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

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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 (10^6 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.

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).

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.

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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