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Indigenous legume fermentation: Effect on some antinutrients and in-vitro digestibility of starch and protein

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Indigenous fermentation of coarsely ground dehulled black-gram dhal slurry at 25, 30, and 35°C for 12 and 18 h reduced the levels of phytic acid and polyphenols significantly ($P < 0.05$). The unfermented legume batter had high amounts of phytic acid (1000 mg/100 g) and polyphenols (998 mg/100 g), and these were reduced to almost half in the product fermented at 35°C for 18 h. *In-vitro* digestibility of starch and protein improved significantly ($P < 0.05$) with increase in the temperature and period of fermentation. A significant ($P < 0.01$) and negative correlation found between the *in-vitro* digestibility and the antinutrient further strengthens these findings.

INTRODUCTION

Over the centuries, traditional methods of processing and cooking legumes have been evolved to give safe, appetizing and nutritious products. Appropriate processing is probably more important for legumes than for any other food group, owing to the high contents of toxins and the indigestible nature of many raw legumes. In developing countries such as India, where the consumption of legumes remains high, many varied and appetizing dishes are prepared by using different methods of processing, including soaking, cooking, sprouting, and fermentation.

Fermentation is probably one of the oldest methods of processing legumes. Generally, the traditional methods of preparing fermentated foods are simple and inexpensive. During the last few years, much interest has been generated in the fermented foods of Asian and African countries, including India, where such foods are still being manufactured according to traditional, technologically less-advanced methods, by using simple equipment and produced on a cottage-industry scale by means of natural microflora.

Idli, dosa, wadies, dhokla, and *khaman* are a few of the popular indigenously fermented legume/cereal–legume products that are commonly prepared and consumed in India. *Wadies,* somewhat like Japanese *miso*, are spicy, hollow, brittle, friable balls, 5–8 cm in diameter, very popular in northern India, and prepared from dehulled green-gram or black-gram cotyledons. No systematic studies have been carried out so far to evaluate the nutritive values of *wadies*. The present communication deals with studies of the levels of phytic acid and polyphenols and *in-vitro* digestibility of starch and protein of black-gram-dhal *wadies*.

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MATERIALS AND METHODS

Materials

For the preparation of an indigenous-legume fermented product, i.e. *wadi*, black-gram dhal (*Phaseolus mungo*) was procured from a local market in a single lot. The legume grains were cleaned of dust, stones, split and wrinkled seeds, and foreign materials.

Wadi manufacture

Soaking and grinding. Dehulled black gram (200 g) was soaked in distilled water (300 ml) for 12 h at 30°C. The soaked dhal was coarsely ground in an electric grinder. The soaking water was not discarded because it was just sufficient to be used for grinding.

Fermentation

The soaked and ground slurry of black-gram cotyledons was kept as such for natural fermentation at 25, 30, and 35°C for 12 and 18 h in a BOD incubator.

Preparation of wadies

At the end of fermentation at a specific temperature,

spices such as salt (2 g/100 g) and black-pepper powder (0·65 g/100 g dhal) were added to the fermented slurry. Small portions of the fermented-legume slurries were taken and shaped into the form of round balls by hand (*wadies*) on polythene sheets. These *wadies* were dried at 60°C for 36 h to a constant weight. The unfermented coarsely ground slurry containing the spices served as control.

The dried *wadies* were finely ground in an electric grinder (Cyclotec M/s Tecator, Höganäs, Sweden) and used for chemical analysis.

Chemical analysis

The phytic acid content was determined by the method of Davies and Reid (1979). The polyphenolic compounds were extracted from the defatted sample by refluxing with 50 ml methanol containing 1% HCl for 4 h and estimated as tannic acid equivalent according to the Folin–Denis procedure (Swain & Hills, 1959). Starch digestibility (*in vitro*) was determined by employing pancreatic amylase and then measuring the maltose liberated by using dinitrosalicylic acid reagent (Singh *et al.*, 1982). *In-vitro* protein digestibility was carried out by the method of Akeson and Stahmann (1964) as modified by Singh and Jambunathan (1981).

Statistical analysis

The data were subjected to analysis of variance in a completely randomized design, and correlation coefficients were derived according to standard statistical methods (Panse & Sukhatme, 1961).

RESULTS AND DISCUSSION

Phytic acid

The unfermented black-gram-dhal *wadi* mixture contained 1000 mg/100 g phytic acid (Table 1). Fermentation of the mixture reduced the phytic acid content significantly ($P < 0·05$). The legume product fermented at 35°C for 18 h had only one-half of the phytic acid content of that present in the unfermented mixture. An increase in the fermentation period, i.e. from 0 to 12 and from 12 to 18 h at different temperatures, resulted in 18–43% decrease in phytic acid content. The higher the temperatures and the longer the period of fermentation, the greater was the reduction in the content of this antinutrient.

A wide range of microflora has been known to possess phytase activity (Daniels & Fisher, 1981; Lopez *et al.*, 1983), which may be partly responsible for reducing the phytic acid content in the fermented products. The inherent phytase activity reported in legumes may also be responsible for decreasing the phytate content during *wadi* fermentation. The optimum temperature for phytase activity from plants and microbial sources has been known to range from 35 to 45°C. This may account for a greater reduction in phytic acid content at 35°C than at 30 or 25°C. A decrease in phytic acid content during fermentation has also been reported in various foods, including *tempeh* (Sutardi & Buckle, 1985) and *soy-rabadi* (Grewal, 1992).

Table 1. Effect of temperature and period of fermentation on phytic acid and polyphenol content of *wadies* prepared from black-gram dhal (mg/100 g on dry-matter basis)

Temperature (°C)	Period of fermentation (h)	Phytic acid	Polyphenol
	0 (Control)	1 004 ± 0·14	998 ± 0·04
25	12	820 ± 0·05 (18·37)	742 ± 0·14 (25·68)
	18	788 ± 0·09 (21·59)	703· ± 0·01 (29·57)
30	12	699 ± 0·06 (30·45)	587 ± 0·02 (41·19)
	18	647 ± 0·11 (35·56)	536 ± 0·07 (46·27)
35	12	626 ± 0·09 (37·66)	516 ± 0·06 (48·33)
	18	576 ± 0·07 (42·58)	487 ± 0·06 (51·20)
	SE (m)	0·47	0·23
	CD ($P < 0·05$)	0·41	0·69

Values are means ± SD of four independent determinations. Figures in parentheses indicate percentage decrease in phytic acid and polyphenol contents over the control value.

Polyphenol

The unfermented legume batter had 998 mg polyphenols per 100 g (Table 1). Significant variations occurred in the polyphenolic contents of black-gram-dhal *wadies* fermented at various temperatures for 12 and 18 h. The extent of polyphenolic loss ranged from 26 to 30, from 41 to 46, and from 48 to 52% over the control values in the products fermented at 25, 30, and 35°C, respectively, for varying periods. Polyphenols were reduced to almost half in the product fermented at 35°C for 18 h.

The diminishing effect of fermentation on polyphenols may be due to the activity of polyphenol oxidase present in legume or microflora. A decrease in polyphenolic content during fermentation has been reported earlier in various fermented foods, including *rabadi* (Dhankher & Chauhan, 1987) and in pearl millet fermented by pure cultures of yeasts and lactobacilli (Khetarpaul & Chauhan, 1990). Contrary to these results, some workers (Goyal, 1991; Grewal, 1991) reported an increase in the polyphenolic content of fermented products.

Starch digestibility (*in vitro*)

Starch digestibility (*in vitro*) expressed as mg maltose released/g flour was 35·7 in unfermented legume batter (Table 2). Fermentation improved the starch digestibility of *wadies* significantly ($P < 0·05$). Starch digestibility seemed to be enhanced significantly with an increase

Table 2. Effect of temperature and period of fermentation on *in-vitro* starch and protein digestibility of *wadies* prepared from black-gram dhal (on dry-matter basis)

Temperature (°C)	Period of fermentation (h)	Starch digestibility (mg maltose released/ g meal)	Protein digestibility (%)
	0 (Control)	35·7 ± 0·08	53·04 ± 0·51
25	12	26·1 ± 0·10 (57·1)	68·1 ± 0·45 (27·5)
	18	56·9 ± 0·03 (59·4)	70·1 ± 0·46 (31·2)
30	12	60·6 ± 0·60 (69·9)	71·9 ± 0·95 (34·6)
	18	61·2 ± 0·49 (63·2)	73·8 ± 0·61 (38·3)
35	12	66·6 ± 0·08 (86·6)	77·3 ± 0·53 (44·7)
	18	67·2 ± 0·07 (88·2)	79·3 ± 0·63 (48·3)
	SE (m)	0·30	0·13
	CD ($P < 0·05$)	0·89	0·38

Values are means ± SD of four independent determinations. Values in parentheses indicate percentage increase over the control value.

in temperature at both the periods of fermentation that were used.

When black-gram-dhal *wadies* were fermented at 25°C for 12 and 18 h, the improvement in starch digestibility ranged from 57 to 59% over the control value. As the temperature of fermentation was raised to 30°C, a further significant ($P < 0·05$) enhancement in starch digestibility was noticed. The maximum improvement in starch digestibility occurred when *wadies* were fermented at 35°C for 18 h; an increase of up to 88% over the control value occurred.

The breakdown of starch to oligosaccharides by fermenting microflora (Cronk *et al.*, 1977) or by the enzyme inherent in legume grains may be responsible for the improvement in starch digestibility during fermentation. Amylolysis has also been reported to be inhibited by phytic acid (Thompson & Yoon, 1984), and hence the reduction in phytate content of black-gram, dhal during fermentation as observed in this study (Table 1) may have accounted for the improvement in starch digestibility of the fermented-legume product. A significant ($P < 0·01$) and negative relationship has been found between phytic acid and starch digestibility of *wadies*.

An improvement in starch digestibility through fermentation has been reported in soybean (Boralkar & Reddy, 1985; Grewel, 1992) and cereal–legume blends (Grewel, 1991). Soni and Sandhu (1990) reported a higher amylase activity in conventional black-gram *wadi* dough after three days' fermentation.

Protein digestibility (*in vitro*)

The unfermented slurry of black-gram dhal had 53% *in -vitro* protein digestibility (Table 2). The relatively low protein digestibility of this legume may be due to the resistance of globulins, the major storage proteins, to proteolytic enzymes and to the presence of a considerable amounts of antinutrients such as phytic acid and polyphenols (Table 1). Phytic acid decreases the solubility and functionality of protein by binding it, and it may also cause protein to be more resistant to proteolytic digestion and may inhibit several proteolytic enzymes (Deshpande & Cheryan, 1984). Tannins have also been known to result in inactivation of digestive enzymes and protein insolubility (Reddy *et al.*, 1985).

Fermentation of black-gram-dhal batter at 25, 30, 35°C for 12 and 18 h improved the protein digestibility considerably (Table 2). The protein digestibility improved gradually and significantly as fermentation progressed at all the temperatures for 12 and 18 h. Both temperatures and time period seemed to have a cumulative effect on the improvement of protein digestibility; the maximum enhancement (48% over the control value) occurred when fermentation was carried out at 35°C for 18 h. The higher the temperature and the longer the period of fermentation, the greater was the extent of the increase in protein digestibility.

An appreciable improvement in protein digestibility as a results of *wadi* fermentation is mainly associated with enhanced proteolytic activity of fermenting microflora. High proteinase activity has frequently been reported in the fermentation of protein foods as in the production of Japanese *miso* and soy sauce (Wang & Hesseltine, 1970). An increase in the amino nitrogen by fermentation signifies a partial breakdown of protein to peptides and amino acids, resulting in improved protein digestibility (Kao & Robinson, 1978).

An increase in protein digestibility has been reported earlier in various fermented products, including *tempeh* and *miso* (Kao & Robinson, 1978), fermented soybean (Boralkar & Reddy, 1985; Grewal, 1992) and fermented rice-defatted soyflour-blend products (Goyal, 1991).

Phytic acid, known to inhibit the proteolytic enzymes (Knuckles *et al.*, 1985), is considerably reduced during *wadi* fermentation (Table 1), which may partly explain the increase in protein digestibility during fermentation. A significant and negative correlation was found between the phytic acid or polyphenol content and protein digestibility.

Hence the traditional indigenous method of *wadi* fermentation is a potential as well as cost-effective method for reducing the contents of phytic acid and polyphenols and improving the digestibility (*in vitro*) of starch and protein of dehulled black-gram cotyledons.

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