

FORMULATION, NUTRITIONAL EVALUATION AND UTILIZATION OF PROBIOTIC FERMENTED COARSE CEREAL BLENDS

By

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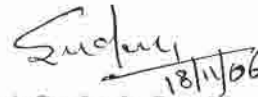
*Dedicated
to
my Pillars....
Bhai*

Sonu & Raja

CERTIFICATE – I

This is to certify that this thesis entitled, “**Formulation, nutritional evaluation and utilization of probiotic fermented coarse cereal blends**” submitted for the degree of **Master of Science** in the subject, **Foods and Nutrition** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms Sonia Arora** under my supervision and guidance 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.



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CERTIFICATE-II

This is to certify that this thesis entitled, "**Formulation, nutritional evaluation and utilization of probiotic fermented coarse cereal blends**", submitted by **Sonia Arora** to the CCS Haryana Agricultural University, Hisar, in partial fulfilment of the requirements for the degree of **Master of Science**, in the subject of **Foods and Nutrition**, has been approved by the Student's Advisory Committee, after an oral examination on the same.


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DEAN, POST-GRADUATE STUDIES

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(Sonia Arora)

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Chapter-1

Introduction

Coarse cereals and millets occupy important place in the grain economy of the world. Coarse grains and millets like barley, rye, oats, sorghum, pearl millet etc. form staple foods for a large segment of the population in India. These are comparable and even superior to major cereals with respect to protein, energy, vitamins, minerals and crude fibre. Besides this, they are rich in fibre and also contain phytochemicals, nutraceuticals and hence, now-a-days termed as neutraceuticals. Among these, barley (*Hordeum vulgare*) is a major world crop. In India, its production is largely confined to Uttar Pradesh, Punjab and Haryana. It is an excellent source of B-complex vitamins, minerals and complex carbohydrates (Kalra and Jood, 2000). Pearl millet (*Pennisetum glaucum*) is an important food crop of arid and semi-arid regions of the country. Pearl millet forms a major source of dietary protein to large section of the population and its nutritive value especially protein, fat and mineral contents are superior to other cereal food grains. Hence, coarse cereals and millets require more cooking time and have relatively poor digestibility and availability of minerals due to presence of inherent antinutritional factors like phytates, polyphenols, tannins, amylase inhibitors etc. In India, various processing methods used in preparation of products from barley and pearl millet include dehulling, cooking, germination and

fermentation. Such methods, especially germination and fermentation have been reported to improve their nutritional quality by lowering the contents of antinutrients (Mahgoub and Elhag, 1998; Pugalenti and Vadivel, 2005).

Malnutrition and deficiency of micro-nutrients are highly prevalent and even increasing in parts of several developing countries. Factors of immediate and direct influence to these nutritional disorder are inadequate food consumption and diseases, which usually interact in a mutually reinforcing manner. In addition, the bioavailability of many nutrients in vegetarian diets is usually low, and this significantly contributes to the nutritional inadequacy. To improve the nutrient intake, food preparation technologies have been advocated that will effectively increase the nutrient availability of vegetarian diets. Animal foods, although of excellent nutritional quality, are not available in enough quantity due to their high cost and certain religious traditions and customs to the population of developing countries like India.

Several methods can be employed to improve the nutritional quality of coarse cereal grains. These include genetic improvement, amino acid fortification and supplementation with protein-rich sources. However, the application of different processing technologies are of equal importance in order to increase the bioavailability of indigenous nutrients in grains such as protein, starch and minerals. Such household level technologies that are widely practised in many developing countries include germination and fermentation techniques.

The process of germination and fermentation are commonly used for production of traditional foods. Germination improves the vitamin contents of the grain and increases the digestibility of protein (Saharan, *et al.*, 2002; Grewal, 2003) and starch (Jood *et al.*, 1998; Grewal, 2003) and availability of minerals (Sharma and Kapoor, 1996; Antony and Chandra, 1998; Grewal, 2003). Germination lowers phytase, tannins and oxalate levels. Due to reduction in antinutrient contents, the activity of proteolytic enzymes increase which improves the protein quality and digestibility.

Fermentation, a simple and convenient domestic technique is one such processing method known to be effective for improving the nutritive value of plant foods. Indigenous fermented foods including curd, *rabadi*, yoghurt, *idli*, *dhokla*, etc. form an important part of human diet in different parts of the world. Such nutritionally superior, acceptable products have been reported to have improved digestibility of starch and protein (Saharan, 1994; Rani and Khetarpaul, 1999), reduced levels of antinutrients (Gupta *et al*, 1992) and enhanced bioavailability of minerals (Goyal and Khetarpaul, 1994). Besides nutrients, these fermented foods are known to contain different micro-organisms including probiotics, such as *L. acidophilus*.

Foods fermented with *Lactobacilli* have been found to be more nutritious than their unfermented counterparts. This improvement can come about in at least three different ways (Potter and Hotchkiss, 1996). Micro-organisms not only are catabolic, breaking down more complex compounds, but they also are anabolic and synthesize several B-complex vitamins and other growth factors. The second important way in which fermented foods can be improved nutritionally has to do with the liberation of nutrients locked into plant structures and cells by indigestible material. A third mechanism by which fermentation can enhance nutritional value, especially of plant materials, involves enzymatic splitting of cellulose, hemicellulose and related polymers that are not digestible by humans into simpler sugars and sugar derivatives.

Microbial cultures are now also being tested for their ability to prevent and cure diseases in animals and humans. Based on these new applications, the word 'probiotics' was used to describe beneficial micro-organisms by Lilley and Stillwell (1965). Fuller (1992) defined a probiotic as 'live microbial feed supplement which beneficially affects the host animal by improving its microbial balance.' Havenaar and Huis (1992) have broadened the definition of probiotics as a mono or mixed culture of live micro-organisms which, when applied to man

or animal as dried cells or as fermented products, affect beneficially the host by improving the properties of the indigenous microflora.

The history recording the probiotic properties of live microbial food supplements is long. A good probiotic is that which is capable of exerting a beneficial effect on host animals and is non-pathogenic and non-toxic. The probiotics should be present as viable cells in large number and capable of surviving and metabolizing in the gut environment. Also, it should be stable and capable of remaining viable for long periods under storage and field conditions.

The majority of probiotics recommended (Fuller, 1992) are bacterial, with the species of *Lactobacillus* being the most common. Some examples include *L. acidophilus*, *L. plantarum*, *L. casei*, *L. GG*, *Streptococcus lactis* etc. More recently there has been interest in use of other strains such as *Saccharomyces boulardii*, non-pathogenic *E. coli* etc. (Conway, 1996).

Literature indicates several potential health and nutritional benefits possible from probiotic foods. Bacterial enzymatic hydrolysis has been shown to enhance the bioavailability of protein and fat (Friend and Shahani, 1984). Bacterial protease can increase the production of free amino acids which can benefit the nutritional status of host particularly if the host has a deficiency in endogenous protease production. Lactic acid bacteria have also been shown to increase the content of the vitamin B-complex in fermented foods (Deeth and Tamime, 1981). Probiotic fermentation has been shown to improve protein and starch digestibility, reduce the level of antinutrients and enhance the bioavailability of minerals (Binita 1995; Khetarpaul, 1998; Chahal, 1999; Sindhu *et al.*, 2005).

Besides nutritional benefits, probiotic organisms have also a role in improving metabolism, reducing constipation, lowering of cholesterol levels in blood, stimulation of immune system, increasing the phenol tolerance and detoxification of potential carcinogens (Smoragicwicz *et al.*, 1993). All these benefits of probiotic fermented foods are due to growth of viable lactic acid bacteria which produce a variety of antimicrobial agents which are reported to

have inhibitory effect on various species of food spoilage and pathogenic bacteria (Shahani *et al.*, 1976; Babel, 1977; Gilliland and Speck, 1977).

Some of the fermented foods such as milk and whey, containing a micro-organism having probiotic property i.e. *L. acidophilus*, have been described to have nutritional as well as therapeutic advantages in the management of diarrhoea. Acidophilus yoghurts, acidophilus yeast milk, acidophilin, sweet acidophilus milk and acidophilus powders etc. have been developed and are consumed as more acceptable means of ingesting live *L. acidophilus* cells. *L. acidophilus* is an organism which is able to implant in intestine and can help the host by restoration of normal intestinal flora, resulting in prevention and cure of many intestinal disorders (Khedekar *et. al.*, 1990, Katelaris, 1996).

Using *L. acidophilus*, fermented products based on milk or whey have been prepared but no such work has been conducted till today on the development of products based on raw and germinated coarse cereals and millets after fermentation with *L. acidophilus*. The cumulative effect of germination and fermentation, especially with probiotic micro-organism i.e. *L. acidophilus* can have added advantages. They may not only improve the nutritional quality of products but also add therapeutic benefits. Further, addition of tomato pulp and whey powder to the coarse cereal and millet blends will add nutrients. With this perspective, the present study was planned with the following objectives:

- i) To develop probiotic fermented coarse cereal and millet based food mixtures
- ii) To carry out the organoleptic and nutritional evaluation of fermented food mixtures
- iii) To develop probiotic fermented products and evaluate them for their organoleptic and nutritional parameters.

Chapter-2

Review of Literature

Coarse grains and millets form staple foods for a large segment of the population in India. Various processing methods used in the preparation of products from pearl millet include dehulling, cooking, germination and fermentation. Such methods especially germination and fermentation have been reported to improve their nutritional quality by lowering the content of antinutrients (Khetarpaul, 1988). The cumulative effect of germination and fermentation especially with probiotic microorganism can have added advantages. They may not only improve the nutritional quality of barley and pearl millet products but may also add therapeutic effects (Klaenhammer and Kullen, 1999; Sharma and Ghosh, 2006).

Probiotics are preparation of live micro-organisms which beneficially affect the host by improving the properties of the indigenous microbes. Probiotics are used to improve intestinal health and stimulate the immune system. The microbes commonly used as probiotics for humans are the lactic acid bacteria. Consuming fermented products containing these organisms allows the continuous passage of these organisms through the gut (Modler *et al.*, 1990).

The available literature pertaining to use of probiotic organism i.e. *L. acidophilus* in the development of fermented foods and changes brought about

in the nutritional composition by fermentation has been reviewed under the following heads and subheads :

- 2.1 Nutritional and health benefits of probiotics
- 2.2 Chemical analysis
 - 2.2.1 pH and titrable acidity
 - 2.2.2 Proximate composition
 - 2.2.3 Carbohydrates
 - 2.2.4 Dietary fibre
 - 2.2.5 β -glucan
 - 2.2.6 B-complex vitamins
 - 2.2.7 Protein fractions
 - 2.2.8 Antinutrients
 - 2.2.9 *In vitro* protein and starch digestibility
 - 2.2.10 Minerals
- 2.3 Organoleptic and nutritional evaluation of fermented food products

2.1 Nutritional and health benefits of probiotics

The term 'probiotic' was first used by Parker (1974) to describe "micro-organisms and substances which contribute to intestinal microbial balance". The term probiotic originated from two Greek words, meaning 'for life' and contrasted with the term antibiotic which means 'against life'. Thus, was born the concept of microbial inoculation based on the principal of competitive exclusion.

The key common factors in successful use of probiotics have been the presence of viable bacteria in sufficient numbers in the probiotics and their capacity to colonise in the gastro-intestinal tract. Probiotics are not effective if one or both of these factors are absent (Klaenhammer and Russel, 2000).

Other workers also stated the following characteristics of probiotics i.e. i) it must be alive ii) it must deliver a measured physiological benefit iii) it need not be

restricted to food applications or oral delivery (iv) it should not limit the mechanism of action but the living of metabolites/products by micro-organisms to the small intestine be consisted as a probiotic activity (Lee and Salminen, 1995; Conway, 1996; Sharma and Ghosh, 2006).

Probiotics have received considerable attention over the past few years for their purported health benefits. *Lactobacillus acidophilus*, and bifidobacteria often referred to as '*bifidus*'. Foods used for dissemination of probiotics are usually fermented foods even if probiotics also could be present in infant formula, fruit drinks, whey drinks and sweet milk. Fermented milk and cheese are the most common foods with probiotics (Gupta and Garg, 1997; Christopher *et al.*, 2006). Fermented foods are produced by a microbial fermentation in which fermentable carbohydrates are transformed into ethanol and/or organic acids mainly acetic, lactic and propionic acid. Yeast and lactic acid bacteria are the microbes commonly used in food fermentation (Sharma and Ghosh, 2006).

Probiotics come in two main forms i.e. foods and dietary supplements. Consumption of probiotics as part of foods such as dairy products has the advantage of having health benefits of foods (minerals, protein, vitamins, bioactive ingredients) increasing compliance (foods are regular part of our day) and improving the chances that the probiotics reach the intestine alive (as buffer for survival). Consuming probiotics as a dietary supplement has the advantage of delivering a high level of bacteria easily, assuming the products are stored properly (Lee and Salminen, 1995).

Food containing probiotic bacteria fall within the functional foods category since they provide health benefits over the basic nutrition (Sindhu *et al.*, 2005).

Health benefits are :

- Improved digestibility
- Decreasing the incidence of diarrhoea.

- Reducing respiratory infections such as sinusitis, bronchitis and pneumonia in children.
- Improving immune system
- Vitamin production, availability of minerals and trace elements
- Cholesterol lowering effects

The functional food is an expanding sector of the food industry. A major development in functional food pertains to foods containing probiotics and prebiotics that enhance health promoting microbial flora in the intestine. A healthy gut microflora may provide protection against gastrointestinal disorders (Sharma and Ghosh, 2006). Probiotic fermentation can also play an excellent role in the production of wholesome foods ranging from fermented fresh vegetables such as cabbage (*Sauerkraut*) and cucumber (*pickles*), fermented cereal buttermilk mixture (*rabadi*), fermented cereal-legume mixture (*dhokla, idli, wadi*), fermented cereal yoghurt (Nigerian *ogi*/Kenyan *uji*), sour dough bread (*puto*), fermented milks (*dahi/yoghurt/cheese*) and fermented meats (European salami). These products are either fermented naturally or with the help of pure cultures of micro-organisms (Soni and Sandhu, 1990; Binita, 1995; Chahal, 1999).

Other workers also reported that the fermentation with probiotics has been improving the starch and protein digestibility (Antony and Chandra, 1998; Binita and Khetarpaul, 1999). Improvement in starch digestibility during fermentation can be related to enzymatic properties of fermenting microflora and that brings about the break down of starch to oligosaccharides. The presence of α -amylase in the fermenting microflora was indicated by Bernfeld (1962). The enzymes bring about the cleavage of amylase and amylo-pectin to maltose and glucose. Reduction in amylase inhibitor activity may also be responsible for increased starch digestibility (Sharma and Kapoor, 1996). Similarly, improved protein digestibility is mainly associated with proteolytic activity of fermenting microflora. This increase in digestibility may also be due to reduced antinutrient

content of the fermented food as antinutrients are known to inhibit amylolysis and proteolytic activity (Knuckles *et al.*, 1985). Reduction in antinutrients may also explain the increased bio-availability of various minerals as indicated by a number of studies (Sharma and Khetarpaul, 1996; Chahal, 1999). In plant foods, minerals like Ca, Fe and Zn are bound with phytic acid and a protein-phytate mineral complex is formed (Prattley *et al.*, 1982). Such a complex inhibits the availability of minerals and therefore, extractability of minerals is low in raw foods. Decrease in level of phytic acid after fermentation, which is possibly through hydrolysis by inherent phytate in fermenting microflora may release these metallic ions in free form and therefore, may account for increased bioavailability of these in fermented products.

Rao and Shahani (1987) reported that during their growth in the manufacture of cultured products, the lactic acid bacteria generally utilize some vitamins. However, yoghurt cultures will increase the amount of some B vitamins during their growth.

Lay and Fields (1981) reported that cornmeal fermented at various temperatures by natural lactic acid fermentation resulted in the increased level of availability lysine, tryptophan and methionine (Sripriya *et al.*, 1997).

2.2 Chemical analysis

2.2.1 pH and titratable acidity :

Fermentation has been reported to cause a decrease in pH with a simultaneous increase in titratable acidity in several fermented products prepared from pearl millet, wheat, barley, rice, corn, cowpea, chickpea, soybean and their blends (Goyal and Khetarpaul, 1994; Binita, 1995; Chaudhary, 1998; Chahal, 1999).

Goyal (1991) reported that the pH of fermented cereal-legume blends i.e. rice-bengal gram *dhal* flour, rice – defatted soy flour, barley – bengal gram flour, and barley defatted soy flour, dropped significantly with a corresponding increase

in titratable acidity at various temperature and durations of fermentations. Yadav (1992) reported similar results in *wadies* prepared from green gram *dhal* and black gram *dhal*.

Gupta and Khetarpaul (1993) carried out *rabadi* fermentation of autoclaved as well as unautoclaved wheat or barley flour and buttermilk mixtures. They found that pH dropped and titratable acidity rose as the fermentation progressed at different temperatures (30, 40 and 40⁰C) and for different time periods (6, 12, 18, 24 and 48 h). Maximum drop in pH was reported when all types of *rabadi* were fermented at 40⁰C for 48 h.

Yadav and Khetarpaul (1993) noticed a significant drop in pH with a corresponding increase in titratable acidity which occurred when slurries prepared from blackgram as well as green gram *dhals* were fermented at different temperatures i.e. 25, 30 and 35⁰C for varying time periods i.e. 12 and 18 h.

Goyal and Khetarpaul (1994) prepared rice-defatted soy flour blends in three different proportions as 50 : 50, 60 : 40 and 70 : 30. When all these blends were fermented at temperature i.e. 25, 30 and 35⁰C for 12, 18 and 24 h, a significant decline in pH with simultaneous increase in titratable acidity was observed. Maximum drop in pH occurred when blends were fermented at 35⁰C for 24 h. Similarly, when maize-cowpea blend was fermented traditionally, pH was dropped from 6.7 - 4.4 per cent (Akpapunam and Sefa, 1995).

Mnkeni *et al.* (1995) fermented two varieties of green beans with or without starter culture. The pH of beans fermented with starter culture decreased to 4.0 after 4 days whereas the samples fermented without starter culture took 8 days. In both cases a sharp increase in acids were observed within the first two days of fermentation but samples fermented without starter culture produced significantly less acids.

Effect of natural fermentation in 4 cereals and 4 decorticated legumes was studied by Agte *et al.* (1997). Fermentation significantly decreased pH with sharp increase in titratable acidity. Urga *et al.* (1997a) observed that pH was decreased and titratable acidity was increased when *tef* flour was mixed with water and was fermented at 22⁰C for 24 h by the action of endogenous microflora in the batter. Similarly the pH of the fermented food prepared from enset (*Enset ventricosum*) was decreased from 5.7 to 3.8 with a concomitant sharp increase in titratable acidity resulting in accumulation of organic acids (Urga *et al.*, 1997b).

A probiotic fermented food mixture containing pearl millet flour, chick pea flour, skim milk powder and fresh tomato pulp was developed by Rani and Khetarpaul (1998). A significant decrease in pH with a corresponding increase in titratable acidity was observed by them. A similar increase in titratable acidity corresponding to a decrease in pH was observed by Chaudhary (1998) who developed two different cereal – legume based food mixtures fermented with three different probiotic organisms.

According to Ghazali and Cheng (1991), soaking and germination had a non-significant effect on pH and titratable acidity of black gram. Fermentation of germinated samples at 30⁰C for 48 h using different cultures and their combinations caused an appreciable increase in acidity and the highest values were observed in naturally fermented samples (Sharma and Kapoor, 1996).

2.2.2 Proximate composition

Pearl millet grain is composed of about 8 per cent of the bran portion (Abdebrahman and Hosney, 1984), which is quite rich in non-protein nitrogen. Non-protein nitrogen (NPN) and true protein content of pearl millet as indicated by Aggarwal (1992) was 39.0 mg/100g and 10.2 per cent, respectively. However, a range of 56-73 mg/100g for NPN and 9.9-12.2 per cent for true protein content was observed by Chaudhary (1993). A value of 51 mg/100g was assessed (Sharma and Kapoor, 1997) for NPN which contributed 10.78 per cent of true protein to

pearl millet grains whereas range of 45- 50 and 43.0 – 48.50 mg/100g or NPN and 11.0 – 12.9 and 12.1 – 12.8 per cent for true protein was given by Archana (1997) and Rekha (1997), respectively.

Oyewole and Odunfa (1989) carried out the fermentation of cassava for production of *fufu*, a pasty food eaten in Africa. Protein content reported was reduced to 20.0 per cent during fermentation. Protein content decreased during the first 72 h, increased rapidly during the last 24 h fermentation. Fat content was found to increase during natural fermentation whereas decreased during pure culture fermentation and ash content did not change (Khetarpaul and Chauhan, 1989).

Grewal (1992) reported a significant increase in protein content, decrease in fat content and non-significant change in ash content during germination of soybean for 12, 24, 36 and 48 h. The decrease in fat contents could be due to the result of metabolism in order to meet the increased energy requirement of the developing plant tissue (Ghazali and Cheng, 1991). During soaking, there is leaching out of minerals which is multiplied by the loss of non-mineral dry matter during germination and sprouts ultimately have same content of minerals matter as raw seeds, hence there is no change in ash content. According to Yadav and Khetarpaul (1993), there was no significant change in crude protein, fat and ash content of green gram and black gram *dhal*.

With an increase in period of germination, no change in protein, but an increase in fat and a decrease in ash content of soybean was noticed by Savitri and Desikacher (1990). Crude protein, fat and ash content of *wadies* prepared by natural fermentation of green gram and black gram *dhal* did not change significantly (Yadav and Khetarpaul, 1993).

Sharma (1994) reported a significant reduction in the protein content of pearl millet when it was fermented with *L. acidophilus*.

Borde and Shamita are the two popular fermented beverages of thick consistency drunk in the Southern part of Ethiopia. These are prepared from maize and barley, respectively. When raw sample was compared with the fermented one, it was found that protein content was high in the fermented one (Ashenafi and Mehari, 1995).

Mnkeni *et al.* (1995) reported a decline in protein content of two varieties of green beans when they were fermented with or without starter culture.

Binita *et al.* (1996) fermented BTMT food mixture containing barley flour, green gram *dhal*, skimmed milk powder and tomato pulp with *L. acidophilus*. Protein content was found to reduce and ash content remained unaltered in fermented products. When the blends of rice and defatted soya flour mixed in three different ratios were fermented, Goyal and Khetarpaul (1994) found that fat and ash remained unchanged whereas crude protein either decreased or remained unchanged. Similarly, Sharma and Khetarpaul (1997) developed cercal legume blends and fermented it at 35⁰C for 18 h. They reported that fermentation did not bring about significant changes in crude protein, fat and ash contents.

Akpapunam and Sefa (1995) observed the effect of traditional lactic acid fermentation and addition of malt on protein content of maize cowpea blends, the products were found to be high in protein content (18.4 to 19.4%).

A decrease of about 42.9 per cent was reported when finger millet was fermented using endogenous grain microflora at 30⁰C (Antony *et al.*, 1996).

Obong and Obizona (1996) carried out the fermentation of African yam bean. They found that fermentation had no effect in crude protein content of the bean.

According to Basappa *et al.* (1997) there was a increase in protein content (7.6 vs. 10.5%) when cooked *ragi* was fermented with *Endonaycopsis fibuligera* and *Saccharomyces cerevisiae*. Similarly Sripriya *et al.* (1997) reported 10 fold

increment in total soluble protein contents when finger millet (*ragi*) was fermented for 32 h.

According to Goldin (1998), bacterial protease increases the production of free amino acids which benefits the nutritional status of host particularly if the host has a deficiency in endogenous protease production.

Decrement in total protein and ash content by 15-16 per cent respectively was observed when enset plants (*Ensete Ventricosum*) was fermented for 7 weeks (Urga *et al.*, 1997b). Similarly, fat content of *tempeh* made from the kocho grass pea combination decreased by approximately one third (Urga *et al.*, 1997a).

According to Chaudhry (1999), protein content decreased significantly in probiotically developed fermented cereal legume based food mixture but moisture, fat and ash remained unaltered.

According to Alonso *et al.* (1998), increase in protein content of 24 h germinated peaseeds was from 19.0 to 19.4 per cent. In increase in soluble protein content was observed when finger millet flour was fermented using endogenous gram microflora. Murkya *et al.* (2000) also noticed that protein content rose steadily during germination, increasing from 6.1 to 7.9 per cent.

2.2.3 Carbohydrates

Bengtssen *et al.* (1990) reported 52.0 to 64.0 per cent starch in barley Newman *et al.* (1990) and Gopalan *et al.* (1991) also reported further higher amount of starch in barley grains.

Hulless barley contains higher starch content than hulled barley (Knuckles *et al.*, 1992). Hulled barley having 61.9 to 64.3 per cent starch while hulless barley has 64.4 to 66.8 per cent. Similar range was also reported by Sundberg *et al.* (1994) in unprocessed barley grain.

Various processing treatments and cooking methods are affective on available carbohydrates of various foods. According to Kataria *et al.* (1990)

hydrolysis of starch to oligosaccharides and that of oligosaccharide to monosaccharides during cooking may be responsible for increased concentration of sugars in pulses.

Khetarpaul and Chauhan (1991a) autoclaved the pearl millet and observed that starch, total soluble sugar, reducing sugars and non-reducing sugars changed from 68.50, 1.76, 0.36 and 1.40 per cent in raw grains to 63.30, 4.52, 1.22 and 3.30 per cent, respectively in autoclaved pearl millet.

Decrease in the content of starch and increase in sugars have been reported in mungbean (Kataria and Chauhan, 1988), amphidiploids (green gram x black gram) (Kataria *et al.*, 1990), soyabean (Grewal, 1992; Liu and Markakis, 1987) and pearl millet (Khetarpaul and Chauhan, 1991b; Sharma, 1994) when these cereals and legumes were cooked or autoclaved. Starch granules may be ruptured followed by hydrolysis of starch on ordinary and pressure cooking (Grewal., 1992).

The total soluble sugars of pearl millet increased during fermentation at 20, 30 and 50⁰C with initial pH 6.5 and 4.0. On the contrary, fermentation at 40⁰C reduced the level of total soluble sugars. Non-reducing sugars also increased after 24 h fermentation.

Khetarpaul and Chauhan (1991b) reported a significant reduction in total soluble, reducing and non-reducing sugar along with starch content during *Lactobacilli* fermentation of pearl millet with increase in fermentation period at all temperatures. Total soluble, reducing and non-reducing sugars as well as starch content were reduced. During fermentation, reduction in total soluble sugars might be due to microbial utilization of released sugars as a ready source of energy.

A sharp decrease in total reducing sugars during fermentation of two varieties of green beans with or without starter culture was observed (Mnkeni *et al.*, 1995). According to Binita (1995), total soluble, reducing and non-

reducing sugars of RSMT food mixture containing rice flour defatted soy flour, skimmed milk powder and tomato pulp increased from 1.30 to 4.11, 0.24 to 0.63 and 1.06 to 3.48 g per 100g, respectively, during autoclaving.

Urga *et al.* (1997b) observed that when enset (*Ensete ventricosum*) was fermented for 17 weeks for 'kocho' production, starch, soluble sugars, reducing sugars and available carbohydrates decreased by 23, 92, 84 and 51 per cent, respectively. Similarly, total and reducing sugars were found to decrease during natural fermentation of *tef* flour mixed with water at 22^oC for 96 h (Urga *et al.*, 1997a).

Total soluble, reducing and non-reducing sugars increased significantly when two different cereal legume blends were autoclaved but fermentation with three probiotics lowered down the level of sugars excepts reducing sugars. Reduction in starch content was observed as a result of autoclaving and fermentation with all the three probiotic organisms i.e. *L. acidophilus*, *L. fermentation* and *B. bifidum* (Chaudhary, 1999).

According to Khetarpaul and Chauhan (1991a), natural fermentation of pearl millet increased the total soluble sugars, reducing sugars and non-reducing sugars and decreased the starch significantly. On the other hand, pure culture and sequential fermentation decreased the total soluble sugars, decrease was more in *Lactobacillus* than yeasts. There was a drastic reduction in total soluble sugars, reducing sugars, non-reducing sugars and starch when sprouted samples were fermented.

Reduction on germination in total soluble sugars, reducing sugars, non-reducing sugars and starch contents by 38.0, 58.0, 36.0 and 37.0 per cent in kidney beans; 24.0, 58.0, 11.0 and 22.0 per cent in Bengal gram and 30.0, 10.0, 34.0 and 35.0 per cent in black gram, respectively was reported by Jood *et al.* (1986). The losses of these nutrients on 24 hour germination were more than in 48 hour

sprouted pulses. On further germination i.e. upto 90 hour, the sugar contents increased while starch content was found to decrease.

Kataria *et al.* (1990) also demonstrated that 12 hour soaked seeds of amphidiploids when germinated showed a gradual increment in concentration of various sugars i.e. total, reducing and non-reducing sugars. According to Obizoba and Atti (1994), starch content of sprouted pearl millet (24 h) was decreased from 3.6 to 2.8 per cent, whereas reducing sugars increased from 0.6 to 3.2 per cent.

Khetarpaul and Chauhan (1991a) observed that total soluble sugars, reducing sugar and non-reducing sugar increased 16.3, 6.54 and 9.80 per cent in germinated from 1.76, 0.36 and 1.40 per cent, respectively in raw pearl millet whereas starch content decreased from 68.5 to 50.2 per cent after germination. An initial increase in reducing sugar level may not be due to amylase activity (Odunfa, 1983).

Taur *et al.* (1984) reported the maximum starch degradation by amylases during fermentation. It resulted in increase in the reducing sugars. There was an inverse relationship between starch content and fermentation period. Murkya *et al.* (2000) also found that starch content decreased slightly over the first 36 hours of germination and sugars increased.

2.2.4 Dietary fibre

Dietary fibre is the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances.

Dietary fibres promote beneficial physiological effects including laxation, lowering of blood cholesterol, lowering of blood glucose level (Anonymous, 2000). According to Food and Nutrition Board (2002) 'total fibre' is defined as the combination of 'dietary' and 'functional' fibre.

Fibres that lower blood cholesterol levels include foods such as apples, barley, beans, legumes, fruits and vegetables, oat meal, oat bran, rice hulls; and purified fibres such as in beet fibre, guar gum, locust bean gum, pectin, *psyllium* seed-husk etc. (Marlett *et al.*, 2002). Both of these fibres sources increase stool weight and improve laxation as well as lower blood cholesterol level (Marlett *et al.*, 2002).

According to Joanne Slavin (2003) dietary fibre consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. The total dietary fibre content of millet ranged from 3.2 to 4.7 per cent which was higher than those reported for other common cereals (Ravindran, 1991).

Navita and Sumathi (1992) reported that NDF, ADF, cellulose, hemicellulose and lignin contents of debranned sorghum, *bajra*, *ragi* and wheat reduced significantly. Kahlon *et al.* (1993) studied composition of dehulled barley and reported 17.2, 6.0 and 11.2 per cent total, soluble and insoluble dietary fibre, respectively. Bhatta *et al.* (1995) observed total, soluble and insoluble dietary fibre in hulless barley 8.7, 2.7 and 4.7. Total dietary fibre of milled millets ranged from 9.6 to 16.2% whereas of whole pearl millet had 16.2 per cent total dietary fibre (Hadimani and Malleshi, 1993). Low content of total dietary fibre, soluble and insoluble dietary fibre in wheat was determined by Hooda (2002). She determined 9.02, 3.82 and 5.16 per cent of total, soluble and insoluble dietary fibre. Kakkar (1992) observed reduction in dietary fibre content after dehusking. Whereas autoclaving resulted in decreased insoluble fibre content.

Cooking has been reported to increase hemicellulose content in various vegetables (Mann *et al.*, 1992) and in chickpea (Kakkar, 1992). On the contrary, Matthee and Appledorf (1978) observed no significant increase in hemicellulose on cooking various vegetables. Matthee and Appledorf (1978) and Mann *et al.* (1992) reported that cellulose content increased on cooking in some plant foods. Starch resistant to hydrolysis may be responsible for slightly higher values of fibre

content especially cellulose fraction. Vidal-Valverde and Frias (1991) observed no difference in cellulose content when cooking water was retained with product.

According to Mann *et al.* (1992), NDF and ADF content of vegetable was increased significantly on cooking. Contrary to this, Kakkar (1992) could not find any significant difference in NDF and ADF values of chickpea on cooking and autoclaving. Similarly, ordinary cooking as well as pressure cooking was observed to have no effect on the dietary fibre constituents of rice bean and faba bean (Saharan, 1994). According to Sharma (1994) when finely or coarsely ground pearl millet was subjected to autoclaving, the autoclaved sample had slightly higher value of NDF than non-autoclaved but differences were non-significant. Similar trend was observed in ADF content.

According to Mugula (1992), dietary fibre content of the *tempeh* prepared by a mixed culture fermentation was reported to be increased by 10.0 per cent when sorghum common bean (40:60) *tempeh* was manufactured by fermenting with a mixed culture of *R. oligosporus* and *R. oryzae* (1:1). Vidal *et al.* (1993) reported that content of NDF was increased when natural fermentation of lentils was carried out for 4 days at 30⁰C.

Soaking reduced total dietary fibre content of maize by 40.0 per cent, which was further decreased on fermentation, reduction being non-significant (Nche *et al.*, 1993). Similarly, NDF and ADF contents of autoclaved pearl millet decreased slightly but non-significantly when it was fermented with *L. acidophilus* (Sharma, 1994) observed that fermentation used for the preparation of fermented products like *idli*, *dosa* and *wadi* from rice bean or faba bean did not affect the various dietary fibre constituents. Sprouting resulted in increase in NDF and ADF content of peas. Sprouting did not result in any change in NDF, ADF, hemicellulose, cellulose, lignin and pectin (Kakkar, 1992). Soaking had no significant role in altering the contents of fibre fractions of chickpea (Kakkar, 1992).

The effect of soaking and cooking on NDF, ADF, cellulose and lignin contents of chickpeas, kidney beans and lentils was studied (Vidal-Valverde and Frias, 1991). Pronounced reduction in all the dietary fibre components (on wet weight basis) in pressure cooked chickpeas and kidney beans was observed when cooking water was removed. In lentils with cooking water embedded, no difference between processes was observed. Amount of dietary fibre components on a dry weight basis increased for chickpeas and beans, except for the hemicellulose content of beans and lentils, which was decreased.

Chitra *et al.* (1996) studied the effect of germination on nutritive value of chickpea and their results showed that total dietary fibre contents decreased from 161.20 to 68.30 g/kg upon germination. Same trend was also observed in sprouted pigeonpea, mungbean, urdbean and soybeans.

Various types of indigenous fermented food mixtures were developed by mixing cereal, pulse, skimmed milk powder / whey powder and banana pulp/ tomato pulp (2:1:1 ,w/w), these mixtures were autoclaved and fermented with *L. acidophilus*. There was non-significant increase in the contents of both NDF and ADF in the autoclaved food mixtures. Fermentation also could not significantly lower down these components of dietary fibre in all the food mixtures (Binita *et al.*, 1996; Chaudhary, 1998; Chahal, 1999).

2.2.5 β -glucan

Although β -glucan occurs in all cereals but their concentration is highest in oats and barley (Hofer, 1986). β -glucan content in barley is 1.8 to 3.8 per cent (Sundberg and Aman, 1994).

Marlett (1991) also observed a very high proportion of soluble β -glucan which made up for 73.0 per cent of total β -glucan in barley. β -glucan content of barley has been reported to range between 3.8 to 8.0 per cent (Newman and Newman, 1991; Sundberg and Aman, 1995). β -glucan contents of hull-less barley

and wheat flour was estimated as 4.5 and 0.4%, respectively by Bhatta (1993) whereas Berglund *et al.* (1994) reported that wheat and rice contains 0.14 and 0.07 per cent total β -glucan.

Knuckles *et al.* (1997) prepared breads by substituting β -glucan rich barley fractions for 5.00, 20.00 and 40.01 per cent standard flour. Breads with 20.01 per cent substitution has 7.6 times total β -glucan. The values for total, soluble and insoluble β -glucan content in control well as supplemented breads were 0.47, 0.31, 0.16 per cent and 7.90, 3.58, 2.44 per cent, respectively as total and insoluble β -glucan was affected on baking.

Mac Gregor *et al.* (1997) observed the early stages of germination and reported that hydrolysis of β -glucan in the crushed cell layer started within 10 h from the initiation of germination. They observed the development of peak activity for (1-3) (1-4) when β -glucanase was preceded by that for alpha-amylase. Total β -glucan content declined by about 50.0 per cent during malting.

Okokon (1992) reported that the β -glucan of whole grains of four sorghum varieties grown in Nigeria, unlike those of barley, is not significantly reduced during malting.

Sluijs *et al.* (1999) reported that total dietary fibre and total β -glucan was slightly higher in uncooked puddings compared to that is boiled and baked puddings containing oatrim. Similarly, Dhingra and Jood (2001) reported that there was significant reduction in total and insoluble β -glucan contents of barley bread.

2.2.6 Vitamin B-complex

Au (1979) observed an increase in thiamine during 4 day fermentation (25°C) of sorghum. However, no difference was observed in levels of riboflavin and in niacin in fermented and non-fermented sorghum meat (at 25°C and 30°C).

Zamora and Fields (1979) reported that thiamine content remained unchanged during fermentation of cowpea and chickpea.

Natural lactic acid fermentation of cereal foods resulted in an increase of riboflavin (Wagner – Jauregg, 1972; Hamad and Fields, 1978; Hamad and Fields, 1979; Kazanas and Fields, 1981). But niacin and thiamine decreased significantly during fermentation (Tongnual and Fields, 1979).

Aliya and Geervani (1981) analysed fermented products of legumes, Bengal, gram *dhal*, green gram *dhal* and millets total pearl millet. Sorghum and range 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 of both legumes and millets increased with increase in fermentation time. Steaming and cooking after fermentation reduced the thiamine and riboflavin content.

Gilay and Fields (1981) reported that sprouts of germinated corn were significantly higher in ascorbic acid, riboflavin and niacin than non-germinated corn. Corn fermented after germination was significantly higher in riboflavin and niacin than non-germinated corn but not germinated corn.

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

2.2.7 Protein fractions

Pioneering work of Osborne and Mendell (1914) led to the development of the concept of solubility of proteins. The four major classes of protein fractions are albumins, globulins, prolamins, and glutelins. In a study conducted by Chen and Bushuk (1970), it was found that in wheats, prolamin was the major fraction which constituted 40.7 per cent of total protein fraction followed by acetic acid soluble fraction (18.3%), albumin (12.2%) and globulin (4.7%).

Konzat (1977) reported that prolamins and glutelins are basic protein fractions of cereals and present in small amount in legumes. According to Mori and Utsmi (1979) albumin was observed to be 3 to 5 per cent in rice bean. Anonymous (1984) showed that legumes contain 7.9 to 11.09 per cent glutelin while prolamins content was 1.00 to 3.00 per cent in rice bean as reported by Hira *et al.* (1988).

When compared with legumes, albumin was 15.0-14.0 per cent of total protein in faba bean and pigeon peas. In legumes, globulin is major storage protein (Singh, 1982). Mori and Utsmi (1979) and Bhatta (1982) reported 30.5 to 42.0 per cent of total protein to be globulin in eight legumes. Prolamine and glutelin form a small fraction of protein. Similar results have been observed by Anonymous (1984) and Hira *et al.* (1988).

Anonymous (2004) studied the effect of fermentation on protein fractions on six pearl millet genotypes. The results indicated that six genotypes have 11.4-16.3 per cent crude protein, 31.1-35.1 per cent globulins + albumins, 35.4-41.2 per cent true prolamins, 5.0-9.6 per cent prolamins like, 2.8-3.6 per cent of glutelin like and 1.3-2.4 per cent insoluble protein residue. There was increase in the globulin plus albumin fraction, a decrease in the prolamins fraction and increase in the glutelin like fraction after fermentation. The true glutelin fraction decreased significantly after 14 h of fermentation.

2.2.8 Phytic acid

Phytic acid is widely distributed in plant foods, particularly in cereals and legumes (Reddy *et al.*, 1982). More than half of the phosphorus in cereals is reported to be present in phytate form (Gopalan *et al.*, 1991) which is not available to the human system.

Phytate-inositol-hexaphosphate is a compound which occurs naturally in many plant food and binds to various essential metals, thus, reducing their

availability for absorption from the diet (Wise, 1983). In acidic medium, phytate forms a binary protein-phytate complex by binding to basic residues of protein. In the presence of cations at alkaline pH, phytic acid forms a ternary protein-minerals-phytate complex (Cheryan, 1980), which inhibits enzymatic degradation of protein (Serraino *et al.*, 1985) and hence, affects the protein digestibility. The activity of digestive enzymes is also inhibited by phytate (Knuckles *et al.*, 1985).

Phytic acid is a powerful chelating agent for divalent cations and interferes with the mineral availability by the formation of insoluble phytates (Reddy and Salunkhe, 1980). According to Wise (1983), the phytate forms more stable complex with zinc followed by calcium, copper, cobalt and manganese. Phytic acid can also affect the digestibility by chelating with calcium or by binding with substrate or proteolytic enzyme (Ologhobo and Fetuga, 1982). Phytase (myo-inositol-hexaphosphate phosphohydrolase) catalyses the hydrolysis of phytic acid (Myo-inositol 1, 2, 3, 4, 5, 6-hexakis dehydrogen phosphate) to myo-inositol and inorganic phosphate, via myoinositol penta to monophosphates. Two phytases have been recognized; 3-phytase which initiates the dephosphorylation of phytate at the 3-position and 6-phytase which first acts on the 6-position of phytate. Eventually both phytases dephosphorylate phytic acid fully.

The phytase activity of micro-organism has also been studied extensively. Fungi (Irving and Cosgrove, 1972), and yeasts (Mayini and Markakis, 1983) exhibit phytase activity. The optimum temperature for the enzymatic hydrolysis of phytic acid varies in the range of 45 to 57⁰C among phytases (Mayini and Markakis, 1983).

Various domestic processing methods such as cooking, autoclaving and fermentation are known to reduce or eliminate phytic acid content of legumes (Grewal, 1992; Bishnoi *et al.*, 1994) and cereals (Khetarpaul and Chauhan, 1990; Sharma, 1994).

More than half of phosphorus in cereals is reported to be present in phytate form (Gopalan *et al.*, 1991) which is not available to the human system. Kakkar (1992) observed that roasting of chickpea reduced phytic acid from 760 to 540 mg/100g.

A loss of 21.0 to 46.0 per cent phytic acid in cowpea (Akinyele, 1989), 20.0 to 26.0 per cent in chickpea and 35.0 to 40.0 per cent in black gram (Duhan *et al.*, 1989) has been observed after cooking. Autoclaving reduced the phytic acid content of pearl millet from 990 to 630 mg/100g (Khetarpaul and Chauhan, 1990).

The phytic acid content of 3 varieties of African yam beans and 2 varieties of bambara groundnut was analysed before and after various processing treatments (cooking, autoclaving and/or roasting). Phytic acid was somewhat lowered by cooking as well as autoclaving processes (Apata and Ologhobo, 1997).

Tchango (1995) reported that mould fermentation of maize-soybean mixture decreased phytate content by 46 per cent. Decreased phytic acid content in fermented yambean was reported by Ene-Obong and Obizoba (1996) too.

Sequential fermentation was more effective than single or mixed culture fermentation by *S. diastaticus*, *S. cerevisias*, *L. brevis* and *L. fermentum*. Fermentation after sprouting eliminated the phytic acid content completely. Yeasts appeared to be more effective than bacteria in lowering the phytate content (Khetarpaul and Chauhan, 1990, 1991b).

Yadav and Khetarpaul (1994) carried out the fermentation of coarsely ground dehulled black gram *dhal* slurry at 25, 30 and 35⁰C for 12 and 18 h. They reported that phytic acid content was reduced significantly. The unfermented legume batter had high amount of phytic acid (1000 mg/100g) and it was reduced to almost half in the product fermented at 35⁰C for 18 h.

Ene-obong and Obizoba (1996) carried out fermentation of African yam bean and reported that phytic acid was significantly reduced during fermentation.

Sharma and Khetarpaul (1997) reported that when fermentation of various rice and black gram *dhal* blends (i.e. 60:40, 70:30 and 80:20 w/w) prepared by mixing them in different proportions at 35°C for 18 h brought about a significant decline in phytic acid content. Phytic acid content in various blends decreased to the extent of 23 to 36 per cent over the control values.

Fermentation of rice-defatted and soy flour was carried out with buttermilk at 25, 30 and 35°C for 12, 18 and 24 h. It was observed that indigenous fermentation at 35°C for 24 h reduced the phytic acid level almost half in all the blends (Goyal and Khetarpaul, 1995).

Rani and Khetarpaul (1997) developed a mixture containing huskless barley flour, green gram *dhal* flour, dried skimmed milk and tomato pulp. This food mixture was autoclaved, cooled and fermented with *L. acidophilus* at 37°C for 24 h. Phytic acid content was reduced from 220.20 to 81.48 mg per 100g.

Urga *et al.* (1997b) prepared *tempeh* by co-fermenting different proportion of *kocho* flour with grass pea (*Lathyrus sativus*) using the traditional inoculum, *usar*. It was observed that fermentation decreased the concentration of phytic acid. When natural fermentation of *tef* flour mixed with water was carried out at 22°C for 96 h, phytic acid was reduced by about 72.0 per cent (Urga *et al.*, 1997a). A reduction in phytic acid content by 49.3 per cent when rapeseed meal was fermented with *L. acidophilus* at 37°C for 30 days (Kaur *et al.*, 1997),.

Vig *et al.* (1997) subjected rapeseed meal to fermentation for 30 days. *Lactobacillus* and *Saccharomyces fermentation* effectively reduced the phytic acid content. Maximum loss of phytic acid occurred in *Lactobacillus* fermentation.

Tef Atmit (thin gruel from *Eragrostis tef*) was fermented by mixed cultures of lactic acid bacterial namely *L. casei*, *L. plantarum*, *L. fermentum* and *Pediococcus pentosaecus* at 30°C for 2 days (Urga and Keshava, 1998). The fermentation brought about a significant decline in phytic acid content, the effects

being more pronounced in Atmit fermented with *L. casei* + *L. plantarum* compared to other cultures. Similarly Antony and Chandra (1998) observed a significant reduction (20%) of phytate at the end of 24 h fermentation of finger millêt flour using endogenous grain microflora.

Mahgoub and Elhag (1998) studied the effect of milling, soaking, malting, heat treatment and fermentation on phytate levels of four Sudanese sorghum cultivars. All treatments investigated caused phytic acid reduction to various extents. Enzymatic methods (malting and fermentation) were found to be most effective.

Rani and Khetarpaul (1999) developed an indigenous cereal legume based nutritious food mixture fermented with probiotic organism. A significant reduction to the extent of 63.0 per cent was noticed in the content of phytic acid due to cumulative effect of autoclaving and fermentation.

Chaudhary (1999) reported a significant reduction (59.0-64.0%) in phytic acid content of two probiotic fermented cereal legume based food mixture. Maximum reduction was observed when the food mixtures were fermented with *L. acidophilus*.

Binita and Khetarpaul (1999) reported the results of probiotic fermentation of food mixture i.e. RSMT containing rice, defatted soyflour, skimmed milk powder and tomato pulp in 2:1:1:1 proportions at 37⁰C for 24 h. A significant reduction in the contents of phytic acid was observed due to the cumulative effect of autoclaving and fermentation to the extent of 63 per cent.

2.2.9 Polyphenols

Polyphenolic compounds which are also called 'tannins' are widely distributed in higher plants used as human food. These are predominantly located in the peripheral area of seed. They impart grey colour and limit protein and starch utilization (Pawar and Parlikar, 1990) either by binding with proteins or by

inhibiting the digestive enzymes especially trypsin and amylase (Griffiths, 1979; Singh, 1984).

Salunkhe *et al.* (1982) also reported that these tannins are known to inhibit the proteolytic enzymes as they form insoluble complexes with food proteins which ultimately lower the digestibility and so also the protein quality. Polyphenols have shown the harmful effects on the availability of minerals and vitamins. The availability of ionizable iron is decreased by tannins due to their natural iron chelating agent action (Rao and Prabhavathi, 1982). Tannins have also been shown to have direct inhibitory effect on digestive enzymes such as trypsin and amylase (Singh, 1984).

Pearl millet contains high amount of polyphenols. The concentration of polyphenols ranging from 285-788 mg/100g has been reported (Mahajan, 1986; Khetarpaul and Chauhan, 1991b; Aggarwal, 1992). Endosperm and bran of pearl millet had 101 and 1148 mg/100g polyphenols respectively as compared to 285 mg/100g in whole pearl millet (Aggarwal, 1992). Whereas Kalra and Jood (2001) reported 420 mg/100g phytic acid in hulles barley.

Various cooking and processing treatments are known to reduce polyhenols. A significant reduction was observed in autoclaved pearl millet which had 693 mg/100g polyphenols as compared to 761 mg/100g in raw pearl millet (Khetarpaul and Chauhan, 1991b). Roasting and autoclaving decreased the tannin content significantly (Kakkar, 1992).

Fermentation reduced the tannin content of pearl millet *rabadi* fermented with buttermilk, polyphenols decreased with increase in the duration of fermentation at different temperatures. At 9 h fermentation, the loss in polyphenol content was the highest and varied from 0-12 per cent at different temperatures. This reduction was attributed to activity of polyphenol oxidase of pearl millet grain or fermenting microflora.

However, Grewal and Chauhan (1993) reported that there was no change or a reduction in polyphenol content when soaked, dehulled and autoclaved soybeans were fermented with skimmed milk curd at 25, 30 and 35⁰C for 12 and 48 h.

Fermentation of dehulled black gram *dhal* slurry was carried out at 25, 30 and 35⁰C for 12 and 18 h. It was reported that unfermented legume batter had high amount of polyphenols (998 mg/100g) and these were reduced to almost half in the product fermented at 35⁰C for 18 h (Yadav and Khetarpaul, 1994). Goyal and Khetarpaul (1995) carried out the fermentation of rice-defatted soy flour and mixed with butter milk at 25, 30 and 35⁰C for 12 h. It was observed that polyphenols increased significantly or remained constant in the fermented blends.

A food mixture containing huskless barley flour, green gram *dhal* flour, dried skim milk and tomato pulp was developed, autoclaved, cooled and fermented with *L. acidophilus* at 37⁰C for 24 h. It was reported that polyphenol content was reduced from 614.58 to 484.37 mg/100 g (Rani and Khetarpaul, 1997). A decrease in tannin content was reported during the natural fermentation of Enset for *kocho* production (Urga *et al.*, 1997b). Antony and Chandra (1998) observed a significant reduction of phenols (20.0%) and tannin (20.0%) during natural fermentation of finger millet.

Chaudhary (1999) reported that two different food mixtures when fermented with *L. acidophilus* or *L. fermentum* or *B. bifidum* resulted in a decrease in polyphenol content of the food mixtures to the extent of 24.0 per cent. Fermentation with *L. acidophilus* and *B. bifidum* brought about the maximum reduction.

Rani and Khetarpaul (1999) developed an indigenous nutritious cereal legume based food mixture. After carrying out the fermentation of autoclaved mixture with *L. acidophilus*, the polyphenol content was found to be reduced to the extent of 19.0 per cent.

Grewal and Jood (2006) reported that heat treatments like cooking and pressure cooking caused significant reduction in polyphenol content of moong bean. Whereas 24 sprouting reduced polyphenol contents i.e. 240.26 mg/100g after 24 h sprouting over raw seeds which contained 353.40 mg/100g.

2.2.10 Amylase inhibitor activity

Most of the cereals including wheat, rice, barley, rye, sorghum, maize, oats, *ragi* and pearl millet are known to possess proteinaceous inhibitors of alpha-amylases (Shivraj and Pattabiraman, 1980; Udupi *et al.*, 1989; Feng *et al.*, 1991) which are effective against human pancreatic amylase, and pig pancreatic amylase but in effective against alpha amylases of *Bacillus subtilis* and *Aspergillus oryzae* (Sharma and Pattabiraman, 1982; Feng *et al.*, 1991).

Kalra (1996) reported that the amylase inhibitor activity of the barley ranged from 132.25 to 160.15 AIU. There was a negative correlation of amylase inhibitor with *in vitro* protein and starch digestibility.

Various processing treatments are known to destroy these inhibitors. Soaking of various varieties of *Lathyrus sativus* reduced the amylase inhibitor activity ranging from 7.0 – 14.0 per cent (Srivastava, 1994). It was further reported that soaking and dehulling caused still more decline in amylase inhibitory activity upto 25.0 per cent.

According to Mulimani and Supriya (1993), cooking of overnight soaked grains of sorghum reduced alpha amylase inhibitor from 113.8 to 0.7 AIU/g after 20 min and eliminated it completely after 25 min. of cooking. Roasting was equally effective in eliminating this activity. Fifty seven per cent reduction after soaking and autoclaving of *Lathyrus sativus* could be observed in amylase inhibitor activity (Srivastava, 1994).

Complete elimination of alpha amylase inhibitors was observed on germination of *ragi* upto 48 hours (Shivraj and Pattabiraman, 1980) and

(Shivraj and Pattabiraman, 1980) and 71.0 per cent reduction could be observed in *Lathyrus sativus* by sprouting (Srivastava, 1994).

2.2.11 *In vitro* Protein digestibility

Plant foods especially cereal, legumes and millets are good sources of protein. Its utilization depends upon the amino acid composition i.e. type of protein and its digestibility.

Cooking and heat treatment have been reported to improve the protein digestibility (Jood *et al.*, 1998; Grewal, 2003). Autoclaving increased the protein digestibility of pearl millet from 51.0 to 59.2 per cent (Khetarpaul and Chauhan, 1991a) and from 52.60 to 71.09 per cent (Sharma, 1994).

Beneficial effect of germination has been reported to improve *in vitro* protein digestibility of various legumes and cereal legume based foods (Saharan, 1994; Negi, 1999; Grewal, 2003). Enhanced proteolytic activity during fermentation is generally associated with improvement in protein digestibility. High proteinase activity has frequently been reported in the fermentation of protein foods as in the production of Japanese *miso*, soysauce and *ogiri* (Kao and Robinson, 1978; Odunfa, 1983). They also reported an increase in the amino nitrogen to signify a partial break down of proteins to peptides and amino acids. It resulted in ultimately increased protein digestibility of *tempeh* and *miso*.

Goyal (1991) reported that as a result of fermentation of blends prepared from rice, barley, bengal gram and defatted soyflour in different proportions, the improvement in protein digestibility ranged from 30.0 to 60.0 per cent, blends containing cereal and legume in 60:40 ratio had higher protein digestibility, followed by 50:50 and 40:60 proportions. A progressive improvement in protein digestibility with longer period of fermentation of soybean batter was also observed (Grewal, 1992). Fermentation caused an increase in protein and starch digestibility (*in vitro*) of wheat flour (Gupta *et al.*, 1992). They reported that after

12 hours of fermentation, protein digestibility (*in vitro*) was from 62.4 to 72.9 per cent and starch digestibility (*in vitro*) was 45.6 to 49.2 mg maltose released/g.

Chaudhary (1993) studied the cumulative effect of germination and fermentation on black gram. The reported that fermentation caused an appreciable enhancement in protein digestibility of different black gram sprout slurries. Increased protein digestibility varied from 53.0 to 83.0 per cent in different sprout slurries fermented at 30°C for 12 h. When the period of fermentation was prolonged i.e. 12 to 24 h protein digestibility almost doubled in 48 h sprouts.

Chitra *et al.* (1996) reported that *in vitro* protein digestibility of chickpeas increased significantly from 75.4 to 86.5 per cent on germination. Same trend was observed in sprouted pigeonpea, mungbean, urdbean and soyabeans. Germination of soaked sorghum also showed significant increase in *in vitro* protein digestibility with increase in germination time; protein digestibility was increase from 80.75 to 95.02, 89.02 to 85.01 per cent when seeds were soaked for 10, 20 and 30 h and then germinated for 72 h (Elmaki *et al.*, 1999). Murkya *et al.* (2000) also showed 55.4 per cent increase in *in vitro* protein digestibility after germination in finger millet.

Uji, a popular thin porridge in Kenya was made from sorghum flour, diluted with water and boiled or directly drum dried. *Uji* was also prepared by using a mixture of malted finger millet flour and sorghum flour slurry. Experiments involved combination of fermentation, malt treatment, boiling and drum drying and it was observed that fermentation improved *in vitro* protein digestibility considerably. Addition of malted flour increased *in vitro* protein digestibility as compared to control (Mbugua *et al.*, 1992).

Fermented gruels or water slurries of flours of non tannin and high tannin varieties of maize, sorghum, finger millet and bulrush millet were prepared by addition of different starter cultures (Lorri and Svanberg, 1993). They observed that lactic fermentation improved ($P < 0.01$) *in vitro* protein digestibility of gruels,

except for the non-tannin maize variety. No difference was observed in protein digestibility between gruels fermented before or after cooking. Unfermented, non-tannin cereal gruels had higher *in vitro* protein digestibility values (61.79%) than unfermented high tannin cereal gruels of sorghum and finger millet (33.0-40.0%). A high tannin variety of bulrush millet, had an average protein digestibility of 7.0 per cent after lactic fermentation, equal to the value for unfermented maize. When fermentation was done with a natural lactic fermentation with dominance of strains from *Lactobacillus*, *Streptococcus* and *Candida* protein digestibility was found to be more as compared with those of pure culture of *L. plantarum*.

Improvement in protein digestibility (*in vitro*) as a result of fermentation of both cereal and legume products was also noted by Goyal and Khetarpaul (1994). Chitra *et al.* (1996) also stated an improvement in protein digestibility (*in vitro*) from 75.4 to 84.5 per cent after fermentation of chickpea *dhal*.

Yadav and Khetarpaul (1994) reported that indigenous fermentation of coarsely ground dehulled black gram *dhal* slurry at 25, 30 and 35⁰C for 12 h significantly improved the *in vitro* protein digestibility. A significant and negative co-relation was found between *in vitro* digestibility and antinutrients.

Hassan and Tinay (1995) studied the two sorghum cultivars – Safra and Cross. Investigations showed that *in vitro* protein digestibility (IBPD) of untreated seeds was 73.6 and 70.7 per cent, respectively. The cultivars were fermented for 14 h. It was found that IVPD increased from 73.6 to 84.9 and from 70.7 to 80.1 per cent of 2 cultivars, respectively.

Rani and Khetarpaul (1999) reported significant rise in *in vitro* protein digestibility due to autoclaving and fermentation of indigenously developed nutritious rice-soy based food mixture.

2.2.12 Starch digestibility

Low digestion of starch may be attributed to the chain length, presence of amylase inhibitors, phytate and polyphenols in the food legumes and cereals (Singh *et al.*, 1982; Thompson and Yoon, 1984). The method involved in processing of cereals and legumes are very effective in improving the starch digestibility as antinutrients responsible for low starch digestibility are reduced considerably by processing technique.

Cooking and fermentation have been reported to improve the starch digestibility of different food grains. Heat treatment increases the rate of amylolysis by three times when compared to that of raw samples of moongbean (Jood *et al.*, 1998; Grewal and Jood, 2006). Enhanced digestibility of cooked legume starches by α -amylase could be attributed to the swelling and rupturing of starch granules which facilitates more randomized configuration of α -amylase to affect hydrolysis, the disintegration of various bean components during cooking and inactivation of amylase inhibitors (Boralkar and Reddy, 1985).

Several workers have reported the beneficial effects of germination and fermentation on starch digestibility (*in vitro*) of moth bean and moong bean (Negi, 1999; Grewal, 2003). During germination, the activity of certain enzymes like amylase present in the seed increases, as a result of this enhanced enzymatic activity, the seeds undergo pronounced metabolic changes and the standard profile of the various organic components is altered (Wang *et al.*, 1997). The improvement or change 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 bacterial 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 five part glucose.

According to Goyal (1991), an improvement occurred in starch digestibility of different cereal legume blends when they were fermented at 25, 30 and 35⁰C for

12, 18 and 24 h. Higher the temperature of fermentation, more was the enhancement in starch digestibility. The increase varied from 14.0 to 39.0 per cent.

Khetarpaul and Chauhan (1991b) reported that single as well as mixed culture fermentation by yeasts (*S. diastaticus*; *S. cerevisiae*) and *Lactobacilli* (*L. brevis*; *L. fermentum*) at 30°C for 72 h improved the starch digestibility (*in vitro*) of pearl millet flour significantly. The flour fermented by *Saccharomyces diastaticus*, a starch hydrolyzing yeast, had the highest starch digestibility.

According to Goyal (1991), an improvement occurred in starch digestibility of different cereal legume blends viz. rice-bengal gram, rice-defatted soy flour, barley-bengal gram and barley-defatted soy flour mixed in different proportions and fermented with buttermilk at 25, 30 and 35°C for 12, 18 and 24 h. the increase ranged from 14.0 to 39.0 per cent over the control values.

Gupta and Khetarpaul (1993) studied the starch digestibility of *rabadi* prepared by mixing unautoclaved or autoclaved wheat/barley flour with buttermilk and allowing it to ferment at 30, 35 and 40°C for 6, 12, 18, 24 and 48 h. A significant improvement occurred in starch digestibility of both the types of *rabadi*; maximum improvement noticed at 40°C for 4 h.

Chaudhary (1993) reported 81.0 per cent increase in starch digestibility over the control value, when the blackgram sprouts were fermented for 24 h at 30°C. Starch digestibility increased to the extent of 53.0 to 72.0 per cent in the fermented products viz., *idli*, *dosa* and *wadi* prepared from ricebean and fababean (Saharan, 1994).

Yadav and Khetarpaul (1995) reported the indigenous fermentation of coarsely ground dehulled black gram *dhal* slurry at 25, 30 and 35°C for 12 and 18 h significantly ($P < 0.05$) improve *in vitro* starch digestibility and antinutrients.

Whey fermentation of various rice and bengal gram *dhal* blends prepared by mixing them in different proportions at 35°C for 18 h improved the starch

digestibilities (Sharma and Khetarpaul, 1997). Improvement in starch digestibility is related to the reduction in phytic acid contents as this antinutrient is known to inhibit amylolysis. A significant negative co-relation was found between phytic acid and starch digestibility.

According to Rani and Khetarpaul (1997) a food mixture containing huskless barley flour, green gram *dhal* flour, dried skim milk and tomato pulp was developed and fermented with *L. acidophilus*. It was reported that *in vitro* digestibility of starch was significantly improved (31.5 vs 63.96) during fermentation. They noticed a significantly negative co-relation between the antinutrients content and starch digestibility.

Various processing treatments (soaking, germination, debranning and autoclaving) significantly improved the starch digestibility of pearl millet which was further improved by its fermentation with *L. acidophilus* and *L. plantarum* (Sharma and Kapoor, 1996). Antony and Chandra (1998) fermented finger millet flour using endogenous grain microflora and reported a significant increase in starch digestibility.

Chaudhary (1999) reported improved starch digestibility when two indigenously developed cereal legume based food mixtures were autoclaved. When these mixtures were fermented with different probiotic organisms, the digestibility of starch was found to increase further. Maximum increase was found to be in food mixtures fermented with *L. acidophilus* (96-110%) followed by those fermented with *B. bifidum* (85-104%) and *L. fermentum* (84-95%).

Rani and Khetarpaul (1999) reported a significant rise in *in vitro* starch digestibility during autoclaving and fermentation of indigenously developed nutritious rice-soy based food mixture.

2.2.13 Minerals

Saibaba (1990) reviewed the mineral composition of barley and reported 26.0, 215.0, 1.67 and 1.20 mg/100 g of calcium, phosphorus, iron and zinc, respectively in barley grains. Gopalan *et al.* (1995) also reported the same amount of calcium, phosphorus and iron contents in barley grains on dry matter basis, calcium and iron content of barley ranged from 23.75 to 28.65 and 1.33 to 1.50 mg/100g, respectively.

Different processing and cooking methods are known to have a pronounced effect on the mineral contents of plant foods especially their availability (Chaudhary, 1999; Chahal, 1999; Grewal and Jood, 2006).

Duhan (1992) noticed no change in calcium and iron contents of pigeonpeas when they were subjected to autoclaving. Ca and Fe contents of fababean and rice bean also remained significantly unchanged upon cooking (Saharan, 1994).

The losses occurring during cooking may be because cooking involves a complex reaction system. Thereby Ca^{++} ions which are concentrated in parenchymal cells migrate into the interior of cell and complex with phytate ion. Further permeability of cell wall is alerted by the presence of Ca^{++} and Mg^{++} ions. More soluble calcium salts might be formed during these changes which get leached out. Bishnoi and Khetarpaul (1996) also noticed a loss of 3.0 to 8.0 per cent Ca when peas were ordinarily cooked.

Germination and fermentation did not alter the total mineral content of various cereals and legumes including barley (Gupta *et al.*, 1993), rice (Sharma, 1994), green gram (Yadav and Khetarpaul, 1994; Grewal, 2003) and cereal legume blends (Goyal, 1991).

Rice and dehulled black gram blends were fermented with buffalo milk whey at 35°C for 18 h and was reported that fermentation did not significantly

change the total amount of calcium, phosphorus and iron present in the blends. Since, no addition or deletion of mineral source occurred during the process of fermentation, the amount of total minerals remained unchanged (Sharma and Khetarpaul, 1997).

No significant change occurred in total Ca, Fe, Na and K content of developed cereal-legume based food mixtures due to autoclaving or fermentation with *L. acidophilus* (Chaudhary, 1999).

Sindhu *et al.* (2005) studied the effect of single culture and sequential culture fermentation in indigenous food mixtures containing rice flour, whey, sprouted green gram paste and tomato pulp (2:1:1:1 w/w) at 37⁰C for 24 h. All the fermentation did not alter the amount of total minerals in the mixture.

2.14 *In vitro* availability of minerals

Extractable minerals in a food are those which are soluble in 0.03 N HCl, the concentration of HCl found in human stomach (Chompreeda and Fields, 1984). The amount of HCl-extractable minerals in food, indicated an index of their bioavailability from the foods. Thus, the solubility of minerals in foods subjected to *in vitro* gastric and intestinal digestion is useful indicator of minerals bioavailability (Rao and Prabhavathi, 1978).

Various processing methods such as soaking, germination, autoclaving and fermentation caused significant improvement in cereals and legumes and their blends (Jood *et al.*, 1998; Grewal, 2003; Sindhu *et al.*, 2005).

The effect of germination on minerals was studied by Giri *et al.* (1981). Their results showed that calcium content decreased during germination of pulses, but total iron and phosphorus contents remained constant. On the other hand, Youssef *et al.* (1987) reported that all the minerals i.e. iron, calcium and phosphorus were increased from 6.1 to 9.2; 30.6 to 63.4 and 369 to 400 mg/100g during germination. Contrary to these studies, a significant decrease in calcium,

iron and phosphorus contents i.e. from 52.2 to 51.3, 21.8 to 17.3 and 406.3 to 331.3 mg/100g, respectively was noted after 24 h of germination (Akinlosotu and Akinyele, 1991).

Fermentation has been reported to increase the availability of all minerals in different plant foods. Fermentation converts the bound form minerals to free form and thus increase for availability (Khetarpaul and Chauhan, 1990b).

Rabadi fermentation of raw as well as autoclaved barley flour at 30, 35 and 40°C for 6, 12, 18, 24 and 48 h brought about a significant increase in HCl-extractability of Fe, Cu, Zn, Mn, Ca and P (Gupta *et al.*, 1993). They observed that increased non-phytate phosphorus and inorganic phosphorus in the fermented product, with a corresponding reduction in the phytate phosphorus, was maximum at 40°C for 48 h and minimum at 30°C for 6 h. Level of HCl-extractability of minerals was significantly and negatively correlated with the level of phytic acid in the fermented products. Similarly, Saharan (1994) reported an increase in the calcium extractability in the fermented rice bean (13.0 to 18.0%) and faba beans (20.0 to 24.0%) products.

Goyal and Khetarpaul (1994) noticed an increase in extractability of calcium, phosphorus and iron with an increase in temperature and period of fermentation in various rice-legume-blends. The loss of phytates and tannins during fermentation may be particularly responsible for increased bioavailability of minerals, as a significant and a negative correlation has been noticed between mineral extractability and the antinutrients by several workers (Khetarpaul and Chauhan, 1991a; Grewal, 1992; Saharan, 1994; Yadav and Khetarpaul, 1994).

Sharma and Khetarpaul (1997) reported that the HCl-extractability of calcium, phosphorus and iron was considerably increased in the fermented rice-black gram-whey blends. The amount of phytate phosphorus decreased significantly. They concluded that the higher HCl-extractability of Ca, Fe and P

may be partly ascribed to a decrease in phytic acid content in the fermented rice-black gram-whey blends.

Basappa *et al.* (1997) found significant amount of available minerals (Ca, P, Fe) in the water extract (chhang), when cooked *ragi* was fermented using either phab, the traditional inoculum or selected starter culture (*Lactobacilli* and *Saccharomyces*) isolated from phab. Similarly, Sripriya *et al.* (1997) also reported an increase in the availability of calcium and iron during traditional fermentation of finger millet.

Antony and Chandra (1998) observed a significant rise in HCl mineral extractability (Ca 20%, P 26%, Fe 27%, Zn 26%, Cu 67% and Mn 10%), when finger millet flour was fermented using endogenous grain microflora.

HCl-extractability of Ca, Fe, Na and K enhanced significantly (23-37, 54-65%, 24-30% and 13-23%, respectively) when two developed cereal legume based food mixtures were fermented with *L. acidophilus*, *L. fermentum* or *B. bifidum*. Maximum increase was brought about after fermentation with *L. acidophilus* (Chaudhary, 1999).

Home made soy products that were prepared after germination and fermentation had higher percentage of dialyzable iron (Latunde-Dada, 1991). Fermentation of autoclaved soybean sprouts or grains enhanced the mineral bioavailability of calcium, iron and zinc (Grewal, 1992). Indigenous fermentation of blackgram sprouts improved the extractability of calcium and iron with an increase in period and temperature of fermentation (Chaudhary, 1993).

Further, with the increase in germination period, phosphorus content increased but iron content decreased; no effect of germination upto 90 hours on calcium content was observed. Significant decrease in calcium (0.47 to 0.45 g/kg), magnesium (1.23 to 1.21 g/kg) and iron (0.06 to 0.05 g/kg) contents were also observed in sprouted chickpea (Chitra *et al.*, 1996). On the other hand, increase in

zinc, copper and iodine contents was noted in 24 hour germinated pearl millet (Obizoba, and Atti, 1994). Murkya *et al.* (2000) also determined that iron and calcium contents increased from 1.81 and 76.9 to 37.3 and 90.2, respectively after germination.

2.3 Organoleptic and nutritional evaluation of fermented food products

Fermented cereal and legume products form an important part of human diet in South-East Asia including India, the middle East and Africa. These are also becoming popular in the developed world due to their high nutritive value and organoleptic characteristics. Fermentation is one of the oldest methods of food preparation and originated centuries ago (Soni and Sandhu, 1990).

Various probiotic microorganisms i.e. *L. acidophilus*, *L. Casei*, *L. plantarum*, *S. boulardii* etc. are most commonly associated with fermentation of cereal legume based products (*idli*, *dosa*, *wadi*, *rabadi*, *papad*) and milk based products (yoghurt, cheese). Such foods are more appetizing, easily digestible and serve as important source of protein calories and certain vitamins in many cases. Fermentation improves the digestibility of ingredients for human consumption, enhances the keeping quality, shelf life and also protects against food poisoning and transmissions of pathogenic organisms.

Idli, *dosa*, *wadi*, *dhokla* and *khaman* are a few of the popular indigenously fermented legume cereal products that are commonly prepared and consumed in India (Yadav and Khetarpaul, 1994).

Wadies prepared from blackgram *dhal* slurries and greengram *dhal* slurries were found acceptable by the judges (Yadav and Khetarpaul, 1994 and 1995; Grewal, 2003).

Sharma and Khetarpaul (1998) reported that the biscuits prepared by incorporating various unfermented as well as fermented rice-dehulled bengal gram-whey and rice-dehulled blackgram-whey blends were found acceptable and

the scores were in the category of 'moderately desirable' to 'desirable'. On the other hand, dried rice-legume whey blends mixed with roasted and ground peanuts and sesame seeds and jaggery were found acceptable in weaning mixtures and their sensory scores fell in the category of 'desirable'.

The average acceptability scores for *dosas* prepared from various fermented cereal-legume blends differed significantly among themselves. The highest average acceptability score for *dosa* was found in case of rice-bengal gram *dhal* followed by barley- bengal gram *dhal* flour, rice-defatted soyflour and barley-defatted soyflour blends (Goyal, 1991). Grewal (2003) prepared green gram *papad* found acceptable and over all acceptability score was found in the category of 'liked moderately'.

A popular wafer like product, *papad* was prepared by substituting 50% of mixture of black gram *dhal* (*Phaseolus mungo*) *dhal papad* for sensory attributes, dough characteristics, rolling properties and nutritional quality. Finger millet *papad* was rich in Ca (102 mg % in roasted and 109 mg % in fried) compared to black gram *dhal papad* (82 mg % in roasted and 99.6 mg % in fried) substitution of finger millet did not affect the quality characteristics of the *papad* (Vidyavati *et al.*, 2004).

Goyal and Mathews (1985) studied the effect of cooking on protein, lysine and sugar contents of cereal pulse combination preparations like *khichari*, *khamman-dhokla* and *missi roti*. They observed no effect of cooking on total protein of different recipes. Losses of lysine, tryptophan and sugar were found to be more in *khichari* preparation due to boiling of rice and green gram *dhal* mixture.

A wide range of microflora has been known to possess phytate activity (Daniels and Fisher, 1981; Lopez *et al.*, 1983) which may be partly responsible for reducing the phytic acid content in fermented products. The inherent phytase activity reported in legume may also be responsible for decreasing the phytate

content during fermentation. Phytic acid content of chickpea *dhal* was also decreased from 9.2 to 5.6 g/kg after fermentation (Chitra *et al.*, 1996).

Fermentation is an important tool for destroying amylase inhibitor activity. Cooked and fermented products like rice *idli*, *dosa* and urd *idli* did not have any inhibitory effect on salivary amylase, though raw ingredients for these products were having (Udupa *et al.*, 1989).

Fermentation of sorghum completely eliminated amylase inhibitor activity (Mulimani and Supriya, 1993). Upto 67% destruction of amylase inhibitor after autoclaving and fermentation of *Lathyrus sativus* could be observed by Srivastava (1994).

Steinkraus (1983) also noted a small amount of TIA in the *idli* as well as *dhokla* prepared in the same manner. Fermentation did not bring about significant changes in crude protein, true protein, fat, ash and dietary essential minerals including Ca, Fe and P of cereal legume blends (Goyal, 1991).

Soni and Sandhu (1990) prepared *wadi* by replacing black gram *dhal*. They observed that fermentation brought an increase in volume and soluble solids whereas reducing sugars decreased significantly. Total nitrogen and total protein did not vary significantly. Most of the changes during *wadi* fermentation caused improvements in digestibility and nutritive value. Mungbean *wadi* dough fermentation showed similar changes as observed in traditional black gram doughs.

Yadav (1992) prepared *wadies* from green gram and black gram *dhals* fermented for 12 and 18 h at 25, 30 and 35⁰C. Fermentation for 12 h at 25⁰C did not bring about a significant change in crude protein and fat content of *wadies*. But as the fermentation time increased, there was a significant loss of protein. Ash content was not altered during fermentation. *In vitro* protein and starch digestibility were also improved. On the other hand, phytic acid and polyphenols were reduced by 50 per cent over the control *wadies*.

Yadav and Khetarpaul (1994); Yadav and Khetarpaul (1995) prepared *wadies* from coarsely ground dehulled blackgram *dhal* and greengram *dhal* slurry fermented at 25, 30 and 35⁰C for 12 h and 18 h reduced the levels of phytic acid and polyphenols significantly. The control *wadi* had high amounts of phytic acid (1000 mg/100g and polyphenols (998 mg/100g), and these were reduced to almost half in the product fermented at 35⁰C for 18 h. *In vitro* protein and starch digestibility improved significantly with increase in temperature and period of fermentation. A significant and negative correlation was found between the *in vitro* digestibility and the antinutrients.

According to Dhankher and Chauhan (1987), temperature had little influence on degradation of either phytic acid or polyphenol content in case of *rabadi* prepared by fermentation of mixture of pearl millet flour and butter milk for three periods of time (3, 6 and 9 h) at four temperatures (35⁰, 40⁰ and 50⁰C).

An indigenous fermented food *rabadi* was prepared by mixing wheat/barley flour with buttermilk, fermented at 30, 35 and 40⁰C for 6, 12, 18, 24 and 48 h and cooking the fermented mixture with continuous stirring. Autoclaved and unautoclaved *rabadi* were analyzed for phytic acid. It was reported that phytic acid was reduced drastically at all the temperatures and period of fermentation in both autoclaved and unautoclaved *rabadi*, however significant improvement also occurred in *in vitro* protein and starch digestibility (Gupta and Khetarpaul, 1993).

Mugula (1992) reported a reduction in phytate content by 67 per cent when maize-soybean (70:30) *tempeh* was manufactured by fermentation with *R. oligosporus* and *R. oryzae* mixed in the ratio of 1:1.

Urga *et al.* (1997a) observed that during natural fermentation of *tef*, tannins were reduced by 55%. Urga *et al.* (1997) prepared *tempeh* by co-fermenting different proportions of *kocho* flour with grass pea (*Lathyrus sativus*) using the traditional inoculum Usar. Fermentation was found to increase the tannin content.

Products prepared from germinated flours showed significant decrease in phytic P and polyphenols with an improvement in starch and protein digestibility (Hira and Sadana, 2004).

Chapter-3

Materials and Methods

The present investigation was carried out in the Department of Foods and Nutrition, I.C. College of Home Science, CCS Haryana Agricultural University, Hisar to study the formulation, utilization and nutritional evaluation of probiotic fermented coarse cereal blends.

This chapter contains relevant information pertaining to the research design and methodological steps used for the present investigation. The research procedure to achieve the foregoing objectives has been distinctly described under the following headings and sub-headings.

- 3.1 Procurement of material
- 3.2 Microbial culture
- 3.3 Development of indigenously developed probiotic fermented coarse cereal food mixtures
- 3.4 Preparation of samples for nutritional evaluation
- 3.5 Titratable acidity and pH
- 3.6 Enumeration of cell counts in the fermented food mixtures
- 3.7 Nutritional evaluation of probiotic fermented food mixtures
 - 3.7.1 Proximate composition

3.7.2 Carbohydrates

3.7.2.1 Available

- 3.7.2.1.1 Total soluble sugar
- 3.7.2.1.2 Reducing sugar
- 3.7.2.1.3 Non-reducing sugar
- 3.7.2.1.4 Starch

3.7.2.2 Non-available

- 3.7.2.2.1 Dietary fibre
- 3.7.2.2.2 β -glucan

3.7.3 B-complex vitamins

- 3.7.3.1 Thiamine
- 3.7.3.2 Riboflavin
- 3.7.3.3 Niacin

3.7.4 Protein fractions

3.7.5 Total lysine

3.7.6 Antinutrients

- 3.7.6.1 Phytic acid
- 3.7.6.2 Polyphenols
- 3.7.6.3 Amylase inhibitor activity

3.7.7 *In vitro* digestibility

- 3.7.7.1 *In vitro* protein digestibility
- 3.7.7.2 *In vitro* starch digestibility

3.7.8 Minerals

- 3.7.8.1 Total minerals (Ca, Fe, Zn)
- 3.7.8.2 Available minerals (Ca, Fe, Zn)

3.8 Development of food products from probiotic fermented food mixtures and their organoleptic evaluation

3.9 Nutritional evaluation of developed food products

3.10 Statistical analysis

3.1 Materials

Barley (hulled var. RD-2052) and pearl millet (HS-B67-2) were procured from KVK, RAU, Bikaner, Rajasthan and Department of Plant Breeding, CCSHAU, Hisar, respectively. Whey powder was provided by Mahaan Proteins Ltd., New Delhi. Tomatoes were purchased from the local market in a single lot. Seedless tomato pulp was obtained by mashing and sieving the blanched tomatoes in a thick strainer. Skimmed milk was obtained from the Department of Animal Products Technology, CCSHAU, Hisar.

3.2 Microbial culture

3.2.1 Collection of culture

The culture of probiotic micro-organism *Lactobacillus acidophilus* (NCDC) was purchased from the Microbial Culture Collection Centre, NDRI, Karnal.

3.2.2 Preparation of stock culture of probiotic organism

The lyophilized cells of *L. acidophilus* were transferred to sterilized reconstituted skimmed milk under aseptic conditions. The inoculated sterilized skimmed milk was incubated at 37°C for 6 h in an incubator. A loopful of *Lactobacilli* growth from the skimmed milk was streaked on the MRS agar plate and again incubated for 24 h at 37°C. After the colonies developed, a single pure colony was picked up and its gram stained slide was examined microscopically. The *L. acidophilus* was found to be gram positive and rod shaped. Purity of the culture was confirmed before use by gram staining technique.

Stock culture of the *L. acidophilus* was maintained on MRS agar by stab inoculation. After incubating at 37°C for 24 h, the stock culture tubes were stored in a refrigerator for further use. For maintenance, the cultures were transferred

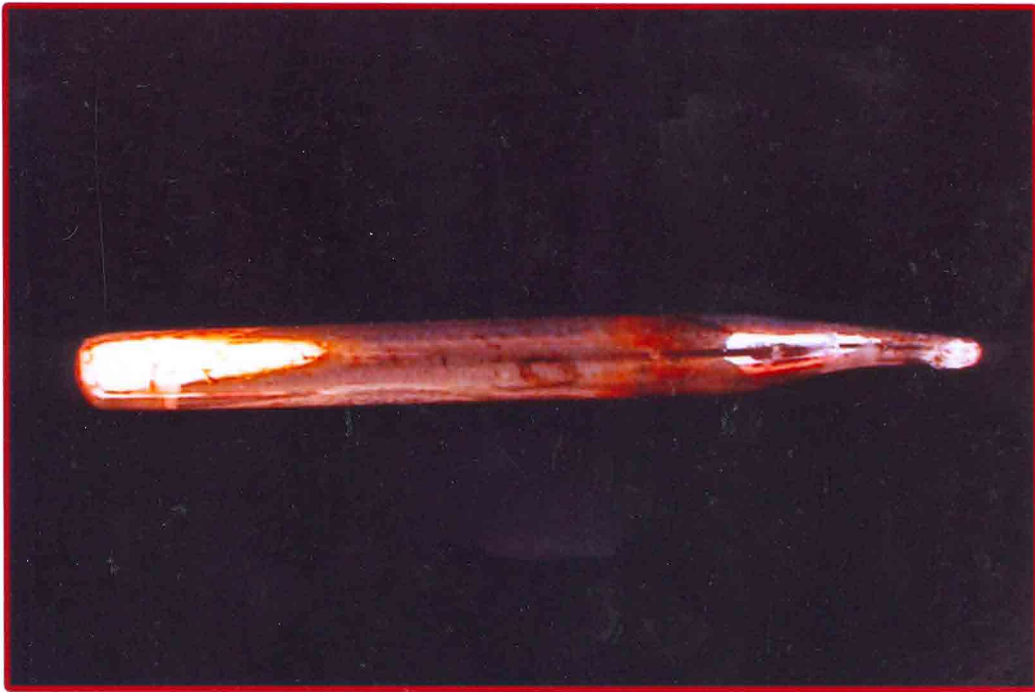


Plate 1 : *Lactobacillus acidophilus* culture

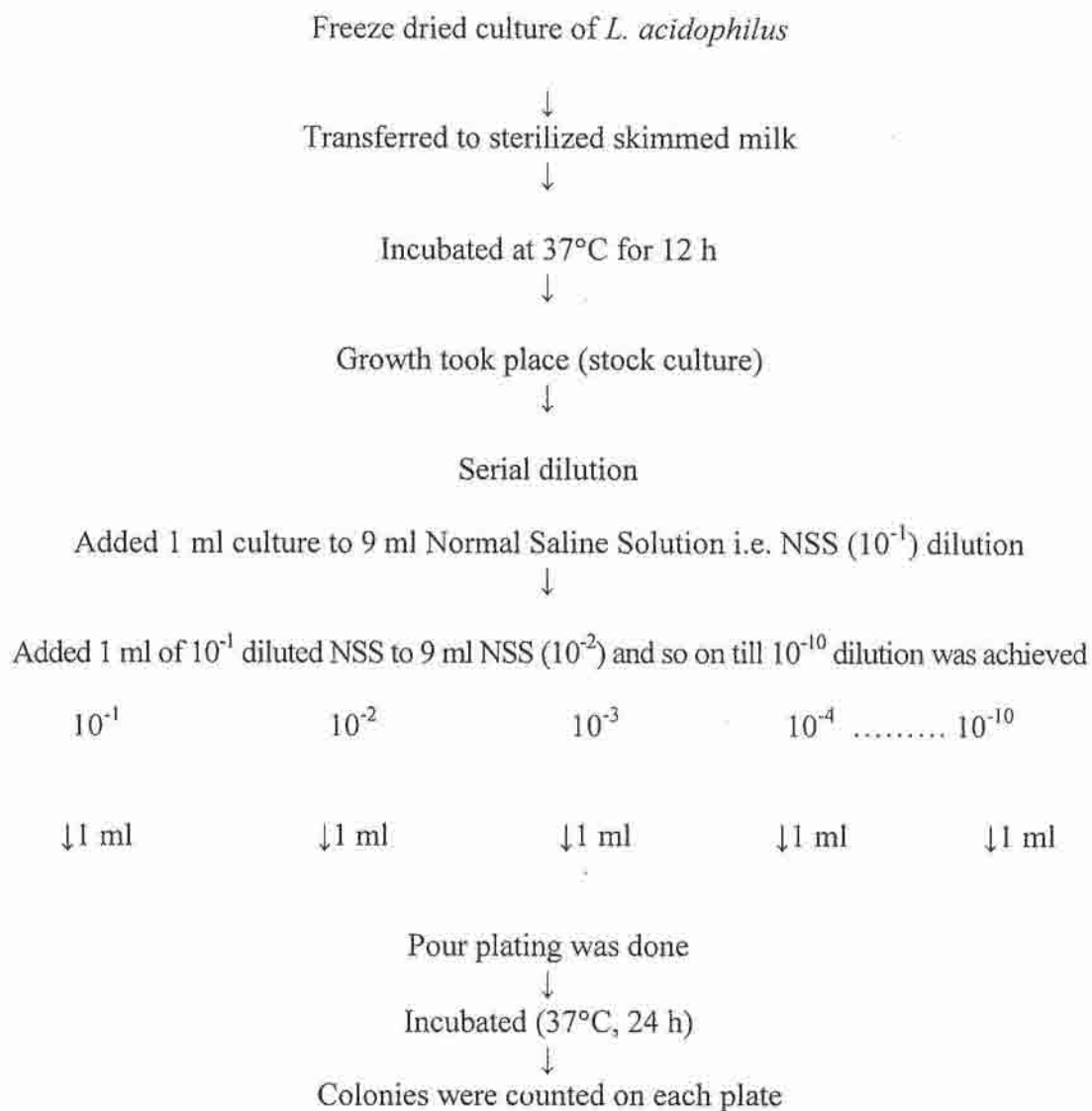
regularly at 2 to 4 weeks intervals. Activation of culture was done by three successive transfers at 24 h intervals in sterilized skimmed milk. The culture was further used as inoculum for preparation of curd.

3.2.2.1 Method used for counting of bacteria

The number of living bacteria or groups of bacteria in a liquid culture or suspension were counted by pour plate method.

Procedure

One ml bacterial suspension was added to nine ml sterile normal saline solution with a sterile one ml delivering pipette. Further dilutions upto 10^{-10} were made using a fresh pipette for each. One ml of each dilution was pour plated in sterilized petri plates. Then, about 10 ml of clear cultured MRS agar, melted and cooled to 45-50°C was poured into each dish and was mixed rapidly moving the plate in a combination of side-to-side and circular movements in different directions for about 10 seconds. These petri plates were incubated at 37°C for 24h. The colonies were counted by using colony counter (Khera instrument Pvt. Ltd., Model No.- KI-256) and multiplied by the dilution factor to obtain the viable count per ml in the original suspension (Fig. 3.1).

**Calculation:**

No. of colonies counted on plate	x	Dilution factor	=	No. of bacteria per ml
100	x	10^4	=	10^6 cells per ml

Fig. 3.1 Flow diagram showing the method used for counting bacteria

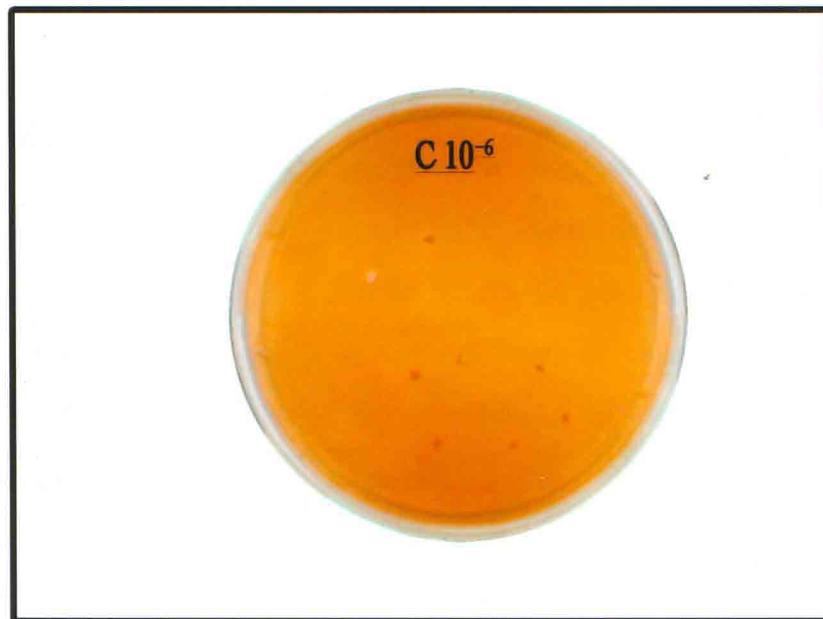
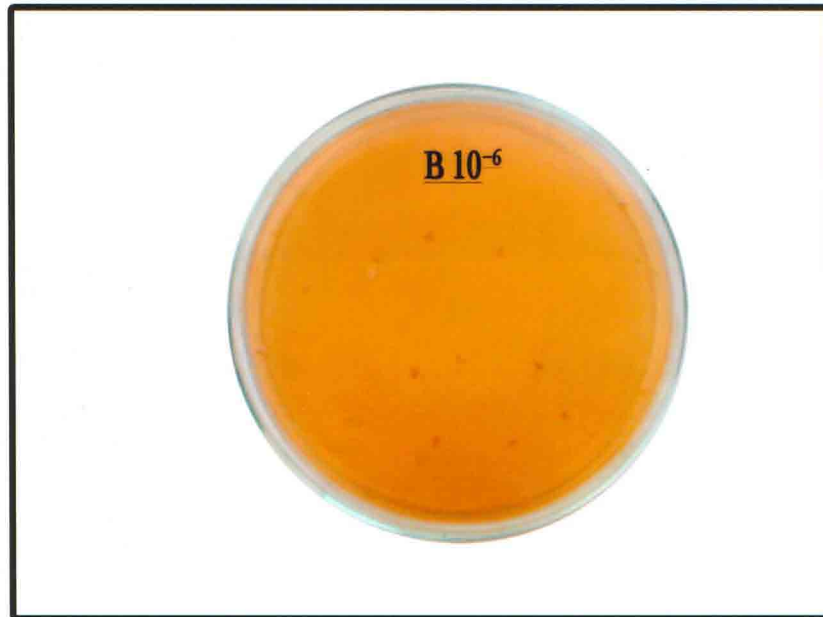
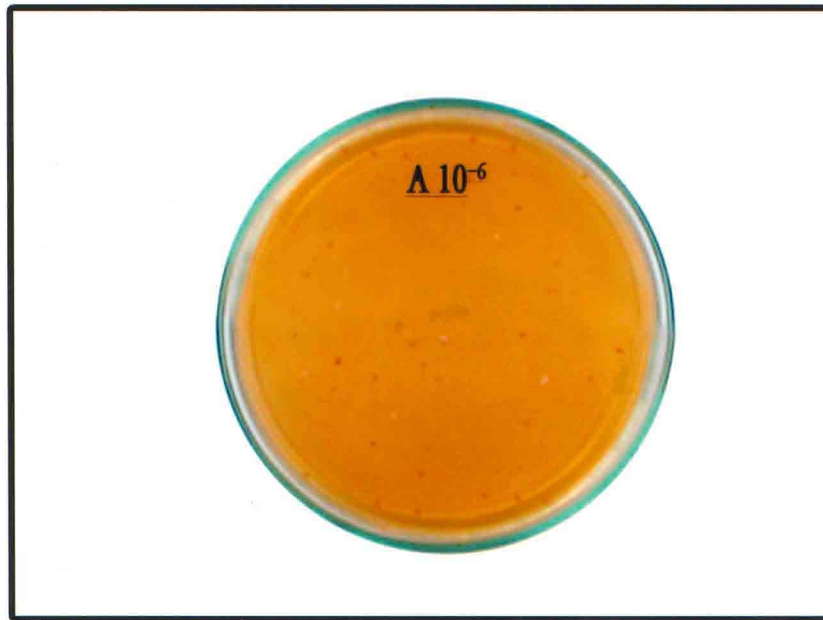


Plate 2 : *L. acidophilus* count

3.2.2.2 Preparation of inoculum

Took 100 ml fresh skimmed milk, autoclaved at 121°C, 1.5 kg/cm² for 15 min and cooled. Added 10⁴ dilution of stock culture in 100 ml sterilized milk to obtain 10⁶ cells/ml. Incubated at 37°C for 12h (Fig 3.2). This inoculum was used for preparation of probiotic curd.

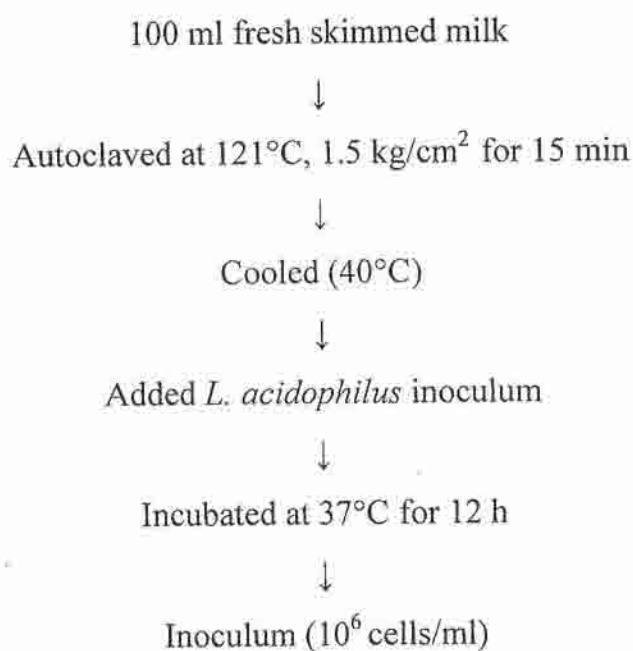


Fig 3.2. Preparation of *L. acidophilus* inoculum

3.2.2.3 Preparation of probiotic curd

Took 100 ml fresh skimmed milk, autoclaved at 121°C, at 1.5 kg/cm² for 15 min and cooled to 40°C. The inoculum prepared under section 3.2.2.2 was used in different proportions of 3, 5 and 10 ml in 100 ml sterilized milk. Fermentation was carried out at 37°C for 6 h. On the basis of organoleptic evaluation, five percent inoculum was selected for preparation of probiotic curd to carry out fermentation in developed food mixtures.

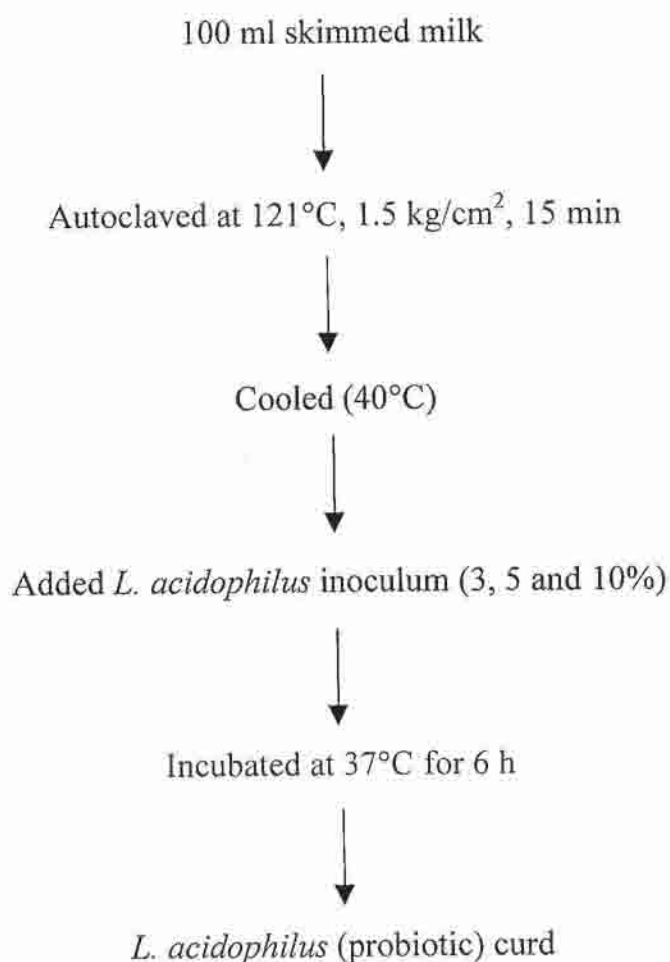


Fig. 3.3 Preparation of *L. acidophilus* curd

3.3 Preparation of indigenously developed probiotic fermented cereal food mixtures

3.3.1 Preparation of raw and germinated flour

Barley and pearl millet seeds were cleaned thoroughly and half of the raw seeds were ground in an electric grinding mill using 1.5 mm sieve size and rest of the seeds were soaked in distilled water for 12 h at room temperature. A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were germinated in sterile petri dishes lined with wet filter paper for 24 h at 37°C with frequent spraying of water. After 24 h, the sprouts were rinsed



Plate 3 : Grains

- I - Raw barley grain**
- II - Germinated barley grain**
- III - Raw pearl millet grain**
- IV - Germinated pearl millet grain**



Plate 4 : Flour

- I - Raw barley flour**
- II - Germinated barley flour**
- III - Raw pearl millet flour**
- IV - Germinated pearl millet flour**

in distilled water and then dried at 55-60°C. The dried samples of germinated seeds were ground to fine powder in an electric grinder and then stored in plastic containers for further use.

3.3.2 Development of food mixtures

Four types of food mixtures were developed using raw and germinated barley and pearl millet flour. The composition of the developed food mixtures was as follows:

- i) Raw barley flour + whey powder + tomato pulp (2:1:1, w/w)
- ii) Raw pearl millet flour + whey powder + tomato pulp (2:1:1, w/w)
- iii) Germinated barley flour + whey powder + tomato pulp (2:1:1, w/w)
- iv) Germinated pearl millet flour + whey powder + tomato pulp (2:1:1, w/w)

Freshly ground raw and germinated flour was mixed with tomato pulp and whey powder in the ratio as mentioned above. Addition of tomato pulp and whey powder in food mixtures provided a media for growth of organism and nutritional enhancement.

3.3.3 Probiotic fermentation of the developed food mixtures

Each of the developed four food mixtures (100 g) was mixed with distilled water (500 ml) (Fig. 3.4) to obtain a homogenous slurry which was subsequently autoclaved at 1.5 kg/cm² for 15 min at 121°C. The autoclaved slurry was cooled and then inoculated with probiotic curd which supplied 10⁶ cells/ml to the slurry to carry out fermentation at 37°C for 12 h in an incubator. The unfermented mixture slurries, before and after autoclaving served as controls.

3.4 Preparation of samples for nutritional analysis

At the end of the fermentation period i.e. 12 hours at 37°C, 100 ml fresh fermented slurry of each food mixtures was taken out for determination of moisture, titratable acidity, pH and *Lactobacilli* count. Rest of the fermented as well as unfermented slurries of different food mixtures were dried at 60°C to a constant weight. The oven dried samples were ground in an electric grinder mill



Plate 5 : Food mixtures

- I - Non-germinated, autoclaved and fermented BWT mixture**
- II - Germinated, autoclaved and fermented BWT mixture**
- III - Non-germinated, autoclaved and fermented PWT mixture**
- IV - Germinated, autoclaved and fermented PWT mixture**

using 1.5 mm sieve size to a fine powder and stored in air tight plastic containers for further nutritional analysis.

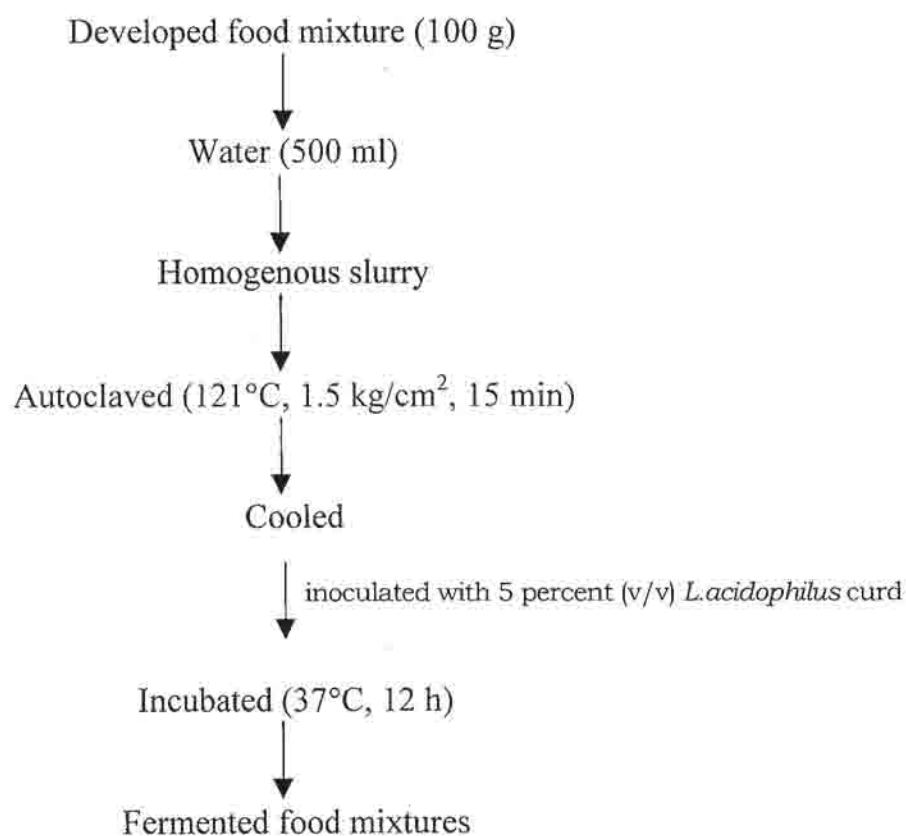


Fig. 3.4 Probiotic fermentation of the developed food mixture

3.5 Titratable acidity and pH

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

Reagents

- i) 0.1 N NaOH: 4 g NaOH was dissolved in water and volume made to one litre.
- ii) Phenolphthalein indicator (1%) in 80% ethyl alcohol

Procedure

Pipetted five ml fresh sample in 250 ml conical flask. Added about 75 ml boiling water and five drops of phenolphthalein indicator. Read the level of 0.1 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. 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 Enumeration of cell count in fermented food mixtures

L.acidophilus present in fermented food mixtures were enumerated using MRS medium. One gram fermented slurry was added to nine ml sterile normal saline solution. Further dilutions upto 10^{-10} were made. Each dilution (1 ml) was pour plated in sterilized petri plates, incubated at 37°C for 24 h and the colonies were counted as per method given in section 3.2.2.1.

3.7 Nutritional evaluation of fermented food products

3.7.1 Proximate composition

3.7.1.1 Moisture

Moisture in the samples was estimated employing the standard method (AOAC, 1995).

Procedure

Five g of fresh sample was weighed in a preweighed petridish and dried in oven at 105°C for 6 h. The sample was reweighed after cooling it in a dessicator.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100$$

3.7.1.2 Crude protein

Crude protein was estimated by standard method of AOAC (1995) using KEL PLUS Automatic Nitrogen Estimation System.

Reagents

- i) Hydrochloric acid (0.1 N)
- ii) Boric Acid : 4%
- iii) Sodium hydroxide : 40%

- iv) Mixed indicator: Bromocresol green solution (3 drops) + methyl red solution (5 drops)
- v) Digestion mixture: K_2SO_4 : $CuSO_4$ (5:1)

Procedure

Digestion :

Set the temperature to 420°C in the controller. Prepared the samples and chemicals. Loaded the tubes with samples + sulphuric acid + digestion mixture (3g) in insert rack. Then placed the manifold over the tubes. Loaded the insert rack + manifold in the digestion block. Opened the water connection. After 1 to 2 h, removed the rack and noted whether all the samples got digested. If not, replaced the tubes in the block and left it for 15 min. End point of digestion was appearance of bluish green colour and flames got reduced. After digestion, removed the insert rack from the block and placed it in the cooling stand. Removed it slowly after 15 min till the tubes got cooled. Then finally closed the water connection. Now the samples were ready for distillation.

Distillation Process

First loaded the macro tube containing digested sample in the space provided in apparatus. Put one empty conical flask on receiver side and ran the programme of the equipment. Boric acid (20 ml) automatically dropped into conical flask. This was pink in color initially. Then 40 ml of 40% NaOH in auto mode was added slowly in the order of 10 ml each time (till the colour in the test tube changed from bluish green to brown precipitate). Then set the process. After 6 min, the colour in the conical flask changed from pink to green. This was the end point of distillation of a sample. Took out the flask for titration.

Titration

Titrated the above solution with 0.1 N HCl till colour changed from green to permanent pale pink colour. This was the end point of titration.

$$\% \text{ of Nitrogen} = \frac{14 \times \text{Titrant value} \times \text{Normality of acid} \times 100}{1000 \times \text{sample weight}}$$

Titrant value = Volume of N/10 HCl used for titration.

3.7.1.3 Crude fat

Crude fat was estimated by standard method of analysis (AOAC, 1995) using the Automatic SOCS Plus Solvent Extraction System.

Procedure

Weighed amount (5 g) of moisture free sample was transferred to a preweighed extraction thimble, and dried overnight. The beaker was washed thoroughly and dried in hot air oven at 60°C and weighed after cooling. The thimble holder along with the sample was kept into the beaker. Took 100 ml of petroleum ether (boiling point 60-80°C) into the beaker. Loaded the beakers into the system and set required temperature 90°C (according to boiling point of solvent) in the controller. The extraction was carried out for one hour at 90°C. After the completion of extraction period, the temperature was raised at 110°C, closed the stopper in order to collect the solvent in the solvent compartment. Removed the beaker along with the fat and kept in hot air oven at 60°C temperature, till a constant weight was obtained. The beaker was weighed after cooling it in a dessicator.

$$\text{Fat (\%)} = \frac{W_2 - W_1 \times 100}{W}$$

where,

W = Weight of sample (g)

W₁ = Weight of empty beaker

W₂ = Weight of beaker with fat

3.7.1.4 Ash

Ash in the sample was estimated by employing the standard method of analysis (AOAC, 1995).

Procedure

Five g oven dried sample was weighed in a preweighed silica crucible. It was ignited till no charred particles remained in the crucible. The crucible was put in muffle furnace at 550⁰C for 5-6 h or till a white ash was obtained. The crucible was cooled in a dessicator and weighed. The loss in weight represented the organic matter and residue represented ash content.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

3.7.1.5 Crude fibre

Crude fibre in the sample was determined by employing the standard method of analysis (AOAC, 1995).

Reagents :

- i) Sulphuric acid stock solution : 10% (v/v)
- ii) Sulphuric acid working solution : 1.25%
- iii) Sodium hydroxide stock solution : 10% (w/v)
- iv) Sodium hydroxide working solution : 1.25%
- v) Antifoam: Two percent silicon antifoam in CCl₄

Procedure

Two g of fat free oven dried sample was weighed in one litre tall beaker containing 200 ml hot H₂SO₄ (1.25%) solution and a few drops of antifoam were added to it. Then it was heated to boiling in the crude fibre apparatus and kept the solution boiling for exactly 30 min under bulb condensers. Beaker was rotated occasionally to mix the contents and the particles were removed from the sides. The contents of the beaker were then filtered through Buchner funnel and then sample was washed back into the tall beaker with 200 ml NaOH (1.25%). It was again brought to boiling point and boiled for exactly 30 min. All the insoluble matter was transferred to a preweighed sintered crucible by means of boiling water till acid free. It was washed thrice with alcohol followed by washing three times

with acetone and dried at 100°C to constant weight. The crucible was put in a muffle furnace at 550°C for one h and then cooled in a dessicator and reweighed.

Calculation

$$\text{Crude fibre (\%)} = \frac{W_2 - W_3}{W_1} \times 100$$

Where,

W_1 = Wt. of sample (g)

W_2 = Wt. of insoluble matter (Wt. of crucible + insoluble matter
– wt. of crucible)

W_3 = Wt. of ash (Wt. of crucible + ash – weight of crucible)

3.7.2 Carbohydrates

3.7.2.1 Available

3.7.2.1.1 Total soluble sugars

Extraction

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

Twenty five ml ethanol (80%) was added to 0.5 g sample in a round bottom flask connected to a condenser and kept on a heating mantle for 30 min with occasional stirring. The extract was cooled, centrifuged at 8000 rpm for 15 min and supernatant was collected. The above procedure was repeated thrice, each time extracting the residue in 25 ml of 80 percent ethanol. The extract in beaker was evaporated to dryness on a boiling water bath. The residue was dissolved in distilled water and volume made to 50 ml.

Estimation

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

Reagents :

- i) Standard sugar solution : Twenty five mg glucose was dissolved in water and volume made to 100 ml. This solution contained 250 µg

glucose per ml. For obtaining a standard curve, different concentrations of this solution were used.

- ii) Anthrone reagent (0.2% anthrone in 70% H₂SO₄): The reagent was allowed to stand for 30-40 min with occasional shaking until it was perfectly clear. The reagent was freshly prepared each day and used within 12 hours.

Ten ml freshly prepared anthrone reagent was pipetted in test tube (150 x 25 mm) and chilled in ice cold water. One ml sugar extract was taken and layered on the acidic anthrone reagent. After cooling for further five min, the contents were thoroughly mixed while still immersed in ice cold water. The contents in the tube were heated vigorously in a boiling water bath for 10 min and then immediately cooled in cold water. The absorbance was then read at 625 nm in UV-VIS spectrophotometer against a suitable blank.

The amount of sugars was then determined by referring to a standard curve previously prepared with glucose.

3.7.2.1.2 Reducing sugars

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

Reagents :

- i) Copper reagent A : Twenty five g anhydrous sodium carbonate, 25 g potassium sodium tartarate, 20 g sodium bicarbonate and 200 g anhydrous sodium sulphate were dissolved in about 800 ml distilled water and diluted to one litre.
- ii) Copper reagent B : Fifteen gram copper sulphate was dissolved in 100 ml distilled water containing two drops of HCl.
- iii) Arsenomolybdate reagent (Nelson's reagent) : Twenty five g ammonium molybdate was dissolved in 450 ml distilled water by warming and 21 ml concentrated sulphuric acid. Three g sodium hydrogen arsenate was dissolved in 25 ml distilled water with stirring

and added to the above solution. The reagent was stored in a glass stoppered brown bottle and kept in an incubator at 37°C for 24 h before use.

- iv) Copper reagents A and B were mixed in the ratio of 25:1 (v/v) before use.
- v) Standard sugar solution : Twenty five mg glucose was dissolved and made to 100 ml with water. This contained 250 µg glucose per ml.

Estimation

One ml test extract was taken in blood sugar tube graduated at 25 ml. One ml mixed copper reagent (iv) was added and then heated for 24 min in a boiling water bath. To this, one ml of arseno-molybdate reagent was added, mixed thoroughly and the contents were diluted to 25 ml. A stable blue colour appeared quickly which was read at 520 nm in UV-VIS spectrophotometer against suitable blank (distilled water). The amount of reducing sugars was then determined by referring to the glucose standard curve.

3.7.2.1.3 Non-reducing sugars

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

3.7.2.1.4 Starch

Starch from the sugar free pellet was estimated by the method of Clegg (1956).

Extraction :

Five ml water was added to aforesaid residue of test material and while stirring, 6.5 ml of 52% perchloric acid was added. The contents were stirred with a glass rod continuously for five min and then occasionally for the next 15 min. To this, 20 ml of water was added and centrifuged at 8000 rpm for 15 min. The supernatant was settled in a 100 ml volumetric flask. Five ml of water was added to the residue and extraction was repeated with 52% perchloric acid, stirring occasionally for the next 30 min. The contents of the tube were washed into the

flask containing the first extract. The combined extracts were diluted to 100 ml with distilled water and filtered, discarding the first five ml of the filtrate. A suitable aliquot of the extract was used for glucose estimation, using anthrone reagent by the method of Yemm and Willis (1954). Starch was calculated by using the following formula:

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

3.7.2.2 Non available

3.7.2.2.1 Dietary fibre

Total, soluble and insoluble dietary fibre constituents were determined by the enzymatic method given by Furda (1981).

Reagents

- i) 0.005 N HCl
- ii) Phosphate buffer (pH 10)
- iii) EDTA
- iv) Enzymes – Alpha amylase and protease enzymes
- v) Ethanol (75% and absolute)
- vi) Acetone

Procedure

1. **Sample preparation:** 0.5 g sample of less than 1 mm particle size food material was defatted on a Soxhlet apparatus.
2. **Extraction of water-soluble material:** The prepared sample weighing about 2.0 g was dispersed in 200 ml of 0.005 N HCl and boiled for 20 min. The suspension was then cooled down to 60°C; 0.3 g of disodium EDTA was added and then adjusted to pH 5.0-6.5 with 12 ml of phosphate buffer pH 10. The extraction was continued for additional 40 min at 60°C to ensure the extraction of pectins with minimal degradation.
3. **Starch and protein hydrolysis:** Adjusted the pH 6.0-6.5 to bring the solution closer to the pH optimum of amylase and protease. Cooled the suspension to 20-30°C before incubation overnight with 10 mg of bacterial

alpha-amylase and 10 mg of bacterial protease. The incubation was accompanied by slow stirring with a magnetic bar.

4. **Isolation of insoluble dietary fibre (IDF):** The suspension was filtered through a coarse-tared Gooch filtering crucible containing glass wool and the insoluble residue was washed with a small amount of water. The filtrate was saved for the next step. The insoluble residue was then washed with water, alcohol and acetone before being dried at 70°C in a vacuum-oven overnight. The dried residue constitutes insoluble dietary fibre (IDF).
5. **Precipitation and isolation of soluble dietary fibre (SDF):** The saved filtrate was acidified with a few drops of concentrated hydrochloric acid to pH 2-3; this pH tended to facilitate the rapid precipitation of polysaccharides. Slowly added four volumes of ethanol and left suspension to stand for about 1 h. Filtered the precipitate on a tared, coarse Gooch crucible containing glass wool, then washed with 75% ethanol, absolute ethanol, and acetone before drying at 70°C in a vacuum oven overnight. The residue was weighed in the crucible to give the soluble dietary fibre (SDF) content of the original material. The SDF fraction was corrected for ash and for-co-precipitated protein.
6. **Total dietary fibre (TDF):** The sum of insoluble dietary fibre and soluble dietary fibre contents were calculated.

$$\text{TDF} = \text{IDF} + \text{SDF}$$

3.7.2.2.2 β -glucan

The total, soluble and insoluble β -glucan were estimated by the method of McCleary and Glennie Holmes (1985) and Megazyme Mixed Linkage β -glucan and glucose test kits were used.



Total β -glucan

Reagents

- (i) Sodium phosphate buffer (20 mM, pH 6.5): Sodium dihydrogen orthophosphate dihydrate (3.12 g) was dissolved in 900 ml of distilled water and the pH was adjusted to 6.5 by the addition of 100 mM sodium hydroxide (4 g/l). The volume was then adjusted to 1 litre. The buffer was stored at 4°C.
- (ii) Sodium acetate buffer (50 mM, pH 4.0): Glacial acetic acid (2.9 ml) was added to 900 ml distilled water and pH was adjusted to 4.0 using 1M sodium hydroxide. Volume was adjusted to 1 litre and stored at 4°C.
- (iii) Glucose oxidase/oxidase reagent (GOPOD): Glucose reagent buffer (stock) obtained from Megazyme glucose test kit contained 1M potassium dihydrogen orthophosphate 200 mM parahydroxybenzoic acid, 0.4% sodium azide and 4.2% sodium hydroxide. Fifty ml of the stock buffer (1 vial) was diluted to 1 litre with distilled water to get working glucose reagent buffer.
- (iv) Glucose reagent mixture: Dissolved contents of one vial of Megazyme glucose determination reagent (obtained from Megazyme glucose test kit) in 1 litre of working glucose reagent buffer. GOPOD reagent thus, obtained was stored in dark bottle at 4°C (Stable for about 3 months at 4°C).
- (v) Lichenase enzyme (50 U/ml in 20 mM phosphate buffer).
- (vi) β -glycosidase enzyme (2 U/ml in 50 mM sodium acetate, pH 4.0)

Procedure

Sample of 120 mg of known moisture content was taken in a test tube. To it, 1 ml aqueous ethanol (50%, v/v), 4 ml sodium phosphate buffer (20 mM, pH 6.5) were added and stirred the tube on an environ-mixer. The contents were then incubated for 60 seconds at 50°C in boiling water bath, mixed vigorously in

environ-mixer and incubated for 2 min in boiling water bath at 100°C. After cooling the contents to 40°C, 0.2 ml of lichenase was added. The tube was capped and incubated at 50°C for 1 h. Added sodium acetate buffer (5.0 ml, 200 mM, pH 4.0) and mixed well. Then, equilibrated the tubes and centrifuged (1000 rpm, 10 min). 0.1 ml of this aliquot was transferred to three test tubes. To one tube, 0.1 ml of acetate buffer (50 mM, pH 4.0) was added (the blank), while to the other two, 0.1 ml of β -glucosidase was added. The tubes were incubated at 50°C for 10 min. Thereafter, 3 ml of GOPOD reagent was added to each tube and then the tubes were again incubated at 50°C for 20 min. The absorbance was read at 510 nm. With each set of determination, glucose standard of 100 μ g and one standard barley flour was also included.

Calculations

$$\begin{aligned} \beta\text{-glucan (\% w/w)} &= \Delta A \times F \times 94 \text{ (or 64)} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} \\ &= \Delta A \times \frac{F}{W} \times 8.46 \text{ (or 5.76)} \end{aligned}$$

Where,

- ΔA = Absorbance after β -glucosidase treatment (reaction) minus reaction blank absorbance
- F = A factor for the conversion of absorbance values to μ g of glucose
 = $\frac{100 \text{ (\mu g of D-glucose)}}{\text{absorbance of 100 } \mu\text{g of D-glucose}}$
- 94 = Volume correction factor (0.1 mL out of 9.4 mL was analysed for cereal samples)
- 64 = Volume correction factor (0.1 mL out of 6.4 mL was analysed for cooked, toasted and extruded cereal products)

$\frac{1}{1000}$	=	Conversion from μg to mg
$\frac{100}{W}$	=	Factor to express β -glucan content as a percentage of dry flour weight
W	=	The calculated dry weight of the sample analysed in mg
$\frac{162}{180}$	=	A factor to convert from free D-glucose, as determined to anhydro-D-glucose, as occurs in β -glucan

Soluble β -glucan

Reagents

As used for total β -glucan estimation.

Procedure

A sample (500 mg) of known moisture content was taken in a test tube, to which 15 ml of distilled water and 5 ml of sodium phosphate buffer (20 mM, pH 6.5) were added. Rest of the procedure was same as for total β -glucan content estimation.

Insoluble β -glucan

The difference between total β -glucan and soluble β -glucan content was calculated as insoluble β -glucan.

3.7.3 B-complex vitamins

3.7.3.1 Thiamine

Thiamine was determined by Fluorometric method of AOAC (1995).

Reagents

- i) Acid KCl solution: 125 g KCl was dissolved in H_2O . To it, added 4.3 ml conc. HCl and diluted to 500 ml with H_2O .

- ii) Sodium hydroxide solution (15%): Dissolved 37.5 g of NaOH in water to make 250 ml.
- iii) Potassium ferricyanide solution (1%): Dissolved 1 g $K_3Fe(CN)_6$ in H_2O to make 100 ml.
- iv) Oxidizing reagents: Mixed 4 ml of 1% $K_3Fe(CN)_6$ with 15% NaOH solution to make 100 ml. Used within 4 hours.
- v) Iso-butyl alcohol: Redistill in glass apparatus.
- vi) Quinine sulphate stock solution: Dissolved 10 mg quinine sulphate in 0.1 N H_2SO_4 to make 1 lit. Stored in amber coloured bottle.
- vii) Quinine sulphate standard solution: Diluted 1 ml of quinine sulphate stock with 39 vol of 0.1 N H_2SO_4 . Stored in amber coloured bottle.
- viii) Thiamine HCl standard solution
 - (a) Stock solution (100 $\mu g/ml$): Fifty ml of thiamine hydrochloride was dissolved in 20% ethanol, adjusted pH 3.5-4.3 with HCl and diluted to 500 ml with the acidified alcohol.
 - (b) Intermediate solution (10 $\mu g/ml$): Diluted 10 ml stock solution to 100 ml with acidified alcohol. Stored at 10°C in coloured bottle.
 - (c) Working solution (1 $\mu g/ml$): To 10 ml intermediate, added 50 ml 0.1 N HCl and digested in autoclave for 30min under 15 lb pressure. Then diluted to 100 ml with 0.1 N HCl to 100 ml.

Procedure

Extraction

Hydrolysis

Weighed 2.5 g of dried and well ground sample and added 25 ml 0.1 N HCl and shaken well. The sample was washed from the sides of flask and autoclaved for 30 min under 15 lb pressure. Cooled, filtered and diluted to 100 ml with 0.1 N HCl.

Oxidation of thiamine to thiochrome

For standard, added 1.5 g NaCl and 5 ml working standard solution to 4 tubes (cap. 40 ml) and mixed. Added 3 ml oxidizing reagent to two tubes and shaken vigorously. To two remaining tubes, added 3 ml 15% NaOH solution similarly (blank). For sample, added 1.5 g NaCl and 5 ml sample aliquot to another set of four tubes and mixed. Added 3 ml oxidizing reagent to two tubes and to other two tubes added 3 ml 15% NaOH (blank). To all tubes, added 13 ml iso-butanol and shaken over whirl-mixer for two min. Pipetted 10 ml of clear supernatant and measured fluorescence of each tube with input T range 865 nm and output T range 435 nm. Standard quinine sulphate was used to govern the reproducibility of fluorometer.

Calculations:

$$\text{Thiamine (mg/100 g)} = \frac{(I - b) \times V}{(S - D) \times 1000 \times W} \times 100$$

Where

W = Weight (g) of sample

V = Volume of aliquot made

S = Reading of standard

D = Reading of standard blank

I = Reading of sample

b = Reading of sample blank

Conc. of standard thiamine working solution = 1 µg/ml

3.7.3.2 Riboflavin

Riboflavin was determined by Fluorometric method of AOAC (1995).

Reagents

- i) HCl (0.1 N): 4.5 ml of conc. HCl was diluted to 500 ml with H₂O.
- ii) KMnO₄ (4%): Dissolved 4 g of KmNO₄ in water to make 100 ml.

- iii) H_2O_2 (3%): Diluted 10 ml of 30% H_2O_2 to 100 ml with water.
- iv) Acetic acid (0.02 N): Diluted 1.1 ml glacial acetic acid to one litre with water.
- v) Riboflavin standard
 - a) Standard solution (100 $\mu\text{g}/\text{ml}$): Fifty mg of riboflavin was dissolved in sufficient 0.02 N acetic acid on steam bath with constant stirring until dissolved. Cooled and added 0.02 N acetic acid to make 500 ml.
 - b) Intermediate solution (10 $\mu\text{g}/\text{ml}$): Diluted 10 ml of stock solution to 100 ml with 0.02 N acetic acid. Stored under toluence at 10°C .
 - c) Working solution (0.1 $\mu\text{g}/\text{ml}$): Diluted 10 ml intermediate solution to 1 litre with water avoiding exposure to strong light.
 - d) Sodium hydrosulfite: $\text{Na}_2\text{S}_2\text{O}_6 \cdot 2\text{H}_2\text{O}$ solid.

Procedure

Extraction

Weighed 2.5 g sample (estimated to contain not less than 10 μg of riboflavin) into a 100 ml conical flask and added 25 ml 0.1 N HCl. Covered the flask with inverted beaker. Autoclaved the sample for 15 lb for 30 min. Cooled the sample, washed into a 100 ml beaker. The pH of the sample solution was set to 6.8 with 40% NaOH. Now added immediately HCl dropwise, whilst stirring to bring pH to 4.5. Transferred the extract to a 100 ml volumetric flask and diluted to 100 ml with water. Filtered the extract with a fast filter paper.

Decolourising the sample

Ten ml aliquot of the sample solution was pipetted into four 25 ml test tubes. Added 2 ml standard riboflavin working solution (0.1 $\mu\text{g}/\text{ml}$) to two tubes. Added 2 ml H_2O to other two tubes. Added 2 ml glacial acetic acid to each tube and mixed. Added 0.5 ml of 4% KMnO_4 and mixed. After 2 min, added 0.5 ml 3% H_2O_2 .

Measurement of fluorescence

Adjusted the fluorometer to give a deflection of 0 against 0.1 N H₂SO₄ and 100 against the sample + standard solution using wavelength 525 nm. The fluorescence of each tube was measured in turn. Then 20 mg of sodium dithionite was added to each tube in turn and mixed. The blank value was read within 10 seconds.

Calculation:

$$\text{Riboflavin (mg/100 g)} = \frac{B - C}{X - B} \times \frac{0.1 \times 0.001 \times V}{U \times W}$$

Where,

W = Weight of sample

V = Volume of extract (100 ml)

U = Aliquot taken for decolorising (10 ml)

X = Reading of sample + standard

B = Reading of sample with water

C = Reading of sample blank

3.7.3.3 Niacin

Niacin contents were estimated according to method using HPLC.

Processing of sample

Five gram of ground sample was taken in 100 ml volumetric flask and 50 ml of 0.1 N H₂SO₄ was added. The contents were autoclaved for 15 min in a pressure cooker. After cooling, the pH of the contents was adjusted to 4.5 with 2 M Sodium acetate. Five ml of 6% Takadiastase and 5 ml Papain (10%) were added and the contents were incubated overnight at 35 ± 1°C. The contents were filtered through Whatman no. 40 filter paper and diluted the contents to 100 ml in a volumetric flask with HPLC water. Samples were filtered through 0.25 mm filter with 0.5 micron porosity.

Mobile phase

The mobile phase was prepared by following method:

Added 2.2 gram heptane sulphuric acid sodium salt and 100 mg EDTA to 1,500 ml HPLC water, followed by 48 ml glacial acetic acid and 10 ml triethylamine. The contents were mixed and pH was adjusted to 3.6 with acetic acid or triethylamine. The solution was diluted to 1,700 ml with HPLC water, followed by 300 ml methanol (HPLC). The solution was filtered and degassed before use.

Injection of sample

Twenty micron of sample was injected through injection port. The flow rate of eluent was 0.8 ml per minute. The absorbance was measured at 254 nm by a tunable absorbance detector. Twenty micron of standard was injected for identification and quantitative estimation of niacin and measured graphically.

3.7.4 Protein fractions

Different fractions viz., albumin (water soluble), globulin (salt soluble), prolamin (alcohol soluble) and glutelin (alkali soluble) were determined by the method of Osborne (1907) as modified by Naik (1968).

Procedure:

Water extract of the flour was prepared by shaking the flour with distilled water for 1.5 h in a shaker. A flour to solvent ratio of 1:5 (w/v) was used. The suspension was centrifuged at 3000 rpm for 15 min and the clear supernatant was collected. The extraction was repeated twice and the supernatant pooled. It represents albumin fraction.

Salt soluble proteins were prepared in the similar manner by extracting the residue (left after extracting albumin fraction) with 1% w/v NaCl solution.

The alcohol soluble prolamin fraction was prepared by intermittently shaking the residue left behind after the extraction of globulin with 60% ethanol at

60°C for 2 h. The supernatant were collected after centrifugation. The residue obtained after prolamin extraction was extracted with 0.4% NaOH solution by shaking for 2 h and centrifuged at 3000 rpm for 15 min. The supernatant collected after repeated washing of residue with 0.4% NaOH represent the glutelin fraction of wheat protein. The N content in protein fractions was determined using KEL PLUS Automatic Nitrogen Estimation System (AOAC, 1995).

3.7.5 Total lysine

Total lysine was estimated as per the method described by Miyahara and Jikoo (1967).

Hydrolysis

One g defatted sample was transferred to 100 ml conical flask, added 12.5 ml of 2 N HCl and mixed well. Then it was autoclaved at 15 kg/cm² for 1 h. Added a pinch of activated charcoal to hydrolysate, heated to boiling and filtered after washing with hot water. The colour free hydrolysate was made to 50 ml.

Reagents

- a) Citrate phosphate buffer (pH 5.6)
 - A) 0.1 M solution of citric acid (19.21 g in 1000 ml)
 - B) 0.2 M dibasic sodium phosphate (53.65 g of Na₂HPO₄.7H₂O in 1000 ml).
Mixed 21 ml of A) + 29 ml of B).
- b) Bromine saturated aqueous solution: It was prepared by adding 3 ml of bromine to 150 ml of water and mixed well.
- c) Aqueous solution of arsenite: Three g of arsenite trioxide was dissolved in 10 ml of 10% aqueous solution of sodium hydroxide and the resultant solution was diluted to 100 ml with distilled water.
- d) 20% aqueous solution of Na₂CO₃
- e) Folin phenol reagent 1 N: Diluted before use from stock 2 N
- f) Standard lysine

Procedure

Two ml of acid hydrolysate which had been adjusted to pH 5.6 using dilute HCl or NaOH, was taken in a test tube and after the addition of 1 ml of buffer and 1 ml of methanol, it was allowed to stand at -10°C for about 15 min in iced water. Two drops of saturated bromine solution were added, and the mixture was allowed to stand again for 20 min at the same temperature. After that, 0.1 ml of arsenite was added to the mixture to decompose excess of bromine and the mixture was incubated in a water bath at 60°C for 10 min. To the contents in the test tubes, added 1.5 ml of 20% aqueous Na_2CO_3 which had already been warmed to 60°C . After addition of 0.5 ml of F.C. reagent, the mixture was shaken quickly and then allowed to stand at room temperature for 30 min. The intensity of blue colour was measured spectrophotometrically. The results were presented as g lysine/100g protein after taking into account dilution involved. The range of lysine used for standard curve was 20 to 100 μg .

3.7.6 Antinutritional factors

3.7.6.1 Phytic acid

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

Reagents

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

Extraction

Finely ground sample (0.5 g) was extracted with 25 ml of 0.2 N HCl for 3 h with continuous shaking in a shaker. There after, it was filtered through Whatman No.1 filter paper.

Procedure

An aliquot (0.5 ml) of the above extract was pipetted into a test tube fitted with a ground glass stopper. One ml ferric ammonium sulphate solution was added. The tube was heated in a boiling water bath for 30 min. The contents of the tube were mixed and centrifuged at 3000 rpm for 30 min. One ml of supernatant was transferred to another test tube and 1.5 ml bipyridine solution was added. The absorbance was measured at 519 nm against distilled water. For plotting a standard curve, different concentrations (0.2 to 1.0 ml) of standard sodium phytate solution containing 40-240 µg phytic acid were taken and made to 1.4 ml with water.

3.7.6.2 Polyphenols

Total polyphenols were extracted by the method of Singh and Jambunathan (1981).

Extraction

Defatted sample (500 mg) was refluxed with 50 ml methanol containing 1% HCl for 4 h. 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 distilled water and refluxed for 2 h, cooled and diluted it to one litre.
- ii) Tannic acid solution: Dissolved 100 mg tannic acid in distilled water and made upto one litre. Twenty ml of this stock solution was further diluted to

100 ml with water to give working standard solution containing 20 μg tannic acid per ml.

- iii) Saturated sodium carbonate solution: Dissolved 350 g sodium carbonate in hot distilled water (70°C to 80°C), cooled and filtered through glass wool to make one litre volume.

Procedure

Test solution (1.5 ml) was diluted with water to 8.5 ml in a graduated test tube. After thorough mixing, added 0.5 ml Folin-Denis reagent and the tubes were well shaken. Exactly after 3 min, one ml of saturated sodium carbonate solution was added and the tubes were thoroughly shaken again. After an hour, 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.

3.7.6.3 Amylase inhibitor activity

Amylase inhibitor activity was determined by following modified method of Bernfeld (1955).

Reagents

- i) 0.1M Phosphate buffer (pH 7.6): Sixteen ml NaH_2PO_4 (0.2M) and 84 ml Na_2HPO_4 (0.2M) were diluted to 200 ml with water and adjusted pH to 7.6.
- ii) 0.02M phosphate buffer (pH 6.9): Fifty ml 0.02M potassium dihydrogen phosphate was added to 46.8 ml of 0.2M disodium hydrogen phosphate and volume was made upto 1000 ml.
- iii) 1% starch: One g starch was dissolved in 100 ml 0.02M phosphate buffer solution.
- iv) Alpha-amylase solution (5 mg/ml, Sigma, USA): Exactly 250 mg pancreatic alpha amylase was dissolved in 50 ml 0.02M buffer (pH 6.9).

- V) 0.25M NaCl solution: NaCl (1.462 g) was dissolved in water and made volume upto 100 ml.

Extraction

Amylase inhibitor was extracted by shaking a finely ground defatted sample (0.1 g) in 10 ml 0.02M phosphate buffer (pH 6.9) for 2 h at room temperature. The suspension was then centrifuged at 10,000 rpm for 15 min. Supernatant was heated for 10 min at 70°C and it was tested for amylase inhibitor activity by carrying out incubation in the following sequence.

Assay

	Test	Control
1% starch	1.0 ml	1.0 ml
Inhibitor extract	0.5 ml	Nil
0.1M phosphate buffer (pH 7.6)	2.0 ml	2.5 ml
Enzyme solution	0.5 ml	0.5 ml
NaCl (0.25M)	1.0 ml	1.0 ml

Contents were incubated at 37°C for 20 min. The extract (0.5 ml) was diluted to 5ml with distilled water. Maltose was determined in 2 ml of diluted extract as well as incubation mixture according to procedure discussed under 3.9.4.

Amylase inhibitor unit: One unit of amylase was defined as the amount of enzyme which converted one mg starch to maltose for 20 min at 37°C at pH 7.6. One unit of inhibitory activity is that which reduces the activity of amylase by one unit under assay condition.

3.7.7 *In vitro* digestibility

3.7.7.1 *In vitro* protein digestibility

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

Reagents

- i) Pepsin: Dissolved 80 mg pepsin (3000 units) in HCl (pH 2.0) prepared by diluting the acid with water and made upto 100 ml.
- ii) 10% and 5% Trichloroacetic acid (TCA)
- iii) 0.1 M Borate buffer (pH 6.8)
 - a) 0.2 M Boric acid: Dissolved 12.4 g boric acid in distilled water and made the volume to one litre.
 - b) 0.05 M Borax solution: Dissolved 19.05 g borax in distilled water and made the volume to one litre. For preparing the borate buffer, added 140 ml of 0.2 M boric acid, 50 ml distilled water and few drops of 0.05 M borax solution to adjust the pH at 6.8.
- iv) 0.1 M 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.
- v) 0.2 N NaOH
- vi) Pancreatin: Dissolved 50 mg pancreatin (Sigma Chemical Company, USA) in 100 ml of 0.1 M borate buffer (pH 6.8) containing calcium chloride.

Procedure

Two hundred mg ground sample was taken in a 50 ml conical flask. Added 5 ml of 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 flask 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 two ml pancreatin solution was added to each flask and incubated at 37°C in a water bath shaker for 24 h. Then seven ml of 10% TCA was added. The contents were centrifuged at 12,000 rpm for 20 min and the residue was 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 were determined using KEL PLUS Automatic Nitrogen Estimation System (AOAC, 1995). The protein of the sample was determined by multiplying N content by a

factor 5.70. The digested protein of the sample was determined by subtracting the residual protein from the total protein of sample. Protein digestibility was calculated by the following formula:

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

3.7.7.2 *In vitro* starch digestibility

In vitro starch digestibility was assessed by following the method of Singh *et al.* (1982).

Reagents

- i) 0.2 M Phosphate buffer (pH 6.9): Fifty ml of 0.2 M (27.28 g/lit.) potassium dihydrogen phosphate was added to 46.8 ml of 0.2 M (35.98 g/lit.) disodium hydrogen phosphate and made upto 200 ml with water.
- ii) Pancreatic amylase: Twenty mg pancreatic amylase (Sigma Chemical Company, USA) was dissolved in 50 ml of 0.2 M phosphate buffer (pH 6.9).
- iii) Dinitrosalicylic reagent: Dissolved 10 g of 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.

Procedure

Twenty-five mg of defatted sample was dispersed in one ml of 0.2 M phosphate buffer (pH 6.9). Added 0.5 ml of pancreatic amylase to sample suspension and incubated in water bath at 37°C for 2 h. After the incubation period, 3 ml of dinitrosalicylic acid reagent was quickly added and mixture was heated in a boiling water bath for 5 min. 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 while 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. O.D. of 0.299 corresponded to 5 mg maltose.

$$\text{Starch digestibility (\%)} = \frac{M \times V \times 100}{W \times V_1 \times 1000}$$

Where,

W = Weight of sample taken (g)

V = Volume of extract made (ml)

V₁ = Volume of extract aliquot taken (ml)

M = Concentration of extract elute obtained from graph

3.7.8 Minerals

3.7.8.1 Total minerals

Acid digestion

To one g ground sample in a 150 ml conical flask, 25-30 ml of diacid mixture (HNO₃:HClO₄::5:1, v/v) was added and kept overnight. The contents were digested by heating till clear white precipitates settled down at the bottom. The crystals were filtered through Whatman No. 42 filter paper. The filtrate was made to 50 ml volume with double distilled water and used for determination of total Ca, Fe, and Zn.

Calcium, Iron and zinc

Calcium, iron and zinc in acid digested samples were determined by Atomic Absorption Spectrophotometer 2380, PERKIN-ELMER (USA) according to the method of Lindsey and Norwell (1969).

$$\text{Minerals (mg/100 g)} = \frac{\text{Reading (conc. mg/ml)} \times \text{Vol. made} \times 100}{\text{Weight of sample (g)} \times 100}$$

3.7.8.2 Availability (*in vitro*) of minerals

3.7.8.2.1 Calcium and zinc availability (*in vitro*)

Available calcium and zinc were extracted by the method of Kim and Zemel (1986).

Reagents

- i) 0.1% Pepsin in 0.1 N HCl
- ii) HCl
- iii) NaHCO₃
- iv) 0.5% pancreatin in 5% bile

Procedure

Two g of finely ground sample was taken in a conical flask and 3 ml distilled water was added to rehydrate it. To this 20 ml of pepsin solution (0.1% pepsin in 0.1 N HCl) was added. The pH was adjusted to 1.5 with dilute HCl. The contents were incubated at 37°C in a shaker-cum-water bath for an hour. Therefore, the pH of the contents was raised to 6.8 with sodium bicarbonate solution. Then 2.5 ml of a suspension containing 0.5% pancreatin in 5% bile were added and the contents were again incubated at 37°C for an hour. Contents were then immediately centrifuged at 1000 rpm for 45 min at 5°C. Supernatants were collected and re-centrifuged at 50000 rpm for 90 min at 5°C. The supernatant was collected, oven dried, digested in the diacid mixture and proceeded for the estimation of calcium and zinc by the atomic absorption spectrophotometric method.

3.7.8.2.2 Iron availability (*in vitro*)

Extraction

Ionizable iron in the samples was extracted according to the procedure of Rao and Prabhavathi (1978).

Two g sample was mixed with 25 ml pepsin HCl (0.5% pepsin in 0.1N HCl) in a conical flask. The pH of the mixture was adjusted to 1.35 with HCl and incubated at 37°C for 90 min in an environmental shaker. After incubation, pH of the contents was adjusted to 7.5 with NaOH and again incubated at 37°C in an environmental shaker for 90 min. Contents of the flasks were centrifuged at 9000 rpm for 30 min and the supernatant was filtered through Whatman No. 44 filter paper. The filtrate was used for determination of ionizable iron.

Ionizable iron

Free form of iron in the filtrate which reacts with α' , α' -dipyridyl was determined as described by AOAC (1995).

Reagents

- i) α' , α' -dipyridyl solution: Dissolved 0.1 g dipyridyl in water and made the volume to 100 ml.
- ii) Hydroxylamine hydrochloride solution (10%)
- iii) Acetate buffer solution: Dissolved 8.3 g anhydrous sodium acetate (dried at 100°C) in water, added 12 ml acetic acid and made the volume to 100 ml with water.
- iv) HCl
- v) Iron standard solution (0.01 mg iron/ml): Dissolved 3.512 g Fe $(\text{NH}_4)_2\text{H}_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ in water, added two drops of HCl and made to 500 ml with water. Ten ml of the solution was further diluted with water and made to 500 ml. This solution contained 0.01 mg iron per ml.

Procedure

Ten ml filtrate was taken in 25 ml volumetric flask and one ml 10% hydroxylamine hydrochloride solution was added. Then five ml acetate buffer solution was added. The contents were mixed and then one ml dipyridyl solution was added. The volume was made to 25 ml with water and the contents were mixed well. The colour intensity was read at 510 nm.

For plotting a standard curve, 10 to 50 ml of iron standard were taken in 100 ml volumetric flask, added 2.0 ml of HCl to each and made the volume to 100 ml with water. Blank was also prepared in similar manner. Ten ml of each of these solutions were taken in 25 ml volumetric flask and proceeded as mentioned above.

3.8 Development of food products from probiotic fermented food mixtures and their organoleptic evaluation

3.8.1 Development of fermented food products

Following fermented food mixture slurries were used for preparation of the products given below and composition of these mixtures are mentioned under section 3.3.2.

BWT I	Fermented mixture containing raw barley flour, whey powder and tomato pulp (2:1:1)
BWT II	Fermented mixture containing germinated barley flour, whey powder and tomato pulp (2:1:1)
PWT I	Fermented mixture containing raw pearl millet flour, whey powder and tomato pulp (2:1:1)
PWT II	Fermented mixture containing germinated pearl millet flour, whey powder and tomato pulp (2:1:1)

3.8.1.1 Buttermilk

Two types of buttermilks were prepared.

Ingredients

	Control buttermilk	Probiotic buttermilk
Curd	150 g	-
Probiotic curd	-	150 g
Salt	to taste	to taste
Cumin powder	a pinch	a pinch
Water	100 ml	100 ml

Method

Control and probiotic curd were mixed with water to obtain desirable consistency. Added salt, cumin powder and served chilled.

3.8.1.2 *Kadhi*

Five types of *kadhies* were prepared from probiotic fermented food mixtures.

Ingredients

	Control	BWT I	BWT II	PWT I	PWT II
Bengal gram flour	25 g	-	-	-	-
Dahi	120 ml	-	-	-	-
Probiotic curd	-	120ml	120ml	120ml	120ml
Garlic paste	5 g	5 g	5 g	5 g	5 g
Onion paste	45 g	45 g	45 g	45 g	45 g
Turmeric powder	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Refined oil	10 ml	10 ml	10 ml	10 ml	10 ml
Salt	to taste	to taste	to taste	to taste	to taste
BWT I	-	120 ml	-	-	-
BWT II	-	-	120 ml	-	-
PWT I	-	-	-	120 ml	-
PWT II	-	-	-	-	120 ml

Method

- For control *kadhi*, dahi and water were mixed with besan with constant stirring till a homogenous slurry was obtained.
- Similarly, other experimental slurries were mixed with probiotic curd and water to obtain desirable consistency.
- Rest of the procedure given below is same for control and experimental *kadhi*.
- Chopped onion and garlic paste were fried till light brown. Added aforementioned slurries to the above paste slowly with constant stirring.
- Then turmeric powder, chilli powder and salt were added according to taste.

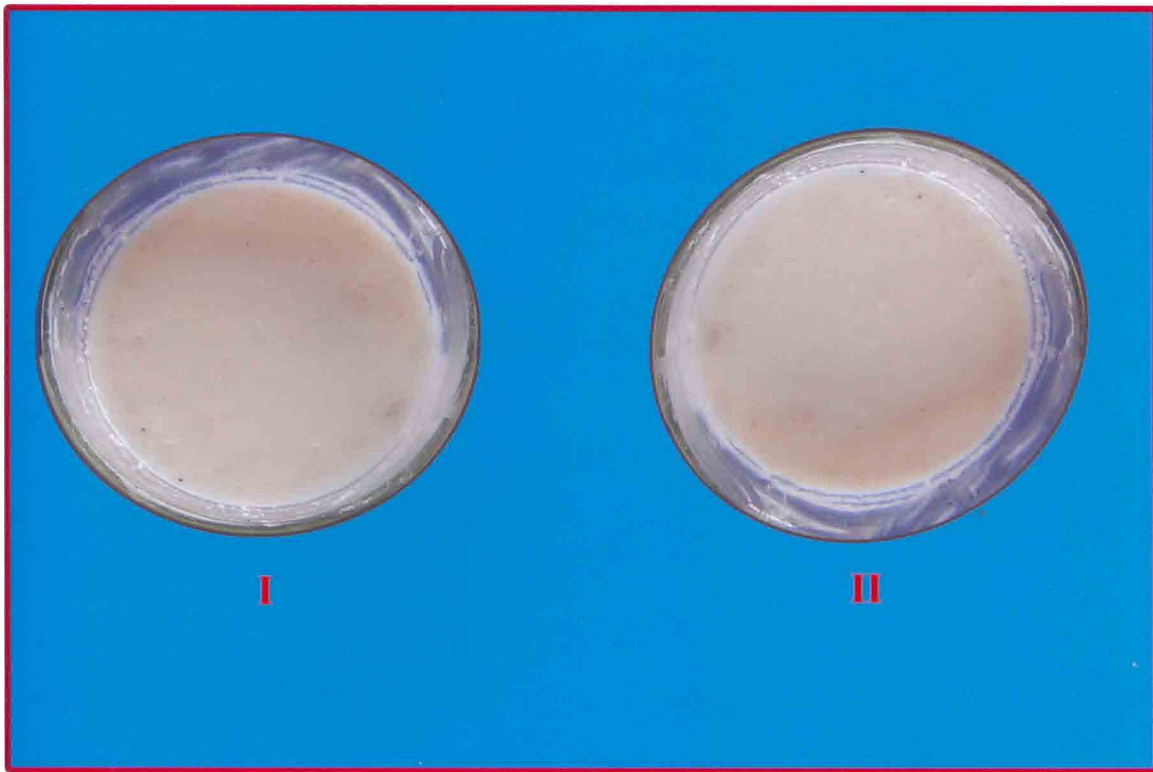


Plate 6 : Buttermilk

- I - Control buttermilk
- II - Probiotic buttermilk

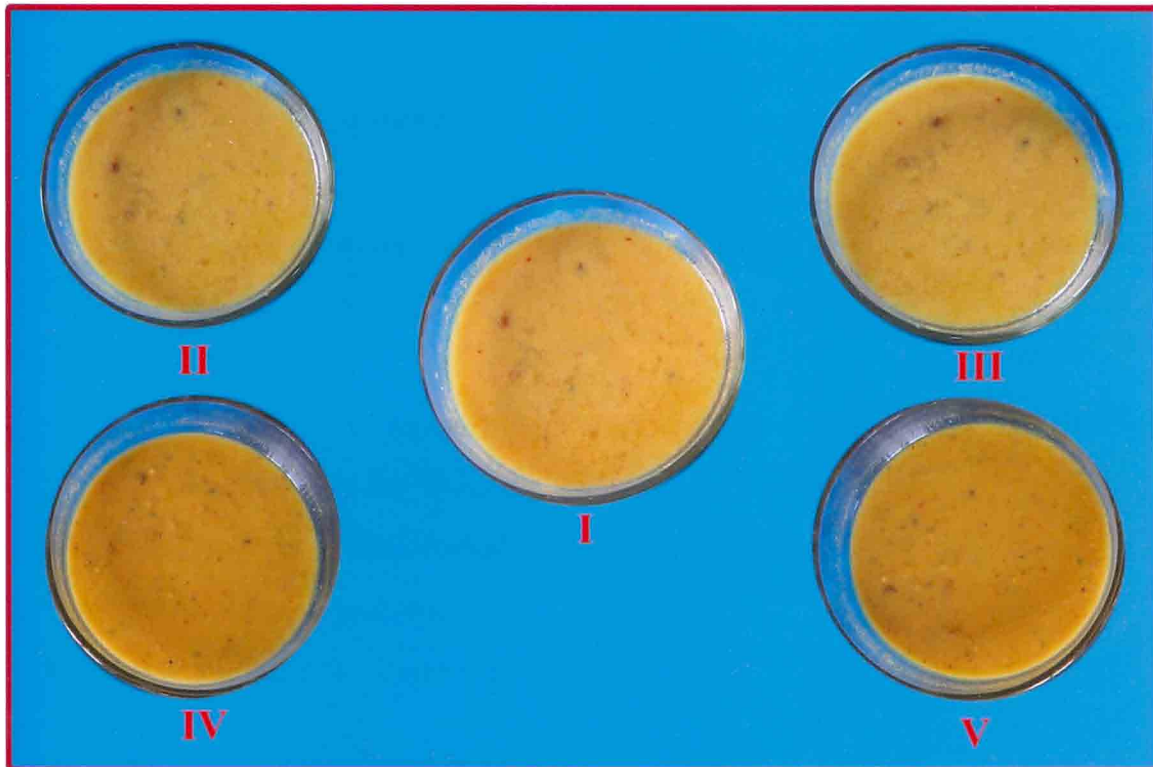


Plate 7 : Kadhi

- I - Control *kadhi*
- II - *Kadhi* prepared from non-germinated, autoclaved, fermented BWT mixture
- III - *Kadhi* prepared from germinated, autoclaved, fermented BWT mixture
- IV - *Kadhi* prepared from non-germinated, autoclaved, fermented PWT mixture
- V - *Kadhi* prepared from germinated, autoclaved, fermented PWT mixture

- After boiling for about 5 min, the *kadhi* was cooked on slow flame till done.

3.8.1.3 Dosa

Five types of *dosas* were prepared using probiotic fermented food mixtures.

Ingredients

	Control	BWT I	BWT II	PWT I	PWT II
Rice	300 g	-	-	-	-
Urd <i>dhal</i>	100 g	-	-	-	-
BWT I	-	120 ml	-	-	-
BWT II	-	-	120 ml	-	-
PWT I	-	-	-	120 ml	-
PWT II	-	-	-	-	120 ml
Salt	to taste	to taste	to taste	to taste	to taste

Method

- For preparation of control *dosa*, rice and *dhal* were soaked separately for overnight.
- Ground the soaked rice and *dhal* separately into thin paste and then mixed together.
- Water was used to make appropriate consistency of the mixture. Salt was added in the prepared slurry and then kept it for fermentation for about 12 h.
- Similarly, in case of experimental *dosa*, salt was added in fresh slurries and allowed to ferment for 12 h.
- Spread the experimental/control slurries on the hot non-stick griddle.
- Shallow frying was done.
- Then folded the *dosa* and served hot.

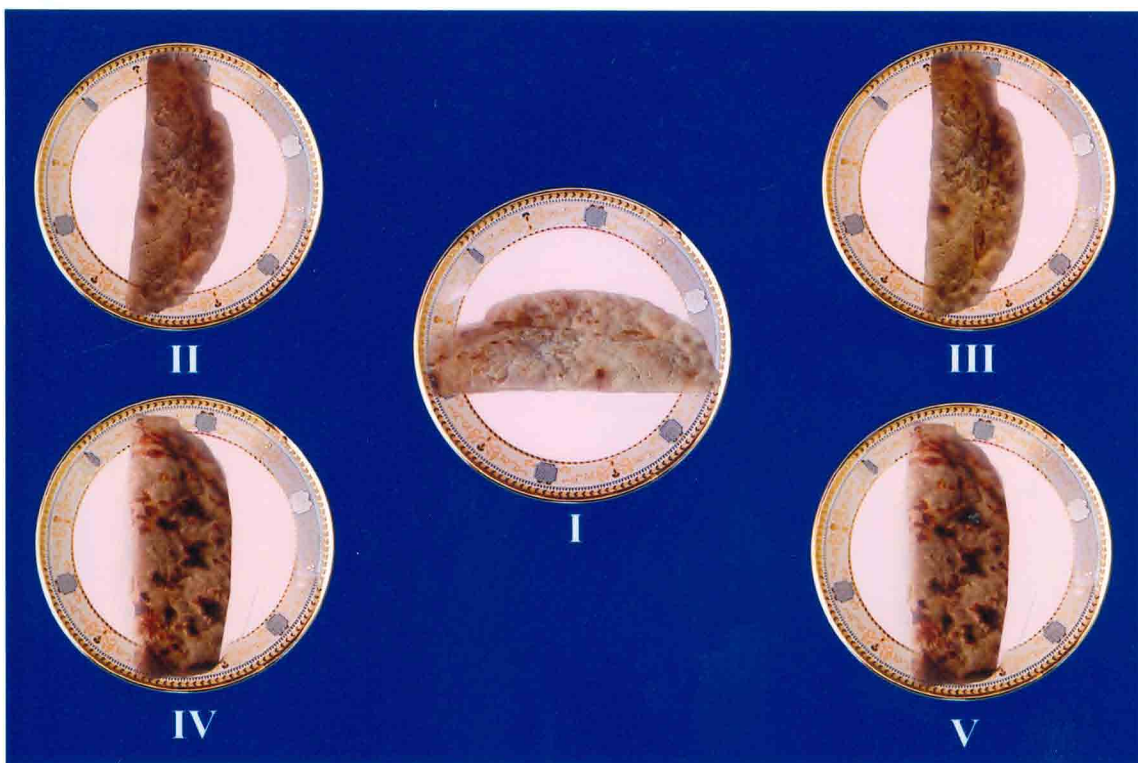


Plate 8 : Dosa

I - Control dosa

II - Dosa prepared from non-germinated, autocalved, fermented BWT mixture

III - Dosa prepared from germinated, autocalved, fermented BWT mixture

IV - Dosa prepared from non-germinated, autocalved, fermented PWT mixture

V - Dosa prepared from germinated, autocalved, fermented PWT mixture

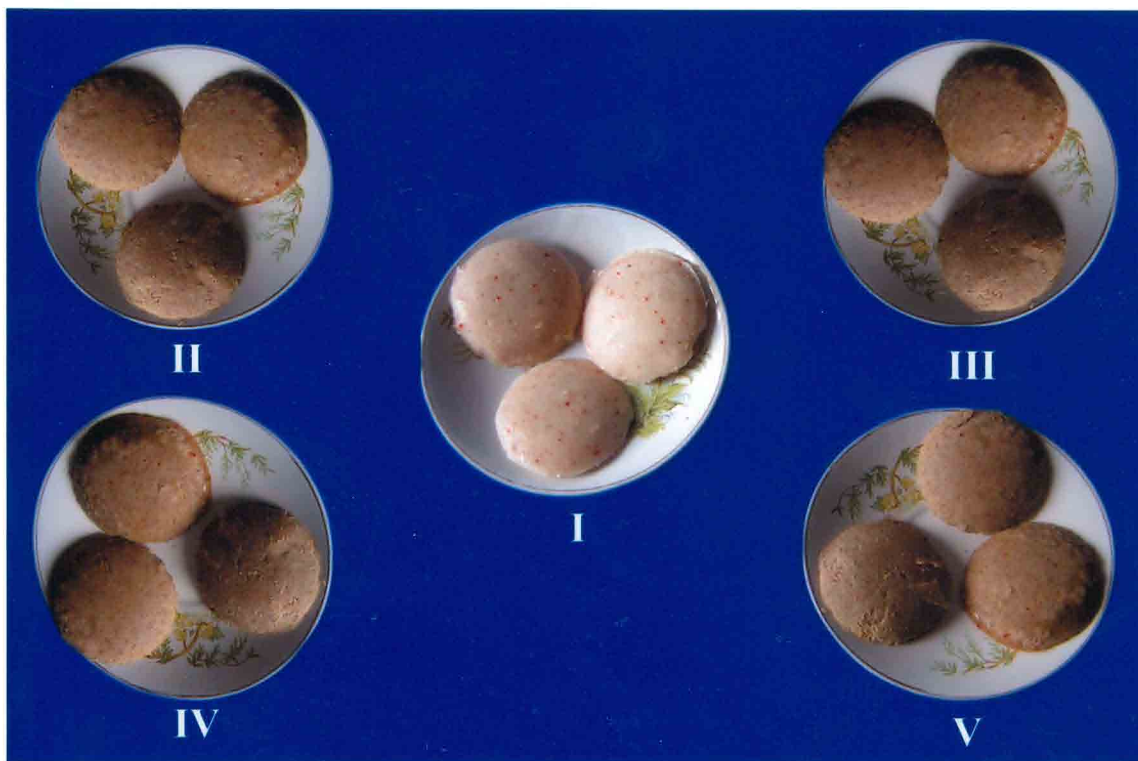


Plate 9 : Idli

I - Control idli

II - Idli prepared from non-germinated, autocalved, fermented BWT mixture

III - Idli prepared from germinated, autocalved, fermented BWT mixture

IV - Idli prepared from non-germinated, autocalved, fermented PWT mixture

V - Idli prepared from germinated, autocalved, fermented PWT mixture

3.8.1.4 Idli

Five types of *idlis* were prepared from probiotic fermented food mixtures.

Ingredients

	Control	BWT I	BWT II	PWT I	PWT II
Rice	200 g	-	-	-	-
Urd <i>dhal</i>	100 g	-	-	-	-
BWT I	-	120 ml	-	-	-
BWT II	-	-	120 ml	-	-
PWT I	-	-	-	120 ml	-
PWT II	-	-	-	-	120 ml
Salt	to taste	to taste	to taste	to taste	to taste

Method

- Rice and *dhal* were soaked separately for overnight.
- Ground *dhal* to a very fine paste and rice little coarsely and mixed together.
- Added salt in control and experimental slurries and allowed to ferment for 12 h.
- Steamed in *idli* moulds till cooked.

3.8.1.5 Papad

Five types of *papads* were prepared from probiotic fermented food mixtures.

Ingredients

	Control	BWT I	BWT II	PWT I	PWT II
Dehulled black gram <i>dhal</i>	100 g	-	-	-	-
Black pepper (coarsely ground)	5 g	5 g	5 g	5 g	5 g
Sodium bicarbonate	6 g	6 g	6 g	6 g	6 g
Cumin seeds	3.5 g	3.5 g	3.5 g	3.5 g	3.5 g

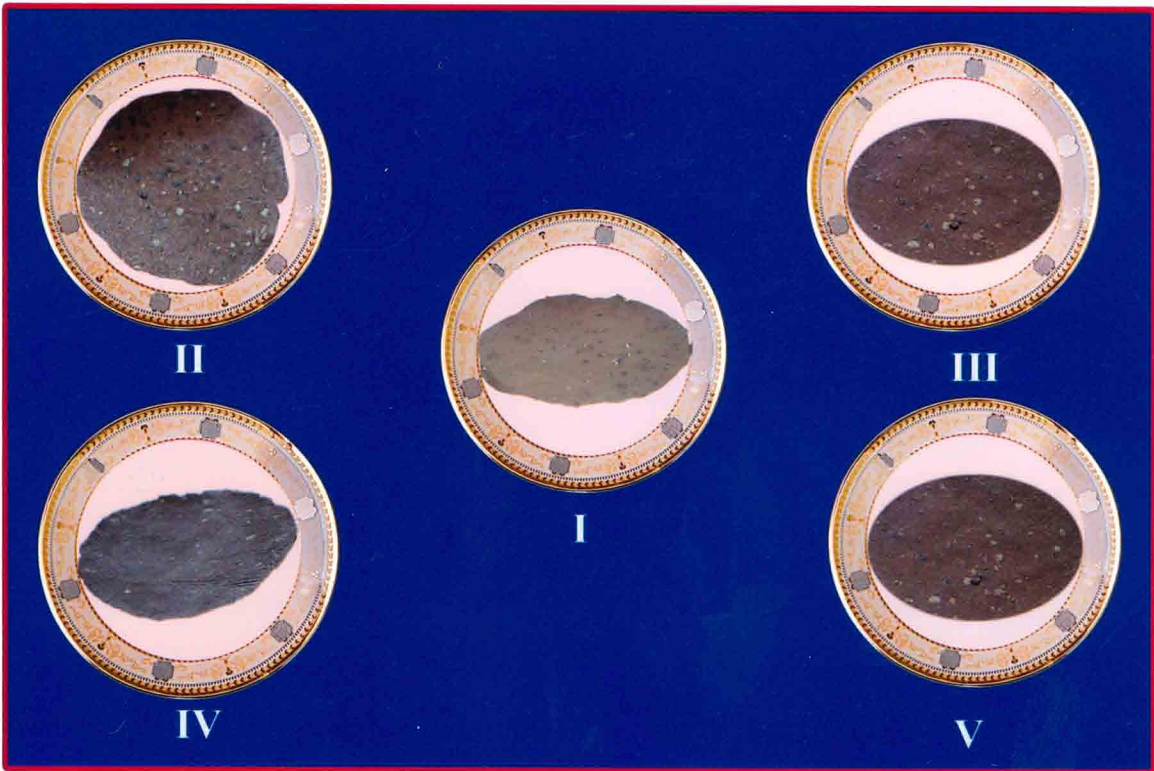


Plate 10 : Uncooked

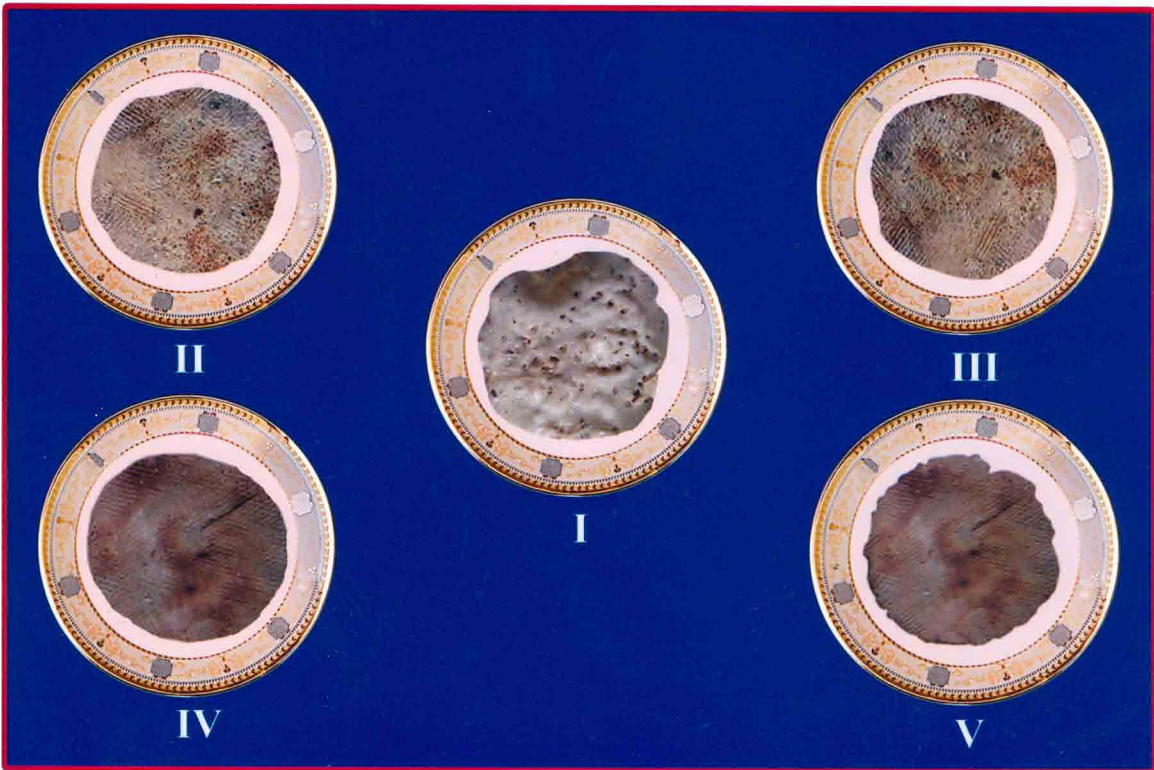


Plate 11 : Cooked *papad*

- I - Control papad
- II - *Papad* prepared from non-germinated, autoclaved, fermented BWT mixture
- III - *Papad* prepared from germinated, autoclaved, fermented BWT mixture
- IV - *Papad* prepared from non-germinated, autoclaved, fermented PWT mixture
- V - *Papad* prepared from germinated, autoclaved, fermented PWT mixture

Salt	8 g	8 g	8 g	8 g	8 g
Mustard oil	5 ml	5 ml	5 ml	5 ml	5 ml
BWT I	-	120 ml	-	-	-
BWT II	-	-	120 ml	-	-
PWT I	-	-	-	120 ml	-
PWT II	-	-	-	-	120 ml

Method

- For preparing control *papad*, mixed all the ingredients to black gram flour.
- Kneaded a hard dough using hot water. Similarly, in case of experimental *papad*, mixed all the ingredients to fermented slurries.
- Kneaded their dough separately and fermented for 12 h.
- Mustard oil was used while kneading so that dough did not stick to the hands.
- Kept the dough for 2 h in a polythene bag. Dough was divided into small balls of 25-30 g and rolled on circular plate having smooth surface with a wooden pin (roller) to give disk of about 0.6 to 0.8 mm thickness and 150-200 cm diameter.
- Dried the *papads* under sun. After drying, roasted the *papads* and served hot.

3.8.1.6 Wadi

Five types of *wadies* were prepared using probiotic fermented food mixtures.

Ingredients

	Control	BWT I	BWT II	PWT I	PWT II
Urd <i>dhal</i>	100 g	-	-	-	-
Dry-coriander powder	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g
Blackpepper (coarsely ground)	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g
Cumin seeds	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g
Heeng powder	a pinch	a pinch	a pinch	a pinch	a pinch
Salt	to taste	to taste	to taste	to taste	to taste
Red chilli powder	to taste	to taste	to taste	to taste	to taste
BWT I	-	120 ml	-	-	-
BWT II	-	-	120 ml	-	-
PWT I	-	-	-	120 ml	-
PWT II	-	-	-	-	120 ml

Method

- For preparing control *wadi*, urd *dhal* was taken and soaked in water for about 12 h.
- The soaked *dhal* was ground in electric grinder to obtain paste.
- For preparing experimental *wadies*, the probiotic slurries were fermented for 12 h.
- Rest of the procedure was same for both control and experimental *wadies*.
- Added all the dry ingredients like black pepper, cumin seeds, dry coriander powder and heeng powder to above slurries.
- Then mixtures were kept aside for fermentation for 5 h. After fermentation, the paste was beaten thoroughly.
- Salt and red chilli powder was added in the fermented paste. Small *wadies* were made and dried in sun.



Plate 12 : Uncooked *wadi*

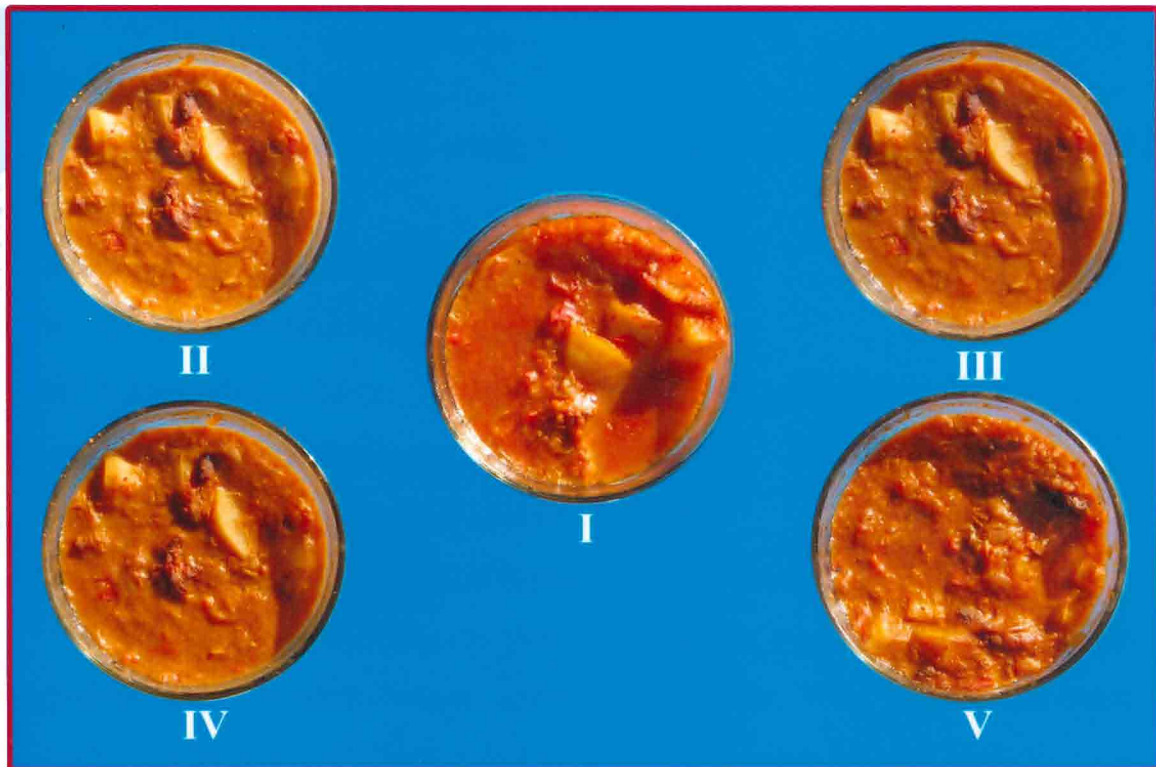


Plate 13 : *Wadi* curry

I - Control *wadi*

II - *Wadi* prepared from non-germinated, autoclaved, fermented BWT mixture

III - *Wadi* prepared from germinated, autoclaved, fermented BWT mixture

IV - *Wadi* prepared from non-germinated, autoclaved, fermented PWT mixture

V - *Wadi* prepared from germinated, autoclaved, fermented PWT mixture

Preparation of *wadi* curry

Ingredients

	Control	BWT I	BWT II	PWT I	PWT II
Urd/experimental <i>wadi</i>	75 g	75 g	75 g	75 g	75 g
Fat	15 g	15 g	15 g	15 g	15 g
Onion	75 g	75 g	75 g	75 g	75 g
Tomato	100 g	100 g	100 g	100 g	100 g
Water	350 ml	350 ml	350 ml	350 ml	350 ml
Ginger	10 g	10 g	10 g	10 g	10 g
Garlic	10 g	10 g	10 g	10 g	10 g
Salt	to taste	to taste	to taste	to taste	to taste
Red chilli powder	to taste	to taste	to taste	to taste	to taste
Turmeric powder	½ tsp	½ tsp	½ tsp	½ tsp	½ tsp
Garam masala	½ tsp	½ tsp	½ tsp	½ tsp	½ tsp

Method

- *Wadies* were soaked in water for 10-15 min in lukewarm water to make them soft.
- Ground onion, garlic and ginger.
- Fried this paste in fat till golden brown.
- Added salt and spices. Added tomato paste and further fried for some time.
- *Wadies* were added to the above masala.
- Added water and pressure cooked for 5 min.

3.8.2 Organoleptic evaluation of fermented food products

The developed products as mentioned above were subjected to sensory evaluation with respect to colour, appearance, flavour, taste, texture and overall acceptability by a semi-trained panel of ten judges using 9-Point Hedonic Scale. The judges were drawn from the Department of Foods and Nutrition, CCSHAU, Hisar.

On the basis of mean scores of sensory evaluation obtained after feeding to the judges, the most acceptable products were selected for further nutritional evaluation.

3.9 Nutritional evaluation of developed food products

All the most acceptable developed food products were nutritionally analysed for the following parameters:

- Crude protein and total lysine (section 3.7.1.2 and 3.7.5)
- Antinutrients (section 3.7.6)
- *In vitro* protein digestibility (section 3.7.7.1)
- *In vitro* starch digestibility (section 3.7.7.2)
- Total and *In vitro* availability of Ca, Fe, and Zn (section 3.7.8.1 and 3.7.8.2)

3.10 Statistical analysis

The data have been analysed using two factor completely randomized design, factor A has two levels, factor B has three levels. Correlation coefficients have also been worked out.

Chapter-4

Results and Discussion

In the present study, efforts were made to develop some nutritious and beneficial blends using various processing treatments like autoclaving, germination and probiotic fermentation. These blends were further utilized for preparation of products. The results pertaining to this study have been presented and discussed under the following heads and subheads:

- 4.1 Organoleptic evaluation of probiotic curd
- 4.2 Cell count of fermented food mixtures
- 4.3 pH and titratable acidity of indigenously developed food mixtures
- 4.4 Nutritional evaluation of indigenously developed food mixtures
 - 4.4.1 Proximate composition
 - 4.4.2 Carbohydrates
 - 4.4.2.1 Available
 - 4.4.2.1.1 Sugars
 - 4.4.2.1.2 Starch
 - 4.4.2.2 Non-available
 - 4.4.2.2.1 Dietary fibre
 - 4.4.2.2.2 β -glucan
- 4.5 B-complex vitamins

- 4.6 Protein fractions
- 4.7 Total lysine
- 4.8 Antinutrients
 - 4.8.1 Phytic acid
 - 4.8.2 Polyphenols
 - 4.8.3 Amylase inhibitor activity
- 4.9 *In vitro* digestibility
 - 4.9.1 *In vitro* protein digestibility
 - 4.9.2 *In vitro* starch digestibility
- 4.10 Minerals
 - 4.10.1 Total minerals (Ca, Fe, Zn)
 - 4.10.2 Available minerals (Ca, Fe, Zn)
- 4.11 Organoleptic evaluation of products developed from probiotic fermented food mixtures
- 4.12 Nutritional evaluation of most acceptable developed food products
 - 4.12.1 Protein and total lysine
 - 4.12.2 Antinutrients
 - 4.12.3 *In vitro* protein and starch digestibility
 - 4.12.4 Total mineral (Ca, Fe, Zn)
 - 4.12.5 Available minerals (Ca, Fe, Zn)

4.1 Organoleptic evaluation of probiotic curd

Three types of curds were prepared using *L. acidophilus* inoculum in three proportions i.e. 3, 5 and 10 per cent each in 100 ml of sterilized skimmed milk and incubated at 37⁰C for 6 h (Table 4.1).

These curds were organoleptically evaluated by a panel of expert judges. It was observed that the probiotic curd prepared with 5 per cent *L. acidophilus* inoculum exhibited significantly highest mean scores of colour (8.50), appearance (8.30), aroma (8.30), texture (8.40), taste (8.23) and overall acceptability (8.30) whereas curd having 10 per cent inoculum had significantly lowest mean scores of

Table 4.1 : Organoleptic evaluation of probiotic curd using different proportions of *L. acidophilus* inoculum

Inoculum (%)	Color	Appearance	Aroma	Texture	Taste	Overall acceptability
3	8.20 ± 0.20	7.90 ± 0.27	8.00 ± 0.21	8.20 ± 0.24	6.80 ± 0.20	7.80 ± 0.20
5	8.50 ± 0.16	8.30 ± 0.21	8.30 ± 0.21	8.40 ± 0.22	8.23 ± 0.21	8.30 ± 0.16
10	7.70 ± 0.30	8.30 ± 0.21	7.80 ± 0.24	8.40 ± 0.22	6.70 ± 0.16	8.00 ± 0.13
SE (m)	0.12	0.10	0.18	0.13	0.11	0.14
CD (P<0.05)	0.36	0.30	0.52	0.39	0.34	0.40

Values are mean ± SE of ten panelists

all organoleptic characteristics. Therefore, on the basis of organoleptic characteristics, probiotic curd with 5 per cent inoculum was further selected for carrying out fermentation in indigenously developed food mixtures as this curd also had desirable cell counts.

4.2 Cell count of fermented food mixtures

All the four autoclaved non-germinated and germinated BWT and PWT food mixture slurries were inoculated with 5 per cent inoculum of *L. acidophilus* curd at a level of 10^6 cells per ml and fermented at 37°C for 12 h.

At the end of fermentation period, the cell count increased in all the four fermenting BWT and PWT food mixture slurries containing probiotic curd (Table 4.2). The growth of *L. acidophilus* in germinated autoclaved and fermented BWT and PWT food mixtures was found to be maximum i.e. 8.88 and 8.64 cfu/g followed by non-germinated autoclaved and fermented BWT and PWT food mixtures. The cell counts were observed 7.75 cfu/g (BWT) and 7.30 cfu/g (PWT), respectively. PWT food mixtures in both cases had higher cell counts as compared to BWT food mixtures. A significant difference was observed in non-germinated and germinated food mixtures.

The growth of this bacteria was due to decrease in pH and increase in acidity. As the optimal temperature for the growth of this organism is 37°C , therefore, this organism grew well at this temperature in food mixtures containing cereal, whey powder, tomato pulp and curd. The ingredients used were the ideal substrates for growth of *L. acidophilus*.

This may be due to hydrolysis of germinated flours which provided better media for growth. Similar findings have been reported earlier by Prajapati *et al.* (1986) who prepared a slurry using neutralized *acidophilus* milk, banana paste, tomato juice concentrate and ground sugar at rate of 40, 10 and 15 per cent, respectively, of the weight of neutralized *acidophilus* milk and observed the *Lactobacilli* count in the final product to be 8.71×10^7 cfu/g.

Table 4.2 : *Lactobacilli* count (log cfu/g) of indigenously developed food mixtures

Processing treatments	BWT* food mixture	PWT* food mixture
(A) Non-germinated		
Raw mixture (control)	---	---
Autoclaved mixture	---	---
Autoclaved and fermented mixture	7.75 ± 0.05	7.30 ± 0.02
(B) Germinated		
Germinated raw mixture (control)	---	---
Germinated autoclaved mixture	---	---
Germinated, autoclaved and fermented mixture	8.88 ± 0.05	8.64 ± 0.03
t' value	3.28*	3.54*

Values are mean ± SE of three independent determinations

Probiotic curd was used as inoculum (3%) for carrying out fermentation in food mixtures

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Similarly, other workers also reported 8.98 to 9.34 cfu/g cell count in food mixtures containing huskless barley flour, pearl millet flour, sprouted green gram, sprouted chickpea flour, skimmed milk powder, banana paste and tomato pulp (Binita, 1995; Chaudhary, 1998 and Chahal, 1999).

4.3 pH and titratable acidity of indigenously developed food mixtures

The pH and titratable acidity of raw (non-germinated) BWT and PWT food mixtures were 6.02 and 6.10, 1.69 and 1.65 g lactic acid/100 ml, respectively. The initial pH and titratable acidity of these mixtures did not vary significantly among each other (Table 4.3, Fig.4.1 and 4.2). After autoclaving at 121⁰C for 15 min at 1.5 kg/cm², a significant decline in pH i.e. 5.82 and 5.88 took place in both BWT and PWT mixtures. Fermentation of autoclaved mixtures with probiotic curd for 12 h at 37⁰C resulted in significant reduction in pH. It was 4.23 and 4.28 in BWT and PWT non-germinated autoclaved and fermented mixtures. A significant decline in pH with a corresponding increase occurred in titratable acidity in autoclaved as well as autoclaved and fermented samples. There was a significant (P<0.05) increase in titratable acidity after fermentation with *L. acidophilus* curd. It increased from 1.69 to 2.60 and 1.65 to 2.68 g lactic acid/100 ml, respectively over control values in both types of mixtures.

However, in case of germinated samples, pH dropped significantly (P<0.05) i.e. 5.14 in BWT and 5.57 in PWT control mixtures. The titratable acidity obtained was 2.00 and 2.15 in both germinated (control) mixtures. The reduction in pH may be due to hydrolysis of starch into sugars during germination which is readily utilized by the organisms and converted to lactic acid. Autoclaving of germinated mixtures caused further decrease in pH with simultaneous increase in titratable acidity was noticed (Table 4.4). Fermentation with *L. acidophilus* curd further decreased pH to the extent of 3.90 and 3.95, respectively with corresponding increase in titratable acidity from 3.10 and 3.05 g lactic acid/100 ml in both types of mixtures. A similar trend in pH and titratable acidity in germinated mixtures and fermented mixtures was also observed by

Table 4.3 : Effect of fermentation with *L. acidophilus* curd on pH and titratable acidity (g lactic acid/100 ml) of indigenously developed BWT* and PWT* food mixtures

Processing treatments	BWT		PWT	
	pH	Titratable Acidity	pH	Titratable Acidity
Food mixtures				
(A) Non-germinated				
Raw mixture (control)	6.02 ± 0.21	1.69 ± 0.05	6.10 ± 0.22	1.65 ± 0.06
Autoclaved mixture	5.82 ± 0.12	1.78 ± 0.02	5.88 ± 0.14	1.74 ± 0.04
Autoclaved and fermented mixture	4.23 ± 0.17	2.60 ± 0.19	4.28 ± 0.22	2.68 ± 0.14
Mean	5.12	2.32	5.19	2.33
(B) Germinated				
Germinated raw mixture (control)	5.14 ± 0.52	2.00 ± 0.13	5.57 ± 0.15	2.15 ± 0.09
Germinated autoclaved mixture	4.90 ± 0.08	2.38 ± 0.01	4.99 ± 0.08	2.42 ± 0.02
Germinated, autoclaved and fermented mixture	3.90 ± 0.11	3.10 ± 0.11	3.95 ± 0.01	3.05 ± 0.04
Mean	5.02	2.28	4.71	2.75
CD (A)	0.44	0.24	0.33	0.11
CD (B)	0.55	0.32	0.39	0.23
CD (A x B)	0.78	0.45	0.56	0.33

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

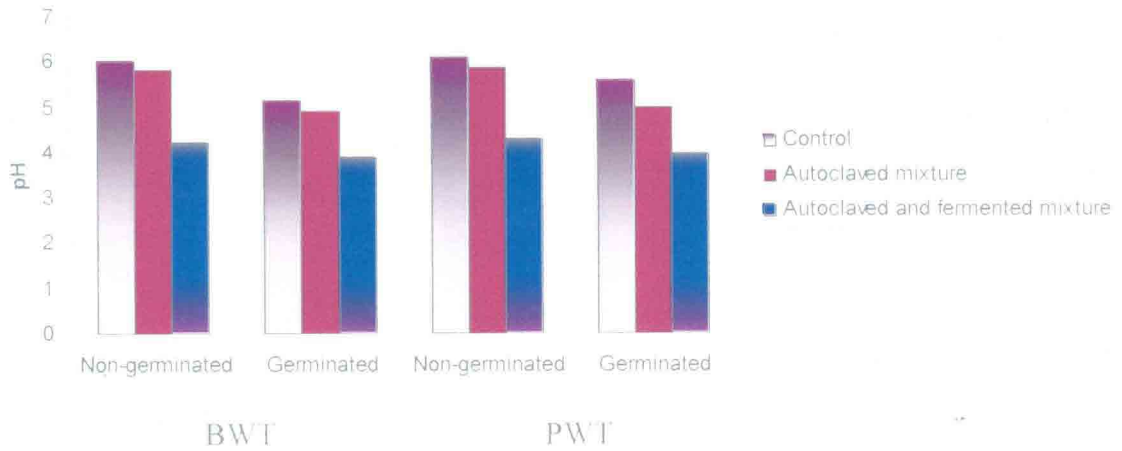


Fig. 4.1. Effect of fermentation with *L. acidophilus* curd on pH of indigenously developed BWT and PWT food mixtures.

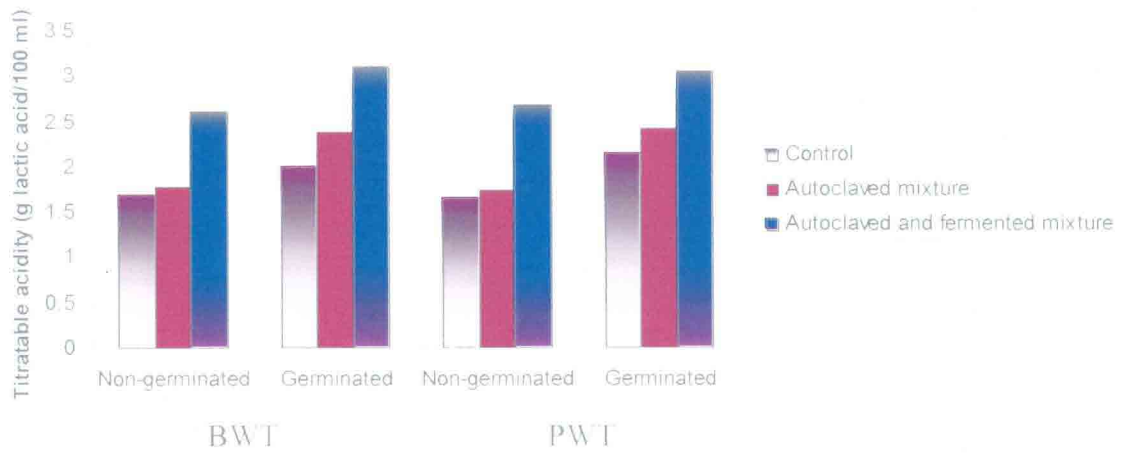


Fig. 4.2. Effect of fermentation with *L. acidophilus* curd on titratable acidity of indigenously developed BWT and PWT food mixtures.

Table 4.4 : Correlation coefficients of pH and tritatable acidity of indigenously developed BWT* and PWT* food mixtures

Processing treatments	pH vs titratable acidity	
	BWT* food mixture	PWT* food mixture
(A) Non-germinated		
Raw mixture (control)	-0.8279*	-0.8075*
Autoclaved mixture	-0.8962*	-0.8675*
Autoclaved and fermented mixture	-0.9279**	-0.9382**
(B) Germinated		
Germinated raw mixture (control)	-0.8969*	-0.8850*
Germinated autoclaved mixture	-0.9012**	-0.9230**
Germinated, autoclaved and fermented mixture	-0.9989**	-0.9990**

*Significant at 5% level

** Significant at 1% level

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

various workers in cereal legume based food mixtures (Khetarpaul and Chauhan, 1989; Antony *et al.*, 1996; Sripriya *et al.*, 1997; Chahal, 1999). The generalized relationship between pH and titratable acidity is explainable with the help of correlation coefficients given in the Table 4.3. In all processed food mixtures, pH had a significantly ($P < 0.05$) negative correlation with titratable acidity. During fermentation, probiotic organisms convert glucose to lactic acid which is responsible for the decline in pH of the developed product. A rapid drop in pH with corresponding to increase in titratable acidity has been reported in cereals and legumes (Agte *et al.*, 1997; Sripriya *et al.*, 1997; Chahal, 1999),

4.4 Nutritional evaluation of indigenously development food mixtures

4.4.1 Proximate composition

The results pertaining to proximate composition of raw, germinated and fermented food mixtures are given in Table 4.5 and 4.6.

4.4.1.1 Moisture

Moisture contents of non-germinated (raw) food mixtures namely, BWT and PWT were 30.45 and 28.47 per cent, respectively (Tables 4.5 and 4.6).

When these mixtures were autoclaved at 121°C for 15 min., no remarkable change occurred in their moisture contents. Further no significant ($P < 0.05$) change took place in moisture content when both autoclaved and cooled mixtures were fermented with *L. acidophilus* curd. The values were 30.45 and 28.47 per cent, respectively in both mixtures.

Similarly, in case of germinated (raw) mixtures, the moisture contents were 30.38 and 28.41 per cent which differed non-significantly among each other. Non-significant change was also observed in germinated + autoclaved mixture as well as germinated + autoclaved + fermented mixtures of BWT and PWT. The values obtained for moisture contents are in line with those obtained earlier for cereal-legume based mixtures (Chaudhary, 1998; Chahal, 1999).

4.4.1.2 Crude protein

Crude protein contents ranged from 13.86 to 14.83 and 13.96 to 14.89 per cent, respectively in non-germinated (raw), autoclaved and autoclaved fermented BWT and PWT food mixtures. Autoclaving did not cause any significant ($P < 0.05$) change in their protein contents, however fermentation of autoclaved mixture caused appreciable change in protein contents (Tables 4.5 and 4.6).

Whereas crude protein contents decreased non-significantly when raw samples were germinated for 24 h. The values were reported as 13.86 and 13.89 per cent, respectively in germinated (raw) mixture of BWT and PWT. But when germinated (raw) mixtures were autoclaved, there was also non-significant change in protein contents. However, a non-significant decrease was observed in germinated + autoclaved and fermented BWT and PWT food mixtures. The values ranged from 13.18 to 13.86 and 13.06 to 13.89 per cent, respectively in both type of mixtures. Thus, it appears that the heat treatment did not affect significantly the protein content of various food mixtures. Similar findings were also observed in various cereal-legume blends (Binita, 1995; Chaudhary, 1998; Chahal, 1999, Grewal, 2003).

A significant reduction in crude protein content of the food mixture as a result of germination and fermentation may be attributed to an increase in protein catabolism by the fermenting micro-organisms which leads to the escaping of the by product of metabolic deamination i.e. ammonia. The results are similar to those reported by Chaudhary (1998) and Chahal (1999) who observed a reduction in protein content of fermented cereal-legume food mixtures by the action of bacteria and yeasts. Sharma (1994) also noticed a significant ($P < 0.05$) reduction in the protein content of pearl millet when it was fermented with *L. acidophilus*. On the contrary, Sripriya *et al.* (1997), Basappa *et al.* (1997) reported that protein content showed marginal increase on germination but marked increase on fermentation for 18 h.

4.4.1.3 Crude fat

The crude fat contents of non-germinated (raw) BWT and PWT food mixtures were 1.95 and 4.29 per cent, respectively (Tables 4.5 and 4.6). Autoclaving as well as fermentation with *L. acidophilus* curd did not cause any significant change in fat content of these two food mixtures. The values obtained in non-germinated autoclaved food mixtures are in agreement with those reported earlier by various workers in autoclaved legumes (Jood *et al.*, 1998; Grewal, 2003). Similarly, no change in fat content due to fermentation with *L. acidophilus* was observed by previous workers in cereal-legume-whey mixture (Sharma and Khetarpaul, 1997), barley flour, sprouted green gram, skimmed milk powder, tomato pulp, banana paste (Chaudhary, 1998).

In case of germinated (raw) BWT and PWT food mixtures, the fat contents did not change significantly on autoclaving. The values were 1.67 and 4.05 per cent, respectively in both mixtures. The slight reduction in fat content may probably be due to the fact that lipid reserves present in the grain get utilized during germination (King and Puwastein, 1987; Grewal, 2003). Similarly, autoclaving and fermentation with *L. acidophilus* curd had non-significant effect on fat contents of both type of food mixtures. On the contrary, some workers reported a significant decrease in fat content of finger millet (Antony and Chandra, 1998) and *tempeh* (urga *et al.*, 1997).

4.4.1.4 Ash

Ash contents of raw BWT and PWT food mixtures were 2.42 and 2.97 per cent, respectively and did not change significantly when these mixtures were autoclaved (121⁰C for 15 min.) and then cooled and fermented with *L. acidophilus* curd (Tables 4.5 and 4.6). Similarly, when the raw samples were germinated, a non-significant change was observed in both type of mixtures. Similar trend was observed when germinated mixtures were autoclaved and then fermented with *L. acidophilus* curd. Since, no addition or deletion of mineral source was involved

Table 4.5 : Effect of fermentation with *L. acidophilus* curd on proximate composition of indigenously developed BWT* food mixture (g/100g, on dry matter basis)

Processing treatments	Moisture*	Crude protein	Fat	Ash	Crude fibre
Food mixtures					
(A) Non-germinated					
Raw mixture (control)	30.45 ± 0.37	14.83 ± 0.58	1.95 ± 0.20	2.42 ± 0.29	2.14 ± 0.10
Autoclaved mixture	30.43 ± 0.30	14.76 ± 0.57	1.91 ± 0.11	2.40 ± 0.05	2.01 ± 0.28
Autoclaved and fermented mixture	30.30 ± 0.73	13.86 ± 0.23	1.83 ± 0.15	2.34 ± 0.08	1.89 ± 0.12
Mean	30.39	14.48	1.89	2.38	2.01
(B) Germinated					
Germinated raw mixture (control)	30.38 ± 0.66	13.86 ± 0.56	1.67 ± 0.17	2.40 ± 0.06	2.10 ± 0.15
Germinated autoclaved mixture	30.16 ± 0.05	13.75 ± 1.17	1.59 ± 0.11	2.32 ± 0.06	2.02 ± 0.17
Germinated, autoclaved and fermented mixture	30.06 ± 0.66	13.18 ± 0.35	1.46 ± 0.18	2.30 ± 0.11	1.88 ± 0.03
Mean	30.20	13.59	1.57	2.34	2.00
CD (A)	NS	0.68	NS	NS	0.32
CD (B)	NS	0.52	NS	NS	0.20
CD (A x B)	NS	1.62	NS	NS	0.26

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

* Moisture on fresh weight basis

Table 4.6 : Effect of fermentation with *L. acidophilus* curd on proximate composition of indigenously developed PWT* food mixture (g/100g, on dry matter basis)

Processing treatments	Moisture*	Crude Protein	Fat	Ash	Crude fibre
Food mixtures					
(A) Non-germinated					
Raw mixture (control)	28.47 ± 0.05	14.89 ± 0.05	4.29 ± 0.05	2.97 ± 0.23	0.80 ± 0.11
Autoclaved mixture	28.44 ± 0.11	14.78 ± 0.05	4.17 ± 0.05	2.97 ± 0.05	0.70 ± 0.08
Autoclaved and fermented mixture	28.43 ± 0.11	13.96 ± 0.17	4.15 ± 0.11	2.90 ± 0.06	0.61 ± 0.05
	28.44	14.54	4.20	2.94	0.70
Mean					
	28.41 ± 0.17	13.89 ± 0.11	4.05 ± 0.02	2.93 ± 0.05	0.79 ± 0.11
(B) Germinated					
Germinated raw mixture (control)	28.39 ± 0.05	13.78 ± 0.05	4.00 ± 0.05	2.90 ± 0.11	0.70 ± 0.11
Germinated autoclaved mixture	28.37 ± 0.05	13.06 ± 0.11	3.89 ± 0.06	2.87 ± 0.11	0.51 ± 0.11
Germinated, autoclaved and fermented mixture	28.39	13.57	3.98	2.90	0.66
Mean	NS	0.67	NS	NS	0.12
CD (A)	NS	0.52	NS	NS	0.10
CD (B)	NS	0.61	NS	NS	0.09
CD (A x B)					

Values are mean ± SE of three independent determinations
 *PWT food mixture contains barley flour, whey powder and tomato pulp
 * Moisture on fresh weight basis

during autoclaving or fermentation, the ash content remained unchanged during these processing methods.

Results of the study are in accordance with those reported by various workers in wheat and barley *rabadi* (Gupta, 1989), *soy rabadi* (Grewal, 1992) and food mixture containing barley flour, green gram flour, skimmed milk powder and tomato pulp which was autoclaved, cooled and fermented with *L. acidophilus* (Binita *et al.*, 1996).

4.4.1.5 Crude fibre

Crude fibre contents of non-germinated (raw) BWT and PWT food mixtures were 2.14 and 0.80 per cent, respectively (Tables 4.5 and 4.6). When raw mixtures were autoclaved, non-significant change in crude fibre contents was exhibited. However, when autoclaved mixtures were fermented with *L. acidophilus* curd, significant ($P < 0.05$) change in crude fibre contents was observed. The values were 1.89 and 0.61 per cent, respectively.

Non-significant change was observed when raw samples were germinated. The values were 2.10 and 0.79 per cent, respectively in BWT and PWT. Autoclaving did not affect significantly the crude fibre contents of BWT and PWT germinated mixtures. But when germinated + autoclaved mixtures were fermented with *L. acidophilus* curd, a significant decrease in crude fibre contents i.e. 1.88 and 0.51 per cent, respectively could be observed. The values obtained in present study are in line with those reported by other workers in cereal-legume based food mixtures (Chaudhary, 1998; Chahal, 1999; Grewal, 2003).

4.4.2 Carbohydrates

4.4.2.1 Available

4.4.2.1.1 Sugars

Non-germinated BWT and PWT food mixtures contained 3.02, 2.90 g/100g total soluble sugars, 0.95, 0.85 g/100g reducing sugars and 2.07, 2.05 g/100 g non-reducing sugars, respectively (Table 4.7 and 4.8). The level of all these sugars increased significantly ($P < 0.05$) in both the food mixtures when they were

subjected to autoclaving. It was found that the level of total and reducing sugars increased significantly almost 2 and 3 folds, respectively in both the mixtures.

As moist heat may cause rupturing of starch granules followed by hydrolysis of starch to oligosaccharides and then to monosaccharides, resulting from autoclaving, may be responsible for increased concentration of sugars in the autoclaved food mixtures. These results bear resemblance to those reported earlier in various cereals and legumes (Negi *et al.*, 2000; Grewal, 2003) and cereal-legume milk whey based food mixtures (Binita, 1995; Chaudhary, 1998; Chahal, 1999).

When the autoclaved BWT and PWT food mixtures were fermented with *L. acidophilus* curd (37°C, 12 h), the contents of total soluble sugar and non-reducing sugars were decreased significantly ($P < 0.05$) whereas reducing sugar contents of both mixtures increased significantly. The values were 5.25 and 6.30 g/100g, respectively.

The concentration of total soluble sugars, reducing sugars and non-reducing sugars increased significantly ($P < 0.05$) when the raw samples of pearl millet and barley were subjected to germination for 24 h, which ultimately increased the level of these sugars in developed mixtures (Tables 4.7 and 4.8). The values were 12.50, 11.00 g/100 total soluble sugars, 5.37, 5.30 g/100g reducing sugars and 7.13, 5.70 g/100g non-reducing sugars, respectively in both (BWT and PWT) food mixtures. When the germinated slurries were further processed for homogenization and autoclaving, further significant ($P < 0.05$) increase in total, soluble and reducing sugars was observed. The increased sugar content of pearl millet (PWT) and barley (BWT) based food mixtures during germination and autoclaving may be due to hydrolysis of starch thereby resulting in higher concentration of soluble sugars. Rise in reducing sugars may be due to mobilization and hydrolysis of seed polysaccharides (Sripriya *et al.*, 1997), leading to more available reducing sugars. Rapid amylolysis might yield significant amount of maltose, a reducing sugar. Increased levels of total soluble sugars, reducing sugars and non-reducing sugars

during germination have been reported earlier in various cereals and pulses (Khetarpaul and Chauhan, 1990; Jood *et al.*, 1998; Chahal, 1999; Grewal, 2003).

4.4.2.1.2 Starch

Starch contents of non-germinated (raw) food mixtures (BWT and PWT) were 42.92 and 48.23 g/100g on dry matter basis, respectively (Tables 4.7 and 4.8). When the raw food mixtures were autoclaved, starch contents significantly ($P < 0.05$) decreased by about 19 per cent in both BWT and PWT food mixtures. Fermentation further resulted in a significant ($P < 0.05$) decline in starch content of BWT and PWT food mixtures. Reduction in starch content was found to be more in PWT food mixture (58%) than BWT food mixture (49%) when these food mixtures were fermented with *L. acidophilus* curd for 12 h at 37°C.

Germinated (raw) BWT and PWT food mixtures contained 30.15 and 34.36 per cent starch contents, respectively. This was significantly lower when compared to starch contents of raw (non-germinated) food mixtures. When these germinated food mixtures were autoclaved, a further significant decrease was observed. Further significant ($P < 0.05$) reduction in starch content was also observed when these germinated and autoclaved slurries were fermented with *L. acidophilus* curd. Reduction in starch contents was 67 and 62 per cent in both types of mixtures.

Autoclaving may cause rupturing of starch granules followed by hydrolysis of starch to oligosaccharides and then to monosaccharides. As a result, starch content decreases. Decrease in starch contents of several plant foods including soybean (Grewal, 1992), pearl millet (Sharma, 1994) and moongbean (Grewal, 2003) have also been reported earlier. As a result of germination and/or fermentation resulted in an increase in total soluble, reducing and non-reducing sugars. Reduction of starch in the fermented products may be attributed to amylolytic action of microorganisms in the fermenting mixture. Fermenting microbes have been reported to possess both α and β -amylases (Bernfeld, 1962). Amylolysis during germination followed by fermentation has been reported in a number of food grains (Khetarpaul and Chauhan, 1990; Sripriya *et al.*, 1997).

Table 4.7 : Effect of fermentation with *L. acidophilus* curd on sugar and starch content of indigenously developed BWT* food mixture (g/100 g, on dry matter basis)

Processing treatments	Total soluble	Reducing	Non-reducing	Starch
Food mixtures				
(A) Non-germinated				
Raw mixture (control)	3.02 ± 0.16	0.95 ± 0.11	2.07 ± 0.11	42.92 ± 0.71
Autoclaved mixture	9.48 ± 0.11	3.95 ± 0.11	5.53 ± 0.05	34.63 ± 0.92 (-19)
Autoclaved and fermented mixture	7.25 ± 0.05	5.25 ± 0.17	2.00 ± 0.05	21.91 ± 2.28 (-49)
Mean	6.58	3.38	3.20	33.15
(B) Germinated				
Germinated raw mixture (control)	12.50 ± 0.05	5.37 ± 0.11	7.13 ± 0.11	30.15 ± 0.91
Germinated autoclaved mixture	17.30 ± 0.11	6.95 ± 0.05	10.35 ± 0.17	20.05 ± 0.87 (-33)
Germinated, autoclaved and fermented mixture	9.50 ± 0.28	8.23 ± 0.11	1.27 ± 0.05	9.98 ± 0.25 (-67)
Mean	13.10	6.85	6.25	20.06
CD (A)	0.27	0.21	0.18	3.62
CD (B)	0.33	0.26	0.22	2.04
CD (A x B)	0.47	0.37	0.32	2.89

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent decrease (-) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

Table 4.8 : Effect of fermentation with *L. acidophilus* curd on sugar and starch content of indigenously developed PWT* food mixture (g/100 g, on dry matter basis)

Processing treatments	Total soluble	Reducing	Non-reducing	Starch
Food mixtures				
(A) Non-germinated				
Raw mixture (control)	2.90 ± 0.11	0.85 ± 0.05	2.05 ± 0.05	48.23 ± 0.95
Autoclaved mixture	8.39 ± 0.22	3.99 ± 0.11	4.40 ± 0.04	39.15 ± 1.08 (-19)
Autoclaved and fermented mixture	7.98 ± 0.13	6.30 ± 0.22	1.63 ± 0.05	20.06 ± 0.93 (-58)
Mean	6.42	3.71	2.69	35.81
(B) Germinated				
Germinated raw mixture (control)	11.00 ± 0.05	5.30 ± 0.17	5.70 ± 0.07	34.36 ± 1.05
Germinated autoclaved mixture	17.55 ± 0.02	6.45 ± 0.02	11.10 ± 0.05	20.08 ± 0.85 (-29)
Germinated, autoclaved and fermented mixture	10.03 ± 0.05	8.67 ± 0.05	1.36 ± 0.11	9.03 ± 0.14 (-62)
Mean	12.86	6.80	6.05	21.15
CD (A)	0.21	0.60	0.16	3.02
CD (B)	0.26	0.32	0.15	2.70
CD (A x B)	0.37	0.33	0.22	2.72

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent decrease (-) over control values

*PWT food mixture contains pearl millet, whey powder and tomato pulp

In the initial stage of fermentation, higher concentration of sugars may be observed but with passage of the period of fermentation, these sugars may be utilized and the fermented product may have sugar level which is lower than the initial concentration of sugars in the germinated and/or fermenting mixture. Hamad and Fields (1979) reported the similar findings that during the natural lactic and fermentation of cereals, the level of reducing sugars increased 4.38 times on the first day but decreased on the second and third days and this increase and decrease was attributed to the action of microflora during fermentation. Similarly, a significant reduction in total soluble and non-reducing sugars was noticed when pearl millet flour was fermented with *L. acidophilus* (Sharma, 1994), Binita (1995), Chaudhary (1998) and Chahal (1999) fermented cereal-legume-milk blend with *L. acidophilus* and reported that the contents of starch, total soluble and non-reducing sugars decreased significantly during fermentation.

Germination as well as its combination with probiotic fermentation brought about a significant change in the profile of available carbohydrates.

4.4.2.2 Non available

4.4.2.2.1 Dietary fibre

Table 4.9 revealed that non-germinated (raw) food mixtures of BWT and PWT contained 5.93, 4.93 g/100g, 2.95, 1.92 g/100 and 2.98, 3.01 g/100g, respectively total, soluble and insoluble dietary fibre contents. Both the mixtures had high amount of dietary fibre. It might be due to high fibre content of barley and pearl millet grains. Comparatively barley based food mixture had higher amount of total and soluble dietary fibre contents than pearl millet based food mixture. When the raw mixtures were subjected to autoclaving at 121⁰C for 15 min, a significant reduction was observed in total (10 and 16%) and insoluble (31 and 41%), whereas soluble fraction increased significantly ($P < 0.05$) after autoclaving.

Heat treatment might have resulted in conversion of insoluble dietary fibre to short length chains or units which could probably be precipitated along with

Table 4.9 : Effect of fermentation with *L. acidophilus* curd on dietary fibre content of indigenously developed BWT* and PWT* food mixtures (g/100g, on dry matter basis)

Processing treatments	BWT			PWT		
	Total	Soluble	Insoluble	Total	Soluble	Insoluble
Food mixtures						
(A) Non-germinated						
Raw mixture (control)	5.93 ± 0.05	2.95 ± 0.08	2.98 ± 0.18	4.93 ± 0.11	1.92 ± 0.04	3.01 ± 0.11
Autoclaved mixture	5.35 ± 0.07 (-10)	3.31 ± 0.11 (+12)	2.04 ± 0.08 (-31)	4.14 ± 1.07 (-16)	2.38 ± 0.21 (+23)	1.76 ± 0.07 (-41)
Autoclaved and fermented mixture	4.93 ± 0.09 (-16)	2.50 ± 0.25 (-15)	2.43 ± 0.05 (-18)	3.92 ± 0.08 (-20)	1.43 ± 0.05 (-25)	2.49 ± 0.05 (-17)
Mean	5.40	2.92	2.48	4.33	1.91	2.42
(B) Germinated						
Germinated raw mixture (control)	4.88 ± 0.17	2.00 ± 0.02	2.88 ± 0.06	3.12 ± 0.05	1.28 ± 0.11	1.84 ± 0.06
Germinated autoclaved mixture	4.16 ± 0.09 (-14)	2.70 ± 0.07 (+35)	1.89 ± 0.07 (-52)	2.84 ± 0.08 (-9)	1.95 ± 0.08 (+52)	0.69 ± 0.06 (-62)
Germinated, autoclaved and fermented mixture	3.88 ± 0.05 (-20)	1.00 ± 0.13 (-50)	2.48 ± 0.04 (-13)	2.61 ± 0.11 (-16)	0.99 ± 0.08 (-22)	1.44 ± 0.11 (-21)
Mean	4.30	1.90	2.41	2.85	1.40	1.32
CD (A)	0.17	0.23	0.14	0.79	0.20	0.15
CD (B)	0.21	0.27	0.15	0.93	0.36	0.18
CD (A x B)	0.27	0.39	0.25	0.32	0.33	0.24

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent increase (+) or decrease (-) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

soluble dietary fibre (Khanum *et al.*, 2000). The results of present study are in resemblance with those reported earlier in cooked vegetables and products where insoluble dietary fibre content decreased, while the soluble fraction was increased slightly (Vidal-Valverde *et al.*, 1992; Maria *et al.*, 1997).

When the autoclaved and cooled food mixtures were subjected to fermentation with *L. acidophilus* curd for 12 h, caused significant ($P < 0.05$) reduction in all dietary constituents of both the mixtures. Reduction was 16, 20 per cent, 15, 25 per cent and 18, 17 per cent, respectively in total, soluble and insoluble dietary fibre contents of BWT and PWT food mixtures.

Germinated (raw) BWT and PWT food mixtures contained total (4.88, 3.12 g/100g), soluble (2.00, 1.28 g/100g) and insoluble (2.88, 1.84 g/100g) dietary fibre contents. Dietary fibre contents of both the mixtures were significantly ($P < 0.05$) lower when compared to the values of non-germinated (raw) food mixtures. It might be due to the fact that on germination, all the dietary fibre components decreased but significant reduction was observed in soluble dietary fibre. This decrease is accompanied by a drop in galactan content. The breakdown of mucilage during germination was previously reported by EL-Mahdy and EL-Sebaiy (1983); Neerja and Rajyalakshmi (1996) and Hooda and Jood (2003). An enzyme β -galactosidase from germinated cereals and pulses partially attacks galactomannan to yield galactose. The decrease in the polysaccharide and mucilage content may be attributed to their breakdown and utilization by the growing sprouts.

It has been reported that soluble fibre is associated with cholesterol lowering and improved diabetic control whereas insoluble dietary fibre is associated with enhanced bowel functions (Yee, 1995). When the germinated BWT and PWT food mixtures were autoclaved, further reduction was observed in total (14 and 9%) and insoluble (52 and 62%), whereas significant ($P < 0.05$) increase was observed in soluble (35 and 52%) dietary fibre contents, respectively. A significant further reduction was observed in all the dietary fibre constituents of

BWT and PWT food mixtures when subjected to fermentation. On fermentation, all the dietary fibre constituents are hydrolyzed due to increased activity of hydrolyzing enzymes like cellulase, α -galactosidase etc.

4.4.2.2.2 β -gluĉan

Total, soluble and insoluble β -glucan contents of non-germinated (raw) BWT and PWT food mixtures were 3.12, 1.11 g/100 g, 1.56, 0.59 g/100g and 1.26, 0.52 g/100, respectively (Table 4.10). Barley based food mixture (BWT) had higher amount of total, soluble and insoluble β -glucan contents as compared to pearl millet based food mixture (PWT). When the raw mixtures were subjected to autoclaving at 121⁰C for 15 min, a significant reduction was observed in total (7 and 2%) and insoluble (12 and 30%) β -glucan contents whereas a significantly increase was observed in soluble β -glucan contents i.e. 15 and 19 per cent, respectively in both types of mixtures. It might be due to that heat treatment is responsible for conversion of total and insoluble β -glucan into soluble β -glucan which ultimately decreased the level of total and insoluble β -glucans. The results of present study are in agreement with those reported earlier (Sluijs *et al.*, 1999; Dhingra and Jood, 2001).

When the autoclaved mixtures were fermented with *L. acidophilus* curd, there was further reduction in total and insoluble β -glucan content. The significant reduction was 45, 33 per cent and 31, 55 per cent, respectively in both types of mixtures. However, soluble β -glucan was also significantly ($P < 0.05$) reduced by 32 and 11 per cent, respectively. It might be due to degradation of total and insoluble β -glucan contents into soluble β -glucan and soluble β -glucan is further hydrolyzed by hydrolyzing enzymes. As due to fermentation, the activity of various enzymes like carboxypeptidases and β -glucanases increased. Finally, degradation into glucose was carried out by the enzymes β -glucosidases, cellobiase and laminaribiase (Palmer *et al.*, 1989; Okokon and EtokAkpan, 1992).

The raw seeds of barley and pearl millet were subjected to germination and then these germinated flours were used for preparation of food mixtures like BWT and PWT. The developed food mixtures had significantly ($P < 0.05$) lower amount of total, soluble and insoluble β -glucans contents. The values were 1.99, 0.59 g/100g, 0.98, 0.33 g/100g and 1.01, 0.32 g/100g, in both type of mixtures. These results are in line with those reported earlier in barley and oats (Ellis *et al.*, 1997; Sluijs *et al.*, 1999). Okokon (1992) and Ellis *et al.* (1999) reported that β -D-glucan from the endosperm cell wall is hydrolyzed by a carboxypeptidase at the onset of germination. The dissolved β -D-glucan, which is of high viscosity is degraded by endo β 1-3, 1-4 and endo β 1-4 glucanases to give products such as cellobiose, laminaribiose and 3-O-D cellobiosyl-D-glucose. These were finally degraded to glucose by the β -glucosidases, cellobiase and laminaribiase.

When the germinated (raw) food mixtures were subjected to autoclaving, there was further decrease in total and insoluble β -glucan and significant ($P < 0.05$) increase in soluble β -glucan contents. The reduction was by about 20, 19 per cent in total β -glucan and 58, 75 per cent in insoluble β -glucan contents, respectively in both the mixtures. However, 15 and 17 per cent increase was observed in soluble β -glucan contents which decreased by about 40 and 33 per cent, respectively on fermentation for 12 h at 37⁰C. Similarly, the total and insoluble β -glucan contents also significantly ($P < 0.05$) decreased on fermentation. On fermentation, further reduction in total and insoluble β -glucan content was observed by about 50, 50 per cent and 60, 78 per cent, respectively in both the mixtures.

The soluble β -glucan has beneficial effect in reducing the level of cholesterol and blood glucose (Knuckles *et al.*, 1997; Kalra and Jood, 2000). It was also reported that soluble β -glucan in boiled and baked preparations was not as effective as in uncooked (Sluijs *et al.*, 1999).

Table 4.10 : Effect of fermentation with *L. acidophilus* curd on β -glucan content of indigenously developed BWT* and PWT* food mixtures (g/100 g, on dry matter basis)

Processing treatments	BWT			PWT		
	Total	Soluble	Insoluble	Total	Soluble	Insoluble
Food mixtures						
(A) Non-germinated						
Raw mixture (control)	3.12 \pm 0.12	1.56 \pm 0.12	1.26 \pm 0.06	1.11 \pm 0.07	0.59 \pm 0.01	0.52 \pm 0.01
Autoclaved mixture	2.90 \pm 0.08 (-7)	1.80 \pm 0.13 (+15)	1.10 \pm 0.08 (-12)	1.09 \pm 0.06 (-2)	0.73 \pm 0.02 (+19)	0.36 \pm 0.01 (-30)
Autoclaved and fermented mixture	2.00 \pm 0.09 (-45)	1.05 \pm 0.09 (-32)	0.95 \pm 0.05 (-31)	0.74 \pm 0.02 (-33)	0.52 \pm 0.00 (-11)	0.23 \pm 0.00 (-55)
Mean	2.67	1.47	1.10	0.98	0.61	0.37
(B) Germinated						
Germinated raw mixture (control)	1.99 \pm 0.12	0.98 \pm 0.04	1.01 \pm 0.02	0.59 \pm 0.06	0.33 \pm 0.01	0.32 \pm 0.01
Germinated autoclaved mixture	1.58 \pm 0.15 (-20)	1.16 \pm 0.08 (+15)	0.42 \pm 0.00 (-58)	0.48 \pm 0.03 (-19)	0.40 \pm 0.02 (+17)	0.08 \pm 0.00 (-75)
Germinated, autoclaved and fermented mixture	0.98 \pm 0.06 (-50)	0.58 \pm 0.01 (-40)	0.40 \pm 0.03 (-60)	0.29 \pm 0.01 (-50)	0.22 \pm 0.01 (-33)	0.07 \pm 0.01 (-78)
Mean	1.51	0.90	0.61	0.45	0.31	0.15
CD (A)	0.27	0.39	0.24	0.15	0.03	0.03
CD (B)	0.39	0.18	0.07	0.14	0.06	0.02
CD (A x B)	0.36	0.33	0.22	0.13	0.06	0.02

Values are mean \pm SE of three independent determinations

Figures in parentheses indicate percent increase (+) or decrease (-) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

4.5 B-complex vitamins

Non-germinated raw (control) BWT and PWT food mixtures contained 0.53, 0.46 mg/100g thiamine, 0.11, 0.13 mg/100g riboflavin and 2.81, 1.25 mg/100g niacin, respectively (Table 4.11). When the raw food mixtures were subjected to autoclaving at 121°C for 15 min, it caused significant ($P < 0.05$) reduction in all the three vitamins. Other workers also reported that autoclaving significantly decreased the B-complex vitamins in fruits and vegetables (Gopalan *et al.*, 1991; Duodu *et al.*, 1999) and legumes (Riddoch *et al.*, 1998). Previous results are in agreement with those reported in present study.

Fermentation for 12 h of the autoclaved BWT and PWT slurries caused increment in all the three vitamins. Increase observed was 10.9 per cent, 8.8 per cent and 5.4 per cent, respectively in thiamine, riboflavin and niacin contents. Among the three vitamins, increment observed was highest in thiamine contents of both type of mixtures.

Germinated (raw) BWT and PWT food mixtures contained significantly ($P < 0.05$) higher amount of thiamine, riboflavin and niacin contents as compared to non-germinated food mixtures. The values were 1.16, 0.86 mg/100g thiamine, 0.32, 0.31 mg/100g riboflavin and 3.98, 2.30 mg/100g niacin, respectively in both types of mixtures. The increase observed was 1-2 folds in thiamine, riboflavin and niacin contents of both mixtures over non-germinated mixtures. When the germinated mixtures were subjected to autoclaving, it caused significant reduction in all the three vitamins. However, when the germinated autoclaved food mixtures were fermented with *L. acidophilus* curd for 12 h at 37°C, it caused further enhancement in thiamine, riboflavin and niacin contents. The increase was 9 and 6 per cent in riboflavin and 7 and 5 per cent in niacin contents of both mixtures whereas comparatively highest reduction was

Table 4.11 : Effect of fermentation with *L. acidophilus* curd on B-complex vitamins of indigenously developed BWT* and PWT* food mixtures (mg/100g, on dry matter basis)

Processing treatments	BWT			PWT		
	Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin
Food mixtures						
(A) Non-germinated						
Raw mixture (control)	0.53 ± 0.01	0.11 ± 0.03	2.81 ± 0.11	0.46 ± 0.01	0.13 ± 0.01	1.25 ± 0.05
Autoclaved mixture	0.48 ± 0.02 (-9)	0.10 ± 0.01 (-9)	2.06 ± 0.05 (-27)	0.40 ± 0.05 (-13)	0.12 ± 0.25 (-8)	1.13 ± 0.07 (-10)
Autoclaved and fermented mixture	0.56 ± 0.01 (+10)	0.12 ± 0.01 (+8)	2.95 ± 0.14 (+5)	0.50 ± 0.02 (+9)	0.14 ± 0.01 (+8)	1.30 ± 0.19 (+4)
Mean	0.41	0.10	2.42	1.06	0.12	1.17
(B) Germinated						
Germinated raw mixture (control)	1.16 ± 0.02	0.32 ± 0.05	3.98 ± 0.05	0.86 ± 0.01	0.31 ± 0.03	2.30 ± 0.14
Germinated autoclaved mixture	1.05 ± 0.01 (-9)	0.29 ± 0.02 (-9)	3.35 ± 0.02 (-16)	0.81 ± 0.05 (-58)	0.29 ± 0.02 (-6)	2.10 ± 0.06 (-9)
Germinated, autoclaved and fermented mixture	1.30 ± 0.02 (+12)	0.35 ± 0.02 (+9)	4.26 ± 0.37 (+7)	0.92 ± 0.11 (+7)	0.33 ± 0.03 (+6)	2.41 ± 0.11 (+5)
Mean	0.92	0.29	3.46	0.69	0.29	2.09
CD (A)	0.02	0.02	0.30	0.09	0.18	0.18
CD (B)	0.03	0.03	0.37	0.09	0.21	0.25
CD (A x B)	0.04	0.03	0.51	0.10	0.30	0.32

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent decrease (-) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

observed in thiamine content i.e. 12 and 7 per cent, respectively in BWT and PWT food mixtures. Similarly, Khetarpaul (1988) also reported decrease in thiamine content of pearl millet by pure culture fermentation of *L.acidophilus* whereas the concentration of thiamine improved almost 2 to 3 fold when fermentation was carried out by *S.diastatiens* and *S. Cervisiae*, respectively.

Gilay and Fields (1981) also reported 1.8 folds increase in thiamine, riboflavin and niacin contents of corn sprouts on germination but when the germinated corn meal was subjected to fermentation, there was non-significant effect on riboflavin, thiamine and niacin contents. On the other hand, Hamad (1978) reported that after fermentation process, the riboflavin content of corn meal stayed the same, whereas the niacin content decreased from 1.97 mg/100g to 1.72 mg/100g. Hamad (1978) used 37⁰C as the fermenting temperature and a 4-days for fermentation period whereas Gilay and Fields (1981) used 25⁰C as fermenting temperature and 5 days for fermentation period, however, in the present study fermentation was carried out for 12 h at 37⁰C. These differences in the previous and present findings can be attributed to the difference in substrate and microflora employed in the fermentation processes.

4.6 Protein fractions

Various protein soluble fractions of indigenously developed BWT and PWT food mixture are given in Tables 4.12 and 4.13. BWT raw food mixture had highest content of prolamins (6.30%) followed by globulin (2.89%), glutelin (2.82%) and albumin (0.99%). Similarly, PWT raw food mixture contained highest amount of prolamin fraction (4.45%) followed by globulin (3.75%), glutelin (1.80%) and albumin (0.45%). After the extraction of various fractions, the protein recovery in both mixtures was 0.35 and 0.25 per cent, respectively. Similarly, Chen and Bushuk (1970) and Dhingra (2001) also observed maximum content of prolamin fraction in barley and wheat flour.

When the raw mixtures were subjected to autoclaving at 121⁰C for 15 min, it caused significant reduction in all protein fractions. The reductions varied from

Table 4.12 : Effect of fermentation with *L. acidophilus* curd on protein fractions of indigenously developed BWT* food mixture (g/100 g, on dry matter basis)

Processing treatments	Albumin	Globulin	Prolamin	Glutelin *	Residue
Food mixtures					
(A) Non-germinated					
Raw mixture (control)	0.99 ± 0.08	2.89 ± 0.10	6.30 ± 0.25	2.82 ± 0.19	0.35 ± 0.01
Autoclaved mixture	0.92 ± 0.03 (-7)	2.75 ± 0.20 (-5)	6.19 ± 0.31 (-2)	2.75 ± 0.25 (-2)	0.30 ± 0.01 (-14)
Autoclaved and fermented mixture	0.89 ± 0.09 (-10)	2.63 ± 0.21 (-9)	4.39 ± 0.12 (-30)	2.72 ± 0.39 (-1)	0.28 ± 0.03 (-20)
Mean	0.93	2.75	5.62	2.76	0.30
(B) Germinated					
Germinated raw mixture (control)	1.09 ± 0.01	2.99 ± 0.03	6.45 ± 0.20	4.99 ± 0.39	0.38 ± 0.01
Germinated autoclaved mixture	1.00 ± 0.01 (-8)	2.89 ± 0.05 (-4)	5.30 ± 0.25 (-18)	4.75 ± 0.42 (-5)	0.30 ± 0.01 (-21)
Germinated, autoclaved and fermented mixture	1.01 ± 0.05 (-7)	2.64 ± 0.12 (-12)	5.15 ± 0.39 (-20)	4.72 ± 0.50 (-6)	0.26 ± 0.02 (-31)
Mean	1.03	2.84	5.63	2.71	0.31
CD (A)	0.18	0.54	0.87	0.75	0.05
CD (B)	0.06	0.32	1.05	1.42	0.03
CD (A x B)	0.15	0.51	0.95	1.41	0.09

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent decrease (-) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

Table 4.13 : Effect of fermentation with *L. acidophilus* curd on protein fractions of indigenously developed PWT* food mixture (g/100 g, on dry matter basis)

Processing treatments	Albumin	Globulin	Prolamin	Glutelin	Residue
Food mixtures					
(A) Non-germinated					
Raw mixture (control)	0.45 ± 0.01	3.75 ± 0.08	4.45 ± 0.12	1.80 ± 0.01	0.25 ± 0.01
Autoclaved mixture	0.42 ± 0.01 (-11)	3.05 ± 0.07 (-18)	4.03 ± 0.08 (-9)	1.70 ± 0.01 (-3)	0.20 ± 0.01 (-20)
Autoclaved and fermented mixture	0.40 ± 0.02 (-15)	3.38 ± 0.10 (-10)	3.29 ± 0.03 (-26)	1.60 ± 0.02 (-11)	0.18 ± 0.01 (-28)
Mean	0.42	3.39	3.92	1.70	0.21
(B) Germinated					
Germinated raw mixture (control)	0.65 ± 0.01	3.95 ± 0.12	4.85 ± 0.10	1.89 ± 0.01	0.29 ± 0.01
Germinated autoclaved mixture	0.63 ± 0.02 (-3)	3.85 ± 0.20 (-3)	4.29 ± 0.22 (-11)	1.75 ± 0.02 (-7)	0.20 ± 0.01 (-31)
Germinated, autoclaved and fermented mixture	0.58 ± 0.03 (-10)	3.40 ± 0.89 (-13)	3.85 ± 0.30 (-20)	1.65 ± 0.05 (-12)	0.21 ± 0.02 (-27)
Mean	0.62	3.73	4.33	1.76	0.23
CD (A)	0.6	0.24	0.27	0.03	0.03
CD (B)	0.6	1.95	0.65	0.09	0.03
CD (A x B)	0.9	2.25	1.15	0.12	0.06

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent decrease (-) over control values

*PWT food mixture contains barley flour, whey powder and tomato pulp

2 to 14 and 3 to 18 per cent, respectively in protein fractions of both mixtures. When the autoclaved mixtures were subjected to fermentation, it caused further reduction in all protein fractions. Among the fractions, prolamin was found to be affected maximum on fermentation with *L. acidophilus* curd in both types of mixtures.

On the other hand, germinated BWT and PWT food mixtures exhibited higher values of all fractions as compared to non-germinated food mixtures. The values were 1.09 and 0.65 per cent (albumin), 2.99 and 3.95 per cent (globulin), 6.45 and 4.85 per cent (prolamin), 4.99 and 1.89 per cent (glutelin), respectively in both mixtures but when the germinated food mixtures were subjected to autoclaving at 121⁰C for 15 min, there was significant ($P < 0.05$) reduction in all protein fractions. Reduction varied from 4 to 18 and 3 to 11 per cent, respectively in both types of mixtures. Maximum reduction was observed in prolamin fraction of both types of mixtures.

Fermentation caused further reduction in all the protein fractions but again maximum reduction was observed in prolamin fraction i.e. 20 per cent in both mixtures. Heat treatment like autoclaving caused reduction in all fractions whereas germination improved the contents of all protein fractions.

Prolamin is the highest fraction in cereals but is deficient in lysine. In the present study, there was maximum reduction in prolamin fractions as compared to other fractions thereby resulting in increase in the lysine content on germination and fermentation.

4.7 Total lysine

Total lysine contents are presented in Table 4.14. The quality of protein is evaluated by its essential amino acids like lysine. The total lysine contents of non-germinated (raw) BWT and PWT food mixtures were 2.98 and 2.53 g/100 g protein, on dry matter basis, respectively. When the raw mixtures were subjected to autoclaving, a significant reduction in total lysine contents was observed. The

Table 4.14 : Effect of fermentation with *L. acidophilus* curd on total lysine content of indigenously developed BWT* and PWT* food mixtures (g/100 g protein, on dry matter basis)

Processing treatments	BWT	PWT
Food mixtures		
(A) Non-germinated		
Raw mixture (control)	2.98 ± 0.10	2.53 ± 0.05
Autoclaved mixture	1.68 ± 0.05 (-44)	1.88 ± 0.15 (-26)
Autoclaved and fermented mixture	3.89 ± 0.11 (+31)	3.92 ± 0.11 (+55)
Mean	2.85	2.77
(B) Germinated		
Germinated raw mixture (control)	5.05 ± 0.06	4.88 ± 0.69
Germinated autoclaved mixture	3.43 ± 0.05 (-32)	3.34 ± 0.57 (-32)
Germinated, autoclaved and fermented mixture	5.89 ± 0.07 (+17)	5.35 ± 0.09 (+10)
Mean	4.79	4.19
CD (A)	0.14	0.17
CD (B)	0.16	0.18
CD (A x B)	0.25	0.28

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent increase (+) or decrease (-) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

reduction was 44 per cent in BWT and 26 per cent in PWT food mixtures. It might be due to denaturation of protein at high temperature.

When these autoclaved slurries were subjected to fermentation, there was significant ($P < 0.05$) increase in total lysine contents in both the mixtures compared to their non-germinated (raw) mixtures. It might be due to the fact that fermenting microbes have capability to synthesize essential amino acids. Similar results were also reported by various workers in processed products (Sharma and Chauhan, 2000; Hooda, 2002). All processing treatments like germination and fermentation caused significant improvement in lysine contents.

Germinated (raw) food mixtures contained significantly ($P < 0.05$) higher total lysine contents as compared to non-germinated (raw) food mixtures. The values were observed as 5.05 and 4.88 g/100 g protein in germinated BWT and PWT food mixtures. When the germinated mixtures were autoclaved, there was significant reduction in total lysine contents i.e. 32 and 31 per cent, respectively in BWT and PWT food mixtures. On the other hand, when germinated and autoclaved mixtures were fermented for 12 h, it caused significant increase in total lysine contents. The increase was 17 and 10 per cent, respectively in both types of mixtures. This may be due to the increase in microbial enzyme activity and protein hydrolysis during fermentation (Hamad and Fields, 1979). The total free amino acids increase rapidly by about 4-5 folds during germination and doubled at 18 h fermentation, reaching a maximum at 36 h of fermentation. The increase in free amino acid content is favourable as the protein quality of food depends not only on its amino acid composition but also on the availability of these amino acids (Hamad and Fields, 1979; Sripriya *et al.*, 1997).

Kazanas and Fields (1981) and Zamora and Fields (1979) observed significant increase in lysine, leucine, isoleucine, methionine and tryptophan contents when sorghum, cowpea and chickpea were fermented. The present data are in agreement with previous findings that fermentation makes amino acids more available by the proteolytic flora in the fermentation.

4.8 Antinutritional factors

4.8.1 Phytic acid

Raw (non-germinated) BWT and PWT food mixtures contained 282.20 and 327.42 mg/100g phytic acid which was significantly reduced over the control values on autoclaving (Tables 4.15 and 4.16, Fig.4.3). Autoclaving reduced phytic acid contents by about 17 and 21 per cent, respectively. Further reduction occurred in the level of phytic acid when these food mixtures were fermented with probiotic curd. The fermented blends had 99.14 and 163.42 mg/100g phytic acid contents, respectively, which means that phytic acid content was reduced by about 64 and 50 per cent, respectively over the control values.

Germinated (raw) BWT and PWT food mixtures contained significantly ($P < 0.05$) lower values of phytic acid as compared to their non-germinated values. The values were 200.15 and 280.24 mg/100g, respectively. This may be due to increased activity of phytase enzyme on germination which may be partly responsible for reducing the phytic acid content in the germinated food mixtures (Sripriya *et al.*, 1997). Autoclaving caused further reduction in germinated food mixtures.

On the other hand, when germinated and autoclaved food mixtures were fermented at 37⁰C for 12 h, it caused significant reduction in phytic acid contents of BWT and PWT fermented food mixtures. The reduction was observed by about 63 and 68 per cent, respectively.

Phytic acid is known to be heat resistant. The reduction in the level of phytic acid in the autoclaved food mixtures may not be due to destruction of the compound but perhaps, due to its ability to form complex with protein and minerals which may not be extractable. The decrease is, therefore, apparent and not true (Kratzer, 1965). Similar reduction in phytic acid content during autoclaving and cooking has been reported in various cereals and legumes (Sharma, 1994; Binita, 1995; Negi, 1999; Grewal, 2003).

Loss of phytic acid during germination may be due to hydrolytic activity of enzyme phytase (Duhan *et al.*, 2002; Grewal and Jood, 2006).

A wide range of microflora have been reported to possess phytase activity which may partly be responsible for reduction in phytic acid content (Lopez *et al.*, 1983). Optimal temperature for phytase activity from plants and microbial sources has been known to range between 35-40°C. As fermentation with pure cultures of *L. acidophilus*, *L. fermentum* and *B. bifidum* was carried out at 37°C, hence this may also be the optimal temperature for microbial phytase to hydrolyse phytic acid content of the autoclaved food mixtures and reduction in the phytic acid content in the fermented product may be attributed to its action. Reduction in the phytic acid content during fermentation has been reported in various fermented blends and their products like *rabadi* prepared from pearl millet (Khetarpaul and Chauhan, 1990a), barley and wheat (Gupta and Khetarpaul, 1993), *wadies* (Yadav and Khetarpaul, 1994) *dosa*, *idli and wadi* prepared from rice bean and fababean (Saharan, 1994), fermented rice defatted soy flour blend (Goyal and Khetarpaul, 1995) and *L. acidophilus* fermented blend containing barley-sprouted green gram, skimmed milk powder, banana paste and tomato pulp (Chaudhary, 1998).

4.8.2 Polyphenols

Polyphenol content of raw non-germinated BWT and PWT food mixtures were 396.21 and 304.66 mg/100g, respectively, which decreased significantly ($P < 0.05$) after autoclaving of raw mixtures. The decrease was observed by about 12 and 15 per cent, respectively (Tables 4.15 and 4.16, Fig.4.4). Fermentation with *L. acidophilus* curd caused further reduction in polyphenol content of BWT and PWT food mixtures. The reduction was 37 and 48 per cent, respectively in both types of mixtures.

On the other hand, when barley and pearl millet germinated flours were mixed with whey powder and tomato pulp (2:1:1 w/w), the developed mixtures exhibited lower values of polyphenols as compared to the raw mixtures (non-germinated). The values were 280.06 and 263.27 mg/100g in germinated mixtures,

which further reduced on autoclaving at 121⁰C for 15 min. The reduction was 15 and 16 per cent, respectively. Reduction observed was almost same in both the mixtures. However maximum reduction was observed when these germinated autoclaved food mixtures were fermented at 37⁰C for 12 h. The reduction was observed by about 50 and 64 per cent, respectively in both type of mixtures. Maximum reduction was due to cumulative effect of germination, autoclaving and fermentation with *L. acidophilus* curd.

A decreased amount of polyphenols in autoclaved food mixtures could result from their reduced extractability, change in chemical reactivity (Satwadhar *et al.*, 1981) due to formation of insoluble complex between tannins and proteins. Cooking and autoclaving has been reported to decrease polyphenol contents of several legumes (Negi, 1999; Grewal, 2003) and cereals and their blends (Sharma, 1994; Chaudhary, 1998; Chahal, 1999).

Decrease in polyphenols during germination may be ascribed to presence of polyphenol oxidase and enzymatic hydrolysis (Jood *et al.*, 1998; Negi, 1999).

The diminishing effect of fermentation on polyphenols may be due to the activity of polyphenol oxidase presenting the food grain or microflora. A reduction in polyphenolic content during fermentation has been reported earlier by various workers in fermented blends (Binita, 1995; Chaudhary, 1998; Chahal, 1999).

4.8.3 Amylase inhibitor activity

Raw mixtures of BWT and PWT contained amylase inhibitor activity i.e. 135.65 and 78.75 AIU/g, respectively (Tables 4.15 and 4.16, Fig.4.5). Pearl millet based food mixture (PWT) has significantly ($P < 0.05$) lower values of amylase inhibitor activity as compared to barley based food mixture (BWT). This might be due to that barley has high amylase inhibitor activity (Kalra, 1996). When these mixtures were autoclaved, the amylase inhibitor activity reduced by about 37 and 38 per cent, respectively. This reduction was further increased on fermentation. The fermented BWT and PWT food mixtures exhibited 35.01 and 28.76 AIU/g,

Table 4.15 : Effect of fermentation with *L. acidophilus* curd on antinutrient content of indigenously developed BWT* food mixture (on dry matter basis)

Processing treatments	Phytic acid (mg/ 100 g)	Polyphenols (mg/ 100 g)	Amylase inhibitor activity (AIU/g)
Food mixtures			
(A) Non-germinated			
Raw mixture (control)	282.20 ± 17.32	396.21 ± 5.63	135.65 ± 6.47
Autoclaved mixture	232.23 ± 11.56 (-17)	348.22 ± 8.51 (-12)	85.60 ± 5.71 (-37)
Autoclaved and fermented mixture	99.14 ± 4.65 (-64)	249.50 ± 11.44 (-37)	35.01 ± 0.48 (-74)
Mean	204.52	331.31	85.42
(B) Germinated			
Germinated raw mixture (control)	200.15 ± 5.81	280.06 ± 48.79	60.38 ± 4.36
Germinated autoclaved mixture	149.53 ± 11.50 (-25)	238.33 ± 6.43 (-15)	39.11 ± 2.30 (-35)
Germinated, autoclaved and fermented mixture	73.23 ± 0.62 (-63)	150.44 ± 2.70 (-50)	12.10 ± 0.17 (-80)
Mean	140.97	216.27	37.19
CD (A)	18.10	17.48	7.23
CD (B)	22.17	15.91	8.85
CD (A x B)	19.72	20.92	12.52

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent decrease (-) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

Table 4.16 : Effect of fermentation with *L. acidophilus* curd on antinutrient content of indigenously developed PWT* food mixture (on dry matter basis)

Processing treatments	Phytic acid (mg/100 g)	Polyphenols (mg/100 g)	Amylase inhibitor activity (AIU/g)
Food mixtures			
(A) Non-germinated			
Raw mixture (control)	327.42 ± 17.17	304.66 ± 10.97	78.75 ± 5.77
Autoclaved mixture	258.48 ± 3.10 (-21)	257.47 ± 18.78 (-15)	48.55 ± 3.17 (-38)
Autoclaved and fermented mixture	163.42 ± 2.86 (-50)	169.38 ± 5.77 (-48)	28.76 ± 1.12 (-64)
Mean	249.77	243.83	52.02
(B) Germinated			
Germinated raw mixture (control)	280.24 ± 5.75	263.27 ± 11.25	49.66 ± 3.17
Germinated autoclaved mixture	178.34 ± 6.18 (-36)	220.18 ± 11.83 (-16)	30.63 ± 2.75 (-38)
Germinated, autoclaved and fermented mixture	88.34 ± 0.51 (-68)	92.28 ± 5.87 (-64)	13.50 ± 2.34 (-73)
Mean	182.31	191.23	31.26
CD (A)	14.23	20.63	5.40
CD (B)	17.43	25.27	6.61
CD (A x B)	24.00	32.40	9.35

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent decrease (-) over control values

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

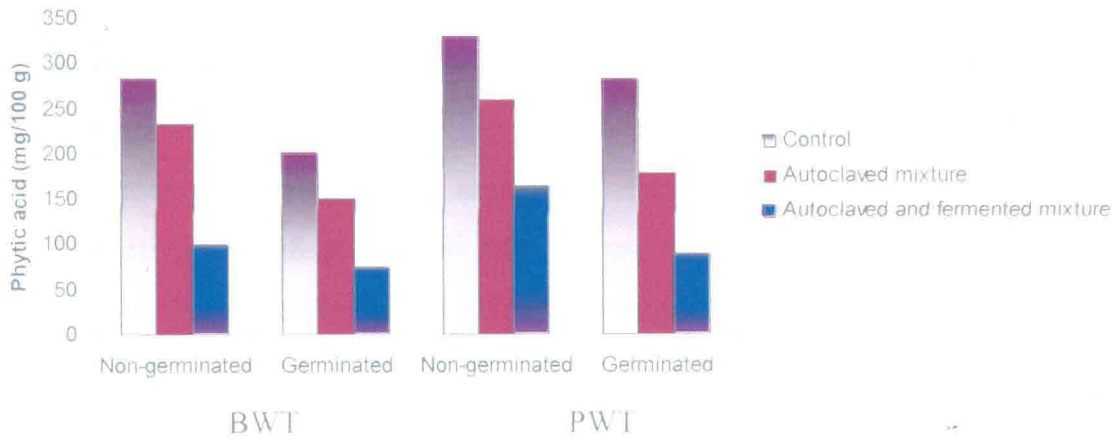


Fig. 4.3. Effect of fermentation with *L. acidophilus* curd on phytic acid of indigenously developed BWT and PWT food mixtures.

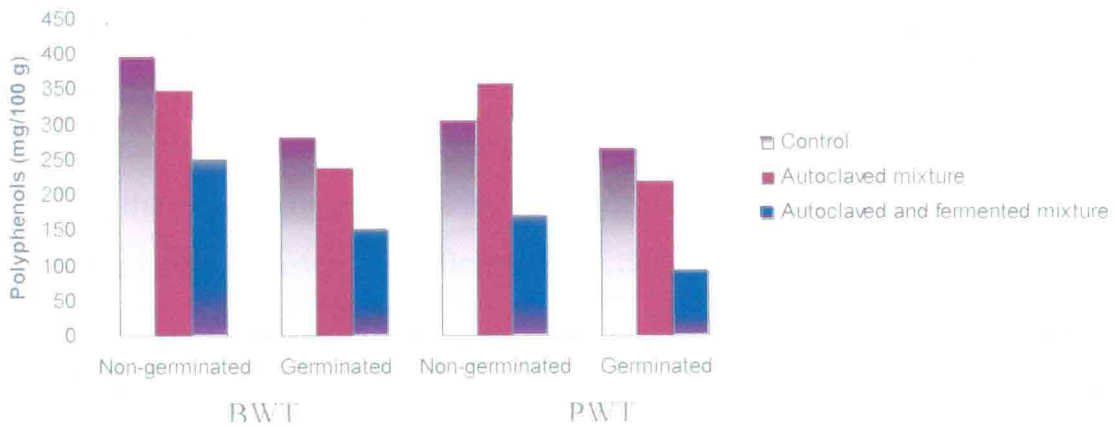


Fig. 4.4. Effect of fermentation with *L. acidophilus* curd on polyphenols of indigenously developed BWT and PWT food mixtures.

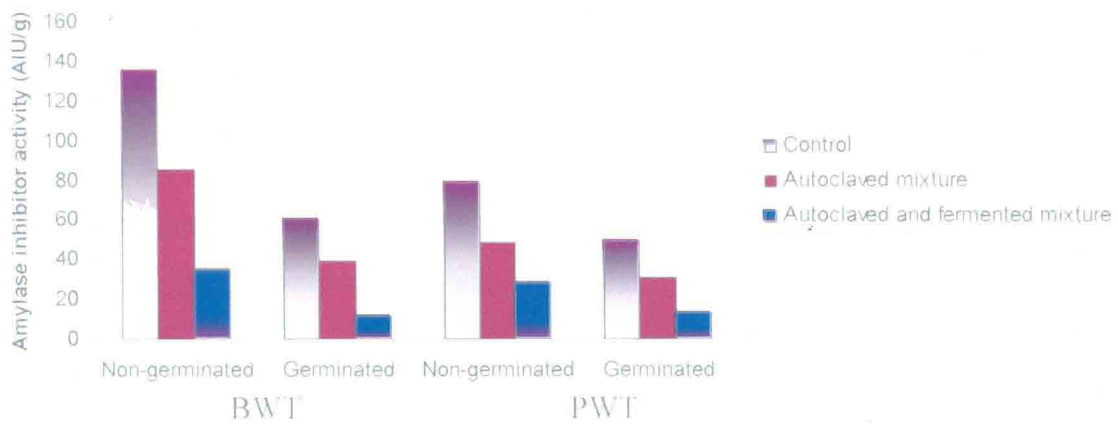


Fig. 4.5. Effect of fermentation with *L. acidophilus* curd on amylase inhibitor activity of indigenously developed BWT and PWT food mixtures.

respectively which were about 74 and 64 per cent lower values as compared to their raw mixtures.

Germinated (raw) BWT and PWT food mixtures contained amylase inhibitor activity 60.38 and 49.66 AIU/g, respectively, which was significantly lower as compared to the values of raw mixtures. When these germinated food mixtures were autoclaved, further reduction was observed. On the other hand, when these autoclaved mixtures were fermented with *L. acidophilus* curd at 37°C for 12 h, it caused further significant reduction in amylase inhibitor activity. Maximum reduction on fermentation may be due to cumulative effect of germination, autoclaving and fermentation. The reduction was 80 and 73 per cent, respectively in both types of mixtures. Among all the antinutrients, fermentation caused maximum reduction in amylase inhibitor activity.

All the processing and cooking methods including germination, autoclaving and fermentation caused significant reduction in amylase inhibitor activity (Bhatty, 1986; Dhingra, 2001).

4.9 *In vitro* digestibility

4.9.1 *In vitro* protein digestibility

The protein digestibility of a raw BWT and PWT food mixtures were 42.05 and 45.30 per cent, respectively (Table 4.17 and Fig 4.6). Autoclaving as well as fermentation significantly ($P < 0.05$) improved the protein digestibility of both the food mixtures. Protein digestibility was increased by about 22 and 19 per cent in autoclaved mixtures and by 62 and 67 per cent, respectively in fermented blends.

Germinated food mixtures contained 66.89 and 71.13 per cent protein digestibility, respectively which was significantly higher as compared to the raw mixtures. Autoclaving caused further improvement in protein digestibility in both the mixtures. The increase was observed by about 9 and 11 per cent, respectively. The enhancement in protein digestibility due to probiotic fermentation in BWT and PWT food mixtures was 36 and 34 per cent, respectively over the control values.

Table 4.17 : Effect of fermentation with *L. acidophilus* on *in vitro* protein (%) and starch (mg maltose released/g meal) digestibility of indigenously developed BWT* and PWT* food mixtures (on dry matter basis)

Processing treatments	BWT		PWT	
	<i>In vitro</i> protein digestibility	<i>In vitro</i> starch digestibility	<i>In vitro</i> Protein digestibility	<i>In vitro</i> starch digestibility
Food mixtures				
(A) Non-germinated				
Raw mixture (control)	42.05 ± 2.3	26.92 ± 0.57	45.30 ± 1.73	20.50 ± 2.77
Autoclaved mixture	52.25 ± 1.93 (22)	39.38 ± 1.15 (46)	53.99 ± 4.04 (19)	30.60 ± 3.54 (50)
Autoclaved and fermented mixture	69.18 ± 1.15 (62)	45.00 ± 0.2 (67)	75.11 ± 2.60 (67)	33.89 ± 2.88 (65)
Mean	54.49	37.10	58.13	28.33
(B) Germinated				
Germinated raw mixture (control)	66.89 ± 2.30	48.97 ± 1.15	71.13 ± 3.48	41.93 ± 5.77
Germinated autoclaved mixture	72.99 ± 5.77 (9)	65.83 ± 1.15 (34)	78.17 ± 5.76 (11)	58.33 ± 1.73 (38)
Germinated, autoclaved and fermented mixture	90.73 ± 11.54 (36)	89.33 ± 5.77 (82)	94.97 ± 5.76 (34)	82.53 ± 4.04 (98)
Mean	76.87	68.04	81.42	60.86
CD (A)	9.80	4.45	7.44	11.48
CD (B)	12.01	4.56	9.11	14.06
CD (A x B)	16.58	7.50	12.55	19.38

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent increase (+) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

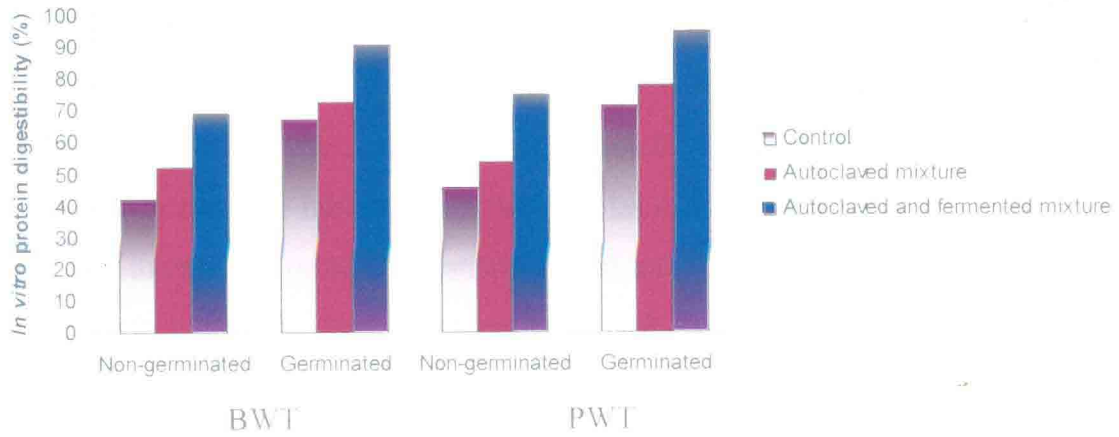


Fig. 4.6. Effect of fermentation with *L. acidophilus* curd on *In vitro* protein digestibility of indigenously developed BWT and PWT food mixtures.

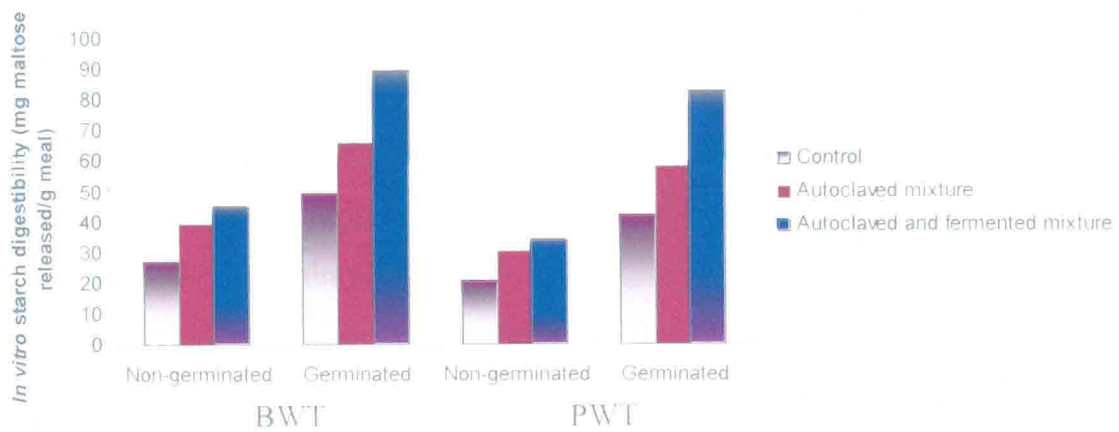


Fig. 4.7. Effect of fermentation with *L. acidophilus* curd on *In vitro* starch digestibility of indigenously developed BWT and PWT food mixtures.

The significant variation in *in vitro* protein digestibility of BWT and PWT food mixtures could be explained by highly significant negative correlation obtained between phytic acid and polyphenols and negative correlation was observed with amylase inhibitor activity (Tables 4.18 and 4.19).

The increase in protein digestibility upon autoclaving of various food mixtures may be attributed to the loss of antinutrients including phytic acid and polyphenols (Grewal, 2003), inactivation or destruction of trypsin inhibitors (Parihar *et al.*, 1993) and opening up of the protein structure through denaturation. Reduction in the polyphenol and phytic acid observed in present study and in previous data may be responsible for improvement in protein digestibility. On autoclaving, there may be degradation of high molecular weight proteins which are easily degraded by enzymes (Negi, 1999; Grewal, 2003).

Germination increases protein digestibility of cereals and pulses (Jood *et al.*, 1998; Chaudhary, 1999; Saharan *et al.*, 2002) as seed proteins are mobilized and antimetabolites including protease inhibitors, phytate, polyphenols etc. are catabolized during germination.

Improvement in protein digestibility of fermented blends is mainly because of that fermenting microflora may produce some proteolytic enzymes during fermentation. High proteinase activity are responsible for breakdown of protein to peptides and amino acid resulting in improved protein digestibility (Kao and Robinson, 1978). As phytic acid known to inhibit the proteolytic enzyme (Knuckles *et al.*, 1985) was considerably reduced during fermentation which may explain partly the improvement in protein digestibility during fermentation.

Similar results were also observed by various workers in germinated and fermented foods (Saharan, 1994; Yadav and Khetarpaul, 1994; Sharma and Khetarpaul, 1997; Uрга *et al.*, 1997b; Chaudhary, 1999).

4.9.2 *In vitro* starch digestibility

The *in vitro* starch digestibility (expressed as mg maltose released/g meal) of unprocessed (raw) BWT and PWT food mixtures were 26.92 and 20.50,

Table 4.18 : Correlation coefficients of *in vitro* digestibility of protein and starch with phytic acid, polyphenol and amylase inhibitor activity of indigenously developed BWT* food mixture

Processing treatments	Phytic acid vs		Polyphenols vs		Amylase inhibitor activity vs	
	Protein digestibility	Starch digestibility	Protein digestibility	Starch digestibility	Protein digestibility	Starch digestibility
Food mixtures						
(A) Non-germinated						
Raw mixture (control)	-0.9625**	-0.9520*	-0.9790**	-0.8396*	-0.8295	-0.9250*
Autoclaved mixture	-0.9980**	-0.9690*	-0.9890**	-0.7387*	-0.7982	-0.9890**
Autoclaved and fermented mixture	-0.9272*	-0.8992*	-0.9736*	-0.8580*	-0.8920	-0.1000**
(B) Germinated						
Germinated raw mixture (control)	-0.9254*	-0.8200*	-0.9825*	-0.8700*	-0.8649	-0.9875*
Germinated autoclaved mixture	-0.8992*	-0.8020*	-0.9438	-0.8928*	-0.7825	-0.9998*
Germinated, autoclaved and fermented mixture	-0.8825*	-0.9020	-0.9672*	-0.8652*	-0.7926	-0.9282*

*Significant at 5 per cent level

**Significant at 10 per cent level

* BWT food mixture contains barley flour, whey powder and tomato pulp

Table 4.19 : Correlation coefficients of *in vitro* digestibility of protein and starch with phytic acid, polyphenols and amylase inhibitor activity of indigenously developed PWT* food mixture

Processing treatments	Phytic acid vs		Polyphenols vs		Amylase inhibitor activity vs	
	Protein digestibility	Starch digestibility	Protein digestibility	Starch digestibility	Protein digestibility	Starch digestibility
Food mixtures						
(A) Non-germinated						
Raw mixture (control)	-0.9234**	-0.9245*	-0.9875**	-0.8925*	-0.7254	-0.8040*
Autoclaved mixture	-0.9872**	-0.8876*	-0.9992**	-0.8870*	-0.6543	-0.8245*
Autoclaved and fermented mixture	-0.9032**	-0.8975*	-0.9645*	-0.8996*	-0.6934	-0.8430*
(B) Germinated						
Germinated raw mixture (control)	-0.9890*	-0.8456*	-0.9672*	-0.8450*	-0.6870	-0.8142*
Germinated autoclaved mixture	-0.8992*	-0.8245*	-0.9315*	-0.8916*	-0.5890	-0.8672*
Germinated, autoclaved and fermented mixture	-0.9872*	-0.9212*	-0.9989*	-0.8240*	-0.6750	-0.8898*

*Significant at 5 per cent level

**Significant at 10 per cent level

* PWT food mixture contains pearl millet flour, whey powder and tomato pulp

respectively (Tables 4.17 and Fig. 4.7). Autoclaving of the raw blend at 121°C for 15 min improved the starch digestibility significantly ($P < 0.05$). The increase was observed by about 46 and 50 per cent, respectively. When the food mixtures were fermented with *L. acidophilus* curd, the digestibility of starch increased further. The increase was about 67 and 65 per cent, respectively in both the mixtures.

On the other hand, germinated food mixtures of BWT and PWT contained significantly higher contents of starch digestibility over the raw mixtures. The values were 48.97 and 41.93 mg maltose released/g meal as compared to raw mixtures which contained 26.92 and 20.50 mg maltose released/g meal. Autoclaving of germinated mixtures caused further improvement. The improvement was observed by the about 34 and 38 per cent, respectively. Whereas, when these mixtures were fermented, it significantly caused further improvement in *in vitro* starch digestibility. It may be due to the cumulative effect of germination, autoclaving and fermentation. The increase was observed on fermentation by about 82 and 98 per cent, respectively, over control values.

A significant ($P < 0.05$) negative correlation of phytic acid and polyphenols with digestibility (*in vitro*) indicates negative effect of these antinutrients on starch digestibility (Table 4.18 and 4.19). A highly significant ($P < 0.05$) negative correlation was observed with amylase inhibitor activity.

Enhanced starch digestibility of cooked cereal and legume starches by α -amylase could be attributed to the swelling and rupturing of starch granules which facilitate more randomized configuration of α -amylase to affect amylolytic hydrolysis, disintegration of various plant food components during cooking and inactivation of α -amylase inhibitors (Boralkar and Reddy, 1985). Improvement in starch digestibility on boiling and autoclaving has been reported by earlier workers in various cereals [wheat and barley (Gupta and Khetarpaul, 1993), products from rice bean and faba bean (Saharan, 1994) and legumes (Jood *et al.*, 1998; Negi, 1999; Grewal and Jood, 2006)].

Increase in starch digestibility on germination may be due to increased activity of α -amylase and elimination of amylase inhibitor activity (Bernfeld, 1962). On fermentation, increase in starch digestibility may be related to enzymatic properties of microbes which ferment the substrate. The presence of α -amylase in the fermenting bacteria was indicated by Bernfeld (1962). The enzyme brings about cleavage of amylose and amylopectin to approximately six parts of maltose and five parts of glucose. Complete elimination of α -amylase inhibitor activity in most of the fermentations contributed to improved starch digestibility. Amylolysis has been reported to be inhibited by phytic acid (Thompson and Yoon, 1984) and hence the reduction in the phytate content of fermented production during probiotic fermentation as observed in this study which may account for improvement in starch digestibility of the fermented blends. Similar results in fermented blends have also been reported by various workers (Yadav and Khetarpaul, 1995; Chaudhary, 1998; Chahal, 1999).

4.10 Minerals

4.10.1 Total minerals

The contents of total calcium, iron and zinc in raw (non-germinated) BWT and PWT food blends were 392.43, 440.82 mg/100g, 1.48, 5.13 mg/100g and 3.66, 3.47 mg/100g, respectively (Table 4.20 and 4.21). Autoclaving as well as fermentation did not alter these contents. On the other hand, in germinated food mixtures, total mineral contents changed non-significantly when compared to raw (non-germinated) food mixtures. Autoclaving of germinated food mixtures and fermentation of autoclaved mixtures did not significantly alter the contents of calcium, iron and zinc.

Similar results were also observed in various legumes (Jood *et al.*, 1998; Grewal and Jood, 2006) and cereals and their fermented blends (Khetarpaul and Chauhan, 1990; Sharma and Khetarpaul, 1997; Chaudhary, 1998 and Sindhu *et al.*, 2005).

Table 4.20 : Effect of fermentation with *L. acidophilus* curd on total (mg/100 g) and *in vitro* availability (%) of minerals of indigenously developed BWT* food mixture (on dry matter basis)

Processing treatments	Calcium		Iron		Zinc	
	Total	Available	Total	Available	Total	Available
Food mixtures						
(A) Non-germinated						
Raw mixture (control)	392.43 ± 1.94	52.92 ± 2.90	1.48 ± 0.05	31.36 ± 0.84	3.66 ± 0.01	53.61 ± 1.64
Autoclaved mixture	392.00 ± 1.42	64.78 ± 3.52 (+22)	1.46 ± 0.01	40.54 ± 0.99 (+29)	3.60 ± 0.02	60.56 ± 1.32 (+13)
Autoclaved and fermented mixture	390.04 ± 1.32	80.79 ± 5.77 (+52)	1.42 ± 0.05	56.59 ± 1.33 (+80)	3.52 ± 0.03	76.71 ± 2.37 (+30)
Mean	391.65	66.16	1.45	42.83	3.59	63.62
(B) Germinated						
Germinated raw mixture (control)	391.39 ± 1.16	67.74 ± 7.53	1.48 ± 0.05	52.31 ± 1.43	3.65 ± 0.05	60.35 ± 4.87
Germinated autoclaved mixture	390.35 ± 1.09	79.91 ± 5.17 (+18)	1.45 ± 0.01	61.64 ± 3.49 (+18)	3.59 ± 0.05	75.64 ± 5.75 (+11)
Germinated, autoclaved and fermented mixture	390.91 ± 1.33	99.54 ± 6.92 (+46)	1.45 ± 0.03	85.35 ± 1.34 (+63)	3.56 ± 0.01	88.47 ± 1.74 (+47)
Mean	390.68	82.39	1.46	66.43	3.60	74.82
CD (A)	NS	9.89	NS	3.20	0.18	10.39
CD (B)	NS	11.79	NS	5.40	NS	12.39
CD (A x B)	NS	16.68	NS	5.40	NS	18.33

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent increase (+) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

NS = Non-significant

Table 4.21 : Effect of fermentation with *L. acidophilus* curd on total (mg/100 g) and *in vitro* availability (%) of minerals of indigenously developed PWT* food mixture (on dry matter basis)

Processing treatments	Calcium		Iron		Zinc	
	Total	Available	Total	Available	Total	Available
Food mixtures						
(A) Non-germinated						
Raw mixture (control)	440.82 ± 3.92	40.54 ± 0.43	5.13 ± 0.17	20.62 ± 0.98	3.47 ± 0.86	36.01 ± 0.17
Autoclaved mixture	440.55 ± 2.60	54.88 ± 2.97 (+35)	5.10 ± 0.10	27.19 ± 1.91 (+32)	3.46 ± 0.12	41.51 ± 6.01 (+14)
Autoclaved and fermented mixture	440.44 ± 4.80	72.76 ± 2.98 (+45)	5.10 ± 0.08	53.51 ± 2.68 (+159)	3.62 ± 0.12	55.23 ± 1.53 (+53)
Mean	440.60	56.06	5.11	33.77	3.51	44.25
(B) Germinated						
Germinated raw mixture (control)	440.56 ± 2.86	50.50 ± 5.53	5.43 ± 0.11	35.65 ± 1.64	3.56 ± 0.08	43.15 ± 3.99
Germinated autoclaved mixture	339.67 ± 2.63	59.78 ± 2.85 (+20)	5.25 ± 0.04	48.46 ± 5.61 (+36)	3.46 ± 0.06	55.96 ± 2.87 (+30)
Germinated, autoclaved and fermented mixture	339.02 ± 8.26	80.57 ± 5.95 (+62)	5.19 ± 0.05	68.41 ± 3.92 (+92)	3.40 ± 0.08	77.97 ± 5.57 (+81)
Mean	373.08	63.61	5.29	50.84	3.47	59.02
CD (A)	NS	6.97	NS	5.69	NS	7.02
CD (B)	NS	8.54	NS	6.97	NS	8.97
CD (A x B)	NS	11.76	NS	9.60	NS	11.85

Values are mean ± SE of three independent determinations

Figures in parentheses indicate percent increase (+) over control values

*BWT food mixture contains barley flour, whey powder and tomato pulp

NS = Non-significant

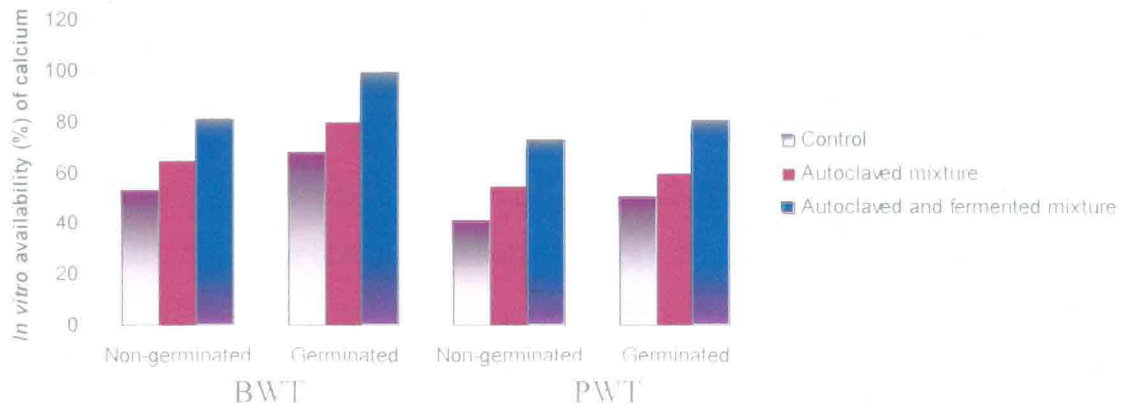


Fig. 4.8. Effect of fermentation with *L. acidophilus* curd on *In vitro* availability of calcium of indigenously developed BWT and PWT food mixtures.

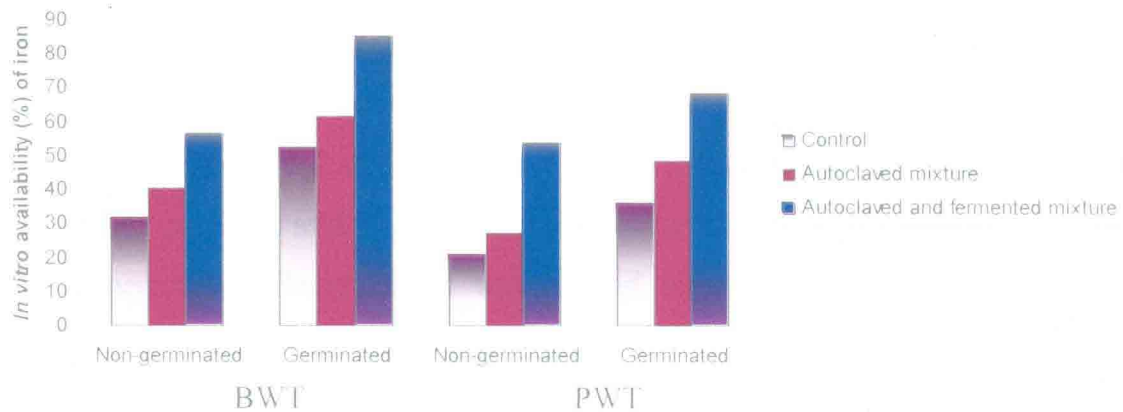


Fig. 4.9. Effect of fermentation with *L. acidophilus* curd on *In vitro* availability of iron of indigenously developed BWT and PWT food mixtures.

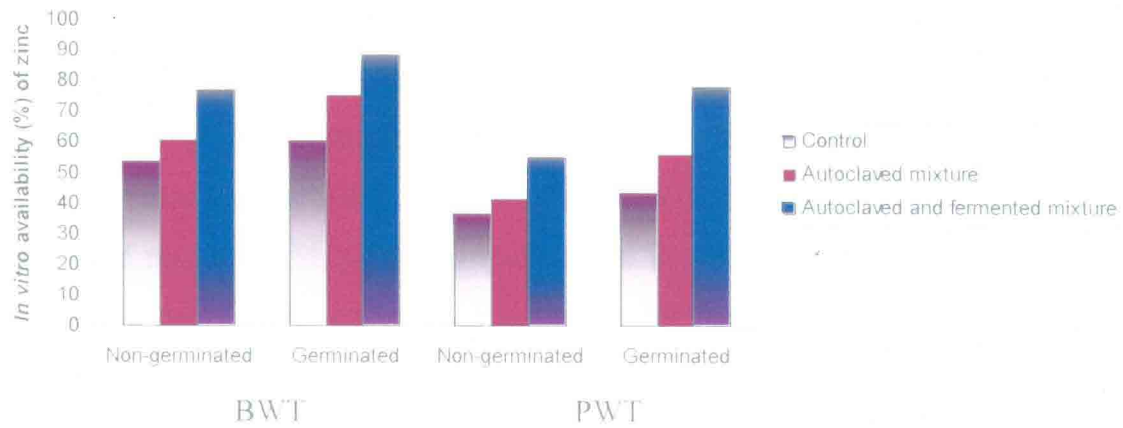


Fig. 4.10. Effect of fermentation with *L. acidophilus* curd on *In vitro* availability of zinc of indigenously developed BWT and PWT food mixtures.

4.10.2 *In vitro* availability of minerals

In vitro availability of calcium, iron and zinc of the raw (non-germinated) BWT and PWT food mixtures were observed to be 52.92, 40.54 per cent, 20.62, 31.36 per cent and 36.01, 53.61 per cent, respectively (Tables 4.20 and 4.21, Fig. 4.8, 4.9 and 4.10). Autoclaving of raw (non-germinated) blend brought about a significant ($P<0.05$) increase in the availability of calcium (22 and 35%), (29 and 32%) and (13 and 14%), respectively over the control values.

Fermentation of non-germinated autoclaved blends caused further improvement in *in vitro* availability of minerals. Among the minerals, maximum improvement was observed in *in vitro* availability of iron in both the mixtures. The increase was observed by about 80 and 159 per cent, respectively.

Germinated BWT and PWT food blends contained 67.74, 50.50 per cent, 52.31, 35.65 per cent and 60.35, 43.15 per cent availability of calcium, iron, and zinc, respectively. These values were found significantly ($P<0.05$) higher as compared to non-germinated food mixtures. BWT food mixture contained significantly higher *in vitro* availability of calcium, iron and zinc after germination as compared to PWT food mixtures. Autoclaving and fermentation also caused significant improvement in all the mineral availability. Germinated autoclaved food mixtures contained 18, 20 per cent, 18, 36 per cent and 11, 30 per cent, respectively *in vitro* availability of calcium, iron and zinc. On the other hand per cent availability of calcium, iron and zinc was 46, 62 per cent, 63, 92 per cent and 47, 81 per cent, respectively in germinated, autoclaved and fermented BWT and PWT food mixtures. Maximum improvement in all the mineral availability was observed in pearl millet based food mixture after fermentation in non-germinated as well as germinated mixtures.

The significant variation in the mineral availability could be explained by highly significant ($P<0.05$) negative correlation obtained between the phytic acid and calcium, iron and zinc availability (*in vitro*) (Table 4.22 and 4.23). Significant and negative correlation was observed between polyphenols and calcium, iron and

Table 4.22 : Correlation coefficients of *in vitro* availability of calcium, iron and zinc with phytic acid, polyphenols and amylase inhibitor activity of indigenously developed BWT* food mixture

Processing treatments	Phytic acid vs			Polyphenols vs			Amylase inhibitor activity vs		
	Calcium	Iron	Zinc	Calcium	Iron	Zinc	Calcium	Iron	Zinc
Food mixtures									
(A) Non-geminated									
Raw mixture (control)	-0.9896**	-0.9920**	-0.9726**	-0.8925*	-0.8625*	-0.8931*	-0.6212	-0.5238	-0.3900
Autoclaved mixture	-0.9585**	-0.1000**	-0.9825**	-0.8820*	-0.8720*	-0.8100*	-0.5810	-0.3815	-0.4215
Autoclaved and fermented mixture	-0.9672**	-0.9998**	-0.9015**	-0.9020*	-0.8420*	-0.8219*	-0.4819	-0.4210	0.6601
(B) Germinated									
Germinated raw mixture (control)	-0.9990*	-0.8632*	-0.9210*	-0.8900*	-0.8210*	-0.8009*	-0.4329	-0.4200	-0.3915
Germinated autoclaved mixture	-0.8990*	-0.8719*	-0.9014*	-0.8601*	-0.8612*	-0.8915*	-0.3925	-0.4615	-0.5325
Germinated, autoclaved and fermented mixture	-0.8889*	-0.9295*	-0.9013*	-0.8219*	-0.7815*	-0.8519*	-0.3819	-0.4109	-0.6215

*Significant at 5 per cent level

**Significant at 1 per cent level

*BWT food mixture contains barley flour, whey powder and tomato pulp

Table 4.23 : Correlation coefficients of *in vitro* availability of calcium, iron and zinc with phytic acid, polyphenols and amylase inhibitor activity of indigenously developed PWT* food mixture

Processing treatments	Phytic acid			Polyphenols			Amylase inhibitor activity		
	Calcium	Iron	Zinc	Calcium	Iron	Zinc	Calcium	Iron	Zinc
(A) Non-germinated									
Raw mixture (control)	-0.1000**	-0.9998**	-0.9295**	-0.8920*	-0.8719*	-0.9100*	-0.4210	-0.5892	-0.4893
Autoclaved mixture	-0.9899**	-0.9697**	-0.9108**	-0.8109*	-0.8420*	-0.9201*	-0.3915	-0.5000	-0.4275
Autoclaved and fermented mixture	-0.9290**	-0.9813**	-0.9345**	-0.8216*	-0.8397*	-0.8912*	-0.6230	-0.4999	-0.5210
(B) Germinated									
Germinated raw mixture (control)	-0.9999*	-0.1000*	-0.9892*	-0.8926*	-0.8818*	-0.8719*	-0.3800*	-0.4200	-0.5100
Germinated autoclaved mixture	-0.9892*	-0.9692*	-0.8996*	-0.8825*	-0.8715*	-0.8929*	-0.3925	-0.4919	-0.4919
Germinated, autoclaved and fermented mixture	-0.9693*	-0.9720**	-0.9432*	-0.8210	-0.8620*	-0.8900*	-0.4210	-0.3915	-0.5219

*Significant at 5 per cent level

**Significant at 1 per cent level

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

zinc where as negative correlation was obtained with amylase inhibitor activity. These results are in conformity with those reported earlier by various workers (Kalra, 1996; Chaudhary, 1999; Chahal, 1999).

Cooking and pressure cooking due to moist heat caused significant enhancement in *in vitro* availability of all the minerals. As phytic acid chelates divalent and trivalent cations like calcium, iron and zinc forming insoluble complexes and thereby decreasing the *in vitro* availability of minerals in humans. The decrease in phytic acid content, possibly through its destruction by heat treatment may result in the divalent and trivalent cations being freed from the phytate mineral complexes thus accounting for the improved availability of minerals in the processed seeds (Hazell and Johnson, 1987).

Germination brings about a considerable translocation of different minerals in the grains (Lorenz, 1980). Increased mineral availability during germination may be due to increased phytase activity resulting in decreased phytate content in sprouts (Michael Eskin and Wiebe, 1983). Other antinutrients like polyphenols, saponins are also known to hinder the availability of minerals are catabolized during germination leading to improvement in mineral availability (Duhan, 2002; Grewal and Jood, 2006).

Since no addition or deletion of total mineral contents took place during all processing methods, the amount of total minerals remained unchanged. In the plant foods, minerals such as Ca, Fe and Zn and other ions remain bound to phytic acid and a protein-phytate-mineral complex is formed which inhibits the availability of minerals, therefore, availability of minerals is low in raw food blends. Decrease in the level of phytic acid after autoclaving and fermentation (Antony and Chandra, 1998; Sindhu *et al.*, 2005), which is possibly through hydrolysis by inherent phytase in fermenting microflora, may release these metallic ions in free form and therefore may allowed for increased availability and/or extractability of these minerals in fermented blends and products (Antony and Chandra, 1998; Chahal, 1999; Sindhu *et al.*, 2005).

4.11 Organoleptic evaluation of fermented products

Various fermented products like *kadhi*, *dosa*, *idli*, *papad* and *wadi* were developed using non-germinated and germinated autoclaved and fermented barley (BWT) and pearl millet (PWT) based food mixtures (blends) whereas *buttermilk* was prepared from control as well as from *L. acidophilus* (probiotic) curd. All the products were organoleptically evaluated. The results of organoleptic characteristics of all developed products are given in tables from 4.24 to 4.29.

Buttermilk

Buttermilk prepared from ordinary skim milk curd served as control. Probiotic buttermilk prepared from *L. acidophilus* curd depicted significantly ($P < 0.05$) higher mean score of colour (8.20), appearance (8.50), aroma (7.60), texture (7.70), taste (8.00) and overall acceptability score (8.00) as compared to control buttermilk. Hence, probiotic buttermilk was 'liked very much' by the panelists, whereas Gupta et al. (1997); Sharma and Ghosh (2006) observed that *Lactobacillus* yoghurt and regular yoghurt were found almost similar in terms of their organoleptic characteristics.

Kadhi

Kadhi prepared by using mixtures of dehulled bengal gram flour and curd served as control. Control *kadhi* exhibited mean scores of colour 8.10, appearance 7.30, aroma 8.80, texture 7.90, taste 8.00 and overall acceptability 7.38, respectively. Whereas experimental *kadhies* (Type-I and Type-II) prepared by using non-germinated and germinated autoclaved and fermented barley flour-whey-tomato pulp slurries were found to be acceptable in terms of colour, appearance, aroma, texture, taste and overall acceptability score. Type-I *kadhi* exhibited maximum mean scores of colour (8.20), appearance (7.90), aroma (8.90), texture (8.00), taste (8.10) and overall acceptability (7.90) followed by Type-II *kadhi* and control *kadhi*. However, mean scores of all organoleptic

characteristics of control as well as experimental *kadhies* (Type-I and Type-II) were in the categories of 'liked moderately' and 'liked slightly'.

Similarly in case of PWT food mixture, Type-I *kadhi* significantly ($P < 0.05$) differed in terms of mean scores of colour, appearance, aroma, texture, taste and overall acceptability as compared to experimental *kadhi* (Type-II) and control *kadhi*. Similar results were also observed by various workers with regard to organoleptic characteristics of *kadhies* prepared from faba bean and greengram (Saharan, 1994; Grewal, 2003).

Dosa

Dosa prepared from fermented mixture of dehulled black gram dal and rice served as control. Control *dosa* exhibited highest mean scores of all organoleptic characteristics followed by Type-I and Type-II (experimental) *dosas*. Type-I and Type-II *dosas* prepared from non-germinated and germinated, autoclaved and fermented BWT slurries were found unacceptable in terms of their organoleptic characteristics. Mean scores of colour, appearance, aroma, texture, taste and overall acceptability of control *dosa* were found in the category of 'liked moderately'. Whereas mean scores of colour, appearance, aroma, texture, taste and overall acceptability of Type-I and Type-II *dosas* were found in the category of 'neither liked nor disliked' and 'disliked slightly'.

Similarly in case of PWT food mixture, Type-I and Type-II *dosas* exhibited significantly ($P < 0.05$) lower mean scores of all organoleptic characteristics as compared to control *dosa*). Mean scores of colour (4.70, 3.60), appearance (4.40, 4.20), aroma (4.00, 3.70), texture (4.10, 3.80), taste (3.50, 4.80) and overall acceptability (4.10, 4.00) of Type-I and Type-II *dosas* were found in the category of 'neither liked nor disliked' and 'disliked slightly'.

It may be inferred from these results that experimental *dosas* which were prepared by incorporating non-germinated and germinated, autoclaved and fermented BWT and PWT food slurries were found unacceptable by the judges in

terms of their colour, texture, appearance, flavour, taste and overall acceptability. Therefore, *dosa* was not selected for further chemical analysis.

Idli

Idli prepared from a fermented mixture of dehulled black gram dal and rice served as control. Control *idli* exhibited significantly ($P < 0.05$) higher mean scores of all organoleptic characteristics such as colour (8.00), appearance (7.50), aroma (7.90), texture (8.10), taste (7.80) and overall acceptability (7.90). On the other hand, experimental *idlis* Type-I and Type-II, prepared by using non-germinated and germinated autoclaved and fermented BWT food mixtures contained significantly ($P < 0.05$) lower mean scores of colour, appearance, aroma, texture, taste and overall acceptability. All the organoleptic characteristics were found in the category of 'neither liked nor disliked' and 'disliked slightly'.

Similarly in case of PWT based *idlis* Type-I and Type-II, their organoleptic characteristics like colour, appearance, aroma, texture, taste and overall acceptability were found significantly ($P < 0.05$) lower as compared to control *idli*.

Hence, in both the cases, experimental *idlis* were found unacceptable in terms of their colour, appearance, aroma, texture, taste and overall acceptability by the judges. Therefore, *idlis* were not selected for further chemical analysis.

Papad

Papad prepared from black gram dal flour served as a control whereas test samples (Type-I and Type-II) were prepared from non-germinated and germinated autoclaved and fermented barley (BWT) and pearl millet (PWT) based food mixtures. Blackgram dal *papad* (control) secured the highest overall acceptability (7.90) score followed by *papad* Type-I (7.80) and Type-II (7.70) prepared from BWT based food mixtures. However, according to panelists in the present study, sensory attributes like colour, appearance, aroma, texture, taste and overall acceptability were as good as those of traditional black gram *papad*.

Similar trend was also found in Type-I and Type-II *papad* prepared from PWT based food mixtures. Type-I *papad* prepared from non-germinated,

autoclaved and fermented food mixture secured higher mean scores of all organoleptic characteristics as compared to Type-II *papad*.

Other workers also prepared *papad* from faba bean, ricebean, greengram and finger millet flour which were found acceptable (Saharan, 1994; Grewal, 2003; Vidyavati *et al.*, 2004).

Wadi

Wadi prepared from coarsely ground fermented legume slurry containing the spices served as control. Control *wadi* as well as experimental *wadies* were incorporated in commonly consumed recipe like *wadi curry* and evaluated for organoleptic characteristics. Control *wadi* secured lower overall acceptability score (7.50) followed by experimental *wadies* namely Type-I and Type-II. Type-I and Type-II *wadies* prepared from BWT non-germinated and germinated autoclaved and fermented mixture differed significantly ($P < 0.05$) in terms of their colour, appearance, aroma, texture, taste and overall acceptability. Type-II *wadi* secured significantly higher mean scores of all organoleptic characteristics as compared to Type-I *wadi*.

Similar trend was also observed in case of *wadies* (Type-I and Type-II) prepared from PWT non-germinated and germinated, autoclaved and fermented food mixture. Type-II *wadi* secured higher mean score of overall acceptability as compared to Type-I *wadi*. Type-II *wadies* in both cases secured highest overall acceptability score followed by Type-I and control *wadi*. Similarly, Yadav and Khetarpaul (1993) prepared *wadies* from fermented legume blends were found acceptable. Whereas Sharma and Khetarpaul (1998) prepared weaning mixtures from blends containing fermented rice dehulled bengal gram in 60:40 and 80:20 proportions were 'moderately desirable'. *Wadies* prepared by other workers from cowpea (Thakur and Puttaraj, 1995) and green gram (Grewal, 2003) were found acceptable

Table 4.24 : Organoleptic evaluation of buttermilk prepared from control and *L. acidophilus* curd

Product	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	7.60 ± 0.26	8.30 ± 0.21	7.50 ± 0.22	7.50 ± 0.21	7.90 ± 0.21	7.76 ± 0.50
Probiotic	8.20 ± 0.24	8.50 ± 0.26	7.60 ± 0.33	7.70 ± 0.15	8.00 ± 0.25	8.00 ± 0.34
t' value	1.76	2.75	1.50	0.70	3.88*	2.58

Values are mean ± SE of ten panelists

*Significant at 5% level

Table 4.25 : Organoleptic evaluation of *kadhi* prepared from indigenously developed BWT* and PWT* fermented food mixtures

Product	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	8.10 ± 0.27	7.30 ± 0.27	8.80 ± 0.29	7.90 ± 0.29	8.00 ± 0.30	7.38 ± 0.40
BWT						
Type-I	8.20 ± 0.57	7.90 ± 0.57	8.90 ± 0.43	8.00 ± 0.47	8.10 ± 0.54	7.90 ± 0.50
Type-II	8.00 ± 0.57	7.30 ± 0.54	8.60 ± 0.38	7.80 ± 0.36	7.90 ± 0.38	7.80 ± 0.51
PWT						
Type-I	8.20 ± 0.63	7.80 ± 0.62	8.10 ± 0.63	7.70 ± 0.61	8.00 ± 0.66	8.00 ± 0.51
Type-II	8.00 ± 0.45	7.20 ± 0.45	7.00 ± 0.47	7.90 ± 0.42	8.10 ± 0.42	7.80 ± 0.24
CD (P<0.05)	NS	NS	1.47	1.45	0.32	1.00

Values are mean ± SE of ten panelists

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Kadhi* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Kadhi* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.26 : Organoleptic evaluation of dosa prepared from indigenously developed BWT* and PWT* fermented food mixtures

Product	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	7.30 ± 0.27	7.40 ± 0.29	7.50 ± 0.30	7.50 ± 0.40	7.50 ± 0.30	7.40 ± 0.31
BWT						
Type-I	5.10 ± 0.42	5.40 ± 0.29	5.00 ± 0.27	4.70 ± 0.86	4.50 ± 0.30	4.90 ± 0.42
Type-II	4.90 ± 0.42	4.50 ± 0.30	4.80 ± 0.24	5.10 ± 0.57	4.40 ± 0.67	4.70 ± 0.32
PWT						
Type-I	4.70 ± 0.61	4.40 ± 0.55	4.00 ± 0.38	4.10 ± 0.57	3.50 ± 0.73	4.10 ± 0.14
Type-II	3.60 ± 0.73	4.20 ± 0.70	3.70 ± 0.81	3.80 ± 0.76	4.80 ± 0.24	4.00 ± 0.24
CD (P<0.05)	1.47	1.26	1.20	1.53	1.34	1.38

Values are mean ± SE of ten panelists

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : Dosa prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : Dosa prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.27 : Organoleptic evaluation of Idli prepared from indigenously developed BWT* and PWT* fermented food mixtures

Product	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	8.00 ± 0.00	7.50 ± 0.30	7.90 ± 0.18	8.10 ± 0.57	7.80 ± 0.24	7.90 ± 0.44
BWT						
Type-I	4.50 ± 0.68	4.40 ± 0.73	4.50 ± 0.82	4.70 ± 0.66	4.40 ± 0.67	4.50 ± 0.51
Type-II	4.80 ± 0.36	4.50 ± 0.40	4.60 ± 0.40	4.60 ± 0.40	4.60 ± 0.40	4.60 ± 0.38
PWT						
Type-I	4.60 ± 0.55	3.00 ± 0.00	3.70 ± 0.47	3.30 ± 0.66	3.20 ± 0.70	3.60 ± 0.14
Type-II	3.60 ± 0.62	3.50 ± 0.68	4.00 ± 0.38	3.70 ± 0.54	3.00 ± 0.38	3.60 ± 0.53
CD (P<0.05)	1.32	1.26	1.36	1.68	1.41	1.66

Values are mean ± SE of ten panelists

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : Idli prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : Idli prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.28 : Organoleptic evaluation of papad prepared from indigenously developed BWT* and PWT* fermented food mixtures

Product	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	8.00 ± 0.40	7.80 ± 0.30	7.50 ± 0.73	7.90 ± 0.18	8.00 ± 0.73	7.90 ± 0.38
BWT						
Type-I	7.80 ± 0.36	8.00 ± 0.27	7.60 ± 0.29	7.60 ± 0.29	7.80 ± 0.24	7.80 ± 0.44
Type-II	7.90 ± 0.28	7.70 ± 0.27	7.50 ± 0.30	7.80 ± 0.24	7.60 ± 0.29	7.70 ± 0.51
PWT						
Type-I	7.80 ± 0.24	8.10 ± 0.58	7.30 ± 0.27	7.50 ± 0.30	7.80 ± 0.24	7.90 ± 0.60
Type-II	7.60 ± 0.57	7.30 ± 0.27	7.40 ± 0.29	7.80 ± 0.24	7.70 ± 0.27	7.80 ± 0.42
CD (P<0.05)	1.12	1.01	NS	0.75	1.06	0.24

Values are mean ± SE of ten panelists

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.29 : Organoleptic evaluation of wadi prepared from indigenously developed BWT* and PWT* fermented food mixtures

Product	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	7.40 ± 0.55	7.70 ± 0.27	7.40 ± 0.29	7.40 ± 0.29	7.70 ± 0.27	7.50 ± 0.38
BWT						
Type-I	7.50 ± 0.56	7.60 ± 0.29	7.50 ± 0.30	7.70 ± 0.38	7.50 ± 0.73	7.60 ± 0.12
Type-II	7.80 ± 0.36	7.80 ± 0.24	7.30 ± 0.27	7.80 ± 0.29	7.80 ± 0.24	7.70 ± 0.11
PWT						
Type-I	7.60 ± 0.55	7.30 ± 0.27	7.30 ± 0.27	7.80 ± 0.24	7.30 ± 0.27	7.50 ± 0.31
Type-II	7.80 ± 0.24	7.30 ± 0.27	7.40 ± 0.29	7.80 ± 0.30	7.50 ± 0.30	7.60 ± 0.44
CD (P<0.05)	NS	NS	NS	NS	NS	NS

Values are mean ± SE of ten panelists

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : Wadi prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : Wadi prepared from germinated autoclaved and fermented BWT and PWT food mixtures

4.12 Nutritional evaluation of most acceptable developed fermented food products

4.12.1 Protein and total lysine

Table 4.30 reveals the data regarding protein, total lysine and *in vitro* protein digestibility contents of control buttermilk (prepared from control skim milk curd) and probiotic curd (prepared from *L. acidophilus* skim milk curd). Protein content was 2.8 per cent in control and 2.9 per cent in probiotic buttermilk whereas lysine content was found to be increased from 0.30 g/g N in control and 0.58 g/g N in probiotic buttermilk. It might be due to the fact that fermenting microbes have capability to synthesize essential amino acids (Hamad and Fields, 1979). Similarly, in case of *in vitro* protein digestibility, there was significant ($P < 0.05$) increase i.e. 50.02 per cent in control buttermilk and 88.50 per cent in probiotic buttermilk.

Increase in protein digestibility of probiotic curd is mainly because of the fact that microflora may produce some proteolytic enzymes during fermentation which are responsible for breakdown of protein to peptides and amino acids (Kao and Robinson, 1978). Similar results were also observed by Dhankher and Chauhan (1987) in pearl millet buttermilk mixture.

Table 4.31 reveals the results regarding protein and total lysine contents of *kadhi*. *Kadhi* prepared from a mixture of bengal gram flour and buttermilk (prepared from skim milk curd) served as control whereas *kadhies* prepared from non-germinated and germinated, autoclaved and fermented BWT and PWT food mixtures served as experimental *kadhies*. (Type-I and Type-II). Control *kadhi* exhibited 18.05 per cent protein contents on dry matter basis. However, there was non-significant change in protein contents of Type-I and Type-II *kadhies* in both cases. The values were 14.25 and 14.00; 14.70 and 13.90 per cent, respectively in Type-I and Type-II *kadhies* prepared from non-germinated and germinated + autoclaved and fermented BWT and PWT food mixtures. It might be due to the fact that germination and fermentation did not cause any significant effect on the

protein contents of food mixtures. Similarly, non-significant change in protein contents was also reported earlier by various workers in sprouted and fermented *idli* and *kadhi* (Saharan, 1994; Grewal, 2003).

On the other hand, total lysine contents of experimental *kadhies* increased significantly. Control *kadhi* contained total lysine i.e. 4.00 g/100g protein which was significantly lower than experimental. Type-I *kadhi* had 4.34 g/100g protein of total lysine which significantly ($P < 0.05$) increased in Type-II *kadhi*. This might be due to the additional effect of fermentation as fermenting microbes produce essential amino-acids which results in increase in lysine contents of fermented products (Hamad and Fields, 1979).

Similar trend was also observed in case of *kadhies* (Type-I and Type-II) prepared from non-germinated and germinated + autoclaved + fermented PWT food mixtures. The values of total lysine were 3.46 and 5.83 g/100 g protein in Type-I and Type-II, respectively.

Control *papad* contained 19.25 per cent protein as it was prepared from blackgram dal. Experimental *papad* Type-I and Type-II exhibited lower protein contents as compared to control (Table 4.32). The values were observed 14.00, 14.25 and 14.72, 13.95 per cent, respectively in Type-I and Type-II *papad* prepared from BWT and PWT food mixtures. All processing treatments like autoclaving, germination and fermentation employed did not cause significant ($P < 0.05$) change in protein contents of food mixtures.

Control *papad* contained 4.33 g/100g protein of total lysine contents. Type-I *papad* exhibited 4.02 g/100g protein total lysine content which significantly improved in Type-II *papad*. This might be due to additional effect of germination. Type-II *papad* had total lysine contents i.e. 5.98 g/100g protein. Similar trend was also observed in case of *papad* prepared from PWT food mixtures.

Similar trend was witnessed in case of *wadi* made from dehulled blackgram *dhal* as well as from fermented mixtures (Table 4.33). Control *wadi* contained 19.29 per cent total protein and 4.61 g/100g protein of total lysine. Whereas

Table 4.30 : Protein (%), total lysine (g/g N) and *in vitro* protein digestibility (%) of buttermilk (on dry matter basis)

Buttermilk	Protein	Total lysine	<i>In vitro</i> protein digestibility
Control	2.8±0.05	0.30±0.01	50.02±0.58
Probiotic	2.9±0.07	0.58±0.02	88.50±0.28
't' value	0.19	0.23*	13.88*

*Significant at 5 per cent level

Buttermilk prepared from skimmed milk curd served as control whereas buttermilk prepared from *L. acidophilus* skimmed milk curd served as experimental

Table 4.31 : Protein (%) and total lysine (g/100g protein) content of *kadhi* prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Protein	Total lysine
Control	18.05 ± 1.29	4.00 ± 0.08
BWT		
Type-I	14.25 ± 2.29	4.34 ± 0.57
Type-II	14.00 ± 2.23	5.29 ± 0.57
Mean	14.12	4.31
CD (P<0.05)	1.02	0.98
PWT		
Type-I	14.70 ± 2.20	4.46 ± 0.26
Type-II	13.19 ± 1.19	5.83 ± 0.23
Mean	14.30	4.64
CD (P<0.05)	0.70	0.62
Overall CD (P<0.05)	1.09	0.98

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Kadhi* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Kadhi* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.32 : Protein (%) and total lysine (g/100g protein) content of *papad* prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Protein	Total lysine
Control	19.25 ± 2.49	4.33 ± 0.18
BWT		
Type-I	14.00 ± 1.32	4.02 ± 0.17
Type-II	14.25 ± 1.00	5.98 ± 0.57
Mean	14.12	5.00
CD (P<0.05)	1.29	0.77
PWT		
Type-I	14.72 ± 3.39	4.03 ± 0.23
Type-II	13.95 ± 2.42	5.60 ± 0.55
Mean	14.33	4.84
CD (P<0.05)	0.92	0.60
Overall CD (P<0.05)	2.00	1.05

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.33 : Protein (%) and total lysine (g/100g protein) content of *wadi* prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Protein	Total lysine
Control	19.29 ± 2.48	4.61 ± 0.31
BWT		
Type-I	13.89 ± 1.20	4.32 ± 0.56
Type-II	13.99 ± 1.22	6.43 ± 0.13
Mean	13.94	5.37
CD (P<0.05)	2.89	0.54
PWT		
Type-I	13.20 ± 1.00	4.70 ± 0.27
Type-II	13.00 ± 0.99	6.39 ± 0.28
Mean	13.10	5.53
CD (P<0.05)	0.89	0.54
Overall CD (P<0.05)	2.01	0.96

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Wadi* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Wadi* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

experimental *wadies* exhibited lower protein contents as these are prepared from cereal-based mixtures.

There was non-significant change in protein contents of Type-I and Type-II *wadies* prepared from BWT and PWT food mixtures. Various processing treatments involved in preparation of food mixtures and *wadi* did not change the protein contents.

According to Goyal (1991), fermentation either decreased or did not alter the protein content of cereal legume blends. Total nitrogen and crude protein did not change although non-protein nitrogen increased during fermentation of mung bean *wadi* (Soni and Sandhu, 1990). Aliya and Geervani (1981) observed a decrease in crude protein of bengal gram and green gram whereas Grewal (2003) showed no change in protein and fat contents during germination and fermentation.

Control *wadi* contained lower amount of total lysine contents as compared to experimental *wadies* (Type-I and Type-II). Total lysine contents of type-II *wadies* prepared from germinated and fermented mixtures of BWT and PWT differed significantly from control and Type-I *wadies*. The values observed were 4.32, 6.43 and 4.70, 6.39 g/100g protein, respectively in Type-I and Type-II *wadies* of BWT and PWT mixtures.

4.12.2 Antinutrients

Control *kadhi* contained significantly ($P < 0.05$) higher content of phytic acid (298.35 mg/100g), polyphenols 222.12 (mg/100g) and amylase inhibitor activity (118.23 AIU/g) as compared to experimental *kadhies* (Table 4.34). In case of BWT *kadhies*, Type-I exhibited higher values of all antinutrients as compared to Type-II. Type-I *kadhi* prepared from non-germinated food mixture whereas Type-II prepared from germinated food mixtures and other processing treatments were same as germination caused significant reduction in antinutrient contents due to hydrolysis of these metabolites (Grewal, 2003).

Type-I *kadhi* had significantly higher content of phytic acid (89.55 mg/100g), polyphenols (200.22 mg/100g) and amylase inhibitor activity (18.30 AIU/g) as compared to Type-II *kadhi*. Further reduction was observed in antinutrient contents of *kadhies* as compared to their respective control food mixtures. Similar trend was also observed in *kadhies* prepared from PWT food mixtures. *Kadhi* prepared from PWT food mixtures contained significantly higher contents of all the three antinutrients as compared to *kadhies* prepared from BWT food mixtures. Similar results were also observed by Sahran (1994) and Grewal (2003) in fermented *idli*, *dosa* and *wadi* prepared from fababean and moongbean.

In the present study, cumulative effect of soaking, germination, autoclaving and fermentation was found to be effective for lowering down all the antinutrient contents. During germination and fermentation, the diminishing effect on polyphenols, phytic acid and amylase inhibitor activity may be due to the activity of phytase, polyphenol oxidase and amylases present in the sprouts and/or fermenting microflora (Saharan, 1994; Yadav and Khetarpaul, 1994; Grewal, 2003).

Control *papad* contained significantly ($P < 0.05$) higher antinutrient contents compared to experimental *papad*. In case of BWT, Type-I *papad* showed significantly ($P < 0.05$) higher phytic acid (94.12 mg/100g), polyphenols (216.08 mg/100g) and amylase inhibitor activity (19.29 AIU/g) as compared to Type-II *papad* which contained 69.99 mg/100g, 120.18 mg/100g and 6.11 AIU/g, respectively (Table 4.35). Similar trend was also observed in *papad* Type-I and Type-II prepared from PWT food mixtures.

Antinutrient contents of control and experimental *wadies* are presented in Table 4.36. Phytic, polyphenols and amylase inhibitor activity were found to be higher in control *wadi* (347.36 mg/100g, 225.33 mg/100g and 96.41 AIU/g, respectively) as against the experimental *wadies*. There was a significant difference in antinutrient contents of the control and experimental *wadies*. However, significant difference was also observed between Type-I and Type-II

Table 4.34 : Antinutrient content of *kadhi* prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Phytic acid (mg/100 g)	Polyphenols (mg/100 g)	Amylase inhibitor activity (AIU/g)
Control	298.35 ± 11.45	222.12 ± 9.54	118.23 ± 5.77
BWT			
Type-I	89.55 ± 4.50	200.22 ± 4.59	18.30 ± 0.55
Type-II	69.32 ± 0.72	110.13 ± 5.88	4.33 ± 0.57
Mean	79.43	112.13	11.31
CD (P<0.05)	14.47	17.24	7.93
PWT			
Type-I	150.43 ± 1.42	152.26 ± 6.84	10.32 ± 1.12
Type-II	73.22 ± 0.54	82.26 ± 5.74	5.36 ± 0.11
Mean	111.82	117.26	7.84
CD (P<0.05)	9.83	12.86	6.00
Overall CD (P<0.05)	11.52	12.00	6.62

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Kadhi* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Kadhi* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.35 : Antinutrient content of papad prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Phytic acid (mg/100 g)	Polyphenols (mg/100 g)	Amylase inhibitor activity (AIU/g)
Control	320.36 ± 5.80	230.85 ± 11.29	125.22 ± 8.69
BWT			
Type-I	94.12 ± 3.46	216.08 ± 9.23	19.29 ± 0.71
Type-II	69.99 ± 0.11	120.18 ± 6.87	6.11 ± 0.05
Mean	82.05	168.13	68.87
CD (P<0.05)	12.56	63.00	6.00
PWT			
Type-I	146.39 ± 2.32	159.27 ± 5.22	18.35 ± 0.58
Type-II	75.44 ± 0.23	84.22 ± 5.77	5.94 ± 1.61
Mean	110.91	121.74	12.14
CD (P<0.05)	16.68	81.86	11.06
Overall CD (P<0.05)	11.25	32.85	9.86

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.36 : Antinutrient content of wadi prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Phytic acid (mg/100 g)	Polyphenols (mg/100 g)	Amylase inhibitor activity (AIU/g)
Control	347.36 ± 11.31	225.33 ± 9.40	96.41 ± 0.57
BWT			
Type-I	84.10 ± 2.66	190.12 ± 5.77	16.18 ± 1.15
Type-II	66.12 ± 0.11	110.22 ± 1.67	3.31 ± 0.25
Mean	75.11	150.17	9.74
CD (P<0.05)	10.02	17.82	13.28
PWT			
Type-I	147.19 ± 2.88	143.07 ± 11.58	10.00 ± 1.15
Type-II	66.51 ± 0.12	78.08 ± 4.58	3.07 ± 5.77
Mean	106.85	110.57	38.96
CD (P<0.05)	14.62	21.86	4.51
Overall CD (P<0.05)	12.30	19.92	6.09

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

wadies prepared from non-germinated and germinated, autoclaved and fermented BWT and PWT food mixtures. In case of BWT, Type-I *wadi* had 84.10 mg/100g, 190.12 mg/100g and 16.18 mg/100g phytic acid, polyphenols and amylase inhibitor activity, respectively which found to be significantly higher as against 66.12 mg/100g, 110.22 mg/100g and 3.31 mg/100g, respectively in Type-II *wadi*. It might be due to the additional effect of germination in reduction of antinutrients contents of mixture. Similar trend was also observed in *wadies* (Type-I and Type-II) prepared from PWT food mixtures.

Similarly, other workers also reported that all the processing methods including soaking, germination, autoclaving, fermentation and cooking caused significant reduction in antinutrient contents of sprouted and fermented products (Yadav and Khetarpaul, 1995; Grewal, 2003).

A wide range of microflora has been known to possess phytase, polyphenol oxidase and amylase activities (Lopez *et al.*, 1983) which may be partly responsible for reducing the antinutrient contents in the fermented products. A decrease in phytic acid and polyphenol contents during fermentation has also been reported in various foods including *soy rabadi* (Grewal, 1992), fababean *wadi* (Saharan, 1994) and fermented blackgram *dhal wadi* (Yadav and Khetarpaul, 1994).

4.12.3 *In vitro* protein and starch digestibility

Control *kadhi* (prepared from a mixture of bengal gram flour and skim milk curd) had 57.19 per cent and 28.98 mg maltose released/g meal protein and starch digestibility, respectively (Table 4.37). *In vitro* digestibility of protein and starch were found to be lower in control *kadhi* as against experimental *kadhies*. However, Type-I and Type-II *kadhies* also differed significantly ($P < .05$) with regard to their digestibility contents in both the cases. Type-I *kadhi* which was prepared from non-germinated, autoclaved and fermented mixture contained significantly lower content of protein (72.80%) and starch (46.86 mg maltose released/g meal) digestibility as compared to Type-II *kadhi* prepared from

germinated, autoclaved and fermented mixture which exhibited 92.92 per cent and 89.74 mg maltose released/g meal protein and starch digestibility, respectively. It might be due to the additional effect of germination involved in preparation of food mixture used for Type-II *kadhi*.

Similar trend was also observed in case of *kadhies* prepared from PWT food mixtures.

These results are in line with those reported earlier in fababean and ricebean *kadhies* (Saharan, 1994).

In vitro protein and starch digestibility of control and experimental *papad* are presented in Table 4.38. Control *papad* contained 50.25 per cent and 31.85 mg maltose released/g meal protein and starch digestibility, respectively, which differed significantly from Type-I and Type-II *papad* prepared from BWT and PWT fermented food mixtures. In both cases, Type-I *papad* exhibited lower values of protein and starch digestibility as compared to Type-II *papad*.

The data regarding *in vitro* protein and starch digestibility of control and experimental *wadies* are present in Table 4.39. Control *wadi* contained 68.50 per cent and 47.42 mg maltose released/g meal protein and starch digestibility, respectively which differed significantly ($P < 0.05$) from Type-I and Type-II *wadies* prepared from BWT and PWT fermented food mixtures. Type-I and Type-II *wadies* in both cases also differed significantly ($P < 0.05$) in terms of their *in vitro* protein and starch digestibility which might be due to additional effect of germination.

An enhancement in protein digestibility upon germination may be because of decline in antinutritional factors due to action of hydrolytic enzymes (Grewal, 2003; Grewal and Jood, 2006).

Similar trend for improvement in protein and starch digestibility was also observed in fermented fababean and moongbean *wadi* and *dhokla* (Saharan, 1994; Grewal, 2003). The cumulative effect of different processing treatments used in

Table 4.37 : *In vitro* protein (%) and starch digestibility (mg maltose released/g meal) of kadhi prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	<i>In vitro</i> protein digestibility	<i>In vitro</i> starch digestibility
Control	57.19 ± 4.10	28.98 ± 2.34
BWT		
Type-I	72.80 ± 2.25	46.86 ± 8.68
Type-II	92.96 ± 1.15	89.74 ± 5.91
Mean	82.88	68.30
CD (P<0.05)	6.89	9.35
PWT		
Type-I	76.82 ± 2.88	40.62 ± 5.89
Type-II	95.86 ± 4.60	83.62 ± 4.74
Mean	86.34	62.12
CD (P<0.05)	10.79	12.02
Overall CD (P<0.05)	5.98	6.53

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Kadhi* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Kadhi* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.38 : *In vitro* protein (%) and starch digestibility (mg maltose released/g meal) of papad prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	<i>In vitro</i> protein digestibility	<i>In vitro</i> starch digestibility
Control	50.25 ± 6.78	31.85 ± 1.12
BWT		
Type-I	74.76 ± 3.29	49.75 ± 5.20
Type-II	92.75 ± 5.77	90.49 ± 5.71
Mean	83.75	70.12
CD (P<0.05)	11.16	6.80
PWT		
Type-I	76.45 ± 11.44	36.51 ± 3.54
Type-II	95.04 ± 5.37	85.68 ± 4.51
Mean	85.85	61.09
CD (P<0.05)	14.04	8.69
Overall CD (P<0.05)	12.32	6.80

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.39 : *In vitro* protein (%) and starch digestibility (mg maltose released/g meal) of wadi prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	<i>In vitro</i> protein digestibility	<i>In vitro</i> starch digestibility
Control	68.50 ± 1.22	47.42 ± 5.80
BWT		
Type-I	74.49 ± 5.76	58.91 ± 6.09
Type-II	95.21 ± 2.86	89.92 ± 6.06
Mean	84.85	74.41
CD (P<0.05)	10.39	8.70
PWT		
Type-I	78.62 ± 3.92	48.61 ± 4.77
Type-II	98.46 ± 5.77	86.33 ± 3.46
Mean	88.54	67.47
CD (P<0.05)	13.33	10.94
Overall CD (P<0.05)	14.22	8.92

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Wadi* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Wadi* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

preparation of food mixtures of *wadi* was found to be responsible for increasing the starch and protein digestibility.

An increase in protein and starch digestibility has been reported earlier in various fermented products, including *tempeh* and *miso* (Kao and Robinson, 1978), fermented soybean (Grewal, 1992) and fermented rice-defatted soyflour blend products (Goyal, 1991).

4.12.4 Total minerals

Table 4.40 reveals the total mineral contents of control and probiotic buttermilk. Control buttermilk (prepared from skim milk curd) contained 120.02, 0.25 and 0.20 mg/100g total calcium, iron and zinc contents, respectively which differed non-significantly ($P < 0.05$) from probiotic buttermilk as *L. acidophilus* fermentation also did not cause any significant change in total mineral contents.

Control *kadhi* contained 143.35, 4.00 and 2.05 mg/100g total calcium, iron and zinc contents, respectively (Table 4.41). These values were found to differ significantly from experimental values. Type-I *kadhi* in both cases contained 390.28, 440.40, 1.42, 5.10, 3.52 and 3.60 mg/100g, total calcium, iron and zinc contents, respectively which differed non-significantly from Type-II *kadhies* in both cases. Type-I and Type-II *kadhies* from pearl millet based food mixtures (PWT) exhibited higher calcium, iron and zinc contents as compared to *kadhies* prepared from BWT based food mixtures.

However, all processing treatments employed did not change total mineral contents. Similarly, other workers also reported non-significant change in mineral contents of fermented *idli*, *dosa* and *wadi* prepared from pearl millet (Khetarpaul and Chauhan, 1990), cereal legume blends (Goyal, 1991), blackgram and greengram (Yadav and Khetarpaul, 1994; Grewal, 2003).

Similar trend was also observed in case of *papad* and *wadies* prepared from control and experimental mixtures (Tables 4.42 and 4.43).

Table 4.40 : Total mineral content of buttermilk* (mg/100g, on dry matter basis)

Product	Calcium	Iron	Zinc
Control	120.02	0.25	0.20
Probiotic	121.03	0.26	0.21
't' value	NS	NS	NS

* Significant at 5% level

*Buttermilk prepared from skimmed milk curd served as control whereas buttermilk prepared from *L. acidophilus* skimmed milk curd served as experimental

Table 4.41 : Total mineral content of *kadhi* prepared from indigenously developed BWT* and PWT* fermented food mixtures (mg/100g, on dry matter basis)

Product	Mineral Content (mg/100g)		
	Calcium	Iron	Zinc
Control	243.35 ± 5.63	4.00 ± 0.12	2.05 ± 0.89
BWT			
Type-I	390.28 ± 4.28	1.42 ± 0.01	3.52 ± 0.30
Type-II	390.00 ± 3.20	1.40 ± 0.09	3.50 ± 0.15
Mean	390.14	1.41	3.51
CD (P<0.05)	10.09	0.28	0.89
PWT			
Type-I	440.40 ± 5.28	5.10 ± 0.10	3.60±0.62
Type-II	339.89 ± 6.20	5.00 ± 0.28	3.58±0.25
Mean	390.14	5.05	3.59
CD (P<0.05)	9.89	0.99	1.00
Overall CD (P<0.05)	22.89	4.69	3.28

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.42 : Total mineral content of papad prepared from indigenously developed BWT* and PWT* fermented food mixtures (mg/100g, on dry matter basis)

Product	Calcium	Iron	Zinc
Control	192.89 ± 9.20	4.20 ± 2.00	2.50 ± 0.09
BWT			
Type-I	390.89 ± 5.89	1.42 ± 0.01	3.52 ± 0.03
Type-II	390.00 ± 6.20	1.40 ± 0.02	3.51 ± 0.15
Mean	390.44	1.41	3.51
CD (P<0.05)	8.98	0.48	0.28
PWT			
Type-I	441.20±7.20	5.12 ± 0.10	3.62 ± 0.52
Type-II	440.00±8.09	5.00 ± 0.18	3.60 ± 0.28
Mean	440.60	5.10	3.61
CD (P<0.05)	7.56	1.02	0.83
Overall CD (P<0.05)	12.86	2.10	1.89

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : Papad prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : Papad prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.43 : Total mineral content of wadi prepared from indigenously developed BWT* and PWT* fermented food mixtures (mg/100g, on dry matter basis)

Product	Calcium	Iron	Zinc
Control	195.00 ± 5.63	4.30 ± 0.29	2.80 ± 0.06
BWT			
Type-I	400.92 ± 8.90	1.43 ± 0.02	3.55 ± 0.42
Type-II	400.00 ± 7.89	1.43 ± 0.03	3.50 ± 0.15
Mean	400.46	1.43	3.52
CD (P<0.05)	11.23	1.03	0.78
PWT			
Type-I	441.89 ± 8.00	5.15 ± 0.10	3.65 ± 0.60
Type-II	440.78 ± 9.28	5.14 ± 0.09	3.62 ± 0.68
Mean	441.33	5.14	3.63
CD (P<0.05)	12.38	1.12	0.89
Overall CD (P<0.05)	14.86	1.82	1.42

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

4.12.5 Available minerals

The data regarding *in vitro* availability of calcium, iron and zinc contents of control as well as probiotic buttermilk are given in Table 4.44. Control buttermilk contained 42.31, 22.06 and 39.83 per cent *in vitro* availability of calcium, iron and zinc, respectively. These values were found to be significantly ($P < 0.05$) lower compared to probiotic buttermilk. Probiotic buttermilk exhibited 62.66, 39.39 and 46.45 per cent *in vitro* availability of calcium, iron and zinc, respectively.

Kadhi prepared from a mixture of bengal gram flour and skim milk curd served as control which contained 51.61, 40.78 and 52.77 per cent *in vitro* availability of calcium, iron and zinc, respectively (Table 4.45). *In vitro* availability of calcium, iron and zinc of control *kadhi* was significantly lower as compared to experimental *kadhi*. Type-I and Type-II *kadhies* from BWT and PWT food mixtures also differed significantly ($P < 0.05$) in terms of their *in vitro* availability of calcium, iron and zinc. Type-II *kadhies* prepared from germinated, autoclaved and fermented BWT and PWT food mixtures exhibited higher values of *in vitro* availability of calcium, iron and zinc followed by Type-I and control *kadhies*. The values observed were 99.90 and 82.37, 89.55 and 70.24, 89.61 and 79.26 per cent, respectively in Type-I and Type-II *kadhies* in both cases.

Similar improvement in *in vitro* availability of minerals was also observed earlier in rice bean and fababean *kadhies* (Saharan, 1994).

Similar trend was also observed in case of *papad* (Table 4.46). Control *papad* showed significantly lower values of *in vitro* availability as compared to experimental *papad*. Type-II *papad* in both cases had significantly ($P < 0.05$) higher *in vitro* availability of calcium, iron and zinc followed by Type-I and control *papad*. Higher values of *in vitro* availability of minerals in Type-II *papad* might be due to the additional effect of germination involved in preparation of food mixture.

Wadies prepared from control mixture and experimental mixture also differed significantly ($P < 0.05$) in terms of their *in vitro* availability of calcium, iron and zinc (Table 4.47). Control *wadi* exhibited 65.29, 50.29 and 52.70 per cent *in vitro* availability calcium, iron and zinc, respectively. Among the experimental *wadies*, Type-II *wadies* in both cases contained significantly ($P < 0.05$) higher values of *in vitro* availability of calcium, iron and zinc, respectively as compared to Type-I *wadies*. Type-I *wadies* contained 82.91 and 74.31, 58.66 and 58.14; 59.82 per cent, *in vitro* availability of calcium, iron and zinc, respectively. Whereas Type-II *wadies* contained 99.95 and 82.95, 89.93 and 72.37, 70.22 and 80.14 per cent calcium, iron and zinc, respectively.

Various processing treatments involved had a cumulative effect for bringing about improvement in mineral extractability/ availability of minerals. Similar enhancement was also observed earlier in fermented products by various workers (Saharan, 1994; Grewal, 1992; Grewal, 2003).

Divalent cations i.e. calcium, iron and zinc etc. are generally present in bound form with phytic acid and a protein-phytate mineral complex is formed. Reduction in phytic acid during germination and fermentation possibly through hydrolysis by inherent phytase in sprouts and fermented microflora may release these metallic ions in free form and therefore, may account for increased *in vitro* availability of minerals in fermented products (Nolan and Duffins, 1987). The phytic acid and other antinutrients in the fermented products were found to have a significant and negative correlation with *in vitro* availability of minerals which ascertain the role of there antinutrients in lowering the availability of mineral in these foods (Saharan, 1994).

Table 4.44 : *In vitro* availability (%) of calcium, iron and zinc of buttermilk* (on dry matter basis)

Buttermilk	Calcium	Iron	Zinc
Control	42.31 ± 0.66	22.06 ± 3.46	39.83 ± 0.57
Probiotic	62.66 ± 0.33	39.39 ± 12.10	46.45 ± 1.73
't' value	28.26*	43.94*	19.78*

* Significant at 5% level

* Buttermilk prepared from skimmed milk curd served as control whereas buttermilk prepared from *L. acidophilus* skimmed milk curd served as experimental

Table 4.45 : In vitro availability (%) of calcium, iron and zinc of *kadhi* prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Calcium	Iron	Zinc
Control	51.61 ± 10.05	40.78 ± 4.04	52.77 ± 5.78
BWT			
Type-I	82.89 ± 11.54	57.66 ± 2.34	79.40 ± 1.74
Type-II	99.90 ± 3.43	89.55 ± 6.08	89.61 ± 2.72
Mean	91.39	73.60	84.50
CD (P<0.05)	12.82	11.46	6.02
PWT			
Type-I	73.59 ± 4.44	58.84±11.54	59.58 ± 3.88
Type-II	82.37 ± 3.52	70.24± 5.97	79.26 ± 4.40
Mean	77.98	64.54	69.42
CD (P<0.05)	15.71	14.43	8.07
Overall CD (P<0.05)	16.63	8.86	5.82

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.46 : In vitro availability (%) of calcium, iron and zinc of papad prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Calcium	Iron	Zinc
Control	43.38 ± 1.25	41.02 ± 5.77	46.61 ± 1.57
BWT			
Type-I	80.32 ± 4.58	56.56 ± 3.06	79.40 ± 2.56
Type-II	99.39 ± 5.84	88.66 ± 7.04	89.30 ± 6.01
Mean	89.85	69.61	84.35
CD (P<0.05)	6.84	9.09	5.76
PWT			
Type-I	72.23 ± 5.77	58.47 ± 2.64	59.34 ± 3.34
Type-II	81.27 ± 1.80	68.39 ± 5.77	78.19 ± 2.85
Mean	76.25	63.43	68.76
CD (P<0.05)	8.61	11.46	7.06
Overall CD (P<0.05)	7.11	5.18	5.96

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I : *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II : *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Table 4.47 : In vitro availability (%) of calcium, iron and zinc of wadi prepared from indigenously developed BWT* and PWT* fermented food mixtures (on dry matter basis)

Product	Calcium	Iron	Zinc
Control	65.29 ± 2.80	50.29 ± 2.80	52.70 ± 3.37
BWT			
Type-I	82.91 ± 5.74	58.66 ± 5.92	59.82 ± 2.31
Type-II	99.95 ± 2.79	89.93 ± 2.78	70.22 ± 3.95
Mean	91.43	74.29	65.02
CD (P<0.05)	7.02	11.28	7.23
PWT			
Type-I	74.31 ± 5.74	58.14 ± 1.71	59.38 ± 5.88
Type-II	82.95 ± 2.79	72.37 ± 5.79	80.14 ± 2.92
Mean	78.63	65.25	69.76
CD (P<0.05)	8.86	12.83	9.10
Overall CD (P<0.05)	10.18	9.56	7.79

Values are mean ± SE of three independent determinations

*BWT food mixture contains barley flour, whey powder and tomato pulp

*PWT food mixture contains pearl millet flour, whey powder and tomato pulp

Type-I: *Papad* prepared from non-germinated autoclaved and fermented BWT and PWT food mixtures

Type-II: *Papad* prepared from germinated autoclaved and fermented BWT and PWT food mixtures

Chapter-5

Summary and Conclusion

Coarse cereals and millets are the staple foods and the majority of population consume these in developing countries like India. If staple food based slurry is prepared from the locally available cereals and millets fermented with probiotic microorganisms, it will be highly advantageous. Besides nutritional benefits, such developed functional foods containing probiotic organism may have therapeutic value too. In the present study also, an attempt has been made to use live cells of probiotic i.e. *L. acidophilus* for development of probiotic curd. The probiotic curd was used for fermenting the food mixtures based on coarse cereals and millets. The following four food mixtures were prepared by mixing raw and germinated (barley/pearl millet flour), whey powder and tomato pulp.

- (i) Raw barley flour + whey powder + tomato pulp (2:1:1 w/w)
- (ii) Germinated barley flour + whey powder + tomato pulp (2:1:1 w/w).
- (iii) Raw pearl millet flour + whey powder + tomato pulp (2:1:1 w/w)
- (iv) Germinated pearl millet flour + whey powder + tomato pulp (2:1:1 w/w)

Three types of curds were prepared using *L. acidophilus* inoculum in three proportions i.e. 3, 5 and 10 per cent each in 100 ml of sterilized skimmed milk. On the basis of their organoleptic characteristics, probiotic curd with 5 per cent inoculum was further selected for carrying out the fermentation in indigenously developed food mixtures.

Each of the above developed food mixtures was mixed with water (1:5 w/v), autoclaved at 121°C for 15 min at 1.5 kg/cm², cooled and inoculated with 5 per cent probiotic curd (prepared from *L. acidophilus* inoculum) and incubated at 37°C for 12 h. The cell count was found to increase in all the four fermented BWT and PWT food mixtures upon probiotic fermentation. Growth of *L. acidophilus* in germinated + autoclaved + fermented BWT and PWT food mixtures was found to be maximum i.e. 8.88 and 8.64 cfu/g as compared to non-germinated + autoclaved + fermented BWT and PWT food mixtures i.e. 7.75 and 7.30 cfu/g, respectively.

The pH of raw (non-germinated and germinated) BWT and PWT food mixtures were 6.02, 5.14 and 6.10, 5.57, whereas titratable acidity were 1.69, 2.00 and 1.65, 2.15 g lactic acid/100 ml, respectively. A significant decline in pH was observed on autoclaving. Fermentation of these blends further brought about significant decline in pH with a corresponding rise in titratable acidity. The maximum increase in titratable acidity i.e. 3.10 and 3.05 g lactic acid/100ml was noticed in germinated + autoclaved + fermented BWT and PWT food mixtures. A significant negative correlation was found between the pH and titratable acidity in all types of food mixtures.

Non-germinated and germinated (control) BWT and PWT food mixtures had moisture 30.45 and 28.47 per cent, respectively on fresh weight basis. Crude protein 14.83 and 14.89, fat 1.95 and 4.29, ash 2.42 to 2.97 and crude fibre 2.14 to 0.80 per cent, respectively on dry matter basis were observed in both BWT and PWT (control) mixtures. No significant change took place in moisture, fat and ash contents when these food mixtures were autoclaved. Fermentation with *L. acidophilus* curd at 37°C for 12 h resulted in a significant ($P < 0.05$) decline in crude protein and crude fibre contents.

Non-germinated BWT and PWT food mixtures contained 3.02 and 2.90 g/100g total soluble sugars, 0.95 and 0.85 g/100g reducing sugars and 2.07 and

2.05 g/100g non-reducing sugars, respectively. Starch content of these mixtures varied from 42.92 to 48.23 g/100g, respectively in BWT and PWT food mixtures. Autoclaving of these mixtures resulted in significant increase in total soluble and reducing sugars and decrease in starch contents. There was significant ($P < 0.05$) improvement in reducing sugars and decrease in total soluble and non-reducing sugars on fermentation. Simultaneously, starch content decreased in fermented food mixtures of BWT and PWT.

BWT and PWT non-germinated food mixtures contained 5.93, 4.93 g/100g, 2.95, 1.92 g/100g and 2.98, 3.01 g/100g, respectively total, soluble and insoluble dietary fibre contents. Autoclaving caused significant reduction in total and insoluble dietary fibre, whereas soluble fraction increased significantly after autoclaving. When the autoclaved food mixtures were subjected to fermentation with *L. acidophilus* curd for 12 h, it caused significant reduction in all dietary fibre constituents of both BWT and PWT mixtures. Germinated (raw) BWT and PWT food mixtures contained total (4.88 and 3.12 g/100g), soluble (2.00 and 1.28 g/100g) and insoluble (2.88 and 1.84 g/100g) dietary fibre contents. Dietary fibre contents of germinated mixtures were significantly ($P < 0.05$) lower when compared to the values of non-germinated food mixtures. Autoclaving caused reduction in total and insoluble dietary fibre whereas fermentation caused significant reduction in all the three dietary fibre constituents.

Non-germinated BWT food mixture contained 3.12, 1.56 and 1.26 per cent whereas non-germinated PWT food mixture contained 1.11, 0.59 and 0.52 per cent of total, soluble and insoluble β -glucan contents. Autoclaving caused significant reduction in total contents (7 and 2%) and in insoluble β -glucan (12 and 30%), respectively in BWT and PWT food mixtures, whereas soluble β -glucan increased by 15 and 19 percent on autoclaving. Fermentation caused significant reduction in total, soluble and insoluble β -glucan contents of non-germinated + autoclaved + fermented BWT and PWT food mixtures. Germinated samples of BWT and PWT

had lower amount of total, soluble and insoluble β -glucan contents as compared to the non-germinated samples as germination caused reduction in β -glucan contents. When the germinated food mixtures were subjected to autoclaving, there was further decrease in total and insoluble β -glucan and increase in soluble fractions of BWT and PWT food mixtures. Whereas fermentation caused significant reduction in total (50 and 50%), soluble (40 and 33%) and insoluble (60 and 78%), respectively. Maximum total, soluble and insoluble β -glucan contents were found in all types of BWT food mixtures.

B-complex vitamins, i.e., thiamine, riboflavin and niacin contents of non-germinated BWT and PWT food mixtures, were 0.53, 0.11, 2.81 mg/100g and 0.46, 0.13, 1.25 mg/100g, respectively. Autoclaving caused significant reduction in all the three vitamins. Fermentation of autoclaved BWT and PWT slurries caused significant reduction in all three vitamins. Among the three vitamins, reduction observed was highest in thiamine contents of both types of mixtures. This decrease was because of consumptions of thiamine by fermenting microbes for their metabolic activity. Germinated BWT and PWT food mixtures contained significantly higher amount of thiamine 1.16 and 0.86 mg/100g, 0.32 and 0.31 mg/100g riboflavin and 3.98 and 2.30 mg/100g niacin, respectively. However, autoclaving and fermentation caused significant reductions in all the three vitamins.

Total lysine contents of BWT and PWT non-germinated food mixtures varied from 2.98 to 2.53 g/100g protein. These values reduced by 44 and 26 per cent on autoclaving, whereas increased by 31 and 55 per cent after fermentation of these autoclaved mixtures. Germination further improved their lysine contents almost 2 folds in both types of mixtures. These decreased to 3.43 and 3.34 g/100g protein on autoclaving whereas increased to 5.89 and 5.35 g/100g protein on fermentation with *L. acidophilus* curd for 12 h.

Prolamin fraction was found highest followed by globulin, glutelin and albumin in both non-germinated BWT and PWT food mixtures. Both treatments like autoclaving and fermentation caused reduction in all protein fractions but prolamin fraction was found to be affected maximum on fermentation. On the other hand, germinated mixtures of BWT and PWT exhibited higher values of all fractions as compared to non-germinated food mixtures. However, autoclaving of germinated food mixtures caused reduction in all fractions. Fermentation of germinated + autoclaved mixtures caused further reduction.

The two mixtures contained considerable amount of phytic acid (282.20 to 327.42 mg/100g), polyphenols (396.21 to 304.66 mg/100g) and amylase inhibitors (135.65 to 78.75 AIU/g), respectively, which decreased significantly after autoclaving, i.e., phytic acid (17 and 21%), polyphenols (12 and 15%) and amylase inhibitor (37 and 38%). Fermentation further caused significant ($P < 0.05$) reduction in these antinutrients. Germinated mixtures exhibited lower values as compared to the raw mixtures (non-germinated). Autoclaving and fermentation of these mixtures diminished the antinutrient contents by about 73.23 and 88.34 mg/100g, 150.44 and 92.28 mg/100g; 12.10 and 13.50 AIU/g, respectively in phytic acid, polyphenols and amylase inhibitor activity

In vitro digestibility of protein and starch of BWT and PWT non-germinated food mixtures varied from 42.05 to 45.30 percent and from 26.92 to 20.50 mg maltose released/g meal whereas germinated BWT and PWT food mixtures varied from 66.89 to 71.13 per cent and 48.97 to 41.93 mg maltose released /g meal. Germinated mixtures exhibited significantly ($P < 0.05$) higher values of *in vitro* protein and starch digestibility. Autoclaving as well as fermentation brought about significant improvement in the digestibilities (*in vitro*) of starch as well as protein.

The contents of calcium (392.43 to 440.82 mg/100g), iron (1.48 to 5.13 mg/100g) and zinc (3.66 to 3.47 mg/100g) of non germinated BWT and PWT food mixtures. The amount of all minerals remained unaltered after autoclaving as well as fermentation. Similar trend was also observed in germinated BWT and PWT food mixtures. On the other hand, availability of all minerals increased significantly ($P < 0.05$) on autoclaving and fermentation. Germination also caused significant improvement in *in vitro* availability of calcium, iron and zinc of BWT and PWT food mixtures.

The non-germinated as well as germinated + autoclaved + fermented blends were used for development of various local recipes like *kadhi*, *dosa*, *idli*, *papad* and *wadi*. Probiotic buttermilk which was prepared from *L.acidophilus* curd secured higher mean scores of all organoleptic characteristics as compared to control buttermilk. Other products like *kadhi*, *dosa*, *idli*, *papad* and *wadi* were developed from four types of non-germinated and germinated + autoclaved + fermented BWT and PWT food mixtures were also organoleptically evaluated.

Overall acceptability scores of *kadhi*, *papad* and *wadi* were found in the category of 'liked moderately' and 'liked slightly' whereas in case of *dosa* and *idli*, overall acceptability scores ranged from 4.00 to 4.90 and 3.60 to 4.50, respectively. These were found in the category of 'neither liked nor disliked' and 'disliked slightly'.

Most acceptable products were nutritionally evaluated. Probiotic buttermilk exhibited higher values of total lysine, *in vitro* protein digestibility, and *in vitro* availability of calcium, iron and zinc over control values, whereas crude protein and total minerals remained unaltered. Control *kadhi*, *papad* and *wadi* contained higher crude protein contents i.e. 18.05, 19.25 and 19.29 per cent, respectively over experimental values as control products are prepared from pulses which are rich in protein. Experimental (Type-I and Type- II) *kadhi*, *papad* and *wadi*

prepared from non-germinated and germinated + autoclaved + fermented mixtures of BWT and PWT had almost similar crude protein contents.

In both cases, Type-I and Type-II *kadhi*, *papad* and *wadi* exhibited significantly higher values of total lysine, *in vitro* protein and starch digestibility and *in vitro* availability of calcium, iron and zinc as compared to their respective food mixtures as well as control products whereas total minerals remained almost unaffected on germination, autoclaving and fermentation. All the developed products contained significantly lower contents of phytic acid, polyphenols and amylase inhibitor activity as compared to their control values and their respective food mixtures.

Hence, combination of germination followed by fermentation is a potential process for developing a food product of improved nutritive value in terms of *in vitro* protein and starch digestibility and *in vitro* availability of minerals.

The food mixtures prepared with barley flour, pearl millet flour, whey powder and tomato pulp were not only organoleptically acceptable and microbiologically safe but also feeding of such fermented mixtures contained live cells of probiotic organisms, i.e. *L. acidophilus*, which could be used to control diseases like diarrhoea. Consumption of probiotic foods can provide protection against GIT infections as well as raise the nutritional status of the masses. Hence, there is a great scope to more research work in this area so as to develop commercially viable health foods.

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APPENDIX-I

Hedonic Rating Test

Name: _____

Dated: _____

Product: _____

Test these samples and check how much you like or dislike each one. Use appropriate scale to show your attitude by assigning points that best describe your feelings about the sample. An honest expression of your feeling will help us.

Sr. No.	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability	Remarks

Rate

Like extremely
Like very much
Like moderately
Like slightly
Neither like nor dislike
Dislike slightly
Dislike moderately
Dislike very much
Dislike extremely

Organoleptic score

9
8
7
6
5
4
3
2
1

Abstract

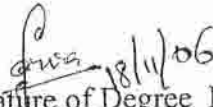
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Key words: Barley, pearl millet, *L. acidophilus* culture, fermentation, probiotic curd food mixtures, development of products, organoleptic, nutritional evaluation

The present investigation was conducted to study the formulation, nutritional evaluation and utilization of probiotic fermented coarse cereal blends. Four different food mixtures namely raw barley flour + whey powder + tomato pulp; germinated barley flour + whey powder + tomato pulp; raw pearl millet flour + whey powder + tomato pulp; germinated pearl millet flour + whey powder + tomato pulp were developed. In these food mixtures, barley/pearl millet flour, whey powder and tomato pulp were added in the ratio of 2:1:1(w/w). Each of these mixtures was mixed with water (1:5 w/v), autoclaved

and fermented with 5 per cent *L. acidophilus* curd as inoculum at 37°C for 12 h. *L. acidophilus* count was found maximum in germinated + autoclaved + fermented BWT and PWT food mixtures as compared to non-germinated mixtures. The pH declined with a corresponding rise in titratable acidity in all the probiotic fermented food mixtures. The maximum increase in titratable acidity was noticed in germinated + autoclaved + fermented BWT and PWT food mixtures. Autoclaving and fermentation did not bring any change in moisture, ash, fat whereas crude protein and crude fibre were reduced non-significantly on germination and fermentation. Total soluble, reducing and non-reducing sugars increased significantly when food mixtures were autoclaved but on germination and fermentation with *L. acidophilus* curd lowered down the level of total and non-reducing sugars and increase in reducing sugars. Starch content of all the food mixtures decreased as a result of germination, autoclaving and fermentation. Autoclaving caused significant reduction in total and insoluble dietary fiber whereas soluble fraction increased significantly. When autoclaved food mixtures subjected to fermentation for 12 h, it caused reduction in all dietary fibre constituents. Germinated food mixtures contained lower contents of all dietary fibre as compared to non-germinated food mixtures. Similar trend was also observed in case of total, soluble and insoluble β -glucan contents of all food mixtures. B-complex vitamins i.e. thiamine, riboflavin and niacin on autoclaving and fermentation showed reduction. Germinated food mixtures contained higher values of all the three vitamins as compared to non-germinated food mixtures of BWT and PWT. Total lysine contents of all food mixtures were found to be decreased on autoclaving but germination and fermentation caused improvement in total lysine. Protein fractions were found to be affected on all treatments. Phytic acid, polyphenols and amylase inhibitor activity significantly reduced after autoclaving and fermentation. Germination also caused reduction in all antinutrients. Simultaneously, a significant improvement *in vitro* protein and starch digestibility and *in vitro* availability of minerals was observed in autoclaved, fermented and germinated food mixtures. Total minerals were found unchanged on autoclaving and fermentation. Developed products namely buttermilk, *kadhi*, *papad* and *wadi* found organoleptically acceptable whereas *dosa* and *idli* were found unacceptable. Probiotic buttermilk found more nutritious as compared to control buttermilk. Similarly, *kadhi*, *papad* and *wadi* contained significantly higher contents of total lysine, *in vitro* protein and starch digestibility and *in vitro* availability of calcium, iron and zinc and lower amount of antinutrients as compared to control products and their respective food mixtures.


Signature of Degree Holder


Major Advisor


Head of the Department

