

**Utilization of *Lactobacillus acidophilus*
and *Bifidobacterium bifidum* to
Produce Fermented Buffaloes' Milk**

BY

FATMA AHMED FATHY MOHAMED

**A thesis submitted in partial fulfillment
of
the requirements for the degree of**

**DOCTOR OF PHILOSOPHY
IN
Agricultural Science
(Dairy Science and Technology)**

**Department of Food Science
Faculty of Agriculture
Ain Shams University**

1994



**Utilization of *Lactobacillus acidophilus*
and *Bifidobacterium bifidum* to
Produce Fermented Buffaloes' Milk**

BY

FATMA AHMED FATHY MOHAMED

A thesis submitted in partial fulfillment
of
the requirements for the degree of

**DOCTOR OF PHILOSOPHY
IN
Agricultural Science
(Food Science and Technology)**

18770

Food Science
Department
v



Approval sheet

***Utilization of *L. acidophilus* and *Bif. bifidum* to produce
Fermented Buffaloes' Milk.***

By

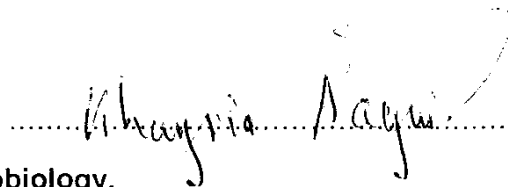
Fatma Ahmed Fathy Mohamed

B.Sc. Agric.(Food Sci.) , 1972 , Ain Shams University

M.Sc. Agric. (Dairying), 1987, Cairo University

**This thesis for Ph.D degree in Dairy Science and Technology has been
approved by:**

Prof. Dr. Kh. M. Naguib



Professor of Dairy Microbiology.

National Research Center , Cairo ,Egypt .

Prof. Dr. A.E.A. Hagra



Professor of Dairy Science.

Faculty of Agric., Ain Shams University , Cairo ,Egypt .

Prof. Dr. M. N. Magdoub



Professor of Dairy Microbiology .

Faculty of Agric.,Ain Shams University , Cairo ,Egypt .(Supervisor)

Date of Examination : 24 / 3 / 1994

***Utilization of *L. acidophilus* and *Bif. bifidum* to produce
Fermented Buffaloes' Milk.***

By

Fatma Ahmed Fathy Mohamed

B.Sc. Agric.(Food Sci.) , 1972 , Ain Shams University

M.Sc.Agric. (Dairying) , 1987 , Cairo University

Under the Supervision of :-

Prof. Dr. M. N. Magdoub

Professor of Dairy Microbiology , Fac. Agric., Ain Shams University.

Prof. Dr. S.A. El-Nokrashi

Professor of Dairy Microbiology. National Research Center .

ABSTRACT

Acidophilus and ABghurt milks are not familiar as fermented milks for the Egyptian consumer. Therefore, this work was undertaken to introduce these products to the Egyptian market through different trials. The effect of six different growth promoters (Yeast extract, Glucose , Lactose , Lactase, Lactulose, Yeast extract+Glucose, Yeast extract + Lactose , Yeast extract + Lactase, Yeast extract + Lactulose, Cysteine, Tyrosine) were studied seperately and as mixtures to attain the highest viable count of *L. acidophilus* and *Bif. bifidum* .

Fortification of Buffaloes' milk with Yeast extract and Yeast extract + Glucose were preferable for producing acidophilus milk .While Lactulose and Yeast

II

extract +Lactulose were preferred for ABghurt. The addition of cysteine had some positive effect on the growth of *Bif. bifidum*.

The inhibitory effect of *L. acidophilus* on *Campylobacter jejuni*, *Listeria monocytogenes*, *E.coli* and *B. cereus* grown in sterile milk at 6 °C was detected.

The effect of different fat contents on the properties of acidophilus and ABghurt buffalo's milks was studied. Also, fortification of buffaloes' milk with WPC up to 15% was studied. Moreover, manufacture of low lactose Acidophilus and ABghurt Milks from buffaloes' milk was performed. The results obtained from the chemical examination and from the organoleptic evaluation the fermented products indicate that buffalo's milk containing 4% fat gave more acceptable product. Fortification with 5% WPC was recommended for acidophilus and ABghurt milks manufacturing. Low lactose product were also prepared using up 14% SNF, which have the longest shelf-life period.

Keyword

Fermented milk, *Lactobacillus acidophilus*, *Bifdobacterium bifidum*, Acidophilus Milk, ABghurt Milk, Antagonistic, Pathogenic bacteria, Whey protein concentrate, Ultrafiltration, Sensory evaluation.

III

ACKNOWLEDGEMENT

The author wishes to express her sincere gratitude and great appreciation to **Pr. Dr. M. N. Magdoub** Professor Dairy Microbiology , Fac. Agric., Ain Shams University (ASU) , and **Prof. Dr. S. A. El-Nokrashi** Professor of Dairying , National Research Center (NRC) , for suggesting the problem, supervising and guiding the work and preparation of this text .

Special thanks and deep obligation extended to **Prof. Dr. M. A. Khorshid** Deputy Chairman Food Technology and Nutrition Division (NRC) and **Dr. M.A. El- Nawawy** , Associate Professor of Dairy Microbiology , Fac. Agric., Ain Shams University , and **Dr. N. F. Tawfik** , Associate Professor of Dairying , National Research Center (NRC) for their kind attention , endless collaboration and reviewing of the manuscript of the thesis.

The author would like to extend her deep gratitude to all members at the Food Science Department , and Cairo **MIRCEN** , Fac. Agric., ASU, as well as to my colleagues at the Department of Food and Dairy Technology , National Research Centre for providing facilities and every possible help.

IV

Contents

	Page
Introduction.....	1
Review of Literature.....	3
1- Utilization of <i>L . acidophilus</i> and <i>Bif. bifidum</i> to produce fermented milk	3
2- Viability of <i>L. acidophilus</i>	11
3- Production of Low Lactose fermented milk	14
4- Antagonistic Effect of <i>L . acidophilus</i>	21
5- Therapeutic Effect of Acidophilus Milk.....	26
 Materials and Methods	 32
A- Materials :.....	32
B- Methods :	35
1- Preparation of Acidophilus milk from Buffaloes'milk	35
2- Preparation of UF - whey protein concentrate.....	35
3- Preparation of retentate	36
4- Bacteriological Procedure:.....	36
a-Inoculum preparation of the pathogen	36
b-Viability of the pathogenic bacteria in acidophilus milk	36
c-Inhibitory Effect of <i>L.acidophilus</i> by the Agar	
Diffusion Technique	38
5- Chemical Analysis	38
6- Sensory evaluation	42
7- Statistical analysis	42

	Page
Results and Discussion	43
Part I : Effect of some additives on the growth and acid production by <i>L. acidophilus</i> and <i>Bif. bifidum</i> and their mixture	41
Section (1) Effect of some additives on the growth and acid production by <i>L. acidophilus</i>	41
a- Effect of Yeast extract	41
b- Effect of Glucose	47
c- Effect of Lactose	52
d- Effect of Lactase	57
e- Effect of Yeast extract and Glucose.....	62
f- Effect of Yeast extract and Lactose.....	67
g- Effect of Yeast extract and Lactase.....	67
h- Effect of Cysteine	76
i- Effect of Tyrosine	82
Effect of additives on the Organoleptic properties of acidophilus buffaloes'milk	87
Section (2) Effect of some additives on the growth and acid production by <i>Bif. bifidum</i>	89
a- Effect of Yeast extract	89
b- Effect of Glucose	90

VI

	Page
c- Effect of Lactose	95
d- Effect of Lactulose	104
e- Effect of Yeast extract and Glucose.....	109
f- Effect of Yeast extract and Lactose.....	114
g- Effect of Yeast extract and Lactuloase.....	114
h- Effect of Cysteine	123
i- Effect of Tyrosine	128
Effect of additives on the Organoleptic properties of Bifidus buffaloes'milk	133
Section (3) Effect of some additives on the growth and acid production by <i>L . acidophilus</i> and <i>Bif. bifidum</i> ..	
a- Effect of Yeast extract	135
b- Effect of Yeast extract and Glucose	141
c- Effect of Lactulose	146
d- Effect of Yeast extract and Lactulose	146
Effect of additives on the Organoleptic properties of ABghurt buffaloes'milk	155
Section (4) Antagonismic Effect of Acidophilus buffaloes'milk.157	
a - Inhibitory effect of <i>L . acidophilus</i> on some pathogenic bacteria	159
b - Effect of different treatment on the antibacteria of cell free filtrated of <i>L. acidophilus</i> against some pathogenic bacteria	163

VII

	Page
Part II : Manufacture of Buffaloes' Acidophilus and ABghurt Milks	166
Section (1) Manufacture of Acidophilus and ABghurt Milks with different fat ratios	166
Section (2) Manufacture of Acidophilus and ABghurt milks from buffaloes'milk fortified with WPC	176
Section (3) Manufacture of Low Lactose Acidophilus and ABghurt milks from buffaloes'milk	186
Summary and Conclusion	195
References	203
Arabic Summary.....	221

VIII

LIST OF TABLES

Table Nr.		Page
1	Effect of adding Yeast Extract on the pH and acidity of buffaloes' acidophilus milk	43
2	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Yeast extract	44
3	Effect of adding Glucose on the pH and acidity of buffaloes' acidophilus milk	48
4	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Glucose	49
5	Effect of adding Lactose on the pH and acidity of buffaloes' acidophilus milk	53
6	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Lactose	54
7	Effect of adding Lactase on the pH and acidity of buffaloes' acidophilus milk	58
8	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Lactase	59
9	Effect of adding Yeast Extract+Glucose on the pH and acidity of buffaloes' acidophilus milk	63
10	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Yeast extract+Glucose	64
11	Effect of adding Yeast Extract+Lactose on the pH and acidity of buffaloes' acidophilus milk	68
12	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Yeast extract+Lactose	69

IX

Table Nr.		Page
13	Effect of adding Yeast Extract+Lactase on the pH and acidity of buffaloes' acidophilus milk	72
14	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Yeast extract+Lactase	73
15	Effect of adding Cysteine on the pH and acidity of buffaloes' acidophilus milk	77
16	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Cysteine	78
17	Effect of adding Tyrosine on the pH and acidity of buffaloes' acidophilus milk	83
18	Viable count of <i>L. acidophilus</i> of buffaloes' acidophilus milk fortified with Tyrosine	84
19	Effect of additives on the organoleptic properties of acidophilus buffaloes' skim milk	88
20	Effect of adding Yeast Extract on the pH and acidity of buffaloes' bifidus milk	91
21	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Yeast extract	92
22	Effect of adding Glucose on the pH and acidity of buffaloes' bifidus milk	96
23	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Glucose	97
24	Effect of adding Lactose on the pH and acidity of buffaloes' bifidus milk	100
25	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Lactose	101

Table Nr.		Page
26	Effect of adding Lactase on the pH and acidity of buffaloes' bifidus milk	105
27	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Lactulose	106
28	Effect of adding Yeast Extract+Glucose on the pH and acidity of buffaloes' bifidus milk	110
29	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Yeast extract+Glucose	111
30	Effect of adding Yeast Extract+Lactose on the pH and acidity of buffaloes' bifidus milk	115
31	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Yeast extract+Lactose	116
32	Effect of adding Yeast Extract+Lactulose on the pH and acidity of buffaloes' bifidus milk	119
33	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Yeast extract+Lactulose	120
34	Effect of adding Cysteine on the pH and acidity of buffaloes' bifidus milk	124
35	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Cysteine	125
36	Effect of adding Tyrosine on the pH and acidity of buffaloes' bifidus milk	129
37	Viable count of <i>Bif. bifidum</i> of buffaloes' acidophilus milk fortified with Tyrosine	130
38	Effect of additives on the organoleptic properties of bifidus buffaloes' skim milk	134

XI

Table Nr.		Page
39	Effect of adding Yeast Extract on the pH and acidity of buffaloes' ABghurt milk	137
40	Viable count of <i>L. acidophilus</i> and <i>Bif. bifidum</i> of buffaloes' ABghurt milk fortified with Yeast extract	138
41	Effect of adding Yeast Extract+Glucose on the pH and acidity of buffaloes' ABghurt milk	142
42	Viable count of <i>L. acidophilus</i> and <i>Bif. bifidum</i> of buffaloes' ABghurt milk fortified with Yeast extract+Glucose	143
43	Effect of adding Lactulose on the pH and acidity of buffaloes' ABghurt milk	147
44	Viable count of <i>L. acidophilus</i> and <i>Bif. bifidum</i> of buffaloes' ABghurt milk fortified with Lactulose	148
45	Effect of adding Yeast Extract+Lactulose on the pH and acidity of buffaloes' ABghurt milk	151
46	Viable count of <i>L. acidophilus</i> and <i>Bif. bifidum</i> of buffaloes' ABghurt milk fortified with Yeast extract+Lactulose	152
47	Effect of additives on the organoleptic properties of ABghurt buffaloes' skim milk	156
48	The counts of Test Organisms inoculated in test medium	158
49	Survival of different bacteria in the precence of <i>L. acidophilus</i> in test medium store at 6 °C	160
50	pH values of acidophilus milk in the presence of some pathogenic bacteria store at 6 °C	162
51	Antibacterial activity of cell free filtrates of <i>L.acidophilus</i> against some pathogenic bacteria	165