

**PROCESS OPTIMISATION AND QUALITY
EVALUATION OF JACKFRUIT BASED PROBIOTIC
FOOD PRODUCTS**

By
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(2016-24-001)



**DEPARTMENT OF COMMUNITY SCIENCE
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2020**

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Community Science

(FOOD SCIENCE AND NUTRITION)

Faculty of Agriculture



Kerala Agricultural University
DEPARTMENT OF COMMUNITY SCIENCE
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA
2020

DECLARATION

I, hereby declare that the thesis entitled “**Process optimisation and quality evaluation of jackfruit based probiotic food products**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed during the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Acknowledgement

The journey was not easy. But I could enjoy it because of the great companions. They helped me, encouraged me and assisted me whenever I was in trouble. Now it is time to take a pause and express my heartfelt gratitude to all of them.

*I would like to express my whole hearted gratitude towards my major advisor **Dr. Sharon. C. L.** Assistant Professor, Dept. Community Science. Her timely advises and support enabled me to complete this task successfully. She always stood behind me as a strong pillar of care, support and motivation and at the same time she never compromised on the quality of my work. I would always like to remember my teacher as the most valuable blessing of my career.*

*I place a deep sense of obligation towards **Dr. Seeja Thomachan Panjikkaran**, Assistant Professor and Head, **Dr. Aneena. E. R.** and **Dr. Lakshmi. P.S.** Assistant Professors, Department of Community Science for their valuable suggestions and support throughout the period of my research.*

*I express my sincere thanks to **Dr. D. Girija**, Professor and Registrar (i/c) KAU, Dept. Agrl. Microbiology and **Dr. Beena. A.K.** Professor and Head, Dept. Dairy Microbiology, College of Dairy Science and Technology, Mannuthy for their expert advises, kind concern and ever willing help, throughout the period of my research.*

*I am deeply indebted to **Dr. S. Krishnan**, Professor, Dept. Agricultural Statistics, for his expert advice, constant motivation, and help he rendered during the statistical interpretations and **Mrs. Norma Xavier C**, Assistant Professor and former Head of the Department, Department of Community Science, for her help advice and motivation.*

*I also avail this opportunity to pay my sincere thanks to **Dr. B. Ajithkumar**, Asst. Professor and Head, Dept. Agricultural Meteorology, **Mrs. Meagle Joseph**, Associate Professor and Head, and **Dr. Saji Gomez**, Assistant Professor, Dept.*

Postharvest Technology, **Dr. V. Ramnath**, Professor and Head, Dept. Veterinary Physiology, **Dr. S.N. Rajkumar**, Head, Dairy plant, Kerala Veterinary and Animal Sciences University for their interest, cooperation and encouragement.

I would also like to remember with great pleasure **Gangadharan sir, Arjun chettan, Sreejith** and all other staff and students of Dept. Agrl. Meteorology, **Dr. Surendra Gopal, Panchami madam, Reshma** and the entire team of Dept. Agrl. Microbiology, **Dr. Sudheer. K.P** Professor and Head, Dept of Agrl. Engineering of College of Horticulture, Vellanikkara.

I am genuinely indebted to the entire staff and students of **Dept. Dairy Microbiology, Dairy Chemistry, Dairy Engineering, Central Instrumentation Laboratory and Dairy plant** of Kerala Veterinary and Animal Sciences University, Mannuthy.

Words cannot really express the friendship I relished from my dear friends **Shahanas. E, Rammya Molu. K, Amitha, Siji, Ajisha, Simla, Athira, Meera, Riya, Aiswarya, Lovely, Sruthy, Reshma, Thrishna, Fadhiya, Reenu, Raji, Jesmin, Krishnapriya, Anirudh and Lakshmy**. They helped me, supported me, encouraged me and gave me enough mental strength to go through all the difficulties of my life and studies.

I express my unreserved gratitude to **Kumari chechi and Shija chechi** for their timely help and cooperation throughout the study.

Special thanks to **Shyama madam, Dr. Jamaludeen, Dr. Chinnu, Sarath Sir and Rajesh Sir** of Kerala Veterinary and Animal Sciences University, Mannuthy and **Aravind chettan and Rajitha chechi** of Students Computer Club, College of Horticulture for rendering necessary help whenever needed.

*I am thankful to the **University Grants Commission** for the financial support for the conduct of the study and **Kerala Agricultural University** for the opportunity to carry out the work.*

*I express my deep sense of grateful and heartfelt thanks to my parents **Mr. Ramachandran** and **Mrs. Geetha**, husband **Mr. Rajan**, sister **Mrs. Reshma**, lovely children **Devdarsh** and **Dhanashyam**, sister in law **Mrs. Reshma** and my whole extended family members for their love, co-operation, encouragement moral support and the unconditional faith in me throughout the study. I am also grateful to the bondless affection, eternal love and the deep concern and prayers of my beloved **grandparents** even though I lost them during the journey. I am genuinely indebted to all my friends, relatives and well-wishers, who supported me in the collection of raw and ripe jackfruits from the nook and corner of the district.*

With immense pleasure, I remember all the staff and students of KAU for the love, and assistance I received during my KAU life.

*Above all, I humbly bow my head before the **Almighty**, for being with me in the form all the above mentioned.*

Remya P.R

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-37
3	MATERIALS AND METHODS	38-65
4	RESULTS	66-201
5	DISCUSSION	202-247
6	SUMMARY	248-254
	REFERENCES	i-xlii
	APPENDICES	
	ABSTRACT	

LIST OF TABLES

Table No.	Titles	Page in between
1.	Proportion of ingredients in the jackfruit based food mixtures	41
2.	Details of oxidation for the assessment of riboflavin content	52
3.	Treatments for the preparation of instant shake mixes	57
4.	Combination of ingredients in the preparation of jackfruit yoghurt	58
5.	Mean score and mean rank scores for the organoleptic qualities of jackfruit (<i>koozha</i>) food mixtures	66-67
6.	Selected combinations of food mixture from each set (<i>koozha</i>)	68
7.	Mean score and mean rank score for the organoleptic qualities of jackfruit (<i>varikka</i>) food mixtures	68-69
8.	Selected combinations of jackfruit based food mixtures (<i>varikka</i>)	70
9.	Viable count of <i>L. acidophilus</i> in food mixtures with different substrate concentrations	71
10.	Viable count of <i>L. acidophilus</i> in food mixtures with different time of incubation	73
11.	Viable count of <i>L. acidophilus</i> in food mixtures at different pH levels	74

12.	Viabile count of <i>L. acidophilus</i> in food mixtures at various temperatures	76
13.	Viabile count of <i>L. acidophilus</i> in food mixtures at various inoculum concentrations	77
14.	Moisture content of fermented food mixtures during storage	78-79
15.	Comparison of moisture content of fermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	79
16.	Moisture content of unfermented food mixtures during storage	80-81
17.	Comparison of moisture content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	80-81
18.	Titratable acidity of fermented food mixtures	81-82
19.	Comparison of titratable acidity of fermented <i>koozha</i> and <i>varikka</i> food mixtures	81
20.	Titratable acidity of un fermented food mixtures	81-82
21.	Comparison of titratable acidity of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	82-83
22.	Protein content of the fermented food mixtures	82-83
23.	Comparison of protein content of fermented food mixtures on storage	83
24.	Protein content of the unfermented food mixtures	83-84

25.	Comparison of protein content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	84
26.	β carotene content of fermented food mixtures	84-85
27.	Comparison of β carotene content of fermented food mixtures <i>koozha</i> and <i>varikka</i> variety	85-86
28.	β carotene content of unfermented food mixtures during storage	85-86
29.	Comparison of beta carotene content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	85-86
30.	Crude fibre content of the fermented food mixtures during storage	86-87
31.	Comparison of crude fibre content of the fermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	86
32.	Crude fibre content of unfermented food mixture during storage	87-88
33.	Comparison of crude fibre content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	87-88
34.	TSS content of fermented food mixtures on storage	87-88.
35.	Comparison of TSS content of fermented food mixtures on storage	88
36.	TSS content of unfermented food mixtures	88-89
37.	Comparison of TSS content of fermented and unfermented food mixtures	88-89

38.	Reducing sugar content of the fermented food mixtures on storage	88-89
39.	Comparison of reducing sugar content of fermented food mixtures	89
40.	Reducing sugar content of the unfermented food mixtures on storage	90-91
41.	Comparison of reducing sugar content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	90-91
42.	Total sugar content of fermented food mixtures on storage	90-91
43.	Comparison of total sugar content of fermented food mixtures	91
44.	Total sugar content of unfermented food mixtures on storage	91-92
45.	Comparison of total sugar content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	91-92
46.	Starch content of fermented food mixtures on storage	91-92
47.	Comparison of starch content of fermented food mixtures	92
48.	Starch content of unfermented food mixtures on storage	92-93
49.	Comparison of total starch content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	93-94

50.	Total ash content of fermented food mixtures on storage	93-94
51.	Comparison of total ash content of fermented food mixtures	93-94
52.	Total ash content of unfermented food mixtures on storage	93-94
53.	Comparison of total ash content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	94-95
54.	Calcium content of fermented food mixtures on storage	94-95
55.	Comparison of calcium content of fermented food mixtures on storage	95
56.	Calcium content of unfermented food mixtures on storage	95-96
57.	Comparison of calcium content of fermented and unfermented food mixtures on storage	95-96
58.	Iron content of fermented food mixtures on storage	95-96
59.	Comparison of iron content of fermented food mixtures on storage	96-97
60.	Iron content of unfermented food mixtures on storage	96-97
61.	Comparison of iron content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	96-97
62.	Potassium content of fermented food mixtures on storage	96-97

63.	Comparison of potassium content of fermented food mixtures	97
64.	Potassium content of unfermented food mixtures on storage	97-98
65.	Comparison of potassium content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	98-99
66.	Thiamine content of fermented food mixtures	98-99
67.	Comparison of thiamine content of fermented food mixtures	98
68.	Thiamine content of unfermented food mixtures	99-100
69.	Comparison of thiamine content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	99-100
70.	Riboflavin content of fermented food mixtures on storage	99-100
71.	Comparison of riboflavin content of fermented food mixtures	100
72.	Riboflavin content of unfermented food mixtures on storage	100-101
73.	Comparison of riboflavin content of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	100-101
74.	<i>In vitro</i> starch digestibility of the fermented food mixtures	100-101
75.	Comparison of starch digestibility of <i>koozha</i> and <i>varikka</i> fermented food mixtures	101

76.	<i>In vitro</i> starch digestibility of the unfermented food mixtures	101-102
77.	Comparison of starch digestibility of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	102-103
78.	<i>In vitro</i> protein digestibility of the fermented food mixtures	102-103
79.	Comparison of starch digestibility of <i>koozha</i> and <i>varikka</i> fermented food mixtures	102
80.	<i>In vitro</i> protein digestibility of the un-fermented food mixtures	103-104
81.	Comparison of protein digestibility of fermented and unfermented food mixtures from <i>koozha</i> and <i>varikka</i> variety	103-104
82.	Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+T)	103-104
83.	Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+P)	104-105
84.	Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+JSF+T)	104-105
85.	Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+JSF+P)	105-106
86.	Viable count of <i>L.acidopilus</i> in fermented food mixtures	105-106

87.	Total bacterial count in fermented food mixtures on storage	106-107
88.	Total bacterial count in unfermented food mixtures on storage	106-107
89.	Correlation between nutrient content of food mixture and weather parameters (<i>koozha</i> variety)	108
90.	Correlation between nutrient content of food mixture and weather parameters (<i>varikka</i> variety)	110
91.	Glycemic indices of jackfruit based food mixtures	111
92.	Comparison of glycemic index of fermented food mixtures	112
93.	GI fermented and unfermented food mixtures	113
94.	Mean scores and mean rank score for the organoleptic qualities of probiotic shake mixes (<i>koozha</i>)	113-114
95.	Mean scores and mean rank score for the organoleptic qualities of probiotic shake mixes (<i>varikka</i>)	114-115
96.	Moisture content of the instant probiotic shake mixes	115
97.	Titrateable acidity of the instant probiotic shake mixes	116
98.	Protein content of the instant probiotic shake mixes	116
99.	β carotene content of the instant probiotic shake mixes	117
100.	Crude fibre content of the instant probiotic shake mixes	118
101.	TSS content of the instant probiotic shake mixes	118

102.	Starch content of the instant probiotic shake mixes	119
103.	Reducing and total sugars of the instant probiotic shake mixes	119-120
104.	Mineral content of the instant probiotic shake mixes	120
105.	Thiamine and riboflavin content of the instant probiotic shake mixes	121
106.	<i>In vitro</i> starch and protein digestibility of the instant probiotic shake mixes	122
107.	Mean scores for organoleptic evaluation of selected instant probiotic shake mixes during storage	123-124
108.	Viable count of <i>L.acidophilus</i> of instant probiotic shake mixes during storage	124
109.	Total bacterial count of instant probiotic shake mixes during storage	125
110.	Mean scores and mean rank score for the organoleptic qualities of jackfruit based yoghurts (<i>koozha</i>)	127-128
111.	Selected combinations of jackfruit bio-yoghurts (<i>koozha</i>)	129
112.	Mean scores and mean rank score for the organoleptic qualities of jackfruit based yoghurts (<i>varikka</i>)	130-131
113.	Selected combinations of jackfruit bio-yoghurts (<i>varikka</i>)	130
114.	Viable count of <i>L.acidophilus</i> in jackfruit bio-yoghurts at different substrate concentrations	132
115.	Viable count of <i>L.acidophilus</i> in jackfruit bio-yoghurts at different time of incubation	133
116.	Viable count of <i>L.acidophilus</i> in jackfruit bio-yoghurts at different pH	134

117.	Viable count of <i>L.acidophilus</i> in jackfruit bio-yoghurts at different temperature	136
118.	Viable count of <i>L.acidophilus</i> in jackfruit bio-yoghurts at 100µl inoculum concentration	137
119.	Moisture content of the bio-yoghurts on storage	139
120.	Comparison of moisture content of HM, SM and HM+SM bio-yoghurts on storage	141
121.	Acidity of bio-yoghurts on storage	143
122.	Comparison of acidity of HM, SM and HM+SM bio-yoghurts on storage	144
123.	Fat content of bio-yoghurts on storage	146
124.	Comparison of fat content of HM, SM and HM+SM bio-yoghurts on storage	147
125.	Reducing sugar content of bio-yoghurts on storage	149
126.	Comparison of reducing sugar content of HM, SM and HM+SM bio-yoghurts on storage	150
127.	Total sugar content of bio-yoghurts on storage	152
128.	Comparison of total sugar content of HM, SM and HM+SM bio-yoghurts on storage	153
129.	TSS content of bio-yoghurts on storage	155
130.	Comparison of TSS content of HM, SM and HM+SM bio-yoghurts on storage	156
131.	Crude fibre content of jackfruit based bio-yoghurts on storage	157
132.	Comparison of crude fibre content of HM, SM and HM+SM bio-yoghurts on storage	159

133.	Protein content of bio-yoghurts on storage	160
134.	Comparison of protein content of HM, SM and HM+SM bio-yoghurts on storage	162
135.	β carotene content of bio-yoghurts on storage	164
136.	Comparison of β carotene content of HM, SM and HM+SM bio-yoghurts on storage	165
137.	Total ash content of bio-yoghurts on storage	167
138.	Comparison of protein content of HM, SM and HM+SM bio-yoghurts on storage	168
139.	Calcium content of bio-yoghurts on storage	170
140.	Comparison of calcium content of HM, SM and HM+SM bio-yoghurts on storage	171
141.	Iron content of bio-yoghurts on storage	173
142.	Comparison of iron content of HM, SM and HM+SM bio-yoghurts on storage	174
143.	Potassium content of bio-yoghurts on storage	176
144.	Comparison of potassium content of HM, SM and HM+SM bio-yoghurts on storage	177
145.	Synerisis of bio-yoghurts on storage	179
146.	Comparison of synerisis of HM, SM and HM+SM bio-yoghurts on storage	180
147.	Water holding capacity of bio-yoghurts on storage	182
148.	Comparison of water holding capacity of HM, SM and HM+SM bio-yoghurts on storage	183

149.	Curd tension of bio-yoghurts on storage	184-185
150.	Comparison of curd tension of HM, SM and HM+SM bio-yoghurts on storage	186
151.	Curd tension of viscosity on storage	188
152.	Comparison of viscosity of HM, SM and HM+SM bio-yoghurts on storage	189
153.	Organoleptic scores of control bio-yoghurts on storage	191
154.	Organoleptic scores of <i>koozha</i> based bio-yoghurts on storage	193-194
155.	Organoleptic scores of <i>varikka</i> based bio-yoghurts on storage	195-196
156.	Viability of <i>L.acidophilus</i> in the bio-yoghurts during storage	197
157.	Total bacterial count of bio-yoghurts on storage	198-199
158.	Total yeast count of bio-yoghurts on storage	199
159.	Cost of production of selected jackfruit based probiotic fermented food products	200

LIST OF FIGURES

Figure No.	Titles	Page in between
1.	Monthly average temperature during the storage period of food mixtures	107-108
2.	Relative humidity during storage the period of food mixtures	107-108
3.	Glycemic response of individuals after consuming <i>koozha</i> based food mixtures	111-112
4.	Glycemic response of individuals after consuming <i>varikka</i> based food mixtures	111-112
5.	Mean scores for overall acceptability of food mixtures (<i>koozha</i> variety)	204-205
6.	Mean scores for overall acceptability of food mixtures (<i>varikkavariety</i>)	204-205
7.	Viable count of <i>L. acidophilus</i> in food mixtures with different substrate concentration	207-208
8.	Viable count of <i>L. acidophilus</i> in food mixtures with different time of incubation	207-208
9.	Viable count of <i>L. acidophilus</i> in food mixtures with different pH levels	207-208
10.	Viable count of <i>L. acidophilus</i> in food mixtures with different temperatures of incubation	207-208
11.	Viable count of <i>L. acidophilus</i> in food mixtures with different inoculum concentrations	207-208
12.	Moisture content of fermented (FM) and unfermented (UFM) food mixtures on storage	211-212

13.	Titrateable acidity of fermented (FM) and unfermented (UFM) food mixtures on storage	211-212
14.	Protein content of fermented (FM) and unfermented (UFM) food mixtures on storage	214-215
15.	Beta carotene content of fermented (FM) and unfermented (UFM) food mixtures on storage	214-215
16.	Crude fibre content of fermented (FM) and unfermented (UFM) food mixtures on storage	215-216
17.	TSS content of fermented (FM) and unfermented (UFM) food mixtures on storage	215-216
18.	Reducing sugar of fermented (FM) and unfermented (UFM) food mixtures on storage	216-217
19.	Total sugar content of fermented (FM) and unfermented (UFM) food mixtures on storage	216-217
20.	Starch content of fermented (FM) and unfermented (UFM) food mixtures on storage	216-217
21.	Total ash content of fermented (FM) and unfermented (UFM) food mixtures on storage	217-218
22.	Calcium content of fermented (FM) and unfermented (UFM) food mixtures on storage	217-218
23.	Iron content of fermented (FM) and unfermented (UFM) food mixtures on storage	217-218
24.	Potassium content of fermented (FM) and unfermented (UFM) food mixtures on storage	217-218
25.	Thiamine content of fermented (FM) and unfermented (UFM) food mixtures on storage	219-220
26.	Riboflavin content of fermented (FM) and unfermented (UFM) food mixtures on storage	219-220

27.	<i>In vitro</i> starch digestibility of fermented (FM) and unfermented (UFM) food mixtures on storage	220-221
28.	<i>In vitro</i> protein digestibility of fermented (FM) and unfermented (UFM) food mixtures on storage	220-221
29.	Mean scores of organoleptic qualities of fermented (FM) and unfermented (UFM) food mixtures	221-222
30.	Mean scores of organoleptic qualities of fermented (FM) and unfermented (UFM) food mixtures	221-222
31.	Viability of <i>L.acidophilus</i> in fermented food mixtures during storage	223-224
32.	Moisture content of instant probiotic shake mixes during storage	228-229
33.	Titratable acidity of instant probiotic shake mixes during storage	228-229
34.	Protein content of instant probiotic shake mixes during storage	229-230
35.	β carotene content of instant probiotic shake mixes during storage	229-230
36.	Crude fibre content of instant probiotic shake mixes during storage	229-230
37.	TSS content of instant probiotic shake mixes during storage	229-230
38.	Starch content of instant probiotic shake mixes during storage	229-230
39.	Reducing and total sugar content of instant probiotic shake mixes during storage	229-230
40.	Mineral content of instant probiotic shake mixes during storage	230-231

41.	Riboflavin content of instant probiotic shake mixes during storage	230-231
42.	<i>In vitro</i> digestibility of instant probiotic shake mixes during storage	231-232
43.	Mean scores of organoleptic qualities of instant probiotic shake mixes	231-232
44.	Viability of <i>L.acidophilus</i> in instant probiotic shake mixes during storage	231-232
45.	Mean scores for overall acceptability of jackfruit based yoghurt (<i>koozha</i> variety)	232-233
46.	Mean scores for overall acceptability of jackfruit based yoghurt (<i>koozha</i> variety)	232-233
47.	Viable count of <i>L. acidophilus</i> in jackfruit bio-yoghurts at different substrate concentrations	233-234
48.	Viable count of <i>L. acidophilus</i> in jackfruit bio-yoghurts at different time of incubation	233-234
49.	Viable count of <i>L. acidophilus</i> in jackfruit bio-yoghurts at different pH	233-234
50.	Viable count of <i>L. acidophilus</i> in jackfruit bio-yoghurts at different temperatures	233-234
51.	Viable count of <i>L. acidophilus</i> in jackfruit bio-yoghurts at 100 µl inoculum concentration	233-234
52.	Moisture content of the bio-yoghurts during storage	234-235
53.	Acidity of bio-yoghurts on storage	234-235
54.	Fat content of bio-yoghurts on storage	236-237

55.	Reducing sugar content of bio-yoghurts on storage	236-237
56.	Total sugar content of bio-yoghurts on storage	237-238
57.	TSS content of bio-yoghurts on storage	237-238
58.	Crude fibre content of the jackfruit based bio-yoghurts on storage	239-240
59.	Protein content of probiotic bio-yoghurts on storage	239-240
60.	β carotene of probiotic bio-yoghurts on storage	241-242
61.	Total ash contents of probiotic bio-yoghurts on storage	241-242
62.	Calcium content of probiotic bio-yoghurts on storage	242-243
63.	Iron content of probiotic bio-yoghurts on storage	242-243
64.	Potassium content of probiotic bio-yoghurts on storage	242-243
65.	Synerisis of probiotic yoghurt on storage	243-244
66.	Water holding capacity of probiotic yoghurt on storage	243-244
67.	Curd tension of probiotic yoghurt on storage	243-244
68.	Viscosity of probiotic yoghurt on storage	243-244

69.	Mean scores of organoleptic qualities of <i>koozha</i> based bio-yoghurts on storage	245-246
70.	Mean scores of organoleptic qualities of <i>varikka</i> based bio-yoghurts on storage	245-246
71.	Viability of <i>L. acidophilus</i> in the bio-yoghurts during storage	245-246
72.	Total bacterial count in the bio-yoghurts during storage	245-246

LIST OF PLATES

Sl. No.	Title	Page No.
1.	Preparation of jackfruit based food mixture	41-42
2.	Probiotic strain sub culturing	42-43
3.	Preparation of freeze dried powder	44-45
4.	Preparation of jackfruit pulp	58-59
5.	<i>L.acidophilus</i> in MRS media	78-79
6.	Fermented food mixture	78-79
7.	Instant probiotic shake mixes in poly ethylene pouches	114-115
8.	Probiotic shake	114-115
9.	Jackfruit based bio-yoghurts	138-139

LIST OF APPENDIX

Sl. No.	TITLE
1.	Score card for the organoleptic evaluation of jackfruit based food mixtures
2.	Score card for the organoleptic evaluation of jackfruit based instant probiotic shake mixes
3.	Score card for the organoleptic evaluation of jackfruit based bio-yoghurts
4.	Weekly temperature and relative humidity of the storage room during storage

A decorative horizontal scroll graphic with a black outline. The scroll is partially unrolled at both ends, with the top corners of the unrolled sections shaded in light gray. The word "Introduction" is centered on the scroll.

Introduction

1. INTRODUCTION

“Leave your drugs in the chemist’s pot if you can heal the patient with food.”

- Hippocrates

Food can be anything that when ingested, satisfies hunger and nourishes the body. But this basic concept of food had changed with the introduction of a new area called ‘functional foods’. The proponents of functional foods gives an additional disease prevention dimension to foods.

Food containing significant levels of biologically active substances that can impart health benefits beyond basic nutrition are generally referred to as functional foods. FFC (2011) defined functional food as “Natural or processed food that contains known or unknown biologically active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease.” Probiotics can be considered as a potential functional food as it improves the quality of life through food.

The term ‘probiotic’ refers to a preparation of defined microorganisms, in sufficient numbers to alter the microflora in the intestinal compartment of the host and bring beneficial effects. Probiotics have been associated with mankind ever since people started consuming fermented milk and fermented foods. However, their health effects came to light, after Metchnikoff (1907) suggested that the gut microflora are associated with human health and longevity.

Probiotics aid in breakdown of proteins and fats in food and help to absorb vitamins minerals and amino acids, efficiently. In addition to these, probiotic bacteria boost immune system and prevent or limit the growth of harmful bacteria like *Salmonella* and *E.coli*. Under natural conditions, the protective gut microflora is

sufficient and there is no much need for bacterial supplements. Various factors that call for the need for probiotics are change in food habits, fast life, unhealthy living conditions and excessive consumption of antibacterial substances like antibiotics.

During the past few decades, there is a consumer driven trend in the probiotic market and this trend is expected to continue, because of the health benefits of probiotic bacteria. The growing demand for healthy food is stimulating the innovation and development of new products nationally and internationally and hence, the current food industry moved progressively towards the development of dietary supplements with probiotic organisms. The most widely used probiotic strains are lactobacilli, bifidobacterium and streptococci.

Even though the majority of probiotic food is dairy based, currently there is a shift in the trend. Increased awareness about conditions like lactose intolerance, milk protein allergy and saturated fatty acid content of milk resulted in the development of non-dairy based probiotic products. The greatest advantage of these non-dairy based probiotics are that they stay for shorter period in the stomach than the milk and move to the colon at a faster rate. Hence, the probiotic strain exposed to the harsh acidic conditions of stomach for a relatively shorter period, reach the colon where they can grow and multiply easily.

The incorporation of probiotics to the underutilised fruits can improve the acceptability and market potential of the fruit crop. Such products may also have better profile of nutrients, acceptability and therapeutic value.

Jackfruit is one of the major underutilised fruit, shown to have numerous culinary uses and high availability during the season. Despite its potential for value addition, majority of the fruits remains wasted during the season. If a probiotic product is developed from the fruit, it would definitely grab consumer attention and improve the economic value of the fruit.

Hence, the present study entitled “Process optimisation and quality evaluation of jackfruit based probiotic food products” was undertaken with the following objectives

1. To standardise different food mixtures with raw jackfruit flour and yoghurt with ripe jackfruit pulp involving probiotic fermentation with *Lactobacillus acidophilus*
2. To develop an instant shake mix based on the probiotic jackfruit food mixture
3. To evaluate the nutritional and organoleptic qualities as well as the storage stability of the developed products



Review of literature

2. REVIEW OF LITERATURE

The literature review of the present study entitled ‘Process optimisation and quality evaluation of jackfruit based probiotic food products’ is discussed under the following headings.

2.1. Jack: The wonder fruit

2.2. Functional food: The horizon of wellness

2.2.1. Definition of functional food

2.2.2. Classification of functional food

2.2.3. Scope of functional food

2.3. Probiotic as functional food

2.4. Mechanism of probiotic action

2.4.1. Competition for nutrient

2.4.2. Production of antimicrobial substance

2.4.3. Immune modulation

2.4.4. Competition for adherence

2.5. Nutritional benefits of probiotics

2.5.1 Lactose

2.5.2. Protein

2.5.3. Vitamins

2.5.4. Minerals

2.5.5. Antinutritional factors

2.6. Fruit based probiotic products

2.7. Statutory aspects of probiotic food

2.1. Jack: The wonder fruit

Research in recent years has focused on the search for common, underutilized and nutritious crops. Jackfruit (*Artocarpus heterophyllus*) belonging to the family *Moraceae* is one such crop (Ocloo *et al.*, 2010).

There are mainly two varieties of jackfruit *koozha*, which is small, fibrous, soft and mushy with sweet carpels and a texture like that of a raw oyster and *varikka* which is crisp, crunchy and not very sweet. The large seeds of this nonleguminous plant are also edible, even though they are difficult to digest (Siddappa, 1957).

The fruit that once enjoyed the status of a heavenly fruit in ancient periods, now lost its status and is one of the most under exploited fruits in the current state scenario. Kerala contributes 551.47 million tons in total jackfruit production (NHB, 2015) but greater per cent of this is wasted because of the lack of processing units and marketing.

Raw jackfruit is composed of nutritional compounds like vitamins, minerals, antioxidants, folates, phytochemicals, dietary fibres and has relatively low calories (Murcia, 2009). According to Tejpal and Amrita (2016) jackfruit is a health boon to mankind due to its multifaceted medicinal properties like antiasthmatic activity, antioxidant, antifungal, anticancer, antimalarial, antidiarrhoeal, antiarthritic, antiviral, antiatherosclerotic and wound healing effect. The consumption of jackfruit helps to fight against wrinkles and helps in getting a glowing complexion and flawless skin.

Jackfruit is a good source of antioxidants and provide about 13.7 mg/100g of vitamin C. Consumption of foods rich in vitamin C helps the body to develop resistance against infectious agents and scavenge harmful free radicals. It is one of the rare fruits, rich in B complex group of vitamins such as pyridoxine, niacin, riboflavin and folic acid. Fresh fruit is a good source of potassium, magnesium, manganese and iron.

Potassium is an important component of cell and body fluids that helps to control heart rate and blood pressure (Baliga and Bhat, 2001).

Jackfruit is a rich source of magnesium and it helps in the calcium absorption, strengthen the bone and prevent bone related disorders such as osteoporosis. The iron present in jackfruit helps to maintain healthy blood circulation and prevent anaemia (Devi *et al.*, 2004). Chandrika *et al.* (2005) identified the carotenoids present in jackfruit, namely β carotene, α carotene, β zeacarotene, α zeacarotene, di carboxylic carotenoids and crocetin. Carotenoids present in jackfruit, fight against certain diseases, especially cardiovascular diseases and age related macular degeneration. Lignans, isoflavones, and saponins, the main phytonutrients seen in jackfruit have the ability to inhibit the formation of cancer cells in the body, lower blood pressure, fight against stomach ulcers and slow down the degeneration of cells, that makes the skin look young and fresh (Soobrattee *et al.*, 2005).

The phenylflavones present in jackfruit act against lipid peroxidation and have strong antioxidant properties. Jackfruit also contains numerous chemical constituents like artocarpin, isoartocarpin, cycloartocarpin, artocarpanone, artocarpetin, cynomacurin, dihydromorin, cyloartocarpin, morin, oxydihydroartocaepesin and cycloartinone (Rao *et al.*, 1973). The study conducted by Wei *et al.* (2005) showed that flavonoids present in jackfruit have antiinflammatory effect by inhibiting the release of inflammatory mediators from the mast cells, neutrophils and macrophages.

Jackfruit seeds contain two lectins namely jacalin and artocarpin. Jacalin has been proved to be useful for the detection of the immune status of patients infected with human immunodeficiency virus 1 (Samaddar, 2002). Jackfruit seed is an important ingredient in antidote preparation for heavy drinkers to overcome the effect of alcohol (Butool and Butool, 2013). Azeez *et al.* (2015) reported that the essential amino acids, fatty acids and trace amount of sugars present in jackfruit seeds make it a

cheap source of dietary nutrients and health snack for overweight people. Jackfruit seed is considered as fat free food, it is suitable for the patients having life style diseases like diabetes, cardiovascular diseases etc.

Jackfruit conforms to the definition of functional food in several ways, because it has valuable compounds in different parts of the fruit that display functional and medicinal effects. The use of standardised jackfruit products offers consumers, a novel way of reaping the broad spectrum of health benefits of this fruit (Swami *et al.*, 2015).

2.2. Functional foods: The horizon of wellness

The therapeutic benefits of food is an evergreen concept. That's why the age old quote by Hippocrates "*Let food be thy medicine and medicine be thy food*" remained as the tenet of modern man. To some extent, all foods are functional as they provide taste, aroma and nutrients. Several investigations are going on to identify the foods with added physiological benefits, which may reduce chronic disease risk or otherwise optimise health. These investigations and experiments have led to the emergence of new food category now recognized as "functional foods" (Hasler, 1998). The link between diet and disease has been well established now and the concept has been quite widely accepted by organisations as well as individuals (Shi *et al.*, 2002).

There is an ever-increasing trend in the number of consumers who are interested in maintaining the quality of life by using the natural products like functional foods (Ernst and Young, 2009). The functional food concept has become a felt need of the health conscious community and has become a popular choice among them, as functional foods are less expensive, beneficial and a more natural alternative (Rao *et al.*, 2011).

Functional foods and nutraceuticals have emerged as a novel concept during the past decade and have become a major trend in the current, consumer-driven market.

Functional foods act by helping the ageing populations to have greater control over their health by delaying the process of ageing, preventing diseases and enhancing well-being as well as performance. This trend is expected to continue, and hence the scientific information regarding all aspects of functional foods is vital to the advancement of this emerging sector (Howard and Kritchevsky, 2007).

2.2.1. Definition of functional food

Functional foods have been defined by many authors in different fashion. IFIC (1995), defined them as foods that may provide additional health benefits beyond basic nutrition. But Coghlan (1996), defined functional foods as ‘everyday foods transformed into a potential lifesaver by the addition of a magical ingredient’.

Terms like designer foods, medicinal foods, therapeutic foods, super foods, foodiceuticals and medifoods are also used synonymously for functional foods (Berner and O’Donnel, 1998).

Functional foods contains adequate amount of one or a combination of components which affects the functions in the body so as to have positive cellular and physiological effects (Roberfroid, 1998). According to Diplock *et al.* (1999), functional foods can be considered to be one step ahead of healthy natural foods as they assist the therapeutic process of the body towards substitution of medicines.

The explanation given to functional foods by FNB (1994), is ‘food that encompasses potentially helpful products including any modified food or food ingredient that may provide a health benefit beyond that of the traditional nutrient it contains’. According to Hilliam (1995), functional foods are ‘food and drink products derived from naturally occurring substances consumed as part of the daily diet and possessing particular physiological benefits when ingested’.

ADA (1999), defined functional foods as ‘whole, fortified, enriched or enhanced food that should be consumed regularly and at effective amounts in order to derive health benefits’.

Sloan (2000) offered a different definition for functional food as “a food or beverage that imparts a physiological benefit that enhances overall health, helps prevent or treat a disease/condition, or improves physical or mental performance via an added functional ingredient, processing modification, or biotechnology.”

According to Chaturvedi (2001), functional food should be a food derived from natural food ingredients and can be consumed as a part of regular diet. Apart from this, functional foods should be able to enhance body’s natural defense system, prevent the onset of chronic diseases, ensure rapid recovery from specific diseases, control physical and mental stress and altogether slow down the process of ageing.

According to Roberfroid (2002), a food can be considered as functional if it is competently manifested to alter beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way which is admissible to either the state of well-being and health or the reduction of the risk of a disease. A food ingredient can be considered as functional, if it affects its host in a targeted manner so as to exert positive effects.

The functional food group comprise (i) conventional foods with natural bioactive substances (eg: dietary fibre) (ii) foods enriched with bioactive substances (eg: probiotics, antioxidants) and (iii) synthesized food ingredients introduced to traditional foods (eg: prebiotics) (Grajek *et al.*, 2005).

As per the view of Berger and Shenkin (2006), functional/medicinal foods play positive roles in maintaining the wellbeing and thus enhancing health, and prevent specific diseases by modulating the immune system.

FUFOSE (2008), developed a working standard for functional foods and it states that ‘functional food as one that is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and wellbeing and/or reduction of risk of disease’.

The FFC (2011) proposed a new definition for functional food and it defined functional foods as ‘natural or processed foods that contains known or unknown biologically active compounds; which in defined amounts provide a clinically proven and documented health benefit for the prevention, management or treatment of chronic disease’.

IFT (2018) opined that ‘functional food is a typical food that has specific nutrients added to it, like vitamins or minerals, fibre, or probiotics or prebiotics. In general, this includes anything added for a specific functional purpose’.

2.2.2. Scope of functional foods

Functional food science is a new branch that is part of nutrition science, which is aimed at stimulating research and development of functional foods by using a function-driven approach (Bellisle *et al.*, 1998). The scope of functional food is staggering, even then the full spectrum of benefits is yet to be investigated.

The consumer demand is increasing for foods that are not only good from a nutritional perspective, but are health promoting i.e., functional foods or nutraceutical (Senorans *et al.*, 2003). Functional food could be targeted to healthy as well as diseased individuals and the functional foods can take up a crucial role in remodeling our food supply (Haesman and Mallentin, 2014).

The vast investigation on the relationship between food and health has widened the scope of functional foods. The functional food concept has now become one of the

most important area of discussion in the food industry worldwide. Functional food is an effective way to improve health, reduce health care costs and it can support the economic growth in the rural communities (Dilip, 2010).

Wildman and Kelley (2007) were of the opinion that the major reasons for the development of the functional food market are current population and their health trends. People can optimise their own health by the way of diet or through supplementation and by consuming foods that have been fortified in order to include health-enhancing factors. Highsmith (2011) reported that the demand for functional food is growing, especially in the developed economies due to increasing awareness towards the health benefits of functional foods.

Kotilainen *et al.* (2006) reviewed that functional foods have entered the global markets with a considerable force in the past decade and were able to rapidly gain the market share than that of organic foods. Thus, apart from the health benefits, functional foods also holds new, economic opportunities. It should be also considered, that the cost of functional food is higher than the conventional foods and thus provides larger profit margins to the manufacturer, which can make the sector more interesting for those engaged in the functional food supply chain.

The market of functional food is undoubtedly expanding in most countries that have an established processed food market (Arai *et al.*, 2002). According to Jones (2002), increased consumer awareness, the trend of health claim approval of functional foods and the widening spectrum of food-disease scientific database, altogether predict a growth in the functional food sector in the future.

The global market size of functional food was estimated to be approximately US\$ 30 -US\$ 60 billion which holds 1 to 3 per cent of the total food market (Kotilainen *et al.*, 2006). Revenue generated by the functional food and allied sectors for the year 2007 in Canada was approximately \$ 3.7 billion (Cinnamon, 2007).

Functional foods remained the fastest-growing sector of North American nutraceuticals market, with a compound annual growth rate (CAGR) of 6.5 per cent during the year 2007-2011 (Anon., 2012). The global nutraceuticals market that include functional foods, functional beverages and dietary supplements is on the track of continuous growth (Industry ARC, 2013). This growth is being supported by the consumers who are conscious about health and healthy eating. The world's largest functional food market is the United States market with sales of \$ 43.9 billion in 2012, +6.9 per cent over 2011 (NBJ, 2013).

Khan *et al.* (2014) published a report on the value of the global market for functional foods. They reported \$168 billion for the year 2013 and forecasts more than \$300 billion for 2020. This growing market perspective has driven several food manufacturers to invest in the research and development of new functional food products.

Functional foods can play a major role in the fiscal growth of many developing countries blessed with rich biodiversity and traditional knowledge of the health effects of indigenous plant species. According to the Global nutraceutical market (2011), functional foods and functional beverages are relatively nascent markets in India, primarily due to the budding middle class that relies on traditional practices such as Ayurveda. Nielsen (2013) was of the opinion that the functional food market in India is expected to have a moderate growth of 70.74 per cent compared to the dietary supplement market in 2017.

Sharma (2005) argued that, apart from the opportunities for product diversification and value addition, farming for the functional foods industry can benefit the primary producers and rural communities in various ways. Opportunities are also there for innovative dairy beverages targeting the functional food trends.

Verschuren (2002) was of the opinion that, the development and marketing of functional foods demands significant research efforts because most markets search for scientific evidence and the proof for functionality. This research requires time, financing and skilled labour, especially for products intended for export markets. Innovation as well as research capacity is required to screen local biodiversity to uncover potential resources for functional foods (William *et al.*, 2006).

According to William *et al.* (2006) it is necessary to build a trust in the consumer regarding the functional food and for that, a clear regulatory system for the production, sales, certification and advertising of functional foods, together with consistent enforcement are critical. Hence, the development of institutions like food research centres, advisory services for producers, educators in food sector marketing and management and authorities approving health claims for functional foods are essential.

In the era of declining health and elevating healthcare expenditure, novel approaches to healthcare delivery are becoming a necessity. Food, especially “functional food,” can be a solution for this problem. During the past decade the importance of preventive medicine improved drastically. The crucial role of nutrition in the prevention of chronic diseases have become more evident. With the entry of functional food concept, individuals became more aware about the role of diet, not only in sustaining life but also in the prevention and reduction of chronic diseases (Lopez-Varela *et al.*, 2002).

As per the opinion of Martirosyan (2015), the field of functional foods science is a rapidly evolving and is being supported by the scientific communities as well as the food industries.

‘Nutrigenomics’ and ‘nutrigenetics’ the two emerging fields of science that can significantly increase the fundamental knowledge of the interaction between diet and

life processes and which in long run can lead to the development of novel functional foods to improve the health status of general population as well as to prevent the onset of nutrition related diseases in genetically predisposed individuals (Mariman, 2006).

2.2.3. Classification of functional foods

Hasler (1998), classified functional foods as plant based functional foods and functional foods from animal sources according to the nature of origin. Whereas, Arai *et al.* (2001) classified functional foods according to their origin as plant derived functional foods, animal derived functional foods, microbial functional foods and miscellaneous functional foods.

Senorans *et al.* (2003) classified functional food according to their different possibilities in food processing. They have divided the health-promoting functional foods into three different categories: 1) those with specific functionalities 2) foods fortified with natural ingredients able to provide a desired functionality (foods enriched with natural ingredients) and 3) probiotics and prebiotics.

From the product point of view, functional foods have been classified as (1) food fortified with additional nutrients such as fruit juices fortified with vitamin C (2) food with an added nutrient or component which was not normally found in that particular food like probiotics or prebiotics (3) food from which a harmful compound has been removed, reduced or replaced by a beneficial component like fibres as fat releasers in ice creams (4) food in which a nutrient or component has been enhanced by natural means, like omega-3 content enhanced eggs (Sloan, 2000; Kotilainen *et al.*, 2006; Spence, 2006).

Makinen - Aakula (2006) classified functional foods on the basis of their aim. According to the author, functional foods can be classified as (1) functional foods that enhances health or improve children's life, like prebiotics and probiotics, (2) foods that

can reduce an existing health problem such as hypercholesterolemia or hypertension, (3) foods that makes life easier, such as lactose-free or gluten-free products.

Ford and Dahl (2012) classified functional foods into two broad categories. The first category consists of foods with naturally occurring functional groups and the second category consists of processed foods in which a component is added to give an additional health benefit.

According to EUFIC (2019), functional foods can be of two types, based upon the health claim. Type A includes foods with “enhanced function” claim and the type B includes foods with “reduction of disease risk” claim.

2.3. Probiotics as functional foods

The last decade witnessed an increase in the development of food ingredients such as probiotics which promise to improve the gut health and confer benefits beyond nutritional value. There is increasing evidence that the composition and metabolic effects of the gastrointestinal microflora are of key importance for human health (Roberfroid, 2000).

Probiotic is a relatively new word which means “for life” and it is currently being used to name bacteria associated with beneficial effects for humans and animals. The positive role played by some selected bacteria was first studied by Eli Metchnikoff, the Russian born Nobel Prize winner working at the Pasteur Institute. He suggested that, "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (Metchnikoff, 1907).

Credit of the term ‘probiotic’ belongs to Werner Kollath who proposed the term ‘Probiotika’ to designate active substances that are essential for a healthy development of life (Vergin, 1954). The term ‘probiotic’ was derived from the Greek and it was first

used by Lilly and Stillwell (1965) to describe substances produced by one protozoan that stimulated the growth of another. Parker (1974) used the term to describe organisms and substances which contribute to intestinal microflora.

According to Huisint and Shortt, (1996) it is a mono or mixed cultures of live microorganisms which when consumed by man or animal, affects beneficially the host by improving the properties of indigenous microflora. The most recent definition of probiotic was drawn by Schrezenmeir and De Verse (2001). They defined probiotics as viable microbial food supplements which beneficially influence the health of the host. Probiotics can be bacteria, mould or yeast among which Lactobacilli, Streptococci, and Bifidobacteria are commonly used groups and they can be defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (WHO, 2001). Guarner and Malagelada (2003) argued that these microorganisms interact with the diet and the host, contributing to protection against intestinal pathogens through colonization, resistance and providing nutritional and health benefits via their metabolic activities

The functional aspects of probiotic food depends upon the viability and persistence of the probiotic strain in the gastro intestinal tract, immunomodulation, antagonistic activity against pathogens and anti-mutagenic properties (Saarela *et al.*, 2000).

The functional benefits from probiotic foods may also be due to the metabolites produced during fermentation. These bioactive metabolites responsible for the functional benefits are certain vitamins, organic acids and bioactive peptides (Stanton *et al.*, 2005).

According to Tripathy (2014), probiotic foods are considered functional because of the several reported health benefits including the maintenance and balancing of the intestinal microflora and increasing the resistance against invading pathogens.

Foods containing live probiotic organisms confers several health benefits to the consumer as they help in maintaining stability and composition of the intestinal microbiota and thus boost the resistance against pathogens. Hence, probiotics can be included in the class of functional foods, as they offer health benefits more than the conventional foods (Begum *et al.*, 2017).

The property of probiotic microbiota to modulate immunity and to stabilize the microbial balance of commensal enteric microorganisms confers the consumer a potent biological alternative to better health than the consumption of therapeutic drugs (Chin and Kailasapathy, 2000).

2.4. Mechanism of probiotic action

The claimed health benefits of probiotic fermented functional foods are expressed either directly or indirectly. The proposed mechanism may vary according to the bacterial species and strains. The action may be directly through the interaction of ingested live microorganisms and the host or it may be due to the action of ingested microbial metabolites produced during the process of fermentation. Even though not fully understood, following are some of the proposed mechanisms of action of probiotics on host (Wilson and Perini, 1988).

2.4.1. Competition for Nutrients

Probiotics may compete for nutrients, which is otherwise consumed by pathogenic organisms. Consumption of monosaccharides by probiotics may reduce the growth of *Clostridium difficile*, which is dependent on monosaccharides for growth. *In vitro* studies revealed that the gut microorganisms compete more efficiently for the monomeric glucose N-acetyl-glucosamine and sialic acid than *C. difficile* (Wilson and Perini, 1988).

Elli *et al.* (2000) reported that the probiotic strain *Lactobacillus delbrueckii* can bind iron to its cellular surface as iron hydroxide making it unavailable to other microbes and thus affecting the growth of pathogenic microbes.

The action of probiotic *E.coli* (EcN) was explained by Grozdano *et al.* (2004) that they will secrete siderophores to chelate the ferric or ferrous forms of iron and expresses the iron uptake system to transport iron to the probiotic bacterial cell. This probiotic *E.coli* (EcN) can compete for iron very effectively because it effectively decodes seven different iron uptake systems.

2.4.2. Production of antimicrobial substances

One of the proposed mechanism of action involved in the health benefits claimed by probiotic microorganisms comprise the formation of low molecular weight compounds like organic acids and the production of antibacterial substances termed as bacteriocins (Bermudez-Brito *et al.*, 2012).

Organic acids, particularly acetic acid and lactic acid, produced by probiotic bacteria were found to have a strong inhibitory effect against gram negative bacteria. These organic acids were considered as the main antimicrobial compounds responsible for the inhibitory activity of probiotics against pathogens (Alakomi *et al.*, 2000).

Lievin *et al.* (2000) reported the production of a potential low molecular weight lipophilic molecule by two bifidobacterium strains. These lipophilic molecules were found to be effective against several pathogens including *Salmonella enterica ser. typhimurium* SL 1344 and *E. coli* C 1845.

Toure *et al.* (2003) conducted a study to examine the production of antibacterial substances by the bifidobacterial isolates against *Listeria monocytogens* and they reported that the bifido bacterial isolates from infant stool, produced heat stable proteinaceous substances which was active against *L. monocytogens*.

Probiotics prevent epithelial invasion either by inducing host cells to produce peptides or by directly releasing peptides that interfere with pathogens. Defensins (hBD protein) and cathelicidins are the antimicrobial peptides expressing antimicrobial activity against a wide variety of bacteria, fungi and some viruses. Certain probiotic strains like *E. coli* strain DSM 17252 G2 and several Lactobacilli species have shown to express certain defensins (host defense peptides). Healthy volunteers who received probiotics had increased fecal hBD protein and remained elevated for 9 weeks after completion of 3 weeks of probiotic treatment (Mondel *et al.*, 2000).

Saulnier *et al.* (2009) reported that the probiotic lactic acid bacteria *Lactobacillus reuteri* is capable of producing reutrin, an antimicrobial substance which has got broad spectrum activity against bacteria, fungi, protozoa and viruses.

Oelschlaeger (2010) reported that the probiotic bacteria are cable of producing deconjugated bile acids, which are derived from bile salts. These de conjugated bile acids exhibits a stronger antimicrobial activity compared to that of the bile salts synthesised by the host organism.

2.4.3. Immune modulation

An enhancement in the non-specific immune phagocytic activity of granulocytes were observed by Schiffrin *et al.* (1995) when the blood samples of volunteers were checked after the regular consumption of *L.acidophilus* and *B.bifidum*.

Perdigon *et al.* (1995) observed that probiotic bacteria can enhance the immune response of the host. The enhancement of immune response may be attributed to the increased secretion of immunoglobulin A (Ig A), increased count of natural killer cells, or the enhanced phagocytic activity of macrophages (Link- Amster *et al.*, 1994; Schiffrin *et al.*, 1995; Hawrelak, 2003).

Solis and Lemonnier (1996), documented the stimulated cytokine production in blood cells followed by the probiotic yoghurt ingestion. Gill (1998) opined that the lactic acid bacteria exerts their immunity enhancing activity by augmenting both specific and non-specific immune responses in the host.

Fuller and Gibson (1997), opined that the increased production of IgA will result in the reduction of pathogens in the gastro intestinal tract which inturn maintain the composition of healthy micro flora in the gut.

L. casei have been shown to augment total and pathogen specific secretory IgA levels upon infection in mice by stimulating B cell class switching to IgA. But the specific antibodies against *L. casei* were not produced, which shows the non-responsiveness of the gut immune system to this beneficial bacterium (Galdeano and Perdigon 2006).

In infant rabbits pretreated with *L. casei*, morbidity of subsequent EHEC (Enterohemorrhagic *E. coli*) infection was reduced due to increased mucosal levels of anti-EHEC and anti-Shiga toxin IgA antibodies compared with that of controls (Ogawa *et al.*, 2007).

2.4.4. Competition for adherence

Lee *et al.* (2003) reported that lactobacilli strains can directly compete with other pathogens, such as Salmonella species, for the adhesion sites on human mucins or Caco-2 cell surfaces. These lactobacilli can also displace pathogens which were bound to the epithelium even though at a lesser extent at slow pace.

Major action of probiotics involves the enhancement of epithelial barrier by increased adhesion to intestinal mucosa, concomitant inhibition of pathogen adhesion resulting in the competitive exclusion of pathogenic microbes (Bermudez-Brito *et al.*, 2012).

Probiotic bacteria are capable of competing with invading pathogens for binding sites of epithelial cells and the overlying mucus layer in a strain-specific manner. The surface layer proteins purified from the strain *L. helveticus* R 0052 inhibited the adhesion of entero hemorrhagic *Escherichia coli* O157:H7 and the subsequent rise in its permeability, without altering the growth of the pathogen (Johnson-Henry *et al.*, 2007). Wu *et al.* (2008) reported that *S. boulardii* secretes a heat labile factor that becomes inactivated at higher temperatures and these factors has been shown to be responsible for the decreased adherence of pathogenic bacteria.

2.5. Nutritional benefits of probiotics

Probiotic bacteria are capable of producing specific beneficial compounds in foods. These functional ingredients are sometimes referred to as ‘nutraceuticals’, a term that was introduced by De Felice (1986). These ingredients can be macronutrients, micronutrients (such as vitamins) or non-nutritive compounds. The proper selection and exploitation of micro-organisms is an interesting strategy to produce novel foods with increased nutritional and/or health-promoting properties (Hugenholtz and Smid 2002).

2.5.1. Lactose

Dairy products containing probiotic cultures shows increased digestibility of lactose due to the intra-intestinal digestion of lactose by β d galactosidase released from the cultures (Saviano *et al.*, 1984). The lactose content of fermented milk is reduced by 25 to 50 per cent during the process of fermentation (Mc Donough *et al.*, 1987).

Yoghurt intake was found to improve the milk tolerance among individuals with cow milk allergy (Kolars *et al.*, 1984). Similarly, Onwulata *et al.* (1989) reported the beneficial effects of acidophilus milk in alleviating the symptoms of lactose malabsorption. The beneficial effects of yoghurts on the management of lactose

intolerance is due to the presence of lactic acid bacteria in yoghurts, which in turn increase the lactase activity in small intestine (Marteau *et al.*, 1990; Pelletier., 2001).

The study conducted by Martini *et al.* (1991) suggested that the consumption of yoghurts will improve lactose digestion. To evaluate the ability of different lactic acid bacteria to digest lactose, yoghurts and fermented milks containing the individual species of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were fed to healthy individuals who cannot digest lactose. Yoghurts shows complete lactose digestion.

The bacterial cultures, *S. thermophilus*, *L.bulgaricus* and other lactobacilli present in the fermented milk products are capable of alleviating the symptoms of lactose malabsorption by providing bacterial lactose to the intestine and stomach (Dairy Council of California, 2000).

The improved lactose tolerance of yoghurts containing the active cultures is due to the inherent beta galactosidase activity of yoghurt cultures, which can act upon and hydrolyse a part of ingested lactose (Kotz *et al.*, 1994). Certain functional foods containing probiotic provide preformed lactase to gut and allow better digestion of lactose (Nagpal *et al.*, 2012).

2.5.2. Protein

Zamora and Fields (1979) opined that the increased protein content during fermentation can be attributed to the microbial synthesis of protein during the life cycle. They synthesis protein from metabolic intermediates. But Oboh and Akindahusi (2003) came up with a different opinion. According to them, the secretion of some extracellular enzymes (like amylases and cellulases) into the food mixtures may be the possible reason behind this.

Lactic acid bacteria (LAB) produce a range of secondary metabolites during fermentation which is associated with health promoting properties. The most important among these are the B vitamins and bio active peptides (Stanton *et al.*, 2005). Wang (2007) in his study reported an increase in the crude protein content in the peanut flour when fermented with *Lactobacillus plantarum* p 9. Sylva *et al.* (2008) conducted a study among preschool children where the students were fed with iron fortified milk supplemented with *L. acidophilus*. The study concluded that the children with the probiotic supplementation have higher RBC status than the control group.

In a study conducted by Onimawo *et al.* (2003), they reported that probiotic fermentation of pumpkin seeds causes increase in the protein content of pumpkin seeds from 28.0 per cent to 39.4 per cent. Kee-Jong *et al.* (2004) evaluated the effect of probiotic fermentation using *Aspergillus oryzae* GB 107 on the nutritional quality of food grade soya beans. The study confirmed that the process of fermentation increased the protein content, eliminated trypsin inhibitors and reduced the peptide size in the beans.

A significant increase ($p < 0.05$) in protein content was reported by Oboh (2003) when cassava peels were fermented with *Lactobacillus delbruckii*, *Lactobacillus coryneformis* and *Saccharomyces cerevisiae*. Similar result was reported by Sharon (2010) who stated that there is a significantly higher ($p < 0.05$) level of protein content in probiotic fermented banana based food mixture compared to the unfermented control sample.

In vitro digestibility of protein increases with probiotic fermentation and it is evident from several studies. The improvement in protein digestibility is due to the proteolytic activity of the fermenting organism (Hesseltine, 1983). According to Chavan *et al.* (1988), during fermentation the degradation of complex storage proteins

takes place and this can be attributed to the increased protein digestibility of the probiotic products.

Rani and Khetarpaul (1999) reported an improvement of *in vitro* digestibility of protein due to the process of fermentation. Fifty per cent increase in the protein digestibility of food mixture containing barley flour, milk co precipitate, sprouted green gram paste and tomato pulp was reported by Sindhu and Khetarpaul (2001) when fermented with *Streptococcus Boulardi* and *Lactobacillus cassei*. A similar result was reported by Sharon (2010) where the *in vitro* protein digestibility of unfermented food mixtures shown an increase from 57.15 per cent to 85.41 per cent when fermented with *L.acidophilus* for 24 hours.

2.5.3. Vitamins

Many enzymes of human body requires B complex vitamins as their co enzymes for the proper functioning. Probiotic bacteria like bifidobacteria are able to produce some of the B group vitamins like B₁, B₆, B₁₂ and folic acid (Deguchi and Morishita, 1985). The *L. acidophilus* can also inhibit the growth thiamine (vitamin B₁) decomposing bacteria (Honma and Ohtani, 1987).

The action of probiotic microbes present in the food has shown to improve the quantity, availability and digestibility of certain nutrients. Probiotic fermentation has proved to improve the folic acid content in yoghurt, bifidus milk and kefir (Shahani and Chandran, 1979). Keuth and Bisping (1993) opined that the elevated levels of B group vitamins in *tempeh* (fermented soya product, popular in Japan) is due to the microbial biosynthesis of the above said vitamins.

In a study published by Crittenden *et al.* (2003) it was found that a combination of two lactic acid bacteria *Streptococcus thermophilus* and *Bifidobacterium animalis* increased the folic acid levels by six fold. The study also revealed that *Lactococcus*

lactis and *Leuconostoc* spp. were capable of producing folate. Sybesma *et al.* (2003) screened a variety of lactic acid bacteria for their ability to produce folate.

The concentration of thiamine in milk was found to be improved by 11 per cent on fermentation with *Bifidobacterium longum* for 48 hours (Zhou *et al.*, 2000). An increase in niacin, riboflavin and thiamine content was recorded by Sunny *et al.* (2004). They obtained an imitation milk from groundnut seeds and prepared a yoghurt like product using a culture pack with the strains *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

In a clinical trial, Fabian *et al.* (2008) reported increased levels of plasma free riboflavin in healthy women volunteers after the consumption of probiotic yoghurt (200 g) for a period of two weeks. The riboflavin levels come to initial levels when the intake of yoghurts were stopped. Champagne *et al.* (2010) reported a slight increase in the thiamine and pyridoxine concentrations in soy as a result of fermentation with the probiotic strains *Streptococcus thermophilus* ST 5 and *Lactobacillus helveticus* R 0052. Fermentation of soy milk with various lactic acid bacteria viz. *Lactobacillus acidophilus* B 4496, *Lactobacillus bulgaricus* CFR 2028, *Lactobacillus casei* B 1922, *Lactobacillus plantarum* B 4495 and *Lactobacillus fermentum* B4655 resulted in an increase in the riboflavin and niacin content of the fermented soymilk than the unfermented sample (Rekha and Vijayalakshmi, 2010).

Jayashree *et al.* (2010) screened for riboflavin producing strains from different fermented milk products of Vellore region of India. In the study, a single strain (*Lactobacillus fermentum* MTCC 8711) was identified as an efficient riboflavin producing strain. The specified strain produced 2.29 mg/l of riboflavin after 24 hrs of growth in the chemically defined medium. The authors put forward the possibility of exploiting this strain for the enhanced production of riboflavin in the fermented food industry. The probiotic cashew apple juice was developed by Kaprasob *et al.* (2018)

with the probiotic strains *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides* and *Bifidobacterium longum*. The fermentation with *L.acidophilus* and *L.casei* increased the B vitamins of cashew apple juice by 19.25 per cent and 23.11 per cent respectively.

In a study Morishita *et al.* (1999) examined the ability of lactic acid bacteria to produce vitamin K₁ and K₂. The strains *Lactococcus lactis* ssp *cremoris*, *Lactococcus lactis* ssp *lactis* and *Leuconostoc lactis* were found to be the higher producers of quinones and they synthesized more than 230 nmol/g quinones of dried cells.

2.5.4. Minerals

Fermentation has been found to increase the bioavailability of all minerals in different plant foods. This is because fermentation converts the bound form minerals to free form and thus increase the availability (Khetarpaul and Chauhan, 1990).

Ghanem *et al.* (2004) reported that the consumption of probiotic yoghurt containing the strains *Lactobacillus casei*, *Lactobacillus ruteri* and *Lactobacillus gasseri* increased calcium absorption in experimental rats. The probiotic culture also increased the bone mineral content (BMC) of the rats. The effect of *L. helvictus* was examined by Narva *et al.* (2004). They noticed that the serum ionised calcium, total calcium, urinary calcium and phosphate levels were higher in women consuming the milk fermented with *L.helveticus* than the control group. Jood and Khetarpaul (2005) also stated that reduction in antinutrients due to fermentation may increase the bioavailability of various minerals but there need not to be any change in the total mineral content in fermented foods.

In a study published by Aljewicz *et al.* (2014) it was reported that the use of probiotic cultures significantly increased the availability of calcium (2.5%), phosphorus (6 %) and magnesium (18 %). The study was conducted on Dutch type

cheese fermented by the probiotic cultures *Lactobacillus rhamnosus* HN 001, *Lactobacillus paracasei* LPC 37, and *Lactobacillus acidophilus* NCFM. Even though there was no increase in the mineral content due to fermentation, the availability showed an increase.

Fermentation of soy milk with various lactic acid bacteria viz. *Lactobacillus acidophilus* B 4496, *Lactobacillus bulgaricus* CFR 2028, *Lactobacillus casei* B 1922, *Lactobacillus plantarum* B 4495 and *Lactobacillus fermentum* B 4655 resulted in an increase in the calcium as well as magnesium levels (Rekha and Vijayalakshmi, 2010).

Hoppe and Larsen (2008) tested the effect of probiotic fermentation on bioavailability of non-haem iron. The study concluded that the mean iron absorption from the fruit drink containing 10^9 cfu/ml of the probiotic strain *Lactobacillus plantarum* 299 v was significantly higher than the control drink. Fermented almond milk was developed by Bernat *et al.* (2015) using the probiotic strains *B. bifidum*, *B. longum* and *L. rhamnosus*. It was reported by them that the process of probiotic fermentation enhanced the uptake of iron by cells and thus improve the bioactivity of developed product.

2.4.5. Antinutritional factors

The diminishing effect of fermentation on phytic acid and polyphenol may be due to the activity of enzymes like phytase and polyphenol oxidase present in fermenting microflora (Lopez *et al.*, 1983). Many fruits and vegetables contain toxins and antinutritional factors naturally (Drewnowski and Carmen, 2000). But probiotic bacteria can remove or reduce these antinutritional compounds through the fermentation process. The diminishing effect of fermentation on phytic acid and polyphenol may be due to the activity of enzymes like phytase and polyphenol oxidase present in fermenting microflora (Lopez *et al.*, 1983).

During the fermentation of coarsely ground dehulled black gram dhal slurry at various temperatures of 25, 30 and 35°C for 12 and 18 hours, Yadav and Khetarpaul (1994) reported a drastic reduction in phytic acid content of the slurry. The phytic acid content of unfermented legume slurry (1000 mg/100 g) was reduced to almost half in the product when fermented at 35°C for 18 h. Ene-obong and Obizoba (1996) also reported a significant reduction in the phytic acid content of African yam bean during fermentation. Rani and Khetarpaul (1997) developed an indigenous food mixture containing husk less barley flour, green gram *dhal* flour, dried skimmed milk and tomato pulp. This was then autoclaved, cooled and fermented with *L. acidophilus* at 37°C for 24 h. The probiotic fermentation resulted in the reduction of phytic acid content of the food mixture from 220.20 to 81.48 mg per 100g.

Rani and Khetarpaul (1999) developed an indigenous nutritious food mixture containing rice, defatted soya flour, skimmed milk powder and tomato pulp in 2:1:1:1 proportion (w/w). Probiotic fermentation was carried out with *L.acidophilus* (10^5 cells/ml) and fermented at 37°C for 24h after autoclaving. A significant ($P<0.05$) reduction in the contents of phytic acid and polyphenols was reported due to the cumulative effect of autoclaving and fermentation to the extent of 63 and 19 per cent respectively.

Jood and Khetarpaul (2005) stated that, due to the reduction in the antinutrient contents during fermentation, there may be increase in the bioavailability of nutrients in fermented food. It was observed by Kostinek *et al.* (2005) that the cyanogen level of cassava roots were reduced drastically when it was fermented by a mixed population of yeasts and lactic acid bacteria.

Lactic acid bacteria was found to reduce the toxic substances of African locust beans and leaves of *Cassava obtusifolia* during the preparation of a Sudanese food called *kawal* (Dirar, 1993). Tamang *et al.* (2016) reported that the lactic acid bacteria

isolated from ethnic fermented vegetables of Himalayas has the capacity to degrade antinutritive factors.

Chaudhary (1998) fermented two different food mixtures fermented with *L. acidophilus*, *L. fermentum* and *B. bifidum*. The fermentation resulted in a decrease in polyphenol content of the food mixtures to the extent of 24 per cent and it was also reported that fermentation with *L. acidophilus* and *B. bifidum* brought about the maximum reduction. In another study conducted by Rani and Khetarpaul (1999), an indigenous nutritious cereal legume based food mixture fermented with *L. acidophilus* was developed and the polyphenol content was found to be reduced to the extent of 19 per cent by this fermentation process.

The study done by Sindhu and Khetarpaul (2001) reported a drastic reduction in the phytic acid, trypsin inhibitor activity and polyphenol content of indigenously developed BCGT food mixture when it was subjected to probiotic fermentation. Two types of fermentations were carried out, single culture fermentation [*L. casei*, *L. plantarum* (37 °C, 24 h)] and sequential culture fermentation [*S. boulardii* (25 °C, 24 h) + *L. casei* (37 °C, 24 h); *S. boulardii* (25 °C, 24 h) + *L. plantarum* (37 °C, 24 h)].

Rani *et al.* (2016) developed an indigenous WPMT food mixture containing wheat, pigeon pea, skim milk powder and tomato pulp in 2:1:1:1 proportion (w/w). The food mixture was fermented with probiotic *L. acidophilus* (10^5 cells/ml) at 37 °C for 24 h. This process markedly reduced the phytic acid and polyphenol content of the food mixture.

Two indigenous fermented food mixtures were developed by Arora *et al.*, (2008) by mixing raw and germinated pearl millet flour, whey powder and tomato pulp in the ratio 2:1:1(w/w) and fermented with *Lactobacillus acidophilus* curd (5 %) which supplied 10^6 cells/ml to the slurry at 37 °C for 12 h. A significant ($P < 0.05$) reduction

in the contents of phytic acid and polyphenols was noticed by the authors due to the cumulative effect of germination, autoclaving and fermentation.

2.6. Fruit based probiotic products

The changing life style and health consciousness of customer had led to the necessity of non-dairy based probiotic food products. The demand for such products are increasing drastically. The wide spectrum of fruits and vegetables and the large number of lactic acid bacteria provide the probiotic industry with new challenges and opportunities of developing and commercialising value added non-dairy fermented probiotic beverages. Several tropical fruits are widely used as substrates for the fermentation by different strains of lactic acid bacteria (Pangahal *et al.*, 2015).

According to Tuorila and Cardellor (2002) fruit juices are an ideal medium for the functional health ingredients because of the beneficial nutrients and taste profiles within them are pleasing to all age groups and perceived as healthy and refreshing.

Heenan *et al.*, (2004) reported that current industrial probiotic foods are basically dairy products, which may represent inconveniences due to their lactose and cholesterol content. The wide varieties of fruits and vegetables as well as the large spectrum of probiotic strains provide new scopes and for the development and commercialization of value added non-dairy based probiotic foods/beverages. The survival of probiotic strains in these non-dairy food matrix is a matter of concern which inturn depend upon factors such as nutrients, pH, temperature and the presence of inhibitors of food matrix. Several tropical fruit juices widely used as substrates for the fermentation by different strains of LAB.

Sindhu and Khetarpaul (2001) developed indigenous probiotic food mixture by mixing rice, whey, green gram and tomato in the ratio of 2:1:1:1 (w/w). The developed food (100 g) was mixed with water (500 ml) and the homogenous slurry was autoclaved

and fermented sequentially using *L. casei*, *L. plantarum* and *S. boulardii*. All the fermented and lyophilized food mixtures were found to be organoleptically acceptable to human palate and maintained adequate cell viability.

Betoret *et al.* (2003) developed vacuum impregnated probiotic enriched dried fruits. The apple cylinders were impregnated with apple juice containing 10^7 or 10^8 cfu/ml *Lactobacillus casei*. The dried product was stored for two months at room temperature and it was observed that at the end of storage viable count of the product was greater than 10^6 cfu/g which was similar to the commercial dairy products.

Yoon *et al.* (2004) determined the suitability of the tomato juice as a raw material for the production of probiotic juice by *Lactobacillus acidophilus*, *L. plantarum*, *L. casei* and *L. delbrueckii*. The viable cell counts of the four lactic acid bacteria in the fermented tomato juice ranged from 10^6 to 10^8 cfu/ml after 4 weeks of cold storage at 4 °C.

Yoon *et al.* (2005) also evaluated the potential of red beets as the substrate for the production of probiotic beet juice by different four species of lactic acid bacteria, the viable cell counts of these bacteria, except for *L. acidophilus*, in the fermented beet juice still remained at 10^6 - 10^8 cfu/ml after 4 weeks of cold storage at 4 °C.

Vijayalakshmi (2005) prepared yoghurt like product with the probiotic culture *Lactobacillus acidophilus* NCDC 14. The product was prepared by incorporating various cereal flours (rice, wheat, corn and oats) and different fruit pulps (mango, banana, apple and sapota). Of all flours, corn flour and among the fruits, mango was selected based on the sensory as well as structural properties.

A probiotic dairy beverage consisting of *B. bifidum* was incorporated with tomato juice, carrot concentrate or pureed pumpkin, straw berries, black mulberries or

red grapes was prepared by Salem *et al.* (2006). Sensory analysis of the developed product revealed that the beverage had an acceptable flavour.

Calvo *et al.* (2007) developed the lactic acid fermented beverage (US patent 4855147) by lactic acid fermentation of tomato juice. The US patent described the production of lactic acid fermented tomato beverage as tomato juice with the sugar content of 5.4 per cent and a pH of 5.2 heated to 110 °C for sterilization and then cooled to 35 °C and then fermented with 24 hour old culture of *L. acidophilus*.

Ding and Shah (2008) investigated the survival of probiotic bacteria on orange and apple juices. The fruit juices with various species of lactobacillus were found to maintain the live bacteria upto five weeks on refrigerated storage.

Hatanaka *et al.* (2008) published a patent on tomato juice containing alcoholic drink and the method of production of the same. The inventors reported that the alcoholic drink contains lactic acid fermented clear tomato juice having one per cent alcohol content with stabilised lycopene.

The sensory characteristics of grape and orange juice with microencapsulated beads of the probiotic strain *Lactobacillus casei* 01 (Chrs. Hansen) were analysed by Krasaekoopt and Kitsawad (2010) among the consumers of Thailand. The study revealed the existence of a potential market for probiotic product as 86 per cent of the participants were willing to purchase the product.

Probiotic tomato juice was developed by Koh *et al.* (2010) using the strains *Bifidobacterium breve*, *B.longum* and *B.infantis*. They added fructo oligosaccharide (FOS) prior to fermentation to the fruit juice as a source of sugar. The study reported that the addition of FOS increased the taste of the juice during fermentation and suggest the probiotic tomato juice as an alternative for the dairy based probiotic drinks.

Pereira *et al.* (2011) developed probiotic fermented cashew apple juice with the probiotic strain *Lactobacillus casei* NRRL B 442. The product was stored under refrigerated condition for a period of 42 days and the probiotic strain was found viable throughout the storage period.

Mousavi *et al.* (2011) examined the efficacy of pomegranate juice as a substrate for the growth probiotic strains. They developed probiotic fermented pomegranate juice with *L. plantarum*, *L. delbruekii*, *L. paracasei* and *L. acidophilus*. The product was able to maintain probiotic viability upto two weeks under the refrigerated condition. Thakur and Sharma (2017) developed a probiotic pomegranate beverage and analysed its physico chemical as well as microbial characters. The product showed better physico chemical properties than the control and the probiotic viability was found to be 6.5×10^6 cfu/ml.

Nagpal *et al.* (2012) fermented grape, orange and tomato juice with *L. plantarum* and *L. acidophilus* and observed that the two strains were not only able to survive in the fruit juice matrices but also utilise the fruit juices for their cell synthesis. This was evident from a decrease in fruit sugar content and increase in pH as well as acidity of the fruit juices after fermentation.

Pineapple juice based probiotic beverage was formulated by Shukla *et al.* (2013) by incorporating pineapple juice with whey in 35:65 proportion. Fermentation of the mixture with *Lactobacillus acidophilus* for five hours resulted in a probiotic beverage with good sensory scores and probiotic viability of more than 10^8 cfu/ml.

Probiotic chocolate was prepared by Panda (2014) using bael fruit powder. *Lactobacillus sporogens* was the probiotic strain used. The probiotic organisms were microencapsulated and added to the milk based and water based chocolate. The milk based was found to be more acceptable because it had a milder flavour.

Silva and Ferrari (2016) conducted a study to develop a probiotic grape juice with *Lactobacillus paracasei* and to evaluate microorganism viability during storage.

The beverage was kept at 4 °C for 28 days and cell viability, pH and Brix were monitored. Cell viability reduced about 3 logarithmic cycles in 14 days and remained about 6 log cfu/ml until the end of storage. The authors concluded that grape juice is a promising matrix for the production of a beverage with probiotic *L. paracasei*.

Tomato juice incorporated probiotic yoghurts were prepared by Kaur *et al.*, (2016) using three strains of lactobacilli namely *L. acidophilus*, *L. plantarum* and *L. casei*. It was found in present study that all the cultures were able to survive in the fermented juices with high acidity and low pH; therefore and hence, the authors advocated that fruit juices could be exploited as a carrier/ medium for the fermentation and delivery of probiotic lactic acid bacteria, and these probiotic fortified fruit products could be used as a functional healthy supplement for the people, especially for those who are allergic or intolerant to milk based products.

Probiotic fermented prickly pears (*Opuntia sp.*) juice using the strain *Lactobacillus fermentum* ATCC 9338 was developed by Panda *et al.* (2017). The developed product was highly acceptable among judges and got a mean score of 7.5 for the overall acceptance in a nine point hedonic scale.

Campanella *et al.* (2017) examined the efficacy of grape marc (pomace) as a substrate for the fermentation of probiotic lactic acid and Bifidobacteria fermentation. They concluded that the grape marc is a suitable substrate for the probiotic fermentation and there was an increase in the antioxidant activity of the fermented grape marc than the unfermented one. Probiotic fermented blueberry juice was developed by Oh *et al.* (2017) using two probiotic strains such as *Bacillus amyloliquefaciens* and *Lactobacillus brevis*. They found out that the fermented products had augmented antimicrobial and antioxidant activity than the control juices.

Takur and Sharma (2017) developed a probiotic pomegranate beverage by inoculating a mixed culture of 10 per cent *L. bulgaricus* and *L. plantarum* (1:1) to pomegranate juice and fermented for 7 hours. The microbiological analysis of developed probiotic beverage showed that prepared beverage contained optimum level of cultures i.e. 6.5×10^9 cfu/ml and was free from any traces of yeast, mold and coliform bacteria.

Wiejemanna and Ravindra (2018) conducted a study to develop a fruit based probiotic drink by the incorporation of amla with probiotic bacterial strain *Lactobacillus acidophilus* MTCC 10307. They concluded that it is possible to develop a probiotic drink with amla fruit with optimum cell count and good sensory properties and can be stored for a period of 12 days.

Probiotic fruit yoghurts were prepared by Meenakshi *et al.* (2018) with banana, sapota and papaya pulps. The probiotic strains used were *Lactobacillus plantarum* NCDC 25, *Lactobacillus casei* NCDC 298 and *Lactobacillus rhamnosus* NCDC 19. Yoghurts with the probiotic strain *Lactobacillus casei* NCDC 298 and incorporation of 10 per cent fruit pulp scored maximum in the sensory evaluation. At the end of 14 days storage period, banana yoghurt was found to contain maximum number of probiotic bacteria.

2.7. Statutory aspects of probiotic foods

The regulatory requirements of probiotics may vary depending upon the intended use of probiotic. If intended to use as a drug, it must satisfy the regulatory guidelines as a drug, similar to that of any other new therapeutic agent and if intended as a dietary supplement, it comes under the category of “foods,” regulated by FDA’s Center for Food Safety and Applied Nutrition (Venugopalan *et al.*, 2010).

The regulations regarding probiotic as a food component may vary from country to country. The Working Group of WHO-FAO (2006) recommends that genus, species and strain designation should be labelled and the strain designation should not mislead consumers about the functionality of the strain. The working group also suggest to mention the minimum viable numbers of each probiotic strain at the end of the shelf-life, the suggested serving size, proposed health claim(s), storage conditions and corporate contact details for consumer information.

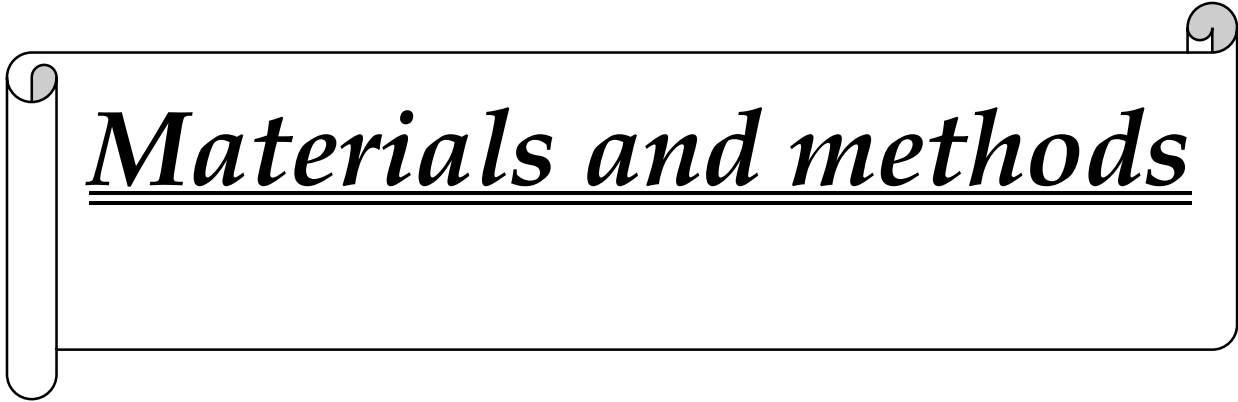
Along with the Department of Biotechnology of the Ministry of Science and Technology, Indian Council of Medical Research proposed the guidelines for evaluation of probiotics in foods in India. These guidelines articulates the base for law governing probiotics in India (Gokhale and Nadkarni, 2007). For any strain or food to be termed as probiotic for marketing in India, it must satisfy the set of guidelines put forward by the ICMR taskforce (ICMR, 2011).

According to FSSAI (2016) a food with probiotic ingredients is any ‘food with live microorganisms beneficial to human health, which when ingested in adequate numbers as single strain or a combination of cultures, confers one or more specified or demonstrated health benefits in human beings’.

As per the FSSAI (2016) regulations, the mandatory regulations for a manufacturer to market a probiotic food in Indian market are (i) the probiotic culture must be approved by the FSSAI from time to time, (ii) the viable number of probiotic organisms in the food should be $\geq 10^8$ cfu/g, (iii) the labelling, presentation or advertisement of probiotic foods shall not claim that it has the property of preventing, curing or treating any human diseases.

FSSAI (2016) also regulate that every probiotic package shall carry the information on the label like (i)“PROBIOTIC FOODS”, (ii) ‘NOT FOR MEDICINAL

USE', (iii) warning or precautions to be taken while consuming the food and (iv) other information like side effects if any, contraindications and product drug interactions.



Materials and methods

3. MATERIALS AND METHODS

The various materials and methods which were used for the thesis entitled 'Process optimisation and quality evaluation of jackfruit based probiotic food products' are discussed under the following heads.

3.1. Collection of raw materials

3.2. Standardisation of food mixtures

3.2.1. Standardising the combination of ingredients in the food mixture

3.2.2. Acceptability of the prepared food mixtures

3.3. Optimisation of conditions for the growth of *L. acidophilus*

3.3.1. Optimisation of substrate concentration

3.3.2. Optimisation of time of incubation

3.3.3. Optimisation of pH

3.3.4. Optimisation of temperature

3.3.5. Optimisation of population of inoculum concentration

3.4. Development of food mixtures

3.4.1. Development of autoclaved fermented food mixtures

3.4.2. Development of autoclaved unfermented food mixture

3.5. Storage studies of the developed food mixtures

3.5.1. Physico-chemical constituents of the food mixture

3.5.2. *In vitro* starch digestibility of food mixtures

3.5.3. *In vitro* protein digestibility of food mixtures

3.5.4. Organoleptic evaluation of the food mixtures

3.5.5. Enumeration of population of *L. acidophilus* in the fermented food mixture

- 3.5.6. Microbial enumeration of total micro flora and insect infestation
- 3.5.7. Temperature and relative humidity
- 3.6. Analysis of glycemic index of the selected food mixtures
- 3.7. Standardisation of instant shake mix from the selected food mixtures
 - 3.7.1. Standardising the combination of ingredients in the instant shake mixes
 - 3.7.2. Acceptability of the prepared instant shake mixes
- 3.8. Storage studies of the developed instant shake mixes
- 3.9. Standardisation of proportion of ingredients in jackfruit yoghurt
 - 3.9.1. Standardising the combination of ingredients in jackfruit yoghurt
 - 3.9.2. Acceptability of the prepared jackfruit yoghurt
- 3.10. Optimisation of conditions for the growth of *L. acidophilus* in yoghurt
 - 3.10.1. Optimisation of substrate concentration
 - 3.10.2. Optimisation of time of incubation
 - 3.10.3. Optimisation of pH
 - 3.10.4. Optimisation of temperature
 - 3.10.5 Optimisation of population of inoculum concentration
- 3.11. Development of jackfruit based bio-yoghurt
- 3.12. Storage studies of jackfruit based bio-yoghurts
 - 3.12.1. Physico-chemical constituents of the food mixture
 - 3.12.2. Organoleptic evaluation of the bio-yoghurts
 - 3.12.3. Enumeration of population of *L. acidophilus* in the bio yoghurts
 - 3.12.4. Microbial enumeration of total micro flora and other insect infestation
- 3.13. Statistical analysis

3.1. Collection of raw materials

Raw as well as ripe jackfruit (both *varikka* and *koozha* type) and seeds were collected from the local households. Raw jackfruit flour and jackfruit seed flour were prepared as per the standard procedures (Pandeay and Ukkuru, 2005 and Kumari *et al.*, 2015).

Tomato, papaya, defatted soya, skimmed milk, sugar and other ingredients needed for the study were purchased from the local market.

The cultures *L. acidophilus*, *L. bulgaricus* and *Streptococcus thermophilus* were needed for the study. Pure cultures of the probiotic strain *L. acidophilus* MTCC 10307 was obtained from Institute of Microbial Technology (IMTECH) Chandigarh. The yoghurt culture was purchased from the Department of Dairy Microbiology, College of Dairy Science and Technology, Kerala Veterinary and Animal Science University, Mannuthy, and the cultures *L. bulgaricus* and *S. thermophilus* were isolated and maintained separately.

3.2. Standardisation of proportion of ingredients in the food mixture

3.2.1. Standardising the combination of ingredients in the food mixture

Food mixtures were prepared using raw jackfruit flour, defatted soy flour, jackfruit seed flour, tomato and papaya pulp using both the *koozha* and *varikka* variety of jackfruit. For the preparation of food mixture, the standardized procedure of Rani and Khetarpaul (1997) was followed with slight modification. Various combinations used for the preparation of food mixtures are given in Table 1.

The food mixtures in their appropriate proportion of ingredients (100 g) were mixed with 500 ml of distilled water and stirred well to obtain a uniform slurry which was then autoclaved at 1.5 kg/cm² for 15 minutes. The autoclaved slurry was then allowed to cool and dried at 60⁰C for 12 hours in hot air oven. The dried mixture was ground and sieved to get a uniform powder.

Table 1. Proportion of ingredients in the jackfruit based food mixtures

Food mixtures (treatments)	Combination of ingredients	Percentage of ingredients				
		JF	DSF	JSF	T	P
T ₁	JF+DSF+T	70	20	-	10	-
		60	30	-	10	-
		50	40	-	10	-
		40	50	-	10	-
T ₂	JF+DSF+P	70	20	-	-	10
		60	30	-	-	10
		50	40	-	-	10
		40	50	-	-	10
T ₃	JF+DSF+JSF+T	70	10	10	10	-
		60	15	15	10	-
		50	20	20	10	-
		40	25	25	10	-
T ₄	JF+DSF+JSF+P	70	10	10	-	10
		60	15	15	-	10
		50	20	20	-	10
		40	25	25	-	10

(JF-Jackfruit flour, DSF- Defatted soy flour, JSF-Jackfruit seed flour, T- Tomato, P-Papaya)

3.2.2. Acceptability of the prepared food mixtures

3.2.2.1. Selection of panel members for the organoleptic evaluation

Triangle test suggested by Jellinek (1985) was carried out in the laboratory. Based on the results of triangle test, a panel of fifteen judges (between 18-35 years) were selected. The acceptability trials of the food mixtures were done by this panel.



Autoclaved food mixture



Food mixture after drying



Powdered food mixture

Plate 1. Preparation of food mixture

3.2.2.2. Score cards for the organoleptic evaluation

The nine point hedonic scale, originally developed by the US Army (Jones *et al.*, 1955) was used for the organoleptic evaluation of the food mixtures by the panel members. The score card is given in Appendix 1.

3.2.2.3. Organoleptic evaluation of prepared food mixtures

The prepared food mixtures underwent a series of sensory evaluation by a panel of 15 selected judges using the nine point hedonic scale. The sensory evaluation were carried out in the morning and quality attributes like appearance, colour, flavor, texture, taste and overall acceptability were evaluated.

3.2.2.4. Selection of the most acceptable combination of food mixture

On the basis of organoleptic scores, the food mixtures with maximum quality attributes were selected for further study.

3.3. Optimisation of conditions for the growth of *L. acidophilus*

3.3.1. Optimisation of substrate concentration

From the selected combinations of food mixtures (four combinations from both *koozha* and *varikka*) 25g, 50g and 75g were weighed and mixed with 150 ml of distilled water to get a slurry. This was autoclaved at 121⁰C for 15 minutes and allowed to cool. The autoclaved slurry was then inoculated with 100 μ l (107×10^9 cfu/ml) of 24 hour old culture of *L. acidophilus*. The samples were incubated at 37⁰C for 24 hours and freeze dried. After freeze drying the mixture was finely powdered and enumerated for the growth of *L. acidophilus*.

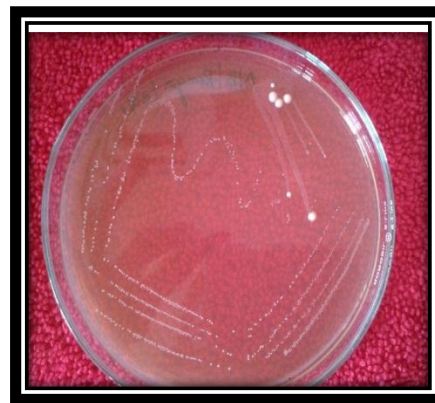
The viability of probiotic organism in the fermented food mixture were assessed using MRS (De Man Rogosa and Sharpe) medium. One gram of the sample was measured and transferred to a test tube containing 9ml sterile distilled water (10^{-1} dilution). This was then serially diluted up to 10^{-9} dilutions. The microbial enumeration was done by pour plate method using MRS agar and the results are expressed as 10^9 cfu/g.



MTCC 10307



MTCC 10307 in milk



MTCC 10307 in MRS media

Plate 2. Probiotic strain sub culturing

3.3.2. Optimisation of time of incubation

The substrate concentration with maximum viability of *L. acidophilus* was taken and mixed with 150 ml of distilled water for preparing the slurry. The slurry was then autoclaved at 121⁰C for 15 minutes, allowed to cool and then inoculated with 100 µl (107×10⁹cfu/ml) of 24 hour old culture of *L. acidophilus*. The samples were then incubated at 37⁰C for 18, 24 and 30 hours. After this, the samples were subjected to freeze drying and the viability of probiotic organism was enumerated.

3.3.3. Optimisation of pH

Slurries were prepared by mixing 150ml of distilled water and substrate concentration with maximum viability of *L. acidophilus*. pH of the samples were adjusted to 4.5, 5.5 and 6.5 using 20 per cent food grade citric acid. The autoclaved samples were inoculated with *L. acidophilus* and incubated at 37⁰C for the optimum period of fermentation. After incubation, the mixtures were freeze dried and enumerated for the viability of *L. acidophilus*.

3.3.4. Optimisation of temperature

The food mixtures with optimum substrate concentration was taken and mixed with 150 ml distilled water and the adjusted optimum pH using 20 per cent food grade citric acid. The samples were autoclaved, inoculated with 100 µl of the 24 hour old culture and incubated at varying temperatures of 37⁰C, 41⁰C and 45⁰C for the optimum period of fermentation. The mixtures were freeze dried after incubation and tested for viability of *L. acidophilus*.

3.3.5. Optimisation of population of inoculum concentration

Each food combinations with best substrate concentration was mixed with 150ml of distilled water and adjusted to optimum pH using 20 per cent food grade citric acid. The autoclaved slurries were then inoculated with 100µl, 200µl and 300µl of 24 hours old culture of *L. acidophilus* and kept for incubation at optimum temperature for optimum period of fermentation. Freeze drying was carried out after incubation. The freeze dried powders were then enumerated for the total number of viable cells of *L. acidophilus*.

3.4. Development of food mixtures

After the process of optimisation of variables, the selected food mixture from each set was fermented under the optimum conditions along with an unfermented control.

3.4.1. Development of autoclaved fermented food mixture

The selected food mixture from each set (50 g) was mixed with 150 ml of distilled water and stirred gently to get a uniform slurry. pH of the slurry was adjusted to 4.5 using 20 per cent food grade citric acid. The slurry was then autoclaved at 121⁰C (1.5 kg/cm²) for 15 minutes and allowed to cool. The autoclaved slurry was then inoculated with 300 µl of 24 hour old culture of *L. acidophilus* and incubated for a period of 24 hours at 37⁰C. The fermented slurry was then freeze dried and powdered. The fermented food mixture was then packed in laminated polyethylene pouches and stored at ambient conditions for a period of six months.

3.4.2. Development of autoclaved unfermented food mixtures

The best food mixture (50 g) from each set was mixed with 150 ml of sterile distilled water and stirred to get a slurry. pH of the slurry was adjusted to 4.5 using 20 per cent food grade citric acid and autoclaved. It was then freeze dried and powdered. The freeze dried powder was stored at ambient condition in laminated polyethylene pouches.

3.5. Storage studies of the developed food mixtures

The eight food mixtures, (four from *koozha* variety and four from *varikka* variety) along with their controls were packed in laminated polyethylene pouches and stored for a period of six months under ambient conditions. The quality evaluation of the stored food mixtures were done at monthly intervals for a period of six months.



Fermented food mixture



Freeze drying



Freeze dried powder

Plate 3. Preparation of freeze dried powder

3.5.1. Physico-chemical constituents of the food mixture

Analysis of each parameter was carried out in three replications and the methods used are discussed below.

3.5.1.1. Moisture

The method suggested by AOAC (1994) was followed to assess the moisture content of the developed food mixtures.

In order to find the moisture content, five gram of the test sample was taken in a petri dish and dried in a hot air oven at 60-70⁰C, cooled in a desiccator and then weighed. The drying and cooling process was repeated until the constant weight was obtained. Moisture content of the sample was calculated from the weight lost during the drying process.

$$\text{Moisture \%} = \frac{I-F}{I} \times 100$$

I- Initial weight of the sample

F- Final weight of the sample

3.5.1.2. Acidity

To determine acidity of the food mixtures, the method suggested by Ranganna (1986) was followed. Titratable acidity was determined by titrating the food mixture extract against 0.1 N sodium hydroxide (NaOH) using one per cent phenolphthalein solutions as indicator. To prepare the extract, a measured quantity of the food mixture was boiled in distilled water. The titre values were recorded when the solution turns pink. Titratable acidity was expressed as percent citric acid equivalent using the formula.

$$\% \text{ titratable acidity} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times \text{Volume made up} \times \text{Equivalent weight of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{Weight of sample taken} \times 1000}$$

3.5.1.3. Total soluble solids

Total soluble solids (TSS) of the food mixtures were determined using a hand refractometer. The readings were taken at room temperature and expressed as degree brix (Ranganna 1986).

3.5.1.4. Starch

The starch content of food mixtures were estimated colorimetrically using anthrone reagent as per the procedure suggested by Sadasivam and Manickam (1992). For the analysis, 100 mg of samples were homogenized in 80 per cent hot ethanol in order to remove sugars. Residues were extracted repeatedly to remove all the sugar content. The residue retained was dried over a water bath. To this dried residue, added 6.5 ml 52 per cent perchloric acid and 5 ml distilled water. This mixture was incubated for a period of 20 min and after that the supernatant was re extracted with perchloric acid. The supernatant was collected and made upto a volume of 100 ml. From this known volume, 0.2 ml aliquot was pipetted out and mixed with 0.8 ml distilled water and 4 ml anthrone reagent .The mixture was boiled for 8 min in a water bath and cooled in ice bath. The colour intensity was measured at 630 nm.

3.5.1.5. Reducing and total sugars

Twenty five gram of food mixture was ground with 100 ml of distilled water and transferred to a conical flask. It was then neutralised with 1N sodium hydroxide solution in the presence of phenolphthalein. Clarification of the neutralised mixture was done by the addition of 2 ml of lead acetate. The excess

amount of lead acetate was removed by adding 2 ml of potassium oxalate. It was then allowed to stand for 10 minutes for the settlement of the precipitate. The solution was filtered through Whatman's No.1 filter paper. It was then made upto 250 ml. Aliquot of the solution was titrated against a boiling mixture of fehling's solution A and B using methylene blue as indicator. End point of the reaction is the appearance of brick red colour (Ranganna, 1986). The reducing sugars present in the food mixtures were computed using the formula as follows.

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's factor} \times \text{dilution} \times 100}{\text{Titre value} \times \text{weight of the sample}}$$

3.5.1.6. Total sugar

The total sugar was determined using the method given by Ranganna (1986). From the clarified solution used for the estimation of reducing sugar, 50 ml was taken. This solution was gently boiled after adding citric acid and water. The volume was made upto 250 ml after neutralizing the solution with sodium hydroxide. The aliquot of this solution was titrated against Fehling's solution A and B. The total sugar content was expressed as percentage.

$$\text{Total sugars (\%)} = \frac{\text{Fehling's factor} \times 250 \times \text{dilution} \times 100}{\text{Titre value} \times 50 \times \text{weight of the sample}}$$

3.5.1.7. Protein

Protein content of the samples were determined by the method suggested by AOAC (1994). The sample 0.5 g was placed in a digestion flask. Five gram of Kjeldahl reagent (9 part K_2SO_4 and 1 part CuSO_4) and 200 ml of conc. H_2SO_4 was

added to this. After digestion, it was diluted with distilled water and 25 ml of 40 per cent NaOH was pumped. The distillate was collected in a receiver containing two per cent boric acid and mixed with indicators and then titrated standard acid (0.2 N HCl) against 40 per cent NaOH.

$$\text{Protein (\%)} = \frac{(A-B) \times N \times 1.4007 \times 6.25}{W}$$

Where

A = Volume (ml) of 0.2 N HCl used in the sample titration

B = Volume (ml) of 0.2 N HCl used in the blank titration

N = Normality of HCl

W = Weight (g) of the sample

1.4007 = Atomic weight of nitrogen

6.25 = The protein-nitrogen conversion factor

3.5.1.8. β carotene

The sample (2 g) was taken in a 100 ml glass stopper flask and added 10 ml of water saturated butanol (WSB). The contents of the flasks were mixed vigorously for 1 minute and kept undisturbed for 16-18 hrs (overnight) at room temperature. Dark condition was maintained for the complete extraction of β -carotene. The contents were again subjected to shaking and filtered completely through the Whatmann no.1 filter paper into a 100 ml volumetric flask. The optical density (O.D) was measured at 440 nm (Sadasivam and Manickam, 1992).

3.5.1.9. Crude fibre

Crude fibre is the organic matter in the dried residue remaining after digesting the sample with dilute sulphuric acid and sodium hydroxide. Two grams of the sample (food mixture) was taken in a crucible and boiled for 30 minutes with H₂SO₄ (200 ml). The sample after boiling was thoroughly washed in boiling water

and was again boiled for 30 minutes with 200 ml of NaOH. After digestion, the sample was washed thoroughly with boiling water, and rinsed in alcohol under vacuum. The weight of the dried crucible was taken and the difference in weight is the weight of crude fibre present in the sample (ASTA, 1968).

$$\text{Crude fibre content (\%)} = \frac{(A-B) \times 100}{W}$$

Where

A = Weight of crucible with dry residue (g)

B = Weight of crucible with ash (g)

W = Weight of the sample

3.5.1.10. Total ash

Total ash was found by the procedure of AOAC (1994). A clean and dry crucible was accurately weighed first and noted down. About two grams of the sample was placed in the crucible and again weighed so as to get the accurate weight of the sample. The crucible containing the sample was placed in an electric burner in a partially open manner for the sample to get charred with initial expulsion of smoke. After this, the crucible was placed in a muffle furnace and heated to 600°C for 2 hours. Crucible was carefully removed from the furnace and cooled to room temperature and weighed again to get the reading.

$$\text{Ash content (\%)} = \frac{(Z-X) \times 100}{(Y-X)}$$

Where,

X- Weight of empty crucible in grams

Y- Weight of crucible + sample in grams

Z- Weight of crucible + ash in grams (after complete ashing)

3.5.1.11. Calcium

Calcium content of the selected food mixtures were estimated by Atomic Absorption Spectrophotometric method using the di acid extract prepared from the sample (Perkin-Elmer, 1982). A sample of 0.20 g was predigested with 10 ml of 9:4 mixture of nitric acid and perchloric acid and made up the volume to 50 ml and used directly in Atomic Absorption Spectrophotometer for the estimation of calcium and expressed in mg 100 g⁻¹ of sample.

3.5.1.12. Iron

Iron content present in selected food mixtures were determined using the method suggested by Perkin-Elmer (1982). One gram of the sample was predigested using 9:4 ratio of nitric and percholoric acid (10 ml). The prepared di acid extract of the food mixture sample was used for estimation of iron in Atomic Absorption Spectrophotometer. Iron content present in the sample was expressed as mg 100 g⁻¹ of the sample.

3.5.1.13. Potassium

The potassium content present in the prepared food mixture was estimated using the procedure suggested by Jackson (1973). The di acid extract of the food mixture was directly read in the flame photometer and the potassium content was expressed in mg 100 g⁻¹ of sample.

3.5.1.14. Thiamine

Thiamine content of the food mixture was estimated by the method of Sadasivam and Manickam (1996). For the estimation, 5.0 g of the sample was weighed in a conical flask and added 100 ml of 0.1N H₂SO₄ slowly. The flask was allowed to stand overnight and shaken vigorously the next day and filtered. Pipetted out 10 ml extract in 100 ml separating funnel. Another 10 ml was pipetted out as working standard. Added 3.0 ml of NaOH (15%) to each funnel separately and further added 4 drops of ferricyanide solution. It was then shaken for 30 seconds.

Iso-butanol (15ml) was added and shook vigorously for another 60 sec and allowed the layers to separate. The bottom layer was discarded. Added one spatula of sodium sulphate, swirled gently, clarified and collected the clean extracts into test tubes. A set of blank sample was prepared by pipetting out 10 ml of the extract and followed the above procedure by omitting addition of ferricyanide. A blank was prepared separately for the standard. The absorbance was read at 366 nm and thiamine content was calculated using the following formula.

$$\text{Thiamine } (\mu\text{g}/100 \text{ g}) = [(0.25 \times 10) / a - a_1] \times [(b - b_1) \times 100 / 10] \times [10/5]$$

Where

a = Reading of standard

a₁ = Reading of standard blank

b = Reading of sample

b₁ = Reading of sample blank

3.5.1.15. Riboflavin

The riboflavin content of the developed food mixtures was estimated by the method suggested by Sadasivam and Manickam (1992). Two grams of the sample was taken into a 250 ml conical flask and mixed thoroughly with 75 ml of 0.1 normal H₂SO₄. The sample was then subjected to autoclaving at 15 lbs for 30 minutes. By shaking every five minutes, the flasks were cooled to room temperature. To this, 5 ml of 2.5 M sodium acetate solution was added, mixed and allowed to stand for one hour. pH of the above solution was adjusted to 4.5, transferred to a 100 ml volumetric flask and made upto the volume with distilled water. Filtered through whatman filter paper No. 2, discarded the first 10-15 ml. In test tubes (one inch diameter) marked A and B oxidation was carried out with stirring bars (Table.2).

Table 2. Details of oxidation for the assessment of riboflavin content

	Low/Blank Tube A	Sample Tube B	High/ Blank Tube A	Sample Tube B
Sample solution (ml)	10	10	10	10
Standard solution (ml)	1	-	1	-
Water (ml)	1	2	-	1
*KMnO ₄ (4%) (ml)	0.5	0.5	1	1
Time laps (min)	2	2	4	4
**Hydrogen peroxide (ml)	0.5	0.5	1	1

*Stir samples after addition of permanganate

**Shake after adding peroxide until foaming is negligible

The fluorimeter was set and the fluorescence of solutions A and B was determined. To solution B in the cuvette, 20 mg sodium hydrosulphite was added, stirred and recorded the blank fluorescence (C). The percent of riboflavin content was calculated by the formula:

$$\text{Riboflavin } \mu\text{g}/100 \text{ g} = \frac{B-C}{A-C} \times \frac{R}{S} \times \frac{V}{V_1} \times 100$$

Where,

A = Reading of the sample plus riboflavin standard

B = Reading of sample plus water

C = Reading after adding sodium hydrosulphite

R = Standard riboflavin added = $\mu\text{g} / V_1$ of sample solution

V = Original volume of sample solution in ml

V₁ = Volume of sample solution taken for measurement (10 ml)

S = Sample weight (g)

In this dilution R = 1, V = 100 and V1 = 10

3.5.2. *In vitro* starch digestibility of food mixtures

The method suggested by Saterlee *et al.*, (1979) was used for assessing the *in vitro* starch digestibility of the food mixture. One gram of the sample was mixed in 100 ml of distilled water and boiled for one hour. From the gelatinized solution, one ml was filtered out and mixed with an equal amount of enzyme solution (saliva diluted with equal quantity of water). After incubating this mixture at 37°C for 1-2 hours, the reaction was stopped by the addition of one ml of sodium hydroxide. The glucose estimation was conducted by the method suggested by Somoygi (1952).

3.5.3. *In vitro* protein digestibility of food mixtures

In vitro protein digestibility of the food mixture was assessed by the method suggested by Sadasivam and Manickam (1992). A multi enzyme system consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase per ml was used. Added 10 ml of distilled water to the powdered sample (amount of sample is adjusted to contain 6.25 g of protein/ml) and allowed it to stand for at least 1 hour at 5°C. Adjusted the pH of the sample and multi enzyme system to 8.0 at 37°C. Added 1ml of the three enzyme solution and stirred while maintaining the temperature at 37°C. Exactly after ten minutes, added the bacterial protease solution and immediately transferred the solution to 55°C water bath. Nine minutes after the addition of bacterial protease, the solution was transferred to 37°C water bath. Measured the pH of the hydrolysate exactly 10 minute after adding the bacterial enzyme. This is called the 20 minute pH.

$$\text{In vitro protein digestibility (\%)} = 234.84 - 22.56X$$

Where

X= pH after 20 minute incubation

3.5.4. Organoleptic evaluation of the food mixtures

The developed freeze dried food mixtures were subjected to organoleptic evaluation by the panel of selected judges. The procedure of organoleptic evaluation is mentioned in 3.2.1.

3.5.5. Enumeration of population of *L. acidophilus* in the fermented food mixture

The viable count of *L. acidophilus* present in the developed freeze dried food mixtures were enumerated by serial dilution and plate count method as detailed by Agarwal and Hasija (1986). For enumerating the probiotic bacteria (*L. acidophilus*), ten grams of the developed food mixture was mixed with 90 ml distilled water and mixed thoroughly. One ml of this mix was transferred to a test tube containing 9 ml of distilled water. This form 10^{-2} dilution. Similarly the dilutions upto 10^{-9} were made. The viable counts of *L. acidophilus* were enumerated as mentioned in 3.3.1.

3.5.6. Microbial enumeration of total micro flora and insect infestation

3.5.6.1. Enumeration of total microflora

The microbial population present in the food mixtures were estimated using serial dilution plate count method as suggested by Agarwal and Hasija (1986). The microbial analysis was carried out in selected food mixtures of each set initially and at monthly intervals of storage.

The sample was prepared by mixing 90 ml of distilled water with 10 g of freeze dried food mixture and shaken well using a shaker to obtain suspension. This is 10^{-1} dilution. The serial dilutions were carried out in the prepared water blank. To 9 ml of water blank transfer one ml of the prepared suspension and this forms a dilution of 10^{-2} . This is then diluted to 10^{-3} followed by 10^{-4} , 10^{-5} and 10^{-6} using serial dilution techniques. Bacteria, fungi and yeast count were assessed using Nutrient Agar (NA) for bacteria, Potato Dextrose Agar (PDA) for fungi and Sabouraud's Dextrose Agar (SDA) media for yeast respectively and results were given as cfu/g.

3.5.6.2. Enumeration of bacterial colony

Total number of bacterial colony was enumerated in 10^{-5} dilution in nutrient agar medium. In a sterile petri dish, pour one ml of 10^{-5} dilution using a micropipette. To petri dish pour about 20 ml of the nutrient agar medium which is uniformly spread in petri dish by rotating in clockwise and anticlockwise directions. For bacterial colony the enumerated petri dishes were incubated for 48 hrs at room temperature. The total number of bacterial colonies were counted and expressed as cfu/g.

3.5.6.3. Enumeration of fungal colony

Total number of fungal colony was enumerated in 10^{-3} dilution in Martin Rose agar medium. In a sterile petri dish, pour one ml of 10^{-3} dilution using a micropipette. To petri dish pour about 20 ml of the Potato Dextrose Agar medium and uniformly spread. For fungal colony enumeration, the petri dishes were incubated for 4 to 5 days at room temperature. The total number of fungal colonies counted and expressed as cfu/g.

3.5.6.4. Enumeration of yeast colony

Total number of yeast colony was enumerated in 10^{-3} dilution in Sabouraud's Dextrose Agar medium. In a sterile petri dish, pour one ml of 10^{-3} dilution using a micropipette. To petri dish pour about 20 ml of the Sabouraud's Dextrose Agar medium which is uniformly spread in the petri dish by rotating. For enumeration of yeast population, the petri dishes were incubated for 4 to 5 days in room temperature. The total number of yeast colonies were counted and expressed as cfu/g.

3.5.6.5. Evaluating insect infestation

The insect infestation of the stored food mixtures were done at monthly intervals. The mixtures were observed under day light and also under microscope. The food mixtures were sieved well before examinations.

3.5.7. Temperature and relative humidity

Maximum and minimum room temperature was measured using the Whirling Psychrometer in the morning and evening. The reading were taken at the same time each day to avoid variations in the reading. From this mean of maximum and minimum temperature was recorded. Relative humidity was calculated with the help of relative humidity chart.

3.5.8. Selection of food mixtures with maximum quality attributes

The food mixtures one from each variety were selected for further studies. The mixtures were selected using geometric mean scores

3.6. Analysis of glycemic index of the selected food mixtures

Glycemic index of the selected food mixtures (*koozha* and *varikka*) were computed with aid of formula suggested by Srilakshmi (2011). The food mixture was given to ten healthy individuals for this purpose. The selection of respondents were done based on glucose tolerance test.

Prior to the test, all the respondents were requested to stop all medications at least 3 days before the test date and to maintain 8 hours fasting prior the test. The fasting blood glucose level of each respondent was measured using one touch glucometer and recorded. A drink was prepared using the food mixture which is equivalent to 50 g of glucose. The blood glucose levels were checked and measured at 30 minutes interval upto 2 hours of drink administration. From the observations, glycemic index of each food mixture was assessed using the formula

$$\text{Glycemic index} = \frac{\text{Incremental area under the 2 hours plasma glucose curve after eating 50 g of CHO from the test food}}{\text{Incremental area under the 2 hours plasma glucose curve after eating 50 g of glucose}}$$

3.7. Standardisation of instant shake mix from the selected food mixtures

3.7.1. Standardising the combination of ingredients in shake mix

Shake mixes were prepared using the selected food mixtures and skimmed milk powder. Ten grams of sugar and two grams of nuts and spices (cashew nut and cardamom) were also added during the preparation. Various combinations used for the preparation of instant shake mixes are given in Table 3.

Table 3. Treatments for the preparation of instant shake mixes.

Treatment	Combinations
T ₁	80% SMP + 20% FM
T ₂	70% SMP +30 % FM
T ₃	60% SMP + 40% FM
T ₄	50% SMP + 50% FM

(SMP- Skimmed milk powder, FM- Food mixture)

3.7.2. Acceptability of the prepared food mixtures

The organoleptic evaluation of the shake mixes were carried out by preparing shakes with these mixes. The reconstituted shakes (shake mixes) were evaluated organoleptically by the judges using a 9 point hedonic scale as explained in section 3.2.2. Organoleptic score card used for the organoleptic evaluation is given in Appendix II. Shake mixes were prepared from the best selected food mixture each from *koozha* and *varikka* variety.

3.8. Storage studies of instant shake mixes

The prepared shake mixes from *koozha* and *varikka* variety were stored in laminated polyethylene pouches for a period of two months. Quality aspects of the

shake mixes were studied throughout the storage period at fifteen days intervals. The methods followed were mentioned in 3.5.

3.9. Standardisation of jackfruit yoghurt

3.9.1. Standardising the combination of ingredients in jackfruit yoghurt

Sweetened fruit yoghurts were prepared as per the standard procedure suggested by Khedkar *et al.* (2015) by incorporating *L. bulgaricus* and *S. thermophilus*. Table 4. narrates the various combination of treatments used in the standardization of jackfruit yoghurt.

Table 4. Combination of ingredients in the preparation of jackfruit yoghurt

Set	Treatments	Ingredients	Percentage of ingredients		
			HM	SM	JP
1	T ₀	HM+JP	100	-	-
	T ₁		90	-	10
	T ₂		80	-	20
	T ₃		70	-	30
2	T ₀	SM+JP	-	100	-
	T ₁		-	90	10
	T ₂		-	80	20
	T ₃		-	70	30
3	T ₀	HM+SM+JP	50	50	-
	T ₁		45	45	10
	T ₂		40	40	20
	T ₃		35	35	30

(HM- Homogenised milk, SM- Skimmed milk, JP- Jackfruit pulp)

3.9.2. Acceptability of the prepared jackfruit yoghurt

The prepared jackfruit yoghurts were subjected to acceptability tests as mentioned in 3.2.2. In each set, yoghurts were prepared from both the *koozha* and



Ripe jackfruit bulbs



Blanching for five minutes



Fruit pulp

Plate 4. Preparation of jackfruit pulp

varikka variety. Based on the sensory evaluation, the best combination from each set was selected for further experiments. The organoleptic score card used is given in appendix III

3.10. Optimisation of conditions for the growth of *L. acidophilus* in yoghurt

3.10.1. Optimisation of substrate concentration

From the selected jackfruit yoghurt combination (best combination from both *koozha* and *varikka*) 25 g, 50 g and 75 g were weighed and inoculated with 200 µl of probiotic culture and 100 µl of yoghurt culture. The mixture was then incubated at 37⁰C for 4 hours.

The viability of probiotic organism in the yoghurts were assessed using MRS medium. One gram of the sample was measured and transferred to test tube containing 9 ml sterile distilled water (10⁻¹ dilution). This was then serially diluted up to 10⁻⁹ dilutions. The microbial enumeration was done by pour plate method using MRS agar and the results expressed as 10⁹ cfu/g.

3.10.2. Optimisation of time of incubation

The substrate concentration with maximum viability was taken and inoculated with 200 µl of probiotic culture and 100 µl of yoghurt culture. The mixture was then incubated for 4, 5 and 6 hours at 37⁰C. After this, the viability of probiotic organism was enumerated.

3.10.3. Optimisation of pH

Jackfruit pulp and milk was mixed and inoculated with the probiotic and yoghurt cultures. The mix was gently stirred and the initial pH was noted down. After this, the samples were incubated at 37⁰C. pH of the samples were analysed hourly. In this way the pH of yoghurt samples were adjusted to 5.0, 4.5 and 4.0. On attaining the respective pH, the samples were taken out and refrigerated. The viability of *L. acidophilus* was enumerated.

3.10.4. Optimisation of temperature

The optimum substrate concentration was taken and mixed probiotic and yoghurt strains and incubated at 37⁰C, 39⁰C and 41⁰C for the optimum period of fermentation and pH. After incubation, the samples were tested for viability of *L. acidophilus*.

3.10.5. Optimisation of population of inoculum concentration

Each yoghurt combinations was taken and mixed with 100 µl, 200 µl and 300 µl probiotic culture and 100 µl of yoghurt culture. This mixture was then incubated at the optimum temperature for the optimum period of fermentation and pH. The prepared yoghurts were then enumerated for the total number of viable cells of *L. acidophilus*.

3.11. Development of jackfruit based bio-yoghurt

The selected combination of ingredients i.e. 70 per cent milk and 30 per cent jackfruit pulp was mixed and inoculated with 200 µl of *L. acidophilus* and 100 µl of yoghurt culture. It was then incubated for 6 hours at 37⁰C. Yoghurts were stored in the refrigerator once it is set.

3.12. Storage studies of jackfruit based bio-yoghurts

The prepared jackfruit based bio-yoghurts were packed in food grade plastic containers and kept at refrigerated condition for a period of 15 days. Quality aspects of the products were studied throughout the storage period. Minerals (calcium, iron and potassium) and crude fibre were analysed initially and finally. All the other parameters were evaluated at 5 days interval. The parameters studied and the methods followed are mentioned below

3.12.1. Physico-chemical constituents of the food mixture

3.12.1.1. Moisture

For analysing the moisture content, the method suggested by AOAC (1994) was followed as mentioned in 3.5.1.

3.12.1.2. Acidity

By following the procedure mentioned in 3.5.1.2, the titratable acidity of yoghurts were estimated. It was expressed as percentage of lactic acid.

3.12.1.3. Fat

Ten grams of the sample was weighed in a small beaker. Concentrated hydrochloric acid (10 ml) was added to this and heated on a Bunsen burner. The sample was stirred continuously with a glass rod until the contents turn dark brown. The contents were then allowed to come to room temperature. The contents were then transferred to a Mojonnier fat extraction flask. Ethyl alcohol (10 ml) was added, first to the beaker and to the Mojonnier fat extraction flask. Mixed well. Similarly 25 ml of ethyl ether was added to the Mojonnier flask, closed with a cork and vigorously shaken for one minute. After adding 25 ml of petroleum ether, shaking was repeated for one more minute. The Mojonnier flask was centrifuged at 600 rpm for 3 minutes. Tip and the stopper of the extraction flask was washed with a mixture containing equal parts of the two solvents (ethyl alcohol and ethyl ether) and the washings were added to the weighing flask. The extraction of liquid remaining in the flask was repeated successively using 15 ml of each solvent. After extraction, the solvent was completely evaporated on water bath (at a temperature that does not cause sputtering or bumping). Fat was dried in an oven at $102 \pm 2^\circ\text{C}$ to a constant weight. The cooled flask was weighed. After the removal of fat completely from the container with warm petroleum ether, the container was weighed again as before (Sadasivam and Manickam, 1992).

$$\text{Fat \% (w/w)} = \frac{100 (W_1 - W_2)}{W_3}$$

Where,

W_1 = Weight in g of contents in the flask before removal of fat.

W_2 = Weight in g of contents in the flask after removal of fat and

W_3 = Weight in g of material taken for the test.

3.12.1.4. Water holding capacity

The water holding capacity was determined according to the procedure suggested by Guzmán-González *et al.*, (1999). A weighed amount of sample (20g), (Y) was centrifuged at 1250 rpm for 10 min at 4 °C. The whey expelled (W) was removed and weighed again. The water holding capacity (WHC, g.kg⁻¹) was calculated as

$$\text{WHC} = \frac{(Y - W)}{Y} \times 100$$

3.12.1.5. Syneresis

The procedure suggested by Gaston *et al.* (2007) was used to assess the syneresis of prepared yoghurts. Yoghurt samples (35g) were centrifuged at 1100 rpm for 10min at 5±2°C. The clear supernatant was poured off and weighed. This was recorded as syneresis (%).

3.12.1.6. Viscosity

Brookfield viscometer model BM type was used to measure the yoghurt viscosity. The reported value was an average of three readings. The readings were taken at 10°C (the temperature at which the yoghurt is consumed). The spindle speed was adjusted according to the firmness of the sample. The specification combination used in this case was speed 12 (revolutions/ second) and spindle number 4. To calculate the final viscosity in centipoises, a factor of 400 was used to multiply the obtained figure.

3.12.1.7. Curd tension

The curd tension was measured by using stainless steel cone penetrometer and was expressed as mm/5sec. A higher penetration value implicates lower hardness or curd tension of the product. The product temperature of 5±2°C was maintained prior to firmness measurement. A cone and test rod (probe) weighing 32 g was allowed to penetrate the sample for a fixed time (5 seconds). The average of three readings was taken as millimeter of penetration.

3.12.1.8. TSS

Total soluble solids (TSS) of the food mixtures were determined using a hand refractometer. The readings were taken at room temperature and expressed as degree brix (Ranganna 1986).

3.12.1.9. Reducing and total sugars

Reducing and total sugars present in the yoghurt samples were estimated as per the procedure suggested by Ranganna (1986). The methods are detailed in 3.5.1.5 and 3.5.1.6.

3.12.1.10. Protein

Protein content of the prepared bio-yoghurts will be estimated by the Kjeldhal method which is explained in section 3.5.1.7. The crude protein content present in the sample is expressed as percentage by mass. The percentage protein content is obtained by multiplying the nitrogen content by 6.38 (the protein - nitrogen conversion factor) (Sadasivam and Manickam, 1996).

$$\text{Protein (\%)} = \frac{(A-B) \times N \times 1.4007 \times 6.25}{W}$$

Where

A = Volume (ml) of 0.2 N HCl used in the sample titration

B = Volume (ml) of 0.2 N HCl used in the blank titration

N = Normality of HCl

W = Weight (g) of the sample

1.4007 = Atomic weight of nitrogen

6.25 = Protein-nitrogen conversion factor

3.12.1.11. β carotene

As mentioned in 3.5.1.8, two grams of the sample was taken in a 100 ml glass stopper flask and added 10 ml of water saturated butanol (WSB). The contents

of the flasks were mixed vigorously for 1 minute and kept undisturbed for 16-18 hrs (overnight) at room temperature. Dark condition was maintained for the complete extraction of β -carotene. The contents were again subjected to shaking and filtered completely through Whatmann no.1 filter paper into a 100 ml volumetric flask. The optical density (O D) was measured at 440 nm.

3.12.1.12. Crude fibre

Crude fibre is the organic matter in the dried residue remaining after digesting the sample with dilute sulphuric acid and sodium hydroxide. The crude fibre content of the prepared yoghurts were assessed by ASTA (1968) method as described in 3.5.1.9.

3.12.1.13. Total ash

Section 3.5.1.10 of this chapter explains how total ash content of the sample was determined.

3.12.1.14. Calcium

Calcium content of the selected food mixtures were estimated by Atomic Absorption Spectrophotometric method using the di acid extract prepared from the sample (Perkin-Elmer, 1982). The procedure is given in section 3.5.1.11.

3.12.1.15. Iron

Iron content present in selected food mixtures were determined using the method suggested by Perkin- Elmer (1982). Section 3.5.1.12 of this chapter explains the method.

3.12.1.16. Potassium

The potassium content present in the prepared food mixture was estimated using the procedure suggested by Jackson (1973) and explained in 3.5.1.13.

3.12.2. Organoleptic evaluation of the developed bio-yoghurts

The prepared bio-yoghurts were organoleptically evaluated at five days interval as mentioned in 3.3.2.

3.12.3. Population of *L. acidophilus* in the bio-yoghurts

The probiotic count was enumerated during the storage period at 5 days interval. The method followed is mentioned in section 3.3.1.

3.12.4. Enumeration of total microflora and insect infestation

The enumeration of total microflora and insect infestation were done using the methods described in 3.5.6.

3.13. Statistical analysis

Statistical analysis of the obtained data were done by applying the techniques like Kendall's coefficient of concordance, Duncan's multiple range test, geometric mean scores and independent sample 't' test.



Results

4. RESULTS

“Without data, you are just another person with an opinion”

- Edwards Deming

4.1 Standardising the proportion of ingredients in the food mixture.

The food mixtures were prepared as per the standard procedure of Rani and Khetarpaul (1997) as mentioned in section 3.2.1 and all the prepared food mixtures were organoleptically evaluated by a panel of fifteen selected judges. The organoleptic qualities like appearance, colour, flavour, texture, taste and overall acceptability of all the food mixtures were evaluated using a nine point hedonic scale. Results of the organoleptic evaluation of different food mixtures are given in Table 5.

Food mixture set 1 was a combination of jackfruit flour (JF), defatted soy flour (DSF) and tomato (T) pulp. The amount of jackfruit flour varied from 40 per cent to 70 per cent and that of defatted soya flour from 20 per cent to 50 per cent. The proportion of tomato pulp was kept constant at 10 per cent. The experiment was repeated with both *koozha* and *varikka* varieties. On observing the mean scores for the organoleptic evaluation of food mixtures of *koozha* variety, it was evident that the treatment T₂ scored maximum score for all the organoleptic qualities. This variation secured a mean score of 8.14, 8.13, 7.99, 7.86, 7.91 and 8.01 for appearance, colour, flavor, texture, taste and overall acceptability respectively and the total score of this treatment was 48.04. The overall acceptability of the food mixtures were in the order of 7.47, 8.01, 7.49 and 7.26 respectively. Among the treatments, T₄ was the least acceptable combination.

In the second set of experiment, tomato pulp was replaced with papaya pulp and the other proportion of ingredients remained same. The mean scores obtained by the food mixtures containing jackfruit flour (JF), defatted soy flour (DSF), and papaya pulp (P) are illustrated in Table 5.

Table 5: Mean score and mean rank scores for the organoleptic qualities of jackfruit (*koozha*) food mixtures

	Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
Set 1	T ₁	7.60 (2.37)	7.73 (2.33)	7.24 (2.00)	7.56 (2.27)	7.22 (2.30)	7.47 (2.63)	44.82
	T ₂	8.14 (3.37)	8.13 (3.33)	7.99 (3.57)	7.86 (3.27)	7.91 (3.87)	8.01 (3.87)	48.04
	T ₃	7.51 (2.13)	7.80 (2.63)	7.40 (2.70)	7.60 (2.67)	7.16 (2.33)	7.49 (2.20)	44.96
	T ₄	7.51 (2.13)	7.47 (1.70)	7.07 (1.73)	7.40 (1.80)	6.87 (1.50)	7.26 (1.30)	43.58
	Kendalls W value	0.27	0.33	0.45	0.30	0.62	0.77	
Set 2	T ₁	8.00 (2.50)	7.98 (2.73)	7.42 (1.80)	7.62 (2.30)	6.96 (1.67)	7.60 (1.87)	45.58
	T ₂	8.16 (3.00)	8.09 (2.97)	7.98 (3.77)	7.87 (3.37)	7.89 (3.70)	8.00 (3.47)	47.99
	T ₃	8.00 (2.27)	7.87 (2.37)	7.64 (2.43)	7.69 (2.47)	7.16 (2.10)	7.67 (2.53)	46.03
	T ₄	7.96 (2.23)	7.73 (1.93)	7.44 (2.00)	7.51 (1.87)	7.31 (2.53)	7.59 (2.13)	45.54
	Kendalls W value	0.11	0.16	0.58	0.30	0.51	0.33	

Table 5. Contd.

Set 3	T ₁	8.00 (2.50)	7.98 (2.73)	7.42 (1.80)	7.62 (2.30)	6.96 (1.67)	7.60 (1.87)	45.58
	T ₂	8.00 (2.27)	7.87 (2.37)	7.64 (2.43)	7.69 (2.47)	7.16 (2.10)	7.67 (2.53)	46.03
	T ₃	8.16 (3.00)	8.08 (2.97)	8.01 (3.77)	7.85 (3.37)	7.89 (3.70)	7.99 (3.47)	47.98
	T ₄	7.96 (2.23)	7.73 (1.93)	7.44 (2.00)	7.51 (1.87)	7.31 (2.53)	7.59 (2.13)	45.54
	Kendalls W value	0.11	0.16	0.57	0.31	0.51	0.33	
Set 4	T ₁	8.20 (2.60)	8.04 (2.27)	7.67 (2.00)	7.76 (2.43)	7.16 (2.03)	7.76 (1.73)	46.59
	T ₂	8.10 (2.43)	8.10 (2.33)	7.67 (1.70)	7.80 (2.33)	7.20 (2.00)	7.77 (1.73)	46.64
	T ₃	8.20 (2.53)	8.11 (2.83)	8.03 (3.50)	7.87 (2.63)	7.84 (3.13)	8.01 (3.63)	48.06
	T ₄	7.97 (2.43)	7.93 (2.17)	7.86 (2.80)	7.87 (2.60)	7.58 (2.83)	7.84 (2.90)	47.05
	Kendalls W value	0.00	0.09	0.51	0.01	0.22	0.56	

*Figures in parenthesis indicates mean rank scores; ** Significant at 1% level

Set 1- T₁-70% JF+ 20% DSF+ 10% T; T₂- 60% JF+ 30% DSF+ 10% T; T₃- 50% JF+ 40% DSF+ 10%T; T₄-40% JF+ 50% DSF+10%T

Set 2- T₁-70% JF+ 20% DSF+ 10% P; T₂- 60% JF+ 30% DSF+ 10% P; T₃- 50% JF+ 40% DSF+ 10% P; T₄-40% JF+ 50% DSF+ 10% P

Set 3- T₁-70% JF+ 10% DSF+ 10% JSF+ 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% T; T₃- 50% JF+ 20% DSF+ 20% JSF + 10% T; T₄- 40% JF+ 25% DSF+ 25% JSF + 10% T

Set 4- T₁-70% JF+ 10% DSF+ 10% JSF 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% P; T₃- 50% JF+ 10% DSF+ 10% JSF + 10%; T₄-40% JF+ 25% DSF+ 25% JSF + 10% P

Table 5 revealed that in the second set also, treatment T₂ was the most acceptable among the judges than the other three treatments. For the food mixtures, the mean scores for overall acceptability was in the order of 7.60, 8.00, 7.67 and 7.59 for the treatments T₁, T₂, T₃ and T₄ respectively. Mean scores for the sensory parameters among the treatment were found to increase upto T₂ and slightly decreased after that. Total score of this treatment was 47.99 and the mean scores for appearance, colour, flavour, texture, taste and overall acceptability of T₂ was in the order of 8.16, 8.09, 7.98, 7.87, 7.89 and 8.00. As evident from the table, treatment T₄ got the least scores for organoleptic parameters.

The set 3 was a combination of jackfruit flour (JF), defatted soy flour (DSF), jackfruit seed flour (JSF) and tomato pulp (T). Here also the quantity of jackfruit flour varied from 40 per cent to 70 per cent and tomato pulp was kept constant at 10 per cent. The amount of defatted soy flour and jackfruit seed flour varied from 10 to 25 per cent.

The mean scores of organoleptic evaluation of the food mixture T₃ of set 3 (*koozha*) variety were in the order of 8.16, 8.08, 8.01, 7.85, 7.89 and 7.99 respectively for appearance, colour, flavour, texture, taste and overall acceptability. The mean scores for overall acceptability of the treatments T₁, T₂, T₃ and T₄ were 7.60, 7.67, 7.99 and 7.59. The maximum total score obtained by the treatment T₃ (47.98). Here, the acceptability of food mixtures tends to increase from T₁ to T₃ and then decreased. Just like the set 1 and 2, here also the treatment T₄ scored the least for organoleptic evaluation.

The combination of jackfruit flour (JF), defatted soy flour (DSF), jackfruit seed flour (JSF) and papaya pulp (P) was worked out in set 4. The proportion of ingredients were similar to that of set 3 except for tomato. Instead of tomato pulp, papaya pulp (10%) was used in this set. The treatment T₃ of *koozha* variety scored maximum points during organoleptic evaluation and secured a total score of 48.06. Mean scores obtained for overall acceptability of the treatments T₁, T₂, T₃ and T₄ were in the

Table 7: Mean score and mean rank scores for the organoleptic qualities of jackfruit (*varikka*) food mixtures

Treatment		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
Set 1	T ₁	8.16 (2.83)	7.69 (2.30)	7.60 (2.37)	7.84 (3.00)	7.69 (2.77)	7.79 (2.97)	46.77
	T ₂	8.17 (3.03)	8.19 (3.43)	7.98 (3.30)	7.94 (3.60)	7.84 (3.23)	8.02 (3.80)	48.14
	T ₃	8.00 (2.30)	7.49 (1.80)	7.62 (2.63)	7.44 (1.97)	7.49 (2.57)	7.60 (2.00)	45.64
	T ₄	7.84 (1.83)	7.43 (2.47)	6.98 (1.70)	7.02 (1.43)	7.11 (1.43)	7.27 (1.23)	43.65
	Kendalls W value	0.27	0.50	0.39	0.66	0.41	0.84	
Set 2	T ₁	8.07 (2.40)	8.00 (2.80)	7.60 (1.60)	7.67 (2.10)	7.13 (2.20)	7.69 (1.70)	46.16
	T ₂	8.13 (2.20)	8.20 (2.10)	8.02 (3.90)	7.91 (2.60)	7.86 (3.20)	8.02 (3.50)	47.96
	T ₃	8.09 (2.50)	8.13 (2.20)	7.82 (1.80)	7.73 (2.60)	7.27 (2.00)	7.80 (2.20)	46.84
	T ₄	8.07 (2.90)	8.11 (2.90)	7.67 (2.70)	7.73 (2.70)	7.20 (2.60)	7.75 (2.60)	46.53
	Kendalls W value	0.05	0.13	0.73	0.06	0.17	0.36	

Table 6. Contd.

Set 3	T ₁	8.00 (2.50)	7.86 (2.37)	7.42 (1.80)	7.62 (2.30)	6.96 (1.67)	7.59 (1.87)	45.45
	T ₂	8.00 (2.27)	7.98 (2.73)	7.64 (2.43)	7.68 (2.47)	7.15 (2.10)	7.71 (2.53)	46.16
	T ₃	8.15 (3.00)	8.14 (2.97)	7.97 (3.77)	7.93 (3.37)	7.88 (3.70)	8.01 (3.47)	48.08
	T ₄	7.95 (2.23)	7.73 (1.93)	7.44 (2.00)	7.51 (1.87)	7.31 (2.53)	7.58 (2.13)	45.52
	Kendalls W value	0.11	0.16	0.58	0.30	0.51	0.33	
Set 4	T ₁	8.07 (2.57)	8.04 (1.97)	7.60 (2.23)	7.67 (2.37)	7.13 (1.73)	7.72 (1.83)	46.23
	T ₂	8.07 (2.27)	8.13 (2.67)	7.94 (1.83)	7.89 (2.40)	7.58 (2.47)	7.90 (2.47)	47.51
	T ₃	8.11 (2.80)	8.16 (2.93)	8.04 (3.27)	7.95 (3.27)	7.90 (3.37)	8.03 (3.53)	48.19
	T ₄	8.04 (2.37)	8.04 (2.43)	7.73 (2.67)	7.69 (2.47)	7.29 (2.43)	7.75 (2.17)	46.54
	Kendalls W value	0.04	0.12	0.28	0.02	0.30	0.37	

*Figures in parenthesis indicates mean rank scores; ** Significant at 1% level

Set 1- T₁-70% JF+ 20% DSF+ 10% T; T₂- 60% JF+ 30% DSF+ 10% T; T₃- 50% JF+ 40% DSF+ 10%T; T₄-40% JF+ 50% DSF+10%T

Set 2- T₁-70% JF+ 20% DSF+ 10% P; T₂- 60% JF+ 30% DSF+ 10% P; T₃- 50% JF+ 40% DSF+ 10% P; T₄-40% JF+ 50% DSF+ 10% P

Set 3- T₁-70% JF+ 10% DSF+ 10% JSF+ 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% T; T₃- 50% JF+ 20% DSF+ 20% JSF + 10% T; T₄- 40% JF+ 25% DSF+ 25% JSF + 10% T

Set 4- T₁-70% JF+ 10% DSF+ 10% JSF 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% P; T₃- 50% JF+ 10% DSF+ 10% JSF + 10%; T₄-40% JF+ 25% DSF+ 25% JSF + 10% P

respective order of 7.76, 7.77, 8.01 and 7.84. The mean scores for appearance, colour, flavour, texture, taste and overall acceptability of T₃ was in the order of 8.20, 8.11, 8.03, 7.87, 7.84 and 8.01. Similar to the above described sets, in set 4 also the treatment T₄ was the least scored variation.

The mean scores obtained by the each treatment of four sets during the organoleptic evaluation were statistically analysed using the Kendall's coefficient of concordance and the mean ranks were worked out. Based on the mean scores and mean rank scores, the best treatment from each of the four sets were selected for the further studies. In set 1 and 2, the treatment T₂ scored the maximum score and selected for further studies. Whereas in set 3 and 4, the treatment T₃ was selected based on the organoleptic evaluation and Kendall's coefficient of concordance. The selected treatments along with their combination of ingredients are given in Table 6.

Table 6. Selected combinations of jackfruit based food mixtures (*koozha*)

Set	Combination	Treatment
1	60% JF+ 30% DSF+ 10% T	T ₂
2	60% JF+ 30% DSF+ 10% P	T ₂
3	50% JF+ 20% DSF+ 20% JSF+ 10% T	T ₃
4	50% JF+ 20% DSF+ 20% JSF+ 10% P	T ₃

JF-Jackfruit flour, DSF- Defatted soy flour, T- Tomato, P- Papaya

The above said experiments were repeated with the *varikka* variety also. The scores given to each treatment by the judges during organoleptic evaluation were tabulated and given in the Table 7.

For the food mixture (set 1) of *varikka* variety, scores for overall acceptability were in the order of 7.79, 8.02, 7.60 and 7.27 respectively for the treatments T₁, T₂, T₃, and T₄. The treatment T₂ was the best scored variation in this set and the scores

Table 14. Moisture content of fermented food mixtures during storage (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	2.73 ^c	2.76 ^c (0.72)	2.79 ^c (1.08)	2.81 ^d (0.71)	2.95 ^b (4.9)	3.01 ^c (3.05)	3.09 ^c 2.65
	JF+DSF+P	2.74 ^b	2.77 ^{bc} (1.08)	2.79 ^c (0.72)	2.82 ^c (1.07)	2.89 ^c (2.42)	3.03 ^{bc} (4.84)	3.07 ^{cd} (1.32)
	JF+DSF+JSF+T	2.77 ^a	2.80 ^a (1.07)	2.83 ^a (1.07)	2.87 ^a (1.41)	3.03 ^a (5.57)	3.07 ^a (1.32)	3.19 ^a (3.9)
	JF+DSF+JSF+P	2.76 ^{ab}	2.79 ^{ab} (1.76)	2.81 ^b (0.71)	2.85 ^b (1.42)	2.98 ^{ab} (4.56)	3.04 ^{ab} (2.01)	3.11 ^b (2.30)
	CD value	0.039	0.039	0.012	0.035	0.052	0.024	0.021
<i>Varikka</i>	JF+DSF+T	2.36 ^c	2.38 ^c (0.84)	2.40 ^c (0.84)	2.44 ^c (1.66)	2.50 ^b (2.45)	2.56 ^c (2.4)	2.59 ^c (1.17)
	JF+DSF+P	2.31 ^d	2.32 ^d (0.43)	2.34 ^d (0.86)	2.37 ^d (1.28)	2.41 ^c (1.68)	2.46 ^d (2.07)	2.51 ^d (2.03)
	JF+DSF+JSF+T	2.49 ^a	2.51 ^a (0.79)	2.53 ^a (0.79)	2.57 ^a (1.58)	2.60 ^a (1.16)	2.64 ^a (1.53)	2.69 ^a (1.89)
	JF+DSF+JSF+P	2.44 ^b	2.46 ^b (0.81)	2.49 ^b (1.21)	2.52 ^b (1.20)	2.55 ^b (1.19)	2.59 ^b (1.56)	2.63 ^b (1.54)
	CD value	0.045	0.045	0.022	0.011	0.049	0.037	0.024

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

obtained for the organoleptic parameters like appearance, colour, flavour, texture, taste and overall acceptability were in the order of 8.17, 8.19, 7.98, 7.94, 7.84 and 8.02 respectively and a total score of 48.14. Treatment T₄ secured the lowest scores for all the above said parameters.

In a similar fashion, the acceptance was maximum for the treatment T₂ in the set 2 of *varikka* variety and the total score of this treatment was 47.96. The mean scores attained by the treatment were 8.13, 8.20, 8.02, 7.91, 7.86 and 8.02 respectively for the appearance, colour, flavour, texture, taste and overall acceptability. Overall acceptability for the treatments T₁, T₂, T₃ and T₄ were 7.69, 8.02, 7.80, and 7.75 respectively. In both set1 set 2 the acceptance of food mixtures tends to increase from T₁ to T₂ and then decreased.

In the third set of experiments with *varikka* variety, the most acceptable treatment among the judges was T₃ (total score 48.08). From Table 7, it is clearly understood that the treatment T₃ secured the highest scores for all the organoleptic parameters. The scores were in the order of 8.15, 8.14, 7.97, 7.93, 7.88 and 8.01 for appearance, colour, flavour, texture, taste and overall acceptability. In this set, the variation T₄ secured least scores for organoleptic qualities.

The set 4 of *varikka* variety showed a similar trend like that of the *koozha* variety. The treatment T₃ was selected as the best combination by the judges and this particular food mixture scored 8.11, 8.16, 8.04, 7.95, 7.90 and 8.03 respectively for appearance, colour, flavour, texture, taste and overall acceptability and a total score of 48.19. The acceptance of the food mixtures tends to increase from T₁ to T₃ and then decreased. The treatment T₄ was the least accepted combination in the fourth set of *varikka* variety.

In the initial two sets, as observed in *koozha* variety, the treatment T₂ was scored as the best combination. When moving to the next two sets, viz, set 3 and 4, the treatment T₃ was the best combination. In all the four sets, the treatment T₄ was least

acceptable for the judging panel. Even though the treatment T₄ was on the last rank, the scores for the organoleptic evaluation was 7-8, which was within the acceptable range.

Kendall's coefficient of concordance was used to statistically analyse the data obtained during the organoleptic evaluation of different food mixture and the mean ranks were worked out and the results are depicted in Table 8. Based on the mean scores and mean rank scores, the best treatment from each of the four sets were selected for the further studies. Just like in the *koozha* variety, here also in set 1 and 2, the treatment T₂ scored the maximum score and selected for further studies. Whereas in set 3 and 4, the treatment T₃ was selected. The selected treatments along with their combination of ingredients are given in Table 8.

Table 8. Selected combinations of jackfruit based food mixtures (*varikka*)

Set	Combination	Treatment
1	60% JF+ 30% DSF+ 10%T	T ₂
2	60% JF+ 30% DSF+ 10%P	T ₂
3	50% JF+ 20% DSF+ 20% JSF+ 10% T	T ₃
4	50% JF+ 20% DSF+ 20% JSF+ 10% P	T ₃

JF-Jackfruit flour, DSF- Defatted soy flour, T- Tomato, P- Papaya

4.2. Optimisation of conditions for the growth of *L. acidophilus*

From the prepared food mixtures, best one from each set was selected for the optimisation process. The selected food mixtures were fermented with the probiotic strain *L. acidophilus* at various conditions and the optimum fermentation conditions were drawn based on the results. Variables such as substrate concentration, time of incubation, pH, temperature and population of *L. acidophilus* for inoculation were optimised.

4.2.1. Optimisation of substrate concentration

Each of the selected food mixtures were taken in three different quantities like 25 g, 50 g and 75 g and fermented as mentioned in section 3.3.1 and were fermented *L. acidophilus*. The fermented food mixtures were freeze dried and were enumerated for the viable count of *L. acidophilus*. Results are given in the Table 9.

Table 9. Viable count of *L. acidophilus* in food mixtures with different substrate concentrations

Quantity of substrates (g)		25	50	75
		Viable counts ($\times 10^9$ cfu/ml)		
<i>Koozha</i>	JF+DSF+T	62 (10.79)	84 (10.92)	29 (10.46)
	JF+DSF+P	61 (10.78)	77 (10.77)	23 (10.36)
	JF+DSF+JSF+T	66 (10.81)	88 (10.94)	36 (10.55)
	JF+DSF+JSF+P	64 (10.80)	83 (10.91)	33 (10.51)
<i>Varikka</i>	JF+DSF+T	54 (10.73)	78 (10.89)	30 (10.47)
	JF+DSF+P	52 (10.71)	74 (10.86)	28 (10.44)
	JF+DSF+JSF+T	58 (10.76)	83 (10.91)	37 (10.56)
	JF+DSF+JSF+P	56 (10.74)	78 (10.89)	34 (10.53)

All values are means of three independent enumerations

JF-Jackfruit flour, DSF- Defatted soy flour, T- Tomato, P- Papaya

Figures in parenthesis indicates log cfu/g

From the Table 9, it can be concluded that 50 g of the substrate concentration showed maximum growth of the probiotic organism. This trend was observed in both the *koozha* and *varikka* varieties. The viable count of probiotic organism varied from 23 (10.36 log cfu/ml) to 88 (10.94 log cfu/ml) $\times 10^9$ cfu/ml, in the *koozha* variety, whereas it was 28 (10.44 log cfu/ml) to 83 (10.91 log cfu/ml) $\times 10^9$ cfu/ml in the *varikka* variety. Minimum probiotic growth was observed in 75 g substrate concentration in both varieties.

The figure 1 given below reveals that the maximum probiotic growth was observed in the JF+DSF+JSF+T combination followed by JF+DSF+ JSF+ P in the *koozha* variety. The viable count of these combinations were 88 and 83 $\times 10^9$ cfu/ml respectively. In *varikka* variety also the above said combinations marked the maximum probiotic activity, with a viable count of 82 and 78 $\times 10^9$ cfu/ml respectively.

4.2.2. Optimisation of time of incubation

As the maximum probiotic growth was observed in 50 g substrate concentration, to get the desired time of incubation, 50 g of substrate from both the varieties were fermented for 18, 24 and 30 hours. After fermentation, it was freeze dried and enumerated for the viable count of *L. acidophilus*. The results of the above said experiment is depicted in the Table 10.

Table 10 represents the viable count of *L. acidophilus* at different time of incubation at 10^9 dilution. The combination JF+DSF+JSF+T followed by the combination JF+DSF+ JSF+P shows the maximum probiotic activity. In *koozha* variety, their viable counts were in the order of 79 and 74 $\times 10^9$ cfu/ml and in *varikka* variety it was 68 and 58 $\times 10^9$ cfu/ml respectively.

Table 10. Viable count of *L. acidophilus* in food mixtures with different time of incubation

Treatment (Food mixtures)		Time (hrs)		
		18	24	30
		Viable counts ($\times 10^9$ cfu/ml)		
<i>Koozha</i>	JF+DSF+T	18 (10.25)	52 (10.71)	26 (10.61)
	JF+DSF+P	14 (10.14)	48 (10.68)	24 (10.38)
	JF+DSF+JSF+T	32 (10.50)	79 (10.89)	39 (10.59)
	JF+DSF+JSF+P	29 (10.46)	74 (10.86)	32 (10.50)
<i>Varikka</i>	JF+DSF+T	24 (10.38)	49 (10.69)	29 (10.46)
	JF+DSF+P	21 (10.32)	36 (10.55)	27 (10.43)
	JF+DSF+JSF+T	29 (10.46)	68 (10.38)	43 (10.63)
	JF+DSF+JSF+P	25 (10.39)	58 (10.76)	31 (10.49)

All values are means of three independent enumerations

JF-Jackfruit flour, DSF- Defatted soy flour, T- Tomato, P- Papaya

Figures in parenthesis indicates log cfu/g

From figure 2, it is clear that in both the varieties, the combination of jackfruit flour along with defatted soy flour, jackfruit seed flour and tomato pulp produced the maximum probiotic growth.

4.2.3. Optimisation of pH

As a part of optimisation process, different pH levels were also standardised. pH levels of 4.5, 5.5 and 6.5 were tried out with the help of 20 per cent food grade citric acid. Fifty grams of the food mixtures from both varieties were adjusted to the above said pH levels and fermented for a period of 24 hours. The fermented food mixtures were freeze dried and enumerated to study their probiotic viability.

Table 11. Viable count of *L. acidophilus* in food mixtures at different pH levels

pH Treatment (Food mixtures)		4.5	5.5	6.5
		Viable counts ($\times 10^9$ cfu/ml)		
<i>Koozha</i>	JF+DSF+T	64 (10.80)	36 (10.55)	28 (10.44)
	JF+DSF+P	55 (10.74)	33 (10.51)	22 (10.34)
	JF+DSF+JSF+T	79 (10.89)	47 (10.67)	28 (10.44)
	JF+DSF+JSF+P	58 (10.76)	38 (10.57)	19 (10.27)
<i>Varikka</i>	JF+DSF+T	55 (10.74)	32 (10.50)	21 (10.32)
	JF+DSF+P	49 (10.69)	25 (10.39)	16 (10.20)
	JF+DSF+JSF+T	62 (10.79)	34 (10.53)	22 (10.34)
	JF+DSF+JSF+P	51 (10.70)	27 (10.43)	19 (10.27)

All values are means of three independent enumerations

JF-Jackfruit flour, DSF- Defatted soy flour, T- Tomato, P- Papaya

Figures in parenthesis indicates log cfu/g

The probiotic activity at 10^9 dilutions were enumerated and tabulated. The results are given in Table 11. From the data, it is clear that the optimum pH was found to be 4.5. At pH 4.5, the maximum growth in the *koozha* variety was 79×10^9 cfu/ml followed by 64×10^9 cfu/ml for the food mixtures JF+DSF+JSF+T and JF+DSF+JSF+ respectively.

In the *varikka* variety also maximum probiotic activity was exhibited by the food mixture JF+DSF+JSF+T followed by JF+DSF+ JSF+ P. The viable counts of these food mixtures were in the order of 62 and 55×10^9 cfu/ml respectively. In both the varieties, probiotic activity showed to decrease with an increase in the pH from 4.5 to 6.5. Figure. 3 gives the graphical representation of viability of the probiotic organism in different food mixtures at pH 4.5.

4.2.4. Optimisation of temperature for fermentation

Fermentation was carried out to decide the optimum temperature for the growth of probiotic bacteria. Three different temperature (37°C , 41°C and 45°C) were tried out. The optimum temperature was found to be 37°C and the results are given in Table 12.

It is clear from Table 12 that, maximum number of probiotic colonies were found in the food mixture JF+DSF+JSF+T followed by JF+DSF+JSF+P. The trend was similar in both the varieties and the viable count varied from 14 to 79×10^9 cfu/ml in the *koozha* variety and 11 to 63×10^9 cfu/ml in the *varikka* variety.

Table 12. Viable count of *L. acidophilus* in food mixtures at various temperatures

Temperature (°C)		37	41	45
		Viable counts ($\times 10^9$ cfu/ml)		
Treatment (Food mixtures)				
<i>Koozha</i>	JF+DSF+T	53 (10.72)	16 (10.20)	0
	JF+DSF+P	51 (10.70)	14 (10.14)	0
	JF+DSF+JSF+T	79 (10.89)	25 (10.39)	0
	JF+DSF+JSF+P	77 (10.88)	18 (10.25)	0
<i>Varikka</i>	JF+DSF+T	46 (10.66)	14 (10.14)	0
	JF+DSF+P	42 (10.62)	28 (10.44)	0
	JF+DSF+JSF+T	63 (10.79)	11 (10.04)	0
	JF+DSF+JSF+P	51 (10.70)	18 (10.25)	0

All values are means of three independent enumerations

JF-Jackfruit flour, DSF- Defatted soy flour, T- Tomato, P- Papaya

Figures in parenthesis indicates log cfu/g

4.2.4. Optimisation of population of *L. acidophilus* for inoculation

Fifty grams of the food mixtures each from *koozha* and *varikka* variety were taken and the pH was adjusted to 4.5, inoculated with 100, 200 and 300 μ l of *L. acidophilus* and incubated at 37°C for 24hours. After fermentation, the food mixtures were freeze dried and again enumerated at 10^9 dilution for the number of probiotic organism. Table 13 describes the results.

The probiotic count ranged from 42 to 89×10^9 cfu/ml in the *koozha* variety and 32 to 86×10^9 cfu/ml in the *varikka* variety. The probiotic count was tend to increase from 100 μ l to 300 μ l and the maximum growth was observed in 300 μ l concentration.

Table 13. Viable count of *L. acidophilus* in food mixtures at various inoculum concentrations

Concentration of inoculum (μ l)		100	200	300
		Viable counts ($\times 10^9$ cfu/ml)		
Treatment (Food mixtures)				
	<i>Koozha</i>	JF+DSF+T	47 (10.61)	65 (10.81)
JF+DSF+P		42 (10.62)	62 (10.79)	84 (10.92)
JF+DSF+JSF+T		54 (10.73)	75 (10.87)	89 (10.94)
JF+DSF+JSF+P		53 (10.72)	70 (10.84)	87 (10.93)
<i>Varikka</i>	JF+DSF+T	39 (10.59)	74 (10.86)	84 (10.92)
	JF+DSF+P	32 (10.50)	65 (10.81)	81 (10.90)
	JF+DSF+JSF+T	51 (10.70)	80 (10.90)	86 (10.94)
	JF+DSF+JSF+P	47 (10.67)	78 (10.89)	85 (10.93)

All values are means of three independent enumerations

JF-Jackfruit flour, DSF- Defatted soy flour, T- Tomato, P- Papaya

Figures in parenthesis indicates log cfu/g

In *koozha* variety, the maximum growth was reported in the food mixture containing JF+DSF+JSF+T followed by JF+DSF+ JSF+ P and their probiotic counts were 89 and 87×10^9 cfu/ml respectively. The same combination of food mixture reported the maximum viability in the *varikka* variant also. Here the viable counts were in the order of 86 and 85×10^9 cfu/ml respectively for JF+DSF+JSF+T ad JF+DSF+ JSF+P. Figure.5 shows the viable count of different food mixtures at the optimum inoculum concentration.

Thus it can be concluded that for all the treatments fermentation with fifty gram substrate concentration at 4.5 pH inoculated with 300µl, inoculated at 37 °C for 24 hours resulted in the production of food mixture with maximum probiotic organisms. This is in line with the desired value of probiotic organisms to be present in any probiotic foods, as specified by FSSAI (2016).

4.3 Development of fermented food mixture

The selected food mixtures from *koozha* and *varikka* variety were fermented at the optimum conditions stated earlier in this chapter. After the fermentation process, the food mixtures were freeze dried and finely powdered. The freeze dried powders were stored in laminated polyethylene pouches and further studies were carried out with this powder. Along with the fermented samples, unfermented samples were also freeze dried and stored as control.

4.3.1 Physico-chemical analysis of the developed food mixtures

4.3.1.1 Moisture content of the developed food mixtures

The developed food mixtures were packed in laminated polyethylene pouches and analysed for their nutritional qualities for a period of six months. Table 14. reveals the moisture content of fermented food mixtures (*koozha* and *varikka*) during storage.

Initially the moisture content of the developed fermented food mixtures (*koozha*) varied from 2.73 per cent to 2.77 per cent whereas that of *varikka* mixtures



Plate 5. *L.acidophilus* in MRS



Plate 6. Fermented food mixture

varied from 2.31 per cent to 2.49 per cent. The maximum moisture content in *koozha* and *varikka* was observed for the sample JF+DSF+JSF+T whereas the minimum was observed in the sample JF+DSF+T for *koozha* and JF+DSF+P for *varikka*. DMRT was done to statistically analyse the moisture content of different food mixtures. Moisture content of the food mixture JF+DSF+JSF+T was found to be significantly higher in both the varieties and the moisture content of JF+DSF+JSF+P and JF+DSF+P of *koozha* variety were comparable.

Moisture content of developed probiotic food mixtures were found to increase during storage. The per cent relative change in moisture was assessed and given in parenthesis. Among the two variants of food mixtures, the *koozha* jackfruit based food mixtures were found to have more moisture content initially as well as during storage. The moisture content of JF+DSF+JSF+T in *koozha* and *varikka* were 2.77 and 2.49 per cent respectively. On storage these values were increased to 3.19 and 2.69 per cent respectively.

Table 15. Comparison of moisture content of fermented food mixtures from *koozha* and *varikka* variety (%)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	2.780	2.780	2.810	2.848	2.953	3.043	3.140
<i>Varikka</i>	2.418	2.418	2.440	2.475	2.515	2.563	2.605
Mean difference	0.362	0.362	0.370	0.373	0.438	0.480	0.535
t value	8.414*	8.414*	8.451*	8.101*	8.966*	10.018*	10.940*
Significance	S	S	S	S	S	S	S

*Significant at 1%

The comparison of moisture content of *koozha* and *varikka* fermented food mixtures were done with the aid of independent 't' test. Results are depicted in Table 15. It is clear from the table that the moisture content of the *koozha* based probiotic food mixtures were significantly higher than the *varikka* food mixtures. The results were statistically significant during the entire storage period.

Table 16, deals with the moisture content of unfermented food mixture during storage. The results of DMRT showed that the moisture content of food mixtures vary significantly (at 5 % level) in both the varieties. The moisture content of unfermented food mixture(*koozha*) ranged from 2.33 per cent to 2.92 per cent initially, in which the moisture content was maximum in the JF+DSF+JSF+T (2.92 %) followed by the JF+DSF+JSF+P (2.68 %). In *varikka* mixtures, moisture varied from 2.01 per cent to 2.62 per cent, and the food mixture JF+DSF+JSF+T reported to have significantly higher moisture (2.62 %) followed by JF+DSF+JSF+P (2.32 %). Here also an increase in the moisture content was observed during storage and the figures in parenthesis indicates the per cent relative change in moisture content during storage.

To examine the effect of probiotic fermentation on moisture content of the food mixtures, independent 't' test was carried out. The test results are given in Table 17. No significant difference could be observed in the moisture content of fermented and unfermented food mixtures.

4.3.1.2 Titratable acidity of the developed food mixtures

Titrate acidity of the selected food mixtures were analysed by titrating against 0.01N NaOH and the maximum acidity was reported for JF+DSF+JSF+T (2.96 %) and minimum was reported for JF+DSF+P (2.32 %) in the fermented *koozha* food mixtures (Table 18). There was significant difference in the acidity of different food mixtures as evident from the results of DMRT. In the *varikka* food mixtures acidity ranged from 2.52 per cent to 2.73 per cent, of which JF+DSF+JSF+T (2.73 %) had significantly higher acidity followed by JF+DSF+JSF+P (2.71 %).

Table 16. Moisture content of unfermented food mixtures during storage (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	2.55 ^c	2.56 ^c (0.39)	2.59 ^c (1.17)	2.64 ^c (1.93)	2.69 ^c (1.89)	2.74 ^b (1.85)	2.81 ^a (2.55)
	JF+DSF+P	2.33 ^d	2.33 ^d (0.00)	2.41 ^d (3.43)	2.46 ^d (2.07)	2.52 ^d (2.43)	2.59 ^b (2.78)	2.64 ^a (1.93)
	JF+DSF+JSF+T	2.92 ^a	2.93 ^a (0.34)	2.97 ^a (1.36)	2.99 ^a (0.67)	3.01 ^a (0.68)	3.03 ^b (0.66)	3.15 ^a (3.96)
	JF+DSF+JSF+P	2.68 ^b	2.69 ^b (0.37)	2.71 ^b (0.74)	2.75 ^b (1.47)	2.81 ^b (2.18)	2.87 ^b (2.13)	2.98 ^a (3.83)
	CD value	0.023	0.014	0.026	0.024	0.025	0.017	0.019
<i>Varikka</i>	JF+DSF+T	2.13 ^c	2.13 ^c (0.00)	2.16 ^c (1.40)	2.20 ^c (1.85)	2.25 ^c (2.27)	2.36 ^c (4.88)	2.39 ^c (1.27)
	JF+DSF+P	2.01 ^d	2.02 ^d (0.49)	2.04 ^d (0.09)	2.17 ^d (6.37)	2.21 ^d (1.80)	2.26 ^d (2.26)	2.31 ^d (2.21)
	JF+DSF+JSF+T	2.62 ^a	2.62 ^a (0.00)	2.65 ^a (1.14)	2.69 ^a (1.50)	2.72 ^a (1.11)	2.75 ^a (1.10)	2.79 ^a (1.45)
	JF+DSF+JSF+P	2.32 ^b	2.32 ^b (0.00)	2.35 ^b (1.29)	2.39 ^b (1.70)	2.45 ^b (2.51)	2.49 ^b (1.63)	2.53 ^b (1.60)
	CD value	0.030	0.026	0.028	0.027	0.041	0.025	0.036

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

Table 17. Comparison of moisture content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	2.780	2.780	2.810	2.848	2.953	3.043	3.140
	UFM	2.620	2.628	2.670	2.705	2.758	2.808	2.895
	Mean difference	0.160	0.152	0.140	0.143	0.195	0.235	0.245
	t value	1.294 ^{NS}	1.214 ^{NS}	1.189 ^{NS}	1.326 ^{NS}	1.829 ^{NS}	2.395 ^{NS}	2.148 ^{NS}
<i>Varikka</i>	FM	2.418	2.418	2.440	2.475	2.515	2.563	2.605
	UFM	2.270	2.273	2.300	2.363	2.408	2.465	2.505
	Mean difference	0.148	0.145	0.140	0.112	0.107	0.098	0.100
	t value	1.057 ^{NS}	1.051 ^{NS}	1.002 ^{NS}	0.883 ^{NS}	0.871 ^{NS}	0.866 ^{NS}	0.894 ^{NS}

FM- Fermented food mixture, UFM- Unfermented food mixture

NS- Non significant

The titratable acidity of the probiotic (fermented) food mixtures during the storage period is given in Table.18. Acidity of all the fermented food mixtures tend to increase during the storage period and the per cent relative change throughout the storage period is indicated in parenthesis. In both the varieties, maximum acidity was observed for the food mixture JF+DSF+JSF+T followed by JF+DSF+JSF+P (initially and during storage). To compare the acidity of *koozha* and *varikka* based fermented food mixtures, independent ‘t’ was performed and the results are given in Table 19. Table 19 clarifies that the acidity of fermented food mixtures do not vary significantly among the two varieties of jackfruit.

Table 19. Comparison of titratable acidity of fermented *koozha* and *varikka* food mixtures (%)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	2.69	2.73	2.79	2.84	2.87	2.92	2.97
<i>Varikka</i>	2.62	2.65	2.77	2.81	2.86	2.91	2.95
Mean difference	0.07	0.07	0.02	0.02	0.01	0.01	0.01
t value	0.47 ^{NS}	0.51 ^{NS}	0.15 ^{NS}	0.16 ^{NS}	0.07 ^{NS}	0.10 ^{NS}	0.09 ^{NS}

NS Non-significant

Table 20 gives the titratable acidity of unfermented samples. As per the results of DMRT, the combinations JF+DSF+JSF+T (1.87 % in *koozha* and 1.63 % in *varikka*) and JF+DSF+JSF+P (1.79 in *koozha* and 1.54 % in *varikka*) were the food mixtures with significantly higher acidity in both varieties. The initial acidity of the food mixture JF+DSF+JSF+T (*koozha* variety) was 1.87 per cent which increased upto 2.13 per cent at the end of storage. The per cent relative change over the months of storage is

Table 18. Titratable acidity of fermented food mixtures (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	2.65 ^c	2.69 ^c (1.50)	2.75 ^c (2.23)	2.81 ^c (2.18)	2.87 ^c (2.13)	2.92 ^c (1.74)	2.98 ^c (2.05)
	JF+DSF+P	2.32 ^d	2.36 ^d (1.72)	2.40 ^d (1.69)	2.43 ^d (1.25)	2.48 ^d (2.05)	2.52 ^d (1.61)	2.55 ^d (1.19)
	JF+DSF+JSF+T	2.96 ^a	2.99 ^a (1.01)	3.03 ^a (1.33)	3.07 ^a (1.33)	3.12 ^a (2.97)	3.17 ^a (1.60)	3.21 ^a (1.26)
	JF+DSF+JSF+P	2.85 ^b	2.89 ^b (1.40)	2.91 ^b (0.69)	2.96 ^b (1.71)	2.99 ^b (1.01)	3.03 ^b (1.33)	3.09 ^b (1.08)
	CD value	0.017	0.015	0.014	0.012	0.021	0.014	0.018
<i>Varikka</i>	JF+DSF+T	2.53 ^c	2.57 ^c (1.58)	2.60 ^c (1.16)	2.64 ^c (1.53)	2.67 ^c (1.13)	2.71 ^c (1.49)	2.76 ^c (1.84)
	JF+DSF+P	2.52 ^d	2.55 ^d (1.19)	2.61 ^d (2.35)	2.66 ^d (1.91)	2.76 ^d (1.50)	2.84 ^d (2.89)	2.98 ^d (4.92)
	JF+DSF+JSF+T	2.73 ^a	2.76 ^a (1.09)	2.84 ^a (2.89)	2.95 ^a (3.87)	3.09 ^a (4.74)	3.14 ^a (1.61)	3.29 ^a (4.77)
	JF+DSF+JSF+P	2.71 ^b	2.74 ^b (1.10)	2.78 ^b (1.45)	2.86 ^b (2.87)	2.95 ^b (3.14)	3.01 ^b (2.03)	3.16 ^b (4.98)
	CD value	0.018	0.019	0.017	0.20	0.022	0.016	0.028

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

Table 20. Titratable acidity of unfermented food mixtures (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	1.36 ^d	1.39 ^d (2.20)	1.43 ^d (2.87)	1.46 ^d (2.09)	1.50 ^d (2.73)	1.55 ^d (3.33)	1.61 ^d (3.87)
	JF+DSF+P	1.45 ^c	1.49 ^c (2.75)	1.53 ^c (2.68)	1.56 ^c (1.96)	1.61 ^c (3.20)	1.67 ^c (3.72)	1.71 ^c (2.39)
	JF+DSF+JSF+T	1.87 ^a	1.90 ^a (1.60)	1.93 ^a (1.57)	1.98 ^a (2.59)	2.02 ^a (2.02)	2.07 ^a (2.47)	2.13 ^a (2.89)
	JF+DSF+JSF+P	1.79 ^b	1.82 ^b (1.67)	1.86 ^b (2.22)	1.90 ^b (2.15)	1.95 ^b (2.63)	2.01 ^b (3.07)	2.07 ^b (2.98)
	CD value	0.018	0.016	0.019	0.022	0.036	0.025	0.019
<i>Varikka</i>	JF+DSF+T	1.29 ^d	1.32 ^d (2.32)	1.36 ^d (3.30)	1.41 ^d (3.67)	1.47 ^d (4.25)	1.52 ^d (3.40)	1.59 ^d (4.60)
	JF+DSF+P	1.36 ^c	1.39 ^c (1.87)	1.43 ^c (2.87)	1.48 ^c (5.59)	1.53 ^c (3.37)	1.59 ^c (3.92)	1.66 ^c (4.40)
	JF+DSF+JSF+T	1.63 ^a	1.66 ^a (1.84)	1.71 ^a (3.01)	1.75 ^a (2.33)	1.80 ^a (2.85)	1.86 ^a (3.33)	1.92 ^a (3.22)
	JF+DSF+JSF+P	1.54 ^b	1.58 ^b (2.59)	1.62 ^b (2.53)	1.67 ^b (3.08)	1.71 ^b (2.39)	1.75 ^b (2.33)	1.81 ^b (3.42)
	CD value	0.020	0.017	0.019	0.021	0.024	0.018	0.017

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

indicated in parenthesis. In *varikka* variety the acidity values of JF+DSF+JSF+T were 1.63 per cent and one per cent initially and finally.

While comparing the titratable acidity of fermented and unfermented food mixtures, it was noticed that the acidity of the fermented food mixture was significantly higher than the unfermented food mixtures. The result was similar in case of *koozha* as well as *varikka* based jackfruit food mixtures. The results are given in Table 21.

4.3.1.3 Protein content of the developed food mixtures

The initial protein content of the fermented food mixtures ranged from 22.84 to 24.03 g/100g in *koozha* and 23.15 and 25.15 g/100g in *varikka* food mixtures. The DMRT analysis revealed that the protein content of different food mixtures vary significantly and the significant difference was observed in both *koozha* and *varikka* food mixtures (Table 22.). Maximum protein content was observed in the food mixture JF+DSF+JSF+T followed by JF+DSF+JSF+P. Unlike moisture and acidity, the protein content of the food mixtures got reduced throughout the storage period. Reduction in the protein content throughout the storage was expressed in percent relative change and indicated in parenthesis.

The maximum initial protein content exhibited by JF+DSF+JSF+T in *koozha* and *varikka* varieties and the values were 24.03 and 25.15 g/100g respectively. At the end of storage, the protein content became 22.86 and 23.98 g/100g respectively.

While comparing the protein contents of fermented food mixtures, it was observed that the *varikka* based food mixtures contain more protein than the *koozha* based, but the difference was not statistically significant. Table 23 gives the comparison of protein content of fermented food mixtures during storage.

Table 21. Comparison of titratable acidity of fermented and unfermented food mixtures from *koozha* and *varikka* variety (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	2.69	2.73	2.77	2.81	2.86	2.91	2.95
	UFM	1.61	1.65	1.68	1.48	1.77	1.82	1.88
	Mean difference	1.07	1.08	1.04	1.33	1.09	1.08	1.07
	t value	5.72*	5.81*	5.91*	5.75*	5.83*	5.74*	5.57*
<i>Varikka</i>	FM	2.62	2.655	2.798	2.845	2.878	2.928	2.973
	UFM	1.45	1.488	1.530	1.578	1.628	1.680	1.745
	Mean difference	1.16	1.167	1.268	1.267	1.25	1.248	1.228
	t value	12.06*	12.06*	10.98*	10.81*	10.81*	10.66*	10.80*

FM- Fermented food mixture, UFM unfermented food mixture

* Significant at 1%

Table 22. Protein content of the fermented food mixtures (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	22.93 ^c	22.80 ^c (0.56)	22.65 ^c (0.65)	22.46 ^b (0.83)	22.22 ^b (1.06)	22.10 ^c (0.54)	21.03 ^d (4.84)
	JF+DSF+P	22.84 ^d	22.71 ^d (0.57)	22.58 ^d (0.57)	22.35 ^c (1.01)	22.19 ^{bc} (0.71)	22.01 ^d (0.81)	21.96 ^c (0.22)
	JF+DSF+JSF+T	24.03 ^a	23.89 ^a (0.58)	23.68 ^a (0.87)	23.47 ^a (0.88)	23.28 ^a (0.80)	23.02 ^a (1.11)	22.86 ^b (0.69)
	JF+DSF+JSF+P	23.98 ^{ab}	23.83 ^{ab} (0.62)	23.65 ^b (0.75)	23.49 ^a (0.67)	23.27 ^a (0.93)	23.18 ^b (0.38)	22.96 ^a (0.94)
	CD value	0.016	0.019	0.021	0.020	0.019	0.016	0.026
<i>Varikka</i>	JF+DSF+T	23.15 ^d	23.04 ^d (0.47)	22.85 ^c (0.82)	22.67 ^d (0.78)	22.42 ^d (1.10)	22.16 ^d (1.15)	22.01 ^c (0.67)
	JF+DSF+P	23.36 ^c	23.23 ^c (0.55)	23.03 ^d (0.86)	22.85 ^c (0.78)	22.62 ^c (1.00)	22.49 ^c (0.57)	22.23 ^b (1.15)
	JF+DSF+JSF+T	25.15 ^a	25.05 ^a (0.39)	24.83 ^a (0.87)	24.61 ^a (0.88)	24.39 ^a (0.89)	24.15 ^a (0.98)	23.98 ^a (0.70)
	JF+DSF+JSF+P	25.06 ^b	24.89 ^b (0.67)	24.64 ^b (1.00)	24.44 ^b (0.81)	24.21 ^b (0.94)	24.03 ^b (0.74)	23.97 ^a (0.24)
	CD value	0.021	0.021	0.020	0.029	0.012	0.018	0.023

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parentesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 23. Comparison of protein content of fermented food mixtures on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	23.44	23.30	23.14	22.94	22.74	22.57	22.20
<i>Varikka</i>	24.18	24.05	23.83	23.64	23.41	23.20	23.04
Mean difference	-0.73	-0.53	-0.69	-0.67	-0.67	-0.63	-0.84
t value	-1.17 ^{NS}	-1.20 ^{NS}	-1.15 ^{NS}	-1.16 ^{NS}	-1.11 ^{NS}	-1.05 ^{NS}	-1.20 ^{NS}

NS- Non significant

Table 24 gives the data of protein content in unfermented food mixtures and according to the DMRT analysis, the protein content of the food mixtures vary significantly. On analysing the protein content of the unfermented food mixtures, it was observed that maximum protein content was observed for the combination JF+DSF+JSF+T (22.09 g/100g in *koozha* and 23.14 g/100g in *varikka*) followed by JF+DSF+JSF+P (21.58 g/100g in *koozha* and 22.37 g/100g in *varikka*). Like that of fermented food mixtures, the unfermented food mixtures also show a decrease in the protein content on storage. The per cent relative reduction of protein during storage is given in the parenthesis of Table 24. Even at the end of six months storage, both the fermented and unfermented food mixtures were reported to contain considerably fair amount of protein. The effect of probiotic fermentation on protein content of the food mixtures can be drawn from the Table 25.

Table 24. Protein content of the unfermented food mixtures (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	21.14 ^d	21.03 ^d (0.52)	20.77 ^c (1.23)	20.53 ^b (1.15)	20.38 ^c (0.73)	20.09 ^c (1.42)	19.86 ^c (1.14)
	JF+DSF+P	21.24 ^c	21.11 ^c (0.61)	19.88 ^d (5.82)	19.59 ^d (1.45)	19.34 ^d (1.27)	19.16 ^d (0.93)	18.04 ^d (5.84)
	JF+DSF+JSF+T	22.09 ^a	21.97 ^a (0.54)	21.76 ^a (0.95)	21.48 ^a (1.28)	21.21 ^a (1.25)	21.09 ^a (0.56)	20.96 ^a (0.61)
	JF+DSF+JSF+P	21.58 ^b	21.43 ^b (0.69)	21.21 ^b (1.02)	21.07 ^c (0.66)	20.88 ^b (0.90)	20.64 ^b (1.14)	20.33 ^b (1.50)
	CD value	0.030	0.032	0.030	0.031	0.036	0.028	0.037
<i>Varikka</i>	JF+DSF+T	21.76 ^c	21.63 ^c (0.59)	21.48 ^c (0.69)	21.21 ^c (1.25)	20.97 ^c (0.95)	20.74 ^c (1.09)	20.55 ^c (0.91)
	JF+DSF+P	21.24 ^d	21.02 ^d (1.03)	20.86 ^d (0.76)	20.75 ^d (0.52)	20.53 ^c (1.06)	20.29 ^d (1.16)	20.03 ^d (1.28)
	JF+DSF+JSF+T	23.14 ^a	23.03 ^a (0.47)	22.87 ^a (0.69)	22.69 ^a (0.78)	22.45 ^a (1.05)	22.29 ^a (0.71)	22.09 ^a (0.89)
	JF+DSF+JSF+P	22.37 ^b	22.19 ^b (0.80)	21.99 ^b (0.90)	21.78 ^b (0.95)	21.54 ^b (1.10)	21.37 ^b (0.78)	21.15 ^b (1.02)
	CD value	0.033	0.034	0.032	0.036	0.031	0.042	0.037

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 25. Comparison of protein content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	23.44	23.30	23.14	22.94	22.74	22.57	22.20
	UFM	21.51	21.38	20.90	20.66	20.45	20.24	19.79
	Mean difference	1.93	1.92	2.23	2.27	2.28	2.33	2.40
	t value	4.97*	5.00*	4.47*	4.43*	4.46*	4.53*	3.11*
<i>Varikka</i>	FM	24.18	24.05	23.83	23.64	23.41	23.20	23.04
	UFM	22.12	21.96	21.80	21.60	21.37	21.17	20.95
	Mean difference	2.05	2.08	2.03	2.03	2.03	2.03	2.09
	t value	3.04*	3.05*	3.03*	3.07*	3.07*	3.02*	3.00*

FM-fermented food mixture, UFM-unfermented food mixture; *Significant at 1%

A significant increase in the protein content was observed after probiotic fermentation in both *koozha* and *varikka* varieties. The fermented samples were found to have significantly higher protein throughout the storage period.

4.3.1.4. β carotene content of the developed food mixtures

β carotene content of the fermented food mixtures are given in Table 26. The results of DMRT analysis revealed that food mixture JF+DSF+JSF+P in both the varieties contain significantly higher β carotene. This combination contains 338.89 and 346.46 $\mu\text{g}/100\text{g}$ β carotene respectively for *koozha* and *varikka* food mixtures and this values were statistically superior to the other treatments. β carotene content was found to be minimum in the food mixture containing jackfruit flour, defatted soya flour and tomato pulp (310.49 and 309.87 $\mu\text{g}/100\text{g}$ for *koozha* and *varikka* respectively).

Table 26. β carotene content of fermented food mixtures ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	310.49 ^d	307.53 ^d (0.95)	292.17 ^d (4.99)	265.65 ^d (9.97)	241.53 ^d (9.07)	227.46 ^d (5.82)	212.15 ^d (6.73)
	JF+DSF+P	324.78 ^c	311.07 ^c (4.22)	296.54 ^c (4.67)	286.03 ^c (3.54)	269.58 ^c (5.75)	243.21 ^c (9.78)	221.91 ^c (8.75)
	JF+DSF+JSF+T	336.56 ^b	329.47 ^a (2.10)	315.67 ^a (4.18)	294.11 ^b (6.82)	274.08 ^b (6.81)	254.62 ^b (7.10)	239.14 ^b (6.07)
	JF+DSF+JSF+P	338.89 ^a	327.33 ^b (3.41)	314.01 ^b (4.07)	301.99 ^a (3.82)	286.53 ^a (5.11)	264.19 ^a (7.79)	241.87 ^a (8.44)
	CD value	7.54	7.94	7.64	6.94	7.28	6.80	7.38
<i>Varikka</i>	JF+DSF+T	309.87 ^d	301.49 ^d (2.70)	296.59 ^c (1.62)	287.64 ^c (3.01)	268.36 ^c (6.70)	242.29 ^d (9.71)	211.03 ^d (12.90)
	JF+DSF+P	315.56 ^c	308.46 ^c (2.24)	299.71 ^d (2.83)	284.59 ^d (5.04)	267.43 ^d (6.02)	245.53 ^c (8.18)	229.57 ^c (6.50)
	JF+DSF+JSF+T	338.18 ^b	324.56 ^b (4.02)	312.95 ^b (3.68)	302.07 ^b (3.36)	296.54 ^a (1.83)	281.32 ^a (5.13)	268.43 ^b (4.58)
	JF+DSF+JSF+P	346.46 ^a	338.73 ^a (2.23)	319.13 ^a (5.78)	304.98 ^a (4.71)	291.35 ^b (4.18)	280.04 ^b (3.88)	271.00 ^a (3.22)
	CD value	7.58	7.65	8.92	7.54	7.39	8.17	6.95

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

The results of Table 26 suggest that the β carotene content of the food mixtures got reduced during storage. In both varieties, the food mixture JF+DSF+JSF+P was containing maximum of the nutrient (β carotene) initially and at end of storage period. The per cent relative decrease in the nutrient content over the previous month is indicated in parenthesis. The β carotene content of JF+DSF+JSF+P (*koozha*) was 338.89 $\mu\text{g}/100\text{g}$ initially and reached 241.87 $\mu\text{g}/100\text{g}$ at the end of storage. The same combination of food mixture of *varikka* variety was found to contain 346.46 $\mu\text{g}/100\text{g}$ initially and end up the storage with a β carotene content of 271.00 $\mu\text{g}/100\text{g}$.

The independent 't' test performed between fermented food mixtures of *koozha* and *varikka* variety showed that there was no significant difference in the β carotene content of the two food mixtures. Results are given in the Table. 27.

In Table 28, the β carotene content of the unfermented food mixtures are given. In the unfermented samples also, the food mixture JF+DSF+JSF+P was reported to have significantly higher β carotene content than the rest of the food mixtures in both the varieties. Similar to that of fermented food mixtures, the nutrient decreased significantly during the storage period and the per cent decrease is indicated in parenthesis. JF+DSF+JSF+P (329.49 $\mu\text{g}/100\text{g}$ in *koozha* and 329.34 $\mu\text{g}/100\text{g}$ in *varikka*) was found to contain maximum β carotene initially and throughout the storage. The β carotene content of the food mixture JF+DSF+JSF+P at the end of storage was 238.84 $\mu\text{g}/100\text{g}$ (*koozha*) and 228.15 $\mu\text{g}/100\text{g}$ (*varikka*) respectively.

The β carotene contents of fermented as well as unfermented food mixtures were compared using the independent 't' test and the results are given in Table 29. It can be concluded from the table that, throughout the storage, the β carotene content was maximum in the fermented samples than the unfermented samples but there was no significant difference between the fermented and unfermented samples with respect to β carotene.

Table 27. Comparison of β carotene content of fermented food mixtures *koozha* and *varikka* variety ($\mu\text{g}/100\text{g}$)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	327.680	318.850	304.598	286.945	267.930	247.370	228.768
<i>Varikka</i>	327.518	318.310	307.095	294.820	280.920	262.295	245.008
Mean difference	0.162	0.540	-2.497	-7.875	-12.990	-14.925	-16.240
t value	0.015 ^{NS}	0.054 ^{NS}	-0.311 ^{NS}	-0.844 ^{NS}	-1.608 ^{NS}	-1.126 ^{NS}	-0.991 ^{NS}

NS- non significant

Table 28. β carotene content of unfermented food mixtures during storage ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	307.48 ^d	305.36 ^d (0.28)	290.97 ^d (4.71)	261.31 ^c (10.19)	239.65 ^d (8.28)	222.12 ^d (7.31)	204.05 ^d (8.13)
	JF+DSF+P	319.93 ^b	310.94 ^b (2.80)	292.12 ^b (6.05)	284.63 ^b (2.56)	262.19 ^c (7.88)	240.14 ^c (8.40)	214.45 ^c (10.69)
	JF+DSF+JSF+T	318.68 ^c	313.43 ^b (1.64)	303.04 ^a (3.31)	296.67 ^a (2.10)	268.96 ^b (9.59)	246.39 ^b (8.12)	229.71 ^b (6.76)
	JF+DSF+JSF+P	329.49 ^a	318.67 ^a (3.28)	309.54 ^a (2.86)	296.31 ^a (4.27)	286.35 ^a (3.36)	266.12 ^a (7.06)	238.84 ^a (10.25)
	CD value	0.92	0.89	1.04	0.91	0.88	1.06	0.95
<i>Varikka</i>	JF+DSF+T	310.64 ^d	301.49 ^c (2.94)	286.59 ^c (4.94)	267.64 ^d (6.61)	238.36 ^d (10.94)	232.29 ^c (2.54)	201.03 ^d (13.45)
	JF+DSF+P	312.33 ^c	296.54 ^d (5.05)	276.03 ^d (6.91)	259.58 ^c (5.95)	243.21 ^c (6.30)	231.91 ^d (4.64)	212.34 ^c (8.43)
	JF+DSF+JSF+T	321.62 ^b	314.01 ^b (2.36)	301.99 ^a (3.82)	286.53 ^a (5.11)	264.19 ^a (7.79)	241.87 ^a (8.44)	225.69 ^b (6.68)
	JF+DSF+JSF+P	329.34 ^a	317.53 ^a (3.58)	292.17 ^b (7.83)	275.65 ^b (5.65)	251.53 ^b (8.75)	237.46 ^b (5.59)	228.15 ^a (3.92)
	CD value	1.39	1.22	1.01	0.93	0.75	0.84	0.95

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 29. Comparison of β carotene content of fermented and unfermented food mixtures from *koozha* and *varikka* variety ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	327.68	318.85	304.59	286.94	267.93	247.37	228.67
	UFM	318.89	312.10	298.91	284.73	264.28	243.69	221.67
	Mean difference	8.78	6.75	5.68	2.21	3.64	3.67	7.00
	t value	1.110 ^{NS}	1.084 ^{NS}	0.760 ^{NS}	0.194 ^{NS}	0.269 ^{NS}	0.306 ^{NS}	0.667 ^{NS}
<i>Varikka</i>	FM	327.51	318.31	307.09	294.82	280.92	262.29	245.00
	UFM	318.48	307.39	289.19	279.82	266.82	253.38	234.30
	Mean difference	9.03	10.91	17.90	15.00	14.09	8.91	10.70
	t value	0.921 ^{NS}	1.123 ^{NS}	2.349 ^{NS}	1.750 ^{NS}	1.369 ^{NS}	0.711 ^{NS}	0.650 ^{NS}

FM- Fermented food mixture, UFM- Unfermented food mixture

NS Non-significant

4.3.1.5 Crude fibre content of the developed food mixtures

The tabulated results of crude fibre content of fermented food mixtures during storage is shown in Table 30. The food mixtures JF+DSF+JSF+P was found to have significantly higher fibre content for both varieties (1.84 and 1.56 g/100 initially for *koozha* and *varikka* respectively) followed by JF+DSF+JSF+T (1.68 and 1.54g/100g respectively for *koozha* and *varikka*). The minimum crude fibre content of *koozha* was observed for JF+DSF+T and JF+DSF+P in *varikka* variety and the results of DMRT shows that the fibre content of different food mixtures differ significantly. It is evident from the table that the fibre content of the fermented food mixtures decreased during storage. There was a reduction in the fibre content of both *koozha* as well as *varikka* based food mixtures during storage. The per cent relative decrease in crude fibre content was calculated and given in parenthesis.

On comparing the crude fibre content of fermented and unfermented food mixtures of *koozha* and *varikka* variety (Table 31), it was observed that the fibre content of *koozha* based food mixtures were significantly high. The *koozha* based fermented food mixture contained significantly higher amounts of fibre initially (1.668 g/100g) and throughout the storage (1.410 g/100g finally).

Table 31. Comparison of crude fibre content of the fermented food mixtures *koozha* and *varikka* variety

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	1.66	1.63	1.60	1.55	1.51	1.46	1.41
<i>Varikka</i>	1.48	1.43	1.39	1.34	1.29	1.25	1.19
Mean difference	0.18	0.20	0.21	0.21	0.22	0.21	0.21
t value	2.48*	2.64*	2.75*	2.68*	2.94*	2.78*	2.98*

*Significant at 1%

Table 30. Crude fibre content of the fermented food mixtures during storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	1.56 ^d	1.52 ^d (2.56)	1.48 ^d (2.63)	1.42 ^d (4.05)	1.38 ^d (2.81)	1.32 ^d (4.34)	1.28 ^d (3.03)
	JF+DSF+P	1.59 ^c	1.56 ^c (1.88)	1.51 ^c (3.20)	1.46 ^c (3.31)	1.42 ^c (2.73)	1.38 ^c (2.73)	1.33 ^c (3.62)
	JF+DSF+JSF+T	1.68 ^b	1.66 ^b (1.19)	1.64 ^b (1.20)	1.60 ^b (2.43)	1.55 ^b (3.12)	1.50 ^b (3.12)	1.45 ^b (3.33)
	JF+DSF+JSF+P	1.84 ^a	1.81 ^a (1.63)	1.78 ^a (1.65)	1.73 ^a (2.80)	1.69 ^a (2.31)	1.64 ^a (2.95)	1.58 ^a (3.65)
	CD value	0.098	0.098	0.018	0.018	0.016	0.020	0.024
<i>Varikka</i>	JF+DSF+T	1.43 ^c	1.38 ^c (3.49)	1.34 ^c (2.89)	1.29 ^c (3.73)	1.25 ^c (3.10)	1.21 ^c (3.2)	1.16 ^c (4.13)
	JF+DSF+P	1.41 ^d	1.36 ^d (3.54)	1.33 ^d (2.20)	1.28 ^d (3.75)	1.24 ^d (3.12)	1.20 ^d (3.22)	1.14 ^d (5.00)
	JF+DSF+JSF+T	1.54 ^b	1.50 ^b (2.59)	1.45 ^b (3.33)	1.39 ^b (4.13)	1.33 ^b (4.31)	1.29 ^b (3.00)	1.23 ^b (4.65)
	JF+DSF+JSF+P	1.56 ^a	1.51 ^a (3.20)	1.45 ^a (3.97)	1.41 ^a (2.75)	1.34 ^a (4.96)	1.30 ^a (2.98)	1.25 ^a (3.84)
	CD value	0.017	0.017	0.032	0.029	0.032	0.016	0.025

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 32, depicts the crude fibre content of unfermented food mixtures. The crude fibre content was found to be within a range of 1.45 g/100g (*varikka*) to 1.94 g/100g (*koozha*) in the unfermented food mixtures. The maximum fibre content was observed in the mixtures containing jackfruit seed flour *i.e.* JF+DSF+JSF+P (1.94 and 1.85g/100g in *koozha* and *varikka* respectively) followed by JF+DSF+JSF+T (1.90 and 1.79 g/100g in *koozha* and *varikka* respectively). The fibre content was found to decrease over the storage period of six months and the per cent relative decrease over the months of storage are shown in parenthesis.

Table 33 is the result of independent 't' test between crude fibre content of fermented and unfermented food mixtures. It is evident from the table that the crude fibre of *koozha* variety is significantly higher than the *varikka* variety in both the fermented and unfermented samples throughout the storage period.

4.3.1.6 TSS content of the developed food mixtures

TSS content of fermented food mixtures (Table 34) were found to range from 12.51 to 13.13 °brix in *koozha* and 12.58 to 13.16 °brix in *varikka* variety and the TSS content of the food mixtures vary significantly, as it is evident from the DMRT analysis. The maximum TSS was reported in JF+DSF+P (13.13 °brix) combination, followed by JF+DSF+JSF+P (12.94 °brix) combination in *koozha* variety whereas in *varikka* variety, it was 13.16 °brix (JF+DSF+P) and 12.91 °brix (JF+DSF+JSF+P). The TSS content of the fermented food mixtures were found to increase during storage and the per cent relative change in TSS over the previous month is indicated in parenthesis. At the end of storage, maximum TSS was observed for JF+DSF+P (13.27 °brix finally) in *koozha* and *varikka* (13.37 °brix) based food mixtures.

Table 35 represents the comparative changes in the TSS contents of fermented *koozha* and *varikka* food mixtures on storage. It is evident from the table that there is no significant difference in the TSS content of *koozha* and *varikka* based food mixtures.

Table 32. Crude fibre content of unfermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSFT	1.87 ^c	1.83 ^c (2.13)	1.79 ^c (2.18)	1.75 ^c (2.23)	1.72 ^c (1.71)	1.68 ^c (2.32)	1.62 ^c (3.57)
	JF+DSF+P	1.76 ^d	1.73 ^d (1.70)	1.68 ^d (2.89)	1.63 ^d (2.97)	1.59 ^d (2.45)	1.54 ^d (3.14)	1.50 ^d (2.59)
	JF+DSF+JSF+T	1.90 ^b	1.87 ^b (1.57)	1.84 ^a (1.60)	1.80 ^a (2.17)	1.76 ^a (2.22)	1.72 ^a (2.27)	1.64 ^b (4.65)
	JF+DSF+JSF+P	1.94 ^a	1.92 ^a (1.03)	1.83 ^b (4.68)	1.79 ^b (2.18)	1.74 ^b (2.79)	1.70 ^b (2.29)	1.65 ^a (2.94)
	CD value	0.021	0.018	0.015	0.018	0.019	0.021	0.025
<i>Varikka</i>	JF+DSFT	1.45 ^d	1.42 ^d (2.06)	1.38 ^d (2.81)	1.34 ^d (2.89)	1.29 ^d (3.73)	1.24 ^d (3.87)	1.18 ^d (4.83)
	JF+DSF+P	1.65 ^c	1.63 ^c (1.21)	1.59 ^c (2.45)	1.55 ^c (2.51)	1.50 ^c (3.22)	1.46 ^c (2.66)	1.43 ^c (2.05)
	JF+DSF+JSF+T	1.79 ^b	1.77 ^b (1.11)	1.74 ^b (1.69)	1.70 ^b (2.29)	1.65 ^b (2.94)	1.63 ^b (1.21)	1.59 ^b (2.45)
	JF+DSF+JSF+P	1.85 ^a	1.82 ^a (1.62)	1.78 ^a (2.19)	1.74 ^a (2.24)	1.69 ^a (2.87)	1.66 ^a (1.77)	1.62 ^a (2.40)
	CD value	0.011	0.013	0.023	0.025	0.018	0.016	0.021

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 33. Comparison of crude fibre content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	1.668	1.638	1.603	1.553	1.510	1.460	1.390
	UFM	1.868	1.838	1.785	1.743	1.703	1.660	1.603
	Mean difference	-0.2	-0.2	-0.182	-0.19	-0.193	-0.2	-0.213
	t value	-2.170*	-2.627*	-2.347*	-2.354*	-2.408*	-2.449*	-2.403*
<i>Varikka</i>	FM	1.485	1.438	1.393	1.343	1.290	1.250	1.195
	UFM	1.685	1.660	1.623	1.583	1.408	1.498	1.445
	Mean difference	-0.2	-0.222	-0.23	-0.24	-0.118	-0.248	-0.25
	t value	-2.070*	-2.276*	-2.838*	-2.485*	-2.572*	-2.476*	-2.496*

FM- Fermented food mixtures, UFM- Unfermented food mixtures

*Significant at 5% level

Table 34. TSS content of fermented food mixtures on storage (⁰ brix)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	12.51 ^d	12.51 ^d (0.00)	12.56 ^d (0.39)	12.60 ^d (0.31)	12.62 ^d (0.15)	12.64 ^d (0.15)	12.67 ^d (0.23)
	JF+DSF+P	13.13 ^a	13.14 ^a (0.07)	13.17 ^a (0.22)	13.19 ^a (0.15)	13.22 ^a (0.22)	13.24 ^a (0.15)	13.27 ^a (0.22)
	JF+DSF+JSF+T	12.80 ^c	12.80 ^c (0.00)	12.81 ^c (0.07)	12.83 ^c (0.15)	12.86 ^c (0.23)	12.89 ^c (0.23)	12.93 ^c (0.31)
	JF+DSF+JSF+P	12.94 ^b	12.94 ^b (0.00)	12.96 ^b (0.15)	12.96 ^b (0.00)	13.01 ^b (0.38)	13.09 ^b (0.61)	13.15 ^b (0.45)
	CD value	0.012	0.017	1.98	0.96	0.018	0.016	1.34
<i>Varikka</i>	JF+DSF+T	12.58 ^d	12.59 ^d (0.07)	12.62 ^d (0.23)	12.65 ^d (0.23)	12.69 ^d (0.31)	12.73 ^d (0.31)	12.77 ^d (0.31)
	JF+DSF+P	13.16 ^a	13.17 ^a (0.07)	13.20 ^a (0.22)	13.24 ^a (0.30)	13.27 ^a (0.22)	13.32 ^a (0.37)	13.37 ^a (0.37)
	JF+DSF+JSF+T	12.75 ^c	12.76 ^c (0.08)	12.79 ^c (0.23)	12.81 ^c (0.15)	12.84 ^c (0.23)	12.87 ^c (0.23)	12.91 ^c (0.31)
	JF+DSF+JSF+P	12.91 ^b	12.91 ^b (0.00)	12.93 ^b (0.15)	12.96 ^b (0.23)	12.99 ^b (0.24)	13.03 ^b (0.30)	13.09 ^b (0.46)
	CD value	0.012	0.012	0.017	0.016	0.023	0.015	0.013

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 35. Comparison of TSS content of fermented food mixtures on storage (⁰brix)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	12.84	12.84	12.87	12.89	12.92	12.96	13.00
<i>Varikka</i>	12.85	12.85	12.88	12.91	12.94	12.98	13.03
Mean difference	-0.00	-0.01	-0.01	-0.02	-0.02	-0.02	-0.03
t value	-0.02 ^{NS}	-0.05 ^{NS}	-0.08 ^{NS}	-0.14 ^{NS}	-0.11 ^{NS}	-0.12 ^{NS}	-0.16 ^{NS}

NS -Non significant

On analysing, it is clear from Table 36 that the TSS content of unfermented food mixtures increase from during storage and the per cent relative change is indicated in parenthesis. The maximum TSS content among the unfermented food mixtures was observed for JF+DSF+P in both varieties (13.64⁰ brix in *koozha* and 13.60⁰ brix in *varikka*). The same food mixture continue to contain the maximum total soluble sugars throughout the storage period. And the results were statistically significant.

The comparison of TSS content of fermented and unfermented food mixtures of both *koozha* and *varikka* based food mixtures were done with independent ‘t’ test and the results are given in Table 37. It is revealed from the table that the unfermented food mixture contain significantly higher amount of TSS.

4.3.1.7 Reducing sugar content of the developed food mixtures

Table 38 gives the reducing sugar of fermented food mixtures on storage. Reducing sugar was found to be within 4.17 to 4.68 g/100g in fermented *koozha* food mixtures and 4.12 to 4.65 g/100g in fermented *varikka* food mixtures (initial levels). DMRT was done to statistically analyse the results and significantly higher reducing sugar was observed in JF+DSF+P (4.68 g/100g in *koozha* and 4.65 g/100g in *varikka*)

Table 36. TSS content of unfermented food mixtures (⁰ brix)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	13.29 ^c	13.29 ^c (0.00)	13.31 ^d (0.15)	13.35 ^d (0.29)	13.38 ^d (0.22)	13.43 ^d (0.37)	13.47 ^d (0.29)
	JF+DSF+P	13.64 ^a	13.64 ^a (0.00)	13.66 ^b (0.14)	13.69 ^b (0.21)	13.71 ^b (0.14)	13.75 ^b (0.29)	13.79 ^b (0.29)
	JF+DSF+JSF+T	13.50 ^b	13.50 ^b (0.00)	13.51 ^c (0.07)	13.54 ^c (0.22)	13.58 ^c (0.29)	13.62 ^c (0.29)	13.66 ^c (0.29)
	JF+DSF+JSF+P	13.11 ^d	13.12 ^d (0.07)	13.14 ^a (0.14)	13.16 ^a (0.14)	13.20 ^a (0.28)	13.25 ^a (0.35)	13.29 ^a (0.28)
	CD value	0.328	0.013	0.032	0.022	0.028	0.016	1.09
<i>Varikka</i>	JF+DSF+T	13.34 ^c	13.34 ^c (0.300)	13.36 ^c (0.14)	13.39 ^c (0.22)	13.43 ^c (0.29)	13.47 ^c (0.29)	13.51 ^c (0.29)
	JF+DSF+P	13.49 ^b	13.51 ^b (0.14)	13.52 ^b (0.07)	13.55 ^b (0.21)	13.57 ^b (0.14)	13.61 ^b (0.29)	13.65 ^b (0.28)
	JF+DSF+JSF+T	13.60 ^a	13.61 ^a (0.07)	13.64 ^a (0.21)	13.66 ^a (0.14)	13.67 ^a (0.07)	13.70 ^a (0.21)	13.71 ^a (0.07)
	JF+DSF+JSF+P	13.15 ^d	13.15 ^d (0.00)	13.17 ^d (0.14)	13.19 ^d (0.14)	13.23 ^d (0.28)	13.26 ^d (0.21)	13.31 ^d (0.35)
	CD value	0.018	0.019	0.017	0.019	0.034	0.016	0.025

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

Table 37. Comparison of TSS content of fermented and unfermented food mixtures (^o brix)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	12.850	12.858	12.855	12.915	12.948	12.988	13.035
	UFM	13.695	13.703	13.723	13.748	13.775	13.810	13.845
	Mean difference	-0.845	-0.845	-0.868	-0.833	-0.827	-0.822	-0.81
	t value	-4.041*	-4.052*	-4.027*	-3.999*	-3.972*	-3.941*	-3.791*
<i>Varikka</i>	FM	12.845	12.848	12.875	12.805	12.968	12.965	13.055
	UFM	13.635	13.638	13.655	13.685	13.718	13.763	13.803
	Mean difference	-0.79	-0.79	-0.78	-0.88	-0.75	-0.798	-0.748
	t value	-3.633*	-3.585*	-3.570*	-3.719*	-3.666*	-3.655*	-3.634*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 5% level

Table 38. Reducing sugar content of the fermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+ T	4.43 ^b	4.43 ^b	4.45 ^b (0.45)	4.48 ^b (0.67)	4.51 ^b (0.67)	4.54 ^b (0.66)	4.60 ^b (1.30)
	JF+DSF+P	4.68 ^a	4.68 ^a	4.70 ^a (0.42)	4.71 ^a (0.21)	4.74 ^a (0.64)	4.76 ^a (0.42)	4.81 ^a (1.03)
	JF+DSF+JSF+T	4.17 ^d	4.17 ^d	4.19 ^d (0.47)	4.22 ^d (0.71)	4.25 ^d (0.71)	4.28 ^d (0.70)	4.33 ^d (1.15)
	JF+DSF+JSF+P	4.28 ^c	4.28 ^c	4.30 ^c (0.46)	4.32 ^c (0.46)	4.35 ^c (0.69)	4.37 ^c (0.45)	4.41 ^c (0.90)
	CD value	0.027	0.019	0.035	0.028	0.043	0.048	0.029
<i>Varikka</i>	JF+DSF+T	4.46 ^b	4.46 ^b	4.48 ^b (0.44)	4.51 ^b (0.66)	4.53 ^b (0.44)	4.58 ^b (1.10)	4.63 ^b (1.07)
	JF+DSF+P	4.65 ^a	4.65	4.67 (0.43)	4.69 (0.42)	4.73 (0.85)	4.76 (0.63)	4.83 ^a (1.44)
	JF+DSF+JSF+T	4.12 ^d	4.13 ^d	4.15 ^d (0.48)	4.17 ^d (0.48)	4.21 ^d (0.95)	4.23 ^d (0.47)	4.26 ^d (0.70)
	JF+DSF+JSF+P	4.37 ^c	4.37 ^c	4.39 ^c (0.45)	4.43 ^c (0.91)	4.46 ^c (0.67)	4.5 ^c (0.89)	4.54 ^c (0.88)
	CD value	0.027	0.027	0.019	0.043	0.027	0.048	0.036

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

followed by JF+DSF+ T (4.43 g/100g in *koozha* and 4.46 g/100g in *varikka*). During storage, the reducing sugar content of the food mixtures were found to increase gradually. The food mixture JF+DSF+P continue to have maximum reducing sugar till the end of storage period. The increase in reducing sugar content of the fermented food mixtures were evident from the second month of storage. The changes in the reducing sugar of the food mixtures during storage was represented in parenthesis as per cent relative change over the previous month.

Table below (Table 39) is the representation of comparison of reducing sugar content of fermented food mixtures (*koozha* and *varikka*) on storage. The reducing sugar content of the fermented food mixtures were compared using independent 't' test. On observing the table, no significant difference was seen in the reducing sugar content of *koozha* and *varikka* based fermented food mixtures. The reducing sugar content tends to increase during storage and throughout the storage period, no significant difference was observed in the total sugar content of the food mixtures.

Table 39. Comparison of reducing sugar content of fermented food mixtures (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	4.39	4.39	4.41	4.43	4.46	4.44	4.53
<i>Varikka</i>	4.40	4.40	4.42	4.45	4.48	4.51	4.56
Mean difference	-0.01	-0.01	-0.01	-0.01	-0.02	-0.07	-0.02
t value	-0.06 NS	-0.0 ^{NS}	-0.08 ^{NS}	-0.11 ^{NS}	-0.13 ^{NS}	-0.19 ^{NS}	-0.14 ^{NS}

NS- Non significant

On observing Table 40, it is clear that the reducing sugar content of the food mixture JF+DSF+P (7.61 g/100g) was found to be significantly higher in *koozha* and that of JF+DSF+JSF+P (7.64 g/100g) was found to be higher in *varikka* based food mixtures. On storage, the reducing sugar content tend to increase and the relative change is indicated in parenthesis.

Table 41 gives the comparison of reducing sugar content of fermented and unfermented food mixtures. It could be concluded from the table that the unfermented food mixtures of both *koozha* and *varikka* variety contained significantly higher amount of reducing sugar. The process of fermentation resulted in the reduction of reducing sugar content. On the other hand, the reducing sugar content of all the food mixtures tends to rise gradually during the storage period.

4.3.1.8 Total sugar content of the developed food mixtures

The total sugar content of fermented food mixtures ranged from 11.58 to 11.80 (*koozha*) and 11.03 to 12.45 g/100g (*varikka*) with maximum total sugar content in JF+DSF+JSF+P. Total sugar contents of all the food mixtures vary significantly as revealed from the statistical analysis (DMRT). From the second month of storage, an increase was observed in the total sugar content of the fermented food mixtures and the percent relative change is indicated in parenthesis. Table 42 shows the results in detail.

The total sugar content of fermented *koozha* and *varikka* food mixtures were compared statistically using the 't' test and there was no significant difference observed in the total sugar content of the *koozha* and *varikka* based fermented food mixtures. The total sugar content of the food mixtures were found to increase throughout the storage period. Table 43 is the comparison of total sugar content of *koozha* and *varikka* based probiotic food mixtures.

Table 40. Reducing sugar content of the unfermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	6.68 ^c	6.72 ^c (0.59)	6.74 ^c (0.29)	6.77 ^c (0.44)	6.81 ^c (0.59)	6.84 ^c (0.43)	6.87 ^c (0.43)
	JF+DSF+P	7.61 ^a	7.63 ^a (0.26)	7.66 ^a (0.39)	7.69 ^a (0.39)	7.72 ^a (0.39)	7.75 ^a (0.38)	7.79 ^a (0.51)
	JF+DSF+JSF+T	5.67 ^d	5.75 ^d (1.41)	5.84 ^d (1.56)	5.17 ^d (11.47)	5.20 ^d (0.58)	5.45 ^d (4.58)	5.66 ^d (3.85)
	JF+DSF+JSF+P	7.43 ^b	7.46 ^b (0.40)	7.49 ^b (0.40)	7.53 ^b (0.53)	7.59 ^b (0.79)	7.62 ^b (0.39)	7.66 ^b (0.52)
	CD value	0.137	0.035	0.019	0.028	0.036	0.029	0.014
<i>Varikka</i>	JF+DSF+T	6.73 ^c	6.76 ^c (0.44)	6.80 ^c (0.59)	6.82 ^c (0.29)	6.86 ^c (0.58)	6.89 ^c (0.43)	6.93 ^c (0.58)
	JF+DSF+P	7.51 ^b	7.53 ^b (0.26)	7.57 ^b (0.53)	7.59 ^b (0.26)	7.61 ^b (0.26)	7.63 ^b (0.26)	7.66 ^b (0.39)
	JF+DSF+JSF+T	6.67 ^d	6.69 ^d (0.29)	6.73 ^d (0.59)	6.75 ^d (0.29)	6.79 ^d (0.59)	6.83 ^d (0.58)	6.85 ^d (0.29)
	JF+DSF+JSF+P	7.64 ^a	7.66 ^a (0.26)	7.69 ^a (0.39)	7.73 ^a (0.52)	7.76 ^a (0.38)	7.80 ^a (0.51)	7.84 ^a (0.51)
	CD value	0.067	0.049	0.058	0.038	0.137	0.035	0.094

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

Table 41. Comparison of reducing sugar content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	4.400	4.403	4.423	4.450	4.483	4.518	4.560
	UFM	7.318	7.160	7.198	7.223	7.255	7.288	7.320
	Mean difference	-2.918	-2.757	-2.775	-2.773	-2.772	-2.77	-2.76
	t value	-9.880*	-10.260*	-10.146*	-10.025*	-10.171*	-10.154*	-9.985*
<i>Varikka</i>	FM	4.390	4.390	4.410	4.443	4.463	4.44	4.538
	UFM	6.848	6.890	6.933	6.790	6.830	6.915	6.995
	Mean difference	-2.458	-2.5	-2.523	-2.347	-2.367	-2.475	-2.457
	t value	-5.404*	-5.653*	-5.869*	-4.024*	-4.019*	-4.508*	-4.907*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 1%

Table 42. Total sugar content of fermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	11.69 ^c	11.69 ^c	11.71 ^c (1.58)	11.74 ^c (0.26)	11.76 ^c (0.35)	11.80 ^c (0.35)	11.84 ^c (0.44)
	JF+DSF+P	11.58 ^d	11.58 ^d	11.59 (0.09)	11.62 (0.27)	11.67 (0.45)	11.71 (0.35)	11.74 (0.26)
	JF+DSF+JSF+T	11.73 ^b	11.73 ^b	11.75 ^b (0.16)	11.77 ^b (0.08)	11.80 ^b (0.25)	11.83 ^b (0.25)	11.87 ^b (0.33)
	JF+DSF+JSF+P	11.80 ^a	11.80 ^a	11.82 ^a (0.17)	11.85 ^a (0.17)	11.89 ^a (0.34)	11.92 ^a (0.43)	11.95 ^a (0.34)
	CD value	0.138	0.178	1.213	0.094	0.186	0.019	0.026
<i>Varikka</i>	JF+DSF+T	11.33 ^c	11.33 ^c	11.37 ^c (0.26)	11.40 ^c (0.26)	11.42 ^c (0.17)	11.45 ^c (0.26)	11.49 ^c (0.34)
	JF+DSF+P	11.03 ^d	11.03 ^d	11.06 ^d (0.27)	11.09 ^d (0.27)	11.11 ^d (0.18)	11.14 ^d (0.27)	11.17 ^d (0.27) ^b
	JF+DSF+JSF+T	11.84 ^b	11.84 ^b	11.86 ^b (0.16)	11.89 ^b (0.25)	11.93 ^b (0.33)	11.97 ^b (0.33)	11.02 (8.62)
	JF+DSF+JSF+P	12.45 ^a	12.45 ^a	12.46 (0.08)	12.49 (0.24)	12.51 (0.16)	12.53 (0.15)	12.55 (0.15)
	CD value	0.144	0.135	0.169	0.178	0.026	0.018	0.035

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

Table 43. Comparison of total sugar content of fermented food mixtures (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	11.70	11.70	11.71	11.74	11.78	11.81	11.72
<i>Varikka</i>	11.66	11.66	11.68	11.72	11.74	11.77	11.56
Mean difference	0.04	0.04	0.03	0.02	0.04	0.04	0.16
t value	0.11 ^{NS}	0.11 ^{NS}	0.09 ^{NS}	0.08 ^{NS}	0.12 ^{NS}	0.13 ^{NS}	0.42 ^{NS}

NS- Non significant

The total sugar content of unfermented food mixtures are given in Table 44. From the table, it is clear that the total sugar content varies from 19.98 to 20.89 g/100g in *koozha* based food mixtures and 20.03 to 21.07 g/100g in *varikka* based food mixtures. The food mixture JF+DSF+JSF+P was found to contain significantly higher total sugar in *koozha* variety (20.89 g/100g). The total sugar content of the food mixture JF+DSF+JSF+P was found to be on par with that of JF+DSF+JSF+T in *varikka* variety. Both varieties of food mixtures shows an increase in the total sugar content during storage. The increase in total sugar content over the months of storage is represented as per cent relative change and given in brackets.

On comparing the total sugar content of fermented and unfermented food mixtures using ‘t’ test, it was noticed that the total sugar content of the unfermented samples were significantly higher. The results are given in Table 45. During the storage period, the total sugar content of fermented as well as unfermented samples were found to increase.

4.3.1.9 Starch content of the developed food mixtures on storage

The results of starch analysis of the fermented food mixtures are given in Table 46. The maximum starch content was observed in JF+DSF+JSF+P (41.97 g/100g in *koozha* variety and 41.85 g/100g in *varikka* variety) and the minimum was

Table 44. Total sugar content of unfermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	20.37 ^c	20.39 ^c (0.01)	20.40 ^c (0.10)	2.43 ^c (0.05)	20.45 ^c (0.025)	20.28 ^c (0.83)	20.52 ^c (1.18)
	JF+DSF+P	20.76 ^b	20.77 ^b (0.04)	20.79 ^b (0.05)	20.82 ^b (0.10)	20.84 ^b (0.24)	20.87 ^b (0.14)	20.90 ^b (0.14)
	JF+DSF+JSF+T	19.98 ^d	20.01 ^d (0.15)	20.04 ^d (0.15)	20.06 ^d (0.15)	20.09 ^d (0.25)	20.11 ^d (0.10)	20.14 ^d (0.15)
	JF+DSF+JSF+P	20.89 ^a	20.90 ^a (0.04)	20.92 ^a (0.05)	20.94 ^a (0.10)	20.97 ^a (0.24)	21.01 ^a (0.19)	21.04 ^a (0.14)
	CD value	0.326	0.024	0.037	0.025	0.019	0.026	0.035
<i>Varikka</i>	JF+DSF+T	20.03 ^c	20.05 ^c (0.09)	20.07 ^c (0.10)	20.09 ^c (0.10)	20.12 ^c (0.25)	20.14 ^c (0.10)	20.17 ^c (0.15)
	JF+DSF+P	20.40 ^b	20.41 ^b (0.04)	20.43 ^b (0.05)	20.45 ^b (0.10)	20.48 ^b (0.24)	20.52 ^b (0.20)	20.55 ^b (0.15)
	JF+DSF+JSF+T	21.05 ^a	21.07 ^a (0.09)	21.09 ^a (0.10)	21.11 ^a (0.09)	21.13 ^a (0.19)	21.16 ^a (0.14)	21.19 ^a (0.14)
	JF+DSF+JSF+P	21.07 ^a	21.08 ^a (0.04)	21.10 ^a (0.05)	21.13 ^a (0.09)	21.16 ^a (0.28)	21.18 ^a (0.09)	21.22 ^a (0.19)
	CD value	0.108	0.016	0.024	0.027	0.019	0.016	0.021

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

Table 45. Comparison of total sugar content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	11.70	11.70	11.72	11.74	11.78	11.81	11.72
	UFM	20.50	20.52	20.54	20.56	20.59	20.56	20.65
	Mean difference	-8.80	-8.82	-8.82	-8.82	-8.81	-8.75	-8.93
	t value	-41.77*	-42.80*	-43.02*	-42.93*	-43.08*	-39.08*	-36.08*
<i>Varikka</i>	FM	11.66	11.67	11.69	11.72	11.74	11.77	11.78
	UFM	20.64	20.65	20.67	20.69	20.72	20.75	20.78
	Mean difference	-8.98	-8.98	-8.98	-8.97	-8.98	-8.98	-9.00
	t value	-22.29*	-22.35*	-22.58*	-22.51*	-22.52*	-22.59*	-21.47*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 1% level

Figure 46. Starch content of fermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	39.36 ^c	38.13 ^c (3.12)	37.04 ^{cd} (2.94)	36.85 ^c (0.51)	35.65 ^c (3.25)	34.47 ^c (3.30)	32.15 ^d (6.73)
	JF+DSF+P	41.89 ^a	40.44 ^{ab} (3.46)	39.36 ^b (2.73)	38.15 ^b (3.17)	37.18 ^b (2.54)	36.77 ^b (1.10)	35.35 ^b (3.86)
	JF+DSF+JSF+T	39.91 ^b	38.95 ^b (2.40)	37.85 ^c (2.90)	36.16 ^d (4.67)	35.36 ^d (2.21)	34.18 ^d (3.33)	32.85 ^c (3.89)
	JF+DSF+JSF+P	41.97 ^a	40.88 ^a (2.59)	40.10 ^a (1.94)	39.57 ^a (1.33)	38.36 ^a (3.05)	37.20 ^a (3.02)	35.85 ^a (3.62)
	CD value	0.723	0.694	1.04	0.786	0.965	1.153	0.843
<i>Varikka</i>	JF+DSF+T	40.11 ^c	39.95 (1.08)	38.87 ^c (2.77)	37.68 ^c (3.15)	36.44 ^d (3.29)	35.26 ^d (3.23)	34.90 ^c (1.02)
	JF+DSF+P	41.24 ^a	41.34 ^a (1.21)	40.76 ^a (1.42)	40.13 ^a (1.57)	39.41 ^a (1.79)	38.63 ^a (1.97)	36.18 ^a (6.34)
	JF+DSF+JSF+T	40.39 ^d	39.11 ^d (2.49)	38.34 ^d (2.01)	37.05 ^d (3.48)	36.87 ^c (0.48)	35.71 ^c (3.14)	34.37 (3.75)
	JF+DSF+JSF+P	41.85 ^b	40.93 ^b (0.75)	40.01 ^b (2.23)	39.67 ^b (0.85)	38.24 ^b (3.60)	37.33 ^b (2.37)	36.05 ^b (3.42)
	CD value	0.192	0.748	0.942	1.92	0.964	1.034	0.786

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

observed in JF+DSF+ T. It was observed from the table that the starch content of the *koozha* based JF+DSF+JSF+P food mixture was on par with JF+DSF+P. There was a gradual reduction was observed in the starch content of the fermented food mixtures on storage. Figures in parenthesis given in the table (Table 46) represents the per cent decrease in the starch content over the periods of storage. The food mixture JF+DSF+JSF+P continue to be the food mixture with maximum starch content throughout the storage period.

Table 47 is the comparison of starch content of fermented food mixtures (*koozha* and *varikka*). From the table, it is clear that there is no significant difference in the starch content of the fermented food mixtures. On storage, the mean starch content of the fermented *koozha* food mixtures got reduced from 54.62 g/100g to 48.63 g/100g. On the other hand, the initial mean starch of the fermented *varikka* food mixture got reduced to 45.76 g/100g from the initial starch of 53.90 g/100g.

Table 47. Comparison of starch content of fermented food mixtures (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	54.62	53.49	52.89	51.89	50.99	49.76	48.63
<i>Varikka</i>	53.90	52.66	52.14	50.08	49.27	47.42	45.76
Mean difference	0.72	0.83	0.75	1.81	1.72	2.34	2.87
t value	0.95 ^{NS}	1.21 ^{NS}	0.92 ^{NS}	1.83 ^{NS}	2.12 ^{NS}	3.12 ^{NS}	4.17 ^{NS}

NS- Non significant

In Table 48, the starch content of unfermented food mixtures are explained. The starch content of *koozha* based unfermented food mixtures ranged from 54.11 to 56.64 g/100g, where the significantly higher starch was reported for the combination JF+DSF+JSF+T (56.64 g/100g) and the minimum was reported for JF+DSF+P (54.11

Table 48. Starch content of unfermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	54.11 ^d	53.73 ^b (0.70)	53.24 ^b (0.92)	52.64 ^b (1.13)	51.52 ^b (2.17)	50.48 ^b (2.06)	49.22 ^b (2.55)
	JF+DSF+P	53.49 ^c	52.11 ^d (2.64)	51.04 ^d (2.09)	50.85 ^c (0.37)	50.65 ^c (0.39)	49.47 ^c (2.38)	48.35 ^c (2.31)
	JF+DSF+JSF+T	54.23 ^b	53.05 ^c (2.22)	52.86 ^c (0.35)	50.72 ^d (4.21)	49.63 ^d (2.19)	48.22 ^d (2.92)	47.18 ^d (2.20)
	JF+DSF+JSF+P	56.64 ^a	55.06 ^a (2.86)	54.44 ^a (1.13)	53.36 ^a (2.02)	52.15 ^a (2.32)	50.88 ^a (2.49)	49.77 ^a (2.23)
	CD value	0.049	0.036	0.025	0.89	0.027	0.041	0.038
<i>Varikka</i>	JF+DSF+T	53.54 ^c	52.19 ^c (2.58)	51.06 ^d (2.21)	49.87 ^b (2.38)	48.66 ^d (2.48)	47.53 ^c (2.37)	45.04 ^d (2.49)
	JF+DSF+P	54.07 ^b	53.28 ^a (1.48)	52.64 ^b (1.21)	51.19 ^a (2.83)	50.63 ^a (1.10)	48.34 ^a (4.73)	46.66 ^a (3.60)
	JF+DSF+JSF+T	53.35 ^d	52.14 ^d (2.32)	51.87 ^c (0.52)	48.06 ^c (7.92)	47.95 ^b (0.22)	46.15 ^d (3.90)	45.19 ^c (2.12)
	JF+DSF+JSF+P	54.64 ^a	53.02 ^b (3.09)	52.98 ^a (0.07)	51.19 ^a (3.49)	49.83 ^c (2.72)	47.66 ^b (4.55)	46.17 ^b (3.22)
	CD value	0.140	0.173	1.23	0.037	0.164	0.024	0.168

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

g/100g). The same food mixtures were reported to have the maximum and minimum starch content in *varikka* variety food mixtures and the results were statistically significant. Here the starch values ranged from 53.35 to 54.64 g/100g. Throughout the storage period significant reduction was observed in the starch content of the unfermented food mixtures and the per cent relative reduction in starch content is represented in parenthesis.

The effect of probiotic fermentation on the starch content of the food mixtures were drawn with the help of independent 't' test and the results are given in Table 49. It is clear from the table that, in both the *koozha* and *varikka* based food mixture, probiotic fermentation could bring down the starch content of the food mixtures.

4.3.1.10 Total ash content of the developed food mixtures

Table 50 shows the changes in the total ash content of the fermented food mixtures on storage. The ash content of the *koozha* based fermented food mixtures were found to range from 3.03 per cent to 3.96 per cent where the maximum was reported for JF+DSF+JSF+P and minimum was reported for JF+DSF+ T and the results of DMRT shows that the treatments were statistically significant. During storage period, the ash content of fermented food mixtures of both *koozha* and *varikka* food mixtures were found to decrease and the per cent decrease from the previous month was indicated in parenthesis. By the end of six months, total ash content was found to be in the range of 2.71 to 3.74 per cent in *koozha* and 2.90 to 3.61 per cent in *varikka*.

Table 51 is the comparison of ash content of the fermented food mixtures. It is clear from the table that there was no significant difference in the ash content of the fermented food mixtures of *koozha* and *varikka* varieties throughout the storage period.

Table 52 gives the result of ash content of unfermented food mixture. On analysing the results using DMRT, it can be concluded that the significantly higher ash content of the unfermented food mixture *koozha* variety was observed for the food mixture JF+DSF+P (3.18 %) and the minimum for JF+DSF+JSF+T (2.89 %). In the

Table 49. Comparison of total starch content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	40.78	39.60	38.59	37.68	36.64	35.66	34.05
	UFM	54.62	53.49	52.89	51.89	50.98	49.76	48.63
	Mean difference	-13.84	-13.89	-14.3	-14.21	-14.34	-14.1	-14.58
	t value	-14.32*	-15.56*	-14.44*	-14.23*	-16.16*	-14.45*	-13.59*
<i>Varikka</i>	FM	40.89	40.33	39.49	38.63	37.74	36.73	35.37
	UFM	53.90	52.66	52.14	50.07	49.27	47.42	45.76
	Mean difference	-13.01	-12.33	-12.65	-11.44	-11.53	-10.69	-10.39
	t value	-26.40*	-21.29*	-18.20*	-10.86*	-12.78*	-11.88*	-17.64*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 1% level

Table 50. Total ash content of fermented food mixtures on storage (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	3.03 ^d	3.00 ^d (0.99)	2.84 ^d (5.33)	2.82 ^d (0.70)	2.79 ^d (1.06)	2.75 ^d (1.43)	2.71 ^d (1.45)
	JF+DSF+P	3.78 ^b	3.75 ^b (0.79)	3.72 ^b (0.80)	3.70 ^b (0.53)	3.66 ^b (1.08)	3.62 ^b (1.09)	3.58 ^b (1.10)
	JF+DSF+JSF+T	3.19 ^c	3.17 ^c (0.62)	3.14 ^c (0.94)	3.11 ^c (0.95)	3.07 ^c (1.28)	3.04 ^c (0.97)	3.00 ^c (1.31)
	JF+DSF+JSF+P	3.96 ^a	3.93 ^a (0.75)	3.90 ^a (0.76)	3.87 ^a (0.76)	3.85 ^a (0.51)	3.81 ^a (1.03)	3.74 ^a (1.83)
	CD value	0.074	0.074	0.034	0.046	0.089	0.904	1.21
<i>Varikka</i>	JF+DSF+T	3.17 ^d	3.15 ^d (0.63)	3.14 ^d (0.31)	3.11 ^d (0.95)	2.98 ^d (4.18)	2.95 ^d (1.00)	2.90 ^d (1.69)
	JF+DSF+P	3.52 ^c	3.50 ^c (0.56)	3.47 ^c (0.85)	3.44 ^c (0.86)	3.40 ^c (1.16)	3.37 ^c (0.88)	3.32 ^c (1.48)
	JF+DSF+JSF+T	3.79 ^a	3.76 ^a (0.79)	3.74 ^a (0.53)	3.73 ^a (0.26)	3.69 ^a (1.07)	3.65 ^a (1.08)	3.61 ^a (1.09)
	JF+DSF+JSF+P	3.72 ^b	3.70 ^b (0.53)	3.67 ^b (0.81)	3.63 ^b (1.08)	3.59 ^b (1.10)	3.54 ^b (1.39)	3.51 ^b (1.09)
	CD value	0.024	0.026	0.027	0.057	1.261	0.986	0.341

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 51. Comparison of total ash content of fermented food mixtures (%)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	3.49	3.46	3.40	3.37	3.34	3.30	3.25
<i>Varikka</i>	3.55	3.52	3.50	3.47	3.41	3.38	3.33
Mean difference	-0.06	-0.06	-0.1	-0.1	-0.07	-0.08	-0.08
t value	-0.22 ^{NS}	-0.24 ^{NS}	-0.37 ^{NS}	-0.36 ^{NS}	-0.24 ^{NS}	-0.24 ^{NS}	-0.26 ^{NS}

NS- Non significant

Table 52. Total ash content of unfermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	2.97 ^c	2.95 ^c (0.67)	2.93 ^c (0.67)	2.90 ^c (1.02)	2.87 ^c (1.03)	2.84 ^c (1.04)	2.80 ^c (1.40)
	JF+DSF+P	3.18 ^a	3.16 ^a (0.62)	3.14 ^a (0.63)	3.11 ^a (0.95)	3.08 ^a (0.96)	3.05 ^a (0.97)	3.02 ^a (0.98)
	JF+DSF+JSF+T	2.89 ^d	2.87 ^d (0.69)	2.84 ^d (1.04)	2.81 ^d (1.05)	2.79 ^d (0.71)	2.75 ^d (1.43)	2.72 ^d (1.09)
	JF+DSF+JSF+P	3.16 ^b	3.14 ^b (0.63)	3.11 ^b (0.95)	3.09 ^b (0.64)	3.05 ^b (1.29)	3.02 ^b (0.98)	3.00 ^b (0.66)
	CD value	0.024	0.026	0.038	0.941	0.035	0.042	0.056
<i>Varikka</i>	JF+DSF+T	2.89 ^c	2.87 ^c (0.69)	2.84 ^c (1.04)	2.81 ^c (1.05)	2.75 ^c (2.13)	2.76 ^c (0.36)	2.73 ^c (1.08)
	JF+DSF+P	3.24 ^a	3.22 ^a (0.61)	3.21 ^a (0.31)	3.19 ^a (0.62)	3.16 ^a (0.94)	3.14 ^a (0.63)	3.11 ^a (0.95)
	JF+DSF+JSF+T	2.83 ^d	2.81 ^d (0.70)	2.79 ^d (0.71)	2.76 ^d (1.07)	2.72 ^d (1.44)	2.70 ^d (0.73)	2.66 ^d (1.48)
	JF+DSF+JSF+P	3.21 ^b	3.19 ^b (0.62)	3.17 ^b (0.62)	3.14 ^b (0.94)	3.11 ^b (0.95)	3.09 ^b (0.64)	3.05 ^b (1.29)
	CD value	0.038	0.039	0.026	0.028	0.015	0.043	0.024

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

varikka based food mixtures, the maximum ash content was observed for JF+DSF+P (3.24 %), followed by JF+DSF+JSF+P (3.21 %). Similar to that of fermented food mixtures, here also, the ash content decreases with the advancement of storage and is given in the parenthesis as per cent relative change.

The ash content of fermented and unfermented food mixtures were compared and the results are given in Table 53. The results of the independent 't' test suggest that no significant variation exist between the ash content of fermented and unfermented samples.

4.3.1.11. Calcium content of the developed food mixtures

Tables 54 to 57 shows the result of calcium content of the developed food mixtures. In Table 54, the calcium content of fermented food mixtures are explained. The food mixture with maximum calcium content was JF+DSF+JSF+P (101.07 mg/100g in *koozha* and 101.34 mg/100g in *varikka*) and the food mixture JF+DSF+T (97.32 mg/100g in *koozha* and 96.03 mg/100g in *varikka*) were reported to have the minimum calcium content. DMRT done within the different food mixtures shows that the calcium content of the mixtures vary significantly. The figures given in parenthesis gives an idea of the per cent relative change in the nutrient over the storage period. Throughout the storage period, the calcium content of the fermented food mixtures were found to decrease. The per cent decrease was minimum during the first two months of storage and increases with time.

In Table 55, the result of independent 't' test conducted between the fermented food mixtures (*koozha* and *varikka*) are given. From the table, it could be concluded that, the calcium content of the fermented food mixtures do not vary significantly between the *koozha* and *varikka* varieties.

Table 53. Comparison of total ash content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	3.49	3.46	3.40	3.37	3.34	3.30	3.25
	UFM	3.05	3.03	3.01	2.97	2.94	2.91	2.88
	Mean difference	0.44	0.43	0.39	0.4	0.4	0.39	0.37
	t value	1.865NS	1.842NS	1.534NS	1.546NS	1.533NS	1.516NS	1.472NS
<i>Varikka</i>	FM	3.55	3.52	3.50	3.47	3.41	3.37	3.33
	UFM	3.04	3.02	3.00	2.97	2.93	2.92	2.88
	Mean difference	0.51	0.5	0.5	0.5	0.48	0.45	0.45
	t value	2.401 ^{NS}	2.405 ^{NS}	2.903 ^{NS}	2.86 ^{NS}	2.459 ^{NS}	2.390 ^{NS}	2.316 ^{NS}

FM- Fermented food mixture, UFM- Unfermented food mixture

NS- Non significant

Table 54. Calcium content of fermented food mixtures on storage (mg/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	97.32 ^d	97.18 ^d (0.14)	96.23 ^d (0.97)	93.94 ^d (2.37)	91.84 ^d (2.23)	90.11 ^d (1.88)	88.81 ^d (1.44)
	JF+DSF+P	99.69 ^b	98.75 ^b (0.94)	97.81 ^b (0.95)	95.56 ^b (2.30)	93.46 ^b (2.19)	91.76 ^b (1.81)	90.46 ^b (1.41)
	JF+DSF+JSF+T	98.01 ^c	97.92 ^c (0.09)	97.03 ^c (0.90)	94.82 ^c (2.27)	92.64 ^c (2.29)	90.93 ^c (1.84)	89.66 ^c (1.39)
	JF+DSF+JSF+P	101.07 ^a	100.37 ^a (0.69)	98.41 ^a (1.95)	96.12 ^a (2.32)	93.87 ^a (2.34)	92.20 ^a (1.77)	90.96 ^a (1.34)
	CD value	0.750	0.841	1.213	0.931	0.234	0.750	0.931
<i>Varikka</i>	JF+DSF+T	96.03 ^d	95.65 ^d (0.39)	93.71 ^d (2.02)	91.46 ^d (2.40)	88.35 ^d (3.40)	85.66 ^d (3.40)	82.33 ^d (3.88)
	JF+DSF+P	98.30 ^c	97.65 ^c (0.66)	95.68 ^c (2.01)	93.46 ^c (2.32)	91.34 ^c (2.26)	89.68 ^c (1.81)	86.44 ^c (3.61)
	JF+DSF+JSF+T	100.98 ^b	99.74 ^b (1.22)	97.52 ^b (2.22)	94.08 ^b (3.52)	91.19 ^b (3.07)	89.43 ^b (1.93)	87.15 ^b (2.54)
	JF+DSF+JSF+P	101.34 ^a	100.07 ^a (1.25)	98.19 ^a (1.87)	96.45 ^a (1.77)	93.84 ^a (2.70)	90.68 ^a (3.36)	88.71 ^a (2.17)
	CD value	0.017	0.021	0.034	0.025	0.046	0.013	0.024

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 55. Comparison of calcium content of fermented food mixtures on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	99.02	98.55	97.37	95.11	92.95	91.25	89.97
<i>Varikka</i>	99.16	98.27	96.27	93.86	91.18	88.86	86.15
Mean difference	-0.14	0.28	1.1	1.25	1.77	2.39	3.82
t value	-0.09 ^{NS}	0.22 ^{NS}	0.98 ^{NS}	1.10 ^{NS}	1.46 ^{NS}	1.99 ^{NS}	2.64 ^{NS}

NS- Non significant

Table 56 gives the calcium content of unfermented food mixtures. Here also the food mixture JF+DSF+JSF+P was found to be the food mixture with significantly higher calcium content in both the *koozha* and *varikka* varieties as per the results of DMRT. The minimum calcium content was observed in JF+DSF+T in both the *koozha* and *varikka* variety. Similar to that of the fermented food mixture, here also calcium content got reduced during storage and the per cent relative reduction is represented in parenthesis.

The compared result of calcium content of fermented and unfermented food mixtures are given in Table 57. From the table it is clear that during probiotic fermentation, calcium content increased significantly. Throughout the storage period, the fermented food mixtures contained maximum calcium content.

4.3.1.12. Iron content of the developed food mixtures

Table 58, shows the iron content of fermented food mixtures. Iron was found to present in the fermented food mixtures in varying proportions. The minimum was 7.86 mg/100g in the *koozha* whereas 7.28 mg/100g in *varikka*. The maximum was 8.05 mg/100g in *koozha* and 8.94 mg/100g in *varikka*. In both varieties the significantly

Table 56. Calcium content of unfermented food mixtures on storage (mg/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	96.03 ^d	95.03 ^d (1.04)	94.25 ^d (0.82)	92.0 ^c (2.38)	89.47 ^c (2.75)	85.91 ^b (3.97)	81.07 ^d (5.63)
	JF+DSF+P	97.23 ^b	96.14 ^b (1.12)	94.59 ^b (1.61)	92.47 ^a (2.24)	89.63 ^b (3.07)	85.36 ^c (4.73)	82.11 ^b (3.80)
	JF+DSF+JSF+T	96.30 ^c	95.91 ^c (0.40)	93.23 ^b (2.79)	91.99 ^d (1.33)	88.72 ^d (3.55)	85.38 ^c (3.76)	81.64 ^c (5.13)
	JF+DSF+JSF+P	97.51 ^a	96.40 ^a (1.13)	94.89 ^a (1.56)	92.04 ^b (3.00)	89.73 ^a (2.50)	86.44 ^a (3.66)	82.19 ^a (4.91)
	CD value	0.027	0.026	0.023	0.019	0.943	1.210	0.024
<i>Varikka</i>	JF+DSF+T	95.09 ^d	94.03 ^d (1.11)	92.22 ^d (1.92)	90.73 ^b (1.61)	87.63 ^d (3.41)	84.52 ^d (3.54)	81.09 ^d (4.05)
	JF+DSF+P	96.98 ^b	95.00 ^c (2.04)	93.68 ^b (1.38)	91.03 ^c (2.82)	89.27 ^a (1.93)	87.67 ^a (1.79)	84.11 ^a (4.06)
	JF+DSF+JSF+T	96.42 ^c	95.43 ^b (1.02)	93.32 ^c (2.21)	90.21 ^d (3.33)	87.10 ^c (3.44)	84.99 ^c (2.42)	81.87 ^c (3.67)
	JF+DSF+JSF+P	97.18 ^a	96.07 ^a (1.14)	94.96 ^a (1.15)	91.85 ^a (3.27)	88.74 ^b (3.38)	85.88 ^b (3.22)	83.91 ^b (2.29)
	CD value	0.038	0.036	0.034	0.025	0.029	0.017	0.019

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 57. Comparison of calcium content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (mg/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	99.02	98.55	97.37	95.112	92.95	91.92	89.97
	UFM	96.76	95.87	93.99	91.6	89.14	85.83	81.94
	Mean difference	2.26	2.68	3.38	3.512	3.81	6.09	8.03
	t value	2.459*	3.597*	5.252*	4.796*	6.935*	10.057*	14.077*
<i>Varikka</i>	FM	99.16	98.27	96.27	93.86	91.18	88.86	86.15
	UFM	96.41	95.13	93.54	90.95	88.18	85.76	82.74
	Mean difference	2.75	3.14	2.73	2.91	3.00	3.1	3.41
	t value	2.062*	2.827*	2.366*	2.683*	2.440*	2.379*	2.197*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 1%

Table 58. Iron content of fermented food mixtures on storage (mg/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	7.86 ^c	7.55 ^d (3.94)	7.37 ^c (2.38)	7.17 ^d (2.71)	7.07 ^d (1.39)	6.87 ^d (2.82)	6.66 ^d (3.05)
	JF+DSF+P	8.05 ^a	7.97 ^a (0.87)	7.76 ^a (2.63)	7.42 ^a (4.38)	7.26 ^a (2.15)	7.04 ^b (3.03)	6.88 ^b (2.27)
	JF+DSF+JSF+T	7.98 ^b	7.73 ^c (3.13)	7.65 ^b (1.03)	7.36 ^b (3.79)	7.16 ^c (2.71)	7.01 ^c (2.09)	6.69 ^c (4.56)
	JF+DSF+JSF+P	8.04 ^a	7.86 ^b (2.36)	7.66 ^b (2.54)	7.33 ^c (4.30)	7.18 ^b (2.04)	7.08 ^a (1.39)	6.93 ^a (2.11)
	CD value	0.047	0.038	0.036	0.042	0.025	1.410	0.973
<i>Varikka</i>	JF+DSF+T	7.44 ^c	7.26 ^c (2.41)	7.05 ^c (2.89)	6.83 ^c (3.12)	6.64 ^c (2.78)	6.39 ^c (3.76)	6.16 ^c (3.59)
	JF+DSF+P	8.94 ^a	8.84 ^a (1.11)	8.69 ^a (1.69)	8.43 ^a (2.99)	8.27 ^a (1.89)	8.18 ^a (1.08)	7.09 ^b (13.32)
	JF+DSF+JSF+T	7.28 ^d	7.01 ^d (3.70)	6.94 ^d (0.99)	6.73 ^d (3.02)	6.60 ^d (1.93)	6.39 ^c (3.18)	6.12 ^d (4.22)
	JF+DSF+JSF+P	8.38 ^b	8.21 ^b (2.02)	8.06 ^b (1.82)	7.95 ^b (1.36)	7.73 ^b (2.76)	7.42 ^b (4.01)	7.15 ^a (3.63)
	CD value	0.020	0.026	0.031	0.057	0.982	0.035	0.024

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

higher amount of iron was observed in JF+DSF+P followed by JF+DSF+JSF+P. As the storage period advances, the iron content was found reducing in the fermented food mixtures and the per cent decrease over the previous month is given in parenthesis.

The results of independent 't' test between iron content of fermented food mixtures are given in Table 59. The results suggest that there is no significant difference exist between the iron content of fermented *koozha* and *varikka* food mixtures.

Table 60 gives the result of iron content of unfermented food mixtures. Here the maximum iron content was exhibited by the food mixture JF+DSF+JSF+P in both *koozha* and *varikka* based food mixture and this was found to contain the significantly higher iron content throughout the storage. The food mixtures were reported to lose iron during storage. The initial values of *koozha* based unfermented food mixtures ranged from 7.12 mg/100g to 7.94 mg/100g and on storage these values were decreased and at the end of storage the values ranged from 5.93 to 7.09 mg/100g. In the *varikka* based food mixtures the initial values were 7.03 to 7.84 mg/100g and the final values ranged from 5.07 to 6.70 mg/100g. The per cent relative change in the iron content of the food mixtures during storage is indicated in parenthesis.

The iron contents of fermented and unfermented food mixtures of *koozha* and *varikka* varieties were compared and the results are depicted in Table 61. The comparison of iron content of fermented and unfermented food mixtures of developed food mixtures shows that there was no significant difference between the iron content of fermented and unfermented samples throughout the storage period.

4.3.1.13. Potassium content of the developed food mixture

From Table 62, the details of the potassium content of the fermented food mixtures can be drawn. The food mixtures were found to have fair amounts of potassium and was expressed in grams per hundred gram. The results of DMRT suggests that the potassium content of different food mixtures vary significantly. The

Table 59. Iron content of fermented food mixtures on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	7.98	7.77	7.61	7.32	7.16	7.00	6.79
<i>Varikka</i>	7.76	7.58	7.43	7.23	7.06	6.84	6.38
Mean difference	0.22	0.19	0.18	0.09	0.1	0.16	0.41
t value	0.560 ^{NS}	0.456 ^{NS}	0.409 ^{NS}	0.210 ^{NS}	0.265 ^{NS}	0.346 ^{NS}	1.665 ^{NS}

NS- Non significant

Table 60. Iron content of unfermented food mixtures on storage (mg/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	7.12 ^d	7.08 ^d (0.56)	6.86 ^d (3.10)	6.71 ^d (2.18)	6.50 ^d (3.12)	6.14 ^d (5.53)	5.93 ^d (3.42)
	JF+DSF+P	7.44 ^c	7.33 ^c (1.47)	7.18 ^c (2.04)	7.01 ^c (2.36)	6.86 ^c (2.13)	6.61 ^c (3.64)	6.46 ^c (2.26)
	JF+DSF+JSF+T	7.82 ^b	7.71 ^b (1.40)	7.62 ^b (1.16)	7.49 ^b (1.70)	7.30 ^b (2.53)	7.14 ^b (2.19)	7.02 ^b (1.68)
	JF+DSF+JSF+P	7.94 ^a	7.82 ^a (1.51)	7.73 ^a (1.15)	7.59 ^a (1.81)	7.37 ^a (2.89)	7.20 ^a (2.30)	7.09 ^a (1.57)
	CD value	0.029	0.026	0.027	0.015	0.029	0.041	0.057
<i>Varikka</i>	JF+DSF+T	7.03 ^d	6.91 ^d (1.70)	6.73 ^d (3.56)	6.49 ^d (3.56)	6.25 ^d (3.69)	6.12 ^d (2.08)	5.07 ^d (1.71)
	JF+DSF+P	7.29 ^c	7.15 ^c (1.92)	7.01 ^c (1.95)	6.84 ^c (2.42)	6.57 ^c (3.94)	6.36 ^c (3.19)	6.24 ^c (1.88)
	JF+DSF+JSF+T	7.65 ^b	7.50 ^b (1.96)	7.36 ^b (1.86)	7.21 ^b (2.03)	7.07 ^b (1.94)	6.84 ^b (3.25)	6.66 ^b (2.63)
	JF+DSF+JSF+P	7.84 ^a	7.69 ^a (1.91)	7.43 ^a (3.38)	7.22 ^a (2.82)	7.08 ^a (1.93)	6.87 ^a (2.96)	6.70 ^a (2.47)
	CD value	0.036	0.038	0.029	0.014	0.057	0.028	0.941

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 61. Comparison of iron content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (mg/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	7.98	7.77	7.61	7.32	7.16	7.00	6.79
	UFM	7.58	7.48	7.34	7.20	7.00	6.77	6.62
	Mean difference	0.4	0.29	0.27	0.12	0.16	0.23	0.17
	t value	2.099 ^{NS}	1.513 ^{NS}	1.204 ^{NS}	0.562 ^{NS}	0.773 ^{NS}	0.899 ^{NS}	0.590 ^{NS}
<i>Varikka</i>	FM	7.76	7.58	7.43	7.23	7.06	6.84	6.38
	UFM	7.45	7.31	7.13	6.94	6.74	6.54	6.16
	Mean difference	0.31	0.27	0.3	0.29	0.32	0.3	0.22
	t value	0.708 ^{NS}	0.584 ^{NS}	0.673 ^{NS}	0.675 ^{NS}	0.702 ^{NS}	0.618 ^{NS}	0.474 ^{NS}

FM- Fermented food mixture, UFM- Unfermented food mixture
NS- Non significant

Table 62. Potassium content of fermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	0.98 ^c	0.97 ^c (1.02)	0.95 ^c (2.06)	0.93 ^c (2.10)	0.91 ^c (2.15)	0.9 ^c (1.09)	0.88 ^c (2.22)
	JF+DSF+P	1.34 ^a	1.33 ^a (0.74)	1.32 ^b (0.75)	1.30 ^a (1.51)	1.28 ^a (1.53)	1.25 ^a (2.34)	1.22 ^a (2.4)
	JF+DSF+JSF+T	0.96 ^d	0.95 ^d (1.04)	0.93 ^d (2.10)	0.92 ^d (1.07)	0.90 ^d (2.17)	0.88 ^d (2.22)	0.86 ^d (2.27)
	JF+DSF+JSF+P	1.10 ^b	1.09 ^b (0.90)	1.07 ^a (1.83)	1.05 ^b (1.86)	1.02 ^b (2.85)	1.00 ^b (1.96)	0.97 ^b (3.00)
	CD value	0.024	0.028	0.026	0.031	0.075	1.230	0.025
<i>Varikka</i>	JF+DSF+T	0.88 ^d	0.87 ^d (1.13)	0.86 ^d (1.14)	0.84 ^c (2.32)	0.81 ^d (3.57)	0.79 ^c (2.49)	0.76 ^d (3.79)
	JF+DSF+P	1.29 ^a	1.27 ^a (1.55)	1.25 ^a (1.57)	1.22 ^a (2.40)	1.20 ^a (1.63)	1.17 ^a (2.50)	1.14 ^a (2.56)
	JF+DSF+JSF+T	0.90 ^c	0.89 ^c (1.11)	0.88 ^c (1.12)	0.85 ^c (3.40)	0.82 ^c (3.52)	0.80 ^c (2.43)	0.77 ^c (3.75)
	JF+DSF+JSF+P	1.18 ^b	1.17 ^b (0.84)	1.15 ^b (1.70)	1.14 ^b (0.86)	1.11 ^b (2.63)	0.98 ^b (11.71)	0.95 ^b (3.06)
	CD value	0.022	0.022	0.221	0.038	0.074	0.041	0.931

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

maximum was reported by the food mixture JF+DSF+P in both the *koozha* (1.34 g/100g) and *varikka* (1.29 g/100g). Minimum potassium was observed in JF+DSF+T in both the food mixtures. On storage, the potassium content was found to decrease considerably in the food mixtures and the per cent relative decrease during storage is indicated in parenthesis.

The table given below (Table 63) is the result of comparison of potassium content of *koozha* and *varikka* based fermented food mixtures. The comparison was made with the aid of independent 't' test. On analysing the data of the table, it is clear that fruit variety did not affected the potassium content of the samples as there is no significant difference between the potassium content of *koozha* and *varikka* based probiotic food mixtures.

Table 63. Comparison of potassium content of fermented food mixtures (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	1.09	1.08	1.07	1.05	1.02	1.00	0.98
<i>Varikka</i>	1.06	1.05	1.03	1.01	0.98	0.93	0.90
Mean difference	0.03	0.03	0.04	0.04	0.04	0.07	0.08
t value	0.24 ^{NS}	0.26 ^{NS}	0.24 ^{NS}	0.28 ^{NS}	0.31 ^{NS}	0.58 ^{NS}	0.63 ^{NS}

NS-Non significant

Table 64 depicts the potassium content of the jackfruit based unfermented food mixtures. In this case also, similar to that of the fermented counter parts, the food mixture JF+DSF+P showed the maximum potassium content. The potassium content of *koozha* based unfermented food mixtures ranged from 0.88 to 1.29g/100g and that of *varikka* were 0.85 to 1.13g/100g. DMRT results revealed that the potassium content of JF+DSF+T of *koozha* variety is on par with the JF+DSF+JSF+T of *koozha*. All the

Table 64. Potassium content of unfermented food mixtures on storage (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	0.88 ^c	0.87 ^c (1.13)	0.85 ^c (2.29)	0.84 ^c (1.17)	0.82 ^c (2.38)	0.80 ^c (2.43)	0.77 ^c (3.75)
	JF+DSF+P	1.29 ^a	1.27 ^a (1.15)	1.26 ^a (2.29)	1.24 ^a (1.58)	1.21 ^a (2.41)	1.19 ^a (1.65)	1.16 ^a (2.52)
	JF+DSF+JSF+T	0.88 ^c	0.86 ^d (2.27)	0.85 ^c (1.16)	0.83 ^d (2.35)	0.81 ^d (2.40)	0.79 ^d (2.46)	0.77 ^d (2.53)
	JF+DSF+JSF+P	0.90 ^b	0.89 ^b (1.11)	0.87 ^b (2.24)	0.85 ^b (2.29)	0.83 ^b (2.35)	0.81 ^b (2.40)	0.78 ^b (3.70)
	CD value	0.019	0.021	0.017	0.026	0.022	0.024	0.036
<i>Varikka</i>	JF+DSF+T	0.85 ^c	0.84 ^c (1.17)	0.83 ^c (1.19)	0.81 ^c (2.40)	0.79 ^c (2.46)	0.76 ^c (3.79)	0.73 ^c (3.94)
	JF+DSF+P	1.13 ^a	1.11 ^a (1.76)	1.10 ^a (0.90)	1.08 ^a (1.81)	1.05 ^a (2.77)	1.02 ^a (2.85)	1.00 ^a (2.85)
	JF+DSF+JSF+T	0.84 ^d	0.83 ^d (1.19)	0.82 ^d (1.20)	0.80 ^d (2.43)	0.77 ^d (3.75)	0.75 ^d (2.59)	0.72 ^d (4.00)
	JF+DSF+JSF+P	0.89 ^b	0.88 ^b (1.12)	0.86 ^b (2.27)	0.84 ^b (2.32)	0.81 ^b (3.57)	0.79 ^b (2.46)	0.76 ^b (3.79)
	CD value	0.017	0.018	0.021	0.034	0.025	0.031	0.019

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

unfermented food mixtures were shown to have decreased potassium content on storage.

The result of ‘t’ between the potassium contents of fermented and unfermented food mixture are given in table 65 and the data showed that probiotic fermentation did not made any significant difference in the potassium content of the food mixtures.

4.3.1.14 Thiamine content of the developed food mixtures

Table 66 gives the thiamine content of fermented food mixtures. The mixtures JF+DSF+JSF+P (0.090 $\mu\text{g}/100\text{g}$) and JF+DSF+P (0.090 $\mu\text{g}/100\text{g}$) were having the maximum of the nutrient among the food mixtures of *koozha* variety. The food mixture with the minimum thiamine content in this variety was JF+DSF+JSF+T (0.064 $\mu\text{g}/100\text{g}$). The thiamine content of the *varikka* variety ranged from 0.060 to 0.080 $\mu\text{g}/100\text{g}$. The food mixtures, JF+DSF+JSF+P and JF+DSF+P was found to have statistically higher thiamine content in this variety. On analysing with DMRT, it was observed that the thiamine content of JF+DSF+P was on par with that of JF+DSF+JSF+P in both the varieties. On storage, the thiamine content of the food mixtures were reported to decrease gradually and the per cent relative decrease is given in brackets.

Table 67. Comparison of thiamine content of fermented food mixtures ($\mu\text{g}/100\text{g}$)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	0.078	0.071	0.173	0.048	0.043	0.032	0.020
<i>Varikka</i>	0.073	0.064	0.055	0.047	0.042	0.027	0.017
Mean difference	0.005 ^{NS}	0.007 ^{NS}	0.118 ^{NS}	0.001 ^{NS}	0.001 ^{NS}	0.005 ^{NS}	0.003 ^{NS}
t value	0.562	0.796	1.054	0.163	0.205	0.782	0.650

NS-Non significant

Table 65. Comparison of potassium content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (g/100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	1.09	1.08	1.07	1.05	1.03	1.00	0.98
	UFM	0.98	0.97	0.95	0.94	0.91	0.89	0.87
	Mean difference	0.11	0.11	0.12	0.11	0.12	0.11	0.11
	t value	0.805 ^{NS}	0.850 ^{NS}	0.815 ^{NS}	0.824 ^{NS}	0.835 ^{NS}	0.850 ^{NS}	0.884 ^{NS}
<i>Varikka</i>	FM	1.06	1.05	1.03	1.01	0.98	0.93	0.90
	UFM	0.92	0.91	0.90	0.88	0.85	0.83	0.80
	Mean difference	0.14	0.14	0.13	0.13	0.13	0.1	0.1
	t value	1.098 ^{NS}	1.125 ^{NS}	1.123 ^{NS}	1.098 ^{NS}	1.088 ^{NS}	0.954 ^{NS}	0.919 ^{NS}

FM- Fermented food mixture, UFM- Unfermented food mixture

NS- Non significant

Table 66. Thiamine content of fermented food mixtures ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSFT	0.072 ^b	0.061 ^b (14.75)	0.050 ^c (22.00)	0.041 ^c (21.95)	0.035 ^{ab} (17.14)	0.021 ^{ab} (66.66)	0.012 ^{ab} (75.00)
	JF+DSF+P	0.090 ^a	0.085 ^a (15.88)	0.067 ^a (26.86)	0.056 ^a (19.64)	0.051 ^a (19.88)	0.044 ^a (15.90)	0.028 ^a (57.14)
	JF+DSF+JSF+T	0.064 ^c	0.054 (11.11)	0.051 (15.88)	0.041 (24.39)	0.035 ^{ab} (17.14)	0.021 ^{ab} (66.66)	0.012 ^{ab} (75.00)
	JF+DSF+JSF+P	0.090 ^a	0.083 ^a (18.43)	0.065 ^b (27.69)	0.054 ^b (20.37)	0.050 ^a (18.00)	0.043 ^a (16.27)	0.026 (65.38)
	CD value	0.006	0.007	0.072	0.004	0.029	0.008	0.005
<i>Varikka</i>	JF+DSFT	0.071 ^b	0.063 ^c (11.11)	0.053 ^c (18.86)	0.043 ^c (23.25)	0.038 ^c (13.15)	0.023 ^d (65.21)	0.012 ^d (91.66)
	JF+DSF+P	0.080 ^a	0.067 ^b (19.40)	0.056 ^b (19.64)	0.051 ^a (9.80)	0.044 (15.90) ^b	0.028 ^b (57.14)	0.019 ^c (47.36)
	JF+DSF+JSF+T	0.060 ^c	0.058 ^d (13.44)	0.051 ^d (13.72)	0.044 ^b (15.9)	0.039 ^c (12.82)	0.026 ^c (50.00)	0.017 ^b (52.94)
	JF+DSF+JSF+P	0.080 ^a	0.069 ^a (15.94)	0.058 ^a (18.96)	0.051 ^a (13.72)	0.046 ^a (10.86)	0.031 ^a (48.38)	0.018 ^a (72.22)
	CD value	0.006	0.005	0.021	0.029	0.004	0.005	0.004

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

In Table 67, the result of 't' test is given. The test was performed to assess the difference in thiamine content of the *koozha* and *varikka* based fermented food mixtures. The results revealed that there is no significant difference between the food mixtures of *koozha* and *varikka* variety.

In Table 68, the thiamine content of unfermented food mixtures are given. In *koozha* variety, the values ranged from 0.050 to 0.080 µg/100 g and in *varikka* food mixtures it ranged from 0.050 to 0.070 µg/100 g. In both the varieties, the food mixture JF+DSF+JSF+P (0.080 µg/100 g in *koozha* and 0.070 µg/100g in *varikka*) was found to contain maximum and JF+DSF+JSF+T (0.05 µg/100 g in *koozha* and *varikka*) was found to contain minimum of thiamine. The thiamine content of *varikka* based JF+DSF+T was on par with that of JF+DSF+JSF+T of same variety.

The comparison of thiamine content of fermented and unfermented food mixtures were done with 't' test and the results were explained in the Table 69. The results suggest that the fermented food mixtures have significantly higher amount of thiamine than the unfermented food mixtures throughout the storage period.

4.3.1.15. Riboflavin content of the developed food mixtures

The presence of riboflavin was analysed and represented in Table 70. In *koozha* food mixtures the riboflavin content ranged from 0.052 to 0.090 µg/100g of the sample. The maximum was observed in JF+DSF+JSF+P (0.090 µg/100g) whereas minimum was reported in the sample JF+DSF+JSF+T (0.048 µg/100g). On analysing the details of *varikka* based fermented food mixtures, it is clear that 0.051 to 0.088 µg/100g riboflavin was present in the samples of which JF+DSF+JSF+P (0.88 µg/100g) showed the maximum. On storage, the nutrient was found reducing and the per cent relative change is indicated in parenthesis.

Table 68. Thiamine content of unfermented food mixtures ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSFT	0.060 ^c	0.058 ^b (3.44)	0.047 ^b (23.40)	0.033 ^b (42.42)	0.028 ^{ab} (37.85)	0.015 ^b (46.66)	0.011 ^c (66.36)
	JF+DSF+P	0.071 ^b	0.058 ^b (20.68)	0.047 ^b (23.40)	0.031 ^b (51.61)	0.023 ^b (34.78)	0.014 ^b (32.35)	0.011 ^c (62.10)
	JF+DSF+JSF+T	0.050 ^d	0.044 ^c (13.63)	0.033 ^c (33.33)	0.021 ^c (47.14)	0.018 ^c (46.66)	0.017 ^a (43.33)	0.017 ^a (64.11)
	JF+DSF+JSF+P	0.080 ^a	0.068 ^a (17.64)	0.057 ^a (19.29)	0.047 ^a (21.27)	0.028 ^a (37.85)	0.017 ^a (44.70)	0.012 ^b (61.66)
	CD value	0.038	0.008	0.004	0.005	0.059	0.006	0.006
<i>Varikka</i>	JF+DSFT	0.050 ^c	0.048 ^c (14.16)	0.037 ^c (29.72)	0.025 ^c (48.00)	0.016 ^c (46.25)	0.005 ^c (53.00)	0.004 ^c (65.00)
	JF+DSF+P	0.064 ^b	0.053 ^b (13.20)	0.050 ^b (16.00)	0.041 ^b (21.95)	0.027 ^b (31.85)	0.019 ^b (42.11)	0.013 ^b (56.15)
	JF+DSF+JSF+T	0.050 ^c	0.044 ^d (13.63)	0.037 ^c (18.91)	0.025 ^c (38.00)	0.016 ^c (46.25)	0.005 ^c (45.45)	0.004 ^c (58.00)
	JF+DSF+JSF+P	0.070 ^a	0.066 ^a (16.06)	0.058 ^a (13.79)	0.047 ^a (23.40)	0.033 ^a (42.42)	0.028 ^a (47.85)	0.019 ^a (57.36)
	CD value	0.006	0.007	0.029	0.004	0.028	0.001	0.004

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 69. Comparison of thiamine content of fermented and unfermented food mixtures from *koozha* and *varikka* variety (μg /100g)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	0.078	0.071	0.063	0.048	0.043	0.032	0.020
	UFM	0.065	0.057	0.046	0.033	0.017	0.009	0.006
	Mean difference	0.013	0.014	0.127	0.015	-0.044	0.023	0.014
	t value	1.263*	1.491*	1.129*	2.232*	0.691*	3.030*	2.498NS
<i>Varikka</i>	FM	0.073	0.064	0.055	0.047	0.042	0.027	0.017
	UFM	0.058	0.053	0.046	0.035	0.023	0.014	0.008
	Mean difference	0.015	0.011	0.009	0.012	0.019	0.013	0.009
	t value	2.216*	2.143*	1.666*	2.116*	4.038*	2.164*	2.226*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 5%

Table 70. Riboflavin content of fermented food mixtures on storage ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSFT	0.052 ^c	0.048 ^c (8.33)	0.041 ^c (17.07)	0.037 ^c (10.81)	0.032 ^c (15.62)	0.030 ^c (6.66)	0.024 ^c (25.00)
	JF+DSF+P	0.076 ^b	0.073 ^b (4.10)	0.069 ^b (5.79)	0.065 ^b (6.15)	0.059 ^b (10.16)	0.053 ^b (11.32)	0.047 ^b (12.76)
	JF+DSF+JSF+T	0.048 ^d	0.045 ^c (6.66)	0.039 ^c (15.38)	0.033 ^d (18.18)	0.028 ^d (17.85)	0.022 ^d (27.27)	0.016 ^d (37.50)
	JF+DSF+JSF+P	0.090 ^a	0.088 ^a (2.27)	0.082 ^a (7.31)	0.075 ^a (9.33)	0.071 ^a (5.63)	0.067 ^a (5.97)	0.061 ^a (9.83)
	CD value	0.004	0.005	0.059	0.004	0.028	0.001	0.032
<i>Varikka</i>	JF+DSFT	0.055 ^c	0.053 ^c (3.77)	0.048 ^c (10.41)	0.041 ^c (17.07)	0.035 ^c (17.14)	0.029 ^c (20.68)	0.022 ^c (31.81)
	JF+DSF+P	0.074 ^b	0.070 ^b (5.71)	0.066 ^b (6.06)	0.059 ^b (11.86)	0.053 ^b (11.32)	0.048 ^b (10.41)	0.043 ^b (11.62)
	JF+DSF+JSF+T	0.051 ^d	0.049 ^d (4.08)	0.042 ^d (16.66)	0.037 ^d (13.51)	0.031 ^d (19.35)	0.027 ^d (14.81)	0.022 ^d (22.72)
	JF+DSF+JSF+P	0.088 ^a	0.084 ^a (4.76)	0.080 ^a (5.00)	0.076 ^a (5.26)	0.073 ^a (4.10)	0.069 ^a (5.79)	0.065 ^a (6.15)
	CD value	0.006	0.046	0.003	0.032	0.005	0.037	0.049

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 71. Comparison of riboflavin content of fermented food mixtures ($\mu\text{g}/100\text{g}$)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	0.067	0.064	0.058	0.053	0.048	0.036	0.035
<i>Varikka</i>	0.069	0.066	0.059	0.052	0.050	0.043	0.038
Mean difference	-0.193	-0.002	-0.001	0.001	-0.002	-0.007	-0.01
t value	-0.038 ^{NS}	-0.038 ^{NS}	-0.091 ^{NS}	0.055 ^{NS}	-0.035 ^{NS}	-0.399 ^{NS}	-0.069 ^{NS}

NS-Non significant

In Table 71, the result of 't' test is given. On comparing the data of *koozha* and *varikka* food mixtures, no significant variations were observed in the riboflavin content of the two food mixtures throughout the storage period.

Table 72 is the riboflavin content of different food mixtures that were not fermented with the probiotic organism. Here also JF+JSF+DSF+P was found to contain the maximum riboflavin among the food mixtures of *koozha* and *varikka* variety. It was also revealed from the data that during the last months of storage, the riboflavin contents reaches a negligible level.

In Table 73, the comparison of riboflavin contents of fermented and unfermented food mixtures are given. The result revealed that during probiotic fermentation, the riboflavin content of the food mixtures increased considerably.

4.3.2.1. *In vitro* digestibility of starch and protein

Table 74 shows the *in vitro* starch digestibility of the fermented food mixtures throughout the storage period of six months.

Statistical analysis with DMRT shows that the food mixtures of both *koozha* and *varikka* variety differ significantly in terms of *in vitro* starch digestibility. There

Table 72. Riboflavin content of unfermented food mixtures on storage ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSFT	0.020 ^b	0.015 ^b (25.00)	0.013 ^b (13.33)	0.010 ^b (23.07)	0.007 ^b (30.00)	0.003 ^b (57.14)	0.000 ^b
	JF+DSF+P	0.030 ^a	0.028 ^a (6.66)	0.022 ^a (21.42)	0.015 ^a (31.81)	0.011 ^a (26.66)	0.007 ^a (36.36)	0.004 ^a (42.85)
	JF+DSF+JST	0.010 ^c	0.007 ^c (30.00)	0.005 ^c (28.57)	0.003 ^c (40.00)	0.00 ^c	0.00 ^c	0.000 ^b
	JF+DSF+JSF+P	0.030 ^a	0.028 ^a (6.66)	0.022 ^a (21.42)	0.015 ^a (31.81)	0.011 ^a (26.66)	0.007 ^a (36.36)	0.000 ^b
	CD value	0.002	0.031	0.002	0.002	0.000	0.000	0.000
<i>Varikka</i>	JF+DSFT	0.010 ^c	0.007 ^c (36.36)	0.005 ^c (28.57)	0.003 ^c (40.00)	0.00 ^d	0.00 ^c	0.000 ^b
	JF+DSF+P	0.021 ^b	0.018 ^b (30.00)	0.015 ^b (16.66)	0.011 ^b (26.66)	0.005 ^b (54.54)	0.003 ^b (40.00)	0.000 ^b
	JF+DSF+JSF+T	0.011 ^c	0.006 ^c (10.00)	0.004 ^c (33.33)	0.003 ^c (25.00)	0.002 ^c (33.33)	0.00 ^c	0.000 ^b
	JF+DSF+JSF+P	0.030 ^a	0.028 ^a (6.66)	0.024 ^a (14.28)	0.021 ^a (12.50)	0.015 ^a (28.57)	0.008 ^a (46.66)	0.005 ^a (37.50)
	CD value	0.021	0.022	0.022	0.002	0.000	0.000	0.000

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative decrease over the previous month

Values with different superscript differ significantly at 5%

Table 73. Comparison of riboflavin content of fermented and unfermented food mixtures from *koozha* and *varikka* variety ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	0.067	0.064	0.058	0.053	0.048	0.036	0.034
	UFM	0.023	0.020	0.016	0.009	0.007	0.004	0.001
	Mean difference	0.044	0.044	0.042	0.044	0.041	0.032	0.036
	t value	3.975*	3.818*	3.720*	3.997*	3.746*	2.187*	3.461*
<i>Varikka</i>	FM	0.067	0.064	0.059	0.053	0.048	0.043	0.037
	UFM	0.018	0.015	0.013	0.010	0.006	0.003	0.001
	Mean difference	0.049	0.049	0.024	0.043	0.042	0.04	0.036
	t value	5.024*	5.133*	0.903*	4.405*	4.179*	4.058*	3.438*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 1%

Table 74. *In vitro* starch digestibility of the fermented food mixtures (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	81.04 ^a	81.06 ^a (0.02)	81.27 ^c (0.25)	82.20 ^a (1.13)	82.60 ^a (0.48)	82.75 ^a (0.18)	83.37 ^a (0.74)
	JF+DSF+P	79.89 ^d	79.92 ^d (0.03)	80.17 ^d (0.31)	81.20 ^d (1.26)	81.40 ^d (0.24)	81.75 ^d (0.42)	82.21 ^d (0.55)
	JF+DSF+JSF+T	80.76 ^b	80.78 ^b (0.02)	81.47 ^a (0.84)	81.75 ^b (0.34)	82.41 ^b (0.80)	82.64 ^b (0.27)	83.23 ^b (0.70)
	JF+DSF+JSF+P	80.17 ^c	80.19 ^c (0.02)	81.40 ^b (1.48)	81.60 ^c (0.24)	81.64 ^c (0.04)	81.78 ^c (0.17)	82.25 ^c (0.57)
	CD value	0.040	0.028	0.026	0.024	0.035	0.231	0.037
<i>Varikka</i>	JF+DSF+T	81.94 ^a	81.95 ^a (0.01)	82.06 ^b (0.13)	82.20 ^c (0.17)	83.27 ^c (1.28)	83.60 ^b (0.35)	84.03 ^b (0.51)
	JF+DSF+P	81.06 ^d	81.08 ^d (0.02)	81.51 ^c (0.52)	81.54 ^d (0.03)	82.06 ^c (0.63)	82.51 ^b (0.54)	83.37 ^c (1.03)
	JF+DSF+JSF+T	81.56 ^b	81.58 ^c (0.02)	81.60 ^d (0.02)	81.75 ^d (0.18)	82.31 ^c (0.68)	82.64 ^b (0.39)	83.13 ^d (0.58)
	JF+DSF+JSF+P	81.47 ^c	81.48 ^b (0.01)	82.12 ^a (0.77)	83.02 ^d (1.08)	83.70 ^c (0.81)	84.01 ^b (0.36)	84.70 ^a (0.81)
	CD value	0.047	0.025	0.027	0.016	0.019	0.023	0.016

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

was a gradual increase in the *in vitro* starch digestibility of the fermented food mixtures during storage and the per cent relative change is indicated in parenthesis. The increase in starch digestibility was observed in both the *koozha* and *varikka* jackfruit based food mixtures. In the *koozha* variety, the food mixture JF+DSF+T (81.04 %) was found have maximum starch digestibility throughout the storage period followed by JF+DSF+JSF+T (80.76 %). In *varikka* food mixtures, JF+DSF+T (81.94 %) followed by JF+DSJSF+T (81.56 %) were having maximum digestibility.

An independent ‘t’ test was carried out to compare the starch digestibility of both variety. From the table, it is clear that there is no significant difference in the starch digestibility of the *koozha* and *varikka* jackfruit based food mixtures. The result of ‘t’ test is given in Table 75.

Table 75. Comparison of starch digestibility of *koozha* and *varikka* fermented food mixtures

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	80.46	80.48	81.07	81.68	82.01	82.23	82.76
<i>Varikka</i>	81.50	81.52	81.82	82.12	82.83	83.19	83.80
Mean difference	-1.04	-1.03	-0.74	-0.44	-0.82	-0.96	-1.04
t value	-2.26 ^{NS}	-2.26 ^{NS}	-2.17 ^{NS}	-1.13 ^{NS}	-1.96 ^{NS}	-2.13 ^{NS}	-2.21 ^{NS}

NS- Non significant

The unfermented food mixtures (Table 76) were also analysed for their *in vitro* starch digestibility. A comparatively lower starch digestibility were observed for the unfermented food mixtures than the fermented samples. As per the results of DMRT, The food mixture JF+DSF+P was found to have significantly higher starch digestibility among the food mixtures of *koozha* and *varikka* variety. The digestibility of this

Table 76. *In vitro* starch digestibility of the unfermented food mixtures (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	63.93 ^b	63.95 ^b (0.03)	63.99 ^b (0.06)	64.17 ^b (0.28)	64.86 ^b (1.06)	64.11 ^b (0.38)	64.97 ^b (1.30)
	JF+DSF+P	64.46 ^a	64.47 ^a (0.01)	64.60 ^a (0.20)	65.13 ^a (0.81)	66.86 ^a (2.58)	67.09 ^a (0.34)	67.85 ^a (1.12)
	JF+DSF+JSF+T	63.58 ^c	63.60 ^c (0.03)	63.74 ^c (0.21)	64.12 ^b (0.59)	64.97 ^b (1.30)	65.01 ^b (0.06)	65.67 ^c (1.00)
	JF+DSF+JSF+P	62.89 ^d	62.91 ^d (0.03)	63.03 ^d (0.19)	63.45 ^c (0.66)	64.27 ^c (1.27)	64.98 ^c (1.09)	65.32 ^d (0.52)
	CD value	0.016	0.034	0.008	0.016	0.021	0.035	0.007
<i>Varikka</i>	JF+DSF+T	64.09 ^d	64.11 ^d (0.03)	64.67 ^d (0.85)	65.14 ^d (0.71)	65.84 ^a (1.04)	66.27 ^c (0.63)	66.77 ^c (0.73)
	JF+DSF+P	64.84 ^a	64.86 ^a (0.03)	64.93 ^a (0.10)	65.09 ^a (0.24)	65.75 ^b (1.00)	66.29 ^b (0.81)	66.95 ^b (0.98)
	JF+DSF+JSF+T	64.73 ^b	64.75 ^b (0.03)	64.83 ^b (0.12)	64.93 ^b (0.15)	65.17 ^d (0.36)	65.53 ^d (0.54)	66.14 ^d (0.92)
	JF+DSF+JSF+P	64.38 ^c	64.40 ^c (0.03)	64.75 ^c (0.54)	64.83 ^c (0.12)	65.36 ^c (0.81)	66.69 ^a (1.99)	67.24 ^a (0.81)
	CD value	0.025	0.016	0.024	0.016	0.018	0.035	0.009

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

particular combination was 64.46 per cent in *koozha* and 64.84 per cent in *varikka* based varieties. Throughout the storage period, the starch digestibility was found to increase in both varieties and expressed as per cent relative change.

The Table 77 depicts the results of ‘t’ test performed between the fermented and unfermented samples. The test results showed that the fermented food mixture had significantly higher digestibility rates throughout the storage period.

4.3.2.2. *In vitro* protein digestibility of the developed food mixtures on storage

The *in vitro* protein digestibility of the fermented food mixtures throughout the storage period is given Table 78. From the second month of storage, significant increase in the protein digestibility were observed in both *koozha* and *varikka* food mixtures. In *koozha* food mixture, JF+DSF+T (83.83 %) had the maximum protein digestibility whereas the least digestibility was observed for JF+DSF+P (77.49 %). In *varikka* food mixtures, JF+DSF+T (82.48 %) had the maximum protein digestibility and the food mixture JF+DSF+P (80.67 %) had the least digestibility. The DMRT results suggest a significant difference in the protein digestibility of different fermented food mixtures.

Table 79. Comparison of starch digestibility of *koozha* and *varikka* fermented food mixtures

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
<i>Koozha</i>	80.72	80.74	80.90	81.15	81.35	81.48	81.90
<i>Varikka</i>	81.34	81.35	81.42	81.51	81.60	81.73	81.94
Mean difference	-0.61	-0.68	-0.52	-0.36	-0.25	-0.25	-0.04
t value	-0.45 ^{NS}	-0.45 ^{NS}	-0.38 ^{NS}	-0.25 ^{NS}	-0.16 ^{NS}	-0.16 ^{NS}	-0.02 ^{NS}

NS non-significant

Table 77. Comparison of starch digestibility of fermented and unfermented food mixtures from *koozha* and *varikka* variety (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	80.465	80.488	81.078	81.688	82.013	82.230	82.765
	UFM	63.715	63.733	63.840	64.218	65.240	65.548	66.203
	Mean difference	16.750	16.755	17.940	17.470	16.773	16.682	16.562
	t value	39.707*	39.965*	38.666*	43.390*	26.521*	28.705*	25.694*
<i>Varikka</i>	FM	81.508	81.523	81.823	82.128	82.835	83.190	83.808
	UFM	64.760	64.780	65.045	65.248	65.780	64.445	67.025
	Mean difference	17.108	16.743	16.778	16.880	17.055	18.745	16.783
	t value	71.844*	72.256*	63.826*	37.856*	31.640*	32.390*	34.218*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant @ 1%

Table 78. *In vitro* protein digestibility of the fermented food mixtures (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	83.83 ^a	83.85 ^a (0.02)	84.02 ^a (0.20)	84.54 ^a (0.61)	84.98 ^a (0.52)	85.16 ^a (0.21)	85.98 ^a (0.96)
	JF+DSF+P	77.49 ^d	77.50 ^d (0.01)	77.52 ^d (0.02)	77.61 ^d (0.11)	77.74 ^d (0.16)	77.79 ^d (0.06)	78.24 ^d (0.57)
	JF+DSF+JSF+T	81.59 ^b	81.61 ^b (0.02)	81.65 ^b (0.04)	81.69 ^b (0.04)	81.74 ^b (0.06)	81.86 ^b (0.14)	82.04 ^b (0.21)
	JF+DSF+JSF+P	79.98 ^c	80.00 ^c (0.02)	80.43 ^c (0.53)	80.78 ^c (0.43)	80.96 ^c (0.22)	81.13 ^c (0.20)	81.35 ^c (0.27)
	CD value	0.019	0.024	0.017	0.035	0.018	0.022	0.018
<i>Varikka</i>	JF+DSF+T	82.48 ^a	82.50 ^a (0.02)	82.59 ^a (0.11)	82.64 ^a (0.06)	82.72 ^a (0.09)	82.95 ^a (0.28)	83.19 ^a (0.29)
	JF+DSF+P	80.67 ^d	80.68 ^d (0.01)	80.74 ^d (0.07)	80.75 ^d (0.01)	80.82 ^d (0.08)	80.88 ^d (0.07)	80.94 ^d (0.07)
	JF+DSF+JSF+T	81.96 ^b	81.99 ^b (0.03)	82.07 ^b (0.09)	82.20 ^b (0.16)	82.35 ^b (0.18)	82.49 ^b (0.17)	82.67 ^b (0.22)
	JF+DSF+JSF+P	81.25 ^c	81.26 ^c (0.01)	81.30 ^c (0.04)	81.47 ^c (0.20)	81.54 ^c (0.08)	81.63 ^c (0.11)	81.98 ^c (0.42)
	CD value	0.11	0.16	0.221	0.024	0.023	0.010	0.017

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

An independent 't' test was performed to compare the protein digestibility of the both *koozha* and *varikka* based fermented food mixtures throughout the storage period (Table 79). There were no significant difference observed in the protein digestibility of the *koozha* and *varikka* based food mixtures.

The observed values of *in vitro* protein digestibility of the unfermented food mixtures are given in Table 80. The protein digestibility of the unfermented food mixtures were relatively lower than the fermented food mixtures. JF+DSF+JSF+T (69.05% in *koozha* and 67.11% in *varikka*) food mixture was reported to have significantly higher protein digestibility among the food mixtures and JF+DSF+P (62.16 % in *koozha* and 64.58 % in *varikka*) had the minimum digestibility. The protein digestibility of the food mixtures tends to increase during the storage period and the per cent relative change during storage is indicated in parenthesis.

To compare the effect of probiotic fermentation on protein digestibility during storage, independent 't' test was performed. The test results reported that throughout the storage period, the protein digestibility was significantly higher for the fermented food mixtures throughout the storage period. The test results are given in the Table 81.

4.3.3. Organoleptic evaluation of the developed food mixtures

The developed food mixtures, both fermented and unfermented were subjected to organoleptic evaluation by a panel of fifteen judges using the nine point hedonic scale throughout the storage period at monthly interval. The results are given in Table 82 to 85.

4.3.3.1. Organoleptic evaluation of the developed food mixture JF+DSF+T

Table 82 gives the results of organoleptic evaluation of the food mixture JF+DSF+T of both varieties. The score for appearance, colour, flavor, texture and taste were 8.20, 8.56, 8.00, 7.13, 7.60 for fermented and 8.33, 8.56, 8.00, 7.05, 7.23 respectively for the unfermented food mixtures of *koozha* variety. In the case of *varikka* variety, it was 8.23, 8.00, 8.25, 7.32, 7.60 and 8.20, 8.00, 8.23, 7.35, 7.23 respectively

Table 80. *In vitro* protein digestibility of the unfermented food mixtures (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	67.95 ^c	67.96 ^c (0.01)	68.04 ^c (0.11)	68.14 ^c (0.14)	68.25 ^c (0.16)	68.67 ^c (0.61)	69.01 ^c (0.49)
	JF+DSF+P	62.16 ^d	62.18 ^d (0.03)	62.22 ^d (0.06)	62.34 ^d (0.19)	62.59 ^d (0.40)	62.71 ^d (0.19)	63.11 ^d (0.63)
	JF+DSF+JSF+T	69.05 ^a	69.05 ^a (0.00)	69.14 ^a (0.13)	69.21 ^a (0.10)	69.35 ^a (0.20)	69.48 ^a (0.18)	69.67 ^a (0.27)
	JF+DSF+JSF+P	68.50 ^b	68.51 ^b (0.01)	68.57 ^b (0.08)	68.64 ^b (0.10)	68.82 ^b (0.26)	68.94 ^b (0.17)	69.11 ^b (0.24)
	CD value	0.017	0.018	0.018	0.021	0.024	0.017	0.026
<i>Varikka</i>	JF+DSF+T	66.13 ^c	66.16 ^c (0.04)	66.19 ^c (0.04)	66.24 ^c (0.07)	66.39 ^c (0.22)	66.47 ^c (0.12)	66.79 ^c (0.48)
	JF+DSF+P	64.58 ^d	64.59 ^d (0.01)	64.63 ^d (0.06)	64.74 ^d (0.17)	64.82 ^d (0.12)	64.91 ^d (0.13)	65.02 ^d (0.16)
	JF+DSF+JSF+T	67.11 ^a	65.13 ^a (0.03)	65.28 ^a (0.23)	65.46 ^a (0.27)	65.64 ^a (0.27)	65.98 ^a (0.51)	66.12 ^a (0.21)
	JF+DSF+JSF+P	66.27 ^b	64.30 ^b (0.04)	64.38 ^b (0.12)	64.49 ^b (0.17)	64.60 ^b (0.17)	64.71 ^b (0.17)	65.12 ^b (0.63)
	CD value	0.026	0.035	0.018	0.022	0.025	0.019	0.021

JF- Jackfruit Flour, DSF- Defatted Soya Flour, T- Tomato, P- Papaya, JSF- Jackfruit Seed Flour

DMRT Column wise comparison

Figure in parenthesis indicate per cent relative increase over the previous month

Values with different superscript differ significantly at 5%

Table 81. Comparison of protein digestibility of fermented and unfermented food mixtures from *koozha* and *varikka* variety (%)

Treatment		Storage period in months						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	FM	80.723	80.740	80.905	81.155	81.355	81.485	81.903
	UFM	66.915	66.925	66.993	67.083	67.253	67.450	67.725
	Mean difference	13.808	13.815	13.912	14.072	14.102	14.035	14.178
	t value	6.623*	6.631*	6.625*	6.572*	6.522*	6.399*	6.393*
<i>Varikka</i>	FM	81.340	81.358	81.425	81.515	81.608	81.738	81.945
	UFM	65.023	65.045	65.120	65.233	65.363	65.518	65.763
	Mean difference	16.317	16.313	16.305	16.282	16.245	16.220	16.182
	t value	37.457*	37.412*	37.855*	39.561*	38.494*	37.207*	37.088*

FM- Fermented food mixture, UFM- Unfermented food mixture

*Significant at 5%

Table 82. Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+T)

Quality attributes		Storage period in months													
		Initial		1		2		3		4		5		6	
		FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM
<i>Koozha</i>	Appearance	8.20	8.33	8.19	8.33	8.18	8.31	8.12	8.26	8.10	8.20	7.85	8.10	7.80	8.00
	Colour	8.56	8.56	8.56	8.56	8.54	8.52	8.50	8.46	8.46	8.40	8.41	8.37	8.35	8.32
	Flavour	8.00	8.00	7.98	7.96	7.93	7.92	7.90	7.90	7.86	7.85	7.83	7.83	7.80	7.78
	Texture	7.13	7.05	7.13	7.05	7.10	7.00	7.08	6.98	7.03	6.95	7.00	6.92	6.95	6.90
	Taste	7.60	7.23	7.55	7.20	7.52	7.18	7.50	7.15	7.48	7.12	7.45	7.10	7.41	7.06
	OAA	8.09	8.03	8.08	8.02	8.05	7.98	8.02	7.95	7.98	7.90	7.90	7.86	7.86	7.81
	Total score	47.58	47.2	47.49	47.1	47.32	46.91	47.12	46.7	46.91	46.42	46.44	46.18	46.17	45.87
<i>Varikka</i>	Appearance	8.23	8.20	8.20	8.18	8.19	8.17	8.16	8.15	8.11	8.10	8.07	8.05	8.05	8.00
	Colour	8.00	8.00	7.98	7.96	7.93	7.92	7.90	7.90	7.86	7.85	7.83	7.83	7.80	7.78
	Flavour	8.25	8.23	8.25	8.23	8.23	8.20	8.20	8.16	8.18	8.15	8.15	8.13	8.12	8.10
	Texture	7.32	7.35	7.31	7.33	7.30	7.31	7.27	7.28	7.25	7.24	7.22	7.21	7.20	7.18
	Taste	7.60	7.23	7.55	7.20	7.52	7.18	7.50	7.15	7.48	7.12	7.45	7.10	7.41	7.06
	OAA	8.08	8.00	8.06	7.98	8.03	7.96	8.01	7.93	7.98	7.89	7.94	7.86	7.92	7.82
	Total score	47.48	47.01	47.35	46.88	47.20	46.74	47.04	46.57	46.86	46.35	46.66	46.18	46.50	45.94

OAA- Overall acceptability, FM- Fermented mixture, UFM- Unfermented mixture

for fermented and unfermented samples. The initial overall acceptability of the food mixtures of *koozha* variety were 8.09 and 8.03 for fermented and unfermented samples respectively. In the case of *varikka*, the scores were 8.08 and 8.00 respectively. The organoleptic attributes of the food mixtures (both fermented and unfermented of *koozha* and *varikka*) shows a gradual decrease on storage. This is evident from the total scores of the samples. The initial total scores of *koozha* variety were 47.58 (FM) and 47.20 (UFM) and on storage, it got reduced to 46.17 (FM) and 45.87 (UFM). In *varikka* based food mixtures, the total scores were 47.48 (FM), 47.01 (UFM) initially and 46.50 (FM), 45.94 (UFM) finally.

4.3.3.2. Organoleptic evaluation of the developed food mixture JF+DSF+P

In Table 83 the details of the food mixture JF+DSF+P is given. Both the fermented and unfermented food mixtures of *koozha* and *varikka* variety were highly acceptable among the judges. With respect to the appearance, colour and flavour the food mixtures scored between 8.20 and 8.56 and for texture and taste, the scores were between 7.05 and 7.50. As revealed from the table, the organoleptic scores declined on storage and as a result, the total score of the food mixture got reduced gradually. From the initial point of 47.89 and 47.59, the total scores of *koozha* based fermented and unfermented food mixtures total scores reduced to 46.88 and 46.35 at the end of six months. Similarly, in the case of *varikka* based food mixtures, the values reached 47.34 (fermented) and 46.81 (unfermented) from the initial values of 48.26 and 47.86 respectively.

4.3.3.3. Organoleptic evaluation of the developed food mixture JF+DSF+JSF+T

The organoleptic evaluation of the food mixtures JF+DST+JSF+T was done along with the unfermented control samples and the experiments were repeated in both varieties. Results are given in the Table 84. The table revealed that the organoleptic attributes of the food mixtures with respect to appearance, colour, flavour and overall acceptability were very much liked by the panelist. In this group of the food mixtures

Table 83. Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+P)

Quality attributes		Storage period in months													
		Initial		1		2		3		4		5		6	
		FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM
<i>Koozha</i>	Appearance	8.56	8.56	8.56	8.56	8.54	8.52	8.50	8.46	8.46	8.40	8.41	8.37	8.35	8.32
	Colour	8.23	8.20	8.20	8.18	8.19	8.17	8.16	8.15	8.11	8.10	8.07	8.05	8.05	8.00
	Flavour	8.32	8.35	8.31	8.33	8.30	8.31	8.27	8.28	8.25	8.24	8.22	8.21	8.20	8.18
	Texture	7.13	7.05	7.13	7.05	7.10	7.00	7.08	6.98	7.03	6.95	7.00	6.92	6.95	6.90
	Taste	7.50	7.33	7.50	7.30	7.48	7.28	7.45	7.25	7.40	7.20	7.38	7.18	7.35	7.06
	OAA	8.15	8.10	8.14	8.08	8.12	8.06	8.09	8.02	8.05	7.98	8.02	7.95	7.98	7.89
	Total score	47.89	47.59	47.84	47.50	47.73	47.34	47.55	47.14	47.30	46.87	47.10	46.68	46.88	46.35
<i>Varikka</i>	Appearance	8.32	8.35	8.31	8.33	8.30	8.31	8.27	8.28	8.25	8.24	8.22	8.21	8.20	8.18
	Colour	8.56	8.56	8.56	8.56	8.54	8.52	8.50	8.46	8.46	8.40	8.41	8.37	8.35	8.32
	Flavour	8.25	8.23	8.25	8.23	8.23	8.20	8.20	8.16	8.18	8.15	8.15	8.13	8.12	8.10
	Texture	7.32	7.35	7.31	7.33	7.30	7.31	7.27	7.28	7.25	7.24	7.22	7.21	7.20	7.18
	Taste	7.60	7.23	7.55	7.20	7.52	7.18	7.50	7.15	7.48	7.12	7.45	7.10	7.41	7.06
	OAA	8.21	8.14	8.20	8.13	8.18	8.10	8.15	8.07	8.12	8.03	8.09	8.00	8.06	7.97
	Total score	48.26	47.86	48.18	47.78	48.07	47.62	47.89	47.40	47.74	47.18	47.54	47.02	47.34	46.81

OAA- Overall acceptability, FM- Fermented mixture, UFM- Unfermented mixture

Table 84. Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+JSF+T)

Quality attributes		Storage period in months													
		Initial		1		2		3		4		5		6	
		FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM
<i>Koozha</i>	Appearance	8.43	8.40	8.40	8.38	8.38	8.38	8.320	8.36	8.28	8.25	8.18	8.18	8.16	8.15
	Colour	8.40	8.40	8.38	8.40	8.35	8.37	8.30	8.34	8.25	8.28	8.14	8.23	8.09	8.19
	Flavour	8.43	8.23	8.41	8.23	8.41	8.20	8.37	8.18	8.33	8.03	8.26	8.00	8.08	7.84
	Texture	8.26	8.35	8.21	8.33	8.20	8.31	8.17	8.28	8.15	8.24	8.12	8.21	8.10	8.18
	Taste	7.40	7.38	7.40	7.30	7.38	7.28	7.35	7.25	7.30	7.20	7.28	7.18	7.25	7.06
	OAA	8.18	8.15	8.16	8.13	8.14	8.11	8.10	8.08	8.06	8.00	8.00	7.96	7.94	7.88
	Total score	49.10	48.91	48.96	48.77	48.86	48.65	48.61	48.49	48.37	48.00	47.98	47.76	47.62	47.30
<i>Varikka</i>	Appearance	8.43	8.23	8.41	8.23	8.41	8.20	8.37	8.18	8.33	8.03	8.26	8.00	8.08	7.84
	Colour	8.40	8.40	8.38	8.40	8.35	8.37	8.30	8.34	8.25	8.28	8.14	8.23	8.09	8.19
	Flavour	8.25	8.23	8.25	8.23	8.23	8.20	8.20	8.16	8.18	8.15	8.15	8.13	8.12	8.10
	Texture	8.32	8.35	8.31	8.33	8.30	8.31	8.27	8.28	8.25	8.24	8.22	8.21	8.20	8.18
	Taste	7.60	7.23	7.55	7.20	7.52	7.18	7.50	7.15	7.48	7.12	7.45	7.10	7.41	7.06
	OAA	8.20	8.09	8.18	8.08	8.16	8.05	8.13	8.02	8.10	7.96	8.04	7.93	7.98	7.87
	Total score	49.20	48.53	49.08	48.47	48.97	48.31	48.77	48.13	48.59	47.78	48.26	47.60	47.88	47.24

OAA- Overall acceptability, FM- Fermented mixture, UFM- Unfermented mixture

also, the scores tend to decrease during storage. Even after the storage of six months, the food mixtures overall acceptability were within acceptable levels (organoleptic scores 7.94 for FM and 7.88 for UFM) which implicates the storage stability of the food mixture.

4.3.3.4. Organoleptic evaluation of the developed food mixture JF+DSF+JSF+P

Food mixtures of this category (Table 85) also showed the similar trends of other food mixtures. The initial overall acceptability of the fermented food mixtures were 8.18 and 8.21 in the *koozha* and *varikka* varieties and unfermented food mixtures scored 8.16 and 8.14 respectively in the *koozha* and *varikka* mixtures. During storage the scores got reduced and reached 7.93 (FM) and 7.87 (UFM) in the *koozha* variety and 8.06 (FM) and 7.97 (UFM) in *varikka* variety. At the end of storage, the total scores were 47.59, 47.23, 48.34 and 47.81 respectively for the fermented and unfermented samples of *koozha* and *varikka* food mixtures.

4.3.4. Viable count of *L. acidophilus* in fermented food mixtures during storage

The viable count of *L. acidophilus* in the fermented food mixtures were enumerated and tabulated. Table 86 represents the results. As revealed from the table, the food mixture JF+DSF+JSF+T (79 in *koozha* and 76×10^9 cfu/g in *varikka*) reported maximum probiotic growth initially in both *koozha* and *varikka* variety. The probiotic count was more in the *koozha* food mixtures than the *varikka* mixtures. As expressed in logs, the probiotic count (initially) of the developed food mixtures ranged from 10.85 to 10.90 log cfu/g. There was a significant reduction in the viable count of *L. acidophilus* throughout the storage period. After six months of storage, the viable count of probiotic organism reduced from the range of 74 to 79×10^9 cfu/g in *koozha* to 24 to 38×10^9 cfu/g and in *varikka* the reduction was from 74 to 76×10^9 cfu/g to 21 to 26×10^9 cfu/g. The viable count of the probiotic bacteria in log cfu are given in parenthesis.

Table 85. Mean scores for organoleptic qualities of fermented and unfermented food mixtures on storage (JF+DSF+JSF+P)

Quality attributes		Storage period in months													
		Initial		1		2		3		4		5		6	
		FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM	FM	UFM
<i>Koozha</i>	Appearance	8.20	8.33	8.19	8.33	8.18	8.31	8.120	8.26	8.00	8.21	7.88	8.13	7.83	8.00
	Colour	8.56	8.56	8.56	8.55	8.55	8.52	8.46	8.46	8.41	8.38	8.35	8.33	8.30	8.28
	Flavour	8.43	8.23	8.41	8.23	8.41	8.20	8.37	8.18	8.33	8.03	8.26	8.00	8.08	7.84
	Texture	8.32	8.35	8.31	8.33	8.30	8.31	8.27	8.28	8.25	8.24	8.22	8.21	8.20	8.18
	Taste	7.40	7.33	7.40	7.30	7.38	7.28	7.35	7.25	7.30	7.20	7.28	7.18	7.25	7.06
	OAA	8.18	8.16	8.17	8.15	8.16	8.12	8.11	8.09	8.06	8.01	8.00	7.97	7.93	7.87
	Total score	49.09	48.96	49.04	48.89	48.98	48.74	48.68	48.52	48.35	48.07	47.99	47.82	47.59	47.23
<i>Varikka</i>	Appearance	8.32	8.35	8.31	8.33	8.30	8.31	8.27	8.28	8.25	8.24	8.22	8.21	8.20	8.18
	Colour	8.56	8.56	8.56	8.56	8.54	8.52	8.50	8.46	8.46	8.40	8.41	8.37	8.35	8.32
	Flavour	8.25	8.23	8.25	8.23	8.23	8.20	8.20	8.16	8.18	8.15	8.15	8.13	8.12	8.10
	Texture	8.32	8.35	8.31	8.33	8.30	8.31	8.27	8.28	8.25	8.24	8.22	8.21	8.20	8.18
	Taste	7.60	7.23	7.55	7.20	7.52	7.18	7.50	7.15	7.48	7.12	7.45	7.10	7.41	7.06
	OAA	8.21	8.14	8.20	8.13	8.18	8.10	8.15	8.07	8.12	8.03	8.09	8.00	8.06	7.97
	Total score	49.26	48.86	49.18	48.78	49.07	48.62	48.89	48.40	48.74	48.18	48.54	48.02	48.34	47.81

OAA- Overall acceptability, FM- Fermented mixture, UFM- Unfermented mixture

Table 86. Viable count of *L .acidophilus* in fermented food mixtures ($\times 10^9$ cfu/g)

Treatment		Viable count						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	78 (10.89)	72 (10.85)	62 (10.79)	53 (10.72)	42 (10.62)	35 (10.54)	24 (10.38)
	JF+DSF+P	74 (10.86)	66 (10.81)	58 (10.76)	49 (10.69)	40 (10.60)	32 (10.50)	30 (10.47)
	JSF+DSF+JSF+T	79 (10.90)	73 (10.86)	67 (10.82)	60 (10.77)	51 (10.70)	43 (10.63)	38 (10.57)
	JF+DSF+JSF+P	75 (10.87)	70 (10.84)	63 (10.79)	57 (10.75)	50 (10.69)	42 (10.62)	31 (10.49)
<i>Varikka</i>	JF+DSF+T	75 (10.87)	68 (10.83)	59 (10.77)	50 (10.69)	41 (10.61)	32 (10.50)	23 (10.36)
	JF+DSF+P	72 (10.85)	63 (10.79)	55 (10.74)	46 (10.66)	38 (10.57)	30 (10.47)	22 (10.34)
	JSF+DSF+JSF+T	76 (10.88)	70 (10.84)	63 (10.79)	54 (10.73)	45 (10.65)	37 (10.56)	26 (10.41)
	JF+DSF+JSF+P	74 (10.86)	69 (10.83)	58 (10.76)	49 (10.69)	38 (10.57)	29 (10.46)	21 (10.32)

Values are mean of three independent enumerations, Figures in parenthesis indicates log cfu/g

4.3.5. Enumeration of total microflora and insect infestation

4.3.5.1. Total microbial population of fermented and unfermented food mixtures

All the food mixtures (both the fermented and unfermented) from both the *koozha* and *varikka* food mixtures were plated in the appropriate media for enumerating the total bacteria, fungi and yeast during each month of storage and the results are presented in tables 87 and 88.

The total bacterial count of fermented food mixtures are given in Table 87. Initially, total bacterial population varied from 81 to 89×10^3 cfu/g in the *koozha* based food mixtures and 77 to 84×10^3 cfu/g in the *varikka* based mixtures. The maximum bacterial count was observed in JF+DSF+JSF+T in the *koozha* and *varikka* based probiotic food mixtures. On storage, there was significant reduction in the total bacterial count of the fermented food mixtures. After six months of storage, the bacterial count of the fermented food mixtures ranged from 40 to 46×10^3 cfu/g in *koozha* and 37 to 41×10^3 cfu/g in *varikka* food mixtures.

Table 88 is the total bacterial count of unfermented food mixtures. Initially, total bacterial population varied from 4 to 8×10^3 cfu/g in *koozha* and 4 to 6×10^3 cfu/g in *varikka* food mixtures. Maximum bacterial count was observed in the food mixture JF+DSF+JSF+P followed by the mixture JF+DSF+JSF+T. There was an increase observed in the total bacterial count of the food mixtures during storage. The minimal bacterial count was observed in JF+DSF+P in *koozha* and JF+DSF+T in *varikka* food mixtures.

4.3.5.2. Yeast and fungal count of fermented and unfermented food mixtures

There were no fungal colonies observed in the fermented and unfermented food mixtures during storage. Similarly no yeast growth also not observed in the food mixtures upto six months.

Table 87. Total bacterial count in fermented food mixtures on storage ($\times 10^3$ cfu/g)

Treatment		Viable count						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	81	79	75	69	63	57	42
	JF+DSF+P	85	80	73	68	59	51	40
	JSF+DSF+JSF+T	89	84	78	70	61	53	46
	JF+DSF+JSF+P	87	82	75	64	59	50	44
<i>Varikka</i>	JF+DSF+T	79	77	70	62	53	48	39
	JF+DSF+P	77	68	63	57	51	42	37
	JSF+DSF+JSF+T	80	75	68	61	54	46	40
	JF+DSF+JSF+P	84	79	72	65	58	49	41

Values are mean of three independent enumerations

Table 88. Total bacterial count in unfermented food mixtures on storage ($\times 10^3$ cfu/g)

Treatment		Viable count						
		Initial	1	2	3	4	5	6
<i>Koozha</i>	JF+DSF+T	5	6	8	10	12	13	14
	JF+DSF+P	4	5	7	8	10	12	13
	JSF+DSF+JSF+T	6	7	10	11	13	17	18
	JF+DSF+JSF+P	8	9	12	14	16	18	19
<i>Varikka</i>	JF+DSF+T	4	5	6	8	10	12	13
	JF+DSF+P	4	5	7	9	10	12	14
	JSF+DSF+JSF+T	5	7	8	9	11	13	15
	JF+DSF+JSF+P	6	8	11	13	14	15	17

Values are mean of three independent enumerations

4.3.5.2. Insect infestation of fermented and unfermented food mixtures on storage

No insects were found in the stored fermented and unfermented food mixtures on storage. The food mixtures were subjected to mere visual observation in day light and microscopic observation was also done. Prior to the microscopic observation, the food mixtures were well sieved first through 60BL sieve and then through 100 BL sieve.

The food mixtures were observed for insect infestation at monthly interval throughout the storage period.

4.3.5.2. Temperature and relative humidity on storage period

Figure 1 and 2 shows the changes in temperature and relative humidity at monthly intervals during storage. The temperature data is attached with Appendix IV.

Figure 1 shows the variation in room temperature at monthly interval. The dry bulb temperatures ranged from 31.54 to 34.07 °C whereas the wet bulb temperatures ranged from 23.07 to 24.21 °C. Figure 7 is the average relative humidity during storage period. Details are attached with Appendix IV.

Relative humidity of the storage room was calculated using the dry and wet bulb temperatures and relative humidity chart. The relative humidity ranged from 43.90 per cent to 50.83 per cent during the storage. Minimum relative humidity was observed during the month of March (43.90 %) and the maximum humidity was observed during the month of January (50.83 %). The correlation between nutrients and weather parameters were analysed using the SPSS software and the results are given in Tables 89 and 90.

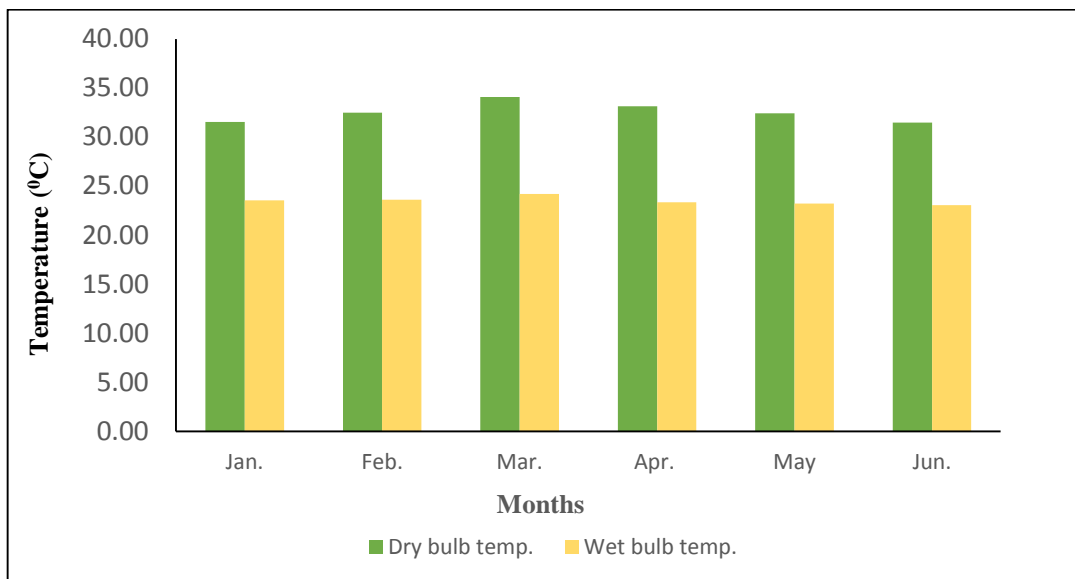


Fig.1. Monthly average temperature during the storage period of food mixtures

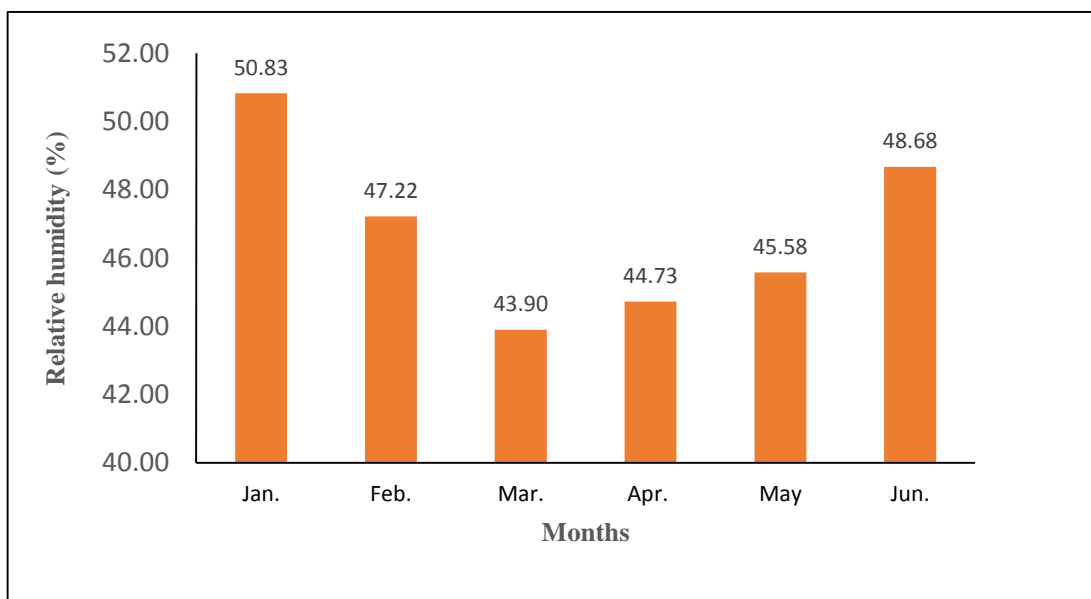


Fig. 2. Relative humidity during storage the storage period of food mixtures

Table 89. Correlation between nutrient content of food mixture and weather parameters (*koozha* variety)

Nutrient	Fermented		Unfermented	
	Dry bulb temperature (°C)	Rh (%)	Dry bulb temperature (°C)	Rh (%)
Titrateable acidity	0.701**	-0.360	-0.506*	0.193
Protein	-0.740**	0.395	-0.748**	0.343
βcarotene	-0.582**	0.215*	-0.696**	0.404
Crude fibre	-0.730**	0.411	-0.717**	0.407
TSS	0.675**	-0.349	0.136*	0.348
Reducing sugar	0.786**	-0.389	0.198*	0.028
Total sugar	0.439*	-0.657**	0.846**	-0.333
Starch	-0.724**	0.443*	-0.707**	0.466*
Total ash	-0.684**	0.404	-0.742**	0.454*
Calcium	-0.730**	0.463*	-0.672**	0.354
Iron	-0.760**	0.394	-0.573**	0.416
Potassium	-0.802**	0.300	-0.710**	0.492*
Thiamine	0.687**	0.500*	-0.110	0.220
Riboflavin	-0.598**	0.453*	-0.755*	0.421
<i>In vitro</i> starch digestibility	0.667**	-0.385	0.729**	-0.512*
<i>In vitro</i> protein digestibility	0.441*	-0.177	0.097**	0.115

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)

Table 89 gives the correlation of nutrients of the fermented and unfermented food mixtures of *koozha* variety with weather parameter. From the analysis it was observed that with increase in temperature the nutrient quality *viz.* protein, β carotene, crude fibre, starch, total ash, calcium, iron, potassium and riboflavin of the fermented food mixtures were found deteriorating in the storage condition. On observing the correlation of nutrients of this food mixture with relative humidity, it was observed that β carotene, starch, calcium, thiamine and riboflavin were found to have a positive correlation whereas the total sugars showed a negative correlation with relative humidity.

It was also clear from the table that the nutrients like titratable acidity, protein, β carotene, crude fibre, starch, total ash, calcium, iron, potassium, thiamine and riboflavin of the unfermented samples were also found decreasing with increased temperature of the storage room. Nutrients like starch, total ash and potassium of the unfermented samples shows a positive and *in vitro* starch digestibility shows negative correlation with relative humidity.

Table 90 is the correlation of nutrients of fermented and unfermented food mixtures of *varikka* variety. On analysing the table, the nutrients like β carotene, starch, total ash, calcium, iron, potassium, and riboflavin were found deteriorate with increase in temperature whereas, TSS was found deteriorating with increase in relative humidity. The correlation between relative humidity and nutrients of unfermented food mixtures, titratable acidity, β carotene, crude fibre, starch, total ash, calcium, iron potassium, thiamine and riboflavin were found deteriorating with increasing storage temperature and the increase in relative humidity resulted in the reduced *in vitro* starch digestibility of the unfermented food mixtures.

Table 90. Correlation between nutrient content of food mixture and weather parameters (*varikka* variety)

Nutrient	Fermented		Unfermented	
	Dry bulb temperature (°C)	Rh (%)	Dry bulb temperature (°C)	Rh (%)
Titratable acidity	0.701**	0.360	-0.710**	0.436
Protein	0.701**	0.360	-0.743**	0.493
β carotene	-0.701**	0.411	-0.708**	0.390
Crude fibre	-0.122	0.398	-0.831**	0.681
TSS	-0.122	-0.558**	0.658**	0.361
Reducing sugar	-0.001	-0.408	0.022*	-0.144
Total sugar	0.399	-0.431	0.665**	0.297
Starch	-0.736**	0.433*	-0.663**	0.315**
Total ash	-0.661**	0.419	-0.729**	0.490*
Calcium	-0.693**	0.413	-0.737**	0.438*
Iron	-0.627**	0.349	-0.731**	0.379
Potassium	-0.657**	0.550**	-0.308**	0.216
Thiamine	-0.426	0.346	-0.739**	0.492
Riboflavin	-0.630**	0.284	-0.736**	0.595
<i>In vitro</i> starch digestibility	0.698**	-0.343	0.787**	-0.128*
<i>In vitro</i> protein digestibility	0.357*	0.103	0.658**	-0.319

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)

4.4. Glycemic index of the selected food mixture

For assessing the glycemic index of the selected food mixtures, the fermented (probiotic) and unfermented food mixture equivalent to 50 g of carbohydrate was given to 10 non diabetic individuals. The average postprandial glycemic response of the individuals after the consumption of jackfruit based food mixtures are represented in Figures 3 and 4

The glycemic index (GI) was calculated using the graphs by plotting the area under curve (AUC). Figure 3 represents the glycemic responses after the ingestion of *koozha* based food mixtures and Figure 4 is the glycemic responses after the ingestion of *varikka* based food mixtures. From the figures, it is clear that the glycemic responses of individuals after the consumption of jackfruit based food mixtures were lesser than the control (50 g glucose). The comparative rise in blood glucose levels on consumption of fermented food mixtures were less than that of unfermented samples. Table 91 shows the glycemic indices of the jackfruit based food mixtures. Along with glycemic index, glycemic load (GL) of the food mixtures were also calculated and the results are furnished in the table.

Table 91. Glycemic indices of jackfruit based food mixtures

Treatments		Quantity (g) (Equivalent to 50g CHO)	Glycemic index	Glycemic load
<i>Koozha</i>	JF+DSF+JSF+P (Fermented)	158.64	45.35	19.03
	JF+DSF+JSF+P (Unfermented)	112.55	51.86	28.12
<i>Varikka</i>	JF+DSF+JSF+P (Fermented)	142.34	47.99	19.79
	JF+DSF+JSF+P (Unfermented)	117.28	54.85	29.97

JF-Jackfruit flour, DSF-Defatted soya flour, JSF-Jackfruit seed flour, T-Tomato, P-Papaya

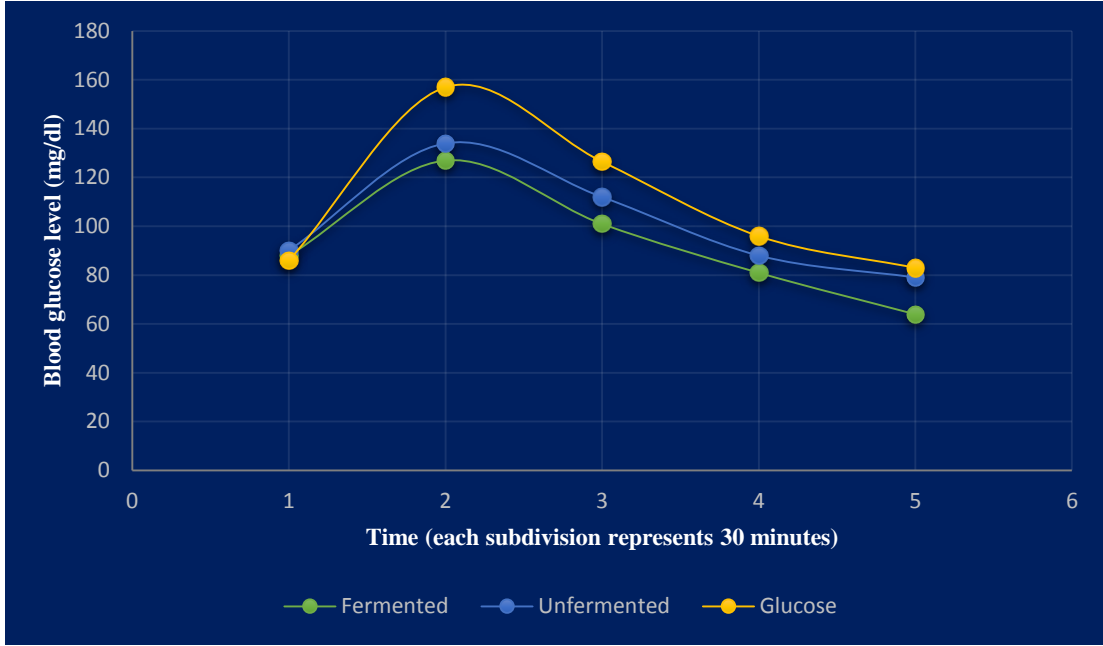


Fig.3. Glycemic response of individuals after consuming *koozha* based food mixtures

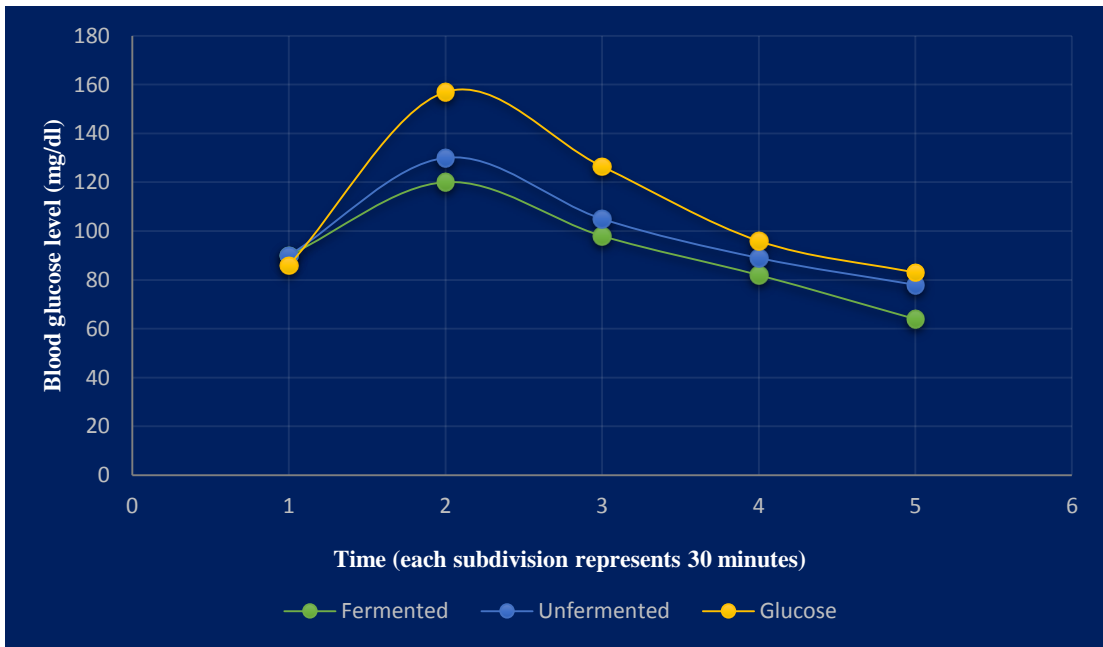


Fig.4. Glycemic response of individuals after consuming *varikka* based food mixture

The glycemic indices of fermented food mixtures were 45.35 and 47.99 respectively for *koozha* and *varikka* based food mixtures. The glycemic indices of unfermented samples were 51.86 (*koozha*) and 54.85 (*varikka*). Whereas the glycemic load (GL) of fermented and unfermented *koozha* food mixtures were 19.03 and 28.12 respectively. On the other hand, GL of the fermented and unfermented *varikka* food mixtures were 19.79 and 29.97 respectively. Both the GI and GL plays an important role in the management of diabetes.

The GI of fermented and unfermented food mixtures of both *koozha* and *varikka* variety were below 55 and hence the food mixtures can be classified as low GI foods. But as revealed from the GL, the fermented food are having medium glycemic load (11-19) and the unfermented food mixtures have high GL (20 or higher). On comparing the glycemic index of the fermented food mixtures (Table 92), it was observed that the GI of *koozha* based food mixtures were found to have minimum GI whereas the *varikka* based had the maximum.

Table 92. Comparison of glycemic index of fermented food mixtures

Treatment	Glycemic index
<i>Koozha</i>	45.365
<i>Varikka</i>	47.975
Mean difference	-2.61
t value	-213.106*

*Significant at 1%

In Table 93, the glycemic index of fermented as well as unfermented food mixtures are given. The table clearly says that the GI of unfermented samples were higher than that of the fermented samples.

Table 93. GI of fermented and unfermented food mixtures

Treatments		Glycemic index
<i>Koozha</i>	Fermented	45.365
	Unfermented	51.870
	Mean difference	-6.505
	t value	-624.980*
<i>Varikka</i>	Fermented	47.975
	Unfermented	54.885
	Mean difference	-8.91
	t value	-405.279*

*Significant at 1%

On concluding the experiment that dealt with the GI index of jackfruit based food mixture, it can be pointed out that the jackfruit based food mixtures come under the category of low glycemic index foods (GI 55 or less). The developed fermented food mixtures had low GL and the unfermented food mixtures had high GL.

4.5. Standardisation of instant shake mixes

Instant probiotic shake mixes were standardized using the best probiotic food mixtures of *koozha* and *varikka* variety. The probiotic food mixture and skimmed milk were incorporated in various proportions during the standardisation procedure. Addition of ten grams of sugar and two grams of nuts and spices were common to all the treatments. Results of the organoleptic evaluation of shake mixes are given in Table 94.

From the table it is clear the organoleptic properties increases from treatment T₁ to T₄. Overall acceptability of the treatments were 7.27, 7.60, 7.79 and 8.02

Table 94. Mean score and mean rank score for the organoleptic qualities of probiotic shake mixes (*koozha*)

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T ₁	8.00 (1.83)	7.43 (2.47)	6.98 (1.70)	7.02 (1.43)	7.11 (1.43)	7.27 (1.23)	43.81
T ₂	8.06 (2.30)	7.49 (1.80)	7.62 (2.63)	7.44 (1.97)	7.49 (2.57)	7.60 (2.00)	45.70
T ₃	8.16 (2.83)	7.69 (2.30)	7.60 (2.37)	7.84 (3.00)	7.69 (2.77)	7.79 (2.97)	46.77
T ₄	8.17 (3.03)	8.19 (3.43)	7.98 (3.30)	7.94 (3.60)	7.84 (3.23)	8.02 (3.80)	48.14
Kendall's W value	0.27	0.50	0.39	0.66	0.41	0.84	

respectively for T₁, T₂, T₃ and T₄. The maximum scores for all the organoleptic attributes were attained by the treatment T₄. This treatment scored 8.17 (3.03), 8.19 (3.43), 7.98 (3.30), 7.94 (3.60), 7.84 (3.32) and 8.02 (3.80) for appearance, colour, flavour, texture, taste and overall acceptability respectively and the total score was 48.14. The mean scores obtained by each treatment for organoleptic evaluation were statistically analysed using the Kendall's coefficient of concordance and the mean ranks were worked out. Based on the mean scores and mean rank scores, the best treatment was selected. Hence from the set of *koozha* based probiotic shake mixes, the treatment T₄ was selected as the best.

The probiotic food mixtures of *varikka* variety was also used for the development of instant shake mixes as explained earlier. This was also subjected to organoleptic evaluation by the selected panel of judges. Table 95 represents the results of organoleptic evaluation of the *varikka* based instant probiotic shake mixes.

In this set also, the organoleptic properties and overall acceptability of the probiotic shake mixes increased from treatment T₁ to T₄ and T₄ scored maximum whereas the treatment T₁ scored minimum. Overall acceptability of the instant probiotic shake mixes were 7.58, 7.59, 7.71 and 8.01 respectively for T₁, T₂, T₃ and T₄. The organoleptic scores of all the treatments were analysed statistically using the Kendall's coefficient of concordance and the mean ranks were worked out. From the group of *varikka* based probiotic shake mixes, the treatment T₄ was selected for further studies which scored a total score of 48.08.

4.5.1. Storage studies of the instant probiotic shake mixes.

From the *koozha* and *varikka* based instant probiotic shake mixes, the treatment T₄ was selected for the storage studies. All the prepared shake mixes were packed in laminated polyethylene pouches and stored at room temperature for a period of two months. The quality aspects were evaluated at 15 days interval and the results are described below.

Table 95. Mean score and mean rank score for the organoleptic qualities of probiotic shake mixes (*varikka*)

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T ₁	7.95 (2.23)	7.73 (1.93)	7.42 (1.80)	7.51 (1.87)	6.96 (1.67)	7.58 (2.13)	45.15
T ₂	8.00 (2.50)	7.86 (2.37)	7.44 (2.00)	7.62 (2.30)	7.15 (2.10)	7.59 (1.87)	45.66
T ₃	8.00 (2.27)	7.98 (2.73)	7.64 (2.43)	7.68 (2.47)	7.31 (2.53)	7.71 (2.53)	46.32
T ₄	8.15 (3.00)	8.14 (2.97)	7.97 (3.77)	7.93 (3.37)	7.88 (3.70)	8.01 (3.47)	48.08
Kendall's W value	0.11	0.16	0.58	0.30	0.51	0.33	



Plate 7. Instant probiotic shake mixes in poly ethylene pouches



Plate 8. Probiotic shake

4.5.1.1. Moisture content of the instant probiotic shake mixes

Table 96 represents the moisture content of instant probiotic shake mixes throughout the storage period. Initially the moisture content of the *koozha* based probiotic shake mix was 2.53 per cent and that of *varikka* based was 2.50 per cent. An independent ‘t’ test was performed to compare the moisture content of *koozha* and *varikka* based probiotic shake mixes and it was observed that throughout the storage period, the moisture content was found higher in the *koozha* based shake mix. Both the food mixtures were found to have minimum moisture content which can contribute to the shelf life of the developed product.

Table 96. Moisture content of the fermented instant probiotic shake mixes (%)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	2.53	2.54	2.56	2.59	2.61
<i>Varikka</i>	2.50	2.52	2.53	2.55	2.58
Mean difference	0.03	0.02	0.03	0.04	0.03
t value	1.98*	7.348*	6.971*	17.146*	10.070

*Significant at 1%

4.5.1.2. Titratable acidity of the instant probiotic shake mixes

In Table 97, the results of titratable acidity of the probiotic shake mixes are given. The initial titratable acidity of 1.33 and 1.32 per cent of *koozha* and *varikka* food mixtures became 1.37 and 1.36 per cent respectively for *koozha* and *varikka* based probiotic shake mixes on storage after 60 days. On analysing it can be concluded no significant variations exist between the acidity of *koozha* and *varikka* based probiotic shake mixes.

Table 97. Titratable acidity of the instant probiotic shake mixes (%)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	1.33	1.32	1.33	1.36	1.37
<i>Varikka</i>	1.32	1.33	1.34	1.35	1.36
Mean difference	0.01	0.01	0.01	0.01	0.01
t value	2.46 ^{NS}	2.46 ^{NS}	0.82 ^{NS}	2.80 ^{NS}	2.32 ^{NS}

NS- Non significant

4.5.1.3. Protein content of the developed shake mixes

Table 98 depicts the protein content of the probiotic shake mixes. The protein content were 26.30 and 26.27 g/100 g respectively for *koozha* and *varikka* based probiotic shake mixes. The protein content of probiotic shake mixes were compared using ‘t’ test. There was no significant difference observed in the protein content of the shake mixes. On storage, like that of the food mixtures, protein content also tends to decrease in the shake mixes also.

Table 98. Protein content of the instant probiotic shake mixes (g /100g)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	26.30	26.30	26.28	26.27	26.25
<i>Varikka</i>	26.27	26.27	26.26	26.24	26.23
Mean difference	0.03	0.03	0.02	0.03	0.02
t value	0.098 ^{NS}	0.098 ^{NS}	0.158 ^{NS}	0.037 ^{NS}	0.021 ^{NS}

NS- Non significant

4.5.1.4. β carotene content of the instant probiotic shake mixes

β carotene content of the developed shake mixes are given in the Table 99. The β carotene content of the *koozha* based shake mix was 313.16 $\mu\text{g}/100\text{g}$ initially which got reduced to 304.40 $\mu\text{g}/100\text{g}$ during the storage. The *varikka* shake mixes contained 312.25 $\mu\text{g}/100\text{g}$ and during storage, it got reduced to 302.67 $\mu\text{g}/100\text{g}$ at the end of storage period. The ‘t’ test conducted between the samples shows that the two shake mixes do not vary significantly with respect to the β carotene content.

Table 99. β carotene content of the instant probiotic shake mixes ($\mu\text{g}/100\text{g}$)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	313.16	311.15	310.28	307.28	304.40
<i>Varikka</i>	312.25	309.16	308.08	306.41	302.67
Mean difference	0.91	1.99	2.20	0.87	1.73
t value	2.63 ^{NS}	2.22 ^{NS}	2.25 ^{NS}	0.94 ^{NS}	1.98 ^{NS}

NS- Non significant

4.5.1.5. Crude fibre content of the instant probiotic shake mixes

On observing the crude fibre content of the shake mixes (Table 100), it can be concluded that shake mixes are a not a good source of dietary fibre. Only negligible amounts were present in the shake mixes. The *koozha* variety contained 0.25g/100 g and *varikka* variety contained 0.22g/100g initially and they got reduced during storage. The ‘t’ test performed between the shake mixes of *koozha* and *varikka* variety showed a significant difference in the crude fibre content of the two.

Table 100. Crude fibre content of the instant probiotic shake mixes (g /100g)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	0.25	0.23	0.22	0.21	0.20
<i>Varikka</i>	0.22	0.21	0.20	0.19	0.17
Mean difference	0.03	0.02	0.02	0.02	0.03
t value	6.78*	2.44 *	1.12 *	2.94 *	2.67*

*Significant at 5% level

4.5.1.6. TSS content of the instant probiotic shake mixes

The table given below (Table 101), shows the TSS content of developed probiotic shake mixes. The initial TSS of *koozha* shake mix was 16.24⁰brix and final TSS was 16.37⁰brix, whereas TSS in the *varikka* variety was 16.39 and 16.55⁰ brix initially and at the end of storage. The results of ‘t’ shows that there is no significant difference between the TSS content of probiotic shake mixes.

Table 101. TSS content of the instant probiotic shake mixes (⁰ brix)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	16.24	16.24	16.28	16.32	16.37
<i>Varikka</i>	16.39	16.40	16.45	16.51	16.55
Mean difference	-0.15	-0.16	-0.17	-0.19	-0.18
t value	-0.184 ^{NS}	-0.196 ^{NS}	-0.208 ^{NS}	0.136 ^{NS}	-0.220 ^{NS}

NS- Non significant

4.5.1.7. Starch content of the instant probiotic shake mixes

The starch content of the developed shake mixes tend to decrease during storage period (Table 102). From the initial values of 15.52 and 16.68 g/100g the starch content became 12.29 and 12.95 g/100 respectively for *koozha* and *varikka* based probiotic shake mixes at the end of storage. An independent 't' test performed between the *koozha* and *varikka* shake mixes do not show any significant difference.

Table 102. Starch content of the instant probiotic shake mixes (g /100g)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	15.52	15.39	14.26	13.93	12.29
<i>Varikka</i>	16.68	16.13	15.40	14.37	12.95
Mean difference	-1.16	-0.74	-1.14	-0.44	-0.66
t value	-0.196 ^{NS}	0.318 ^{NS}	-0.707 ^{NS}	-0.707 ^{NS}	-0.808 ^{NS}

NS- Non significant

4.5.1.8. Reducing and total sugars content of the instant probiotic shake mixes

The reducing sugar content was also found increasing during storage. The reducing sugar of the *koozha* shake mix was 8.21g/100g and *varikka* shake mix was 8.25 g/100g initially. On storage, the reducing sugar content of *koozha* and *varikka* shake mixes increased to 8.27 and 8.31 g/100g respectively. Results of both total and reducing sugars are given in the following table. Similarly the total sugar content of the probiotic shake mixes (Table 103) were 52.55 and 52.03 g/100g respectively for *koozha* and *varikka* respectively. During storage these values increased and at the end of storage the total sugar contents of *koozha* and *varikka* shake mixes were 55.71 and 54.98 g/100g respectively.

Table 103. Reducing and total sugars of the fermented instant probiotic shake mixes (g /100g)

Treatment		Storage period in days				
		Initial	15	30	45	60
Total sugar	<i>Koozha</i>	52.55	53.98	54.89	55.19	55.71
	<i>Varikka</i>	52.03	52.32	53.84	54.27	54.98
	Mean difference	0.52	1.66	1.05	0.92	0.73
	t value	0.021 ^{NS}	2.033 ^{NS}	1.245 ^{NS}	0.516 ^{NS}	2.173 ^{NS}
Reducing sugar	<i>Koozha</i>	8.21	8.22	8.24	8.26	8.27
	<i>Varikka</i>	8.25	8.25	8.26	8.28	8.31
	Mean difference	-0.04	-0.03	-0.02	-0.02	-0.04
	t value	-1.220 ^{NS}	-0.012 ^{NS}	-0.008 ^{NS}	-0.008 ^{NS}	-1.503 ^{NS}

NS- Non significant

4.5.1.9. Mineral contents (total ash, calcium, iron and potassium) of the instant probiotic shake mixes

The mineral contents of the probiotic shake mixes are given in the Table 104. The total ash content of *koozha* and *varikka* food mixtures were 2.76 and 2.77 per cent respectively which implicates a good mineral status in the shake mixes.

Table 104. Mineral contents of the instant probiotic shake mixes

Treatment		Storage period in days				
		Initial	15	30	45	60
Total ash (%)	<i>Koozha</i>	2.76	2.75	2.74	2.72	2.70
	<i>Varikka</i>	2.77	2.77	2.74	2.75	2.73
	Mean difference	-0.01	-0.02	0	-0.03	-0.03
	t value	0.078 ^{NS}	0.063 ^{NS}	0.731 ^{NS}	0.940 ^{NS}	0.024 ^{NS}
Calcium (g/100g)	<i>Koozha</i>	0.57	0.57	0.55	0.53	0.51
	<i>Varikka</i>	0.57	0.56	0.54	0.52	0.50
	Mean difference	0	0.01	0.01	0.01	0.01
	t value	-	0.024 ^{NS}	0.731 ^{NS}	0.241 ^{NS}	0.032 ^{NS}
Iron (mg/100g)	<i>Koozha</i>	0.091	0.091	0.090	0.089	0.086
	<i>Varikka</i>	0.090	0.090	0.089	0.088	0.085
	Mean difference	0.001	0.001	0.001	0.001	0.001
	t value	0.070 ^{NS}	0.070 ^{NS}	0.055 ^{NS}	0.055 ^{NS}	1.768 ^{NS}
Potassium (g/100g)	<i>Koozha</i>	0.68	0.68	0.67	0.64	0.61
	<i>Varikka</i>	0.70	0.69	0.66	0.63	0.60
	Mean difference	-0.02	-0.01	0.01	0.01	0.55
	t value	-0.632 ^{NS}	-0.632 ^{NS}	1.22 ^{NS}	-1.25 ^{NS}	0.50 ^{NS}

NS- Non significant

The calcium was reported to be 0.57g/100g in both the *koozha* and *varikka* shake mixes. At the end of storage 0.51 g/100g calcium was present in *koozha* and 0.50 g/100g was present in *varikka* based probiotic shake mixes. The iron content of the shake mixes were 0.091mg/100g and 0.090 mg/100g respectively for *koozha* and *varikka* varieties. Initially the potassium contents were 0.68g and 0.70 g respectively in hundred grams of *koozha* and *varikka* shake mixes. On storing, all the minerals were found to decrease from the initial points and subsequent reduction was also observed for total ash content also. The results of 't' test showed no significant difference between the mineral content of *koozha* and *varikka* based instant shake mixes.

4.5.1.10. Thiamine and riboflavin content of the instant probiotic shake mixes

Table 105 shows the thiamine and riboflavin content of the developed probiotic shake mixes during storage. It was found that a fair amount of riboflavin (94.06 and 94.18 mg/100g for *koozha* and *varikka* respectively) was detected in the shake mixes.

Table 105. Thiamine and riboflavin content of instant probiotic shake mixes ($\mu\text{g}/100\text{g}$)

Treatment		Storage period in days				
		Initial	15	30	45	60
Thiamine	<i>Koozha</i>	ND	ND	ND	ND	ND
	<i>Varikka</i>	ND	ND	ND	ND	ND
	t value	-	-	-	-	-
Riboflavin	<i>Koozha</i>	94.06	93.98	93.66	93.11	92.90
	<i>Varikka</i>	94.18	93.87	93.76	93.36	93.03
	Mean difference	-0.12	0.11	-0.1	-0.25	-0.13
	t value	-0.147 ^{NS}	0.135 ^{NS}	0.538 ^{NS}	-0.36 ^{NS}	0.310 ^{NS}

NS- Non significant

The result of 't' test suggest that there is no significant difference between the thiamine content of the prepared shake mixes. Thiamine was absent in both the *koozha* and *varikka* based probiotic shake mixes.

4.5.1.11. *In vitro* digestibility of starch and protein of the instant probiotic shake mixes

The table below (Table 106) represents the *in vitro* digestibility of the probiotic shake mixes. The *in vitro* starch digestibility of *koozha* and *varikka* based food mixtures do not vary significantly.

Table 106. *In vitro* starch and protein digestibility of probiotic shake mixes (%)

Treatment		Storage period in days				
		Initial	15	30	45	60
<i>In vitro</i> starch digestibility	<i>Koozha</i>	81.66	81.71	82.26	82.44	83.62
	<i>Varikka</i>	81.70	81.90	82.40	83.33	84.33
	Mean difference	-0.04	-0.49	-0.14	-0.89	-0.71
	t value	1.38 ^{NS}	0.94 ^{NS}	0.59 ^{NS}	2.49 ^{NS}	-1.57 ^{NS}
<i>In vitro</i> protein digestibility	<i>Koozha</i>	83.56	84.57	84.85	86.12	86.91
	<i>Varikka</i>	83.55	84.58	85.20	86.93	87.17
	Mean difference	0.01	-0.01	-0.35	-0.81	-0.26
	t value	1.99 ^{NS}	-1.34 ^{NS}	0.67 ^{NS}	0.67 ^{NS}	0.64 ^{NS}

NS- Non significant

The starch digestibility of the shake mixes were 81.66 and 81.70 per cent initially for *koozha* and *varikka* varieties. On storage, these increased upto 83.62 and 84.33 per cent respectively. A similar trend was observed for protein digestibility also. The protein digestibility of *koozha* shake mix increased from 83.56 per cent to 86.91 per cent and that of *varikka* increased from 83.55 per cent to 87.17 per cent. On comparing the *in vitro* digestibility of the instant probiotic shake mixes, no significant

difference was observed between the starch as well as the protein digestibility of *koozha* and *varikka* based shake mixes.

4.5.1.12. Organoleptic evaluation of the instant probiotic shake mixes

The prepared instant probiotic shake mixes (25 g) were reconstituted with chilled milk (100g) and served chilled for organoleptic evaluation. The sensory attributes were analysed throughout the storage period at 15 days interval and the results are given in Table 107.

The prepared shake mixes scored organoleptic scores of above eight, which means that it was highly acceptable among the judges. For appearance, the mean scores obtained were 8.51 and 8.53 respectively for *koozha* and *varikka* based shake mixes. Even though the scores for appearance decreased during storage, much variation wasn't observed. The mean scores for appearance at the end of storage was 7.86 and 7.78 respectively. The colour of *koozha* and *varikka* shake mixes could obtained a mean score of 8.23 and 8.25 respectively. During storage, minimal variations were observed for the mean scores and at the end of storage, the mean score of *koozha* shake mix was 8.00 and *varikka* was 8.08.

The appearance and flavour of probiotic shake mixes were very good as evident from the mean scores. The initial mean score was 8.33 and 8.43 respectively for *koozha* and *varikka* food mixtures. On storage the flavour tend to decrease and the mean scores obtained at the end of storage were 7.26 and 7.38 respectively for *koozha* and *varikka* based probiotic shake mixes. The texture also scored good points during the organoleptic evaluation and the *koozha* food mixture obtained 8.23 in the initial assessment and 7.73 during the final judgment. The *varikka* shake mix scored 8.15 and 7.71 respectively during the initial and final organoleptic evaluations.

The taste was also highly acceptable and the mean scores for taste of the *koozha* and *varikka* based shake mixes were 8.21 and 8.23 respectively. Even though during storage the taste of shake mixes decreased, it was within the acceptable levels. At the

Table 107. Mean scores for organoleptic evaluation of selected instant probiotic shake mixes during storage

Parameters	<i>Koozha</i>					<i>Varikka</i>				
	Initial	15 days	30 days	45 days	60 days	Initial	15 days	30 days	45 days	60 days
Appearance	8.51	8.51	8.08	8.00	7.86	8.53	8.53	8.05	8.00	7.78
Colour	8.23	8.23	8.20	8.15	8.00	8.25	8.25	8.20	8.17	8.08
Flavour	8.33	8.27	7.91	7.37	7.26	8.43	8.31	7.88	7.46	7.38
Texture	8.23	8.13	7.89	7.82	7.73	8.15	8.10	7.83	7.80	7.71
Taste	8.21	8.21	8.08	8.00	7.86	8.23	8.23	8.05	8.00	7.78
Overall acceptabilty	8.23	8.23	8.05	8.00	7.78	8.23	8.23	8.05	8.00	7.78

end of storage the scores for taste were 7.86 and 7.78 respectively for *koozha* and *varikka*.

The overall acceptability of the *koozha* and *varikka* based instant probiotic shake mixes were 8.22 and 8.23 in the initial organoleptic evaluation and as time elapsed, the overall acceptability decreased and scored 7.80 (*koozha*) and 7.78 (*varikka*) at the end of storage.

4.5.1.13. Viable count of *L. acidophilus* in instant probiotic shake mixes during storage.

Table 108. Viable count of *L. acidophilus* of instant probiotic shake mixes during storage ($\times 10^9$ cfu/ml)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	150 (11.19)	138 (11.13)	127 (11.10)	119 (11.07)	105 (11.02)
<i>Varikka</i>	141 (11.14)	131 (11.11)	124 (11.09)	112 (11.04)	98 (10.99)
Mean difference	15.00	7.00	3.00	7.00	7.00
t value	13.59 ^S	3.67 ^S	3.16 ^S	6.95 ^S	7.34 ^S

Figure in parenthesis indicate bacterial count in log cfu/ml

Values are mean of 3 independent enumerations

Significant at 5% level

From the Table 108, it is clear that the developed shake mixes were able to maintain the viable cells of *L. acidophilus* during the storage period. Maximum number of probiotic cells were observed in the *koozha* based shake mixes compared to the *varikka* based shake mixes. A significant reduction was observed in the probiotic count of shake mixes during storage. The viable count of *koozha* shake mix

reduced from 156 to 105×10^9 cfu/ml on storage and for *varikka* it was from 141 to 98×10^9 cfu/ml.

4.5.1.14. Total microbial population in the instant probiotic shake mixes during storage

The instant probiotic shake mixes were enumerated for total bacteria, fungi, and yeast during the storage period at 15 days interval. The results are presented in Table 109.

4.5.1.15. Total bacterial count in the instant probiotic shake mixes on storage.

Table 109. Total bacterial count of probiotic shake mixes during storage ($\times 10^9$ cfu/ml)

Treatment	Storage period in days				
	Initial	15	30	45	60
<i>Koozha</i>	159 (2.20)	144 (2.15)	135 (2.13)	126 (2.10)	115 (2.06)
<i>Varikka</i>	147 (2.16)	136 (2.13)	124 (2.09)	119 (2.07)	109 (2.03)
Mean difference	12.00	8.00	11.00	7.00	6.00
t value	12.64 ^S	7.27 ^S	11.70 ^S	7.34 ^S	4.11 ^S

Figure in parenthesis indicate bacterial count in log cfu/ml

Values are mean of 3 independent enumerations

Significant at 5% level

The initial total bacterial count of the probiotic shake mixes were 159 and 147 ($\times 10^9$ cfu/ml). The maximum bacterial count was observed in *koozha* based shake mix and minimum observed for *varikka* based shake mix. On storage, the bacterial count of the probiotic shake mixes were found decreasing. After 2 months storage, the total bacterial count of the shake mixes were 115 and 109 ($\times 10^9$ cfu/ml).

4.5.1.16. Fungal count of the instant probiotic shake mixes during storage

During the storage of period of two months, no fungal colonies were observed in the probiotic shake mixes.

4.5.1.17. Yeast count in the instant probiotic shake mixes on storage

There were no yeast colonies found in the probiotic shake mixes during the two month period when it was plated on SDA media.

4.5.1.18. Insect infestation of the stored instant probiotic shake mixes

No insects were found in the stored instant probiotic shake mixes. The shake mixes were subjected to mere visual observation in day light and microscopic observation was also done. Prior to the microscopic observation, the shake mixes were well sieved first through 60BL sieve and then through 100 BL sieve.

The probiotic shake mixes were observed for insect infestation at 15 days interval throughout the storage period (2 months).

On concluding the experiment that dealt with the standardisation and storage studies of the jackfruit based instant probiotic shake mixes, it was observed that it is possible to develop an instant probiotic shake mix with the jackfruit based probiotic food mixtures. The developed shake mixes had good nutritional profile and was shelf stable during the storage period (2 months). Even at the end of storage period, the organoleptic acceptability of the shake mixes were liked moderately by the evaluators. The probiotic shake mixes also maintained the viability of probiotic organism during storage period.

4.6. Standardisation of yoghurt

Jackfruit based sweetened bio-yoghurts were prepared as per the standard procedure of Khedkar *et al.* (2015) by incorporating *L. bulgaricus* and *S. thermophilus*. All the prepared jackfruit bio-yoghurts were organoleptically evaluated by a panel of fifteen selected judges. The organoleptic qualities like appearance,

colour, flavour, texture, taste and overall acceptability of all the bio-yoghurts were evaluated using a nine point hedonic scale. Results of the organoleptic evaluation of different bio-yoghurts are given in Table 110 and 111.

Bio-yoghurts in set 1 was a combination of homogenised milk (HM) and jackfruit pulp (JP) in varying proportions. The amount of jackfruit pulp varied from 10 per cent to 30 per cent and that of homogenized milk from 70 to 90 per cent. Yoghurt prepared with 100 per cent HM served as the control. The experiment was repeated with both *koozha* and *varikka* varieties. Table 110 gives the results of organoleptic properties of *koozha* jackfruit based bio-yoghurts. On observing the mean scores for the organoleptic evaluation of bio-yoghurts of the *koozha* variety, it was evident that the control yoghurt (T_0) scored maximum points for all the organoleptic qualities. The control yoghurt prepared with HM was liked very much by the panelist and the total score obtained was 54. On moving to the jackfruit based bio-yoghurts, treatment T_3 was mostly accepted. This variation secured a mean score of 8.6, 9.00, 8.80, 8.87, 8.87, 8.87 for the organoleptic properties like appearance, colour, flavor, texture, taste and overall acceptability and a total score of 53.01. The treatment T_1 scored least in organoleptic evaluation and the total score of this yoghurt was 46.33. The overall acceptability of the food mixtures T_1 , T_2 and T_3 were in the order of 7.33, 8.67 and 8.87 respectively. Acceptability of the fruit bio-yoghurts tend to increase from T_1 to T_3 .

In the second set of bio-yoghurts, homogenized milk was replaced with skimmed milk (SM) and the control yoghurt was prepared with 100 per cent skimmed milk (SM). The proportion of jackfruit pulp remained the same.

Table 110 reveal that in the second set also, treatment T_0 was most acceptable among the judges and the total score of this treatment was 45.85. The treatment T_3 scored maximum overall acceptability among the jackfruit bio-yoghurts. The mean scores for overall acceptability of the bio-yoghurts were in the order of 7.07, 7.40 and 7.54 for the treatments T_1 , T_2 and T_3 respectively and their total scores in their

Table. 110. Mean scores and mean rank scores for the organoleptic qualities of jackfruit (*koozha*) based yoghurts

Treatment		Appearance	Colour	Flavour	Texture	Taste	OAA	Total score
HM+JP (Set 1)	T ₀	9.00 (3.90)	9.00 (3.43)	9.00 (3.73)	9.00 (4.00)	9.00 (3.70)	9.00 (3.27)	54.00
	T ₁	8.00 (1.60)	8.00 (1.50)	8.00 (1.83)	7.00 (1.50)	8.00 (1.67)	7.33 (1.63)	46.33
	T ₂	8.20 (2.03)	8.87 (2.73)	8.27 (1.63)	8.67 (3.10)	8.67 (3.10)	8.67 (2.83)	51.35
	T ₃	8.60 (3.10)	9.00 (3.23)	8.80 (3.50)	8.87 (3.63)	8.87 (3.27)	8.87 (3.53)	53.01
	Kendalls W value	0.98	0.90	0.70	1.00	0.97	0.84	
SM+JP (Set 2)	T ₀	8.02 (3.47)	7.87 (2.67)	7.40 (2.33)	7.62 (2.73)	7.47 (2.07)	7.47 (2.37)	45.85
	T ₁	7.11 (1.33)	7.11 (1.00)	7.02 (1.63)	7.00 (1.00)	7.02 (1.33)	7.07 (1.00)	42.33
	T ₂	7.33 (1.50)	7.40 (2.43)	7.60 (1.83)	7.33 (2.00)	7.60 (2.07)	7.40 (2.43)	44.66
	T ₃	7.67 (2.93)	7.79 (3.03)	7.32 (2.60)	7.52 (3.00)	7.44 (3.00)	7.54 (2.90)	45.28
	Kendalls W value	0.69	0.4	0.33	0.71	0.42	0.37	

Table 110. Cntd.

HM+SM+JP (Set 3)	T ₀	8.87 (3.70)	8.93 (3.13)	8.33 (2.47)	8.73 (3.00)	8.83 (3.42)	8.96 (3.27)	52.65
	T ₁	7.78 (1.50)	7.80 (1.10)	8.00 (1.73)	7.30 (1.23)	7.95 (1.67)	7.76 (1.63)	46.59
	T ₂	8.10 (1.90)	8.84 (2.60)	8.00 (2.30)	8.33 (2.13)	8.27 (2.10)	8.67 (2.83)	51.45
	T ₃	8.67 (3.03)	8.84 (3.20)	8.33 (3.40)	8.67 (3.13)	8.73 (3.23)	8.78 (3.37)	52.02
	Kendalls W value	0.66	0.72	0.47	0.64	0.42	0.77	

*Figures in parenthesis indicates mean rank scores

** Significant at 1% level

HM- Homogenised milk, SM- Skimmed milk, JP- Jackfruit Pulp, OAA- Overall acceptability

respective orders were 42.33, 44.66 and 45.28. Mean scores for the sensory parameters were found to have an increase from T₁ to T₃. The mean scores for appearance, colour, flavour, texture, taste and overall acceptability of T₃ was in the order of 7.67, 7.79, 7.32, 7.52, 7.44 and 7.54.

The experiments was repeated with preparing bio-yoghurts using equal amounts of homogenized milk (HM) and skimmed milk (SM). Proportion of jackfruit pulp (JP) remained the same as in the previous two sets. Hence in set 3, there was a combination of HM+SM+JP. In this set, the yoghurt with 50 per cent HM and 50 per cent SM served as the control.

The mean scores of overall acceptability of the bio-yoghurts were in the order of 8.96, 7.76, 8.67 and 8.78 respectively for T₀, T₁, T₂ and T₃. Here also, the acceptability of control bio-yoghurts were higher than the jackfruit bio-yoghurts. The total scores of the bio-yoghurts T₀, T₁, T₂ and T₃ were 52.65, 46.59, 51.45 and 52.02 respectively. The acceptability of jackfruit bio-yoghurts tends to increase from T₁ to T₃. The mean scores for organoleptic qualities of the treatment T₃ was 8.67, 8.84, 8.33, 8.67, 8.73 and 8.78 respectively for appearance, colour, flavour, texture, taste and overall acceptability. Similar to that of set 1, and set 2 here also the treatment T₁ scored the least for organoleptic evaluation.

The mean scores obtained by the each treatment of three sets during the organoleptic evaluation were statistically analysed using the Kendall's coefficient of concordance and the mean ranks were worked out. Based on the mean scores, mean rank scores and total scores, the best treatment from each sets were selected for the further studies. In set 1, 2 and 3, the treatment T₃ scored the maximum points and selected for further studies. The selected treatments along with their combination of ingredients are given in Table 111.

Table. 111. Selected combinations of jackfruit bio-yoghurts (*koozha*)

Set	Combination	Treatment
1	30 % JP+ 70 % HM	T ₃
2	30 % JP+ 70 % SM	T ₃
3	30 % JP+ 35 % HM+35 % SM	T ₃

JP- Jackfruit Pulp, HM- Homogenised milk, SM- Skimmed milk

The above said experiments were repeated with the *varikka* variety also. The scores given to each treatment by the judges during organoleptic evaluation were tabulated and given in Table 112.

For the jackfruit bio-yoghurts (HM+JP) of *varikka* variety scores for overall acceptability was in the order of 7.40, 8.67 and 8.82 respectively for the treatments T₁, T₂ and T₃ and their total scores were 47.40, 51.48 and 53.35. The control yoghurt prepared with 100 per cent HM scored maximum for the organoleptic properties (9.00) for appearance, colour, flavour, texture, taste and overall acceptability. Among the JP incorporated bio-yoghurts, treatment T₃ was the best scored variation in this set and the scores obtained for the organoleptic parameters like appearance, colour, flavour, texture, taste, overall acceptability and total score were in the order of 9.00, 9.00, 8.8, 9.00, 8.73, 8.82 and 53.35 respectively. Treatment T₁ secured the lowest scores (total score 47.40) for all the above said parameters.

In a similar fashion, the acceptance was higher for the treatment T₃ in the set 2 of *varikka* variety which replaced HM with SM. The control yoghurt was one with 100 per cent SM. It scored the highest in the group for organoleptic properties. The mean scores for all organoleptic parameters were maximum for treatment T₃ among the jackfruit bio-yoghurts. The mean scores attained by the treatment T₃ were 7.67, 7.81, 7.32, 7.52, 7.34, 7.53 and 45.85 respectively for the appearance, colour, flavour, texture, taste, overall acceptability and total score respectively. Overall

acceptability for the treatments T₁, T₂ and T₃ were 7.07, 7.40 and 7.53 respectively. In this set also T₃ was selected as the best yoghurt.

The third set of experiments were done by incorporating equal amounts of HM and SM. In the third set of experiment with *varikka* variety, the most acceptable treatment among the judges was T₃ and it was revealed by the total score of this treatment (52.27). From the Table 112 it could be clearly understood that the treatment T₃ secured the maximum scores for all the organoleptic parameters among the jackfruit bio-yoghurts. The scores were in the order of 8.67, 8.87, 8.47, 8.87, 8.67 and 8.82 for appearance, colour, flavour, texture, taste and overall acceptability. In this set, the variation T₁ secured least scores for organoleptic properties.

Kendall's coefficient of concordance was used to statistically analyse the data obtained during the organoleptic evaluation of different bio-yoghurts of *varikka* variety and the mean ranks worked were indicated in the parenthesis of the table. Based on the mean scores, mean rank scores and total scores, the best treatment from each of the three sets were selected for the further studies. Just like in the *koozha* variety, here also in set 1, 2 and 3, the treatment T₃ scored the maximum scores and selected for further studies. Based on the mean scores and mean rank scores, it can also be concluded that the acceptability of the jackfruit from the three sets were in the order of HM+JP > H+SM+JP > SM+JP. The selected treatments along with their combination of ingredients are given in table 113.

Table 113. Selected combinations of food mixture (*varikka*)

Set	Combination	Treatment
1	30 % JP+ 70 % HM	T ₃
2	30 % JP+ 70 % SM	T ₃
3	30 % JP+ 35 % HM+ 35 % SM	T ₃

JP- Jackfruit Pulp, HM- Homogenised milk, SM- Skimmed milk,

Table. 112 Mean score and mean rank scores for the organoleptic qualities of jackfruit (*varikka*) based yoghurts

Treatment		Appearance	Colour	Flavour	Texture	Taste	OAA	Total score
HM+JP (Set 1)	T ₀	9.00 (3.70)	9.00 (3.13)	9.00 (3.53)	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)	54.00
	T ₁	8.00 (1.77)	8.20 (1.80)	8.13 (1.80)	7.67 (1.73)	8.00 (1.67)	7.40 (1.67)	47.40
	T ₂	8.67 (2.33)	8.87 (2.73)	8.33 (2.63)	8.67 (3.10)	8.27 (3.10)	8.67 (2.83)	51.48
	T ₃	9.00 (3.03)	9.00 (3.43)	8.80 (3.50)	9.00 (3.63)	8.73 (3.27)	8.82 (3.50)	53.35
	Kendalls W value	0.98	0.90	0.70	1.00	0.97	0.84	
SM+JP (Set 2)	T ₀	8.02 (2.93)	7.87 (2.67)	7.40 (2.33)	7.62 (2.73)	7.47 (2.07)	7.47 (2.37)	45.85
	T ₁	7.11 (1.33)	7.11 (1.10)	7.02 (1.63)	7.00 (1.00)	7.02 (1.57)	7.07 (1.00)	42.33
	T ₂	7.33 (1.90)	7.40 (2.33)	7.60 (2.20)	7.33 (2.03)	7.60 (1.10)	7.40 (2.43)	44.66
	T ₃	7.67 (2.30)	7.81 (2.93)	7.32 (2.43)	7.52 (3.00)	7.34 (3.03)	7.53 (2.93)	45.19
	Kendalls W value	0.69	0.4	0.33	0.71	0.42	0.37	

Table 112. Cntd.

HM+SM+JP (Set 3)	T ₀	8.87 (3.47)	8.93 (2.93)	8.33 (2.47)	8.73 (3.00)	8.53 (2.97)	8.96 (3.27)	52.35
	T ₁	7.93 (1.60)	8.00 (1.50)	8.00 (1.73)	7.00 (1.23)	7.40 (1.60)	7.33 (1.57)	45.66
	T ₂	8.18 (2.03)	8.67 (2.43)	8.27 (2.30)	7.93 (2.13)	7.93 (2.90)	8.67 (2.73)	49.65
	T ₃	8.67 (2.93)	8.87 (3.03)	8.47 (3.40)	8.87 (3.13)	8.57 (3.23)	8.82 (3.50)	52.27
	Kendalls W value	0.66	0.72	0.47	0.64	0.42	0.77	

*Figures in parenthesis indicates mean rank scores

** Significant at 1% level

HM- Homogenized milk, SM- skimmed milk, JP- Jackfruit pulp

4.7. Optimisation of conditions for the growth of *L. acidophilus*

From the jackfruit bio-yoghurts, best one from each set (30 % JP +70 % milk) was selected for the optimisation process. Probiotic bio-yoghurts were prepared with the incorporation of *Lactobacillus acidophilus* (MTCC 10307) at various conditions and the optimum fermentation conditions were drawn based on the results. As in the case of fermented food mixtures, here also variables such as substrate concentration, time of incubation, pH, temperature and population of *L. acidophilus* for inoculation were optimised.

4.7.1. Optimisation of substrate concentration

The best combination of jackfruit based yoghurts (30 % JP + 70 % milk) from all the three sets were used for optimisation studies. Twenty five, fifty and seventy five grams of the yoghurt combination were taken and fermented for 4 hours at 37 °C with 100 µl of *L. acidophilus*. Viable count of *L. acidophilus* were enumerated and the results are given in Table 114.

The experiment showed that the substrate concentration of 25 g resulted in a good curd. On serial dilution also the substrate concentration of 25 g gave more colonies in the MRS medium. The probiotic growth was minimum in the 75g substrate concentration. The number of colonies were maximum in the SM milk combination (75 and 67× 10⁹cfu/ml in *koozha* and *varikka* respectively). The minimum colonies were reported in the HM combination (69 and 60×10⁹cfu/ml for *koozha* and *varikka* respectively). Figures in parenthesis indicates the number of bacteria in log cfu/ml.

Table 114. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different substrate concentrations

Quantity of substrates (g)		25	50	75
		Viable counts ($\times 10^9$ cfu/ml)		
Treatment (Jackfruit bio-yoghurts)				
<i>Koozha</i>	HM+JP	69 (10.83)	57 (10.75)	45 (10.65)
	SM+JP	75 (10.87)	63 (10.75)	59 (10.77)
	HM+SM+JP	72 (10.85)	60 (10.79)	43 (10.63)
<i>Varikka</i>	HM+JP	60 (10.77)	45 (10.65)	38 (10.57)
	SM+JP	67 (10.82)	59 (10.77)	47 (10.67)
	HM+SM+JP	65 (10.81)	56 (10.74)	44 (10.64)

All values are means of three independent enumerations
 JP-Jackfruit pulp, HM- Homogenised milk, SM-Skimmed milk
 Figures in parenthesis indicates log cfu/ml

4.7.2. Optimisation of time of incubation

As the maximum number of probiotic colonies were observed in the 25 g concentration, this was selected for further studies. This was inoculated with 100 μ l of *L. acidophilus* at 37 °C for four, five and six hours. Viable count of *L. acidophilus* were enumerated and the results are given in the Table 115.

Table 115. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different time of incubation

Time of incubation (hrs)		4	5	6
		Viable counts ($\times 10^9$ cfu/ml)		
Treatment (Jackfruit bio-yoghurts)				
<i>Koozha</i>	HM+JP	69 (10.83)	75 (10.87)	84 (10.92)
	SM+JP	72 (10.85)	88 (10.94)	99 (10.99)
	HM+SM+JP	70 (10.84)	86 (10.93)	97 (10.98)
<i>Varikka</i>	HM+JP	60 (10.77)	78 (10.89)	86 (10.93)
	SM+JP	68 (10.82)	81 (10.91)	90 (10.95)
	HM+SM+JP	63 (10.79)	80 (10.90)	88 (10.94)

All values are means of three independent enumerations

JP-Jackfruit pulp, HM- Homogenised milk, SM-Skimmed milk

Figures in parenthesis indicates log cfu/ml

The number of probiotic colonies increased with the incubation period, and maximum number of organism was observed in the plates with the samples of bio-yoghurts incubated for six hours. The number of colonies in the *koozha* variety after six hours incubation were 84, 99 and 97 $\times 10^9$ cfu/ml for HM, SM and HM+SM respectively. The number of colonies were minimum in the *varikka* variety when

compared with the *koozha*. Here the number of probiotic colonies after six hours of incubation were 86, 80 and 88 × 10⁹cfu/ml (10.98, 10.95 and 10.94 log cfu/ml).

4.7.3. Optimisation pH

As the maximum number of probiotic colonies were observed in the 25 g concentration when incubated for six hours, this was selected for further studies. The pH was then optimised. For this 25 g substrate, 100 µl of the probiotic culture was inoculated and incubated. Viable count of *L. acidophilus* were enumerated and the results are given in the Table 116.

Table 116. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different pH

pH Treatment (Jackfruit bio-yoghurts)		5	4.5	4
		Viable counts (× 10 ⁹ cfu/ml)		
<i>Koozha</i>	HM+JP	11 (10.04)	40 (10.60)	79 (10.89)
	SM+JP	14 (10.14)	44 (10.64)	86 (10.93)
	HM+SM+JP	12 (10.07)	40 (10.60)	82 (10.91)
<i>Varikka</i>	HM+JP	10 (10.00)	38 (10.57)	67 (10.82)
	SM+JP	12 (10.07)	41 (10.61)	80 (10.90)
	HM+SM+JP	11 (10.04)	30 (10.47)	88 (10.94)

All values are means of three independent enumerations
 JP-Jackfruit pulp, HM- Homogenised milk, SM-Skimmed milk
 Figures in parenthesis indicates log cfu/ml

The initial pH of the samples before the incubation was six. Change in pH was monitored hourly. When the desired pH attained, the samples were moved to refrigerator to arrest the process of fermentation. From Table 116, it is clear that the maximum number of probiotic colonies were observed in the pH 4. It was also seen from the table that the number of bacterial colonies increases with time. The number of probiotic bacteria present in the yoghurt at pH four were 79, 86 and 82×10^9 cfu/ml for HM, SM and HM+SM of *koozha* bio-yoghurts and 67, 80 and 88×10^9 cfu/ml for HM, SM and HM+SM of *varikka* bio-yoghurts. The bacterial counts expressed in log cfu/ml are given in parenthesis.

4.7.4. Optimisation of temperature of incubation

As the maximum number of probiotic colonies were observed in the 25 g concentration when incubated for six hours and when the pH was four, this was selected for further studies. The temperature of incubation was optimised in the next step. For this 25 g substrate was inoculated with 100µl Of the probiotic culture and incubated for six hours at 38 °C, 40 °C and 42 °C. The maximum number of colonies were observed when the incubation was carried out at 38 °C. Viable count of *L. acidophilus* were enumerated and the results are given in the Table 117.

Table117 shows the viable count of *L. acidophilus* at varying temperatures of 38, 40 and 42 °C. From the data, it is clear that the favorable temperature for the probiotic bacteria were 38 °C rather than 40 or 42 °C. The minimum number of colonies were observed in the temperature of 42 °C. The figures in parenthesis of the table indicates the bacterial cunt in log cfu/ml.

Table 117. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different temperatures

Temperature (°C)		38	40	42
		Viable counts ($\times 10^9$ cfu/ml)		
Treatment (Jackfruit bio-yoghurts)				
<i>Koozha</i>	HM+JP	79 (10.89)	40 (10.60)	24 (10.38)
	SM+JP	84 (10.92)	44 (10.64)	18 (10.25)
	HM+SM+JP	82 (10.91)	40 (10.60)	22 (10.34)
<i>Varikka</i>	HM+JP	70 (10.84)	38 (10.57)	18 (10.25)
	SM+JP	72 (10.86)	41 (10.61)	13 (10.11)
	HM+SM+JP	71 (10.85)	30 (10.47)	15 (10.17)

All values are means of three independent enumerations

JP-Jackfruit pulp, HM- Homogenised milk, SM-Skimmed milk

Figures in parenthesis indicates log cfu/ml

4.7.5. Optimisation of inoculum concentration

As the maximum number of probiotic colonies were observed in the 25 g concentration when incubated at 38 °C for six hours and when the pH was four, this was selected for further studies. The inoculum concentrations were also needed to be

optimised. For this 25 g substrate was inoculated with 100 µl, 200 µl and 300 µl of the probiotic culture and incubated for six hours at 38 °C. Inoculation with 200 and 300 µl of probiotic strains caused over fermentation. Hence, the inoculum concentration of 100 µl was selected. The probiotic count is given in Table 118.

The inoculum concentration of 100 µl was selected for the development of probiotic bio-yoghurts. And the viability of *L. acidophilus* at 100µl concentration satisfies the FSSAI (2016) requirements of probiotic products.

Table 118. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at 100 µl inoculum concentration

Treatment (Jackfruit bio-yoghurts)		Inoculum concentration (µl)
		100
<i>Koozha</i>	HM+JP	79 (10.89)
	SM+JP	84 (10.92)
	HM+SM+JP	82 (10.91)
<i>Varikka</i>	HM+JP	70 (10.84)
	SM+JP	72 (10.86)
	HM+SM+JP	71 (10.85)

All values are means of three independent enumerations
 JP-Jackfruit pulp, HM- Homogenised milk, SM-Skimmed milk
 Figures in parenthesis indicates log cfu/ml

4.8. Development of bio-yoghurts

After optimisation, the yogurts were developed as per the conditions optimised in the previous section of this chapter. To 25 g of the milk and jackfruit pulp combination, 100 µl of probiotic culture and 25 µl of yoghurt culture was added. This mixture was then incubated at 38 °C for six hours. After six hours, the pH was checked whether it attained the desirable pH. After incubation, the products were stored under refrigeration temperature for fifteen days for the further studies.

4.9. Physico-chemical analysis of the developed bio-yoghurts

4.9.1. Moisture content of the developed bio-yoghurts

The prepared probiotic bio-yoghurts were analysed for their nutritional qualities. All the determined parameters are given in the following tables. Table 119 reveals the moisture content of the probiotic bio-yoghurts during storage.

Table 119 describes the moisture content of jackfruit based probiotic bio-yoghurts along with control bio-yoghurts on storage. On analysing the results, the minimum moisture content was reported by the control sample of each set, next to that is the probiotic yoghurt (bio yoghurt) with *varikka* jackfruit and lastly the bio yoghurt with *koozha* jackfruit. The DMRT analysis of the data showed significant difference in the moisture content of HM, SM and HM+SM bio-yoghurts.

In the first group, the bio-yoghurts with homogenized milk (HM) was explained. Moisture content of the HM bio yoghurt was 75.29 per cent, whereas the fruit based bio-yoghurts were 78.52 per cent (*koozha*) and 76.03 per cent (*varikka*) of moisture. On storage, the moisture content of all the bio-yoghurts were found increasing. The per cent increase of moisture content was higher in the control yoghurt followed by *koozha* and *varikka* based bio-yoghurts.



Plate 9. Jackfruit based bio-yoghurts

Table119. Moisture content of the bio-yoghurts during storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM	75.29 ^c	75.73 ^c (0.58)	76.39 ^c (0.87)	77.28 ^c (1.16)
	HM+JP (<i>koozha</i>)	78.52 ^a	79.28 ^a (0.96)	80.28 ^a (1.26)	81.63 ^a (1.68)
	HM+JP (<i>varikka</i>)	76.03 ^b	76.53 ^b (0.65)	77.24 ^b (0.92)	78.23 ^b (1.28)
	CD value	0.030	0.020	0.034	0.028
Set 2	SM	81.93 ^c	82.79 ^c (1.04)	83.93 ^c (1.37)	85.02 ^c (1.32)
	SM+JP (<i>koozha</i>)	83.85 ^a	84.76 ^a (1.09)	85.93 ^a (1.39)	87.14 ^a (1.42)
	SM+JP (<i>varikka</i>)	82.19 ^b	83.07 ^b (1.07)	84.21 ^b (1.37)	85.35 ^b (1.35)
	CD value	0.32	0.29	0.34	0.31
Set 3	HM+SM	80.67 ^c	81.19 ^c (0.64)	81.92 ^c (0.89)	82.91 ^c (1.20)
	HM+SM+JP (<i>koozha</i>)	83.00 ^a	83.81 ^a (0.97)	84.95 ^a (1.36)	86.42 ^a (1.73)
	HM+SM+JP (<i>varikka</i>)	81.84 ^b	82.41 ^b (0.69)	83.21 ^b (0.97)	84.29 ^b (1.29)
	CD value	0.26	0.28	0.14	0.29

DMRT coloumn wise comparison, * significant at 5% level

Figures in parenthesis indicates per cent change over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

The bio-yoghurt with SM served as the control in the second set. Here also the maximum moisture content was observed for *koozha* bio-yoghurts 83.85 per cent. On storage, this was increased and reached a level of 87.14 per cent at the end of storage. The initial moisture contents were 82.19 and 81.93 per cent for *varikka* and control bio-yoghurts. In jackfruit based bio-yoghurts also, increased moisture was observed during storage. The figure mentioned in parenthesis of Table 118 is the per cent increase in moisture content during storage.

In the third set of bio-yoghurts, JP was incorporated with equal proportions (1:1) of HM and SM. Here also the jackfruit *koozha* bio-yoghurt had the maximum moisture content (83.00 %) followed by *varikka* (81.84 %) and control (80.67 %) bio bio-yoghurts. During storage, the moisture content of bio-yoghurts were reported to increase and the maximum increase was recorded in the 10-15 days interval. At the end of storage, the moisture contents were 86.42, 84.29 and 82.91 % per cent for *koozha*, *varikka* and control bio- yoghurts.

Table 120 compares the moisture content of bio-yoghurts in a different fashion i.e. based on the nature of milk used for the preparation of bio-yoghurts. In all the three sets of Table 120, the bio-yoghurt prepared with skimmed milk was found to have significantly higher moisture content.

Table 120. Comparison of moisture content of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	75.29 ^c	75.73 ^c (0.58)	76.39 ^c (0.87)	77.28 ^c (1.16)
	SM control	81.93 ^a	82.79 ^a (1.04)	83.93 ^a (1.37)	85.02 ^a (1.32)
	HM+SM control	80.67 ^b	81.19 ^b (0.64)	81.92 ^b (0.89)	82.91 ^b (1.20)
	CD value	0.030	0.031	0.034	0.028
Set 2	HM+JP (<i>koozha</i>)	78.52 ^c	79.28 ^c (0.96)	80.28 ^c (1.26)	81.63 ^c (1.68)
	SM+JP (<i>koozha</i>)	83.85 ^a	84.76 ^a (1.09)	85.93 ^a (1.39)	87.14 ^a (1.42)
	HM+SM+JP (<i>koozha</i>)	83.00 ^b	83.81 ^b (0.97)	84.95 ^b (1.36)	86.42 ^b (1.73)
	CD value	0.039	0.042	0.030	0.028
Set 3	HM+JP (<i>varikka</i>)	76.03 ^c	76.53 ^c (0.65)	77.24 ^c (0.92)	78.23 ^c (1.28)
	SM+JP (<i>varikka</i>)	82.19 ^a	83.07 ^a (1.07)	84.21 ^a (1.37)	85.35 ^a (1.35)
	HM+SM+JP (<i>varikka</i>)	81.84 ^b	82.41 ^b (0.69)	83.21 ^b (0.97)	84.29 ^b (1.29)
	CD value	0.019	0.021	0.017	0.025

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

4.9.1.2. Titratable acidity content of the developed bio-yoghurts

Table 121 gives the titratable acidity of bio-yoghurts on storage. The initial acidity of set 1 bio-yoghurts ranged from 0.60 to 0.62 per cent. Jackfruit based bio-yoghurts were reported to have less acidity than the control yoghurt. On storage, the acidity of the developed yoghurt were found increasing. The per cent increase during storage at five days interval is given in parenthesis. The acidity of HM bio-yoghurt at the end of 15 days storage was 0.93 per cent and that of the jackfruit based yogurts were 0.91 (*koozha*) and 0.90 (*varikka*) per cent. Within the jackfruit based bio-yoghurts, *koozha* yoghurt were found to have significantly higher acidity than the *varikka* bio-yoghurts.

In the second set of bio-yoghurts, HM was replaced with SM. Here the acidity of control yoghurt was 0.75 per cent which increased to 0.87, 0.98 and 1.29 per cent on 5th, 10th and 15th day of storage respectively. On observing the jackfruit bio-yoghurts, the acidity were 0.73 and 0.71 per cent respectively for *koozha* and *varikka*. Both the bio-yoghurts were found to have increased acidity during storage and at the 15th day the acidity were 1.27 and 1.01 per cent respectively for *koozha* and *varikka* based bio bio-yoghurts.

In the third set of bio-yoghurts, where equal proportions of HM and SM were used, the acidity ranged from 0.62 to 0.64 per cent. The initial acidity of control, *koozha* and *varikka* bio-yoghurts were in the order of 0.64, 0.63 and 0.62 per cent respectively. On storage, acidity of all the bio-yoghurts increased gradually and on the 15th day of storage, the reported acidity were 0.95, 0.93 and 0.91 per cent respectively. The per cent relative change in the titratable acidity during storage is indicated in parenthesis.

Table 121. Acidity of bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM	0.62 ^a	0.73 ^a (17.74)	0.81 ^a (10.95)	0.93 ^a (14.81)
	HM+JP (<i>koozha</i>)	0.61 ^b	0.70 ^b (14.75)	0.83 ^b (18.57)	0.91 ^b (19.63)
	HM+JP (<i>varikka</i>)	0.60 ^c	0.69 ^c (12.85)	0.80 ^c (13.92)	0.90 ^c (7.77)
	CD value	0.14	0.17	0.21	0.24
Set 2	SM	0.75 ^a	0.87 ^a (18.46)	0.98 ^a (12.98)	1.29 ^a (12.64)
	SM+JP (<i>koozha</i>)	0.73 ^b	0.85 ^b (19.04)	0.96 ^b (14.66)	1.27 ^b (12.79)
	SM+JP (<i>varikka</i>)	0.71 ^c	0.82 ^c (12.32)	0.94 ^c (14.63)	1.01 ^c (7.44)
	CD value	0.24	0.31	0.36	0.29
Set 3	HM+SM	0.64 ^a	0.75 ^a (17.18)	0.86 ^a (14.66)	0.95 ^a (10.46)
	HM+SM+JP (<i>koozha</i>)	0.63 ^b	0.73 ^b (17.74)	0.85 ^b (16.43)	0.93 ^b (9.41)
	HM+SM+JP (<i>varikka</i>)	0.62 ^c	0.81 ^c (20.64)	0.92 ^c (13.58)	0.91 ^c (7.60)
	CD value	0.19	0.14	0.17	0.14

DMRT coloumn wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 122. Comparison of acidity of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	0.62 ^c	0.73 ^c (17.74)	0.81 ^c (10.95)	0.93 ^c (14.81)
	SM control	0.75 ^a	0.87 ^a (18.46)	0.98 ^a (12.98)	1.29 ^a (12.64)
	HM+SM control	0.64 ^b	0.75 ^b (17.18)	0.86 ^b (14.66)	0.95 ^b (10.46)
	CD value	0.16	0.19	0.21	0.17
Set 2	HM+JP (<i>koozha</i>)	0.61 ^c	0.70 ^c (14.75)	0.83 ^c (18.57)	0.91 ^c (19.63)
	SM+JP (<i>koozha</i>)	0.73 ^a	0.85 ^a (19.04)	0.96 ^a (14.66)	1.27 ^a (12.79)
	HM+SM+JP (<i>koozha</i>)	0.63 ^b	0.73 ^b (17.74)	0.85 ^b (16.43)	0.93 ^b (9.41)
	CD value	0.24	0.19	0.16	0.17
Set 3	HM+JP (<i>varikka</i>)	0.60 ^c	0.69 ^c (12.85)	0.80 ^c (13.92)	0.90 ^c (7.77)
	SM+JP (<i>varikka</i>)	0.71 ^a	0.82 ^a (12.32)	0.94 ^a (14.63)	1.01 ^a (7.44)
	HM+SM+JP (<i>varikka</i>)	0.62 ^b	0.81 ^b (20.64)	0.92 ^b (13.58)	0.91 ^b (7.60)
	CD value	0.39	0.27	0.14	0.22

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

On comparing the acidity of HM, SM, HM+SM bio-yoghurts, it was observed that the acidity was maximum for SM bio-yoghurts, followed by HM+SM and then SM bio-bio-yoghurts. The per cent increase of acidity on storage was also maximum in SM bio-yoghurts.

4.9.1.3. Fat content of the developed bio-yoghurts

Table 123 represents the fat contents of different bio-yoghurts. The bio-yoghurts prepared from HM reported maximum fat content than the other two groups. The initial fat content of HM control yoghurt was 3.42 per cent and that of SM and HM+SM were 0.59 and 1.07 per cent respectively. The DMRT analysis showed significant difference in the fat content of bio-yoghurts.

In set 1, the fat content of control, *koozha* and *varikka* bio-yoghurts in their respective orders were 3.42, 2.40 and 2.39 per cent. Among the bio-yoghurts of set 1, the control bio-yoghurts reported significantly higher fat content than the jackfruit based bio-yoghurts. On storage, the fat content was found reducing and the per cent reduction over the storage period is indicated in parenthesis.

In set 2 of Table 123, the fat content of SM based bio-yoghurts are explained. The control bio-yoghurt was reported to contain significantly higher fat content than the jackfruit bio-yoghurts, as it is evident from the DMRT results. The fat content of control, *koozha* and *varikka* based bio-yoghurts were 0.59, 0.52 and 0.54 per cent respectively. During storage, the fat content was found reducing and the per cent relative change is indicated in parenthesis.

The fat content of SM+HM based bio-yoghurts are given in set 3 of Table 123. Here also, the control bio-yoghurts were found to have significantly higher fat content initially and throughout the storage period. The control yoghurt was found to have 1.07 per cent fat initially and on storage, it reduced to 0.96 per cent. In the case of *koozha* based bio-yoghurts, the fat content was 1.02 and 0.94 per cent initially and finally and in the case of *varikka*, it was 1.01 and 0.92 per cent respectively.

Table 123. Fat content of bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM	3.42 ^a	3.38 ^a (1.16)	3.32 ^a (1.77)	3.24 ^a (2.40)
	HM+JP (<i>koozha</i>)	2.40 ^b	2.37 ^b (1.25)	2.33 ^b (1.68)	2.29 ^b (1.71)
	HM+JP (<i>varikka</i>)	2.39 ^b	2.36 ^b (1.26)	2.33 ^b (1.27)	2.30 ^b (1.28)
	CD value	0.391	0.243	0.014	0.213
Set 2	SM	0.59 ^a	0.57 ^a (3.38)	0.53 ^a (7.01)	0.50 ^a (5.66)
	SM+JP (<i>koozha</i>)	0.52 ^b	0.50 ^b (3.84)	0.47 ^b (6.00)	0.43 ^b (8.51)
	SM+JP (<i>varikka</i>)	0.54 ^c	0.52 ^c (3.70)	0.49 ^c (5.76)	0.45 ^c (8.16)
	CD value	0.025	0.164	0.042	0.037
Set 3	HM+SM	1.07 ^a	1.03 ^a (3.73)	1.00 ^a (2.91)	0.96 ^a (4.00)
	HM+SM+JP (<i>koozha</i>)	1.02 ^b	1.00 ^b (1.96)	0.97 ^b (1.71)	0.94 ^b (3.09)
	HM+SM+JP (<i>varikka</i>)	1.01 ^b	0.99 ^b (1.98)	0.96 ^b (3.03)	0.92 ^c (4.16)
	CD value	0.014	0.012	0.025	0.27

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 124. Comparison of fat content of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	3.42 ^a	3.38 ^a (1.16)	3.32 ^a (1.77)	3.24 ^a (2.40)
	SM control	0.59 ^c	0.57 ^c (3.38)	0.53 ^c (7.01)	0.50 ^c (5.66)
	HM+SM control	1.07 ^b	1.03 ^b (3.73)	1.00 ^b (2.91)	0.96 ^b (4.00)
	CD value	0.019	0.021	0.017	0.025
Set 2	HM+JP (<i>koozha</i>)	2.40 ^a	2.37 ^a (1.25)	2.33 ^a (1.68)	2.29 ^a (1.71)
	SM+JP (<i>koozha</i>)	0.52 ^c	0.50 ^c (3.84)	0.47 ^c (6.00)	0.43 ^c (8.51)
	HM+SM+JP (<i>koozha</i>)	1.02 ^b	1.00 ^b (1.96)	0.97 ^b (1.71)	0.94 ^b (3.09)
	CD value	1.03	0.193	0.012	0.913
Set 3	HM+JP (<i>varikka</i>)	2.39 ^a	2.36 ^a (1.26)	2.33 ^a (1.27)	2.30 ^a (1.28)
	SM+JP (<i>varikka</i>)	0.54 ^c	0.52 ^c (3.70)	0.49 ^c (5.76)	0.45 ^c (8.16)
	HM+SM+JP (<i>varikka</i>)	1.01 ^b	0.99 ^b (1.98)	0.96 ^b (3.03)	0.92 ^c (4.16)
	CD value	0.264	0.012	0.025	0.013

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 124 compares the fat content of HM, SM and HM+SM bio-yoghurts. Among the control bio-yoghurts, HM based yoghurt had significantly higher fat content whereas SM based bio-yoghurts contains the least. Among the jackfruit bio-yoghurts, the fat contents of *koozha* bio-yoghurts were 2.40 (HM), 0.52 (SM) and 1.02 per cent (HM+SM). The *varikka* bio-yoghurts reported fat in their respective order of 2.39, 0.54 and 1.01 per cent for HM, SM and HM+SM.

During the storage of 15 days, gradual decrease was noticed in the fat content of control as well as jackfruit bio-yoghurts. The decrease in fat content was analysed and given in the parenthesis as per cent differences

4.9.1.4. Reducing sugar content of the developed bio-yoghurts

Table 125 is representing the reducing sugar content in the developed bio-yoghurts. In set 1, bio-yoghurts prepared with HM are given. In this set, the maximum reducing sugar was observed in the *varikka* based yogurt (9.42 g/100g) and the minimum was seen in control yoghurt (7.86 g/100g). On storage, the reducing sugar contents shows reduction and this can be understood from the per cent changes given in parenthesis.

In set 2, bio-yoghurts of SM are given. Here the reducing sugar contents of bio-yoghurts were 7.53, 8.49 and 9.06 g/100g respectively for control, *koozha* and *varikka* bio-yoghurts. The significantly higher reducing sugar was observed in *varikka* bio-yoghurts, followed by *koozha* and control bio-yoghurts.

In the third set, the combination of HM and SM was used and here also the results were similar to that of set 1 and 2. The reducing sugar of control, *koozha* and *varikka* bio-yoghurts were in the order 7.71, 8.67 and 9.34 g/100g respectively. As seen in in the set 1 and 2, here also *varikka* jackfruits reported significantly higher reducing sugar and control bio-yoghurts reported the minimum. On storage of 15 days, the reducing sugar of all the bio-yoghurts got reduced significantly and this is represented in parenthesis of the table.

Table 125. Reducing sugar content of bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM	7.86	7.82 (0.50)	7.73 (1.15)	7.67 (0.77)
	HM+JP (<i>koozha</i>)	8.82	8.78 (0.43)	8.69 (1.02)	8.63 (0.69)
	HM+JP (<i>varikka</i>)	9.42	9.20 (2.30)	9.11 (1.01)	9.02 (0.98)
	CD value	0.041*	0.032*	0.036*	0.021*
Set 2	SM	7.53	7.48 (0.66)	7.39 (1.20)	7.30 (1.21)
	SM+JP (<i>koozha</i>)	8.49	8.44 (0.58)	8.35 (1.06)	8.26 (1.07)
	SM+JP (<i>varikka</i>)	9.06	8.98 (0.88)	8.57 (4.55)	8.42 (1.75)
	CD value	0.046*	0.021*	0.041*	0.036*
Set 3	HM+SM	7.71	7.67 (0.51)	7.58 (1.17)	7.49 (1.18)
	HM+SM+JP (<i>koozha</i>)	8.67	8.63 (0.46)	8.54 (1.04)	8.45 (1.05)
	HM+SM+JP (<i>varikka</i>)	9.34	9.30 (0.42)	9.24 (0.64)	9.19 (0.54)
	CD value	0.021*	0.041*	0.036*	0.037*

DMRT coloumn wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 126. Comparison of reducing sugar content of HM, SM and HM+SM bio-yoghurts on storage (g/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM control	7.86	7.82 (0.50)	7.73 (1.15)	7.67 (0.77)
	SM control	7.53	7.48 (0.66)	7.39 (1.20)	7.30 (1.21)
	HM+SM control	7.71	7.67 (0.51)	7.58 (1.17)	7.49 (1.18)
	CD value	0.032*	0.046*	0.021*	0.016*
Set 2	HM+JP (<i>koozha</i>)	8.82	8.78 (0.43)	8.69 (1.02)	8.63 (0.69)
	SM+JP (<i>koozha</i>)	8.49	8.44 (0.58)	8.35 (1.06)	8.26 (1.07)
	HM+SM+JP (<i>koozha</i>)	8.67	8.63 (0.46)	8.54 (1.04)	8.45 (1.05)
	CD value	0.021*	0.041*	0.036*	0.037*
Set 3	HM+JP (<i>varikka</i>)	9.34	9.30 (0.42)	9.24 (0.64)	9.19 (0.54)
	SM+JP (<i>varikka</i>)	9.06	8.98 (0.88)	8.57 (4.55)	8.42 (1.75)
	HM+SM+JP (<i>varikka</i>)	9.42	9.20 (2.30)	9.11 (1.01)	9.02 (0.98)
	CD value	0.026*	0.045*	0.364*	0.247*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

In Table 126, all the control bio-yoghurts, *koozha* bio-yoghurts and *varikka* bio-yoghurts are grouped and results are explained. From the table it is clear that, bio-yoghurts with HM had maximum reducing sugar content than SM or HM+SM. The minimum reducing sugar was observed for the SM bio-yoghurts of each group.

4.9.1.5. Total sugar content of the developed bio-yoghurts

The total sugar content of prepared bio-yoghurts are given in Table 127. Set 1 which includes HM based bio-yoghurts and the maximum total sugar was seen in HM+JP *koozha* yoghurt (18.79 g/100g) followed by HM+JP *varikka* (17.82 g/100g) and the minimum was in the control yoghurt (11.86 g/100g). On storage, all the three bio-yoghurts of this set tend to decrease gradually and the per cent decrease is given in parenthesis.

Second group comprise of SM based bio-yoghurts and here also the jackfruit based bio-yoghurts were reported to significantly higher total sugar and the minimum was reported for the control yoghurt. Among the jackfruit bio-yoghurts of set 2, the *koozha* yoghurt contained maximum total sugar (18.38 g/100g) and the *varikka* yoghurt contained minimum (17.49 g/100g). On storage, the total sugar content of both the control and jackfruit bio-yoghurts tend to decline. The total sugar content of control *koozha* and *varikka* bio-yoghurts of set to reduces from 11.53, 18.38 and 17.49 g/100g to 11.30, 18.18 and 17.27 g/100g respectively on storage. Figures in parenthesis represents the relative per cent change in the total sugar content.

In the third set total sugar content of HM+SM bio-yoghurts are given. Here also the maximum sugar content of 18.57 g/100g for HM+SM+JP (*koozha*) yoghurt followed by HM+SM+JP (*varikka*) yoghurt and the minimum was seen in control yoghurt (11.71 g/100g). The reduction in total sugar content during storage was also observed in this set.

Table 127. Total sugar content of bio-yoghurts on storage (g/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM	11.86 ^c	11.82 ^c (0.33)	11.73 ^c (0.76)	11.67 ^c (0.511)
	HM+JP (<i>koozha</i>)	18.79 ^a	18.74 ^a (0.26)	18.67 ^a (0.37)	18.59 ^a (0.42)
	HM+JP (<i>varikka</i>)	17.82 ^b	17.78 ^b (0.22)	17.70 ^b (0.44)	17.61 ^b (0.50)
	CD value	0.034	0.024	0.091	1.32
Set 2	SM	11.53 ^c	11.48 ^c (0.433)	11.39 ^c (0.78)	11.30 ^c (0.79)
	SM+JP (<i>koozha</i>)	18.38 ^a	18.35 ^a (0.16)	18.26 ^a (0.49)	18.18 ^a (0.43)
	SM+JP (<i>varikka</i>)	17.49 ^b	17.45 ^b (0.22)	17.36 ^b (0.52)	17.27 ^b (0.51)
	CD value	1.211	0.091	0.084	0.076
Set 3	HM+SM	11.71 ^c	11.67 ^c (0.34)	11.58 ^c (0.77)	11.49 ^c (0.77)
	HM+SM+JP (<i>koozha</i>)	18.57 ^a	18.54 ^a (0.26)	18.48 ^a (0.32)	18.39 ^a (0.48)
	HM+SM+JP (<i>varikka</i>)	17.67 ^b	17.63 ^b (0.22)	17.57 ^b (0.34)	17.48 ^b (0.51)
	CD value	1.118	0.973	0.021	0.241

DMRT coloumn wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 128. Comparison of total sugar content of HM, SM and HM+SM bio-yoghurts on storage (g/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM control	11.86 ^a	11.82 ^a (0.33)	11.73 ^a (0.76)	11.67 ^a (0.511)
	SM control	11.53 ^c	11.48 ^c (0.433)	11.39 ^c (0.78)	11.30 ^c (0.79)
	HM+SM control	11.71 ^b	11.67 ^b (0.34)	11.58 ^b (0.77)	11.49 ^b (0.77)
	CD value	0.013	0.084	0.062	0.018
Set 2	HM+JP (<i>koozha</i>)	18.79 ^a	18.74 ^a (0.26)	18.67 ^a (0.37)	18.59 ^a (0.42)
	SM+JP (<i>koozha</i>)	18.38 ^c	18.35 ^c (0.16)	18.26 ^c (0.49)	18.18 ^c (0.43)
	HM+SM+JP (<i>koozha</i>)	18.57 ^b	18.54 ^b (0.26)	18.48 ^b (0.32)	18.39 ^b (0.48)
	CD value	0.094	0.025	0.043	0.039
Set 3	HM+JP (<i>varikka</i>)	17.82 ^a	17.78 ^a (0.22)	17.70 ^a (0.44)	17.61 ^a (0.50)
	SM+JP (<i>varikka</i>)	17.49 ^c	17.45 ^c (0.22)	17.36 ^c (0.52)	17.27 ^c (0.51)
	HM+SM+JP (<i>varikka</i>)	17.67 ^b	17.63 ^b (0.22)	17.57 ^b (0.34)	17.48 ^b (0.51)
	CD value	0.021	0.014	0.022	0.039

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 128 classify all the control bio-yoghurts together, *koozha* bio-yoghurts and *varikka* bio-yoghurts. So in set 1, the control bio-yoghurts with HM, SM and HM+SM are given. Similarly in the second and third set, *koozha* and *varikka* based bio-yoghurts are given.

In all the three sets, the HM bio-yoghurts were found to have significantly higher total sugar followed by HM+SM and minimum was seen in SM bio-yoghurts. The total sugar contents of control bio-yoghurts were in their respective order of 11.86, 11.53 and 11.71 g/100g for HM, SM and HM+SM. Also the per cent decrease was maximum in SM bio-yoghurts than the HM or HM+SM bio-yoghurts.

4.9.1.6. TSS content of the developed bio-yoghurts

The TSS contents of bio-yoghurts are given in Table 129. In set 1, TSS ranged from 18 to 22 °brix and the DMRT results showed a significant difference in the TSS content of bio-yoghurts with maximum in the jackfruit *koozha* based yoghurt followed by the *varikka* and minimum was reported in control yoghurt. On storage, the TSS content got reduced and became 15, 19 and 17.5 °brix respectively for control, *koozha* and *varikka* bio-yoghurts. The per cent reduction in TSS on storage is represented in parenthesis.

In the second set, bio-yoghurts with SM are given. Here the TSS of control yoghurt was 15 °brix (minimum) and that of *koozha* and *varikka* were 19 °brix (maximum) and 17 °brix. On storage, the TSS of control yoghurt of this set reduced to 14, 13.5 and 13 °brix on 5th, 10th and 15th days of storage. A similar result was also observed in the case of *koozha* as well as *varikka* bio-yoghurts. Here the TSS became 15 and 14.5 °brix on 15th the day of storage. Set 3 gives the TSS of HM+SM bio-yoghurts. Here control yoghurt was found to have a TSS of 16 °brix, *koozha* bio yoghurt 20 °brix and the *varikka* bio yoghurt 18 °brix. *Koozha* yoghurt had significantly higher TSS initially and throughout storage. At the end of storage study, its TSS was

17 °brix. Similar to that of reducing and total sugars, TSS of the bio-yoghurts also found decreasing on storage.

Table 129. TSS content of bio-yoghurts on storage (° Brix)

Treatments		Days			
		1	5	10	15
Set 1	HM	18	17 (5.55)	16 (5.88)	15 (6.25)
	HM+JP (<i>koozha</i>)	22	21 (4.54)	20 (4.76)	19 (5.00)
	HM+JP (<i>varikka</i>)	20	19 (5.26)	18.5	17.5 (2.94)
	CD value	0.004*	0.025*	0.037*	0.031*
Set 2	SM	15	14 (6.66)	13.5 (3.57)	13 (3.70)
	SM+JP (<i>koozha</i>)	19	18 (5.26)	17 (5.55)	15 (11.76)
	SM+JP (<i>varikka</i>)	17	16 (5.88)	15.5 (3.12)	14.5 (6.45)
	CD value	0.021*	0.031*	0.046*	0.028*
Set 3	HM+SM	16	15 (6.25)	14 (6.66)	12 (14.28)
	HM+SM+JP (<i>koozha</i>)	20	19 (5.00)	18 (5.26)	17 (5.55)
	HM+SM+JP (<i>varikka</i>)	18	17 (5.55)	16.5 (2.94)	15.5 (6.06)
	CD value	0.043*	0.021*	0.045*	0.037*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 130. Comparison of TSS of HM, SM and HM+SM bio-yoghurts on storage (⁰brix)

Treatments		Days			
		1	5	10	15
Set 1	HM control	19 ^a	18 ^a (5.55)	17 ^a (5.88)	16 ^a (6.25)
	SM control	15 ^c	14 ^c (6.66)	13.5 ^c (3.57)	13 ^c (3.70)
	HM+SM control	16 ^b	15 ^b (6.25)	14 ^b (6.66)	12 ^b (14.28)
	CD value	0.041*	0.021*	0.031*	0.046*
Set 2	HM+JP (<i>koozha</i>)	22 ^a	21 ^a (4.54)	20 ^a (4.76)	19 ^a (5.00)
	SM+JP (<i>koozha</i>)	19 ^c	18 ^c (5.26)	17 ^c (5.55)	15 ^c (11.76)
	HM+SM+JP (<i>koozha</i>)	20 ^b	19 ^b (5.00)	18 ^b (5.26)	17 ^b (5.55)
	CD value	0.043*	0.021*	0.045*	0.037*
Set 3	HM+JP (<i>varikka</i>)	19 ^a	18 ^a (5.55)	17 ^a (5.88)	16 ^a (6.25)
	SM+JP (<i>varikka</i>)	17 ^c	16 ^c (5.88)	15.5 ^c (3.12)	14.5 ^c (6.45)
	HM+SM+JP (<i>varikka</i>)	18 ^b	17 ^b (5.55)	16.5 ^b (2.94)	15.5 ^b (6.06)
	CD value	0.038*	0.061*	0.021*	0.021*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

From the Table 130, it can be concluded that the TSS content of bio-yoghurts varied significantly and TSS was maximum for control bio-yoghurts whereas minimum in *varikka* jackfruit incorporated bio-yoghurts in all the three sets. During storage TSS found to decrease and the per cent relative reduction is given in parenthesis.

4.9.1.5. Crude fibre content of the developed bio-yoghurts

Table 131 gives the fibre content of bio- yoghurts. As the control bio-yoghurts comprises only milk, fibre was absent in this group. Hence, in this table the crude fibre contents of jackfruit bio-yoghurts are given.

Table 131. Crude fibre content of the jackfruit based bio-yoghurts on storage (g/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM+JP (<i>koozha</i>)	0.52	0.50	0.47	0.44
	HM+JP (<i>varikka</i>)	0.49	0.47	0.43	0.41
	t value	53.47*	47.25*	36.99*	10.04*
Set 2	SM+JP (<i>koozha</i>)	0.51	0.48	0.44	0.41
	SM+JP (<i>varikka</i>)	0.47	0.44	0.41	0.38
	t value	25.10*	37.95*	28.43*	24.49*
Set 3	HM+SM+JP (<i>koozha</i>)	0.53	0.50	0.47	0.43
	HM+SM+JP (<i>varikka</i>)	0.48	0.45	0.42	0.38
	t value	84.43*	20.65*	61.23*	48.51*

*Significant at 1% level

In set1, the fibre contents of HM based bio-yoghurts are represented. *Koozha* based bio-yoghurts had significantly higher fibre content whereas, *varikka* had the

minimum fibre content of 0.52 and 0.49 g/100g respectively. On storage, the fibre content of the bio-yoghurts were found decreasing gradually. Independent 't' test was used to compare the fibre content of *koozha* and *varikka* bio-yoghurts in Table 131. The test result showed that the fibre content of *koozha* and *varikka* bio-yoghurts differ significantly and significant higher amounts of fibre was present in the *koozha* bio-yoghurts throughout the storage period.

Set 2 shows the fibre content throughout storage period of SM based bio-yoghurts. Initially, the *koozha* bio-yoghurts were found to contain 0.51 g/100g and the *varikka* bio-yoghurts were containing 0.47 g/100g fibre. At the end of storage period, the fibre content of *koozha* and *varikka* bio-yoghurts were 0.41 and 0.38 g/100 respectively. The 't' test results showed that in this set also fibre content of *koozha* bio-yoghurts were significantly higher initially and also on storage.

In the third set, the fibre content of HM+SM bio-yoghurts are given. The initial fibre content of HM+SM+JP yoghurt of *koozha* and *varikka* were in their respective order of 0.53 and 0.48 g/100g. During storage, both the *koozha* and *varikka* bio-yoghurts were found to have reduction in the fibre content and on the 15th day of storage, the fibre content of *koozha* bio yoghurt was 0.43 and that of *varikka* bio yoghurt was 0.38 g/100g respectively. The comparison of both the bio-yoghurts with the aid of independent 't' test revealed that the fibre content of the two samples differ significantly and the *koozha* bio-yoghurts were found to have maximum fibre content. Table 131 shows the data in detail.

Table 132 is the comparison of fibre content of bio-yoghurts of HM, SM and HM+SM. In set 1, the *koozha* based bio-yoghurts and in set 2, the *varikka* based bio-yoghurts are compared. In set 1, yoghurt prepared with HM+SM was found to have maximum fibre than the other two. Minimum was observed for yoghurt with SM (0.51 g/100g) based bio-yoghurts. On storage, all the bio-yoghurts were found to have decreased fibre content and the per cent relative change over the previous storage interval is given in parenthesis.

The second set deals with the *varikka* bio-yoghurts prepared from HM, SM and HM+SM. Here, the maximum fibre content was seen in HM based yoghurt (0.49 g/100g) and the minimum was observed in SM based yoghurt (0.47g/100g). The crude fibre reduction on storage is represented in parenthesis.

Table 132. Comparison of crude fibre content of HM, SM and HM+SM bio-yoghurts on storage (g/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM+JP (<i>koozha</i>)	0.52 ^b	0.50 ^a (5.76)	0.47 ^a (6.00)	0.44 ^a (6.38)
	SM+JP (<i>koozha</i>)	0.51 ^c	0.48 ^b (5.88)	0.44 ^b (8.33)	0.41 ^b (6.81)
	HM+SM+JP (<i>koozha</i>)	0.53 ^a	0.50 ^a (5.66)	0.47 ^a (0.60)	0.43 ^a (8.51)
	CD value	0.002*	0.005*	0.003*	0.002*
Set 2	HM+JP (<i>varikka</i>)	0.49 ^a	0.47 ^a (4.08)	0.43 ^a (8.51)	0.41 ^a (4.65)
	SM+JP (<i>varikka</i>)	0.47 ^c	0.44 ^c (6.38)	0.41 ^c (6.81)	0.38 ^b (7.31)
	HM+SM+JP (<i>varikka</i>)	0.48 ^b	0.45 ^b (6.25)	0.42 ^b (6.66)	0.38 ^b (9.52)
	CD value	0.002*	0.002*	0.002*	0.003*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

4.9.1.7. Protein content of the developed bio-yoghurts

Table 133. Protein content of bio-yoghurts on storage (g/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM	3.60 ^a	3.53 ^a (1.94)	3.46 ^a (1.98)	3.39 ^a (2.03)
	HM+JP (<i>koozha</i>)	3.03 ^b	2.99 ^b (1.32)	2.95 ^b (1.33)	2.91 ^b (1.35)
	HM+JP (<i>varikka</i>)	3.05 ^b	3.01 ^b (1.31)	2.97 ^b (1.32)	2.92 ^b (1.68)
	CD value	0.188*	0.231*	0.197*	0.094*
Set 2	SM	3.53 ^a	3.49 ^a (1.13)	3.42 ^a (2.00)	3.37 ^a (1.46)
	SM+JP (<i>koozha</i>)	3.06 ^b	3.02 ^b (1.30)	2.98 ^b (1.32)	2.91 ^b (2.34)
	SM+JP (<i>varikka</i>)	3.04 ^b	3.00 ^b (1.31)	2.96 ^b (1.33)	2.89 ^b (2.34)
	CD value	0.173*	0.094*	0.058*	0.072*
Set 3	HM+SM	3.59 ^a	3.55 ^a (1.11)	3.50 ^a (1.40)	3.42 ^a (2.28)
	HM+SM+JP (<i>koozha</i>)	3.03 ^b	2.98 ^b (1.65)	2.92 ^b (2.01)	2.86 ^b (2.05)
	HM+SM+JP (<i>varikka</i>)	3.04 ^b	3.01 ^b (0.98)	2.94 ^b (2.32)	2.87 ^b (2.38)
	CD value	0.031*	0.032*	0.037*	0.083*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Protein content of the developed bio-yoghurts are described in Table 132. In set 1 the protein content of HM based bio-yoghurts are given.

From the table it is clear that the control samples have higher protein content than the jackfruit bio-yoghurts. The protein content of *koozha* and *varikka* bio-yoghurts were comparable and the protein contents of control, *koozha* and *varikka* bio-yoghurts were in the order of 3.60, 3.03 and 3.05 g/100g respectively. On storage, the protein gets hydrolysed and this is evident from the reduced protein contents of bio-yoghurts on storage. On storage, the protein reduces gradually and reached a final value of 3.39 (control), 2.91 (*koozha*) and 2.92 g/100g (*varikka*). The figures that are given in parenthesis is the per cent relative reduction in the protein contents of bio-yoghurts on storage.

In set 2, the protein content of SM based bio-yoghurts are given and the control bio-yoghurts were found to have significantly higher protein. The protein content of *koozha* yoghurt was in par with that of *varikka* yoghurt and on storage, protein was found to reduce. Figure in parenthesis gives the per cent relative change in protein during storage.

In set 3 also, the control bio-yoghurts were containing maximum protein 3.59 g/100g and the jackfruit bio-yoghurts contain comparable amount of protein. During the storage period of 15 days, a gradual reduction in protein was observed and reported as per cent relative change (indicated in parenthesis).

Table 134. Comparison of protein content of HM, SM and HM+SM bio-yoghurts on storage (g/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM control	3.60 ^a	3.53 ^a (1.94)	3.46 ^a (1.98)	3.39 ^a (2.03)
	SM control	3.53 ^b	3.02 ^b (1.30)	2.98 ^b (1.32)	2.91 ^b (2.34)
	HM+SM control	3.59 ^a	3.55 ^a (1.11)	3.50 ^a (1.40)	3.42 ^a (2.28)
	CD value	0.035*	0.021*	0.024*	0.019*
Set 2	HM+JP (<i>koozha</i>)	3.03 ^{NS}	2.99 ^a (1.32)	2.95 ^a (1.33)	2.91 ^a (1.35)
	SM+JP (<i>koozha</i>)	3.03 ^{NS}	2.98 ^b (1.65)	2.92 ^b (2.01)	2.86 ^b (2.05)
	HM+SM+JP (<i>koozha</i>)	3.03 ^{NS}	2.98 ^b (1.65)	2.92 ^b (2.01)	2.86 ^b (2.05)
	CD value	-	0.931*	0.383*	0.076*
Set 3	HM+JP (<i>varikka</i>)	3.05 ^a	3.01 ^a (1.31)	2.97 ^a (1.32)	2.92 ^a (1.68)
	SM+JP (<i>varikka</i>)	3.04 ^b	3.00 ^b (1.31)	2.96 ^b (1.33)	2.89 ^b (2.34)
	HM+SM+JP (<i>varikka</i>)	3.04 ^b	3.01 ^a (0.98)	2.94 ^{ab} (2.32)	2.87 ^b (2.38)
	CD value	0.241*	0.026	0.037	0.039

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

On observing the Table 134, it is clear that the protein content of HM of set 1 was on par with that of HM+SM. The minimum protein was seen for SM based bio-yoghurts of set 1. On the other hand, the initial protein contents of set 2 (*koozha*) bio-yoghurts does not showed a significant difference. In set 3, the protein contents of SM and HM+SM were comparable. Hence it can be concluded from the table that the type of milk used does not have an impact on the protein content of the bio-yoghurts.

4.9.1.8. β carotene content of the developed bio-yoghurts

Table 135 is the result of β carotene analysis of the prepared bio-yoghurts. In set1, the β carotene content ranged from 2.63 to 3.20 $\mu\text{g}/100\text{ml}$ with maximum in *koozha* based yoghurt (3.20 $\mu\text{g}/\text{ml}$). Among the bio-yoghurts of set 1, the bio-yoghurt with *koozha* jackfruit pulp contained significantly higher β carotene. The β carotene content of HM bio-yoghurts were observed to be decreasing on storage and the percent relative reduction is given in parenthesis. The β carotene content of the bio-yoghurts reached 2.32, 2.83 and 2.61 $\mu\text{g}/100\text{ml}$ respectively for control, *koozha* and *varikka* on 15th day of storage.

In set 2, the β carotene content of SM based bio-yoghurts are given. The β carotene content of the bio-yoghurts were found to vary significantly. In this set, the maximum β carotene was observed in *koozha* yoghurt (3.08 $\mu\text{g}/100\text{ml}$) and the minimum was observed in the control yoghurt (1.80 $\mu\text{g}/100\text{ml}$). During storage, the β carotene content decreased and on 15th day of storage, the values were 1.42, 2.68, 1.98 $\mu\text{g}/100\text{ml}$ respectively for control, *koozha* and *varikka* bio-yoghurts. In the third set, which deals with the results of HM+SM bio-yoghurts, the jackfruit bio-yoghurts were having maximum β carotene (3.11 $\mu\text{g}/\text{ml}$ for *koozha* and 2.65 $\mu\text{g}/\text{ml}$ for *varikka*). The minimum β carotene was seen in control yoghurt i.e. 2.47 $\mu\text{g}/100\text{ml}$. In this set also, the β carotene content was found decreasing on storage. The per cent relative change in β carotene content is given in the parenthesis of the table (Table 135).

Table 135. β carotene of bio-yoghurts on storage ($\mu\text{g}/100\text{ml}$)

Treatments		Days			
		1	5	10	15
Set 1	HM	2.63 ^c	2.51 ^c (4.56)	2.43 ^c (3.18)	2.32 ^c (4.52)
	HM+JP (<i>koozha</i>)	3.20 ^a	3.10 ^a (3.12)	2.97 ^a (4.19)	2.83 ^a (4.71)
	HM+JP (<i>varikka</i>)	2.95 ^b	2.89 ^b (2.03)	2.75 ^b (4.84)	2.61 ^b (5.09)
	CD value	.0381*	0.671*	0.179*	0.982*
Set 2	SM	1.80 ^c	1.69 ^c (6.11)	1.54 ^c (8.87)	1.42 ^c (7.79)
	SM+JP (<i>koozha</i>)	3.08 ^a	2.97 ^a (3.57)	2.82 ^a (5.05)	2.68 ^a (4.96)
	SM+JP (<i>varikka</i>)	2.36 ^b	2.27 ^b (3.81)	2.16 ^b (4.84)	1.98 ^b (8.33)
	CD value	0.022*	0.031*	0.013*	0.021*
Set 3	HM+SM	2.47 ^c	2.35 ^c (4.85)	2.24 ^c (4.68)	2.11 ^c (5.80)
	HM+SM+JP (<i>koozha</i>)	3.11 ^a	3.01 ^a (3.21)	2.85 ^a (5.31)	2.72 ^a (4.56)
	HM+SM+JP (<i>varikka</i>)	2.65 ^b	2.54 ^b (4.15)	2.43 ^b (4.33)	2.31 ^b (4.93)
	CD value	0.026*	0.025*	0.023*	0.021*

DMRT coloumn wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 136. Comparison of β carotene content of HM, SM and HM+SM bio-yoghurts on storage ($\mu\text{g}/100\text{ml}$)

Treatments		Days			
		1	5	10	15
Set 1	HM control	2.63 ^a	2.51 ^a (4.56)	2.43 ^a (3.18)	2.32 ^a (4.52)
	SM control	1.80 ^c	1.69 ^c (6.11)	1.54 ^c (8.87)	1.42 ^c (7.79)
	HM+SM control	2.47 ^b	2.35 ^b (4.85)	2.24 ^b (4.68)	2.11 ^b (5.80)
	CD value	0.027*	0.048*	0.046*	0.023*
Set 2	HM+JP (<i>koozha</i>)	3.20 ^a	3.10 ^a (3.12)	2.97 ^a (4.19)	2.83 ^a (4.71)
	SM+JP (<i>koozha</i>)	3.08 ^c	2.97 ^c (3.57)	2.82 ^c (5.05)	2.68 ^c (4.96)
	HM+SM+JP (<i>koozha</i>)	3.11 ^b	3.01 ^b (3.21)	2.85 ^b (5.31)	2.72 ^b (4.56)
	CD value	0.039*	0.032*	0.025*	0.065*
Set 3	HM+JP (<i>varikka</i>)	2.65 ^a	2.54 ^a (4.15)	2.43 ^a (4.33)	2.31 ^a (4.93)
	SM+JP (<i>varikka</i>)	2.36 ^c	2.27 ^c (3.81)	2.16 ^c (4.84)	1.98 ^c (8.33)
	HM+SM+JP (<i>varikka</i>)	2.95 ^b	2.89 ^b (2.03)	2.75 ^b (4.84)	2.61 ^b (5.09)
	CD value	0.053*	0.039*	0.047*	0.039*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 136 compare the β carotene content of HM, SM and HM+SM bio-yoghurts on storage. Within the sets, the β carotene content of bio-yoghurts vary significantly and in all the three sets, the bio-yoghurts with SM were found to have the minimum β carotene content. The HM based bio-yoghurts reported maximum β carotene content when compared with the SM and HM+SM counterparts.

4.9.1.9. Total ash content of the developed bio-yoghurts

Table 137 gives the total ash content of the bio bio-yoghurts. The ash content of HM based bio-yoghurts were 0.88, 0.81 and 0.82 per cent respectively. The maximum ash content was observed in the control yoghurt, and the result of *koozha* and *varikka* jackfruit based bio-yoghurts were on par. The *koozha* yoghurt had 0.81 and *varikka* had 0.82 per cent of total ash. On storage, the ash content was observed to decrease and the per cent relative change in the ash content is represented in parenthesis of the table.

The second set represents SM based bio-yoghurts. In this set also, the control yoghurt had maximum ash content (0.84 %) and the ash content of *koozha* (0.80 %) and *varikka* (0.79 %) bio-yoghurts were comparable. At the end of storage period, *i.e.* on 15th day, the ash content of control, *koozha* and *varikka* bio-yoghurts were 0.78 per cent, 0.75 per cent and 0.73 per cent respectively.

Set 3 comprise of bio-yoghurts containing HM+SM+JP. In this set, ash content varied from 0.83 per cent to 0.86 per cent. The jackfruit bio-yoghurts were found to be on par. A gradual reduction was observed in the ash content of bio-yoghurts as the storage period advances. On 15th day of storage, the ash content of HM+SM bio-yoghurts were 0.80, 0.78 and 0.77 per cent for control, *koozha* and *varikka* respectively.

Table 137. Total ash contents of bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM	0.88 ^a	0.87 ^a (1.13)	0.85 ^a (2.29)	0.83 ^a (2.35)
	HM+JP (<i>koozha</i>)	0.81 ^b	0.80 ^b (1.23)	0.79 ^b (1.25)	0.77 ^b (2.53)
	HM+JP (<i>varikka</i>)	0.82 ^b	0.81 ^b (1.21)	0.80 ^b (1.23)	0.78 ^b (2.5)
	CD value	0.002*	0.004*	0.002*	0.003*
Set 2	SM	0.84 ^a	0.82 ^a (2.38)	0.80 ^a (2.43)	0.78 ^a (2.5)
	SM+JP (<i>koozha</i>)	0.80 ^b	0.78 ^b (2.5)	0.77 ^b (1.28)	0.75 ^b (2.59)
	SM+JP (<i>varikka</i>)	0.79 ^b	0.76 ^b (3.79)	0.75 ^b (1.31)	0.73 ^b (2.66)
	CD value	0.005*	0.003*	0.002*	0.002*
Set 3	HM+SM	0.86 ^a	0.84 ^a (2.32)	0.82 ^a (2.38)	0.80 ^a (2.43)
	HM+SM+JP (<i>koozha</i>)	0.83 ^b	0.81 ^b (2.40)	0.80 ^b (1.23)	0.78 ^b (2.50)
	HM+SM+JP (<i>varikka</i>)	0.83 ^b	0.81 ^b (2.40)	0.79 ^b (2.46)	0.77 ^b (2.53)
	CD value	0.002*	0.003*	0.002*	0.002*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 138. Comparison of total ash content of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	0.88 ^a	0.87 ^a (1.13)	0.85 ^a (2.29)	0.83 ^a (2.35)
	SM control	0.84 ^c	0.82 ^c (2.38)	0.80 ^c (2.43)	0.78 ^c (2.5)
	HM+SM control	0.87 ^b	0.84 ^b (2.32)	0.82 ^b (2.38)	0.80 ^b (2.43)
	CD value	0.003*	0.024*	0.009*	0.005*
Set 2	HM+JP (<i>koozha</i>)	0.81 ^b	0.80 ^a (1.23)	0.79 ^a (1.25)	0.77 ^a (2.53)
	SM+JP (<i>koozha</i>)	0.80 ^c	0.78 ^b (2.5)	0.77 ^b (1.28)	0.75 ^b (2.59)
	HM+SM+JP (<i>koozha</i>)	0.82 ^a	0.81 ^a (2.40)	0.80 ^a (1.23)	0.78 ^a (2.50)
	CD value	0.053*	0.035*	0.003*	0.002*
Set 3	HM+JP (<i>varikka</i>)	0.82 ^b	0.81 ^a (1.21)	0.80 ^a (1.23)	0.78 ^a (2.5)
	SM+JP (<i>varikka</i>)	0.79 ^c	0.76 ^b (3.79)	0.75 ^c (1.31)	0.73 ^c (2.66)
	HM+SM+JP (<i>varikka</i>)	0.83 ^a	0.81 ^a (2.40)	0.79 ^b (2.46)	0.77 ^b (2.53)
	CD value	0.043*	0.032*	0.006*	0.004*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 138 compares the ash content of milk based, jackfruit *koozha* and jackfruit *varikka* based yoghurts in set 1, 2 and 3. On analysing the results, it was noticed that the ash content of bio-yoghurts in set 1 were in the order of $HM > HM+SM > SM$. In the jackfruit based bio-yoghurts of set 2 and 3, total ash content was significantly higher for $HM+SM+JP$ yoghurts followed by HM based yoghurts.

4.9.1.10. Calcium content of the developed bio-yoghurts

On analysing the calcium content of bio-yoghurts given in the Table 139, it is clear that the control samples had maximum calcium content than the fruit based samples. The calcium content of the bio-yoghurts prepared with HM were 130.23, 98.52 and 98.93 mg/100g respectively. On storage, the calcium content was found decreasing and the per cent change relative to the previous storage is indicated in parenthesis.

In set 2 and set 3 also, the control samples having maximum calcium contents. The calcium content of control samples were 130.23 mg/100g (HM), 128.76 mg/100g (SM) and 127.38 mg/100g ($HM+SM$). On storage, the calcium content was found to decrease in all the three sets. On the 15th day analysis, the calcium content of SM based jackfruit bio-yoghurts were 95.39 and 94.11 mg/100g (*koozha* and *varikka* respectively). The $HM+SM$ based jackfruit yoghurt contain 95.67 and 94.10 mg/100 (*koozha* and *varikka* respectively).

Table 139. Calcium content of bio-yoghurts on storage (mg/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM	130.23 ^a	129.67 ^a (0.43)	128.54 ^a (0.87)	126.97 ^a (1.22)
	HM+JP (<i>koozha</i>)	98.52 ^c	97.14 ^c (1.40)	96.24 (0.92)	95.19 (1.09)
	HM+JP (<i>varikka</i>)	98.93 ^b	97.83 ^b (1.11)	96.49 (1.36)	95.29 (1.24)
	CD value	0.034*	0.049*	0.038*	0.027*
Set 2	SM	128.76 ^a	127.94 ^a (0.63)	126.53 ^a (1.10)	125.33 ^a (0.94)
	SM+JP (<i>koozha</i>)	98.84 ^b	97.49 ^b (1.36)	96.28 ^b (1.24)	95.39 ^b (0.92)
	SM+JP (<i>varikka</i>)	97.76 ^c	96.54 ^c (1.24)	95.38 ^c (1.20)	94.11 ^c (1.33)
	CD value	0.037*	0.029*	0.045*	0.041*
Set 3	HM+SM	127.38 ^a	126.49 ^a (0.69)	125.78 ^a (0.56)	124.37 ^a (1.21)
	HM+SM+JP (<i>koozha</i>)	97.93 ^b	96.48 ^b (1.48)	95.22 ^c (1.30)	95.67 ^b (0.47)
	HM+SM+JP (<i>varikka</i>)	97.41 ^c	96.37 ^c (1.06)	95.99 ^b (0.39)	94.10 ^c (1.06)
	CD value	0.041*	0.034*	0.049*	0.059*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 140. Comparison of calcium content of HM, SM and HM+SM bio-yoghurts on storage (mg/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM control	130.23 ^a	129.67 ^a (0.43)	128.54 ^a (0.87)	126.97 ^a (1.22)
	SM control	128.76 ^b	127.94 ^b (0.63)	126.53 ^b (1.10)	125.33 ^b (0.94)
	HM+SM control	127.38 ^c	126.49 ^c (0.69)	125.78 ^c (0.56)	124.37 ^c (1.21)
	CD value	0.031*	0.039*	0.028*	0.053*
Set 2	HM+JP (<i>koozha</i>)	98.52 ^a	97.14 ^a (1.40)	96.24 ^a (0.92)	95.19 ^a (1.09)
	SM+JP (<i>koozha</i>)	98.84 ^b	97.49 ^b (1.36)	96.28 ^b (1.24)	95.39 ^c (0.92)
	HM+SM+JP (<i>koozha</i>)	97.93 ^c	96.48 ^c (1.48)	95.22 ^c (1.30)	95.67 ^b (0.47)
	CD value	0.049*	0.049*	0.030*	0.037*
Set 3	HM+JP (<i>varikka</i>)	98.93 ^a	97.83 ^a (1.11)	96.49 ^a (1.36)	95.29 ^a (1.24)
	SM+JP (<i>varikka</i>)	97.76 ^b	96.54 ^b (1.24)	95.38 ^c (1.20)	94.11 ^b (1.33)
	HM+SM+JP (<i>varikka</i>)	97.41 ^c	96.37 ^c (1.06)	95.99 ^b (0.39)	94.10 ^b (1.06)
	CD value	0.041*	0.038*	0.039*	0.027*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Set 1 of Table 140 deals with the calcium content of control bio-yoghurts whereas set 2 and 3 represent the *koozha* and *varikka* bio-yoghurts. In all the three sets, the calcium content of control bio-yoghurts were significantly higher followed by *koozha* and *varikka* bio-yoghurts. During storage, calcium was also found to decrease gradually.

4.9.1.11. Iron content of the developed bio-yoghurts

Table 141 gives the iron content of probiotic bio-yoghurts on storage. All the bio-yoghurts were found to have least amount of iron. In set 1, the iron content varied from 0.016 mg/100g to 0.025 mg/100g. The maximum amount was seen in the jackfruit *koozha* yoghurt (0.025 mg/100g) whereas the minimum was observed in control yoghurt (0.016 mg/100g). On storage iron content reduced gradually and the reduction is expressed as per cent relative change and is indicated in parenthesis of the table.

On observing the set 2 and set 3 of Table 141 which respectively dealt with SM and HM+SM based bio-yoghurts, the minimum iron content was reported in the control bio-yoghurts. While the SM *koozha* and *varikka* bio-yoghurts contain 0.023 and 0.024 mg/100g iron respectively. The control bio yoghurt reported only 0.015 mg/100g of iron. In a similar fashion, HM+SM *koozha* and *varikka* bio-yoghurts contain 0.023 and 0.024 mg/100g iron respectively whereas the control sample contain only 0.016 mg/100g.

On storage, the bio-yoghurts were found to loose iron and at the end of storage, the iron contents of SM, HM+SM bio-yoghurts were 0.011 and 0.013 mg/100g. The SM, HM+SM *koozha* yoghurt reported to have 0.020 mg/100g iron and SM, HM+SM *varikka* bio-yoghurts have 0.022 and 0.021 mg/100g iron respectively.

Table 141. Iron content of bio-yoghurt on storage (mg/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM	0.016 ^b	0.016 ^b (0.00)	0.015 ^b (6.25)	0.014 ^b (7.16)
	HM+JP (<i>koozha</i>)	0.025 ^a	0.024 ^a (4.16)	0.022 ^a (4.34)	0.021 ^a (4.76)
	HM+JP (<i>varikka</i>)	0.023 ^a	0.022 ^a (4.34)	0.021 ^a (4.54)	0.020 ^a (4.54)
	CD value	0.003*	0.004*	0.003*	0.003*
Set 2	SM	0.015 ^b	0.014 ^b (6.66)	0.013 ^b (7.14)	0.011 ^b (15.38)
	SM+JP (<i>koozha</i>)	0.023 ^a	0.022 ^a (4.34)	0.021 ^a (4.54)	0.020 ^a (4.76)
	SM+JP (<i>varikka</i>)	0.024 ^a	0.023 ^a (4.00)	0.022 ^a (4.16)	0.022 ^a (4.34)
	CD value	0.002*	0.003*	0.001*	0.002*
Set 3	HM+SM	0.016 ^b	0.015 ^b (6.25)	0.014 ^b (6.66)	0.013 ^b (7.14)
	HM+SM+JP (<i>koozha</i>)	0.023 ^a	0.022 ^a (4.34)	0.021 ^a (4.16)	0.020 ^a (4.76)
	HM+SM+JP (<i>varikka</i>)	0.024 ^a	0.023 ^a (4.16)	0.022 ^a (4.54)	0.021 ^a (4.54)
	CD value	0.003*	0.002*	0.001*	0.003*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 142. Comparison of iron content of HM, SM and HM+SM bio-yoghurts on storage (mg/100g)

Treatments		Days			
		1	5	10	15
Set 1	HM control	0.016 ^a	0.016 ^a (0.00)	0.015 ^a (6.25)	0.014 ^a (7.16)
	SM control	0.015 ^b	0.014 ^c (6.66)	0.013 ^c (7.14)	0.011 ^c (15.38)
	HM+SM control	0.016 ^a	0.015 ^b (6.25)	0.014 ^b (6.66)	0.013 ^b (7.14)
	CD value	0.000*	0.001*	0.000*	0.001*
Set 2	HM+JP (<i>koozha</i>)	0.024 ^a	0.023 ^a (4.16)	0.022 ^a (4.34)	0.021 ^a (4.17)
	SM+JP (<i>koozha</i>)	0.023 ^b	0.022 ^b (4.34)	0.021 ^b (4.54)	0.020 ^b (4.76)
	HM+SM+JP (<i>koozha</i>)	0.023 ^b	0.022 ^b (4.34)	0.021 ^b	0.020 ^b (4.76)
	CD value	0.001*	0.002*	0.001*	0.001*
Set 3	HM+JP (<i>varikka</i>)	0.025 ^a	0.024 ^a (4.34)	0.022 ^a (4.54)	0.021 ^a (4.34)
	SM+JP (<i>varikka</i>)	0.024 ^b	0.023 ^b (4.00)	0.022 ^b (4.16)	0.021 ^b (4.34)
	HM+SM+JP (<i>varikka</i>)	0.024 ^b	0.023 ^b (4.16)	0.022 ^b (4.54)	0.021 ^b (4.54)
	CD value	0.002*	0.001*	0.001*	0.002*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

On comparing the iron content of HM, SM and HM+SM given in Table 141, it is clear that among the control bio-yoghurts, the SM based yoghurt had minimum iron content (0.015 mg/100g) and the iron content of HM and HM+SM were comparable. Among the *koozha* bio-yoghurts given in set 2 of the table, HM based *koozha* yoghurt had maximum iron content (0.024 mg/100g) and that of SM and HM+SM were similar. Likewise in the third set of bio-yoghurts also, HM based yoghurt had maximum iron content (0.025 mg/100g) followed by SM, HM+SM.

4.9.1.12. Potassium content of the developed bio-yoghurts

As given in the set 1 of Table 143, the potassium content of HM based bio-yoghurts varied significantly and it ranged from 132.86 to 187.09 mg/100g. The maximum was reported for *varikka* based yoghurt (187.09 mg/100g) and minimum reported for control yoghurt (132.86 mg/100g). The potassium contents of jackfruit bio-yoghurts were on par. Among the SM bio-yoghurts given in set 2, potassium content of the jackfruit bio-yoghurts were on par (187.03 mg/100g for *koozha* and 186.06 mg/100g for *varikka*). In the third category of bio-yoghurts also where the HM+SM was used for the preparation of yoghurt, the control yoghurt reported minimum potassium (132.00 mg/100g).

In the HM, SM and HM+SM bio-yoghurts, the control bio-yoghurts were reported minimum potassium content and the jackfruit bio-yoghurts had comparable level of potassium throughout the storage. Throughout the storage period, the reduction in potassium content is expressed as per cent relative change and it is given in the parenthesis.

Table 143. Potassium content of bio- yoghurt on storage (mg/100g)

	Treatments	Days			
		1	5	10	15
Set 1	HM	132.86 ^b	131.74 ^b (0.84)	130.62 ^b (0.85)	129.19 ^b (1.09)
	HM+JP (<i>koozha</i>)	188.65 ^a	187.43 ^a (0.65)	186.86 ^a (0.30)	185.56 ^a (1.01)
	HM+JP (<i>varikka</i>)	187.09 ^a	186.49 ^a (0.32)	185.36 ^a (0.69)	184.18 ^a (0.63)
	CD value	1.502*	1.007*	1.030*	2.011*
Set 2	SM	131.58 ^b	130.28 ^b (0.98)	129.86 ^b (0.32)	128.87 ^b (0.76)
	SM+JP (<i>koozha</i>)	187.03 ^a	186.86 ^a (0.09)	185.39 ^a (0.60)	184.19 ^a (0.64)
	SM+JP (<i>varikka</i>)	186.06 ^a	185.11 ^a (0.51)	184.38 ^a (0.39)	183.88 ^a (0.27)
	CD value	1.117*	1.241*	2.001*	1.963*
Set 3	HM+SM	132.00 ^b	131.17 ^b (0.62)	130.29 ^b (0.67)	129.83 ^b (0.35)
	HM+SM+JP (<i>koozha</i>)	188.34 ^a	187.39 ^a (0.50)	186.64 ^a (0.40)	185.15 ^a (0.79)
	HM+SM+JP (<i>varikka</i>)	187.85 ^a	186.54 ^a (0.69)	184.39 ^a (1.15)	183.33 ^a (0.57)
	CD value	1.847*	1.003*	1.037*	1.569*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 144. Comparison of potassium content of HM, SM and HM+SM bio-yoghurts on storage (mg/100g)

	Treatments	Days			
		1	5	10	15
Set 1	HM control	132.86 ^a	131.74 ^a (0.84)	130.62 ^a (0.85)	129.19 ^a (1.09)
	SM control	131.58 ^c	130.28 ^{ab} (0.98)	129.86 ^{ab} (0.32)	128.87 ^{ab} (0.76)
	HM+SM control	132.00 ^b	131.17 ^b (0.62)	130.29 ^b (0.67)	129.83 ^b (0.35)
	CD value	0.029*	0.034*	0.032*	0.031*
Set 2	HM+JP (<i>koozha</i>)	188.65 ^a	187.43 ^a (0.65)	186.86 ^a (0.30)	184.19 ^a (1.01)
	SM+JP (<i>koozha</i>)	187.03 ^{ab}	186.86 ^b (0.09)	185.39 ^{ab} (0.60)	184.19 ^b (0.64)
	HM+SM+JP (<i>koozha</i>)	188.34 ^b	187.39 ^b (0.50)	186.64 ^b (0.40)	185.15 ^b (0.79)
	CD value	0.017*	0.021*	0.019*	0.014*
Set 3	HM+JP (<i>varikka</i>)	187.09 ^a	186.49 ^a (0.32)	185.36 ^a (0.69)	184.18 ^a (0.63)
	SM+JP (<i>varikka</i>)	186.06 ^b	185.11 ^{ab} (0.51)	184.38 ^b (0.39)	183.88 ^{ab} (0.27)
	HM+SM+JP (<i>varikka</i>)	187.85 ^a	186.54 ^a (0.69)	184.39 ^b (1.15)	183.33 ^b (0.57)
	CD value	0.018*	0.023*	0.019*	0.017*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 144 compares the potassium content of bio-yoghurts of HM, SM HM+SM based bio-yoghurts. The potassium content of control bio-yoghurts were in the order of 132.86, 131.58, 132.00 mg/100g for HM, SM and HM+SM respectively. Among the *koozha* bio-yoghurts, the maximum potassium was seen in the HM based one (188.65 mg/100g) and followed by SM and HM+SM based yoghurts. In the *varikka* bio-yoghurts, maximum potassium content was seen in the HM yoghurt initially and throughout the storage.

4.9.1.13. Synerisis content of the developed bio-yoghurts

The Tables 145 to 151 gives the rheological properties of prepared bio-yoghurts on storage. Table 145 shows the synerisis of bio-yoghurts during storage. Among the bio-yoghurts of HM, SM and HM+SM bio-yoghurts, the control bio-yoghurts were reported to have maximum synerisis (4.25, 5.00 and 4.65 % respectively for HM, SM and HM+SM) and the *varikka* jackfruit incorporated bio-yoghurts had minimum synerisis (3.55, 4.75 and 4.10 % respectively for HM, SM and HM+SM). It was observed from the table that the addition of fruit pulp caused reduction in the synerisis. Among the jackfruit bio-yoghurts, the *varikka* variety bio-yoghurts had minimum synerisis.

Synerisis of bio-yoghurts as given in the Table 145 was found to follow the order of control > *koozha* > *varikka* bio-yoghurts. The figures given in the parenthesis shows the per cent relative change in synerisis of the yoghurt samples during storage. During the storage of 15 days, the bio-yoghurts were reported to have increase in synerisis. Increase in synerisis was noticed in all the stored bio-yoghurts.

Table 145.Synerisis of bio- yoghurt on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM	4.25 ^a	4.30 ^a (1.17)	4.35 ^a (1.16)	4.50 ^a (3.44)
	HM+JP (<i>koozha</i>)	3.70 ^b	3.80 ^b (2.70)	3.90 ^b (2.63)	4.00 ^b (2.56)
	HM+JP (<i>varikka</i>)	3.55 ^c	3.60 ^c (1.40)	3.65 ^c (1.38)	3.70 ^c (1.36)
	CD value	0.117*	0.113*	0.114*	0.117*
Set 2	SM	5.00 ^a	5.20 ^a (4.00)	5.25 ^a (0.96)	5.30 ^a (0.95)
	SM+JP (<i>koozha</i>)	4.80 ^b	4.85 ^b (1.04)	4.90 ^b (1.03)	4.95 ^b (1.02)
	SM+JP (<i>varikka</i>)	4.75 ^c	4.80 ^c (1.05)	4.85 ^c (1.04)	4.90 ^c (1.03)
	CD value	0.118*	0.117*	0.119*	0.115*
Set 3	HM+SM	4.65 ^a	4.70 ^a (1.07)	4.72 ^a (0.42)	4.75 ^a (0.63)
	HM+SM+JP (<i>koozha</i>)	4.15 ^b	4.20 ^b (1.20)	4.23 ^b (0.71)	4.30 ^b (1.65)
	HM+SM+JP (<i>varikka</i>)	4.10 ^c	4.12 ^c (0.48)	4.15 ^c (0.72)	4.20 ^c (1.20)
	CD value	0.115*	0.114*	0.114*	0.112*

DMRT coloumn wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 146. Comparison of synerisis of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	4.25 ^c	4.30 ^c (1.17)	4.35 ^c (1.16)	4.50 ^c (3.44)
	SM control	5.00 ^a	5.20 ^a (4.00)	5.25 ^a (0.96)	5.30 ^a (0.95)
	HM+SM control	4.65 ^b	4.70 ^b (1.07)	4.72 ^b (0.42)	4.75 ^b (0.63)
	CD value	0.044*	0.038*	0.046*	0.049*
Set 2	HM+JP (<i>koozha</i>)	3.70 ^c	3.80 ^c (2.70)	3.90 ^c (2.63)	4.00 ^c (2.56)
	SM+JP (<i>koozha</i>)	4.80 ^a	4.85 ^a (1.04)	4.90 ^a (1.03)	4.95 ^a (1.02)
	HM+SM+JP (<i>koozha</i>)	4.15 ^b	4.20 ^b (1.20)	4.23 ^b (0.71)	4.30 ^b (1.65)
	CD value	0.039*	0.052*	0.031*	0.048*
Set 3	HM+JP (<i>varikka</i>)	3.55 ^c	3.60 ^c (1.40)	3.65 ^c (1.38)	3.70 ^c (1.36)
	SM+JP (<i>varikka</i>)	4.75 ^a	4.80 ^a (1.05)	4.85 ^a (1.04)	4.90 ^a (1.03)
	HM+SM+JP (<i>varikka</i>)	4.10 ^b	4.12 ^b (0.48)	4.15 ^b (0.72)	4.20 ^b (1.20)
	CD value	0.035*	0.044*	0.038*	0.037*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

As shown in Table 146, synerisis of the bio-yoghurts vary significantly within the group. Among the control bio-yoghurts of SM, HM and HM+SM given in set 1, the HM yoghurt had the minimum (4.25 %) and SM bio-yoghurts had the maximum (5.00 %) synerisis. In a similar fashion, among the *koozha* and *varikka* bio-yoghurts (set 2 and 3) prepared with SM, HM and HM+SM the minimum synerisis was observed in HM+JP bio-yoghurts (3.70 and 3.55 % for *koozha* and *varikka* respectively) and maximum in SM (4.80 and 4.75 % respectively for *koozha* and *varikka*).

4.9.1.14. Water holding capacity (WHC)

The water holding capacity (WHC) of the bio-yoghurts are given in Table 147. Significant difference was observed in the WHC of the bio-yoghurts of each set. The maximum WHC in set 1 was exhibited by bio-yoghurts prepared with HM. Within this set, the maximum WHC was observed in HM+JP *varikka* (96.45 %) followed by HM+JP *koozha* (96.30 %) and the minimum was seen in HM control (95.75 %).

In the second and third sets also, the *varikka* based bio-yoghurts were reported to have maximum WHC and the control samples were reported to have minimum. The observed rank of bio-yoghurts in the descending order of WHC was *varikka* based >*koozha* based>control bio-yoghurts. Throughout the storage period of 15 days, the WHC was assessed at five days interval and the per cent relative change (decrease) was reported in parenthesis. During the storage period all the bio-yoghurts were found to have a gradual reduction in the WHC.

Table 147. Water holding capacity of bio- yoghurt on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM	95.75 ^c	95.50 ^c (0.26)	95.45 ^c (0.05)	94.00 ^c (1.51)
	HM+JP (<i>koozha</i>)	96.30 ^b	96.20 ^b (0.10)	96.00 ^b (0.20)	95.50 ^b (0.52)
	HM+JP (<i>varikka</i>)	96.45 ^a	96.40 ^a (0.05)	96.35 ^a (0.05)	96.30 ^a (0.05)
	CD value	0.297*	0.063*	0.054*	0.038*
Set 2	SM	95.00 ^c	94.80 ^c (0.21)	94.75 ^c (0.05)	94.70 ^c (0.05)
	SM+JP (<i>koozha</i>)	95.20 ^b	95.15 ^b (0.05)	95.10 ^b (0.06)	95.05 ^b (0.06)
	SM+JP (<i>varikka</i>)	95.25 ^a	95.20 ^a (0.05)	95.15 ^a (0.05)	95.10 ^a (0.05)
	CD value	0.351*	0.028*	0.043*	0.052*
Set 3	HM+SM	95.35 ^c	95.30 ^c (0.06)	95.28 ^c (0.02)	95.25 ^c (0.03)
	HM+SM+JP (<i>koozha</i>)	95.85 ^b	95.80 ^b (0.05)	95.77 ^b (0.03)	95.60 ^b (0.17)
	HM+SM+JP (<i>varikka</i>)	95.90 ^a	95.88 ^a (0.02)	95.85 ^a (0.03)	95.80 ^a (0.05)
	CD value	0.276*	0.038*	0.042*	0.031*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 148. Comparison of water holding capacity of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	95.75 ^a	95.50 ^a (0.26)	95.45 ^a (0.05)	94.00 ^a (1.51)
	SM control	95.00 ^c	94.80 ^c (0.21)	94.75 ^c (0.05)	94.70 ^c (0.05)
	HM+SM control	95.35 ^b	95.30 ^b (0.06)	95.28 ^b (0.02)	95.25 ^b (0.03)
	CD value	0.068*	0.043*	0.052*	0.043*
Set 2	HM+JP (<i>koozha</i>)	96.30 ^a	96.20 ^a (0.10)	96.00 ^a (0.20)	95.50 ^a (0.52)
	SM+JP (<i>koozha</i>)	95.20 ^c	95.15 ^c (0.05)	95.10 ^c (0.06)	95.05 ^c (0.06)
	HM+SM+JP (<i>koozha</i>)	95.85 ^b	95.80 ^b (0.05)	95.77 ^b (0.03)	95.60 ^b (0.17)
	CD value	0.038*	0.039*	0.041*	0.037*
Set 3	HM+JP (<i>varikka</i>)	96.45 ^a	96.40 ^a (0.05)	96.35 ^a (0.05)	96.30 ^a (0.05)
	SM+JP (<i>varikka</i>)	95.25 ^c	95.20 ^c (0.05)	95.15 ^c (0.05)	95.10 ^c (0.05)
	HM+SM+JP (<i>varikka</i>)	95.90 ^b	95.88 ^b (0.02)	95.85 ^b (0.03)	95.80 ^b (0.05)
	CD value	0.042*	0.048*	0.021*	0.018*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

From Table 148, it can be concluded that among the control, *koozha* and *varikka* based bio-yoghurts of set 1, 2 and 3 the bio-yoghurts with HM was found to have maximum WHC than the SM and HM+SM. The ascending order of WHC was SM < SH+SM < HM.

4.9.1.15. Curd tension

The curd tension of bio-yoghurts are described in Table 149. The curd tension of HM based bio-yoghurts are discussed in set 1 of the table. DMRT shows a significant difference in the curd tension of control, *koozha* and *varikka* based bio-yoghurts. Curd tension of set 1 bio-yoghurts varied from 0.115 to 0.128 N where the minimum was observed in the control and maximum was seen in the *varikka* jackfruit incorporated bio-yoghurts. Among the control and fruit based bio-yoghurts, curd tension was maximum in the *varikka* jackfruit incorporated bio-yoghurts and minimum was observed in control bio-yoghurts. The curd tension of control bio-yoghurts were in their respective order of 0.115, 0.108 and 0.114 N for SM, HM and HM+SM. On storage, the curd tension of the bio-yoghurts were decreasing gradually and is indicated as per cent relative change and given in parenthesis.

Table 149. Curd tension of bio- yoghurt on storage (N)

Treatments		Days			
		1	5	10	15
Set 1	HM	0.115 ^c	0.113 ^c (1.73)	0.111 ^c (1.76)	0.108 ^c (2.70)
	HM+JP (<i>koozha</i>)	0.128 ^b	0.127 ^b (0.78)	0.126 ^b (0.78)	0.125 ^b (0.79)
	HM+JP (<i>varikka</i>)	0.157 ^a	0.156 ^a (0.63)	0.155 ^a (0.64)	0.154 ^a (0.64)
	CD value	0.002*	0.002*	0.001*	0.003*

Table 149. Contd.

Set 2	SM	0.108 ^c	0.106 ^c (1.85)	0.104 ^c (1.88)	0.102 ^c (1.92)
	SM+JP (<i>koozha</i>)	0.115 ^b	0.114 ^b (0.86)	0.112 ^b (1.75)	0.110 ^b (1.78)
	SM+JP (<i>varikka</i>)	0.135 ^a	0.134 ^a (0.74)	0.133 ^a (0.74)	0.131 ^a (1.50)
	CD value	0.003*	0.003*	0.002*	0.001*
Set 3	HM+SM	0.114 ^c	0.113 ^c (0.87)	0.112 ^c (0.88)	0.110 ^c (1.78)
	HM+SM+JP (<i>koozha</i>)	0.118 ^b	0.117 ^b (0.84)	0.116 ^b (0.85)	0.114 ^b (1.72)
	HM+SM+JP (<i>varikka</i>)	0.143 ^a	0.141 ^a (1.39)	0.140 ^a (0.70)	0.139 ^a (0.71)
	CD value	0.002*	0.003*	0.003*	0.002*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

The comparison of curd tension of bio-yoghurts of control, *koozha* and *varikka* varieties of HM, SM and HM+SM are given in Table 150. From the table, it is clear that similar to that of water holding capacity, bio-yoghurts prepared with HM have maximum curd tension where it was minimum for the SM based bio-yoghurts. The curd tension of HM based bio-yoghurts were 0.115 N (control), 0.128 N (*koozha*) and 0.157 N (*varikka*) on the other hand, the SM based bio-yoghurts were noticed to have curd tension 0.108, 0.115, 0.135 N respectively for control, *koozha* and *varikka* bio-yoghurts.

Table 150. Comparison of curd tension of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	0.115 ^a	0.113 ^a (1.73)	0.111 ^a (1.76)	0.108 ^a (2.70)
	SM control	0.108 ^c	0.106 ^c (1.85)	0.104 ^c (1.88)	0.102 ^c (1.92)
	HM+SM control	0.114 ^b	0.113 ^b (0.87)	0.112 ^b (0.88)	0.110 ^b (1.78)
	CD value	0.003*	0.002*	0.002*	0.001*
Set 2	HM+JP (<i>koozha</i>)	0.128 ^a	0.127 ^a (0.78)	0.126 ^a (0.78)	0.125 ^a (0.79)
	SM+JP (<i>koozha</i>)	0.115 ^c	0.114 ^c (0.86)	0.112 ^c (1.75)	0.110 ^c (1.78)
	HM+SM+JP (<i>koozha</i>)	0.118 ^b	0.117 ^b (0.84)	0.116 ^b (0.85)	0.114 ^b (1.72)
	CD value	0.004*	0.003*	0.003*	0.002*
Set 3	HM+JP (<i>varikka</i>)	0.157 ^a	0.156 ^a (0.63)	0.155 ^a (0.64)	0.154 ^a (0.64)
	SM+JP (<i>varikka</i>)	0.135 ^c	0.134 ^c (0.74)	0.133 ^c (0.74)	0.131 ^c (1.50)
	HM+SM+JP (<i>varikka</i>)	0.143 ^b	0.141 ^b (1.39)	0.140 ^b (0.70)	0.139 ^b (0.71)
	CD value	0.002*	0.002*	0.003*	0.001*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 150 clarifies that, among the control bio-yoghurts of HM, SM and HM+SM bio-yoghurts, the HM bio-yoghurts were having maximum curd tension of 0.115 N and SM had the minimum (0.108 N). Similarly HM *koozha* (0.128 N), HM *varikka* (0.157 N) had the maximum whereas SM *koozha* (0.115 N), SM *varikka* (0.135 N) have the minimum curd tension.

4.9.1.16. Viscosity

Table 151 gives the viscosity of control as well as jackfruit based bio-yoghurts. On analysing the data given in table, it is clear that the control bio-yoghurts had minimum viscosity and the maximum was reported for *varikka* based bio-yoghurts. Incorporation of jackfruit pulps were found to increase the viscosity of bio-yoghurts. The viscosity of HM based bio-yoghurts were 27200, 28800 and 29200 cP respectively for control, *koozha* and *varikka* bio-yoghurts. A similar trend was also seen in the SM and HM+SM based bio-yoghurts. The viscosity tends to increase in the ascending order of control < *koozha* < *varikka*.

DMRT done within the sets showed a significant difference in the viscosity of the developed bio-yoghurts. The *varikka* based bio-yoghurts had significantly higher viscosity whereas the control bio-yoghurts reported to have minimum. Similar to the results of curd tension and WHC, here also a gradual reduction in the viscosity of bio-yoghurts observed. The reduction of viscosity was assessed in per cent relative change throughout the storage period and indicated in the parenthesis.

Table 151. Viscosity of bio-yoghurt on storage (cP)

Treatments		Days			
		1	5	10	15
Set 1	HM	27200 ^c	25900 ^c (4.77)	23600 ^c (8.88)	21500 ^c (8.89)
	HM+JP (<i>koozha</i>)	28800 ^b	27300 ^b (5.20)	25800 ^b (5.49)	23400 ^b (9.30)
	HM+JP (<i>varikka</i>)	29200 ^a	28850 ^a (1.19)	26400 ^a (8.49)	24950 ^a (5.49)
	CD value	210.64*	215.98*	195.87*	201.03*
Set 2	SM	20200 ^c	19200 ^c (4.95)	17650 ^c (8.07)	15430 ^c (12.57)
	SM+JP (<i>koozha</i>)	22500 ^b	21850 ^b (2.88)	20150 ^b (7.78)	19850 ^b (1.48)
	SM+JP (<i>varikka</i>)	24900 ^a	23650 ^a (5.02)	21400 ^a (8.49)	20200 ^a (5.60)
	CD value	186.32*	195.28*	204.87*	213.54*
Set 3	HM+SM	22750 ^c	21350 ^c (6.15)	20500 ^c (3.98)	19650 ^c (4.14)
	HM+SM+JP (<i>koozha</i>)	24300 ^b	23550 ^b (8.88)	22350 ^b (5.09)	22100 ^b (1.11)
	HM+SM+JP (<i>varikka</i>)	26200 ^a	25750 ^a (8.49)	24900 ^a (3.30)	22950 ^a (7.83)
	CD value	227.97*	218.37*	205.54*	211.08*

DMRT column wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent decrease over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 152. Comparison of viscosity of HM, SM and HM+SM bio-yoghurts on storage (%)

Treatments		Days			
		1	5	10	15
Set 1	HM control	27200 ^a	25900 ^a (4.77)	23600 ^a (8.88)	21500 ^a (8.89)
	SM control	20200 ^c	19200 ^c (4.95)	17650 ^c (8.07)	15430 ^c (12.57)
	HM+SM control	22750 ^b	21350 ^b (6.15)	20500 ^b (3.98)	19650 ^b (4.14)
	CD value	224.09*	232.57*	219.49*	204.67*
Set 2	HM+JP (<i>koozha</i>)	28800 ^a	27300 ^a (5.20)	25800 ^a (5.49)	23400 ^a (9.30)
	SM+JP (<i>koozha</i>)	22500 ^c	21850 ^c (2.88)	20150 ^c (7.78)	19850 ^c (1.48)
	HM+SM+JP (<i>koozha</i>)	24300 ^b	23550 ^b (8.88)	22350 ^b (5.09)	22100 ^b (1.11)
	CD value	254.81*	218.41*	238.45*	213.87*
Set 3	HM+JP (<i>varikka</i>)	29200 ^a	28850 ^a (1.19)	26400 ^a (8.49)	24950 ^a (5.49)
	SM+JP (<i>varikka</i>)	24900 ^c	23650 ^c (5.02)	21400 ^c (8.49)	20200 ^c (5.60)
	HM+SM+JP (<i>varikka</i>)	26200 ^b	25750 ^b (8.49)	24900 ^b (3.30)	22950 ^b (7.83)
	CD value	256.49*	234.19*	269.95*	247.35*

DMRT coloumn wise comparison (significant at 5% level)

Figures in parenthesis indicates per cent increase over the previous storage

HM-Homogenised milk, SM-Skimmed milk, JP-Jackfruit pulp

Table 152 depicts the viscosity of HM, SM and HM+SM based bio-yoghurts. From the table, it is clear that among bio-yoghurts of set 1, significantly higher viscosity was reported for bio-yoghurts with HM (27200 cP for control). In set 2 and 3, the addition of *koozha* and *varikka* jackfruit pulp added to the gel strength and the viscosity increased further (28800 cP *koozha* and 29200 cP *varikka*). Throughout the storage period, the viscosity was found to decrease and the per cent relative decrease in viscosity is given in parenthesis of the table.

4.10. Organoleptic evaluation of bio-yoghurts on storage

The developed bio-yoghurts were stored at refrigerated condition and organoleptically evaluated at five days interval for a period of 15 days. The organoleptic properties like appearance, colour, flavour, texture, taste and overall acceptability of the products were assessed and the results are given below.

4.10.1. Organoleptic scores of control bio-yoghurts

Among the control bio-yoghurts, HM based bio-yoghurts were the most acceptable by the judges. It is evident from the table (Table 153) that, the control bio-yoghurt was liked extremely by the panel and got a mean score of nine for all the sensory parameters and the total score of this control yoghurt was 54. Even after the storage of fifteen days, the HM based bio-yoghurts were liked very much by the panelists, and the overall acceptability score was eight. During storage, the total scored got reduced and on the 15th day, it was 48.

The organoleptic scores of SM based bio-yoghurts are given in the second set. Initially the overall acceptability was 8.04 which implicates that the product is in the 'liked very much' category. On the subsequent evaluations of 5th, 10th and 15th day, the overall acceptability were 7.91, 7.54 and 7.23. As the scores of organoleptic attributes tends to decrease, the total score also reduced. The initial total score of SM based control bio-yoghurts were 48.26, which on the 15th day became 43.40.

Table 153. Organoleptic scores of control bio-yoghurts on storage

Treatments		Storage period in days			
		1	5	10	15
HM (Set 1)	Appearance	9.00	9.00	8.00	8.00
	Colour	9.00	9.00	9.00	9.00
	Flavour	9.00	9.00	8.00	8.00
	Taste	9.00	9.00	8.00	7.00
	Texture	9.00	9.00	8.00	8.00
	Overall acceptability	9.00	9.00	8.20	8.00
	Total score	54.00	54.00	49.20	48.00
SM (Set 2)	Appearance	8.11	8.02	7.40	7.00
	Colour	8.11	8.11	8.00	7.92
	Flavour	8.00	7.87	7.38	7.22
	Taste	7.98	7.73	7.64	7.03
	Texture	8.02	7.84	7.32	7.00
	Overall acceptability	8.04	7.91	7.54	7.23
	Total score	48.26	47.48	45.28	43.40
HM+SM (Set 3)	Appearance	8.87	8.21	7.46	7.20
	Colour	8.93	8.93	8.64	8.50
	Flavour	8.33	8.21	8.14	8.00
	Taste	8.73	8.68	8.41	8.20
	Texture	8.83	8.71	8.45	8.00
	Overall acceptability	8.73	8.54	8.22	7.98
	Total score	52.42	51.28	49.32	47.88

HM-Homogenised milk, SM-Skimmed milk

In the third set, the organoleptic scores of HM+SM bio-yoghurts are given. The bio-yoghurts of this group were more acceptable than the SM based bio-yoghurts. The overall acceptability of this yoghurt was 8.73 initially, which reduced to 8.54, 8.22 and 7.98 on the 5th, 10th and 15th days of storage. The initial total score of HM+SM yoghurt was 52.42 and on 15th day it became 47.88. The overall acceptability of HM+SM based bio-yoghurts after the storage period was in the range of 7.98. From the Table 151, it can be concluded that the acceptability of control bio-yoghurts were in the order of HM > HM+SM > SM.

4.10.2. Organoleptic evaluation of *koozha* based bio-yoghurts on storage

In Table 154, the mean scores of organoleptic evaluation of *koozha* based bio-yoghurts are given. Here also, the bio-yoghurts prepared with the incorporation of HM was the most acceptable one. The acceptability was least for the SM based yogurts.

The organoleptic scores of HM based *koozha* bio-yoghurts are given in first set of Table 154. The organoleptic scores during the initial evaluation revealed that the developed *koozha* based bio-yoghurts were liked very much by the panelists (mean score for overall acceptability 8.53). Total score of this bio-yoghurts initially were 51.21 which reduced to 50.26, 47.78 and 46.40 on the subsequent evaluation conducted on 5th, 10th and 15th days of storage.

In the second set, SM based *koozha* bio-yoghurts are given. It was the least acceptable bio-yoghurts among the panel members and the initial overall acceptability of these bio-yoghurts were in the range of 7.05 which means that it was liked moderately by the panelists. From the initial total score of 42.31, the total score reduced to 38.60 on the 15th day of storage. All the organoleptic attributes continue to decrease on storage and on the 10th and 15th day evaluation, the overall acceptability score of the SM based bio-yoghurts were 6.84 and 6.43.

The set 3 of Table 154 deals with the organoleptic evaluation scores of HM+SM based *koozha* bio-yoghurts. HM+SM based *koozha* bio-yoghurts were more acceptable

than the SM based *koozha* bio-yoghurts. The initial scores of HM+SM *koozha* bio-yoghurts were 8.20, 8.62, 8.20, 8.50, 8.63 and 8.49 for appearance, colour, flavour, taste, texture and overall acceptability respectively. The initial total score of the HM+SM *koozha* bio-yoghurts were 50.94 and was found reducing on the later evaluations conducted on 5th, 10th and 15th days of storage to 47.5, 45.28 and 44.65 respectively.

From Table 155, it is clear that the *koozha* bio-yoghurts were acceptable among the judges and here also the acceptability were maximum for the HM based bio-yoghurts followed by HM+SM and minimum for SM based bio-yoghurts.

Table 154. Organoleptic scores of *koozha* based bio-yoghurts on storage

Treatments		Storage period in days			
		1	5	10	15
HM (set 1)	Appearance	8.50	8.10	7.34	7.14
	Colour	8.87	8.84	8.51	8.31
	Flavour	8.27	8.00	7.98	7.65
	Taste	8.67	8.43	7.78	7.54
	Texture	8.67	8.52	7.21	8.03
	Overall acceptability	8.53	8.37	7.96	7.73
	Total score	51.21	50.26	47.78	46.40
SM (set 2)	Appearance	7.11	7.00	6.40	6.00
	Colour	7.11	7.11	7.00	6.92
	Flavour	7.02	7.00	6.88	6.22
	Taste	7.00	7.00	6.64	6.03
	Texture	7.02	6.98	7.32	7.00
	Overall acceptability	7.05	7.01	6.84	6.43
	Total score	42.31	42.10	41.08	38.60

Table 154. Contd.

HM+SM (set 3)	Appearance	8.20	7.8	7.66	7.57
	Colour	8.62	8.48	8.31	8.22
	Flavour	8.20	7.98	7.08	7.00
	Taste	8.50	7.79	7.54	7.34
	Texture	8.63	7.54	7.15	7.08
	Overall acceptability	8.49	7.91	7.54	7.44
	Total score	50.94	47.50	45.28	44.65

HM-Homogenised milk, SM-Skimmed milk

4.10.3. Organoleptic evaluation of *varikka* based bio-yoghurts on storage

The organoleptic scores for the HM *varikka* bio-yoghurts are given in the set 1 of Table 155. The HM *varikka* bio-yoghurts were liked very much by the panelists as evident from its total score of 52.08. On subsequent storage of 5th, 10th and 15th days the overall acceptability tends to decrease and the total scores were 51.38, 47.40 and 46.09 respectively. The initial total score of the HM *varikka* yoghurt was more than its counterpart of HM *koozha*. The total score of HM *koozha* was 51.21 initially whereas that of HM *varikka* was 52.08.

The set 2 gives the organoleptic evaluation results of SM *varikka* bio-yoghurts, which was the least acceptable combination. The scores obtained for the sensory attributes by SM *varikka* bio-yoghurts initially were 7.67, 7.89, 7.82, 7.82, 7.64 and 7.77 respectively for appearance, colour, flavour, taste, texture and overall acceptability. The total score obtained for the SM *varikka* bio-yoghurts were 46.61, 46.19, 45.54 and 44.65 respectively on initial, 5th, 10th and 15th days of storage.

The third set comprised of HM+SM *varikka* bio-yoghurts. The results of the table reveal that, the acceptability of HM+SM *varikka* bio-yoghurts were higher than that of the SM *varikka* bio-yoghurts. This is clear from the overall acceptability scores

of the two bio-yoghurts. The total score of HM+SM bio-yoghurts were 51.25. On storage, the scores of the sensory attributes of HM+SM *varikka* bio-yoghurts showed a reduction in the total scores. On the subsequent evaluation of 5th, 10th and 15th days of storage, the total scores of HM+SM *varikka* bio-yoghurts were 50.15, 49.22 and 47.94 respectively.

Table 155. Organoleptic scores of *varikka* based bio-yoghurts on storage

Treatments		Storage period in days			
		1	5	10	15
HM (set 1)	Appearance	8.67	8.54	7.78	7.62
	Colour	8.93	8.86	7.97	7.73
	Flavour	8.33	8.21	7.98	7.75
	Taste	8.73	8.58	7.89	7.68
	Texture	8.74	8.63	7.88	7.63
	Overall acceptability	8.68	8.56	7.90	7.68
	Total score	52.08	51.38	47.40	46.09
SM (set 2)	Appearance	7.67	7.58	7.49	7.38
	Colour	7.89	7.74	7.65	7.49
	Flavour	7.82	7.75	7.69	7.5
	Taste	7.82	7.83	7.62	7.41
	Texture	7.64	7.59	7.5	7.43
	Overall acceptability	7.77	7.70	7.59	7.44
	Total score	46.61	46.19	45.54	44.65

Table 155. Contd.

HM+SM (set 3)	Appearance	8.33	8.00	7.76	7.6
	Colour	8.74	8.65	8.58	8.47
	Flavour	8.20	8.03	7.98	7.86
	Taste	8.63	8.51	8.39	8.16
	Texture	8.74	8.60	8.31	7.86
	Overall acceptability	8.54	8.36	8.20	7.99
	Total score	51.25	50.15	49.22	47.94

HM-Homogenised milk, SM-Skimmed milk

It can be concluded from the organoleptic evaluations of bio-yoghurts that the control bio-yoghurts were the most acceptable. Among the *koozha* and *varikka* jackfruit based bio-yoghurts, the *varikka* bio-yoghurts obtained higher scores and was more acceptable than the *koozha* bio-yoghurts. The acceptability was higher for the HM based bio-yoghurts of all groups followed by HM+SM and then SM. The developed jackfruit based bio-yoghurt were able to maintain the acceptability during storage period of 15 days.

4.11. Viability of *L. acidophilus* during storage.

All the prepared bio-yoghurts were evaluated at 5th, 10th and 15th days of storage. The bio-yoghurts were serially diluted to 10⁹ dilutions and plated on MRS agar. The results are given in Table 156. The bacterial count are represented in log cfu/ml and given in parenthesis of the table.

Table 156. Viability of *L. acidophilus* in the bio-yoghurts during storage ($\times 10^9$ cfu/ml).

Treatments		Storage period in days			
		1	5	10	15
Set 1	HM control	57 (10.75)	44 (10.64)	31 (10.49)	25 (10.40)
	SM control	62 (10.79)	53 (10.71)	40 (10.60)	29 (10.46)
	HM+SM control	59 (10.77)	48 (10.68)	35 (10.54)	27 (10.43)
Set 2	HM+JP (<i>koozha</i>)	42 (10.62)	31 (10.49)	27 (10.43)	16 (10.20)
	SM+JP (<i>koozha</i>)	51 (10.71)	46 (10.66)	33 (10.52)	21 (10.32)
	HM+SM+JP (<i>koozha</i>)	48 (10.68)	37 (10.57)	25 (10.40)	19 (10.28)
Set 3	HM+JP (<i>varikka</i>)	38 (10.58)	29 (10.46)	17 (10.23)	12 (10.08)
	SM+JP (<i>varikka</i>)	46 (10.66)	35 (10.54)	23 (10.36)	18 (10.26)
	HM+SM+JP (<i>varikka</i>)	43 (10.63)	32 (10.51)	21 (10.32)	15 (10.18)

HM-Homogenised milk, SM-Skimmed milk, JP- Jackfruit pulp

Figures in parenthesis indicates log cfu/ml

The viability of *L. acidophilus* was maximum in the SM based bio-yoghurts followed by HM+SM and HM bio-yoghurts of all the three sets. The initial bacterial count of SM control bio-yoghurts were 62×10^9 cfu/ml (10.79 log cfu/ml) and that of HM+SM and SM were 59×10^9 cfu/ml (10.77 log cfu/ml) and 57×10^9 cfu/ml (10.75 log cfu/ml). During storage the number of bacteria was found decreasing and on 15th day of storage, the counts were 29, 27 and 25×10^9 cfu/ml (10.46, 10.43 and 10.40 log cfu/ml) respectively for SM, HM+SM and HM. Similarly in the jackfruit based bio-yoghurts also, the SM based bio-yoghurts were found to have maximum number of probiotic organism followed by HM+SM and HM. Among the jackfruit based bio-yoghurts, the maximum probiotic viability was observed in the *koozha* based bio-yoghurts. The number of viable cells of *L. acidophilus* of *koozha* bio-yoghurts varied from 42 to 51×10^9 cfu/ml (10.62 to 10.71 log cfu/ml) and that of *varikka* it varied from 38 to 46×10^9 cfu/ml (10.58 to 10.66) log cfu/ml.

4.12. Enumeration of total microflora and insect infestation

4.12.1. Enumeration of total microflora

The total bacterial count of the developed probiotic bacteria were enumerated in the nutrient agar (NA) medium and the results are explained in Table 157. The bacterial counts ranged from 128 to 131×10^5 cfu/ml. The bacterial count was found to be minimum in jackfruit bio-yoghurts than the control.

Table 157. Total bacterial count of *L. acidophilus* ($\times 10^5$ cfu/ml)

Treatments		Storage period in days			
		1	5	10	15
Set I	HM control	128	132	135	146
	SM control	131	136	142	149
	HM+SM control	129	133	139	147

Table 157. Contd.

Set 2	HM+JP (<i>koozha</i>)	113	119	123	129
	SM+JP (<i>koozha</i>)	120	125	131	136
	HM+SM+JP (<i>koozha</i>)	118	123	129	132
Set 3	HM+JP (<i>varikka</i>)	108	111	119	120
	SM+JP (<i>varikka</i>)	115	119	123	129
	HM+SM+JP (<i>varikka</i>)	113	118	121	126

HM-Homogenised milk, SM-Skimmed milk, JP- Jackfruit pulp

4.12.2. Enumeration of yeast count in the developed bio-yoghurts

The developed bio-yoghurts were tested for yeast during the storage period at 5 days interval and the results are given below.

Table 158. Total yeast count of the probiotic bio-yoghurts ($\times 10^2$ cfu/ml)

Treatments		Storage period in days			
		1	5	10	15
Set 1	HM control	ND	ND	ND	ND
	SM control	ND	ND	ND	ND
	HM+SM control	ND	ND	ND	ND
Set 2	HM+JP (<i>koozha</i>)	ND	ND	ND	1
	SM+JP (<i>koozha</i>)	ND	ND	ND	1
	HM+SM+JP(<i>koozha</i>)	ND	ND	ND	1
Set 3	HM+JP (<i>varikka</i>)	ND	ND	ND	1
	SM+JP (<i>varikka</i>)	ND	ND	ND	1
	HM+SM+JP(<i>varikka</i>)	ND	ND	ND	1

ND not detected

The yeast counts were not detected in the control bio-yoghurts throughout the storage. In the jackfruit bio-yoghurts, minimum yeast colonies were observed on the 15th day of storage.

4.12.3. Enumeration of fungal colonies

No fungal colonies were detected in the stored bio-yoghurts throughout the storage period.

4.12.4. Insect infestation

Bio-yoghurts were examined at 5 days interval for the insect infestation and no insects were found in the bio-yoghurts on storage. The bio-yoghurts were subjected to visual observation in day light and microscopic observation was also done.

4.13. Cost of production for selected jackfruit based probiotic fermented food products

Table 159. Cost of production for selected jackfruit based probiotic fermented food products

Jackfruit based probiotic products	Cost (/100g)
Probiotic food mixture (JF+DSF+JSF+P)	260.37
Probiotic Instant shake mix	138.54
Probiotic yoghurt	18.56-19.56

JF- Jackfruit flour, DSF- Defatted soya flour, JSF- Jackfruit seed flour, P- Papaya

The cost of production for the selected jackfruit based probiotic fermented food products (probiotic food mixture, probiotic instant shake mix and probiotic yoghurt) were calculated by considering the material cost, labour charges,

fuel and electricity costs and the cost of freeze drying. The cost was calculated per 100 g and presented in Table 156.

The production cost of probiotic food mixture (JF+DSF+JSF+P) was found to be 260 Rs/100g and that of probiotic instant shake mix was 138.54Rs/100g. Among the prepared jackfruit probiotic products, cost of the production of probiotic yoghurt (18.56 to 19.56 Rs/100g) was observed to be lowest.



Discussion

5. DISCUSSION

5.1. Standardisation of ingredients in the food mixture

The last decade has seen an increase in the development of foods that satisfies hunger and provide some additional benefits other than nutrients. This search lead to the development of a new area called functional foods and the ingredients such as probiotics and prebiotics comes under the category of functional foods. Probiotics promise to improve the gut health and confer benefits beyond nutritional value and several scientific reports support this statement. Probiotics are often used in combination with foods known as prebiotics and such mixtures are called synbiotics. The activity of probiotics can be enhanced if they are given an adequate growth environment (Anderson *et al.*, 2001).

Probiotic fermentation was carried out with a variety of the fruits and vegetables but the possibility of jackfruit as a substrate for probiotic fermentation has not investigated. Hence, in this study, raw jackfruit flour was tried out to test the efficacy of jackfruit flour as a substrate for probiotic fermentation. Along with the raw jackfruit flour, defatted soy flour, jackfruit seed flour, papaya and tomato pulp were also used in varying proportions. The prepared food mixtures were dried, powdered and subjected to organoleptic evaluation.

The matrix of food substrate is of great importance in the production of any probiotic food. The food matrix act as a medium for the microbes to achieve the desirable growth level of at least 10^9 cfu/g or ml (WHO, 2006).The selection of ingredients were based on the review of literature detailing their contribution to the growth and survival of probiotic organism.

Defatted soy flour was selected as a protein source in the food mixture and this selection was substantiated by the opinion of Saarela *et al.* (2002) that soya is a good substrate for probiotic bacteria.

Rani and Khetarpaul (1999) used defatted soya flour as a protein source along with skimmed milk powder while developing the rice based probiotic food mixture. In another study, which was focused on developing a banana based probiotic food mixture, Sharon (2010) also made use of defatted soy flour as a source of protein for the probiotic organism *L. acidophilus*.

Apart from the proteins, the presence of soyabean oligosaccharides (SOS) also enhance the growth of probiotic strains like bifido bacteria (Hayakawa *et al.*, 1990). Soyabean oligosaccharides are oligosaccharides present in soybeans, which consist of raffinose and stachiose (Gibson, 2004). These oligosaccharides can withstand enzymatic digestion of stomach and small intestine and hence, they become available for the fermenting microflora of large intestine for hydrolysis.

Prajapathi *et al.* (1987) suggested the incorporation of fruits as a prebiotic substrate during probiotic fermentation with *L. acidophilus*. Several studies suggested that fruit juices especially tomato and papaya can enhance the growth and activity of *Lactobacilli*. Babu *et al.* (1992) proved that tomato as well as papaya pulp is a good substrate for the growth of *L. acidophilus*. Sindhu and Khetarpaul (2001) developed barley based indigenous food mixture containing barley flour, milk co precipitate, sprouted green gram paste and tomato pulp in the ratio of 2:1:1:1 (w/w). While developing a pearl millet based probiotic food mixture with *Lactobacillus acidophilus*, Rani (2016) included chickpea, skimmed milk powder and tomato pulp (2:1:1:1 w/w).

Jackfruit contains 1.90 per cent protein on fresh weight basis and 14.55 per cent on dry weight basis. Jackfruit bulbs are rich in sugars and contain fair amount of carotene, protein and minerals (Sadasivam and Neelakantan, 1976). Jackfruit seeds are good source of carbohydrate (79%), protein (13.5%) and dietary fibre (3.2%) (Singh *et al.*, 1991). Jackfruit seed flour consist fair amount of indigestible polysaccharide which is capable of selectively stimulating the growth of three probiotics, *L. acidophilus*, *L. plantarum* and *B. bifidum* (Thammarutwasik *et al.*,

2009). All these components are crucial for the survival of probiotic organisms. Hence, with the assumption that jackfruit can be a substrate for the probiotic fermentation, the present study was formulated.

In the present study, jackfruit flour (JF), defatted soya flour (DSF), jackfruit seed flour (JSF), tomato pulp (T) and papaya pulp (P) were used in different combinations. All the experiments were repeated with both the *koozha* and *varikka* variety of jackfruit. From set 1 and 2, treatment T₂ was selected for further studies based on the organoleptic properties, and from set 3 and 4 the treatment T₃ was selected. The same trend was observed in both the varieties. Figures 5 and 6 depicts the sensory attributes of selected food mixtures from *koozha* and *varikka* varieties. The selected combinations contains 50-60 per cent of raw jackfruit flour, 20-30 per cent defatted soya flour, 0-20 per cent jackfruit seed flour and 10 per cent of the fruit pulp (either papaya or tomato). The prepared food mixtures of both varieties were organoleptically acceptable.

Rani and Khetarpaul (1998) developed an acceptable probiotic drink incorporating pearl millet flour, chick pea flour, skim milk powder and fresh tomato pulp in the ratio 2:1:1:1, w/w with *L. acidophilus*. In another study that dealt with the lipid lowering effect of probiotic organism, Sindhu and Khetarpaul (2003) developed an experimental diet containing barley flour, spouted green gram paste, milk coprecipitate and tomato pulp in the ratio 2:1:1:1 (w/w) and the food mixtures were found to be organoleptically acceptable to human palate and maintained adequate cell viability.

Lavanya (2008) developed an indigenous food mixture by incorporating bajra flour, defatted soya flour and skimmed milk powder in the ratio of 2:1:1 and it was found to be organoleptically acceptable.

Sharon (2010) standardised the proportion of ingredients for the development of an acceptable banana based probiotic food mixture. The selected food mixtures

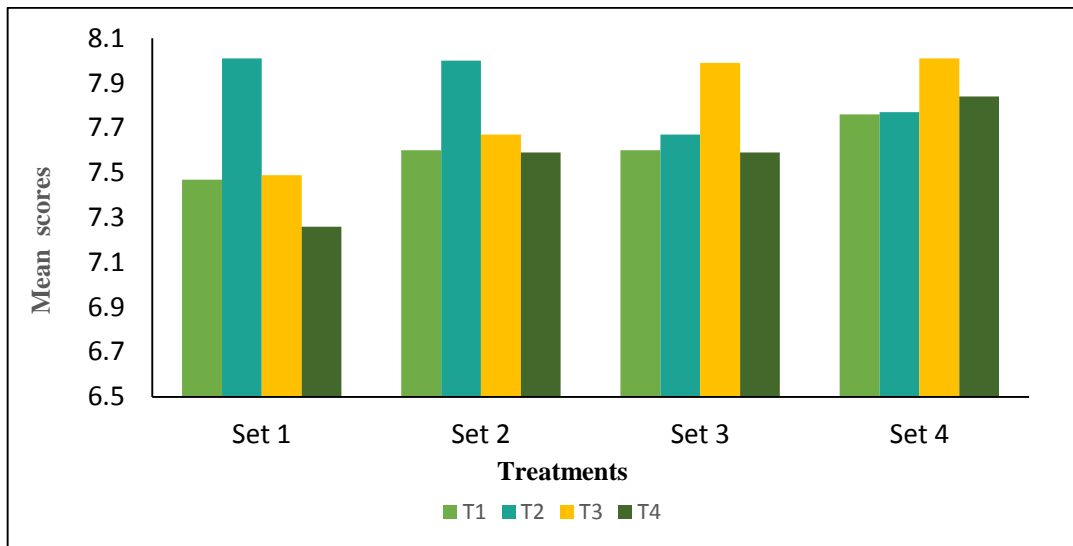


Fig. 5. Mean scores for overall acceptability of food mixtures (*koozha* variety)

Set 1- T₁-70% JF+ 20% DSF+ 10% T; T₂- 60% JF+ 30% DSF+ 10% T;
T₃- 50% JF+ 40% DSF+ 10% T; T₄-40% JF+ 50% DSF+10% T

Set 2- T₁-70% JF+ 20% DSF+ 10% P; T₂- 60% JF+ 30% DSF+ 10% P;
T₃- 50% JF+ 40% DSF+ 10% P; T₄-40% JF+ 50% DSF+ 10% P

Set 3- T₁-70% JF+ 10% DSF+ 10% JSF+ 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% T;
T₃- 50% JF+ 20% DSF+ 20% JSF + 10% T; T₄- 40% JF+ 25% DSF+ 25% JSF + 10% T

Set 4- T₁-70% JF+ 10% DSF+ 10% JSF 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% P;
T₃- 50% JF+ 10% DSF+ 10% JSF + 10%; T₄-40% JF+ 25% DSF+ 25% JSF + 10% P

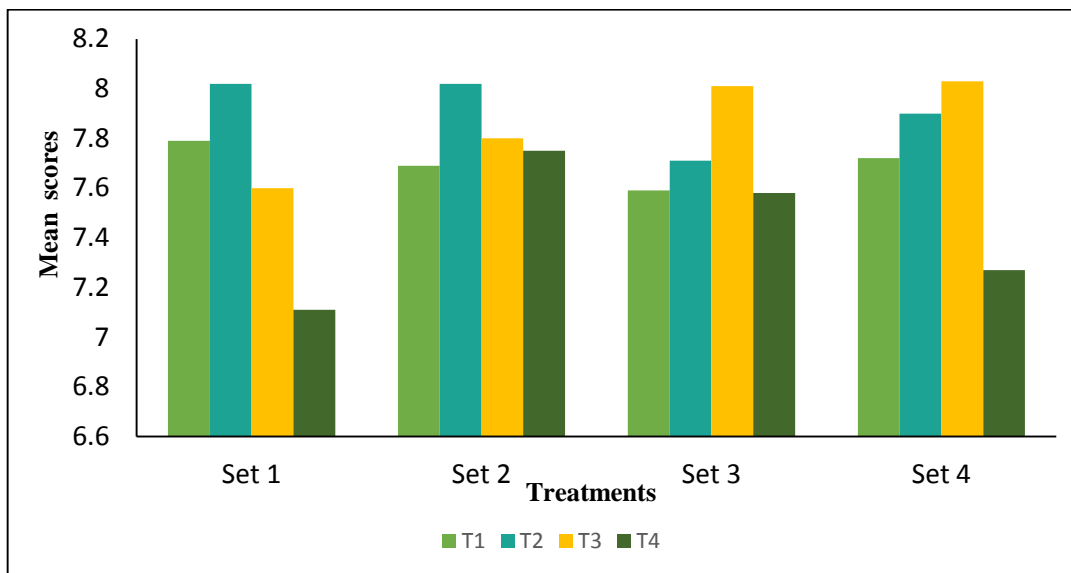


Fig. 6. Mean scores for overall acceptability of food mixtures (*varikka* variety)

Set 1- T₁-70% JF+ 20% DSF+ 10% T; T₂- 60% JF+ 30% DSF+ 10% T;
T₃- 50% JF+ 40% DSF+ 10% T; T₄-40% JF+ 50% DSF+10% T

Set 2- T₁-70% JF+ 20% DSF+ 10% P; T₂- 60% JF+ 30% DSF+ 10% P;
T₃- 50% JF+ 40% DSF+ 10% P; T₄-40% JF+ 50% DSF+ 10% P

Set 3- T₁-70% JF+ 10% DSF+ 10% JSF+ 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% T;
T₃- 50% JF+ 20% DSF+ 20% JSF + 10% T; T₄- 40% JF+ 25% DSF+ 25% JSF + 10% T

Set 4- T₁-70% JF+ 10% DSF+ 10% JSF 10% T; T₂- 60% JF+ 15% DSF+ 15% JSF + 10% P;
T₃- 50% JF+ 10% DSF+ 10% JSF + 10%; T₄-40% JF+ 25% DSF+ 25% JSF + 10% P

contained 60-70 per cent banana flour as the major ingredient, 20 percent defatted soy flour or green gram flour and 10-20 per cent fruit pulps viz papaya and tomato. A similar result was reported by Jood *et al.*, (2012) during the development of an indigenous food mixture using barley flour, whey powder and tomato pulp in the ratio 2:1:1.

A novel, cereal based probiotic product was prepared by using food grade lactic acid bacteria by incorporating wheat grain (150g), flax seeds (50g) and chia seed (2g) with distilled water and the prepared product scored 7.02 to 8.44 on a nine point hedonic scale (Gautam and Sharma, 2014). Ogunremi *et al.* (2015) developed a probiotic cereal based multi mix with the strain *Pichia kudriavzevii* OG32 with the incorporation of pearl millet, red sorghum, white sorghum and wheat in the ratio of 1:1:1:1 and found to have an acceptability score of 5.8 out of 7.

Baruah *et al.* (2018) developed a functional multimix for probiotic fermentation using lactic acid bacteria with the incorporation of rice, rice bean, foxtail millet, flax seed and tomato pulp in the ratio 3:4:1:1:1:1. The food mixtures were found to be of better nutrient profile and storage stability of 30 days. A probiotic drink was developed by Chavan *et al.* (2018) by incorporating barley flour, finger millet and moath bean in the ratio 2.5:1.5:1 which was then mixed with distilled water, soy milk and coconut milk and the drink prepared with coconut milk was found to be of better sensorial properties.

5.2. Optimisation of conditions for the growth of *L.acidophilus*

The probiotic strain *Lactobacillus acidophilus* MTCC 10307 was used as the probiotic entity throughout the research. Several researchers used the same strain as a probiotic entity in their studies. Pradhan *et al.* (2016) tested the probiotic properties of *L. acidophilus* MTCC 10307 *in vitro* with respect to its toxicity to immunological cells, modulation of innate immune genes, increasing the survivability of primed immune cells against *Salmonella* induced cytotoxicity. Based on the results

they suggested it as a probiotic and also successfully tested its efficacy as a probiotic strain to clear Salmonella infection in mouse model.

Nath *et al.* (2015) developed a probiotic honey beverage containing *L. acidophilus* MTCC 10307 which was superior to the non-probiotic honey beverage nutritionally and organoleptically. Wiejemanna and Ravindra (2018) formulated a probiotic amla drink with *L. acidophilus* MTCC 10307 and *Saccharomyces boulardii*.

In any fermentation process, the medium and condition of fermentation plays a critical role because they effect the product quality and yield and thus effecting the overall process economics. Therefore it is important to optimise the fermentation process, in order to maximise the benefits from fermentation (Schmidt, 2005).

In a fermentation process, different combinations and sequence of fermentation conditions and medium components are to be optimised to determine the growth conditions that produces the end product with the best physiological state (Stanbury *et al.*, 1997).

In this study also optimisation of growth conditions of *L. acidophilus* MTCC 10307 was carried out with regard to substrate concentration, pH, temperature of incubation, time of incubation and inoculum concentration.

The study revealed that 50 g substrate concentration reported the maximum number of probiotic cells in both the *koozha* (88×10^9 cfu/ml) and *varikka* (82×10^9 cfu/ml) varieties (Fig. 7). The optimum fermentation period reported was 24 hours (Fig. 8) and pH was found to be 4.5 (Fig. 9). The maximum probiotic count was observed in the food mixture JF+DSF+JSF+T *koozha* variety (79×10^9 cfu/ml) followed by the *varikka* variety (62×10^9 cfu/ml). While analysing the effect of temperatures on the probiotic culture, the maximum viable numbers of *L. acidophilus* was observed at 37⁰ C in both the varieties (Fig. 10).

The maximum probiotic count was observed when 50g substrate was inoculated with 300µl (Fig. 11) of the 24 hour old culture of *L.acidophilus* MTCC 10307.

Optimisation studies were done by several researchers for the development of probiotic products. Santos and Soccol (2003) maximised the growth of *L.casei* and *L.acidophilus* in cassava flour based probiotic beverage. According to them, the optimum conditions for the growth of probiotic bacteria were 20 percent cassava flour with four percent inoculum, fermented at 35⁰C for 16 hours. Angelov *et al.* (2006) developed an oat based probiotic drink in which the whole oats substrate was inoculated with 5 percent *L. plantarum* and fermented for 6 hours to produce 7.5×10¹⁰ cfu/ml of the probiotic organism.

A similar result was stated by Sharon *et al.* (2015) when optimising the growth conditions for *L.acidophilus* (MTCC 447) in banana based food mixture. The study reported maximum cell count at 25 g substrate concentration fermented for 24 hours, at a pH 4.5 and temperature of 37⁰C and an inoculum concentration of 300µl. A probiotic food mixture developed by Jood *et al.*(2012) using *L.acidophilus* NCDC 16 was fermented at 37⁰C for 12 hours. During the development of a probiotic honey beverage, Nath *et al.* (2015) developed a probiotic honey beverage inoculated with *L.acidophilus* MTCC 10307 at 37⁰C for 6 hours. Ashrafuzzaman *et al.* (2015) optimised the temperature of growth for *L.acidophilus* and they reported the maximum activity of the bacteria at 37⁰C and no growth at 45⁰C.

Kaur *et al.* (2016) developed a probiotic fermented tomato juice using probiotic lactic acid bacteria in which there was a faster decrease in sugar, pH and increase in acidity during the first 24 hours indicating the maximum growth of probiotic organism in the first 24 hours. In the next 24 hours of fermentation, fermentation was carried out at a slower pace which may be due to the harmful

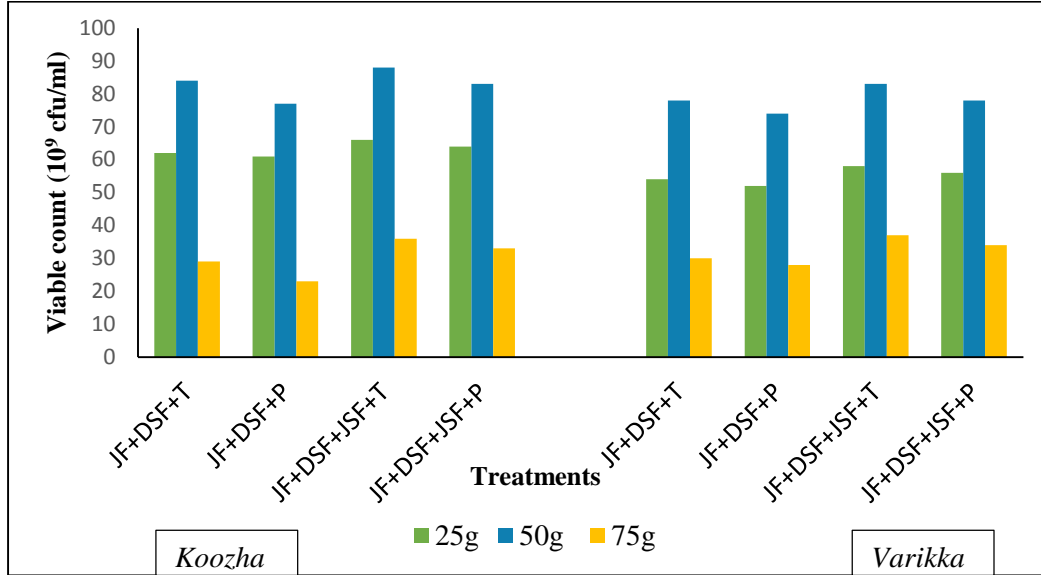


Fig.7. Viable count of *L. acidophilus* in food mixtures with different substrate concentration

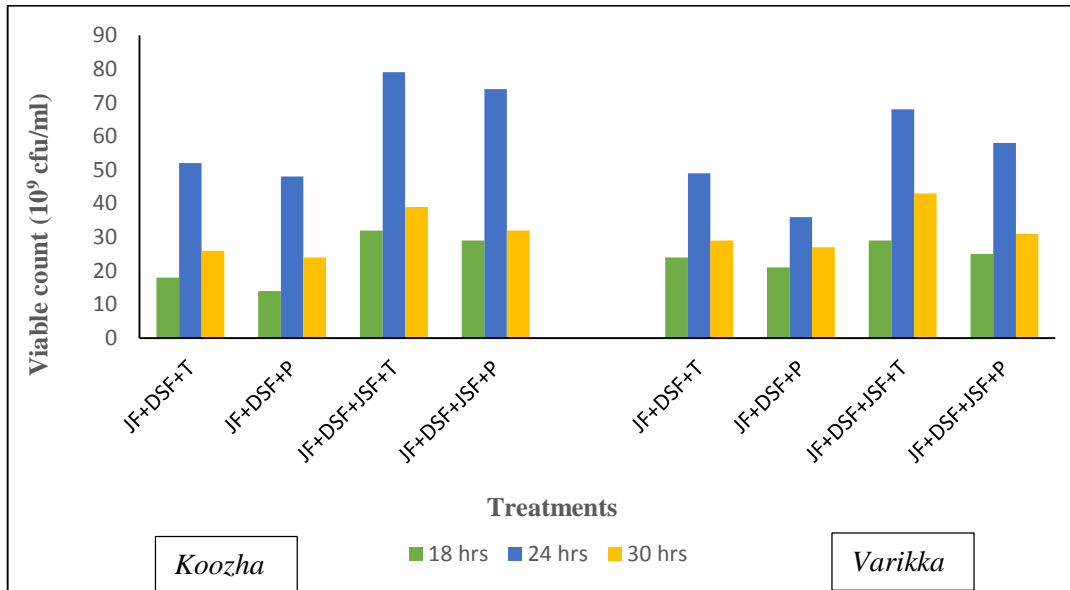


Fig. 8. Viable count of *L. acidophilus* in food mixtures with different time of incubation

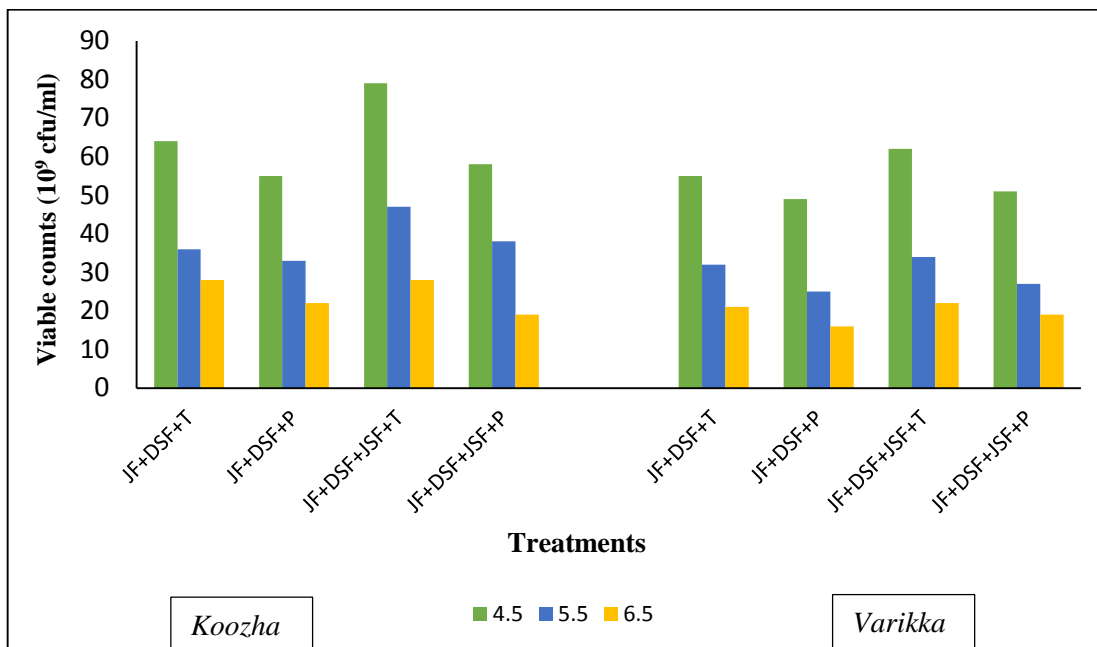


Fig. 9. Viable count of *L. acidophilus* in food mixtures with different pH levels

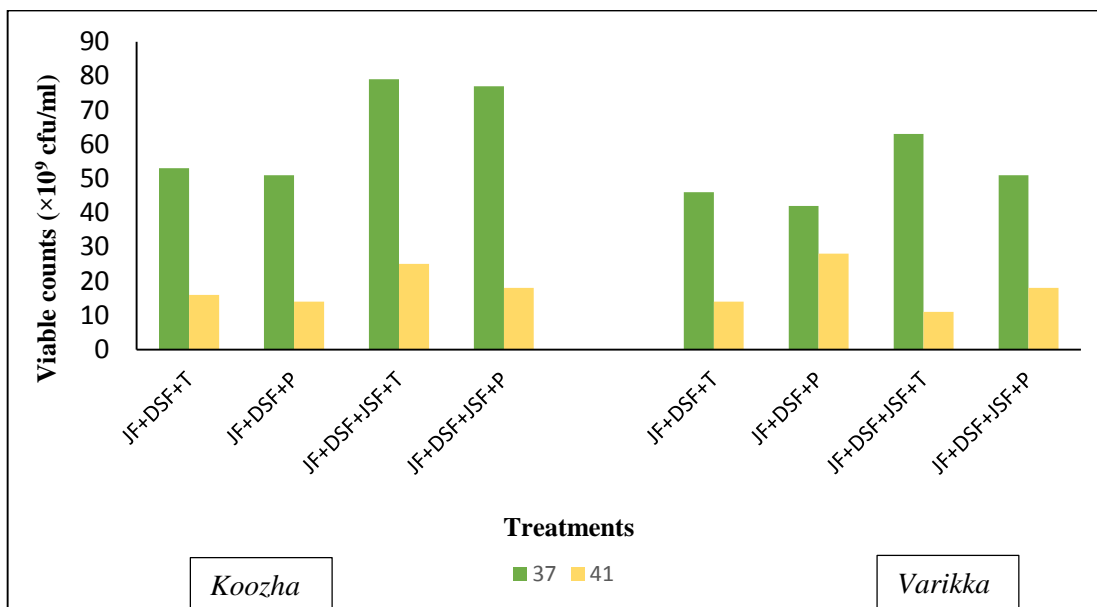


Fig. 10. Viable count of *L. acidophilus* in food mixtures with different temperatures ($^{\circ}$ C) of incubation

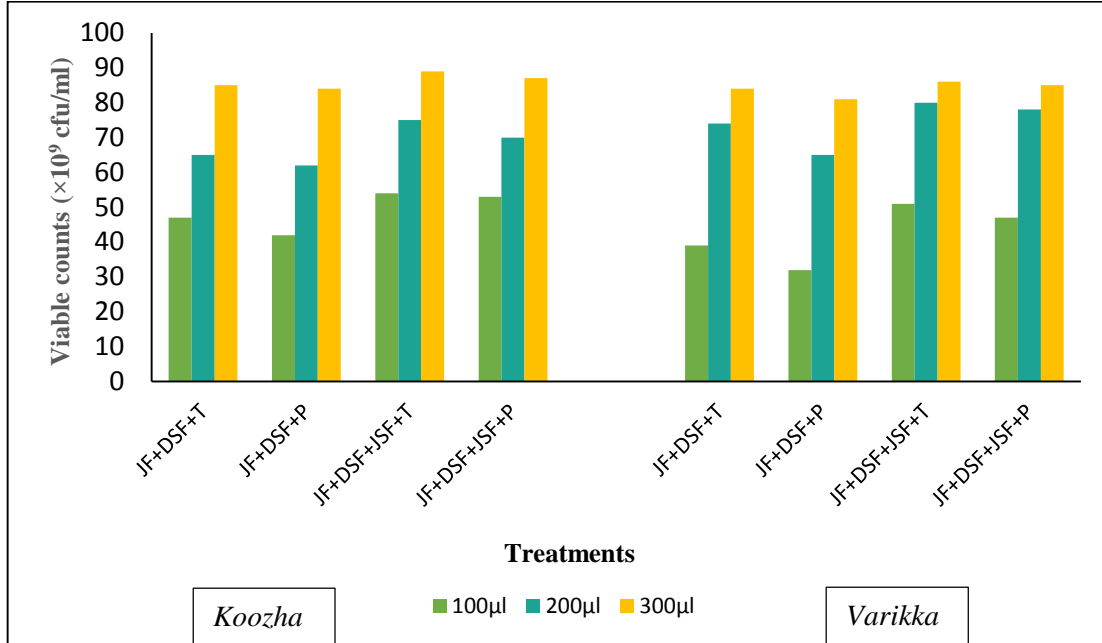


Fig. 11. Viable count of *L. acidophilus* in food mixtures with different inoculum concentrations (μl)

effect extremely low pH and high acidity, achieved during the initial 24 hours of fermentation.

Non-dairy based probiotic products are gaining attention because of their low fat content. These products are also safe for consumers suffering from lactose intolerance. The data obtained from the present study reveals that, jackfruit is a suitable substrate for the growth of probiotic bacteria. Using indigenous crops for developing probiotic product will help in reducing the gap between probiotic products and consumers.

5.3 Development of food mixtures

The process of fermentation was carried out at the optimum conditions. The selected combination of ingredients were mixed with distilled water and this slurry was autoclaved prior to fermentation process. Autoclaving was done in the food mixture to make it aseptic. Autoclaving is the essential and standard procedure for sterilizing any bacterial medium. As the medium for the probiotic growth in this study was the food mixture, it was subjected to autoclaving to prevent the growth of unwanted organisms.

An unfermented sample was also made after autoclaving so as to have a relative comparison of changes during fermentation. Both the fermented and unfermented samples were freeze dried and packed in laminated polyethylene pouches. As the substances are not exposed to high temperature during freeze drying, the dried products preserve their initial nutrient properties (Wilkowska *et al.*, 2016).

Compared to other methods of food drying, freeze drying method yields final product of highest quality. This process also protect the primary structure and shape of the product with minimal reduction in volume (Ratti, 2001). Freeze drying (lyophilization) is considered as a suitable method for drying heat sensitive substances like pigments, flavonoids, nutrients, microorganisms etc. During this procedure, the core materials (*L. acidophilus*) and matrix solutions (autoclaved food

mixture) are homogenised and then colyophilised, resulting in a dry material (Laokuldilok and Kanha, 2015).

Freeze drying process provide stability to the probiotic organism upon storage. The activity of the cultures can be improved by incorporating it into a gel matrix. By this way, it is also possible to increase the stability of probiotic organism in the gastro intestinal tract and thereby increase the efficacy of the product (Chavarri *et al.*, 2012).

In any food industry, the prime importance is given to safe and wholesome food products. The use of proper packaging material is necessary to protect the food from detrimental effects of the surrounding environment. When it comes for the packaging of probiotic foods, it is important to note the material used for packaging as well as the storage conditions of the food. Both can have an impact on the viability of probiotic organism, through the permeability to oxygen as well as temperature (Shah and Ravula, 2000; Miller *et al.*, 2002).

The food mixtures prepared as a part of this particular study was successfully stored for a period of six months in laminated polyethylene pouches under ambient condition. Laminated polyethylene pouches provided effective protection to the food mixtures from the surrounding environment.

Packaging can influence the product quality, as improper packaging may reduce product acceptability, increase oxidation and off-flavors, increase waste, and result in lowering overall product quality and shelf life (Meiron and Saguy, 2007). Plastic packaging can help to increase the storage life of perishables and processed products (Suryawanshi, 2008). Food packaging is an important component of food industry, as it prolongs the food storage period, prevent wastage and ensures that the food is in the desirable condition throughout the storage period (Robertson, 2014).

5.4 Quality evaluation of food mixtures

Fermentation is an age old process. The process brings about unique change in the organoleptic as well as nutritional properties of the food. Lactic acid bacteria is one of the most commonly used probiotic organism and owns a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic and shelf-life characteristics (Wood and Holzapfel, 1995; Leroy and De Vuyst, 2004).

Lactic acid bacteria cause rapid acidification of food product through the production of organic acids, mainly lactic acid. In addition, they also produces acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes of importance. Lactic acid bacteria is the most commonly used starter culture in the food industry. In this study *L.acidophilus* (MTCC 10307) was used as the fermenting organism.

5.4.1 Moisture content of the developed food mixtures

Moisture is an important parameter in any food product. It may vary from product to product. The moisture content is the crucial factor which determines the shelf life of a developed product. Lower the moisture content, greater will be its stability on storage.

The moisture content of the developed fermented food mixtures of *koozha* variety varied from 2.76 per cent to 2.80 per cent whereas in *varikka* mixtures, it varied from 2.32 per cent to 2.51 per cent. The moisture content of the unfermented food mixtures (*koozha*) ranged from 2.33 per cent to 2.92 per cent in which the moisture content was maximum in the JF+DSF+JSF+T (2.92%) followed by the JF+DSF+JSF+P (2.68%). Similarly in *varikka* mixtures also these food mixtures contained maximum moisture than the JF+DSF+T and JF+DSF+P. Even though

there was no significant difference in the moisture content of fermented and unfermented food mixtures, the moisture content of the fermented *koozha* mixture was significantly higher than *varikka* mixtures (Fig. 12). Similar observations were reported when probiotic fermentation of barley based food mixture was done (Sindhu and Khetarpaul, 2004) and when banana based food mixture was fermented with probiotic *L.acidophilus* (Sharon, 2010).

Different authors like Goyal and Khetarpaul (1995) and Sharma and Khetarpaul (1997) also reported that there is no difference in the moisture content of fermented as well as unfermented food mixtures.

In this study, high moisture content was reported for the *koozha* variety than the *varikka* and the combination containing jackfruit seed flour than the other combinations. The *koozha* variety of jackfruit contains 1.22g/100g fibre whereas *varikka* variety contains 1.03g.100g. Fibre is reported to absorb more water and this may have contributed to the increased moisture content of *koozha* based food mixtures. Jackfruit seed contains 3.01 per cent fibre (Singh *et al.*, 1991) which may have contributed to the moisture content of this particular combination and apart from this, it should also be noted that jackfruit seed flour has good water absorption capacity of 205 per cent (Thulyathan *et al.*, 2002).

5.4.2 Titratable acidity of the food mixtures

The titratable acidity values for fermented food mixtures ranged from 2.32 to 2.96 per cent for *koozha* food mixtures and 2.52 per cent and 2.73 per cent for *varikka* food mixtures. A significantly lower values were obtained for the unfermented food mixtures, 1.36 to 1.79 in *koozha* and 1.29 to 1.63 in *varikka* (initial values).

During fermentation, the probiotic organism utilise glucose and convert it to lactic acid. The homo fermentative *L. acidophilus* converts glucose to lactic acid which is responsible for the decrease in pH of the product. *Lactobacillus* spp. is more

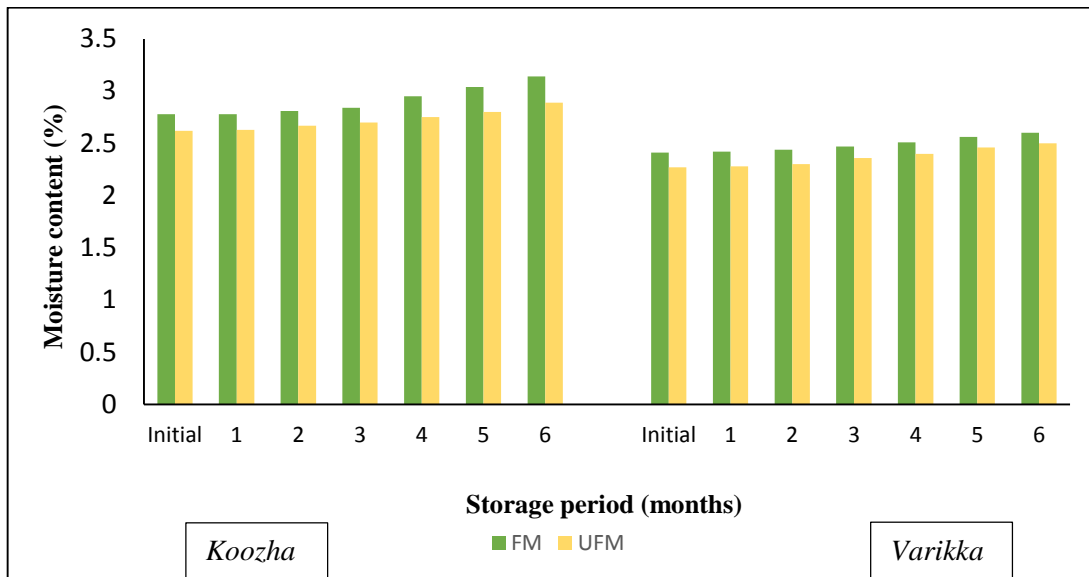


Fig. 12. Moisture content of fermented (FM) and unfermented (UFM) food mixtures on storage

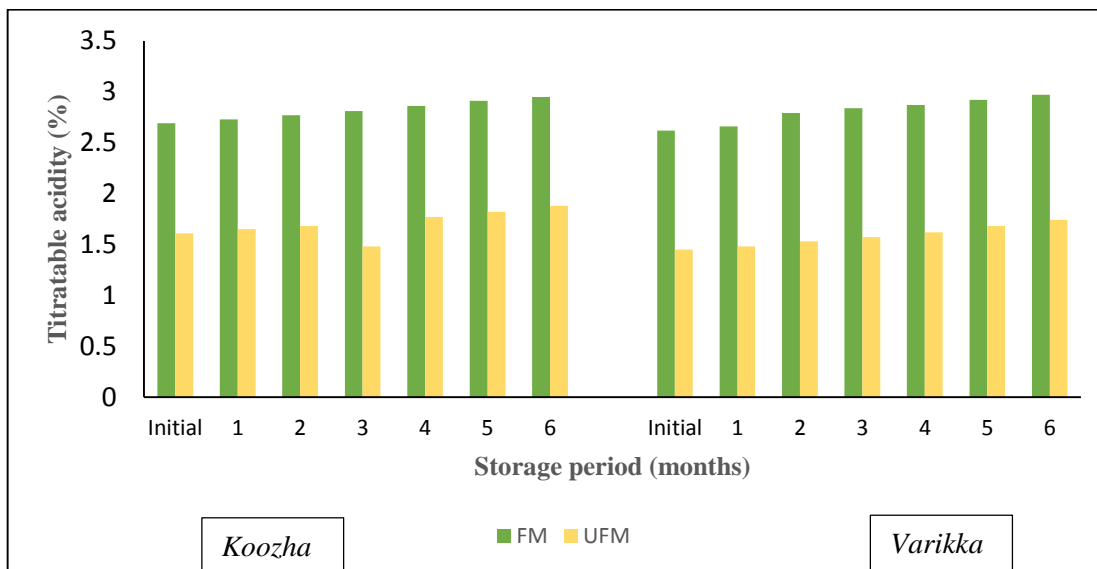


Fig. 13. Titratable acidity of fermented (FM) and unfermented (UFM) food mixtures on storage

effective in bringing down pH than yeasts and combination of microbes (Gautam and Sharma, 2014).

Khetarpaul and Chauhan (1990) observed a rapid drop in pH and a corresponding increase in the titratable acidity of pearl millet based food mixture after lactic acid fermentation. Similar results were reported by Sripriya *et al.*, (1997) where the titratable acidity of the finger millet based food mixture increased after probiotic fermentation and a corresponding drop in pH of the food mixture. Arora *et al.* (2008) reported the titratable acidity of raw non-germinated pearl millet based food mixture increased from 1.65 per cent to 2.68 per cent on fermentation with *L. acidophilus* curd. In another probiotic study, Sharon (2010) reported a significant increase in the titratable acidity of banana based food mixture after probiotic fermentation with *L. acidophilus*.

A rapid drop in pH with corresponding increase in titratable acidity has been reported in lactic acid fermentation of a number of foods (Agte *et al.*, 1997; Urga *et al.*, 1997) like sorghum-green gram blend (Chavan *et al.*, 1998), indigenous food mixtures containing cereals, legumes, skimmed milk powder and fresh tomato pulp (Rani and Khetarpaul, 1998; Sindhu and Khetarpaul, 2001).

Throughout the storage period, the acidity of food mixtures, both fermented and unfermented was found to increase. The initial mean value of *koozha* fermented food mixture was 2.65 per cent which became 2.98 per cent at the end of storage period. In a similar fashion, the *varikka* variety also exhibited increase in titratable acidity on storage. The increase in titratable acidity can be attributed to the accumulated organic acids especially lactic acid by the action of probiotic bacteria in the fermented food mixture. Figure 13 shows the changes in titratable acidity during the storage of six months.

5.4.3 Protein content of the developed food mixtures

Protein content of the fermented food mixtures ranged from 22.93 to 23.98 g/100g in *koozha* and 23.15 and 25.15 g/100g in *varikka* food mixtures. But it ranged from 21.14 to 22.09g/100g for *koozha* 21.24 to 23.14g/100g in *varikka* unfermented food mixtures (Fig. 14). It was also observed in the study that probiotic fermentation resulted in significant increase in the protein content of the food mixture.

The increased protein content during fermentation can be attributed to the microbial synthesis of protein during life cycle. They synthesis protein from metabolic intermediates. Kee-jong *et al.* (2004) evaluated the effect of probiotic fermentation using *Aspergillus oryzae* GB-107 on the nutritional quality of food grade soya beans. The study confirmed that the process of fermentation increased the protein content. In a study of Onimawo *et al.*, (2005) probiotic fermentation of pumpkin seeds increased the protein content of pumpkin seeds from 28.0 per cent to 39.4 per cent.

A significant increase in protein content ($p < 0.05$) was reported by Oboh (2006) when cassava peels were fermented with *Lactobacillus delbruckii*, *L.coryneformis* and *Saccharomyces cerevvisae*. Wang (2007) in his study reported an increase in the crude protein content in the peanut flour when fermented with *Lactobacillus plantarum* p9. Similarly Sharon (2010) reported that a there is a significantly higher ($p < 0.05$) level of protein content in fermented banana based probiotic food mixture compared to the unfermented control.

On the contrary, a few authors (Sharma, 1994; Binita *et al.*, 1996; Sindhu and Kheatapaul, 2004; Arora *et al.*, 2008) reported reduction in the crude protein content of the food item on fermentation.

Shahzad *et al.*, (2005) reported a decrease in the protein content of composite flours on storage and they explained this phenomenon as due to the absorption of moisture by the flour, which in turn increased the proteolytic activity.

As per the observations of Goldin *et al.*, (1998) decreased protein content may be due to the increased uptake of moisture by stored flour and the subsequent increase in proteolytic activity. Authors like Sharon *et al.* (2015) and Lakshmy (2012) also reported a decrease in the protein content of fermented food items on storage.

5.4.4 β carotene content of the food mixtures

The β carotene content of the fermented food mixtures were observed to be within the range of 309.87 and 346.46 $\mu\text{g}/100\text{g}$. It was found to be higher in the food mixture JF+DSF+JSF+P in both the varieties and least in the food mixture containing jackfruit flour, defatted soya flour and tomato pulp. When it comes to unfermented food mixtures, the values vary from 307.48 to 329.49 $\mu\text{g}/100\text{g}$ in *koozha* mixtures and 310.64 to 329.34 $\mu\text{g}/100\text{g}$ in *varikka* food mixtures. Probiotic fermentation does not resulted in significant improvement in the β carotene content of the developed food mixtures.

De Faria *et al.* (2009) reported the β carotene content of young jackfruit as 29.55 $\mu\text{g}/100\text{g}$ on fresh weight basis whereas Ranasinghe *et al.*, (2019) reported the β carotene content of 175-540 $\mu\text{g}/100\text{g}$ in the mature fruit.

Probiotic fermentation did not improve the β carotene content of the food mixture and this finding go in line with that of Li *et al.*, (2007) who reported no significant variation in the β carotene content of fermented and unfermented maize porridges. Similarly, Sharon (2010) reported a non-significant change in the banana based probiotic food mixture and their unfermented counterpart.

Figure 15 shows that, on storage, the mean β carotene content of *koozha* based fermented food mixtures reduced from 327.680 $\mu\text{g}/100\text{g}$ to 228.768 $\mu\text{g}/100\text{g}$ whereas

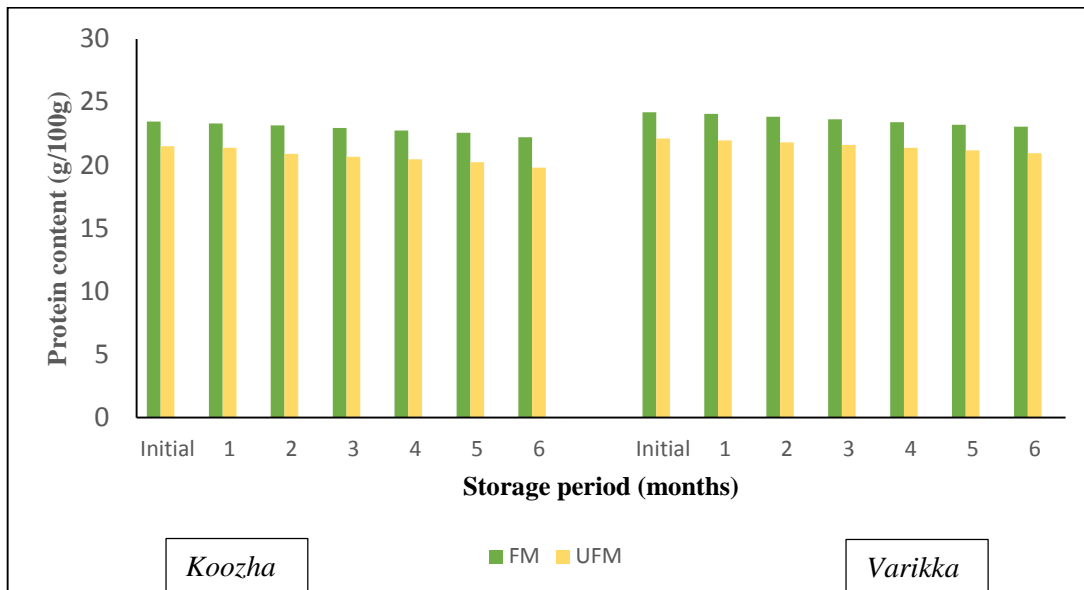


Fig. 14. Protein content of fermented (FM) and unfermented (UFM) food mixtures on storage

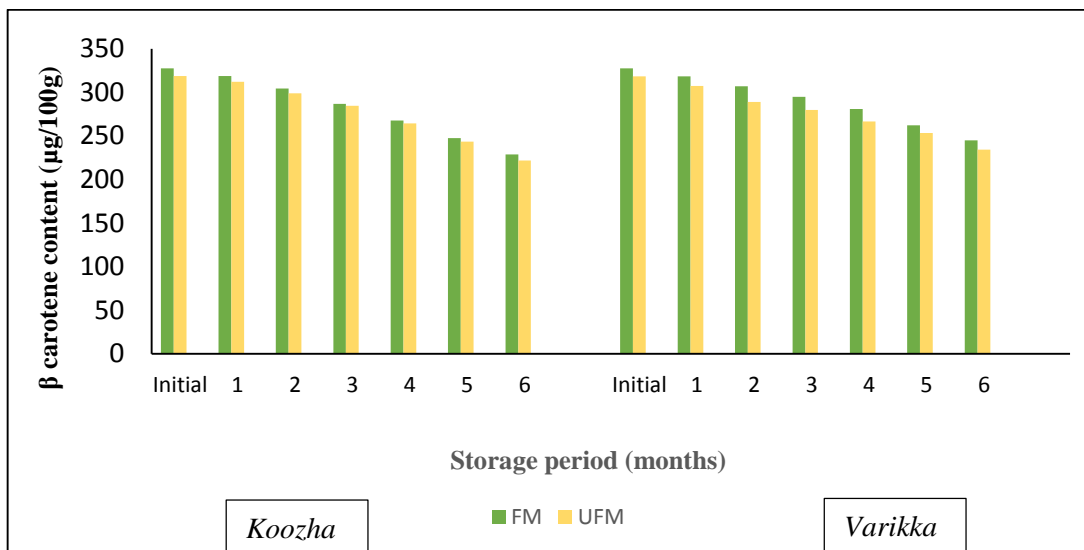


Fig. 15. β carotene content of fermented (FM) and unfermented (UFM) food mixtures on storage

that of *varikka* food mixture was reduced from 327.518 µg/100g to 245.008 µg/100g. A similar reduction was also reported in the case of unfermented food mixtures.

The reduction in β carotene content of the food mixture on storage was well explained by Gloria *et al.*, (1995). They reported that, β carotene absorbs oxygen and gives rise to colourless oxidative products.

5.4.5. Crude fibre content of the food mixtures

The crude fibre content of the fermented food mixture was found to vary from 1.56 to 1.84g/100g in *koozha* and 1.41 to 1.56g/100g in *varikka* with maximum value observed for JF+DSF+JSF+P combination in both the varieties. A drop in the crude fibre content was observed on fermentation. Figure 16 depicts the change in crude fibre content of fermented and unfermented food mixtures.

Raimbault and Tewe (2001) opined that during fermentation, carbohydrates like cellulose, pectin, lignocellulose and starch are broken down by the fermenting organisms and thereby reduce the fibre content of the food sample. According to Oboh and Akindahunsi (2014) decrease in fibre content during fermentation may be due to the ability of the lactic acid bacteria to metabolize the available fibre enzymatically and utilizing them as a source of carbon.

In a fermentation work conducted by Sindhu and Khetarpaul (2004) a significant reduction in the crude fibre content of the fermented sample than the unfermented sample was observed. Arora *et al.* (2008) reported a significant ($P < 0.05$) decrease in crude fibre content of food mixtures fermented with *L acidophilus* when compared to the raw unfermented sample.

Technologically, Lactobacilli strains adheres well to fibres present in the food matrix for their growth and multiplication, resulting in decreased fibre content in cereal and legume based probiotic food products (Roberts and Knorr, 2008). Baruah *et al.* (2018) found that crude fibre content decreased in multi mix containing rice, rice

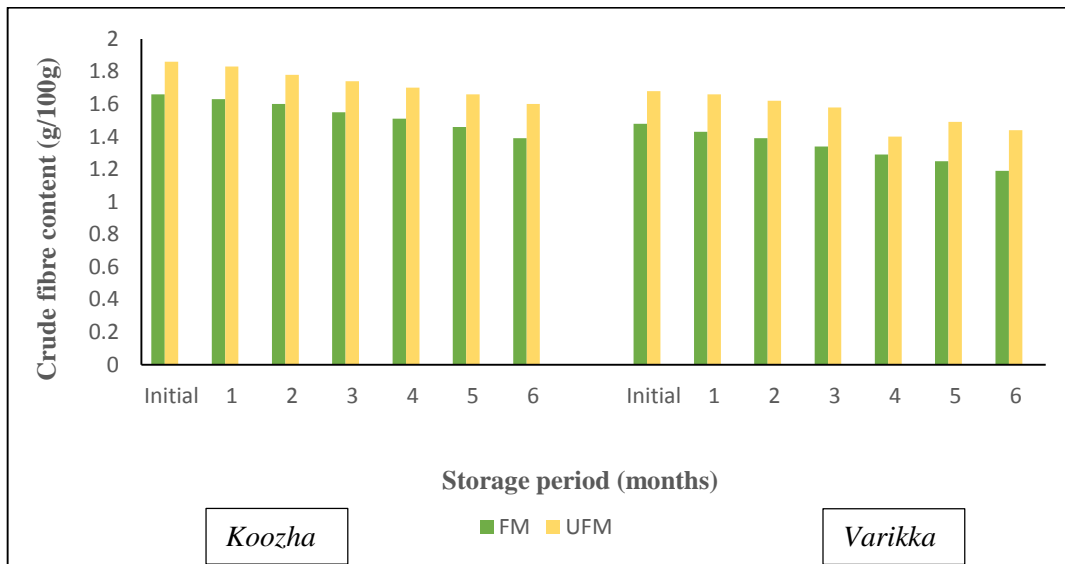


Fig. 16. Crude fibre content of fermented (FM) and unfermented (UFM) food mixtures on storage

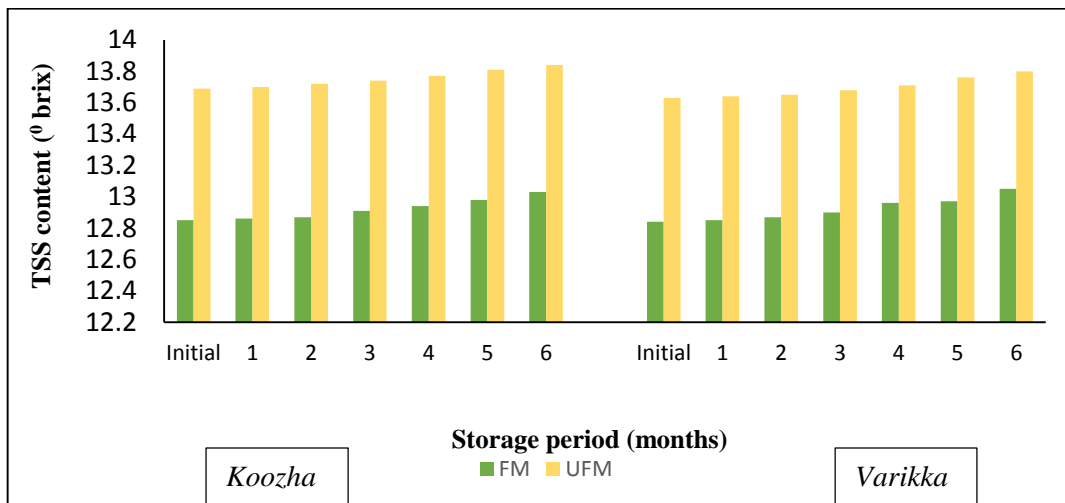


Fig. 17. TSS content of fermented (FM) and unfermented (UFM) food mixtures on storage

bean, foxtail millet, flax seed and tomato on fermentation with lactobacilli strains. Similar results were also observed by Ogodo *et al.*, (2018) on bombara groundnut seed flour that the fibre content significantly decreased during fermentation with lactic acid bacteria from 11.02 ± 0.05 to 10.44 ± 0.12 g per 100 g.

On storage, the crude fibre content of the food mixtures were found to reduce significantly. This may be due to the degradation of complex polysaccharides into simpler forms. The increased moisture content of the food mixture may also have contributed to this degradation of fibre and Ahmad (1996) was of the same opinion.

5.4.6. TSS, reducing sugar, total sugar and starch content of developed food mixtures

TSS is an index of soluble solids concentration in fruit. In the present study the TSS of the fermented food mixtures ranged from 12.51 to 13.13 °brix and that of unfermented samples ranged from 13.29 to 14.15° brix. The TSS content of both the fermented and unfermented food mixtures were found to increase significantly during the storage period. The unfermented food mixtures were found to have more TSS than the fermented samples (Fig. 17).

The reducing sugar content of the food mixture decreased from 5.67-7.64 g/100g to 4.12-4.68 g/100g on fermentation (Fig.18). Similarly, the total sugar was also reported to reduce from 20.03- 21.07 g/100g to 11.03-12.45g/100g (Fig. 19). Figure 20 shows the changes in the starch content of fermented and unfermented food mixtures on storage. The starch content of the food mixture was also found reduced on fermentation.

During fermentation, the probiotic bacteria produces lactic acid by hydrolyzing starch. This can be the reason for decreased TSS as well as starch in fermented samples (Adams and Moss, 2008). This metabolic activity will bring down starch to fermentable simple sugars which is used up by the fermenting organisms.

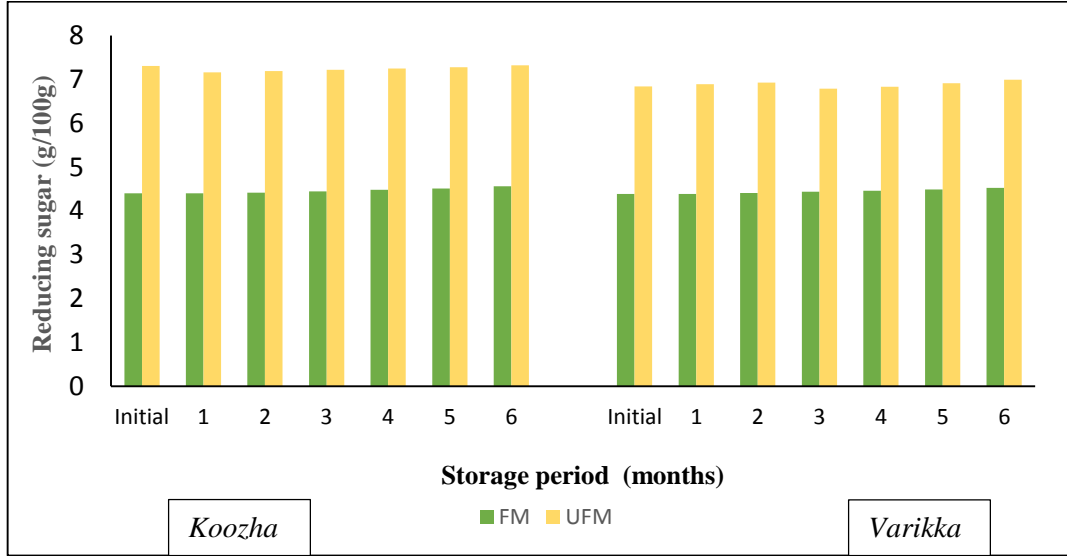


Fig. 18. Reducing sugar of fermented (FM) and unfermented (UFM) food mixtures on storage

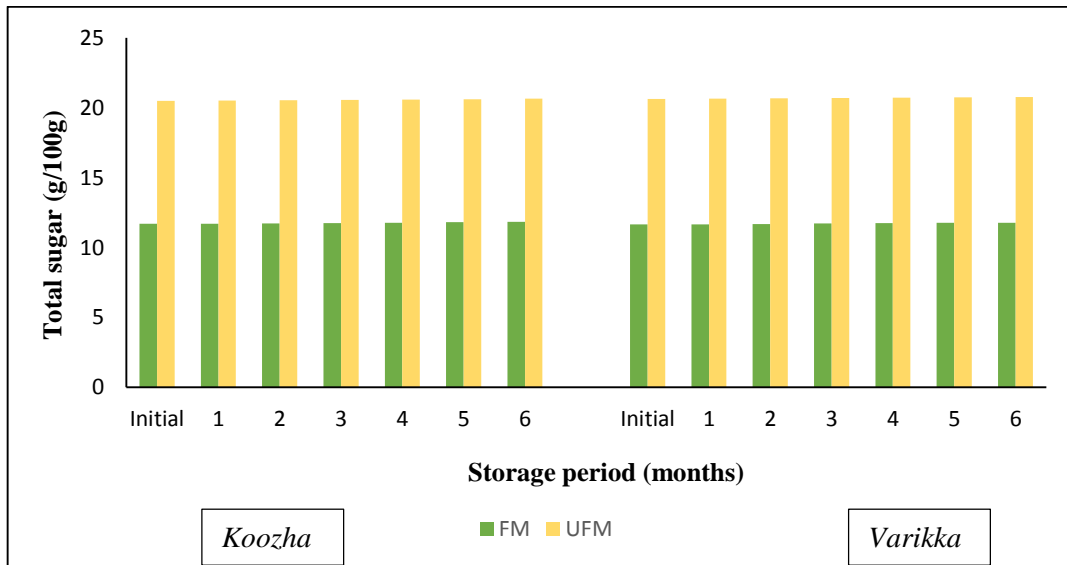


Fig. 19. Total sugar content of fermented (FM) and unfermented (UFM) food mixtures on storage

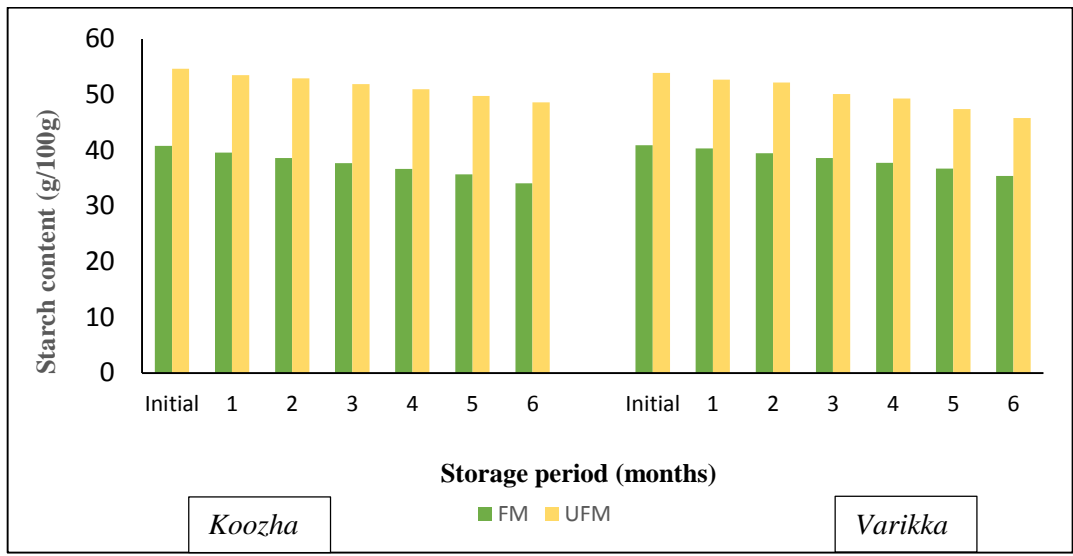


Fig. 20. Starch content of fermented (FM) and unfermented (UFM) food mixtures on storage

The findings of the present study agrees with that of Sefa-Dedeh and Sakyi-Dawson (2001) who reported decreased carbohydrate content of cereal/legume blend in the fermented sample when compared with unfermented ones. In another study Yoon *et al.*, (2004) observed that the sugar content of the tomato pulp fermented with Lactic acid cultures reduced from an initial value of 34mg/ml to 25.2, 21.0 and 19.3 mg/ml after 24, 48 and 72 hours fermentation respectively. Similarly when cassava flour was fermented with *L. plantarum*, the sugar content was reduced from 5.21 percent to 4.41 per cent (Sobowale *et al.*, 2007).

Similar results were reported by Sindhu *et al.* (2005) during the development of an indigenous fermented food mixture using rice flour, whey, sprouted green gram paste and tomato pulp. Sharon *et al.*(2015) on developing a banana based probiotic food mixture reported a significant reduction in the total sugar, reducing sugar and starch content on fermentation.

During storage period, there was an increase observed in the quantity of TSS, reducing and total sugars in the stored food mixtures, whereas the starch content decreased significantly. This may be because, the relatively larger starch molecules underwent hydrolysis and produced simple sugars like sucrose, glucose and fructose. According to Thilagavathi *et al.* (2015), the increased level of moisture and air in the formulated food product can fasten the process of starch degradation.

5.4.7. Total ash and mineral content of the developed food mixtures

5.4.7. Total ash and mineral content of the developed food mixtures

The study reported non significant changes in the mineral contents viz, calcium, iron, potassium and total ash of the fermented and unfermented food mixtures (Fig. 21-24).

Several authors have reported that probiotic fermentation increase the bioavailability of minerals but not the mineral content. This is because fermentation

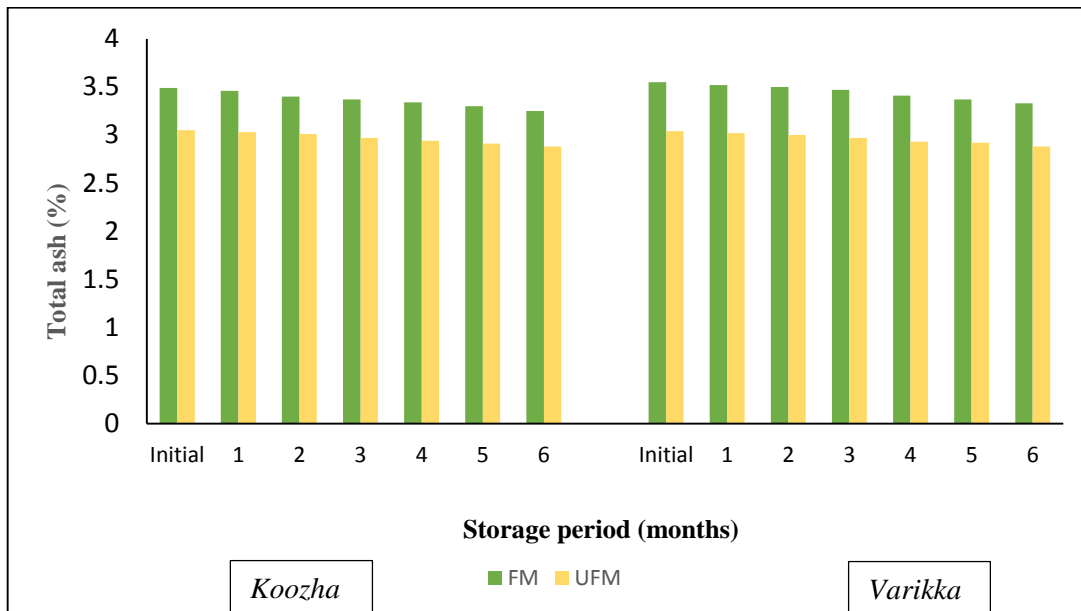


Fig. 21. Total ash content of fermented (FM) and unfermented (UFM) food mixtures on storage

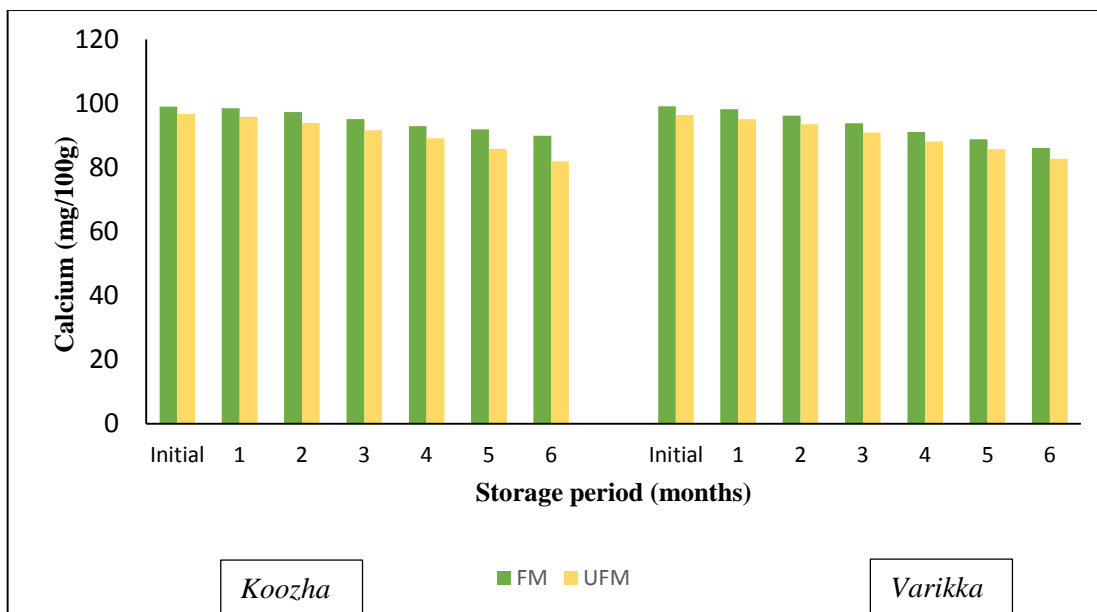


Fig. 22. Calcium content of fermented (FM) and unfermented (UFM) food mixtures on storage

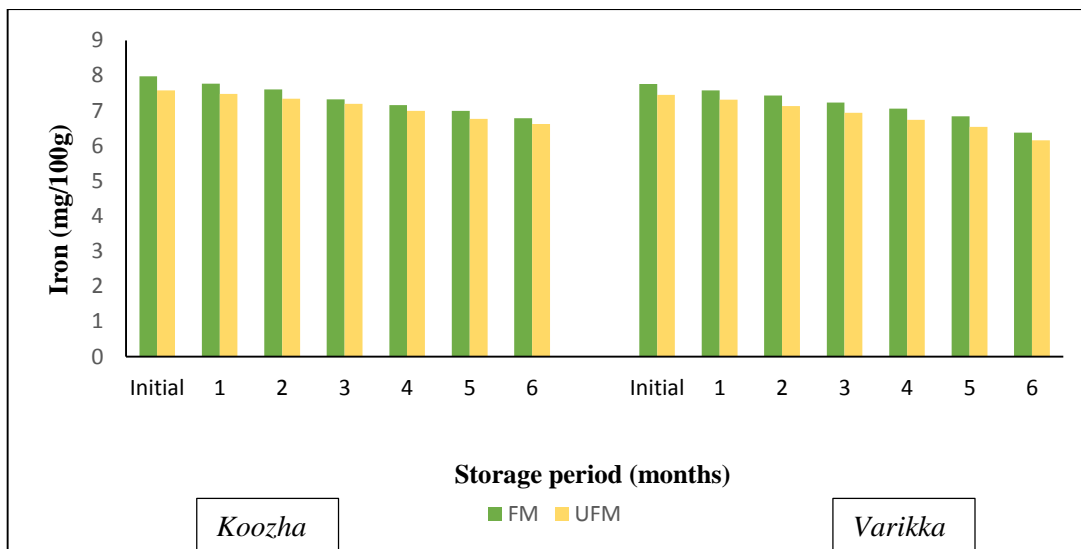


Fig. 23. Iron content of fermented (FM) and unfermented (UFM) food mixtures on storage

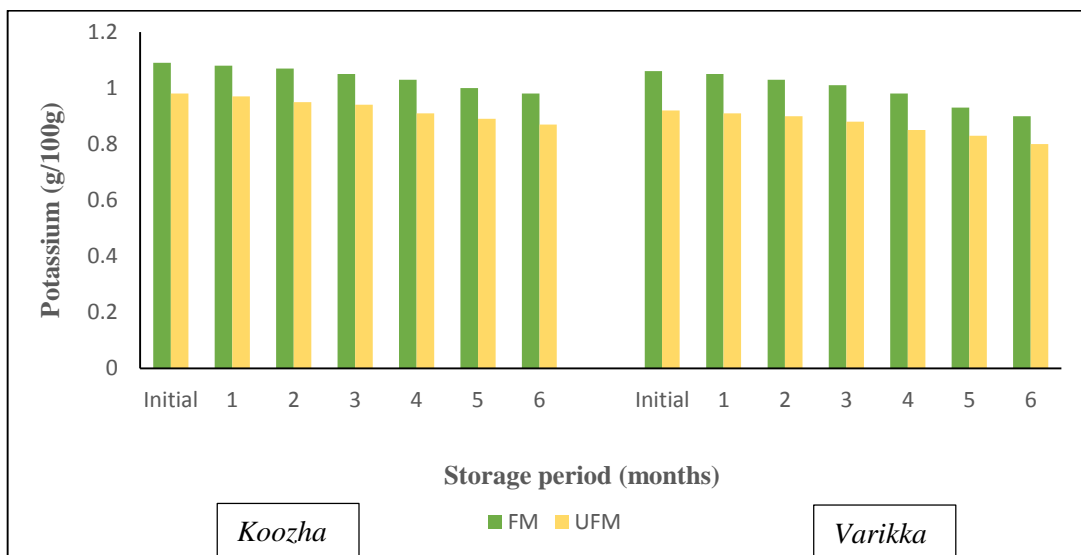


Fig. 24. Potassium content of fermented (FM) and unfermented (UFM) food mixtures on storage

converts the bound form minerals to free form and thus increase the availability (Khetarpaul and Chauhan, 1990). Jood and Khetarpaul (2005) also stated that reduction in antinutrients due to fermentation increase the bioavailability of various minerals but there need not be any change in the total mineral content in fermented foods. In a study published by Aljewicz *et al.* (2014) it was reported that the use of probiotic cultures significantly increased the availability of calcium (2.5%), phosphorus (6%) and magnesium (18 %) in Dutch type cheese fermented by the probiotic cultures *Lactobacillus rhamnosus* HN001, *Lactobacillus paracasei* LPC-37 and *Lactobacillus acidophilus* NCFM.

Sharma and Khetarpaul (1997) during the fermentation of rice dehulled black gram paste with whey, reported increased HCl extractability of calcium, iron and phosphorus and fermentation did not cause change in the above said minerals.

During the storage period, both the mineral content as well as the ash contents of the fermented and unfermented food mixtures were reduced. Similar trend of gradual decrease was observed during the storage of fermented foods by Sharon (2010) and Lakshmy (2012).

The reduction in the mineral content and total ash content may be due to the changes in storage temperature. It was observed in this study that there is a significant negative correlation exist between the calcium, iron, potassium and total ash with dry bulb temperature.

Kramer (1977) suggested that the rate of nutrient losses is usually proportional to the storage temperature. Several factors contribute to the loss of food quality especially in terms of nutrients of which respiration and transpiration are of prime importance (Osunde and Orhevba, 2009).

The effect of storage condition and storage period on the nutritional qualities of stored yam tubers were studied by Osunde and Orhevba (2009) and the study reported a decrease in the calcium and potassium content of the stored yam during the six month storage. This calcium and potassium contents of stored yam tubers

were found to be inversely proportional to the storage temperature. Krishnaja (2014) reported a reduction in the calcium, iron and potassium contents of developed fermented functional food supplements during storage. Similarly Nath (2015) reported a decrease in the chemical and sensory qualities of probiotic drink during storage at ambient condition when compared with the refrigerated ones. The result suggest the role of storage temperature on the quality of stored food item.

5.4.8. Thiamine and riboflavin content of the developed food mixtures

The action of probiotic microbes present in the food has been shown to improve the quantity, availability and digestibility of certain nutrients. B vitamins are one among them. Probiotic fermentation increased the thiamine and riboflavin contents in the food mixtures. Figures 25 and 26 shows the changes in the thiamine and riboflavin content of both the fermented and unfermented food mixtures on storage.

Keuth and Bisping (1993) opined that the elevated levels of B group vitamins in *temph* (fermented soya product, popular in Japan) is due to the microbial biosynthesis of the above said vitamins. The concentration of thiamine in milk was found to improve by 11 percent on fermentation with *Bifidobacterium longum* for 48 hours (Hou *et al.*, 2000). Sunny *et al.*, (2004) prepared a yoghurt like product with imitation milk from groundnut seeds with the strains *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and an increase in niacin, riboflavin and thiamine content was recorded.

Fermentation of soy milk with various lactic acid bacteria viz. *Lactobacillus acidophilus* B4496, *Lactobacillus bulgaricus* CFR2028, *Lactobacillus casei* B1922, *Lactobacillus plantarum* B4495 and *Lactobacillus fermentum* B4655 resulted in an increase in the riboflavin and niacin content of the fermented soymilk than the unfermented sample (Rekha and Vijayalakshmi, 2010). Jood *et al.* (2012) reported

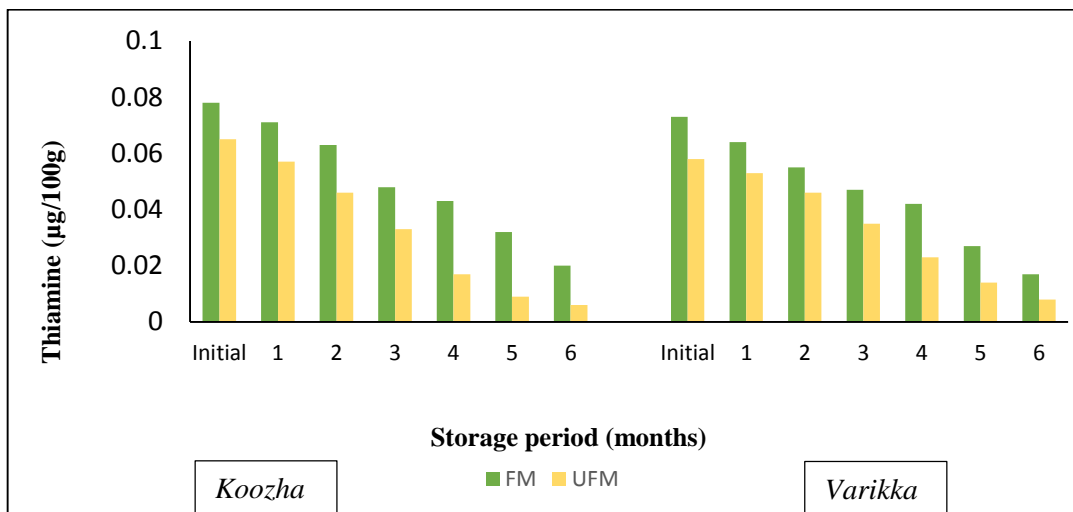


Fig. 25. Thiamine content of fermented (FM) and unfermented (UFM) food mixtures on storage

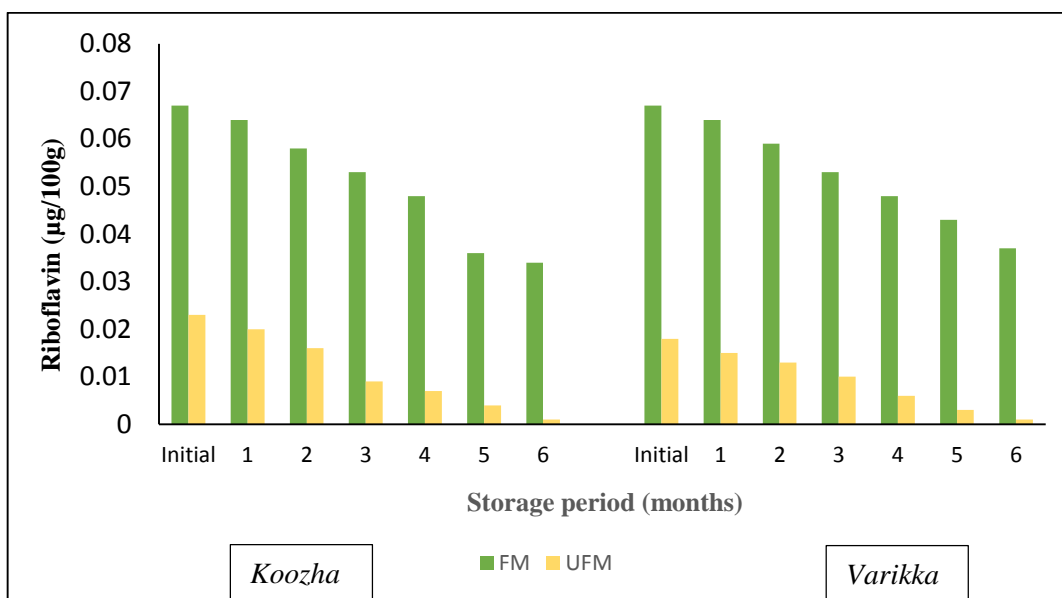


Fig. 26. Riboflavin content of fermented (FM) and unfermented (UFM) food mixtures on storage

that the thiamine, riboflavin and niacin content improved in sorghum based food mixture on probiotic fermentation with *L. acidophilus*.

During storage, the B vitamins found to decrease gradually in fermented and unfermented food mixtures. As the unfermented food mixtures were found to have relatively lower levels of B vitamins, at the end of storage, it reached negligible levels. Rangaswamy and Bagyaraj (2000) were also of the same opinion and reported that microorganisms present in the food will utilize the B vitamins, causing its gradual degradation.

5.4.9. *In vitro* starch and protein digestibility of the developed food mixtures

From the figure 27 and 28, it is clear that probiotic fermentation resulted in significant increase in the *in vitro* digestibility of starch as well as protein. *In vitro* protein digestibility increased upon autoclaving and fermentation due to the significant reduction in antinutrients, which are responsible for inhibiting the activity of proteolytic enzymes (Goyal and Khetarpaul, 1995; Jood and Khetarpaul, 2005). Starch digestibility may be increased on fermentation and this can be related to enzymatic properties of microbes, which ferment the substrate (Rani, 2016).

Similar findings were reported by Rani and Khetarpaul (1998) where the starch digestibility of unfermented autoclaved RSMT mixture was improved from 62.65 per cent to 78.33 per cent upon fermentation. It was reported by Sindhu and Khetarpaul (2001) that, on fermenting the indigenous food mixture containing tomato pulp using *L. casie* and *L. plantarum*, both the starch and protein digestibility were improved.

Arora *et al.* (2008) reported that the *in vitro* protein digestibility of pearl millet based indigenous food mixture was found to increase from 43.30 to 50.99 per cent after fermentation with *L. acidophilus*. During the development of banana based probiotic food mixture, Sharon *et al.*, (2015) observed that the *in vitro* starch digestibility of unfermented and fermented food mixtures were 54.41 to 56.34 per cent and 78.57 to 83.60 per cent respectively.

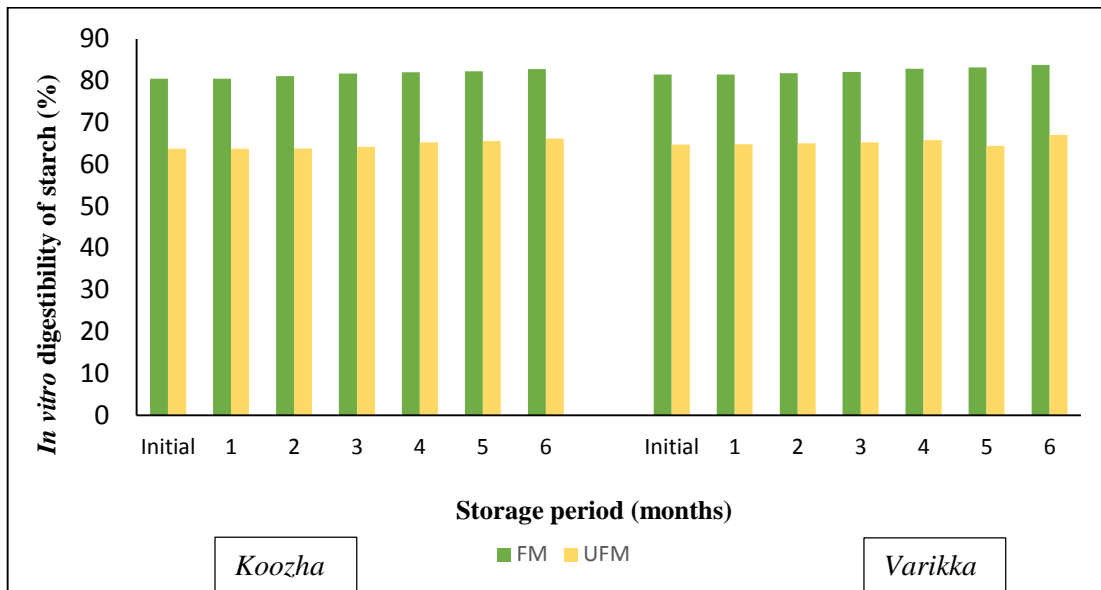


Fig. 27. *In vitro* starch digestibility of fermented (FM) and unfermented (UFM) food mixtures on storage

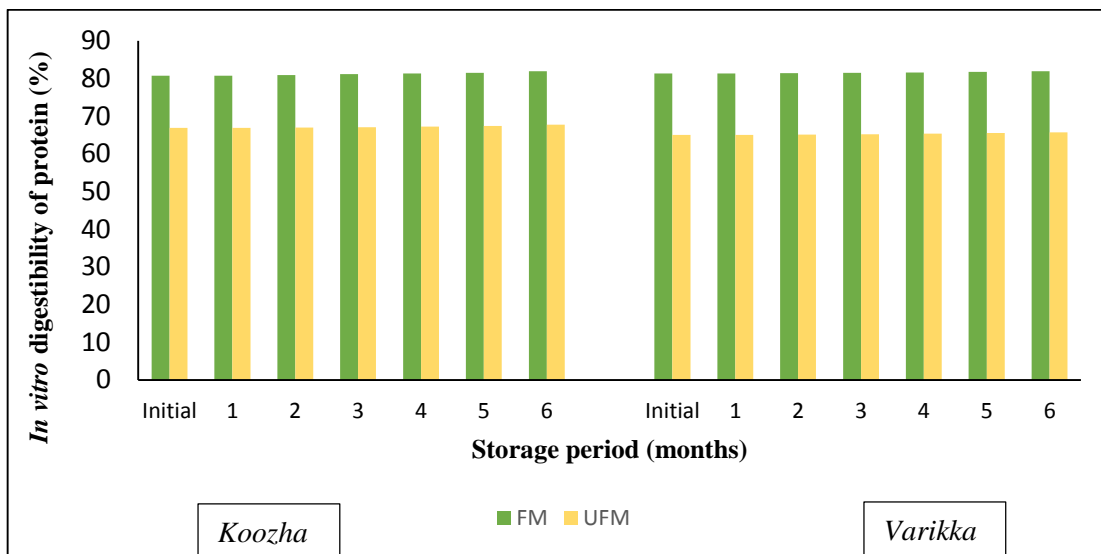


Fig. 28. *In vitro* protein digestibility of fermented (FM) and unfermented (UFM) food mixtures on storage

Rani (2016) reported a significant increase in the *in vitro* starch and protein digestibility of indigenous food mixture (containing pearl millet, chickpea, skim milk powder and tomato pulp) on fermentation with *L. acidophilus*. The starch digestibility of the food mixture increased from 48.97 to 59.95 per cent and the protein digestibility increased from 56.11 to 68.91 per cent after fermentation.

During storage, the digestibility of starch as well as protein was found to decrease considerably. The findings were in agreement with Sharon (2010) and Chandraprabha (2017).

5.4.10. Organoleptic evaluation of the developed food mixtures

From the study, it is clear that there were no significant differences in the appearance, colour, flavour and texture of the fermented and unfermented food mixtures. But the taste and overall acceptability were significantly higher for the fermented food mixtures. Figure 29 represents the overall acceptability of the fermented and unfermented food mixtures *koozha* variety and figure 30 represents the results of *varikka* variety. From the figures it is evident that the fermented food mixtures were more acceptable among the judges than the unfermented samples.

Lactic acid bacteria are found to produce unique aroma and flavour for fermented products. As a result of the lactic acid production, these bacteria create a tangy lactic acid taste. Further, upon the bioconversion of amino acids by the proteolytic enzymes, they produce aromatic compounds (Williams *et al.*, 2001; Yvon and Rijnen, 2001; van Kranenburg *et al.*, 2002). Apart from causing acidification of the raw material through the production of organic acids, lactic acid bacteria also produce acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several important enzymes. In this way they enhance microbial safety, contribute to shelf life, improve texture and create the pleasant sensory profile of the end product (Leory and De Vyust, 2004).

Blandino *et al.* (2003) suggested that the presence of diacetyl, acetic and butyric acid are responsible for making the cereal based fermented products more appetizing.

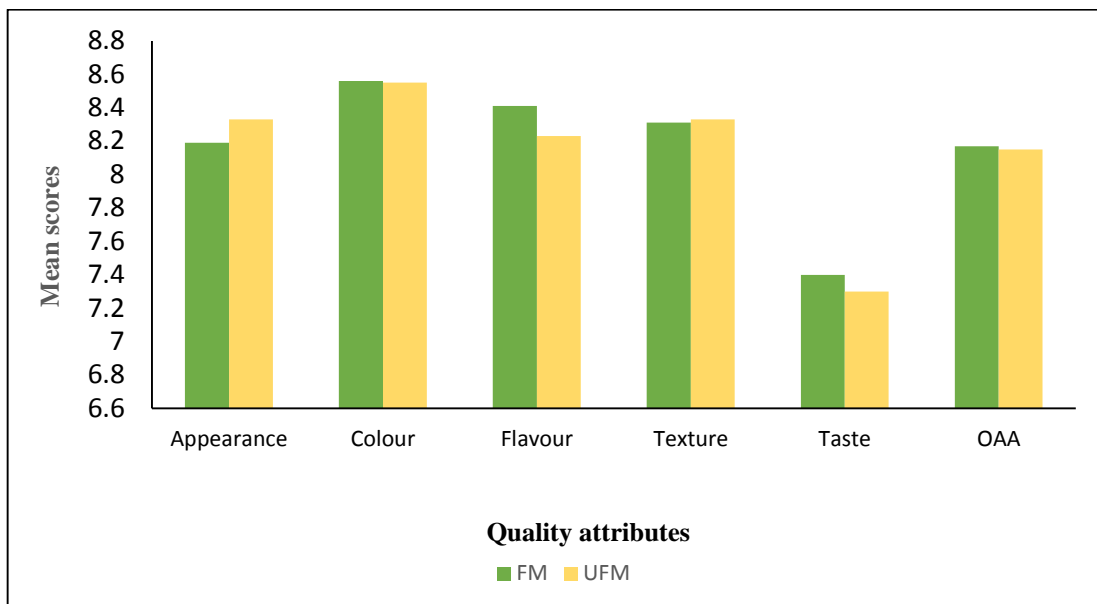


Fig. 29. Mean scores of organoleptic qualities of fermented (FM) and unfermented (UFM) food mixtures (*koozha*)

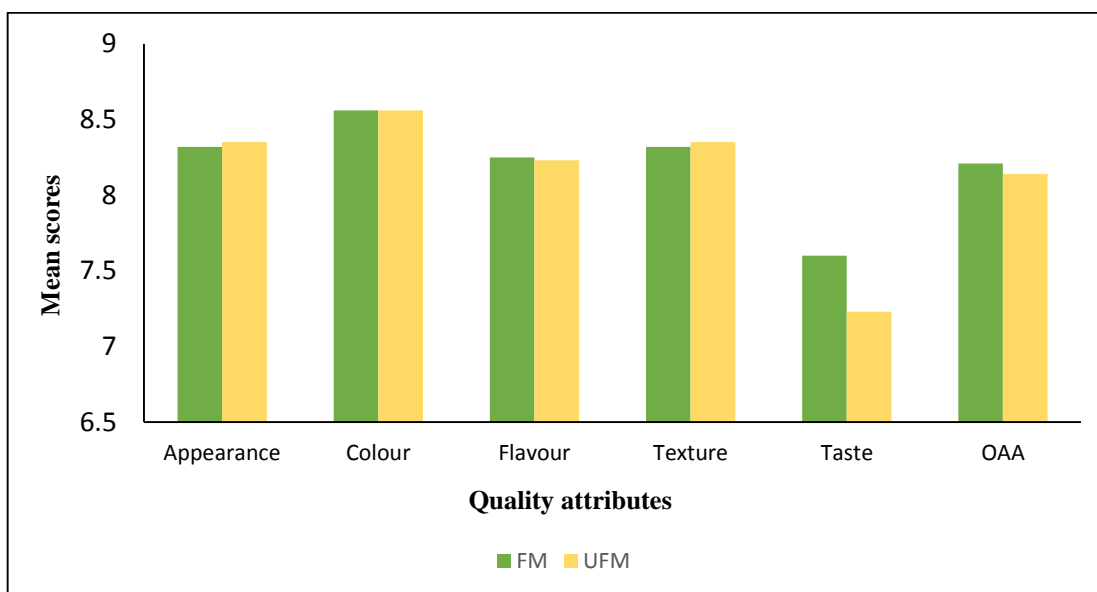


Fig. 30. Mean scores of organoleptic qualities of fermented (FM) and unfermented (UFM) food mixtures (*varikka*)

Leory and De Vyust (2004) observed that the homo fermentative lactic acid bacteria convert sugar to lactic acid to produce energy and this can lead to the generation of many metabolites such as acetate, ethanol, diacetyl and acetaldehyde which contribute to the typical flavour of fermented products, such as sourdough (determined by the lactate/acetate ratio), kefir and koumiss (ethanol), butter and buttermilk (diacetyl) and yoghurt (acetaldehyde).

Rani and Khetarpaul (1998) developed an acceptable probiotic food mixture by incorporating pearl millet flour, chick pea flour, skimmed milk powder and tomato pulp. The fermentation was carried out with *L. acidophilus* at 37⁰C for 24 hours. The BCGT (Barley flour, milk co precipitate, sprouted green gram paste, and tomato pulp) food mixture developed by Sindhu and Khetarpaul (2004) was found to be more acceptable than the unfermented food mixture.

The banana based probiotic food mixture developed by Sharon (2010) was more acceptable than the unfermented food mixture. The probiotic mixture was 'liked very much' (organoleptic scores 8-9) by the judges whereas the unfermented samples were in the 'nor liked neither disliked' (organoleptic scores 5-6) category. Baruah *et al.* (2018) developed a functional multi mix with rice, rice bean, foxtail millet, flax seed and tomato pulp in the ratio 3:4:1:1:1:1 using lactic acid bacteria and the developed product was organoleptically acceptable to the human palate.

5.4.11 Viable count of *L. acidophilus* in fermented food mixtures

Any probiotic food must meet several criteria for recognising as a marketable probiotic food item. The most important requirements among them is the ability of the concerned bacteria to survive in sufficient numbers in the product. It should maintain the viability till the product reaches the consumer.

The viability of the organism is given much importance because it must survive in the food matrix during the storage and during the transit through the varying destructive conditions of gastro intestinal tract (Playne, 1994).

Previous references suggest the probiotic viability of a probiotic product as 10^6 cfu/ml or 10^6 cfu/g (Kurmman and Rasic, 1991; Shah, 2001). But as per the latest recommendations given by FSSAI (2016), a probiotic food must contain 10^9 cfu/ml of the fermenting organism. In the present study, the viability of jackfruit based probiotic food mixtures were found to vary from 74 to 79×10^9 cfu/g initially and found reducing during storage (Fig.31).The food mixtures maintained an optimum viability even after six months of storage.

The BCGT probiotic food mixture developed by Sindhu and Khetarpaul (2001) reported a probiotic viability of $9.88 \log$ cfu/g. Angelov *et al.* (2006) developed oats based probiotic drink with lactic acid bacteria and observed a probiotic viability of 9.3×10^9 cfu/g. When tested for the probiotic capacity of peanut flour with different lactic acid bacteria, Wang (2007) found that *L.plantarum* p9 grew to the highest cell population ($9.48 \log$ cfu/g) in peanut flour after 72 hours of fermentation at 37°C .

Arora *et al.* (2008) developed probiotic food mixtures based on raw as well as germinated pearl millet flour and the authors could observe a significantly higher probiotic viability in the germinated flour (8.64×10^8 cfu/g) based probiotic food. Sharon (2010) evaluated the probiotic capacity of banana based food mixture and found out that after fermentation, the powdered food mixture contained $9.45 \log$ cfu/g of *L. acidophilus*.

5.4.12. Microbial enumeration and insect infestation of the developed food mixtures

During storage, the total bacteria as well as the probiotic bacteria were found to be reduced in the fermented food mixtures. On the other hand, the total bacterial count of the unfermented food mixtures increased gradually. No fungal and yeast

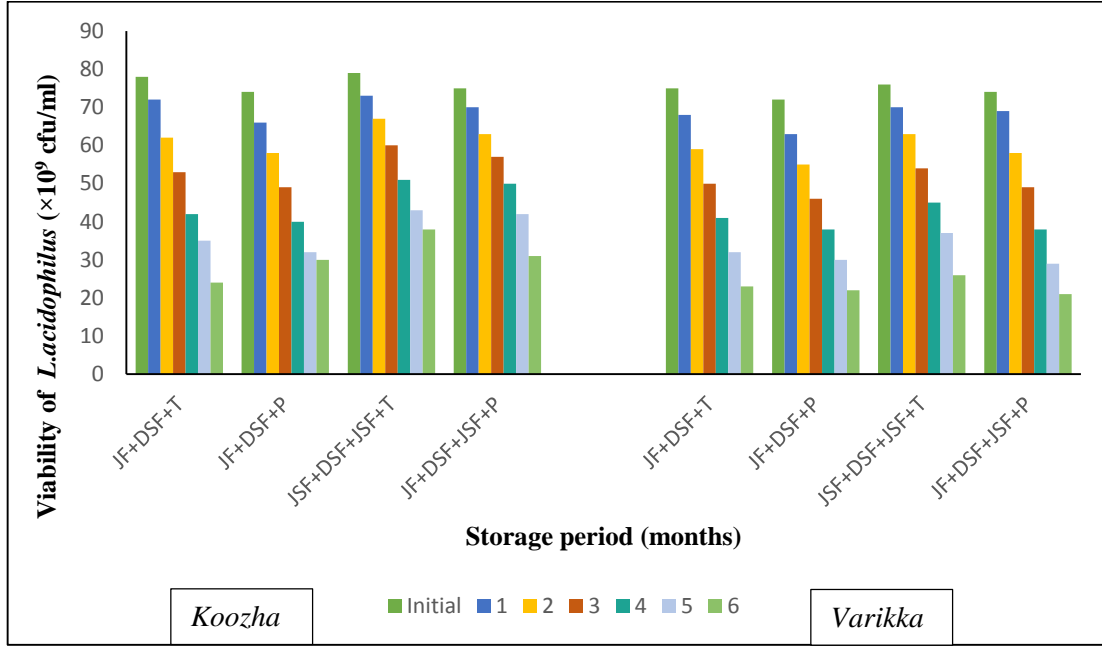


Fig. 31. Viability of *L. acidophilus* in fermented food mixtures during storage

colonies were reported in the food mixtures and during the storage period. Insect infestation was also absent. The developed food mixtures were also free of insect infestation during the storage period of six months

Sharon (2010) reported a decrease in the total bacterial count in the banana based probiotic food mixture. Lakshmy (2012) also reported a gradual decrease in the bacterial count of the freshly prepared '*tempeh*' on storage. As per the FSSAI (2010) guidelines, the bacterial count (aerobic plate count) is not applicable in the case of fermented fruit and vegetable products. The presence of yeast and fungi were not there in the freeze dried food mixtures and this is in agreement with the specifications of FSSAI (2010) which specifies the safer limit of yeast and molds in fermented fruit and vegetable products as 1×10^2 cfu/g. Hence, it can be concluded that the product was shelf stable upto six months of storage and was microbiologically safe for consumption even after six months of storage.

5.5. Temperature and relative humidity

Relative humidity is the amount of moisture in the air compared to what the air can "hold" at that temperature. When the air can't "hold" all the moisture, it condenses as dew (Anon., 2018). Relative humidity will reach minimal values when the temperatures are high.

Powders that are stored under humid environments can adsorb unintended surface moisture, which creates liquid bridges between particles that can adversely affect flow. In extreme cases, this can lead to powder caking (i.e., agglomeration) and reduced or free flow of the powders. Armstrong *et al.* (2014) demonstrated that powder properties relevant to process performance can change significantly when a material is exposed to changes in humidity. Lu *et al.* (2017) reported both the flow and dispersion properties of lactose blends deteriorate after being stored at 85 per cent RH, but improved after being conditioned at 58 per cent RH.

During the conduct of the present study the relative humidity of the stored condition was below 60 percent, which might have protected the food mixture from deterioration due to moisture. The low relative humidity may also be one of reason for the shelf life of the developed food mixtures upto six months.

5.6. Glycemic index of the food mixtures

According to Wolever *et al* (2006), Glycemic index is the incremental area under the blood glucose response curve elicited by a 50 g available carbohydrate portion of a test food expressed as a percentage over the response after 50 g anhydrous glucose taken by the same subject. Itam *et al* (2012), says that glycemic index (GI) is a measure of the potential of foods containing the same amount of carbohydrate to raise glucose concentration in the blood after a meal. It compares the hyperglycemic effect of a meal with pure glucose or bread.

As per the opinion of Franz (2001), glycemic index was developed as an effective way of classifying foods on the basis of glycemic response. When foods with high glycemic index (GI) produce a higher peak in postprandial blood glucose and a greater overall blood glucose response during the first two hour after consumption, the foods with low GI cause comparatively mild responses. Despite of the controversies at the earlier stages, GI is now widely recognized as a reliable, physiologically based classification of foods according to their postprandial glycemic effect (Powell *et al.*, 2002).Based on the GI, foods are classified into three category like the high GI foods (> 70), intermediate GI foods ($>55 - < 70$) and low GI foods (< 55). Glycemic Load (or GL) combines both the quantity and quality of carbohydrates. It can be considered as the best way to compare blood glucose values of different types and amounts of foods (GIF, 2017). Both the GI and GL have great impact on the glycemic response (GR) of a particular meal.

In the present study, the jackfruit based food mixtures were found to have low GI on the basis of their glycemic responses. This result is in line with the findings of

Hettiaratchi *et al.* (2011). They studied the effect of a 'jackfruit meal' in ten healthy individuals and found that the supplemented 'jackfruit meal' have low GI. The presence of α -D Glactose specific lecithin reported in jack fruit seeds are capable of binding with mono and oligo saccharides (Kumar *et al.*, 1982).

The probiotic food mixtures had low GI compared to that of unfermented food mixtures in the present study. This can be better explained with the higher GL of unfermented food mixtures (28.12 and 29.97 respectively for *koozha* and *varikka*). GL of a particular food is the product of glycemic index and available CHO in one gram of the food. As GL increases, glycemic responses also increase. As revealed from the present study, the starch as well as sugar content of the probiotic food mixtures (41.97g/100g starch and 11.80 g/100g total sugar in *koozha*; 41.24 g/100gstarch and 12.45 g/100 total sugar in *varikka*) were lower than that of the unfermented samples (56.64g/100g starch and 20.89g/100 total sugar in *koozha*; 54.64 g/100g starch and 21.07 g/100g total sugar in *varikka*) which may have made the probiotic food mixtures, a low GL food.

Apart from the GL, total protein content of a food can also regulate the post prandial blood glucose response (GIF, 2017). The protein content of fermented food mixtures were 23.98 and 25.06 g/100g respectively for *koozha* and *varikka* whereas the protein content of unfermented food mixtures were 21.58 and 22.37g/100g for *koozha* and *varikka*. Protein will stimulate additional insulin secretion, resulting in lower blood glucose levels. Protein tend to delay stomach emptying, thereby slowing the rate at which carbohydrate can be digested and absorbed. Both the food mixtures gave mild glycemic responses than the control (glucose) may be because of these factors.

The present study revealed that the probiotic fermentation could significantly increase protein content of the food mixtures. Hence, the relatively higher amount of protein, combined with the low starch and total sugar contents of the probiotic

fermented food mixtures than the unfermented ones may be the reason for low GI of fermented food mixtures. Along with this, the live probiotic bacteria may also interfere with the glucose response of the probiotic food mixtures. Several authors (Calcinaro *et al.*, 2005; Yadav *et al.*, 2008; Ostadrahimi *et al.*, 2015) concluded in their studies that the probiotic organism have antidiabetic as well as hypoglycemic effects.

The present study concluded that the *koozha* based food mixtures were found to have low GI than the *varikka* based food mixtures. *Koozha* based food mixtures were reported to have higher amounts of crude fibre than the *varikka* based mixtures and the fibre is capable of lowering the glyceemic responses of foods. Higher the fibre content, lower will be the rise in blood glucose. Rahman *et al.* (1991) reported higher percentage of free sugars and starch in the firm (*varikka*) variety of jackfruit than the soft (*koozha*) variety.

5.7. Standardisation and quality evaluation of instant shake mix

The multifaceted concept of convenience is often listed as the most important factor that determine the food of choice apart from the cost, health, sensory acceptability and related concerns (McIntosh *et al.*, 1996; Rappoport *et al.*, 1993; Steptoe *et al.*, 1995; Scholderer and Grunert, 2005). To a great extent convenience decides what to eat, when to eat, how to eat and from where to eat foods (Costa *et al.*, 2007). As a consequence, in this convenient driven society the demand of ready to eat or ready to cook minimally processed products has noticeably increased during the recent years (Kilinc *et al.*, 2007; Lee *et al.*, 2007).

In order to achieve the best product formulations, food manufacturers now use scientific approaches (Granato *et al.*, 2011). In the present study, the shake mixes with 50 per cent incorporation of fermented food mixtures were selected as the best treatment from both varieties (*koozha* and *varikka*) by the panel members. This is in agreement with the findings of Howard *et al.* (2009), who reported that the most

acceptable formulation for the instant peanut beverage included equal amounts of peanut flour, sugar, and non-fat dry milk. As the amount of jackfruit in food mixture increases, it gives body and consistency to the product which contributed greatly to the acceptability of the product.

Satter *et al.* (2013) developed an instant weaning mix by incorporating 50 per cent jackfruit pulp with various proportions of wheat, soya flour and milk powder and the mixes were highly acceptable among judges. Remya *et al.* (2017) developed jackfruit based instant shake mix and the shake mix with 50 per cent pre gelatinised jackfruit flour and 50 per cent skimmed milk powder was reported to be the best combination. During the development of protein enriched instant soup mix, the soup mix with 55 per cent fish powder got maximum scores in the organoleptic evaluation (Islam *et al.*, 2018).

The moisture content of the shake mixes were 2.53 and 2.5 per cent respectively for *koozha* and *varikka* based shake mixes. During storage, the moisture content was found to increase (Fig. 32). The moisture content is relatively minimum and can be considered as the positive side of the developed product. The moisture content of an instant *anjeer* (fig) shake mix was reported to be 2.8 per cent (Bhatnagar, 2002). Moisture provides a measure of the water content of the sample and for that matter its total solid content. It is also an index of storage stability of the flour. The lower the moisture content of flour, the better its shelf stability and the quality. According to FSSAI (2010), the moisture content of fruit based beverage mix/powdered fruit based beverage should not be more than 5.0 per cent. The moisture content of the probiotic shake mixes were within the limit. The reduced moisture content observed in the shake mix make it suitable for long term storage.

Moisture content (%) of instant probiotic shake mixes did not show much variation. With progressive storage, there was slight increase in the moisture content of instant shake mixes and the increase was within safe limit. Butt *et al.* (2004)

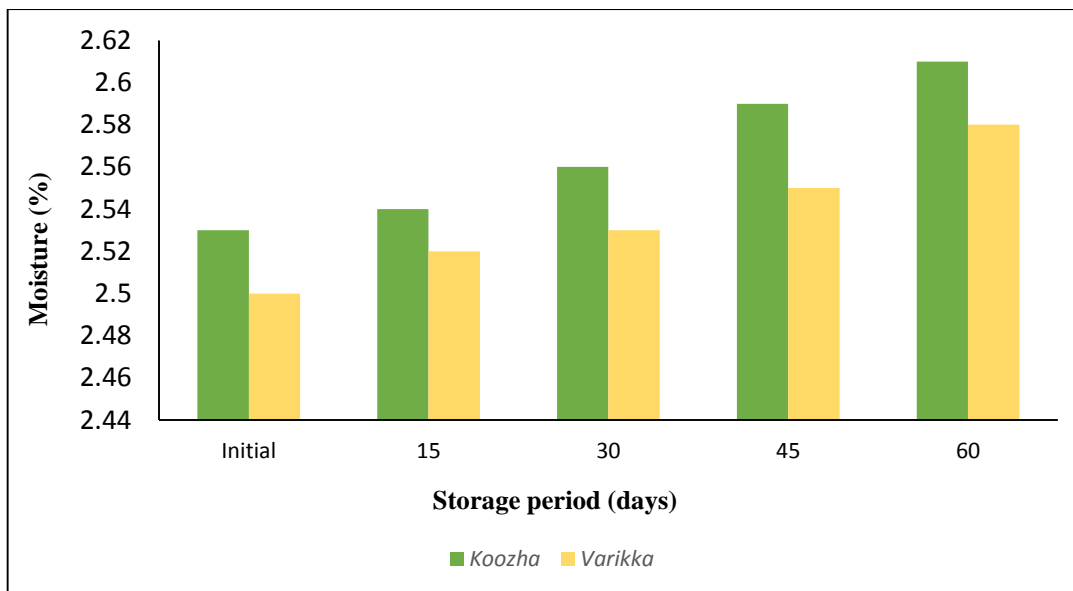


Fig. 32. Moisture content of instant probiotic shake mixes during storage

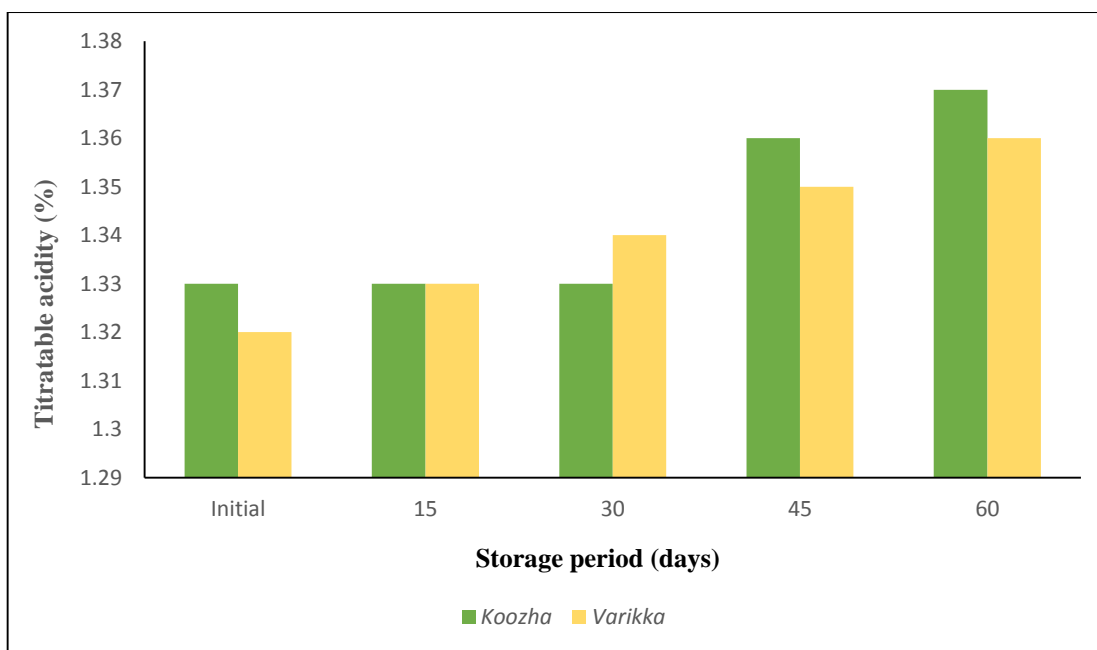


Fig.33. Titratable acidity of instant probiotic shake mixes during storage

explained the increase in moisture content during storage may be affected due to storage, treatments and packaging conditions and may also be due to the hygroscopic nature of the product. Nithiya *et al.* (2014) found that the moisture content of prawn incorporated instant soup mix increased from 3.21per cent to 3.25per cent after 2 months of storage. Figure 33 is the pictorial representation of the titrable acidity of the probiotic shake mixes during storage. There is gradual increase in the acidity of the probiotic shake mixes and this may be due the conversion of glucose into lactic acid by the probiotic organism for their survival.

The protein of probiotic shake mixes were 26.30 and 26.67 g/100g, and found decreasing during the storage time (Fig. 34). The protein content of raw jackfruit varies from 2.00 to 2.60 g/100g and that of ripe fruit from 1.20 to 1.90 g/100g (Ko *et al.*, 1998). The protein value of skimmed milk is 35g/100g (Anon., 2005) and that of jackfruit flour is 1.05g as reported by Munishamanna (2012). The presence of deffated soya flour in the food mixture may also have contributed to the protein value of shake mix.

Dhiman *et al.* (2017) reported a protein content of 12.65per cent in corn starch incorporated pumpkin seed based instant shake mix and during storage of six months, the protein content decreased from 12.65per cent to 12.25per cent. Sarkar *et al.*, (2019) developed an instant chicken soup mix with a protein content of 25.93g/100g.

Initially, the β carotene content of the probiotic shake mixes were 313.16 μ g/100g and 312.25 μ g/100g for *koozha* and *varikka* respectively and during storage, this nutrient was also found decreasing (Fig. 35). The changes in crude fibre content of instant probiotic shake mixes are given in figure 36. On storage, the fibre content of the shake mixes were found decreasing and this may also be due to the action of fermenting organism. Remya *et al.* (2017) reported a crude fibre content of 0.18per cent in jackfruit based instant shake mix and Shahanas *et al.* (2017) reported a crude fibre content of 0.061per cent in jackfruit based instant pudding mix.

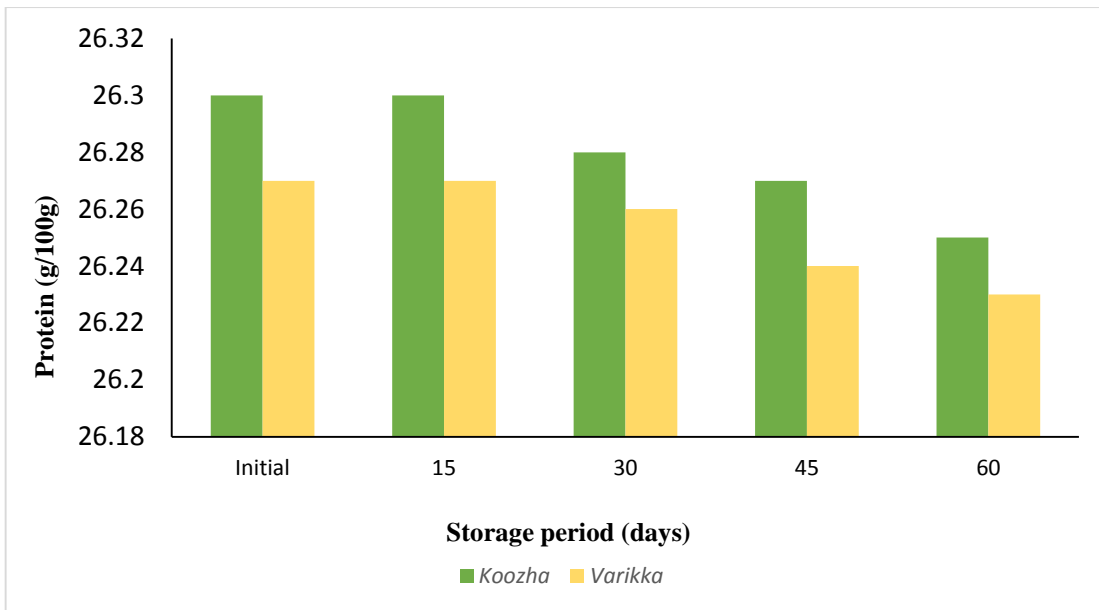


Fig.34. Protein content of instant probiotic shake mixes during storage

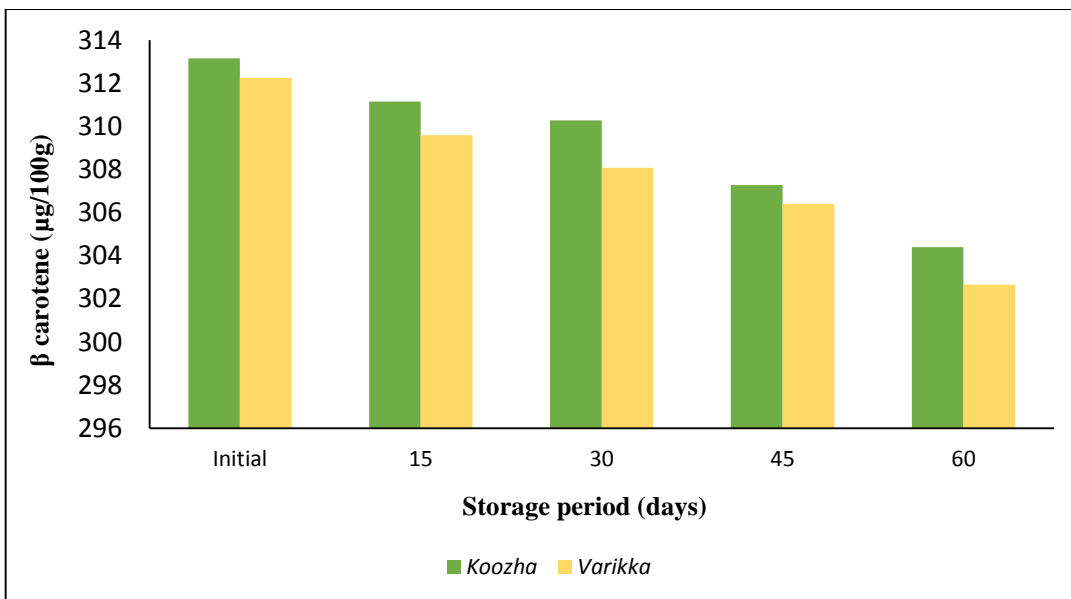


Fig.35. β carotene content of instant probiotic shake mixes during storage

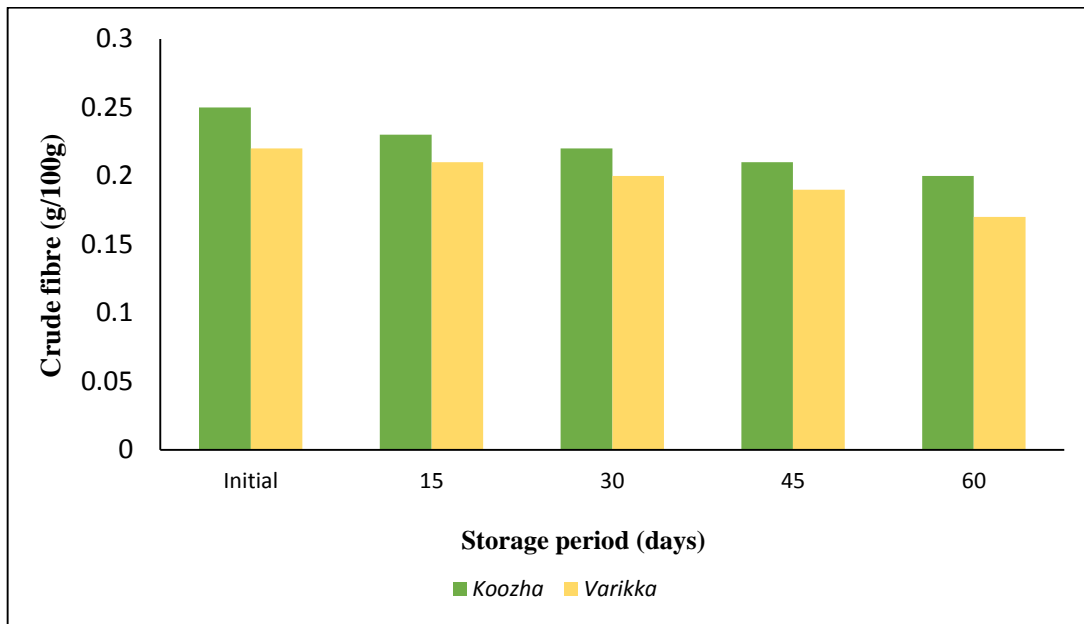


Fig.36. Crude fibre content of instant probiotic shake mixes during storage

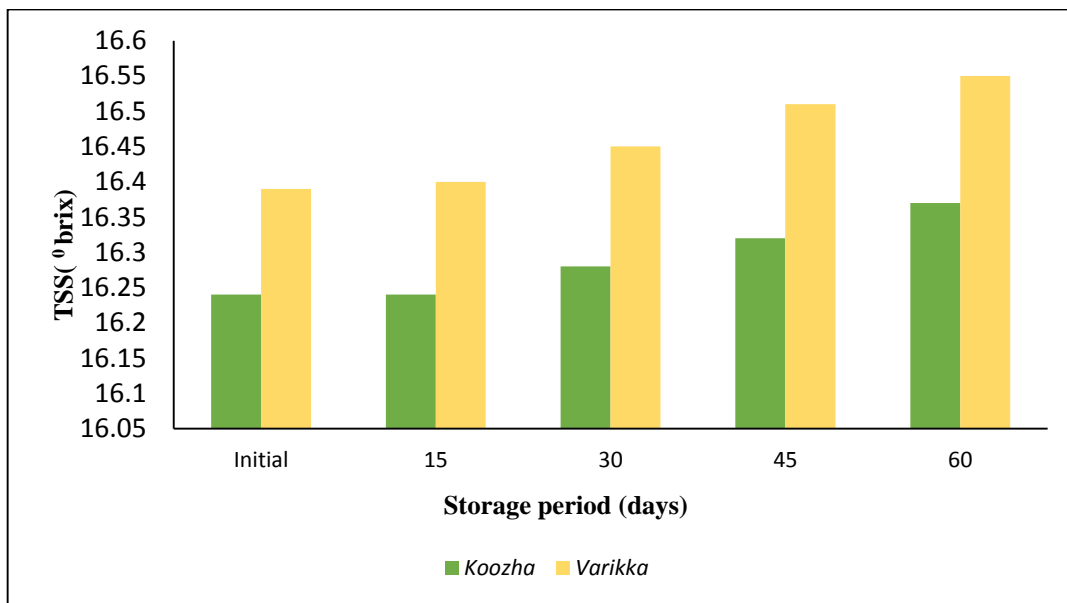


Fig.37. TSS content of instant probiotic shake mixes during storage

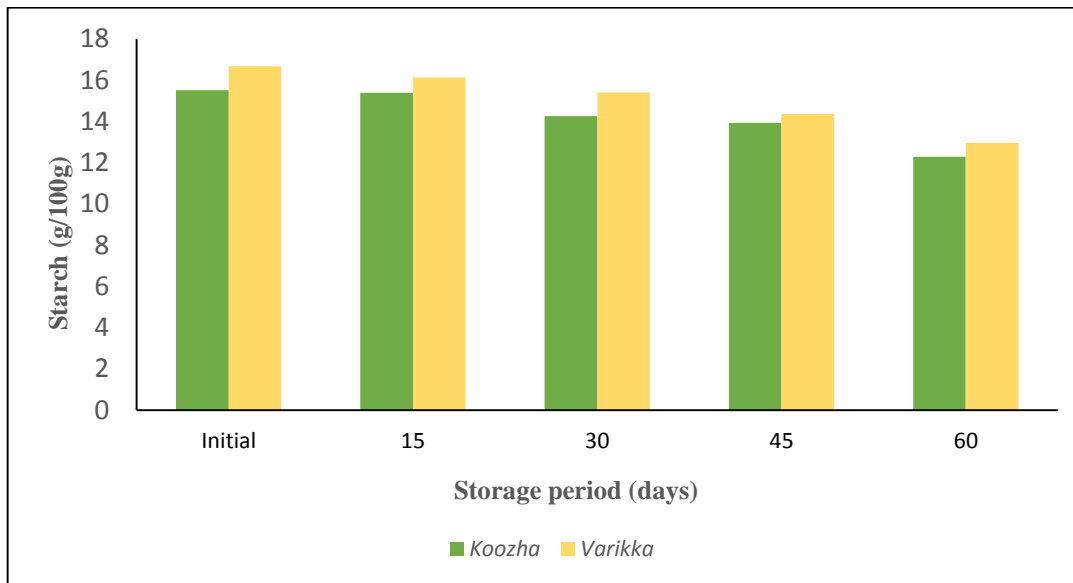


Fig.38. Starch content of instant probiotic shake mixes during storage

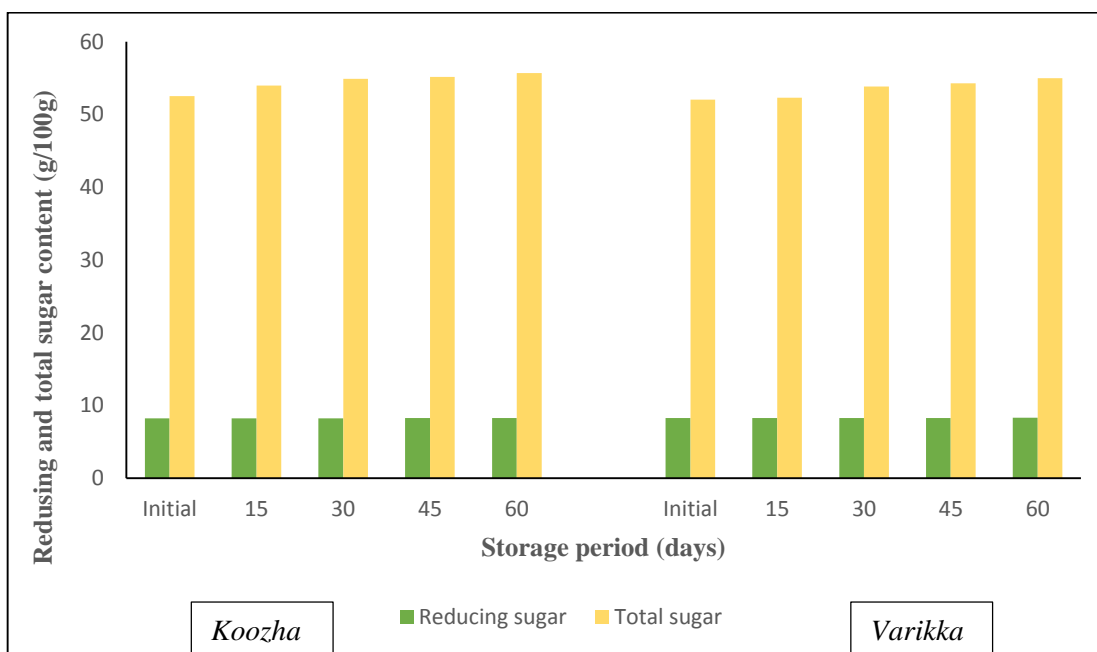


Fig.39. Reducing and total sugar content of instant probiotic shake mixes during storage

The TSS content of developed probiotic shake mixes were 16.24 (initial) and 16.37⁰brix (final) in *koozha* variety, and 16.39 (initial) to 16.55 ⁰brix (final) *varikka* variety (Fig. 37). This value is in accordance with the FSSAI (2010) specification of 10per cent TSS for RTS beverages. The TSS content of custard apple soya milk shake was found to be 16 ⁰ brix (Avhad *et al.*, 2017).

Unlike the TSS content, the starch content of the developed shake mixes tend to decrease during storage period. From the initial values of 15.22 and 16.68 g/100g the starch content became 12.29 and 12.95 g/100 respectively for *koozha* and *varikka* based probiotic shake mixes at the end of storage (Fig.38). Similarly the reducing and total sugar content also increased during storage (Fig. 39). Dhiman *et al.* (2017) reported a reducing sugar of 12.31 and total sugar of 39.85g/100g in cornstarch based instant soup mix with dehydrated pumpkin seed and on storage, the reducing and total sugar contents were found to be increased.

Ash is important in terms of nutrition because it tells how dense the minerals are in a particular food sample. Generally, low ash content indicates that the food product analysed is not a rich source of minerals. Both the jackfruit shake mixes contain considerable amount of ash. There was no significant difference in the ash contents of shake mixes, and the values were 2.76 per cent and 2.77 per cent respectively for *koozha* and *varikka*. The ash content of skimmed milk powder ranged from 8.20 to 8.60 per cent (USDEC, 2005) where as that of jackfruit bulb flour is 7.16 per cent (Swami *et al.*, 2015). This may be the reason for the fair ash value of control shake mixes.

During the analysis of the instant shake mix, it was noticed that it contains fair amount of calcium and potassium but not iron. Iron content was relatively less (Fig. 40). This may be because the fact that the skimmed milk powder was a good source of calcium (1.2 g/100 and 1.6g/100g) and potassium (Anon., 2005). Bhatnagar (2002)

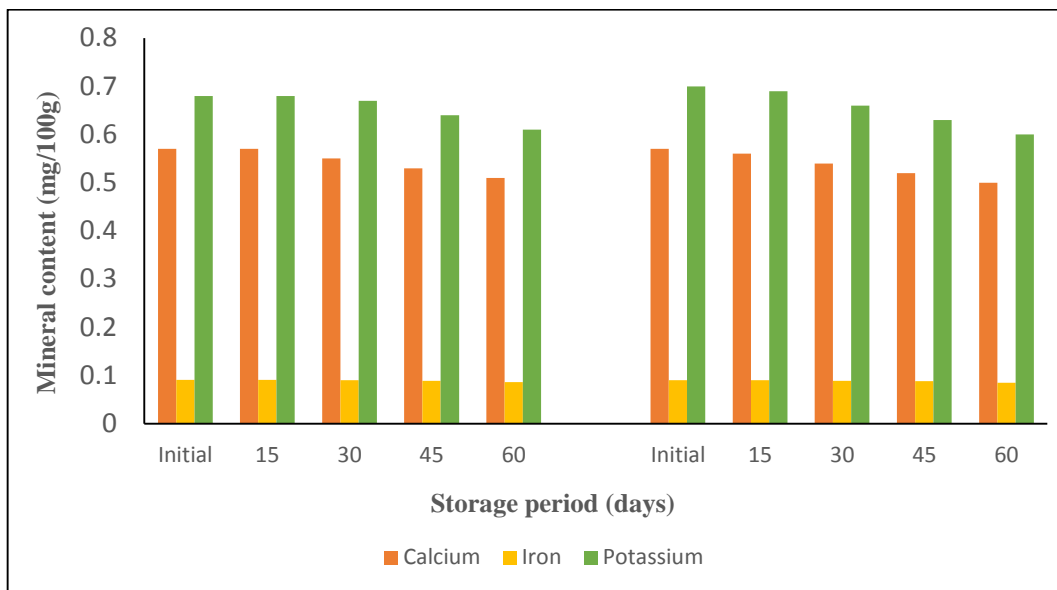


Fig.40. Mineral content of instant probiotic shake mixes during storage

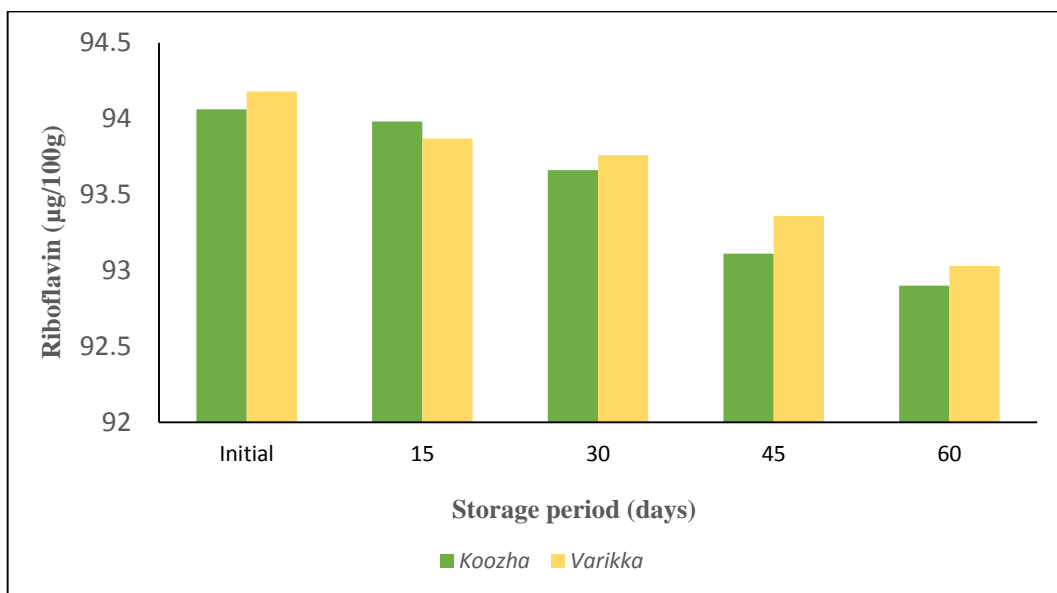


Fig.41. Riboflavin content of instant probiotic shake mixes during storage

developed an instant *anjeer* (fig) shake mix and reported calcium (833.4mg/100g) and potassium (991.0 mg/100g) but not iron (1.2mg/100g) in the final product. The jackfruit based instant weaning mix also reported fair amount of calcium (450.63 mg/100g) (Satter *et al.*, 2013).

On analysing the B vitamins, riboflavin was detected and thiamine was not detected in the shake mix (Fig. 41). This may be due to the fact that probiotic food mixtures contain only 0.073-0.080 µg/100g of thiamine and the skimmed milk powder also do not contain much of the nutrient, whereas the skimmed milk powder contain 1.7mg/100g of riboflavin (Anon., 2005). On storage, the nutrients undergoes decomposition and hence there was decrease in the riboflavin content.

The *in vitro* digestibility of both starch and protein increased during storage (Fig. 42) and this is in line with the findings of Sharon (2010), who reported an increase in starch and protein digestibility of sorbitol and rice bran incorporated banana based probiotic food mixtures on storage.

The developed shake mixes were liked very much by the panelist during the initial evaluation for their sensory parameters. At the end of storage the sensory parameters showed minimal decrease and the overall acceptability of the developed products were in the range of 'liked moderately' (organoleptic scores 6-7) by the panelists (Fig. 43).

Yadav (2016) developed an instant '*mangodi*' mix and stored it for a period of 60 days under ambient conditions and during storage, the overall acceptability of the developed product decreased and attained a final score of 8.35 from the initial score of 8.90. The results of the present study is in agreement with the findings of Ojo and Enujiugha (2018), who reported the overall acceptability score of a probiotic fermented '*ogi*' gruel as 8.67. Mehta and Jood (2018) in their study reported the overall acceptability of the oats based gluten free instant '*dhokla*' as 7.55. They also

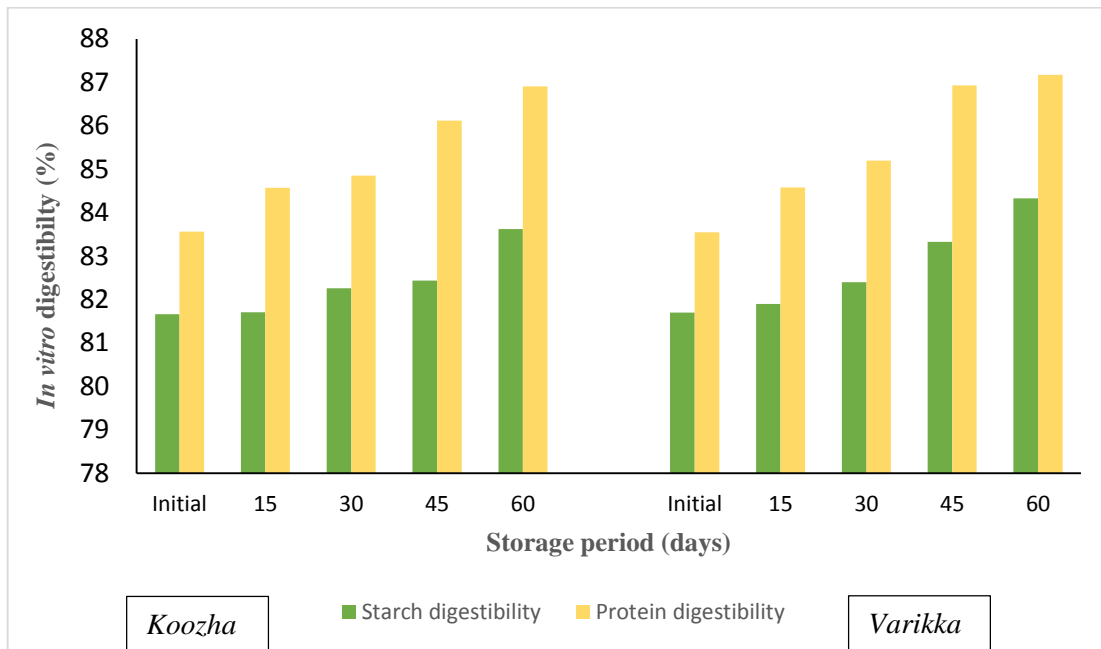


Fig.42. *In vitro* digestibility of instant probiotic shake mixes during storage

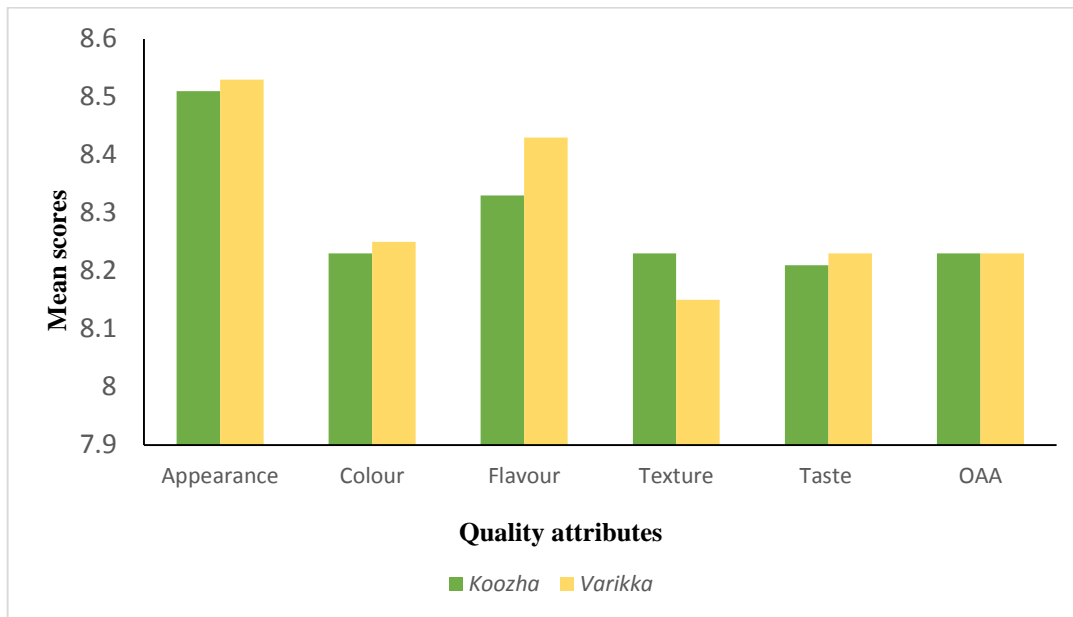


Fig. 43. Mean scores of organoleptic qualities of instant probiotic shake mixes during storage

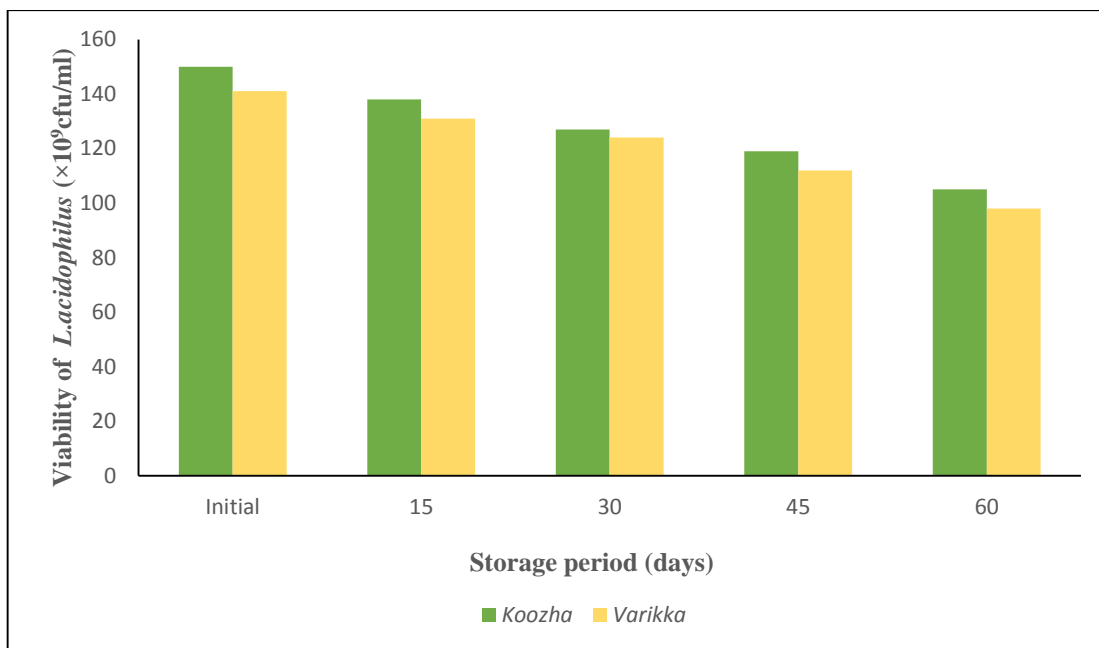


Fig. 44. Viability of *L. acidophilus* in instant probiotic shake mixes during storage

reported that the overall acceptability of the developed instant '*dhokla*' reduced during storage and maintained the acceptability upto three months.

5.7.1. Microbial enumeration and insect infestation of the instant shake mixes

During storage, the total bacteria as well as the probiotic bacteria was found to be reduced in the instant shake mixes (Fig. 44). No fungal and yeast colonies were reported in the shake mixes and during the storage period insect infestation was also absent. Sharon (2010) reported a decrease in the total bacterial count in the banana based probiotic food mixture. The developed food mixtures were also free of insect infestation during the storage period of 6 months. Lakshmy (2012) also reported a gradual decrease in the bacterial count of the freshly prepared '*temph*' on storage. The presence of yeast and fungi were not present in the probiotic shake mixes and this is in agreement with the specifications of FSSAI (2010) which specifically says an absence of yeast and moulds in evaporated products. Hence, it can be concluded that the product was shelf stable upto 2 months of storage and was microbiologically safe for consumption even after two months of storage.

5.8. Standardisation of jackfruit yoghurts

In the present study, jackfruit yoghurts with 30 per cent incorporation of jackfruit pulp (JP) was selected for further studies based on organoleptic evaluation. The organoleptic acceptance of the jackfruit yoghurts were observed to increase with the increase in amount of JP. The mean scores for overall acceptability of the jackfruit yoghurts from the two varieties are represented in Figure 45 and 46.

Ndife *et al.* (2014) prepared functional yoghurt with 10, 20 and 30 per cent incorporation of coconut milk slurry and the one with 30 per cent got the maximum overall acceptability. Findings of the present study was in agreement with that of Kumar and Mishra (2003) who dealt with the preparation of mango pulp fortified yoghurt and the study reported that, the overall acceptability of the product increases as the concentration of fruit pulp increases. Gad *et al.* (2015) prepared functional

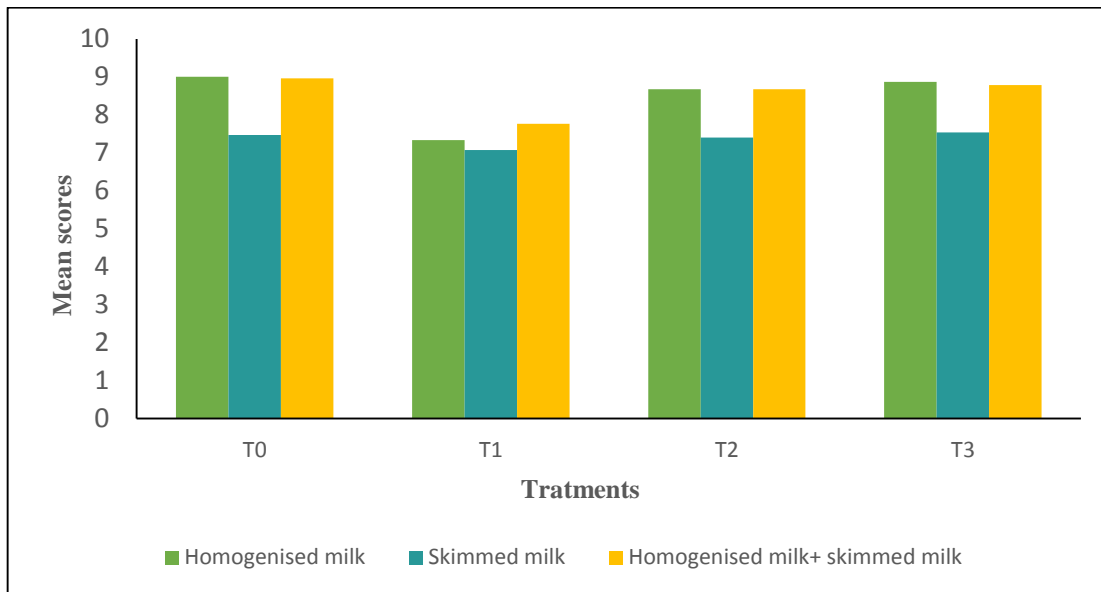


Fig. 45. Mean scores for overall acceptability of jackfruit based yoghurt (*koozha* variety)

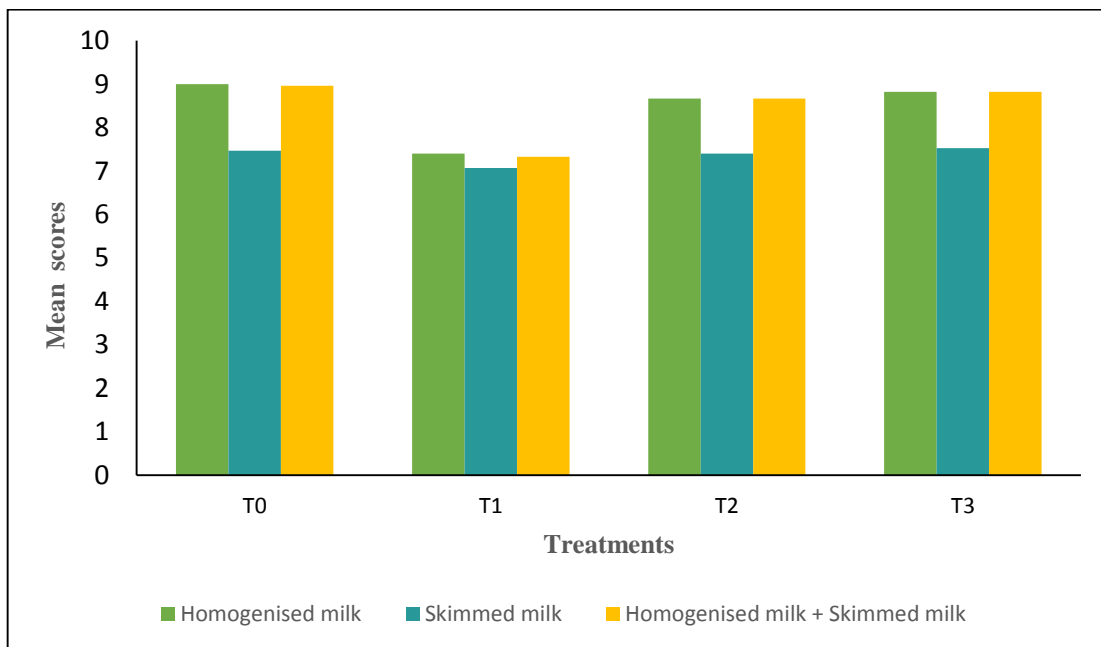


Fig. 46. Mean scores for overall acceptability of jackfruit based yoghurt (*koozha* variety)

yoghurts fortified with carrot and cantaloupe juice and the sensory evaluation of fruit yogurts concluded that fruit juice incorporation improved the acceptability of the yoghurts. From the figure, it is clear that the overall acceptability was higher for the *varikka* based yoghurts than the *koozha* based.

The yoghurt prepared with the *varikka* variety was more acceptable with reference to flavour and texture. Increased flavour of *varikka* yoghurts can be attributed to the presence of increased volatile compounds in the *varikka* variety. The major aroma concentrates of the jackfruit varieties are isopentyl isovalerate and butyl isovalerate. Isopentyl isovalerate of *varikka* variety was found to be 28.4 per cent and butyl isovalerate was 25.60 per cent, where as that of *koozha* variety was 18.3 per cent and 12.9 per cent respectively and these flavour compound are responsible for the enhanced flavour of *koozha* variety (Maia *et al.*, 2004). *Varikka* variety got good texture than the *koozha* variety and this may be due to the increased water content and juiciness of *koozha* (soft fleshed) jack fruit. Gad *etal.* (2015) opined that increased water content of fruit juice will lead to pronounced decrease in the body and texture of fruit enriched yoghurts. These factors might have contributed to the increased overall acceptability of *varikka* yoghurts than the *koozha* yoghurts.

From the results, it was also concluded that the yoghurts with HM were more acceptable than the HM+SM as well as SM. The acceptability were in the order of HM+JP> HM+HM+JP> SM+JP. Milk is the major ingredient in the production of yoghurt and the type of milk will decide the type of yoghurt and viz versa (Lopez *et al.*, 1997). Depending upon the variations in the type of milk used, the quality of yoghurt will vary. Milk with high fat content will produce rich creamy yoghurt with excellent mouth feel compared with low fat and skimmed milk (Robinson and Tamime, 1991).

The selected yoghurts were optimised for the growth of maximum probiotic organism and the maximum probiotic growth was observed at 25g substrate

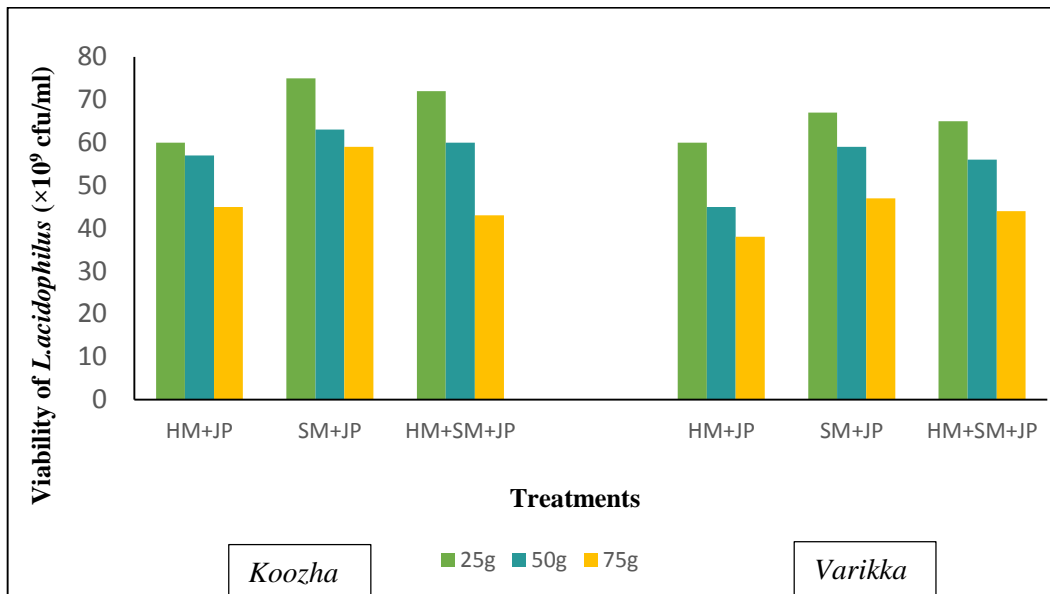


Fig.47. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different substrate concentrations

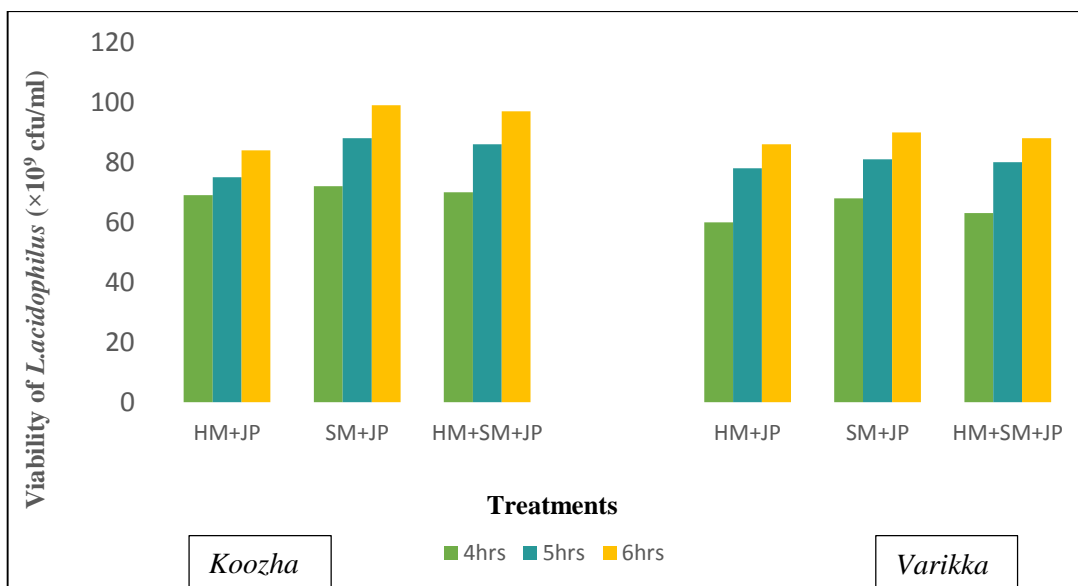


Fig.48. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different time of incubation

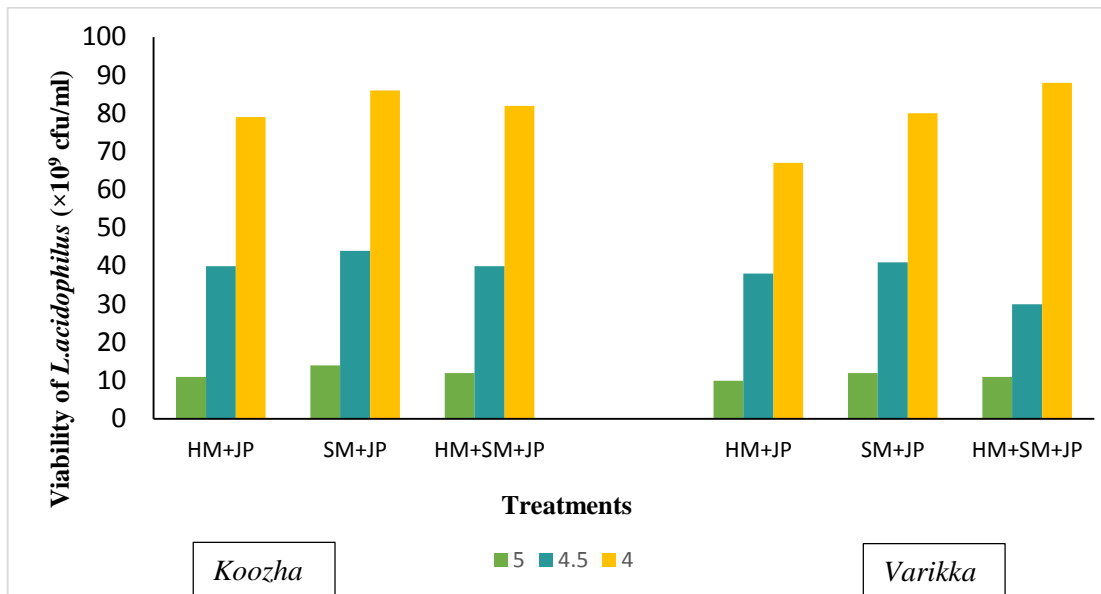


Fig.49. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different pH

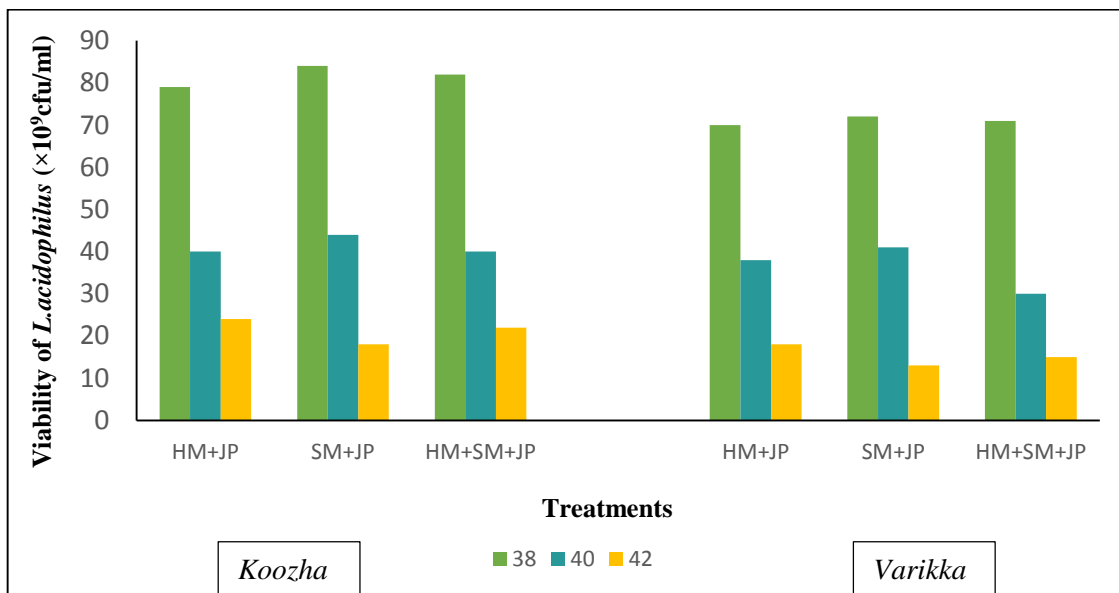


Fig.50. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at different temperatures ($^{\circ}$ C)

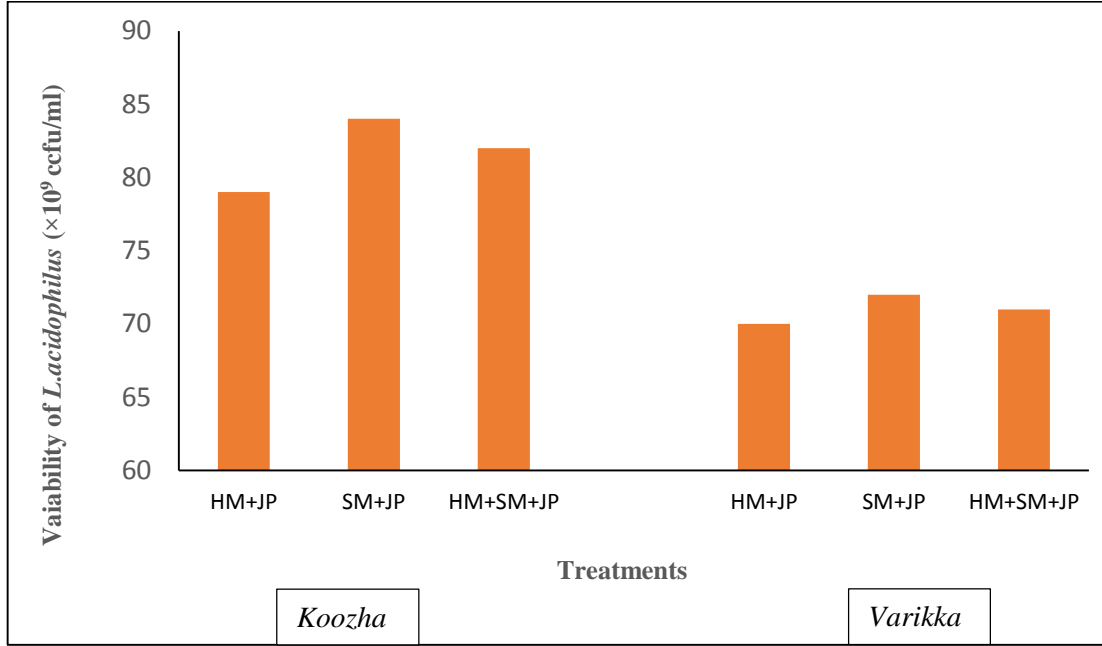


Fig.51. Viable count of *L. acidophilus* in jackfruit bio-yoghurts at 100 μ l inoculum concentration

concentration, when inoculated with 100µl of *L. acidophilus* and fermented at 38⁰C for 6hrs and the pH was 4 (Fig. 47-51).

5.9. Quality evaluation of yoghurts.

Moisture

The moisture content of the yoghurts were analysed and it was observed that moisture content was minimum for the control yoghurts of each group. The moisture contents of HM, SM and HM+SM control yoghurts were in the order of 75.29, 81.93 and 80.67 per cent respectively. A similar result was observed in the case of jackfruit based yoghurts also. Within the group of jackfruit yoghurts, the *koozha* based yoghurts were reported to have the maximum moisture content. The moisture content of all the prepared bio yoghurts were found increasing significantly during the storage (Fig. 52).

Moisture content of the fruit based yoghurts were higher than the control yoghurts may be because of the incorporation of jackfruit pulp during yoghurt preparation. The *koozha* jackfruit based yoghurts were having more moisture than the *varikka* due to the higher moisture content in *koozha* pulp. Pandey and Ukkuru (2005) reported the moisture content of *varikka* jackfruit pulp as 77.98 per cent and that of *koozha* as 79.03per cent.

The findings of the present study is in line with several other authors. Hossain *et al.* (2012) reported that the incorporation of 15 per cent strawberry juice during preparation increased the moisture content of yoghurt from 74.03 to 74.29 per cent. Matter *et al.* (2016) developed cactus pear and papaya yoghurts and the moisture content of the plain, cactus pear and papaya yoghurts were 84.21, 89 and 85.12 per cent respectively. Barakat and Hassan (2017) also reported a similar result during the moisture content analysis of plain and pumpkin incorporated yoghurts.

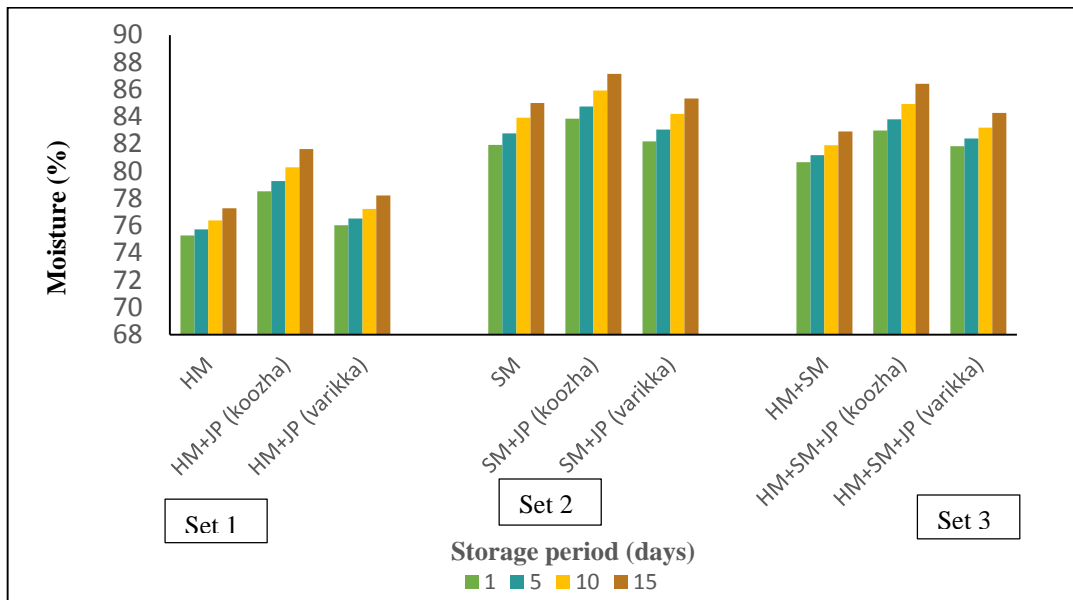


Fig.52. Moisture content of the bio-yoghurts during storage

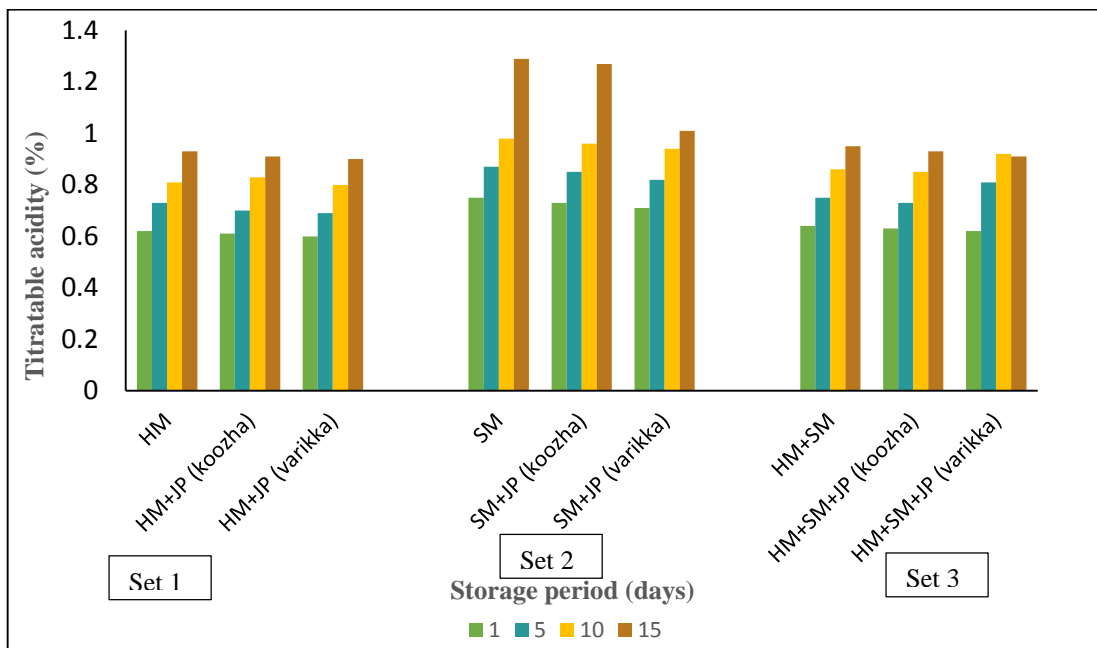


Fig.53. Acidity of bio-yoghurts on storage

Moisture content of the yoghurts were found increasing during storage, and this can be explained with the help of physical properties of yoghurt. On storage, the water holding capacity of the curd (yoghurt) decreases, which in turn resulted in increased syneresis and moisture content of the yoghurt (Andleeb *et al.*, 2008). The authors also observed a gradual increase in the moisture content of homemade as well as commercial yoghurts on storage.

Titrateable acidity

Acidity was maximum for the control bio yoghurts than the jackfruit yoghurts in each set. Acidity of the control yoghurts were 0.62, 0.75 and 0.64 per cent for HM, SM and HM+SM yoghurts. In the case of *koozha* yoghurts, the acidity were 0.61 (HM), 0.73 (SM) and 0.63 per cent (HM+SM) and for *varikka* it was 0.60 (HM), 0.71 (SM) and 0.62 per cent (HM+SM). The *koozha* based jackfruit yoghurts were having maximum acidity followed by *varikka* and the minimum for control yoghurts. On analysing the acidity of yoghurts of different milk composition, SM was the one with maximum acidity, followed by HM+SM and then HM (Fig. 53).

During fermentation, acidity increased due to the production of lactic acid by lactic acid bacteria during fermentation (Elke *et al.*, 2013). As the lactose content was more in the control yoghurts than the fruit based yoghurts, it may have led to the increased acidity in control yoghurts as more lactic acid is produced.

The plain yoghurt was more acidic than the fruit yoghurts and this result is in accordance with the findings of Ndife *et al.* (2014), who reported a low pH value of 4.32 for plain yoghurt and 4.50 for the coconut enriched yogurt. Nazni and Komathi (2014) compared the physicochemical properties of plain as well as fruit yoghurts and found out that the fruit yoghurts were less acidic (6.3) when compared with the plain yoghurts (4.50).

The nature of fruit pulp added will affect the acidity of the end product. Because of the comparable acidity of pulps of *koozha* and *varikka* jackfruits (Pandey

and Ukkuru, 2005), acidity of the *koozha* and *varikka* based bio-yoghurts were also comparable.

On comparing the acidity of HM, SM, HM+SM yoghurts, it was observed that the acidity was maximum for SM yoghurts, followed by HM+SM and then SM. In the case of *koozha* and *varikka* based bio yoghurts also, this was observed. The percentile increase of acidity on storage was also maximum in SM yoghurts. This is because milk fat has an impact on the fermentation process. The fat content affects acidity of yoghurts *i.e.* lesser the fat content more will be acidity (Sfakianakis and Tzia, 2014). The findings of the present study is in agreement with Tavakoili *et al.* (2019) who reported the pH of fat free, semi fat and full fat probiotic yoghurts in the order of 4.22, 4.3 and 4.34 respectively.

In the present study, a constant increase in acidity was observed in control yoghurt, followed by probiotic jackfruit yoghurts during storage period. This might be attributed to the utilisation of residual carbohydrate by viable microorganisms present in yoghurt and production of lactic acid. Lactic acid can affect the acidity of yoghurt. If more lactic acid is produced, an increase in acidity will be reported. Several researchers have reported different degrees of increase in acidity and decrease in pH under different storage conditions (Yeganehzad *et al.*, 2007; Akpan *et al.*, 2007; Viljeon *et al.*, 2003). Meenakshi *et al.* (2016) also reported an increase in the acidity of control, probiotic banana and probiotic sapota yoghurts.

Fat

On analysis, it was observed that the maximum fat was for HM yoghurts whereas minimum was for SM yoghurts. The HM used for preparation of yoghurts had a fat content of 3.5 per cent on the other hand it was 0.5 per cent skimmed milk. No other fat sources was added as an ingredient during yoghurt preparation. The findings of the present study is comparable with the FSSAI (2017) regulation which

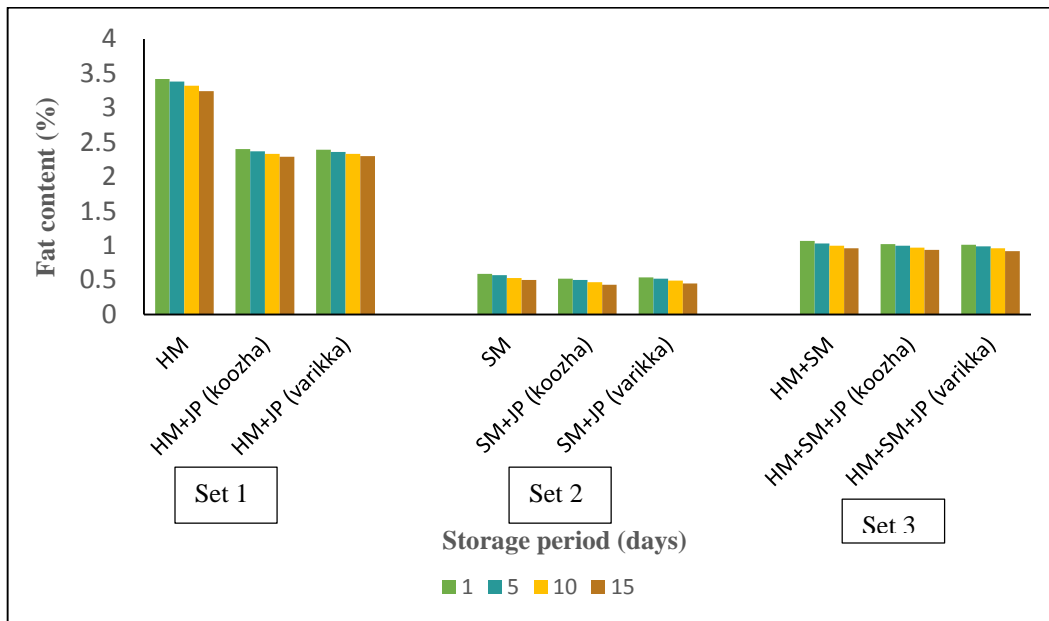


Fig.54. Fat content of bio-yoghurts on storage

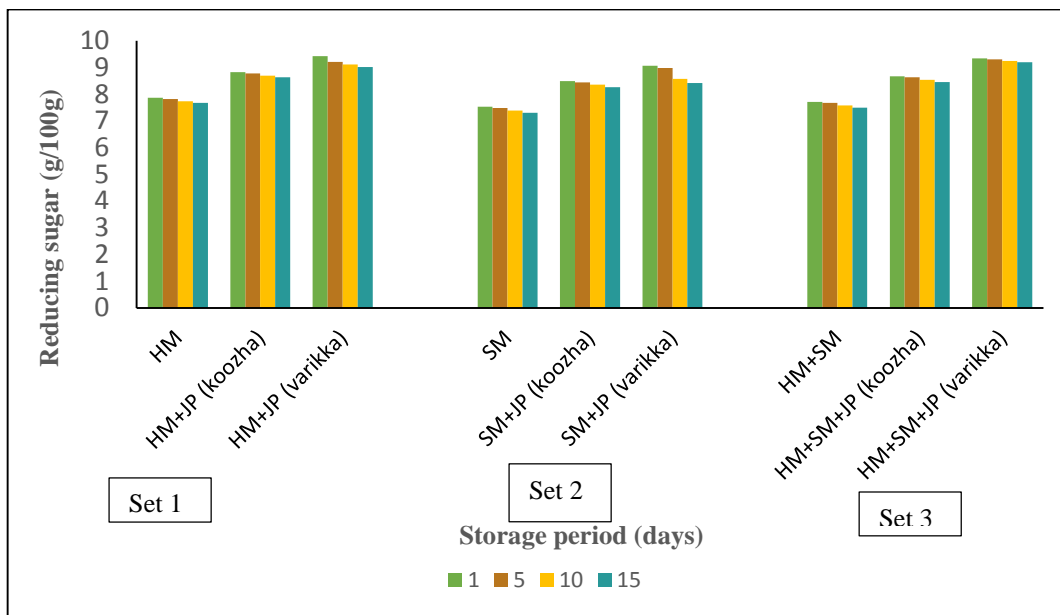


Fig.55. Reducing sugar content of bio-yoghurts on storage

specify the fat content of HM yoghurt as 3 per cent, SM yoghurt as 0.5 per cent (maximum) and partly skimmed milk in the range of 0.5-3.0 per cent (Fig. 54).

Kanchan (2016) developed low fat frozen yoghurts with the incorporation of milk of varying fat content of 0.5, 1, 1.5 and 2 per cent and fruits like banana, lychee and mango pulp. Tavakoli *et al.* (2019) also developed fat free, low fat and full fat yoghurts by adjusting the milk fat to 0, 2 and 3.5 per cent.

Reducing and total sugars

The reducing as well as total sugar content of the bio yoghurts were analysed and it was observed that both reducing and total sugar content was maximum in the case of jackfruit yoghurts and minimum for control yoghurts (Fig. 55 and 56). Again the reducing sugar was reported to be maximum in *varikka* yoghurts (9.34 g/100g in HM, 9.06 g/100g in SM and 9.42 g/100g in HM+SM) whereas the total sugar content was maximum in the *koozha* yoghurts (8.82 g/100g in HM, 8.49 g/100g in SM and 8.67 g/100g in HM+SM).

The reducing sugar content was maximum in *varikka* jackfruit bulb (5.71 g/100 g) and pulp (5.11) but the *koozha* jackfruit bulb (22.83 g/100g) and pulp (24.33 g/100g) have maximum total sugar than *varikka* variety (Pandey and Ukkuru, 2005). This may be the reason for the increased reducing and total sugar content of the jackfruit yoghurts. On the other hand milk contains lactose as the reducing sugar which is used up more efficiently by the fermenting organism than the fruit sugars. Hence, the reducing and total sugar content of the control yoghurts remained minimum.

Within the different milk based yoghurts, the SM yoghurts were found to have minimum sugar content than the HM+SM and HM. This can be correlated to the higher acidity of SM yoghurts (0.73 g/100g) than HM and HM+SM. An increase in acidity will result in the reduction of reducing and total sugar. Walia *et al.* (2013) reported a reduction in the reducing sugar content of mango soy fortified yoghurt with increasing acidity. Illias kutty (2005) reported a reducing sugar content of 3.74

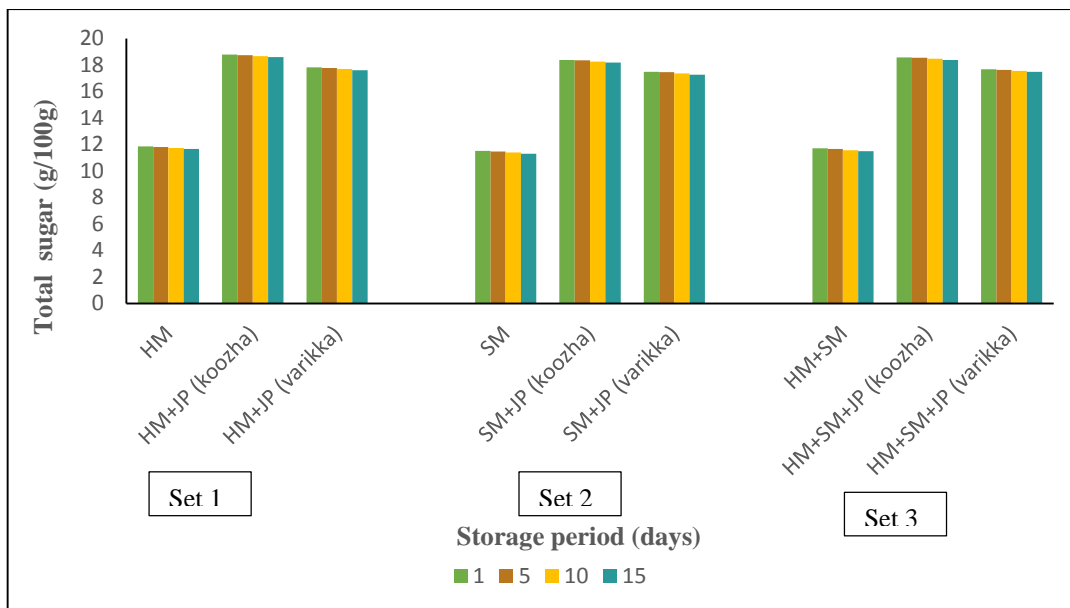


Fig.56. Total sugar content of bio-yoghurts on storage

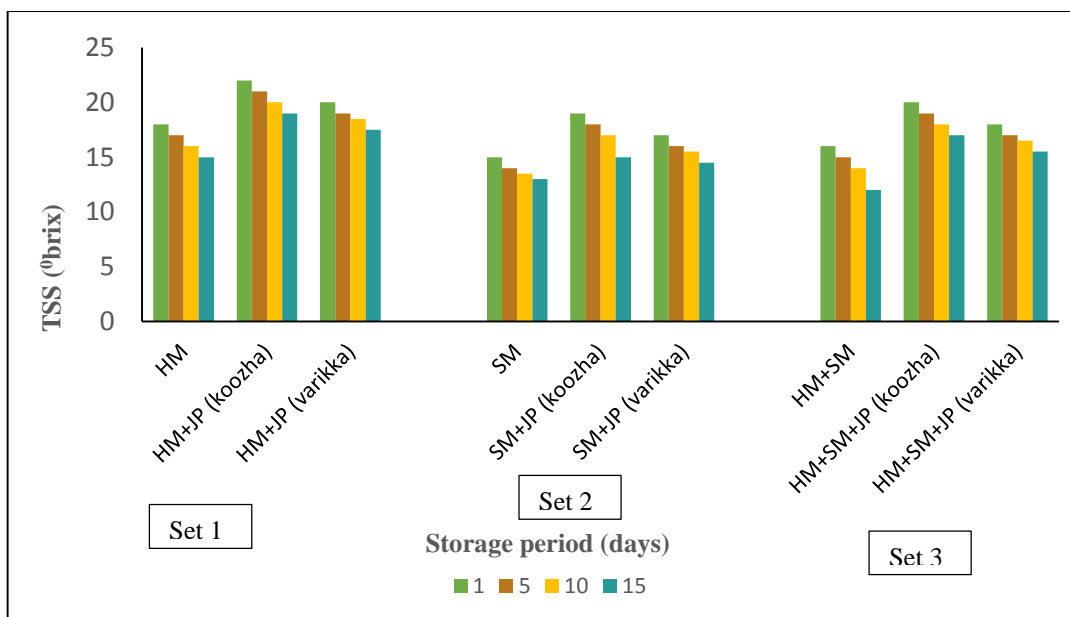


Fig.57. TSS content of bio-yoghurts on storage

g/100g and total sugar content of 13.22 g/100g in coconut yoghurt. Kale *et al.* (2008) observed the reducing and total sugar content of pomegranate enriched yoghurt as 5.40 and 11.73g/100g and that of control sample as 5.86g/100 (as control was prepared without the addition of cane sugar).

TSS

The yoghurts with HM were having the maximum TSS followed by HM+SM and minimum was seen in SM. When compared to the control yoghurts of each category, the jackfruit bio yoghurts were having more TSS. Within the jackfruit yoghurts, the maximum TSS was observed for *koozha* than the *varikka* yoghurts. Similar to that of reducing and total sugar contents, the TSS of the yoghurts were also reported to be decreasing on storage (Fig. 57).

TSS is directly related to the sugar content and as the total sugar contents were maximum in *koozha* yoghurts followed by *varikka*, the TSS also was maximum for *koozha* yoghurts. It was observed that the maximum TSS was for HM yoghurts and minimum for SM yoghurts. This can be explained on the basis of maximum moisture as well as acidity of the SM yoghurts and the minimum reducing as well as total sugars. On storage, TSS reduced because of the reduced sugar contents, increased acidity and moisture.

The results of the present study are in accordance with the results of Vasiljevic and Jelen (2002) and Wang *et al.* (2002). The authors observed a decrease in total soluble solids in yoghurts from 7.33 °brix to 6.83 °brix and 15.33 °brix to 14.93 °brix in corn milk and cow milk yoghurts respectively. It was also been reported that these reductions are due to the utilisation of sugar by the starter cultures.

Mittal and Bajwa (2012) reported a reduction in the TSS content of control as well as low calorie milk drink on storage. Kaur *et al.* (2015) developed an alovera yoghurt and the TSS observed was 13 °brix. Meenakshi *et al.* (2018) reported the TSS

of control yoghurt as 15 °brix and that of the probiotic sapota yoghurt as 16 °brix and during storage, TSS was found to decrease gradually.

Crude fibre

The control samples contained no fibre because milk is a poor source of fibre. Figure 58 describes that the crude fibre content of the *koozha* (0.52, 0.51 and 0.53 g/100g respectively in HM, SM and HM+SM) yoghurts were significantly higher than the *varikka* (0.49, 0.47 and 0.48 g/100g respectively in HM, SM and HM+SM). This is because the higher levels of crude fibre content in the *koozha* variety of jackfruit than the *varikka*. Pandaey and Ukkuru (2005) reported a crude fibre content of 1.22 g/100g in *koozha* jackfruit and 1.03g/100g in *varikka* jackfruit.

The incorporation of jackfruit pulp made the presence of fibre in yoghurts which will protect the probiotic bacteria from the harsh conditions of gastrointestinal tract so that more number of beneficial bacteria will reach the colon. The presence of fibre will enhance the fermentation process which provide health benefits to the host.

As reported by Siddappa and Bhatia (1955), the fibre content of jackfruit was 2.89 per cent while, Tojal (1975) reported 0.68 per cent fibre in jackfruit. Anon (1979) reported the fibre content of ripe jackfruit as 1.0 g/100.

The variations in composition of fibre was well explained by Samaddar and Yadav (1970) and according to them varietal difference, climatic and soil conditions may influence the chemical composition of jackfruit. Ndife *et al.* (2014) also reported an increased crude fibre content in the plain yoghurt when incorporated with 10, 20 and 30 per cent coconut cake.

Reduction in fibre content was observed throughout the storage period. This may be because the action of fermenting bacteria present in the yoghurt. Lactic acid produced during fermentation will cause hydrolysis of fibre and the presence of moisture will enhance this process. SM yoghurts were found to have minimum fibre

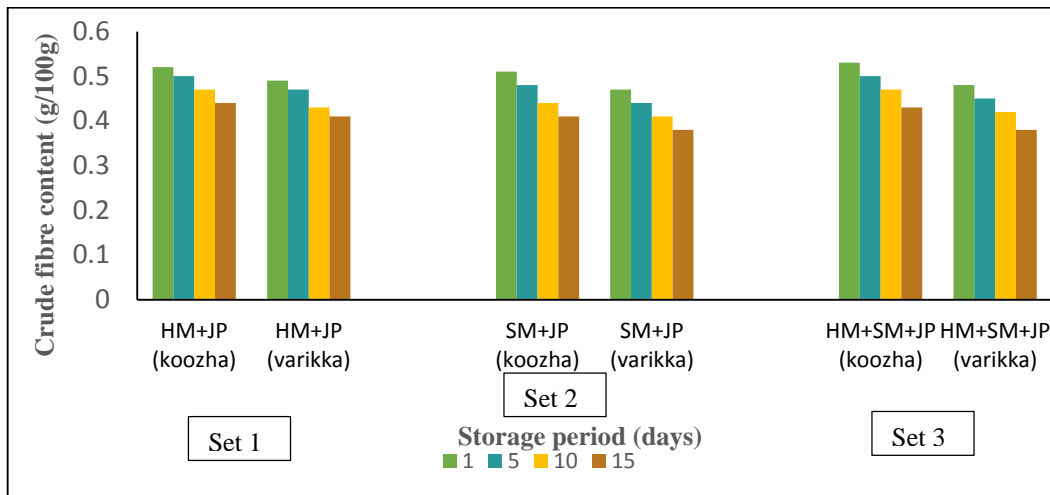


Fig.58. Crude fibre content of the jackfruit based bio-yoghurts on storage

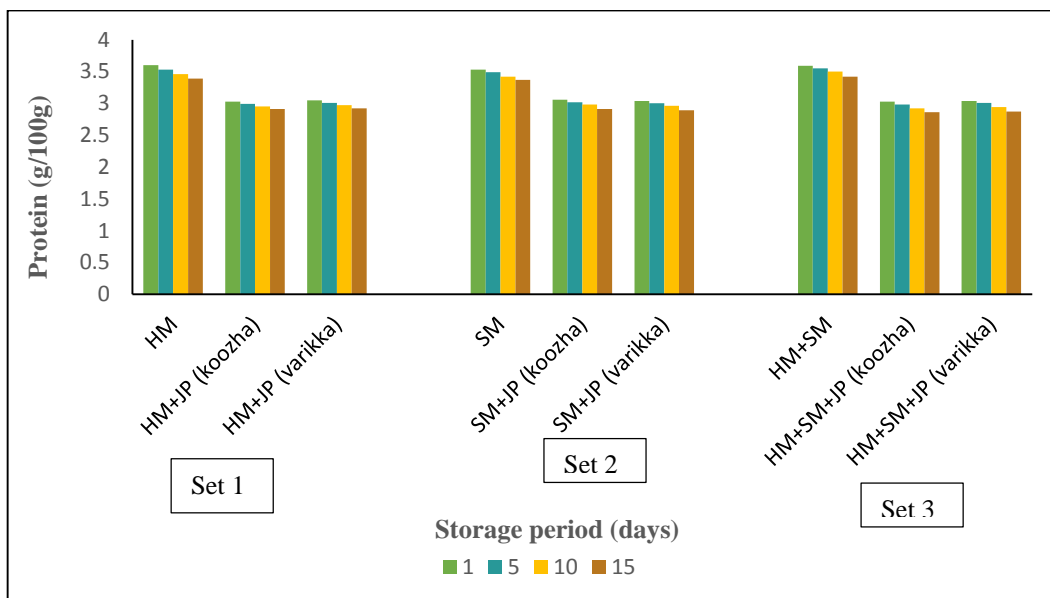


Fig.59. Protein content of probiotic bio-yoghurts on storage

contents (0.51 and 0.47 g/100g for *koozha* and *varikka* bio yoghurts) and this can also be explained with the acidity of SM yoghurts as the acidity was maximum for SM based yoghurts.

Protein

The protein content of control yoghurts were found to be maximum in the HM (3.60 g/100g) followed by HM+SM groups (3.05 g/100g). Protein content was found to be more in control yoghurt than the fruit yoghurt and this can be attributed to the incorporation of fruit pulp (Fig. 59). Milk is a good source of protein that contain 3.2g/100g whereas the ripe jackfruit contains 1.9g/100g protein (Ranasinghe *et al.*, 2019).

Roy *et al.* (2015) reported that protein content of fruit yoghurts tend to decrease with increase in the fruit content of yoghurt, and the papaya yoghurt containing 5, 10 and 15 per cent fruit pulp have a protein content of 3.76, 3.73 and 3.68 per cent respectively.

Roy *et al.* (2015) reported a protein content of 3.80 per cent in control and 3.53 per cent in 15 per cent water melon incorporated yoghurt samples. Meenakshi *et al.* (2018) reported a protein content of 3.55g/100g in plain yoghurt and 3.40g/100g in than the sapota based probiotic yoghurt.

The yoghurts with SM was found to have the minimum protein content among the control as well as jackfruit yoghurts and on storage, the protein content of prepared yoghurts were found decreasing. The results can be explained on the basis of acidity, aslactic acid hydrolyse the larger protein molecules to simpler amino acids and thereby increasing protein digestibility making it easier to be utilized by the fermenting lactic acid bacteria. Tzvetkova *et al.* (2007) supported this and reported that, during fermentation process, milk proteins are acidified by lactic acid and are hydrolyzed by proteases and peptidases from bacteria.

β carotene

The most common carotenoid of fruits and vegetables is β carotene and it can promote health when taken at dietary levels (Stahl and Sies, 1996). The β carotene content of the jackfruit yoghurts were found to be higher than the control samples and is given in Figure 60. This is because of the incorporation of jackfruit pulp which contain more β carotene than milk. Jackfruit is considered as a cheap but valuable source of β carotene. Gopalan *et al.* (1999) reported β carotene content of ripe jackfruit as 175 μg , while Hossain and Haque (1979) had reported β carotene content between 250-1740 μg and 100g. The HM, SM, HM+SM yoghurts contained 2.63, 1.80 and 2.47 $\mu\text{g}/100\text{g}$ β carotene whereas the *koozha* yoghurts contained 3.20, 3.08 and 3.11 $\mu\text{g}/100\text{g}$ β carotene. On the other hand, the β carotene content of milk varies from 0.076 $\mu\text{g}/100\text{g}$ to 0.08 $\mu\text{g}/100\text{g}$ (Strusinska *et al.*, 2010).

Among the jackfruit yoghurts, the *koozha* yoghurts were found to have more carotene than the *varikka*. This is because carotene content was found to be more in *koozha* variety (178.36 μg) compared to *varikka* (163 .66 μg).

From figure 60, it is clear that the yoghurt prepared with SM have the least β carotene content. This can be attributed to the process of skimming fat from milk. β carotene of milk is usually associated with butterfat and the removal of this butterfat during skimming process result in the substantial loss of β carotene (Musara and Nyagura, 2017).

The present study also observed a significant reduction in the β carotene content of yoghurts on storage. This decline may be due to the increased β -carotene degradation during storage as stated by Vàsquez-Caicedo *et al.*, (2007). This finding is in agreement with that of Gad *et al.*, (2015), who reported a decrease in the β carotene content of fresh yoghurt on 12 storage. The reduction observed was from 8.69 $\mu\text{g}/\text{ml}$ initially to 5.30 $\mu\text{g}/\text{ml}$ at the end of storage. Gad *et al.* (2015) opined that even in the refrigerated condition, β carotene is subjected to rapid degradation and

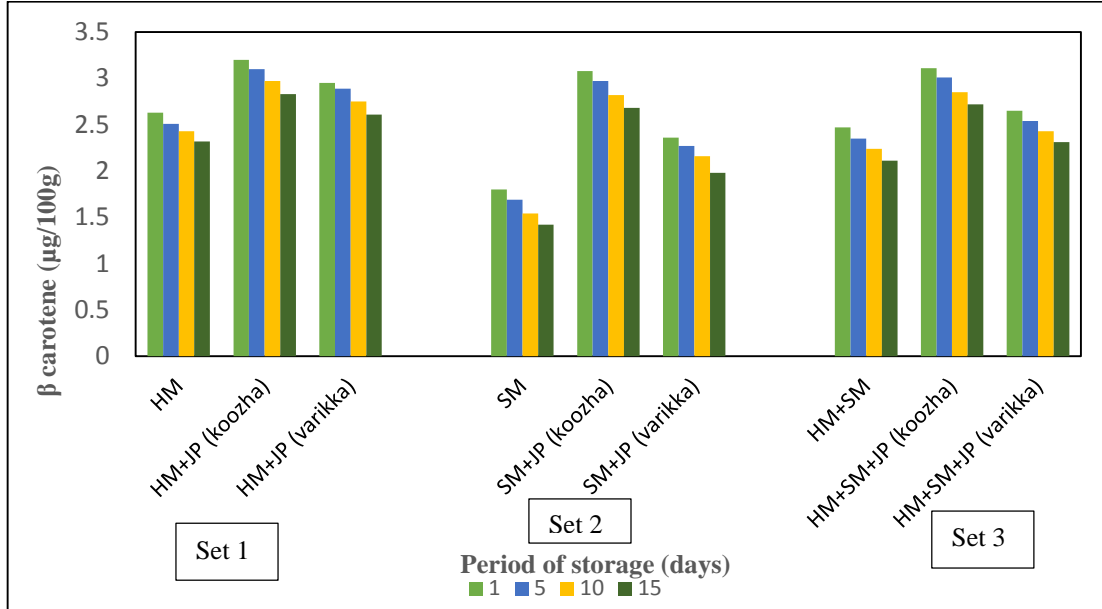


Fig.60. β carotene of probiotic bio-yoghurts on storage

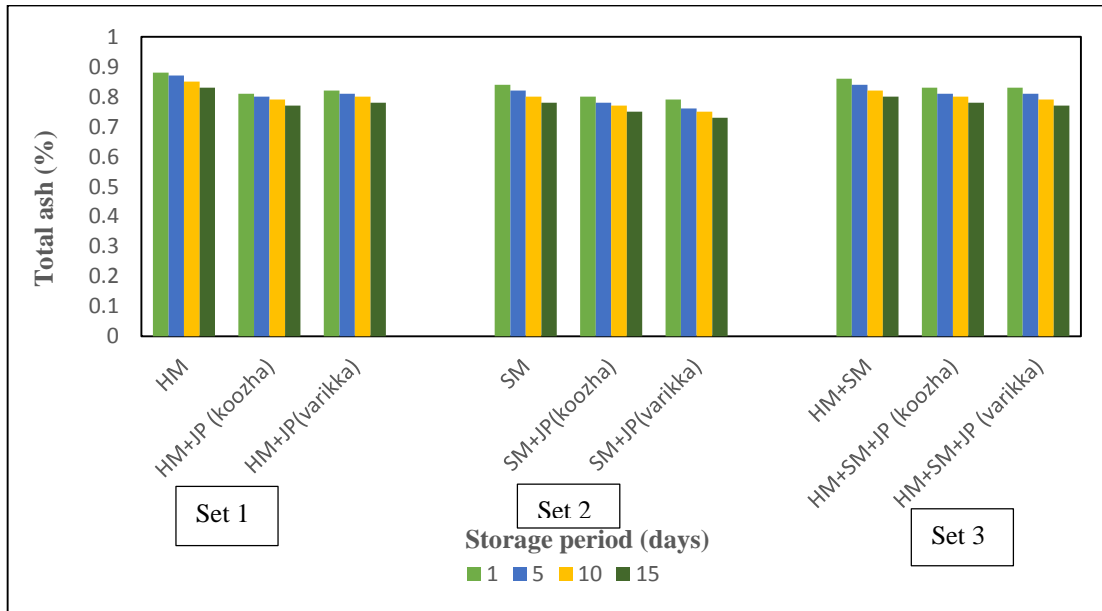


Fig.61. Total ash contents of probiotic bio-yoghurts on storage

fermenting organism present in the yoghurts may consume. to enhance the shelf stability of this vital nutrient, techniques like microencapsulation is needed.

Mineral status of the yoghurts

The total ash content was observed to be maximum in the control samples (0.88% in HM, 0.84% in SM and 0.86% in HM+SM) than the jackfruit based yoghurts. Also, the ash content of the yoghurts were in the order of HM>HM+SM>SM (Fig. 61). In a similar fashion, the calcium, iron and potassium contents were reported to be maximum in the control yoghurts than the jackfruit pulp incorporated counterparts. The calcium content of control samples were ranged from 127.38 mg/100g to 130.23mg/100 and on the other hand the calcium of jackfruit based yoghurts were found to be within the range of 97.41mg/100g to 98.93mg/100g (Fig. 62). The iron and potassium levels of the jackfruit yoghurts were found to be comparable and also the potassium content was found maximum in the jackfruit yoghurts than the control yoghurts (Fig. 63 and 64).

Ash value represents the mineral status of food. Hence higher the ash value, higher the mineral status (Trachoo and Mistry, 1998). Igbabul *et al.* (2014) reported varying ash values of commercial yoghurts from 04% to 1.26%. As the jackfruit pulp is not a good source as milk, the incorporation of jackfruit pulp made the ash value of jackfruit yoghurts to decrease. This finding is in agreement with the findings of Hossain *et al.*, (2012). They reported a decrease in the ash content of fruit based yoghurts when compared with plain. On adding 15 per cent straw berry juice, the ash content decreased from 0.71 per cent to 0.62 per cent.

Calcium content was found to be higher in the plain yoghurt whereas the iron and potassium were found to be maximum in jackfruit yoghurts. This is due to the variation in the mineral content of milk and jackfruit. Jackfruit is classified as a high potassium food and contain 370mg/100g of potassium (Aong, 2013) whereas the potassium content of cow's milk ranged from 144-178mg/100g (Zamberlin *et al.*,

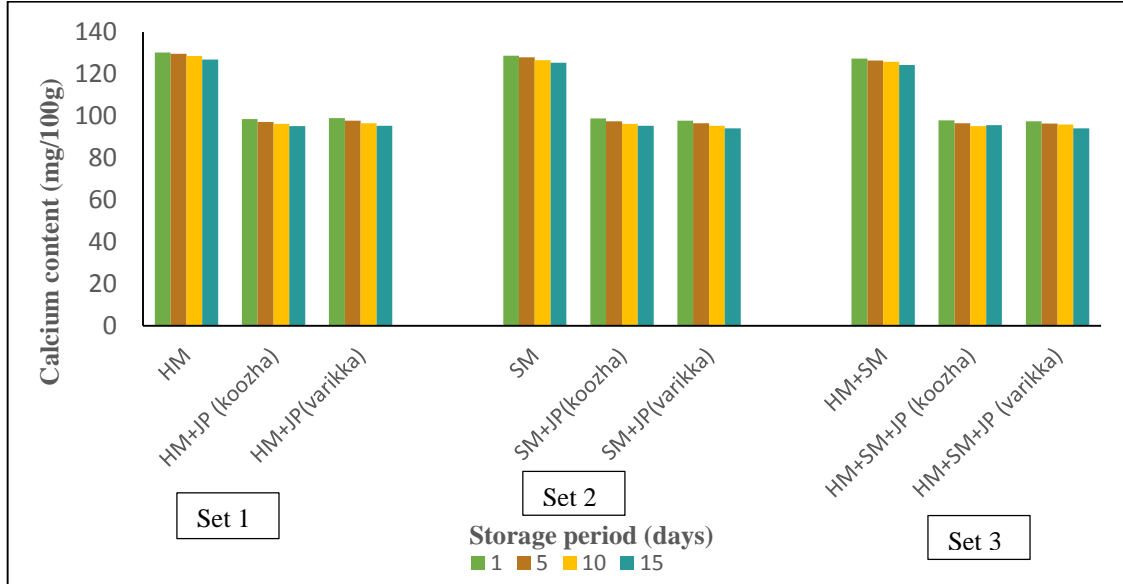


Fig.62. Calcium content of probiotic bio-yoghurts on storage

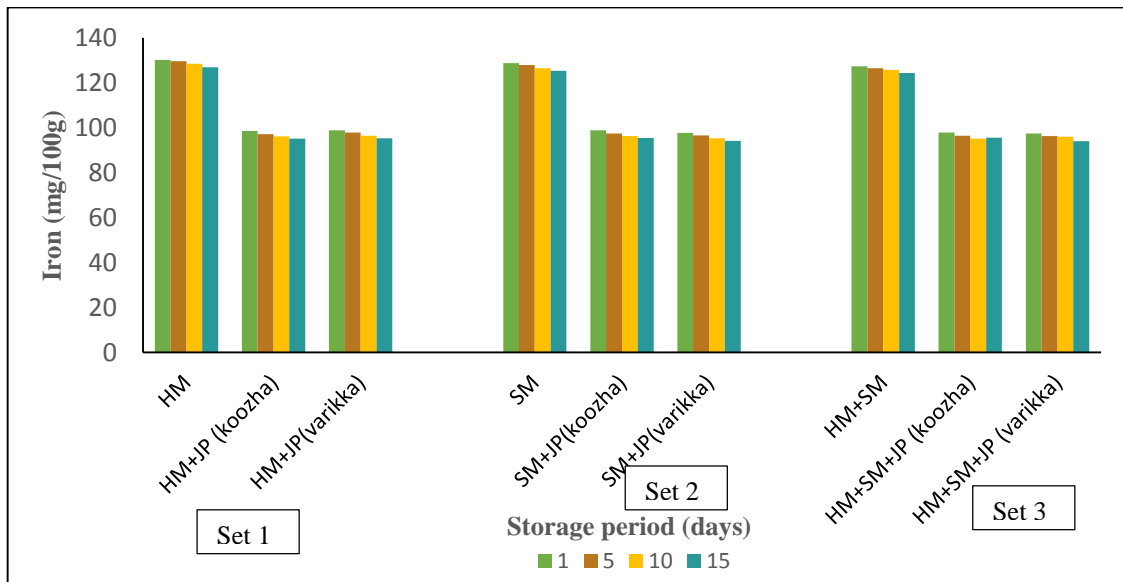


Fig.63. Iron content of probiotic bio-yoghurts on storage

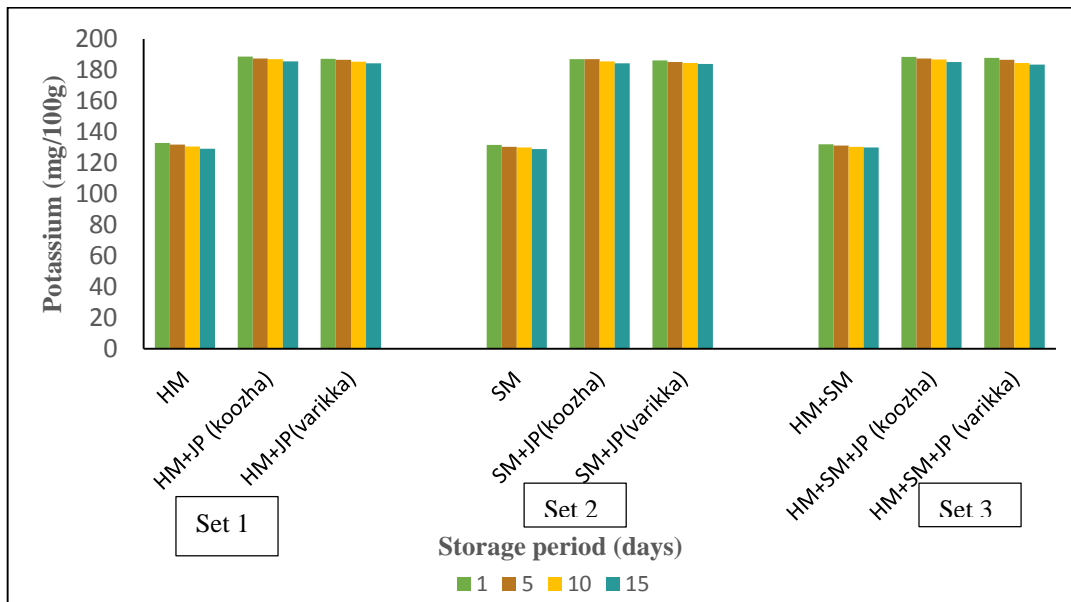


Fig.64. Potassium content of probiotic bio-yoghurts on storage

2012). The iron content of ripe jackfruit vary from 0.5 to 1.1 mg/100g (Arkroyd *et al.*, 1969) whereas the iron content of milk is only 0.07mg/100g.

Hence, the iron content was also maximum in jackfruit bio yoghurts. Even the iron and potassium content were maximum in jackfruits yogurts, the presence of relatively higher percentages of calcium along with fair amount of potassium and iron in the control yoghurt is responsible for the higher ash values of control yoghurts.

Synerisis, water holding capacity, curd tension and viscosity of bio yoghurts

The rheological properties of the bio yoghurts were assessed at five days interval throughout the storage period of 15 days. The parameters like synerisis, water holding capacity (WHC), curd tension and viscosity were assessed. All these parameters plays a crucial role in the quality of yoghurts and influence the consumer acceptance to a great extent.

The study revealed that the yoghurts prepared with HM was of superior quality, as the curd tension, water holding capacity and viscosity were maximum whereas synerisis was minimum in this group. The addition of jackfruit pulp was found to increase the curd tension, WHC and viscosity and decreases the synerisis of the yoghurts. On storage, synerisis of the yoghurts were found increasing gradually whereas curd tension, WHC and viscosity decreased (Fig. 65-68).

Yogurt is a complex gel network composed of denatured protein and milk fat globule, and the fat globules will directly influence the final strength yoghurt (Xu *et al.*, 2008). When the fat content of yoghurt decreases, a more fragile gel network structure of yoghurt forms, and this also leads to less desirable rheological properties, texture characteristics, taste, and flavor (Lobato-Calleros *et al.*, 2014). In the present study also the yoghurts with SM were found to have minimum gel strength as evident from the curd tension, WHC and synerisis of the SM yoghurts.

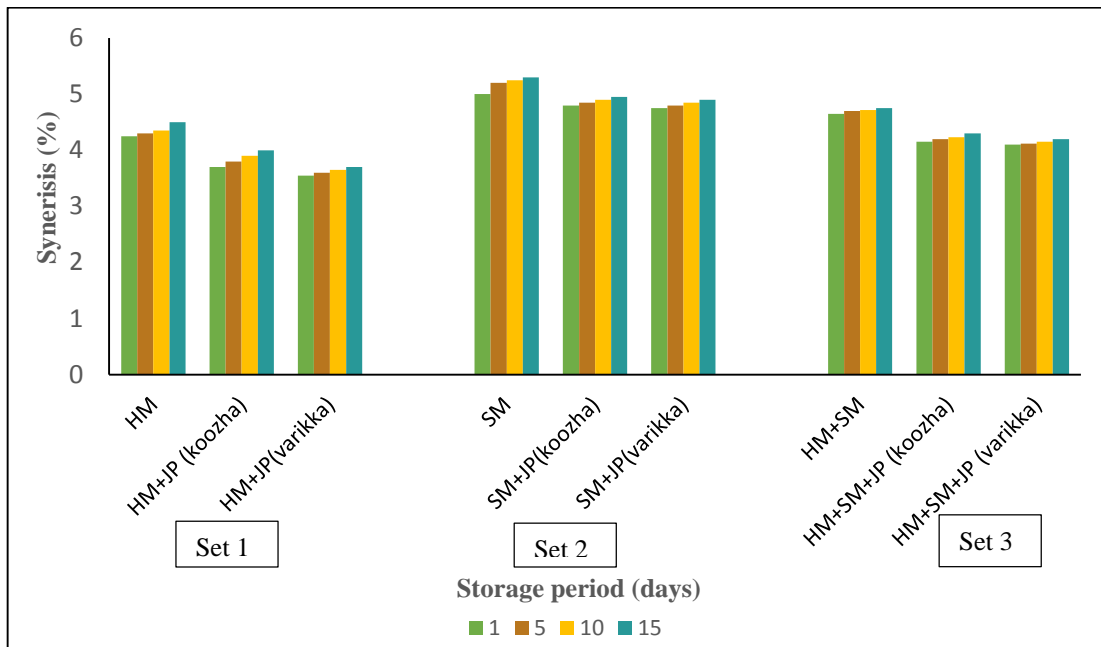


Fig.65. Synerisis of probiotic yoghurt on storage

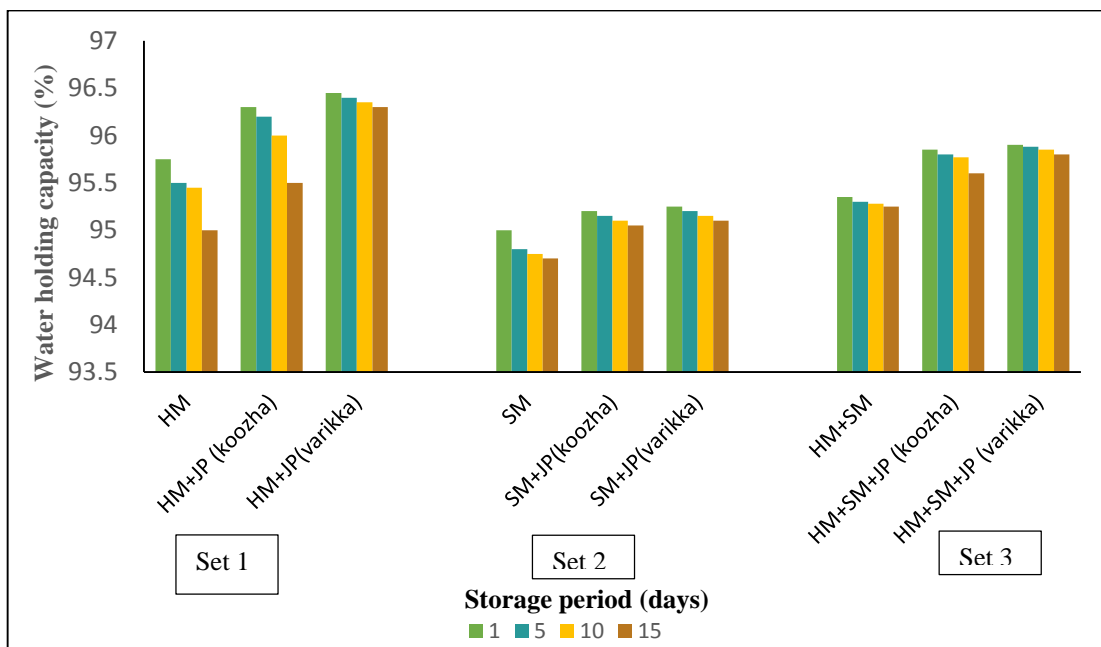


Fig.66. Water holding capacity of probiotic yoghurt on storage

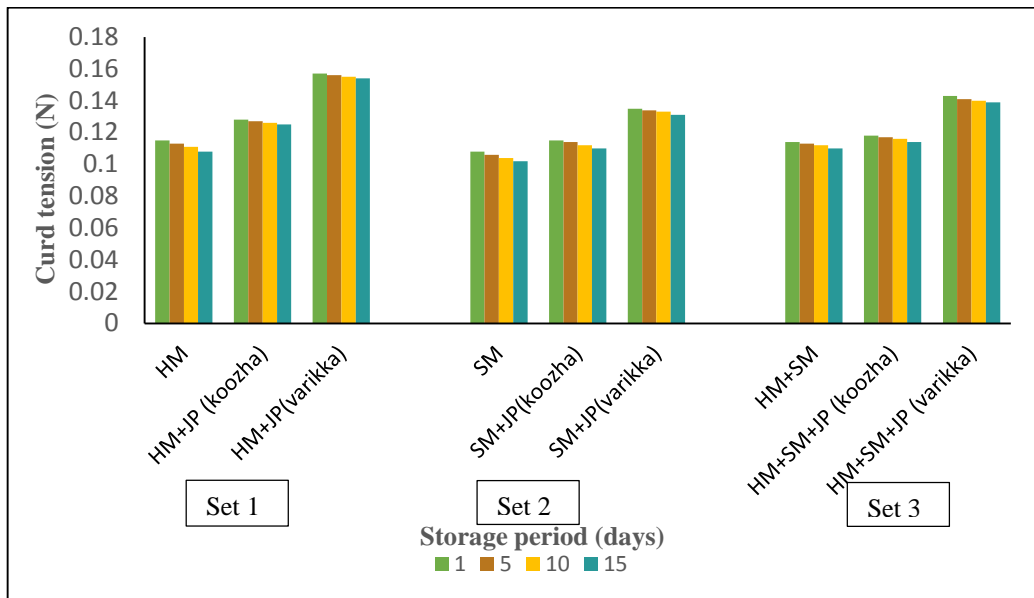


Fig.67. Curd tension of probiotic yoghurt on storage

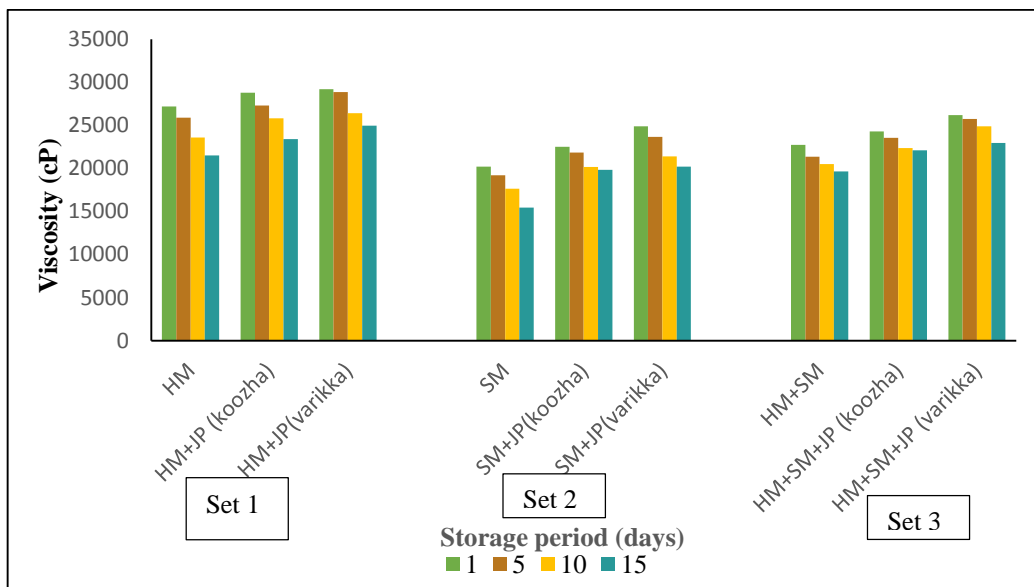


Fig.68. Viscosity of probiotic yoghurt on storage

Meenakshi *et al.*, (2015) developed fruit based banana yoghurts with the incorporation of sapota and banana pulp and the study reported an improvement in the physical properties of the fruit based probiotic yoghurts than the control sample. The syneresis of control plain yoghurt was 30% whereas that of banana and sapota yoghurts were 6.2 and 21.4% respectively. Viscosity was also improved by the addition of fruit pulp and the viscosity of plain, banana and sapota yoghurts were 4450, 11400 and 5200cP respectively.

On storage, the syneresis was increased which is an indication of reduction in the WHC and curd tension of yoghurts. Kucukoner and Tarakci(2003) evaluated syneresis in fruit flavoured yoghurts (Cornelian, Morello Chery and Rose hip marmalade, grape molasses, date pulp, and control) and the study reported a significant increase in syneresis after 6 days of storage.

Salvador and Fiszman (2004) also concluded that syneresis increased with storage time due to the shrinkage of gel during storage. Syneresis was observed to be increasing during storage of date pulp incorporated yoghurt (Kucukoner and Tarakci, 2003). Similarly, Salwa *et al.* (2004) also reported an increase in syneresis of carrot juice incorporated yoghurts (from 7.2 to 17.2 %) during 21 days of storage.

5.10. Organoleptic qualities of bio yoghurts

The Organoleptic evaluations indicated that yoghurt produced from HM was more acceptable among the judges than the SM and HM+SM yoghurts. The higher value for HM yoghurt may be due to long time familiarity with products from HM and also because of more fat content which improves the taste, appearance, texture and flavor of yoghurts. This can be supported by the report of Sahan *et al.* (2008) that most consumers of diary product are conscious of the positive impact of yoghurt from skimmed milk but sacrifice their health to taste, texture and flavour. Domagla (2009) observed that the texture characteristics of full fat milk was superior to that of

skimmed milk as well as low fat milk. This may also be a reason for the increased acceptability of HM based yoghurts over the other two.

The organoleptic scores of control yoghurts were superior to the fruit yoghurts and this result is in agreement with the findings of Dey *et al.*, (2014). In this study, yoghurts were prepared with incorporation of jackfruit juice and the overall acceptability of plain yoghurt was 8.09 whereas that of 15 per cent jackfruit juice incorporated yoghurt was 7.09. Roy *et al.*, (2015) in their study reported that the overall acceptability was higher for the control samples than the fruit juice incorporated yoghurts. The study also reported a reduction in the sensory scores of yoghurts on storage which may be due to the reduced textural properties during storage.

In the present study, the sensory scores of *varikka* yoghurts were superior to the *koozha* yoghurts and this may be due to the higher moisture content of *koozha* variety (Fig. 69 and 70). Increased moisture of fruit pulp will result in a product with increased moisture which can affect the overall textural properties of the final product. A similar result was reported by Roy *et al.* (2015) that the sensory scores of 15 percent papaya incorporated probiotic yoghurts were 8.46 whereas that of a yoghurt with 15 per cent watermelon incorporated yoghurt was 7.09. Similarly Meenakshi *et al.* (2015) reported a mean score of 8.60 for 15 per cent banana pulp incorporated yoghurt and 7.80 for the yoghurt with 15 per cent incorporation of sapota pulp.

5.11. Viability of *L. acidophilus*

The viability of *L. acidophilus* were found to be maximum in the control yoghurts than the fruit based yoghurts. The lactose in milk favours the growth of lactic acid bacteria like *L. acidophilus*. Replacement of 30 per cent milk by jackfruit pulp made a reduction in the lactose content which might have contributed to the lesser number of viable cells in the jackfruit yoghurts (Fig. 71). Meenakshi *et al.*

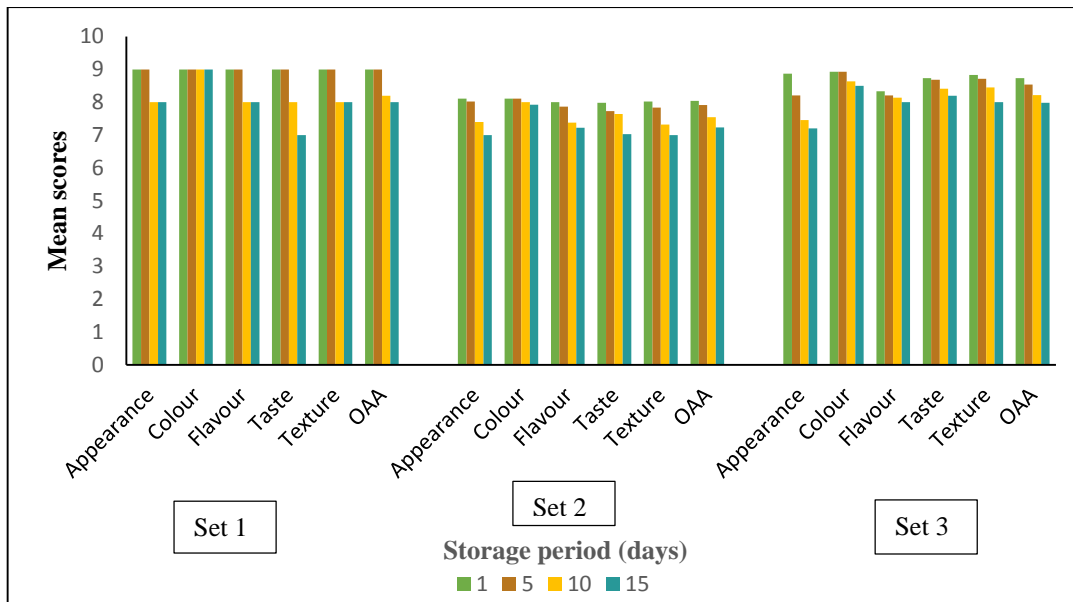


Fig. 69. Mean scores of organoleptic qualities of *koozha* based bio-yoghurts on storage

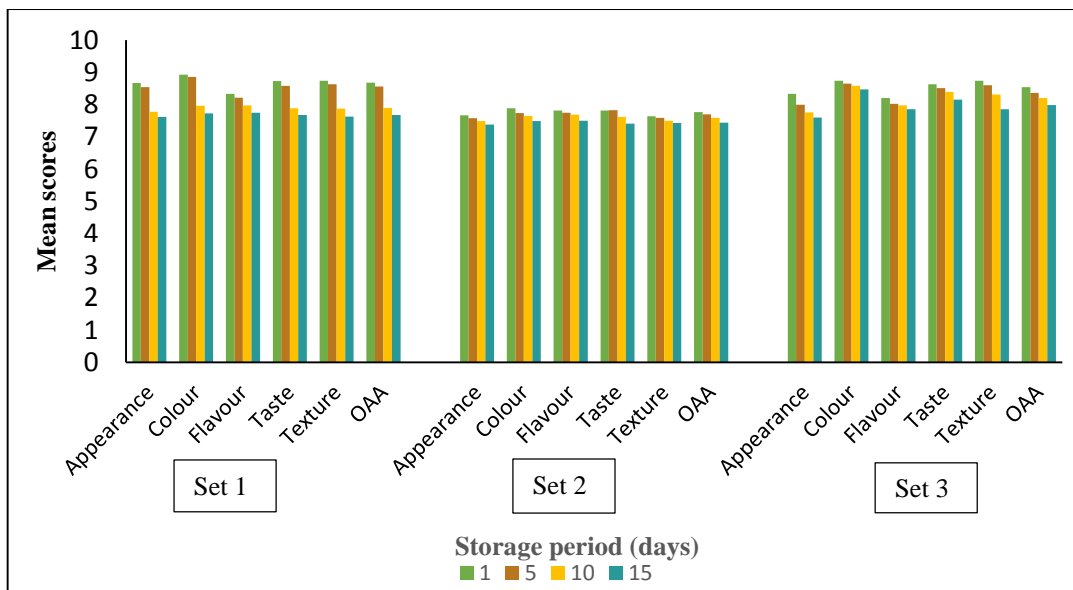


Fig. 70. Mean scores of organoleptic qualities of *varikka* based bio-yoghurts on storage

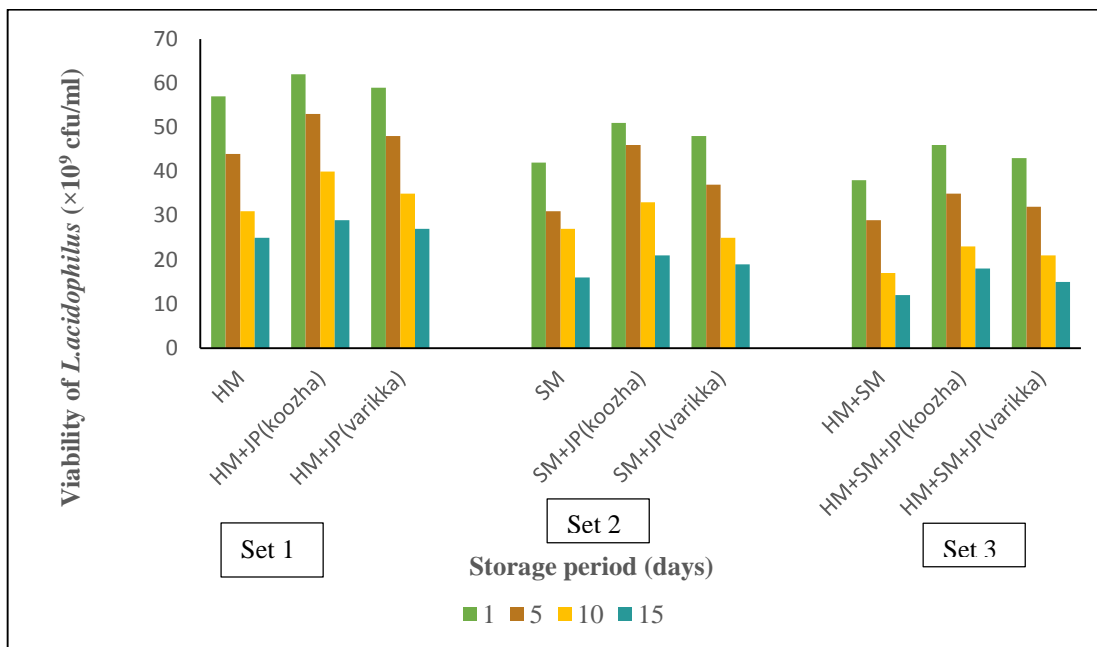


Fig.71. Viability of *L. acidophilus* in the bio-yoghurts during storage

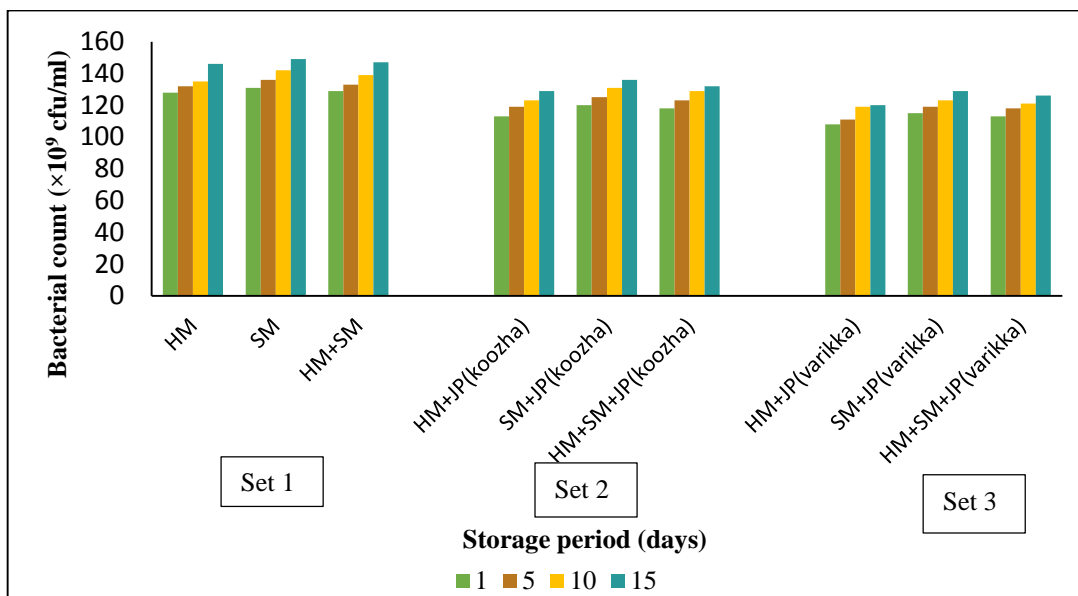


Fig.72. Total bacterial count in the bio-yoghurts during storage

(2015) reported the probiotic count in control yoghurts as 57×10^{12} cfu/ml whereas probiotic banana and sapaota yoghurts were reported to have 42 and 34×10^{12} cfu/ml respectively.

The probiotic activity was found maximum in the *koozha* based yogurt than the *varikka* based and this may be due to the higher fibre content of *koozha* variety than the *varikka* (Pandey, and Ukkuru 2005). Fibre act as a prebiotic for the growth of probiotic organism. This result is in line with that of Hassani *et al.* (2017) who reported an increase in the probiotic count of *L. acidophilus* with increase in the barley bran content. The probiotic viability was 7.22log cfu/ml on 0.3per cent barley bran which increased to 7.77 log cfu/ml when the barley bran was increased to 1.2 per cent.

The probiotic viability was found maximum in the SM based yoghurts. A similar result was reported by Tavakoli *et al.* (2019), on observing the survival of *L.acidophilus* in full fat, semi fat and low fat yoghurts were assessed. The initial bacterial count of full,semi and low fat yoghurts were 7.10, 7.50 and 7.92log cfu/ml respectively and the study reported a decrease in the probiotic activity during storage.

5.12 Microbial evaluation of yoghurts

Total bacterial count of the probiotic yoghurts are given in Figure 72. The microbial studies suggested no fungal colonies in the yogurts and minimum (1×10^2) yeast colonies in the stored product. This is in agreement with the guidelines of FSSAI 2016 for fermented milk products. No insects were found in the yoghurt samples throughout the storage period.

5.13. Cost of production

The cost of production of the probiotic food mixture was Rs 260/kg. The cost was increased because of the freeze drying process. The cost is on higher side since it involves the process of freeze drying. However, compared to other freeze dried

products available in the market, the price of the prepared food mixture is more competitive and comparable. The cost of 37 g of freeze dried raspberry powder is 443.91 rupee and that of a freeze dried dragon fruit powder is Rs 1218/kg (Anon., 2019).

The cost of production of instant shake mix was 138.54/100g. The cost of this product is on the lower side when compared with that of commercially available products. The cost of commercially available probiotic smoothie mix is more than 712.46 Rs for a 200g packet (Anon., 2019). The cost of production of bio-yoghurt with the incorporation of jackfruit pulp was 18.56 -19.56 Rs/100 ml. The cost of a commercially packed yoghurt ranges from 27-40 RS/100 ml (Anon., 2019).



Summary

6. SUMMARY

The study entitled 'Process optimisation and quality evaluation of jackfruit based probiotic food products' was carried out with the objective of developing different food mixtures with raw jackfruit flour and yoghurt with ripe jackfruit pulp involving the probiotic fermentation with *L.acidophilus* MTCC 10307. The study was also aimed at developing an instant shake mix with probiotic fermented jackfruit food mixture and to evaluate the nutritional, organoleptic and shelf life qualities of the developed products.

Probiotic food mixtures were developed with the incorporation of raw jackfruit flour, defatted soya flour, jackfruit seed flour, tomato and papaya in various proportions. The proportion of ingredients were standardised with four sets of treatments and from each set, one food mixture with maximum organoleptic scores were selected. The experiment was repeated for both *koozha* and *varikka* varieties. The food mixture containing 60 per cent raw jackfruit flour was selected from set 1 and 2, whereas food mixture containing 50 per cent raw jackfruit flour and 20 per cent jackfruit seed flour was selected from set 3 and 4.

Theses food mixtures were then optimised for maximising the growth of *L. acidophilus* MTCC 10307. Fifty grams of the food mixture (pH 4.5) fermented with 300 µl of inoculum at 37 °C for 24 hours gave the maximum viable count of *L. acidophilus* ranging from 10.90 to 10.94 log cfu/g. All the fermented food mixtures were freeze dried along with their respective control samples, powdered and packed in laminated polyethylene pouches and stored for a period of six months under the ambient conditions.

The physico chemical evaluation revealed that titratable acidity (2.32 to 2.96 %), protein (22.84 to 25.16 g/100g), thiamine (0.064 to 0.090 µg/100g), riboflavin (0.048 to 0.088 µg/100g), *in vitro* starch digestibility (79.89 to 81.94 %) and *in vitro* protein digestibility (77.49 to 83.83 %) were significantly higher in the fermented food mixtures when compared with the unfermented samples. The probiotic count of the food mixtures ranged from 10.85 to 10.90 log cfu/g. On

storage, physico chemical properties like moisture, titratable acidity, TSS, reducing sugars, total sugars and *in vitro* digestibility of starch as well as protein of the fermented and unfermented food mixtures were found increasing. On the other side, constituents like protein, β carotene, crude fibre, starch, total ash, minerals (calcium, iron and potassium) and vitamins like thiamine and riboflavin were found decreasing in both the fermented and unfermented food mixtures on storage.

The developed fermented food mixtures were shelf stable upto six months. No evidence of fungal infection and other insect infestation were found during the storage period. The food mixtures from both the *koozha* and *varikka* varieties were able to maintain the probiotic viability throughout the storage period.

Based on the nutritive, sensory and probiotic viability, the food mixtures with 50 per cent raw jackfruit flour, 20 per cent defatted soya flour, 20 per cent jackfruit seed flour and 10 per cent tomato pulp (JF+DSF+JSF+T) were selected from both *koozha* and *varikka* varieties for further analysis.

Glycemic index of the selected food mixtures (both fermented and unfermented) were assessed in ten non diabetic individuals. The food mixture were given to the individuals and their blood glucose response after the ingestion of food mixtures were recorded and compared with 50 grams of standard glucose. The fermented and unfermented food mixtures were found to have low glycemic indices. The glycemic index observed for the fermented food mixtures were 45.35 for *koozha* and 47.99 for *varikka*. The glycemic index of unfermented food mixtures were 51.86 and 54.85 for *koozha* and *varikka* respectively.

Using the best probiotic food mixture (JF+DSF+JSF+T) from *koozha* and *varikka* varieties, two instant shake mixes were prepared and standardised. The mostly accepted shake mixes (T₄) contain 50 per cent fermented food mixtures and 50 per cent skimmed milk powder along with other ingredients (sugar, nuts and spices). The shake mixes were packed in laminated polyethylene pouches for a period of two months and the quality (nutritional, organoleptic and shelf life) aspects were analysed at 15 days interval.

The moisture content of the *koozha* and *varikka* based shake mixes were 2.53 and 2.5 per cent respectively. The protein contents were 26.30 and 26.67 g/100g. The TSS content ranged from 16.24 (initial) to 16.37⁰brix (final) in *koozha* variety and 16.39 (initial) to 16.55⁰brix (final) in *varikka* variety. Similar to that of the fermented food mixtures, nutrients like protein, crude fibre, total ash, vitamins and minerals were found decreasing during storage. Both the jackfruit shake mixes contain considerable amount of ash. During the analysis of the instant shake mix, it was noticed that it contain fair amount of calcium (0.57 mg/100g in both varieties) and potassium (0.68 and 0.70 mg/100g in *koozha* and *varikka*) but iron was comparatively low. On analysing the B vitamins, riboflavin was detected and thiamine was not detected in the shake mixes. On storage the nutrients undergoes decomposition and hence there was decrease in the riboflavin content. The *in vitro* digestibility of both starch and protein increased during storage.

Both the shake mixes were nutritionally and organoleptically acceptable without the presence of fungi, yeast and insect infestation throughout the storage period. The probiotic count of the developed instant shake mixes varied from 10.14 to 10.19 log cfu/g and it maintained the probiotic viability throughout the storage period.

Jackfruit incorporated probiotic yoghurts were standardised and the yoghurt with 30 per cent jackfruit pulp incorporation was found to be the most acceptable. Yoghurts were prepared using homogenised milk (HM), skimmed milk (SM) and a combination of both (HM+SM). In the selected combination of yoghurts of both *koozha* and *varikka* variety, the conditions for the growth of *L. acidophilus* were maximized.

Twenty five grams of the yoghurt, fermented with 100 µl of inoculum at 38⁰C (pH 4.5) gave the maximum total viable count of *L. acidophilus* ranging from 10.84 to 10.92 log cfu/g. Using the jackfruit pulp of both *koozha* and *varikka* variety, bio-yoghurts were developed at the optimum conditions and stored for a period of 15 days under refrigerated condition. The quality aspects (physico

chemical, organoleptic and shelf life) were studied at five days interval for a period of 15 days

The moisture content of the yoghurts were analysed and it was observed that moisture content was minimum for the control yoghurts of each group. The moisture contents of HM, SM and HM+SM control yoghurts were in the order of 81.63, 78.23 and 77.28 per cent respectively. A similar result was observed in the case of jackfruit based yoghurts also. Within the group jackfruit yoghurts, the *koozha* based yoghurts were reported to have the maximum moisture content. The moisture content of all the prepared bio-yoghurts were found increasing significantly during the storage.

Acidity was maximum for the control bio yoghurts than the jackfruit yoghurts of each set. Acidity of the control yoghurts were 0.70, 0.73 and 0.72 per cent for HM, SM and HM+SM yoghurts respectively. In the case of *koozha* yoghurts, the acidity were 0.62 (HM), 0.65 (SM) and 0.64 (HM+SM) per cent and for *varikka* it was 0.61 (HM), 0.63 (SM) and 0.62(HM+SM) per cent. The *koozha* based jackfruit yoghurts were having maximum acidity followed by *varikka* and the minimum for control yoghurts. On analysing the acidity of yoghurts of different milk composition, SM was the one with maximum acidity, followed by HM+SM and then HM.

On analysing the fat content, it was observed that the maximum fat was for HM yoghurts whereas minimum was for SM yoghurts. As there was no added fat other than milk as an ingredient during yoghurt preparation, the fat content of prepared yoghurts were containing fat similar to milk.

The reducing as well as total sugar content of the bio yoghurts were analysed and it was observed that both reducing and total sugar content was maximum in the case of jackfruit yoghurts and minimum for control yoghurts. The reducing sugar was reported to be maximum in *varikka* yoghurts (9.34 g/100g in HM, 9.06 g/100g in SM and 9.42 g/100g in HM+SM) whereas the total sugar content was maximum in the *koozha* yoghurts (8.82 g/100g in HM, 8.49 g/100g in SM and 8.67 g/100g in HM+SM).

The yoghurts with HM were having the maximum TSS followed by HM+SM and minimum was seen in SM. When compared to the control yoghurts of each category, the jackfruit bio yoghurts were having more TSS. Within the jackfruit yoghurts, the maximum was observed for *koozha* then the *varikka* yoghurts. Similar to that of reducing and total sugar contents, the TSS of the yoghurts were also reported to be decreasing on storage.

The crude fibre content of the *koozha* (0.52, 0.51 and 0.53 g/100g in HM, SM and HM+SM respectively) yoghurts were significantly higher than the *varikka* (0.49, 0.47 and 0.48 g/100g in HM, SM and HM+SM respectively). The protein content of control yoghurts were found to be maximum in the HM and HM+SM groups (3.60 and 3.05 g/100g respectively). The protein content of SM based control was on par with that of jackfruit based bio yoghurts. Protein content was found to be more in control yoghurt than the fruit yoghurt. Among the jackfruit yoghurts, the *koozha* yoghurts were found to have more β carotene than the *varikka*.

The total ash content was observed to be maximum in the control samples (0.88 % in HM, 0.84 % in SM and 0.86 % in HM+SM) than the jackfruit based yoghurts. Also, the ash content of the yoghurts were in the order of HM > HM+SM > SM. In a similar fashion, the calcium, iron and potassium contents were reported to be maximum in the control yoghurts than their jackfruit pulp incorporated counterparts. The iron and potassium levels of the jackfruit yoghurts were found to be comparable and also the potassium content was found maximum in the jackfruit yoghurts than the control yoghurts.

The rheological properties of the bio yoghurts were assessed at five days interval throughout the storage period of 15 days. The parameters like syneresis, water holding capacity (WHC), curd tension and viscosity were assessed.

The study revealed that the yoghurts prepared with HM was of superior quality as the curd tension, water holding capacity and viscosity were maximum whereas syneresis was minimum. The addition of jackfruit pulp was found to increase the curd tension, water holding capacity (WHC), viscosity of the yoghurts

and the synerisis was found reducing. On storage, synerisis of the yoghurts were found increasing gradually whereas curd tension, WHC and viscosity decreased.

The organoleptic evaluations indicated that yoghurt produced from HM was more acceptable among the judges than the SM or HM+SM yoghurts. The higher value for HM yoghurt may due to long time familiarity with products from HM and because fat improves the taste, appearance, texture and flavor of yoghurts. In the present study, the sensory scores of *varikka* yoghurts were superior to the *koozha* yoghurts and this may be due to the lesser moisture content of *varikka* variety. Increased moisture of fruit pulp will result in a product with increased moisture which can affect the overall textural properties of the final product.

The viability of *L. acidophilus* was maximum in the SM based yoghurts followed by HM+SM and HM yoghurts of all the three sets. The initial bacterial count of SM control yoghurts were 10.79 and that of HM+SM and SM were 10.77 and 10.75 log cfu/ml. During storage the number of bacteria was found decreasing and on 15th day of storage, the counts were 10.46, 10.43 and 10.40 log cfu/ml for SM, HM+SM and HM respectively. Similarly in the jackfruit based yoghurts also, the SM based yoghurts were found to have maximum number of probiotic organism followed by HM+SM and HM. Among the jackfruit based yoghurts, the maximum probiotic viability was observed in the *koozha* based yoghurts. The number of viable cells of *L. acidophilus* of *koozha* yoghurts varied from 10.62 to 10.71 log cfu/ml and that of *varikka* it varied from 10.58 to 10.66 log cfu/ml.

The cost of production for the selected jackfruit based probiotic fermented food products (probiotic food mixture, probiotic instant shake mix and probiotic yoghurt) were calculated for 100 g. The production cost of probiotic food mixture (JF+DSF+JSF+P) containing 50 per cent Jackfruit flour, 25 per cent defatted soya flour and 10 per cent was found to be 260 Rs/100g and that of probiotic instant shake mix was (138.54 Rs/100g). Among the prepared jackfruit probiotic products, cost of the production of probiotic yoghurt (18.56-19.56 Rs/100g) was observed to be lowest.

The study revealed that jackfruit can be a suitable substrate for probiotic fermentation and the probiotic food mixtures, instant shake mixes and probiotic yoghurt can be successfully developed. Further research can be done for the development of innovative probiotic products from jackfruit.



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Appendices

APPENDIX – I

Score card for the organoleptic evaluation of jackfruit based food mixtures

Name:

Date:

Signature

Parameters	Treatments			
	T ₁	T ₂	T ₃	T ₄
Appearance				
Colour				
Flavour				
Texture				
Taste				
Overall acceptability				

9 point hedonic scale

Like extremely	- 9	Dislike slightly	- 4
Like very much	- 8	Dislike moderately	- 3
Like moderately	- 7	Dislike very much	- 2
Like slightly	- 6	Dislike extremely	- 1
Neither like or dislike	- 5		

APPENDIX – II

Score card for the organoleptic evaluation of jackfruit based instant probiotic shake mixes

Name:

Date:

Signature

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Appearance				
Colour				
Flavour				
Texture				
Taste				
Overall acceptability				

9 point hedonic scale

- | | | | |
|-------------------------|-----|--------------------|-----|
| Like extremely | - 9 | Dislike slightly | - 4 |
| Like very much | - 8 | Dislike moderately | - 3 |
| Like moderately | - 7 | Dislike very much | - 2 |
| Like slightly | - 6 | Dislike extremely | - 1 |
| Neither like or dislike | - 5 | | |

APPENDIX – III

Score card for the organoleptic evaluation of jackfruit based bio-yoghurts

Name:

Date:

Signature

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Appearance				
Colour				
Flavour				
Texture				
Taste				
Overall acceptability				

9 point hedonic scale

Like extremely	- 9	Dislike slightly	- 4
Like very much	- 8	Dislike moderately	- 3
Like moderately	- 7	Dislike very much	- 2
Like slightly	- 6	Dislike extremely	- 1
Neither like or dislike	- 5		

APPENDIX IV

Weekly temperature and relative humidity of the storage room during storage

Week	Dry bulb (°C)	Wet bulb (°C)	RH (%)
1	31.00	27.00	73.2
2	31.5	27.5	73.4
3	31	25	61.3
4	31.00	26.00	67.1
5	32.00	28.40	76.1
6	31	26	67.1
7	30.5	27.5	79.3
8	30	27	79.1
9	31.50	26.00	64.5
10	30.00	27.00	67.4
11	31.5	26.5	79.1
12	31	27	73.2
13	32.50	28.00	70.9
14	32.00	25.00	56.5
15	31.5	28.5	79.7
16	31	25	61.3
17	31.00	25.50	64.2

18	31.50	26.00	64.5
19	30	27	79.1
20	31.5	26.5	67.4
21	31.50	25.50	61.6
22	32.50	27.50	68
23	32	28	73.7
24	30	27	79.1
25	31	25	61.3

**PROCESS OPTIMISATION AND QUALITY
EVALUATION OF JACKFRUIT BASED
PROBIOTIC FOOD PRODUCTS**

**By
REMYA P.R.**

**ABSTRACT OF THE THESIS
Submitted in partial fulfilment of the
requirement for the degree of**

**Doctor of Philosophy in Community Science
(FOOD SCIENCE AND NUTRITION)
Faculty of Agriculture
Kerala Agricultural University**



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ABSTRACT

The concept of food has changed from its basic definition of satisfying hunger and nourishing the body, to health maintenance and prevention of diseases. Probiotics are one among such foods. The incorporation of probiotics to locally available foods may help to develop its nutritional profile and therapeutic value. Hence, the study entitled “Process optimisation and quality evaluation of jackfruit based probiotic food products” was undertaken with the objective of standardising probiotic food mixtures with raw jackfruit flour, instant shake mixes with the probiotic food mixture, probiotic yoghurt with ripe jackfruit and also to evaluate the nutritional, organoleptic and shelf life qualities of these developed food products.

Probiotic food mixtures were developed with the incorporation of raw jackfruit flour, defatted soya flour, jackfruit seed flour, tomato and papaya in various proportions. The proportion of ingredients were standardised with four sets of treatments and from each set, one food mixture with maximum organoleptic scores were selected. The experiment was repeated for both *koozha* and *varikka* varieties. The food mixture containing 60 per cent raw jackfruit flour was selected from set 1 and 2 whereas food mixture containing 50 per cent raw jackfruit flour and 20 per cent jackfruit seed flour was selected from set 3 and 4.

For all the selected food mixtures, the conditions were optimised for attaining the maximum viable count of *L. acidophilus*. Fifty grams of the food mixture at pH 4.5 fermented with 300 µl of inoculum for 24 hours at 37⁰C gave the maximum viable count of *L. acidophilus* ranging from 10.90 to 10.94 log cfu/g. The selected food mixtures from each set along with their respective unfermented samples were freeze dried and packed in laminated polyethylene pouches and kept for storage studies under ambient conditions for a period of six months.

Titrateable acidity (2.32 to 2.96 %), protein (22.84 to 25.16 g/100g), thiamine (0.064 to 0.090 µg/100g), riboflavin (0.048 to 0.088 µg/100g), *in vitro*

starch digestibility (79.89 to 81.94 %) and *in vitro* protein digestibility (77.49 to 83.83 %) were significantly higher in the fermented food mixtures when compared with the unfermented samples. The probiotic count of the food mixtures ranged from 10.85 to 10.90 log cfu/g.

Based on the nutritive, sensory and probiotic viability, the food mixtures with 50 per cent raw jackfruit flour, 20 per cent defatted soya flour, 20 per cent jackfruit seed flour and 10 per cent tomato pulp were selected from both *koozha* and *varikka* varieties for further analysis. Glycemic index of the food mixtures were assessed and a low glycemic index of 45.35 for *koozha* and 47.99 for *varikka* was obtained.

Using the best probiotic food mixture one each from *koozha* and *varikka* varieties, two instant shake mixes were prepared. The developed shake mixes contain 50 per cent fermented food mixtures along with other ingredients. The shake mixes were packed in laminated polyethylene pouches for a period of two months and the quality (nutritional, organoleptic and shelf life) aspects were analysed at 15 days interval. Both the shake mixes were nutritionally and organoleptically acceptable without the presence of fungi, yeast and insect infestation throughout the storage period. The probiotic count of the developed instant shake mixes varied from 10.14 to 10.19 log cfu/g.

Jackfruit incorporated probiotic yoghurts were standardized and the yoghurt with 30 per cent jackfruit pulp was found to be the most acceptable. Yoghurts were prepared using homogenized milk (HM), skimmed milk (SM) and a combination of both. The conditions for the growth of *L.acidophilus* were optimised for all the selected yoghurts. Twenty five grams of the yoghurt, fermented with 100 µl of inoculum at 38 °C gave the maximum total viable count of *L.acidophilus* ranging from 10.84 to 10.92 log cfu/g.

The prepared yoghurts were kept under refrigeration for a period of 15 days for quality evaluation. The probiotic yoghurts were found to be acceptable with a

mean score of more than seven even at the 15th day of storage and the probiotic viability ranged from 10.62 to 10.79 log cfu/g.

The cost of probiotic fermented food mixture was Rs. 260.31 /100g, instant shake mix was Rs. 138.54 /100g and that of probiotic yoghurt was Rs. 18.56-19.56 /100 ml.

The study revealed that jackfruit can be a suitable substrate for probiotic fermentation and the probiotic food mixtures, instant shake mixes and probiotic yoghurt can be successfully developed. Further research can be done for the development of innovative probiotic products from jackfruit.