In vitro hypoglycemic effects and starch digestibility characteristics of wheat based composite functional flour for diabetics

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Abstract The associations between chronic feeding of high level of soluble/insoluble fibers and low serum glucose levels have been well documented. In the present study, composite flours were formulated using psyllium, barley and oat at two different levels [WPOB-I = wheat flour (75 %), psyllium (5 %), oat (10 %) and barley (10 %), WPOB-II = wheat flour (60 %), psyllium (10 %), oat (15 %) and barley (15 %)]. Chapaties were prepared from all formulations and various starch fractions were analyzed using controlled enzymatic digestion. The digestibility characteristics were studied using amylolysis kinetics employing porcine pancreatic α -amylase in vitro. Results showed that both the variations (WPOB-I & WPOB-II) had acceptable sensory qualities and had significantly lower ($p \leq 0.05$) values for total starch (TS), rapidly digestible starch (RDS), resistant starch (RS), starch digestibility index (SDI) and rapidly available glucose (RAG) compared to control. Between the two variations, WPOB-I showed better starch digestibility characteristics with significantly lower ($p \leq 0.05$) starch digestibility index (SDI). In case of amylolysis kinetics, both the variations significantly ($p \leq 0.05$) inhibited α -amylase as reflected by lower glucose diffusion and significantly higher ($p \leq 0.05$) glucose dialysis retardation index (GDRI) compared to control. It is inferred that, consumption of the composite flours might be helpful in establishing stable blood glucose pattern due to the

redistribution of nutritionally important starch fractions and inhibition of carbohydrate digestion in the gastrointestinal tract.

Keywords Barley · Composite flours · Oat · Psyllium · Starch digestibility

Introduction

Diabetes is a chronic metabolic disorder with disturbances in carbohydrate, protein and lipid metabolism often associated with micro- and macro-vascular complications resulting in significant morbidity and mortality (Rang et al. 1991). The increasing number of ageing population, consumption of calorie-rich diet, obesity and sedentary life style have led to a tremendous increase in the incidence of diabetes worldwide. In India, it is estimated that the number of diabetics will rise to 57 million by the year 2025 making it a diabetic capital of the world (King et al. 1998; Boyle et al. 2001). Thus, it is necessary to look for an urgent and viable solution to prevent its incidence and manage the existing diabetes essentially by dietary and lifestyle modifications.

In recent years, disease specific functional foods are gaining attention from the researchers worldwide. Several studies have shown that, glycemic and insulin responses to different foods are influenced by both the amount of carbohydrate consumed and its source (Aarathi et al. 2003). Low glycemic index (GI) foods with added dietary fiber, have been shown to have reduced postprandial blood glucose and insulin responses thereby improving overall blood glucose and lipid concentrations in normal and diabetic subjects (Jenkins et al. 1987; Brand et al. 1991; Fontvieille et al. 1992; Wolever et al. 1992). In the last decade, much research has been devoted for development of high fiber and protein flours which have served as excellent carriers for fiber and protein enrichment,

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because of their centralized production and the convenience of admixing functional ingredients in the production (De Ruiter 1974). Many of these efforts have been successful in obtaining nutritionally valuable flours, but their utilization in the production of human food is only in its very beginning.

In India, 80 % of wheat is consumed in the form of chapati, which are the most popular unleavened flat breads consumed during almost every meal of the day (Rao et al. 1986). Traditionally, wheat is milled into whole-wheat flour on a power driven stone mill and dough is prepared by hand mixing of flour with water. About 60–100 g of the dough is rounded between the palms and sheeted manually into a disk, 2–3 mm thick using a wooden rolling pin. It is then immediately baked on a preheated iron griddle at a temperature of 230 °C for about 1 min on each side. As chapatis are thin, they are susceptible to moisture loss and stalling after baking, thus they are prepared fresh for both lunch and dinner (Sidhu et al. 1988).

With this background, since chapatis are widely consumed in most parts of India, the present study was planned to formulate low cost wheat based composite chapati flours containing barley, oat and psyllium as functional ingredients. Redistribution of starch fractions and the hypoglycemic potential of the composite flours chapatis were studied using in vitro techniques.

Materials and methods

Food ingredients and chemicals

Wheat flour, oat, barley and psyllium were purchased from a local store. Amyloglucosidase, porcine pancreatic α -amylase, heat stable α -amylase were purchased from Sigma Aldrich, Bangalore, India. Invertase (Himedia, Mumbai, India), glucose oxidase-peroxidase reagent kit (Agappe Diagnostics, Ernakulam, India) were used. All the chemicals and the reagents used in the study were of extra pure analytical grade.

Formulation of composite flours and in vitro evaluation of the product

Wheat flour (WF) was blended with psyllium (PS), oat (OA) and barley (BA) at two levels (Table 1). The blends were mixed well and sieved through a 40 mesh sieve for uniform mixing.

Using the above blends of the composite flours, chapatis were prepared according to the method of Rao et al. (1986) with slight modifications. Chapati dough was prepared by mixing 100 g flour and water equivalent to chapati water absorption in a laboratory scale planetary mixer for 3 min. The dough was rested for 10 min and then divided into two

Table 1 Formulation of composite flours

	Wheat flour (%)	Psyllium (%)	Oat (%)	Barley (%)
Control	100	–	–	–
WPOB-I	75	5	10	10
WPOB-II	60	10	15	15

^a WOPB-I: wheat based composite flour variation-I, WOPB-II: wheat based composite flour variation-II

pieces. The dough was then rounded between the palms and sheeted manually into a disk of about 15 cm diameter using a wooden rolling pin. It is then immediately baked on a preheated iron griddle at a temperature of 230 °C for about 1 min on each side (Sidhu et al. 1988) and subjected to sensory evaluation. Samples were served in containers coded with three digit random numbers, to the panelists. Plain water was served as palate cleanser, along with the samples. Sensory analysis of the samples was carried out in “Sensory Booths” under white fluorescent light, with the booth area maintained at a temperature of 20 ± 2 °C and RH 50 ± 5 %. Descriptors for the quality of chapatis were generated by ‘Free choice profiling’ and suitable ones were listed on the score card developed.

Sensory analysis of the three chapatis sample was carried out by a trained panel of 10 members. “Quantitative Descriptive Analysis” (QDA) method was employed for this purpose, using an end as ‘Low’ and ‘High’ representing ‘Recognition Threshold’ and ‘Saturation threshold’ respectively. Panelist were asked to mark the perceived intensity of each attribute listed on the score card by drawing a vertical line on the scale and writing the code number. The scores for each attribute for a given sample were tabulated, representing the judgment of individual panelists. Finally, mean value was taken for each attribute of a sample, representing the panel’s verdict about the sensory quality of the product. The product was also analyzed for functional properties like water and oil absorption capacities using standard methods (Sosulki 1962).

Determination of dietary fiber content of the product

The chapatis were dried and were finely powdered to pass through a sieve of 100 mesh. This fine powder was used for the estimation of soluble (SDF), insoluble (IDF) and total dietary fiber (TDF) contents by the method of Asp et al. (1983).

Measurement of nutritionally important starch fractions in the product

Total starch (TS) and the different starch fractions—SDS (slowly digestible starch), RDS (rapidly

Fig. 1 Summary of the analytical strategy for measurement of starch fractions

digestible starch), RS (resistant starch) and RAG (rapidly available glucose) were measured by the method of Englyst and Cummings (1996) and Englyst et al. (1992) in the prepared chapatis in three replicates after incubation with invertase (to hydrolyze sucrose),

pancreatin and amyloglucosidase at 37 °C in capped tubes immersed in a shaker water bath. Since foods normally require chewing they were minced using a commercial Waring blender. The incubation tubes contained glass beads for disrupting the food particles

Table 2 Dough characteristics and physical properties of chapati prepared from composite flour

Variation	Water used (mL)	Weight of dough (g)	Weight of chapati (g)	Moisture (%)	WAC (g/g)	OAC (g/g)
Control	75	82	66	41.7	3.1	0.06
WPOB-I	90	93	72	45.6	3.5	0.02
WPOB-II	100	99	80	48.6	4.6	0.01

^a Control: wheat flour (100 %), WPOB-I: wheat based composite flour variation-I, WPOB-II: wheat based composite flour variation-II, WAC, water absorption capacity; OAC, oil absorption capacity

Table 3 Dietary fiber content of chapaties (% on dry basis)

Variations	IDF	SDF	TDF
Control	0.5 ^a ±0.21	1.1 ^a ±0.30	1.6 ^a ±0.35
WPOB-I	6.6 ^b ±0.93	2.2 ^b ±0.37	8.8 ^b ±1.45
WPOB-II	6.7 ^b ±0.84	3.9 ^c ±0.53	10.6 ^b ±0.98

^a Control: wheat flour (100 %), WPOB-I: wheat flour (75 %), psyllium (5 %), oat (10 %) and barley (10 %), WPOB-II: wheat flour (60 %), psyllium (10 %), oat (15 %) and barley (15 %)

^b IDF, insoluble dietary fiber; *SDF*, soluble dietary fiber; *TDF*, total dietary fiber

^c Mean values carrying different superscript letters a and b in columns differ significantly ($p \leq 0.05$)

and guar gum was added to standardize the viscosity of the incubation mixture. A value for RAG was obtained as the glucose released after 20 min (G_{20}). A second measurement (G_{120}) was obtained as glucose released after further 100 min incubation. A third measurement (total glucose; TG) was obtained by gelatinization of the starch in boiling water and treatment with 7 M aqueous KOH at 0 °C, followed by complete enzymatic hydrolysis with amyloglucosidase. Resistant starch was measured as the starch remained unhydrolyzed after 120 min incubation. Free glucose (FG) was also determined by treating the sample with acetate buffer and placing the tube in a water bath at 100 °C for 30 min. Simultaneous tests were run in a similar manner with glucose standard. A blank tube containing buffer, glass beads and guar gum was also included to correct for the glucose present in the amyloglucosidase solution. A summary of the analytical strategy used is shown in Fig. 1. Glucose was determined in all the samples using a glucose oxidase-peroxidase diagnostic kit.

Treatment of data

The values for TS, RDS, SDS and RS were calculated from the values of G_{20} , G_{120} , FG and TG as follows.

$$TS = (TG - FG) \times 0.9$$

$$RDS = (G_{20} - FG) \times 0.9$$

$$SDS = (G_{120} - G_{20}) \times 0.9$$

$$RS = TS - (RDS - SDS) \text{ or } (TG - G_{120}) \times 0.9$$

Determination of in vitro hypoglycemic potential of the product

Amylolysis kinetics and glucose diffusion retardation index (GDRI) were determined by the method of Ou et al. (2001). Briefly, one gram of each product was added to 25 mL of phosphate buffer (0.05 M, pH 6.5) into dialysis membrane tubing (12 KD) along with 100 mg of α -amylase and dialyzed against 200 mL of distilled water at 37 °C in a shaker water bath. The amount of glucose diffused into the dialysate was measured at 30, 60, 120 and 180 min using glucose oxidase-peroxidase assay kit. Glucose dialysis retardation index (GDRI) was calculated with the following formula.

$$GDRI = 100 - \frac{\text{Glucose content in sample}}{\text{Glucose content in control}} \times 100$$

Statistical analysis

All determinations were carried out in triplicates. Data were analyzed by ANOVA followed by Turkey's multiple comparisons test for significant differences. The correlations between the hypoglycemic effect of the test food and starch digestibility were computed by Pearson's correlation using SPSS 17.0 software. The values were considered significant at the level $p \leq 0.05$.

Results

Physicochemical properties

The dough characteristics and physical properties of chapaties are shown in Table 2. The amount of water required for dough preparation was higher in WOPB-II followed by WOPB-I and

Table 4 Sensory profile of chapaties made of composite flours

	Appearance	Color	Texture	Aroma	Taste	Mouth feel	Overall quality
Control	9.5±0.4	9.3±0.4	9.4±0.3	9.1±0.3	9.3±0.3	9.5±0.4	9.1±0.5
WPOB-I	8.7±0.3	8.5±0.3	8.1±0.3	8.5±0.3	8.5±0.4	7.9±0.5	8.5±0.7
WPOB-II	8.4±0.4	8.1±0.3	8.1±0.3	8.4±0.3	8.3±0.4	7.5±0.5	7.8±0.5

^a Scores: 9–10=excellent; 7–8=good; 4–6=fair; 1–3=poor

^b Control: wheat flour (100 %), WPOB-I: wheat flour (75 %), psyllium (5 %), oat (10 %) and barley (10 %), WPOB-II: wheat flour (60 %), psyllium (10 %), oat (15 %) and barley (15 %)

Fig. 2 Amylolysis kinetics characteristics of chapaties. **a** Control: wheat flour (100 %), WPOB-I: wheat flour (75 %); psyllium (5 %); oat (10 %); barley (10 %), WPOB-II: wheat flour (60 %); psyllium (10 %); oat (15 %); barley (15 %). **b** Bars carrying different superscript letters a, b, c differ significantly ($p \leq 0.05$). **c** Values over each bar represents GDRI

control. Similar trend was observed in moisture content, weights of dough and chapaties. The water absorption capacity of WPOB-II was higher than WPOB-I followed by control, but an inverse effect was observed in case of oil absorption capacity. The functional properties of the products were in response to the ratio of the presence of commercial dietary fiber sources oat, barley and psyllium. The dietary fiber content of chapaties is given in Table 3. WPOB-II and WPOB-I contained significantly higher ($p \leq 0.05$) total, insoluble and soluble dietary fiber than that of control. However, no significant ($p \leq 0.05$) differences were observed between the dietary fiber contents of the two variations (WPOB-I & WPOB-II).

Results of sensory analysis are presented in Table 4. Chapaties prepared from both composite flours (WPOB-I and WPOB-II) had an appealing light brown color and did not have any perceptible no off taste or off aroma compared to that of control. Both the variations were neither tough nor hard to chew and had optimum chewing properties thereby scoring well in taste and texture compared control. The taste and aroma scores of both WPOB-I and WPOB-II were comparable with that of control. WPOB-I had desirable tearing strength comparable with that of control. Although, both the

variations (WPOB-I & WPOB-II) were acceptable in all the sensory attributes, WPOB-I showed higher acceptability than WPOB-II compared to control indicating the suitability of the composite flours for regular human consumption.

Effect on amylolysis kinetics and GDRI

The amylolysis kinetics and GDRI of two variations are presented in Fig. 2. Compared to control chapaties, the diffusion rate of glucose in WPOB-I and WPOB-II, were significantly low ($p \leq 0.05$) at each interval of time. The diffusion rate of glucose decreased in the variations with increased fiber levels. WPOB-II showed significantly ($p \leq 0.05$) higher GDRI values than WPOB-I followed by control at all time intervals, and GDRI diminished over the time.

Starch digestibility characteristics

The total starch and its fractions, RDS, SDS and RS in the product are shown in Table 5. The starch fraction profile differed according to the composition of the product. Compared to control, both the variations (WPOB-I & WPOB-II)

Table 5 Starch digestibility characteristics of chapaties made of composite flours (%)

Sample	Dry matter	TS	RDS	SDS	RS	SDI	RAG
Control	58.3	39.85 ^c ±2.87	7.07 ^b ±0.37	5.86 ^b ±0.98	26.91 ^b ±2.30	19.15 ^b ±1.03	9.96 ^a ±1.03
WPOB-I	54.4	33.88 ^b ±2.85	5.22 ^a ±0.74	6.21 ^b ±0.57	22.69 ^a ±1.97	14.67 ^a ±1.17	7.82 ^a ±1.11
WPOB-II	51.3	28.19 ^a ±1.71	4.78 ^a ±0.71	4.75 ^a ±0.35	18.95 ^a ±1.77	16.88 ^b ±1.35	7.72 ^a ±0.98

^a Control: wheat flour (100 %), WPOB-I: wheat flour (75 %); psyllium (5 %); oat (10 %); barley (10 %), WPOB-II: wheat flour (60 %); psyllium (10 %); oat (15 %); barley (15 %)

^b Mean values carrying different superscript letters a, b, c..... in columns differ significantly ($p \leq 0.05$)

had significantly lower ($p \leq 0.05$) values for TS, RDS, RS, SDI and RAG. Between the two variations, WPOB-I showed better starch digestibility characteristics with significantly low ($p \leq 0.05$) SDI. Measurement of different starch fractions provides a means for predicting the rate and extent of starch digestion in the human small intestine. Functional foods with such a nutritional profile are suitable for diabetics as absorption of glucose will be delayed resulting in lower glycemic responses consequently blunting the postprandial glycemia.

Discussion

The present study reports the analysis of dietary fiber content, GDRI and nutritionally important starch fractions in composite flours (WPOB-I and WPOB-II) along with control. The significantly lower GDRI of WPOB-I and WPOB-II compared to control, may be attributed to the presence of soluble and insoluble dietary fibers from oat, β -glucan from barley and viscous polysaccharide from psyllium, all of which acts as functional bioactive ingredients. Cereal β -glucans display all the physiological properties that have been attributed to dietary fiber like water holding capacity, swelling, diffusion-suppressing ability (viscous, gel formation) and binding properties. Therefore, grains with high levels of soluble β -glucans, such as oat and barley are generally more effective in controlling blood glucose levels, improving insulin responses and reducing serum cholesterol levels than wheat, which contains predominantly insoluble dietary fiber. Diets containing foods enriched with oat and barley β -glucans have reduced glycemic index in clinical trials (Biliaderis and Marta 2007). Psyllium's mechanism of action for glucose reduction in diabetic patients is probably similar to that of other soluble fibers because psyllium forms a viscous gel in aqueous solution or delay gastric emptying or may be due to sequestration of carbohydrates ingested with the meal thereby retarding carbohydrate access to digestive enzymes. Reports indicate that psyllium can exert these effects hours after its administration and can produce a significant reduction in blood glucose after a second meal (Layce et al. 1991). Studies also suggest that the inhibition of glucose diffusion in the small intestine is due to the adsorption or inclusion of the smaller sugar molecules within the structure of the fiber particles (Nishimune et al. 1991; Lopez et al. 1996). Ahmed et al. (2010) reported a significant decrease in the levels of glucose by administering psyllium fibers to the diabetic rats. Similarly, reduction in glucose and cholesterol levels was observed in diabetic patients without significant adverse effects (Rodriguez et al. 1998).

The lower GDRI is may be due to the inhibition of α -amylase, thereby limiting the release of glucose from the starch. The inhibition of α -amylase activity might be attributed to several possible factors such as fiber concentration, the

presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample, thereby reducing accessibility of starch to the enzyme and direct adsorption of the enzyme on fibers leading to decreased amylase activity (Ou et al. 2001). Inhibitors of carbohydrate hydrolyzing enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (Bailey 2002). It is likely that the mechanism by which β -glucans and viscous polysaccharides decrease the postprandial glucose response is the result of not only high viscosity in the gastrointestinal track, but also the reduction of starch digestion by α -amylase (Layce et al. 1991; Biliaderis and Marta 2007). Clinical studies have demonstrated that β -glucan decreases plasma glucose and insulin concentrations following a single meal in both healthy individuals and individuals with type 2 diabetes (Braaten et al. 1994; Wood et al. 1994; Battilana et al. 2001).

Conclusion

The formulated composite flours have the potential to be used as a supplement in the regular diet of normal as well as diabetic subjects. The hypoglycemic effects of composite flours are might be due to changes in the adsorption of glucose or inhibition of α -amylase or both of the mechanisms.

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