Food Chemistry 119 (2010) 779-784

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of germination and probiotic fermentation on nutrient composition of barley based food mixtures

Sonia Arora, Sudesh Jood *, N. Khetarpaul

Department of Foods and Nutrition, CCS Haryana Agricultural University, Hisar 125004, India

ARTICLE INFO

Article history: Received 7 January 2009 Received in revised form 24 May 2009 Accepted 20 July 2009

Keywords: Food mixtures Germination Lactobacillus acidophilus pH Titratable acidity Proximate composition Vitamins Lysine Available carbohydrates Dietary fibre

1. Introduction

In recent years interest has been renewed in health promotion and disease prevention by incorporation of probiotic bacteria into foods to counteract harmful bacteria in the intestinal tract. Probiotic organisms have been known to have a role in improving metabolism, lowering of cholesterol levels in blood, stimulation of the immune system, detoxification of potential carcinogens etc. (Nomoto, 2005; Smoragiewicz, Bielecka, Babuchowski, Boutard, & Dubeau, 1993). Literature indicates that probiotic foods not only have several potential health benefits but also have nutritional benefits (Sharma & Ghosh, 2006). Bacterial enzymatic hydrolysis has been shown to enhance the bioavailability of proteins by increasing 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 B-complex vitamins in fermented foods (Friend & Shahani, 1984). During the process of fermentation, acids and alcohols are produced which inhibit the growth of common pathogenic microbes. As a result of fermentation, pH is lowered, which helps to improve the shelf life of fermented foods (Sindhu & Khetarpaul, 2005).

ABSTRACT

Food mixtures formulated from non-germinated and germinated barley flour, whey powder and tomato pulp (2:1:1w/w) were autoclaved, cooled and fermented with 5% *Lactobacillus acidophilus* curd (10⁶ cells/ml) at 37 °C for 12 h. The cell count was found significantly higher (8.88 cfu/g) in the fermented food mixture formulated from germinated flour as compared to the non-germinated barley based food mixture. A significant drop in pH with corresponding increase in titratable acidity was found in the germinated barley flour based food mixture. Processing treatments like germination, autoclaving and probiotic fermentation did not bring about any significant change in ash and fat contents, but significant decrease was noticed in crude protein, crude fibre, starch, total and insoluble dietary fibre contents. The combined processing caused significant improvement in reducing sugar, thiamine, niacin, lysine and soluble dietary fibre contents of barley based food mixtures. In conclusion, a combination of germination and fermentation is a potential process for enhancing the nutritional quality of food mixtures based on coarse cereals.

A number of fermented products based on milk or curd have been prepared by using probiotic micro-organism, but until now, much less work has been done on the development of probiotic fermented products based on coarse cereals which constitute the staple diet of the majority of population in developing countries like India. Among these, barley (*Hordeum vulgare*) is a major world crop which is an excellent source of B-complex vitamins, minerals and complex carbohydrates (Kalra & Jood, 2000). Hence, coarse cereals require more cooking time and have relatively poor digestibility and availability of minerals, so various processing methods including dehulling, cooking, germination etc. have been reported to improve their nutritional quality (Pugalenthi & Vadivel, 2005).

In the present study, an attempt has been made to report the cumulative effect of germination and fermentation; especially with probiotic micro-organisms i.e. *Lactobacillus acidophilus*, on nutrient composition of indigenously developed barley based food mixtures.

2. Materials and methods

2.1. Materials

Huskless barley seeds were procured from the Department of Plant Breeding, Rajasthan Agricultural University, Bikaner, India.



^{*} Corresponding author. Tel.: +91 1662 244123; fax: +91 1662 234952. *E-mail address:* ramjood@rediffmail.com (S. Jood).

^{0308-8146/\$ -} see front matter \circledcirc 2009 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodchem.2009.07.035

Whey powder was provided by Mahaan Proteins Ltd., New Delhi, India. Tomatoes were purchased from the local market in a single lot. Seedless tomato pulp was obtained by mashing and sieving blanched and peeled tomatoes in a thick strainer. Skimmed milk was obtained from the Department of Animal Products Technology, CCSHAU, Hisar, India.

A pure culture of *probiotic* micro-organism *L*. *acidophilus* (NCDC-16) was collected from the Microbial Culture Collection Centre, NDRI, Karnal, India. The stock culture of *L*. *acidophilus* was added to 100 ml sterilized skimmed milk to obtain 10^6 cells/ml, incubated at 37 °C for 12 h and 5% inoculum was used for preparation of probiotic curd which was used further for probiotic fermentation of food mixtures.

2.2. Development of food mixtures

Barley seeds were cleaned thoroughly and half of the raw seeds were ground in an electric grinding mill using a 1.5 mm sieve size and the 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 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.

Two types of food mixtures were formulated from non-germinated and germinated barley flour along with whey powder and tomato pulp in the ratio of 2:1:1 w/w. Addition of tomato pulp and whey powder in food mixtures not only added nutrients, but also provided an optimum medium for growth of *L. acidophilus*.

2.3. Probiotic fermentation of the developed food mixtures

Each of the developed food mixtures (100 g) was mixed with distilled water (500 ml) to obtain homogenous slurry which was subsequently autoclaved at 1.5 kg cm⁻² for 15 min at 121 °C. It was then cooled and inoculated with 5% probiotic curd which supplied 10^6 cells/ml of broth to the slurry to carry out fermentation at 37 °C for 12 h in an incubator. The unfermented autoclaved slurries served as controls. At the end of fermentation period, 100 ml fresh fermented slurry of each food mixture was taken out for determination of titratable acidity, pH and cell counts.

2.4. Enumeration of Lactobacilli count

L. acidophilus present in fermented food mixtures were enumerated using DeMan–Rogosa–Sharpe (MRS) medium. One gram of fermented slurry was added to 9 ml sterile normal saline solution. Further dilutions up to 10^{-10} were made. Each dilution (1 ml) was pour plated in sterilized petriplates, incubated at 37 °C for 24 h and the colonies were counted by pour plating method using a colony counter.

2.5. Chemical analysis

2.5.1. Titratable acidity and pH

Titratable acidity was determined as lactic acid per 100 ml by using the standard method (Amerine, Berg, & Cruess, 1967). The pH was measured by a digital pH metre.

2.5.2. Proximate composition

Moisture and ash was estimated by using the standard methods of AOAC (2000). Crude protein, crude fat and crude fibre were estimated using the Autometic KEL PLUS, SOCS PLUS and FIBRA PLUS instruments, Pelican Equipments, Chennai, India by employing the standard methods of AOAC (2000).

2.5.3. B-complex vitamins and lysine

Thiamine was analysed using a Fluorometer (Toshniwal, Instruments, Pvt. Ltd., Ajmer, India) and niacin was analysed by a colorimetric method using a double beam spectrophotometer, 2203 (Systronics, Ambalal Sarabhai Enterprises Ltd., New Delhi, India) by following the standard methods of the AOAC (2000). Lysine content was estimated according to the method described by Miyahara and Jikoo (1967).

2.5.4. Available carbohydrates and dietary fibre

Total soluble sugars were extracted by refluxing in 80% ethanol (Cerning & Guilbot, 1973). Starch from the sugar-free pellet was extracted in 52% perchloric acid at room temperature (Clegg, 1956). Quantitative determination of total soluble sugars and starch was carried out according to the colorimetric method (Yemm & Willis, 1954). Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945). Non-reducing sugars were determined by calculating the difference between total soluble sugars and reducing sugars. Total, soluble and insoluble dietary fibre contents were determined by following the enzymatic method (Furda, 1981). The sum of insoluble dietary fibre and soluble dietary fibre contents were calculated as total dietary fibre.

2.6. Statistical analysis

The data were statistically analysed for analysis of variance in a completely randomized design to determine the critical difference (CD) among treatments. The difference of two means between the treatments exceeding this value is significant (Panse & Sukhatme, 1961).

3. Results and discussion

3.1. Cell count

Autoclaved non-germinated and germinated barley based food mixture slurries were inoculated with 5% 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 the fermented barley based food mixture slurries containing probiotic curd (Table 1). The growth of *L. acidophilus* in fermented food mixture formulated from germinated barley flour was found to be significantly higher (8.88 log cfu/g) as compared to the non-germinated food mixture (7.75 log cfu/g).

As the optimal temperature for the growth of probiotic organism was used, it appears that the food mixtures containing cereal, whey powder and tomato pulp supported the growth of *Lactobacilli* well. In the germinated food blend the increase in *Lactobacilli* count might be due to hydrolysis of germinated flours, which also provided better media for growth (Sripriya, Usha, & Chandra, 1997). Sindhu and Khetarpaul (2005) also reported 8.90 log cfu/g cell count of *Lactobacilli* as compared to yeast (7.63 log cfu/g) in fermented rice-pulse-milk coprecipitate based slurry.

3.2. pH and titratable acidity

Non-germinated (unprocessed) barley based food mixture had initial pH 6.02 and titratable acidity 1.69 g lactic acid/100 ml (Table 1), and on autoclaving, no significant change was noticed. However, a significant decrease in pH (4.23) and corresponding increase in titratable acidity (2.60 g lactic acid/100 ml) was observed when

Table 1

Effect of fermentation with *L. acidophilus* curd on *Lactobacilli* count (log cfu/g), pH and titratable acidity (g lactic acid/100 ml) of indigenously developed barley based food mixtures.^a

Food mixtures ^b	Lactobacilli count	рН	Titratable acidity
Non-germinated			
Unprocessed food mixture	-	6.02 ± 0.21	1.69 ± 0.05
Autoclaved food mixture	-	5.82 ± 0.12	1.78 ± 0.02
Autoclaved and fermented food mixture	7.75 ± 0.05	4.23 ± 0.17	2.60 ± 0.19
Germinated			
Unprocessed germinated food mixture	-	5.14 ± 0.52	2.00 ± 0.13
Germinated autoclaved food mixture	-	4.90 ± 0.08	2.38 ± 0.01
Germinated, autoclaved and fermented food mixture	8.88 ± 0.05	3.90 ± 0.11	3.10 ± 0.11
CD (P < 0.05)	-	0.78	0.45
'ť-Value	3.28 [*]	-	-

^a Values are mean ± SD of three independent determinations.

^b Barley based food mixture contains barley flour, whey powder and tomato pulp.

* Significant at 5% level.

the autoclaved food mixture was fermented with *L. acidophilus* curd.

A similar trend was also observed in pH and titratable acidity of food mixtures formulated from germinated flour (Table 1). However, decrease in pH and increase in titratable acidity was significantly higher in germinated food mixtures as compared to nongerminated food mixtures. The reduction in pH may be due to hydrolysis of starch into sugars during germination, which is readily utilised by the organisms and converted to lactic acid (Sripriya et al., 1997). A slight change was observed in pH and titratable acidity of autoclaved germinated food mixture. Whereas after probiotic fermentation a significant decrease in pH and increase in titratable acidity was measured.

A rapid drop in pH with corresponding increase in titratable acidity has been reported in lactic acid fermentation of a number of foods including finger millet (Sripriya et al., 1997) and cereal-legume blend (Sindhu & Khetarpaul, 2005). The homo-fermentative *L. acidophilus* converts glucose to lactic acid, which is responsible for the decline in pH of the product. It was also reported that *Lac*-*tobacillus* spp. is more effective in lowering pH than yeast and a combination of microbes (Sangeeta & Khetarpaul, 2001).

3.3. Nutritional evaluation

3.3.1. Proximate composition

The moisture, fat and ash contents of non-germinated and germinated (unprocessed) food mixtures (30.45% and 30.38%, 1.95% and 1.67%, 2.42% and 2.40%, respectively) did not vary significantly (P < 0.05) after germination, autoclaving and probiotic fermentation (Table 2). The findings of the present study are in agreement with those of previous workers (Sharma & Khetarpaul, 1997; Sindhu & Khetarpaul, 2005) who observed no change due to single and sequential culture fermentation in the fat and ash contents of cereal-legume food blends. On contrary, a few others reported a significant decrease in fat content of millets (Antony & Chandra, 1998; Elkhalifa, El Tinay, & Ali, 2007).

Crude protein and crude fibre contents of non-germinated (unprocessed) food mixture formulated from non-germinated (unprocessed) barley flour were 14.83% and 2.14%, respectively which decreased slightly after autoclaving but significantly after fermentation i.e. 7% and 12%, respectively (Table 2). On the other hand, food mixture formulated from germinated (unprocessed) barley flour contained significantly lower protein (13.86%) and fibre (2.10%) contents as compared to food mixture containing unprocessed non-germinated flour. Autoclaving and then fermentation of food mixtures may have caused reduction in crude fibre contents due to solubilisation of fibre (Sindhu & Khetarpaul, 2005). Similarly, Akubor and Obiegbuna (1999) reported reduction in total protein content and improvement in protein extractability of germinated finger millet. The protein reserves of the millet endosperm may have been used in the formation of new protoplasm and to meet the needs of the growing seedling. Again, autoclaving of this mixture did not have a significant effect, but fermentation caused further reduction by about 5% in total protein content of the food mixture formulated from germinated flour. Similarly, previous workers also reported that the bacteria used in fermentation of *natto* and *tempe* caused significant increase in the level of free amino acids, since during fermentation the micro-organisms secrete hydrolytic enzymes which degrade complex proteins into simpler proteins, peptides and amino acids (Ahmad, Mubarak, & EL-Beltagy, 2008; Akubor & Obiegbuna, 1999). On the contrary, increased protein content due to fermentation has also been reported in millets (Antony, Sripriya, & Chandra, 1996; Elkhalifa et al., 2007).

Table 2

Effect of fermentation with L. acidophilus curd on proximate composition of indigenously developed barley based food mixture (g/100 g, on dry matter basis).^a

Food mixtures ^b	Moisture ^c	Crude protein	Fat	Ash	Crude fibre
Non-germinated					
Unprocessed food mixture	30.45 ± 0.37	14.83 ± 0.58	1.95 ± 0.20	2.42 ± 0.29	2.14 ± 0.10
Autoclaved food mixture	30.43 ± 0.30	14.76 ± 0.57	1.91 ± 0.11	2.40 ± 0.05	2.01 ± 0.28
Autoclaved and fermented food mixture	30.30 ± 0.73	13.86 ± 0.23	1.83 ± 0.15	2.34 ± 0.08	1.89 ± 0.12
Germinated					
Unprocessed germinated food mixture	30.38 ± 0.66	13.86 ± 0.56	1.67 ± 0.17	2.40 ± 0.06	2.10 ± 0.15
Germinated autoclaved food 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 food mixture	30.06 ± 0.66	12.18 ± 0.35	1.46 ± 0.18	2.30 ± 0.11	1.88 ± 0.03
CD (<i>P</i> < 0.05)	NS	1.62	NS	NS	0.26

^a Values are mean ± SE of three independent determinations NS = non-significant.

^b Barley based food mixture contains barley flour, whey powder and tomato pulp.

^c Moisture on fresh weight basis.

Table 3

Effect of fermentation with *L. acidophlus* curd on B-complex vitamin (mg/100 g) and lysine (g/100 g protein) contents of indigenously developed barley based food mixtures (on dry matter basis).^a

Food mixtures ^b	Thiamine	Niacin	Lysine
Non-germinated			
Unprocessed food mixture	0.53 ± 0.01	2.81 ± 0.11	2.98 ± 0.10
Autoclaved food mixture	$0.48 \pm 0.02(-9)$	$2.65 \pm 0.05(-6)$	$2.50 \pm 0.05(-16)$
Autoclaved and fermented food mixture	$0.59 \pm 0.01(+11)$	2.95 ± 0.14(+5)	3.89 ± 0.11(+31)
Germinated			
Unprocessed germinated food mixture	1.16 ± 0.02	3.98 ± 0.05	5.05 ± 0.06
Germinated autoclaved food mixture	$1.05 \pm 0.01(-9)$	$3.35 \pm 0.02(-8)$	$4.17 \pm 0.05(-17)$
Germinated, autoclaved and fermented food mixture	1.32 ± 0.02 (+14)	4.39 ± 0.37 (+10)	$6.77 \pm 0.07(+34)$
CD (<i>P</i> < 0.05)	0.04	0.51	0.25

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

^a Values are mean ± SE of three independent determinations.

^b Barley based food mixture contains barley flour, whey powder and tomato pulp.

3.3.2. B-complex vitamins

Unprocessed non-germinated food mixture contained 0.53 mg/ 100 g thiamine and 2.81 mg/100 g niacin, respectively (Table 3). When the raw food mixture was subjected to autoclaving, it caused significant (P < 0.05) reduction in these vitamin contents. Other workers also reported that autoclaving significantly decreased the B-complex vitamins in legumes and millets (Khetarpaul & Chauhan, 1989 Ibanoglu, Ainsworth, & Hayes, 1997). Whereas fermentation of autoclaved food mixture with probiotic curd, caused significant improvement in thiamine and niacin contents by about 11% and 5%, respectively. These results are in agreement with those reported in previous studies (Khetarpaul & Chauhan, 1989; Sanni, Onilude, & Ibidapo, 1999).

Unprocessed germinated food mixture contained significantly (P < 0.05) higher amount of thiamine and niacin contents as compared to unprocessed non-germinated food mixture (Table 3). The values were 1.16 and 0.53 mg/100 g thiamine and 3.98 and 2.81 mg/100 g niacin, respectively. The increase was by about 1-2 folds in thiamine, and niacin contents of germinated food mixture. Similarly, Gilay and Field (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. In the present study, when the germinated mixture was subjected to autoclaving, it caused significant reduction in these vitamins. However, when the germinated autoclaved food mixture was fermented with probiotic curd, it caused 14% and 11% enhancement in thiamine and niacin contents. Similarly, Khetarpaul and Chauhan (1989) reported marginal increase in thiamine content of pearl millet by pure culture fermentation of L. acidophilus, whereas the concentration of thiamine improved almost 2-3 folds when fermentation was carried out by Saccharomyces diastaticus and Saccharomyces cervisiae, respectively.

3.3.3. Lysine

Lysine content of unprocessed non-germinated food mixture was 2.98 g/100 g protein, on a dry matter basis. When this mixture was subjected to autoclaving, 16% reduction in lysine content was observed (Table 3). However, it is known that heat processing can cause a decrease in the availability of essential amino acids through non-enzymatic browning reactions. As lysine become unavailable on heat treatment due to formation of Maillard reaction products between reducing sugars and amino acids (Ibanoglu et al., 1997). However, when the autoclaved food mixture was further subjected to fermentation, a significant (P < 0.05) increase by about 31% in lysine content was noticed. This may be due to the increase in microbial enzyme activity and protein hydrolysis during fermentation (Elkhalifa et al., 2007; Sripriya et al., 1997).

Germinated (unprocessed) food mixture contained significantly (P < 0.05) higher 5.05% lysine content as compared to non-germinated (unprocessed) food mixture. When the germinated mixture was autoclaved, 17% reduction was observed in lysine content. On the other hand, when germinated and autoclaved mixture was further fermented with L. acidophilus curd, it caused significant increase (34%) in lysine content. The present data are in agreement with previous findings that fermentation increased the contents and availability of lysine. According to Sripriya et al. (1997) the total free amino acids increased rapidly by about 4-5 folds during germination and doubled at 18 h fermentation. Similarly, Elkhalifa et al. (2007) also reported a significant increase in the content of lysine and methionine as a result of fermentation. Whereas Hamad, Bocker, Vogel, and Hammes (1992) reported no significant change in the amino acid composition of sorghum dough during fermentation.

3.3.4. Available carbohydrates

Unprocessed non-germinated barley based food mixture contained 3.02 g/100 g total soluble sugar, 0.95 g/100 g reducing sugar, 2.07 g/100 g non-reducing sugar and 42.92 g/100 g starch, respectively (Table 4). Autoclaving of the food mixture resulted in a significant increase in total soluble, reducing and non-reducing sugar contents and decrease in starch content up to the extent of 19%. Moist heat may cause rupturing of starch granules followed by hydrolysis of starch to oligosaccharides and then to monosaccharides, which caused significant increase in concentration of sugars (Grewal & Jood, 2009). When the autoclaved food mixture was fermented with L. acidophilus curd, a marked change in the profile of available carbohydrates was observed. Fermentation of food mixture reduced the contents of total soluble sugar, non-reducing sugar and starch by about 24%, 64% and 37%, respectively and these changes were significant when compared to the carbohydrate profile of the autoclaved unfermented food mixture. In contrast, reducing sugars of the autoclaved + fermented food mixture increased by about 4-fold over the unprocessed food mixture.

In the case of germinated food mixtures, the concentration of total (12.50 g/100 g), reducing (5.37 g/100 g) and non-reducing (7.13 g/100 g) sugars increased and starch content decreased (30.15 g/100 g) significantly (P < 0.05) as compared to non-germinated (unprocessed) food mixture, and this might be due to enzymatic hydrolysis of starch to simpler sugars during germination. When the germinated food blend was autoclaved, further significant decrease in starch content and corresponding increase in total soluble, reducing and non-reducing sugars was also observed. This increase could result from starch solubilisation during autoclaving. However, starch content was further decreased by about 50% when autoclaved food mixture was fermented with probiotic curd which resulted in a 5-fold increase in reducing sugars and significant

Effect of fermentation with L. acidophilus curd on sugar and starch contents of indigenously developed barley based food mixtures (g/100 g, on dry matter basis).^a

Food mixtures ^b	Total soluble	Reducing	Non-reducing	Starch
Non-germinated				
Unprocessed food mixture	3.02 ± 0.16	0.95 ± 0.11	2.07 ± 0.11	42.92 ± 0.71
Autoclaved food mixture	9.48 ± 0.11	3.95 ± 0.11	5.53 ± 0.05	34.63 ± 0.92
Autoclaved and fermented food mixture	7.25 ± 0.05	5.25 ± 0.17	2.00 ± 0.05	21.91 ± 2.28
Germinated				
Unprocessed germinated food mixture	12.50 ± 0.05	5.37 ± 0.11	7.13 ± 0.11	30.15 ± 0.91
Germinated autoclaved food mixture	17.30 ± 0.11	6.95 ± 0.05	10.35 ± 0.17	20.05 ± 0.87
Germinated, autoclaved and fermented food mixture	9.50 ± 0.28	8.23 ± 0.11	1.27 ± 0.05	9.98 ± 0.25
CD (P < 0.05)	0.47	0.37	0.32	2.89

^a Values are mean ± SE of three independent determinations.

^b Barley based food mixture contains barley flour, whey powder and tomato pulp.

decrease in total soluble and non-reducing sugar contents. Soluble sugars in the fermenting mixture may be utilized by the microflora as a carbon source and the fermented product may ultimately contain a level of sugars lower than that of the autoclaved food blend. It was reported that in the initial stages of fermentations, higher concentration of the sugars may be observed but with increased period of fermentation, the sugars may be utilized by the fermenting microflora and the fermenting product may contain lower levels of sugar than the initial concentration of sugars in the fermenting mixture. Reduction in starch in the fermented product may be attributed to hydrolysis of polysaccharides by fermenting microbes which possess both alpha and beta amylases (Sindhu & Khetarpaul, 2005; Sripriya et al., 1997).

3.3.5. Dietary fibre

Non-germinated food mixture (unprocessed) exhibited total, soluble and insoluble dietary fibre contents of 5.93, 2.95 and 2.98 g/100 g, respectively (Table 5). When the raw mixture was autoclaved, a significant reduction was observed in total and insoluble dietary fibre i.e. 10% and 31%, respectively, whereas soluble fraction was increased by about 12% 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 soluble dietary fibre. The results of present study are in agreement with those reported earlier in cooked vegetables and products where insoluble dietary fibre content decreased, while the soluble fraction was increased (Vidal-Valverde & Frias, 1992). When the autoclaved food mixture was further subjected to fermentation with probiotic curd, it caused again significant (P < 0.05) reduction in total and insoluble dietary constituents by about 16% and 65%, respectively, whereas further improvement was noticed in soluble fraction by about 32%.

On the other hand, food mixture formulated from germinated flour contained 4.88, 2.00 and 2.88 g/100 g total, soluble and insol-

uble dietary fibre contents, which were significantly lower as compared to food mixture formulated from unprocessed nongerminated barley flour (Table 5). It might be due to the fact that germination caused significant reduction in all the dietary fibre components. An enzyme β-galactosidase from germinated cereals and pulses partially attacks galactomannan to yield galactose. Therefore, the decrease in the polysaccharide and mucilage content may be attributed to their breakdown and utilisation by the growing sprouts (El-Mahdy & El-Sebiy, 1983; Hooda & Jood, 2003). In the present study, when the germinated barley based food mixture was autoclaved, further reduction was observed in total and insoluble fractions by about 14% and 49%, respectively, whereas significant (P < 0.05) increase i.e. 35% was observed in soluble fraction. Simultaneously, further reduction was also observed in total and insoluble dietary fibre and improvement in soluble fibre of fermented food mixture, due to increased activity of hydrolysing microbial enzymes (El-Mahdy & El-Sebiy, 1983). The results of this study are in accordance with those reported for cereal-legume based food mixtures fermented with single or sequential culture fermentation with yeast and Lactobacilli (Sindhu & Khetarpaul, 2005).

4. Conclusion

It may be concluded that a combination of germination followed by fermentation with probiotic organism of indigenously developed food mixtures is a potential process for developing food products of improved nutritional quality. This type of fermented food product not only offers unique nutritional value but also has therapeutic value. The consumption of such food mixtures may be useful in controlling diarrhoea induced by pathogens or antibiotics. To authenticate such therapeutic claims, the results of feeding trials carried out on mice in our lab shall be reported in future communications.

Table 5

Effect of fermentation with L. acidophilus curd on dietary fibre contents of indigenously developed barley based food mixtures (g/100 g, on dry matter basis).^a

Food mixtures ^b	Total	Soluble	Insoluble
Non-germinated			
Unprocessed food mixture	5.93 ± 0.05	2.95 ± 0.08	2.98 ± 0.18
Autoclaved food mixture	$5.35 \pm 0.07(-10)$	3.31 ± 0.11(+12)	$2.04 \pm 0.08(-31)$
Autoclaved and fermented food mixture	$4.93 \pm 0.09(-16)$	$3.90 \pm 0.25(+32)$	$1.03 \pm 0.05(-65)$
Germinated			
Unprocessed germinated food mixture	4.88 ± 0.17	2.00 ± 0.02	2.88 ± 0.06
Germinated autoclaved food mixture	$4.16 \pm 0.09(-14)$	2.70 ± 0.07(+35)	$1.46 \pm 0.07(-49)$
Germinated, autoclaved and fermented food mixture	$3.88 \pm 0.05(-20)$	$3.15 \pm 0.13(+58)$	$0.73 \pm 0.04(-74)$
CD (P < 0.05)	0.27	0.39	0.25

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

^a Values are mean ± SE of three independent determinations.

^b Barley based food mixture contains barley flour, whey powder and tomato pulp.

References

- Ahmad, G. N., Mubarak, A. E., & EL-Beltagy, A. E (2008). Nutritional potential and functional properties of tempe produced from mixture of different legumes. 1: Chemical composition and nitrogenous constituent. *International Journal of Food Science and Technology*, 43, 1754–1758.
- Akubor, P. I., & Obiegbuna, J. E. (1999). Certain chemical and functional properties of ungerminated and germinated millet flour. Journal of Food Science and Technology, 36, 241–243.
- Amerine, M. A., Berg, H. W., & Cruess, W. V. (1967). The technology of wine making. Westport, Connecticut: The AVI Publishing Company Inc. (pp. 692–694).
- Antony, U., & Chandra, T. S. (1998). Antinutrient reduction and enhancement in protein, starch and mineral availability in fermented flour of finger millet (Eleusine coracana). Journal of Agricultural and Food Chemistry, 46, 2578–2582.
- Antony, U., Sripriya, G., & Chandra, T. S. (1996). The effect of fermentation on the primary nutrients in foxtail millet (*Setaria italica*). Food Chemistry, 56, 381– 384.
- AOAC (2000). Official methods of analysis of the association of official agricultural chemists. Washington, DC: Association of Analytical Chemists (pp. 125–139).
- Cerning, J., & Guilbot, J. (1973). Change in carbohydrates composition during maturation of wheat and barley kernels. *Cereal Chemistry*, 50, 220–222.
- Clegg, K. M. (1956). The application of the anthrone reagent to the estimation of starch in cereal. *Journal of Science and Food Agriculture*, 7, 40–45.
- Elkhalifa, A. O., El Tinay, A. H. & Ali, A. M. (2007). Fermented sorghum foods of Sudan - A review. *Journal of Food Science and Technology*, 44, 343–349. El-Mahdy, A. R., & El-Sebiy, L. A. (1983). Changes in phytate and minerals during
- El-Mahdy, A. R., & El-Sebiy, L. A. (1983). Changes in phytate and minerals during germination and cooking of fenugreek seeds. *Food Chemistry*, *9*, 149–158.
- Friend, B. A., & Shahani, S. (1984). Nutritional and therapeutic aspects of Lactobacilli. Journal of Applied Nutrition, 36, 125–153.
- Furda, I. (1981). Simultaneous analysis of soluble and insoluble dietary fibre. In W. P. T. James & O. Theander (Eds.), *The analysis of dietary fibre* (pp. 163–172). New York: Marcel Dekker, Inc..
 Gilay, M. M., & Field, M. L. (1981). Nutritive value of germinated corn and corn
- Gilay, M. M., & Field, M. L. (1981). Nutritive value of germinated corn and corn fermented after germination. *Journal of Food Science*, 46, 835–841.
- Grewal, A., & Jood, S. (2009). Chemical composition and digestibility (*in vitro*) og green gram as affected by processing and cooking methods. *British Food Journal*, 111, 235–242.
- Hamad, S. H., Bocker, G., Vogel, R. F., & Hammes, W. P. (1992). Microbiological and chemical analysis of fermented sorghum dough for *Kisra* production. *Applied Microbiology and Biotechnology*, 37, 728–731.
- Hooda, S., & Jood, S. (2003). Effect of soaking and germination on nutrient and antinutrient contents of fenugreek (*Trigonella foenum graecum*). Journal of Food Biochemistry, 27, 165–176.

- Ibanoglu, S., Ainsworth, P., & Hayes, G. D. (1997). In vitro protein digestibility and content of thiamine and riboflavin in extruded tarhana a traditional Turkish cereal food. Food Chemistry, 58, 141–144.
- Kalra, S., & Jood, S. (2000). Cholesterol-lowering effect of dietary barley β-glucan in rats. *Journal of Cereal Science*, 31, 141–145.
- Khetarpaul, N., & Chauhan, B. M. (1989). Effect of fermentation on protein, fat, minerals and thiamine contents of pearl millet. *Plant Foods and Human Nutrition*, 39, 169–178.
- Miyahara, S., & Jikoo, K. (1967). Examination of the method of calorimetric determination of lysine. Journal of the Japanese Society for Food Science and Technology, 14, 512–513.
- Nomoto, K. (2005). Review: Prevention of Infections by probiotics. Journal of Bioscience and Bioengineering, 100, 583–592.
- Panse, Y. G., & Sukhatme, P. V. (1961). Statistical methods of agricultural coworkers (2nd ed.). New Delhi: Indian Council of Agricultural Research (pp. 87–89).
- Pugalenthi, M., & Vadivel, V. (2005). Nutritional evaluation and the effect of processing methods on antinutritional factors of sword bean (*Canavalia* gladiata). Journal of Food Science and Technology, 42, 510–516.
- Sangeeta, C., & Khetarpaul, N. (2001). Probiotics: Nutritional and health benefits. Life Science Reporter, 3, 1–10.
- Sanni, A. I., Onilude, A. A., & Ibidapo, O. T. (1999). Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean. *Food Chemistry*, 65, 35–39.
- Sharma, A., & Khetarpaul, N. (1997). Effect of fermentation on phytic acid content and *in vitro* digestibility of starch and protein of rice-black gram dhal – whey blends. Journal of Food Science and Technology, 34, 20–22.
- Sharma, G., & Ghosh, B. C (2006). Probiotic dairy foods and probiotics for health benefits. Indian Food Industry, 25, 68–73.
- Sindhu, S. C., & Khetarpaul, N. (2005). Development, acceptability and nutritional evaluation of an indigenous food blend fermented with probiotic organisms. *Nutrition and Food Science*, 35, 20–27.
- Smoragiewicz, W., Bielecka, M., Babuchowski, A., Boutard, A., & Dubeau, H. (1993). Probiotic bacteria. Canadian Journal of Microbiology, 39, 1089–1095.
- Somogyi, M. (1945). A new reagent for the determination of sugars. Journal of Biological Chemistry, 160, 61.
- Sripriya, G., Usha, A., & Chandra, T. C. (1997). Changes in carbohydrates, free amino acid, organic acid, phytate and HCl-extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). Food Chemistry, 58, 345–350.
- Vidal-Valverde, C., & Frias, J. (1992). Legume processing effects in dietary fibre components. Journal of Food Science, 56, 1350–1352.
- Yemm, E. W., & Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, 57, 508–509.