Study of effect of natural fermentation on the resistant starch content of legume based fermented foods

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Resistant starch (RS) was originally described as starch, which escapes digestion in the small intestine and passes into the colon. RS formation was studied in the *wadies* and *idli*, two of the popular legume based indigenous fermented food products in North and South India, respectively. Blackgram *wadies*, greengram *wadies* and *idli* were prepared by natural fermentation carried out at temperatures of 25, 30 and 30°C for 12, 18 and 24 h at each temperature. Fermentation decreased the RS content in all the fermented products significantly ($P \leq 0.05$). The maximum reductions in the RS content were 74.4, 81.7 and 85.1% for blackgram *wadies*, greengram *wadies* and *idli*, respectively at 37°C for 24 h. The effect of fermentation temperature in decreasing the RS content was found to be more pronounced as compared to the effect of time of fermentation. The reductions in RS content after 24 h of fermentation were not significant when compared to the values after 18 h of fermentation for each product ($P \leq 0.05$). Fermentation at 37°C was most effective in reducing the RS content. The reduced content of RS in fermented food can be ascribed to a loss in the structural integrity of the starch granules, breakdown of starch in to simpler sugars and oligosaccharides by the enzymes of the fermenting microflora and loss of some antinutrients like phytic acid and polyphenols. The phytic acid content was found to decrease significantly by 46-47% in all products ($P \leq 0.05$). The polyphenols content was also reported to reduce by 44, 50 and 52% in blackgram *wadies*, green gram *wadies* and *idli*, respectively.

Key words: Resistant starch, natural fermentation, polyphenols

Introduction

RS is defined as the total amount of starch and products of starch degradation that resist digestion in the small intestine of healthy people and fermented in the colon (Asp, 1992). Although resistant starch is present in small amounts in food, it is considered a physiologically important indigestible

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starch fraction. The various beneficial effects of RS includes reduced post-prandial glycemia and insulinemia (Raben *et al.*, 1994; Behall and Hallfrisch, 2002; Kim *et al.*, 2003), decreased serum cholesterol (Cheng and Lia, 2000; Lopez *et al.*, 2001), increased fecal bulk (Cummings *et al.*, 1996; Topping and Clifton, 2001), protection against colon cancer (Cassidy, 1994) and prebiotic effect (Wang *et al.*, 1999; Champ *et al.*, 2003). The causes of enzyme resistance of starch are diverse and include its native granular structure (RS1), physical inaccessibility to enzymes (RS2), amylose retrogradation (RS3), chemical modification of starch (RS4) and antinutritional factors. Different food processing treatments affect the RS content of different foods. Fermentation is probably one of the oldest methods of legume processing. The naturally fermented legume products are simple and inexpensive and hence such foods are still manufactured in India with traditional and simple methods. *Wadies* and *idli* are the common fermented legume products in North and South India respectively. The present study was undertaken with a view to understand the effect of natural fermentation on the RS content of these foods.

Materials and methods

Chemicals and enzymes

All the chemicals used were of analytical grade. The enzymes used for analysis were pepsin (Merck No. 7190, 2002 FIP U/G), pancreatic alpha amylase (Sigma A-3176), Amyloglucosidase from *Aspergillus niger* (Fluka 10115), glucose oxidase from *Aspergillus niger* (SRL 074040) and peroxidase from Horseradish (Himedia RM 664).

Materials

Black gram (*Phaseolus mungo*), green gram (*Vigna radiata*) and rice (*Oryza sativa*) were procured from local market. The legumes were milled to dhal in the locally available mills. Blackgram *wadies*, greengram *wadues* and *idli* were prepared by the traditional and indigenous methods of natural fermentation. The unfermented products prepared immediately after grinding the batter with similar composition served as control.

Analytical methods

The raw crops were analyzed for their moisture, ash, fat and proteins by employing the standard methods of analysis (AOAC, 1984). Phytic acid was

determined by the method of Haugh and Lantzsch (1983). The amounts of phenolic compounds were estimated as tannic acid equivalent according by Folin-Denis procedure (Swain and Hills, 1959). The RS was calculated as glucose x 0.9 by the enzymatic method as given by Goni *et al.* (1996). The method involved estimation of RS in terms of glucose after enzymatic hydrolysis and removal of the digestible starch followed by solubilization of RS in strong alkali and its hydrolysis by amyloglucosidase. The glucose thus obtained was estimated by glucose oxidase-peroxidase method.

Statistical analysis

The data were analyzed statistically by using one factor ANOVA and two factors ANOVA in a completely randomized design by using Opstat.

Results and discussion

The resistant starch content of the unfermented controls varied from 2.16 to 2.93%, unfermented batter for greengram *wadies* showing maximum value of 2.93%. Significant reductions were observed in all the three fermented products ($P \leq 0.05$). Significant reductions in RS content were also observed with increasing temperature and time of fermentation at 5% level of significance. The maximum decrease was obtained at 37°C for 24h of fermentation for each product. At each temperature when fermentation period was increased from 12 to 18h, a significant decrease ($P \leq 0.05$) was observed in RS contents in comparison to controls and between two time periods also as reported in Table 1, 2 and 3. However, for each fermentation temperature the decrease in RS content after 24h of fermentation was not significant ($P \leq 0.05$) when compared to values at 18h of fermentation period. The effect of fermentation temperature was found to be more significant in comparison to effect of time period of fermentation. For blackgram *wadies* a maximum decrease of about 54% was observed when fermented at 25°C for 24h. However, a maximum decrease of about 86% was observed when the blackgram *wadies* were fermented at 37°C for 24h. The decrease in RS content was highest at 37°C for any time period (Table 1). In case of greengram *wadies* also a maximum decrease of about 78% was observed from control at 37°C for 24h (Table 2), which was highly significant ($P \leq 0.05$). However the decrease after 24h of fermentation was not significant from values of RS after 18h of fermentation period at 30 and 37°C. In case of *idli* also almost similar pattern was observed in the amounts RS content as shown in Table 3. A maximum

decrease of about 85% was reported at 37°C for 24h, which was highly significant when compared to control value (P≤0.05).

Table 1. RS content (%dry matter basis) of blackgram *wadies* fermented for different time and temperatures.

Fermentation temperature(°C)	Control	Fermentation periods (hours)		
		12	18	24
25	2.74±0.41	1.60±0.25 (35.2)	1.25±0.14 (49.3)	1.14±0.18 (53.4)
30	2.47±0.41	1.41±0.21 (42.9)	0.93±0.22 (62.3)	0.78±0.17 (68.4)
37	2.47±0.41	0.83±0.13 (66.3)	0.43±0.03 (82.5)	0.35±0.06 (81.7)

The values are mean± SD of three independent determinations at 5% level of significance.
The values in parentheses show the percentage decrease over the control values.

Table 2. RS content (%dry matter basis) of greengram *wadies* fermented for different time and temperatures.

Fermentation temperature(°C)	Control	Fermentation periods (hours)		
		12	18	24
25	2.93±0.21	2.37±0.27 (19.1)	1.67±0.08 (43.0)	1.43±0.12 (51.2)
30	2.93±0.21	1.74±0.12 (40.6)	1.27±0.07 (56.6)	1.15±0.10 (60.7)
37	2.93±0.21	1.35±0.09 (53.9)	0.79±0.17 (73.0)	0.65±0.11 (74.4)

The values are mean± SD of three independent determinations at 5% level of significance.
The values in parentheses show the percentage decrease over the control values.

Table 3. RS content (%dry matter basis) of *idli* fermented for different time and temperatures**.**

Fermentation temperature(°C)	Control	Fermentation periods (hours)		
		12	18	24
25	2.16±0.21	1.74±0.09 (19.4)	1.35±0.13 (37.5)	1.31±0.09 (39.3)
30	2.16±0.21	1.25±0.12 (42.1)	0.94±0.12 (56.4)	0.80±0.05 (62.9)
37	2.16±0.21	0.72±0.09 (66.6)	0.38±0.17 (82.4)	0.32±0.11 (85.1)

The values are mean± SD of three independent determinations at 5% level of significance.
The values in parentheses show the percentage decrease over the control values.

Kavita *et al.* (1998) also reported a decrease of about 88 to 90% decrease in RS content of *idli* and about 71 to 80% in *dhokla.* The *in vitro* digestibility of starch in fermented *wadies* was also reported to increase from about 53 to 85% (Yadav and Khetarpaul, 1995). Thus the fermentation reduced the RS content significantly making more starch digestible. Part of this reduction can be attributed to breakdown of starch in to simpler sugars and oligosaccharides

by the enzymes of the fermenting microflora. The reduced content of the RS in the fermented products may also be due to a loss in the structural integrity of starch granules, a change in interaction between starch and fiber and because of inactivation of some antinutrients like phytic acid and polyphenols. The phytic acid content was found to decrease significantly ($P \leq 0.05$) for each fermented product when measured after 24h of fermentation (Table 4). The reductions in the phytic acid contents were 46-47% for different products (Table 4). A wide range of microflora has been known to possess phytase activity (Daniel and Fischer, 1981; Lopez *et al.* 1993), which may be partly responsible for reducing the phytic acid content in the fermented products. Reductions from 18 to 43% were obtained in the phytic acid content of fermented blackgram *wadies* (Yadav and Khetarpaul, 1995). The unfermented batters of bengalgram *wadies*, greengram *wadies* and *idli* had 932.00, 875.33 and 833.00 mg/100g polyphenols respectively as shown in Table 5. The polyphenol content was also reported to reduce by 44, 50 and 52% in bengalgram *wadies*, greengram *wadies* and *idli* respectively (Table 5). A decrease in polyphenols content during fermentation has also been reported earlier in indigenous fermented foods like *rabadi* (Dhakher and Chauhan, 1987), and *wadies* (Yadav and Khetarpaul, 1995). The diminishing effect of fermentation on polyphenols may be due to activity of polyphenol oxidase present in legumes or microflora. Phytic acid and polyphenols may affect starch digestibility through interaction with amylase enzyme (Yoon *et al.* 1983; Thompson and Yoon, 1984). The phytic acid may also bind to calcium, which is known to catalyze amylase activity (Yoon *et al.* 1983). Amylolysis has been reported to be inhibited by phytic acid (Thompson and Yoon, 1984). Thus the natural fermentation in *wadies* and *idli* is very effective in decreasing the RS content by degrading the complex starch in to simpler units and reducing the antinutients like phytic acid and polyphenols. Overall natural fermentation is very inexpensive and effective in increasing the nutritional value of foods by enhancing their starch digestibility.

Table 4. Phytic acid contents (mg/100g of dry matter basis) of the *wadies* and *idli* fermented at different temperatures for 24h.

Product	Control	Fermentation temperature (°C)		
		25	30	37
Bengalgram *wadi*	1180.00±17.32	871.00±18.50 (26.1)	705.66±14.44 (40.2)	636.66±15.45 (46.0)
Greengram *wadi*	833.00±6.42	697.67±1.76 (16.2)	562.00±3.78 (32.5)	444.00±5.19 (46.6)
Idli	971.33±4.91	798.00±6.24 (17.8)	601.67±14.24 (38.0)	514.33±10.89 (47.0)

The values are mean± SD of three independent determinations at 5% level of significance.
The values in parentheses show the percentage decrease over the control values.

Table 5. Polyphenol contents (measured as tannins, mg/100g of dry matter basis) of the *wadies* and *idli* fermented at different temperatures for 24h**.**

Product	Control	Fermentation temperature (°C)		
		25	30	37
Bengalgram *wadi*	932.00±14.73	685.33±7.62 (26.4)	547.00±12.85 (41.3)	516.66±6.93 (44.5)
Greengram *wadi*	875.33±12.99	730.00±9.29 (16.6)	513.00±5.29 (41.3)	434.00±12.12 (50.4)
Idli	833.00±6.42	646.66±4.09 (22.3)	534.33±4.80 (35.8)	394.00±3.78 (52.7)

The values are mean± SD of three independent determinations at 5% level of significance.
The values in parentheses show the percentage decrease over the control values.

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