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Effect of Germination and Probiotic Fermentation on pH, Titratable Acidity, Dietary Fibre, β-Glucan and Vitamin Content of Sorghum Based Food Mixtures

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Abstract

Sorghum is an ancient, drought resistant cereal grain, often grows in semi-arid conditions where other cereal grains cannot. Lactic acid fermentation has been shown to improve the nutritional value of sorghum and the low pH generated during fermentation protects these foods against the growth of pathogenic bacteria. The present study has been planned to see the effect of germination and probiotic fermentation in enhancing the nutritional value of sorghum.

Two types of food mixtures were developed using raw and germinated sorghum flour along with whey powder and tomato pulp in 2:1:1 proportion (w/w). The developed food mixtures were mixed with water, autoclaved, cooled and fermented at 37°C for 12 h with *Lactobacillus acidophilus* curd containing 10^6 cells/ml. These food mixtures were analysed for pH, titratable acidity, dietary fiber, β-glucan and vitamin content.

Fermentation significantly reduced the pH and simultaneously increased the titratable acidity of germinated as well as non germinated food samples. Insoluble and total dietary fiber content increased significantly by fermentation ($p<0.05$), however, soluble fiber content decreased significantly. β-glucan content reduced significantly ($p<0.05$) in fermented sorghum based food mixture. The thiamine, riboflavin and niacin content increased significantly on fermentation ($p<0.05$) in both germinated and non germinated food mixture. This study has significance in terms of improving the nutritional quality of sorghum through probiotic fermentation.

Keywords: Sorghum; Germination; Probiotic fermentation; *Lactobacillus acidophilus*

Introduction

Sorghum is an annual erect plant that bears a cereal seed that is used by the world over as a food and feed and for a long list of other uses as well. The sorghum grain is an important cereal grain that is said to be the staple food of the poor in many countries. India has ever been among the major producers of sorghum in the world. The 2005-06 Indian sorghum production figures were 8 million metric tons. Area wise, India accounts for around 20% of the world total area used for the crop production. The major states in the country where this cereal grain is produced are Maharashtra, Karnataka, Gujarat, Madhya Pradesh, Andhra Pradesh, Rajasthan and Uttar Pradesh. Maharashtra produces the maximum sorghum in India. Sorghum ranks third between wheat and maize in production. While, sorghum has traditionally been used as animal feed in the U.S., approx. 40% of worldwide production is used for human consumption in Africa, India, etc. It is also known with different names depending upon the geographical area that includes Durra, Egyptian millet, Guinea corn, Jowar, Juwar, Milo, Shallu and Sudan grass. The grain sorghum is similar to that of maize but having more fats and proteins. This proves beneficial for the livestock and hence is the reason of the popularity of the crop as a feed.

Various processing methods used in the preparation of products from sorghum include dehulling, cooking, germination and fermentation. Recently numerous researchers proposed germination and fermentation as ways to improve nutritional quality of cereals. The cumulative effect of germination and fermentation especially with probiotic microorganism can have added advantages. They may not only improve the nutritional quality of sorghum products but may also add therapeutic effects [1,2]. In general fermentation means the conversion of organic substances to simpler substances. Fermentation has been shown to increase the level of vitamin B complex especially that of thiamine and riboflavin [3]. These changes are important since most of Indian diets are comprised of cereals, millets and legumes and the incidence of protein and vitamin B deficiencies is high [4]. The objective of this investigation was to study the effect of germination and probiotic fermentation on dietary fiber, β-glucan and vitamin content of sorghum based food mixtures.

Materials and Methods

Materials

Sorghum (HS-B67-2) was procured from Department of Plant Breeding, CCSHAU, Hisar. 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.

Microbial culture

The culture of probiotic micro-organism *Lactobacillus acidophilus*

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

Preparation of raw and germinated sorghum flour

Sorghum 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 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.

Development of food mixtures

Two types of food mixtures were developed using raw and germinated sorghum flour along with whey powder and tomato pulp. The composition of the developed food mixtures was as follows:

(a) Raw sorghum flour + whey powder + tomato pulp (2:1:1, w/w)

(b) Germinated sorghum 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.

Probiotic fermentation of the developed food mixtures

Each of the developed food mixture (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. The autoclaved slurry was cooled and then inoculated with probiotic curd which supplied 10^6 cells/ml to the slurry to carry out fermentation at 37°C for 12 h in an incubator. The unfermented mixture slurries, before autoclaving served as controls.

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 and pH. Rest of the fermented as well as unfermented slurries were dried at 60°C to a constant weight. The oven dried samples were ground in an electric grinder mill using 1.5 mm sieve size to a fine powder and stored in air tight plastic containers for further nutritional analysis.

Chemical analysis

Moisture, pH and titratable acidity: Fermented slurry was analysed for moisture [5], pH (by digital pH meter) and titratable acidity (g lactic acid/100 ml) according to the method of Amerine et al. [6].

Dietary fibre: Soluble and insoluble dietary fibre constituents were determined by the enzymatic method given by Furda [7]. Total dietary fibre content was calculated according to formula given below:

Total dietary fibre=Insoluble Dietary Fibre (IDF)+Soluble Dietary Fibre (SDF)

β-glucan: The total and soluble β-glucan content was estimated by the method of McCleary and Holmes [8] and Megazyme Mixed Linkage β-glucan and glucose test kits. Insoluble β-glucan content was calculated as the difference between total β-glucan and soluble β-glucan content.

B-complex vitamins: Thiamine was determined by Fluorometric method of AOAC [5]. Riboflavin was determined by Fluorometric method of AOAC [5]. Niacin content was estimated according to method using HPLC.

Statistical analysis

The data were subjected to analysis of variance in a completely randomized design according to the standard methods [9].

Result and Discussions

Moisture, pH and titratable acidity

The moisture content of non-germinated sorghum based food mixture was 26.32 percent on fresh weight basis (Table 1). When this mixture was autoclaved and fermented, no significant change in the moisture content was observed. A similar trend was found in germinated samples.

The pH of non-germinated raw food mixture was 6.23 (Table 1). After autoclaving and fermentation, a significant decline in pH took place. The titratable acidity of food mixture was found to have inverse relationship with pH. As pH decreased in autoclaved and fermented food mixture, a significant (P<0.05) increase in titratable acidity was found. It increased from 1.71 (control) to 1.88 (autoclaved) and 2.22 (fermented) (g lactic acid/100 ml).

In case of germinated food mixture, the pH of raw sample dropped from 5.24 to 3.65 in fermented sample with corresponding increase in titratable acidity. The probiotic organism used for fermentation convert glucose to lactic acid which is responsible for the decline in pH of the food product.

The comparison of pH and titratable acidity in non-germinated and germinated food mixtures reveals that latter had significantly lower pH and higher titratable acidity as compared to non-germinated one. The reason being that during germination starch is hydrolyzed into sugars which is readily utilized by the organisms and converted to lactic acid.

Type of food mixture	Moisture	pH	Titratable acidity (g lactic acid/ml)
Non-germinated			
U	26.32 ± 0.62	6.23 ± 0.02	1.71 ± 0.05
A	26.17 ± 1.38	5.61 ± 0.04	1.88 ± 0.03
A+F	26.16 ± 0.99	4.43 ± 0.06	2.22 ± 0.04
CD (P<0.05)	NS	0.46	0.26
Germinated			
U	26.90 ± 0.39	5.24 ± 0.01	2.04 ± 0.04
A	26.61 ± 0.41	4.78 ± 0.03	2.43 ± 0.02
A+F	26.59 ± 0.47	3.65 ± 0.02	3.14 ± 0.04
CD (P<0.05)	NS	0.56	0.34
Overall CD (P<0.05)	NS	0.79	0.46

U-Unprocessed, A- Autoclaved, A+F- Autoclaved + fermented
Values are mean ± SE of three independent determinations
*Sorghum based food mixtures contain sorghum flour: tomato pulp: whey powder (2:1:1)

Table 1: Effect of germination and probiotic fermentation on pH, titratable acidity (g lactic acid/ml), *Lactobacilli* count (log cfu/g) and moisture content of indigenously developed sorghum based food mixtures* (on fresh matter basis).

Type of food mixture	Dietary fibre Insoluble Soluble Total		
Non-germinated			
U	12.55 ± 0.95	2.23 ± 0.18	14.78 ± 0.76
A	11.04 ± 0.49 (-12.03)	2.60 ± 0.08 (+16.50)	13.64 ± 0.54 (-7.71)
A+F	12.73 ± 0.87 (+1.43)	1.13 ± 0.04 (-49.30)	13.86 ± 0.92 (-6.22)
CD (P<0.05)	2.82	0.43	2.68
Germinated			
U	8.95 ± 0.05	1.38 ± 0.01	10.33 ± 0.07
A	11.60 ± 0.73 (+29.60)	1.23 ± 0.07 (-10.80)	12.83 ± 0.72 (+24.20)
A+F	10.73 ± 0.28 (+19.88)	0.43 ± 0.01 (-68.80)	11.25 ± 0.17 (+8.90)
CD (P<0.05)	1.60	0.15	1.52
Overall CD (P<0.05)	2.03	0.28	1.92

U-Unprocessed, A- Autoclaved, A+F- Autoclaved + fermented
Values are mean ± SE of three independent determinations
Figures in parentheses indicate percent increase (+) or decrease (-) over control
*Sorghum based food mixtures contain sorghum flour: tomato pulp: whey powder (2:1:1)

Table 2: Effect of germination and probiotic fermentation on dietary fibre contents of indigenously developed sorghum based food mixtures* (g/100 g, on dry matter basis).

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 [10]. According to Food and Nutrtion Board [11] 'total fibre' is defined as the combination of 'dietary' and 'functional' fibre. The results pertaining to total, soluble and insoluble dietary fibre has been presented below:

Total dietary fibre

The total dietary fibre content of non-germinated unprocessed, autoclaved and fermented food mixture was found to range between 12.16-13.38%. Autoclaving resulted in significant (P<0.05) decrease (8%) in total dietary fibre content. Fermentation resulted in 9% reduction in total dietary fibre content as compared to unprocessed raw mixture (Table 2).

A similar trend was observed in the germinated food mixtures; however the values of dietary fibre contents were significantly lower as compared to non-germinated food mixtures.

Insoluble dietary fibre

The non-germinated raw food mixture contained 10.55% insoluble dietary fibre. Autoclaving resulted in significant reduction in insoluble dietary fibre content. When this food mixture was fermented with *Lactobacillus* curd, the insoluble dietary fibre content decreased significantly as compared to control, but increased significantly as compared to autoclaved food mixture.

A similar trend was found in germinated food mixture. The insoluble dietary fibre content of raw, autoclaved and fermented food mixtures was found to be 8.95, 7.25 and 8.05%, respectively.

Soluble dietary fibre

The soluble dietary fibre content of non-germinated raw food mixture was 2.83%. Autoclaving resulted in significant (P<0.05) increase (13%) in soluble dietary fibre content of food mixture. However, when autoclaved sample was fermented with *Lactobacillus* curd, a significant decrease in soluble dietary fibre content was found. A similar trend was found in the germinated food mixture. However, the values of soluble dietary fibre of germinated raw, autoclaved and fermented food mixtures were significantly less as compared to their corresponding non-germinated food mixture.

The reduction in total and insoluble fibre and increase in soluble fibre during autoclaving might be due to conversion of insoluble dietary fibre to short length chains or units which could probably be precipitated along with soluble dietary fibre. The decrease in soluble, insoluble and total dietary fibre content during fermentation might be due to increased activity of hydrolyzing enzymes like cellulose, α-galactosidase etc. which hydrolyze the dietary fibre constituents and lead to conversion of insoluble dietary fibre into soluble dietary fibres during fermentation process. Chitra *et al.* [12] also showed the reduction in total dietary fibre content of chickpea dhal from 161.2 to 82.4 g/kg after fermentation.

The germinated food mixture had significantly lower dietary fibre values as compared to non-germinated food mixture. It might be due to the fact that on germination, activity of enzyme alpha-galactosidase increases which leads to reduced levels of dietary fibre during germination. Hooda and Jood [13] also reported that an enzyme β-galactosidase from germinated cereals and pulses 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.

Prior to germination, the sorghum seeds were soaked in water for 12 hrs followed by discarding of soaked water and germination under controlled conditions. Soaking might have caused a decrease in total and insoluble dietary fibre and increase in soluble dietary fibre due to leaching out of soluble dietary fibre into the soaking medium and conversion of some insoluble dietary fibre into the soluble dietary fibre.

β-glucan

The non-germinated raw sorghum based food mixture contained 5.32 g/100 g total β-glucan. Autoclaving and fermentation resulted in 11 and 21 percent reduction in total β-glucan content, respectively (Table

Type of food mixture	β-glucan Soluble Insoluble Total		
Non-germinated			
Raw (control)	3.14 ± 0.02	2.18 ± 0.04	5.32 ± 0.06
Autoclaved	4.01 ± 0.06 (+28)	0.74 ± 0.02 (-66)	4.75 ± 0.08 (-11)
Autoclaved + fermented	3.10 ± 0.06 (-1)	1.10 ± 0.05 (-50)	4.20 ± 0.02 (-21)
CD (P<0.05)	0.18	0.13	0.20
Germinated			
Raw (control)	2.56 ± 0.05	1.45 ± 0.10	4.01 ±
Autoclaved	3.21 ± 0.04 (+25)	0.51 ± 0.03 (-65)	3.72 ± (-7.23)
Autoclaved + fermented	1.02 ± 0.08 (-60)	0.22 ± 0.02 (-85)	1.24 ± (-69)
CD (P<0.05)	0.21	0.21	0.35
Overall CD (P<0.05)	0.17	0.15	0.25

U-Unprocessed, A- Autoclaved, A+F- Autoclaved + fermented
Values are mean ± SE of three independent determinations
Figures in parentheses indicate percent increase (+) or decrease (-) over control
*Sorghum based food mixtures contain sorghum flour: tomato pulp: whey powder (2:1:1)

Table 3: Effect of germination and probiotic fermentation on β-glucan contents of indigenously developed sorghum based food mixtures* (g/100 g, on dry matter basis).

Type of food mixture	Thiamine (mg/100 g)	Riboflavin (mg/100 g)	Niacin (mg/100 g)
Non-germinated			
U	0.15 ± 0.02	0.06 ± 0.01	1.71 ± 0.04
A	0.14 ± 0.02	0.05 ± 0.01	1.69 ± 0.02
A+F	0.23 ± 0.07 (53.30)	0.10 ± 0.01 (66.60)	2.20 ± 0.02 (28.60)
CD (P<0.05)	0.06	0.02	0.10
Germinated			
U	0.34 ± 0.01	0.19 ± 0.01	2.84 ± 0.05
A	0.33 ± 0.02	0.17 ± 0.01	2.82 ± 0.01
A+F	0.51 ± 0.02 (50.00)	0.32 ± 0.01 (68.40)	3.10 ± 0.02 (9.15)
CD (P<0.05)	0.06	0.04	0.11
Overall CD (P<0.05)	0.05	0.03	0.09

U-Unprocessed, A- Autoclaved, A+F- Autoclaved + fermented
Values are mean ± SE of three independent determinations
Figures in parentheses indicate percent increase over control
*Sorghum based food mixtures contain sorghum flour: tomato pulp: whey powder (2:1:1)

Table 4: Effect of germination and probiotic fermentation on B-complex vitamin contents of indigenously developed sorghum based food mixtures* (on fresh matter basis).

3). A similar trend was also observed in insoluble β-glucan content. This might be due to heat treatment which is responsible for conversion of total and insoluble β-glucan into soluble β-glucan. As a result, the soluble β-glucan content of autoclaved food mixture was increased significantly (P<0.05) by 28%.

When the autoclaved mixtures were fermented with *L. acidophilus* curd, there was no significant change in soluble β-glucan, however, the total and insoluble β-glucan content decreased significantly (P<0.05) by 21 and 50 percent, respectively when compared with raw food mixture. The apparent decrease in total and insoluble β-glucan observed after fermentation might be due to enhanced activity of enzymes like β-glucanases and carboxypeptidases which cause degradation of total and insoluble β-glucan content into soluble β-glucan. Further due to fermentation activity of other enzymes like β-glucosidases, cellobiose etc. increase which further hydrolyse the soluble β-glucan into glucose. This leads to significant reduction in soluble β-glucan after fermentation [14].

A similar trend was found in the germinated raw, autoclaved and fermented food mixture. However, the values of β-glucan of germinated food mixture were significantly lower as compared to their respective non-germinated food mixture.

B-complex vitamins

The data regarding the B-complex vitamins namely thiamine, riboflavin and niacin have been presented in Table 4. The unprocessed non-germinated food mixture contained 0.15 mg/100 g thiamine, 0.06 mg/100 g riboflavin and 1.71 mg/100 g niacin. No significant change was observed in B-complex vitamins after autoclaving of the food mixture. When the autoclaved non-germinated food mixture was fermented with *L. acidophilus* curd, a significant increase in thiamine (53%), riboflavin (67%) and niacin (29%) content was observed.

A similar trend was found in the germinated raw, autoclaved and fermented food mixtures; however, the values of germinated food mixtures were higher than their respective non-germinated food mixture. Aliya and Geervani [15] observed an increase in thiamine and riboflavin content in fermented legume and millet product. Khetarpaul and Chauhan [16] observed 2 to 3 fold increase in thiamine content of pearl millet when fermentation was carried out by *S. diastatiens* and *S. cervisiae*, respectively.

Conclusion

The thiamine, riboflavin and niacin content increased significantly on fermentation (p<0.05) in both germinated and non germinated food mixture. This study has significance in terms of improving the nutritional quality of sorghum through probiotic fermentation.

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