

IMPROVEMENT OF NUTRITIONAL VALUE OF PEARL MILLET BY
FERMENTATION AND UTILISATION OF THE FERMENTED PRODUCT

By

NEELAM KHETARPAUL

Dissertation submitted to the Haryana Agricultural University
in partial fulfilment of the requirements for the degree of:

DOCTOR OF PHILOSOPHY

in

FOODS AND NUTRITION



College of Home Science
Haryana Agricultural University
HISAR

ACKNOWLEDGEMENTS

I feel great pride and privilege to express profound sense of gratitude and infinite indebtedness to my esteemed mentor Dr.B.M.Chauhan, Scientist, Department of Foods and Nutrition, under whose close supervision and invaluable guidance, this study has been completed. I am also extremely grateful for his sublime suggestions and keen interest during the course of the investigation and preparation of this manuscript.

Earnest thanks are due to Dr.A.C. Kapoor, Dr.P.C. Panda, Dr.L.S. Kaushik and Dr.D.S.Dahiya, the members of my Advisory Committee, for their healthy criticism and lofty suggestions for the improvement of the manuscript and timely assistance throughout the course of the present study.

I am extremely grateful to Dr.D.S. Dahiya, Dr.B.S. Yadav and Dr.S.S. Dhamija, Department of Microbiology, for providing laboratory facilities for conducting the microbiological work. This research work was impossible without the co-operation of the Department of Microbiology.

It gives me immense gratification to venerate the teaching, research as well as office staff of the Department of Foods and Nutrition, for their valuable co-operation.

Finally, I snatch this opportunity in bowing affectionately to my parents, my husband Dr. N.K. Khetarpaul, my son Vipul, Chander Mohan, Asha and Sudha who sacrificed their comforts for my sake, rendered great inspiration and kept me in high spirits throughout the period of this scientific study.

Place: Hisar

Neelam Khetarpaul
(NEELAM KHETARPAUL)

Dated: 5th March, 1988

CERTIFICATE I

This is to certify that this dissertation entitled "Improvement of nutritional value of pearl millet by fermentation and utilisation of the fermented product" submitted for the degree of Ph.D. in the subject of Foods and Nutrition of the Haryana Agricultural University, Hisar is a bonafide research work carried out by Mrs. Neelam Khetarpaul under my supervision and that no part of this dissertation has been submitted for any other degree.

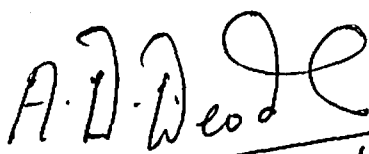
The assistance and help received during the course of investigation have been fully acknowledged.


(Dr. B. M. Chauhan)

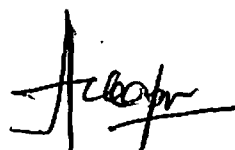
Scientist,
Deptt. of Foods and Nutrition,
I.C. College of Home Science,
H.A.U., Hisar.

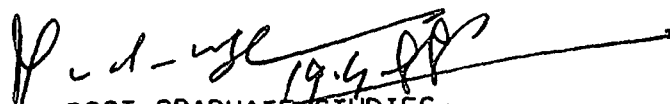
CERTIFICATE II

This is to certify that this dissertation entitled "Improvement of nutritional value of pearl millet by fermentation and utilisation of the fermented product" submitted by Mrs. Neelam Khetarpaul to the Haryana Agricultural University, Hisar in partial fulfilment of the requirements for the degree of Ph.D. in the subject of Foods and Nutrition, has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.


EXTERNAL EXAMINER 15/4/88


MAJOR ADVISOR


HEAD OF THE DEPARTMENT


DEAN, POST-GRADUATE STUDIES

CONTENTS

<u>CHAPTER</u>		<u>PAGE</u>
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-34
	2.1 Microbiology of fermentation	6
	2.2 Fermentative changes	10
	2.2.1 pH and titratable acidity	10
	2.2.2 Total protein, fat and ash	12
	2.2.3 Available carbohydrates	14
	2.2.4 Starch digestibility	17
	2.2.5 Protein digestibility	19
	2.2.6 Phytic acid	21
	2.2.7 Polyphenols	25
	2.2.8 Minerals	27
	2.2.9 Thiamine	30
	2.2.10 Biological utilisation	32
	2.2.11 Organoleptic value	34
III	MATERIALS AND METHODS	35-77
	3.1 Materials	35
	3.2 Selection of fermentation temperatures	35
	3.3 Fermentation	36
	3.3.1 Natural lactic fermentation	36
	3.3.2 Pure culture fermentation	36
	3.4 Microbial analysis	42
	3.5 Titratable acidity and pH	44
	3.6 Nutritional evaluation	44
	3.6.1 Crude protein, fat and ash	44
	3.6.2 Available carbohydrates	46
	3.6.3 Starch digestibility (<u>in vitro</u>)	49

<u>CHAPTER</u>		<u>PAGE</u>
	3.6.4 Protein digestibility(<u>in vitro</u>)	50
	3.6.5 Antinutritional factors	51
	3.6.6 Mineral composition	53
	3.6.7 Thiamine	56
	3.7 Animal experiment	58
	3.7.1 Test animals	58
	3.7.2 Composition of basal diet	58
	3.7.3 Biological evaluation	60
	3.7.4 Body fluids and organs	64
	3.8 Development and utilisation of fermented products	71
	3.8.1 Pearl millet cutlets	71
	3.8.2 Pearl millet <u>pakoras</u>	72
	3.8.3 Pearl millet <u>chappaties</u>	73
	3.8.4 Fried pearl millet vermicilli	74
	3.8.5 Weaning mixtures	75
	3.8.6 Pearl millet biscuits	76
	3.8.7 Organoleptic evaluation	77
	3.9. Statistical analysis	77
IV	RESULTS AND DISCUSSION	78-157
	4.1 Sensory evaluation of naturally fermented pearl millet flour	79
	4.2 pH and titratable acidity	79
	4.3 Microbial composition	89
	4.4 Total protein, fat and ash	92
	4.5 Available carbohydrates	98
	4.6 Digestibility (<u>in vitro</u>)	105

<u>CHAPTER</u>		<u>PAGE</u>
	4.7 Antinutritional factors	111
	4.8 Mineral extractability	117
	4.9 Thiamine	128
	4.10 Biological evaluation	133
	4.11 Weight and composition of organs	142
	4.12 Serum analysis and haematological examination	144
	4.13 Histopathology	149
	4.14 Utilisation for human consumption	149
V	SUMMARY AND CONCLUSIONS	158-168
VI	LITERATURE CITED	i-xviii
	APPENDICES	

LIST OF TABLES

Table No.	Particulars
1.	Composition of lactobacillus broth
2.	Composition of YEPDA medium
3.	Composition of experimental diets
4.	Composition of salt mixture
5.	Composition of Vitamin mixture
6.	Sensory evaluation of naturally fermented pearl millet flour
7.	Changes in pH and titratable acidity (g lactic acid per 100 ml) during natural fermentation of pearl millet flour
8.	Changes in pH and titratable acidity (g lactic acid per 100 ml) during natural fermentation of autoclaved pearl millet flour
9.	Changes in pH and titratable acidity (g lactic acid per 100 ml) during pure culture fermentation of autoclaved pearl millet flour
10.	Changes in pH and titratable acidity (g lactic acid per 100 ml) during fermentation of the sprouted pearl millet
11.	Correlation coefficient of group means of pH with titratable acidity
12.	Microbial composition of the fermented pearl millet flour (non-autoclaved)
13.	Microbial composition of the fermented pearl millet flour (autoclaved)
14.	Effect of natural fermentation on total protein, crude fat and ash content of pearl millet flour (g/100, on dry matter basis)
15.	Effect of pure culture fermentation on total protein, crude fat and ash content of pearl millet flour. (g/100 g, on dry matter basis)

List of Tables (contd...)

Table No.	Particulars
16.	Effect of mixed fermentation on total protein, crude fat and ash content of sprouted pearl millet (g/100 g, on dry matter basis)
17.	Effect of natural fermentation on total soluble sugars, reducing sugars non-reducing sugars and starch content of pearl millet flour (g/100 g, on dry matter basis)
18.	Effect of pure culture fermentation on total soluble sugars, reducing sugars, non-reducing sugars and starch content of pearl millet flour (g/100 g, on dry matter basis)
19.	Effect of mixed fermentation on total soluble sugars reducing sugars, non-reducing sugars and starch content of sprouted pearl millet (g/100 g, on dry matter basis)
20.	Effect of natural fermentation on <u>in vitro</u> starch digestibility (mg maltose released per g flour) and <u>in vitro</u> protein digestibility(%) of pearl millet flour (on dry matter basis)
21.	Effect of fermentation by pure cultures on <u>in vitro</u> STARCH DIGESTIBILITY (mg maltose released per g flour) and <u>in vitro</u> protein digestibility (%) of pearl millet flour (on dry matter basis)
22.	Effect of mixed fermentation on <u>in vitro</u> starch digestibility (mg maltose released per g flour) and <u>in vitro</u> protein digestibility (%) of sprouted pearl millet (dry matter basis)
23.	Changes in polyphenol and phytic acid contents during natural fermentation of pearl millet flour (mg/100 g, on dry matter basis)
24.	Changes in polyphenol and phytic acid contents during fermentation by pure cultures of pearl millet flour (mg/100 g, on dry matter basis)
25.	Changes in polyphenol and phytic acid contents during mixed fermentation of sprouted pearl millet mg/100 g, on dry matter basis)

List of Tables (contd...)

Table No.	Particulars
26.	Correlation coefficient of group means of <u>in vitro</u> starch and protein digestibility with phytic acid content of fermented pearl millet flour
27.	Effect of natural fermentation on HCl-extractability(%) of Ca, Fe, Zn, Cu and Mn of pearl millet flour (on dry matter basis)
28.	Effect of pure culture fermentation on HCl-extractability (%) of Ca, Fe, Zn, Cu and Mn of pearl millet flour on dry matter basis)
29.	Effect of mixed fermentation on HCl-extractability(%) of Ca, Fe, Zn, Cu and Mn of sprouted pearl millet (on dry matter basis)
30.	Effect of natural fermentation on phytate P, non-phytate P, inorganic P (% of total P) and HCl-extractability of P (%) of pearl millet flour
31.	Effect of pure culture fermentation on phytate P, non-phytate P, inorganic P(% of total P) and HCl-extractability of P(%) of pearl millet flour
32.	Effect of fermentation on phytate P, non-phytate P, inorganic P(% of total P) and HCl-extractability of P(%) of sprouted pearl millet
33.	Correlation coefficient of group means of inorganic phosphorus, extractable zinc, copper, iron, manganese, phosphorus and calcium with phytic acid content of fermented pearl millet flour
34.	Changes in thiamine content during natural fermentation of pearl millet flour ($\mu\text{g}/100\text{ g}$, on dry matter basis)
35.	Changes in thiamine content during pure culture fermentation of pearl millet flour ($\mu\text{g}/100\text{ g}$, on dry matter basis)
36.	Changes in thiamine content during mixed fermentation of sprouted pearl millet ($\mu\text{g}/100\text{ g}$, on dry matter basis)

List of Tables (contd...)

Table No.	Particulars
37.	Food intake, protein intake, gain in body weight, feed efficiency ratio(FER) and protein efficiency ratio (PER) of fermented pearl millet flour
38.	Apparent digestibility, true digestibility, biological value, net protein utilisation and utilisable protein of fermented pearl millet flour
39.	Correlation coefficient of group means of apparent and true digestibility with phytic acid content of fermented pearl millet flour
40.	Correlation coefficient of group means of <u>in vitro</u> protein digestibility with apparent and true digestibility
41.	Net protein retention and protein retention efficiency in five days by rats fed on fermented pearl millet flour
42.	Fresh weights of liver (g), heart (mg) and kidney (mg) of albino rats fed fermented pearl millet flour
43.	Total protein and lipid content of the liver of the rats fed on fermented pearl millet flour
44.	Total lipid content of the kidney of rats fed on fermented pearl millet flour
45.	Serum protein, albumin, SGOT and SGPT concentrations of rats fed on fermented pearl millet flour
46.	Haematological examination of rats fed different diets
47.	Organoleptic evaluation of fermented pearl millet cutlets
48.	Organoleptic evaluation of fermented pearl millet <u>pakora</u>
49.	Organoleptic evaluation of weaning mixtures prepared from fermented pearl millet
50.	Organoleptic evaluation of fermented pearl millet <u>chappaties</u>
51.	Organoleptic evaluation of fried vermicilli (bhujia) prepared from fermented pearl millet
52.	Organoleptic evaluation of biscuits prepared from fermented pearl millet

LIST OF FIGURES

<u>Figure No.</u>	<u>Description</u>
1	Relative cell count of the microorganisms during natural fermentation of raw and autoclaved pearl millet flour (Expressed as percent of total count)
2	Effect of natural fermentation on phytate P, non-phytate P, inorganic P(% of total P) and HCl-extractability of P(%) of pearl millet flour
3	Effect of pure culture fermentation on phytate P, non-phytate P, inorganic P (% of total P) and HCl-extractability of P (%) of pearl millet flour
4	Effect of fermentation on phytate P, non-phytate P, inorganic P (% of total P) and HCl-extractability of P (%) of sprouted pearl millet

LIST OF ABBREVIATIONS

Sd	<u>Saccharomyces diastaticus</u>
Sc	<u>Saccharomyces cerevisiae</u>
Lb	<u>Lactobacillus brevis</u>
Lf	<u>Lactobacillus fermentum</u>
SdLb	Mixed fermentation by <u>S.diastaticus</u> and <u>L.brevis</u>
SdLf	Mixed fermentation by <u>S.diastaticus</u> and <u>L.fermentum</u>
ScLb	Mixed fermentation by <u>S.cerevisiae</u> and <u>L.brevis</u>
ScLf	Mixed fermentation by <u>S.cerevisiae</u> and <u>L.fermentum</u>
Sd+Lb	<u>S.diastaticus</u> fermentation followed by <u>L.brevis</u>
Sd+Lf	<u>S.diastaticus</u> fermentation followed by <u>L.fermentum</u>
Sc+Lb	<u>S.cerevisiae</u> fermentation followed by <u>L.brevis</u>
Sc+Lf	<u>S.cerevisiae</u> fermentation followed by <u>L.fermentum</u>
Zf	Zero hour fermentation
Rp	Raw pearl millet
F20	Natural fermentation at 20°C
F25	Natural fermentation at 25°C
F30	Natural fermentation at 30°C
G	Germinated
GSdLb	Germinated and mixed fermented as SdLb
GSdLf	Germinated and mixed fermented as SdLf
GScLb	Germinated and mixed fermented as ScLb
GScLf	Germinated and mixed fermented as ScLf
RNV	Relative nutritive value
EA	Egg albumen

CHAPTER-I

INTRODUCTION

Pearl millet (Pennisetum typhoideum) plays a very important role in the agriculture of many developing countries. It is widely grown in Africa and Asia as a food grain. Although yields of the millet are generally low in comparison to those of other grains, the millet will yield better than most cereals under adverse hot, low fertility and limited rainfall conditions.

In India, pearl millet is grown as a grain crop next to rice, wheat and sorghum. Since it is drought tolerant and is able to grow on comparatively infertile sandy soils, Rajasthan and Haryana form the major pearl millet producing states of India. The production of pearl millet grain in India was over six million tonnes during 1984-85, out of which over half a million tonnes were contributed by Haryana.

Pearl millet is the staple food and forms a major source of dietary nutrients to a large section of Indian population. Pearl millet is equal or superior to corn, sorghum and rice in protein and oil content. It contains more iron and is almost similar in phosphorus and calcium contents to cereals (Desai and Zende, 1979).

Like other cereals, the quality of pearl millet protein is low. Protein content of pearl millet varies from 8 to 12 per cent and protein digestibility ranges from 53 to 68 per cent (Chauhan et al., 1986; Mahajan, 1986). Carbohydrates of pearl millet consist of starch and smaller amounts of soluble sugars including pentoses and hexoses. Amylopectin (67.9%) is the major constituent of pearl millet starch (Desai and Zende, 1979). The starch digestibility of pearl millet is low as it has been shown to be relatively more resistant to attack by pancreatic amylase (Sullins and Ronney, 1977). Pearl millet contains

only marginal amounts of essential vitamins, thus prolonged consumption of this millet based diet may lead to deficiency of niacin, B₁₂, riboflavin etc.

No doubt pearl millet has a relatively better mineral profile but owing to certain inherent factors the bioavailability of these minerals to human system is low (Mahajan, 1986). More than half of the phosphorus in pearl millet is present in the phytate form which is not available to the human system (Daniels and Fisher, 1981). Besides, dietary phytate is known to render minerals, especially divalent cations, unavailable as well as to inhibit the proteolytic and amylolytic enzymes (Sutardi and Buckle, 1985). The formation of a ternary protein-mineral-phytate complex is believed to obstruct or inhibit the enzymatic degradation of protein and results in lower digestibility of protein (Serraino et al., 1985; Knuckles et al., 1985). In the presence of phytate, starch digestibility is significantly reduced (Yoon et al., 1983). Fairly high amounts of polyphenols are present in pearl millet (Hulse, 1979) which may also adversely affect the minerals availability and protein and carbohydrate digestibility of the food grain (Bressani and Elias, 1980; Deshpande and Salunkhe, 1982; Thompson et al., 1984; Aw and Swanson, 1985).

Improved irrigation facilities in the last decade seem to have markedly changed the cropping pattern and dietary habits of people in semiarid areas of Haryana. Pearl millet has been replaced by other preferred foods and when people can afford a choice, they go for the other cereals like wheat and rice. As a result, during bumper harvest, there are not many buyers of the pearl millet and it fetches poor returns to the growers.

Recently, there has been a great deal of interest and concern among the food scientists and technologists for using microorganisms in the processing of food. Generally, this type of research is directed towards processing

to upgrade agricultural commodities to make them more pleasant in colour, aroma, taste, appearance and texture. This is further aimed at changing the nutritional properties of the commodities, making the product safe i.e. free from spoilage and toxins and modifying the process to make a commercially viable product. Use of fermentation technology has resulted in the development of several foods from corn, sorghum, wheat, soybean and other food legumes. Pearl millet may also be fermented to develop new foods having better digestibility, acceptability and low amount of antinutrients.

Fermentation of food grains has now been well recognised to be an effective method of improving their nutritive value. Fermentation improves the starch and protein digestibility (Boralkar and Reddy, 1985; Dhankher and Chauhan, 1987b), increases the bioavailability of minerals (Harland and Harland, 1980; Dhankher, 1985; Mahajan, 1986) and biological utilisation (Vass et al., 1984). Fermentation also brings down the levels of antinutrients like phytic acid (Dhankher and Chauhan, 1987c; Mahajan, 1986; Sutardi and Buckle, 1985) and polyphenols (Dhankher and Chauhan, 1987c) of plant foods. Fermented foods having acidic pH are microbiologically safe and may not encourage the growth of toxin producing microflora.

Fermentation is inexpensive, does not require sophisticated equipment or technology and consumes little fuel energy. The fermented products supply protein and enrich the predominantly starchy diets with vitamins and other nutrients needed by millions of people subsisting on marginal incomes. In a world where millions of people are underfed, fermentation offers an effective approach to improved utilisation of scarce nutritional resources.

In some instances, the fermentation process produces a food of convenience. For instance, in desert areas where people travel from one place to another for water and forage of their animals, the ready to eat and unperishable fermented foods prepared from locally available foodstuffs can be conveniently transported from one area to another.

Preparation of a commercially viable product from pearl millet may lead to better utilisation and increased demand for this lowly placed food grain. This will ultimately promise better returns to the growers.

With these perspectives, the present study was carried out with the following objectives:

- i) To carry out fermentation of pearl millet flour
- ii) To assess the nutritive value of the fermented product
- iii) To determine the levels of antinutritional factors in the fermented product
- iv) To devise methods of utilisation of the fermented product

CHAPTER-II

REVIEW OF LITERATURE

Fermentation of food for preservation, enhancement of nutritive value and improvement of flavour is being practised since prehistoric times. It is one of the oldest and economical methods of producing and preserving the foods highly acceptable to man.

Microorganisms play an excellent role in the production of wholesome foods ranging from fermented fresh vegetables such as cabbage (sauerkraut) and cucumbers (pickles), fermented cereal yoghurt (Nigerian ogi/Kenyan uji), sour dough bread and breadlike products without the use of wheat or rye flours (Indian idli/Phillipine puto), to fermented milks (yoghurt/cheeses) and fermented meats (European salami).

Fermentation is known to improve the nutritional quality of various food grains like corn, sorghum and various food legumes. The information available on the improvement of nutritive value of various food grains can be suitably reviewed under the following heads:

- 2.1 Microbiology of fermentation
- 2.2 Fermentative changes
 - 2.2.1 pH and titratable acidity
 - 2.2.2 Total protein, fat and ash
 - 2.2.3 Available carbohydrates
 - 2.2.4 Starch digestibility
 - 2.2.5 Protein digestibility
 - 2.2.6 Phytic acid
 - 2.2.7 Polyphenols
 - 2.2.8 Minerals
 - 2.2.9 Vitamins
 - 2.2.10 Biological utilisation
 - 2.2.11 Organoleptic value

2.1 Microbiology of fermentation

Nearly all food fermentations are the result of activity of more than one species of microorganisms. An organism that initiates a fermentation will develop until its by-products of growth inhibit further growth and fermentation. During this initial growth period, other organisms develop and in turn are followed by other more tolerant species. In general, growth is initiated by bacteria, followed by yeasts and then moulds, if conditions are suitable for growth of these microorganisms. Heterofermentative species of the *Lactobacillus* convert glucose to about 50% lactic acid, 25% acetic acid and ethyl alcohol, and 25% CO₂. The heterofermentative species like *Lactobacillus brevis* and *Lactobacillus fermentum* are the mannitol formers and some species produce dextran from sucrose. The fermentative yeasts produce ethyl alcohol and CO₂ from sugars.

Raw foods harbour miscellaneous, primarily aerobic and facultative microflora which one might expect from the more or less aerobic habitat. The degree of contact of different foods with air, soil, water, animal bodies, equipment and other foods will affect the microflora. Houston et al. (1957) reported that the population of actinomycetes, bacteria and yeasts in rice stored for 215 days and moisture contents and relative humidities upto 16.8 and 95 per cent, respectively and at temperatures of 21 or 32°C, always decreased. Mould population also decreased at 13.8 per cent moisture and 70 per cent relative humidity or below at either temperature. At 15 per cent moisture and above, mould populations increased. Such trends suggest that the rice stored for one year below 70 per cent relative humidity would have even smaller population of microorganisms than the 4.5×10^5 yeasts, 1.0×10^3 moulds, 2.3×10^7

actinomycetes, 2.9×10^7 aerobic bacteria and 3×10^5 anaerobic bacteria per g observed after 215 days (Houston et al., 1957).

In idli and dosa, lactic acid fermentation by Leuconostoc mesenteroides and Streptococcus faecalis developed concomitantly during soaking and reached more than 1×10^9 /g after 13 h fermentation. These two species predominated until 23 h batter fermentation. L. mesenteroides is the microorganism essential for leavening of batter and along with S. faecalis is also responsible for acid production. Both functions are essential for producing a satisfactory idli (Mukherjee et al., 1965). Ramakrishnan et al. (1976) pointed out in soy idli fermentation that the organisms that were present in the batter in appreciable numbers to start with, were P. cerevisiae, Microbacterium flavum, Lactobacillus spp and Bacillus spp. L. fermenti, L. delbrueckii and Leuconostoc mesenteroides seemed to be probable soy idli fermenters. All these organisms became predominating at the later stages of fermentation. Yajurvedi (1980) reported that the microorganisms present in the ingredients as well as the environmental contaminants determine the nature of organisms involved in idli fermentation. He reported a number of wild yeasts along with different lactic bacteria in the fermenting batters. Venkatasubbaiah et al. (1984) reported an increase in the counts of Lactobacillus, coliforms and moulds with increase in the period of idli fermentation. Venkatasubbaiah et al. (1985) inoculated various types of combinations of bacteria and yeast viz., Leuconostoc mesenteroides and Hansenula anomala, L. mesenteroides and Candida glabrata in the idli batter. Lactic and yeast counts increased with the period of fermentation. The role of lactic acid bacteria was to reduce the pH of the batter to a level (4.1-4.5) which is optimum for yeast activity.

Lactobacillus spp and a yeast were the essential microbes in sour dough as reported by Kline and Sugihara (1971). Saccharomyces spp of yeast was isolated and identified in the fermented sour dough leavened.

Tongananta (1971) in his study on fermented rice puto reported that during all of the processing steps involving an incubation with any rice variety, the yeasts and microaerophilic bacteria increased in number with time. Aerobic bacteria present at the beginning disappeared. The predominant organism was always L.mesenteroides, followed by S. faecalis and Saccharomyces cerevisiae. At the end of any stage of fermentation, L. mesenteroides comprised from 45 to 89 per cent of the total microbial population and was responsible for most of the acid and gas production. S.cerevisiae made up as much as 18 per cent of the total population. Hence, yeast and L.mesenteroides played an important role in leavening the batter.

Merican (1977) reported that non salime-forming strains of L. mesenteroides were the early dominant microorganisms in the production of fermented Malaysian pickled cucumbers. They were followed by heterofermentative lactobacilli L. brevis, L. fermentum and the homofermentative L. plantarum. The lactic acid bacteria count was very low (6×10^2 per ml) on the first day of pickling. After the initial lag stage, however, a rapid increase of 2×10^8 organisms per ml at 15 days was observed. Yeast count progressively increased from 3×10^3 organisms per ml on the third day to 5×10^7 organisms per ml at the end of fermentation.

During the preparation of sauerkraut (fermented cabbage) fermentation was found to be initiated by L. mesenteroides and completed by Lactobacillus plantarum (Mukherjee et al., 1977).

Odunfa (1985) reported that microflora of traditionally produced ogi-baba were dominated by Lactobacillus spp. followed by Streptococcus spp, yeast (Debarymyces hansenii) was also present. Gashe (1985) reported the involvement of Leuconostoc mesenteroides, S. faecalis, coliforms, Pediococcus cerevisiae, Lactobacillus spp and yeasts in the fermentation of tef, an Ethiopian fermented food. The enterobacteriaceae appeared to be the most predominant microflora during the first 18 h of fermentation. After this, coliforms were replaced by L. mesenteroides and S. faecalis. The lactic acid bacteria viz., Lactobacillus brevis, L. plantarum and L. fermentum appeared in significant numbers at about pH 4.7. The heterofermenters produced leavening action as well as acidification in the dough. Dhankher (1985) reported that before fermentation of rabadi, the pearl millet flour-buttermilk mixture contained three major groups of microorganisms, namely, lactobacillus, yeast and coliform. Yeast was predominant and fungi were not detected. At the end of nine h of fermentation, lactobacilli dominated and accounted for more than 99 per cent of the total microbes. Maximum growth of lactobacillus was observed at 40°C and lesser at 35, 45 and 50°C. Mahajan (1986) carried out the natural fermentation of pearl millet by mixing freshly ground pearl millet flour with water. The fermentation occurred at temperatures 20,30,40 and 50°C at initial pH of 6,5,4 and 3 for 24, 48, 72 and 96 h. She found that yeasts and lactobacilli were the two most dominating microflora present in the pearl millet flour. Maximum growth of yeast occurred when fermentation was carried out for 72 h at 30°C with initial pH 6. Whereas the lactobacillus count was the highest when fermentation was conducted at 30 or 40°C with an initial pH 6 or 5. Coliforms were not present in an appreciable number. Fungal cells had the lowest count. Optimum temperature and pH for the cell count of coliform and fungus were 20°C with 6 and 5 pH and 20°C with 4 and 5 pH, respectively.

Thiam (1977) reported that millet flour stored at 30°C and 90-95% relative humidity in a controlled atmosphere showed a significant increase in bacterial and yeast counts. Hamad (1978) isolated seven strains of lactic acid bacteria from fermenting wheat meal and five strains from fermenting corn meal. Lactobacillus fermentum was most abundant in both fermented and whole corn and wheat meal. Zamora (1978) isolated Pediococcus acidilactici, Lactobacillus casei, Lactobacillus plantarum from fermented cowpea and Lactobacillus helveticus from chickpea along with the previous species. Nanson and Fields (1982) studied the effect of L. fermentum, Bacillus subtilis, B. cereus and Pseudomonas maltophilia singly and in combination on the relative nutritive value (RNV) of the fermented corn meal and found that any single microorganism was ineffective in improving the RNV of corn meal.

2,2 Fermentative changes

2.2.1 pH and titratable acidity

Tongananta (1971) studied the microbiological and chemical changes in the Philippine fermented rice cake puto made from three rice varieties and found a decrease in pH from 6.3 to 4.2 while the corresponding titratable acidity increased from 0.03 to 0.64 per cent during 24 h of fermentation.

Arroya et al. (1977) observed that during rice shrimp fermentation (balao), the initial pH of the fermenting mixture fell from 7.56 to 3.52 after 9 days, in the presence of 3 per cent salt. Titratable acidity also increased most rapidly reaching 1.75 per cent as lactic acid.

Morcos (1977) noticed the changes in pH and titratable acidity of fresh and pickled Egyptian vegetables. In case of cucumber pickle, pH dropped from 7.0 to 3.2 while titratable acidity increased from 0.03 to 0.78 per cent as lactic acid. Mukherjee et al. (1977) studied chemical and microbial changes in cabbage during fermentation and found a sharp change in pH and acidity during the initial 21 days of fermentation and relatively little changes thereafter. Production

of acetic acid as well as lactic acid by heterofermenters during the initial phase of fermentation resulted in a sharp decrease of pH. The pH was nearly minimum while total acid was maximum at 60 days of fermentation.

During the fermentation of cassava to prepare gari (Ogunsua, 1977), pH decreased from 6.2 to 4.0 and titratable acidity increased from 1.2 to 3.4 mg NaOH/g after third day of fermentation.

Zamora and Fields (1979) found that titratable acidity increased sharply as the fermentation of food legumes continued from the first to the third day. With the abrupt increase in titratable acidity, there was a corresponding drop in pH. After natural fermentation at room temperature (25°C) for 4 days, pH dropped to 4.12 and 3.92 in case of cowpea and chickpea, respectively while titratable acidity values went up to the level of 1.32 and 1.02 per cent as lactic acid in case of chickpea and cowpea, respectively.

Banigo and Muller (1972) found that in the traditional process of preparation of ogi, the pH of the fermenting maize grain decreased from 5 to 4 during 72 h of steeping while the corresponding titratable acidity increased from 80 to 301 mg NaOH/100 g grain. In the preparation of Nigerian agidi, a fermented product of corn, Umoh and Fields (1981) reported a drop in pH from 6.7 to 4.7 and a corresponding increase in titratable acidity from 0.09 to 0.38 per cent (as lactic acid).

The pH of fermented sorghum changed from 6.7 to 3.8 after two days of fermentation (Paul and Fields, 1981). Concomitantly with the drop in pH, there was a rise in the titratable acidity of medium. At the end of fifth day, the pH of corn meal was 3.8 and the titratable acidity was about 1.0 per cent indicating that normal lactic acid fermentation occurred (Lopez et al., 1983). Baseler and Fields (1984) found a drop in pH from 6.38 to 3.78 after 72 h fermentation of corn meal by Candida tropicalis whereas titratable acidity increased from

0.06 to 0.91 per cent (as lactic acid). According to Nanson and Fields (1984) the pH of fermenting cornmeal dropped from 6.25 to 4.75, 4.40, 3.45 and 3.75 after 24, 48, 72 and 96 h lactic acid fermentation, respectively whereas titratable acidity increased from 0.03 to 1.02 after 96 h fermentation.

Fermentation of idli batter brought about a drop in pH from 6.10 to 4.70 and from 5.80 to 4.10 after 18 h fermentation at room temperature (Venkatasubbaiah et al., 1984; Venkatasubbaiah et al., 1985). Titratable acidity as lactic acid increased from 0.90 to 3.18 per cent after 18 h. Tef injera a pancake like acidic food was prepared from tef flour and a decrease in pH to about 5.8 after 18 h and 3.8 after 72 h fermentation was observed by Gashe (1985).

Mahajan (1986) carried out natural lactic acid fermentation of pearl millet flour and reported that the pH of fermented product dropped and titratable acidity rose as the fermentation progressed at all the temperatures and pH of fermentation except at an initial pH 3 in which case the fermented product had nonsignificantly low pH. Decrease in pH and increase in titratable acidity was more pronounced when fermentation was carried out at 40 or 50°C with an initial pH 6 for 48 h or more.

Dhankher and Chauhan (1987a) reported a drop in pH of the rabadi, a fermented pearl millet and buttermilk product. The pH was the lowest at 45°C and the titratable acidity was the highest at 40 and 45°C when fermentation was carried out for nine h. The corresponding decrease in pH at 35, 40, 45 and 50°C was from 4.3 to 3.6, 4.1 to 3.3, 4.4 to 2.9 and 4.1 to 3.3, respectively.

2.2.2 Total protein, fat and ash

Rama Rao and Swaminathan (1953) studied six varieties of pearl millet and reported protein content to range from 12.50 to 14.35 per cent and fat from 5.10 to 5.81 per cent. A sample of pearl millet grain in South Texas was reported to contain 17.4 per cent protein, 4.9 per cent oil and 61.5 per cent starch on dry matter basis (Burton and Powell, 1968).

While studying the Indian varieties of pearl millet, Sawhney and Naik (1969) found a wide range of variability in protein content (7.9 to 20.1%). Lipid content was found to vary from 4.6 to 6.4 percent and ash from 2.0 to 3.2 per cent. Similarly, in 58 Indian inbred entries of pearl millet, protein content ranged from 9.39 to 14.40 per cent, phosphorus 0.70 to 0.96 per cent, calcium from 0.025 to 0.046 per cent, ash from 1.58 to 2.46 per cent, fat from 2.81 to 7.76 per cent (Goswami et al., 1970a; Goswami et al., 1970b). Singh and Popli (1973) reported protein content of pearl millet to range from 8.4 to 14.5 per cent and total carbohydrates from 65.4 to 71.2 per cent.

Burton et al. (1972) found 9.2 to 16.1 per cent protein and 4.5 per cent fat in several pearl millet varieties. Desikachar (1977) reported 12.5 per cent protein, 5.6 per cent lipids and 2.8 per cent ash in whole grain of pearl millet. According to Perten (1977) pearl millet contained 8.9 per cent protein, 6.9 per cent fat and 2.1 per cent ash. Chaudhary and Kapoor (1984) reported that protein, ether extract, sugars and ash contents ranged from 10.41 to 13.20, 6.03 to 8.05, 2.51 to 2.59 and 1.8 to 2.48 per cent, respectively in three varieties of pearl millet. Batra (1985) reported 10.6 per cent protein, 5.6 per cent ether extract and 1.7 per cent ash content in pearl millet. Seeds of eight high yielding varieties of pearl millet were assessed for total protein by Chauhan et al. (1986). They observed that crude protein and fat ranged from 10.50 to 14.80 and 8.32 to 10.40 per cent, respectively.

Akinrele and Bassir (1967) found that fermented ogi, a maize fermented product, contained 9.21 per cent crude protein and 5.13 per cent fat. The amount was found to be higher than that present in the unfermented maize. Wang et al. (1968) reported that the fermentation of cooked and cracked wheat and soybean for 24 h with Rhizopus oligosporus increased the protein content slightly at the expense of carbohydrates.

Changes in protein and fat content of locust bean after fermentation were noticed by Eka (1980). Protein increased from 30.6 to 38.5 per cent and fat from 15.2 to 31.2 per cent. According to Aliya and Geervani (1981) the fermentation decreased the crude protein contents of legume, bengalgram and green gram products by 6 to 8 per cent and those of pearl millet and ragi by 4 to 6 per cent, whereas the sorghum products were unaffected.

Ezeala (1984) indicated a decrease in protein content of fermented cassava meal. Microorganisms including bacteria and yeast in the traditional fermented food resulted in decreased cassava protein when no external source of nitrogen was added.

Mahajan (1986) found no effect on fat content of fermented pearl millet flour irrespective of temperature, pH and period of fermentation. But protein content of the fermented product either decreased or remained unchanged depending upon pH, temperature and period of fermentation; decrease being more distinct at 40°C. According to Dhankher and Chauhan (1987a) rabadi fermentation did not show any change in protein and fat content of the fermenting mixture.

Opoku et al, (1981) reported that raw pearl millet and grain germinated at 25°C for 84 h contained 8.6 and 11.8 per cent protein, 7.5 and 2.5 per cent lipid, 4.1 and 3.2 per cent ash and 53.9 and 48.5 per cent carbohydrates, respectively.

2.2.3 Available carbohydrates

Pearl millet sugars consist of fructose, glucose, sucrose and maltose along with two higher oligosaccharides. Total carbohydrates ranged from 65.4 to 71.2 per cent and starch constituted 56.3 to 63.7 per cent (Singh and Popli, 1973). Total sugars in pearl millet varieties ranged from 244 to 331 mg maltose/10 g while the reducing the nonreducing sugars ranged from 39 to 57 and 181 to 233 mg/10 g, respectively. Starch is the main carbohydrate in pearl millet.

Sugars are present in relatively less amounts i.e. 1.2 per cent. Starch is composed of amylose (32.1%) and amylopectin (67.9%). The amylose content of pearl millet varied from 18.32 to 24.58 per cent (Desai and Zende, 1979). Subramanian et al. (1981) reported that the pearl millet consists of stachyose 0.06-0.13 per cent (3.6), raffinose 0.65 to 0.84 (27.9), sucrose 1.32 to 1.82 per cent (64.3), glucose and fructose 0.08 to 0.16 per cent (4.2) and total soluble sugars 2.16 to 2.78 per cent. The values in parentheses indicate per cent of total soluble sugars. The total soluble sugars, reducing sugars, non-reducing sugars and starch content of pearl millet were reported to be 2.5, 0.30, 2.20 and 55 g/100 g, respectively (Mahajan, 1986).

Tongananta (1971) reported that during the puto fermentation, non-reducing sugars decreased while the reducing sugars increased and the sugars mainly consisted of glucose, sucrose, galactose, maltose, raffinose, fructose and maltotriose.

Watson et al. (1975) indicated a decrease in starch content of fermented beans. They found that although carbohydrates constituted 11.15 per cent of unfermented beans, no starch was present in the fermented beans. Ekmon and Nagodawithana (1977) reported that the primary biochemical change that took place in the hopper fermentation (Steam baked acid fermented dough made from rice or wheat flour and coconut using S.cerevisiae as inoculum) was the utilisation of the added sugar by the yeast, with the concomitant release of CO₂ and some ethanol. The small proportion of flour amylases converted starch to dextrin and mono- or disaccharides which were quickly consumed by the yeast.

Ogunsua (1977) found an increase i.e. 3.1 to 6.2 mg/g in the reducing sugar content of fermented cassava mash on the first day of fermentation which decreased to 4.4 mg/g on the second day and remained only 2.8 mg/g on the third day of fermentation due to utilisation by the fermenting microorganisms.

Autoclaving and fermentation increased the levels of reducing sugars in corn meal by about four times (Wang and Fields, 1978) when it was germinated and fermented with Candida tropicalis 1369, the species capable of utilising the maltose and soluble starch. Hamad and Fields (1979) reported that during the natural lactic acid fermentation of cereals, the level of reducing sugars increased 4.38 times on the first day but decreased on the second and third day. The increase and decrease were attributed to the action of microflora during fermentation.

Zamora and Fields (1979a) reported starch degradation by amylases during fermentation and increase in the reducing sugar content. Total available carbohydrate content in beans decreased during fermentation (Eka, 1980 and Miller, 1980). Sugar values of locust beans decreased to a large extent during iru fermentation.

During the fermentation of sorghum meal, reducing sugars increased (Kazanas and Fields, 1981). Increased sugars provided a carbon source for microorganisms during fermentation. This indicated that fermentation process enhanced the power of takadiastase enzyme by increasing the starch availability in fermented product as compared to non-fermented meal. Significant increase in starch availability was supported by significant increase in reducing sugar content of fermented sorghum.

Odunfa (1983a) reported an initial increase in the reducing sugar level and explained that this increase may not be due to the amylase activity. The reducing sugars might be produced from the hydrolysis of oligosaccharides present in unfermented bean and easily utilised by Bacillus spp and Staphylococcus spp involved in fermentation.

Taur et al. (1984b) reported a maximum starch degradation by amylases during fermentation. The maximum degradation of starch resulted in increase in the reducing sugars. There was an inverse relationship between starch content and fermentation period. It was also reported that the starch content in germinated meal further got degraded by amylases during fermentation which causes further increase in starch availability. Taur et al. (1984c) found maximum starch degradation by amylases during germination too and noted an inverse relationship between germination and per cent starch degradation. With increase in germination period, the starch content significantly decreased from 75.36 to 23.76 per cent. Sheorain and Wagle (1973) and Jain and Date (1975) had reported the similar findings.

Total soluble sugars of pearl millet increased when fermentation occurred at 20, 30 and 50°C with initial pH 6.5, and 4 (Mahajan, 1986). On the contrary, fermentation at 40°C reduced the level of total soluble sugars. Non-reducing sugars also increased after 24 h fermentation. The increase noticed after 24 h declined when fermentation was continued.

2.2.4 Starch digestibility

Starch digestibility of pearl millet is low. Mahajan (1986) reported that the starch digestibility of pearl millet (mg maltose released/g) was 12.

Several workers have reported the beneficial effect of fermentation on starch digestibility (In vitro). The improvement or changes in starch digestibility in the fermented product are perhaps related to enzymatic properties of microbes which ferment the substrate. The presence of two amylases in the fermenting bacteria was indicated by Bernfeld (1962). These two enzymes are responsible for bringing about a cleavage of amylose and amylopectin to approximately six parts maltose and one part glucose.

Kazanas and Fields (1981) studied the nutritional improvement of sorghum by fermentation and reported an increase in the per cent starch availability i.e. from 16.70 to 31.82. Ramachandran and Bolodia (1984) reported an improvement in the starch availability of tef due to partial breakdown of soluble starch.

Oyewole (1984) found that out of 45 strains of microbes isolated from iru, 31 were amylolytic, which improved the starch digestibility. Taur et al. (1984b) reported an increase in the starch digestibility as a result of germination followed by natural lactic acid fermentation of sorghum due to degradation of starch by amylases.

Boralkar and Reddy (1985) studied the effect of different periods of roasting, germination and fermentation on the digestibility of soybean starch (in vitro) and reported progressive improvement in the per cent starch digestibility with increasing periods of germination and fermentation.

Wood (1983) emphasised the commensalism between I.holmii that utilises only glucose and Lactobacillus spp that preferentially utilises maltose releasing glucose so that both develop in a dough where the amylase hydrolyzes starch to maltose. With a yeast that can only grow on glucose, a bacterium which produces glucose as a product of maltose metabolism, and a medium where maltose, produced by amylases acting on starch in the dough, is the major fermentable carbohydrate, there is the basis for balanced growth of the two organisms in the dough.

Yoon et al. (1983) reported an increase in glycemic index as a result of improved starch digestibility which was attributed to the reduction of phytic acid during fermentation. There was an improvement in the starch digestibility (in vitro) of pearl millet following fermentation (Mahajan, 1986).

Initial fermentation pH 6 and 3 and 40°C temperature appeared to be the most effective in raising the starch digestibility. Longer the period of fermentation better was the starch digestibility. Improvement in starch digestibility was attributed to breakdown of starch to oligosaccharides by fermenting microflora (Cronk et al., 1977) or by the enzymes inherent in the pearl millet flour.

Rabadi had better starch digestibility (in vitro) than the initial fermentation mixture. Digestibility increased gradually at all the temperatures as the fermentation prolonged (Dhankher and Chauhan, 1987b). Nine h fermentation gave the highest starch digestibility; at 35°C, 40°C, 45°C and 50°C, it increased by 44, 50, 58 and 50 per cent, respectively. Levels of phytate and polyphenols in cereal foods, which inhibit amylases (Thompson and Yoon, 1984) are decreased during fermentation (Lopez et al., 1983; Dhankher, 1985). This may partly contribute to better starch digestibility of fermented products.

2.2.5 Protein digestibility

Protein digestibility of pearl millet has been reported to vary from 53 to 68 per cent (Chauhan et al., 1986). Improvement of protein digestibility as a result of fermentation is mainly associated with enhanced proteolytic activity. High proteinase activity has frequently been reported in the fermentation of protein foods as in the production of Japanese miso, soysauce (Wang and Hesseltine, 1970; Steinkraus et al., 1965) and ogiri (Odunfa, 1983b). The strong ammonia smell, characteristic of the later stages of iru fermentation, may also be an end product of proteinase activity as illustrated by Whitaker (1978).

Kao and Robinson (1978) reported an increase in the amino nitrogen to signify a partial breakdown of proteins to peptides and amino acids. It

resulted ultimately in increased protein digestibility. The percentage of water soluble proteins to total protein increased from 22.04 to 56.87 per cent and 16.67 to 66.67 per cent in the fermented products, tempeh and miso, respectively.

Zamora and Fields (1979a) reported that the bacteria produced during fermentation increased the proteolytic activity and degraded protein into peptides and amino acids which were readily utilisable by the bacteria. In addition, bacteria may also synthesize amino acids from metabolic intermediates during their growth cycle. Numerous investigators found a large increase in essential amino acids in cereal and legume grains during fermentation. Kazanas and Fields (1981) reported an increase in the protein availability (8.33%) of the fermented sorghum meal while the protein availability from the unfermented sorghum meal was 1.42 per cent only. Paul and Fields (1981) reported increased relative nutritive value (%RNV) of sorghum fermented at 25° and 35°C.

Neumann et al. (1984) reported that fermentation of corn gluten meal with Aspergillus oryzae and Rhizopus oligosporus resulted in rapid increase in proteolytic activity. Oyewole (1984) found that of 46 strains of microbes in ogiri, all were proteolytic. In the fermentation of ogiri, a Nigerian melon fermented product, the most significant change during fermentation was an increase in soluble products, particularly amino acids. This improved the protein digestibility of locust bean which in its unfermented state was not normally used as food (Odunfa, 1983a).

Boralkar and Reddy (1985) studied the effect of roasting, germination and fermentation on the digestibility of protein present in soybean and found a progressive improvement in the protein digestibility with longer periods of germination and fermentation. Fermentation of soybean batters for varying

period (8, 12 and 16 h) brought about significant increase in in vitro protein digestibility.

Mahajan (1986) reported a significant improvement in the protein digestibility during natural lactic fermentation of pearl millet. Maximum improvement in protein digestibility could be achieved by fermenting at 50°C for 48 h with initial pH 6. Fermentations at 30°C for 72 h and at 40°C for 72 h were equally effective.

Dhankher and Chauhan (1987b) reported gradual increase in protein digestibility of pearl millet flour-buttermilk mixture (rabadi) at all the temperatures as the duration of fermentation increased. During 3, 6 and 9 h of fermentation, the increase in the digestibility ranged from 9 to 23, 25 to 33 and 40 to 51 per cent, respectively. Microflora may produce some proteolytic enzymes during fermentation (Hesseltine, 1983) which may be responsible for increased protein digestibility of rabadi. In addition, antinutrients like phytate and polyphenols present in cereals that affect protein digestibility adversely (Tan et al., 1984; Knuckles et al., 1985) were also decreased during fermentation (Lopez et al., 1983; Dhankher and Chauhan, 1987c).

2.2.6 Phytic acid

More than half of the phosphorus in cereals is reported to be present in the form of phytic acid (Gopalan et al., 1981) which is not available to the human system. Chauhan et al. (1986) reported a wide variation in phytate content (594 to 1040 mg/100 g) among pearl millet varieties.

Phytic acid binds to protonated basic residues of protein at acidic pH forming a binary protein-phytate complex. At alkaline pH, in the presence of cations, phytic acid forms a ternary protein-mineral-phytate complex (Cheryan, 1980). Such a complex formation is believed to obstruct or inhibit

the enzymatic degradation of protein (Serraino et al., 1985; O'Dell and De Boland, 1976; Singh and Krikorian, 1982; Ranhotra, 1972). Knuckles et al. (1985) studied the effect of phytate and partially hydrolyzed phytate on in vitro protein digestibility. The effects of sodium phytate and partially hydrolyzed sodium phytate (0 to 82% hydrolyzed) on pepsin digestion of casein and bovine serum albumin were evaluated by an in vitro procedure using dialysates of pepsin digestion over a period of 0-23 h. The inhibitory effect of phytate differed with substrate and increased with dose level.

Yoon et al. (1983) studied the effect of phytic acid on starch digestibility in vitro and related to glycemic index in healthy volunteers. The glycemic index was found to correlate negatively with the phytic acid content of the food tested. In the presence of sodium phytate, the rate of digestion of raw wheat starch reduced significantly by 50 per cent.

Phytate forms insoluble complexes with iron, zinc, calcium and copper (Oberleas et al., 1966). Addition of sodium phytate at the rate of 2.5 g/day decreased the serum iron levels in human beings (Reinhold, 1975). When sodium phytate was added to semisynthetic diet (10g/kg) and fed to rats for 21 days, their whole body retention of iron fell (Davies and Nightingale, 1975). Phytate (inositol hexaphosphate) is a compound which occurs naturally in many foods derived from plants (Maga, 1982; Reddy et al., 1982) which binds various essential metals and reduces their availability for absorption from the diet (Cheryan, 1980; Maga, 1982; Reddy et al., 1982; Wise, 1983) and reabsorption after their secretion in digestive juices (Davies and Nightingale, 1975). Phytin, the mixed Ca-Mg salt of phytic acid, reduces of bioavailability of dietary minerals, especially zinc (Cummings, 1978; Davies and Reid, 1979; Daniels and Fisher, 1981). In the presence of Ca^{2+} and Zn^{2+} , an insoluble

Zn-Ca-phytate complex is formed at pH 6. The formation of such a complex in vivo in the upper gastro-intestinal tract of single stomached animals is thought to be the mechanism by which phytate reduces zinc availability.

Griffiths and Thomas (1981) also reported that phytic acid forms insoluble chelates with various trace and macroelements such as Zn, Fe and Ca. Wise (1983) reported the following decreasing order of stability of the metal-phytate complex: $Zn^{++} > Cu^{++} > Co^{++} > Mn^{++} > Ca^{++}$.

The nutritional contribution of the plant foods could be significantly increased if the phytate in these foods were reduced (Ranhotra, 1972; Reinhold, 1975). Phytate content reduced as a result of idli fermentation (Rajalakshmi and Vanaja, 1967) and in rice-sorghum fermented batter (Ramakrishnan et al., 1976). Chang (1977) noted that the incubation of presoaked beans in water at 60°C for 10 h lowered their phytate content by 90 per cent. Incubation in pH 5.5 buffer at 50°C for 24 h lowered it by 62 per cent. Kazanas (1979) reported that phosphorus was released from bound state during third and fourth day of natural lactic acid fermentation of sorghum. Reddy and Salunkhe (1980) found that unprocessed, soaked and fermented cotyledons of black gram had 4.80, 4.66 and 4.16 mg/g phytate, respectively.

Daniels and Fisher (1981) emphasised the importance of yeast phytase and concluded that high fibre bread with a lower phytate content than usual could be best obtained by increasing yeast levels and fermenting times. Fermentation reduced the phytate content with the fastest decrease occurring during the first 30 min. Breads prepared after 120 min fermentation sustained a 72-77 per cent loss of phytate (Nayini and Markakis, 1983). As the phytate content decreased, the inorganic phosphate content increased. Tangkongchitr et al. (1981) could account for almost all the phytate P loss by the increase in inorganic P, but this occurred after 8 h fermentation by which time perhaps all of the

intermediate inositol phosphates were dephosphorylated. Fermentation of bread containing bran reduces the phytic acid content and increases zinc absorption from such bread (Navert and Sandstrom, 1985). The phytic acid content of bread was reduced to about 40 per cent and 50 per cent after 2 h and 2 days leavening, respectively. One method for decreasing the phytate intake is the introduction of leavens and fermentation into rural breadmaking methods which increases the availability of zinc and calcium in bread and their uptake by the intestine is increased several folds. As a result, the likelihood of the development of mineral deficiencies would be decreased (Reinhold, 1975).

The phytate content of locust beans decreased from 51 mg in unfermented samples to 31 mg/100 g in fermented samples (Eka, 1980). A significant difference was observed in the non-fermented and fermented corn meal samples in their phytate phosphorus contents. The amount of phytate phosphorus decreased by 65 per cent by the fifth day of fermentation (Lopez et al., 1983). The reduction of phytate phosphorus was probably due to phytase elaborated by the bacteria of the fermentation since bacteria isolated during fermentation were later shown to breakdown phytic acid. Chompreeda and Fields (1984) found that natural lactic acid fermentation of corn meal decreased phytate phosphorus by 77.8 per cent.

Sutardi and Buckle (1985) reported reduction in phytic acid levels in soybeans during tempeh production, storage and frying. Tempeh was prepared from soybeans inoculated with the traditional Indonesian inoculum (usar) and two pure culture strains of the mould Rhizopus oligosporus. The phytic acid content of soaked soybeans was halved during tempeh fermentation and was further reduced when tempeh was stored for 72 h at 5 and 30°C. Deep fat frying of tempeh in peanut oil further halved the phytic acid content. Less than 10 per cent of the phytic acid remained after tempeh fermentation, storage

and frying. The reduction of phytic acid can be accounted for by the activity of phytase elaborated by the mould responsible for the fermentation (Lolas and Markakis, 1977).

Phytic acid in pearl millet was drastically reduced during natural lactic fermentation at all the temperatures, pH and periods of fermentation (Mahajan, 1986). First 24 h fermentation brought about most of the reduction; 40 and 50°C with 6 pH and 5 pH were the optimum conditions for phytic acid removal from pearl millet flour. Dhankher and Chauhan (1987c) reported that as the period of rabadi fermentation increased, a significant decrease in phytic acid content of the fermenting mixture was observed at all the temperatures. The decrease was maximum (27-30%) after nine h fermentation. Enzymatic hydrolysis of phytic acid by endogenous phytase of pearl millet (Mahajan and Chauhan, 1987) and that of fermenting microflora in rabadi may account for the loss of the antinutrient during fermentation. The low pH of the fermented product and the temperature of fermentation may provide favourable conditions for phytase activity.

Reduction of phytic acid also took place as a result of germination. After 2 days of germination, phytic acid decreased by 13 per cent in rapeseeds (Thompson and Serraino, 1985). This study suggested that phytase is present in rapeseed and its activity increases during germination.

2.2.7 Polyphenols

Polyphenolic compounds that are interchangeably called "tannins" by numerous investigators (Maxson and Rooney, 1972; Singh and Jambunathan, 1981; Deshpande and Salunkhe, 1982 and Griffiths and Moseley, 1980) are powerful reducing agents that are widely distributed in higher plants used as human food. Mahajan (1986) reported an appreciable amount of polyphenols (788 mg/100 g) in the pearl millet flour which may partly account for its relatively low nutritive value.

Polyphenols can complex with dietary proteins, reducing their digestibility and hence protein quality (Haslam, 1974; Bressani and Elias, 1980). They have also been shown to inhibit the activity of digestive enzymes especially of trypsin and amylase (Griffiths, 1979; Tamir and Alumot 1969; Singh, 1984). Davis and Hoseney (1979) suggested the selective adsorption of polyphenolic substances by starch and Deshpande and Salunkhe (1982) demonstrated the lowered in vitro starch digestibility due to their tannin adsorption. Thompson et al. (1984) observed a negative correlation between glycemic index and concentration of total polyphenols. The addition of tannic acid and phytic acid reduced the starch digestibility by 13 and 60 per cent after 5 h, respectively. Combined tannic and phytic acid reduced the digestibility at a level (63%) which did not differ significantly from that with only phytic acid.

Elias et al. (1979) studied the possible effects of seed coat polyphenolics on the nutritional quality of a protein. The results obtained suggest that the pigments present in the seed coat contained high levels of tannins and other related polyphenols. Polyphenols can react with protein, decreasing their digestibility and therefore, their quality. The findings of Aw and Swanson (1985) had shown that bean tannins adversely affected the nutritive value of black beans by decreasing the digestibility of the bean proteins. Protein efficiency ratio was inversely related to the tannin content.

Polyphenol content of rabadi decreased with an increase in the time of fermentation at all the temperatures (Dhankher and Chauhan, 1987c). At nine h fermentation, the loss in polyphenol content was the highest and varied from 10-12 per cent at different temperatures. The diminished levels of polyphenols may be due to activity of polyphenol oxidase of pearl millet grain or fermenting microflora. On the contrary, natural lactic fermentation

of pearl millet increased the polyphenols. The amount of polyphenols in the fermented product increased with increase in fermentation period except at 40°C where it decreased (Mahajan, 1986). Of all the initial pH (6,5,4 and 3), 3 was less effective. Increase in polyphenols could be limited to the lowest when fermentation was carried out with 3 pH at 40°C for 96 h or at 40°C at an initial pH 6 for 96 h.

Khokhar and Chauhan (1986) studied the effect of methods of domestic processing and cooking on the antinutritional factors in moth bean and reported reduction in phytic acid and polyphenols after sprouting of the moth beans.

2.2.8 Minerals

Calcium and phosphorus content of pearl millet varied from 69 to 83 and 274 to 302 mg/100 g, respectively (Dhillon et al., 1982). Kumar and Kapoor (1984) reported the calcium, manganese, copper, iron and zinc content of five different varieties of pearl millet as 0.90, 0.25, 0.40 to 0.67, 5.48 to 7.35, 1.52 to 2.04 and 1.90 to 2.50 mg/100 g, respectively. Chauhan et al. (1986) reported the calcium and phosphorus content of pearl millet to vary from 52 to 65 mg and 300 mg to 390 mg/100 g, respectively. According to Mahajan (1986), pearl millet contained 48.5, 302, 2.7, 16, 1.9 and 1.24 mg/100 g Ca, P, Zn, Fe, Mn and Cu, respectively.

Several investigators have reported that solubility of minerals in foodstuffs subjected to in vitro gastric or gastrointestinal digestion is indicative of their bioavailability from these foodstuffs (Lock and Bender, 1980; Miller et al., 1981; Rao and Prabhavathi, 1978; Schricker and Miller, 1982; Schricker et al., 1982; Schwartz et al., 1982; Wien and Schwartz, 1983; Wien and Schwartz, 1985).

Miller et al. (1981) described an in vitro method for estimating food iron availability. The method involved stimulated gastrointestinal digestion followed by measurement of soluble, low molecular weight iron. Mixture of foods (meals) were homogenized and exposed to pepsin at pH 2. Dialysis was used to adjust the pH to intestinal levels and digestion was continued after the addition of pancreatin and bile salts. Iron from the digestive mixture which diffused across a 6 to 8000 molecular weight cutoff semipermeable membrane was used as an indicator of available iron.

Kim and Zemel (1986) compared calcium solubility from sea mustard to that of spinach and milk in an in vitro digestive system with normal and reduced gastric acid concentrations. Aliquots of each were subjected to peptic and pancreatic digestions and ionic and total soluble calcium levels were measured as indices of potential availability.

According to Chompreeda and Fields (1984) extractable minerals are those soluble in 0.03 N HCl, the concentration of HCl found in stomach of adult. They incubated the food samples in a 37°C waterbath for 3 h with constant stirring to simulate conditions that occur in the human stomach (Peterson et al., 1943) and after filtering through an ashless filter paper, analyzed the filtrate for minerals. The HCl extractable minerals presented an index of their availability from the foods. Thus, the solubility or extractability of minerals in food subjected to in vitro gastric and intestinal digestions may prove to be a useful indicator of mineral bioavailability.

Harland and Harland (1980) tabulated the values for Ca, P, Mg, Cu, Fe, Mn and Zn in the fermented bread and found that the whole wheat fermented bread had more P, Mg, Fe, Mn and Zn. On the contrary, no effect of fermentation on total content of Ca, Mg, Zn and Fe was reported by Reddy

and Salunkhe (1980). Tangkongchitr et al. (1981) indicated that loss of phytate phosphorus by fermentation for 8 h was accounted by an increase in inorganic phosphorus.

When breads prepared from the fermented dough were fed, bioavailability of zinc improved significantly and rats showed highest weight gains and femur zinc content (Faridi et al., 1983). Fermentation increased the bioavailability of zinc from bread blended with bran in human beings (Navert and Standstrom, 1985).

Fermentation brought about conversion of the bound form of minerals to free form thus increasing the amount of extractable minerals (Ramakrishnan et al., 1976; Kunitarao, 1961). Phosphorus and iron were more extractable from corn meals after fermentation (Chompreeda and Fields, 1984). Fermentation of tef increased the dialysable portion of their Fe, P and Zn contents from 9, 16 and 7 per cent respectively, to 24, 60 and 43 per cent (Ramachandran and Bolodia, 1984). The increase in dialysable iron and hence its bioavailability may explain the rarity of Fe deficiency anaemia among tef eating population in Ethiopia.

Fermentation of rabadi did not alter the total contents of Ca, P, Fe, Cu, Zn and Mn. Period and temperature of fermentation, however, had significant effects on the HCl-extractability of phosphorus, calcium, zinc, iron and copper, but not of manganese. Iron was affected to the maximum at 45°C for nine h of fermentation when its extractability was increased by more than two and a half fold. Fermentation with isolated culture of lactobacillus also affected significantly the extractability of iron, phosphorus, calcium and zinc (Dhankher, 1985).

Mahajan (1986) reported that natural lactic fermentation had no effect on the total content of minerals but phosphorus and calcium extractability of

pearl millet increased significantly. Longer the period of fermentation, the higher was the extractability. Fermentation also brought about a significant increase in extractability of trace minerals including iron, zinc, copper and manganese in the pearl millet flour.

Rao and Deosthale (1983) studied the effect of malting on availability of iron and zinc. Malted samples of a varieties of pearl millet and six varieties of ragi were analysed for availability of iron and zinc. The availability of iron and zinc in the millet improved several folds on malting. The values for ionisable iron (mg/100 g) in raw and malted grain were 0.64 and 7.20 in millet and 0.29 to 2.98 in ragi; values for zinc were 2.04 and 5.25 in millet and 2.15 and 3.24 in ragi. The reduction in phytate on malting partly explained the improved availability of iron and zinc. Beal et al. (1984) reported improved zinc bioavailability from the pea sprouts. The decreased phytate resulting from germination was responsible for the enhanced response from the pea sprouts.

2.2.9 Vitamins (Thiamine)

Pearl millet contains 283 to 450 $\mu\text{g}/100\text{ g}$ thiamine (Rama Rao and Swaminathan, 1953). Simwemba (1984) reported a different level of thiamine i.e., 5.26 to 5.84 $\mu\text{g}/\text{g}$.

Thiamine content increased from 0.21 to 0.58 mg/100 g during the fermentation of idli. In khaman, thiamine content increased from 0.53 to 0.79 mg/100 g (Rajalakshmi and Vanaja, 1967). On the contrary, no change was found in the thiamine content during fermentation of idli, possibly because of different technique of fermentation employed by Ananthachar and Desikachar (1962). Lee et al. (1980) reported that fermentation of rice in water at room temperature for 4 days brought about an increase in the riboflavin content whereas thiamine content decreased by 50 per cent. During the fermentation, riboflavin doubled, niacin increased 7 times, and

vitamin B₁₂ activity increased 33 times. According to Steinkraus et al. (1967) thiamine decreased from 10 µg in soybean to 4 µg/g in tempeh.

Quantitative nutritive changes occur in milk during fermentation. Rao and Basu (1952) demonstrated that the vitamin content of dahi depends upon the type of organism used for fermentation. They found that a mixture culture of L.bulgaricus and L.cremoris decreased thiamine, whereas, single culture of S.lactis and S.cremoris raised the thiamine concentration from 2 to 20 per cent over the milk.

Natural lactic acid fermentation of cereal foods resulted in an increase of riboflavin (Wagner-Jauregg, 1972; Hamad and Fields, 1979; Tongnual and Fields, 1979; Kazanas and Fields, 1981). But niacin and thiamine decreased significantly during fermentation (Tongnual and Fields, 1979). Paul (1979) found that thiamine decreased at 25°C during the 4th day of fermentation of sorghum. Murdock and Fields (1984) studied the B-vitamin content of corn meal after 24, 48, 72 and 96 h of natural lactic acid fermentation. Compared to the non-fermented control, all fermented samples had significantly higher level of vitamin B₁₂, riboflavin and folacin. Samples fermented for 96 h had a significant increase in pantothenic acid content. Samples fermented for 24 h had lower levels of thiamine.

Aliya and Geervani (1981) analysed fermented products of legumes, bengal gram dhal, green gram dhal and millets like pearl millet, sorghum and ragi for vitamin B content. The thiamine and riboflavin content of both legumes and millets increased with increase in fermentation time. Steaming and cooking after fermentation reduced the thiamine and riboflavin content.

Robinson and Kao (1977) observed an increase in the water soluble vitamins except thiamine and cobalamine during the fermentation of tempeh and miso prepared from chickpea, horsebean and soybean. Zamora and Fields (1979a) reported that thiamine content remained unchanged during fermentation of cowpea and chickpea.

Dhankher and Chauhan (1987a) reported a significant increase in the level of thiamine, riboflavin and niacin during the rabadi fermentation; maximum increase occurred at 35°C.

2.2.10 Biological utilisation

Kappuswamy et al. (1957) found that feeding of pearl millet diets to rats at 5 to 6 and 9 to 11 per cent level of protein gave protein efficiency ratio of 1.2, and 1.4 to 1.8 whereas BV was 83 and 84 per cent, respectively. Naryana Rao et al. (1961) also observed 1.2 PER at 5 to 6 per cent level of protein of pearl millet. Kurien et al. (1961) studied the effect of partial or complete replacement of rice in poor vegetarian diets by pearl millet and reported that at 10 per cent level of protein intake, PER was 1.43 for millet as compared to 1.20 for wheat. Hariharan et al. (1965) reported that PER of pearl millet was 1.67 at 11 per cent protein intake. Pushpamma et al. (1972) reported 1.84 PER at 11 per cent of pearl millet protein.

Hesseltine and Shibasaki (1961) prepared miso using pure culture of Saccharomyces rouxii and reported the net protein utilisation (NPU) of miso to be about 72 which was higher than the NPUs of its ingredients (rice, 70; barley, 60 and soybean, 61). Tempeh prepared from soybean was harvested after 0,12,24,36,48,60 and 72 h fermentation, freeze dried, and fed to rats (10% protein level) in an attempt to determine the amount of fermentation needed to develop optimum nutritive value. The rats, found tempeh less palatable as they consumed less food. However, apparent digestion coefficient (%) and PER remained very nearly the same throughout the course of the fermentation (Hackler et al., 1964). Zamora and Veum (1979) reported an increase in the average daily weight gain for weanling rats fed soybean

fermented with R. oligosporus compared with the daily weight gains for rats fed soybeans given the same heat treatment (121°C for 30 min) but not fermented. The fermented soybeans also had a greater apparent biological value and net protein utilisation.

Khader (1983) did nutritional studies on fermented, germinated and baked soybean preparations. The feeding tests on rats showed that the processing of soybean considerably increased its PER to a level still below that of casein. Khader (1982) fed a fermented product like miso prepared from rice, bengal gram and curds or yeasts and reported an increase in the PER values. Khader (1979) fed dhokla to rats at 8 per cent protein level and reported 2.97 PER and 92.5 per cent digestibility. Liver weights of the rats given skim milk as a control milk were slightly greater than those given dhokla while no difference was found in the weight of other organs.

Weanling rats fed on idli and khaman were found to be superior to those fed on the corresponding unfermented product with regard to weight gain and nitrogen retention. When the soy ogi was fed to rats, PER increased three folds (Akinrele and Edward, 1971). Weight gain and liver protein level of rats given fermented idli were higher than the rats fed unfermented soy idli (Akolkar and Parekh, 1983).

Klopfenstein et al. (1985) fed rats diets consisting of sorghum grain supplemented with minerals and vitamins, pearl millet grains supplemented with minerals and vitamins, unsupplemented sorghum and pearl millet, pearl millet weaning food (cooked milled flour with skim milk powder, vegetable oil and sugar). They reported that supplemented pearl millet gave better weight gains than did supplemented sorghum. Lin et al. (1986) fed fermented bread with Candida utilis to rats and found improved PER i.e. 2.28 while that of unfermented bread was 1.31.

Chaudhary and Atreja (1985) fed shrikhand a fermented milk product to rats at 10 per cent protein level. Streptococcus lactis, S. thermophilus and Lactobacillus bulgaricus were used. The BV, digestibility coefficient, PER, NPU of chakkas were similar to that of casein. Vass et al. (1984) fed fermented milk products viz., Yoghurts, kefir, sour milk to rats and found the maximum increments of body mass, highest value of the protein utilisation index and PER. It was attributed to the better digestibility of these fermented milk products.

2.2.11 Organoleptic values

Khader (1982) prepared a fermented food similar to miso from rice, bengal gram and curd or yeast. The fermented foods when fried were well accepted by a tasting panel.

Taur et al. (1984a) did sensory evaluation of the chips made from control, germinated and germinated-fermented grain sorghum. They soaked 100g cleaned sample in 300 ml tap water for overnight. After that germination was carried out at 25°C for 120 h. Samples were washed with water twice to prevent mould growth. To 50g germinated, cleaned and ground samples, added 200 ml tap water and allowed natural lactic acid fermentation for 24 h at 25°C. Thereafter the samples were dried at 50°C for 40-48 h. Samples were ground and used for preparing chips. The sorghum chips were prepared from non-germinated fermented (control), 120 h germinated and 120 h germinated - 24 h fermented sorghum meals. Ingredients included 100 g of sorghum meal, 3 g salt, 4 g sugar, 60 ml water and 1 per cent baking powder. A dough was prepared and rolled thinly (1/8 inch thick) with a rolling pin. The dough was divided into strips of about 1 x 1 inch and deep fried in safflower oil at 160°C for one min. Chips were organoleptically evaluated for colour, flavour, taste, texture and overall acceptability. The chips made from germinated and germinated-fermented grain sorghum varieties were in "neither like nor dislike" category in overall acceptability.

CHAPTER-III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Grains

Pearl millet grains, used for fermentation, were procured from the local market in a single lot. It was cleaned of broken seeds, dust and other foreign material. The grains were coarsely ground on the day of fermentation by an electric grinder using 2.5 mm sieve.

3.1.2 Microbial cultures

Four cultures namely, Saccharomyces cerevisiae, Saccharomyces diastaticus, Lactobacillus brevis and Lactobacillus fermentum were obtained from the Director, National Chemical Laboratory, Poona.

3.2 Selection of fermentation temperatures

Distilled and sterile water (400 ml) was added to the freshly ground (150 g) pearl millet flour and mixed by stirring sufficiently with a glass rod. The slurry was allowed to ferment at 20,25, 30,35, 40,45 and 50°C for 72 h. Sensory evaluation of the fermented samples for aroma and colour was conducted by a semi-trained panel of judges by employing a 9 point hedonic scale. The judges were drawn from different constituent colleges of the Haryana Agricultural University, Hisar. The score sheet of the taste panel was as follows:

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 nor dislike	5		

A typical proforma used for organoleptic analysis is given in Appendix 1.

On the basis of sensory evaluation, three temperatures of natural lactic fermentation (20,25 and 30°C) which gave acceptable product were selected.

3.3 Fermentation

Various types of the fermentations conducted in the study included natural lactic fermentation of pearl millet flour and pure culture fermentation of the flour as well as sprouts.

3.3.1 Natural lactic fermentation

Natural lactic fermentation of freshly ground pearl millet flour, as discussed above (3.2), was carried out at 20,25 and 30°C in a B.O.D. incubator for 72 h. The fermented product was analysed for microbial composition (3.4), pH and titratable acidity.

In another experiment, the pearl millet flour (100 g) was mixed with distilled water (900 ml) in a conical flask and autoclaved at 15 psi for 15 min. After cooling the autoclaved mixture, 10 g freshly ground pearl millet was added as inoculum. Fermentation was then carried out at 20,25 and 30°C in a B.O.D. incubator for 72 h. After the expiry of the fermentation period about 50 ml of the slurry was taken out for determining pH, titratable acidity and the composition of fermenting microflora. The remaining fermented product was oven dried in petriplates for 48-72 h at 65°C to a constant weight. The dry fermented sample was finely ground in a cyclone mill using 0.5 mm sieve size and stored in air tight plastic containers at room temperature for further analysis.

3.3.2 Pure culture fermentation

Two types of fermentations were carried out with pure cultures:

- i) Fermentation of pearl millet flour
- ii) Fermentation of pearl millet sprouts

3.3.2.1 Preparation of stock cultures

3.3.2.1.1 Lactobacillus

The lyophilised cells in ampules were transferred to lactobacillus broth (composition given in Table 1) under aseptic conditions and the inoculated broth was incubated at 37°C for 48 h in a B.O.D. incubator. A loopful of lactobacilli growth from the broth was streaked on the lactobacillus agar plates and again incubated for 48 h at 37°C. After the colonies developed, a single pure colony was picked up and its gram stained slide was examined microscopically. The confirmation for lactobacilli was also made by performing catalase test. These lactobacilli cultures were found to be gram +ve, rod shaped and catalase negative.

Stock cultures of the lactobacilli were maintained on lactobacilli agar by stab inoculation. After incubating at 37°C for 24-48 h, the stock culture tubes were stored in a refrigerator. For maintenance, the cultures were transferred regularly at 2-4 week intervals.

3.3.2.1.2 Yeast

A loopful of yeast growth from the slants of yeasts obtained from NCL, Poona, were transferred to YEPDA broth (composition given in Table 2) and incubated at 30°C for 24-48 h. The inoculated broth was streaked on the YEPDA plates and the colonies were isolated. A single isolated colony was picked up and examined microscopically. After microscopic confirmation, these isolated and purified colonies of the yeasts were maintained on YEPDA slants. After incubation at 30°C for 48 h, the tubes were stored in refrigerator. Subculturing was done once a month.

Table 1

Composition of lactobacillus broth*

INGREDIENTS	AMOUNT
Glucose	500mg
Lactose	500mg
Liver extract	600mg
Sodium acetate	600mg
Yeast extract	500mg
Salt A	0.5 ml
Salt B	0.5 ml
Volume	100 ml
pH	7.6
Salt A	
KH_2PO_4	25 g
K_2HPO_4	25 g
Dissolved the above salts in water and made up the volume to 250 ml.	
Salt B	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	10 g
NaCl	500 mg
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	500 mg
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	500 mg
Dissolved the salts in water and made up the volume to 250 ml.	

*As supplied by the Director, National Chemical Laboratory, Poona, alongwith the cultures.

Table 2

Composition of YEPDA medium*

INGREDIENTS	AMOUNT
Malt extract	0.2g
Glucose	1.0g
Yeast extract	0.3g
Peptone	0.5g
Volume	100 ml
pH	6.4-6.8
Agar	1.5 g/100 ml

*As supplied by the Director, National Chemical Laboratory, Poona, along with the cultures.

3.3.2.2 Preparation of inoculum

The day prior to use, actively growing cells from the stock culture were transferred to the Roux bottle having a slant of the MRS/YEPDA medium. After incubating at 37°C for lactobacillus and 30°C for yeast for 24 h, the microbial growth in the Roux bottle was harvested with measured amount of sterile saline containing glass beads and transferred under aseptic conditions to a conical flask having a known volume of sterile saline. The optical density of the microbial suspension was measured and the cell number was counted after giving appropriate dilutions. Optical density of the microbial suspension corresponding to 10^5 cells/ml was determined in case of yeast and lactobacillus. Inoculum containing 10^5 cells/ml was prepared in case of both yeast and lactobacillus and used for various types of pure culture fermentations. The inoculum having 10^5 cells/ml corresponded to 0.111, 0.137, 0.343 and 0.311 OD in case of S. diastaticus, S. cerevisiae, L. brevis and L. fermentum, respectively. This formed the basis of using the amount of inoculum for the fermenting pearl millet flour. The number of cells in the fermenting mixture after adding the inoculum was estimated in order to confirm that the pearl millet flour contained the desirable initial count (10^5 cells of lactobacillus/yeast per ml) necessary for further fermentation.

3.3.2.3 Preparation of pure culture fermented samples

3.3.2.3.1 Pure culture fermentation of pearl millet flour

Pearl millet flour (100g) was mixed with distilled water (900 ml) and autoclaved at 15 psi for 15 min. After cooling, it was inoculated with the microflora and incubated at 30°C for 72 h. Pure cultures of S. diastaticus, S. cerevisiae, L. brevis and L. fermentum were used for carrying out single, mixed and sequential fermentations. For single culture fermentation, the

inoculum of any one of the microorganisms viz., S. diastaticus, S. cerevisiae, L. brevis and L. fermentum which supplied 10^5 cells/ml to the fermenting mixture was added and incubated at 30°C for 72 h.

In case of mixed fermentation, a combination of yeast (10^5 cells/ml) and lactobacillus (10^5 cells/ml) was inoculated together. Four different types of mixed fermentations included S. diastaticus and L. brevis (SdLb), S. cerevisiae and L. brevis (ScLb), S. diastaticus and L. fermentum (SdLf) and S. cerevisiae and L. fermentum (ScLf).

In case of sequential fermentation, firstly, yeast inoculum providing 10^5 cells/ml of the fermenting mixture was used and the samples were fermented at 30°C for 72 h. After the expiry of the first fermentation, the same sample was inoculated with Lactobacillus spp (10^5 cells/ml) and again fermented at 30°C for 72 h more. Thus the total fermentation time was 144 h. Four types of samples were prepared by sequential fermentation i.e. S. diastaticus followed by L. brevis (Sd+Lb), S. cerevisiae followed by L. brevis (Sc+Lb), S. diastaticus followed by L. fermentum (Sd+Lf) and S. cerevisiae followed by L. fermentum (Sc+Lf).

3.3.2.3.2 Pure culture fermentation of pearl millet sprouts

Pearl millet grains were washed and soaked in distilled water overnight. The soaked grains were sprouted at 30°C for 24 h in a B.O.D. incubator. The sprouts (100g) were mixed with water (200 ml), ground to paste in an electric blender and autoclaved at 5 psi for 20 min. After cooling, it was inoculated with inoculum giving 10^5 cells of yeast and 10^5 cells of the lactobacillus per ml of the fermenting medium as in the case of mixed fermentation of pearl millet flour. The mixture was fermented at 30°C for 72 h in a B.O.D. incubator.

After the expiry of the fermentation period, about 50 ml slurry was taken out for studying the microbial composition and determining pH and titratable acidity. Rest of the sample was oven dried in petriplates at 65°C to a constant weight. It was finely ground in the cyclone mill using a 0.5 mm sieve and stored in plastic containers for further analysis.

3.4 Microbial analysis

The naturally fermented samples were analysed for standard plate count (SPC 30°C), lactobacillus, yeast, fungus and coliforms.

3.4.1 Standard plate count (SPC 30°C)

Standard plate count (SPC 30°C) was done by pour plate method employing Plate Count Agar medium. One ml fermented pearl millet slurry was added to nine ml sterile water blank. Further dilutions from 10^{-2} to 10^{-7} were made. The various dilutions were pour plated and incubated at 30°C for 48 h. The colonies developed were counted.

3.4.2 Lactobacillus

Lactobacilli were enumerated using MRS medium. One ml fermented slurry was added to nine ml sterile water blank. Further dilutions upto 10^{-7} were made. Each dilution (0.1 ml) was inoculated in petriplates containing solid MRS medium. Plates were incubated at 37°C for 24 h and the colonies were counted.

3.4.3 Coliform

Coliform was counted by using MacConkey's Agar. One ml fermented slurry was added to nine ml sterile water blank and thoroughly mixed. Further dilutions upto 10^{-7} were made. Each dilution (1 ml) was pour plated in sterilized petri dishes. The dishes were incubated at 30°C for 48 h and the brick red colour colonies were counted.

3.4.4 Yeast

Yeast count was done by pour plate method using Potato Dextrose Agar (PDA) medium (pH 3.5). One ml of the fermented slurry was added to nine ml sterile water blank and mixed thoroughly. Further dilutions upto 10^{-7} were made and each dilution was pour plated. The petridishes were incubated at 25°C for 72 h and the developed colonies were counted.

3.4.5 Fungus

Fungi were enumerated using Potato Dextrose Agar (PDA) medium by pour plate method as mentioned above for yeast.

3.4.6 Composition of media (Harrigan and McCance, 1976)

i) MRS Agar

Peptone	10g	Meat extract	10g
Yeast extract	5g	D-glucose	20g
Tween 80	1ml	Dipotassium Hydrogen phosphate	2g
Sodium acetate	5g	Triammonium citrate	2g
Magnesium sulphate	0.2g	Manganese sulphate	0.5g
Distilled water	1 L	Agar	20g
pH	6.2-6.6(adjusted with HCl)		

ii) MacConkey's Agar

Peptone	20g	Bile salts	5g
Sodium chloride	5g	Lactose	10g
Bromocresol purple (1% solution)	1ml	Distilled water	1 L
Agar	20g	pH	7.4(Adjusted with HCl)

iii) Potato Dextrose Agar (PDA)

Potato peeled and diced	200g	D-glucose	20g
Agar	20g	Distilled water	1 L
pH	3.5(Adjusted with tartaric acid)		

iv) Plate Count Agar (PCA)

Tryptone	5g	Yeast extract	25g
Dextrose	1g	Agar	15g
Distilled water	1L	pH	7(Adjusted with HCl)

3.5 Titratable acidity and pH

Titratable acidity was determined as g lactic acid per 100 ml by the method of Amerine et al. (1967).

Reagents:

- i) 0.01 N NaOH: 0.4 g NaOH dissolved in water and made to one litre.
- ii) Phenolphthalein indicator (1%) in 80% ethyl alcohol.

Estimation:

Pipetted five ml sample in 250 ml conical flask. Added about 75 ml boiling water and five drops of phenolphthalein indicator. Read the level of 0.01 N NaOH in the burette. Added NaOH slowly to the sample until finally one drop gave a pink colour lasting for a minute or longer. Read the burette again. The amount of alkali in ml used times 0.150 equalled the total acidity expressed as g lactic acid per 100 ml.

The pH was measured by a pH meter against a standard buffer of 4.0 pH.

3.6 Nutritional evaluation

3.6.1 Crude protein, fat and ash

3.6.1.1 Crude protein

Reagents:

- i) N/100 H_2SO_4
- ii) Boric acid (4%)
- iii) Mixed indicator solution: Took 0.5 g bromocresol green and 0.1 g methyl red and dissolved in 100 ml 95% ethanol and adjusted the solution with drops of dilute NaOH or HCl to bluish purple colour.

iv) NaOH (45%)

v) Digestion mixture: 10 g K_2SO_4 , 0.5g $CuSO_4 \cdot 5H_2O$ and 2 g $FeSO_4$

Procedure:

Took 1 g sample and digested with 25 ml conc H_2SO_4 and a pinch of digestion mixture. The nitrogen in ammonical salt was distilled with 45 per cent NaOH in a Kjeldahl apparatus. The ammonia liberated was absorbed in 10ml boric acid solution containing a few drops of mixed indicator and titrated against standard H_2SO_4 (N/100). The end point was indicated by the change of colour. A factor 6.25 was applied to convert the amount of nitrogen to crude protein.

3.6.1.2 Crude fat

Crude fat was estimated by employing the standard method of analysis (AOAC, 1980) using the Soxhlet extraction apparatus.

Procedure:

Weighed about 5 g of dry sample and transferred it to an extraction thimble dried overnight at $105^\circ C$. Placed the thimble in a Soxhlet extractor fitted with a condenser and flask containing sufficient petroleum ether. The extraction was carried out for 6 h. After the extraction, thimble was removed with the sample from the extraction apparatus and dried in the hot air oven to a constant weight. Cooled in a desiccator to room temperature and weighed. The loss in the weight of the thimble was the estimate of the ether extract in the sample.

3.6.1.3 Ash

Ash in the sample was estimated by employing the standard method of analysis (AOAC, 1980).

Procedure:

Took 5 g oven dried sample in the weighed crucible. Ignited it till no charred particles remained in the crucible. Then put the

crucible in muffle furnace (550°C) for 5-6 h or till a white ash was obtained. Cooled the crucible in a desiccator and weighed. The loss in weight represented the organic matter and residue, the ash content.

3.6.2 Available carbohydrates

Total soluble sugars other than starch were extracted according to the procedure of Cerning and Guilhot (1973).

Added 25 ml ethanol (80%) to 500 mg sample in a round bottomed flask. The flask was connected to a condenser and kept on a heating mantel for 30 min with occasional stirring. The extract was cooled, centrifuged at 8000 rpm for 15 min and the supernatant collected. The above procedure was repeated twice, each time extracting the residue in 25 ml 80% ethanol. The combined extract in the beaker was evaporated to dryness on a boiling waterbath. The residue was dissolved in distilled water and made to 50 ml.

3.6.2.1 Total soluble sugars

Total soluble sugars were estimated by the method of Yemm and Willis (1954).

Reagents:

- i) Standard sugar solution: Dissolved 25 mg glucose in water and made to 100 ml. This solution contained 250 µg glucose per ml. For obtaining a standard curve, 0.1 ml to 1.0 ml of this solution was used.
- ii) Anthrone reagent (0.2% anthrone in 70% H_2SO_4): This reagent was prepared fresh daily and allowed to stand for 30 -40 min before use.

Estimation:

Freshly prepared 10 ml anthrone reagent was pipetted in a test tube (150x25 mm), chilled and kept in ice cold water. One ml of the sugar extract was taken and diluted to 10 ml with water. Out of the diluted sugar extract, one ml was taken and was layered on the anthrone reagent. After cooling for

3-5 min, the contents were thoroughly mixed while still immersed in ice cold water. The contents in the tube were heated vigorously in a boiling waterbath for 10 min and then immediately cooled in cold water. The absorbance was then read at 625 nm against a suitable blank. 0.108 OD in the standard curve corresponded to 50 μ g glucose.

3.6.2.2 Reducing sugars

Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945).

Reagents:

- i) Copper reagent A: Dissolved 25 g anhydrous sodium carbonate, 25g potassium sodium tartarate, 20g sodium bicarbonate and 200 g anhydrous sodium sulphate in about 800 ml distilled water and diluted to one litre.
- ii) Copper reagent B: Dissolved 15 g CuSO_4 in 100 ml distilled water containing two drops of HCl.
- iii) Arsenomolybdate reagent: Dissolved 25g ammonium molybdate in 450 ml distilled water by warming. Added 21 ml conc H_2SO_4 with stirring. Three g sodium hydrogen arsenate dissolved in 25 ml distilled water was added with stirring. The solution was kept in an incubator at 37°C for 24 h before use. This reagent in a glass stoppered brown bottle was stored in refrigerator.
- iv) Copper A and B were mixed in the ratio of 25: 1(v/v) before use.
- v) Standard sugar solution: Dissolved 25 mg glucose and made to 100 ml with water. This contained 250 μ g glucose/ml.

Estimation:

One ml test extract obtained in 3.6.2 was taken in a blood sugar

tube graduated at 25 ml. One ml mixed copper reagent (iv) was added and then heated for 20 min in a boiling waterbath. To this one ml arsenomolybdate reagent was added, mixed thoroughly and the contents diluted to 25 ml. A stable blue colour appeared quickly which was read at 520 nm against a suitable blank. The amount of reducing sugar was then determined by referring to the glucose standard curve.

3.6.2.3 Non-reducing sugars

The amount of non-reducing sugars was calculated as the difference between total soluble sugars and reducing sugars.

3.6.2.4 Starch

Starch from the sugar-free pellet obtained after centrifugation in 3.6.2 was estimated by the method of Clegg (1956).

Extraction:

Added 5 ml water to the aforesaid residue of test material and while stirring added 6.5 ml of 52% perchloric acid. Stirred the contents continuously for five min and then occasionally for next 15 min. Added 20 ml water and centrifuged. Collected the supernatant in a 100 ml volumetric flask. Added 5 ml water to the residue and repeated the extraction with 52% perchloric acid, stirring occasionally for next 30 min. Washed the contents of the tube into a volumetric flask containing the test extract and made it to 100 ml with water. It was then filtered discarding first 5 ml of filtrate. A suitable aliquot of the extract was used for glucose estimation using anthrone reagent by the method of Yemm and Willis (1954) as described under 3.6.2.1. Starch was calculated using the following formula:

$$\text{Starch} = \text{Glucose} \times 0.9$$

3.6.3 Starch digestibility (in vitro)

In vitro starch digestibility was assessed by employing pancreatic amylase (Singh et al., 1982).

Reagents:

- i) 0.2 M Phosphate buffer (pH 6.9): Fifty ml 0.2 M potassium dihydrogen phosphate was added to 46.8 ml 0.2 M disodium hydrogen phosphate and made up to 200 ml.
- ii) Pancreatic amylase: Twenty mg pancreatic amylase (Sigma Chemical Company, USA) was dissolved in 50 ml 0.2 M phosphate buffer (pH 6.9).
- iii) Dinitrosalicylic reagent: Dissolved 10 g 3,5-dinitrosalicylic acid, 300 g sodium-potassium tartarate and 16 g NaOH in carbon dioxide-free water and made to 1000 ml. The reagent was stored in brown bottle and protected from carbon dioxide.
- iv) Standard maltose solution: Dissolved 100 mg maltose monohydrate in water and made upto 100 ml.

Estimation:

A suitable amount of defatted sample (25 mg) was dispersed in one ml 0.2 M phosphate buffer, pH 6.9. Added 0.5 ml pancreatic amylase to sample suspension and incubated in water bath at 37°C for two h. After the incubation period was over, 2 ml dinitrosalicylic acid reagent was quickly added and the mixture was heated for 5 min in a boiling water bath. After cooling, the solution was made to 25 ml with distilled water and filtered prior to measurement of absorbance at 350 nm. A blank was run simultaneously by incubating the sample; the dinitrosalicylic acid reagent was added before addition of the enzyme solution. Maltose was used as standard and values were expressed as mg maltose released per g defatted sample. Standard curve was prepared by taking 0.8 to 8 mg maltose from a standard maltose solution. 0.140 OD corresponded to 1.6 mg maltose.

3.6.4 Protein digestibility (in vitro)

In vitro digestibility of protein was carried out by the method of Akeson and Stahmann (1964) as modified by Singh and Jambunathan (1981).

Reagents:

- i) Pepsin: Dissolved 40 mg pepsin (3000 unit) in HCl (pH 2.0, prepared by diluting the acid with water) and made upto 100 ml.
- ii) 10% Trichloroacetic acid (TCA)
- iii) 5% TCA
- iv) 0.1 M Borate buffer (pH 6.8):
 - a) 0.2 M Boric acid: Dissolved 12.4 g boric acid in water and made the volume to one litre.
 - b) 0.05 M Borax solution: Dissolved 19.05 g borax in water and made the volume to one litre.

For preparing the borate buffer, added 140 ml 0.2 M boric acid, 50 ml distilled water and a few drops of 0.05 M borax solution to adjust the pH at 6.8.
- v) 0.1M Borate Buffer (pH 6.8) containing 0.025 M calcium chloride was prepared by dissolving 2.75 g calcium chloride in one litre of the buffer.
- vi) Pancreatin: Dissolved 50 mg pancreatin (Sigma Chemical Company, USA) in 100 ml 0.1 M borate buffer (pH 6.8) containing calcium chloride.
- vii) 0.2 N NaOH

Procedure:

Took 200 mg ground sample in a test tube, added 5 ml pepsin solution to it and incubated the contents at 37°C for 16 h in a water bath shaker. A few drops of toluene were added to each tube to check the growth of microbes

After incubating for 16 h, the pH was adjusted to 7.0 with 0.2 N sodium hydroxide solution. Then 2 ml pancreatin solution was added to each tube and incubated at 37°C in a water bath shaker for 24 h. Then 7 ml 10% trichloroacetic acid was added. The contents were centrifuged at 12,000 rpm for 20 min and the residue washed twice with 5 ml of 5% TCA. The supernatants were pooled and the volume was made upto 25 ml with 5% TCA. Five ml aliquot was taken and dried at 80-90°C in a hot air oven and the nitrogen contents (digestible) determined by MicroKjeldahl method (AOAC, 1980). The digested protein of the sample was determined by multiplying N content by 6.25 factor. Protein digestibility was calculated by the following formula:

$$\text{Protein Digestibility(\%)} = \frac{\text{Digested protein} \times 100}{\text{Total protein}}$$

3.6.5 Antinutritional factors

3.6.5.1 Phytic acid

Phytic acid was determined by the method of Haug and Lantzsch (1983).

Reagents:

- i) Phytate reference solution: Dissolved 30.54 mg sodium phytate (5.5% water, 97% purity and containing 12 Na/mole) in 100 ml 0.2 N HCl which gave a solution containing 200 µg phytic acid per ml.
- ii) Ferric ammonium sulphate solution: Dissolved 0.2 g ferric ammonium sulphate in 100 ml 2 N HCl and made to 1000 ml with water.
- iii) Bipyridine solution: Dissolved 10 g 2-2' bipyridine and 10 ml thioglycollic acid in water and made to 1000 ml.

The solutions are stable for several months at room temperature.

Extraction:

One g finely ground sample was extracted with 25 ml 0.2 N HCl for three h with continuous shaking in a shaker. After proper shaking, it was

filtered through Whatman # 1 filter paper.

Estimation:

Pipetted 0.5 ml of the above extract into a test tube fitted with a ground glass stopper. Added one ml ferric ammonium sulphate solution. Heated the tube in a boiling water bath for 30 min. Mixed the contents of the tube and centrifuged for 30 min at 3000 rpm. Transferred one ml supernatant to another test tube and added 1.5 ml bipyridine solution. Measured the absorbance at 519 nm against distilled water. For plotting a standard curve different concentrations i.e., 0.2 to 1.0 ml of standard sodium phytate solution containing 40 - 200 µg phytic acid were taken and made to 1.4 ml with water. 0.5 OD corresponded to 120 µg phytic acid.

Phytate phosphorus was derived by using the following formula:

$$\text{Phytate phosphorus(mg)} = \frac{A \times 28.18}{100}$$

where, A = Phytate content (mg).

3.6.5.2 Polyphenols

The polyphenolic compounds were extracted from the defatted sample (500 mg) by refluxing with 50 ml methanol containing 1% HCl for four h (Singh and Jambunathan, 1981). The extract was concentrated by evaporating methanol on a boiling water bath and brought its volume to 25 ml with methanol-HCl. The amounts of phenolic compounds were estimated as tannic acid equivalent according to Folin-Denis procedure (Swain and Hills, 1959).

Reagents:

- i) **Folin-Denis Reagent:** Added 100 g sodium tungstate, 20 g phosphomolybdic acid, 50 ml phosphoric acid to 750 ml water and refluxed for 2h, cooled and diluted it to one litre.
- ii) **Tannic acid solution:** Dissolved 100 mg tannic acid in water and made upto one litre. Twenty ml of this stock solution were further diluted

to 100 ml with water to give working standard solution which contained two μg tannic acid per ml.

- iii) Saturated sodium carbonate solution: Dissolved 350 g sodium carbonate in one litre of water at 70°C to 80°C, cooled, seeded and filtered through glass wool.

Estimation:

Test solution (0.2 ml) was diluted to 8.5 ml with water in a test tube. After thorough mixing, added 0.5 ml Folin-Denis reagent and the tube was well shaken. Exactly after three min, one ml saturated sodium carbonate solution was added and the tube was thoroughly shaken again. After one h the absorbance was read at 725 nm using a suitable blank. If the solution was cloudy or precipitates appeared, it was centrifuged before readings were taken.

A standard curve was plotted by taking 0.5 ml to 4.0 ml working tannic acid standard solution (containing 10 μg to 80 μg tannic acid). 0.425 OD corresponded to 50 μg tannic acid.

3.6.6 Mineral composition

3.6.6.1 Digestion

One g ground sample was taken in a 150 ml conical flask. To this 25-30 ml diacid mixture (HNO_3 : HClO_4 ::5:1 v/v) was added and kept overnight. Next day it was digested by heating till clear white precipitates settled down at the bottom. The crystals were dissolved by diluting in double distilled water. The contents were filtered through Whatman # 42 filter paper. The filtrate was made to 50 ml with double distilled water and was used for determination of total Ca, P, Zn, Cu, Fe and Mn.

3.6.6.2 Calcium

Calcium in the digested sample was determined by titration method of Vogel (1962).

Reagents:

- i) Standard CaCO_3 (N/100). Dissolved 0.5 g CaCO_3 in 6N HCl and made the volume to one litre with water.
- ii) EDTA (N/100): Dissolved 1.86 g EDTA (Disodium salt) in water and made the solution to 1000 ml.
- iii) NaOH solution (1%)
- iv) Hydroxylamine hydrochloride solution(5%).
- v) Triethanolamine
- vi) Calcon indicator: Dissolved 0.4 g calcon in 100 ml methanol.
- vii) Polyvinyl alcohol: one per cent solution in water.

Procedure:

Pipetted a suitable volume of aliquot (10ml) in a conical flask and added about 10 ml water. Added 10% NaOH drop by drop to adjust the pH to neutrality. Added ten drops of 5% hydroxylamine hydrochloride solution with continuous stirring. Added ten drops of triethanolamine followed by 2.5 ml of 1% polyvinyl alcohol. Added 2 ml 10% NaOH. Finally added a few drops of calcon indicator. A violet colour appeared which was then titrated against N/100 EDTA solution to give bluish green end point.

Calculations:

$$\text{Ca meq./lt} = \frac{\text{EDTA used} \times \text{NEDTA} \times 1000}{\text{Aliquot taken}}$$

$$\text{mg Ca/100 g} = \frac{\text{Ca(meq/lt)} \times 20 \times 50 \times 100}{1000}$$

where 50 was the volume of digested material made from one g sample.

3.6.6.3 Phosphorus

Phosphorus was determined colorimetrically by the method of Chen et al. (1956).

Reagents:

- i) Ascorbic acid (10%)
- ii) Ammonium molybdate (2.5%)
- iii) Reagent C: Mixed 6 N H_2SO_4 , water, 2.5% ammonium molybdate and 10% ascorbic acid in the ratio of 1:2:1:1 (v/v), respectively. This reagent was prepared fresh every day.
- iv) Standard phosphorus solution: Dissolved 0.351 g pure and dry anhydrous monopotassium dihydrogen orthophosphate in a few ml of water and 10 ml 10N H_2SO_4 . The volume was made to one litre with water. This stock solution contained 80 μg P/ml. Diluted 25 ml stock solution to one litre which served as working standard solution. It contained 2 μg P/ml. Two to three drops of chloroform were added for preserving the solution.

Procedure:

Pipetted a suitable aliquot (1 ml) of the mineral extract in a test tube and made the volume to 4 ml with water. Added 4 ml Reagent C and mixed well. Incubated the contents at 37°C in a water bath for 90 min. Removed and allowed to cool to room temperature and read absorbance at 820 nm against a suitable blank. Standard curve was plotted using one to eight μg P. 0.2 OD corresponded to 2 μg P.

3.6.6.4 Inorganic phosphorus

One g sample was extracted in 20 ml water by shaking on a rotary shaker at room temperature for 3 h. The suspension was filtered in a Whatman # 42 filter paper with water rinsings and made to 50ml. Inorganic P

in the filtrate was determined by the method of Chen et al. (1956) as described in 3.6.6.3.

3.6.6.5 Trace minerals

Trace minerals including iron, zinc, copper and manganese in acid digested samples were determined by the Atomic Absorption Spectrophotometer AA 120, in the Department of Soils, Haryana Agricultural University, Hisar according to the method of Lindsey and Norwell (1969).

3.6.6.6 HCl extractable minerals

Minerals including calcium, phosphorus, iron, zinc, copper and manganese were extracted in 0.03 N HCl (Peterson et al., 1943) and estimated to assess the bioavailability of these minerals in the fermented product.

To one g sample, 50 ml 0.03 N HCl was added. The mixture was incubated at 37°C in a shaker-cum-waterbath for three h to simulate conditions that occur in human stomach. The mixture was then filtered through an ashless filter paper (Whatman # 42). The filtrate was oven dried, digested in the diacid mixture and proceeded for the determination of individual minerals as detailed above (Ca 3.6.6.2, P 3.6.6.3; Trace minerals 3.6.6.5).

3.6.7 Thiamine

Thiamine in the sample was determined by the thiochrome method (AOAC, 1980).

Reagents:

- i) Acetate buffer (pH 4.0-4.2): Mixed 30 ml of 1 M sodium acetate solution with 70 ml of 1N acetic acid and made upto 500 ml.
- ii) Enzyme suspension: 150 mg takadiastase and 75 mg papain were dissolved in 5 ml acetate buffer.

- iii) Basic lead acetate solution: Dissolved 180 g lead acetate in about 700 ml distilled water and boiled the solution. To the boiling solution, added 180 g lead oxide and the solution was boiled for 30 min. It was then cooled, made upto 1 litre with distilled water and filtered.
- iv) Standard thiamine solution: Dissolved 25 mg thiamine (Sigma Chemical Company, USA) in 250 ml 0.01 N HCl. One ml of this stock solution was diluted to 100 ml to get a working standard containing 1 µg/ml.

Extraction:

Ten g well mixed ground sample was taken and treated with 100 ml acetate buffer (pH 4.0-4.2) and the mixture blended in a waring blender. To the slurry, 5 ml enzyme suspension was added. An enzyme blank containing the buffer and the enzymes but without the sample was also run simultaneously. A few drops of toluene were added and the mixture was incubated overnight at 37°C. The enzymes were inactivated by heating in a water bath at about 80°C for 5-10 min, the solution cooled, the mixture centrifuged and the clear centrifugate collected. Aliquots of this solution were used for estimating thiamine.

Procedure:

Treated 20 ml vitamin extract with 10 ml basic lead acetate solution, mixed well and centrifuged. A suitable aliquot of the supernatant (20 or 25 ml) was treated in another centrifuge tube with 3 ml 30% H_2SO_4 and 12 ml water (or enough H_2O to make the total volume to 40 ml), mixed well and centrifuged. Two ml 40% NaOH added to 3, 100 ml separating funnels (one of them serving as a blank, the other as the experimental and the third as recovery). Added 0.8 ml 2% potassium ferricyanide solution to the experimental and recovery, mixed well and 10ml thiamine extract was added to all the separating funnels. To the recovery, one µg of pure thiamine standard solution was added and the contents allowed to stand for 2 min after shaking. Then

15 ml isobutyl alcohol (water saturated) was added and shaken for 2 min. The aqueous layer was rejected. If the alcohol layer was turbid, one ml of ethyl alcohol was added to clear the solution. The fluorescence of the isobutyl alcohol was measured in a fluorimeter using the thiamine filter. The calculation was done after making due allowance for the dilution and the blank.

Calculation:

$$\text{Thiamine } (\mu\text{g}/100 \text{ g sample}) = \frac{\text{Experiment} - \text{Blank}}{\text{Recovery-experiment}} \times \text{dilution} \times \frac{100}{\text{wt of sample(g)}}$$

3.7 Animal experiment

3.7.1 Test animals

Young albino rats weighing 28 ± 5 g were obtained from the Disease and Germ Free Small Animal House, Haryana Agricultural University, Hisar. The rats were randomly divided into ten groups, each consisting of eight rats. The rats were housed individually in polypropylene cages kept in air conditioned room maintained at $21 \pm 1^\circ\text{C}$ with 12 h light and dark cycle. Food and water were given ad libitum.

3.7.2 Composition of basal diet

Composition of the basal diet which contained unfermented and fermented pearl millet flour so as to provide protein at 8 per cent level is given in Table 3. Naturally fermented and mixed pure culture fermented pearl millet samples were biologically evaluated. The diet of the control group of the rats had egg albumen as the source of protein at 8 per cent level.

For the preparation of diets, ingredients were homogenized and passed through 70 mesh sieve to ensure uniform distribution of vitamins and minerals. The composition of mineral and vitamin mixtures recommended by BARR were used

TABLE 3
Composition of the experimental diets

Dietary groups	Dietary components (g/kg diet)									
	Egg albumen	Fermented product	Raw millet flour	Zero hour fermented flour	Mineral mixture*	Vitamin mixture*	Sucrose	Fat*	Cellulose	Starch
EA	103	-	-	-	40.0	10	50	100.0	50	647.0
Rp	-	-	800.8	-	23.9	10	50	48.6	-	66.7
Zf	-	-	404.0	404.0	23.8	10	50	48.1	-	60.1
SdLb	-	430.1	430.1	-	22.8	10	50	45.2	-	11.8
SdLf	-	413.5	413.5	-	23.5	10	50	50.0	-	39.5
ScLb	-	430.1	430.1	-	22.8	10	50	46.8	-	10.2
ScLf	-	431.0	431.0	-	23.8	10	50	50.3	-	3.9
F20	-	404.0	404.0	-	23.9	10	50	44.7	-	63.4
F25	-	431.0	431.0	-	22.8	10	50	40.5	-	14.7
F30	-	435.5	435.5	-	22.6	10	50	40.0	-	6.4

* The fat and mineral content of the diets was made to contain 10 and 4 per cent, respectively, after taking into account the level of these constituents in the fermented products. Refined and deodorised peanut oil was the source of the fat.

and has been given in tables 4 and 5, respectively.

The diets made exclusively from the fermented samples were not accepted by the rats. Thus it was interpreted that low pH of the sample imparted sourness leading to refusal of the diet by animals. In order to overcome this difficult, the fermented samples were mixed with equal amount of raw pearl millet flour and then incorporated in the diets. The diets for one week were prepared at one time and stored in refrigerator. Different parameters such as feed efficiency ratio (FER), protein efficiency ratio (PER), apparent digestibility (AD), true digestibility (TD), biological value (BV), net protein utilisation (NPU), utilisable protein (UP), net protein retention (NPR) and protein retention efficiency (PRE) were worked out.

3.7.3 Biological evaluation

3.7.3.1 Protein efficiency ratio (PER) and Food efficiency ratio (FER)

PER was determined by the method of Chapman et al. (1959). The rats were initially weighed and then weighed on every alternate day. The weighed diet was daily given and the unconsumed diet was collected, dried and weighed. Food and water were given ad libitum. The rats fed on different experimental and control diets were finally weighed after four weeks and the gain in weight of rats during this period was recorded. The amount of food and protein intake during this period was calculated on dry matter basis.

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein consumed (g)}}$$

$$\text{FER} = \frac{\text{Gain in body weight (g)}}{\text{Food consumed (g)}}$$

3.7.3.2 Apparent digestibility (AD), True digestibility (TD) and Biological Value (BV)

These were determined by the method of Chick et al. (1935). After

TABLE 4

Composition of mineral mixture (AIN-76A)^a

Ingredients	g/kg mineral mixture
Calcium phosphate (dibasic)	500.00
Sodium chloride	74.00
Potassium citrate monohydrate	220.00
Potassium sulphate	52.00
Magnesium oxide	24.00
Ferric citrate	6.00
Manganese carbonate	3.50
Zinc carbonate	1.60
Cupric carbonate	0.30
Potassium iodate	0.01
Sodium selenite, pentahydrate	0.01
Chromic potassium sulphate dodecahydrate	0.55
Starch	118.03

^a Based on the National Academy of Science recommended levels for rats (BARR Committee on Animal Nutrition, 1972)

Table 5
Composition of vitamin mixture (AIN-76A)^a

Ingredients	g per kg diet
Thiamine hydrochloride	0.6
Riboflavin	0.6
Pyridoxine hydrochloride	0.7
Nicotinic acid	3.0
Calcium pantothenate	1.6
Folic acid	0.2
Biotin	0.02
Retinol acetate	0.001
Cholecalciferol	0.0025
D- α -Tocopherol	To provide 5000 IU of Vit E activity
Menadione	0.005
Cyanocobalamine	0.001
Starch, finely powdered	To make 1000g

^a Based on the National Academy of Science recommended levels for rats (BARR Committee on Animal Nutrition, 1972).

28 days of feeding, the rats were transferred to metabolic cages and fed the experimental diets. Another group of rats of the same weight and age was fed on a nitrogen free diet. The experiment was conducted for nine days; during the first four days, the rats got acclimatised; the remaining five days, diet residue, urine and faeces were separately collected from each rat. Daily weight gain by the animals was recorded. A few drops of H_2SO_4 (10%) were added to urine so as to prevent any loss of ammonia. The faeces were dried at $70^\circ C$, weighed and powdered. The urine samples were filtered and transferred to volumetric flasks and volumes were made to 50 ml. Diet residue samples were dried in hot air oven to a constant weight, cooled in desiccator and weighed. Nitrogen in each sample of urine and faeces was determined by the MicroKjeldahl method (AOAC, 1980). AD, TD and BV were derived from the following formulae:

$$AD = \frac{N \text{ intake} - FN}{N \text{ intake}} \times 100$$

$$TD = \frac{N \text{ intake} - (FN - MFN)}{N \text{ intake}} \times 100$$

$$BV = \frac{N \text{ intake} - (FN - MFN) - (UN - EUN)}{N \text{ intake} - (FN - MFN)} \times 100$$

where,

FN = Faecal nitrogen

MFN = Metabolic faecal nitrogen

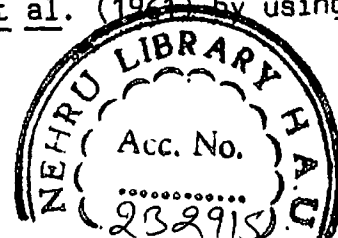
UN = Urinary nitrogen

EUN = Endogenous urinary nitrogen

3.7.3.3 Net protein utilisation (NPU)

NPU was determined by the method of Platt et al. (1961) by using the following formula:

$$NPU = \frac{BV \times TD}{100}$$



3.7.3.4 Net protein retention(NPR) and Protein retention efficiency(PRE)

The initial and final weights of rats after the experimental period of five days of metabolic study were recorded. Rats fed on test diets showed an increase and those fed on nitrogen-free diet (control) showed a decrease in the body weight.

The values of NPR were calculated according to the method of Bender and Doell (1957) as given below:

$$\text{NPR} = \frac{\text{Gain in weight of test animals} + \text{Loss in weight of control animals}}{\text{Protein consumed by test animals}}$$

$$\text{PRE} = \text{NPR} \times 16.$$

3.7.3.5 Utilisable protein (UP)

The utilisable proteins were estimated by the method of Gupta et al. (1979) as mentioned below:

$$\text{Utilisable protein} = \frac{\text{NPU} \times \text{N (per cent on dry matter basis)} \times 6.25}{100}$$

3.7.4 Body fluids and organs

At the end of the experiment, the rats were lightly anaesthetised with diethyl ether and blood was collected by cardiac puncture and the serum was separated out by centrifuging the blood at 3000 rpm for 15 min. Serum was stored in screw capped vials kept in deep freeze until used for further analysis. The liver, kidney, heart, thymus and adrenal were excised, cleaned of adhering matter, blotted in filter paper, weighed and stored in screw capped vials in deep freeze until used for further analysis.

3.7.4.1 Tissue lipids

3.7.4.1.1 Extraction and purification of lipids

Liver and kidney tissues were homogenized in an electrically operated Potter- Elvehjem homogeniser, held in icebath, for 10 min with 19 volume of cold chloroform-methanol mixture (2:1 v/v). After filtration through Whatman # 41 filter paper saturated with the solvent mixture, the homogenate residue was again treated with 10 volumes of chloroform-methanol mixture (1:2 v/v) containing 5 per cent water. Both filtrates were combined and an appropriate amount of chloroform was added to yield a final concentration of chloroform methanol of 2:1 (v/v). This filtrate contained practically all lipids of the tissue and also contaminating non-lipid material like peptides, free amino acids, sugars etc. and the residue contained proteins and nucleic acids.

The lipids in the filtrate were freed from contaminating material by method of Folch et al. (1957) and Suzuki (1965). The filtrate in the stoppered cylinders was mixed thoroughly and agitated by adding 2 volumes of 0.88 per cent aqueous KCl. Gangliosides and non-lipid materials were partitioned into the upper aqueous phase by keeping the cylinder undisturbed for 12 h at room temperature. The upper phase was removed leaving the lower phase undisturbed by sucking it out with the help of a sharply pointed pipette fitted to a vacuette. The lower phase was gently washed twice with the theoretical upper phase (chloroform: methanol: water :: 3: 48:47 v/v) containing no salt. If the lower phase, after removing whole of upper phase became turbid or cloudy, it was made clear by the addition of a few drops of methanol. The lower phase was evaporated to dryness in vacuum oven at a temperature less than 45°C. The dried lipids were dissolved in 5 ml of chloroform and kept in well stoppered volumetric flasks (5 ml). These

flasks were stored in the deep freeze.

3.7.4.1.2 Total lipids

Total lipids in the tissues were determined gravimetrically. 0.5 ml of the purified and concentrated lipid extract (5 ml) was taken in a micropetri plate and the contents were dried of the solvent to a constant weight by keeping the petriplates at 90°C in hot air oven for 12 h. The weight of the empty petriplates was recorded, and the difference in the weight of petriplates with dried lipids and empty plate gave the amount of lipids present in 0.5 ml of the lipid extract of tissue. The figure multiplied by 10 gave the lipids in the liver and kidney tissues.

3.7.4.2 Tissue proteins

The residue left as in 3.7.4.1.1 contained protein and nucleic acids. The residue was dissolved in 10 ml 1N NaOH by keeping the beakers in water bath at 60°C. The dissolved proteins were made to 50 ml volume with distilled water and stored in deep freeze for further protein estimation.

Total quantity of proteins dissolved in NaOH (3.7.4.2) was assayed colorimetrically by the modified method of Lowry et al. (1951).

Reagents:

- i) Reagent A: Dissolved 2 g sodium carbonate in 0.1 N NaOH and volume made to 100 ml.
- ii) Reagent B: 0.5 per cent $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1 per cent sodium potassium tartarate.
- iii) Reagent C (Alkaline-copper solution): 50 ml reagent A was mixed with one ml of reagent B. Mixture was used only when prepared fresh, after 24 h it was discarded. The reagent was used for standard sample only where protein was dissolved in water, whereas reagent D was used for the samples which were dissolved in NaOH.

- iv) Reagent D (Carbonate-copper solution): The reagent was prepared similarly as reagent C except for the omission of NaOH in the preparation of reagent A. This reagent was used for the samples when proteins were dissolved in NaOH.
- v) Reagent E: 2N Folin- Ciocâtleau reagent was diluted to 1 N with water and used.
- vi) Protein standard: Standard solution of bovine serum albumin containing 250 µg per ml was prepared and used for making a standard curve. For establishing a standard curve, standard solution 0.1 to 1.0 ml (25 µg - 250 µg) was taken. 0.1 OD corresponded to 50 µg of protein.

Procedure:

One ml of test protein solution in NaOH was taken and diluted to 10 ml with water. To 0.2 ml of this diluted solution, 0.8 ml of water was added. Then 5 ml of Reagent C (for standard solution) or Reagent D (for sample) were added to it and mixed well. After allowing to stand for 10 min or longer at room temperature, 0.5 ml Reagent E was added very rapidly and mixed within a second or two. After 30 min or longer, the blue colour so developed was measured at 510 nm.

3.7.4.3 Serum analysis

Serum protein, serum albumin, SGOT and SGPT activities were analysed by Ames Blood Analyzer employing the Sera-Pak reagents supplied by M/s. Miles India Ltd., Baroda.

3.7.4.3.1 Serum protein

It was based on the method of Strickland et al. (1961).

Reagents:

- i) Biuret reagent: Diluted the contents of the bottle with 158 ml distilled water.

- ii) Surfactant
- iii) Solution I: Mixed the reagent (ii) with reagent (i) gently to avoid foaming and kept tightly closed.

Procedure:

Took two cuvettes referred as sample and standard. To the sample cuvette only added 0.05 ml serum and to the standard cuvette added 0.05 ml of the standard solution. Added 3 ml solution I to each cuvette. Mixed by inverting the cuvettes two times. Incubated the cuvettes in the incubator at 37°C for 12 min. Removed the cuvettes from the incubator and inverted once. Put the instrument switch in the 'ON' position. Inserted the cuvette containing the standard in the test well and calibrated to read 6 g/dL. Replaced this cuvette with test cuvette and read the result in g/dL. Normal value of serum protein ranges between 6.6 and 8.3 g/dL.

3.7.4.3.2 Serum albumin

Reagents:

- i) Albumin standard (5.0 g/dL)
- ii) Saline (0.9%)
- iii) Prepared a 2.5 g/dL albumin standard by mixing equal volumes of the 5.0 g/dL albumin standard and 0.9% saline.
- iv) BCG reagent

Procedure:

Took three cuvettes 2.5 g/dL standard (1), 5.0 g/dL standard (2) and test (3). Added 0.02 ml sample serum and 3 ml BCG reagent (iv) to the test cuvette. To 2.5 g/dL standard (1) cuvette, added 0.02 ml of 2.5 g/dL standard and 3 ml BCG reagent while to 5.0 g/dL standard (2) cuvette, added 0.02 ml standard (5.0 g/dL) and 3 ml BCG reagent. Mixed by inverting the cuvettes two times and allowed to stand for one min at room temperature.

Put the instrument switch in the 'ON' position, inserted the cuvette containing 2.5 g/dL standard in the test well and calibrated with the calibration knob to read 2.50 g/dL. Replaced this cuvette with the cuvette containing the 5.0 g/dL standard and set the meter pointer to 5.0 g/dL using the back panel slope control. Removed the cuvette. Inserted the test cuvette in the test well and read the result in g/dL. Normal value for serum albumin is 3.5-5.0 g/dL.

3.7.4.3.3 SGOT activity (Serum glutamate oxaloacetate transaminase)

It based on the method of Henry (1968).

Reagents:

- i) L-Aspartate buffer
- ii) Alpha-Ketoglutaric acid buffer
- iii) Aniline buffer
- iv) Potassium hydroxide
- v) Vanillin in ethylene glycol and ethylene glycol monomethyl ether

Procedure:

In the blank cuvette, added 0.1 ml L-aspartate buffer and 0.1 ml alpha-ketoglutaric acid buffer. To the test cuvette, added 0.2 ml serum, 0.1 ml each of L-aspartate buffer and alpha-ketoglutaric acid buffer. Mixed the contents well. Incubated the cuvettes in the incubator at 37°C for 30 min. After incubation, added 0.1 ml aniline buffer and 0.2 ml serum sample in the blank cuvette while only 0.1 ml aniline buffer was added to the sample cuvette. Mixed the contents well and incubated for 5 min. Added 0.1 ml of potassium hydroxide and vaniline to both the blank and test cuvettes. Mixed and incubated the cuvettes in the incubator for 15 min. Removed the cuvettes from the incubator. Added 2 ml distilled water to the blank and test cuvettes. Mixed by inverting the cuvettes twice. Put the instrument

switch in the 'ON' position, inserted the blank cuvette in the test well and calibrated to read zero IU/L on the dial scale. Replaced this cuvette with the test cuvette and read the result in IU/L. The colour of the reaction was stable for 30 min.

3.7.4.3.4 SGPT activity (Serum glutamate pyruvate tranaminase)

It based on the method of Henry (1968).

Reagents:

- i) L-Alanine buffer
- ii) Alpha-ketoglutaric acid buffer
- iii) Potassium hydroxide
- iv) Vanillin in ethylene glycol and ethylene glycol monomethyl ether

Procedure:

Pipetted 0.1 ml each of reagent (i) and (ii) in the blank cuvette while 0.1 ml each of reagent (i) and (ii) and 0.2 ml of serum was pipetted in the sample cuvette. Mixed well and incubated the cuvettes in the incubator at 37°C for 15 min. Removed the cuvettes and added 0.1 ml each of reagents(iii) and (iv) to both the blank and sample cuvettes whereas 0.2 ml of serum sample was added to the blank cuvette only. Mixed thoroughly and again incubated for 15 min. Added 2 ml distilled water to both the cuvettes. Put the instrument switch in the 'ON' position, inserted the blank cuvette in the test well and calibrated to read zero IU/L on the dial scale. Replaced this cuvette with the sample cuvette and read the result in IU/L. The colour of the reaction was stable for 30 min.

3.7.4.4 Histopathology

Liver, kidney, thymus and adrenals were examined histopathologically. Tissues were preserved in 10 per cent formaline solution. Tissues were thoroughly washed in running tap water for 12 h, dehydrated in acetone,

cleaned in benzene and embedded in paraffin wax (melting point 60-62°C). Sections (5 microns in thickness) were cut and stained with routine haematoxylin and eosin method for histopathological studies as described by Luna (1968).

3.7.4.5 Haematology

Slides were prepared for total leucocyte count (TLC) and differential leucocyte count (DLC) and examined microscopically.

3.8. Development and utilisation of the fermented products

Various types of products were prepared from the naturally and mixed fermented pearl millet flour.

3.8.1 Cutlets

3.8.1.1 Cutlet A

Ingredients:

Rice(boiled)	50g	Fermented pearl millet flour	50g
Potato	100g	Onion	10g
Green chillies	1-2	Salt and other spices	To taste
Hydrogenated vegetable oil	To fry		

Method:

Mashed the boiled potatoes. Cut the onion, green chillies into small pieces. Mixed the boiled and mashed potatoes, rice, fermented pearl millet flour, onion, green chillies, salt and other spices. Took a small portion of the mixture and shaped into the form of a cutlet. Deep fried it till golden brown in colour and served hot.

3.8.1.2 Cutlet B

Ingredients:

Fermented pearl millet flour	100g	Potato	100 g
Bread	4-5 slices	Salt and other spices	To taste
Hydrogenated vegetable oil	To fry		

Method:

Boiled and mashed the potatoes. Soaked the bread slices in water for a few minutes. Squeezed out the water of soaked bread slices. Mixed the mashed potatoes, bread and fermented pearl millet flour. Added salt and spices to the above mixture. Took a small portion of the mixture and shaped into the form of a cutlet and deep fried it.

3.8.2 Pakora

Three types of pearl millet pakoras were tried out.

3.8.2.1 Pakora A

Ingredients:

Fermented pearl millet flour	50g	Besan	40g
Spinach	50g	Onion	20g
Salt and spices	To taste	Baking powder	$\frac{1}{2}$ tsp
Hydrogenated vegetable oil	To fry		

Method:

Washed and cut the spinach and onion. Mixed fermented pearl millet flour, besan, spinach and onion. Added baking power, salt and spices. Prepared the batter of a desirable consistency with the help of the water, so that pakoras can be fried.

3.8.2.2 Pakora B

Ingredients:

Fermented pearl millet flour	50g	Besan	30g
Salt and spices	To taste	Baking powder	$\frac{1}{2}$ tsp
Hydrogenated vegetable oil	To fry	Onion	30g

Method:

Cut onions into pieces. Mixed fermented pearl millet flour, besan, onion, baking powder, salt and spices. Added water to make the batter of pakora consistency. Took a small portion of the batter and formed it into a pakora and deep fried it in the hot oil.

3.8.2.3 Pakora C

Ingredients:

Fermented pearl millet flour	75 g	Besan	25g
Onion	60 g	Salt and spices	To taste
Baking powder	$\frac{1}{2}$ tsp	Hydrogenated vegetable oil	To fry

Method:

Cut onions into pieces. Mixed fermented pearl millet flour, besan, onion, baking powder, salt and spices. Added water to make a batter of pakora consistency. Took small portion of the batter and formed into pakora and deep fried.

3.8.3 Chappaties

3.8.3.1 Chappati A

Ingredients:

Fermented pearl millet flour	60 g	Raw pearl millet flour	40 g
Water	To knead the dough	Salt	To taste

Method:

Mixed the fermented and raw pearl millet flour. Added salt to it. Added water to knead the dough, rolled into chappati and baked it on the hot tawa.

3.8.3.2 Chappati B

Ingredients:

Fermented pearl millet flour	75g	Wheat flour (whole)	25g
Water	To knead the dough	Salt	To taste

Method:

Mixed the fermented pearl millet and whole wheat flour. Added salt to it. Added water to knead the dough. Rolled into chappati and baked it on the hot tawa.

3.8.3.3 Chappati C

Ingredients:

Fermented pearl millet flour	60g	Wheat flour(whole).	20g
Besan	20g	Water	To knead the dough
Salt	To taste		

Method:

Mixed fermented pearl millet flour, whole wheat flour, besan and salt. Added water to knead the dough. Rolled into chappati and baked it on the hot tawa.

3.8.4 Fried vermicilli

Ingredients:

Fermented pearl millet flour	50g	Besan	50g
Water	To knead the dough	Salt	To taste
Hydrogenated vegetable oil	To fry		

Method:

Mixed fermented pearl millet flour, besan and salt. Added water to make a stiff dough. Extruded the stiff dough through a mould by pressing the piston of the machine while keeping it above the karahi containing hot oil. Fried the vermicillies in the hot oil.

3.8.5 Weaning mixtures

3.8.5.1 Weaning mixture A

Ingredients:

Fermented pearl millet flour	55g	Green gram dal	15g
Peanut	10g	Sesame	5g
Sugar	30g		

Method:

Roasted green gram dal, sesame and peanuts separately. Ground and mixed the roasted green gram dal, sesame, peanuts and fermented pearl millet flour. Added sugar to the above mixture.

3.8.5.2 Weaning mixture B

Ingredients:

Fermented pearl millet flour	50g	Roasted bengal gram	15g
Wheat flour (whole)	15g	Peanut	15g
Jaggery	35g		

Method:

Ground the roasted wheat flour and peanuts. Mixed the fermented pearl millet flour, ground and roasted bengal gram, wheat flour and peanuts. Added jaggery to the above mixture.

3.8.5.3 Ingredients weaning mixture C

Fermented pearl millet flour	50g	Roasted bengal gram	15g
------------------------------	-----	---------------------	-----

Wheat flour(whole)	10g	Peanut	10g
Sesame	10g	Sugar	35g

Method:

Roasted and ground the peanuts, sesame and whole wheat flour separately. Mixed the fermented pearl millet flour, whole wheat flour, ground bengal gram, peanuts and sesame. Mixed sugar to the above mixture.

3.8.6. Pearl millet biscuits

3.8.6.1 Biscuits A

Ingredients:

Weaning mixture(c)	50g	Wheat flour	50g
Sugar	75g	Hydrogenated vegetable oil	50g
Water	30ml	Ammonia powder	$\frac{1}{2}$ tsp

Method:

Ground the sugar finely in an electric mixer.. Creamed sugar and hydrogenated vegetable oil. Sieved wheat flour and added baking powder to it. To the creamed sugar and oil mixture, added wheat flour,mixture(c) and made a dough with the help of water. Added ammonia powder to the dough. Rolled it and cut into the shape of biscuits with the help of a cutter. Baked biscuits in the oven at 150°C for 10-15 min.

3.8.6.2 Biscuits B

Ingredients:

Fermented pearl millet flour	50g	Wheat flour	50g
Sugar	75g	Hydrogenated vegetable oil	50g
Water	30ml	Ammonia powder	$\frac{1}{2}$ tsp

Method:

Ground the sugar finely in an electric grinder. Creamed sugar and hydrogenated vegetable oil. Sieved wheat flour and added baking powder to it. To the creamed sugar and oil mixture, added maida and made a dough with the help of milk. Added ammonia powder to the dough. Rolled it and cut into the shape of biscuits with the help of a cutter. Baked the biscuits in the oven at 150°C for 10-15 min.

3.8.6.3 Biscuits C

Biscuits C were prepared in the same manner as biscuits B; instead of adding sugar, common salt was added.

3.8.7 Organoleptic evaluation

The products developed from the fermented pearl millet flour were evaluated for colour, flavour, taste, texture and appearance by a semitrained panel of judges deploying a 9 - point hedonic scale as given in the Appendix-I. Average of the scores for all these characteristics was expressed in terms of overall acceptability.

3.9 Statistical analysis

The data were subjected to statistical analysis for analysis of variance and correlation coefficients in a completely randomised design according to standard methods (Panse and Sukhatme, 1961).

CHAPTER-IV

RESULTS AND DISCUSSION

The results of the study have been presented and discussed under the following heads and subheads:

- 4.1 Sensory evaluation of naturally fermented pearl millet flour
- 4.2 pH and titratable acidity
- 4.3 Microbial composition
- 4.4 Total protein, fat and ash
 - 4.4.1 Protein
 - 4.4.2 Fat
 - 4.4.3 Ash
- 4.5 Available carbohydrates
- 4.6 Digestibility (in vitro)
 - 4.6.1 Starch digestibility (in vitro)
 - 4.6.2 Protein digestibility (in vitro)
- 4.7 Antinutritional factors
 - 4.7.1 Phytic acid
 - 4.7.2 Polyphenols
- 4.8 Mineral extractability
- 4.9 Thiamine
- 4.10 Biological evaluation
- 4.11 Weight and composition of organs
- 4.12 Serum analysis and haematological examination
- 4.13 Histopathology
- 4.14 Utilisation for human consumption

4.1 Sensory evaluation of naturally fermented pearl millet flour

Freshly ground pearl millet flour was mixed with sterilised water and allowed to be fermented by the native microflora of the flour. After the fermentation for 72 h at 20,25,30,35,40,45 and 50°C temperatures, the fermented product was subjected to organoleptic evaluation by a panel of judges using a 9-point hedonic scale. This was aimed at finding out the temperatures of fermentation giving an acceptable fermented product for studying it further.

The data on the aroma and colour evaluation of the naturally fermented pearl millet flour are presented in Table 6. For colour, the product was acceptable at 20, 25, 30 and 35°C and for the aroma, at 20, 25 and 30°C. On this basis, the fermentation temperatures were confined to 20,25 and 30°C only.

4.2 pH and titratable acidity

After 72 h fermentation of pearl millet at 20, 25 and 30°C, the pH dropped from 6.40 to 3.98, 3.72 and 3.46 with corresponding increase in titratable acidity from 1.33 to 5.55, 8.85 and 10.3 g lactic acid per 100 ml, respectively. The pH was the lowest and titratable acidity was the highest when fermentation was carried out at 30°C. The lower the temperature, the higher was the pH and the lower was the titratable acidity (Table 7). These changes in pH and titratable acidity were noted when the freshly ground pearl millet flour without autoclaving was fermented. But when the pearl millet flour after autoclaving was inoculated with 10 per cent raw pearl millet flour and allowed to ferment for the same period at the same temperatures as above, the changes in pH and titratable acidity were slightly different from those observed when unautoclaved flour was fermented (Table 8). In both the

TABLE 6

Sensory evaluation of naturally fermented pearl millet flour

Fermentation	Aroma	Colour
F20	6.9±0.5	6.5±0.5
F25	6.4±0.8	6.6±0.7
F30	5.9±0.9	6.9±0.8
F35	4.6±1.7	6.4±0.7
F40	4.0±1.3	5.1±1.6
F45	3.9±1.0	3.9±1.6
F50	3.5±1.0	3.6±1.6
SE(m)	±0.36	±0.38
CD(P < 0.05)	1.03	1.08

Values are means ± SD of twelve replicates.

TABLE 7

Changes in pH and titratable acidity (g lactic acid per 100 ml) during natural fermentation of pearl millet flour

Fermentation	pH	Titratable acidity
F20	3.98±0.01	5.55±0.02
F25	3.72±0.01	8.85±0.36
F30	3.46±0.01	10.3 ±0.07
Zf	6.40±0.01	1.33±0.02
SE(m)	±0.005	±0.08
CD(P<0.05)	0.01	0.24

Values are means ± SD of six independent determinations.

TABLE 8

Changes in pH and titratable acidity (g lactic acid per 100 ml) during natural fermentation of autoclaved pearl millet flour

Fermentation	pH	Titratable acidity
F20	4.35±0.01	0.90±0.05
F25	4.01±0.01	2.85±0.09
F30	3.55±0.01	4.35±0.11
ZF	6.42±0.00	0.55±0.01
SE(m)	±0.002	±0.034
CD(P< 0.05)	0.01	0.10

Values are means ± SD of six independent determinations.

cases the lowest pH and the highest titratable acidity were recorded at 30°C but the values were a little different. Higher pH and lower titratable acidity were found at a relatively lower temperature. The most marked difference was noticed in titratable acidity. The autoclaved flour fermented at 30°C for 72 h had 4.35 titratable acidity where as the corresponding value for non-autoclaved fermented sample was 10.3 (g lactic acid/100 ml). Likewise, the titratable acidity at other temperatures was also substantially low.

A significant decrease in pH and simultaneous increase in titratable acidity was recorded when the pearl millet flour was fermented by pure cultures of yeast and lactobacilli at 30°C for 72 h (Table 9). The initial pH of 6.42 declined to pH values varying from 3.73 to 5.65 after 72 h of pure culture fermentations. In case of single culture fermentation, Lactobacillus fermentum had the highest pH lowering effect followed by Lactobacillus brevis, S. diastaticus and S. cerevisiae. The highest titratable acidity in single culture fermentation group was brought about by Lactobacillus fermentum followed by Lactobacillus brevis, S. diastaticus and S. cerevisiae. When one species of yeast and lactobacillus each was combined and used for mixed culture fermentation, the pH after 72 h fermentation ranged from 4.04 to 4.39 and the corresponding titratable acidity from 1.95 to 1.31. The lowest pH and the highest titratable acidity were observed in SdLf fermented groups. Other mixed culture fermentations were significantly different from SdLf in respect of pH and titratable acidity values. There was no significant difference between the pH of the product fermented by SdLb and ScLb. The values of titratable acidity in the mixed culture fermentation groups were significantly different from one another; the highest in SdLf and the lowest in SdLb groups.

TABLE 9

Changes in pH and titratable acidity (g lactic acid per 100 ml) during pure culture fermentation of autocalved pearl millet flour

Fermentation	pH	Titratable acidity
<u>Single fermentation</u>		
Sd	4.80±0.01	1.12±0.04
Sc	5.65±0.04	0.82±0.08
Lb	4.54±0.01	1.18±0.02
Lf	4.41±0.01	1.50±0.05
<u>Mixed fermentation</u>		
SdLb	4.39±0.01	1.31±0.04
SdLf	4.04±0.01	1.95±0.04
ScLb	4.38±0.01	1.35±0.00
ScLf	4.36±0.02	1.48±0.01
<u>Sequential fermentation</u>		
Sd+Lb	3.99±0.01	1.95±0.00
Sd+Lf	3.73±0.02	2.74±0.04
Sc+Lb	4.26±0.00	1.58±0.03
Sc+Lf	4.02±0.00	1.97±0.00
<u>Control</u>		
Zf	6.42±0.00	0.55±0.00
SE(m)	±0.01	±0.02
CD (P<0.05)	0.03	0.06

Values are means ± SD of four independent determinations.

In the sequential culture fermentation, the final pH and titratable acidity varied from 3.73 to 4.26 and 1.58 to 2.74 g lactic acid per 100 ml, respectively. The lowest pH was in Sd+Lf group followed by Sd+Lb, Sc+Lf and Sc+Lb groups in ascending order. In the similar fashion, the Sd+Lf group had the highest titratable acidity followed by Sc+Lf, Sd+Lb and Sc+Lb groups. The Sc+Lf and Sd+Lb groups had titratable acidity not significantly different from one another.

From the single, mixed and sequential culture fermentation, it can be visualised that S. diastaticus, L. fermentum and their combinations in mixed or sequential culture fermentations were responsible for relatively low pH and corresponding high titratable acidity of the fermented product. Similarly, S. cerevisiae and L. brevis and their combinations appeared to result in relatively higher pH and lower titratable acidity.

On the whole, sequential culture fermentation yielded the lowest pH followed by mixed and single culture fermentations. The lowest pH and the highest titratable acidity are anticipated because of relatively longer periods of fermentation. It may be mentioned here that single or mixed culture fermentations were conducted for 72 h whereas sequential culture fermentation continued for 144 h. In case fermentation was carried for 72 h, the mixed culture fermentation seemed to be producing lower pH and higher titratable acidity than single culture fermentation.

The pearl millet sprouts were ground and made to slurry which had a pH of 5.89 (Table 10). This starting pH of fermentation dropped to as low as 3.62 when fermentation at 30°C for 72 h was carried out with a mixture of S. diastaticus and L. fermentum. The fermentation carried out with three remaining combinations of pure cultures yielded significantly higher pH; the highest being in GSclb group. The average pH of the fermented sprouts appeared to be the lowest among all pure culture fermentations including single,

TABLE 10
Changes in pH and titratable acidity (g lactic acid
per 100 ml) during fermentation of the sprouted pearl millet

Fermentation	pH	Titratable acidity
GSdLb	3.67±0.01	2.10±0.00
GSdLf	3.62±0.01	3.31±0.02
GScLb	3.91±0.00	2.02±0.00
GScLf	3.85±0.01	3.23±0.01
G	5.89±0.01	1.27±0.01
SE(m)	±0.005	±0.008
CD (P < 0.05)	0.015	0.024

Values are means ± SD of four independent determinations.

mixed and sequential fermentations of pearl millet flour. Different mixed pure culture fermentations of the sprouts did not give that wide variation in pH as the other types of pure culture fermentations.

Relatively low pH of the fermented sprouts may be due to starting pH (5.89) which was lower than that of the other fermentations (6.42). The titratable acidity of the fermented sprouts varied from 2.02 to 3.31 g lactic acid per 100 ml. As in other fermentations, the low pH corresponded to high titratable acidity and vice versa, the same was observed in case of fermented sprouts too. The generalised relationship between pH and titratable acidity is explainable with the help of correlation coefficients given in Table 11. In all the fermentations, pH had a significantly negative correlation with titratable acidity.

The hetrofermentors like L. fermentum and L. brevis convert glucose to equimolar mixture of lactic acid, ethanol and CO_2 . These heterofermentative lactobacilli, ferment glucose aerobically, reoxidising NADH at the expense of O_2 by means of a flavoprotein enzyme. Due to the production of acid by the lactobacilli, pH of the fermented product is lowered down.

A rapid drop in pH with corresponding increase in titratable acidity has been reported in lactic acid fermentation of corn (Baseler and Fields, 1984; Lopez et al., 1983; Nanson and Fields, 1984), sorghum (Paul and Fields, 1981), pearl millet (Mahajan, 1986), food legumes (Zamora and Fields, 1979a) and in fermented products like idli (Venkatasubbaiah et al., 1984), puto (Tongananta, 1971), ogi (Banigo and Müller, 1972), pickled Egyptian vegetables (Morcos, 1977), gari (Ogunsua, 1977), tef injera (Gashe, 1985) and rabadi (Dhankher and Chauhan, 1987a).

TABLE 11
Correlation coefficients of group means of pH with
titratable acidity

Fermentation	pH vs Titratable acidity
Natural fermentation	- 0.7032*
Pure culture fermentation	- 0.8573**
Pure culture fermentation of sprouts	- 0.8653*

* Values are significant at 5% level

** Values are significant at 1% level

4.3 Microbial composition

The microbial composition of the pearl millet flour after natural fermentation underwent a significant qualitative and quantitative change (Tables 12 and 13). When the freshly ground pearl millet flour was fermented without prior autoclaving, the SPC, yeasts, lactobacilli, fungi and coliforms increased substantially (Table 12). Coliforms were in the highest number in the fermenting mixture followed by yeast, lactobacillus and fungus. As the fermentation continued, all the microflora grew in number but the maximum increase in cell number was noticed in lactobacillus at all the temperatures (Fig 1a). The lactobacilli cells grew to the maximum at 30°C followed by their number at 25°C and 20°C in descending order. The growth of yeast like that of lactobacillus was the highest at 30°C and less at the remaining temperatures; lowest being at 20°C. Fungal cells grew marginally during fermentation; the growth was maximum at 30°C. Fungal growth at 20 and 25°C was the same but apparently less than that at 30°C. Coliforms showed an ample growth at all the temperatures; the highest at 30°C and lowest at 20°C.

When the pearl millet flour was autoclaved and inoculated with 10 per cent freshly ground raw pearl millet for fermentation, the initial as well as final counts of all types of the microflora seemed to be less than that of freshly ground pearl millet flour after fermentation without autoclaving. The trends of qualitative and quantitative changes in the microbial growth were the same whether the pearl millet flour was fermented with or without autoclaving (Fig 1b). The temperature effect also appeared to be the same in both types of natural fermentations i.e., the growth of lactobacillus, yeast, fungus and coliform was the highest at 30°C and the lowest at 20°C.

TABLE 12

Microbial composition of the fermented pearl millet flour (non-autoclaved)

Fermentation	SPC	Yeast	Lactobacillus	Fungus	Coliform
F20	1.15×10^8	2.0×10^4	3.6×10^7	2.0×10^2	3.0×10^6
F25	1.43×10^8	3.0×10^5	6.2×10^7	2.0×10^2	2.6×10^7
F30	1.48×10^9	2.3×10^6	8.5×10^7	2.0×10^3	5.0×10^7
Zf	2.00×10^5	2.5×10^2	2.0×10^2	1.3×10^2	5.0×10^4

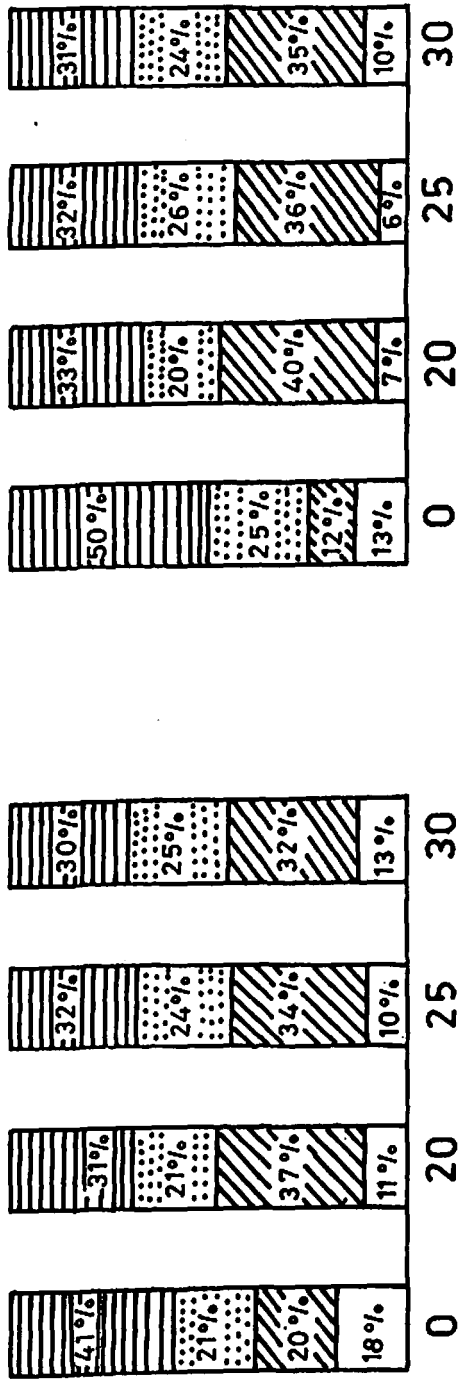
Values are means of six determinations expressed as cell number/ml of the fermented product.

TABLE 13
Microbial composition of the fermented pearl millet flour (autoclaved)

Fermentation	SPC	Yeast	Lactobacillus	Fungus	Coliform
F20	6.6×10^7	4.5×10^3	2.7×10^7	2.0×10^1	1.2×10^6
F25	1.5×10^8	2.5×10^5	3.6×10^7	2.0×10^1	5.0×10^6
F30	1.6×10^8	3.0×10^5	7.1×10^7	1.4×10^2	7.2×10^6
Zf	1.6×10^4	1.0×10^2	1.0×10^1	1.0×10^1	1.0×10^4

Values are means of six determinations expressed as cell number/ml of the fermented product.

Fungus
 Lactobacillus
 Yeast
 Coliform



——— Temperatures of Fermentation ———
 FIG 1(a) (°C) FIG 1(b)

FIG. 1. RELATIVE CELL COUNT OF THE MICROORGANISMS DURING NATURAL
 FERMENTATION OF RAW (FIG.1(a)) AND AUTO CLAVED (FIG.1(b)) PEARL
 MILLET FLOUR (EXPRESSED AS PERCENTAGE OF TOTAL COUNT)

According to Mahajan (1986) the yeast and lactobacilli were the dominating microorganisms in the pearl millet flour fermented at 30°C for 96 h. Maximum growth of both lactobacilli and yeasts occurred when fermentation was carried out at 30°C for 72 h. In her study, lactobacillus dominated in the initial cell count as oppose to our results where coliforms were in the larger number at the initial stage of fermentation. The difference in the environment and source of procurement of pearl millet flour may account for the above variation. Odunfa (1985) reported that the microflora of traditionally produced ogi-baba were dominated by lactobacillus. Gashe (1985) found that coliform was the predominant microflora during the first 18 h of tef fermentation. The utilisation of sugars resulted in low pH of the growing medium which is known to be favourable for the growth of lactobacillus and yeast. This explains the growth of these two dominating microorganisms in the naturally fermented pearl millet flour.

4.4 Total protein, fat and ash

4.4.1 Crude protein

Natural fermentation brought about a marginal change in the protein content of the pearl millet flour (Table 14). The only significant difference in the protein content was noticed when raw pearl millet flour and the fermented pearl millet at 30°C were compared. This fermented product did not vary significantly from the zero h control in respect of their protein content. The temperature of fermentation did not seem to affect the protein content of the fermented product significantly (Table 14).

Single, mixed and sequential pure culture fermentations for 72 h either decreased or did not change the protein content of pearl millet flour (Table 15). The protein content of S. cerevisiae fermented pearl millet flour was significantly higher as compared to the flour fermented by S. diastaticus,

TABLE 14

Effect of natural fermentation on total protein, fat and ash content of pearl millet flour (g/100 g, on dry matter basis)

Fermentation	Protein	Fat	Ash
F20	9.90±0.46	6.85±0.06	1.99 ±0.10
F25	9.28±0.50	6.90±0.10	2.00±0.16
F30	9.18±0.45	6.90±0.12	2.00±0.17
Rp	9.99±0.35	6.42±0.02	2.01±0.16
Zf	9.90±0.42	6.42±0.02	2.00±0.07
SE(m)	±0.26	± 0.05	± 0.08
CD (P<0.05)	0.78	0.15	NS

Values are means ± SD of four independent determinations.

NS Non-significant at 5% level

TABLE 15

Effect of pure culture fermentation on total protein, fat and ash content of pearl millet flour (g/100 g, on dry matter basis)

Fermentation	Protein	Fat	Ash
<u>Single fermentation</u>			
Sd	9.42±0.35	5.96±0.45	2.00±0.16
Sc	9.99±0.19	5.41±0.63	2.01±0.15
Lb	9.30±0.24	5.17±0.18	2.01±0.16
Lf	9.26±0.22	5.79±0.39	2.00±0.18
<u>Mixed fermentation</u>			
SdLb	9.30±0.41	6.37±0.25	2.00±0.16
SdLf	9.67±0.33	6.04±0.64	1.99±0.13
ScLb	9.30±0.24	6.19±0.33	2.00±0.10
ScLf	9.28±0.76	5.88±0.33	2.00±0.20
<u>Sequential fermentation</u>			
Sd+Lb	9.89±0.40	5.63±0.19	2.01±0.15
Sd+Lf	9.26±0.11	5.35±0.35	1.99±0.06
Sc+Lb	9.90±0.56	5.62±0.14	2.00±0.15
Sc+Lf	9.38±0.08	5.65±0.35	1.99±0.12
<u>Control</u>			
Rp	9.99±0.35	6.42±0.18	2.01±0.16
Zf	9.90±0.42	6.42±0.20	2.00±0.07
SE(m)	±0.18	±0.20	± 0.08
CD (P<0.05)	0.54	0.60	NS

Values are means ± SD of four independent determinations.

NS Non-significant at 5% level

L. brevis and L. fermentum. Except S.cerevisiae, the protein contents of fermented pearl millet flour with S.diastaticus, L.brevis and L.fermentum were significantly less as compared to that of raw pearl millet flour. The protein contents of the mixed culture fermented pearl millet flours were not significantly different from each other. All the four mixed culture fermentation combinations except SdLf, had significantly lower protein values than both raw pearl millet flour and zero hour control. In case of sequential culture fermentation, only Sd+Lf and Sc+Lf fermented pearl millet flours had significantly lower protein content than that of raw pearl millet and zero h control. The protein content of Sd+Lf fermented pearl millet flour was significantly less as compared to that of Sd+Lb or Sc+Lb fermented flour. Pure culture fermentation did not change the protein content of the pearl millet sprouts (Table 16).

Thus it appears that the fermentation of pearl millet either naturally or with pure cultures did not bring about any improvement in protein content of this coarse grain. Increased protein catabolism by fermenting microorganisms may account for loss of protein by escaping ammonia, a by-product of metabolic deamination. Some strains of bacteria are known to possess deaminases. The strong ammonia smell, characteristic of the later stages of iru fermentation, was observed by Whitaker(1978). Aliya and Geervani (1981) reported that the fermentation decreased the crude protein contents of bengalgram and greengram products by 6 to 8 per cent and those of pearl millet and ragi by 4 to 6 per cent whereas the sorghum products were unaffected. Ezeala (1984) indicated a decrease in protein content of fermented cassava meal. Microorganisms including bacteria and yeast in traditional fermented food resulted in decreased cassava protein when no external source of nitrogen was added. Mahajan (1986) also reported that

TABLE 16
Effect of mixed fermentation on total protein, fat and ash content of sprouted
pearl millet (g/100 g, on dry matter basis)

Fermentation	Protein	Fat	Ash
GSdLb	9.59±0.32	6.21±0.22	2.01±0.16
GSdLf	9.75±0.26	5.92±0.38	2.00±0.13
GSCLb	9.28±0.18	5.61±0.22	2.01±0.11
GSCLf	9.89±0.40	6.55±0.22	2.00±0.10
G	9.90±0.56	6.55±0.36	2.00±0.16
SE(m)	±0.22	±0.17	±0.08
CD (P < 0.05)	0.66	0.51	NS

Values are means±SD of four independent determinations.

NS Non-significant at 5% level

the protein content either decreased or remained unaffected when pearl millet flour was naturally fermented.

4.4.2 Crude fat

Natural lactic fermentation improved the fat content of pearl millet significantly (Table 14). Temperature of natural fermentation did not influence the fat content of the fermented product.

Pure culture fermentation either decreased or did not alter the fat content of pearl millet flour (Table 15). The pearl millet flour fermented with S. cerevisiae, L. brevis and L. fermentum had significantly less fat as compared to raw pearl millet flour and zero h control. S. diastaticus fermented pearl millet flour did not vary significantly from the controls in fat content. Lowest fat content in single culture fermentation was observed when the pearl millet flour was fermented by L. brevis. These values were significantly lower than that of S. diastaticus and L. fermentum fermented pearl millet. The S. cerevisiae or L. brevis fermented products had almost the same amount of fat. The fat content of the product of mixed fermentation was not significantly different from the unfermented controls. The types of combinations in the mixed culture fermentation did not seem to make any significant effect on the fat content of the fermented product (Table 15). The fermentations carried out by yeasts and lactobacilli reduced the fat content of the pearl millet flour significantly in all the sequential culture fermentations. But the type of combinations made no significant difference in the fat content of the fermented product and all the four products varied non-significantly among themselves.

Pure culture fermentation either decreased or did not change the fat content of pearl millet sprouts. Fermentation of the sprouts by GSdLf and GSclb lowered the fat contents significantly but the fermentation by

GSdLb and GScLf did not change the fat content (Table 16). Among fermentation combinations, GScLf yielded the highest fat content in the fermented sprouts.

Some yeast strains are known to be fat producing and their likely participation in the natural fermentation may explain the increased amount of fat of the fermented pearl millet through natural fermentation. Eka (1980) reported that fermentation increased the fat content of the locust beans from 15.2 to 31.2 per cent.

4.4.3 Ash

Natural fermentation and pure culture fermentation could not significantly change the ash content of pearl millet flour and sprouts (Tables 14, 15 and 16). Since no addition or deletion of mineral source was involved in the process of fermentation, no change in the ash content of the fermented product was expected. Mahajan (1980) carried out the natural lactic fermentation of pearl millet flour at different temperatures and periods of fermentation and found no change in the ash content.

4.5 Available carbohydrates

Pearl millet contained 68.5 per cent starch which on autoclaving was reduced to 63.3 per cent. This amount of starch was further reduced to 63.3 per cent. This amount of starch was further reduced to 31.4, 28.5 and 24.6 per cent when the pearl millet flour was naturally fermented at 20, 25 and 30°C, respectively (Table 17). It appeared from the data that higher the temperature, the more was the reduction in starch content of the fermented pearl millet flour. Autoclaving of raw pearl millet flour raised the level of total soluble sugars, reducing sugars and non-reducing sugars significantly. All these values were further raised when the fermentation continued for 72 h at all the temperatures. The extent of increase seemed to be the highest at 30°C and the lowest at 20°C (Table 17).

TABLE 17

Effect of natural fermentation on total soluble sugars, reducing sugars, non-reducing sugars and starch content of pearl millet flour (g/100 g, on dry matter basis)

Fermentation	Total soluble sugars	Reducing sugars	Non-reducing sugars	Starch
F20	9.87±0.70	9.44±0.40	0.43±0.48	31.4±2.12
F25	12.10±0.62	9.50±0.00	2.55±0.62	28.5±1.43
F30	12.40±0.60	9.75±0.02	2.65±0.59	24.6±0.38
Rp	1.76±0.06	0.36±0.02	1.40±0.07	68.5±0.32
Zf	4.52±0.08	1.22±0.02	3.30±0.08	63.3±1.55
SE(m)	0.29	0.11	0.25	0.81
CD(P < 0.05)	0.87	0.33	0.75	2.44

Values are means ± SD of four independent determinations.

Pure culture fermentation of autoclaved pearl millet flour reduced the starch content and as a result, the fermented product contained starch which varied from 50.4 to 60.2 per cent in single culture fermentation; from 50.8 to 57.6 per cent in mixed culture fermentations and 27.1 to 37.6 per cent in sequential culture fermentation (Table 18). The greater reduction during sequential culture fermentation may be due to longer period of fermentation as compared to mixed and single culture fermentations. Of all the single culture fermentations, S. diastaticus brought about the highest reduction followed by S. cerevisiae, L. brevis and L. fermentum. Of the two yeasts, S. diastaticus seemed to be more amylolytic than S. cerevisiae. L. brevis and L. fermentum did not show any significant difference in starch utilisation. When yeasts and lactobacilli were compared, yeasts as expected utilised starch to a greater extent. In mixed fermentation, Sd combinations had more disparaging effect on starch content than Sc combinations. Pure culture fermentations for 72 h lowered the total soluble sugars during single culture fermentation; the resultant total soluble sugar content was the highest in S. diastaticus followed by S. cerevisiae, L. brevis and L. fermentum. The reducing sugar content in the fermented product was maximum in Sd group and the lowest in Lb group whereas the Sc group contained the highest amount of non-reducing sugars.

Mixed culture fermentations by the yeasts and lactobacilli combinations, also brought down the total soluble sugar content of autoclaved pearl millet flour significantly; maximum reduction was in Sc combination and the lowest in Sd combinations. Among pure culture fermentations of the pearl millet flour, the lowest content of soluble sugars was seen in sequential culture fermentation. The concentrations of reducing sugars as well as non-reducing sugars were found to be the lowest in sequential culture fermentation. During the mixed fermentation, SdLf, ScLb and ScLf combinations

TABLE 18

Effect of pure culture fermentation on total soluble sugars, reducing sugars, non-reducing sugars and starch content of pearl millet flour (g/100 g, on dry matter basis)

Fermentation	Total soluble sugars	Reducing sugars	Non-reducing sugars	Starch
<u>Single fermentation</u>				
Sd	3.09±0.10	2.01±0.00	1.08±0.10	50.4±1.63
Sc	2.82±0.02	0.66±0.03	2.16±0.04	54.2±3.60
Lb	1.82±0.00	0.24±0.02	1.58±0.02	59.3±1.68
Lf	1.77±0.00	0.75±0.03	1.02±0.01	60.2±0.32
<u>Mixed fermentation</u>				
SdLb	4.27±0.27	1.27±0.03	3.00±0.28	50.8±1.82
SdLf	4.11±0.13	0.90±0.01	3.21±0.12	51.0±0.22
ScLb	2.71±0.07	0.90±0.02	1.81±0.06	56.7±1.60
ScLf	2.62±0.01	0.61±0.03	2.01±0.05	57.6±2.20
<u>Sequential fermentation</u>				
Sd+Lb	0.94±0.02	0.09±0.00	0.85±0.02	27.1±0.67
Sd+Lf	0.66±0.01	0.13±0.01	0.53±0.02	37.1±0.52
Sc+Lb	0.85±0.05	0.04±0.00	0.81±0.05	36.1±2.13
Sc+Lf	1.79±0.14	0.22±0.01	1.57±0.13	37.6±2.65
<u>Control</u>				
Rp	1.76±0.06	0.36±0.02	1.40±0.07	68.5±0.32
Zf	4.52±0.08	1.22±0.02	3.30±0.08	63.3±1.55
SE(m)	±0.06	±0.01	±0.06	±1.03
CO(P < 0.05)	0.18	0.03	0.18	3.09

Values are means ± SD of four independent determinations.

had lower amounts of reducing sugars whereas SdLb combination had higher amount of reducing sugars when compared to autoclaved pearl millet flour. After fermentation, all mixed fermentation groups except SdLf had significantly lower concentration of non-reducing sugars than zero hour control.

There was a marked decrease in starch content when the pearl millet grains were sprouted for 24 h. This reduction in starch content was still higher when the sprouted grains were fermented with mixed pure cultures (Table 19). The fermented sprouts contained starch which varied from 38.5 to 42.8 per cent; maximum and minimum being in GScLb and GSdLb groups, respectively. Starch content of the Sd combinations had more reduction than Sc combinations. Sd and Sc combinations did not produce any significant variations as far as starch content of the fermented products was concerned. The pearl millet sprouts had lower concentration of soluble sugars than total soluble sugars after pure culture fermentation of 72 h. GSdLb group had the highest concentration of total soluble sugars which was significantly different from the other three combinations. Likewise, reducing as well as non-reducing sugars were in their highest concentrations when the pearl millet sprouts were fermented by GSdLb combination.

Reduction of starch in the fermented product may be attributed to amylolytic action of microorganisms in the fermenting mixture. Fermenting microbes have been reported to possess both alpha and beta amylases (Bernfeld, 1962). Natural lactic acid fermentation has been reported to reduce the starch content of non-autoclaved pearl millet flour after 96 h at different temperatures; maximum reduction being at 40°C (Mahajan, 1986). Dhankher (1985) also found a reduction in starch content of rabadi fermenting mixture after 9 h at all the temperatures studies. Taur et al. (1984b) reported a maximum starch degradation by amylases during fermentation

TABLE 19

Effect of mixed fermentation on total soluble sugars, reducing sugars, non-reducing sugars and starch content of sprouted pearl millet (g/100 g, on dry matter basis)

Fermentation	Total soluble sugars	Reducing sugars	Non-reducing sugars	Starch
GSdLb	11.8±0.14	7.25±0.28	4.55±0.42	38.5±0.49
GSdLf	4.2±0.47	0.51±0.04	3.69±0.48	39.6±0.24
GSclb	5.8±0.28	2.84±0.04	2.96±0.33	42.8±0.98
GSclf	4.0±0.71	1.55±0.04	2.45±0.74	41.4±1.31
G	16.3±0.87	6.54±0.12	9.80±0.88	50.2±0.62
SE(m)	±0.33	±0.08	±0.35	±0.47
CD (P<0.05)	0.99	0.24	1.05	1.41

Values are means ± SD of four independent determinations.

and found an inverse relationship between starch content and fermentation period. Similar reduction in starch content of food legumes during fermentation have been reported (Watson et al., 1975; Ogunsua, 1977; Zamora and Fields, 1979a; Odunfa, 1983a). Inherent amylases of pearl millet grain may be partly responsible for starch hydrolysis during germination which may account for the reduced level of starch in the pearl millet sprouts. Amylolysis during germination have been reported in a number of foodgrains including pearl millet (Sheorain and Wagle, 1973), sorghum (Taur et al., 1984c). Further reduction of starch in the sprouts could be due to amylolytic action of the microflora in the fermenting autoclaved pearl millet flour as discussed above. Taur et al. (1984c) found maximum degradation of starch by amylases during germination followed by fermentation of sorghum.

Moist heating of pearl millet flour might degrade starch and lower the starch content in the autoclaved flour and thereby increase the amount of total soluble sugars. Similarly, germination also may result in higher concentration of sugars in the pearl millet sprouts. These soluble sugars may further be utilised by the growing microorganisms in the fermentation mixture as a source of energy.

In the initial stages of fermentation, higher concentration of the soluble sugars may be seen but with the passage of time in fermentation, these sugars may be utilised and the fermented product may contain the sugar level which may be lower than the initial concentration of sugars in the fermenting mixture. Hamad and Fields (1979) reported that during the natural lactic acid fermentation of cereals, the level of reducing sugars increased 4.38 times on the first day but decreased on the second and third day. The increase and decrease were attributed to the action of the microflora during fermentation. Odunfa (1983a) and Mahajan (1986) also reported an initial increase in the reducing sugar level due to hydrolysis of oligosaccharides

present in the unfermented sample and decrease in later stages of fermentation due to utilisation of sugars by the fermenting microflora.

4.6 Digestibility

4.6.1 Starch digestibility (in vitro)

Starch digestibility increased as a result of natural fermentation at all the temperatures; highest being at 30°C (Table 20). Fermentation carried out at 20 and 25°C improved the starch digestibility but the difference between the two was non-significant.

Pure culture fermentation increased the starch digestibility of the pearl millet flour significantly in all types of fermentations (Table 21) except Lb fermentation. Fermentation by yeasts appeared to be resulting in higher starch digestibility than lactobacilli. S. diastaticus fermented product had significantly higher starch digestibility than S. cerevisiae fermented product. Mixed culture fermentation improved the starch digestibility and the fermented product was found to possess the starch digestibility varying from 28.5 in ScLf to 35.6 in SdLb fermentation. Sequential culture fermentation appeared to have the highest starch digestibility when compared with mixed and single culture fermentations. Sequential culture fermented Sd+Lb group had the starch digestibility which was significantly higher than Sd+Lf, Sc+Lb and Sc+Lf groups. The latter three groups had the starch digestibility varying non-significantly from one another. Among all the microorganisms studied, S. diastaticus appeared to be a better organism for improving the starch digestibility.

There was more than three fold increase in starch digestibility when pearl millet grains were sprouted for 24 h (Table 22). This increase was further enhanced when the sprouts were fermented by pure culture combinations of yeasts and lactobacilli. The starch digestibility was the

TABLE 20

Effect of natural fermentation on in vitro starch digestibility
(mg maltose released per g flour) and in vitro protein digestibility
(%) of pearl millet flour (on dry matter basis)

Fermentation	Starch digestibility	Protein digestibility
F20	90.9±0.72	64.3±0.21
F25	90.5±0.93	75.5±7.00
F30	110.1±0.74	83.3±0.14
Rp	17.8±1.00	51.0±0.04
Zf	18.7±0.25	59.2±0.00
SE(m)	±0.45	±1.80
CD (P < 0.05)	1.35	5.42

Values are means ± SD of four independent determinations.

TABLE 21

Effect of pure culture fermentation on in vitro starch digestibility (mg maltose released per g flour) and in vitro protein digestibility(%) of pearl millet flour (on dry matter basis)

Fermentation	Starch digestibility	Protein digestibility
<u>Single fermentation</u>		
Sd	38.3±0.79	67.6±0.11
Sc	35.6±0.83	81.6±5.27
Lb	19.6±0.65	68.4±0.33
Lf	20.5±0.83	68.7±0.10
<u>Mixed fermentation</u>		
SdLb	35.6±0.36	72.2±0.16
SdLf	29.7±0.74	72.4±3.53
ScLb	30.1±0.22	75.3±3.75
ScLf	28.5±0.50	75.5±0.20
<u>Sequential fermentation</u>		
Sd+Lb	41.1±0.65	64.4±9.10
Sd+Lf	38.7±0.18	65.0±0.25
Sc+Lb	37.0±1.00	70.8±3.50
Sc+Lf	36.6±0.65	69.8±5.45
<u>Control</u>		
Rp	17.8±1.00	51.0±0.04
Zf	18.7±0.25	59.2±0.00
SE(m)	±0.38	±2.07
CD(P<0.05)	1.14	6.21

Values are means ± SD of four independent determinations.

TABLE 22

Effect of mixed fermentation on in vitro starch digestibility
(mg maltose released per g flour) and in vitro protein digestibility
(%) of sprouted pearl millet (on dry matter basis)

Fermentation	Starch digestibility	Protein digestibility
GSdLb	82.2±0.54	79.7±0.21
GSdLf	65.3±1.08	78.6±0.19
GScLb	65.7±0.82	82.4±0.14
GScLf	61.7±0.43	90.1±0.19
G	55.0±1.14	77.2±0.28
SE(m)	±0.49	±0.12
CD(P < 0.05)	1.48	0.36

Values are means± SD of four independent determinations.

the highest i.e. 82.2 mg maltose released per g flour when pearl millet sprouts were fermented with GSdLb. The starch digestibility under all other combinations was relatively low. Improvement in the starch digestibility was similar when the sprouts were fermented by SdLf, ScLb and ScLf combinations.

Improvement in starch digestibility during fermentation and germination may be due to breakdown of starch to oligosaccharides by fermenting microflora (Cronk et al., 1977) or by the enzyme inherent in pearl millet grains (Sheorain and Wagle, 1973; Jain and Date, 1975). Phytic acid has been known to inhibit the amylolysis (Thompson and Yoon, 1984) and the reduction in the phytate content of the pearl millet flour or grains during fermentation or germination as observed (Tables 23, 24 and 25) in this study and earlier (Lopez et al., 1983; Dhankher and Chauhan 1987c) may account for improvement in the starch digestibility of the fermented products and the sprouts.

An improvement in starch digestibility through fermentation has been reported in case of sorghum (Taur et al., 1984b), pearl millet (Mahajan, 1986) soybean (Boralkar and Reddy, 1985), tef (Ramachandran and Bolodia, 1984), iru (Oyewole, 1984), rabadi (Dhankher and Chauhan, 1987b).

4.6.2 Protein digestibility (in vitro)

A significant increase in protein digestibility was also noticed when pearl millet flour was autoclaved at 15 psi for 15 min. Protein digestibility (in vitro) improved significantly as a result of fermentation carried out at all the temperatures except 20°C; temperature having a positive relationship with improvement in protein digestibility (Table 20). Protein digestibility at 30°C was significantly higher than that at 25°C or 20°C. Similarly at 25°C, the protein digestibility was significantly more than that at 20°C.

Protein digestibility was enhanced in all the pure culture combinations (Table 21). In single culture fermentation, S. cerevisiae product had the highest protein digestibility which was significantly more than the protein digestibility of the product fermented by other microorganism. There was no significant difference in the protein digestibility when the pearl millet flour was fermented by S.diastaticus, L.brevis and L.fermentum. Leaving aside S. cerevisiae fermented product, mixed culture fermentation seemed to result in better protein digestibility of pearl millet flour. The protein digestibility of mixed fermented product varied from 72.2 to 75.5 per cent. Sequential culture fermented product had a relatively low protein digestibility which varied from 64.4 in Sd+Lb to 70.8 per cent in Sc+Lb combination.

Germination along brought about a pronounced increase in protein digestibility (Table 22). Of all fermentations, the fermented sprouts seemed to have all time high protein digestibility which varied from 78.6 in GSdLf to 90.1 per cent in GSclF combination.

Improvement of protein digestibility as a result of fermentation is mainly associated with enhanced proteolytic activity. High proteinase activity has frequently been reported in the fermentation of protein foods as in the production of Japanese miso, soysauce (Wang and Hesseltine, 1970; Steinkraus et al., 1965) and ogiri (Odunfa, 1983 b). An increase in the amino nitrogen by fermentation signifies a partial breakdown of proteins to peptides and amino acids resulting in improved protein digestibility (Kao and Robinson, 1978).

Increased protein digestibility in various fermented products including tempeh and miso (Kao and Robinson, 1978), fermented sorghum meal

(Kazanas and Fields, 1981; Paul and Fields, 1981), germinated and fermented soybean (Boralkar and Reddy, 1985) and rabadi (Dhankher and Chauhan, 1987b) has been reported earlier. Phytic acid known to inhibit the proteolytic enzymes (Tan et al., 1984; Knuckles et al., 1985), is considerably reduced during the natural as well as pure culture fermentations (Tables 23, 24 and 25) which may explain partly the increase in protein digestibility during fermentation.

4.7 Antinutritional factors

4.7.1 Phytic acid

Autoclaving lowered the phytic acid content of the pearl millet flour considerably from 990 to 630 mg/100 g. When this autoclaved flour was fermented by natural lactic fermentation, the phytate content was further reduced and the flour fermented product at 20 and 25°C contained 240 and 113 mg phytic acid per 100 g, respectively (Table 23). The fermentation at 30°C eliminated the phytic acid completely. Thus, higher the temperature, the more was the loss of phytic acid during fermentation.

As a result of pure culture fermentation, the fermented pearl millet flour contained phytic acid significantly lower than the zero h control. Of the three types of pure culture fermentations of pearl millet flour, sequential culture fermentation appeared to have the most pronounced phytate lowering effect followed by mixed and single culture fermentation (Table 24). Sequential fermentation by Sd+Lb combination could completely eliminate the phytic acid from the pearl millet flour.

Germination of pearl millet grain for 24 h reduced the phytic acid content drastically from 990 in pearl millet grain to 375 mg/100 g in sprouts. Fermented sprouts had either little or negligible amount of phytic acid (Table 25).

TABLE 23

Changes in polyphenol and phytic acid contents during natural fermentation of pearl millet flour (mg/100 g, on dry matter basis)

Fermentation	Polyphenols	Phytic acid
F20	816±13.5	240±1.4
F25	851±14.5	113±0.6
F30	860±13.4	ND*
Rp	761±3.2	990±3.5 ,
Zf	693±2.4	630±10.6
SE(m)	±6.3	±2.9
CD(P < 0.05)	18.8	8.8

Values are means±SD of four independent determinations.

* Not detected

TABLE 24

Changes in polyphenol and phytic acid contents during pure culture fermentations of pearl millet flour (mg/100 g, on dry matter basis)

Fermentation	Polyphenol	Phytic acid
<u>Single fermentation</u>		
Sd	641±16.9	585±0.7
Sc	719± 4.7	570±1.4
Lb	634± 4.4	555±0.0
Lf	561± 3.3	525±3.5
<u>Mixed fermentation</u>		
SdLb	678± 3.2	488±2.6
SdLf	618± 5.6	480±1.5
ScLb	644± 2.1	580±2.9
ScLf	648± 7.5	510±3.5
<u>Sequential fermentation</u>		
Sd+Lb	677± 7.8	ND*
Sd+Lf	650± 8.7	225±0.0
Sc+Lb	596± 6.7	450±3.5
Sc+Lf	624± 8.9	488±2.8
<u>Control</u>		
Rp	761± 3.2	990±3.5
Zf	693± 2.4	630±10.6
SE(m)	±4.14	±2.12
CO(P < 0.05)	12.4	3.36

Values are means±SD of four independent determinations.

* Not detected

TABLE 25

Changes in polyphenol and phytic acid contents during mixed fermentation of sprouted pearl millet (mg/100 g, on dry matter basis)

Fermentation	Polyphenols	Phytic acid
GSdLb	841±5.6	ND*
GSdLf	658±1.5	15±4.1
GScLb	761±5.5	ND*
GScLf	771±4.6	ND*
G	753±4.8	375±2.7
SE(m)	±3.97	±1.25
CD(P < 0.05)	11.9	3.76

Values are means±SD of four independent determinations.

* Not detected

As discussed earlier, the decrease in phytic acid content of pearl millet flour during fermentation may account for improved protein as well as starch digestibility under all different types of fermentations as phytic acid had a significant negative correlation with in vitro starch and protein digestibility (Table 26).

A wide range of microflora have been known to possess phytase activity (Daniels and Fisher, 1981; Lopez et al., 1983) which may be partly responsible for reducing its contents in the fermenting food. The inherent phytase activity in pearl millet grain (Mahajan and Chauhan, 1987) may be responsible for lowering the phytate content of pearl millet grain during germination and fermentation of sprouts may have complementary effect on phytic acid of the sprouts.

Phytate reduction during fermentation has been reported in various foods including rabadi (Dhankher and Chauhan, 1987c), fermented pearl millet (Mahajan and Chauhan, 1987), fermented corn meal (Lopez et al., 1983; Chompreeda and Fields, 1984), tempeh (Sutardi and Buckle, 1985) and leavened bread (Navert and Sandstrom, 1985).

4.7.2 Polyphenols

Autoclaving of pearl millet flour lowered the total polyphenols significantly. This reduced level of polyphenols was further raised when the natural lactic fermentation was carried out at 20, 25 and 30°C. A gradual increase in the level of polyphenols occurred with increase in the temperature of fermentation (Table 23).

Polyphenol content of pure culture fermented pearl millet product was significantly lower than that of raw pearl millet flour. When compared with zero h control, the significant difference was noticed in all the fermentations except single culture fermentation by S.cerevisiae (Table 24).

TABLE 26

Correlation coefficients of group means of in vitro
starch and protein digestibility with phytic acid content
of fermented pearl millet flour

Fermentation	Protein digestibility	Starch digestibility
Natural fermentation	- 0.9399*	- 0.9458**
Pure culture fermentation	- 0.1057	- 0.5610*
Pure culture fermentation of sprouts	- 0.9423**	- 0.8758**

*Values are significant at 5% level

**Values are significant at 1% level

Germination could significantly increase the concentration of polyphenols in pearl millet grains (Table 25). The amount of polyphenols of the sprouts decreased when fermentation was carried out by SdLf combination. On the other hand, the fermentation carried out by GScLf and GSdLb combinations increased the polyphenols content significantly. GScLb combination for the fermentation of sprouts did not change the polyphenol content significantly.

The increased amount of polyphenols in the germinated or fermented product may be due to metabolic activity in the grain or microflora during germination and fermentation, respectively. The metabolic changes may also enhance extraction of polyphenols from pearl millet flour or sprouts which may lead to estimation of higher amounts of polyphenols in the fermented products (Mahajan, 1986). The diminishing effect on polyphenols may be due to the activity of polyphenol oxidase of pearl millet grain or fermenting microflora (Dhankher and Chauhan, 1987c). Natural lactic fermentation has been reported to increase the polyphenol content of pearl millet flour (Mahajan, 1986). A decrease in polyphenol content during rabadi fermentation was observed by Dhankher and Chauhan (1987c).

4.8. Mineral extractability

Fermentation as well as germination did not change the total amount of the minerals in the pearl millet flour. The HCl extractability of the minerals, an index of their bioavailability to human system, changed significantly to varying extent as a result of different types of fermentations. The raw pearl millet flour had 36 per cent calcium extractability which was raised significantly to 44.8 per cent as a result of autoclaving. Calcium extractability of zero h control was considerably improved by natural fermentation at all the temperatures; the extractability was higher at higher temperatures of fermentation (Table 27). A similar trend in zinc

TABLE 27

Effect of natural fermentation on HCl-extractability(%) of Ca, Fe, Zn, Cu and Mn of pearl millet flour (on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
F20	75.0±0.41	52.3±1.70	58.8±2.15	84.5±1.20	75.4±0.00
F25	77.8±2.27	52.2±4.75	61.5±0.00	85.7±0.00	81.7±0.00
F30	82.6±5.10	72.3±4.39	64.6±0.00	86.6±0.00	81.7±0.00
Rp	36.0±5.22	19.4±1.66	42.2±3.21	35.2±8.34	52.8±2.85
Zf	44.8±3.49	20.5±2.95	44.5±1.81	37.4±0.46	55.7±0.00
SE(m)	± 2.17	± 2.58	±1.10	±2.17	±0.74
CD(P < 0.05)	6.53	7.74	3.30	6.51	2.23

Values are means±SD of four independent determinations.

extractability was observed during natural fermentation. Iron extractability was increased more than three times by natural fermentation at 30°C; the fermentations at 20 and 25°C were relatively less effective and at both the temperatures, the iron extractability was the same. Copper extractability during natural fermentation was more than double at all the temperatures. Variation in the fermentation temperature did not cause a significant impact on copper extractability. Similarly, manganese extractability was significantly improved (Table 27). The zero h control had 55.7 per cent manganese extractability which was raised to 75.4 per cent at 20°C and 84.70 per cent at both 25 and 30°C.

Zero h fermentation control had higher calcium extractability than raw pearl millet flour (Table 28). Mixed culture fermentation appeared to bring about the improvement in calcium extractability significantly followed by single and sequential culture fermentations. Types and combinations of microflora in the pure culture fermentations did not seem to have any significant effect on calcium extractability of the pearl millet flour. Zero h control had significantly better extractability of zinc and manganese as compared to raw pearl millet flour. The extractability of iron, zinc, copper and manganese during pure culture fermentations improved significantly and sequential culture fermentation was found to give better extractability of all these minerals (Table 28). Single and mixed culture fermentations gave similar extractability of the minerals.

The sprouting of pearl millet grains improved the extractability of all the minerals (Table 29). But when the sprouts were fermented with pure cultures, the extractability either improved or remained unchanged. Calcium extractability was improved significantly by GSdLf and GSCLb; iron by GSdLb and GSdLf; zinc by GSdLb and GSCLb; copper by all the combinations and manganese by GSdLb and GSCLb combinations only.

TABLE 28

Effect of pure culture fermentation on HCl-extractability(%) of Ca,
Fe, Zn, Cu and Mn of pearl millet flour(on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
<u>Single fermentation</u>					
Sd	80.0±3.30	27.1±4.27	46.1±4.77	66.0±0.00	66.8±5.60
Sc	80.0±1.93	29.0±0.46	49.8±1.12	61.5±3.38	66.8±0.00
Lb	84.3±2.36	35.7±5.55	52.8±2.69	62.2±7.15	75.2±4.80
Lf	78.4±3.72	36.9±2.01	48.4±1.34	61.5±11.3	66.8±0.00
<u>Mixed fermentation</u>					
SdLb	89.3±2.32	23.9±3.48	50.7±1.55	66.6±0.00	68.7±6.84
SdLf	89.0±6.71	27.5±2.42	49.2±0.00	66.6±0.00	66.8±0.00
ScLb	86.0±6.92	30.5±4.47	50.0±0.75	66.6±0.00	75.2±4.80
ScLf	85.0±2.66	41.6±4.27	48.4±1.34	66.6±0.00	72.4±0.00
<u>Sequential fermentation</u>					
Sd+Lb	72.0±9.93	47.5±8.16	62.5±3.24	88.7±6.85	77.9±7.88
Sd+Lf	76.0±3.12	36.0±5.19	55.3±3.05	74.9±5.86	72.4±3.92
Sc+Lb	73.4±1.84	54.9±6.64	52.3±2.15	74.9±5.86	66.8±1.90
Sc+Lf	76.2±4.55	32.9±1.83	50.7±1.55	66.6±0.00	66.8±1.90
<u>Control</u>					
Rp	36.0±5.22	19.4±1.66	42.2±3.21	35.2±8.34	52.8±2.85
Zf	44.8±3.49	20.5±2.95	44.5±1.81	37.4±0.46	55.7±0.00
SE(m)	± 2.72	-2.50	-1.36	2.99	- 2.31
CD(P < 0.05)	8.16	7.50	4.08	8.97	6.93

Values are means ± SD of four independent determinations.

TABLE 29

Effect of mixed fermentation on HCL-extractability(%) of Ca,Fe,Zn, Cu and Mn of sprouted pearl millet (on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
GSdlb	78.0±1.41	56.4±1.30	58.4±3.10	83.8±1.20	75.4±0.00
GSdlf	78.3±1.87	58.8±4.91	52.3±0.00	76.8±5.90	69.1±0.00
GSclb	80.8±4.64	44.7±10.50	55.3±0.00	85.7±0.00	75.4±0.00
GSclf	77.2±3.91	40.3±0.91	52.8±0.00	76.8±3.18	70.6±4.10
G	72.5±2.50	38.7±0.00	50.3±1.32	71.4±0.49	66.0±5.45
SE(m)	±1.80	±3.03	±0.97	±1.80	±1.76
CD(P < 0.05)	5.42	9.09	2.92	5.42	5.28

Values are means±SD of four independent determinations.

Distribution of various forms of phosphorus in the fermented pearl millet flour was also studied (Tables 30, 31 and 32) and a correlation between phytic acid and the extractable phosphorus and Pi was also established (Table 33).

Phosphorus extractability in HCl increased significantly as a result of autoclaving well as fermentations of the autoclaved pearl millet flour. After 72 h fermentation at 30°C, the phosphorus extractability was more than doubled. The extractability at 20 and 25°C was less than that at 30°C. There seemed to be a positive relationship between phosphorus extractability at higher temperatures. The increase in phosphorus extractability corresponded with a proportional increase in non-phytate phosphorus as well as inorganic phosphorus (Fig. 2, 3 and 4). This showed that hydrolytic reduction of phytic acid during fermentation may release more inorganic phosphorus which may be contributing towards extractable phosphorus (Table 33).

The phosphorus extractability increased significantly as a result of pure culture fermentation of pearl millet flour (Table 31). The resultant phosphorus extractability in single, mixed and sequential culture fermentations varied from 39.8 to 45.7, 41.0 to 46.8 and 43.1 to 60.6 per cent, respectively. Except Sd+Lb fermentation, there did not appear to be much of variation in phosphorus extractability under various pure culture fermentation combinations. Higher the non-phytate phosphorus, the more seemed to be the phosphorus extractability but inorganic phosphorus appeared to have a little different pattern (Fig. 3). Correlation coefficients showed a significant negative correlation between phytate and inorganic and extractable phosphorus ($P < 0.01$). Indirectly, it can be said that higher the non-phytate phosphorus, more was the inorganic phosphorus as well as extractable phosphorus (Table 33).

TABLE 30

Effect of natural fermentation on phytate P, non-phytate P, inorganic P(% of total P) and HCl-extractability of P (%) of pearl millet flour (on dry matter basis)

Fermentation	Phytate P	Non-phytate P	Inorganic P	Extractability
F20	21.5±0.18	78.5±0.20	27.5±0.07	57.0±0.62
F25	9.6±0.05	90.4±0.07	30.4±0.12	62.7±0.60
F30	0.0±0.0	100.0±0.0	47.6±0.61	84.3±0.36
Rp	80.4±0.27	19.6±0.27	6.2±0.15	32.5±0.26
Zf	57.1±0.63	42.9±0.63	9.3±0.10	34.8±0.29
SE(m)	±0.19	±0.19	±0.17	±0.26
CD(P < 0.05)	0.57	0.57	0.51	0.78

Values are means±SD of four independent determinations.

 Non-phytate P
  Phytate P
  Extractable P
  Inorganic P

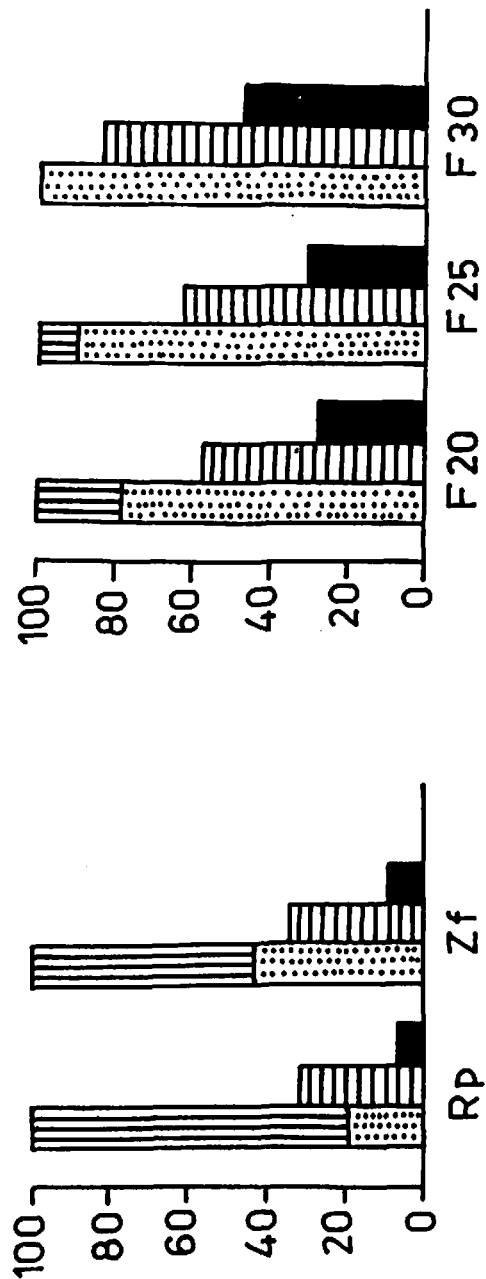


FIG. 2. EFFECT OF NATURAL FERMENTATION ON PHYTATE P, NON-PHYTATE P, INORGANIC P (% OF TOTAL P) AND HCL-EXTRACT-ABILITY OF P (%) OF PEARL MILLET FLOUR

TABLE 31

Effect of pure culture fermentation on phytate P, non-phytate P, inorganic P(% of total P) and HCl-extractability of P(%) of pearl millet flour (on dry matter basis)

Fermentation	Phytate P	Non-phytate P	Inorganic P	Extractability
<u>Single fermentation</u>				
Sd	47.9±0.21	52.1±0.21	15.2±0.07	44.0±0.15
Sc	50.2±0.10	49.8±0.10	12.8±0.00	45.7±0.65
Lb	46.7±0.10	53.3±0.10	17.3±0.10	44.0±0.70
Lf	47.0±0.45	53.0±0.45	13.9±0.18	39.8±0.33
<u>Mixed fermentation</u>				
SdLb	41.8±0.07	58.2±0.07	18.8±0.08	46.8±0.53
SdLf	41.1±0.28	58.9±0.28	12.9±0.11	45.2±0.33
ScLb	44.1±0.68	55.9±0.68	12.7±0.25	41.0±0.45
ScLf	44.1±0.31	55.9±0.31	15.9±0.11	41.2±0.40
<u>Sequential fermentation</u>				
Sd+Lb	00.0±0.00	100.0±0.00	30.8±0.10	60.6±0.47
Sd+Lf	19.4±0.08	80.6±0.08	17.7±0.10	47.6±0.23
Sc+Lb	38.9±0.28	61.1±0.28	21.1±0.10	45.0±0.34
Sc+Lf	43.6±0.25	56.4±0.25	19.5±0.31	43.1±0.21
<u>Control</u>				
Rp	80.4±0.27	19.6±0.27	6.20±0.15	32.5±0.26
Zf	57.1±0.63	42.9±0.63	9.30±0.10	34.8±0.29
SE(m)	±0.19	±0.19	±0.09	±0.23
CD(P<0.05)	0.57	0.57	0.27	0.69

Values are means± SD of four independent determinations.

TABLE 32

Effect of fermentation on phytate P, non-phytate P, inorganic P (% of total P) and HCl-extractability of P(%) of sprouted pearl millet (on dry matter basis)

Fermentation	Phytate P	Non-phytate P	Inorganic P	Extractability
GSdLb	0.0±0.00	100.0±0.00	38.8±0.12	73.8±0.52
GSdLf	1.4±0.36	98.6±0.35	30.7±0.22	63.6±0.36
GScLb	0.0±0.00	100.0±0.00	40.7±0.07	74.9±0.21
GScLf	0.0±0.00	100.0±0.00	39.8±0.45	70.2±0.21
G	34.9±0.10	65.1±0.12	22.0±0.18	52.4±0.29
SE(m)	±0.10	±0.10	±0.14	±0.17
CD(P < 0.05)	0.30	0.30	0.42	0.51

Values are means ± SD of four independent determinations.

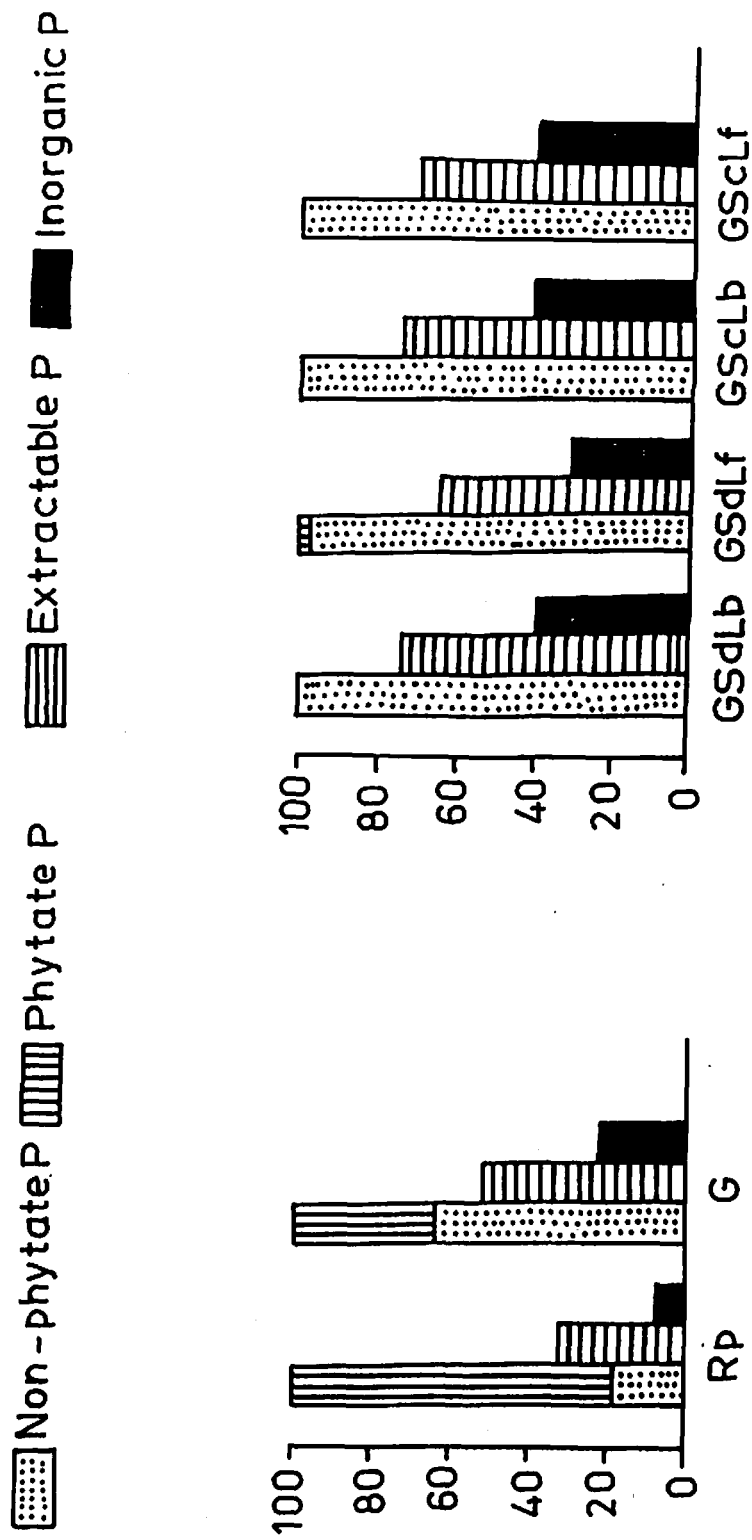


FIG. 4. EFFECT OF FERMENTATION ON PHYTATE P, NON-PHYTATE P, INORGANIC P (% OF TOTAL P) AND HCL-EXTRACTABILITY OF P (%) OF SPROUTED PEARL MILLET

TABLE 33

Correlation coefficients of group means of inorganic phosphorus, extractable zinc, copper, iron, manganese, phosphorus and calcium with phytic acid content of fermented pearl millet flour

Minerals	Natural fermentation	Pure culture fermentation	Pure culture fermentation of sprouts
Inorganic P	- 0.9129*	- 0.8609**	- 0.9498**
Extractable Zn	- 0.9642**	- 0.8800**	- 0.8985*
Extractable Cu	- 0.7125	- 0.8644**	- 0.9067*
Extractable Fe	- 0.9409*	- 0.5843*	- 0.7640
Extractable Mn	- 0.9424*	- 0.7000**	- 0.8622*
Extractable P	- 0.8850*	- 0.8280**	- 0.9422**
Extractable Ca	- 0.9751**	- 0.4427	- 0.9387**

* Values are significant at 5% level

** Values are significant at 1% level

Germination for 24 h increased the phosphorus extractability of pearl millet grains from 32.5 to 52.4 per cent (Table 32). The phosphorus extractability of sprouts was further significantly raised after pure culture fermentation; different combinations had significantly different values for phosphorus extractability. With increase in extractability, there was an associated increase in inorganic phosphorus (Fig. 4). Reduction in phytate leading to increased non-phytate phosphorus had most likely an elevating effect on phosphorus extractability and inorganic phosphorus levels in the fermented products (Table 33). A significant negative correlation was found between phytic acid and inorganic phosphorus and phosphorus extractability ($P < 0.01$).

Iron, zinc, calcium etc., the divalent cations in plant foods, are generally present in association with the phytic acid which may be responsible for lower extractability of these divalent cations. Decrease in the level of phytic acid (Tables 23, 24 and 25) by fermentation, possibly through hydrolysis by phytase of the pearl millet grain or the fermenting microflora may release these metallic ions in free form and may account for their increased HCl extractability in the fermented product. The phytic acid in the fermented products was found to have a significant negative correlation with extractable zinc, copper, iron, manganese, calcium and phosphorus (Table 33) which ascertained the role of phytic acid in lowering the extractability of the divalent cations in the plant foods.

Fermentation has been found to bring about conversion of the bound form of minerals to free form, thus, increasing the amount of extractable minerals (Ramakrishnan et al., 1976; Nolan and Duffin, 1987). Tangkongchitr et al. (1981) indicated that loss of phytate phosphorus by fermentation for 8 h was accounted by an increase in inorganic phosphorus. Fermentation increased the bioavailability of zinc from breads blended with bran in human

beings (Navert and Sandstrom, 1985). Ramachandran and Bolodia (1984) reported an increase in dialysable portion of iron during fermentation of tef and wheat from 9 to 24 per cent. Chompreeda and Fields (1984) found an increase in extractability of iron in fermented corn meal. An increased extractability of manganese and copper during fermentation of bread was reported by Ramakrishnan et al. (1976) and Harland and Harland (1980). Dhankher (1985) and Mahajan (1986) too reported an improvement in extractability of calcium, phosphorus, copper, iron, zinc and..... manganese during natural lactic fermentation of pearl millet flour and rabadi fermentation.

4.9 Thiamine

Autoclaving could lower the thiamine content of pearl millet significantly (Table 34). Natural fermentation at 20 and 25°C raised the thiamine content of autoclaved pearl millet flour significantly; values at 20°C were significantly higher than those at 25°C. But the fermentation at 30°C lowered the thiamine content significantly and the amount was less than 50 per cent of the amount present at 20 and 25°C.

Pure culture fermentations by lactobacilli alone or in combination with yeasts lowered the thiamine content of the pearl millet flour significantly (Table 35). But the concentrations of thiamine improved almost two fold and three fold when the fermentation was carried by S. diastaticus and S. cerevisiae, respectively.

As expected, germination improved the thiamine content of the pearl millet grains significantly. But when the sprouts were fermented with pure culture fermentations, then the fermented sprouts had lower thiamine content than the unfermented sprouts (Table 36).

TABLE 34
Changes in thiamine content during natural fermentation
of pearl millet flour ($\mu\text{g}/100\text{ g}$, on dry matter basis)

Fermentation	Thiamine
F20	336 $\pm$ 3.16
F25	330 $\pm$ 3.54
F30	154 $\pm$ 3.74
Rp	321 $\pm$ 2.12
Zf	197 $\pm$ 1.58
SE(m)	$\pm$ 1.70
CD(P $\leq$ 0.05)	5.10

Values are means $\pm$ SD of four independent determinations.

TABLE 35

Changes in thiamine content during pure culture fermentation of pearl millet flour ($\mu\text{g}/100\text{ g}$, on dry matter basis)

Fermentation	Thiamine
<u>Single fermentation</u>	
Sd	576 $\pm$ 2.54
Sc	840 $\pm$ 3.74
Lb	54 $\pm$ 2.68
Lf	148 $\pm$ 1.87
<u>Mixed fermentation</u>	
SdLb	70 $\pm$ 4.41
SdLf	142 $\pm$ 1.65
ScLb	118 $\pm$ 2.54
ScLf	201 $\pm$ 2.55
<u>Sequential fermentation</u>	
Sd+Lb	149 $\pm$ 1.00
Sd+Lf	170 $\pm$ 3.80
Sc+Lb	202 $\pm$ 2.00
Sc+Lf	257 $\pm$ 3.08
<u>Control</u>	
Rp	321 $\pm$ 2.12
Zf	197 $\pm$ 1.58
SE(m)	$\pm$ 1.56
CD(P< 0.05)	4.68

Values are means $\pm$ SD of four independent determinations.

TABLE 36
Changes in thiamine content during mixed fermentation
of sprouted pearl millet ($\mu\text{g}/100\text{ g}$, on dry matter basis)

Fermentation	Thiamine
GSdLb	260 $\pm$ 3.81
GSdLf	315 $\pm$ 1.00
GScLb	278 $\pm$ 1.58
GScLf	312 $\pm$ 2.45
G	344 $\pm$ 2.16
SE(m)	$\pm$ 1.38
CD ($P < 0.05$)	4.15

Values are means $\pm$ SD of four independent determinations.

Yeasts are known to synthesise thiamine whereas lactobacilli need an external source of thiamine for their growth. This is why single culture fermentation by yeasts could improve the thiamine content and the lactobacilli perhaps consumed the thiamine in the fermentation media and resulted in low amount of the vitamin in the fermented product. In sequential culture fermentation also during the first 72 h fermentation, the yeasts might have raised the thiamine which would have been later utilised by the lactobacilli and the resultant level was even lower than the zero h control. Germination is also known to increase the level of B-vitamins including thiamine; the germination of pearl millet grains for 24 h raised the level of this vitamin in the pearl millet grain.

Lee et al. (1980) reported that fermentation of rice in water at room temperature for four days brought about an increase in the riboflavin content whereas thiamine content decreased by 50 per cent. According to Steinkraus et al. (1967), thiamine decreased from 10 µg in soybean to 14 µg/g in tempeh. Rao and Basu (1952) reported that vitamin content of the dahi depends upon the type of organisms used for fermentation. They found that a mixed culture of L. bulgaricus and L. cremoris decreased thiamine whereas, single cultures of S. lactis and S. cremoris raised the thiamine concentrations from 2 to 20 per cent over that of milk. Paul (1979) found that thiamine decreased at 25°C during the fourth day of fermentation of sorghum. Murdock and Fields (1984) studied the effect of natural lactic fermentation of cornmeal on B-vitamins and reported decrease in the thiamine content after 24 h fermentation whereas other B-vitamins increased. Dhankher and Chauhan (1987a) demonstrated a significant increase in the level of thiamine during rabadi fermentation.

4.10 Biological evaluation

Diets providing 8 per cent protein derived from different fermented products, raw pearl millet, zero h control and egg albumen were fed to the weanling rats and their food intake, protein intake, gain in body weight, food efficiency ratio (FER) and protein efficiency ratio (PER) were estimated (Table 37). For these estimations, only products of natural fermentation and mixed culture fermentation were used for biological evaluation. Raw pearl millet flour, zero h control and egg albumen diets formed the control groups. When the natural and mixed fermented diets were fed, they were not found to be acceptable to the rats. Thus, in order to increase the acceptability, the fermented products and zero h control were mixed with equal amounts of raw pearl millet flour and then used in preparing the experimental diets.

Food intake was the highest in egg albumen group and the lowest in the F30 group and these values were significantly different from those for raw pearl millet and zero h control groups. Food intake in F25, F20, SdLf, SdLb, ScLb and ScLf groups were significantly lower than that of the egg albumen group. Similar trends were seen in protein intake under different dietary groups. The diets containing fermented and unfermented pearl millet resulted in a gain in body weight of the rats which was significantly lower than that of the egg albumen control group. The poorest gain in body weight was encountered in F30 group. All the remaining fermented groups and the raw pearl millet and zero h control groups showed better gain in body weights. Mixed fermentation improved the body weight of the rats to a significantly higher level than the raw pearl millet, zero h control and the naturally

Food intake, protein intake, gain in body weight, feed efficiency ratio(FER) and protein efficiency ratio (PER) in rats fed fermented pearl millet flour

Dietary groups	Food intake(g)	Protein intake(g)	Gain in body weight(g)	Food efficiency ratio	Protein efficiency ratio
EA	205.1±12.7	16.4±1.02	42.9±5.11	0.209±0.02	2.62±0.28
Rp	115.3±12.7	9.22±1.02	12.8±1.80	0.111±0.01	1.38±0.12
Zf	114.8±4.8	9.18±0.38	13.0±0.81	0.113±0.01	1.41±0.06
ScLf	123.0±10.2	9.84±0.80	18.8±3.82	0.152±0.02	1.91±0.24
ScLb	107.6±7.2	8.60±0.57	16.4±2.77	0.152±0.02	1.90±0.28
SdLf	119.3±8.5	9.54±1.90	18.0±4.29	0.150±0.03	1.88±0.32
SdLb	120.8±12.0	9.66±0.96	17.4±3.26	0.144±0.01	1.80±0.24
F20	109.7±6.2	8.77±0.49	9.64±1.40	0.087±0.01	1.09±0.15
F25	112.7±9.4	9.01±0.74	9.76±2.50	0.086±0.02	1.08±0.27
F30	99.8±6.0	7.98±0.48	6.30±1.47	0.063±0.01	0.789±0.15
SE(m)	±4.18	±0.35	±1.14	±0.007	±0.08
CD(P<0.05)	12.5	1.05	3.42	0.02	0.24

Values are means±SD of eight rats fed for four weeks.

fermented dietary groups. In the similar way, FER and PER were the highest in egg albumen dietary group, followed by mixed and natural fermentation dietary groups. Among the naturally fermented groups, the lowest PER and FER were observed in F30 and the values were significantly lower than F20 and F25 groups. FER and PER in all the naturally fermented groups were significantly lower than raw pearl millet and zero h control groups. Mixed fermented dietary groups had significantly higher PER and FER values than the naturally fermented pearl millet and zero h control groups. PER among various mixed fermented groups did not vary significantly whereas FER in SdLb group was significantly lower than that in the remaining mixed fermentation groups.

Uncontrolled fermentations allowed the growth of diverse types of microorganisms, some of which perhaps resulted in the production of unpalatable flavour and taste. The fermentation at 30°C might allow the growth of a wide spectrum of the microflora and this may result in even the maximum growth of undesirable microorganisms. Thus, the resultant fermented product at 30°C might be relatively less acceptable. This is even indicated by the sensory evaluation of the naturally fermented products (Table 6), according to which the product fermented at 30°C had the lowest acceptable aroma. This could have caused low palatability of the diets and resultantly low food intake by the rats. Since protein intake by the animals depended upon their food intake, groups having higher food intake showed the high protein intake too. Similarly, FER and PER depended on the food intake, protein intake and gain in body weight. Thus, the differences in values of the determining factors could be instrumental in differences of these ratios. Since F30 group had the lowest food intake, protein intake and gain in body weight, this group had the lowest FER and PER values too.

Kappuswamy et al. (1957) found that feeding of pearl millet diets to rats at 5 to 6 per cent and 9 to 11 per cent of protein gave PER values of 1.2 and 1.4 to 1.8, respectively. Narayana Rao et al. (1961) observed 1.2 PER at 5-6 per cent level of protein of pearl millet. Pushpamma et al. (1972) reported 1.84 PER at 11 per cent of pearl millet protein. Khader (1983) fed a fermented product like miso prepared from rice, bengalgram and curds or yeast and found an increase in the PER values. Weanling rats fed on idli and khaman were found to be superior to those fed on corresponding unfermented product and PER values increased three folds (Akinrele and Edwards, 1971). Klopfenstein et al. (1985) fed supplemented pearl millet flour to rats and reported an increase in the PER value. Vass et al. (1984) fed yoghurt, kefir, sour milk etc. to rats, PER values increased significantly because of better digestibility of these fermented milk products.

Egg albumen group had the highest true digestibility and as compared to it, all other fermented and control groups had significantly lower true digestibility values (Table 38). All the fermented groups had the similar true digestibility which did not vary significantly among themselves. All the fermented groups except SdLf had significantly higher true digestibility when compared to that of raw pearl millet group. Similar trend was observed in apparent digestibility. Biological value of egg albumen was much higher than that of the fermented and unfermented pearl millet control groups. No significant difference was observed among the fermented pearl millet groups (Table 38). Biological value under ScLf and SdLb was significantly higher than that under the raw pearl millet group. The remaining fermented groups did not vary significantly from the raw pearl millet groups for biological values. The unfermented and fermented groups had

TABLE 38

Apparent digestibility, true digestibility, biological value, net protein utilisation, and utilisable protein of fermented pearl millet flour

Dietary groups	Nitrogen consumed (mg/day)	Nitrogen absorbed (mg/day)	Apparent digestibility (%)	True digestibility (%)	Nitrogen retained (mg/day)	Biological value (%)	Net protein utilisation (%)	Utilisable protein (%)
EA	87.4±2.95	83.8±3.1	92.1±1.7	95.8±1.7	78.2±4.0	93.3±1.9	89.3±3.2	7.64±0.27
Rp	59.9±3.66	48.9±2.9	76.4±2.4	81.6±2.4	37.4±2.8	76.4±3.6	62.3±3.9	5.33±0.37
Zf	61.4±4.45	50.3±4.2	76.8±4.6	81.9±4.4	39.0±3.4	77.6±0.78	63.5±4.0	5.43±0.43
ScLf	63.0±19.9	55.3±17.8	82.3±2.6	87.7±2.7	45.5±16.1	82.2±5.20	72.1±4.6	6.17±0.43
ScLb	61.6±3.20	54.0±2.3	82.7±3.0	87.6±3.2	43.4±1.5	80.3±3.6	70.3±4.5	6.01±0.42
SdLf	62.4±6.96	52.7±5.3	79.1±3.1	84.4±3.3	41.7±5.6	79.4±3.4	66.7±4.6	5.77±0.39
SdLb	70.2±9.40	61.9±7.4	82.6±1.9	86.8±2.2	49.3±6.4	80.8±1.9	70.1±0.9	6.00±0.08
F20	63.7±9.80	56.3±11.2	82.5±6.2	88.3±5.3	43.9±9.9	77.9±2.9	68.7±5.8	5.88±0.51
F25	71.0±5.20	61.7±5.7	82.3±2.5	86.9±2.4	49.2±5.7	79.7±3.7	69.2±3.7	5.92±0.29
F30	60.9±5.00	52.7±3.8	81.4±2.6	86.5±2.8	41.2±4.0	78.1±3.3	67.5±1.4	5.77±0.12
SE(m)	±3.84	±3.51	±1.50	±1.45	±3.21	±1.45	±3.05	±0.14
CD (P<0.05)	11.50	10.50	4.50	4.35	9.63	4.35	9.15	0.42

Values are means±SD of six albino rats kept in metabolic cages for five days after acclimatisation.

significantly lower NPU values than egg albumen group. When compared to raw pearl millet and zero h control groups, only ScLf group had significantly higher NPU whereas other fermented groups did not exhibit any significant variation from control values. Utilisable protein was the highest in the egg albumen group whereas all other groups had significantly lower values. The fermentation of pearl millet flour improved the utilisable protein significantly. The variations for utilisable protein among fermentation groups were, however, not significant.

An attempt was made to correlate apparent and true digestibility with phytic acid content of the pearl millet flour, as an association of in vitro digestibility with phytate content of the plant foods has been known to exist. In natural fermentation, phytate had a significant negative correlation with apparent and true digestibility of the fermented product (Table 39). This correlation was negative but not significant in pure culture fermentation groups.

In vitro digestibility of the fermented products could be used as a yardstick for assessing the apparent digestibility of the fermented food as a significant positive correlation between the two was established (Table 40). A similar correlation of in vitro protein digestibility with true digestibility was observed in pure culture fermentation while in natural fermentation only positive but non-significant correlation existed.

Net protein retention and protein retention efficiency were significantly lower when egg albumen in the diets was replaced by raw pearl millet, zero h control and the fermented products. The pearl millet flour fermented by SdLb and naturally fermented at 25 and 30°C had the same NPR as the raw pearl millet flour or zero h control. The remaining fermented groups had significantly higher NPR than that of the raw pearl millet flour group. Similar trend was observed for PRE (Table 41).

TABLE 39

Correlation coefficients of group means of apparent and true digestibility with phytic acid content of fermented pearl millet flour

Fermentation	Apparent digestibility	True digestibility
Natural fermentation	- 0.9403*	- 0.8784*
Pure culture fermentation	- 0.7698	- 0.7601

*Values are significant at 5% level

TABLE 40

Correlation coefficient of group means of in vitro
protein digestibility with apparent and true
digestibility

Fermentation	Apparent digestibility	True digestibility
Natural fermentation	0.7821*	0.6548
Pure culture fermentation	0.9446**	0.8121*

* Values are significant at 5% level

** Values are significant at 1% level

TABLE 41

Net protein retention and protein retention efficiency in rats fed on fermented pearl millet flour

Dietary groups	Gain in weight (g)	Loss in weight (g)	Protein consumed (g)	Net protein retention	Protein retention efficiency
EA	11.9±0.83	2.30	2.55±0.09	5.58±0.20	89.2±3.2
Rp	2.20±0.22	2.30	1.75±0.11	2.57±0.20	41.1±3.3
Zf	2.43±0.28	2.30	1.79±0.05	2.64±0.21	42.2±3.4
ScLf	3.82±1.13	2.30	1.83±0.58	3.34±0.61	53.4±9.8
ScLb	3.32±0.22	2.30	1.79±0.09	3.13±0.23	50.0±3.8
SdLf	3.35±0.40	2.30	1.82±0.20	3.10±0.28	49.6±4.5
SdLb	3.30±1.00	2.30	2.04±0.27	2.74±0.52	53.8±8.4
F20	3.81±0.74	2.30	1.85±0.28	3.30±0.71	52.8±11.4
F25	3.31±0.62	2.30	2.07±0.15	2.71±0.23	43.4±3.8
F30	2.45±1.00	2.30	1.75±0.15	2.71±0.48	43.4±7.8
SE(m)	±0.32		±0.11	±0.18	±2.97
CD (P<0.05)	0.96		0.33	0.45	8.91

Values are mean±SD of six albino rats kept in metabolic cages for five days after acclimatisation.

Khader (1979) fed dhokla to rats at 8 per cent protein level and reported 92.5 per cent true digestibility. Chaudhary and Atreja (1985) fed shrikhand, a milk product fermented by S.lactis, S.thermophilus and L.bulgaricus, to rats and reported BV, digestibility coefficient, NPU values similar to that of casein. Zamora and Veum (1979) reported an increase in the average daily weight gain for weanling rats fed soybean fermented with R. oligosporus compared with the daily weight gain for rats fed soybean given the same heat treatment (121°C for 30 min) but not fermented. The fermented soybeans had a greater apparent digestibility, BV and NPU values.

4.11 Weight and composition of organs

Different dietary groups were evaluated for their effect on the weight of liver in the growing rats (Table 42). Except ScLf group, liver weight of the egg albumen group was significantly higher than that of the all other groups. Fermentation of the pearl millet flour could not result in improved weight of the liver when the diets prepared from the fermented products were fed to the growing rats; ScLf was the only exception. Fermentation of pearl millet flour seemed to increase the heart weight in experimental rats only when fermentation was carried out with ScLf and natural fermentation at 20°C. These values of heart weight were also significantly lower than those found in egg albumen fed rats. Kidney weights seemed to be non-significantly different from those of the egg albumen fed group when the rats were given ScLf and F20 fermented diets. Kidney weight in the rest of the fermented groups did not vary significantly (Table 42).

Liver of the rats fed with fermented pearl millet diets had significantly higher level of protein than the animals fed with raw pearl

TABLE 42

Fresh weights of liver(g), heart (mg) and kidney (mg) of albino rats fed fermented pearl millet flour

Dietary groups	Liver	Heart	Kidney
EA	3.20±0.31	304±20	561±50
Rp	1.95±0.28	193±10	385±50
Zf	2.03±0.07	205±30	440±26
SclF	2.86±0.64	268±40	524±60
Sclb	2.23±0.19	221±30	459±50
SdLf	2.25±0.35	215±29	468±60
SdLb	2.28±0.21	221±28	458±34
F20	1.98±0.32	239±17	511±58
F25	2.23±0.39	227±20	439±40
F30	1.98±0.23	198±16	420±38
SE(m)	±0.15	±11.4	±21.4
CD(P < 0.05)	0.45	34.2	64.2

Values are means±SD of six rats.

millet diet (Table 43). Among the fermented groups, feeding of F30 resulted in the lowest amount of protein in the liver. Feeding of ScLb diet caused the highest increase in protein content of the liver but these values were significantly lower than those in the egg albumen group. The amount of lipids in the liver was minimum when naturally fermented pearl millet flour was fed to the rats, the values not being significantly different from those in the pearl millet fed rats. A relatively higher amount of lipids was found in the mixed fermented groups and were significantly better than the pearl millet controls (Table 43).

By feeding fermented diets, the protein in the kidney increased significantly when compared with the protein contents of the kidney of the raw pearl millet fed rats. Mixed culture fermentation and natural fermentation seemed to behave almost similarly except ScLf group which resulted in relatively higher amount of protein in the kidney (Table 44). Lipids in the kidney behaved like proteins in the liver when the rats were fed with diets containing different fermented products. Mixed culture fermentation seemed to be superior to natural fermentation as far as the amount of total lipids in the kidney of the experimental rats was concerned.

4.12 Serum analysis and haematological examination

Feeding of fermented products did not result in any abnormality in the SGOT, SGPT activities, protein and albumin contents of the serum. These values were within the normal range (Table 45).

TLC, DLC and RBC counts were also within the normal range. The values for TLC and RBC counts ranged from 8000 to 8950/ cmm and 7.5 to 8.6 millions, respectively (Table 46). It signifies that the feeding of fermented and control diets was biologically safe and did not cause any serum abnormality i.e. infection, anaemia etc.

TABLE 43

Total protein and lipid content of the liver of the rats fed on fermented
pearl millet flour

Dietary groups	Protein		Lipids	
	mg/organ	g/100g	mg/organ	g/100g
EA	509.4±13.1	15.90±0.40	105.0±0.16	3.29±0.01
Rp	134.7±10.3	6.90±0.53	68.2±0.24	3.49±0.01
Zf	155.4±8.8	7.66±0.43	72.2±0.84	3.56±0.04
ScLf	233.5±2.8	10.47±0.13	84.4±2.94	2.95±0.10
ScLb	285.4±3.6	9.98±0.30	89.0±4.38	3.99±0.20
SdLf	188.3±1.5	8.37±0.06	89.7±6.45	3.98±0.28
SdLb	204.7±10.0	8.98±0.44	82.0±10.8	3.59±0.47
F20	180.3±10.7	9.11±0.54	64.2±0.32	3.24±0.02
F25	194.2±14.9	8.71±0.66	62.1±2.19	2.78±0.09
F30	163.9±8.4	8.28±0.42	62.2±4.20	3.14±0.21
SE(m)	±5.41	±0.24	±2.60	±0.12
CO(P < 0.05)	16.23	0.72	7.80	0.36

Values are means±SD of six rats.

TABLE 44

Total protein and lipid content of the kidney of rats fed on fermented pearl millet flour

Dietary groups	Protein		Lipids	
	mg/organ	g/100g	mg/organ	g/100g
EA	78.9±1.55	14.06±0.27	16.1±0.07	2.86±0.01
Rp	26.9±0.29	6.99±0.07	4.8±0.31	1.25±0.08
Zf	31.8±1.74	7.22±0.39	6.9±0.29	1.57±0.06
ScLf	52.8±0.92	10.09±0.17	11.8±0.14	2.25±0.03
ScLb	43.3±3.20	9.44±0.69	12.6±0.22	2.74±0.05
SdLf	37.6±2.10	8.05±0.44	10.1±0.15	2.17±0.03
SdLb	34.9±1.40	7.63±0.30	9.58±0.21	2.09±0.05
F20	38.1±4.30	7.46±0.85	8.50±0.14	1.67±0.03
F25	41.3±5.10	9.42±1.16	6.10±0.16	1.40±0.04
F30	38.4±0.90	9.16±0.21	6.93±0.25	1.65±0.06
SC(m)	±1.51	±0.32	±0.12	±0.03
CD(P < 0.05)	4.53	0.96	0.35	0.09

Vales are means ± SD of six rats.

TABLE 45

Serum protein and albumin concentration and SGOT and SGPT activity in rats fed on fermented pearl millet flour

Dietary groups	Serum protein (g/100 ml)	Serum albumin (g/100 ml)	SGOT (IU/L)	SGPT (IU/L)
EA	6.46±0.50	3.00±0.25	16.0±1.41	15.0±3.53
Rp	4.80±0.40	2.05±0.07	16.0±3.74	11.0±1.00
Zf	4.85±0.22	2.00±0.10	14.0±2.95	13.0±1.58
ScLf	5.50±0.21	2.50±0.35	17.5±1.85	8.0±1.41
ScLb	4.90±0.21	2.10±0.16	10.0±1.41	8.0±1.41
SdLf	5.50±0.07	2.20±0.37	19.0±2.16	20.0±3.54
SdLb	5.40±0.14	3.80±0.14	11.0±1.00	13.0±1.00
F20	4.83±0.55	2.16±0.11	10.4±0.35	16.0±1.00
F25	5.80±0.14	2.06±0.29	15.3±0.75	12.9±0.71
F30	5.50±0.31	2.06±0.54	11.7±1.48	14.6±0.83
SE(m)	±0.18	±0.16	±1.14	±1.09
CD(P<0.05)	0.54	0.48	3.42	3.27
Normal value	4.5-9.0	2.0-4.8	Rat < 45 Mouse < 35	Rat < 20 Mouse < 35

Values are means±SD of six rats.

TABLE 46

Haematological examination of rats fed different diets

EA	Rp	Zf	Dietary groups					F20	F25	F30	Normal values
			SdLb	SdLf	ScLb	ScLf					
Total leucocyte count (TLC)	8,736	8,000	8,600	8,350	8,475	8,270	8,038	8,067	8,950	8,100	$8 \times 10^3 - 11 \times 10^3$
Differential leucocyte count (DLC)											
Polymorphs(P)	29	34	33	34	34	39	34	26	22	25	15-40
Lymphocytes(L)	68	64	64	64	64	59	63	72	75	72	50-80
Monocytes(M)	2	1	2	1	1	1	2	1	2	1	2-7
Eosinophils(E)	1	1	1	1	1	1	1	1	1	2	0-4
Red Blood cells count (RBC)	8.6	7.8	7.5	8.1	8.3	8.1	8.0	7.9	7.9	7.8	7-10

TLC is expressed as count per cubic millimeter

DLC is expressed as per cent of total leucocyte counts

RBC is expressed as millions per cubic millimeter

Values are averages of six replicates.

4.13 Histopathology

Histopathological examination of liver, kidney, adrenals and thymus of the rats fed on different dietary groups did not reveal any abnormality. The feeding of fermented products did not affect the organs adversely.

4.14 Utilisation for human consumption

The pearl millet flour was fermented naturally and by mixed cultures, and then dried to a constant weight in the hot air oven kept at 65°C. The dried powder was ground in an electric grinder and used for preparing different recipes in combination with other food items. Their acceptability in terms of colour, flavour, taste, appearance, bitterness, sourness and texture was adjudged by a panel. The average values for overall acceptability are given in Tables 47 to 52.

The cutlets prepared in combination with boiled rice and potatoes (cutlet A) and soaked bread and boiled potatoes (cutlet B) were found to be acceptable (Table 47). When naturally fermented product was incorporated in cutlets A and B, the cutlets were found to be 'slightly acceptable'. Cutlet A prepared from mixed fermented pearl millet flour was 'moderately liked'. Cutlets B prepared from ScLb were 'slightly liked' whereas those prepared from other mixed fermentations were 'moderately liked'.

The fermented pearl millet flour was incorporated with besan, spinach and onions for the preparation of pakora A; besan and onions for pakora B; with besan and onions (a slightly different proportion of besan from that used for pakora B) for pakora C. Pakora A containing mixed fermented and naturally fermented pearl millet flours at 20 and 25°C were 'moderately liked' whereas those prepared from naturally fermented pearl millet flour at 30°C were 'slightly liked'. Pakora B incorporating SdLb, SdLf and ScLf products were 'moderately liked' whereas those having ScLb,

TABLE 47
Overall acceptability of fermented pearl millet cutlets*

Fermentation	Cutlet	
	A	B
SdLb	7.58±0.42	7.19±0.51
SdLf	7.02±0.48	6.94±0.52
ScLb	7.14±0.71	6.54±0.81
ScLf	7.14±0.62	7.12±0.54
F20	6.66±0.38	6.78±0.29
F25	6.92±0.34	6.70±0.36
F30	6.16±0.82	6.26±0.44
SE(m)	±0.18	±0.17
CD(P < 0.05)	0.54	0.51

*Average score of seven characteristics (colour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point Hedonic scale.

Values are means±SD of ten replicates.

TABLE 48

Overall acceptability of fermented pearl millet pakora*

Fermentation	Pakora		
	A	B	C
SdLb	7.10±0.68	6.66±0.80	6.92±0.85
SdLf	7.24±0.53	6.62±0.72	6.86±0.77
ScLb	7.16±0.56	6.38±0.87	6.74±0.69
ScLf	7.38±0.60	7.00±0.51	7.08±0.42
F20	6.88±0.43	6.38±0.56	6.26±0.59
F25	6.90±0.31	5.94±0.43	6.20±0.51
F30	6.42±0.38	5.62±0.60	6.16±0.45
SE(m)	±0.17	±0.22	±0.21
CD(P < 0.05)	0.51	0.66	0.63

* Average score of seven characteristics (colour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point Hedonic scale.
Values are means±SD of ten replicates.

TABLE 49
Overall acceptability of weaning mixtures prepared from fermented
pearl millet*

Fermentation	Weaning mixture		
	A	B	C
SdLb	7.32±0.42	6.94±0.79	6.90±0.52
SdLf	7.08±0.50	6.38±0.71	7.00±0.59
ScLb	7.20±0.59	6.44±0.74	7.12±0.57
ScLf	7.02±0.52	6.70±0.69	6.98±0.50
F20	6.00±0.81	4.92±0.51	5.67±0.88
F25	5.15±1.01	4.07±0.50	5.70±0.94
F30	4.49±0.58	3.37±0.30	5.24±0.59
SE(m)	±0.22	±0.21	±0.22
CD(P < 0.05)	0.66	0.63	0.66

* Average score of seven characteristics (colour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point Hedonic scale.

Values are means±SD of ten replicates. .

TABLE 50
Overall acceptability of fermented pearl millet chappati*

Fermentation	Chappati		
	A	B	C
Sdlb	6.74±0.47	6.56±0.33	6.80±0.55
Sdlf	6.37±0.64	6.75±0.39	6.84±0.35
ScLb	6.66±0.52	6.45±0.50	6.83±0.29
ScLf	6.12±0.70	6.29±0.48	6.58±0.56
F20	5.28±0.81	5.46±0.74	5.80±0.48
F25	4.72±1.13	4.82±0.50	5.06±0.59
F30	3.76±0.86	3.84±0.64	3.88±0.71
SE(m)	±0.25	±0.18	±0.17
CD(P < 0.05)	0.75	0.54	0.51

*Average score of seven characteristics (colour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point Hedonic scale.
Values are means±SD of ten replicates.

TABLE 51
Overall acceptability of fried vermicilli prepared
from fermented pearl millet*

Fermentation	Fried vermicilli
SdLb	6.52±0.47
SdLf	6.64±0.47
ScLb	6.70±0.51
ScLf	6.48±0.40
F20	5.56±0.39
F25	5.40±0.92
F30	4.26±0.63
SE(m)	±0.19
CD(P < 0.05)	0.57

*Average score of seven characteristics (colour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point Hedonic scale.

Values are means±SD of ten replicates.

TABLE 52

Overall acceptability of fermented pearl millet biscuits*

Fermentation	Biscuit		
	A	B	C
SdLb	6.95±0.57	6.60±0.67	6.08±0.65
SdLf	6.84±0.69	7.10±0.64	5.30±1.13
ScLb	7.09±0.49	6.96±0.58	6.16±0.46
ScLf	7.05±0.41	6.86±0.59	6.34±0.54
F20	6.20±0.32	6.28±0.38	5.27±0.70
F25	5.49±0.46	5.31±0.57	4.88±0.55
F30	4.57±0.65	4.13±0.68	4.14±0.61
SE(m)	±0.17	±0.20	±0.23
CD(P< 0.05)	0.51	0.60	0.69

* Average score of seven characteristics (colour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point Hedonic scale. Values are means±SD of ten replicates.

F20, F25 and F30 products were in the 'slightly liked' category. Pakoras C had a significant variation in acceptability score when mixed fermented and naturally fermented pearl millet flours were incorporated. Pakora C from mixed fermentation was 'moderately liked' while those from naturally fermented pearl millet flour were 'slightly liked' (Table 48).

Weaning mixtures containing roasted wheat flour, green gram dal, chickpea, groundnut, sesame, sugar and jaggery in addition to the fermented pearl millet flour were prepared. Weaning mixtures A and C were 'moderately liked' when mixed fermented pearl millet flour was incorporated for their preparation (Table 49). Weaning mixture having mixed fermentation product was 'slightly liked' whereas those prepared from F20, F25 and F30 were 'slightly disliked', 'slightly disliked' and 'moderately disliked', respectively. Weaning mixtures A from F20, F25 and F30 were slightly liked, 'neither liked nor disliked' and 'disliked slightly, respectively. Weaning mixtures C from F20 and F25 were 'neither liked nor disliked' while those from F30 were 'slightly disliked'.

Three different types of chappaties were prepared after the addition of raw pearl millet flour (Chappati A), whole wheat flour (Chappati B) and whole wheat flour and besan (Chappati C) to the fermented pearl millet flour. The chappaties prepared from mixed culture fermented pearl millet flour were 'slightly liked' (Table 50). Chappaties A, B and C were 'neither liked nor disliked' when F20 pearl millet flour was used; 'slightly disliked' when F25 was used for chappaties A and B and 'neither liked nor disliked' when used for chappati C. Chappaties A, B and C were 'moderately disliked' when prepared from F30 fermented flour.

A fried, vermicilli product using besan in combination with the fermented flour was prepared and the product was 'slightly liked' when mixed fermented pearl millet flour was used. F20 and F25 pearl millet flour

produced 'neither liked nor disliked' product and F30 fermented flour yielded fried vermicilli which was 'slightly disliked' (Table 51).

Biscuits containing pearl millet flour, weaning mixture C (Biscuit A), refined wheat flour and sugar (Biscuit B), refined wheat flour and common salt (Biscuit C) were prepared and their acceptability was assessed (Table 52). All the mixed fermented flour biscuits (A and B) were 'moderately liked' whereas those prepared from F20, F25 and F30 were 'slightly liked', 'neither liked nor disliked' and 'slightly disliked', respectively. Biscuits (C) were 'slightly liked' when SdLb, ScLb and ScLf fermented products were incorporated; 'neither liked nor disliked' when SdLf and F20 products were used and 'slightly disliked' when F25 and F30 products were included for biscuit making.

Most of the overall acceptability results indicated that naturally fermented product was less acceptable than the mixed fermented product. Mixed fermented pearl millet flour products were generally acceptable when incorporated in various recipes, although a slight variation in their overall acceptability score was seen here and there. The difference in the overall acceptability of the products prepared from naturally fermented pearl millet flour appeared to base on their characteristic aroma as evaluated earlier (Table 6). Naturally fermented products were found to be acceptable satisfactorily when they were incorporated in the deep fried recipes.

Khader (1982) prepared a fermented food similar to miso from rice, bengal gram and curd or yeast. The fermented foods when fried were well accepted by the tasting panel. Taur et al. (1984a) did sensory evaluation of the chips made from germinated-fermented sorghum and they were 'neither liked nor disliked' by the panel.

CHAPTER-V

SUMMARY AND CONCLUSIONS

In India, pearl millet is the fourth important grain crop, being only next to rice, wheat and sorghum. Pearl millet is the staple food and forms a major source of dietary nutrients to a large section of Indian population. It has limited use and low nutritive value. This study was carried out to improve its nutritive value and to diversify its uses. Natural as well as pure culture fermentations of pearl millet were conducted. Freshly ground pearl millet flour (100 g) was mixed with distilled water (900 ml) and autoclaved at 15 psi for 15 min. After autoclaving and cooling, 10 g freshly ground pearl millet flour was added as inoculum and was fermented at 20, 25 and 30°C for 72h. Single, mixed and sequential fermentations of pearl millet flour as well as mixed fermentations of the sprouts were carried out by using pure cultures of S.diastaticus, S.cerevisiae, L.brevis and L. fermentum. Pearl millet flour (100 g) was mixed with distilled water (900 ml) and autoclaved at 15 psi for 15 min. After cooling, it was inoculated with the microflora (10^5 cells/ml) and fermented at 30°C for 72 h. The soaked pearl millet grains were sprouted at 30°C for 24 h. The sprouts (100g) were mixed with water (200 ml), ground to paste and autoclaved at 5 psi for 20 min. After cooling, it was mixed fermented by the yeasts and lactobacilli combinations at 30°C for 72h.

Natural lactic fermentation at 20, 25 and 30°C produced the fermented product which was acceptable for aroma and colour. The pH of the fermented product dropped and titratable acidity rose as the fermentation of raw as well as autoclaved flour progressed at all the temperatures. The pH was the lowest and titratable acidity was the highest when fermentation of the raw and autoclaved pearl millet flour was carried out at 30°C. A significant decrease in pH and simultaneous increase in titratable acidity was noticed in

the pearl millet flour fermented by pure cultures of yeast and lactobacilli, at 30°C for 72 h. In case of single culture fermentation, Lactobacillus fermentum had the highest lowering effect followed by L.brevis, S.diastaticus and S.cerevisiae. Sequential fermentation yielded the lowest pH followed by mixed and single culture fermentations. In case of fermentation of the pearl millet sprouts, the initial pH (5.89) dropped to as low as 3.62 (GSdLf group).

Lactobacilli were the most dominating microflora present in the naturally fermented product. SPC, yeasts, Lactobacilli, fungi and coliforms increased substantially when the fresh pearl millet flour was fermented without prior autoclaving. As the fermentation progressed, all the microflora grew in number; the maximum increase being in lactobacillus followed by coliform and yeast at all the temperatures. Fungal cells grew marginally during fermentation; growth being maximum at 30°C. Coliforms showed an ample growth at all the temperatures. When the pearl millet flour was first autoclaved and then inoculated with pearl millet flour, lactobacilli were the dominating microflora of the fermented product and the growth of all the microorganisms was the highest at 30°C and the lowest at 20°C.

Protein content of the fermented pearl millet products was either decreased or remained unchanged. Natural fermentation brought about a marginal reduction in the protein content of the pearl millet flour. The temperature of fermentation did not affect the protein content significantly. The protein content of S.cerevisiae fermented pearl millet flour was significantly higher as compared to the flour fermented by S.diastaticus, L.brevis and L.fermentum. All the mixed fermentation combinations except SdLf, had significantly lower protein values than raw pearl millet flour and zero h control. In case of sequential fermentation, only Sd+Lf and Sc+Lf groups decreased the protein content of pearl millet flour significantly. Pure culture fermentations did not change the protein content of the pearl millet sprouts.

Natural lactic fermentation improved the fat content of pearl millet significantly; temperature did not seem to influence the fat content of the fermented product. Pure culture fermentation either decreased or did not alter the fat content. Single culture fermentation improved the fat content; the lowest amount was found in Lb group. Mixed culture fermentation did not alter the fat content of the flour significantly. Sequential fermentations reduced the fat content of the pearl millet flour; the type of combinations had no significant effect. Fermentation of the sprouts by GSdLf and GSclb lowered the fat contents significantly but in GSdLb and GSclf fermentations, there was no change in the fat content. Natural fermentation and pure culture fermentations of the flour as well as sprouts could not significantly alter the ash content of the pearl millet.

Level of total soluble sugars, reducing and non-reducing sugars increased significantly following autoclaving of the pearl millet flour. All these values were further raised when the autoclaved flour was naturally fermented for 72 h at all the temperatures. The extent of increase seemed to be the highest at 30°C and the lowest at 20°C. Temperature of natural fermentation influenced the starch content of the flour; the higher the temperature the more was the reduction in starch content. Pure culture fermentations reduced the starch content significantly; the greatest reduction was observed during sequential fermentation. S. diastaticus brought about the highest reduction in starch content followed by S. cerevisiae, L. brevis and L. fermentum. In mixed fermentation of the flour as well as the sprouts, Sd combinations had relatively more disparaging effect on the starch content. Pure culture fermentations for 72h lowered the total soluble and reducing sugars. The concentrations of reducing as well as non-reducing sugars were found to be the lowest in sequential fermentation.

There was an improvement in digestibility (in vitro) of starch as well as protein following fermentation. Starch digestibility increased significantly as a result of natural fermentation at all the temperatures; the highest being at 30°C. Pure culture fermentations increased the starch digestibility of the pearl millet flour significantly except the fermentation by L.brevis. Fermentation by yeasts appeared to result in higher starch digestibility than lactobacilli. S.diastaticus fermented product had significantly higher starch digestibility than S.cerevisiae fermented product. Sequential fermentation appeared to result in the highest starch digestibility when compared with mixed and single culture fermentations. GSdLb fermented sprouts had the highest starch digestibility among all the fermented sprout groups. Of all the microorganisms studied, S.diastaticus appeared to be a better organism for improving the starch digestibility.

Protein digestibility (in vitro) improved significantly as a result of natural fermentation carried out at all the temperatures except 20°C; temperature having a positive relationship with improvement in protein digestibility. In single culture fermentation, Sc had the highest protein digestibility. Leaving aside Sc fermented product, mixed fermentation seemed to result in better protein digestibility of pearl millet flour. Sequential culture fermented product had a relatively low protein digestibility which varied from 64.4 per cent in Sd+Lb to 70.8 per cent in Sc+Lb combination. Germination alone brought about a pronounced increase in protein digestibility. Of all the pure culture fermentations, the fermented sprouts seemed to have the highest protein digestibility which varied from 78.6 per cent in GSdLf to 90.1 per cent in GScLf.

There was a drastic reduction in phytic acid content during natural fermentation; a greater reduction at a higher temperature. The natural fermentation at 30°C eliminated the phytic acid completely. Among pure culture

fermentations, sequential fermentation appeared to have the most pronounced phytate lowering effect followed by mixed and single culture fermentation; Sd+Lb combination could completely eliminate the phytic acid from the pearl millet flour. Germination of pearl millet grain for 24h reduced the phytic acid substantially from 990 in pearl millet grain to 375 mg/100 g in sprouts. Fermented sprouts had negligible amount of phytic acid.

A gradual increase in the level of polyphenols occurred with increase in the temperature of natural fermentation. Pure culture fermentation significantly lowered the polyphenol content of the pearl millet flour whereas germination increased the concentration of polyphenols in the pearl millet grains. Polyphenols of the sprouts decreased when fermentation was carried out by GSdLf combination. On the other hand, fermentation by GSclf and GSdLb combination increased the polyphenol content significantly.

Fermentation and germination did not change the total minerals content of pearl millet. The HCl extractability of the minerals, an index of their bioavailability to human system, changed significantly as a result of different types of fermentations. Calcium extractability improved following natural fermentation; the higher the temperature of fermentation, the more was the mineral extractability. A similar trend was observed for zinc and iron extractability. Iron extractability was increased more than three times by natural fermentation at 30°C. Variation in the fermentation temperature did not cause a significant impact on copper extractability. Manganese extractability was significantly improved at all the fermentation temperatures.

Mixed fermentation appeared to bring about the maximum improvement in calcium extractability followed by single and sequential culture fermentations. Types and combinations of microflora in the pure culture

fermentation did not seem to have any significant effect on the calcium extractability of the pearl millet flour. The extractability of zinc, iron, copper and manganese improved significantly during pure culture fermentations of pearl millet flour. Sequential fermentation was found to give better extractability of all these minerals. Single and mixed culture fermentations gave almost similar extractability of the minerals. The sprouting of pearl millet grains improved the extractability of Ca, Zn, Fe, Cu and Mn. But when the sprouts were mixed fermented with pure cultures, the extractability either improved or remained unchanged. Calcium extractability was improved significantly by GSdLf and GSCLb; iron by GSdLb and GSdLf; zinc by GSdLb and GSCLb; copper by all the combinations and manganese by GSdLb and GSCLb combinations only.

The phosphorus extractability improved significantly as a result of all types of fermentations. The phosphorus extractability was more than doubled when the flour was naturally fermented; a higher phosphorus extractability at a higher temperature. Pure culture fermentation of the pearl millet flour as well as of the sprouts improved the HCl-extractability of phosphorus. In all types of fermentations, the increase in phosphorus extractability was reflected in a proportional increase in non-phytate phosphorus as well as inorganic phosphorus. A significant negative correlation was found between phytic acid and inorganic phosphorus and phosphorus extractability of naturally as well as pure culture fermented pearl millet. Hydrolysis of phytic acid during fermentation may release more inorganic phosphorus which may be contributing towards more extractable phosphorus.

Thiamine content of the pearl millet was reduced significantly as a result of autoclaving. Natural fermentation at 20 and 25°C raised the thiamine content significantly. Thiamine content present in the naturally fermented product at 30°C was less than 50% of the amount present at 20 and

25°C. Pure culture fermentations by lactobacilli alone or in combination with yeasts lowered the thiamine content of the pearl millet flour significantly. The concentrations of thiamine improved almost two fold and three fold when the fermentation was carried out by S.diastaticus and S.cerevisae, respectively. Germination improved the thiamine content while the mixed fermentation of the sprouts lowered the thiamine content. Yeasts are known to synthesize thiamine whereas lactobacilli need an external source of thiamine for their growth.

The fermented pearl millet products were fed to the rats and their biological utilisation was compared. Protein and feed efficiency ratio were the highest in egg albumen group, followed by mixed and natural fermentation groups. Mixed fermented dietary groups had significantly higher PER and FER values than the naturally fermented pearl millet and zero h control groups. PER among various mixed fermented groups did not vary significantly whereas FER in SdLb group was significantly lower than that in the remaining mixed fermentation groups. Among the naturally fermented groups, the lowest PER and FER were observed in F30 group. FER and PER values in all the naturally fermented groups were significantly lower than those of the raw pearl millet and zero h control groups.

Egg albumen group: had the highest true digestibility value. All the fermented groups except SdLf had significantly higher true digestibility than raw pearl millet group. Biological value of egg albumen was much higher than that of the fermented and unfermented pearl millet control groups. No significant difference in biological value was observed among the fermented pearl millet groups. Biological value under ScLf and SdLb was significantly higher than that of the raw pearl millet group. The biological value of the remaining fermented groups did not vary significantly from that of the raw pearl millet group. The unfermented and fermented groups had significantly lower NPU value than the egg albumen group. Only ScLf group had significantly

higher NPU when compared to NPU of the raw pearl millet and zero h control groups. The fermentation of pearl millet flour improved the utilisable protein significantly. The variations for utilisable protein among fermentation groups were, however, not significant. An attempt was made to correlate apparent and true digestibility with phytic acid content of the pearl millet flour. In natural fermentation, phytate had significant negative correlation with apparent and true digestibility of the fermented product. A significant positive correlation between in vitro protein digestibility and true digestibility was observed in pure culture fermentation.

The pearl millet flour fermented by SdLb and naturally fermented at 25 and 30°C had the same NPR as raw pearl millet and zero h control groups. The remaining fermented groups had significantly higher NPR than that of the raw pearl millet group. Similar trend was observed for protein retention efficiency.

Feeding of the fermented pearl millet flour did not result in improved weights of the livers; SclF being the only exception. Fermentation seemed to increase the heart weights in experimental rats fed on SclF and F20 diets. Kidney weights were not significantly affected by feeding different fermented product diets. Liver of the rats fed fermented pearl millet diets had significantly higher level of protein than the animals fed with raw pearl millet diet. Feeding of F30 diet resulted in the lowest amount of protein in the liver while feeding of Sclb diet caused the highest increase in liver protein. The amounts of lipids in the liver were minimum when naturally fermented pearl millet diets were fed to the rats; the values were not significantly different from those in the fresh pearl millet fed rats. A relatively higher amount of lipids was found in the mixed fermented groups which were significantly better than the pearl millet controls. Feeding of the mixed and natural fermented products did not change the protein content of kidney except SclF group which resulted in higher amount of kidney proteins.

Feeding of fermented products did not result in any abnormality in the SGOT, SGPT activities, protein and albumin contents of the serum. All these values were within the normal range. Feeding of the fermented products did not bring about any physiological, histopathological and haematological changes in rats.

Various recipes including cutlets, parkoras, chappaties, weaning mixtures, fried vermicilli and biscuits were prepared from the naturally and mixed fermented pearl millet flour. The cutlets prepared in combination with boiled rice and potatoes (cutlet A) and soaked bread and boiled potatoes (cutlet B) were found to be acceptable. Cutlet A prepared from the mixed fermented pearl millet flour was 'moderately liked' and cutlet B prepared from the mixed culture fermentations except ScLb was in the category of 'moderately liked'. The naturally fermented products when incorporated in cutlets A and B were found to be 'slightly liked'. The fermented pearl millet flour was incorporated with besan, spinach and onions for the preparation of pakora A; besan and onions for pakora B; with besan and onions (a slightly different proportions of besan from that used for pakora B) for pakora C. Pakoras A prepared from F20 and F25 products were 'moderately liked' whereas those prepared from F30 were 'slightly liked'. Pakoras B and C incorporating F20, F25 and F30 products were in the 'slightly liked' category. Pakoras A prepared from mixed fermented pearl millet flour had better overall acceptability as compared to that of the pakoras B and C. Pakoras B and C, incorporating SdLb, SdLf and ScLf were 'moderately liked'.

Weaning mixtures containing roasted wheat flour, green gram dal, chickpea, groundnut, sesame, sugar and jaggery in addition to the fermented pearl millet flour were prepared. Weaning mixtures prepared from the mixed fermented pearl millet flour were found to be more acceptable as compared to those prepared from the naturally fermented pearl millet flour. Among the

weaning mixtures, weaning mixture A prepared from the mixed fermented pearl millet flour had overall better acceptability. Weaning mixtures A and C were 'moderately liked' while B was 'slightly liked' when mixed fermented pearl millet flour was incorporated for their preparation. Weaning mixtures B and C were found to be 'slightly liked' or 'neither liked nor disliked' when prepared from F20 and F25 products, respectively. When F30 was incorporated in various types of weaning mixtures, it was 'slightly disliked'.

Three different types of chappaties were prepared after the addition of raw pearl millet flour (Chappati A), whole wheat flour (Chappati B) and whole wheat flour and besan (Chappati C) to the fermented pearl millet flour. Chappaties A, B and C were in the 'slightly liked' category when prepared from mixed fermented pearl millet flour. All the three types of chappaties were 'neither liked nor disliked' when prepared from F20 product while those prepared from F25 and F30 products were not liked. Fried vermicilli prepared from the mixed fermented pearl millet flour was 'slightly liked'. F20 and F25 products yielded 'neither liked nor disliked' fried vermicilli whereas the fried vermicilli prepared from F30 was not liked. Biscuits containing pearl millet flour, weaning mixture C (Biscuit A), refined wheat flour and sugar (Biscuit B) and refined wheat flour and common salt (Biscuit C) were prepared. All the types of biscuits prepared from the mixed fermented pearl millet flour were in the 'moderately/slightly liked' category. Biscuits prepared from the F20 and F25 were 'slightly liked' or 'neither liked nor disliked' but F30 biscuits were not found to be acceptable.

Overall, naturally fermented F30 product was not found to be acceptable in various types of preparations made. Naturally fermented products were found to be acceptable satisfactorily when they were incorporated in the deep fried recipes. Mixed fermented pearl millet products were generally acceptable when incorporated in various recipes although a slight variation in their overall acceptability score was seen here and there.

The study indicated that there was significant improvement in the nutritional quality of pearl millet flour following fermentation. The natural as well as pure culture fermented flour/grain had better protein and starch digestibility, low levels of phytic acid, better carbohydrate profile and significant improvement in HCl-extractability of minerals than the raw pearl millet. Biological evaluation of naturally fermented product did not show any significant improvement over control which may be ascribed to low acceptability of the fermented product by the rats. PER, FER, BV, AD, TD, NPU, NPR, PRE and UP values were higher in case of pure culture fermented products than the raw pearl millet flour. By and large, sequential fermentation and fermentation of the sprouts except in some cases were found to be the best for enhancing the nutritional value of raw pearl millet. Natural fermentation improved the nutritional value of pearl millet significantly but for human consumption, except deep fried recipes, other products prepared from naturally fermented flour were not found to be acceptable. The various recipes prepared from the pure culture fermented pearl millet were found to be acceptable.

Fermentation is therefore, a potential processing method for improving the nutritive value of pearl millet. Controlled pure culture fermentations seem to be more advantageous than the natural fermentation. The pure culture fermented product could be safely included in the diet of the people for improving their nutritional status; particularly for better bioavailability of minerals and proteins from the pearl millet. Yeast fermentation is very simple and less expensive technique and can be adopted at household level for better utilisation of the pearl millet flour for human nutrition. For future studies some more pure cultures of microbes, known for fermentative improvement of foods, can be tried for carrying out fermentation of pearl millet flour or sprouts.

CHAPTER-VI

LITERATURE CITED

- Akeson, W.E. and Stahmann, M.A. 1964. A pepsin-pancreatin digest index of protein quality evaluation. *J. Nutr.* 83:257-259.
- Akinrele, I.A. 1964. Fermentation of cassava. *J. Sci. Food Agric.* 9:589-594.
- Akinrele, I.A. and Edwards, C.C.A. 1971. An assessment of the nutritive value of maize-soya mixture, Soya-ogi, as a weaning food in Nigeria. *Brit. J. Nutr.* 26:177-185.
- Akinrele, I.A. and Bassir, O. 1967. The nutritive value of ogi, Nigerian infant food. *J. Trop. Med. Hyg.* 70: 279-280.
- Akolkar, P.N. and Parekh, L.J. 1983. Nutritive value of soy idli. *J. Food Sci. Technol.* 20:1-4.
- Aliya, S. and Geervani, P. 1981. An assessment of the protein quality and vit. B.contents of commonly used fermented products of legumes and millets. *J. Sci. Food Agric.* 32:837-842.
- Amerine, M.A., Berg, H.W. and Cruess, W.V. 1967. *The Technology of Wine Making.* The AVI Publishing Company Inc. Westport, Connecticut. pp.692.
- Ananthachar, T.K. and Desikachar, H.S.R. 1962. Effect of fermentation on the nutritional value of idli. *J. Sci. Indust. Res.* 21C:191-192.
- AOAC, 1980. *Official Methods of Analysis of the Association of Official Agricultural Chemists, Association of Analytical Chemists,* Washington, D.C. pp.125-139.
- Arroya, P.T., Pelayo, L.A., Solidum, H.T., Chiu, Y.N., Lero, M. and Alcantara, E.E. 1977. Studies on rice shrimp fermentation: balao-balao. *Phil. J. Food Sci. and Tech.* 2:106-125.
- Aw, T.L. and Swanson, B.G. 1985. Influence of tannin on Phaseolus vulgaris protein digestibility and quality. *J. Food Sci.* 50:67.
- Banigo, E.O.I. and Muller, H.G. 1972. Manufacture of ogi (A Nigerian fermented cereal porridge): comparative evaluation of corn, sorghum and millet. *Can. Inst. Food Sci. Technol. J.* 5:217-221.
- Baseler, M.E. and Fields, M.L. 1984. Use of Candida tropicalis ATCC 9968 to adjust the pH of a natural lactic acid fermentation of corn meal. *J. Food Sci.* 49:1198-1199.

- Batra, R. 1985. Effect of different treatments on keeping quality of bajra flour. M.Sc. thesis. Haryana Agricultural University, Hisar, India.
- Beal, L., Finney, P.L. and Mehta, T. 1984. Effects of germination and calcium on zinc bioavailability from peas. J. Food Sci. 49:637-638.
- Bender, A.E. and Doell, B.H. 1957. Biological evaluation of proteins; A new aspect. Brit. J. Nutr. 11:140-148.
- Bernfeld, P. 1962. In: Comparative Biochemistry (Florkin, M. and Mason, H.W. eds.) Academic Press, New York. Vol. III. pp.355.
- Boralkar, M. and Reddy, N.S. 1985. Effect of roasting, germination and fermentation on the digestibility of starch and protein present in soybean. Nutr. Reports International. 3:833-836.
- Bressani, R. and Elias, L.G. 1980. The nutritional role of polyphenols in beans. In: Hulse, J.H. (ed.). Polyphenols in cereals and legumes: Ottawa, Canada. pp.61-68.
- Burton, G.W. and Powell, J.B. 1968. Pearl millet breeding and cytogenetics. Adv. Agron. 20:49.
- Burton, G.W., Wallace, A.T. and Rachie, K.O. 1972. Chemical composition and nutritive value of pearl millet grain. Crop Sci. 12:187-190.
- Cerning, J. and Guilhot, J. 1973. Change in carbohydrates composition during maturation of wheat and barley kernels. Cereal Chem. 50:220.
- Chang, R. 1977. Phytate: Removal from whole dry beans by enzymatic hydrolysis and diffusion. J. Food Sci. 42: 1098-1101.
- Chapman, D.G., Castillo, R. and Campbell, J.A. 1959. Evaluation of protein in foods. Can. J. Biochem. Physiol. 37:679.
- Chaudhary, A. and Atreja, S.K. 1985. Nutritional quality of proteins of shrikand. J. Food Sci. Technol. 22: 411-414.
- Chaudhary, P. and Kapoor, A.C. 1984. Nutritive value of bajra flour as influenced by storage conditions. M.Sc. thesis. Haryana Agricultural University, Hisar, India.
- Chauhan, B.M., Suneja, N. and Bhat, C.M. 1986. Nutritional value and fatty acid composition of some high yielding varieties of barja. Bull. Grain Technol. 21:441-442.

- Chen, P.S., Tosibara, T.Y. and Warner, H. 1956. Micro-determinations of phosphorus. *Anal. Chem.* 28: 1756-1759.
- Cheryan, M. 1980. Phytic acid interaction in food systems. *CRC Crit. Rev. Food Sci. Nutr.* 13:297-335.
- Chick, H., Hutchinson, J.C.D. and Jackson, H.M. 1935. The biological value of protein VI. Further investigation of balance sheet method. *Biochem. J.* 29:1702-1711.
- Chompreeda, P.T. and Fields, M.L. 1984. Effect of heat and fermentation on the extractability of minerals from soybean meal and corn meal blends. *J. Food Sci.* 49:566-568.
- Clegg, K.M. 1956. The application of anthrone reagent to the estimation of starch in cereals. *J. Sci. Food Agric.* 7:40.
- Cronk, T.C., Steinkraus, K.H., Hackler, L.R. and Mattick, L.R. 1977. Indonesian tape ketan fermentation. *Appl. Environ. Microbiol.* 33:1067-1073.
- Cummings, J.H. 1978. Nutritional implication of dietary fibre. *Am. J. Clin. Nutr.* 31:521.
- Daniels, D.G.H. and Fisher, N. 1981. Hydrolysis of the phytate of wheat flour during bread making. *Brit. J. Nutr.* 46:1-6.
- Davies, N.T. and Nightingale, R. 1975. The effects of phytate on intestinal absorption and secretion of zinc, whole body retention of zinc, copper, iron and manganese in rats. *Brit. J. Nutr.* 34:243-246.
- Davies, N.T. and Reid, H. 1979. An evaluation of phytate, zinc, copper, iron and manganese contents of, and zinc availability from soy-based textured vegetable protein meat substitutes or meat extenders. *Brit. J. Nutr.* 41:579-589.
- Davis, A.B. and Moseney, R.C. 1979. Grain sorghum condensed tannins. I. Isolation, estimation and selective adsorption by starch. *Cereal Chem.* 56:310-314.
- Desai, B.B. and Zende, G.K. 1979. Role of bajra (Pennisetum typhoides) in human and animal nutrition. *Indian J. Nutr. Dietet.* 16:390-396.

- Deshpande, S.S. and Salunkhe, D.K. 1982. Interaction of tannic acid and catechin with legume starches. *J. Food Sci.* 47:2080-2081.
- Desikachar, H.S.R. 1977. Processing of sorghum and millets for versatile food uses in India. Proceedings of the, symposium on 'Sorghum and Millets for Human Food.' International Association for Cereal Chemists, Vienna, 11 May, 1976: Tropical products Institute, London, England.
- Dhankher, N. 1985. Studies on the nutritional value of rabadi - a fermented bajra product. M.Sc. thesis. Haryana Agricultural University, Hisar, India.
- Dhankher, N. and Chauhan, B.M. 1987a. Preparation, acceptability and B. vitamin content of rabadi - a fermented pearl millet food. *Int. J. Food Sci. Technol.* 22: 173-176.
- Dhankher, N. and Chauhan, B.M. 1987b. Effect of temperature and period of fermentation on protein and starch digestibility (*in vitro*) of rabadi - a pearl millet fermented food. *J. Food Sci.* 52:489-490.
- Dhankher, N. and Chauhan, B.M. 1987c. Effect of temperature and fermentation time on phytic and polyphenol content of rabadi - fermented pearl millet food. *J. Food Sci.* 52: 828-829.
- Dhillon, S., Popli, S. and Dhindsa, K.S. 1982. Chemical composition and protein fractions of some high yielding varieties of bajra. *Bull. Grain Technol.* 20:155.
- Eka, O.U. 1980. Effect of fermentation on the nutrient status of locust beans. *Food Chem.* 5:303-308.
- Ekmon, T.D. and Nagodawithana, T. 1977. Fermented foods of Sri Lanka. Symposium on 'Indigenous Fermented foods', Bangkok, Thailand.
- Elias, L.G., De Fernandez, D.G. and Bressani, R. 1979. Possible effects of seed coat polyphenolics on the nutritional quality of bean protein. *J. Food Sci.* 44:524-527.
- Ezeala, D.O. 1984. Changes in nutritional quality of fermented cassava tuber meal. *J. Agric. Food Chem.* 32:467-469.

- Faridi, H.A., Finney, P.L. and Rubenthaler, G.L. 1983. Iranian flat breads; Relative bioavailability of zinc. *J. Food Sci.* 48:107-110.
- Folch, J., Less, M. and Sloane-Stanley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:407.
- Gashe, B.A. 1985. Involvement of lactic acid bacteria in the fermentation of tef, an Ethiopian fermented food. *J. Food Sci.* 50:800-801.
- Gopalan, C., Rama Shastri, B.V. and Balasubramanian, S.C. 1981. The nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research, New Delhi. pp. 83.
- Goswami, A.K., Sehgal, K.L. and Gupta, K.P. 1970a. Chemical composition of bajra grains. III. Indian inbreds. *Indian J. Nutr. Dietet.* 7:5-9.
- Goswami, A.K., Sharma, K.P. and Sehgal, K.L. 1970b. Chemical composition of bajra grains. IV Indian varieties. *Indian J. Nutr. Dietet.* 7:67-70.
- Griffiths, D.W. 1979. Inhibition of digestive enzymes by extracts of field beans (Vicia- faba). *J. Sci. Food Agric.* 30:458-462.
- Griffiths, D.W. and Moseley, G. 1980. The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of rats. *J. Sci. Food Agric.* 31: 255-259.
- Griffiths, D.W. and Thomas, T.A. 1981. Phytate and total phosphorus content of field beans. *J. Sci. Food Agric.* 32:187.
- Gupta, H.O., Lodha, M.L., Mehta, S.I., Rastogi, D.K. and Singh, J. 1979. Effect of amino acid(s) and pulse supplementation on nutritional quality of normal and modified Opaque-2 maize. *J. Agric. Food Chem.* 27:787-790.
- Hackler, L.R., Steinkraus, K.H., Buren, J.P. and Hand, D.B. 1964. Studies on the utilization of tempeh protein by weanling rats. *J. Nutr.* 82:452-456.

- Hamad, A.M. 1978. Evaluation of natural fermentation and germination to improve the nutritive value of cereals. Ph.D. thesis. University of Missouri, Columbia.
- Hamad, A.M. and Fields, M.L. 1979. Evaluation of the protein quality and available lysine of germinated and fermented cereals. J. Food Sci. 44:456.
- Hariharan, K., Rajurs, T.S.S., Desai, B.L.M., Venkatrao, S. Rajalakshmi, D., Swaminathan, M. and Parpia, H.A.B. 1965. Effect of supplementing poor Indian diets based on Kaffir, corn, pearl millet and maize with vitamins and minerals and fortified and unfortified groundnut flour on the nutritive value of diets as judged by the growth of rats and on the protein efficiency ratio. Indian J. Nutr. Dietet. 2:196-201.
- Harland, B.F. and Harland, J. 1980. Fermentative reduction of phytate in rye, wheat and whole wheat bread. Cereal Chem. 57:226-229.
- Harrigan, W.F. and McCance, M.E. 1976. Laboratory Methods in Food and Dairy Microbiology, Academic Press, Inc. United States ed. III, Fifth Avenue, New York.
- Haslam, E. 1974. Polyphenol-protein interactions. Biochem. J. 139:285-289.
- Haug, W. and Lantzsch, H.J. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34:1423-1426.
- Henry, R.J. 1968. Glutamic-oxaloacetic transaminase: Clinical Chemistry: Principles and Techniques. pp. 511-518.
- Hesseltine, C.W. and Shibasaki, K. 1961. Miso III. Pure culture fermentation with Saccharomyces rouxii. Appl. Micro. 9:515.
- Hesseltine, C.W. 1983. The future of fermented foods. Nutr. Rev. 41:293-301.
- Hoseney, R.C., Variano-Marston, E. and Dendy, D.A.V. 1981. Sorghum and millet advances. Cereal Sci. Technol. 4:71.

- Houston, D.F., Straka, R.P., Hunter, I.R. Roberts, R.L. and Kester, E.B. 1957. Changes in rough rice of different moisture content during storage at controlled temperatures. *Cereal Chem.* 34: 444-456.
- Hulse, J.H. 1979. Polyphenols in Cereals and Legumes. Proceedings of a symposium held during the 36th annual meeting of the Institute of Food Technologists, St. Louis, Missouri.
- Jain, A.K. and Date, W.B. 1975. Relative amylase activity of some malted cereal grains. *J. Food Sci. Technol.* 12:131.
- Kao, C. and Robinson, R.J. 1978. Nutritional aspect of fermented foods from chickpea, horsebean and soybeans. *Cereal Chem.* 55:512.
- Kappuswamy, S., Joseph, K., Rao, K., Rao, M.N., Rao, G.R., Sankaran, A.K. and Swaminathan, M. 1957. Supplementary value of Indian multipurpose food to poor vegetarian diets based on different cereals and millets. *Food Sci.* 6:84-86.
- Kazanas, H. 1979. Natural fermentation of sorghum. Ph.D. thesis. University of Missouri, Columbia.
- Kazanas, H. and Fields M.L. 1981. Nutritional improvement of sorghum by fermentation. *J. Food Sci.* 46: 819-820.
- Khader, V. 1979. Studies on nutritive value of dhokla based on rice, bengalgram and curd. *Indian J. Nutr. Dietet.* 16:316-319.
- Khader, V. 1982. Preparation and nutritional evaluation of fermentation of foods similar to miso. *Nutr. Reports. Int.* 26:17-24.
- Khader, V. 1983. Nutritional studies on fermented, germinated and baked soybean preparation. *J. Plant Foods.* 5:31-37.
- Khokhar, S. and Chauhan, B.M. 1986. Antinutritional factors in moth bean: varietal differences and effects of methods of domestic processing and cooking. *J. Food Sci.* 51:591-594.
- Kim, H. and Zemel, M.B. 1986. In vitro estimation of the potential bioavailability of calcium from sea mustard, milk and spinach under simulated normal and reduced gastric acid conditions. *J. Food Sci.* 51: 957-959.

- Kline, L. and Sugihara, T.C. 1971. Microorganisms of the San Francisco sour dough bread, process, isolation and characterisation of undescribed bacterial species responsible for the souring activity. *Appl. Microbiol.* 21: 459-465.
- Klopfenstein, C.F., Hosney, R.C., Leipold, H.W. 1985. Nutritional quality of pearl millet and sorghum grain diets and a pearl millet weanling food. *Nutr. Reports Int.* 31:287-297.
- Krueckles, B.E., Kuzmicky, D.D. and Betschart, A.A. 1985. Effect of phytate and partially hydrolyzed phytate on in vitro protein digestibility. *J. Food Sci.* 50:1080-1082.
- Kumar, V. and Kapoor, A.C. 1984. Trace mineral composition of different varieties of cereals and legumes. *Indian J. Nutr. Dietet.* 21:137-143.
- Kunitarao, A. 1961. Proc. meeting on Protein Needs of Infants and Pre-school Children. *Nat. Acad. Sci. Nat. Res. Coun. Pune.* No.848. pp.269.
- Kurien, P.P., Swaminathan, M. and Subrahmanyam, V. 1961. The metabolism of nitrogen, calcium, phosphorus in undernourished children. 7. The effect of partial or complete replacement of rice in poor vegetarian diets by pearl millet. *Brit. J. Nutr.* 15: 345-347.
- Lee, B.Y., Man, P.J., Kim, Y.B., Fields, M.L. and Tongvel, P.K. 1980. Studies on the effects of fermentation on the nutritive value of korean rice. Research reports of the office of Rural Development, S. Korea. 22:36-42.
- Lin, J.C.M., Chastain, M.F., Strength, D.R. 1986. Sensory and nutritional evaluation of wheat bread supplemented with single cell protein from torula yeast (Candida utilis). *J. Food Sci.* 51: 647.
- Lindsey, W.L. and Norwell, M.A. 1969. A new DPTA-TEA soil test for zinc and iron. *Agron. Absts.* 61:84.
- Lock, S. and Bender, A.E. 1980. Measurement of chemically available iron in foods by incubation with human gastric juice in vitro. *Brit. J. Nutr.* 43:413.

- Lolas, G.M. and Markakis, P. 1977. The phytase of navy beans (Phaseolus vulgaris). J. Food Sci. 42: 1094-1106.
- Lopez, Y., Gordon, D.I. and Fields, M.L. 1983. Release of phosphorus from phytate by natural lactic acid fermentation. J. Food Sci. 48:953-954.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193: 265-268.
- Luna, L.G. 1968. Manual of histological staining methods of Armed Forces Institute of Pathology. McGraw Hill Book Company, New York.
- Maga, J.A. 1982. Phytate, its chemistry, occurrence, food interaction, nutritional significance and methods of analysis. J. Agric. Food Chem. 30:1-9.
- Mahajan, S. 1986. Studies on improvement of nutritional value of bajra through fermentation. M.Sc. thesis. Haryana Agricultural University, Hisar, India.
- Mahajan, S. and Chauhan, B.M. 1987. Phytic acid and extractable phosphorus of pearl millet flour as affected by natural lactic acid fermentation. J. Sci. Food Agric. 41:381-382.
- Maxson, E.D. and Rooney, L.M. 1972. Evaluation of methods for tannin analysis in sorghum grain. Cereal Chem. 49:719-729.
- Merican, Z. 1977. Malaysian pickles. Symposium on Indigenous Fermented Foods, Bangkok, Thailand.
- Miller, M.W. 1980. Yeast In: Read, G. (ed.). Prescott and Dunn's Industrial Microbiology. AVI., Pub. Co. Inc. Westport. pp.37.
- Miller, D.D., Schrickler, B.R., Rasmussen, R.R. and Van Campen, D. 1981. An in vitro method for estimation of iron availability from meals. Am. J. Clin. Nutr. 34: 2248-2256.
- Morcos, S.R. 1977. Egyptian fermented foods. Symposium on Indigenous fermented Foods, Bangkok, Thailand.
- Mukherjee, S.K., Albury, M.N., Pederson, C.S., Vanveen, A.G. and Steinkraus, K.H. 1965. Role of Leuconostoc measenteroides in leavening of batter of idli, fermented food of India. Appl. Microbiol. 13:227-231.

- Mukherjee, S.K., Chaudhri, D.R. and Gangopadhyay, H. 1977. Studies on sauerkraut as a fermented food of India. Symposium on 'Indigenous Fermented Foods', Bangkok, Thailand.
- Murdock, F.A. and Fields, M.L. 1984. B-Vitamins content of natural lactic acid fermented corn meal. J. Food Sci. 49: 373-375.
- Nanson, N.J. and Fields, M.L. 1982. Effect of L. fermentum, Bacillus subtilis, B. cereus and Pseudomonas maltophilia singly and in combination on the RNV of fermented corn meal. J. Food Sci. 47:1294-1295.
- Nanson, N.J. and Fields, M.L. 1984. Influence of temperature of fermentation on the nutritive value of lactic acid fermented corn meal. J. Food Sci. 49:958-959.
- NarayanaRao, M., Kurien, P.P., Swaminathan, M. and Subrahmanyam, V. 1961. Nutritive value of certain cereal diets consumed in India. Food Sci. 10: 163-175.
- Navert, B. and Sandstrom, B. 1985. Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. Brit. J. Nutr. 53:47-53.
- Nayini, N.R. and Markakis, P. 1983. Effect of fermentation time on the inositol phosphates of bread. J. Food Sci. 48:262-263.
- Neumann, P.E., Walker, C.E. and Wang, H.L. 1984. Fermentation of corn gluten meal with Aspergillus oryzae and Rhizopus oligosporus. J. Food Sci. 49:1200-1201.
- Nolan, K.B. and Duffin, P.A. 1987. Effect of phytate on mineral bioavailability. In vitro studies on Mg^{2+} , Ca^{2+} , Fe^{3+} , Cu^{2+} and Zn^{2+} solubilities in the presence of phytate. J. Sci. Food Agric. 40:79-83.
- Oberleas, D., Muhrer, M.E. and O'Dell, B.L. 1966. Dietary metal-complexing agents and zinc availability in the rat. J. Nutr. 90:56.
- O'Dell and DeBoland, A. 1976. Complexation of phytate with proteins and cations in corn germ and oilseed meals. J. Agric. Food. Chem. 24:804.

- Odunfa, S.A. 1983a. Carbohydrate changes in fermented locust bean (Parkia filicoidea) during iru preparation. Qualitas Plantarum Plant Foods for Human Nutrition. 32:3-10.
- Odunfa, S.A. 1983b. Biochemical changes during production of ogiri and fermented melon product. Qualitas Plantarum Plant Foods for Human Nutrition. 32:11-18.
- Odunfa, S.A. 1985. Biochemical changes in fermenting African locust bean (Parkia biglobosa) during iru fermentation. J. Food Technol. 20:295-303.
- Ogunsua, A.D. 1977. Fermentation of cassava tubers. Symposium on 'Indigenous Fermented Foods', Bangkok, Thailand.
- Opoku, A.R., Ohenhen, S.O. and Ejiofor, N. 1981. Nutritent Composition of pearl millet grains and malt. J. Agric. Food Chem. 29:1247-1248.
- Orillo, C.A. and Pederson, C.S. 1968. Lactic acid bacterial fermentation of burong dalag. Appl. Microbiol. 16:1669-1671.
- Oyewole, O.B. 1984. Studies on micro-organisms associated with African Locust Bean fermentation. M.Sc. thesis. University of Ibadan, Nigeria.
- Panse, Y.G. and Sukhatme, P.V. 1961. Statistical Methods of Agricultural Workers. 2nd Edn. Indian Council of Agricultural Research, New Delhi. pp. 12,87.
- Paul, P. 1979. Study of fermentation of grain sorghum to improve its amino acid and vitamin content. M.Sc. thesis. University of Missouri, Columbia.
- Paul, M. Au and Fields, M.L. 1981. Nutritive quality of fermented sorghum. J. Food Sci. 46:652-654.
- Perten, H. 1977. In: Proceedings of a symposium on 'Sorghum and Millets' for Human food.' Tropical Products Institute, London, England.

- Peterson, W.H., Skinner, J.T. and Strong, F.M. 1943. Elements of Food Biochemistry. Prentice Hall, Inc. New York. pp. 73.
- Platt, B.S., Miller, D.S. and Payne, P.R. 1961. Protein values of human foods. In: Brock, J.F. (ed.). Recent Advances in Human Nutrition. London. pp.351.
- Pushpamma, S., Parrish, D.B. and Deyow, C.W. 1972. Improving protein quality of millet, sorghum and maize diets by supplementation. Nutr. Rep. Int. 5:93-100.
- Rajalakshmi, R. and Vanaja, K. 1967. Chemical and biological evaluation of the effects of fermentation on the nutritive value of foods prepared from rice and gram. Brit. J. Nutr. 21:467-473.
- Ramachandran, K. and Bolodia, G. 1984. The effect of fermentation on the iron, phosphorus and zinc content of tef (Eragrostis tef). Ethiopian Med. J. 22:45-48.
- Ramakrishnan, C.V., Parekh, L.J., Aliokar, P.N., Rao, G.S. and Bhandari, S.D., 1976. Studies on soy idli fermentation. Plant Foods Man. 2: 15-33.
- Ramakrishnan, C.V. 1979. Studies on Indian fermented food. Baroda. J. Nutr. 6:1-57.
- Rama Rao and Swaminathan, M. 1953. Chemical composition of different varieties of bajra. Bull. Central Food Technol. Inst. Mysore. 3:68.
- Ranhotra, G.S. 1972. Hydrolysis during breadmaking of phytic acid in wheat protein concentrate. J. Food Sci. 37: 12-13.
- Rao, R.V. and Basu, K.P. 1952. Effect of dahi formation on the thiamine, riboflavin and nicotinic acid contents of milk. Indian J. Dairy Sci. 5:1-8.
- Rao, B.S.N. and Prabhavathi, T. 1978. An in vitro method for predicting the bioavailability of iron from foods. Am. J. Clin. Nutr. 31:169.
- Rao, D.S. and Deosthale, Y.G. 1983. Mineral composition, ionisable iron and soluble zinc in malted grains of pearl millet and ragi. Food Chem. 11:217-223.

- Reddy, N.R. and Salunkhe, D.K. 1980. Effect of fermentation on phytate, phosphorus and minerals content in black gram, rice and blackgram and rice blends. *J. Food Sci.* 43:1094-1097.
- Reddy, N.R., Sathe, S.K. and Salunkhe, D.K. 1982. Phytates in legumes and cereals. *Adv. Food Res.* 28:1-9.
- Reinhold, J.G. 1975. Phytate destruction by yeast fermentation in whole wheat meals. *J. Am. Dietet. Assoc.* 66:38-42.
- Robinson, R.J. and Kao, C. 1977. Tempeh and miso from chickpea, horsebean and soybean. *Cereal Chem.* 54:1192.
- Sawhney, S.K. and Naik, M.S. 1969. Amino acid composition of protein fraction of pearl millet and the effect of nitrogen fertilization on its proteins. *Indian J. Genet. Pl. Breeding.* 29:395-406.
- Schricker, B.R. and Miller, D.D. 1982. In vitro estimation of iron availability in breads and meals containing different forms of fortification iron. *J. Food Sci.* 47: 723-724.
- Schricker, B.R., Miller, D.D. and Van Campen, D. 1982. In vitro estimation of iron availability in meals containing soy products. *J. Nutr.* 112:1696-1698.
- Schwartz, R., Belko, A.Z. and Wien, E.M. 1982. An in vitro system for measuring intrinsic dietary mineral exchangeability: Alternative to intrinsic isotopic labelling. *J. Nutr.* 112: 497.
- Serraino, M.R., Thompson, L.U., Savoie, L. and Parent, G. 1985. Effect of phytic acid on the in vitro rate of digestibility of rapeseed protein and amino acids. *J. Food Sci.* 50:1689-1692.
- Sheorain, V.S. and Wagle, D.S. 1973. β amylase activity in germinated bajra and barley varieties. *J. Food Sci. Technol.* 10:184.
- Simwemba, C.G. 1984. Certain B-vitamin and phytic acid contents of pearl millet. *J. Agric. Food Chem.* 32:31-34.
- Singh, M. and Krikorian, A.D. 1982. Inhibition of trypsin activity in vitro by phytate. *J. Agric. Food Chem.* 30:199.

- Singh, R. and Popli, S. 1973. Amylose content and amylolytic studies on high yielding varieties of bajra. J. Food Sci. Technol. 10:31-33.
- Singh, U. and Jambunathan, R. 1981. Studies on Desi and kabuli chickpea cultivars. The levels of protease inhibitors, levels of polyphenolic compounds and in vitro digestibility. J. Food Sci. 46:1364.
- Singh, U., Kherdekar, M.S. and Jambunathan, R. 1982. Studies on Desi and Kabuli chickpea cultivars. The levels of amylase inhibitors, level of oligosaccharides and in vitro starch digestibility. J. Food Sci. 47:510.
- Singh, U. 1984. The inhibition of digestive enzymes by polyphenols of chickpea and pigeon pea. Nutr. Rep. Int. 29:745.
- Somogyi, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160:61.
- Steinkraus, K.H., Lee, C.Y. and Buck, P.A. 1965. Soybean fermentation by the Ontjom and Neurospora. Food Technol. 19:1302-1307.
- Steinkraus, K.H., Van Veen, A.G. and Thiebeau, D.B. 1967. Studies on idli- and Indian fermented blackgram-rice food. Food Technol. 21: 110-113.
- Strickland, R.D., Freeman, M.L. and Gurule, F.T. 1961. Copper binding by proteins in alkaline solution. Anal. Chem. 33.
- Subramanian, V., Jambunathan, R. and Suryaprakash, S. 1981. Sugars of pearl millet. J. Food Sci. 46:1614-1615.
- Sullins, L.W. and Ronney, R.D. 1977. In: Proceedings of a symposium on 'Sorghum and Millets for Human Food'. Tropical Products, institute, London, pp.91.
- Sutardi and Buckle, K.A. 1985. Reduction in phytic acid levels in soybeans during tempeh production, storage and frying. J. Food Sci. 50: 260-261.
- Suzuki, K. 1965. The pattern of mammalian brain gangliosides III. Regional and developmental differences. J. Neurochem. 12:969.

- Swain, J. and Hills, W.E. 1959. The phenolic constituents *Pramus Domestical*. The qualitative analysis of phenolic constituents. *J. Sci. Food Agric.* 10:63.
- Tamir, M. and Alumot, E. 1969. Inhibition of digestive enzymes by condensed tannins from green and ripe carobs. *J. Sci. Food. Agric.* 20:199-202.
- Tangkongchitr, U., Sieb, P.A. and Hosney, R.C. 1981. Phytic acid. II. Its fate during breadmaking. *Cereal Chem.* 58:229-233.
- Tan, N., Wong, K. and de Lumen, B.D. 1984. Relationship of tannin levels and trypsin inhibitor activity with in vitro protein digestibility of raw and heat treated winged bean. *J. Agric. Food Chem.* 32:819.
- Taur, A.T., Pawar, V.D. and Ingle, U.M. 1984a. Sensory evaluation of chips made from control, germinated and germinated- fermented sorghum. *Indian J. Nutr. Dietet.* 21:89-96.
- Taur, A.T., Pawar, V.D. and Ingle, U.M. 1984b. Effect of fermentation on nutritional improvement of grain sorghum. *Indian J. Nutr. Dietet.* 21:129-136.
- Taur, A.T., Pawar, V.D. and Ingle, U.M. 1984c. Nutritional improvement of grain sorghum by germination. *Indian J. Nutr. Dietet.* 21:168-174.
- Thiam, A.A. 1977. Contribution to the study of the biochemical phenomena of millet and sorghum flour deterioration (lecture). *Tropical Products Institute, London.* pp.69-72.
- Thompson, L.U. and Yoon, J.H. 1984. Starch digestibility as affected by polyphenol and phytic acid. *J. Food Sci.* 49:1228-1229.
- Thompson, L.U., Yoon, J.H., Jenkins, J.A., Wolever, T. and Jenkins, A.L. 1984. Relationship between polyphenol intake and blood glucose response of normal and diabetic individuals. *Am. J. Clin. Nutr.* 39:745-751.

- Thompson, L.U. and Serraino, M.R. 1985. Effect of germination on phytic acid, protein and fat content of rapeseed. *J. Food Sci.* 50:1200-1201.
- Tongananta, Q. 1971. Studies on the Philippine fermented rice cake puto. M.S. thesis. University of the Philippines. College, Laguna.
- Tongnual, P. and Fields, M.L. 1979. Fermentation and relative nutritive value of rice meal and chips. *J. Food Sci.* 44:1784-1785.
- Umoh, V. and Fields, M. 1981. Fermentation of corn for Nigerian agidi. *J. Food Sci.* 46:903-904.
- Varriano, Marston, E. and Hoseney, R.C. 1980. Note on mineral content and location in pearl millet. *Cereal Chem.* 57:150-152.
- Vass, A., Szakaly, S. and Schmidt, P. 1984. Experimental study of the nutritional biological characters of fermented milks. *Nutr. Abst. Rev.* 55:77.
- Venkatasubbaiah, P., Dwarkanath, C.T. and Sreenivasa Murthy, V. 1984. Microbiological and physico chemical changes in idli batter during fermentation. *J. Food Sci. Technol.* 21:59-62.
- Venkatasubbaiah, P., Dwarkanath, C.T. and Sreenivasa Murthy, V. 1985. Involvement of yeast flora in idli batter fermentation. *J. Food Sci. Technol.* 22:88-90.
- Vogel, A. 1962. Text Book of Quantitative Inorganic Analysis. 3rd edn. Longmans.
- Wagner-Jauregg, T. 1972. Riboflavin chemistry in: The Vitamin. Vol.V (Serbrell, W.H. and Harris, R.S. eds.) Academic Press, New York, pp. 3.
- Wang, H.L., Ruttle, D.I. and Hesseltine, C.W. 1968. Protein quality of wheat and soybean after Rhizopus oligosporous fermentation. *J. Nutr.* 90:109-114.
- Wang, H.L. and Hesseltine, C.W. 1970. Safu and tao-chao. *J. Agric. Food Chem.* 18:572-575.
- Wang, Y.D. and Fields, M.L. 1978. Feasibility of home fermentation to improve the amino acid balance of corn meals. *J. Food Sci.* 43:1105-1106.

- Watson, J.D., Dako, D.Y. and Amo-akawa -Adu. 1975. Available carbohydrate in Ghanaian foodstuffs. Part 2. Sugars and starch in staple and other foodstuffs. *Plant Foods for Man*. pp169-176.
- Whitaker, J.R. 1978. Biochemical changes occurring during fermentation of high protein foods. *Food Technol.* 32:175-188.
- Wien, E.M. and Schwartz, R. 1983. Comparison of in vitro and in vivo measurements of dietary Ca exchangeability and bioavailability. *J. Nutr.* 113:388.
- Wien, E.M. and Schwartz, R. 1985. Dietary calcium exchangeability and bioavailability. Evaluation and potential use of an in vitro digestion procedure. In "Nutritional Bioavailability of Calcium". C. Kies (Ed). American Chemical Society, Washington, D.C.
- Wise, A. 1983. Dietary factors determining the biological activities of phytate. Review in clinical Nutrition. *Nutr. Abst. Rev.* 53:791.
- Wood, B.J.B. 1983. Lactobacillus/Yeast interactions, their contribution to fermented food stuffs especially traditional bread. pp.179. In:K.H. Steinkraus (Ed.) *Handbook of Indigenous Fermented Foods*-Marcel Dekker Inc., New York.
- Yajurvedi, R.P. 1980. Microbiology of idli fermentation. *Indian Food Packer.* 34:33.
- Yemm, E.W. and Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* 57: 508.
- Yoon, J.H., Thompson, L.U. and Jenkins, D.J.A. 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *Am. J. Clin. Nutr.* 38:835-842.
- Zamora, A.F. 1978. Nutritional evaluation of fermented cowpeas (Vigna senesis) and chickpea (Cicer arietinum). Ph.D. thesis. University of Missouri-Columbia.
- Zamora, A.F. and Fields, M.L. 1979a. Nutritive quality of fermented cowpea and chickpeas. *J. Food Sci.* 44:234.
- Zamora, A.F. and Fields, M.L. 1979b. Microbiological and toxicological evaluation of fermented cowpeas and chickpeas. *J. Food Sci.* 44:928.

- Zamora, R.G. and Veum, T.L. 1979. The nutritive value of dehulled soybeans fermented with Aspergillus oryzae or Rhizopus oligosporus as evaluated by rats. J. Nutr. 109:1333-1339.
- Zemel; M.B. 1984. In vitro evaluation of the effects of ortho-tripoly- and hexametaphosphate on zinc, iron and calcium bioavailability. J. Food Sci. 49:1562-1565.

APPENDICES

APPENDIX I

Score sheet for taste panel/data under hedonic scale

Name _____

Dated: _____

Product _____

Test these samples and check how much you like or dislike each one.

Use appropriate scale to show your attitudes by assigning points that best describe your feeling about the sample. An honest expression of your feeling will help us.

Code No.	Colour	Appear- ance	Flavour	Texture	Taste	Bitterness	Sourness	Overall accept- ability	Total remarks
----------	--------	-----------------	---------	---------	-------	------------	----------	-------------------------------	------------------

Rate

Organoleptic scope

Like extremely

9

Like very much

8

Like moderately

7

Like slightly

6

Neither like nor dislike

5

Dislike slightly

4

Dislike moderately

3

Dislike very much

2

Dislike extremely

1.

Note: Please rinse your mouth before and after tasting each product.

Signature.

APPENDIX II

Table 1

Effect of natural fermentation on total Ca, Fe, Zn, Cu and Mn content of pearl millet flour (mg/100 g, on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
F20	48.0±1.78	17.4±2.12	2.65±0.28	0.926±0.07	1.32±1.0
F25	48.8±0.56	17.6±3.16	2.60±0.35	0.997±0.14	1.32±1.3
F30	49.0±2.82	18.4±3.80	2.60±0.42	0.997±0.28	1.49±1.3
Rp	50.0±3.53	18.0±0.28	2.70±0.00	1.21±0.07	1.57±0.85
Zf	49.5±2.16	17.0±0.14	2.65±0.03	1.14±0.01	1.49±0.0
SE(m)	±1.38	±1.39	±0.16	±0.11	±0.60
CD (P < 0.05)	NS	NS	NS	NS	NS

Values are means±SD of four independent determinations.

APPENDIX III

Table 2

Effect of pure culture fermentation on total Ca, Fe, Zn, Cu and Mn content of pearl millet flour (mg/100 g, on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
<u>Single fermentation</u>					
Sd	50.0±1.41	18.4±2.8	2.60±0.0	0.85±0.0	1.49±1.3
Sc	50.0±1.41	17.2±2.8	2.65±0.3	0.93±0.5	1.49±0.0
Lb	49.8±1.60	17.6±2.8	2.65±0.3	0.99±0.0	1.49±0.0
Lf	49.2±3.69	17.6±3.1	2.60±0.0	0.93±0.5	1.49±1.3
<u>Mixed fermentation</u>					
SdLb	49.0±2.04	18.8±5.6	2.60±0.0	0.85±0.0	1.57±0.8
SdLf	49.4±0.64	18.2±2.8	2.60±0.0	0.85±0.0	1.49±0.0
ScLb	50.0±3.53	18.0±1.0	2.60±0.0	0.85±0.0	1.49±0.0
ScLf	48.0±1.78	19.2±2.8	2.60±0.0	0.85±0.0	1.49±0.0
<u>Sequential fermentation</u>					
Sd+Lb	50.0±3.53	18.4±3.2	2.62±0.2	0.85±0.0	1.49±1.3
Sd+Lf	48.9±0.96	17.2±2.8	2.60±0.0	0.85±0.0	1.49±0.0
Sc+Lb	49.0±1.78	18.2±2.8	2.60±0.0	0.85±0.0	1.49±0.8
Sc+Lf	47.2±1.92	18.2±2.8	2.60±0.0	0.85±0.0	1.49±0.8
<u>Control</u>					
Rp	50.0±3.53	18.0±2.8	2.70±0.0	1.21±0.7	1.57±0.8
Zf	49.6±1.78	17.0±1.4	2.65±0.3	1.14±0.01	1.49±0.0
SE(m)	±1.36	±1.90	±0.09	±0.10	±1.01
CD(P < 0.05)	NS	NS	NS	NS	NS

Values are means±SD of four independent determinations.

Table 3

Effect of mixed fermentation on total Ca, Fe, Zn, Cu and Mn
content of sprouted pearl millet (mg/100 g, on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
GSdLb	50.0±5.0	12.4±2.8	2.60±0.0	0.997±0.14	1.32±0.8
GSdLf	46.0±2.92	13.6±1.4	2.60±0.0	0.926±0.07	1.32±0.0
GSCLb	47.0±2.38	13.4±0.0	2.60±0.0	0.997±0.14	1.32±0.0
GSCLf	44.0±3.53	12.4±2.8	2.65±0.3	0.926±0.20	1.41±0.8
G	48.0±2.00	11.6±0.0	2.62±0.1	0.997±0.28	1.32±0.8
SE(m)	±1.90	±1.10	±0.10	±0.11	±0.30
CD(P < 0.05)	NS	NS	NS	NS	NS

Values are means±SD of four independent determinations.

APPENDIX V

Table 4

Effect of natural fermentation on HCl-extractable Ca, Fe, Zn, Cu and Mn content of pearl millet flour (mg/100 g, on dry matter basis).

Fermentation	Ca	Fe	Zn	Cu	Mn
F20	36.0±1.41	9.1±1.06	1.56±0.04	0.783±0.07	0.996±0.0
F25	38.0±1.47	9.2±0.00	1.60±0.00	0.855±0.00	1.079±0.0
F30	40.4±0.00	13.3±0.70	1.68±0.00	0.863±0.00	1.079±0.0
Rp	18.0±1.78	3.5±0.35	1.14±0.08	0.427±0.00	0.830±0.0
Zf	22.0±1.78	3.5±0.50	1.18±0.03	0.427±0.00	0.830±0.0
SE(m)	±0.84	±0.36	±0.026	±0.03	±0.0003
CD(P < 0.05)	2.52	1.08	0.078	0.09	0.0009

Values are means±SD of four independent determinations.

Table 5

Effect of pure culture fermentation on HCl-extractable Ca, Fe, Zn, Cu and Mn content of pearl millet flour (mg/100 g, on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
<u>Single fermentation</u>					
Sd	40.0±1.08	5.0±0.70	1.20±0.12	0.57±0.0	0.996±0.08
Sc	40.0±0.70	5.0±0.00	1.32±0.04	0.57±0.0	0.996±0.00
Lb	42.0±1.41	6.3±1.08	1.40±0.06	0.64±0.1	0.120±0.07
Lf	38.6±1.29	6.5±0.35	1.26±0.03	0.57±0.1	0.996±0.00
<u>Mixed fermentation</u>					
SdLb	44.0±2.27	5.0±0.70	1.32±0.04	0.57±0.0	1.079±0.05
SdLf	44.0±2.82	5.0±0.35	1.28±0.00	0.57±0.0	0.996±0.00
ScLb	43.0±2.54	5.5±0.81	1.30±0.02	0.57±0.0	1.120±0.07
ScLf	40.8±0.73	8.0±0.70	1.26±0.03	0.57±0.0	1.079±0.00
<u>Sequential fermentation</u>					
Sd+Lb	36.0±4.24	8.7±1.47	1.64±0.08	0.76±0.1	1.162±0.11
Sd+Lf	37.2±1.81	6.2±0.88	1.44±0.08	0.64±0.1	1.079±0.05
Sc+Lb	36.0±1.08	10.0±2.54	1.36±0.05	0.64±0.1	0.996±0.00
Sc+Lf	36.0±2.17	6.0±0.35	1.32±0.04	0.57±0.0	0.996±0.08
<u>Control</u>					
Rp	18.0±1.78	3.5±0.35	1.14±0.08	0.43±0.1	0.830±0.00
Zf	22.0±1.78	3.5±0.50	1.18±0.03	0.43±0.0	0.830±0.00
SE(m)	±1.18	±0.56	±0.04	±0.03	±0.03
CD(P < 0.05)	3.54	1.68	0.12	0.09	0.09

Values are means±SD of four independent determinations.

APPENDIX VII

Table 6

Effect of mixed fermentation on HCl-extractable Ca, Fe, Zn, Cu and Mn content of sprouted pearl millet (mg/100 g, on dry matter basis)

Fermentation	Ca	Fe	Zn	Cu	Mn
GSdLb	39.0±0.70	7.0±0.0	1.52±0.08	0.836±0.12	0.996±0.00
GSdLf	36.0±1.41	8.0±0.70	1.36±0.00	0.712±0.00	0.913±0.00
GSCLb	38.0±1.41	6.0±1.41	1.44±0.00	0.855±0.00	0.996±0.00
GSCLf	34.0±1.00	5.0±0.00	1.40±0.04	0.712±0.10	0.996±0.00
G	34.8±0.25	4.5±0.00	1.32±0.04	0.712±0.14	0.871±0.07
SE(m)	±0.61	±0.41	±0.03	±0.05	±0.02
CD(P < 0.05)	1.83	1.23	0.09	0.15	0.06

Values are means±SD of four independent determinations.

IMPROVEMENT OF NUTRITIONAL VALUE OF PEARL MILLET BY FERMENTATION AND UTILISATION OF THE FERMENTED PRODUCT

by
NEELAM KHETARPAUL

MAJOR ADVISOR: DR.B.M.CHAUHAN
Scientist
Department of Foods and Nutrition
Haryana Agricultural University.
Hisar.

(An abstract of the thesis presented in partial fulfilment of the requirements for the degree of Ph.D. in Foods and Nutrition, Haryana Agricultural University, Hisar).

Natural lactic fermentation of pearl millet flour was carried out at 20, 25 and 30°C for 72h. Pure culture fermentations of the pearl millet flour as well as sprouts were carried out at 30°C for 72h by employing S.diastaticus, S.cerevisiae, L.brevis and L. fermentum in single, mixed and sequential culture fermentations. Natural and pure culture fermentations lowered the pH and raised the titratable acidity. Naturally fermented product contained lactobacillus as the dominating microflora followed by coliform, yeast and fungus. The fermentations either decreased or did not change the protein content. Fat content of the naturally fermented product increased whereas that of the pure culture fermented product decreased. Fermentation of sprouts did not change the fat content. Ash content of all the types of fermented products remained unaltered. Starch content was lowered following fermentation. Maximum reduction was observed in sequential fermentation followed by fermentation of sprouts, mixed and single culture fermentation. Starch content decreased and soluble sugars including reducing and non-reducing sugars increased significantly following natural fermentation at all the temperatures. Protein and starch

digestibility (in vitro) improved significantly as a result of fermentations. Sd appeared to be a better organism for improving the starch digestibility. Sc fermented products had the higher protein digestibility. A drastic reduction and even complete elimination of phytate contents was observed while polyphenols rose in the naturally fermented products. Germination and fermentation of the sprouts eliminated the phytate in pearl millet. The decrease in phytic acid had significantly improving effect on extractability of minerals and digestibility of starch and protein in the fermented products. All the types of pure culture fermentation especially sequential fermentation and mixed fermentation of sprouts had a significant improving effect on the HCl-extractable minerals. Single culture fermentation by yeasts increased the thiamine whereas other fermentations decreased the thiamine content. Feeding of the fermented products did not bring about any physiological, histopathological and haematological changes in rats. PER and FER values of the naturally fermented products were less than in the raw pearl millet fed rats whereas AD,TD,NPU and BV were higher in case of naturally fermented pearl millet flour. PER,FER,BV,AD,TD,NPU,NPR,PRE and UP values in case of pure culture fermented products were higher than the raw pearl millet flour. Overall, F30, sequential fermentation and fermentation of the sprouts were found to be the best for enhancing the nutritional value of raw pearl millet. Pure culture mixed fermented products were liked in all the types of recipes i.e. weaning mixtures, pakoras, cutlets, biscuits, fried vermicilli and chappaties whereas naturally fermented products were liked when incorporated in deep fried recipes. Controlled fermentations instead of natural fermentation appeared to be more advantageous for improving the nutritional value of the pearl millet flour for human consumption.

