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Organoleptic and nutritional evaluation of wheat breads supplemented with soybean and barley flour

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Abstract

Supplementations of soy (full fat and defatted) and barley flours to wheat flours at 5, 10, 15 and 20% levels were carried out to test the effects on organoleptic and nutritional evaluation of the supplemented bread. Additions of 15% barley flour, 10% soy flour (full fat and defatted), 15% barley plus full fat soy flour and 15% barley plus defatted soy flour to wheat flour produced acceptable breads. However, substitution of soy (full fat and defatted) and barley flours to wheat flour separately and in combinations at 20% levels did not produce organoleptically acceptable bread. Various nutritional parameters, such as protein, fat, total lysine, protein digestibility (in vitro), sugars, starch digestibility (in vitro), total and available minerals, antinutrients, dietary fibre and β-glucan were determined in supplemented and control bread. Increasing the level of substitution from 5 to 10% of full fat and defatted soy flour to wheat flour significantly ($P<0.05$) increased protein (from 12.1 to 13.7 and 12.4 to 13.8%), lysine (from 2.74 to 3.02 and 2.76–3.05 mg/100 g protein) and total calcium (from 70.2 to 81.4 and 71.9–81.8 mg/100 g) contents. However, there was also an increase in phytic acid (238–260 and 233–253 mg/100 g), polyphenol (324–331 and 321–329 mg/100 g) and trypsin inhibitor activity (193–204 and 193–198 TIU/g). When barley flour was substituted separately, and in combinations, with full fat and defatted soy flour up to 15%, this significantly increased the contents of protein, total lysine, dietary fibre and β-glucan. It may be concluded that breads supplemented with barley and defatted soy flour, up to a 15% level, are organoleptically and nutritionally acceptable.

Keywords: Bread; Wheat; Soybean; Barley; Nutritional composition

1. Introduction

Wheat (*Triticum aestivum*) is the world's most important cereal crop in terms of production and consumption (Shewry & Tatham, 1994). In India, wheat is being cultivated in an area of 25.40 million ha, with the production of 68.70 million t (FAO, 1998). About 90% of the area in India is under bread wheat (*Triticum aestivum*), 9% area is occupied by macaroni wheat (*Triticum durum*) and the remaining 1% area is covered by quality wheat (*Triticum dicoccum*), which has a high commercial value (Reddy, Nirmala, Rao, Srinivasan, & Hanchinal, 1998). Traditionally 85–90% of the wheat is consumed mainly in the form of homemade products, such as *Chapati* and porridge. However, in recent years, with the advancement in baking technology and changing food habits, wheat is milled into flour which finds use for the preparation of a variety of products, such as breads, which are now becoming popular in urban and semi-urban areas of the country (Sharma, Sekhon, & Nagi, 1999).

Apart from being a good source of calories and other nutrients, wheat is considered nutritionally poor, as the cereal proteins are deficient in essential amino acids such as lysine and threonine. Grain legumes contribute significantly towards protein, mineral and B-complex vitamin needs of people in developing countries. Therefore, supplementation of wheat flour with inexpensive staples, such as cereals and pulses, helps in improving the nutritional quality of wheat products (Sharma et al., 1999).

In the present study, efforts have been made to supplement wheat flour with soybean and barley flours to develop nutritionally rich functional foods such as bread. In recent years, considerable interest has been generated in the development and consumption of such foods. Barley flour has a high concentration of total β-D glucans (especially soluble β-glucan) which have been studied for hypocholesterolemic effects in animals and humans (Hecker, Meier, Newman, & Newman,

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1998; Kalra & Jood, 2000). Soybean flour has high protein (38–40%), fat (18–20%) and lysine (5–6%) contents, which have great potential in overcoming protein-calorie malnutrition (Rastogi & Singh, 1989). No systematic study of the effect of barley- and soybean-supplemented bread has yet been reported. The present investigation was, therefore undertaken to study the organoleptic and nutritional evaluation of wheat supplemented bread.

2. Materials and methods

2.1. Procurement of material

The samples of commercially grown varieties of wheat (WH-423), and soybean (PK-416) were obtained from the Department of Plant Breeding, CCS Haryana Agriculture University, Hisar, India. The barley variety (Dolma) was procured from the Department of Plant Breeding, Himachal Pradesh Krishi Vishva Vidyalaya, Palampur, India. All the grains were cleaned and made free from dust and other foreign materials.

The grain samples of wheat and barley were ground on a junior mill to pass through a 60 mesh sieve and stored in air-tight containers until used. The soybean grains were dehulled in dehusking and a splitting machine (Central Institute of Post Harvest Engineering and Technology, Ludhiana, India) and then ground to fine flour on a junior mill. Soy flour was defatted by using the standard method of AOAC (1995). Two hundred grammes of soy flour were suspended in 50 ml of petroleum ether and shaken for 3 h, the sample left overnight and filtered, dried at room temperature to evaporate petroleum ether and the defatted sample stored in a deep freezer.

2.2. Preparation of plain and blended flours

Barley flour, full fat soy flour, defatted soy flour, barley plus full fat soy flour (50:50) and barley plus defatted soy flour (50:50) were blended with wheat flour at different levels (5, 10, 15 and 20%, respectively).

2.3. Preparation of breads

The bread-making performances of flours (control and blends) were determined using straight dough AACC method (1984) with the remixing procedure of Irvine and McMullan (1960) with the slight modification that dough was mixed using the desired baking absorption for optimum dough handling.

2.4. Organoleptic evaluation

Most acceptable blended breads were selected for nutritional evaluation. Their organoleptic characteristics were determined by a panel of 10 judges for crust colour, appearance, flavour, crust texture, taste and overall acceptability using a nine-point Hedonic Rating Scale ranging from like extremely (9) to dislike extremely (1) for each organoleptic characteristic, as suggested by Austin and Ram (1971).

2.5. Nutritional evaluation of acceptable breads

2.5.1. Protein, fat and ash

Protein, fat and ash contents were estimated by employing standard methods of analysis (AOAC, 1995).

2.5.2. Protein digestibility

Protein digestibility (in vitro) was assessed by employing pepsin and pancreatin (Akeson & Stahmann, 1964). The nitrogen contents of the sample and the undigested residue were determined by the micro-Kjeldahl method (AOAC, 1995). The digested protein of the sample was calculated by subtracting residual protein from total protein of the sample.

$$\text{Protein digestibility } (\%) = \frac{\text{Digested protein}}{\text{Total protein}} \times 100$$

2.5.3. Total lysine

Total lysine was estimated according to the method described by Miyahara and Jikoo (1967).

2.5.4. Sugars and starch digestibility

Total soluble sugars were extracted by refluxing in 80% ethanol (Cerning & Guilbot, 1973). Quantitative determination of total soluble sugars was carried out according to a colorimetric method (Yemm & Willis, 1954). Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945). Non-reducing sugars were determined by calculating the difference between total soluble sugars and reducing sugars. In vitro starch digestibility was assessed by employing pancreatic amylase and incubating at 37 °C for 2 h. Liberated maltose was measured colorimetrically by using dinitro-salicylic acid reagent (Singh, Khedekar, & Jambunathan, 1982).

2.5.5. Total and available minerals

The samples were wet acid-digested, using a nitric acid and perchloric acid mixture (HNO_3:$HClO_4$, 5:1w/v). The total amounts of Ca, Fe and Zn in the digested samples were determined by atomic absorption spectrophotometry (Lindsey & Norwell, 1969). Ionizable Fe in the samples was extracted according to the procedure of Rao and Prabhavathi (1978) and Ca and Zn were extracted by the method of Kim and Zamel (1986). Available Ca, Fe and Zn was determined by atomic

absorption spectrophotometry (Lindsey & Norwell, 1969).

2.5.6. Antinutritional factors

Phytic acid was determined by the method of Haug and Lantzsch (1983). Total polyphenols were extracted the method of Singh and Jambunathan (1981), and estimated as tannic acid equivalents, according to the Folin-Denis procedure (Swain & Hills, 1959). Trypsin inhibitor activity was determined by the modified method of Roy and Rao (1971). Amylase inhibitor activity was determined by following the modified method of Bernfeld (1955).

2.5.7. Dietary fibre and β-glucan

Total, soluble and insoluble dietary fibre contents were determined by following the enzymatic method (Furda, 1981). The sum of insoluble dietary fibre and soluble dietary fibre contents were calculated as total dietary fibre. Total, soluble and insoluble β-glucan contents were estimated by using Megazyme Mixed Linkage β-glucan and glucose test kits, as per the method of McCleary and Glennie (1985).

2.6.. Statistical analysis

The data were statistically analysed in complete randomized design for analysis of variance and correlation coefficients according to the standard method (Panse & Sukhatme, 1961).

3. Results and discussion

3.1. Organoleptic evaluation

Organoleptic evaluation of breads revealed that, as the level of barley and soy flour was increased, the crust colour of the breads changed from creamy white to dull brown. However, no significant difference was observed in crust colour up to 10% blending with non-wheat flours. Thereafter, a significant difference in crust colour was observed in all the blended breads (Table 1). The darker crust colour may be due to Maillard reaction between reducing sugars and protein (Raidi & Klein, 1983). Appearance score for control bread was 7.75

Table 1
Organoleptic characteristics of bread prepared from wheat and various cereal-pulse blends[a]

Breads	Crust colour	Appearance	Flavour	Crust texture	Taste	Overall acceptability
Control (Wheat)	7.75±0.46	7.75±046	7.75±0.46	7.62±0.51	7.62±0.51	7.70±0.53
W+B						
95:05	7.75±0.88	7.75±0.88	7.87±0.8	7.37±0.91	7.50±0.92	7.65±0.84
90:10	7.75±0.88	7.75±0.88	7.87±0.83	7.25±0.70	7.25±0.70	7.57±0.66
85:15	7.50±0.92	7.62±0.91	7.25±0.70	6.75±0.46	6.62±0.74	7.15±0.58
80:20	6.25±0.46	5.87±0.35	5.12±0.99	5.75±0.70	5.00±1.06	5.60±0.47
W+SF						
95:05	7.75±0.46	7.75±0.88	7.62±0.51	7.62±0.51	7.62±0.51	7.67±0.53
90:10	7.62±0.74	7.62±0.74	7.50±0.75	7.25±0.70	7.00±0.92	7.40±0.65
85:15	5.62±0.51	5.37±0.51	4.75±1.16	4.75±0.46	3.87±1.24	4.87±0.36
80:20	5.62±0.51	5.37±0.51	4.00±1.19	4.50±0.53	3.62±1.06	4.62±0.36
W+DSF						
95:05	7.50±0.53	7.75±0.46	7.75±046	7.62±0.74	7.75±0.46	7.67±0.48
90:10	7.50±0.53	7.37±0.74	7.50±0.75	7.37±0.74	7.37±0.74	7.42±0.60
85:15	6.00±0.00	5.37±0.51	5.00±1.30	5.12±0.64	4.62±1.40	5.22±0.67
80:20	6.00±0.00	5.25±0.46	4.50±1.19	4.75±0.46	4.12±0.99	4.92±0.43
W+B+SF						
95:05	7.87±0.83	7.87±0.64	7.87±0.83	7.50±0.92	7.75±0.70	7.77±0.71
90:10	7.87±0.35	7.75±0.46	6.62±0.74	7.50±0.92	6.37±0.51	7.22±0.47
85:15	7.50±0.75	7.37±0.74	6.62±0.74	6.25±0.70	6.00±0.53	6.75±0.56
80:20	5.75±0.70	5.62±0.74	5.00±1.30	4.87±0.64	4.37±1.06	5.12±0.52
W+B+DSF						
95:05	7.75±046	7.50±0.75	7.75±0.46	7.75±0.46	7.87±0.35	7.72±0.46
90:10	7.50±0.75	7.37±0.91	7.37±0.74	7.12±1.24	7.00±0.75	7.27±0.82
85:15	7.37±0.74	6.87±0.64	7.00±0.75	6.50±0.75	6.62±09.74	6.87±0.57
80:20	6.12±0.64	5.50±0.53	5.12±1.45	5.37±0.74	4.87±155	5.40±0.77
SE (*m*)	0.21	0.22	0.49	0.24	0.48	0.13
CD ($P<0.05$)	0.59	0.63	1.36	0.69	1.34	0.38

W, Wheat flour; B, Barley flour; SF, Soy flour; DSF, Defatted soy flour; NS, non-significant.
[a] Values are means±SD of three independent determinations.

which decreased significantly upon increasing the blending levels, i.e. at 15 and 20%, with soy and barley flours.

Flavour of breads increased on increasing the level of barley and soy flour up to 10% level, indicating better flavour rating, and thereafter it decreased at 15 and 20% levels of blending. The flavour of soy flour-blended breads might be affected due to the beany flavour of soybean flour (Grewal, 1992).

The crust texture was related to the external appearance of the bread top, i.e. smoothness or roughness of the crust. Crust texture score also decreased with increase in substitution of barley and soy flour in wheat flour as compared with control bread (7.62). Among the blended breads, maximum score was observed in breads containing both barley and defatted soy flour (7.75) at the 5% level and minimum score was in full fat soy-blended bread.

Taste score also decreased on increasing the level of substitution of barley and soy flours. Breads containing 20% of full fat soy flour was rated poorest in taste (3.62). This might be due to the beany flavour of soy flour (Rastogi & Singh, 1989).

From the overall acceptability rating, it was concluded that breads up to 15% level of barley, 10% level of full fat and defatted soy flour and 15% level of barley and soy flour in combinations could be baked with satisfactory performance.

3.2. Nutritional evaluation

3.2.1. Protein, fat and ash

The wheat bread had 11.5% protein which decreased gradually among barley supplemented breads (Table 2). However, a significant increase was observed in full fat soy-(13.7%) and defatted soy-(13.8%) supplemented breads at the 10% level. Breads containing barley plus full fat soy and barley plus defatted soy flours manifested intermediate protein contents. Several workers have also reported an increase in protein content of breads supplemented with non-wheat flours (Rathna & Neelakantan, 1995; Sharma & Chauhan, 2000).

The fat content of whole wheat flour bread (control) was 5.44%, which increased significantly on blending with full fat soy flour (Table 2). The full fat soy flour-supplemented bread at 10% level had the highest fat content (6.83%). This was due to the higher fat content of full fat soy flour than other flours. There was a non-significant difference in fat content of breads made from barley-, defatted soy-, barley plus full fat soy- and barley plus defatted soy-supplemented flours as compared with control. The breads containing barley, barley plus full fat soy and barley plus defatted soy flour, at 15% level, had 5.58, 6.27 and 5.51% fat contents, respectively. Sharma and Chauhan (2000) also observed an increase in fat content (from 0.90 to 1.50%) in

Table 2
Chemical composition and in vitro protein digestibility of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Protein digestibility (%)	Total lysine (g/100 g protein)
Control (Wheat)	11.5±1.95	5.44±0.45	2.10±0.44	74.0±0.61	2.36±0.22
W+B					
95:05	11.4±0.88	5.49±0.50	2.14±0.37	74.1±0.60	2.52±0.44
90:10	11.0±0.98	5.55±0.45	2.21±0.21	74.2±0.06	2.71±0.60
85:15	10.6±1.23	5.58±0.52	2.33±0.41	74.3±1.47	2.80±0.47
W+SF					
95:05	12.1±0.95	6.32±0.41	2.28±0.36	70.6±0.59	2.74±0.50
90:10	13.7±0.81	6.83±0.58	2.40±0.33	69.1±0.29	3.02±0.09
W+DSF					
95:05	12.4±0.44	5.43±0.23	2.23±0.59	72.1±1.59	2.76±0.28
90:10	13.8±1.02	5.38±0.43	2.35±0.29	70.7±0.69	3.05±0.52
W+B+SF					
95:05	11.7±1.15	5.78±0.78	2.17±0.62	72.7–0.61	2.68±0.50
90:10	12.3±0.32	6.10±0.61	2.31±0.12	71.3±1.74	2.83±0.68
85:15	12.5±1.33	6.27±0.55	2.44±0.38	69.8±0.65	3.02±0.46
W+B+DSF					
95:05	12.0±0.77	5.44±0.30	2.13±0.37	73.0±0.27	2.68±0.53
90:10	12.4±0.48	5.47±0.39	2.26±0.15	71.9±1.00	2.85±0.52
85:15	12.8±0.80	5.51±0.22	2.42±0.31	70.7±0.92	3.05±0.46
SE (*m*)	0.30	0.27	0.32	0.49	0.14
CD ($P<0.05$)	0.89	0.78	NS	1.43	0.46

W, Wheat flour; B, Barley flour; SF, Soy flour; DSF, Defatted soy flour; NS, non-significant.
[a] Values are means±SD of three independent determinations.

breads supplemented with fenugreek flour (0.5–9% level).

The ash content of control bread was 2.10% and it remained almost the same in various supplemented breads at all the levels (Table 2).

3.2.2. Protein digestibility (in vitro) and total lysine

Wheat bread had 74.0% in vitro protein digestibility, which did not change significantly in barley-supplemented breads at all the levels. However, upon blending with soy flours (full fat and defatted) in vitro protein digestibility decreased significantly. This decrease could be due to the high content of trypsin inhibitors and other anti-nutrients in soybean flour (Saxena, Singh, & Mital, 1994). Trypsin inhibitor is responsible for inhibiting the activity of proteolytic enzymes, whereas phytic acid and polyphenols are known to associate with proteins to form insoluble complexes, thus affecting the in vitro digestibility of proteins (Feng, Chen, Kramer, & Reeck, 1991). At 10% level of substitution, in vitro protein digestibilities were 74.2, 69.1, 70.7, 71.3 and 71.9% in breads made from barley-, full fat soy, defatted soy-, barley plus full fat soy- and barley plus defatted soy-blended flours, respectively. Grewal (1992) also reported low protein digestibility of soy flours.

A highly significant ($P<0.05$) and negative correlation was obtained between phytic acid, polyphenols and trypsin inhibitor activity with protein digestibility, whereas non-significant and negative correlation was observed with amylase inhibitor activity (Table 3). Some workers also observed a significantly negative correlation between tannins and phytates and protein digestibility (Jood & Kalra, 2001).

Total lysine contents are presented in Table 2. The quality of protein is evaluated by lysine content. The total lysine content of control bread was 2.36 g/100 g protein. In barley-supplemented breads, the total lysine contents were found at par with the control at all the levels of supplementation. In soy- (full fat and defatted) supplemented breads at 10% substitution, the total lysine contents increased significantly to 3.02 and 3.05 g/100 g protein, respectively. The increase was found to be 28 and 29%, respectively. Similarly, in breads made from barley plus full fat soy- and barley plus defatted soy-supplemented flour at 15% level, total lysine content increased by 28 and 29%, respectively. This might be due to the high content of total lysine in soy flour. Gayle, Knight, Adkins, and Harland (1986) also reported an increase in lysine content of breads substituted with pigeonpea flour at 25% substitution.

3.2.3. Sugars and starch digestibility (in vitro)

Wheat bread contained 20.7% (total soluble), 8.33% (reducing) and 12.4% (nonreducing) sugars, respectively (Table 4). Total and reducing sugar contents of supplemented bread at different levels did not change significantly compared with the control. However, significant ($P<0.05$) change was observed in non-reducing sugar contents of various supplemented breads. The highest non-reducing sugar contents were observed in barley- plus defatted soy flour-supplemented bread at 15% level while the lowest were found in barley plus full fat soy-supplemented bread at 5% level.

Table 3
Correlation coefficients of in vitro digestibility of protein and starch and in vitro availability of Ca, Fe and Zn with antinutrients

Antinutients	In vitro digestibility		In vitro availability		
	Protein	Starch	Ca	Fe	Zn
Phytic acid	−0.881[a]	−0.892[a]	−0.863[a]	−0.889[a]	−0.878[a]
Polyphenols	−0.887[a]	−0.663[a]	−0.889[a]	−0.900[a]	−0.876[a]
Amylase inhibitor activity	−0.240	−0.992[a]	−0.223	−0.269	−0.455
Trypsin inhibitor activity	−0.934[a]	−0.340	−0.409	−0.555	−0.530

[a] Significant at 5% level.

Table 4
Sugar contents and in vitro starch digestibility of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Total soluble sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Starch digestibility (mg maltose released/g meal)
Control (Wheat)	20.7±0.36	8.33±0.38	12.4±0.74	42.8±1.62
W+B				
95:05	21.1±0.70	8.33±0.50	12.8±1.20	41.8±0.51
90:10	21.4±0.55	8.26±0.53	13.1±0.02	41.06±0.30
85:15	21.8±0.26	8.16±0.21	13.7±0.47	40.38±1.58
W+SF				
95:05	21.0±0.54	8.29±0.30	12.7±0.24	43.00±0.34
90: 1()	21.5±0.38	8.24±0.58	13.3±0.91	43.20+1.00
W+DSF				
95:05	21.3±0.58	8.31±0.37	12.9±0.21	43.2±0.36
90:10	21.7±0.46	8.27±0.67	13.4±1.10	43.4±0.54
W+B+SF				
95:05	21.0±0.49	8.31±0.64	12.7±1.13	42.3±0.66
90:10	21.4±0.31	8.25±0.33	13.2±0.02	42.1±0.47
85:15	21.9±0.14	8.19±0.63	13.7±0.49	41.9±1.00
W+B+DSF				
95:05	21.1±0.54	8.31±0.38	12.8±0.92	42.4±1.11
90:10	21.5±0.39	8.26±0.52	13.2±0.13	42.2±0.72
85:15	21.9±0.48	8.19±1.00	13.7±0.52	42.0±0.91
SE (*m*)	0.25	0.28	0.40	0.60
CD ($P<0.05$)	0.72	NS	1.18	1.75

W, Wheat flour; B, Barley flour; SF, Soy flour; DSF, Defatted soy flour; NS, non-significant.

[a] Values are means±SD of three independent determinations.

The slight increase in sugar contents may be partially due to addition of sugar in flours and partially due to hydrolysis of starch during fermentation. Rastogi and Singh (1989) also reported an increase in reducing and non-reducing sugars in blends containing 20% full fat soy flour.

The in vitro starch digestibilities of whole wheat (control) bread and other cereal-pulse-blended breads are presented in Table 4. The in vitro starch digestibility decreased significantly ($P<0.05$) at 15% level in the breads made from barley-supplemented flour (40.4 mg maltose released/g meal) as compared with control (42.8 mg maltose released/g meal. Poor starch digestibility of barley-supplemented breads was due to the binding property of inhibitors with amylase enzymes (Kalra, 1996). The starch digestibility of breads prepared from frill fat soy (43.2 mg maltose released/g meal) and defatted soy (43.4 mg maltose released/g meal) flours, at 10% substitution level, were at par with that of the control. In the case of barley plus full fat soy- and barley plus defatted soy-supplemented breads, at 15% level, the starch digestibilities were 41.9 and 42.0 mg maltose released/g meal, respectively.

A highly significant ($P<0.05$) and negative correlation was obtained between phytic acid, polyphenols and amylase inhibitor activity and starch digestibility whereas non-significant and negative correlation was observed with trypsin inhibitor activity (Table 3).

3.2.4. Total and available Ca, Fe and Zn

Wheat bread had 57.1 mg/100 g (calcium), 8.17 mg/100 g (iron) and 3.75 mg/100 g (zinc) contents, respectively (Table 5). However, barley-supplemented breads, at 5, 10 and 15% levels, exhibited non-significantly lower contents of total calcium, iron and zinc as compared with control bread. This might be attributed to slightly higher phytic acid and polyphenol contents than wheat (Feng et al., 1991). Among the soy flour-supplemented breads, full fat soy-supplemented bread contained significantly ($P<0.05$) higher calcium (70.2–81.4 mg/100 g) and iron (8.70–8.89 mg/100 g) at 5 and 10% levels than control bread. A similar trend was also observed in defatted soy-supplemented bread. High calcium and iron contents of soy-supplemented breads might be due to high calcium and iron contents of soybean flour (Misra, Usha, & Surjain Singh, 1991; Ologhobo, 1989; Suibaba, 1990). However, there was no significant variation in total zinc contents of supplemented bread at different levels of supplementation, whereas the breads containing barley plus full fat soy and barley plus defatted soy flours exhibited intermediate contents of calcium, iron and zinc.

Table 5
Total and per cent availability of minerals of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Total Ca (mg/100 g)	Ca availability (mg/100 g)	Total Fe (mg/100 g)	Fe availability (%)	Total Zn (mg/100 g)	Zn availability (%)
Control (Wheat)	57.1±0.22	48.3±0.23	8.52±0.41	50.2±1.00	3.75±0.35	52.0±0.30
W+B						
95:05	55.2±0.44	47.1±0.51	8.17±0.28	48.8±0.63	3.62±0.21	50.8±0.53
90:10	53.9±0.19	46.6±0.29	7.82±0.27	48.1±0.29	3.50±0.39	49.1±1.22
85:15	52.6±0.42	46.4±0.26	7.51±0.46	47.4±0.43	3.45±0.34	48.4±1.41
W+SF						
95:05	70.2±0.21	46.8±0.38	8.70±0.29	47.9±0.43	3.75±0.22	49.1±0.41
90:10	81.4±0.13	45.7±0.23	8.89±0.26	47.8±0.47	3.80±0.32	47.4±0.85
W+DSF						
95:05	71.9±0.32	46.9±0.28	8.78±0.16	48.2±0.12	3.75±0.15	49.2±0.80
90:10	81.8±0.58	45.8±0.43	8.92±0.68	47.9±0.53	3.83±0.29	47.8±0.85
W+B+SF						
95:05	61.6±0.55	46.9±0.45	8.42±1.00	48.2±0.56	3.70±0.33	49.7±0.25
90:10	67.2±1.00	46.2±0.20	8.30±0.65	47.8±0.35	3.68±0.11	48.4±0.53
85:15	72.8±0.48	45.7±0.24	8.23±0.53	47.0±0.65	3.63±0.22	46.8±0.30
W+B+DSF						
95:05	63.3±0.73	47.0±0.34	8.45±0.42	48.4±0.51	3.74±0.45	50.0±0.27
90:10	68.1±0.73	46.3±0.60	8.32±0.86	48.0±0.27	3.70±0.23	48.4±0.24
85:15	73.1±0.46	45.8±0.33	8.29±0.57	47.28±0.36	3.76±0.26	47.4±0.52
SE (*m*)	0.45	0.31	0.49	0.63	0.17	0.36
CD ($P<0.05$)	0.77	0.55	0.85	1.08	NS	1.05

W, Wheat flour; B, Barley flour; SF, Soy flour; DSF, Defatted soy flour; NS, non-significant.
[a] Values are means±SD of three independent determinations.

Wheat bread showed per cent in vitro availability of calcium to be 48.3%, iron 50.2% and zinc 52.0%, (Table 5). But there was a significant ($P<0.05$) decrease in per cent availability of calcium, iron and zinc in various supplemented breads at 5, 10 and 15% levels. In vitro calcium availability decreased significantly upon increasing the levels of barley and soy flours in wheat bread. A similar trend was also observed for iron and zinc availability. This might be attributed to higher contents of antinutrients in barley (Jood & Kalra, 2001) and soybean (Grewal, 1992).

Significant negative correlation was observed between the availability of Ca, Fe and Zn and phytic acid and polyphenol contents (Table 3). However, non-significant and negative correlation was obtained with amylase inhibitor activity and trypsin inhibitor activity. Phytate, the major phosphorus-bearing, compounds in cereals and pulses, chelates divalent and trivalent cations, such as Ca, Fe and Zn, forming insoluble complexes and thereby decreases the in vitro availability of minerals (Haug & Lantzsch, 1983).

3.2.5. *Antinutrients*

Anti-nutritional factors, i.e. phytic acid, polyphenols, trypsin inhibitors and amylase inhibitors, in control and various supplemented breads are presented in Table 6.

The control (whole wheat flour) bread had 226 mg/100 g phytic acid and it increased significantly with rise in the levels of barley and soy flours. The maximum increase of 20% in phytic acid over control was found in bread containing barley plus full fat soy flour at 15% level (270 mg/100 g) while minimum increase of 2% was found in barley-supplemented bread at 5% level (230 mg/100 g) over the control. The phytic acid contents of full fat- and defatted soy-supplemented breads at 10% level were 260 and 253 mg/100 g, respectively. In the breads containing barley plus full fat soy and barley plus defatted soy flours, the phytic acid contents were 253 and 250 mg/100 g at 10% level of supplementation. The increased phytate content of the breads seems to be the direct effect of replacement of wheat flour with barley and soybean flours, which have high contents of phytic acid. Gayle et al. (1986) also suggested a similar increase in phytate concentration from 160 to 250 mg/100 g in pigeonpea flour-supplemented breads at 5, 10, 15 and 25% levels.

The polyphenolic content of control bread was 315 mg/100 g which increased progressively and significantly ($P<0.05$) with increasing the proportions of barley and soy flours (full fat and defatted). In barley-supplemented breads, at 5% level, the polyphenol content was 327 mg/100 g and it increased to 346 mg/100 g at 15% level. Whereas defatted soy-supplemented bread, at 10% level, manifested the minimum value of polyphenol compared with other supplemented breads. The reason could be dehulling of soybean which reduces the activity of tannins (Reddy, Pierson, Sathe, & Salunkhe, 1985).

Control bread showed a trypsin inhibitor activity 185 TIU/g which did not change significantly in barley supplemented breads at all the levels. Maximum trypsin inhibitor activity (204 TIU/g) was found in breads containing full fat soy flour at 10% level. Significant variation in the level of trypsin inhibitor activity was also noticed in barley plus full fat soy- and barley plus defatted soy-supplemented breads at all the levels. At 15% level, there was an increase of 8 and 4% in the trypsin inhibitor activity as compared with control. Grewal (1992) and Saxena et al. (1994) also reported high contents of trypsin inhibitors in raw soybean flour.

Control bread had low levels of amylase inhibitor activity (68.3 AIU/g). In barley-supplemented bread at 5% level, the value was 71.4 AIU/g which increased to 73.2 AIU/g at 15% supplementation level. In soy-supplemented breads (full fat and defatted) at 10% level, the amylase inhibitor activity decreased to 66.9 and 66.5 AIU/g, respectively. Amylase inhibitor activities in breads made from barley plus full fat soy- and barley plus defatted soy-supplemented flours, at all the levels,

Table 6
Antinutrient contents of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Phytic acid (mg/100 g)	Polyphenols (mg/100 g)	Trypsin inhibitor activity (TIU/g)	Amylase inhibitor activity (AIU/g)
Control (Wheat)	225.6±0.82	315±0.59	185±0.76	68.3±0.75
W+B				
95:05	230±0.51	328±0.62	184±0.84	71.4±0.71
90:10	244±0.69	339±0.58	184±0.16	72.3±0.36
85:15	268±0.58	346±0.52	184±1.08	73.2±0.52
W+SF				
95:05	238±0.45	324±0.44	197±1.56	68.0±0.73
90:10	260±0.55	331±0.22	204±0.22	66.8±0.74
W+DSF				
95:05	233±0.75	321±0.47	193±0.37	67.9±0.49
90:10	253.2±0.65	329±0.48	198±1.00	66.5±0.44
W+B+SF				
95:05	236±0.55	325±0.62	189±0.40	69.3±0.33
90:10	253±0.69	338±0.47	195±0.46	69.7±0.47
85:15	270±0.66	344±0.22	200±0.85	70.2±1.00
W+B+DSF				
95:05	232±0.57	323±0.32	185±1.00	69.7±0.80
90:10	250±0.57	337±0.48	189±0.71	69.9±0.54
85:15	269±0.52	341±0.76	192±0.85	70.5±0.53
SE (*m*)	0.38	0.29	0.37	0.46
CD ($P<0.05$)	1.10	0.89	1.08	1.33

W, Wheat flour; B, Barley flour; SF, Soy flour; DSF, Defatted soy flour; NS, non-significant.

[a] Values are means±SD of three independent determinations.

were intermediate between that of barley- and soy-supplemented breads. Results showed that amylase inhibitor activity in barley-supplemented breads was highest and this might be due to the high activity of amylase inhibitors in barley flour (Henry, Battershell, Bennan, & Oono, 1992; Jood & Kalra, 2001).

3.2.6. Dietary fibre and β-glucan

The results regarding total, soluble and insoluble dietary fibre are given in Table 7. Whole wheat bread contained 8.90, 3.95 and 4.95% total, soluble and insoluble dietary fibre, respectively. All the dietary fibre components increased significantly ($P<0.05$) on increasing the supplementation of wheat with barley. It was found that, at 15% level of supplementation with barley flour, there was an increase of 10, 13 and 7% in total, soluble and insoluble dietary fibre contents of bread as compared to control. On the other hand, breads made from blends containing 10% soy flour (full fat and defatted) showed a decrease in total (8.52 and 8.60%) and insoluble (4.50 and 4.55%) dietary fibre whereas increase was found in soluble (4.02 and 4.05%) dietary fibre contents. This might be because in the present study, dehulled soybean was used and insoluble fibres are mainly present in hulls and soluble fibres are in cotyledons. It has also been reported that soluble fibre is associated with cholesterol-lowering and improved diabetic control whereas insoluble fibre is associated with enhanced bowel functions (Yee, 1995). Non-significant change was observed in total and insoluble dietary fibre contents, whereas significant change was found in soluble dietary fibre contents of wheat bread-supplemented with barley plus full fat soy and barley plus defatted soy flours.

Total, soluble and insoluble β-glucan contents of control and various supplemented breads are presented in Table 7. Wheat bread had 0.41% total, 0.22% soluble and 0.19% insoluble β-glucan contents. Breads containing barley flour at different levels showed significantly ($P<0.05$) higher contents of β-glucan. At 5, 10 and 15% supplementation levels, there was an increase in total (2-, 4- and 5-fold), soluble (4-, 5- and 6-fold) and insoluble (1-, 2- and 3-fold) β-glucan contents as compared with control bread which could be attributed to high β-glucan in hull-less barley variety (Kalra, 1996). It has been also been reported that β-glucan, especially soluble β-glucan, has hypoglycaemic (Knuckles, Hudson, & Sayre, 1997) and hypocholesterolemic (Kalra & Jood, 2000) effects.

Table 7
Total soluble and insoluble dietary fibre and β-glucan contents of breads prepared from wheat and various cereal-pulse blends (% dry matter basis)[a]

Breads	Dietary fibre			β-glucan		
	Total	Soluble	Insoluble	Total	Soluble	Insoluble
Control (Wheat)	8.90±0.55	3.95±0.84	4.95±0.29	0.41±0.20	0.22±0.12	0.19±0.08
W+B						
95:05	9.05±0.32	4.00±0.09	5.05±0.41	1.54±0.28	1.17±0.23	0.36±0.04
90:10	9.37±0.43	4.20±0.22	5.17±0.21	1.83±0.22	1.26±0.16	0.57±0.06
85:15	9.80±0.35	4.48±0.51	5.32±0.16	2.20±0.38	1.38±0.43	0.81±0.05
W+SF						
95:05	8.79±0.15	4.12±0.25	4.67±0.10	0.47±0.11	0.28±0.06	0.19±0.04
90:10	8.52±0.26	4.02±0.28	4.50±0.54	0.52±0.09	0.32±0.04	0.20±0.04
W+DSF						
95:05	8.81±0.16	4.13±0.43	4.67±0.59	0.49±0.17	0.29±0.22	0.21±0.05
90:10	8.60±0.34	4.05±0.30	4.55±0.04	0.53±0.23	0.33±0.26	0.23±0.03
W+B+SF						
95:05	8.91±0.03	4.14±0.34	4.77±0.37	1.11±0.37	0.83±0.42	0.28±0.07
90:10	8.95±0.19	4.17±0.43	4.78±0.24	1.50±0.37	1.13±0.42	0.36±0.05
85:15	9.10±0.24	4.21±0.46	4.89±0.70	1.65±0.41	1.19±0.37	0.45±0.03
W+B+DSF						
95:05	8.93±0.35	4.16±0.34	4.77±0.69	1.16±0.11	0.87±0.07	0.28±0.03
90:10	8.98±0.24	4.18±0.28	4.80±0.04	1.50±0.24	1.13±0.21	0.36±0.02
85:15	9.13±0.08	4.23±0.12	4.90±0.04	1.66±0.38	1.20±0.35	0.45±0.03
SE (*m*)	0.15	0.10	0.21	0.14	0.14	0.03
CD ($P<0.05$)	0.46	0.24	NS	0.44	0.41	0.09

W, Wheat flour; B, Barley flour; SF, Soy flour; DSF, Defatted soy flour; NS, non-significant.
[a] Values are means±SD of three independent determinations.

In breads containing soy (full fat and defatted) flours at 5 and 10% level, the contents of total, soluble and insoluble β-glucans were found to be little higher than control bread. However, in breads containing both barley and soy (full fat and defatted) flours at 5, 10 and 15%, a significant ($P < 0.05$) increase was found in total, soluble and insoluble β-g1ucan contents. At 15% supplementation level, increase was observed in total (3 times), soluble (4 times) and insoluble (2 times) β-glucan contents of barley plus full fat soy- and barley plus defatted soy-supplemented breads. The increase was more in soluble β-glucan than in insoluble β-glucan, in all the supplemented breads. The soluble β-glucans are present primarily in the endospermic cell walls of barley grain (Jood & Kalra, 2001) which has been reported to be useful in regulating cholesterol level and blood glucose levels (Kalra & Jood, 2000; Knuckles et al., 1997). Results presented here are in conformity with those reported by earlier workers. Knuckles et al. (1997) also found that total, soluble and insoluble β-glucan contents increased several times in breads containing a β-glucan rich barley fraction at different levels, as compared with control bread.

4. Conclusion

It may be inferred from the present study that soy flour (full fat and defatted) and barley flour could be added to bread flour up to levels of 10 and 15%, respectively, separately and in combinations without any significant change in organoleptic characteristics. Breads made from both barley and defatted soy flours, up to 15% level, were considered as most acceptable, organoleptically and nutritionally as they contained appreciable amount of protein, total lysine, dietary fibre, β-glucan and minerals. Development (and consumption) of such functional foods not only improves the nutritional status of the general population but also helps those suffering from degenerative diseases associated with today's changing life styles and environment.

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