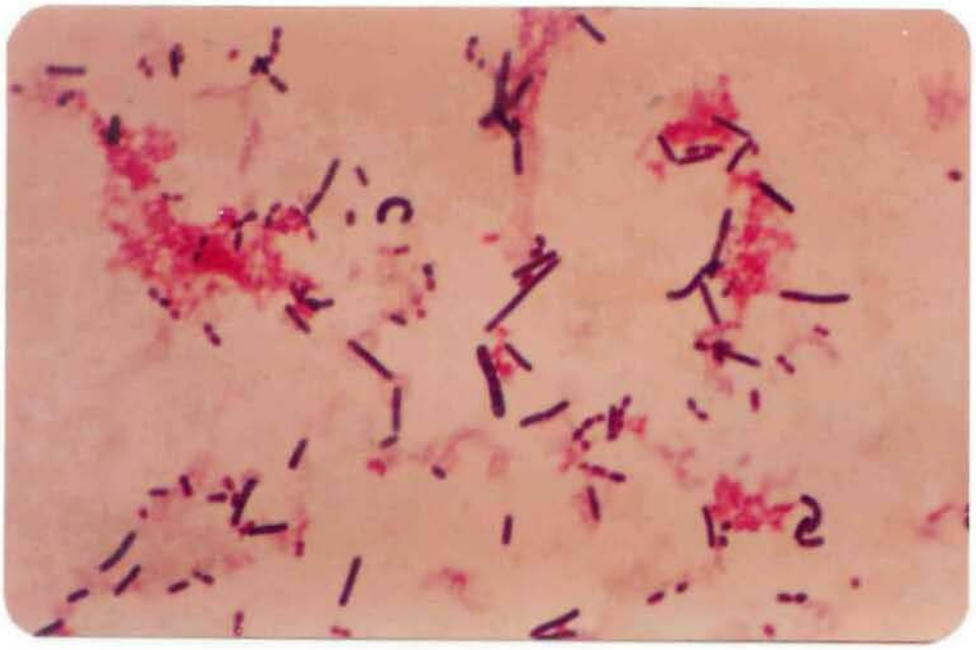


Plate.4. Microscopic examination of dahi prepared with bulk freeze-dried dahi culture DAB

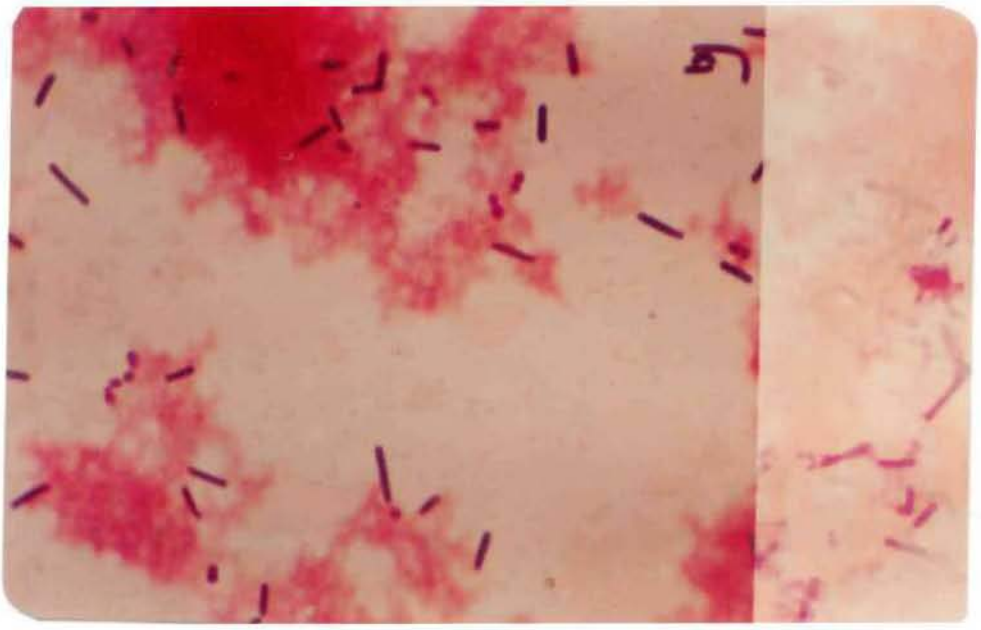


Plate.5 Microscopic examination of yoghurt prepared with yoghurt culture YAB

Table-27: Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried yoghurt culture Y during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)
0*	11.049	1.26	11.049	1.26	11.049	1.26	11.049	1.26
15	11.033 (96.3)	1.26	--	--	11.039 (97.7)	1.26	--	--
30	11.026 (94.8)	1.27	11.025 (94.6)	1.26	11.035 (96.8)	1.26	11.042 (98.4)	1.26
45	11.008 (91.0)	1.27	--	--	11.004 (90.2)	1.27	--	--
60	10.977 (84.7)	1.27	11.010 (91.4)	1.27	10.984 (86.1)	1.27	11.022 (93.9)	1.26
75	10.946 (78.9)	1.28	--	--	10.978 (85.0)	1.28	--	--
90	10.898 (70.6)	1.28	10.968 (83.0)	1.27	10.968 (83.0)	1.28	11.011 (91.6)	1.27

Values are averages of three trials in duplicate.
*Immediately after freeze-drying
Values in parentheses indicate percent survival

Table-28: Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried yoghurt culture Y during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)
0*	0.53	1.64	0.53	1.64	0.53	1.64	0.53	1.64
15	0.53	1.64	--	--	0.53	1.64	--	--
30	0.52	1.63	0.53	1.63	0.52	1.63	0.53	1.64
45	0.52	1.62	--	--	0.52	1.63	--	--
60	0.51	1.62	0.52	1.63	0.52	1.63	0.53	1.63
75	0.51	1.61	--	--	0.51	1.62	--	--
90	0.50	1.61	0.52	1.62	0.51	1.62	0.52	1.63

Values are averages of three trials in duplicate
*Immediately after freeze-drying

Table-29: Influence of sealing atmosphere and temperature on acetalydehyde content and proteolytic activity of bulk freeze-dried yoghurt culture Y during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)
0*	28.60	0.42	28.60	0.42	28.60	0.42	28.60	0.42
15	28.58	0.41	--	--	28.58	0.42	--	--
30	28.57	0.41	28.60	0.42	28.58	0.41	28.58	0.42
45	28.55	0.38	--	--	28.55	0.41	--	--
60	28.53	0.38	28.54	0.39	28.55	0.38	28.58	0.41
75	28.51	0.36	--	--	28.52	0.38	--	--
90	28.50	0.36	28.54	0.39	28.52	0.38	28.55	0.41

Values are averages of three trials in duplicate
*Immediately after freeze-drying

It is clear from the values given in Table 27 to 29 that the storage temperature and sealing atmosphere have little or no effect on the biochemical activity and residual moisture content of freeze-dried culture Y over the period of 90 days storage.

4.3.1.4 Bulk freeze-dried yoghurt culture YAB

A perusal of the table 30 reveals lower survival rate (73.3%) for air sealed cultures when stored at 4°C. Storing at -20°C increased the survival rate by 6%. Similar trend was observed with vacuum sealed cultures stored at 4 and -20°C for a period of 90 days. It is interesting to note that the rate of cell death was doubled during the storage in air compared to vacuum sealed cultures irrespective of the storage temperature. It may be inferred that the viability loss was rapid in cultures sealed under air when compared to that of the vacuum sealed cultures. The loss was more in samples stored for longer period especially at higher temperatures of storage.

The residual moisture content and biochemical performance measured in terms of culture activity, acetaldehyde content, titratable acidity and proleolytic activity of freeze-dried yoghurt culture YAB were little or not at all influenced by the conditions of storage employed in the study (Table 30 to 32).

The data obtained with regard to viable cell counts of the bulk freeze-dried culture combinations of dahi and yoghurt, during storage were analysed statistically by three-way ANOVA method. However, the values of samples stored at 4°C at 15, 45 and 75 day intervals were omitted for uniformity sake in the analysis. The variations in viable cell counts with regard to sealing atmosphere, temperature of storage and storage period were found to be highly significant (Appendix VIII) at one percent level. As the storage period increased, viable cell counts decreased at a faster rate with increasing storage temperature, indicating significant ($P < 0.01$) interaction effect of the two variables.

Table-30: Influence of sealing atmosphere and temperature on viable cell count and residual moisture content of bulk freeze-dried yoghurt culture YAB during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)	Viable cell count (log cfu/g)	Residual moisture content(%)
0*	11.071	1.38	11.071	1.38	11.071	1.38	11.071	1.38
15	11.052 (95.7)	1.39	--	--	11.066 (98.9)	1.38	--	--
30	11.040 (93.1)	1.39	11.055 (96.4)	1.38	11.060 (97.5)	1.39	11.068 (99.3)	1.38
45	11.018 (88.5)	1.40	--	--	11.044 (94.0)	1.39	--	--
60	11.007 (86.3)	1.40	11.016 (88.1)	1.39	11.025 (90.0)	1.39	11.041 (93.4)	1.38
75	10.906 (78.5)	1.40	--	--	11.008 (86.5)	1.40	--	--
90	10.938 (73.3)	1.41	10.976 (80.4)	1.39	10.996 (84.2)	1.40	11.036 (92.3)	1.39

Values are averages of three trials in duplicate.
*Immediately after freeze-drying
Values in parentheses indicate percent survival

Table-31: Influence of sealing atmosphere and temperature on activity and titratable acidity of bulk freeze-dried yoghurt culture YAB during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)	Culture activity factor	% Titratable acidity (expressed as lactic acid)
0*	0.54	1.46	0.54	1.46	0.54	1.46	0.54	1.46
15	0.54	1.45	--	--	0.54	1.465	--	--
30	0.53	1.45	0.54	1.46	0.53	1.45	0.54	1.46
45	0.53	1.44	--	--	0.53	1.45	--	--
60	0.52	1.44	0.53	1.44	0.52	1.44	0.54	1.45
75	0.51	1.43	--	--	0.52	1.44	--	--
90	0.51	1.42	0.52	1.43	0.51	1.43	0.53	1.45

Values are averages of three trials in duplicate
*Immediately after freeze-drying

Table-32: Influence of sealing atmosphere and temperature on acetalydehyde content and proteolytic activity of bulk freeze-dried yoghurt culture YAB during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)
0*	35.0	0.35	35.0	0.35	35.0	0.35	35.0	0.35
15	34.98	0.33	--	--	34.99	0.35	--	--
30	34.97	0.33	35.0	0.35	34.97	0.33	34.97	0.34
45	34.95	0.32	--	--	34.96	0.33	--	--
60	34.95	0.32	34.98	0.34	34.96	0.31	34.95	0.34
75	34.94	0.31	--	--	34.95	0.31	--	--
90	34.93	0.30	34.98	0.34	34.95	0.30	34.95	0.33

Values are averages of three trials in duplicate
*Immediately after freeze-drying

Table-32: Influence of sealing atmosphere and temperature on acetalydehyde content and proteolytic activity of bulk freeze-dried yoghurt culture YAB during storage

Period of storage (days)	Sealing atmosphere							
	Air				Vacuum			
	Storage temperature				Storage temperature			
	4°C		-20°C		4°C		-20°C	
	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)	Acetaldehyde content (ppm)	Proteolytic activity (mg tyrosine/g)
0*	35.0	0.35	35.0	0.35	35.0	0.35	35.0	0.35
15	34.98	0.33	--	--	34.99	0.35	--	--
30	34.97	0.33	35.0	0.35	34.97	0.33	34.97	0.34
45	34.95	0.32	--	--	34.96	0.33	--	--
60	34.95	0.32	34.98	0.34	34.96	0.31	34.95	0.34
75	34.94	0.31	--	--	34.95	0.31	--	--
90	34.93	0.30	34.98	0.34	34.95	0.30	34.95	0.33

Values are averages of three trials in duplicate
*Immediately after freeze-drying

It has been found (Maister *et al.*, 1958 and Decowski, 1961) that both over drying and under drying affect the survival of freeze-dried cultures. It was also demonstrated that there is an optimal residual moisture content for maximum stability (Grieff, 1971). Higher mortality rate, when the residual moisture dropped to less than 0.5%, was shown by Font de Valdez *et al.* (1985). The residual moisture content recorded in the present study (1.20-1.41%) for dahi and yoghurt cultures is within the limit of moisture level recommended (0.5-2.0%) for higher survival rate of freeze-dried lactic acid bacteria (Habaj, 1964; Gehriger, 1968 and Sinha, 1989).

4.3.2 *Prediction of Shelf Life of Bulk Freeze-dried Cultures of Dahi and Yoghurt*

For the preparation of good quality fermented milk products, direct-vat-inoculation (bulk freeze-dried) cultures must have sufficient number of organisms to accomplish the fermentation. However, during the storage of freeze-dried cultures, the viable counts were observed to decrease, possibly to a number which falls below the optimum for obtaining good quality product. The decrease in viable count is a function of several parameters including residual moisture content, temperature and period of storage, atmosphere of sealing etc. These parameters also showed interaction effect. It is, therefore, possible to mathematically predict the viable count at any given storage period as a function of the above parameters.

The storage data obtained with regard to variables such as residual moisture (RM), culture activity factor (CAF), titratable acidity (TA), diacetyl (D) or acetaldehyde (AD) production, proteolytic activity (PA) and storage period (SP) of the samples sealed under vacuum were subjected to multiple regression analysis in log-linear and linear-linear forms. The latter equation was found to be better model which explained the contribution of each viarable to the change in the viable cell count (Y). The regression equations obtained to predict the

viable cell counts are as follows:

1) Dahi culture D sealed under vacuum and stored at 4°C:

$Y = (-164.15 + 18.38\ RM + 43.79\ CAF + 22.72\ TA + 36.12\ D - 42.17\ PA - 0.03\ SP) \times 10^{10}$ $(R^2 = 0.9859)$

2) Dahi culture D sealed under vacuum and stored at -20°C

$Y = (-89.48 + 13.71\ RM + 33.16CAF + 24.42\ TA + 11.42\ D + 42.04\ PA - 0.08\ SP) \times 10^{10}$ $(R^2 = 0.9885)$

3) Dahi culture DAB sealed under vacuum and stored at 4°C

$Y = (-6.64 + 11.02\ RM + 9.91CAF + 4.10\ TA - 1.60\ D + 3.43\ PA - 0.08\ SP) \times 10^{10}$ $(R^2 = 0.9963)$

4) Dahi culture DAB sealed under vacuum and stored at -20°C

$Y = (12.74 - 8.09\ RM - 1.53\ CAF + 9.16\ TA + 1.97D - 11.37\ PA - 0.06\ SP) \times 10^{10}$ $(R^2 = 0.9916)$

5. Yoghurt culture Y sealed under vacuum and stored at 4°C

$Y = (35.90 + 10.19\ RM - 4.71\ CAF + 9.27\ TA - 1.96\ AD + 14.01\ PA - 0.02\ SP) \times 10^{10}$ $(R^2 = 0.9756)$

6. Yoghurt culture Y sealed under vacuum and stored at -20°C

$Y = (-26.10 + 4.66\ RM + 3.44\ CAF + 0.07\ TA + 1.09\ AD - 4.22\ PA - 0.01\ SP) \times 10^{10}$ $(R^2 = 0.9947)$

7. Yoghurt culture YAB sealed under vacuum and stored at 4°C

$Y = (36.63 - 29.40\ RM + 3.13\ CAF + 4.07\ TA + 0.83\ AD - 59.24\ PA + 0.07\ SP) \times 10^{10}$ $(R^2 = 0.8234)$

8. Yoghurt culture YAB sealed under vacuum and stored at -20°C

$$Y = (253.87 - 3.06\ RM - 13.49\ CAF + 0.65\ TA - 6.42\ AD - 19.31\ PA - 0.02\ SP) \times 10^{10}$$

$$(R^2 = 0.9280)$$

The R^2 values of all the equations ranged from 0.9280 to 0.9963 except in case of yoghurt culture YAB sealed under vacuum and stored at 4°C, where the R^2 value was 0.8234 which was comparatively low, yet adequate enough to reasonably predict the viable counts. The high R^2 values in all the equations indicate that the mathematical prediction of viable counts and thereby shelf life, is possible under a set of given variables.

4.4 Use of Bulk Freeze-dried Cultures of Dahi and Yoghurt

To confirm the successful use of bulk freeze-dried cultures prepared in the present investigation, dahi and yoghurt were prepared by directly inoculating the bulk freeze-dried culture into milk. Prior to this the rate of inoculum needed and culture activity and biochemical performance in cow and buffalo milk samples were also determined and the results are presented in this section.

4.4.1 *Determination of Inoculum Size*

The observations pertaining to the trials made to ascertain the size of inoculum needed for direct inoculation of milk for product preparation are presented in Table-33.

It was observed from the results that use of lower levels of inoculum size (0.001 and 0.005% w/v) was unsuitable as it produced insufficient quantities of acidity and improper body and texture. Higher levels of inoculum size i.e. 0.05 and 0.1% were also found to be unsuitable for product preparation as the level of titratable acidity produced was too high and the curd thus formed was hard.

Table-33: Influence of inoculum size of bulk freeze-dried dahi and yoghurt cultures on titratable acidity and body & texture of standardized buffalo milk (4.0% fat) curd

Sl. No.	Culture	Inoculum size (% w/v)	% Titratable acidity (expressed as lactic acid)		Body & Texture of the curd
1.	Dahi culture D	0.001	0.46	(14)	Loose body
		0.005	0.82		Semiglossy and semifirm
		0.010	**0.95**		**Glossy and firm**
		0.050	1.14		Glossy and hard
		0.100	1.30		Semiglossy and hard
	Control:Liquid culture	**1.0 (v/v)**	**0.98**	**(16)**	**Glossy and firm**
2.	Dahi culture DAB	0.001	0.52	(14)	No body
		0.005	1.05		Semiglossy and firm
		0.010	**1.24**		**Glossy and firm**
		0.050	1.42		Semiglossy and hard
		0.100	1.58		Glossy and hard
	Control:Liquid culture	**1.0 (v/v)**	**1.20**	**(16)**	**Glossy and firm**
3.	Yoghurt culture Y	0.001	0.60	(12)	Loose body
		0.005	1.36		Semiglossy and semifirm
		0.010	**1.64**		**Glossy and firm**
		0.050	1.75		Glossy and hard
		0.100	1.84		Glossy and hard
	Control:Liquid culture	**1.0 (v/v)**	**1.66**	**(14)**	**Glossy and firm**
4.	Yoghurt culture YAB	0.001	0.54	(12)	Loose body
		0.005	1.28		Glossy and semifirm
		0.010	**1.40**		**Glossy and firm**
		0.050	1.52		Semiglossy and hard
		0.100	1.68		Semiglossy and hard
	Control:Liquid culture	**1.0 (v/v)**	**1.44**	**(14)**	**Glossy and firm**

Values are averages of five trials in duplicate
Values in parentheses indicate the time (hrs) taken to attain the acidity

The inoculum size, which gave almost same acidity with respect to control samples prepared with 1.0% liquid cultures of respective culture combinations, was 0.01% (w/v) for all combinations of dahi and yoghurt cultures. The titratable acidity and textural characters of the curd samples were also found to be satisfactory at this rate of inoculum.

4.4.2 *Culture activity and Biochemical Performance of Bulk Freeze-dried Cultures of Dahi and Yoghurt in Cow and Buffalo Milk*

Most of the dairy products, in dairy industry are manufactured from mixed milk. However, certain products like Cheddar cheese, Mozzarella cheese etc. are invariably prepared from either cow or buffalo milk. Cow milk has been reported to be congenial for starter growth but buffalo milk owing to certain inherent properties is known to have some effect on starter proliferation. Since bulk freeze-dried starter cultures developed were envisaged to be used with both cow and buffalo milk, the performance of the cultures in both types of milk was determined.

Dahi culture D was found to be active both in buffalo and cow milk samples with (activity factor ranged from 0.45 to 0.52) when subjected to Horrall and Elliker test. The titratable acidity after 16 hrs of incubation at 30°C was in the range of 0.92 to 0.97 in cow and buffalo milk samples. Slightly less quantity of volatile acidity developmentcan be seen in cow milk curd (29.20-29.50 ml of 0.01 N NaOH/50g curd) compared to that produced in buffalo milk (31.80 to 32.50 ml). Diacetyl content was almost same in cow and buffalo milk curds with a concentration of 3.71 to 4.00 ppm. Appreciable difference in proteolytic activity in cow and buffalo milk curd was not observed. The tyrosine content was in the range of 0.43-0.44 mg in cow milk samples and the corresponding value for buffalo milk samples was 0.45-0.47 (Table-34).

Table-34: Culture activity and biochemical performance of 0.01 % (w/v) bulk freeze-dried dahi culture D in skimmed and standardized milk (4.0% fat) of cow and buffalo after 14 hrs incubation at 30°C.

Sl. No.	Biochemical tests	Cow milk		Buffalo milk	
		Skimmed milk	Standardized milk	Skimmed milk	Standardized milk
1.	Culture activity factor (Horrall & Elliker test)	0.45	0.48	0.52	0.50
2.	% Titratable acidity (expressed as lactic acid)	0.92	0.94	0.95	0.97
3.	Volatile acidity (ml of 0.01 N NaOH/50g curd)	29.50	29.20	31.80	32.50
4.	Diacetyl content (ppm)	3.71	3.78	3.92	4.00
5.	Proteolytic activity (mg tyrosine/g curd)	0.43	0.44	0.45	0.47

Values are averages of five trials in duplicate.

There was no difference in the performance of dahi culture combination DAB either in cow or buffalo milk, after 16 hrs of incubation at 37°C. The culture was active both in cow and buffalo milk samples. The titratable acidity produced in two types of milks was comparable. The difference in volatile acidity developed in cow and buffalo milk was meagre which ranged upto a quantity of 0.3 ml of 0.01 N NaOH per 50 g of curd. The diacetyl content and tyrosine content of cow and buffalo milk curds were in the same order (Table-35).

The performance of yoghurt cultures Y and YAB in buffalo milk was comparable to that of cow milk samples (Tables 36 and 37). Though minor differences in the biochemical activity of both the cultures in cow and buffalo milk curds can be observed, they are not of significance. Both the culture combinations (Y and YAB) exhibited slightly more volatile acid production in buffalo milk than in cow milk as was observed by Jain (1979) who has reported similar observation with mixed cultures of yoghurt.

As noted in earlier section, it may be observed that no appreciable difference was noticed in the activity and biochemical performance of all the four cultures either in skimmed or standardized milk irrespective of the type of milk (cow or buffalo) used. This observation was in agreement with the findings of Khanna and Singh (1978) who had reported similar observation with yoghurt starters in buffalo and cow milk samples. The present finding clearly indicated that the bulk freeze-dried culture combinations of dahi and yoghurt can be used in any type of milk to prepare good quality products.

4.4.3 *Analysis of Dahi and Yoghurt*

4.4.3.1 Lactic count and biochemical quality

The results pertaining to lactic count and biochemical quality of dahi and yoghurt samples prepared using freeze-dried cultures are presented in Tables 38 and 39, respectively. The

Table-35: Culture activity and biochemical performance of 0.01 % (w/v) bulk freeze-dried dahi culture DAB in skimmed and standardized milk (4.0% fat) of cow and buffalo after 14 hrs incubation at 37°C.

Sl. No.	Biochemical tests	Cow milk		Buffalo milk	
		Skimmed milk	Standardized milk	Skimmed milk	Standardized milk
1.	Culture activity factor (Horrall & Elliker test)	0.42	0.45	0.46	0.48
2.	% Titratable acidity (expressed as lactic acid)	1.08	1.14	1.16	1.20
3.	Volatile acidity (ml of 0.01 N NaOH/50g curd)	15.86	16.02	15.94	16.12
4.	Diacetyl content (ppm)	2.64	2.70	2.72	2.79
5.	Proteolytic activity (mg tyrosine/g curd)	0.35	0.38	0.37	0.39

Values are averages of five trials in duplicate.

Table-36: Culture activity and biochemical performance of 0.01 % (w/v) bulk freeze-dried yoghurt culture Y in skimmed and standardized milk (4.0% fat) of cow and buffalo after 12 hrs incubation at 37°C.

Sl. No.	Biochemical tests	Cow milk		Buffalo milk	
		Skimmed milk	Standardized milk	Skimmed milk	Standardized milk
1.	Culture activity factor (Horrall & Elliker test)	0.48	0.51	0.50	0.52
2.	% Titratable acidity (expressed as lactic acid)	1.53	1.56	1.58	1.60
3.	Volatile acidity (ml of 0.01 N NaOH/50g curd)	7.54	7.78	7.60	7.85
4.	Acetaldehyde content (ppm)	27.10	27.20	27.20	27.50
5.	Proteolytic activity (mg tyrosine/g curd)	0.31	0.34	0.33	0.36

Values are averages of five trials in duplicate.

Table-37: Culture activity and biochemical performance of 0.01 % (w/v) bulk freeze-dried yoghurt culture YAB in skimmed and standardized milk (4.0% fat) of cow and buffalo after 12 hrs incubation at 37°C.

Sl. No.	Biochemical tests	Cow milk		Buffalo milk	
		Skimmed milk	Standardized milk	Skimmed milk	Standardized milk
1.	Culture activity factor (Horrall & Elliker test)	0.45	0.49	0.48	0.51
2.	% Titratable acidity (expressed as lactic acid)	1.28	1.32	1.31	1.35
3.	Volatile acidity (ml of 0.01 N NaOH/50g curd)	8.26	8.45	8.55	8.60
4.	Acetaldehyde content (ppm)	32.40	32.80	33.08	33.20
5.	Proteolytic activity (mg tyrosine/g curd)	0.28	0.31	0.32	0.34

Values are averages of five trials in duplicate.

microscopic view of the cultures in dahi and yoghurt prepared with DAB and YAB was shown in Plates 4 and 5, respectively.

The lactic counts (excluding the counts of *Lb. acidophilus* and *Bif. bifidum*) of dahi samples prepared with cultures D and DAB were in the range of 7.210 to 8.330 log cfu per gram product, whereas the yoghurt samples prepared with cultures Y and YAB had the lactic cultures in the range of 8.104 to 8.524 log cfu per gram product. The lactic cell count in both dahi and yoghurt was found to be satisfactory to have a good quality product.

The titratable acidity (0.98%) diacetyl content (4.14 ppm) and proteolytic activity (0.49 mg tyrosine) of dahi prepared with freeze-dried culture D was found to be slightly more than the corresponding values of dahi prepared with liquid culture of the same combination, whereas only slight reduction in volatile acidity of dahi prepared with freeze-dried culture combination was noticed.

In terms of biochemical activity, dahi prepared with freeze-dried culture DAB was found to be as good as dahi samples prepared with liquid cultures of the same combination. Similarly, yoghurt samples prepared with freeze-dried cultures Y and YAB were found to be more active in terms of biochemical properties compared to those prepared with respective liquid cultures of the same combinations.

The titratable acidity production in the yoghurt was slightly retarded with the incorporation of *Lb. acidophilus* and *Bif. bifidum* in the culture combination of Y. Similarly, yoghurt prepared with culture combination YAB had less tyrosine content. The present observations are in agreement with the results of Sharma and Prasad (1986) and Kisza *et al.* (1978) with acidophilus yoghurt and Yuguchi *et al.* (1989) with yoghurt containing bifidus culture.

Table-38: Comparison of lactic count and biochemical properties of dahi prepared with bulk freeze-dried dahi cultures using standardized (4.0% fat) buffalo milk

Sl. No.	Biochemical property	Culture D (30°C)*		Culture DAB (37°C)*	
		Liquid culture+ (16 hrs)**	FD culture+ (14 hrs)**	Liquid culture+ (16 hrs)**	FD culture+ (14 hrs)**
1.	Lactic count (log cfu/g)	8.33	8.24	7.45	7.21
2.	% Titratable acidity (expressed as lactic acid)	0.98	0.96	1.20	1.22
3.	Volatile acidity (ml of 0.1N NaOH/50g curd)	36.10	35.80	16.00	16.20
4.	Diacetyl content (ppm)	4.10	4.14	2.70	2.82
5.	Proteolytic activity (mg tyrosine/g curd)	0.46	0.49	0.38	0.40

Values are averages of five trials in duplicate.
* = Temperature at which product was prepared.
** = Period taken to set the milk.
+ = Inoculum size for liquid cultures : 1.0% (v/v) and bulk freeze-dried cultures : 0.01% (w/v)

Table-39: Comparison of lactic count and biochemical properties of yoghurt prepared with liquid and bulk freeze-dried yoghurt cultures using standardized (4.0% fat) buffalo milk at 37 °C

Sl. No.	Biochemical property	Culture Y		Culture YAB	
		Liquid culture+ (14 hrs)*	FD culture+ (12 hrs)*	Liquid culture+ (14 hrs)*	FD culture+ (12 hrs)*
1.	Lactic count (log cfu/g)	8.52	8.31	8.44	8.10
2.	% Titratable acidity (expressed as lactic acid)	1.64	1.62	1.40	1.38
3.	Volatile acidity (ml of 0.1N NaOH/50g curd)	7.80	7.90	8.60	8.80
4.	Acetaldehyde content (ppm)	27.50	27.80	32.80	33.20
5.	Proteolytic activity (mg tyrosine/g curd)	0.36	0.38	0.32	0.35

Values are averages of five trials in duplicate.
* = Period taken to set the milk
+ = Inoculum size for liquid cultures : 1.0% (v/v) and bulk freeze-dried cultures : 0.01% (w/v)

However, the variations in biochemical properties observed in dahi or yoghurt, prepared with liquid and freeze-dried cultures were not significant. It was also observed that all the freeze-dried cultures were able to set the products comparatively in shorter time period (14 hrs with dahi and 12 hrs with yoghurt cultures) compared to 16 hrs with dahi and 14 hrs with yoghurt of incubation required for liquid cultures of respective combinations. This clearly indicated the usability of bulk freeze-dried culture combinations in the preparation of dahi and yoghurt routinely either in small scale or at industrial level.

4.4.3.2 Viability of *Bif. bifidum* and *Lb. acidophilus*

To have the therapeutic benefit, there is a need to maintain a minimum number of cells of *Lb. acidophilus* and *Bif. bifidum* in cultured products. Table-40 indicates the viable cell counts of *Bif. bifidum* and *Lb. acidophilus* in dahi and yoghurt prepared by using liquid as well as freeze-dried cultures.

The viable cell counts of *Lb. acidophilus* in dahi and yoghurt prepared with respective liquid cultures of DAB and YAB were found to be 7.928 and 7.654 log cfu per gram whereas the counts in the products prepared using respective freeze-dried cultures were 7.812 and 7.586 log cfu per gram in dahi and yoghurt, respectively. The viable cell counts of *Bif. bifidum* in dahi and yoghurt prepared with respective liquid cultures of DAB and YAB were found to be 7.579 and 7.122 log cfu per gram whereas the counts in the products prepared using respective freeze-dried cultures were 7.440 and 7.092 log cfu/g in dahi and yoghurt, respectively.

From the results, it may be observed that the counts of acidophilus were more than that of bifidobacteria in all the products prepared. The counts of both bifidobacteria and acidophilus were more in the products prepared with liquid cultures when compared to the products prepared using respective freeze-dried cultures. The difference may be attributed to the

Table-40: Viable cell counts of *Bif.bifidum* I and *Lb.acidophilus* R in dahi and yoghurt prepared with liquid and bulk freeze-dried cultures of dahi (DAB) and Yoghurt (YAB)

Sl. No.	Product prepared	Type of culture	Viable cell counts of *Bif.bifidum* I	Viable cell counts of *Lb.acidophilus* R
			---------(log cfu/g)---------	
1.	Dahi culture DAB	Liquid	7.579	7.928
2.	Dahi culture DAB	Freeze-dried	7.440	7.812
3.	Yoghurt culture YAB	Liquid	7.122	7.654
4.	Yoghurt culture YAB	Freeze-dried	7.092	7.586

Values are average of six trials in duplicate.

loss in the viability during freeze-drying process and during storage of the cultures.

In any case, the viable cell count was more than the 'therapeutic minimum' (10^6 cells/g) which has been proposed as essential to effect some noticeable nutritional, prophylactic and/or therapeutic benefit when consumed (Gilliland, 1989; Klupsch, 1989 and Kurmann and Rasic, 1991). The results are comaprable to the observations of Klupsch (1985) who reported the presence of 10^7-10^8 cells of *Lb. acidophilus* and 10^6-10^7 cells of *Bif. bifidum* per gram in the product and Yuguchi *et al.* (1989) who have demonstrated the presence of 10^6 cells of bifidobacteria in yoghurt fermented with bifidobacteria.

4.4.3.3 Sensory evaluation of dahi and yoghurt

On the basis of organoleptic evaluation, dahi prepared with culture DAB was rated as "very good" with overall scores of 17.58 and 17.36 for products prepared with liquid and freeze-dried cultures (Table-41) whereas dahi samples prepared with liquid and freeze-dried cultures of D were considered "excellent" with scores of 18.48 and 18.18, respectively. In contrast, yoghurt samples prepared with Y and YAB were rated as "excellent"

The analysis of variance (Appendix IX) pertaining to the sensory evaluation of dahi samples prepared with liquid and freeze-dried dahi culture D revealed that the scores allotted were significantly higher compared to the scores allotted to dahi samples prepared with culture DAB at one percent level. The scores allotted to dahi samples prepared with liquid cultures (D and DAB) were significantly higher at 5% level compared to the scores of dahi prepared with freeze-dried cultures (D and DAB).

The sensory scores of yoghurt prepared with culture combination Y and YAB were found to be non-significant at 1% level, which indicated that the addition of *Lb. acidophilus* and *Bif. bifidum* has not changed the sensory properties of yoghurt.

Table-41: Sensory evaluation of dahi and yoghurt prepared with liquid and bulk freeze-dried cultures of dahi and yoghurt.

Sl. No.	Product	Observations	Maximum Scores allotted	Scores obtained	
				Liquid	Freeze-dried
1.	Dahi (with culture D)	a) Appearance	5	4.48	4.73
		b) Body & Texture	5	4.45	4.83
		c) Flavour	10	9.55	9.62
		Overall score	20	18.48	18.18
2.	Dahi (with culture DAB)	a) Appearance	5	4.82	4.61
		b) Body & Texture	5	4.76	4.73
		c) Flavour	10	8.02	8.02
		Overall score	20	17.58	17.36
3.	Yoghurt (with culture Y)	a) Appearance	5	4.70	4.82
		b) Body & Texture	5	4.80	4.64
		c) Flavour	10	9.30	9.82
		Overall score	20	18.80	19.29
4.	Yoghurt (with culture YAB)	a) Appearance	5	4.68	4.84
		b) Body & Texture	5	4.58	4.66
		c) Flavour	10	9.38	9.80
		Overall score	20	18.64	19.30

Values are averages of five samples.

The reason for low flavour scores (8.02) of dahi prepared with culture DAB when compared to the scores of dahi prepared with culture D (9.52 and 9.62) may be due to the high acidity which might have masked the original flavour of dahi and variations of individual liking of the judges.

The observations made with regard to yoghurt samples were in accordance with the findings of Yuguchi *et al.* (1989) and Klupsch (1989).

4.5 Evaluation of Nutritional and Therapeutic Values of Dahi and Yoghurt

The composition of various diets is presented in Table-42. There was no significant difference in fat and cholesterol content among various diets.

4.5.1 *Effect on Body Weight Gain of Male Albino Rats*

The male albino rats were fed with standardized buffalo milk, dahi and yoghurt prepared with freeze-dried culture combinations of dahi and yoghurt, for a period of 30 days and the means of body weight gain are shown in Fig.12.

During the first week, the rats fed with rat chew (control group) grew slightly faster than those fed with cultured products. However, after four weeks the control group had lowest weight gain (84 g) compared to the rats fed with other diets (90-130g). The rats fed with standardized buffalo milk had lower weight gain (99.66 g) when compared to the rats fed with dahi or yoghurt, the values being 107.16 and 119.83, respectively.

Yoghurt prepared with culture Y had more growth promoting activity in rats with a mean body weight gain of 119.83 g than did dahi prepared with culture D with a mean body weight gain of 107.16 g. However, the effect was reversed in the rats fed with

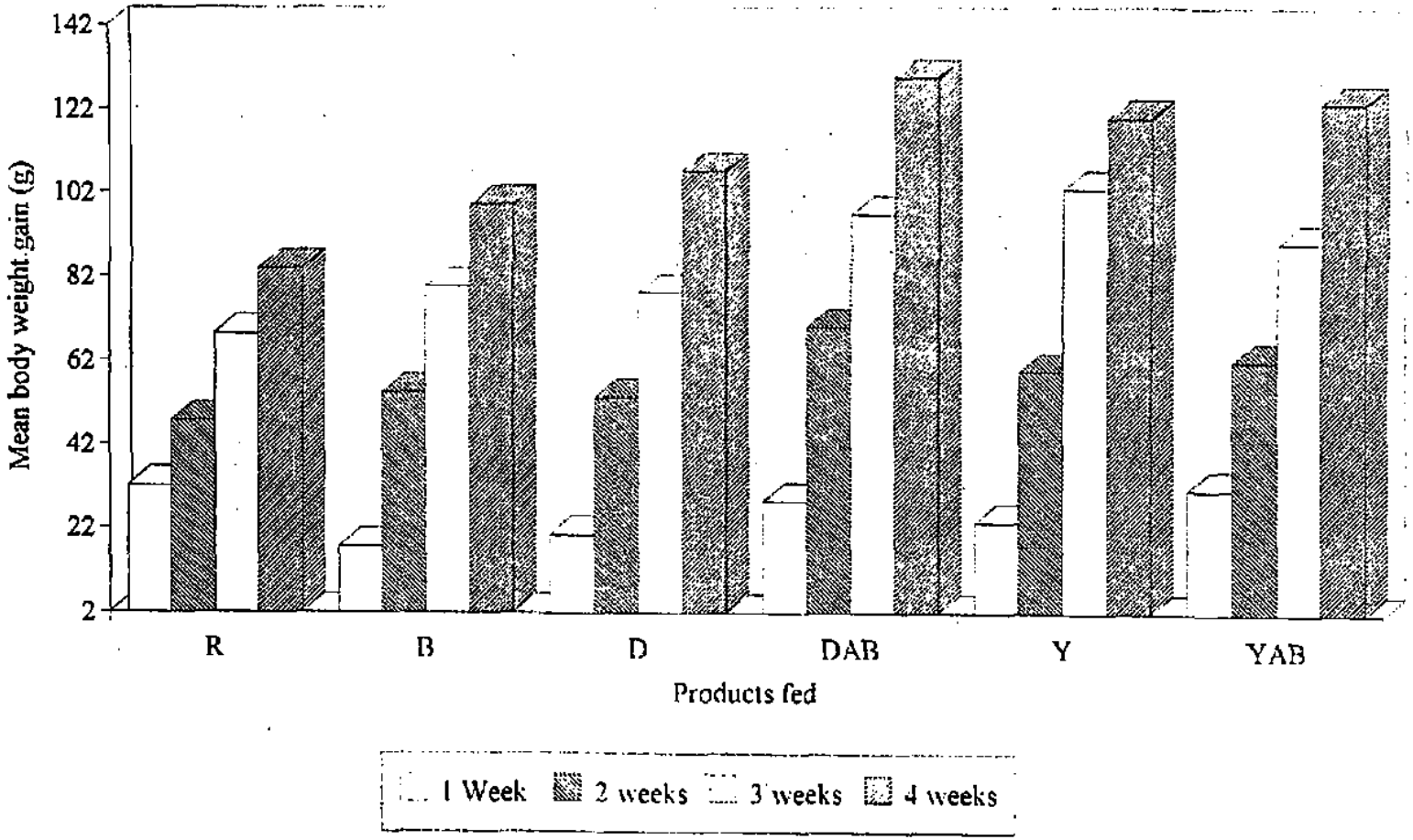
Table-42: Chemical analysis of various products fed to male albino rats

Sl. No.	Type of the diet	Fat content (%)	Cholesterol (mg/100g)	%Titratable acidity (expressed as lactic acid)
1.	Standardized buffalo milk	4.05	11.10	0.15
2.	Dahi with culture D	4.02	11.00	0.92
3.	Dahi with culture DAB	3.98	10.80	1.16
4.	Yoghurt with culture Y	3.88	10.60	1.66
5.	Yoghurt with culture YAB	3.80	10.45	1.36

Values are averages of six estimations.

R	Rat chew
B	Standardized buffalo milk
D	Dahi prepared with culture D
DAB	Dahi prepared with culture DAB
Y	Yogurt prepared with culture Y
YAB	Yogurt prepared with culture YAB

Fig. 12 Effect of dahi and yoghurt prepared by using different culture combinations on mean body weight gain of albino rats.



the products prepared with the combinations of DAB and YAB with a body weight gain of 129.33 and 123.33 g, respectively.

It is interesting to note that the addition of *Bif. bifidum* and *Lb. acidophilus* to the cultures of dahi and yoghurt helped in improving the body weight in both the groups and this effect was significantly more with dahi than with yoghurt samples. The data obtained in the experiment disclosed that the differences observed in body weight gain among the rats fed with various diets was found to be significant at one percent level (Appendix X).

From the data, it may be inferred that fermented milk products definitely have a role in improving the body weight and the present observation is comaparable to the that of Hangroove and Alford (1978). Similar observation was recorded earlier in other animals (McDonough *et al.*, 1982; Hitchins *et al.*, 1983 and Pollman, 1984). The present observations are contradicting the results of Chawla (1982) which may be due to variations in the experimental design, differences in the composition of diet or the ratio of the strains present in the products.

4.5.2 *Effect on Serum Cholesterol Level of Male Albino Rats*

The levels of serum cholesterol in male albino rats fed with dahi and yoghurt prepared with freeze-dried culture combinations are presented in Table 43 and Fig.13.

The experimental animals in Group A became hypercholesterolemic and the serum cholesterol level rose from 58 to 72 mg per deciliter on feeding the standardized buffalo milk for 30 days. The serum cholesterol level of rats fed with dahi had gone upto 65 mg from the initial level of 60 mg per 100 ml of serum at the end of the experiment, whereas an increase of only 2.4 mg cholesterol per 100 ml was observed in control group fed exclusively with rat chew. The rise in serum cholesterol is mainly due to the milk fat present in the diets fed.

R	Rat chew
B	Standardized buffalo milk
D	Dahi prepared with culture D
DAB	Dahi prepared with culture DAB
Y	Yogurt prepared with culture Y
YAB	Yogurt prepared with culture YAB

Fig. 13 Effect of dahi and yoghurt prepared by using different culture combinations on serum cholesterol level of albino rats.

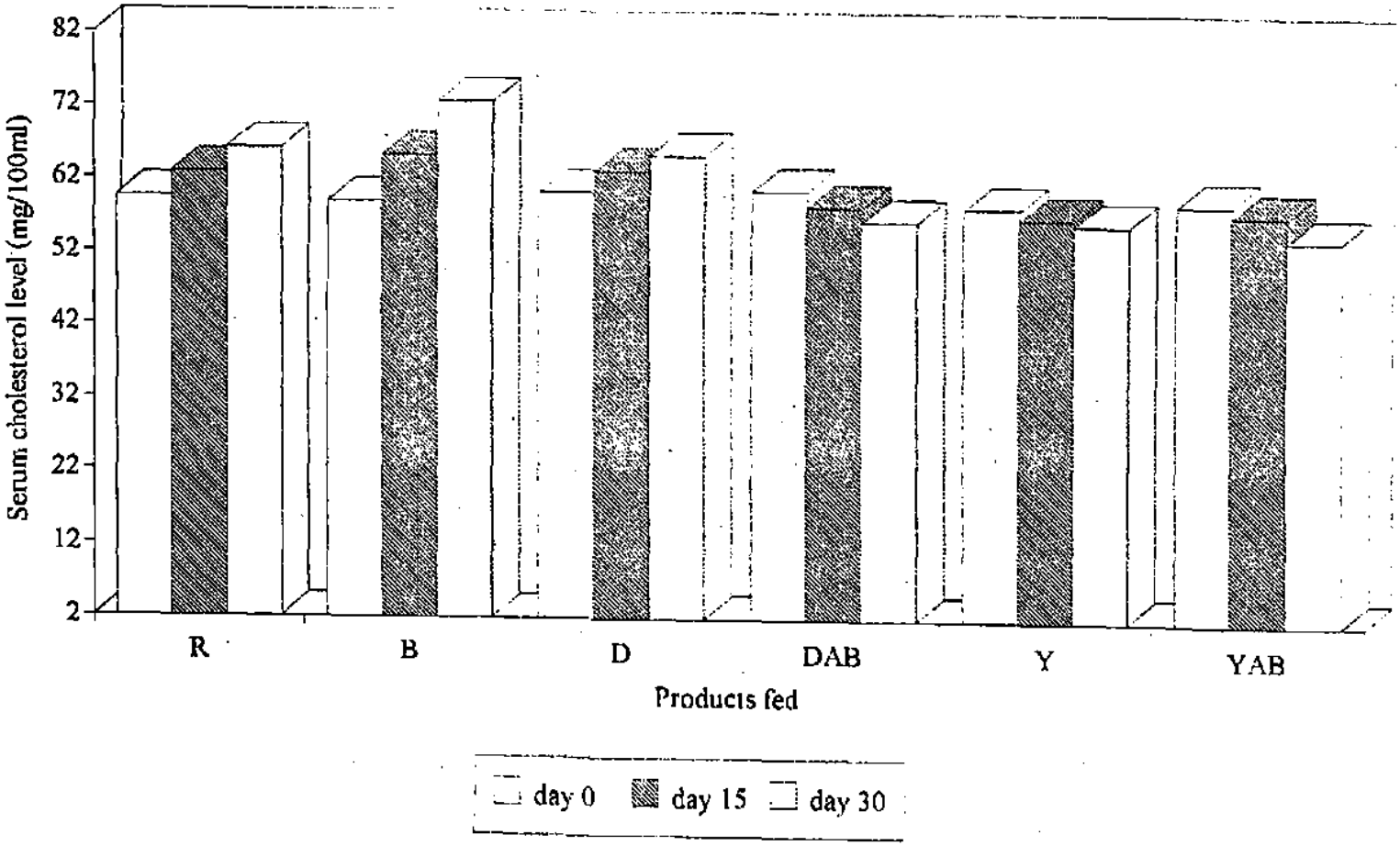


Table-43: Effect of feeding dahi and yoghurt prepared with bulk freeze-dried cultures of dahi and yoghurt on serum cholestrol level of male albino rats

Group	Major Diet Fed	Period (days)		
		0	15	30
		Serum cholestrol level (mg/100 ml)		
	Rat chew	59.55	61.85	62.06
A	Standardized buffalo milk	58.92	65.18	72.54
	Dahi with culture D	60.28	63.18	65.41
	Dahi with culture DAB	60.56	58.46	56.55
B	Yoghurt with culture Y	58.38	56.98	55.75
	Yoghurt with culture YAB	59.21	57.75	54.53

Values are averages of six observations

The rats in Group B fed with yoghurt (prepared with culture Y), dahi and yoghurt containing bifidobacteria and *Lb. acidophilus* registered a steady and gradual decrease in serum cholesterol till the end of experimental period. The decrease was in the order of 2.63, 4.1 and 4.68 mg/100 ml in the rats fed with yoghurt (with culture Y), dahi (with culture DAB) and yoghurt (with culture DAB), respectively.

It is interesting to note that feeding dahi containing therapeutic cultures has decreased the serum cholesterol level considerably more than when dahi prepared with culture D was fed. Similar observations were noted with yoghurt prepared with cultures Y and YAB.

The analysis of the data obtained for group A revealed that the increase in serum cholesterol level was significant at one percent level as shown in the ANOVA Table (Appendix XI) whereas the decrease in serum cholesterol level in rats fed with dahi prepared with culture DAB and yoghurt prepared with cultures Y and YAB was found to be significant ($P \leq 0.01$).

The present findings are in agreement with the reports of Kansal and Chawla (1984), who reported reduction in plasma cholesterol level in rats fed with dahi and acidophilus dahi over a period of 90 days, Hepner *et al.* (1979), Gorbach *et al.* (1988). The experiments of Grunewald (1982) and Ishida and Kubo (1985) are also supporting the present findings of lowering effect of fermented milks containing *Lb. acidophilus*. Similarly, Danielson *et al.* (1989) reported reduction in cholesterol level in boars fed with *Lb. acidophilus* 16.

4.5.3 *Evaluation of Antibacterial Activity*

All the products showed antibacterial activity to a variable degree against all the three test organisms viz. *E. coli*, *S. typhimurium* and *Sh. dysenteriae*. It is evident from the data

Table-44: Antibacterial activity of dahi and yoghurt prepared with bulk freeze-dried cultures of dahi and yoghurt

Sl. No.	Product	Test organism		
		Escherichia coli K 12	*Salmonella typhimurium* A-KIGM	*Shigella dysenteriae* KIGM
		Inhibition zone diameter (mm)*		
1.	Control	10.50	10.00	10.00
2.	Dahi with culture D	12.00	10.34	11.08
3.	Dahi with culture DAB	16.00	14.12	14.20
4.	Yoghurt with culture Y	13.50	12.50	14.00
5.	Yoghurt with culture YAB	18.50	16.10	17.60

Values are averages of five trials in duplicate.
*Values are inclusive of 6mm bore diameter.
Very strong inhibition : >17 - 20 mm
Strong inhibition : >14 - 17 mm
Moderate inhibition : >11 - 14 mm
No inhibition : <11 mm

(Table-44 and Plate-6) that dahi prepared with culture DAB had more antibacterial activity with a strong inhibition of all the test organisms. The inhibition was maximum against *E. coli* with an inhibitory zone diameter of 16 mm. On the contrary, dahi prepared with culture combination D exhibited moderate inhibition against *E. coli* and *Sh. dysenteriae* with a zone diameter of 12.00 and 11.08 mm, respectively.

Yoghurt prepared with culture YAB exhibited very strong inhibition against all the test organisms (16.10 to 18.50 mm of inhibition zone) and yoghurt prepared with culture Y showed antibacterial activity in the range of 12.50 to 14.00 mm. From the results, it is concluded that yoghurt samples with and without therapeutic cultures have strong inhibitory activity compared to that of dahi prepared with culture D or DAB. The greater inhibitory activity observed with dahi and yoghurt compared to control (2% lactic acid) indicates the presence of inhibitory substances other than lactic acid.

The higher inhibitory activity of yoghurt compared to dahi can be attributed to the presence of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. salivarius* ssp. *thermophilus* in yoghurt culture which synthesize the antibacterial compounds. (Abou Donia *et al.*, 1980 and Dave *et al.*, 1992).

The enhanced antibacterial activity of dahi and yoghurt containing *Bif. bifidum* and *Lb. acidophilus* may be due to the high antibiotic nature of *Bif. bifidum* (Anand *et al.*, 1984; Tojo *et al.*, 1987 and Tomada *et al.*, 1988) and that of *Lb. acidophilus* (Vakil and Shahani 1965; Sandine 1979; Sharma, 1982; Keating, 1985; Attaie *et al.*, 1987; Rao and Gandhi, 1988; Ratnakar, 1989 and Khedkar *et al.*, 1990). The present observation is in complete agreement with Rao (1993) who reported stronger antibacterial activity of yoghurt ice-cream supplemented with *Lb. acidophilus* and *Bif. bifidum* than the product prepared with yoghurt cultures.

SUMMARY AND CONCLUSIONS

5.0 SUMMARY AND CONCLUSIONS

5.1 Twelve combinations of lactic cultures were formulated using ten strains of lactic acid bacteria. From these, dahi and yoghurt culture formulations were selected on the basis of better biochemical performance and body and texture of the resultant curds.

To the selected dahi and yoghurt cultures selected strains of *Lb. acidophilus* and *Bif. bifidum* were combined on the basis of total performance of these cultures.

The promising two dahi cultures and two yoghurt cultures developed in this study were designated as dahi culture D, dahi culture DAB, yoghurt culture Y and yoghurt culture YAB and consisted of the following strains of lactic acid bacteria:

Dahi culture D has *Lc. lactis* ssp. *lactis* C10, *Lc. lactis* ssp. *cremoris* C1 and *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC1. Dahi culture DAB has *Lb. acidophilus* R and *Bif. bifidum* I along with the combined strains of dahi culture D. Yoghurt culture Y has *Str. salivarius* ssp. *thermophilus* HST and *Lb. delbrueckii* ssp. *bulgaricus* RTS. Yoghurt culture YAB has *Lb. acidophilus* R and *Bif. bifidum* I along with the strains of yoghurt culture Y.

Dahi culture D had a viable cell count of 8.556 log cfu/g, titratable acidity of 0.93% LA, volatile acidity of 36.0 ml of 0.01N NaOH/50g curd, diacetyl content of 4.05 ppm and proteolytic activity, in terms of 0.46 mg tyrosine liberated, after 18 hrs at 30°C in buffalo skim milk. Dahi culture DAB had a viable count 7.450 log cfu/g, titratable acidity of 1.18% LA, volatile acidity of 16.5 ml of 0.01 N NaOH/50g curd, diacetyl content of 2.68 ppm and proteolytic activity, in terms of 0.36mg tyrosine liberated, after 18 hrs at 37°C in buffalo skim milk.

Yoghurt culture Y had a viable cell count of 8.447 log cfu/g, titratable acidity of 1.68% LA, volatile acidity of 8.2 ml of 0.01N NaOH/50g curd, acetaldehyde content of 28.40 ppm and proteolytic activity of 0.38 mg tyrosine liberated after 18 hrs at 37°C in buffalo skim milk. Yoghurt culture YAB had a viable cell count of 8.380 log cfu/g, titratable acidity of 1.44% LA, volatile acidity of 8.9 ml, acetaldehyde content of 34.60 ppm and proteolytic activity, in terms of tyrosine liberated of 0.33 mg, after 18 hrs at 37°C in buffalo skim milk.

5.2 Eleven skim milk based and seven cheese whey based media were formulated for the biomass production of dahi cultures D and DAB and yoghurt cultures Y and YAB. Skim milk based medium (Medium one) with the ingredients of skim milk (5%), yeast autolysate (1%) and glucose (1%) with a pH of 6.5 for dahi cultures D and DAB. Another skim milk medium (Medium two) with the composition of skim milk (50%), tryptone (1%) and glucose (0.1%) with a pH of 6.0 for yoghurt culture Y and with a pH of 6.5 for yoghurt culture YAB were found to be the best on the basis of the viable count and titratable acidity.

5.3 Medium one with a pH of 6.5 for dahi cultures D and DAB with a respective cell yield of 9.708 and 9.686 (log cfu/g) and medium two with a pH of 6.0 for yoghurt culture Y and with a pH of 6.5 for yoghurt culture YAB, which yielded 9.882 and 9.502 log cfu/g, respectively were found to be the best.

Dahi cultures performed best after neutralization with viable cell counts of 9.708 and 9.686 log cfu/g for D and DAB, respectively, at the end of 18 hrs of incubation under the conditions of pH 6.5, temperature 30°C for D and 37°C for DAB with 1% inoculum rate. For yoghurt culture a pH 6.0 for Y and 6.5 for YAB, temperature 30°C and 1% inoculum rate showed maximum viable cell count (9.88 and 9.502 log cfu/g, respectively for Y and YAB).

5.4 Out of three formulated cryoprotective agents the cryoprotective agent consisting of 10% buffalo milk, 1.5% gelatin, 7% sucrose and 1M monosodium glutamate was found to have maximum protective effect with least reduction in viable cell counts; of dahi culture D (13%), dahi culture DAB (3.8%), yoghurt culture Y (11.6%) and yoghurt culture YAB (11.26). This newly formulated suspending medium was the best among all the cryoprotective agents so far reported.

5.5 The survival of dahi and yoghurt cultures, packed in 25g quantities in laminated aluminium foil pouches (12 micron polyethylene + 12 micron aluminium foil + 150 gaugage polypropylene) sealed under vacuum was found to be maximum when stored at -20°C. The difference in survival rate between dahi and yoghurt cultures sealed under vacuum and stored at -20°C was because of the characteristic of mesophilic and thermophilic nature of the respective cultures. Sealing atmosphere of air and higher temperature of storage (4°C) were found to be deleterious for the viability and activity of cultures.

The residual moisture content of freeze-dried dahi and yoghurt cultures (in the range of 1.20-1.41%) had not showed any deleterious effect on survival. Therefore, the moisture content limit below 1.5% is recommended for freeze-dried lactic acid bacteria.

5.6 The regression equations derived with the parameters of residual moisture content, titratable acidity, diacetyl/acetaldehyde content, proteolytic activity and storage period were found to give the shelf-life of bulk freeze-dried dahi and yoghurt cultures.

5.7 The inoculum rate of 0.01% (w/v) of four cultures was equal to 1.0% of liquid cultures. The biochemical performance of freeze-dried cultures was slightly better than the routinely used liquid cultures. The incubation period was shortened by two hrs

with freeze-dried cultures. A pack of 25g culture can be used to prepare 250 kg of dahi or yoghurt.

5.8 Biochemical performance and starter activity of bulk freeze-dried cultures of dahi and yoghurt were in the same order irrespective of the type of the milk, cow skim milk (9.6% TMS), buffalo skim milk (10.20% TMS), cow milk (4% fat, 13.4% TMS) and buffalo milk (4% fat, 15.7% TMS). The performance of dahi and yoghurt cultures was slightly better in buffalo milk than other milks. Bulk freeze-dried cultures of dahi and yoghurt are equally good to manufacture good quality dahi and yoghurt in all types of milk.

Dahi prepared with culture DAB contained 7.440 and 7.812 (log cfu/g) of *Bif. bifidum* and *Lb. acidophilus*, respectively and yoghurt prepared with yoghurt culture YAB had 7.092 and 7.586 log cfu/g, respectively.

Dahi and yoghurt prepared using bulk freeze-dried cultures were rated from very good to excellent. The score was similar to those obtained by the products prepared with liquid cultures. Addition of *Lb. acidophilus* and *Bif. bifidum* to yoghurt has no effect on the sensory acceptance. However, dahi prepared with DAB scored 4.5% less, yet it was well-accepted.

On the basis of the data obtained in the present study and keeping in view of the general hygienic condition of the dairy plants in our country, the following microbiological standards are suggested:

Dahi with and without acidophilus and bifidobacterium should have the lactic acid bacteria count of 10^7 cfu/g, titratable acidity of 0.8 to 1.2%and diacetyl content of 2.0-3.5 ppm. Yoghurt with and without the therapeutic cultures should contain the lactic acid bacterial count of 10^7 cfu/g, titratable acidity of 1.4-1.6 and 15-20 ppm of acetaldehyde. The minimum counts of

Lb. acidophilus and *Bif. bifidum* should be 10^6 cfu/g in dahi and yoghurt.

5.9 Feeding of dahi and yoghurt to albino rats for a period of four weeks increased the mean body weight but feeding of the products containing therapeutic cultures enhanced the mean body weight gain by 20.68 and 2.92%, respectively. Feeding of standardized buffalo milk (4.0% fat) raised the serum cholesterol level from 58.92 to 72.54 mg percent. Dahi feeding too increased the serum cholesterol content significantly from 60.28 to 65.41 mg. However, dahi incorporated with therapeutic cultures had a decreasing effect on serum cholesterol level. The reduction being 6.68%. Plain yoghurt also reduced the cholesterol level (4.5%), however, presence of therapeutic cultures in yoghurt reduced the serum cholesterol content further (7.9%).

Dahi and yoghurt prepared with DAB and YAB cultures had shown strong antibacterial activity against *E.coli*, *Sal. typhimurium* and *Sh.dysenteriae*. Dahi and yoghurt prepared with D and Y cultures showed moderate inhibition.

CONCLUSIONS

The most important finding of the study is the designation of the promising starter cultures for dahi and yoghurt preparation with uniformity in the quality from batch to batch. Production of concentrated freeze-dried cultures are expected to bring about significant changes in the trend of dahi and yoghurt manufacture in the country.

The present investigation clearly demonstrated the benefits with the incorporation of therapeutic cultures in the cultures of dahi and yoghurt. Therefore, there is every need to use these two cultures to enhance the nutritional and therapeutic values of the products and to maintain health.

*****

BIBLIOGRAPHY

BIBLIOGRAPHY

Abd-El Gawad, I.A., Metwally, M.M., Mehriz, A.M. and El Dieb, S.M. (1988). The hypocholesterolaemic effect of butter milk. II. The effect of buttermilk and cholesterol in the diets on rats' serum and hepatic lipids. Egyptian J. Dairy Sci., 16(2): 185-190.

Abou Donia, S.A., Ismail, A.A., Salma, F.A., Kader, A.E. and Abou-Zeid, N.A. (1980). The inhibitory effect of some fermented milk flora on the growth of some pathogenic bacteria. Monoufeia J. Agril. Res. 3: 225-236. Cited in: Dairy Sci. Abstr., 45(1): 446.

Accolas, J.P., Bloquel, R., Didienne, R. and Regnier, J. (1977). Acid producing properties of thermophilic lactic acid bacteria in relation to yoghurt manufacture. Le Lait, 57: 1-23.

Alaeddinoglu, G., Guven, A. and Ozilgen, M. (1989). Activity-loss kinetics of freeze-dried lactic acid starter cultures. Enz. and Micro. Technol., 11(1): 765-769.

Anand, S.K., Srinivasan, R.A. and Rao, L.K. (1984). Antibacterial activity associated with *Bifidobacterium bifidum* I. Cult. Dairy Prod. J., 19(9): 6-8.

Anand, S.K., Srinivasan, R.A. and Rao, L.K. (1985). Antibacterial activity associated with *Bifidobacterium bifidum* II. Cult. Dairy Prod. J., 20(1): 21 and 23.

Ashton, T.R. (1963). Cultured milk products. J. Soc. Dairy Technol., 16(2): 68-69.

Astrup, P. (1977). Diet and heart disease. Meierirosten, 66: 477-479; 519-522. Cited in: Dairy Sci. Abstr., 39(2): 739.

Attaie, R., Whalen, P.J., Shahani, K.M. and Amer, M.A. (1987). Inhibition of growth of *Staphylococcus aureus* during production of acidophilus yoghurt. J. Food Prot. 50(3): 224-228.

Ayunts, K.M., Vegapetyan, K.V. and Terkazaryan, S.S.H. (1979). Effect of cultivation conditions on yield of biomass of

lactic acid bacteria. Izvestiya Vysshikh Uchebnykh Zavedenii, Pischevaya Tekhnologiya No. 2: 56-57. Cited in: Dairy Sci. Abstr., 42(3): 1700 (1980).

Babel, F.J. (1977). Antibiosis by lactic culture bacteria. J. Dairy Sci., 60: 815-821.

Baisya, R.K. and Bose, A.N. (1974). Studies on dehydration of dahi. J. Food Sci. Technol., 11(3): 128-131.

Baisya, R.K. and Bose, A.N. (1975). Role of inoculating organisms on the Physico-chemical changes of milk and on final dahi quality. Indian J. Dairy Sci., 28(1): 179-183.

Bakshi, S.K. (1982). Studies on the preparation of yoghurt from recombined milk. M.Sc. Dissert., Kurukshetra University, Kurukshetra.

Bannikova, L.A. and Lagoda, I.V. (1970). Production of dried starter cultures from bacterial concentrate. XVIII Int. Dairy Congr.,1E: 277.

Barach, J.T. and Kamara, B.J. (1986). Process to preserve acid producing bacteria and compositions produced thereby. European Patent Application. EP 0 196 593 A2. Cited in: Dairy Sci. Abstr., 49(7): 4563.

Barbour, E.A. and Priest, F.G. (1986). The preservation of lactobacilli: a comparison of three methods. Lett. Appl. Microbiol., 2(4): 69-71.

Belova, G.A., Medvedeva, Z.P., Kornelguk, A.N., Klimova, L.I., Bratsilo, T.E. and Lymer, V.G. (1977). Improvement of technology of bacterial preparation No. 2 for cheese. Trudy No. 21: 16-21. Cited in: Dairy Sci. Abstr., 42(1): 349 (1980).

Belova, A.N. Belova, L.P., Krivorotova, T.L. and Guseva, N.M. (1993). Influence of protective medium composition on freeze-drying and storage of concentrates of thermophilic lactic acid bacteria. Moloch. prom. 1: 25-26. Cited in: Food Sci. Technol. Abstr., 25(11): p39.

Bergere, J.L. (1968). Production massive de cellules de *Streptocoques lactiques*. Le lait, 48: 131.

Berridge, N.J., Newton, G.G.F. and Abraham, E.P. (1952). Purification and nature of the antibiotic Nisin. Biochem. J., 52: 529-535.

Bhatt, N.N. (1976). Utilization of *Streptococcus diacetylactis* mutant for preparation of dahi. M.Sc. dissert., B.N.C. University, Kurukshetra.

Bianchi-Salvadori, B., Camschella, P. and Cislaghi, S. (1988). Effect of yoghurt lactic acid bacteria and bifidobacteria on translocation of *E.coli* in the lymph system. Int. Symp. Intest. Microecol. Alghera. Cited in: IDF (1991).

Bily, R.R. (1987). Starter cultures of improved activity for dairy products and process of making same. US Patent US 4 713 341. Cited in: Dairy Sci. Abstr., 50(8): 4608.

Bishagranta (1907). Sushrut Samhita. 10, Kashi Ghosh Lane, Calcutta. Cited by: Laxminarayana, H. and Iya, K.K. (1952).

Blaine, J.W. (1972). Preparation and preservation of lactic acid starter culture concentrate. Dissert. Abstr. Int., B-33: 340.

Boekhout, F.W.J. and Ott devries J.J. (1920). Verslag. Landb. Onderzoek. Rijksland bouwprofsta, 24: 1-12. Cited by Hammer, B.W. and Babel F.J. (1943). J. Dairy Sci., 46(2): 80-168.

Boyaval, P., Corre, C. and Therre, S. (1987). Continuous lactic acid fermentation with concentrated product recovery by ultrafiltration and electrodialysis. Biotechnol. Lett., 9(3): 207-212.

Bozoglu, T.F., Ozilgen, M. and Bakir, V. (1987). Survival kinetics of lactic acid starter cultures during and after freeze-drying. Enz. Microbiol. and Technol., 9: 531.

Branen, A.L., Go, H.C. and Genska, R.P. (1975). Purification and properties of antibacterial substances produced by *Str. diacetylactis* and *Leu. citrovorum*. J. Food Sci., 40(3): 446-450.

Briggs, M., Tull, G., Newland, L.G.M. and Briggs, C.A.E. (1955). The preservation of lactobacilli by freeze-drying. J. Gen. Microbiol., 12(3): 503-512.

Broussalian, J. and Westhoff, D. (1983). Influence of lactose concentration of milk and yoghurt on growth rate of rats. J. Dairy Sci., 66(3): 438-443.

Champagne, C.P., Detournay, H. and Hardy, M.J. (1991a). Effect of medium on growth and subsequent survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* to freeze-drying. J. Indl. Microbiol., 7(2): 147-150.

Champagne, C.P., Gardner, N., Brochu, E. and Beauliev, Y. (1991b). The freeze-drying of lactic acid bacteria: A reivew. Can. Inst. Sci. Technol. J., 24(3/4): 118-128.

Chawla, K. (1982). Comparative studies on the hypocholestermic effect of milk, dahi, acidophilus milk and acidophilus milk powder. M.Sc. dissert. Kurukshetra University, Kurukshetra.

Cheng, C.C. and Nagasawa, T. (1983). Associative relationships between bifidobacteria and lactobacilli. Jpn. J. Zootech. Sci., 54(11): 740-747.

Christopherson, A.T. and Zottola, E.A. (1989). Growth and activity of mesophilic lactic acid streptococci in ultrafiltered skim milk and in reconstituted non-fat dry milk of different total solid contents. J. Dairy Sci., 72(11): 2856-2861.

Ciblis, F. (1966). Beitrag Zur Gefriertrocknung von Milk saurebak tarien. XVII Int. Dairy Congr., C: 287.

Cogan, T.M., Buckley, D.J. and Condon, S. (1971). Optimum growth parameters of lactic streptococci used for the production of concentrate cheese starter cultures. J. Appl. Bacteriol., 34(2): 403-409.

Collins, E.B. and Aramaki, K. (1980). Production of hydrogen peroxide by *Lactobacillus acidophilus*. J. Dairy Sci., 63(3): 353-357.

Collins, E.B. and Hall, B.J. (1984). Growth of bifidobacteria in milk and preparation of *Bifidobacterium infantis* for a dietary adjunct. J. Dairy Sci., 67(7): 1376-1380.

Czulak, J. and Hammond, L.A. (1954). Compatibility amongst strains of lactic streptococci. Aust. J. Dairy Technol., 9(1): 15-18.

Daeschel, M.A. (1989). Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol., 43(1): 164-167.

Dahiya, R.S. and Speck, M.L. (1966). Associative growth of *Lactobacillus casei* and *Streptococcus lactis* in milk. XVII Int. Dairy Congr. B: 459-465.

Dalai, S. (1983). Studies on bulk freeze-dried starter cultures. M.Sc. Dissert. Kurukshetra University, Kurukshetra.

Daly, C. (1991). Lactic acid bacteria and milk fermentations. J. Chemical Technol. Biotechnol., 51(4): 544-548.

Damjanovic, V. and Radulovic, D. (1967). Survival of *Lactobacillus bifidus* after freeze-drying. Cryobiol., 4(1): 30-32.

Danielson, A.D., Peo Jr., E.R., Shahani, K.M., Lewis, A.J., Whalem, P.J. and Amer, M.A. (1989). J. Anim. Sci., 67(4): 966-974.

Dave, R.I., Dave, J.M. and Sannabhadti, S.S. (1992). Antibacterial activity of buffalo milk dahi prepared using *S. thermophilus*. Indian J. Dairy Sci., 45(11): 607-610.

Decowski, M. (1961). Folica Morphologica 12: 89.

Deeth, H.C., and Tamime, A.Y. (1981). Yoghurt: Nutritive and therapeutic aspects. J. Food Prot., 44(1): 78-86.

Driessen, F.M and de Boer, R. (1989). Fermented milks with selected intestinal bacteria: A healthy trend in new products. Neth. Milk Dairy J., 43(4): 367-382.

Efstathiou, J.D., McKay, L.L., Morris, H.A. and Zottola, E.A. (1975). Growth and preservation parameters for preparation of a mixed species culture concentrate for cheese manufacture. J. Milk Food Technol., 38(8): 444-448.

El-Sadek, G.M., Shchata, A.E. and Shassaw, A.A. (1975). The effect of freeze-drying on viability and activity of lactic streptococci cultures. Egyptian J. Dairy Sci., 3(1): 38-42.

El-Soda, M.A., Abou-Donia, S.A., El-Shafy, H.K., Mashaly, R. and Ismail, A.A. (1986). Metabolic activities and symbiosis in Zabady isolated cultures. Egyptian J. Dairy Sci., 14(1): 1-10.

Ervol'der, T.M. and Gudkov, A.V. (1982). Dried concentrate of bifidobacteria. XXI Int. Dairy Congr., 1(2): 300-301.

Fernandes, C.F., Shahani, K.M. and Amer, M.A. (1986). Effect of bile salts and calcium on growth and antimicrobial activity of *Lactobacillus acidophilus*. J. Dairy Sci., 69(Suppl. 1): 53 (Abstr.).

Ferras, E., Minier, M. and Goma, G. (1986). Acetonobutylic fermentation: Improvement of performances by coupling continuous fermentation and ultrafiltration. Biotechnol. and Bioengg., 28(4): 523-533.

Ferreira, C.L. and Del, I. (1987). Premature death phase of *L. acidophilus* NCFM growing at pH 6.0. Dissert. Abstr. Int. B. 47(9): 3641-3642.

Font de Valdez, G., Savey De Giori, G., Pesce De Ruiz Holgado, A. and Oliver, G. (1983a). Comparative study of the efficiency of some additives in protecting lactic acid bacteria against freeze-drying. Cryobiol., 20: 560-566.

Font de Valdez, G., Savey De Giori, G., Pesce De Ruiz Holgado, A. and Oliver, G. (1983b). Protective effect of Adonitol on lactic acid bacteria subjected to freeze-drying. Appl. Environ. Microbiol., 45(1): 302-304.

Font de Valdez, G., Savey De Giori, G., Pesce De Ruiz Holgado, A. and Oliver, G. (1985). Effect of drying medium on residual

moisture content and viability of freeze-dried lactic acid bacteria. Appl. Environ. Microbiol., 49(2): 413-415.

Frank, J.F. and Marth, E.H. (1977). Inhibition of enteropathogenic *E. coli* by homofermentative lactic acid bacteria in skim milk.II. Comparison of lactic acid bacteria and enumeration methods. J. Food Prot., 40(11): 754-759.

Fuller, R. (1989). Probiotics in man and animals. J. Appl. Bacteriol., 66(5): 365-378.

Galesloot, T.E., Hassing, F. and Verenga, A.A. (1968). Symbiosis in Yoghurt. I. Stimulation of *L. bulgaricus* by a factor produced by *S. thermophilus*. Neth. Milk Dairy J., 22(1): 50-63.

Gandhi, D.N. (1979). Studies on *Lactobacillus acidophilus* based fermented milk products. Ph.D. Thesis, Kurukshetra University, Kurukshetra.

Gandhi, D.N. and Nambudripad, V.K.N. (1975). A note on antimicrobial properties of dahi. Indian J. Dairy Sci., 28(1): 72-73.

Gandhi, D.N. and Sahani, K.M. (1994). Survival of *Lactobacillus acidophilus* in freeze-dried acidophilus powder using two different media. Ind. J. Microbiol., 34(1): 45-47.

Garcia-pina, X., Sancho, J. and Guinea, J. (1979). Control of physical and biological parameters in the process of freeze-drying. Ciencia Y Industria Farmacevtica, 11(2): 51-56. Cited in: Dairy Sci. Abstr., 43(11): 7675 (1981).

Garg, S.K. (1988). Dahi - fermented indigenous milk product. Indian Dairyman, 49(2): 57-60.

Garg, A.R. and Jain, S.C. (1980). Studies on the texteural characteristics of curd. 1. Effect of time temperature combinations for pasteurization and fat and protein content of milk. Milchwissenschaft, 35(12): 738-742.

Gehriger, G. (1968). Freeze-dried *L. helveticus* and *S.thermophilus* and their use in Emmental cheese making. Edig. Tech. Hochsh Zurich. Diss. Nr. 4193, 82pp. Cited in: Dairy Sci. Abstr., 30(12): 4205.

Ghosh, J. and Rajorhia, G.S. (1990). Selection of starter culture for production of indigenous fermented milk product (Mistidahi). Lait, 70(2): 147-154.

Gilliland, S.E. (1976). Preparation and storage of concentrated cultures of lactic streptococci. J. Dairy Sci., 60(5): 805-809.

Gilliland, S.E. (1989). Acidophilus milk products: A review of potential benefits to consumers. J. Dairy Sci., 72(10): 2483-2494.

Gilliland, S.E. and Speck, M.L. (1969). Biological response of lactic streptococci and lactobacilli to catalase. Applied Microbiol., 17(6): 797-800.

Gilliland, S.E. and Speck, M.L. (1977). Antagonistic action of *L. acidophilus* towards intestinal and food borne pathogens in associative culture. J. Food Prot., 40(11): 820-823.

Gilliland, S.E., Anna, E.D. and Speck, M.L. (1970). Concentrated cultures of *Leuconostoc citrovorum*. Appl. Microbiol., 19(6): 890-893.

Gold, B.L. and Samuel, I. (1965). Effect of yoghurt on serum cholesterol. J. Am. Diet. Assoc., 47: 192-193. Cited in: Nutr. Abstr. Rev., 36: 3236 (1966).

Goldin, B.R. and Gorbach, S.L. (1984). The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. Am. J. Clin. Nutr., 39(5): 755-761.

Gorbach, S.L., Vapaetelo, H. and Saminen, S. (1988). Studies on *Lactobacillus* GG in humans. Int. Symp. Intest. Microcol. Alghero. Cited in: IDF (1991).

Grandi, J.C., Lopes-Andrade, E.C. and Andrade, E.C. Lopes (1988). Effect of storage time and number of culture transfers on activity of a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Informe-Agro Peceario 13(155): 40-43. Cited in: Dairy Sci. Abstr. 51(1): 348(1989).

Greiff, D. (1971). Protein structure and freeze-drying the effects of residual moisture and gases. Cryobiol. 8: 145.

Grudzinskaya, E.S. (1982). Collection of lactic acid bacteria and cultures. Moloch. Prom. No.8, 44-45. Cited in: Dairy Sci. Abstr., 45(4): 2313.

Grunewald, K.K. (1982). Serum Cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci., 47(6): 2078-2079.

Grunewald, K.K. (1985). Influence of bacterial starter cultures on nutritional value of foods: Effects of *Lactobacillus acidophilus* fermented milk on growth and serum cholesterol in laboratory animals. Cult. Dairy Prod. J., 20(2): 24-27.

Grunewald, K.K. and Mitchell, L.K. (1983). Growth of mice fed milk fermented with *Lactobacillus acidophilus*. J. Food Prot., 46(4): 315-317.

Gupta, R.C. and Tiwari, M.P. (1985). Microbiological aspects of manufacturing modified varieties of Yoghurt - the liquid and stirred. NDRI Annual Report. p.32.

Gupta, R.C. and Tiwari, M.P. (1988). Microbiological aspects of manufacturing modified varieties of Yoghurt - the liquid and stirred. NDRI Annual Report. p.39.

Gupta, R.K. and Prasad, D.N. (1989). Use of Nisin in dairy industry. Indian Dairyman, 41(5): 229-233.

Habaj, B. (1964). Freeze-drying of pure cultures of lactic acid bacteria. prezegl. Mlecz., 11: 7. Cited in: Dairy Sci. Abstr., 27(6): 1817.

Hall, R.J. and Antonvcci, W.T. (1988). Quantitative evaluation of pH controlled cheese starter production in skim milk. New Zealand J. Dairy Sci. Technol., 23(4): 291-303.

Hamdan, I.Y. and Mikolajcik, E.M. (1973). Growth, viability and antimicrobial activity of *Lactobacillus acidophilus*. J. Dairy Sci., 56(5): 638 (Abstr.).

Hamdan, I.Y., Kunsman, Jr., J.E. and Deane, D.D. (1971). Acetaldehyde production by combined yoghurt cultures. J. Dairy Sci., 54(7): 1080-1082.

Hammer, B.W. (1935). The creatinine test for acetylmethylcarbinol plus diacetys in butter cultures. J. Dairy Sci., 18(9): 579-582.

Hammer, B.W. and Bailey, F.J. (1919). Iowa Agr. Expt. sta. Res. Bul. No. 55. Cited in: Hammer, B.W. and Babel, F.J. (1943). J. Dairy Sci., 26(2): 80-168.

Hargrove, R.E. and Alford, A.J. (1978). Growth rate and feed efficiency of rats fed yoghurt and other fermented milks. J. Dairy Sci., 61(1): 11-19.

Hawrylewiez, E., Gowdey, B. and Ehrlich, R. (1962). Nature: London, 193: 497. Cited by: Dalai, S. (1983).

Heap, H.A. and Lawrence, R.C. (1988). Culture systems for the dairy industry. In: Developments in Food Microbillogy. Vol. 4, Robinson, R.K. (Ed.). Elsevier Appl. Sci. Publishers Ltd., London, U.K. pp.149-186.

Heckly, R.J. (1961). Preservation of bacteria by lyophilization. In: Adv. Appl. Microbiol., 3. (Wayne, W.V. ed.) Academic Press, London, pp. 1-76.

Heckly, R.J. and Quay, J. (1983). Adventitious chemistry at reduced water activities, free radicals and polyhydroxy agents. Cryobiol., 20: 613.

Hempenius, W.L. and Liska, B.J. (1968). Method of determining volatile acids in cultured dairy products. J. Dairy Sci., 51(2): 221-222.

Henning, D.R., Vedamuthu, E.R. Sandine, W.E. and Elliker, P.R. (1964). Citrate utilization and overgrowth by *Streptococcus diacetylactis* in mixed strain starter cultures. J. Dairy Sci., 47(6): 674-675 (Abstr.).

Hepner, G.R., Fried, S.St.Jeor, Fustei, L. and Morin, R. (1979). Hypocholesterolemic effect of yoghurt and milk. Amer. J. Clin. Nutr., 32(1): 19-24.

Hirsch, A. and Wheater, B.H. (1951). The production of antibiotic by streptococci. J. Dairy Res., 18(3): 193-197.

Hitchins, A.D., McDonough, F.E., Wong, N.P. and Hargrove, R.E.(1983). Biological and biochemical variables affecting the relative values for growth and feed efficiency of rats fed yoghurt or milk. J. Food Sci., 48(6): 1836-1840.

Homma, N. (1988). Bifidobacteria as a resistance factor in human beings. *Bifidobacteria and Microflora*, 7(1): 35. Cited in: Hughes, D.B. and Hoover, D.G. (1991).

Horrall, B.E. and Elliker, P.R. (1950). An activity test for Cheddar and Cottage cheese starters. J. Dairy Sci., 33(5): 245-249.

Hughes, D.B. and Hoover, D.G. (1991). Bifidobacteria: Their potential for use in American dairy products. Food Technol., 45(4): 74, 76, 78-80.

Hull, M.E. (1947). Studies on milk proteins.II. Colorimetric determinations of the partial hydrolysis of proteins in milk. J. Dairy Sci., 30(11): 891-900.

Humphreys, C.L. and Plunkett, M. (1969). Yoghurt - a review of its manufacture. Dairy Sci. Abstr., 31(11): 607-622.
IDF (1983): Cultured dairy foods in human nutrition, Bulletin: 159, International Dairy Federation, Brussels, Belgium.
IDF (1983) cultured dairy products in human nutrition. Bulletin: 159.

IDF (1991). Cultured dairy products in human nutrition, Bulletin: 255, International Dairy Federation, Brussels, Belgium.

Ishida, M. and Kubo, H. (1985). Effects of yoghurt, kefir and butter milk on serum lipids of rats. Rep. Miyagi Agric. Coll., 33: 43-47. Cited by: Kaul, J. (1984).

Ishibashi, N., Tatematsu, T., Shimamura, S., Tomita, M. and Okonogi, S. (1985). Effect of water activity on the viability of freeze-dried bifidobacteria and lactic acid bacteria. Refrig. Sci. Technol., 1: 227-232. Cited in: Dairy Sci. Abstr., 50(2): 1316 (1988).

ISI (1967). IS: 4079. Extraction of fat using Mojonnier fat extraction apparatus. Bureau of Indian Standards, Manak Bhawan, New Delhi.

ISI (1973). IS: 7035. Specification for fermented milk products. Bureau of Indian Standards, Manak Bhavan, New Delhi-110001.

ISI (1977). IS:1479. Methods of test for dairy industry. Part.III Bureau of Indian Standards, Manak Bhawan, New Delhi.

ISI (1981). SP:1818, Part.XI. ISI Hand Book of Food Analysis, Dairy Products. Bureau of Indian Standards, Manak Bhawan, New Delhi. pp.15.

Jain, L.K. (1979). Studies on activity of yoghurt starters in buffalo skim milk. M.Sc. Dissert., Kurukshetra University, Kurukshetra.

Jay, J.M. (1982). Antimicrobial properties of diacetyl. Appl. Environ. Microbiol., 44(2): 525-532.

Jonas, L.L., Ernstrom, C.A. and Richardson, G.H. (1977). Major savings realized in the production of bulk lactic culture with whey based phage inhibitory medium provided with pH control. Cult. Dairy Prod. J., 12(2): 12-14.

Jonsson, H. and Pettersson, H.E. (1977). Studies on the citric acid fermentation in lactic starter cultures with special interest in - acetolactic acid. Milchwissenschaft., 32(9,10): 513-516, 587-594.

Kang, K.H., Shin, H.J., Park, Y.H. and Lee, T.S. (1989). Studies on antibacterial substances produced by lactic acid bacteria. Purification and some properties of the antibacterial substance bifilong produced by *Bifidobacterium longum*. Korean J. Dairy Sci., 11(3): 204-216. Cited in: Dairy Sci. Abstr., 52(4): 2630 (1990).

Kansal, V.K. and Chawla, K. (1984). Comparative studies on the depression in hepatic cholesterol genesis in rats fed the full cream cow's milk, dahi and acidophilus milk. Indian J. Nutr. Diet., 21(2): 54-57.

Kapoor, J.P. (1976). Process for production of bacterial concentrates especially of a symbiont culture concentrate for yoghurt production. German Federal Republic Patent Application No.2 455 833. Cited in: Dairy Sci. Abstr., 39(9): 5192 (1977).

Kaviratna, A.C. (1922). The Kalpasthana of Charaka Samhita 200, Cornwellis St. Calcutta. Cited by: Laxminarayana, H. and Iya, K.K. (1952). Studies on "dahi" - I. Introduction and historical review. Indian J. Vet. Sci. Anim. Husb., 22(1): 1-11.

Kaul, J. (1984). A study on the hypocholesteremic effect of yoghurt in rats. Ph.D. Thesis, Kurukshetra University, Kurukshetra.

Keating, K. (1985). The role of cultured dairy products in the prevention of stomach cancer. Cult. Dairy Prod. J., 20(2): 13-14.

Kebary, K.M.K., and Kamaly, K.M. (1991). Susceptibility of starter and non-starter organisms to nisin in stirred yoghurt during storage. Egyptian J. Dairy Sci., 19(1): 157-167.

Kegal, M.A. and Wallace, D.L. (1989). Use of stabilizing agents in culture media for growing acid producing bacteria. US Patent 4 806 479. Cited in: Dairy Sci. Abstr., 51(9): 3512.

Khamagaeva, I.S. and Stolyarova, S. (1993). Freeze-dried starter cultures of bifidobacteria. Moloch. Prom. 3: 23-24. Cited in: Food Sci. Tech. Abstr., 25(11): p.96.

Khambatta, J.S. (1950). Studies on the changes in the chemical composition of milk during souring and nutritive value of dahi. M.Sc. Dissert., University of Bombay, Bombay.

Khanna, A.K. and Singh, J. (1979). A comparison of yoghurt starter in cow and buffalo milk. J. Dairy Res., 46(4): 681-686.

Khedkar, G.D., Dave, J.M. and Sannabhadti, S.S. (1990). Antibacterial activity of human strains of *Lactobacillus*

acidophilus grown in milk against selected pathogenic and spoilage type bacteria. Cult. Dairy Prod. J., 25(4):29-31.

Khedkar, C.D., Garge, R.D., Mantri, J.M., Kulkarni, S.A. and Khedkar, G.D. (1993). Effect of feeding acidophilus milk on serum cholesterol in human volunteers of 50-60 years. J. Dairy. Foods Home Sci., 12(1): 33-38.

Kilara, A., Shahani, K.M. and Das, N.K. (1976). Effect of cryoprotective agents on freeze-drying and storage of lactic culture. Cult. Dairy Prod. J., 11(2): 8-10.

Kim, H.S. (1988). Characterization of lactobacilli and bifidobacteria as applied to dietary adjunts. Cult. Dairy Prod. J. 23(3): 6-9.

Kim, K.S. (1990). Study on the characteristics of mixed lactic culture bacteria. Res. Rept. RAD(L), 32(1) 13-17.

Kim, J.M. Baek, Y.J. and Kim, H.U. (1987). Freeze drying and survival of *Lactobacillus casei* YIT 9018. Korean J. Dairy Sci., 9(2): 84-92. Cited in: Dairy Sci. Abstr., 50(3): 1323.

Kisza, J., and Panfil-Kuncewiz, H. (1989). Characterisitc of butter starters propagated in milk with increased dry matter content. Acta Academiae Agriculturae at Technicae Olstenensis, *Technologia Alimentorum* No. 23: 15-25. *Cited* in: Dairy Sci. Abstr., 52(5): 3500.

Kisza, J., Zbikowski, Z. and Kolend, A.H. (1978). Biochemical and rheological properties of yoghurt with added *Bifidobacterium bifidum*. XX Int. Dairy Congr., 1E: 545-546.

Klaenhammer, T.R. (1988). Bacteriocins of lactic acid bacteria. Biochimie, 70: 337-349.

Klupsch, H.J. (1984). Modified bifidus blood agar. Cited in: Rasic, J.ji (1990). Culture media for detection and enumeration of bifidobacteria in fermented milk products. Int. Dairy Fed. Bull., 252: pp.27.

Klupsch, H.J. (1985). Man and Microflora-Bioghurt, Biograde, South African J. Dairy Technol., 17(4): 153-156. Cited in: Dairy Sci. Abstr., 49(1): 428.

Klupsch, H.J. (1989). The technology for producing fermented milk products using *L. acidophilus* and bifidus bacteria. European Dairy Magazine 1: 14-21.

Kneifel, W., Maria, K., Angela, F. and Ulberth, F. (1992a). Screening of commercially available mesophitic dairy starter cultures: Biochemical, sensory and microbiological properties. J. Dairy Sci., 75(11): 3158-3166.

Kneifel, W., Ulberth, F., Erhard, F. and Jaros, D. (1992b). Aroma profiles and sensory properties of yoghurt and yoghurt related products.I. Screening of commercially available starter cultures. Milchwissenschaft, 47(6): 362-365.

Kondratenko, M.S. and Kondareva, S.Z. (1978). Selection of the most suitable nutrient medium for making bacterial concentrates for sour milk. XX Int. Dairy Congr.,5: 576-577.

Kornacki, K., Rymaszewski, J., Ozimek, G. and Smietana, Z. (1974). Production and preservation of lactic acid bacteria biomass. XIX. Int. Dairy Congr., 1E: 436.

Kornacki, K., Rymaszewski, J. and Poznanski, S. (1977). Preparation of concentrate cultures of lactic and bacteria and their use in the dairy industry. Dairy Sci. Abstr., 39(7): 3882.

Kostrova, I.E., Krasnikova, L.V., Sharobaiko, V.I., Motaeva, T.V. and Ignatyuk, O.A. (1983). Method of preparing a concentrate of lactic acid bacteria for producing large cheeses. USSR Patent SU 1 002 355. Cited in: Dairy Sci. Abstr., 46(1-3): 1260.

Kothari, S.L. (1970). Associative growth of lactic acid bacteria in starter cultures. *Ph.D. Thesis, Udaipur Univ., Udaipur.*

Kujawa, K., Roczniak, B., Drewek, Z., Warminska, Radyko, I., and Kornacka, D. (1987). Surviving ability of *Lactobacillus acidophilus* populations as influenced by the method of preservation. Technologia Alimentorum 22: 53-64. Cited in: Dairy Sci. Abstr., 50(10): 5707.

Kurmann, J.A. (1967). Studies of the production of a mild and aromatic yoghurt. III. Selection of mother cultures and strains by technological criteria. Schweiz. Milchzig (Lait

Kujawa K, Roczniak B. Drewek Z. Warminska-Radyko I and Kornacka D (1987). Surviving ability of L. acidophilus populations as influenced by the method of preservation. Abstr. in : Dairy Sci. Abstr. 50(10) 5707 (1988).

Romand), 93(67): 508-509. Cited in Dairy Sci. Abstr., 31(5): 1652.

Kurmann J.A. (1986). Developments and future trends in fresh milk products manufacture. Dte. Molk. Ztg., 107(44): 1470-78. Cited in: Dairy Sci. Abstr., 49(6): 3433 (1987).

Kurmann, J.A. (1988). Starters with selected intestinal bacteria. Int. Dairy Fed. Bull., 227:pp. 41-55.

Kurmann, J.A. and Rasic, J.Lj (1988). Technology of fermented special products. Int. Dairy Fed. Bull., 227:pp 101-109.

Kurmann, J.A. and Rasic, J.Lj (1991). The health potential of products containing bifidobacteria. In: Threrapeutic properties of fermented milks. Ed. R.K. Robinson, Elsevier Sci. Publishers, London, pp. 117-158.

Labropoulos, A.E., Collins, W.F. and Stone, W.K. (1982). Starter culture effects on yoghurt fermentation. Cult. Dairy Prod. J., 17(2): 15-17.

Lagoda, I.V. and Bannikova, L.A. (1978a). Factors affecting the production of stable *Lactobacillus acidophilus* concentrate. XX Int. Dairy Congr., E: 579.

Lagoda, I.V. and Bannikova, L.A. (1978b). Technology of production of stable bacterial concentrate and its use in the dairy industry. Molch. Prom., 11: 12-15. Cited in: Dairy Sci. Abstr., 42(4): 2123 (1980).

Lagoda, I.V., Priidak, T.A. and Bannikova, L.A. (1978). Nutrient medium for accumulating a biomass of the lactic acid bacteria, *S. lactis*, *S. cremoris* and *L. acidophilus*. USSR Patent SU 591 499. Cited in: Dairy Sci. Abstr., 41(2): 992.

Lagoda, I.V., Bannikova, L.A., Babina, N.A. and Kosareva, G.V. (1981). Improving the production of bacterial concentrate for Tvorog Snetana and other cultured milk products. Molch. Prom. 1: 3-7 Cited in: Dairy Sci. Abstr., 46(9): 61-74.

Lagoda, I.V., Bannikova, L.A., Babina, N.A. and Kosareva, G.A. (1982). Dried bacterial concentrates for manufacture of

cultured milk products. Moloch. Prom., 9:13-14. Cited in: Dairy Sci. Abstr., 45(11): 7934.

Laroia, S. and Martin, J.H. (1990). Bifidobacteria as possible dietary adjuncts in cultured dairy products - A review. Cult. Dairy Prod. J., 25(4): 81-82.

Laxminarayana, H., Nambudripad, V.K.N., Lakshmi, N.V., Anantaramaiah, S.N. and Sreenivasa Murthy, V. (1952). Studies on 'dahi'. II. General survey of the quality of market 'dahi'. Indian J. Vet. Sci. & Ani. Husb., 22(1): 13-25.

Lee, S.H., Koo, Y.J. and Shih, D.H. (1988). Physio-chemical and bacteriological properties of yoghurt made by single or mixed cultures of *L. bulgaricus* and *S. thermophilus*. Korean J. Food Sci. Technol., 20(2): 140-147. Cited in: Dairy Sci. Abstr., 52(2): 1188.

Lin, W., Shih, C.H. and Su, H.P. (1986). Studies on natural antimicrobial agents of lactic acid bacteria. I. Primary screening of lactic cultures for antibacterial activity. J. Chinese Agri. Chem. Soc., 24(4): 384-391. Cited in: Dairy Sci. Abstr., 49(10): 6523.

Lindsay, C. and Day, E.A. (1965). Rapid quantitative method for determination of acetaldehyde in lactic starter cultures. J. Dairy Sci., 48(6): 665-669.

Lloyed, G.T. and Pont, E.G. (1973). Some properties of frozen concentrated starters produced by continuous culture. J. Dairy Res., 40(2): 157-168.

Mahdi, H.A., Tamime, A.Y. and Davies, G. (1990). Some aspects of the production of Labneh by ultrafiltration using cow's, ship's, and goat's milk. Egyptian J. Dairy Sci., 18(2): 345-367.

Maister, H.G., Heger, E.N. and Bogart, W.M. (1958). Continuous freeze-drying of *Serratia marcescens*. Ind. Eng. Chem., 50(4): 623-626.

Mann, G.V. (1977). A factor in yoghurt which lowers cholesteremia in man. Artherosclerosis, 26(3): 335-340. Cited in: Dairy Sci. Abstr., 40(5): 2567.

Mann, G.V. and Spoerry, A. (1974). Studies of a surfactant and cholesteremia in Masai. Am. J. Clin. Nutr., 27(3):464-469.

Mantere-Alhonen, S. (1986). Zur Verwendung Von Propionibakterien Als Probioten - Eln U Berblick. ME.Jerit. Aikakausk, 44: 76-81. Cited in: IDF (1991).

Marshall, V.M. (1987). Fermented milks and their future trends. I. Microbiological aspects. J. Dairy Res., 54(4): 559-574.

Marshall, V.M. and Mabbit, L.A. (1980). The use of single starter organisms for yoghurt manuflacture. J. Soc. Dairy Technol., 30(3): 129-130.

Marshall, V.M., Cole, W.M. and Mabbitt, L.A. (1982). Fermentation of specially formulated milk with single strains of bifidobacteria. J. Soc. Dairy Telchnol. 35(4): 143-144.

Martin, D.R. and Gilliland, S.E. (1980). Inhibition of psychrotrophic bacteria in refrigerated milk by lactobacilli isolated from yoghurt. J. Food Prot., 43(9): 675-678.

Mattick, A.T.R. and Hirsch, A. (1949). The streptococci and antibiotics. Int. Dairy Cong, 2: 546-550.

McDonough, F.E., Hitchins, A.D. and Wong, N.P. (1982). Effects of yoghurt and freeze-dried yoghurt on growth stimulation of rats. J. Food Sci., 47(5): 1463-1465.

McDonough, F.E., Wong, N.P., Wells, P., Hitchings, A.D. and Bodwell, C.E. (1985). Stimulation of rat growth by yoghurt - effect of vitamins and minerals. Nutr. Rep. Int. 31: 1237-1245. Cited in: IDF (1991).

Mehanna, N.M. and Mehanna, A.S. (1989). On the use of stabilizer for improving some properties of cow's milk yoghurt. Egyptian J. Dairy Sci., 17(2): 289-296.

Metwally, M.M., Abd El Gawad, I.A., Mehriz, A.M. and El Dieb, S.M. (1988). The hypocholesterolaemic effect of butter

milk.1. The effect of butteroil, buttermilk, casein and cream on rat's serum and hepatic lipids. Egyptian, J. Dairy Sci., 16(2): 175-184.

Mikolajcik, E.M. and Hamdan, I.Y. (1975). *L. acidophilus* II. Antimicrobial agents. Cult. Dairy Prod. J., 10(1): 18-20.

Misra, A.K. (1978). Effect of cryoprotective agents on the survival of lactic cultures during freeze-drying. M.Sc. Dissert., Kurukshetra University, Kurukshetra.

Misra, A.K. and Kuila, R.K. (1991). Growth response of *Bifidobacterium* in semi and synthetic and milk based media. Indian J. Dairy Sci., 44(3): 226-231.

Mitchell, S.L. and Gilliland, S.E. (1983). Pepsinized sweet whey medium for growing *Lactobacillus acidophilus* for frozen concentrated cultures. J. Dairy Sci., 66(4): 712-718.

Mohammed, F.O. and Younis, Y.A. (1990). Effect of yoghurt cultures on some pathogenic microorganisms in fermented milks. Egyptian J. Dairy Sci., 18(2): 369-375.

Moon, N.J. and Reinbold, G.W. (1976). Commensalism and competition in mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. J. Milk Food Technol., 38(5): 337-341.

Morichi, T. (1970). Nature and action of protective solutes in freeze-drying of bacteria. In: Proceedings of the First International Conference of Culture Collections. p. 121. University Park Press, Tokyo, Japan. Cited by: Champagne *et al.* (1991a).

Morichi, T. (1972). In: Mechnaism and prevention of cellular injury in lactic acid bacteria, subjected to freezing and drying. IDF Scientific Conference, Japan. Cited by: Tamine, A.Y. (1990).

Morichi, T. (1974). Freezing and freeze-drying of dairy micro-organisms. Jpn. J. Dairy Sci., 22(4): A125-A136.

Mulhens, K. and Stamer, H. (1969). Supplementing yoghurt culture with *Lactobacillus acidophilus* and *Lactobacillus bifidus*. Milchwissenschaft, 24(1): 25-28.

Murunova, G.V. and Klimovskii, I.I. (1980). Development of aroma forming streptococci in a citrate medium. Molch. Prom. No. 4, 21-22, 47. Cited in: Dairy Sci. Abstr., 45(5): 2925.

Nagmoush, M.R., Girgis, E.S., Guirguis, A.H. and Fahmi, A.H. (1978). Effect of storage on the behaviour of some freeze-dried lactic acid cultures variously tested. Egyptian J. Dairy Sci., 6(1): 39-47.

Nair, C.R. and Mann, G.V. (1977). A factor in milk which influences cholesteremia in rats. Artherosclerosis, 26(3): 363-368. Cited in: Dairy Sci. Abstr., 39(11): 6548.

Nei, T., Souzu, H. and Araki, T. (1966). Effect of residual moisture content on the survival of freeze-dried bacteria during storage under various conditions. Cryobiol., 2(5): 276-279.

Nikolova, N. (1978). Freeze drying of starters for yoghurt and of *Lactobacillus bulgaricus* in protective media. XX Int. Dairy Congr., E: 589.

Obayashi, Y., Ota, S. and Arai, S. (1961). Some factors affecting preservability of freeze dried bacteria. J. Hyg. Camb., 59: 77-91.

Osborne, R.J.W. (1977). Production of frozen concentrated cheese starters by diffusion culture. J. Society of Dairy Technol., 30(1): 40-49.

Ozlap, E. and Ozlap, G. (1978). Studies on the production of yoghurt cultures. Ankara Universitesi Veteriner Fakultesi Dergisi, 25(2): 315-322. Cited in: Dairy Sci. Abstr., 41(12): 7857 (1979).

Pack, M.Y., Sandine, W.E., Elliker, P.R. Day, E.A. and Lindsay, R.C. (1964). Owades and Jakovac method for diacetyl determination in mixed strain starters. J. Dairy Sci., 47(9): 981-986.

Pack, M.Y. Vedamuthu, E.R. Sandine, W.E., Elliker, P.R. and Leesment, H., (1968). Effect of temperature on growth and diacetyl production by aroma bacteria in single and mixed strain lactic cultures. J. Dairy Sci., 51(3): 339-344.

Panday, P. (1985). Manufacture of "dahi" and yoghurt in Mauritius. Indian Dairyman, 37(6): 270-273.

Payens, W., Rethans, E.J.M. and De Waard, H. (1976). The influence of composition of large quantities of yoghurt or milk on the serum cholesterol concentration. Milchwissenschaft, 31(9): 525-530.

Peebles, M.M., Gilliland, S.E. and Speck, M.L. (1969). Preparation of concentrated lactic *Streptococcus* starters. Appl. Microbiol., 17(6): 805-810.

Peterkova, L. (1980). Selection of milk for pure dairy cultures. Prumysl Potravin, 31(10): 566-568. Cited in: Dairy Sci. Abstr., 43(7): 4676.

Pette, J.W. and Lolkema, H. (1950a). Yoghurt.II. Growth stimulating factors for *Str. thermophilus*. Neth. Milk Dairy J., 4(3): 209-224.

Pette, J.W. and Lolkema, H. (1950b). Yoghurt. III. Acid production and aroma formation in yoghurt. Neth. Milk Dairy J., 4(4): 261-274.

Pettersson, H.E. (1975). Preservation of mixed species lactic starter concentrates by freezing and lyophilization methods. Milchwissenschaft, 30(9): 539-548.

Piard, J.C., Cuminai, C. and Desmazeaud, M.J. (1989). Partial characterization of lactacin 481. A plasmid borne bacteriocin produced by *L. lactis* CNRZ 481. J. Dairy Sci., 72(Suppl.1), 114 (Abstr.).

Pollman, D.S. (1984). Additives, flavours, enzymes and probiotics in animal feeds. Proc. 22nd Anu. Nutr. Conf., Univ. Guelph. Cited in: IDF (1991).

Pont, E.G. and Halloway, G.L. (1968). A new approach to the production of cheese starter. Aust. J. Dairy Technol., 23(1): 22-29.

Porubcan, R.S. and Sellars, R.L. (1975). Stabilized dry cultures of lactic acid producing bacteria. US Patent No. 3 097 307. Cited in: Dairy Sci. Abstr., 38(7): 4405(1976).

Prajapati, J.B. and Dave, R.I. (1994). Protective effect of tomato juice and pectin on *Lactobacillus acidophilus* during freeze-drying. Ind. J. Dairy Sci., 47(1): 68-71.

Prigent, C., Corre, C. and Boyaval, P. (1988). Production of concentrated *Streptococcus salivarius* ssp. *thermophilus* by coupling continuous fermentation and ultrafiltration. J. Dairy Res., 55(4): 569-577.

Priidak, T.A. and Bannikova, L.A. (1982). Selection of lactic acid bacteria for production of culture concentrate. XXI Int. Dairy Congr. 1, Book 2. Cited in: Dairy Sci. Abstr., 45(6): 3808 (1983).

Priidak, T.A., Bauina, N.A. and Bannikova, L.A. (1982). Improving a nutrient medium for the manufacture of bacterial concentrate. Moloch. Prom., 4:33. Cited in: Dairy Sci. Abstr., 45(1): 505 (1983).

Puhan, Z. (1990). Developments in the technology of fermented milk products. Cult. Dairy Prod. J., 25(2): 4-9.

Pulusani, S.R. and Rao, D.R. (1984). Stimulation by formate of antimicrobial activity of *Lactobacillus bulgaricus* in milk. J. Food Sci., 49(2): 652-653.

Pulusani, S.R., Rao, D.R. and Sunki, G.R. (1979). Antimicrobial activity of lactic cultures: Partial purification and characterization of antimicrobial compound(s) produced by *Streptococcus thermophilus*. J. Food Sci., 44(2): 575-578.

Pyatkova, N.P. and Lukonikova, L.A. (1979). Development of *Lactobacillus acidophilus* in skim milk. Moloch Prom. No. 2 36-38. Cited in: Dairy Sci. Abstr., 42(4): 2124.

Radulovic, D. (1971). The effect of some factors on survival of *Lb. acidophilus* during and after freeze-drying. Mikrobiologia, 8: 189-195. Cited from: Dairy Sci. Abstr., 37(11): 7178 (1975).

Rajagopal, S.N. and Sandine, W.E. (1990). Associative growth and proteolysis of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in skim milk. J. Dairy Sci., 73(4): 894-899.

Rajagopal, S.N., Sandine, W.E. and Ayres, J.W. (1990). Whey based bacteriophage inhibitory Italian bulk starter medium. J. Dairy Sci., 73(4): 881-886.

Rakesh, K. (1979). Studies on certain factors affecting preparation and storage of yoghurt starter concentrate. M.Sc. Dissert., Kurukshetra University, Kurukshetra.

Ram, J. (1984). Preparation and preservation of cheese starter concentrate. Ph.D., Thesis, Kurukshetra University, Kurukshetra.

Rao, M.S. (1993). Studies on the manufacture of yoghurt ice cream - milk shake. M.Sc. dissert., NDRI (Deemed University), Karnal.

Rao, S.M. and Gandhi, D.N. (1988). Studies on various quality characteristics of acidophilus sour milk from buffalo milk. Cult. Dairy Prod. J. 22: 22.

Rasic, J.L. and Kurmann, J.A. (1978). Yoghurt. Scientific grounds, technology, manufacture and preparations. Technical Dairy Publishing House, Denmark.

Ratnakar, A.K. (1989). Antibacterial activity of fermented milk products and culture capsules. M.Sc. Dissert., Kurukshetra University, Kurukshetra.

Ray, H.P. (1970). Use of micro-organisms for preparation of indigenous fermented milk products. M.Sc. Dissert., Punjab University, Chandigarh.

Reddy, N.S. (1980). Studies on the antimicrobial substance produced by *S. lactis* subsp. *diacetylactis*. Ph.D. Thesis, Kurukshetra University, Kurukshetra.

Reddy, N.S. and Ranganathan, B. (1983). Preliminary studies on antimicrobial activity of *S. lactis* subsp. *diacetylactis*. J. Food Prot., 46: 222-225.

Richardson, G.H. (1978). Solving bulk culture problems through use of active lactic concentrates. Dairy and Ice cream Field, 161(9): 80 A - 80 D. Cited in: Dairy Sci. Abstr., 41(6): 3252.

Robinson, R.K. (1981). Freeze-dried starter concentrate-Part I. Their characteristics and potential application to the production of cheese and yoghurt. Dairy Indus. Int., 46(10): 15-21.

Robinson, R.K. (1988). Cultures for yoghurt - their selection and use. Dairy Indus. Int., 53(7): 15-17, 19.

Robinson, R.K. and Tamime, A.Y. (1975). Yoghurt - A review of the product and its manufacture. J. Soc. Dairy Technol., 28(3): 149-163.

Robinson, R.K. and Tamime, A.Y. (1989). In: Dairy Microbiology, Vol. 2, Robinson, R.K. (Ed). Elsevier Applied Sci. Publishers Ltd., London, U.K., P. 291.

Rogers, L.A. (1914). The preparation of dried cultures. J. Infect. Dis., 14: 1000.

Rottigni, C. (1987). Innovation in the preparation and use of starters for the manufacture of milk products and cheeses. Technique-Laitiere and Marketing No. 1021, 29-31. Cited in: Dairy Sci. Abstr., 50(3): 1364.

Sandine, W.E. (1979). Role of *Lactobacillus* in the intestinal tract. J. Food Prot. 42(3): 259-262.

Sandine, W.E., Muralidhara, K.S., Elliker, P.R. and England, D.C. (1972). Lactic acid bacteria in food and health. A review with special reference to entero-pathogenic *E. coli* as well as certain enteric diseases and their treatment with antibiotics and lactobacilli. J. Milk Food. Technol., 35(12): 691-702.

Schellaass, S.M. and Morris, H.A. (1985). Rheological and scanning electron microscopic examination of skim milk gels obtained by fermenting with ropy and non-ropy strains of lactic acid bacteria. Food Microstructure, 4(2): 279-287. Cited in: Dairy Sci. Abstr., 48(5): 2726.

Searles, M.A., Argyle, P.J., Chandan, R. and Gordon, J.E. (1970). Lipolytic and proteolytic activities of lactic cultures. XVIII Int. Dairy Congr., 1E: 111.

Senedcor, W.G. and Cochran, G.W. (1967). Statistical methods. 6th Edn. Oxford-IBH Publishing Co., New Delhi.

Shah, R.K., Prajapati, J.B. and Dave, J.M. (1987). Packaging materials to store a spray-dried acidophilus malt preparation. Indian J. Dairy Sci., 40(2): 287-291.

Shahani, K.M. and Chandan, R.C. (1979). Nutritional and healthful aspects of cultured and culture containing dairy foods. J. Dairy Sci., 62(10): 1685-1694.

Shankar, P.A. and Davies, F.L. (1977). Amino acid and peptide utilization by *S. thermophilus* in relation to yoghurt manufacture. J. Appl. Bacteriol., 43(1): VIII (Abstr.).

Sharma, D.K. (1982). Behaviour of *Lactobacillus acidophilus* with yoghurt starters. M.Sc. Dissert. Kurukshetra University, Kurukshetra.

Sharma, D.K. and Singh, J. (1982). Yoghurt starters in skim milk. II. Behaviour of *Lactobacillus acidophilus* with yoghurt starters. Cult. Dairy Prod. J., 17(4): 10.

Sharma, D.K. and Prasad, D.N. (1986). Yoghurt starters in skim milk III. Biochemical performance and growth and *Lactobacillus acidophilus* in yoghurt. Cult. Dairy Prod. J., 21(2): 13-14.

Sheikh, N.M., Haroon, S.N. and Khatoon, M. (1970). Bacteriology of 'dadhi' (Dahi) 1. Qualitative and quantitative studies of microflora of dadhi. Pakistan Council for Sci. & Ind. Res., 7(1): 1-8. Cited in: 'Literature on Indian dairy Products'.

Sangwan, R.B. and Sharma, M.M. (1984). Metropolitan Book Co., New Delhi.

Shukla, F.C., Jain, S.C. and Sandhu, K.S. (1987). Technological and physio-chemical aspects of yoghurt and fruit yoghurt - A review. Indian J. Dairy Sci., 40(1): 1-9.

Shurda, G.C. (1980). Influence of profound oxidation (freezing) on certain properties of lactic acid bacteria. J. Appl. Biochem. Microbiol., 16(1):11-16. Cited in: Dairy Sci. Abstr., 45(3): 1725.

Sidorova, I.N., Grinevich, A.G. and Zaborskikh, E.I. (1983). Freeze-drying of lactic streptococci mutants. Cited in: Dairy Sci. Abstr., 45(1): 464.

Siegenthaler, E., Hanley, H.B., Schmid, F. and Berger (1960). Aco yoghurt. Schwiiz Michzlg, 86: 537-44. Cited in: Dairy Sci. Abstr., 22(9): 2470.

Simatos, D., Blond, G., Dauvois, P. and Sauvageot, F. (1974). La Lyophilisation, P. 350. Collection de L' A.N.R.T., Brevatome, Paris. Cited by: Champagne *et al.* (1991a).

Singh, J., Khanna, A. and Chander, H. (1979). Antibacterial activity of yoghurt starter in cow and buffalo milk. J. Food Prot., 42(8): 664-665.

Singh, J., Sharma, D.K. and Jain, L.K. (1982). Acid and flavour production and proteolytic activity by yoghurt starters. Egyptian J. Dairy Sci., 10(1): 125-128.

Sinha, R.N. (1984). Factors influencing quality of dahi and yoghurt. Indian Dairyman, 36(6): 285-295.

Sinha, R.N. (1989). Storage factors affecting viability of freeze-dried *Streptococcus lactis* C2. Asian J. Dairy Sci., 8(4): 205-212.

Sinha, R.N., Nambudripad, V.K.N., Dudani, A.T. and Laxminarayana, H. (1972). Effect of suspending media on the viability of freeze-dried cultures of *S. lactis*. J. Food. Sci. Technol., 9(2): 85-89.

Sinha, R.N., Nambudripad, V.K.N. and Dudani, A.T. (1970). Studies of some factors affecting the viability of freeze-dried starter cultures during storage. XVIII Int. Dairy Congr., 1E: 128.

Sinha, R.N., Dudani, A.T. and Ranganathan, B. (1974). Protective effect of fortified skim milk as suspending medium for freeze-drying of different lactic acid bacteria. J. Food Sci., 39: 641-642.

Speck, M.L. (1976). Interactions among *lactobacilli* and man. J. Dairy Sci., 59(2): 338-343.

Speckman, C.A. (1975). Preparation, evaluation and use of lyophilized concentrated dairy starter cultures in cheese and yoghurt manufacture. Ph.D. Thesis, Oregon State University, Dissert. Abstr. Int. B35: 3474.

Speckman, C.A., Sandine, W.E. and Elliker, P.R. (1974). Lyophilized lactic acid starter culture concentrates. Preparation and use in inoculation of vat milk for cheddar and cottage cheese. J. Dairy Sci., 57(2): 165-173.

Spelhaug, S.R. and Harlander, S.K. (1989). Inhibition of food borne bacterial pathogens by bacteriocins from *L.lactis* and *Pediococcus pentosaceous*. J. Food Prot., 52: 856-862.

Spork, A.C. (1990). Research in lactic acid bacteria cultures. Scand. Dairy Informat., 4(3): 76-77.

Stadhouders, J., Jansen, L.A. and Hup, G. (1969). Preservation of starters and mass production of starter bacteria. Neth. Milk Dairy J., 23(3): 182-199.

Sunita, S. (1986). Activity of certain cheese starters in buffalo milk. M.Sc. Dissert., Kurukshetra University, Kurukshetra.

Suzuki, I., Watanabe, M., Kitada, T., Kato, S. and Morichi, T. (1980). Effect of *Str. thermophilus* on organoleptic features of fermented skim milk produced by *Lb. bulgaricus* cultures. Jpn. J. Zootech. Sci., 51(5): 342-346.

Tamime, A.Y. (1977). Some aspects of the production of yoghurt and condensed yoghurt. Ph.D. Thesis, University of Reading, U.K. Cited by: Tamime, A.K. (1990).

Tamime, A.Y. (1990). Microbiology of 'starter' cultures'. In: Dairy Microbiology, Vol.2, 2nd edn., Robinson, R.K. (ed), Elsevier Appl. Sci. Publ. Ltd., London, U.K.

Tamime, A.Y. and Robinson, R.K. (1985). Yoghurt Science and Technology. Pergamon Press Ltd., Oxford, U.K.

Tamime, A.Y. and Robinson, R.K. (1988). Fermented Milks Science and Technology. Int. Dairy Fed. Bull.: 227.

Tamime, A.Y., Kalab, M. and Davies, G. (1984). Micro structure of set - style yoghurt manufactured from cow's milk fortified by various methods. Food Microstructure, 3(1): 82-92. Cited in: Dairy Sci. Abstr., 46(11): 7356.

Tamura, Z. (1983). Nutriology of bifidobacteria. Bifidobacteria & Microflora, 2(1): 3-16.

Tojo, M., Oikawa, T., Morikawa, Y., Yamashita, N., Iwata, S., Satoh, Y., Hanada, J. and Tanaka, R. (1987). The effects of *Bifidobacterium breve* administration on *Campylobacter enteritis*. Acta. Paediati, Jpn., 29: 160. Cited by: Hughes, D.B. and Hoover, D.G. (1991).

Tomar, S.K. (1988). Nutritive value of yoghurt. Indian Dairyman, 40(8): 429-432.

Tomada, T., Yasua, N. and Kageyama, T. (1988). Intestinal candida overgrowth candida infection in patients with leukemia. Effects of *Bifidobacterium* administration. Bifidobacteria and Microflora, 7(2): 71. Cited by: Hughes, D.B. and Hoover, D.G. (1991).

Toyoda, S., Oki, Y. and Yoshioka, Y. (1987). Preparation of frozen concentrated starters and their use in cheese making. Reports of the research laboratory, Snow Brand Milk Products Co., Japan, 85: 23-32. Cited in: Dairy Sci. Abstr., 50(5): 2560.

Tsvetkov, T.S. and Nikolova, N. (1974). Freeze-drying of bacterial cultures for yoghurt. Khranitelna promishlenost, 23(10): 15-16. Cited in: Dairy Sci. Abstr. 37(10): 6391.

Vakil, J.R. and Sahani, K.M. (1965). Partial purification of antibacterial activity of *Lactobacillus acidophilus*. Bact. Proc., 43: 9. Cited in: Dairy Sci. Abstr. 27(7): 2176.

Vedamuthu, E.R. (1991). The yoghurt story - past, present and future. Part - IV. Dairy, Food Environ. Sanit. 11(7): 371-374.

Vedamuthu, E.R., Hauser, B.A., Henning, D.R., Sandine, W.E., Elliker, P.R. and Day, E.A. (1966). Competitive growth of *Streptococcus diacetylactis* in mixed strain lactic cultures and cheese. XVII Int. Dairy Congr., D(2): 611-618.

Veringa, H.A., Galesloot, T.E. and Davelaar, H. (1968)a. Symbiosis in yoghurt(II) Isolation and identification of a growth factor for *Lactobacillus bulgaricus* produced by *Streptococcus thermophilus*. Neth. Milk Dairy J., 25(5): 114-120.

Webb, S.J. (1969). The effect of oxygen on the possible repair of dehydration damage by *Escherichia coli*. J. Gen. Microbiol., 58(3): 317-326.

Wheather, D.M., Hirsch, A. and Mattick, A.T.R. (1952). Lactobacillin an antibiotic from lactobacilli. Nature, 168: 659.

Willrett, D.L. and Comotto, M. (1989). Bulk starter medium. US Patent US 4 851 347. Cited in: Dairy Sci. Abstr., 52(1): 488.

Woodward, G.J. (1980). The use of modified lactic agar for estimating freeze-thaw injury in cultures and concentrates of lactic streptococci. Dissert. Abstr. Int., B.41: 880.

Wright, C.T. and Klaenhammer, T.R. (1981). Calcium induced alteration of cellular morphology affecting the resistance of *Lactobacillus acidophilus* to freezing. Appl. Environ. Microbiol., 41(3): 807-815.

Wright, S.L. and Richardson, G.H.(1982). Optimization of whey-based or non-fat dry milk based media for production of pH controlled bulk lactic cultures. J. Dairy Sci., 65(10): 1882-89.

Yang, N.L. and Sandine, W.E. (1978). Activity of freeze-dried lactic starter concentrates in bulk starter and simulated Cheddar cheese manufacture. J. Dairy Sci., 61(Suppl.1): 121(Abstr.).

Yang, N.L. and Sandine, W.E. (1979). Acid producing activity of lyophilized lactic streptococcal cheese starter concentrates. J. Dairy Sci., 62(6): 908-915.

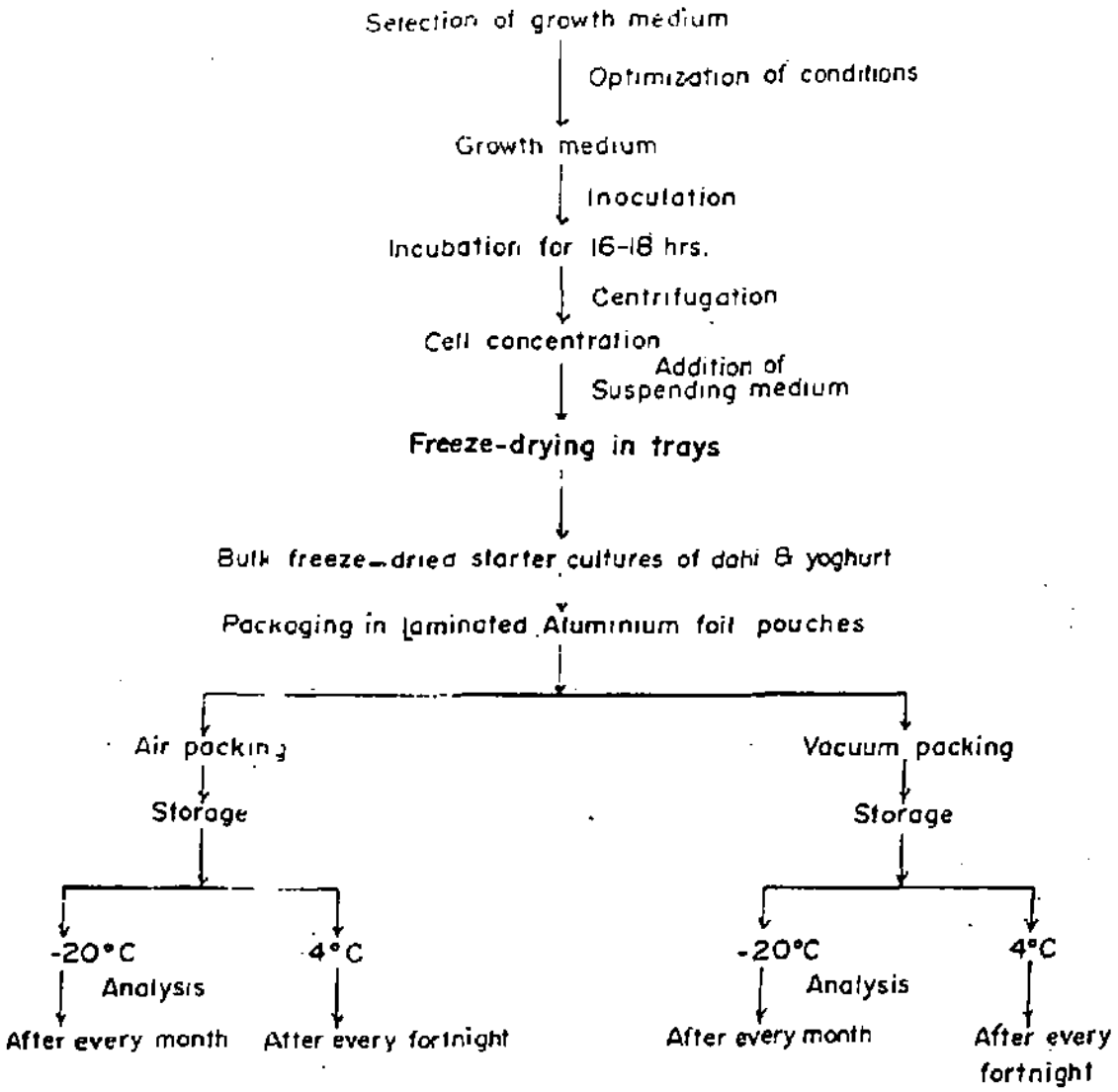
Yano, N., Morichi, T., Irie, R. and Kembo, H. (1960). Studies on the lyophilization of lactic starter organisms. I. Survival in the lyophilization process and death of the dried bacteria during storage. J. Agri. Chem. Soc. Jpn., 34(12): 1046-1049. Cited in: Dairy Sci. Abstr., 23(8): 2313.

Yu, J.H. and Nakanishi, T. (1975). Studies on production of flavour constituents by various lactic acid bacteria. V. Effect of milk fat on production of volatile carbonyl compounds by mixed lactic acid bacteria starter. Jpn. J. Dairy Sci., 24(3): A117-A120.

Yuguchi, H., Hiramatsu, A., Doi, K., Ida, C. and Okonogi, S. (1989). Studies on the flavour of yoghurt cultured with bifidobacteria: significance of volatile components and organic acids in the sensory acceptance of yoghurt. Jpn. J. Zootech. Sci., 60(8): 734-741.

Zlatkis, A., Zak, B. and Boyle, G.J. (1953). Estimation of serum cholesterol. J. Lab. Clin. Med., 41: 486. Cited in: "Practical Clinical Biochemistry" by Varley, H. 4th Edn., Arnold Heinemann. pp313.

APPENDICES



Flow diagram showing different processes in bulk freeze-drying of mixed dahi and yoghurt cultures and their storage.

APPENDIX-II

Score Card for Sensory Evaluation of Dahi/Yoghurt

Dairy Microbiology Division
National Dairy Research Institute
Karnal-132 001

Date of manufacture:
Name of the Judge:
Date of Judging:
Code No:

Observations	Score Maximum	Score Alloted	Defects
			(Put / against defect, if any)
a) Appearance	5		i) Unnatural colour ii) Wheying off iii) Surface discolouration iv) Foreign matter
b) Body & Texture	5		i) Too thin ii)Lumpy/Granular iii) Wheying off iv) Gassy
c) Flavour	10		i) High acid ii)Bitter iii) Yeasty iv)Unclean v) Burnt vi)chalky vii) Powdery flavour in yoghurt
Overall score	20		

Suggested rating	Remarks, if any
Excellent	
Very good	
Good	
Fair	
Poor	

Signature of the Judge

Appendix-III: ANOVA of standardization of growth medium and growth conditions for dahi culture D after 16 hrs of incubation at 30°C

Source of effect	D.F	Mean sum of squares				
		Selection of growth medium	Optimization of temperature	Optimization of pH	Effect of inoculum level	Effect of cryoprotective agent
Replication	5	0.834e-04**	0.269e-04NS	0.270e-04NS	0.845e-04NS	0.903e-02NS
Effect	2	0.196e-01**	0.247**	0.444**	2.535**	0.742**
Error	10	0.838e-05	0.434	0.242e-06	0.111e-03	0.345e-03
CD at 1% level		0.493e-02	0.121e-01	0.900e-02	0.193e-01	0.340e-02

NS = Not Significant :: **Significant at 1% level (P < 0.01)

Appendix-IV: ANOVA of standardization of growth medium and growth conditions for dahi culture DAB after 18 hrs of incubation at 37°C

Source of effect	D.F	Mean sum of squares					
		Optimization of temperature	Optimization of pH	Effect of inoculum level	Effect of cryoprotective agent	DF	Selection of growth medium
Replication	5	0.353e-04NS	0.341e-04NS	0.419e-04NS	0.309e-03NS	5	0.780e-04NS
Effect	2	0.765**	0.222**	2.422**	0.585**	4	0.421e-01**
Error	10	0.375e-04	0.943e-05	0.395e-04	0.389e-03	20	0.468e-01
CD at 1% level		0.112e-02	0.992e-02	0.115e-01	0.361e-01		0.112e-01

NS = Not Significant :: **Significant at 1% level (P < 0.01)

Appendix-III: ANOVA of standardization of growth medium and growth conditions for dahi culture D after 16 hrs of incubation at 30°C

Source of effect	D.F	Mean sum of squares				
		Selection of growth medium	Optimization of temperature	Optimization of pH	Effect of inoculum level	Effect of cryoprotective agent
Replication	5	0.834e-04**	0.269e-06NS	0.270e-04NS	0.845e-04NS	0.903e-03NS
Effect	2	0.196e-01**	0.247**	0.444**	2.535**	0.742**
Error	10	0.838e-05	0.434	0.242e-06	0.111e-03	0.345e-03
CD at 1% level		0.493e-02	0.131e-01	0.900e-02	0.193e-01	0.340e-02

NS = Not Significant :: **Significant at 1% level (P < 0.01)

Appendix-IV: ANOVA of standardization of growth medium and growth conditions for dahi culture DAB after 18 hrs of incubation at 37°C

Source of effect	D.F	Mean sum of squares					
		Optimization of temperature	Optimization of pH	Effect of inoculum level	Effect of cryoprotective agent	DF	Selection of growth medium
Replication	5	0.353e-04NS	0.341e-06NS	0.419e-04NS	0.309e-03NS	5	0.780e-04NS
Effect	2	0.765**	0.222**	2.422**	0.585**	4	0.421e-01**
Error	10	0.375e-04	0.943e-05	0.395e-04	0.389e-03	20	0.468e-01
CD at 1% level		0.112e-02	0.992e-02	0.115e-01	0.361e-01		0.112e-01

NS = Not Significant :: **Significant at 1% level (P < 0.01)

Appendix-V: ANOVA of standardization of growth medium and growth conditions for yoghurt culture Y after 16 hrs of incubation at 37°C

Source of effect	D.F	Mean sum of squares			
		Selection of growth medium	Optimization of pH	Effect of inoculum level	Effect of cryoprotective agent
Replication	5	0.417e-04NS	0.192e-04NS	0.673e-04**	0.180e-02**
Effect	2	0.305e-01**	0.385**	0.112**	1.574**
Error	10	0.176e-04	0.175e-04	0.522e-05	0.212e-03
CD at 1% level		0.768e-02	0.767e-02	0.418e-01	0.266e-01

NS = Not Significant :: **Significant at 1% level (P < 0.01)

Appendix-VI: ANOVA of standardization of growth medium and growth conditions for yoghurt culture YAB after 16 hrs of incubation at 37°C

Source of effect	D.F	Mean sum of squares				
		Optimization of pH	Influence of inoculum level	Effect of cryoprotective agent	DF	Selection of growth medium
Replication	5	0.608e-04**	0.168e-06**	0.120e-03NS	5	0.864e-04*
Effect	2	0.241**	0.116**	0.430**	3	0.147**
Error	10	0.109e-04	0.199e-06	0.682e-03	15	0.208e-04
C.D. at 1% level		0.604e-01	0.816e-02	0.477e-01		0.776e-02

NS = Not Significant :: **Significant at 1% level (P < 0.01)

Appendix-VII: ANOVA of the effect of neutralization of growth medium to maintain the initial pH on viable cell count of dahi and yoghurt cultures

Source of effect	DF	Mean sum of squares			
		Dahi culture combination D	Dahi culture combination DAB	Yoghurt culture combination Y	Yoghurt culture combination YAB
Replication	5	0.124e-03**	0.195e-04**	0.399e-04**	0.433e-04**
Effect	3	13.451**	10.074**	15.054**	11.476**
Error	15	0.534e-05	0.419e-05	0.741e-05	0.216e-05
CD at 1% level		0.393e-02	0.348e-02	0.463e-02	0.250e-02

NS = Not Significant :: **Significant at 1% level (P < 0.01)

Appendix-VIII: ANOVA of the effect of storage conditions on bulk freeze-dried cultures of dahi and yoghurt

Source of effect	DF	Mean sum of squares			
		Dahi culture combination D	Dahi culture combination DAB	Yoghurt culture combination Y	Yoghurt culture combination YAB
Replication	2	0.131e-05NS	0.660e-04NS	0.127e-05NS	0.210e-04NS
A	1	0.278e-01**	0.813e-02**	0.492e-02**	0.390e-02**
B	1	0.269e-01**	0.498e-02**	0.644e-02**	0.105e-02**
C	3	0.179e-01**	0.539e-02**	0.175e-01**	0.126e-01**
A X B	1	0.132e-01**	0.312e-02**	0.367e-04**	0.500e-03**
A X C	3	0.518e-02**	0.139e-02**	0.206e-02**	0.595e-03**
B X C	3	0.635e-02**	0.672e-03**	0.207e-02**	0.120e-03**
A X B X C	3	0.314e-02**	0.433e-03**	0.243e-03**	0.532e-03**
Error	30	0.209e-04	0.169e-04	0.245e-04	0.188e-04
CD at 1% level					
A		0.363e-02	0.327e-02	0.393e-02	0.345e-02
B		0.363e-02	0.327e-02	0.393e-02	0.345e-02
C		0.513e-02	0.462e-02	0.556e-02	0.487e-02

NS = Not Significant :: **Significant at 1% level (P < 0.01)
A = Sealing atmosphere :: B = Storage temperature :: C = Storage period

Appendix-IX: ANOVA of the sensory evaluation of dahi and yoghurt

Source of effect	DF	Mean sum of squares	
		Dahi	Yoghurt
Replication	4	0.613e-01NS	0.218e-01NS
Culture combinations	1	9.249**	0.253e-01NS
Type of culture	1	0.289*	1.265**
All effect interaction	1	1.056**	0.393e-01NS
Error	12	0.341e-01	0.576e-01
CD at 1% level		0.2520	0.3280
CD at 5% level		0.1644	--

NS = Not Significant :: *Significant at 5% level (P < 0.05)
**Significant at 1% level (P < 0.01)

Appendix-X: ANOVA of the effect of feeding dahi and yoghurt on body weight gain of male albino rats

Source	DF	Sum of squares	Mean sum of squares	F value	CD0.01
Replication	5	2175.313	435.06	4.026**	
Period	3	145206.600	48402.21	447.945**	
Treatment	5	10410.560	2082.11	19.269**	7.85
Interaction	15	6118.250	407.88	3.774**	
Error	115	12426.190	108.05		
Total	143	176336.900		C.F.: 705180.06	

** Significant at P<0.01.

Appendix-XI: ANOVA of the effect of feeding dahi and yoghurt on serum cholesterol level of male albino rats

		Group A				Group B			
Source	DF	SS	MSS	F	CD	SS	MSS	F	CD
Replication	5	11.74	2.35	0.83NS	--	10.88	2.18	2.29NS	--
Treatment	2	84.90	42.45	14.92**	1.45	24.69	12.35	13.00**	0.84
Period	2	638.78	319.39	112.39**	1.02	128.22	64.11	67.48**	0.59
Error	44	125.09	2.84			41.9	0.95		
Total	53	860.51				205.69			

** = Significant at P<0.01
NS = Not significant