



Effect of Probiotic Fermentation on Antinutrients and the *In vitro* digestibilities of Starch and Protein of Pearl Millet Based Food Mixture

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ABSTRACT

The consumers over whelming interest for functional foods, including probiotics have resulted in attempts to develop an indigenously developed food mixture containing pearl millet, chickpea, skim milk powder and tomato pulp (2:1:1:1 w/w). The mixture was autoclaved, cooled and fermented with a probiotic *Lactobacillus acidophilus*-R at 37° C for 24 h. Both the antinutrients i.e. phytic acid and polyphenols were reduced significantly after autoclaving as well as fermentation whereas *in vitro* digestibility of starch and protein was significantly ($P < 0.05$) improved. A significant negative relationship was obtained between the content of antinutrients and digestibilities.

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INTRODUCTION

Many foods, particularly those of plant origin contain a wide range of antinutritional factors (Ganguli *et al.*, 2014). Unrefined cereals and millets are the richest source of phytic acid and polyphenols which contributes to poor digestion and absorption of different nutrients present in the foods. If a food mixture is developed from the commonly used cereals and legumes and then fermented with a probiotic organism, It may have a better profile of nutrients. But no such study has been conducted till today on the development of probiotic fermented products based on cereals, legumes or their blends. Therefore, in the present investigation, an attempt was made to develop an indigenous food mixture and study the effect of probiotic fermentation with *L. acidophilus*-R on its contents of antinutrients and *in vitro* digestibility of starch and protein.

MATERIALS AND METHODS

Pearl millet was procured from the Department of plant Breeding, Haryana Agricultural University, Hisar, India. Chickpea and tomato were procured from the local market while skim milk powder and *L. acidophilus*-R culture were collected from NDRI, Karnal, India. Seedless tomato pulp was obtained by mashing and sieving the blanched tomatoes in a thick strainer. The developed food mixture contained freshly ground pearl millet and chickpea along with skim milk powder and tomato pulp (2:1:1:1 w/w). The developed food mixture (100 gm) was mixed with distilled water (600 ml), stirred sufficiently to obtain a homogenous slurry, autoclaved at 121°C for 15 min., cooled, inoculated with *L. acidophilus*-R (10^5 cells/ml) and fermented (37°C, 24 hour) in triplicates. The unfermented slurry before and after autoclaving served as controls. These slurries were dried at 60°C in a hot air oven to a constant weight. The oven dried samples were ground to a fine powder (0.5 mm sieve) and used for chemical analysis. Phytic acid content was determined by the method of Haug and Lantzsch (1983). Total polyphenols, extracted in methanol

containing 1% HCl (Singh and Jambunathan, 1981) were estimated as tannic acid equivalent according to Folin-Denic procedure. Starch digestibility (*in vitro*) was estimated by employing pancreatic amylase (Singh *et al.*, 1982). The maltose so liberated was measured colorimetrically by using dinitrosalicylic acid reagent. Protein digestibility (*in vitro*) was determined by using pepsin and pancreatin (Akeson and Stahmann, 1964) and calculated by the following formula [Eq. 1]:

$$\text{Protein digestibility (\%)} = \frac{\text{Digested proteins} \times 100}{\text{Total proteins}} \text{ [Eq.1]}$$

The data were analyzed statistically in a completely randomized design to test the significant differences among treatments and correlation coefficients were also derived (Panse and Sukhatme, 1961).

RESULTS AND DISCUSSION

The content of phytic acid and polyphenols in the developed food mixture were 307 mg and 544.27 mg per 100 gm respectively on dry matter basis. When food mixture was autoclaved at 121°C for 15 min, a significant reduction ($P < 0.05$) in phytic acid (upto 27%) and polyphenols (upto 11%) was observed (Table 1). Upon fermentation of the autoclaved slurry with *L. acidophilus*-R (37°C, 24 h), phytic acid content came down from 307 mg to 133.08 mg per 100 gm. A significant ($P < 0.05$) decline to the extent of 18% occurred in polyphenols content when the mixture was autoclaved, cooled and then fermented with probiotic micro-organism. A decreased amount of phytic acid and polyphenols in autoclaved mixture may be due to the formation of insoluble complex with protein and minerals (Ekfenyong, 1985). Reduction of phytic acid content of the fermented mixture may be attributed to phytase from the microbial source (Lopez *et al.*, 1983). Similar results has been observed in rice defatted soyflour blends (Goyal and Khetarpaul, 1993). The

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diminishing effect of fermentation on polyphenols level may be due to the activity of polyphenol oxidase present in the foodgrains or microflora (Sindhu and Khetarpaul, 2001). A

reduction in polyphenolic content of pearl millet during culture fermentation with *L. brevis* or *L. fermentum* has been reported earlier (Khetarpaul and Chauhan, 1990).

Table 1: Changes in phytic acid and polyphenol contents during fermentation (mg/100mg, on dry matter basis)

| Processing treatments | Phytic acid | Polyphenols |
|--------------------------------|-----------------|------------------|
| Raw blend (control) | 307.00±7.43 | 544.27±13.28 |
| Unfermented autoclaved mixture | 223.25±6.02(27) | 485.67±14.56(11) |
| Fermented autoclaved mixture | 133.08±4.7(57) | 446.61±12.28(18) |
| SE m (±) | 5.08 | 5.99 |
| CD(P<0.05) | 15.23 | 17.96 |

* Values are means ± SD of three independent determinations.

Figure in parenthesis indicate percent decrease (-) over control values.

In vitro starch digestibility (expressed as mg maltose released per g sample) of unprocessed food mixture was 30.12gm (Table 2) and it improved significantly (P<0.05) upon autoclaving (121°C, 15 min.). Probiotic fermentation improved it further and doubled it in the fermented mixture. Enhanced digestibility of cereal and legume starch 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, the disintegration of various plant food components during cooking and inactivation of α -amylase inhibitors (Subbulakshmi *et al.*, 1976). Increase in starch digestibility of fermented products may be related to enzymatic properties of microbes, which ferment the substrate. The presence of α -amylase in the fermenting bacteria was noticed by Bernfeld (1962) and Soni and Sandhu(1990).

Table 2: Effect of fermentation with *L. acidophilus*-R on *in vitro* starch digestibility (mg maltose released/g mixture) and *in vitro* protein digestibility (%) of the food mixture (on dry matter basis)

| Processing treatments | Starch digestibility | Protein digestibility |
|--------------------------------|----------------------|-----------------------|
| Raw blend (control) | 30.12±1.95 | 47.64±0.71 |
| Unfermented autoclaved mixture | 48.97±3.51(63) | 56.11±1.58(18) |
| Fermented autoclaved mixture | 59.95±2.72(99) | 68.91±2.49(45) |
| SEm (±) | 1.16 | 0.78 |
| CD(P<0.05) | 3.48 | 2.34 |

* Values are means ± SD of three independent determinations.

Figure in parenthesis indicate percent increase (+) over control values.

A significant difference between the protein digestibility of raw and autoclaved food blend was noticed (Table 2). Fermentation further improved the protein digestibility as it could enhance it to the extent of 45 in the fermented blend, when compared to the control. The increase in protein digestibility on autoclaving may be attributed to the loss of phytic acid and inactivation of polyphenols or destruction of trypsin inhibitors (Parihar *et al.*, 1993). Better protein digestibility of fermented products is mainly associated with

the proteolytic activity of fermenting microflora (Sindhu and Khetarpaul, 2003). In addition, phytic acid, which is known to inhibit the proteolytic enzyme (Knuckles *et al.*, 1985), was considerably reduced during fermentation, resulting in the improvement of protein digestibility of the fermented mixture. A significant (P<0.05) negative correlation existed between phytic acid and protein digestibility of the fermented mixture (Table 3).

Table 3: Correlation coefficients of *in vitro* digestibilities of starch and polyphenol content of fermented food mixture

| Food mixture | Starch digestibility Vs. Phytic acid | Protein digestibility Vs. Phytic acid | Starch digestibility Vs. polyphenol | Protein digestibility Vs. polyphenol |
|--------------|--------------------------------------|---------------------------------------|-------------------------------------|--------------------------------------|
| PCMT | -0.8956** | -0.4532* | -0.9573** | -0.5559* |

* Values are significant at 5% level.

** Values are significant at 1% level.

CONCLUSION

It may be concluded that probiotic fermentation of the mixture containing pearl millet, chickpea, skim milk powder and tomato pulp with *L. acidophilus*-R reduced the content of phytic acid and polyphenols and brought a considerable

enhancement in the digestibilities of starch and proteins. Thus, the substrate comprising underutilized raw materials can substitute as a base for low cost probiotic foods with a very high nutritional value.

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