PHYSICOCHEMICAL, FUNCTIONAL AND NUTRITIONAL PROPERTIES OF CEREAL PULSE BLENDS IN BREAD MAKING QUALITY

By

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2001

DEDICATED

TO

MY

LOVING PARENTS

CERTIFICATE – I

This is to certify that this thesis entitled, "Physicochemical, functional and nutritional properties of cereal pulse blends in bread making quality", submitted for the degree of Master of Science, in the subject of Foods & Nutrition of CCS Haryana Agricultural University, Hisar, is a bonafide research work carried out by Shfali Dhingra under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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MAJOR ADVISOR

CERTIFICATE – II

This is to certify that this thesis entitled, "Physicochemical, functional and nutritional properties of cereal pulse blends in bread making quality", submitted by Shfali Dhingra to the CCS Haryana Agricultural University, Hisar in partial fulfilment of the requirements for the degree of Master of Science in the subject of Foods and Nutrition, has been approved by the Student's Advisory Committee after an oral examination on the same.

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CHAPTER-I

INTRODUCTION

Since time immemorial, cereals have played an important role in human nutrition. They have been grown by man for thousands of years beginning long before the Christian era. Botanically cereals belong to grass family (Gramineae) that are grown for the edible grains and supply the basic nutritional need of much of the mankind (Belderok, 2000). On the world wide basis, India ranks third as far as total cereal production is concerned covering 1216.05 lac hectare area and 182 million tonnes total production of various cereals (India, 1994). Cereals which are considered economized source of energy in our diet, constitute 70-80 per cent of the daily energy intake, apart from having moderate (6-12%) protein contents and fairly good source of minerals and vitamins.

Among cereals, wheat is the world's most important cereal crop in terms of production and human consumption (Shewry and Tatham, 1994). Some characteristics of the grain like low water activity, ease of transport, processing and storage qualities have made this crop the staple food of 35 per cent population of the world. In India, wheat is being cultivated in an area of 25.40 million hectares with the production of 68.70 million tonnes (FAO, 1998). About 90 per cent of area in India is under bread wheat (*Triticum aestivum*), 9 per cent area is occupied by macroni wheat (*T. durum*) and remaining 1 per cent area is covered by quality wheat, popularly known as 'emmer' wheat (*T. dicoccum*) which has high commercial value (Reddy *et al.*, 1998).

In India, Haryana is next to Punjab and U.P. in wheat production and it has increased its production to 86 lac tonnes in 1998-99 (Yunus, 2000). Wheat forms a major source of protein and calories to a large section of Indian population. Traditionally, 85-90 per cent of the wheat is consumed mainly in the form of home made products like chapattis (unleavened breads), porridge, biscuits, cakes etc. However, in recent years, with the advancement in baking technology and changing food habits, wheat is milled to flour which finds use for preparation of variety of products like breads in urban and semi-urban areas of the country (Sharma *et al.*, 1999).

Apart from 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. Therefore, composite flours have received great emphasis for improving the nutritional quality of wheat products. Grain legumes contribute significantly towards protein, minerals and B-complex vitamins need of people globally and especially in developing countries. Supplementation of wheat flour with inexpensive staples such as cereals or legumes helps in improving the nutritional quality of wheat products (Sharma *et al.*, 1999).

In view of above, blends of cereals and legumes have an important place in our traditional dietary pattern. Some of the important meals based on cereal legume blends are *idli, dhokla, khichadi*. Among baked products, most widely consumed is bread. Bread making is one of the most eminent methods of cereal processing. Bread, according to Encyclopedia Americana is "A food made from a wide variety of plant substances that are ground into flour, mixed with water in dough and then usually baked into loaves or cakes." Whole wheat flour may be an alternate raw material for manufacturing breads and it may find ready acceptance as it offers better nutrition and familiar wholesome wheat taste to consumers (Indrani and Rao, 1992). Whole wheat flour can be blended with β-glucan rich barley and lysine rich soybean flour to have more healthful benefits as today's consumers are conscious of their diets and are concerned about eating foods with healthful benefits.

Barley (*Hordeum vulgare*) due to its high dietary fibre content, is a highly desirable cereal grain today. It is the worlds' fourth most important cereal after wheat, rice and maize (Newman and Newman, 1991). In India, its production is largely confined to U.P., Punjab and Haryana. The annual production of grain in India is 1.70 million tonnes (FAO, 1998). Barley is an excellent source of B-complex vitamins, minerals and complex carbohydrates. It is unique among cereals because of high concentration of mixed linked $(1\rightarrow3)$, $(1\rightarrow4)$ β-D glucans (β-glucans) which are known to have cholesterol lowering effect, gastrointestinal functions, cancer prevention, etc.

Among legumes, soybean (*Glycine max* L.) is very rich source of essential nutrients and one of the most versatile food stuffs. It is an excellent source of quality protein. It provides an alternative source of proteins for people who are allergic to the proteins in cow's milk (Riaz, 1999). India has a production of 2.3 million tonnes (FAO, 1998). One of the most promising uses of soybean is fortification of cereal based products such as baked products because the profile of essential amino acid in soybean is complementary to that of most cereals (Rastogi and Singh, 1989). Soybean health benefits include reduction of cholesterol levels and menopause symptoms and reduction of cancer, renal disease and osteoporosis (Riaz, 1999). On account of 38-40 per cent protein and 18-20 per cent fat, soybean has great potential in overcoming protein calorie malnutrition (Rastogi and Singh, 1989).

Therefore, the consumption of breads enriched with protein and fibre will improve nutritional status of population and reduce the risk of heart disease in adults. But the information is scanty on supplementation of wheat flour with barley and soy flour and their affect on bread making and nutritional quality.

Keeping these facts in view, the present investigation was undertaken with the following objectives:

(i) To determine the physicochemical and functional properties of wheat, barley and soy flours and their blends.

(ii) To determine the rheological characteristics of dough.

(iii) To prepare breads from cereal-pulse blends and to study the nutritional and organoleptic properties and shelf life of acceptable products.

2. REVIEW OF LITERATURE

Among cereal crops, only wheat flour has been extensively and widely used for the preparation of various bakery products throughout the world. This has been attributed to the inherited property of wheat flour to form dough and retain gases. The quantity and diversity of enjoyable and satisfying products made from wheat are remarkable and these include breads, biscuits, cakes, doughnuts, breakfast cereals etc. Apart from good source of calories and other nutrients, wheat is considered nutritionally poor because of deficiency of certain essential amino acids. Therefore, efforts have been made to supplement wheat flour with other staple cereal and pulse flours to upgrade the quality and quantity of dietary protein of these products. In present study also, efforts were made to supplement wheat flour with barley and soy flours for enhancing the protein, total lysine and dietary fibre contents of bread.

The research work done so far, relevant to the present study has been compiled and summarised under the following headings:

2.1 Physicochemical and functional characteristics

2.2 Rheological and baking characteristics.

2.3 Nutritional composition.

2.4 Shelf life.

2.1 Physicochemical and functional characteristics

Shurpalekar *et al.* (1976) reported 1000 kernel weight as 40.0 g in 17 aestivum Indian wheat varieties. Popli and Dhindsa (1980) also evaluated 7 wheat varieties commonly grown in Haryana and Punjab. The protein and sedimentation values ranged from 8.88-12.09% and 74.14-79.06%, respectively. Similarly, Adsule *et al.* (1985) studied 6 wheat varieties for their grain weight which ranged from 39-45 g. Bhatty (1986) reported water hydration capacity of scout (1.76 ml/g) and tupper (1.62 ml/g) barley flours.

Singh and Paliwal (1986) also studied two Indian durum wheat varieties and revealed that hardness of both varieties were in range of 13.00-13.25 kg/grain whereas 1000 kernel weight was higher than reported by earlier workers and dry gluten content was 10%. They also evaluated 13 wheat varieties for physical characteristics and reported 1000 kernel weight ranged from 40.3-53.2g. Varieties with 1000 kernel weight less than 45 g indicated comparatively smaller grain size. Most of the varieties have kernel hardness more than 10 kg/grain. Bakshi and Bains (1987) noticed wet (27.9%) and dry (9.9%) gluten in wheat flour whereas Pena and Ballance (1987) reported dry gluten in Fedrick (8.6%) and Marquis (12.6%) varieties of wheat.

Rastogi and Singh (1989) studied the effect of addition of full fat soy flour on quality characteristics and bread-making quality of white flour of UP-262 at different levels viz., 5, 10, 15 and 20%. Protein, wet and dry gluten, sedimentation value and water absorption capacity of blends of white flour and full fat soy flour ranged from 15.98-20.24; 36.62-30.86%; 12.31-10.28%; 27.9-24.1 ml and 56.0-60%. The water absorption capacity of white flour was reported to be 55%. Chaudhary and Weber (1990) studied the composition of different flours and reported moisture as 10.2% and protein as 14.8% in whole wheat flour. On the other hand, Singh *et al.* (1990) examined some U.P. hill wheats and reported that hill wheat i.e. Lalmisri were smaller in size and has lower 1000 kernel weight (28.6 g) and had highest kernel hardness (10.43 kg/grain), thus it was classified as hard wheat whereas UP-301 had highest 1000 grain weight (48.5 g). Values for wet and dry gluten, moisture and protein were in the range of 27.67-42.98%; 8.22-11.85%; 11.3-13.2% and 8.50-12.32%. Sedimentation value of Lalmisri was highest i.e. 38.0 cc.

Misra *et al.* (1991) revealed that blending of defatted soy flour at different levels i.e. 2, 4, 6, 8 and 10% to wheat flours of UP-319 and RR-21 varieties showed decrease in sedimentation value. The gluten content of both wheat varieties was 10.41 and 8.52% which decreased upon blending with soy flour. Moisture content of wheat flour was 10.90% and on blending it decreased to 10.80%. Also Newman and Newman (1991) evaluated the waxy hull-less barley flour and reported protein content as 11.9%. Whereas Sundberg and Aman (1994) reported 10% protein in barley fine flour.

Boyacioglu and D'Appolonia (1994) studied the utilization of durum wheat for bread making. The results indicated that durum wheat flour had higher wet and dry gluten and lower sedimentation value than bread wheat flour which may be attributed to disproportionately higher content of gliadins and low proportion of glutenin in durum flour. Nemeth *et al.* (1994) evaluated Canadian, Australian and United States soft wheats for 1000 kernel weight which ranged between 28.0-42.7g. Saxena *et al.* (1994) evaluated 6 new varieties of soybean and showed 1000 grain weight ranged from 94.3-145.6 g; grain hardness in vertical position to be 5.6-7.6 kg/grain; and in horizontal position 13.0-19.8 kg/grain. The colour of varieties varied from light yellow to golden yellow. The moisture and protein content of varieties were 9.8 to 11.8% and 36.1 to 40.7%.

Rao and Rao (1997) incorporated sorghum flour to wheat flour at 5, 10, 15 and 20% levels. Wheat flour had 9.6% protein, 9.0% gluten and sedimentation value of 37 ml. A decrease was reported in protein, gluten and sedimentation value as the percentage of sorghum flour increased in blends.

Reddy *et al.* (1998) evaluated 3 wheat varieties viz., *T. dicoccum, T. aestivum* and *T. durum* for physical characteristics and reported 1000 kernel weight as 32.5-44.5 g. Sharma *et al.* (1999) also reported 1000 kernel weight of durum wheat as 47.9 g. The biochemical attributes of four varieties of wheat i.e. Sonalika, Agrahyani, Akbar and Kanchan were studied by Alauddin *et al.* (1999). Results showed that moisture and protein contents were in the range of 5.4-7.85% and 10.95-12.05%.

Anjum and Walker (2000) tested 8 Pakistani wheat varieties for protein, dry and wet gluten and showed that protein ranged between 11.99-13.80%, dry and wet gluten ranged between 8.88-10.91% and 27.60-35.15%. Also the physicochemical characteristics of fenugreek supplemented wheat flour were evaluated by Sharma and Chauhan (2000). The supplementation of fenugreek flour to wheat flour showed increase in protein from 10.20 to 12.00% and dry gluten from 6.60-7.20%. Sharma (2000) also studied different wheat varieties for physicochemical parameters and estimated 23-34 ml sedimentation value; 6.6-11.3% dry gluten; 10.7-11.9% protein and grain hardness of 8.5-10.7 kg/grain.

2.1.1 Protein fractions

Pioneering work of Osborne and Mendell (1914) led to the development of the concept of solubility fractions of proteins. The four major classes of protein fractions are albumins, globulins, prolamins and glutelins. In a study conducted by Chen and Bushuk (1970), it was found that in wheat, prolamin was the major fraction which constituted 40.7% of total protein fraction, followed by acetic acid soluble fraction (18.3%), albumin (12.2%) and globulin (4.7%).

Katiyar *et al.* (1989) studied the nutrient composition of a traditional soybean cultivar of Kashmir Valley. He found that among the simple proteins, glutelin was the major fraction (91 g/kg) while prolamin constituted the least (3 g/kg). Globulin constituted 41 g/kg of the protein.

2.2 Rheological and baking characteristics

2.2.1 Rheological characteristics

Bhatty (1986) studied the rheological characteristics of wheat flour as well as composite flours containing barley flour at different levels and showed that mixing time for wheat flour was 3.20 min. and it decreased to 3.10 min. at 20% blending with hull-less barley flour which showed that wheat flour had strong gluten. Also the height and area under the curve decreased from 7.1 cm and 69.5 cm^2 for wheat flour to 5.4 cm and 56.8 cm^2 at 20% blending with barley flour.

Singh *et al.* (1990) in their study on mixographic and alveographic characteristics of some UP hill wheat concluded that Lalmishri and UP-301 were found to be of medium strong gluten flour and Thumri, Dutee and Sonalika were weak gluten flour. Whereas Misra *et al.* (1991) studied the effect of blending wheat flour with defatted soy flour at different levels and reported that mixing time increased as the level of defatted soy flour increased in the blends. Indrani and Rao (1992) evaluated whole wheat flour for rheological characteristics and showed that the wheat was medium strong for bread making based on farinograph and mixograph area values. The peak time and peak height of whole wheat flour was reported as 2 min. and 7 cm. Boyacioglu and D'Appolonia (1994) also showed that time to peak height for durum flour and bread flour was 2.0 and 3.5 min.

Mixographic properties for 17 wheat varieties were peak time, 0.8-5.5 min; peak height 32-48.5 min, stability 1.4-9.5 min and tolerance 3.0-11.5 mm as reported by Yamamoto *et al.* (1996). Similarly, Hanslas (1997) also examined 56 wheat varieties in India for mixographic, alveographic and farinographic characteristics. Mixographic studies divided the varieties into strong and poor gluten wheat on the basis of mixing time, peak development area and width of peak. Anjum and Walker (2000) studied the rheological and baking characteristics of 8 Pakistani wheat varieties. The mixogram revealed higher peak time (5.0 min) and peak height for wheat cultivar, Barani-83, which showed that Barani-83 possessed strong gluten. Sharma and Chauhan (2000) also studied the effect of fenugreek supplementation on rheological characteristics of wheat flour.

2.2.2 Baking characteristics

Selvaraj and Shurpalekar (1982) studied effect of incorporating defatted soy flour in wheat flour found that on increasing the level of defatted soy flour in wheat flour adversely affects the loaf volume, crust colour and crumb characteristics. Loaf volume decreased from 640 cc for refined wheat flour to 440 cc at 16% blending with soy flour. Results showed that addition of soy flour upto 10% produced good quality breads. Raidi and Klein (1983) also studied the effect of substituting 5, 10 and 15% field pea or defatted soy flour for wheat flour in chemically leavened quick bread. Significant difference was observed in loaf volume and sensory characteristics specially colour as it darkens with increase in level of substitution. Results indicated that defatted soy flour can be successfully substituted for wheat flour at levels upto 15%. Adsule *et al.* (1985) studied some varieties of wheat for bread characteristics and showed that total and specific loaf volume of breads ranged from 570-690 ml and 1.63-1.77 cc/g.

Bhatty (1986) supplemented barley flour to wheat flour at different levels and showed a decrease in loaf volume of control bread from 845 cc to 545 cc at 25% blending with barley flour. He suggested that 5% or possibly 10% barley flour can be added to wheat flour without seriously affecting the loaf volume and bread appearance.

Bakshi and Bains (1987) compared 10 cultivars of durum and two of bread wheat for baking quality. Loaf volume of durum (260-360 ml) was lower than that of bread wheat flour (415 and 480 ml). Also the bread making characteristic of Mexican wheat flour were examined by Paredes-Lopez *et al.* (1987). Loaf volume of breads ranged between 425-585 cc. Pomeranz *et al.* (1988) also studied the bread making properties of hard red winter (HRW) and hard red spring (HRS) wheats by measuring water absorption, mixograph characteristics, loaf volume and crumb grain in bread. Average loaf volume of HRS wheat flour was higher than that of HRW wheat flour.

Rastogi and Singh (1989) reported that on supplementing the white flour with 5-20% of full fat soy flour increased the loaf weight from 127 g for control to 140 g at 20% blending whereas loaf volume decreased from 420 cc for control to 300 cc at 20% blending. Organoleptic evaluation revealed that blending at 5% level produced satisfactory quality bread without affecting the taste and flavour.

Ereifej and Shibli (1993) conducted a study on baking quality of flour of Landrace and durum wheat cultivars grown in Jordan. The weight of bread loaves ranged from 150.1-162.8 g; loaf volume 315-750 cc and specific loaf volume from 1.9-5.0 cc/g. Similarly, Boyacioglu and D'Appolonia (1994) compared baking properties of bread wheat flour and durum wheat flour. Loaf volume of durum and bread flour ranged between 420-640 cc and 620-780 cc. Breads made from durum and bread flour were similar in external appearance, however, crust colour of durum flour bread was somewhat darker and texture was coarse, dense and rough.

Rathna and Neelakantan (1995) reported that substitution of puffed bengal gram flour beyond 20% to wheat flour resulted in decrease in loaf volume and deterioration in crumb grain and texture. Similarly, Rao and Rao (1997) reported that replacement of wheat flour with sorghum flour upto 15% produced acceptable breads without affecting the loaf volume, crust colour and crumb texture.

Knuckles *et al.* (1997) produced breads by substituting barley flour fractions for 5, 20 and 40% of standard flour. Replacement upto 20% was judged acceptable, though loaf volume was reduced and colour was slightly darker than control bread. Carson *et al.* (2000) studied the sensory characteristics of bread made from 50% sorghum based composite flour and found slight sourness and astringency in the crumb and top crust flavours. The overall acceptability score was 6.9 in the sorghum based composite breads. Sharma (2000) prepared breads from various wheat varieties and reported 150-155.8 g loaf weight, 400-460 cc loaf volume and 2.63-3.05 cc/g specific loaf volume.

2.3 Nutritional characteristics

2.3.1 Protein, fat, ash and sugar content

The contents of protein, fat and ash in wheat ranged between 10.2-11.7; 1.3-2.8 and 1.8-2.1% (Chawla and Kapoor, 1982; Vaidehi, 1984). The contents of non-reducing and total sugars in wheat were estimated to be 178 and 208 mg/10 g wheat flour (Sekhon *et al.*, 1980). The protein, ash, reducing and non-reducing sugar contents ranged between 8.5 to 12.3%; 1.14 to 1.50%; 15.0 to 34.0 mg/10 g and 128 to 221.7 mg/10 g in some UP hill wheats *(Singh et al., 1990). Similarly,* Indrani and Rao (1992); Rao and Rao (1997) recorded 11.92 and 1.54% protein and ash in whole wheat flour. Chemical analysis of some wheat varieties revealed that protein, fat, ash and total sugars ranged between 10.95 to 12.05; 1.88-3.15; 1.6 to 1.8 and 4.1-4.3%, respectively (Alauddin *et al.*, 1999; Sharma *et al.*, 1999).

On an average raw soybean contained 43.00; 21.50 and 4.60% protein, fat and ash, respectively (Savitri and Desikarhar, 1990; Saxena *et al.*, 1994). Akingbala *et al.* (1995) also studied the composition of full fat and defatted soy flours and found that defatted soy flour had 57.4% protein, 1.9% fat and 5.5% ash whereas full fat soy flour had 47.7, 23.5 and 4.5% protein, fat and ash, respectively. Protein, fat and ash content of dehulled barley ranged between 12.5-12.7; 1.8-2.1 and 1.8-3.1% respectively (Bhatty, 1993; Sundberg and Aman, 1994).

Gayle *et al.* (1986) studied the chemical composition of wheat breads supplemented with pigeon pea flour at 0 to 25% levels. Increasing the level of pigeonpea flour increased the protein content from 9.19 to 13.0% and fat and ash contents ranged from 5.12 to 5.93 and 0.89 to 1.19%, respectively.

Rastogi and Singh (1989) evaluated the proximate composition of blends of white flour and full fat soy flour at 5 to 20% and reported that fat, protein, reducing and non-reducing sugars increased from 2.81 to 6.74 g; 15.98 to 20.24 g; 46.92 to 51.48 mg/10 g and 96.27 to 102.46 mg/10 g at 20% substitution.

Chaudhary and Weber (1990) analysed the composition of breads containing 15% fibre ingredients. Wheat bran and oat bran increased the bread's protein content from 9.65 to 9.79%. They also reported the protein, fat and ash contents of wheat flour as 14.8, 2.16 and 1.3%, respectively. Rathna and Neelakantan (1995) studied the composition of breads supplemented with puffed bengal gram flour at 5 to 25% levels and revealed that protein content of control bread (7.59%) increased at 20% substitution (9.44%).

Knuckles *et al.* (1997) gave the composition of breads prepared by substituting β-glucan rich barley fraction for 5, 20 and 40% of standard flour. Control bread had 15.22, 2.50 and 1.61% protein, fat and ash whereas at 20% substitution protein, fat and ash contents were 14.93., 2.29 and 2.13%, respectively.

Sharma and Chauhan (2000) examined the chemical composition of fenugreek supplemented breads and concluded that protein, reducing, non-reducing and total sugars (mg/10 g flour) in control wheat bread were 8.60% and

365, 26 and 383 mg/10 g, respectively. At 9% substitution with fenugreek flour, the values were 10.55% and 255, 27 and 282 mg/10 g, respectively.

2.3.2 Antinutrients

2.3.2.1 Phytic acid

Phytic acid (myoinositol dihydrogen phosphate) is widely distributed in cereals and cereal products (Reddy *et al.*, 1982). Phytic acid in acidic medium binds to protein and forms protein-mineral-phytate complex (Cheryan, 1980) thus, enzymatic degradation of proteins is inhibited and hence protein digestibility is affected (Knuckles *et al.*, 1985). Phytic acid is known to decrease the bioavailability of zinc and other trace elements to human and monogastric animals (Haug and Lantzsch, 1983). Phytic acid is mainly present in outer seed coat, hence dehulling is an effective means of its reduction. Cooking and canning resulted in significant reduction in phytic acid (Tabekhia and Luh, 1980).

Lalos *et al.* (1976) reported that phytate content in barley, wheat and oats ranged between 0.97 to 1.16; 0.73 to 1.35 and 0.82 to 1.01%, respectively. Singh and Reddy (1977) analysed phytic acid content in whole wheat grain as 0.5 to 1.9 mg/g. Gayle et al. (1986) showed a increase in phytate concentration from 160 to 250 mg/100 g in pigeon pea supplemented breads at 5, 10, 15 and 25% levels. The phytic acid content in three soybean varieties ranged between 1.07 to 1.7 g/100g (Vander Riet *et al.*, 1987). Kulshreshtha and Usha (1992) observed 238 mg/100 g phytic acid in wheat. Also Kalra (1996) analysed phytic acid in barley and found it to be 335 to 1030 mg/100 g.

Sharma and Chauhan (2000) also observed increase in phytic acid content in fenugreek supplemented breads at different levels as compared to control bread.

2.3.2.2 Polyphenols

Polyphenols or tannins are widely distributed in plant foods. These are predominantly located in peripheral area of seed. They limit the protein and starch utilization (Pawar and Parlikar, 1990) by binding with proteins or by inhibiting the digestive enzymes specially trypsin and amylase (Singh, 1984). There is a detrimental effect of polyphenols on the availability of minerals and vitamins. Processing of grains such as dehulling, soaking, cooking and germination can reduce the antinutritional activity of tannins (Reddy *et al.*, 1985).

Among cereals barley has been reported to contain low levels of polyphenols i.e. less than 0.1% by weight (Bhatty, 1986). Tannin content of food legumes ranges from 45 mg/100 g to 2000 mg/100 g in soybean and fababean (Reddy *et al.*, 1985). Hira *et al.* (1991) showed that polyphenol content in 19 wheat varieties ranged from 225 to 337 mg/100 g. Jood and Kapoor (1992) also reported that polyphenol content in wheat was 482 mg/100 g. According to Grewal (1992) dry mature soybean had 0.3% polyphenols and Saxena *et al.* (1994) reported 575-720 mg/100 g total phenols in soybean. Kalra (1996) found that polyphenol content in barley ranged between 380-925 mg/100 g.

2.3.2.3 Trypsin inhibitors

Trypsin and chymotrypsin inhibitors are widely distributed in plant kingdom. They have the ability to inhibit the trypsin activity of the stomach. Trypsin inhibitors functions by combining with active enzymes to form a tightly bound enzyme substrate complex which is very stable (Ryan and Santarins, 1976).

Gallardo *et al.* (1974) investigated trypsin inhibitor activity of raw and processed seeds of field beans, broad beans, peas, lentils and soybeans and found that soybean had highest trypsin inhibitor activity. Liu and Markakis (1987) found 58.7 to 64.3 units/mg of trypsin inhibitors in raw soybean which were fully eliminated upon steaming and cooking for 20 min. The trypsin inhibitor activity in

33 varieties of wheat were estimated to be 236 to 365 units/g by Duhan *et al.* (1986). Grewal (1992) revealed that soybean has 2370 units/g of trypsin inhibitors which reduced gradually upon germination. Saxena *et al.* (1994) also reported high trypsin inhibitor activity in soybean.

2.3.2.4 Amylase inhibitors

Proteinaceous inhibitors of alpha amylases are widely distributed in cereals, legumes and other plants. Most of the cereals including wheat, rice, barley, rye, sorghum, maize, oats, ragi are known to possess these inhibitors (Shivraj and Pattabiraman, 1980; Henry *et al.*, 1992). Various processing treatments are known to destroy these inhibitors. It was found that soaking and dehulling caused a decline in amylase inhibitor activity upto 25% (Srivastava, 1994). It has been reported that this antinutrient is heat labile and gets destroyed at 100^{0}C within 10-20 minutes (Shivraj *et al.*, 1979; Singh *et al.*, 1982). Kalra (1996) estimated 132.25 to 160.15 amylase inhibitors units/g in barley cultivars.

2.3.3 *In vitro* digestibility

2.3.3.1 Protein digestibility

The nutritional quality of cereals and legumes is determined not only by their protein and energy content but it also depends upon the digestibility of proteins. In raw state, antinutrients like saponins, tannins and phytates lower the digestibility of proteins (Anonymous, 1973). Legumes are known to possess low protein digestibility. Cooking and heat treatment has been known to increase the protein digestibility (Gupta, 1994). According to Jood and Kapoor (1992) protein digestibility of wheat, maize and sorghum was 72.92, 70.96 and 64.50%, respectively whereas Mouliswar *et al.* (1993) estimated less protein digestibility i.e. 62 and 66% in raw maize and wheat. Kalra (1996) estimated protein digestibility in 16 barley cultivars and concluded that it ranged from 58.28 to 66.65%, respectively.

2.3.3.2 Starch digestibility

The digestibility of starch is limited by the cell wall structural features and antinutrients such as amylase inhibitors, phytates and tannins (Yadav and Khetarpaul, 1994). Starch digestibility is varied in different cereals. According to Jood and Kapoor (1992), the starch digestibility of wheat, maize and sorghum was 31.62, 40.26 and 36.95 mg maltose released/g. Heat processing causes the rupturing of starch granules followed by its hydrolysis enhancing the amylolytic processes. Dry heat treatment was less effective in improving the starch digestibility than moist heat (Chowdhury, 1993). The starch digestibility in 16 barley cultivars was poor and ranged from 20.28 to 28.13 mg maltose released/g which may be due to the binding property of inhibitors with amylase enzymes, thus affecting the starch digestibility (Kalra, 1996).

2.3.4 Minerals and total lysine content

2.3.4.1Minerals

Iron and zinc contents (mg/100 g) of barley, wheat, maize and oats were reported to be 7.92, 2.97; 5.17, 2.83; 4.41, 2.69 and 7.89, 3.01, respectively (Knizhko *et al.*, 1974). Lockhart (1978) studied the mineral content of wheat, rye and triticale. The contents of calcium, iron and zinc in wheat were 50, 10 and 3.1 mg/100 g, respectively. It was found that the mineral profile of soybean was superior to that of the other pulses and contents of calcium and iron were 197-251 and 4.49-7.20 mg/100 g (Ologhoba, 1989). Suibaba (1990) reviewed the mineral composition of barley and found 26, 215, 1.67 and 1.20 (mg/100 g) calcium, phosphorus, iron and zinc, respectively. Misra *et al.* (1991) studied the effect of blending defatted soy flour in wheat flour upto 10% level and found a remarkable increase in calcium content of blended flour. The calcium and iron contents in wheat ranged between 28.3-37 and 2.1-4.3 mg/100 g (Ranhotra, 1991; Sharma *et al.*, 1999). The hull-less barley genotypes were evaluated by Bhatty (1993) and he found 0.2, 76.4 and 44.4 mg/g calcium, iron and zinc. Calcium and iron in soybean cultivars were estimated to be 200-307 and 10.3-14.0 mg/100 g (Saxena *et al.*, 1994).

The availability of any element to meet the requirements cannot be assessed by finding the total amount of that element present in the food item. This may be attributed to the fact that absorption and subsequent utilization of any element is influenced by many factors (Rao and Prabhavati, 1978). Ionizable iron which is considered to be an indicator of available iron in some wheat varieties ranged from 2.1 to 4.6 mg and constituted 28.4 to 54.8% of total iron (Hira *et al.*, 1991).

2.3.4.2Total lysine

Gayle *et al.* (1986) studied the effect of substituting pigeon pea flour to wheat flour at 0 to 25% levels and found an increase in lysine content of bread from 0.3 to 171.0 mg lysine/16gN at 25% substitution. Rathna and Neelakantan (1995) also reported that lysine content increased to 309 mg/100 g in 20% puffed bengal gram flour substituted bread in contrast to 134 mg/100 g in control wheat flour bread.

2.3.5 Dietary fibre

Dietary fibre is the sum of polysaccharides and lignins not digested by the endogenous secretions of the human gastrointestinal digestive tract (Trowell, 1974). It is also popularly described as the portion of plant cells that cannot be digested by human digestive enzymes and therefore, cannot be absorbed from human small intestine. It may be categorized as total, soluble and insoluble dietary fibre. Cereals and legumes are known to contain an appreciable amount of fibre which has an important role in human therapeutics (Slavin, 1987). Kakker (1992) observed reduction in dietary fibre content after dehusking. Also increased period of autoclaving resulted in decreased insoluble fibre content. Maria *et al.* (1987) reported that the total dietary fibre content of wheat and barley were 10.5

and 21.7%, respectively. Chaudhary and Weber (1990) also reported 10.7% total dietary fibre in wheat flour. Kahlon *et al.* (1993) studied composition of dehulled barley and reported 17.2, 6.0 and 11.2% total, soluble and insoluble dietary fibre, respectively. Bhatty *et al.* (1993) observed total, soluble and insoluble dietary fibre in hull-less barley and wheat as 8.7, 2.7, 4.7 and 4.4, 2.2, 1.4%, respectively.

2.3.6 β-glucan

Seventy five per cent of endospermic cell wall is β-glucan while the corresponding value in aleurone cell walls is less than 10% (Forrest and Wainwright, 1977). β-D-glucans contain a mixture (30:70) of β (1→3) and β (1→4) glucosyl bonds, hence they are less tightly folded and partially soluble in water. It can be categorized as total, soluble and insoluble β-glucan. Although β-glucan occurs in all cereals but their concentration is highest in oats and barley (Hofer, 1986).

The soluble β-glucan are a major component of soluble fibres and constitutes higher percentage of total β-glucans (Frolich and Nyman, 1988). Sundberg and Aman (1994) observed 1.8 to 3.8% soluble β-glucan content in barley. Marlett (1991) also observed a very high proportion of soluble β-glucan which made up for 73% of total β-glucan in barley. β-glucan content of barley has been reported to range between 3.8 to 8% (Newman and Newman, 1991; Sundberg and Aman, 1995). Bhatty (1993) estimated β-glucan content of hull-less barley and wheat flour as 4.5 and 0.40%, respectively whereas Berglund *et al.* (1994) reported that wheat and rice contains 0.14 and 0.07% total β-glucan.

Knuckles *et al.* (1997) prepared breads by substituting β-glucan rich barley fractions for 5, 20 and 40% standard flour. Breads with 20% substitution has 7.6 times total β-glucan. The values for total, soluble and insoluble β-glucan content in control as well as supplemented breads were 0.47, 0.31, 0.16% and 7.90, 3.58, 2.44%, respectively.

2.4 Shelf life

Gopaldas *et al.* (1982) studied the keeping quality of multimixtures of roasted and malted wheat, bengal gram and groundnut in 4:1:1 ratio for a period of 4 months and found 28 days shelf life of roasted mixture and 7 days of malted one. Sehgal (1988) stored the weaning mixtures developed from wheat, bengal gram, green gram and jaggery for 40 days and found a significant increase in peroxide value and fat acidity during storage periods.

The storability of roasted and malted supplements were also studied by Dahiya and Kapoor (1994) for a period of 30 days. Results indicated an increase in level of moisture, peroxide value, fat acidity and alcoholic acidity in malted mixtures than the roasted ones. Gahlawat and Sehgal (1994) also reported an increase in peroxide value and fat acidity with increase in storage period. Gupta (1994) found that fat acidity was higher in soybean and green gram flour Panjiri than that of Bengal gram flour Panjiri. She also reported that fat acidity increased significantly with increase in supplementation with soy flour.

3. MATERIAL AND METHODS

The present investigation was carried out in the Department of Foods & Nutrition, I.C. College of Home Science, CCS Haryana Agricultural University, Hisar to study the 'Physicochemical, functional and nutritional properties of cereal pulse blends in bread making quality.'

This chapter contains relevant information pertaining to the research designs and methodological steps used for the present investigation. The research procedures to achieve the foregoing objectives has been distinctly described under the following headings and sub-headings:

3.1 Procurement of material

3.2 Processing of material

3.3 Preparation of blends

3.4 Physicochemical and functional properties

3.4.1 Moisture content

3.4.2 1000-grain weight

3.4.3 Grain colour

3.4.4 Grain hardness

3.4.5 Protein

3.4.6 Protein fractions

3.4.7 Gluten content

3.4.8 Sedimentation value

3.4.9 Hydration capacity

3.4.10 Water absorption capacity

3.5 Rheological characteristics

3.6 Preparation of breads

3.7 Physical characteristics of breads

3.8 Organoleptic evaluation of prepared breads

3.9 Nutritional evaluation of acceptable breads

3.9.1 Protein

3.9.2 Ash

3.9.3 Fat

3.9.4 Starch digestibility (in vitro)

3.9.5 Protein digestibility (in vitro)

3.9.6 Sugars

I. Total soluble sugars

II. Reducing sugars

III. Non-reducing sugars

3.9.7 Minerals

I. Total minerals (Ca, Fe, and Zn)

II. Minerals availability (Ca, Fe and Zn)

3.9.8 Total lysine

3.9.9 Antinutrients

I. Phytic acid

II. Polyphenols

III. Trypsin inhibitors

IV. Amylase inhibitors

3.9.10 Dietary fibre (total, soluble and insoluble)

3.9.11 β-glucan (total, soluble and insoluble)

3.10 Shelf life of acceptable breads

3.10.1 Organoleptic evaluation of stored breads

3.10.2 Peroxide value

3.10.3 Fat acidity

3.11 Statistical analysis

3.1 Procurement of material

The bulk samples of commonly grown varieties of wheat viz., WH-423 and soybean viz., PK-416 were obtained from the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. The barley variety viz., Dolma was procured from the Department of Plant Breeding, Himachal Pradesh Krishi Vishva Vidyala, Palampur. All the grains were cleaned, made free from dust and other foreign material.

3.2 Processing of material

The grain samples of wheat and barley were ground on a junior mill to pass through 60 mesh sieve and were stored in air tight containers until used. The soybean grains were dehulled in dehusking and splitting machine (Central Institute of Post Harvest Engineering and Technology, Ludhiana) and then were ground to fine flour on junior mill. Soy flour was defatted by using the standard method of AOAC (1995). Two hundred g of soy flour was suspended in 500 ml of petroleum ether and shaken for 3 hours, left the sample overnight and filtered, dried at room temperature to evaporate petroleum ether and then stored the defatted sample in deep freezer.

3.3 Preparation of blends

Barley flour, soy flour (full fat and defatted) and both barley and soy flour (full fat and defatted) in the ratio of 50:50 were blended separately with wheat flour at different levels viz., 0, 5, 10, 15 and 20%, respectively.

3.4 Physicochemical and functional properties

3.4.1 Moisture: Moisture in the samples was estimated by employing the standard method of analysis (AOAC, 1995).

3.4.2 1000-grain weight: It was determined by AACC (1984) method. One thousand seeds were counted thrice and were weighed in grams.

3.4.3 Grain colour: It was observed visually.

3.4.4 Grain hardness: It was measured by pressing ten average sized well filled grains under the grain hardness tester (Manufactured by Kiya Seisakusho Ltd., Japan). Force was applied to crush grain by turning the knob. The force in kg displayed on dial at the time of crushing the grain was noted.

3.4.5 Protein: Protein was estimated by Microkjeldahl method (AOAC, 1995).

3.4.6 Protein fractions: Different fractions viz., albumin (water soluble), globulin (salt soluble), prolamin (alcohol soluble) and glutelin (alkali soluble) were determined by the method of Osborne (1907) as modified by Naik (1968).

Procedure: Water extract of the flour was prepared by shaking the flour with distilled water for 1.5 h in a shaker. A flour to solvent ratio of 1:5 (w/v) was used. The suspension was centrifuged at 3000 rpm for 15 min and the clear supernatant was collected. The extraction was repeated twice and the supernatant pooled. It represents albumin fraction.

Salt soluble proteins were prepared in the similar manner by extracting the residue (left after extracting albumin fraction) with 1% w/v NaCl solution.

The alcohol soluble prolamin fraction was prepared by intermittently shaking the residue left behind after the extraction of globulin with 60% ethanol at 60°C for 2h. The supernatant were collected after centrifugation. The residue obtained after prolamin extraction was extracted with 0.4% NaOH solution by shaking for 2 h and centrifuged at 3000 rpm for 15 min. The supernatant collected after repeated washing of residue with 0.4% NaOH represent the glutelin fraction of wheat protein. The N content in protein fractions was determined by micro Kjeldahl's method (AOAC, 1995).

3.4.7 Gluten content: AACC method (1984) was used for wet gluten estimation. Weighed sample (25 g) was transferred into a clean dry mixing bowl and 13.5 ml water was added. The contents were made to a stiff dough ball. The dough ball was dipped into water for half an hour and then washed with hands under the tap water until free from starch. The gluten thus obtained was weighed and expressed in percentage wet gluten. This was then dried in an oven (100°C) and weighed. Results were reported as percent dry gluten.

$$\text{Wet gluten (\%)} = \frac{\text{Wt. of moist gluten}}{\text{Wt. of sample taken}} \times 100$$

$$\text{Dry gluten (\%)} = \frac{\text{Wt. of dry gluten}}{\text{Wt. of sample taken}} \times 100$$

3.4.8 Sedimentation value: Lactic acid-sodium dodecyl sulphate (SDS) solution was prepared by dissolving 20 g SDS in 1 litre of distilled water, 20 ml of stock dilute lactic acid was added to it (1 part by volume of 88% lactic acid + 8 part by volume of distilled water). Sedimentation value in wheat flour was determined according to procedure given by Misra *et al.* (1998). Weighed sample (5 g) was transferred into 100 ml stoppered graduated cylinder. Distilled water (50 ml) was added to it and cylinder shaken horizontally i.e. left-right, for time specified in table and then kept for few minutes. After this lactic-acid-SDS solution (50 ml) was added and again mixed. The volume of sediments left was noted after 5 minutes and was reported as sedimentation value (ml).

3.4.9 Hydration capacity: Hydration capacity was assessed by the method of William *et al.* (1983). Fifty g seeds were counted and transferred to a 250 ml measuring cylinder and 50 ml water was added. The cylinder was stoppered and left overnight at room temperature. Next day the grains were dried by removing superfluous water with absorbent paper and swollen seeds reweighed. Hydration capacity per seed was determined by the following formula:

$$\text{Hydration capacity (per seed)} = \frac{\text{Wt. of soaked seed (g)} - \text{Wt. of seeds before soaking (g)}}{\text{Number of seeds}}$$

3.4.10 Water absorption capacity: Water absorption capacity was assessed by following the method of Quin and Paton (1983). To estimate water absorption capacity, five g flour was weighed in 50 ml centrifuge tube, 30 ml water was added and stirred with glass rod for 5 min, then allowed to stand for 30 min, at ambient conditions then centrifuged at 4500 rpm for 25 min. The volume of free liquid was measured and retained volume was expressed as ml of water absorbed per gram of sample on dry basis.

3.5 Rheological characteristics

Mixing time: Dough properties of flours were studied using 10 g direct drive recording Mixograph (National Manufacturing Division, TMCO, Lincoln, NE, USA) according to standard AACC procedure (1984).

Procedure: Weighed ten g of flour sample (control as well as blends) into mixing bowl at 65% water absorption (14% moisture basis) and run for 8 min. Computer software was used for interpreting mixograms.

3.6 Preparation of breads

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

The formula (based on flour weight) for straight dough method used to bake 100 g loaves of bread was as follows:

Ingredients	Quantity (g)
Flour	100
Compressed yeast	3.5
Sugar	18
Shortening	5
Salt	1.75
Water	Optimum for desirable consistency

The dough was mixed manually in a bowl, rounded to form dough of desirable consistency. The baking schedule was:

Mixing	(Optimum; Room temperature)
↓	
Fermentation	(1 h 40 min; $30+1^{\circ}$C)
↓	
Remixing	(25 sec; Room temperature)
↓	
Recovery (ferment)	(25 min; $30+1^{\circ}$C)
↓	
Proofing	(55 min; $30+1^{\circ}$C)
↓	
Baking	(20 min; $23+1^{\circ}$C)
↓	
Cooling	(25 min; Room temperature)

3.7 Physical characteristics of bread

Breads from control and supplemented flours were baked in replicates. After removing from the oven, loaves were immediately weighed and then placed on a wire grid for about 2 h before volumes were determined. Loaf volumes were measured by rapeseed displacement method. Specific loaf volumes were calculated by dividing the loaf volume by loaf weight and the results were expressed as ml/g.

3.8 Organoleptic evaluation:

Organoleptic evaluation of prepared breads was carried out by a panel of ten judges for crust colour, appearance, flavour, crust texture, taste and overall acceptability using nine-point Hedonic Rating Scale as given in Appendix I.

3.9 Nutritional evaluation of acceptable breads

3.9.1 **Protein**: Protein was estimated by MicroKjeldahl method (AOAC, 1995).

3.9.2 **Ash**: Ash in the samples was estimated by employing the standard method of analysis (AOAC, 1995).

3.9.3 **Fat**: Fat was estimated by employing the standard method of analysis (AOAC, 1995) using the soxhlet extraction apparatus.

3.9.4 **Starch digestibility (*in vitro*)**: *In vitro* starch digestibility was assessed by following the method of Singh *et al.* (1982).

Reagents

(i) **0.2 M phosphate buffer (pH 6.9)**: Fifty ml of 0.2M (27.28 g/lit.) potassium dihydrogen phosphate was added to 46.8 ml of 0.2 M (35.59 g/litre) disodium hydrogen phosphate and made upto 200 ml with water.

(ii) **Pancreatic amylase**: Twenty mg pancreatic amylase (Sigma Chemical Company, USA) was dissolved in 50 ml of 0.2 M phosphate buffer (pH 6.9).

(iii) **Dinitrosalicylic reagent**: Dissolved 10 g of 3,5 dinitrosalicylic acid, 300 g sodium-potassium tartarate and 16 g NaOH in carbon dioxide free water and made to 1000 ml. The reagent was stored in brown bottle and protected from carbon dioxide.

(iv) **Standard maltose solution**: Dissolved 100 mg maltose monohydrate in water and made upto 100 ml.

Estimation

Twenty five mg of defatted sample was dispersed in one ml of 0.2 M phosphate buffer (pH 6.9). Added 0.5 ml of pancreatic amylase to sample suspension and incubated in water bath at 37°C for 2 h. After the incubation period, 3 ml of dinitrosalicylic acid reagent was quickly added and mixture was heated in a boiling water bath for 5 minutes. After cooling, the solution was made to 25 ml with distilled water and filtered prior to measurement of absorbance at 550 nm. A blank was run simultaneously while incubating the sample. The dinitrosalicylic acid reagent was added before addition of the enzyme solution. Maltose was used as standard and values were expressed as mg maltose released per g defatted sample. Standard curve was prepared by taking 0.8 to 8 mg maltose from a standard maltose solution.

3.9.5 **Protein digestibility (*in vitro*)**: *In vitro* digestibility of protein was determined by the method of Akeson and Stahmann (1964) as modified by Singh and Jambunathan (1981).

Reagents

(i) **Pepsin**: Dissolved 80 mg pepsin (3000 units) in HCl (pH 2.0) prepared by diluting the acid with water and made upto 100 ml.

(ii) **10% and 5% trichloroacetic acid (TCA)**

(iii) **0.1 M borate buffer (pH 6.8)**

a) **0.2 M boric acid**: Dissolved 12.4 g boric acid in distilled water and made the volume to one litre.

b) **0.05 M borax solution**: Dissolved 19.05 g borax in distilled water and made the volume to one litre. For preparing the borate buffer, added 140 ml of 0.2 M boric acid, 50 ml distilled water and few drops of 0.05 M borax solution to adjust the pH at 6.8.

(iv) **0.1M borate buffer** (pH 6.8) containing 0.025M calcium chloride was prepared by dissolving 2.75 g calcium chloride in one litre of the buffer.

(v) **0.2N NaOH.**

(vi) **Pancreatin**: Dissolved 50 mg pancreatin (Sigma Chemical Company, USA) in 100 ml of 0.1 M borate buffer (pH 6.8) containing calcium chloride.

Procedure

Two hundred mg ground sample was taken in a 50 ml conical flask, added 5 ml of pepsin solution to it and incubated the contents at 37°C for 16 h in a water bath shaker. A few drops of toluene were added to each flask to check the growth of microbes. After incubating for 16 h, the pH was adjusted to 7.0 with 0.2 N sodium hydroxide solution. Then 2 ml pancreatin solution was added to each flask and incubated at 37°C in a water bath shaker for 24 h. Then 7 ml of 10% TCA was added. The contents were centrifuged at 12,000 rpm for 20 min and the residue was washed twice with 5 ml of 5% TCA. The supernatants were pooled and the volume was made upto 25 ml with 5% TCA. Five ml aliquot was taken and dried at 80-90°C in a hot air oven and the nitrogen contents were determined by microkjeldahl method (AOAC, 1995). The protein of the sample was determined by multiplying N content by a factor 5.70. The digested protein of the sample was determined by subtracting the residual protein from the total protein of sample. Protein digestibility was calculated by the following formula:

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

3.9.6 Sugars

I. **Total soluble sugars:** Total soluble sugars were determined by the ferricyanide method of Hulme and Narain (1931) as described below:

Extraction

One hundred mg of fat free powdered sample was taken in a test tube (1" wide x 7" long) and 10 ml of distilled water was added. The test tube was kept in a boiling water bath for about 30 min, filtered and filtrate was collected in a 100 ml volumetric flask. The extraction procedure was repeated 3-4 times till there was no soluble sugars in the residue when tested with anthrone. The final volume was made to 50 ml with distilled water.

Hydrolysis

Ten ml of sugar extract and 2 ml of concentrated HCl were taken in 50 ml volumetric flask and kept for one hour on a water bath maintained at 68°C. After hydrolysis, the acid was neutralized by adding small quantities of sodium carbonate till the effervescence stopped. The volume was made to 50 ml with distilled water.

Estimation of total soluble sugars

Reagents

(i) **Potassium ferricyanide solution**: Prepared by dissolving 8.25 g of potassium ferricyanide and 10 g of anhydrous sodium carbonate in 500 ml water and volume made to one litre with distilled water. The solution was filtered and kept in dark bottles and was used after three days.

(ii) **Potassium iodide solution**: This solution was prepared by dissolving 12.5 g of potassium iodine, 25 g of zinc sulphate and 125 g of sodium chloride in one litre of distilled water.

(iii) **Starch solution**: One g starch was shaken well with 20 ml distilled water and the mixture was poured in 60 ml of boiling water. After boiling the mixture for about 2 min, 20 g of sodium chloride was added. The mixture was cooled and volume made to 100 ml.

(iv) **5 per cent acetic acid solution.**

(v) **0.01N sodium thiosulphate.**

Method

Five ml of potassium ferricyanide and 10 ml of hydrolysed sugar extract was taken in a test tube and kept for 15 min in boiling water bath. The tubes were cooled under running tap water. Then 5 ml of potassium iodide solution followed by 3 ml of 5 per cent acetic acid were added in each test tube. The liberated iodine was titrated with 0.01N sodium thiosulphate solution using starch as indicator. The disappearance of blue colour and appearance of milky colour indicated the end point. A blank with 10 ml of distilled water was also run simultaneously. Total soluble sugars were calculated by using the following formula:

mg of total soluble sugars $= (V_1 - V_2 + 0.05) \times 0.34$

V_1 = Volume of 0.01N sodium thiosulphate used in the blank

V_2 = Volume of 0.01N sodium thiosulphate used in the sample.

II. **Reducing sugars**: The reducing sugars were estimated from the sugar extract by the same method as used for total sugars.

III. **Non-reducing sugars**: The amount of non-reducing sugars was calculated by subtracting the reducing sugars from the total soluble sugars.

3.9.7 Minerals

I. Total Ca, Fe and Zn

Acid digestion

To one g ground sample in a 150 ml conical flask, 25-30 ml of diacid mixture (HNO_3:$HClO_4$::5:1, v/v) was added and kept overnight. The contents were digested by heating till clear white precipitates settled down at the bottom. The crystals were filtered through Whatman No. 42 filter paper. The filtrate was made to 50 ml volume with double distilled water and used for determination of total Ca, Fe and Zn.

Calcium, iron and zinc in acid digested samples were determined by Atomic Absorption Spectrophotometer 2380, PERKIN-ELMER (USA) according to the method of Lindsey and Norwell (1969).

II. Availability (*in vitro*) of minerals

Calcium and zinc availability (*in vitro*)

Reagents

(i) **0.1% pepsin in 0.1N HCl**

(ii) **HCl**

(iii) **$NaHCO_3$**

(iv) **0.5% pancreatin in 5% bile.**

Available calcium and zinc were extracted by the method of Kim and Zemel (1986).

Procedure

Two g of finely ground sample was taken in a conical flask and 3 ml distilled water was added to rehydrate it. To this 20 ml of pepsin solution (0.1% pepsin in 0.1N HCl) was added. The pH was adjusted to 1.5 with dilute HCl. The contents were incubated at 37°C in a shaker-cum-water bath for an hour. Therefore, the pH of the contents was raised to 6.8 with sodium bicarbonate solution. Then 2.5 ml of a suspension containing 0.5% pancreatin in 5% bile were added and the contents were again incubated at 37°C for an hour. Then the contents were taken out and total volume was made to 50 ml with distilled water. Contents were then immediately centrifuged at 500 x g for 45 min at 5°C. Supernatants were collected and re-centrifuged at 25,000 x g for 45 minutes at 5°C. The supernatant was collected, oven dried, digested in the diacid mixture and proceeded for the estimation of calcium and zinc by the atomic absorption spectrophotometric method.

Iron availability (*in vitro*)

Extraction

Ionizable iron in the samples was extracted according to the procedure of Rao and Prabhavathi (1978).

Two g sample was mixed with 25 ml pepsin HCl (0.5% pepsin in 0.1N HCl) in a conical flask. The pH of the mixture was adjusted to 1.35 with HCl and incubated at 37°C for 90 min in an environmental shaker. After incubation, pH of the contents was adjusted to 7.5 with NaOH and again incubated at 37°C in an environmental shaker for 90 min. Contents of the flasks were centrifuged at 9000 rpm for 30 min and the supernatant was filtered through Whatman No. 44. The filtrate was used for determination of ionizable iron.

Ionizable iron

Free form of iron in the filtrate which reacts with α, α-dipyridyl was determined as described by AOAC (1995).

Reagents

(i) **α, α-dipyridyl solution**: Dissolved 0.1 g dipyridyl in water and made the volume to 100 ml.

(ii) **Hydroxylamine hydrochloride solution (10%)**

(iii) **Acetate buffer solution**: Dissolved 8.3 g anhydrous sodium acetate (dried at 100°C) in water, added 12 ml acetic acid and made the volume to 100 ml with water.

(iv) **HCl**

(v) **Iron standard solution (0.01 mg iron/ml)**: Dissolved 3.512 g Fe $(NH_4)_2H_2SO_4.6H_2O$ in water, added two drops of HCl and made to 500 ml with water. Ten ml of the solution was further diluted with water and made to 500 ml. This solution contained 0.01 mg iron per ml.

Procedure

Ten ml filtrate was taken in 25 ml volumetric flask and one ml 10% hydroxylamine hydrochloride solution was added. Then five ml acetate buffer solution was added. The contents were mixed and then one ml dipyridyl solution was added. The volume was made to 25 ml with water and the contents were mixed well. The colour intensity was read at 510 nm.

For plotting a standard curve 10 to 50 ml of iron standard were taken in 100 ml volumetric flask, added 2.0 ml HCl to each and made the volume to 100 ml with water. Blank was also prepared in similar manner. Ten ml of each of these solutions were taken in 25 ml volumetric flask and proceeded as mentioned above.

3.9.8 Total lysine

Total lysine was estimated as per the method described by Miyahara and Jikoo (1967).

Hydrolysis

One g defatted sample was transferred to 100 ml conical flask, added 12.5 ml of 2N HCl and mixed well. Then it was autoclaved at 15 kg/cm^2 for 1 h. Added a pinch of activated charcoal to hydrolysate, heated to boiling and filtered after washing with hot water. The colour free hydrolysate was made to 50 ml.

Reagents

(a) **Citrate phosphate buffer** (pH 5.6)

(A) **0.1 M solution of citric acid** (19.21 g in 1000 ml)

(B) **0.2M dibasic sodium phosphate** (53.65 g of $Na_2HPO_4.7H_2O$ in 1000 ml).

Mixed 21 ml of A + 29 ml of B.

(b) **Bromine saturated aqueous solution**: It was prepared by adding 3 ml of bromine to 150 ml of water and mixed well.

(c) **Aqueous solution of arsenite**: Three g of arsenite trioxide was dissolved in 10 ml of 10% aqueous solution of sodium hydroxide and the resultant solution was diluted to 100 ml with distilled water.

d) **20% aqueous solution of Na_2CO_3**

e) **Folin phenol reagent 1N:** Diluted before use from stock 2N

f) **Standard lysine**

Procedure

Two ml of acid hydrolysate which had been adjusted to pH 5.6 using dilute HCl or NaOH, was taken in a test tube and after the addition of 1 ml of buffer and 1 ml of methanol to it, it was allowed to stand at $-10^{o}C$ for about 15 min in iced water. Two drops of saturated bromine solution were added, and the mixture was allowed to stand again for 20 min at the same temperature. After that 0.1 ml of arsenite was added to the mixture to decompose excess of bromine and the mixture was incubated in a water bath at $60^{o}C$ for 10 min. To the contents in the test tubes, added 1.5 ml of 20% aqueous Na_2CO_3 which had already been warmed to $60^{o}C$. After addition of 0.5 ml of F.C. reagent, the mixture was shaken quickly and then allowed to stand at room temperature for 30 min. The intensity of blue colour was measured spectrophotometrically. The results are presented as g lysine/100 g protein after taking into account dilution involved. The range of lysine used for standard curve was 20 to 100 ug.

3.9.9 Antinutritional factors

Phytic acid: Phytic acid was determined by the method of Haug and Lantzsch (1983).

Reagents

i) **Phytate reference solution**: Exactly 30.54 mg sodium phytate (5.5% water, 97% purity and containing 12 Na/mole) was dissolved in 100 ml of 0.2N HCl which gave a solution containing 200 ug phytic acid per ml.

i) **Ferric ammonium sulphate solution**: Ferric ammonium sulphate (0.2 g) was dissolved in 100 ml of 2N HCl and made the volume of 1000 ml with distilled water.

ii) **Bipyridine solution**: Ten g 2-2 bipyridine and 10 ml thioglycollic acid were dissolved in distilled water and volume was made to 1000 ml.

These solutions are stable for several months at room temperature.

Extraction

Finely ground sample (0.5 g) was extracted with 25 ml of 0.2N HCl for 3 h with continuous shaking in a shaker. Thereafter, it was filtered through Whatman No. 1 filter paper.

Estimation

An aliquot (0.5 ml) of the above extract was pipetted into a test tube fitted with a ground glass stopper. One ml of ferric ammonium sulphate was added. The tube was heated in a boiling water bath for 30 minutes. The contents of the tube were mixed and centrifuged at 3,000 rpm for 30 minutes. One ml of supernatant was transferred to another test tube and 1.5 ml of bipyridine solution was added. The absorbance was measured at 519 nm against distilled water. For plotting a standard curve different concentrations (0.2 to 1.0 ml) of standard sodium phytate solution containing 40-200 ug phytic acid were taken and made to 1.4 ml with water.

I. **Polyphenols**: Total polyphenols were extracted by the method of Singh and Jambunathan (1981).

Extraction

Defatted sample (500 mg) was refluxed with 50 ml methanol containing 1% HCl for 4 h. The extract was concentrated by evaporating methanol on a boiling water bath and brought its volume to 25 ml with methanol HCl. The amounts of phenolic compounds were estimated as tannic acid equivalent according to Folin-Denis procedure (Swain and Hills, 1959).

Reagents

i) **Folin-Denis reagent**: Added 100 g sodium tungstate, 20 g phosphomolybdic acid, 50 ml phosphoric acid to 750 ml distilled water and refluxed for 2 h, cooled and diluted it to one litre.

ii) **Tannic acid solution**: Dissolved 100 mg tannic acid in distilled water and made upto one litre. Twenty ml of this stock solution was further diluted to 100 ml with water to give working standard solution containing 20 μg tannic acid per ml.

iii) **Standard sodium carbonate solution**: Dissolved 350 g sodium carbonate in hot distilled water (70°C to 80°C), cooled and filtered through glass wool to make one litre volume.

Procedure

Test solution (1.5 ml) was diluted with water to 8.5 ml in a graduated test tube. After thorough mixing, added 0.5 ml Folin-Denis reagent and the tubes were well shaken. Exactly after 3 minutes, one ml of saturated sodium carbonate solution was added and the tubes were thoroughly shaken again. After an hour, the absorbance was read at 725 nm using a suitable blank. If the solution was cloudy or precipitates appeared, it was centrifuged before readings were taken.

A standard curve was plotted by taking 0.5 ml to 4.0 ml working tannic acid standard solution containing 10 μg to 80 μg tannic acid.

l. **Trypsin inhibitor activity**: Trypsin inhibitor activity was determined by the modified method of Roy and Rao (1971).

Reagents

i) **0.1M phosphate buffer (pH 7.6)**; 16 ml NaH_2PO_4 (0.2M) and 84 ml Na_2HPO_4 (0.2M) were diluted to 200 ml with distilled water and pH adjusted to 7.6

ii) **0.05M phosphate buffer (pH 7.0)**; 50 ml 0.1M phosphate buffer was diluted to 100 ml with water and the pH adjusted to 7.0.

iii) **Casein solution (2%)**: A suspension of 2 g casein was prepared with phosphate buffer (0.1M, pH 7.6) and dissolved by warming and shaking in a steam water bath for about 10 minutes. The solution was cooled and made to 100 ml with phosphate buffer and stored in the refrigerator.

iv) **Trypsin solution (5 mg/ml)**: Trypsin (125 mg, 20000 units/g) was dissolved in 25 ml phosphate buffer (0.1M, pH 7.6).

v) **0.001N HCl**: Added 8.88 ml concentrated HCl to distilled water and made the volume to one litre with distilled water. Pipetted 10 ml of this 0.1N HCl and made to one litre with water to give 0.001N HCl.

vi) **Trichloroacetic acid (5%)**: Trichloroacetic acid (5 g) was dissolved in distilled water and volume made to 100 ml.

Extraction

One g sample was taken in a 150 ml conical flask and added 25 ml of 0.05M phosphate buffer (pH 7.0) to it. The contents were shaken at room temperature for 3 h and centrifuged at 10,000 rpm for 20 minutes. The following sets of incubation mixtures were prepared.

(i) **0.05M phosphate buffer (pH 7.0)**: 50 ml 0.1M phosphate buffer was diluted to 100 ml with water and the pH adjusted to 7.0.

(ii) **Casein solution (2%)**: A suspension of 2 g casein was prepared with phosphate buffer (0.1M, pH 7.6) and dissolved by warming and shaking in a steam water bath for about 10 minutes. The solution was cooled and made to 100 ml with phosphate buffer and stored in the refrigerator.

(iii) **Trypsin solution (5 mg/ml)**: Trypsin (125 mg, 20,000 units/g) was dissolved in 25 ml phosphate buffer (0.1M, pH 7.6).

(iv) **0.001N HCl**: Added 8.88 ml concentrated HCl to distilled water and made the volume to one litre with distilled water. Pipetted 10 ml of this 0.1N HCl and made to one litre with water to give 0.001N HCl.

(v) **Trichloroacetic acid (5%)**: Trichloriacetic acid (5 g) was dissolved in distilled water and volume made to 100 ml.

Extraction

One g sample was taken in a 150 ml conical flask and added 25 ml of 0.05M phosphate buffer (pH 7.0) to it. The contents were shaken at room temperature for three hours and centrifuged at 10,000 rpm for 20 minutes. The following sets of incubation mixtures were prepared.

	Test	**Control**	**Blank**
Phosphate buffer (0.1M, pH 7.6)	1.0 ml	1.1 ml	1.0 ml
Trypsin solution (5 mg/ml)	0.5 ml	0.5 ml	0.5 ml
HCl (0.001 N)	0.4 ml	0.4 ml	0.4 ml
TCA (5%)	-	-	6.0 ml
Casein (2%)	2.0 ml	2.0 ml	2.0 ml
Extract	0.1 ml	-	0.1 ml
Incubated at 37°C for 20 min			
CA (5%)	6.0 ml	6.0 ml	-

After incubation and addition of TCA, the contents were centrifuged at 10,000 rpm for 10 min. TCA soluble proteins in supernatant were determined by the method of Lowry *et al.* (1951).

Reagents

(i) **2% Na_2CO_3 in 0.1N NaOH**

(ii) **0.5% $CuSO_4.5H_2O$ in 1% sodium citrate.**

(iii) **Alkaline $CuSO_4$** : 50 parts of solution (i) and one part of solution (ii) were mixed just before use.

(iv) **1N Folin** –Ciocalteau phenol reagent.

(v) **Working casein standard solution (1 mg/ml)**: Diluted 5 ml 2% casein solution to 100 ml with phosphate buffer (0.1M, pH 7.6)

Estimation

To 0.5 ml supernatant, 5 ml alkaline $CuSO_4$ solution was added. It was mixed thoroughly and allowed to stand at room temperature for 10 min. Then 0.5 ml 1N Folin-Ciocalteau phenol reagent was added and again immediately mixed. After 30 min., the colour intensity was read at 520 nm against a blank.

For preparing standard curve, 0.1 ml to 0.5 ml of the working standard casein solution was taken.

Trypsin inhibitor units : One unit of trypsin was defined as the amount of enzyme which converted one mg casein to TCA soluble components at 37°C for 20 minutes at pH 7.6. One unit of inhibitory activity is that which reduces the activity of trypsin by one unit under the assay conditions.

V. **Amylase inhibitor activity**: Amylase inhibitor activity was determined by following modified method of Bernfeld (1955).

Reagents

(i) **0.1M Phosphate buffer** (pH 7.6): Sixteen ml NaH_2PO_4 (0.2M) and 84 ml Na_2HPO_4 (0.2M) were diluted to 200 ml with water and adjusted pH to 7.6.

(ii) **0.02M phosphate buffer** (pH 6.9): Fifty ml 0.02M potassium dihydrogen phosphate was added to 46.8 ml of 0.2M disodium hydrogen phosphate and volume was made upto 1000 ml.

(iii) **1% starch**: One g starch was dissolved in 100 ml 0.02M phosphate buffer solution.

(iv) **Alpha-amylase solution (5 mg/ml, Sigma, USA)**. Exactly 250 mg pancreatic alpha amylase was dissolved in 50 ml 0.02M buffer (pH 6.9).

(v) **0.25M NaCl solution**: NaCl (1.462 g) was dissolved in water and made volume upto 100 ml.

Extraction

Amylase inhibitor was extracted by shaking a finely ground defatted ;ample (0.1 g) in 10 ml 0.02M phosphate buffer (pH 6.9) for 2 h at room emperature. The suspension was then centrifuged at 10,000 rpm for 15 min. upernatant was heated for 10 min at 70°C and it was tested for amylase inhibitor :tivity by carrying out incubation in the following sequence.

ssay

	Test	Control
6 starch	1.0 ml	1.0 ml
ıibitor extract	0.5 ml	Nil
M phosphate buffer (pH 7.6)	2.0 ml	2.5 ml
:yme solution	0.5 ml	0.5 ml
:l (0.25M)	1.0 ml	1.0 ml

Contents were incubated at 37°C for 20 min. The extract (0.5 ml) was diluted to 5 ml with distilled water. Maltose was determined in 2 ml of diluted extract as well as incubation mixture according to procedure discussed under 3.9.4.

Amylase inhibitor unit: One unit of amylase was defined as the amount of enzyme which converted one mg starch to maltose for 20 min at 37°C at pH 7.6. One unit of inhibitory activity is that which reduces the activity of amylase by one unit under assay conditions.

3.9.10 Dietary fibre: Total, soluble and insoluble dietary fibre constituents were determined by the enzymatic method given by Furda (1981).

Reagents

(i) **0.005N HCl**

(ii) **Phosphate buffer (pH 10)**

(iii) **EDTA**

(iv) **Enzymes** – Alpha amylase and protease enzymes were obtained from Sigma Chemical Company, USA.

(v) **Ethanol** (75% and absolute)

(vi) **Acetone**

Procedure

1. **Sample preparation:** 0.5 g sample of less than 1 mm particle size food material was defatted on a Soxhlet or Goldfish apparatus.

2. **Extraction of water-soluble material:** The prepared sample weighing about 2.0 g was dispersed in 200 ml of 0.005N HCl and boiled for 20 min. The suspension was then cooled down to 60°C; 0.3 g of disodium EDTA was added and then adjusted to pH 6.0-6.5 with 12 ml of phosphate buffer

pH 10. The extraction was continued for an additional 40 min at 60°C to ensure the extraction of pectins with minimal degradation.

3. **Starch and protein hydrolysis**: Adjusted the pH 6.0-6.5 to bring the solution closer to the pH optimum of amylase and protease. Cooled the suspension to 20-30°C before incubation overnight with 10 mg of bacterial alpha-amylase and 10 mg of bacterial protease. The incubation was accompanied by slow stirring with a magnetic bar.

4. **Isolation of insoluble dietary fibre (IDF)**: The suspension was filtered through a coarse-tared Gooch filtering crucible containing glass wool and the insoluble residue was washed with a small amount of water. The filtrate was saved for the next step. The insoluble residue was then washed with water, alcohol and acetone before being dried at 70°C in a vacuum-oven overnight. The dried residue constitutes insoluble dietary fibre (IDF).

5. **Precipitation and isolation of soluble dietary fibre (SDF)**: The saved filtrate was acidified with a few drops of concentrated hydrochloric acid to pH 2-3; this pH tends to facilitate the rapid precipitation of polysaccharides. Slowly added four volumes of ethanol and left suspension to stand for about 1 h. Filter the precipitate on a tared, coarse Gooch crucible containing glass wool, then washed with 75% ethanol, absolute ethanol, and acetone before drying at 70°C in a vacuum oven overnight. The residue was weighed in the crucible to give the soluble dietary fibre (SDF) content of the original material. The SDF fraction was corrected for ash and for co-precipitated protein.

. **Total dietary fibre** (TDF): The sum of insoluble dietary fibre and soluble dietary fibre contents were calculated.

TDF = IDF + SDF

3.9.11 β-glucan : The total, soluble and insoluble β-glucan were estimated by the method of McCleary and Glennie Holmes (1985) and Megazyme Mixed Linkage β-glucan and glucose test kits were used.

Total β-glucan

Reagents

(i) Sodium phosphate buffer (20 mM, pH 6.5) : Sodium dihydrogen orthophosphate dihydrate (3.12 g) was dissolved in 900 ml of distilled water and the pH was adjusted to 6.5 by the addition of 100 mM sodium hydroxide (4 g/lt). The volume was then adjusted to 1 litre. The buffer was stored at 4°C.

(ii) Sodium acetate buffer (50 mM, pH 4.0) : Glacial acetic acid (2.9 ml) was added to 900 ml distilled water and pH was adjusted to 4.0 using 1M sodium hydroxide. Volume was adjusted to 1 litre and stored at 4°C.

(iii) Glucose oxidase/peroxidase reagent (GOPOD): Glucose reagent buffer (stock) obtained from Megazyme glucose test kit contained 1M potassium dihydrogen orthophosphate 200 mM parahydroxybenzoic acid, 0.4% sodium azide and 4.2% sodium hydroxide. Fifty ml of the stock buffer (1 vial) was diluted to 1 litre with distilled water to get working glucose reagent buffer.

(iv) Glucose reagent mixture:

Dissolved contents of one vial of Megazyme glucose determination reagent (obtained from Megazyme glucose test kit) in 1 litre of working glucose reagent buffer. GOPOD reagent thus, obtained was stored in dark bottle at 4°C (Stable for about 3 months at 4°C).

(v) Lichenase enzyme (50 U/ml in 20 mM phosphate buffer).

(vi) β-glycosidase enzyme (2 U/ml in 50 mM sodium acetate, pH 4.0)

Procedure

Sample of 500 mg of known moisture content was taken in a test tube. To it, 1 ml aquecus ethanol (50%, v/v), 5 ml sodium phosphate buffer (20 mM, pH 6.5) were added and stirred the tube on an environ-mixer. The contents were then incubated for 45 seconds in boiling water bath, mixed vigorously in environ-mixer and incubated for 45 seconds in boiling water bath. After cooling the contents to 40°C, 0.2 ml of lichenase was added. The tube was capped and incubated at 40°C for 1 h. Thereafter, the volume was adjusted to 30 ml by addition of distilled water (in the samples of hulled genotypes 24 ml distilled water was added while in the samples of hullless genotypes, 19 ml distilled water was added). After thorough mixing, the contents were filtered through Whatman No. 41 filter paper. Of this aliquot (0.1 ml) was transferred to three test tubes. To one tube, 0.1 ml of acetate buffer (50 mM, pH 4.0) was added (the blank), while to the other two 0.1 ml of β-glucosidase was added. The tubes were incubated at 40°C for 15 min. Thereafter, 3 ml of GOPOD reagent was added to each tube and then the tubes were again incubated at 40°C for 20 min. The absorbance was read at 510 nm. With each set of determination, glucose standards of 50 μg and 100 μg and one standard barley flour were also included.

β-glucan (% w/w) =

$$\text{Absorbance (reaction)} - \text{Absorbance (blank)} \times \frac{100}{\text{Absorbance of 100 } \mu\text{g of glucose}} \times \frac{27}{\text{Dry wt. of sample (mg)}}$$

Soluble β-glucan

Reagents

As used for total β-glucan estimation.

Procedure

A sample (500 mg) of known moisture content was taken in a test tube, to which 115 ml of distilled water and 5 ml of sodium phosphate buffer (20 mM, pH 6.5) were added. Rest of the procedure was same as for total β-glucan content estimation.

Insoluble β-glucan

The difference between total β-glucan and soluble β-glucan content was calculated as insoluble β-glucan.

3.10 Shelf life of acceptable breads

Acceptable breads were packed in polythene bags and stored for 0, 2 and 4 days at room temperature (37°C) as well as at refrigeration temperature. The following parameters were determined in stored products.

3.10.1 Organoleptic evaluation

Organoleptic evaluation of stored breads was periodically carried out by a panel of ten judges for crust colour, appearance, flavour, crust texture, taste and overall acceptability using a nine point Hedonic Rating Scale.

3.10.2 Peroxide value : Peroxide value was determined by the method of AOAC (1995).

Reagents

(i) **Acetic acid**: Chloroform solution (3:2, v/v)

(ii) **Saturated potassium iodide solution**

(iii) **0.01N sodium thiosulphate solution**

(iv) **Starch solution**: One g soluble starch was dissolved in cold distilled water to make thin paste, then boiling distilled water was added and boiled for one min while stirring. When completely dissolved, the volume was made to 100 ml.

Procedure

Twenty ml of lipid extract was made to 100 ml. To each flask 30 ml of acetic acid-chloroform mixture was added and swirled to dissolve. Then 0.5 ml of saturated potassium iodide solution was added, kept for 1 min with occasional shaking and 30 ml of distilled water was added. This was slowly titrated with 0.01N sodium thiosulphate with vigorous shaking until yellow colour almost disappeared. Then 0.5 ml of 1% starch solution was added and titration continued shaking vigorously to release all iodine from chloroform layer until blue colour just disappeared. The blank was run in the similar way.

Peroxide value (meq/kg sample) = S x N x 100 x g sample

Where,

S = ml sodium thiosulphate (blank corrected)

N = Normality of sodium thiosulphate solution.

3.10.3 Fat acidity: The fat acidity was determined by the method of AOAC (1995).

Reagents

(i) **Benzene-alcohol-phenolpthalein solution (0.02%)** : To one litre benzene, one litre alcohol and 0.4 g phenolpthalein was added and mixed.

(ii) **Potassium hydroxide solution (0.0178N)**

Procedure

Ten g sample was extracted with petroleum ether on Soxhlet apparatus. The solvent of the extract was completely evaporated on steam bath. The residue was dissolved in extraction flask with 50 ml benzene-alcohol-phenolpthalein solution and titrated with standard potassium hydroxide (1 g/lt) to orange pink colour. Blank titration was made on 50 ml benzene-alcohol-phenolpthalein and this value was substracted from titration value of the sample.

Fat acidity was calculated as mg of potassium hydroxide required to neutralize free fatty acids from 100 g flour.

Fat acidity = 10 x (T-B)

Where,

T = ml KOH required to titrate sample extract

B = ml KOH required to titrate blank.

3.11 Statistical Analysis

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

4. RESULTS AND DISCUSSION

In the present study, efforts were made to supplement the whole wheat flour with barley and soy flours for enhancing the nutritional quality, particularly protein, total lysine and dietary fibre contents of wheat bread. The results pertaining to this study have been presented and discussed under the following headings:

4.1 Physicochemical and functional characteristics

4.2 Baking characteristics

4.3 Organoleptic characteristics

4.4 Nutritional composition

4.5 Shelf life

4.1 Physicochemical and functional characteristics

Various physical characteristics of wheat, barley and soybean grains are presented in Table 1.

Moisture

Moisture content of wheat, barley and soybean grains varied significantly ($P<0.05$) from each other. The whole wheat grain had 8.35% moisture whereas barley and soybean grain had 10.54 and 6.58% moisture, respectively. Similar results were also reported by various workers in wheat and soybean grains (Singh *et al.*, 1990; Misra *et al.*, 1991; Saxena *et al.*, 1994).

1000-grain weight

The values for 1000 grain weight of wheat, barley and soybean were statistically different. Soybean grain had highest 1000-grain weight (71.70g) followed by wheat (40.84g) and barley (30.92g). This variation could be due to difference in grain size and it predicts the milling quality of grain. The values for 1000-grain weight in present study are consistent to those reported by Nemeth *et al.* (1994), Saxena *et al.* (1994), Reddy *et al.* (1998) and Sharma (2000).

Grain hardness

The grain hardness of wheat, barley and soybean was statistically different. The hardness value of wheat, barley and soybean were 8.23±1.00, 17.28 ±0.99 and 11.27±1.07 kg/grain, respectively. This could be attributed to variation in compactness of endosperm cell components. The protein content also influences the hardness (NABIM, 1996). The values of present study were in agreement with those reported by Saxena *et al.* (1994) and Sharma (2000). However, Singh and Paliwal (1986) reported somewhat higher grain hardness in wheat varieties due to varietal differences.

Hydration capacity

Hydration capacity of wheat (0.02 ml/seed), barley (0.04 ml/seed) and soybean grain (0.05 ml/seed) varied significantly ($P<0.05$) from each other. This variation may be due to difference in permeability of seed coat to water (Jood *et al.*, 1998).

Table 1. Physical characteristics of wheat, barley and soybean grains (on dry matter basis)[a]

Grains	Moisture (%)	1000-grain wt. (g)	Grain hardness (kg/grain)	Hydration capacity (ml/seed)	Grain colour
Wheat	8.35±0.97	40.84±0.29	8.23±1.00	0.02±0.01	Light golden yellow
Barley	10.54±0.51	30.92±1.11	17.28±0.99	0.04±0.01	Golden yellow
Soybean	6.58±0.53	71.70±1.56	11.27±1.07	0.05±0.02	Light yellow
SE (m)	0.86	0.71	0.64	0.003	
CD ($P<0.05$)	2.73	2.24	2.04	0.01	

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

Grain colour

Colour of grains varied from light yellow to golden yellow for soybean and barley grains. Wheat had light golden yellow colour. Saxena *et al.* (1994) also observed similar grain colour in soybean grain.

Various physicochemical and functional characteristics of wheat flour and other cereal-pulse blends are presented in Table 2.

Moisture

Whole wheat flour contained moisture content of 10.12% moisture which increased significantly ($P<0.05$) upon blending with barley flour at 20% level (10.60%), whereas it decreased on increasing the blending with full fat soy flour as well as defatted soy flour. Wheat flour blended with full fat soy flour at 20% exhibited minimum moisture content (9.59%). Whereas moisture content of blends containing both barley and soy flours (full fat & defatted) at all the levels i.e. 5, 10, 15 and 20% was at par with that of wheat flour. Chaudhary and Weber (1990) observed 10.2% moisture in whole wheat flour. Singh *et al.* (1990) also reported 11.3 to 13.2% moisture in UP hill wheats.

Gluten

Wheat flour exhibited wet and dry gluten content of 30.60 and 10.30%, respectively which did not change significantly ($P<0.05$) upon blending with barley, full fat soy and both barley and soy flours (full fat and defatted) upto 10% level. Thereafter, a significant reduction was observed in gluten content. Wheat flour blended with defatted soy flour at 20% level had minimum wet and dry gluten (22.57 and 7.68%). Wheat flour blended with barley, full fat soy flour and both barley and soy flours (full fat and defatted) at 20% substitution level had 23.00, 22.81, 22.87 and 22.72% wet gluten and 8.00, 7.88, 7.91 and 7.80% dry gluten contents, respectively. The dry gluten content is a direct indicator of flour strength and bread making potentialities. However, the quantity and quality of gluten which is responsible for better gas production and retention capacity forms cellular network of crumb which imparts desirable characteristics to bread (Anjum and Walker, 2000; Beldeork, 2000). Various workers also observed that the wet and dry gluten decreased on increasing the levels of soy flour in white wheat flour (Rastogi and Singh, 1989; Singh *et al.*, 1990; Misra *et al.*, 1991).

Sedimentation value

Sedimentation test is used to assess the gluten quality and bread-making potential of the flour (Beldeork, 2000). Wheat flour showed highest sedimentation value of 34.25 ml. In barley blended flours, there was a significant decrease in sedimentation value at 15 and 20% levels and the values were 31.75 and 30.00 ml, respectively. However, in blends containing soy flour (full fat and defatted) and both barley and soy flours (full fat and defatted), there was a significant reduction in sedimentation value and maximum reduction was found in defatted soy blended flour at 20% level (26.25 ml). Rastogi and Singh (1989) also stated that since gluten content in blends decreased with increase in the level of soy flour, the corresponding sedimentation value also decreased. Misra *et al.* (1991) and Sharma (2000) also reported similar values in wheat flour and soy-wheat blends.

Water absorption capacity

The water absorption capacity of wheat flour was 70.00% which increased upon increasing the levels of barley and soy flours (full fat and defatted) in wheat flour and maximum value was found in full fat soy blended flour at 20% level (81.85%) followed by defatted soy blended flour at 20% (81.68%). In barley and both barley and soy (full fat and defatted) blended flours at 20% level, the water absorption capacity was 80.70, 81.37 and 81.25%, respectively. This increase might be due to more water retention by these blends as compared to wheat flour. Selvaraj and Shurpalekar (1982) also showed that water absorption increased by about 1% for every 2% increase of soy flour in wheat flour.

Table 2. Physicochemical and functional characteristics of wheat flour and various cereal-pulse blends (on dry matter basis)[a]

Flours	Moisture (g/100 g)	Wet gluten (g/100 g)	Dry gluten (g/100 g)	Sedimentation value (ml)	Water absorption capacity (%)
Wheat flour	10.12±0.23	30.60±1.62	10.30±0.64	34.25±0.25	70.00±2.00
W + B					
95:05	10.20±0.15	28.48±2.28	9.75±1.03	33.12±1.00	71.80±0.22
90:10	10.41±0.27	26.45±0.74	9.23±0.78	32.50±0.50	74.35±0.11
85:15	10.53±0.05	24.76±3.44	8.25±0.75	31.75±1.50	77.40±0.05
80:20	10.60±0.21	23.00±1.60	8.00±0.89	30.00±1.00	80.70±0.06
W+SF					
95:05	10.00±0.22	28.31±2.56	9.43±1.53	30.80±1.40	72.30±0.05
90:10	9.78±0.10	26.39±1.57	9.05±1.13	29.60±1.12	75.80±0.12
85:15	9.70±0.21	24.64±2.26	8.13±0.57	28.50±0.50	78.36±0.35
80:20	9.59±0.22	22.81±3.49	7.88±1.24	27.01±0.02	81.85±0.06
W+DSF					
95:05	10.11±0.12	28.33±3.12	9.46±1.36	31.00±2.00	72.25±0.07
90:10	9.80±0.06	26.42±3.03	9.10±1.20	29.00±1.00	75.50±0.14
85:15	9.75±0.11	24.70±1.90	8.54±1.46	27.50±1.00	78.00±0.25
80:20	9.62±0.14	22.57±5.61	7.68±1.08	26.25±0.25	81.68±0.20
W+B+SF					
95:05	10.12±0.23	28.48±3.77	9.59±1.57	31.75±0.75	72.08±0.13
90:10	10.15±0.15	26.41±4.01	9.12±1.68	30.75±1.25	75.10±0.26
85:15	10.20±0.21	24.69±4.13	8.20±1.28	29.00±0.00	77.63±0.15
80:20	10.27±0.38	22.87±2.49	7.91±0.85	28.23±2.27	81.37±0.22
W+B+DSF					
95:05	10.15±0.13	28.45±4.35	9.52±1.63	32.00±2.00	71.95±0.07
90:10	10.19±0.17	26.44±0.44	9.05±1.86	31.25±1.75	74.98±0.14
85:15	10.23±0.15	24.72±3.08	8.11±1.73	30.49±1.00	77.54±0.18
80:20	10.31±0.20	22.72±1.00	7.80±1.38	29.00±0.00	81.25±0.25
SE (m)	0.11	1.62	0.69	0.63	0.47
CD ($P<0.05$)	0.32	4.65	1.99	1.81	1.35

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

Protein fractions

The protein content and various protein solubility fractions of wheat, barley and soy flours (full fat and defatted) are given in Table 3. Protein contents of wheat, barley, full fat soy and defatted soy flours were in the range of 13.11%, 11.15%, 40.80% and 44.60%, respectively. As far as various solubility fractions are concerned, wheat flour had highest content of prolamins (3.08%) followed by glutelins (2.80%), albumins (1.74%) and globulins (1.45%), respectively. Barley flour also exhibited maximum concentration of prolamins (2.71%) followed by glutelins (2.58%), albumins (1.80%) and globulins (1.36%). In soy flours (full fat and defatted), glutelins (12.29 and 13.89%) were the major fraction followed by globulins (5.53 and 6.78%), albumins (3.65 and 3.68%) and prolamins (0.40 and 0.60%), respectively. After the extraction of various fractions, the protein recovery in wheat, barley and soy (full fat and defatted) flours was in the range of 12.27, 10.50, 39.10 and 43.21%, respectively. Chen and Bushuk (1970) also observed the maximum content of prolamins and minimum content of globulins in wheat flour whereas Katiyar *et al.* (1989) reported that the glutelin was the major fraction in soybean flour.

The protein solubility fractions of various cereal pulse blends are presented in Table 4. The protein content of the blends containing barley flour at all the levels was at par with that of wheat flour. However, protein content increased significantly in blends containing full fat soy (17.17%), defatted soy (17.29%), both barley and full fat soy (15.00%) and both barley and defatted soy (15.58%) flours at 20% blending level, respectively.

Albumin content of blends containing barley and both barley and soy (full fat and defatted) at all the levels was statistically similar to wheat flour. However, in soy (full fat and defatted) blended flours at 20% level, albumin content increased to 2.05 and 2.13%, respectively. Globulin content was found to be

Table 3. Distribution of various protein solubility fractions of wheat, barley and soy flours (% dry matter basis)[a]

Flours	Protein	Albumin	Globulin	Prolamin	Glutelins	Residue	Recovery
Wheat flour	13.11±0.49	1.74±0.16	1.45±0.22	3.08±0.20	2.80±0.12	3.20±0.42	12.27±0.41
Barley flour	11.15±0.74	1.80±0.09	1.36±0.28	2.71±0.20	2.58±0.33	2.05±0.54	10.50±0.42
Soy flour	40.80±0.70	3.65±0.14	5.53±0.30	0.40±0.19	12.29±0.34	17.23±0.18	39.10±0.78
Defatted soy flour	44.60±0.79	3.68±0.10	6.78±0.30	0.60±0.33	13.89±0.42	18.26±0.46	43.21±0.52
SE (m)	0.41	0.07	0.16	0.14	0.19	0.25	0.33
CD (P<0.05)	1.30	0.23	0.52	0.44	0.60	0.79	1.04

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

Table 4. Distribution of various protein solubility fractions of wheat flour and various cereal-pulse blends (% dry matter basis)[a]

Flours	Protein	Albumin	Globulin	Prolamin	Glutelin	Residue	Recovery
Wheat flour	13.11±0.49	1.74±0.16	1.45±0.22	3.08±0.20	2.80±0.12	3.20±0.42	12.27±0.41
W+B							
95:05	13.05±0.66	1.75±0.16	1.44±0.13	3.00±0.14	2.76±0.38	3.05±0.23	12.00±0.21
90:10	12.79±0.16	1.75±0.07	1.41±0.29	2.97±0.24	2.74±0.33	3.05±0.46	11.92±0.30
85:15	12.63±0.39	1.78±0.15	1.38±0.18	2.95±0.27	2.69±0.14	2.88±0.41	11.62±0.30
80:20	12.54±0.31	1.78±0.10	1.38±0.29	2.90±0.46	2.67±0.14	2.88±0.41	11.62±0.30
W+SF							
95:05	14.45±0.51	1.85±0.17	1.66±0.22	2.94±0.25	3.25±0.20	3.85±0.42	13.55±0.32
90:10	15.60±0.32	1.94±0.17	1.97±0.25	2.85±0.23	3.43±0.22	4.20±0.17	13.99±0.48
85:15	16.49±0.43	1.98±0.10	2.09±0.22	2.70±0.32	3.70±0.16	5.06±0.15	15.53±0.33
80:20	17.17±0.30	2.05±0.10	2.16±0.22	2.66±0.49	3.88±0.22	5.28±0.23	16.03±0.42
W+DSF							
95:05	14.89±0.38	1.89±0.16	1.71±0.22	3.00±0.47	3.31±0.54	3.90±0.03	13.81±0.31
90:10	15.98±0.26	1.98±0.15	2.08±0.15	2.92±0.39	3.48±0.21	4.52±0.14	14.98±0.54
85:15	16.88±0.34	2.10±0.11	2.13±0.28	2.73±0.35	3.73±0.39	5.17±0.22	15.86±0.36
80:20	17.29±0.26	2.13±0.26	2.19±0.24	2.69±0.29	3.92±0.10	5.33±0.15	16.16±0.65
W+B+SF							
95:05	13.0±0.52	1.78±0.46	1.53±0.30	2.98±0.30	3.08±0.41	3.38±0.27	12.69±0.46
90:10	14.16±0.33	1.82±0.35	1.64±0.33	2.90±0.25	3.12±0.34	3.56±0.33	13.13±0.35
85:15	14.69±0.10	1.87±0.12	1.69±0.22	2.80±0.61	3.20±0.32	4.00±0.27	13.56±0.35
80:20	15.00±0.20	1.93±0.15	1.77±0.15	2.77±0.29	3.25±0.36	4.11±0.19	13.83±0.25
W+B+DSF							
95:05	13.73±0.29	1.81±0.12	1.58±0.43	3.00±0.19	3.11±0.29	3.43±0.56	12.93±0.31
90:10	14.16±0.33	1.82±0.35	1.64±0.33	2.90±0.25	3.12±0.34	3.56±0.33	13.13±0.35
85:15	14.86±0.23	1.91±0.35	1.79±0.51	2.83±0.35	3.23±0.18	4.68±0.43	13.83±0.35
80:20	15.58±0.29	1.98±0.33	1.82±0.17	2.79±0.27	3.30±0.42	4.13±0.23	14.14±0.15
SE (m)	0.2	0.11	0.15	0.18	0.15	0.19	0.19
CD ($P<0.05$)	0.62	0.33	0.43	NS	0.45	0.56	0.50

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

maximum at 20% level in defatted soy (2.19%) followed by full fat soy (2.16%), both barley and defatted soy (1.82%), both barley and full fat soy (1.77%) and barley (1.38%) blended flours. Prolamin content of various blended flours ranged from 2.66 to 3.08% and were found statistically similar to wheat flour (3.08%). Blends containing defatted soy flour at 20% level had maximum content of glutelin (3.92%). Whereas the glutelin content in barley blended flour at all levels was at par with that of wheat flour (2.80%). At 20% level, glutelin content was 3.25 and 3.30% in both barley and soy (full fat and defatted) blended flours.

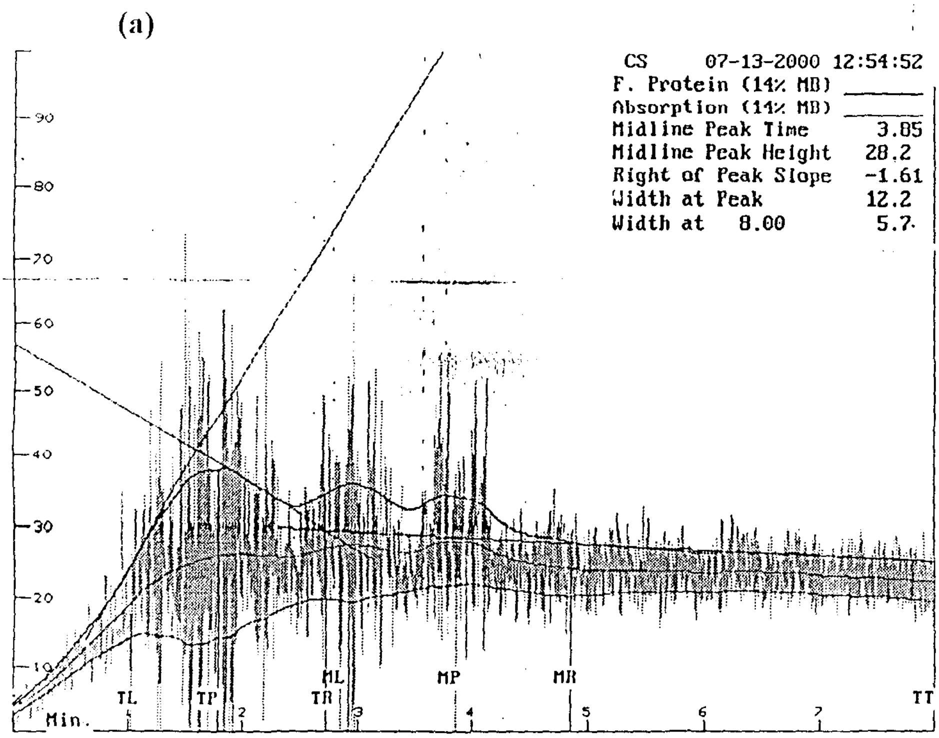
4.2 Baking Characteristics

Results of mixing time, loaf weight, loaf volume and specific loaf volume are given in Table 5.

Mixing time

Mixing time is the time when all the components of flour have been hydrated and thus the height of the mixing curve gradually increase to peak dough. The mixograms of whole wheat flour and various blends (Fig. 1-11) clearly showed that the mixing peak for various doughs occurred at different times. To reach the optimum development, different flours have different work input to mix a dough. It may be due to different composition of flours and quality as well as quantity of gluten (Hoseney, 1994).

Mixing time of different blended flours revealed that whole wheat flour lough (control) had highest mixing time (3.85 min.) which decreased upon blending with barley and soy flours (full fat and defatted) (Fig. 12). The decrease n mixing time was statistically non-significant upto 10% blending with barley lour thereafter, it decreased significantly ($P<0.05$) to 2.90 min. at 20% blending evel. Whereas the dough containing both barley and full fat soy flour at 15 and 0% level exhibited minimum mixing time (2.13 min). This may be due to that



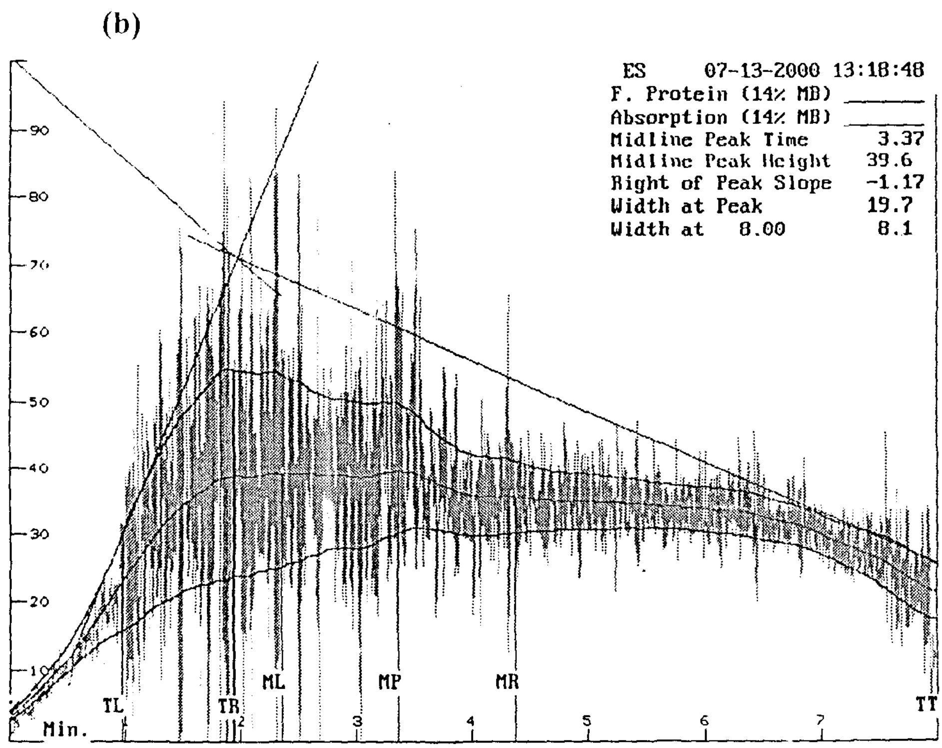
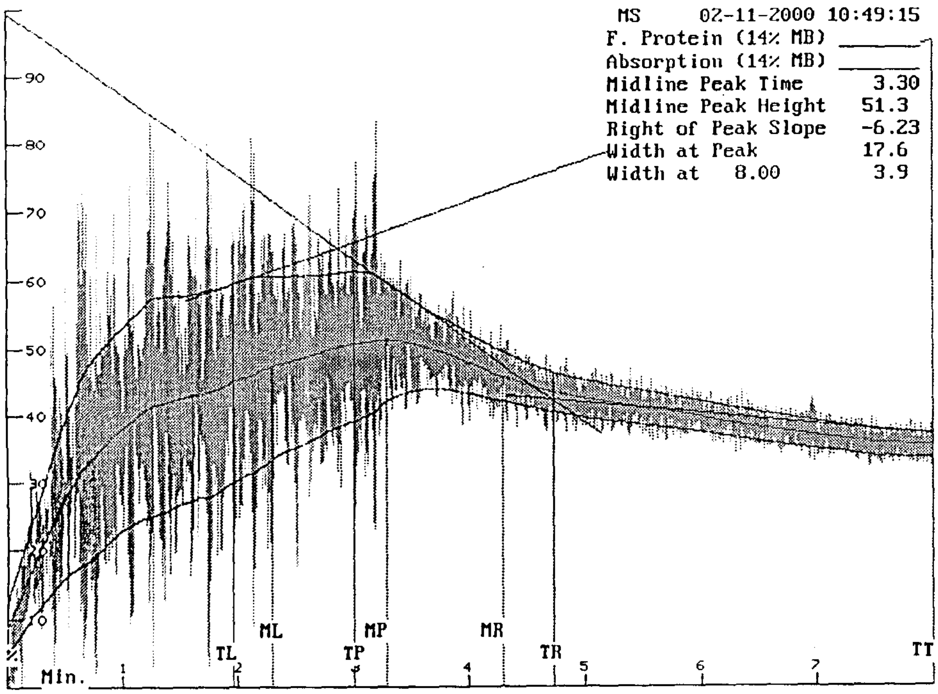


Fig. 1 Mixograms of wheat flour, control (a) and 5% blending of wheat flour with barley flour (b).

(a)



(b)

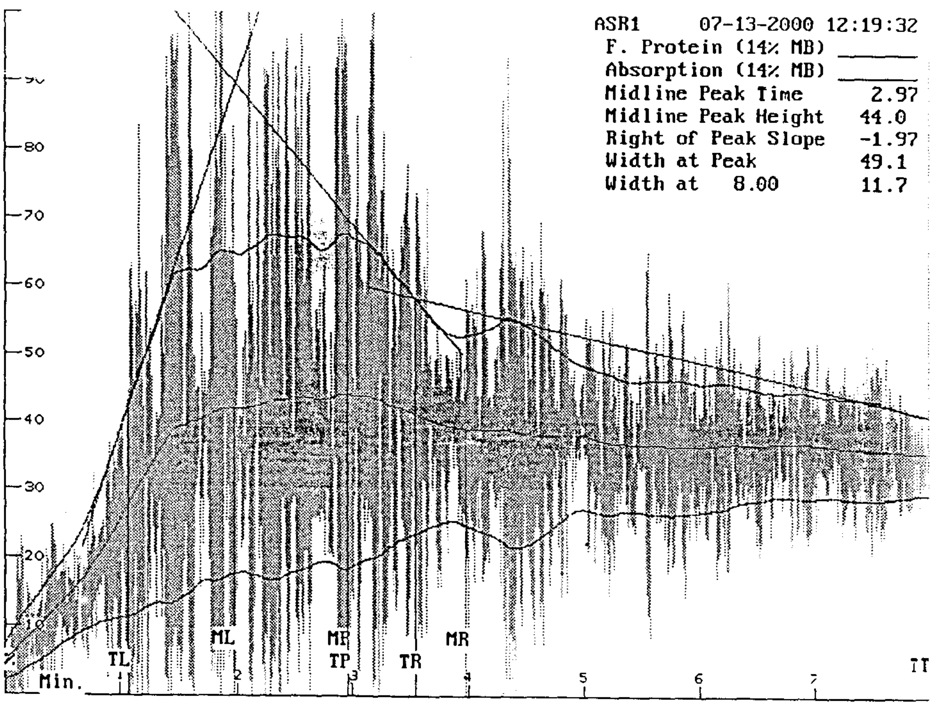
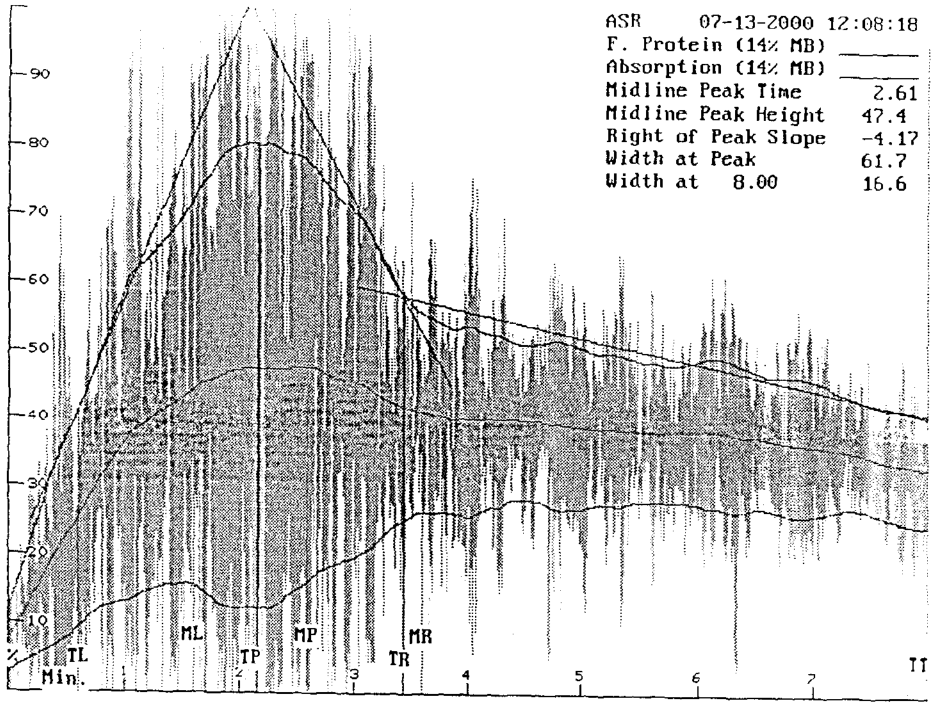


Fig. 2 Mixograms of 10% (a) and 15% (b) blending of wheat flour with barley flour.

(a)



(b)

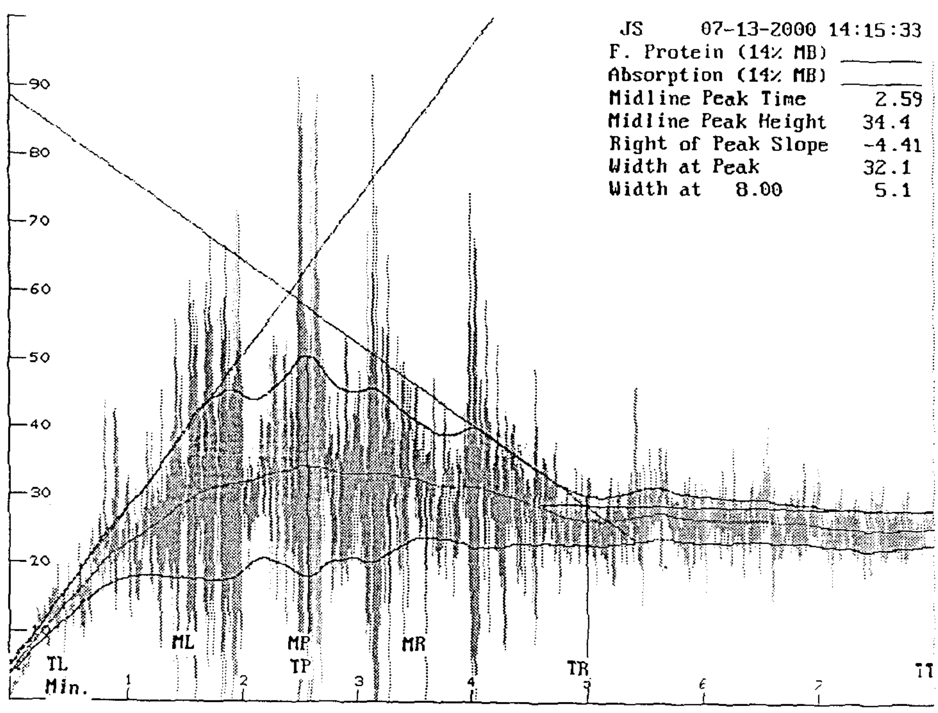
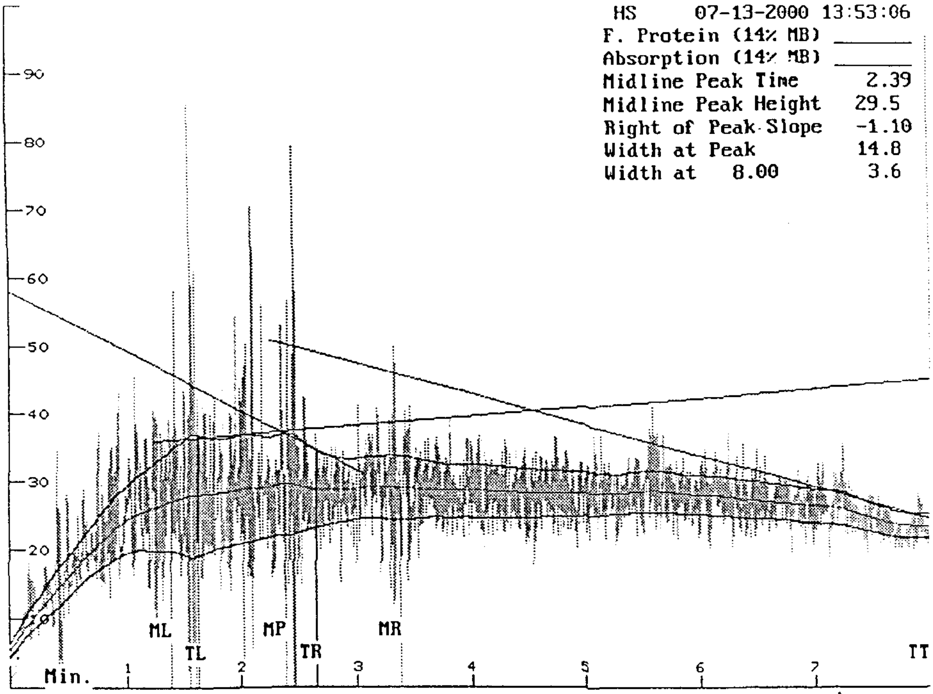


Fig. 4 Mixograms of 10% (a) and 15% (b) blending of wheat flour with soy flour.

(a)



(b)

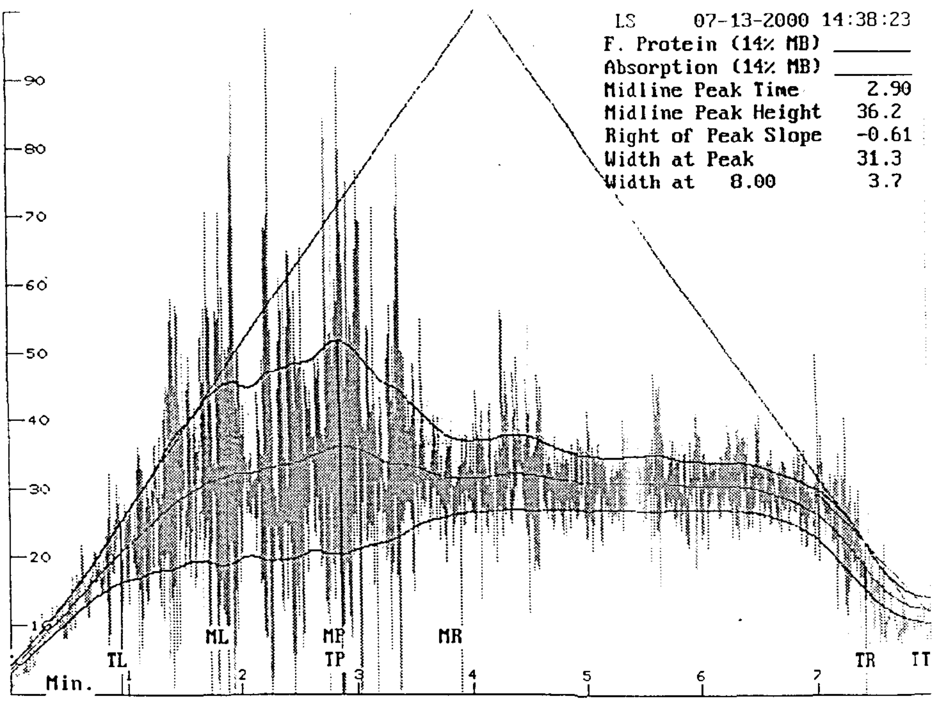
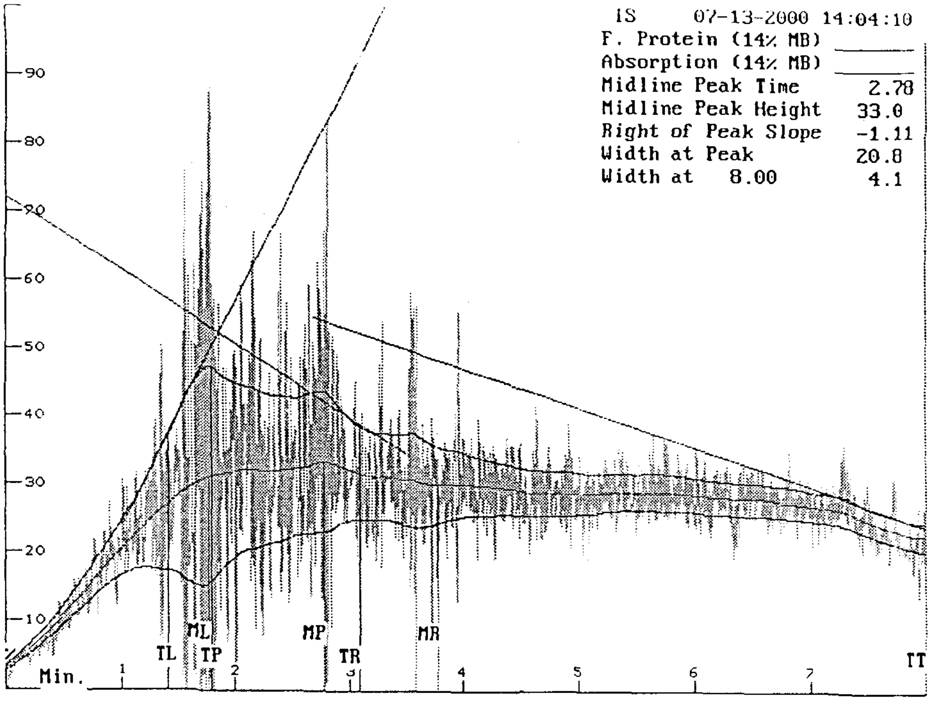


Fig. 5 Mixograms of 20% blending of wheat flour with soy flour (a) and 5% blending of wheat flour with defatted soy flour (b).

(a)



(b)

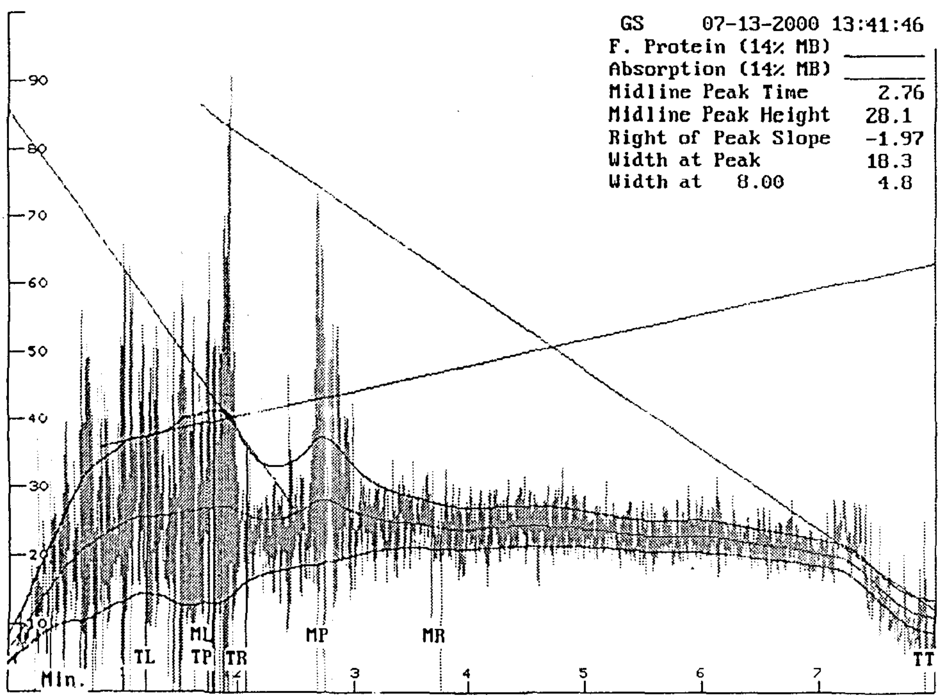
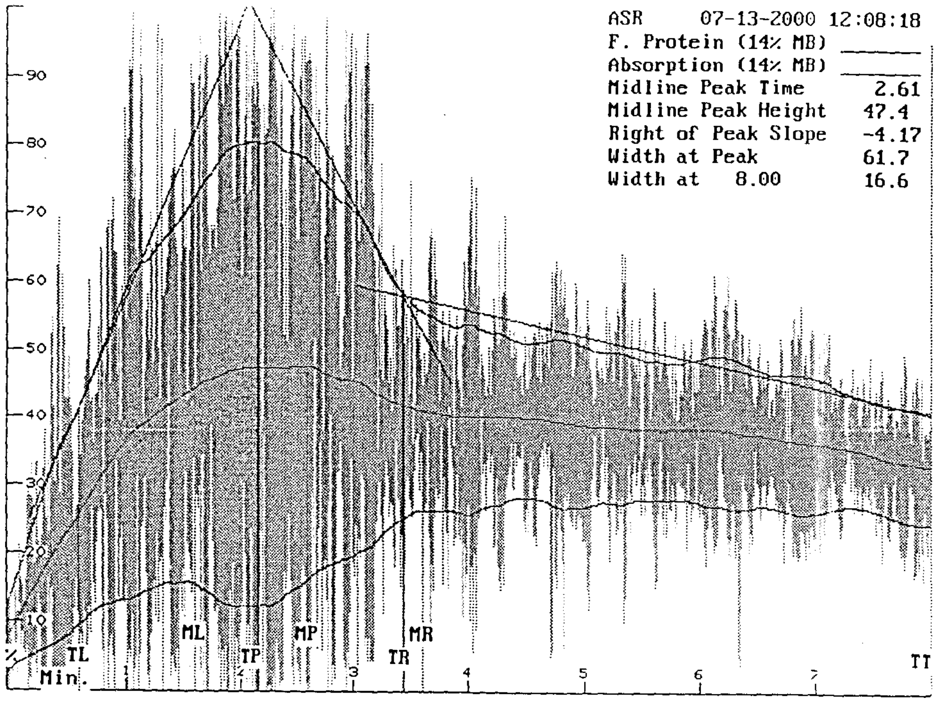


Fig. 6 Mixograms of 10% (a) and 15% (b) blending of wheat flour with defatted soy flour.

(a)



(b)

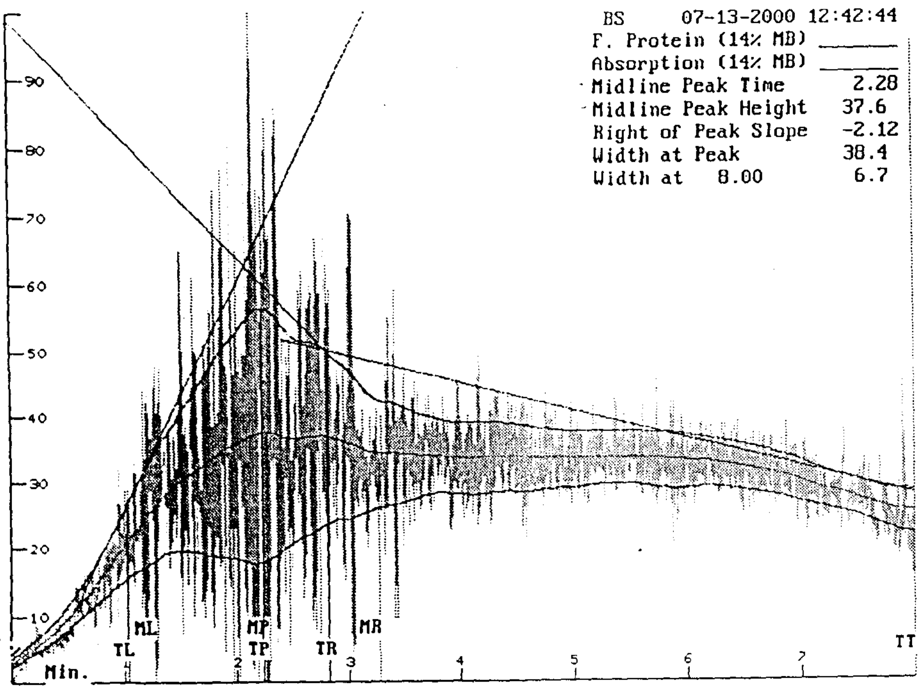
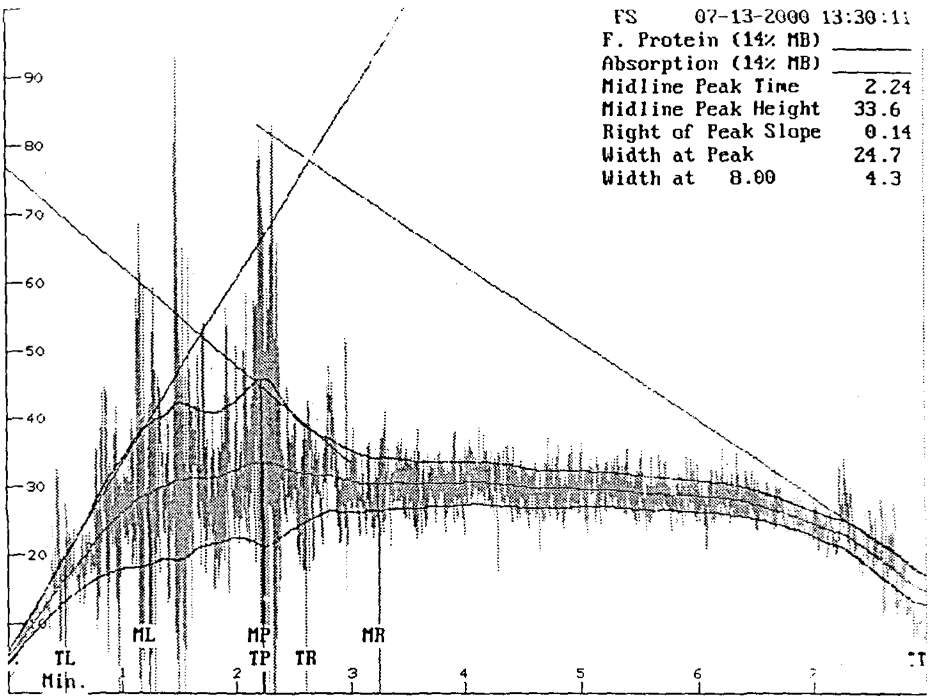


Fig. 7 Mixograms of 20% blending of wheat flour with defatted soy flour (a) and 5% blending of wheat flour with barley + soy flour (b).

(a)



(b)

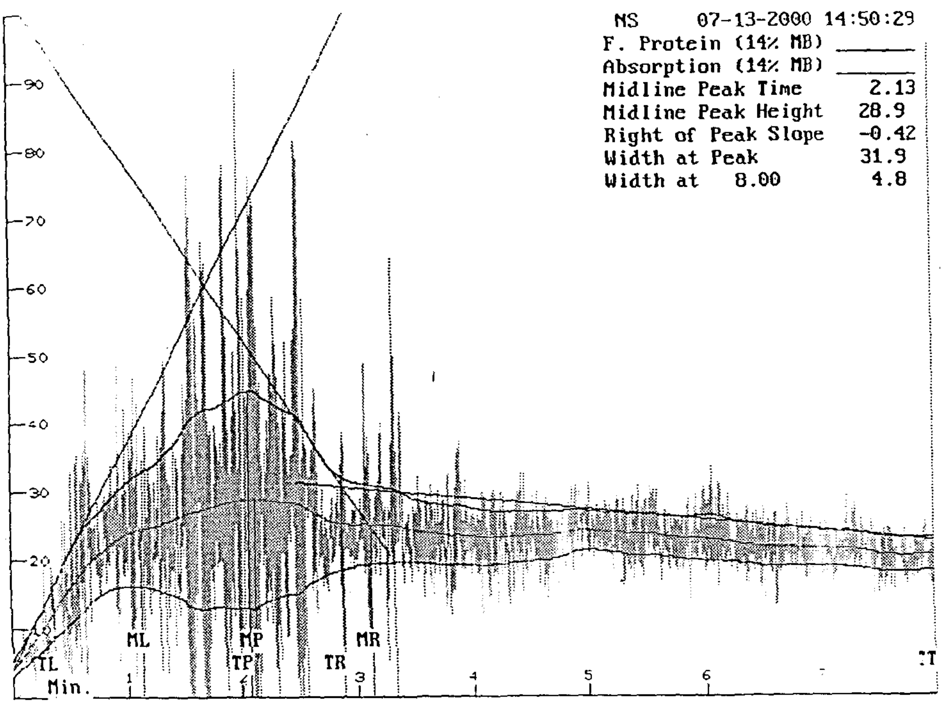
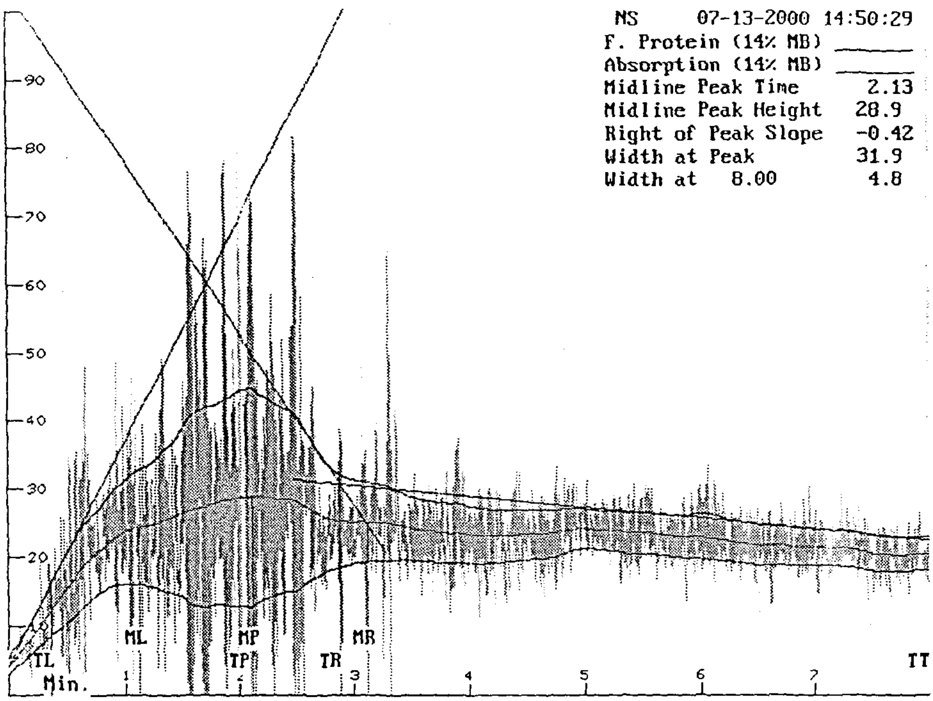


Fig. 8 **Mixograms of 10% (a) and 15% (b) blending of wheat flour with barley + soy flour.**

(a)



(b)

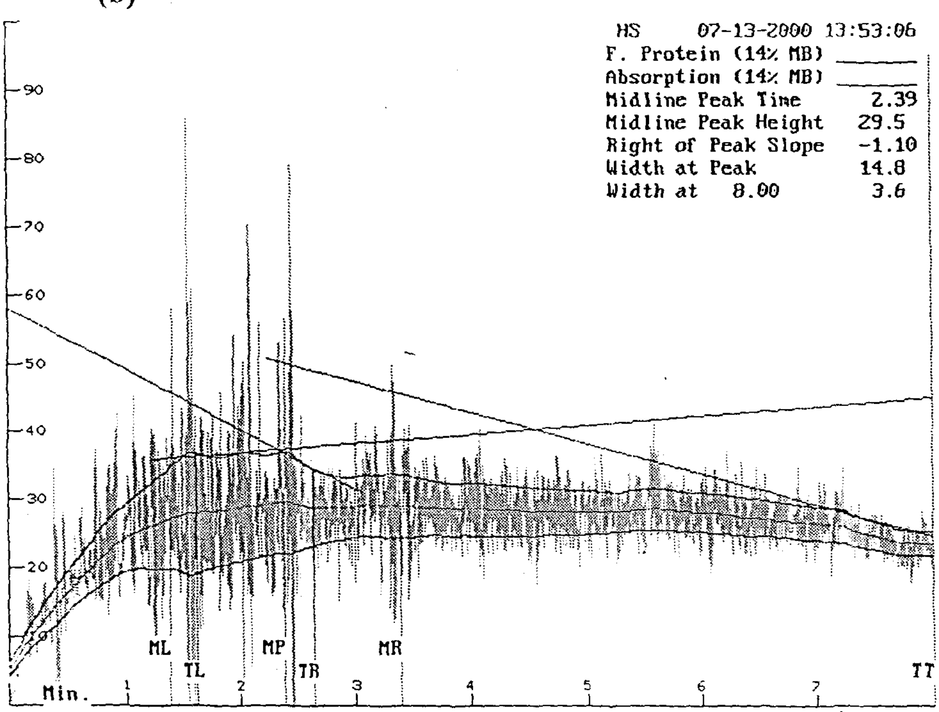
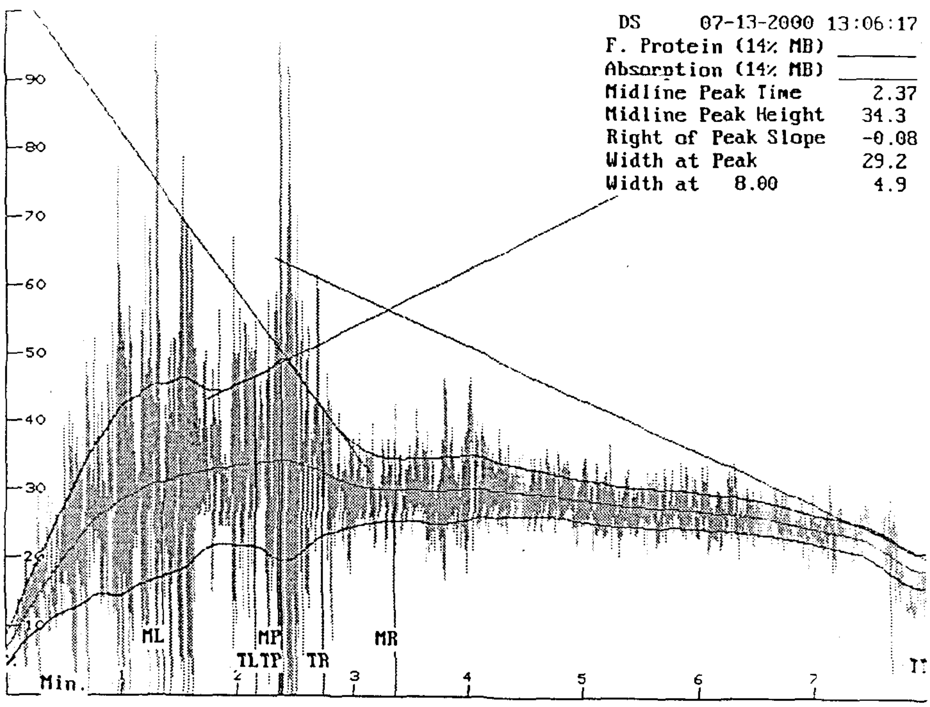


Fig. 9 Mixograms of 20% blending of wheat flour with barley + soy flour (a) and 5% blending of wheat flour with barley + defatted soy flour (b).

(a)



(b)

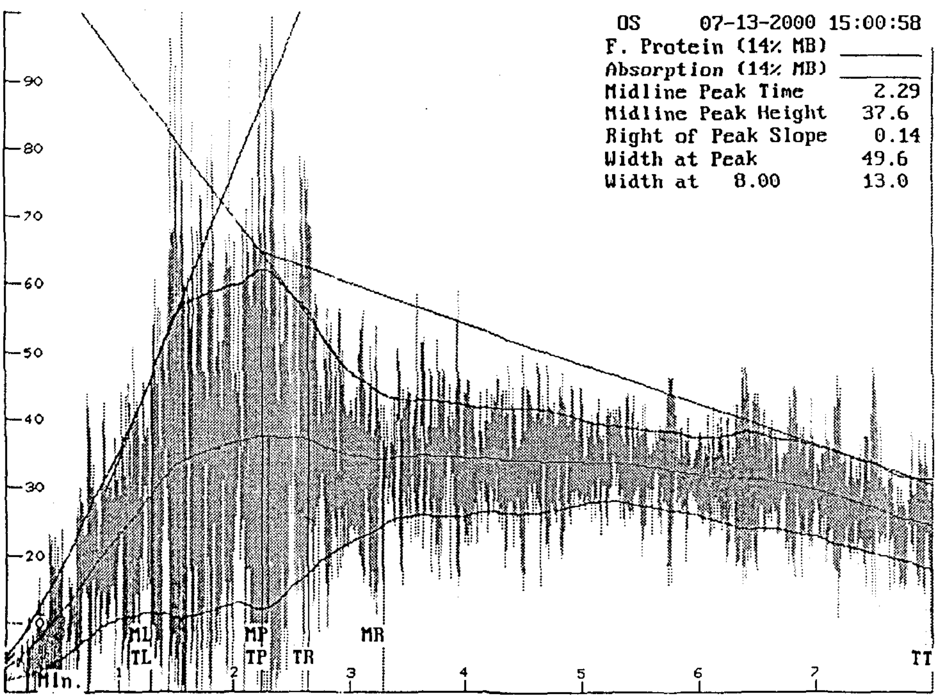


Fig. 10 Mixograms of 10% (a) and 15% (b) blending of wheat flour with barley + defatted soy flour.

(a)

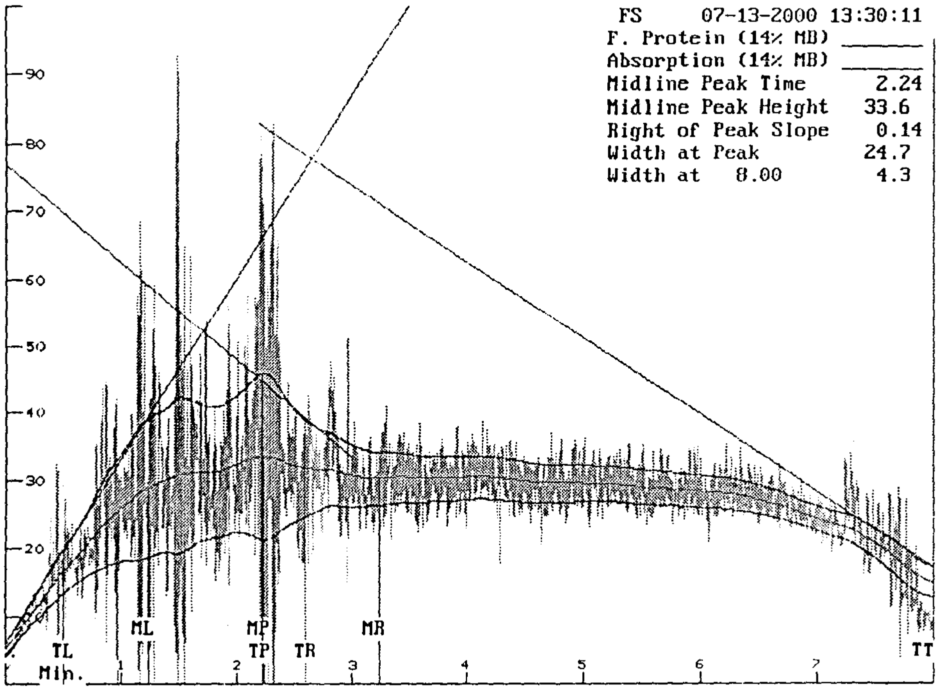


Fig. 11 Mixogram of 20% blending of wheat flour with barley + defatted soy flour (a).

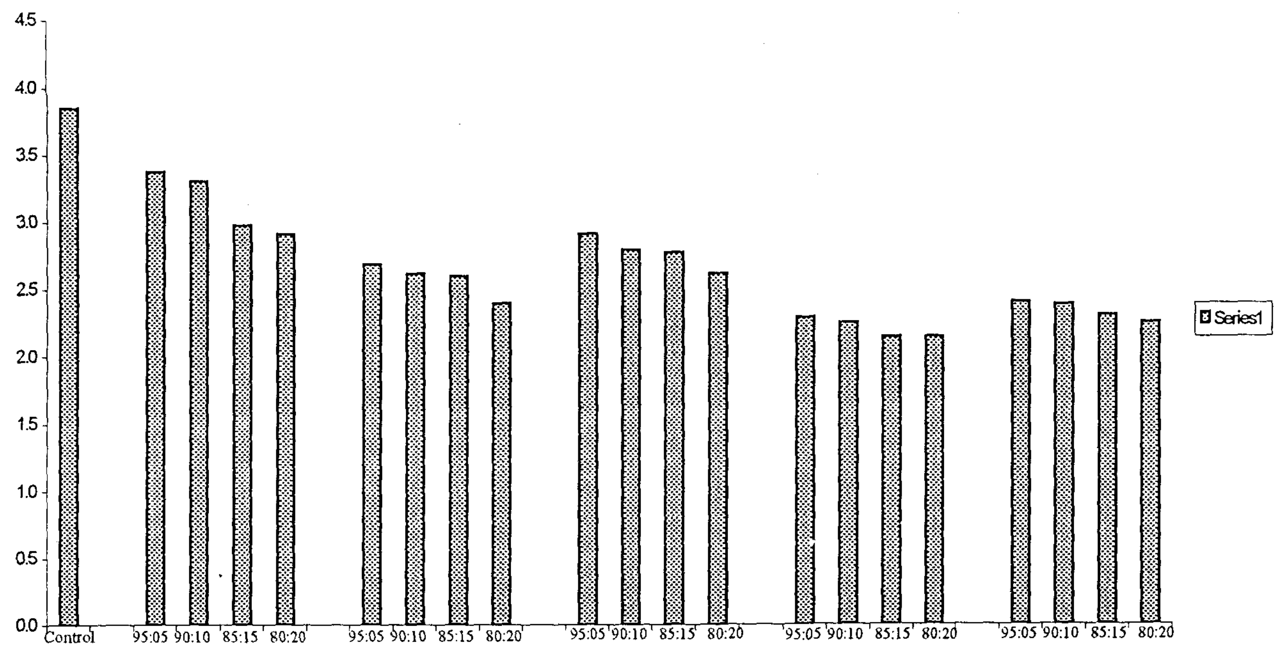


Fig. 12: Mixing time (min.) of the doughs prepared from control and various cereal pulse blends.

blended flours had poor quality and quantity of gluten. Similar results were also reported by Anjum and Walker (2000).

Loaf weight

Loaf weight of control bread was 160g and it did not change significantly upto 5% level of substitution of wheat flour with barley and defatted soy flour. However, a significant ($P<0.05$) increase in loaf weight was observed with each increment of non wheat flour indicating that an extra amount of water was retained in breads after baking (Dreese and Hoseney, 1982; Rao and Hemamalini, 1991). The loaf weight was found maximum (186 g) in full fat soy flour supplemented bread at 20% level. The loaf weight was found to increase on increasing the levels of barley and soy flours may be because of less retention of gas in the blended doughs, hence providing a dense texture to bread. Values of loaf weight observed in present study were similar as reported by earlier researchers (Ereifej and Shibli, 1993; Sharma and Chauhan, 2000).

Loaf volume

The loaf volume of control bread (whole wheat flour) was 520 ml. However, a significant reduction in loaf volume was observed as the level of substitution with barley and soy (full fat and defatted) flours increased. Maximum reduction in loaf volume (444.7 ml) was in bread made from wheat flour blended with full fat soy flour at 20% level whereas defatted soy supplemented bread exhibited 469.0 ml loaf volume at 20% level. It could be the dilution effect on gluten with the addition of non wheat flour to wheat flour and less retention of CO_2 gas which caused depression in loaf volume (Sharma and Chauhan, 2000). The loaf volume of breads prepared from wheat flour blended with barley alone and both barley and soy flours (full fat and defatted) at 20% level were 494.0, 456.5 and 484.0 ml, respectively. Similar values were also reported by Ereifej and Shibli (1993) in whole wheat flour breads. The other reason for decrease in loaf volume could be the presence of relatively high concentration of low molecular weight thiols, especially reduced glutathione which activates proteolytic enzymes, thereby causing detrimental effect on loaf volume (Indrani and Rao, 1992).

Specific loaf volume

Specific loaf volume is obtained by dividing the loaf volume by loaf weight. The results indicated a decrease in specific loaf volume on increasing the levels of non-wheat flours (i.e. barley and soy) as compared to control (3.24 ml/g). The loaf volume decreased to 2.92 ml/g in barley; 2.40 ml/g in full fat soy, 2.71 ml/g in defatted soy and 2.52 and 2.81 ml/g in both barley and soy (full fat and defatted) blended breads at 20% substitution levels (Fig. 13). The low specific loaf volume of various cereal-pulse blended breads might be due to their poor quality and quantity of gluten which is responsible for retention of CO_2 gas in the fermented dough. Similar observations have also been reported by Paliwal and Singh (1986); Indrani and Rao (1992) and Ereifej and Shibli (1993) in breads prepared from wheat flour.

Correlation coefficient of different physicochemical and functional parameters with bread quality (Table 6) revealed that sedimentation value showed significant ($P<0.05$) positive correlation with dry gluten and loaf volume whereas a negative correlation with loaf weight. Mixing time also showed a significantly ($P<0.05$) positive correlation with dry gluten and loaf volume whereas a negative correlation with loaf weight. Water absorption capacity was found to have a significant ($P<0.05$) positive correlation with loaf weight and a negative correlation with both dry gluten and loaf volume.

4.3 Organoleptic Characteristics

The blending of wheat flour with barley and soy flour at different levels altered the organoleptic properties of different blended breads. Data on crust colour, appearance, flavour, crust texture, taste and overall acceptability are presented in Table 7.

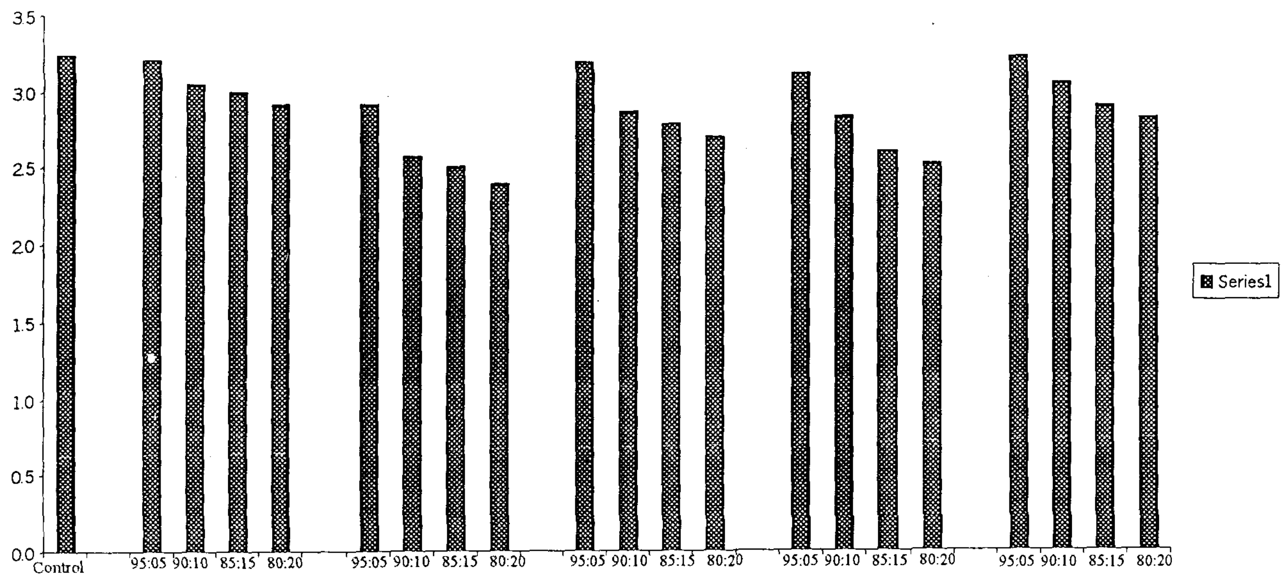


Fig. 13: Specific loaf volume (ml/g) breads prepared from control and various cereal pulse blends.

Table 5. Effect of cereal-pulse blending on mixing time[a] and the quality of breads (on dry matter basis)[b]

Breads	Mixing time (min)	Loaf weight (g)	Loaf volume (ml)	Specific loaf volume (ml/g)
Control (Wheat)	3.85±0.13	160.00±2.00	520.00±2.00	3.24±0.02
W+B				
95:05	3.37±0.14	160.50±0.50	515.00±3.00	3.20±0.02
90:10	3.30±0.06	164.00±1.00	502.00±4.00	3.05±0.04
85:15	2.97±0.12	166.00±1.00	498.00±2.00	2.99±0.00
80:20	2.90±0.04	168.50±0.50	494.00±1.00	2.92±0.00
W+SF				
95:05	2.68±0.13	165.00±0.00	482.73±3.25	2.92±0.02
90:10	2.61±0.13	176.00±4.00	454.56±2.50	2.58±0.04
85:15	2.59±0.06	180.00±1.00	450.50±2.00	2.50±0.01
80:20	2.39±0.18	186.00±2.00	444.70±1.00	2.40±0.02
W+DSF				
95:05	2.90±0.16	160.00±0.50	512.50±7.50	3.19±0.03
90:10	2.78±0.08	167.50±1.50	481.73±3.25	2.87±0.01
85:15	2.76±0.18	170.00±1.00	473.00±1.00	2.78±0.01
80:20	2.61±0.19	172.50±0.50	469.00±2.00	2.71±0.02
W+B+SF				
95:05	2.28±0.18	161.50±1.50	503.50±5.50	3.11±0.00
90:10	2.24±0.19	170.00±1.00	485.00±0.02	2.84±0.01
85:15	2.13±0.19	176.00±2.00	460.00±3.00	2.61±0.05
80:20	2.13±0.18	181.00±2.00	456.50±1.00	2.52±0.02
W+B+DSF				
95:05	2.39±0.28	160.00±1.00	518.00±3.00	3.23±0.00
90:10	2.37±0.21	163.73±1.25	501.00±6.00	3.05±0.01
85:15	2.29±0.29	169.00±1.00	489.50±7.50	2.89±0.03
80:20	2.24±1.00	171.50±0.50	484.00±2.00	2.81±0.00
SE (m)	0.09	0.88	0.94	0.01
CD (P<0.05)	0.29	2.54	5.62	0.03

[a]Observed in dough. [b]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

Table 6. Correlation coefficient of different physicochemical and functional parameters with bread quality

	Dry gluten	Loaf weight	Loaf volume
Sedimentation value	0.588*	-0.741*	0.826*
Mixing time	0.489*	-0.611*	0.653*
Water absorption capacity	-0.621*	0.823**	-0.764**

* Significant at 5% level.
** Significant at 1% level.

Crust colour

As the level of barley and soy flour in blends was increased, the crust colour of the breads changed from creamy white to dull brown. However, no significant difference was observed in crust colour upto 10% blending with non-wheat flours. Thereafter, a significant difference in crust colour was observed in all the blended breads. The data suggested that the darkest colour was observed in breads prepared from full fat soy (15 and 20% level) as well as both barley and full fat soy (20%) blended flours with the score of 5.62±0.51, 5.62±0.51 and 5.75±0.70, respectively. The darker crust colour may be due to maillard reaction between reducing sugars and proteins (Raidi and Klein, 1983).

Appearance

Appearance score for control bread was 7.75 which decreased significantly upon increasing the blending levels i.e. at 15 and 20% with soy flour. At 15% barley blended breads appearance score was in the category of 'like moderately' (7.62±0.91). The appearance score for different breads at 20% level ranged from 5.25 to 5.62 for defatted soy, full fat soy, both barley and soy (full and defatted) blended breads, respectively. Among the blended breads, highest appearance score (7.87) was observed in both barley and full fat soy blended bread at 5% level whereas lowest score (5.25) was observed in defatted soy blended bread (20% level). Gayle *et al.* (1986) and Hoseney (1994) suggested that the appearance of bread was an important sensory characteristic on which the acceptability of bread depends.

Flavour

Results pertaining to flavour of breads revealed that flavour increased on increasing the level of barley flour upto 10% level indicating better flavour rating and thereafter it decreased, at 20% barley blended breads, it was 5.12. Flavour of

Table 7. Organoleptic acceptability of breads 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±0.46	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±0.83	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±0.46	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±0.46	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±1.55	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

[a]Values are mean ± SD of ten penalists. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

soybean supplemented breads did not differ significantly upto 10% level. The breads made from wheat and full fat soy and wheat and defatted soy flour at 20% level had flavour score of 4.00 and 4.50, respectively. The flavour of soy flour blended breads might be affected due to beany flavour of soybean flour. A similar decrease in flavour of breads with increase in supplementation levels of fenugreek flour was also noticed by Sharma and Chauhan (2000).

Crust texture

The crust texture was related to external appearance of 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 to control bread (7.62). Among the blended breads, maximum score was observed in breads containing both barley and defatted soy flour (7.75) at 5% substitution level and minimum score was in full fat soy blended breads (4.50) at 20% level. Our results suggested that in both barley and defatted soy supplemented breads at 5% level, the smoothness was maximum and it ranked highest. The deterioration in crust texture of breads with puffed bengal gram beyond 20% substitution level was also observed (Rathna and Neelakantan, 1995). Similar deterioration in texture of wheat bread on supplementation was observed by Sharma *et al.* (1999).

Taste

Taste evaluation suggested that control and various supplemented breads had most satisfactory taste scores at 5% level. However, wheat and soy flour supplemented breads had satisfactory taste score upto 10% level of substitution. Thereafter, the taste became bland and bread was considered tasteless. Results indicated that taste score decreased with increased level of substitution with soy flour (full fat and defatted) as compared to control bread (7.62±0.51). Wheat flour substituted with full fat soy flour at 20% level was rated poorest in taste (3.62±1.06) may be due to beany flavour of soy flour. Similarly with fenugreek flour substituted breads, the taste deteriorated progressively (Sharma and Chauhan, 2000).

Overall acceptability

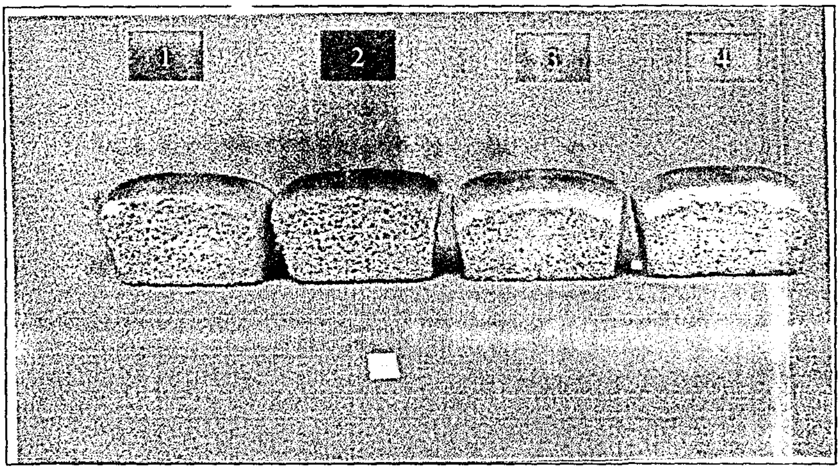
Overall acceptability was the mean score of all the organoleptic characteristics in the present study. The results showed that overall acceptability score of all the supplemented breads at 5% level was at par with control (7.70±0.53). Breads made from wheat and barley flour upto 15% level was found acceptable but at 20% the overall acceptability score was significantly reduced as compared to control. The overall acceptability score of full fat and defatted soy supplemented breads did not change upto 10% level from that of control and considered as acceptable. In both barley and soy flour (full fat and defatted) supplemented breads at 10% level there was a significant decrease in overall acceptability score (7.22 and 7.27) but still found in the category of 'like moderately' (Plate 1-6). Among different supplemented breads, soy (full fat and defatted) supplemented breads at 15 and 20% had poorest overall acceptability score and found to be unacceptable. Similarly, both barley and soy (full fat and defatted) supplemented breads at 20% level had poor overall acceptability score (5.12 and 5.40) and hence were considered unacceptable.

4.4 Nutritional Composition

The data regarding nutritional composition of various breads is discussed below.

Fat

The fat content of whole wheat flour bread (control) was 5.44% which increased significantly on blending with full fat soy flour (Table 8). The full fat soy flour supplemented bread at 10% level had highest fat content i.e. 6.83%. This was due to higher fat content of full fat soy flour as compared to other flours.



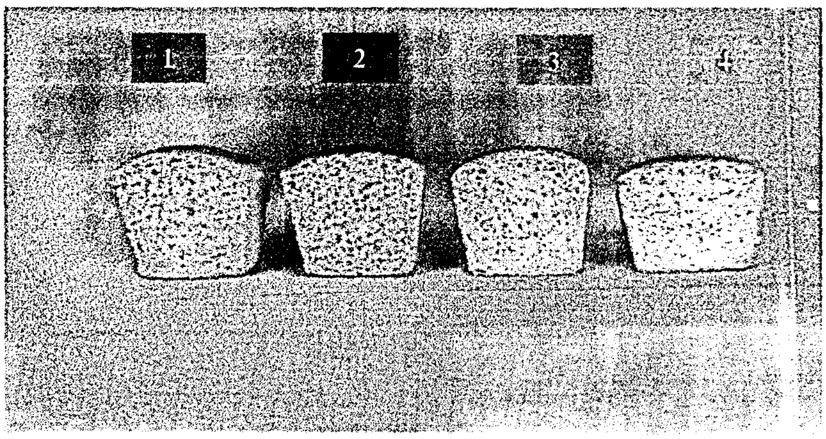


Plate 1:Whole and sliced bread loaves prepared from wheat flour and barley supplemented flours; where (1) control (100% wheat flour)(2)W+B (95:05); (3) W+B (90:10); (4) W+B (85:15)

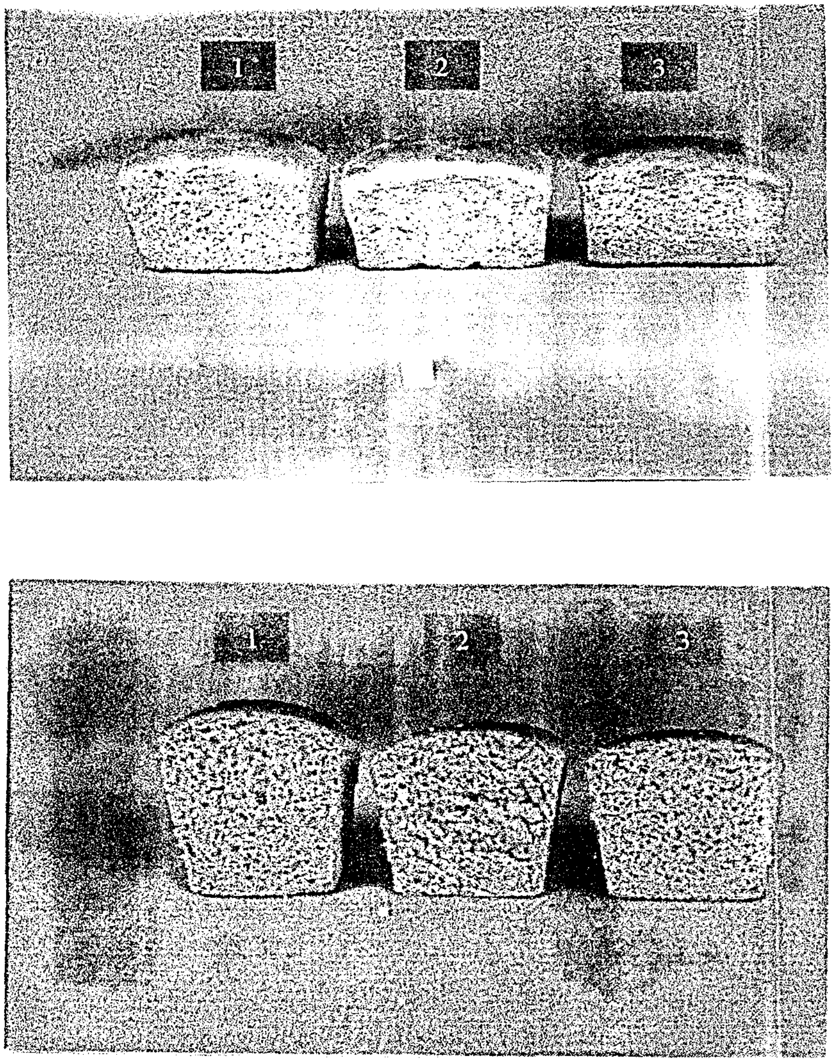


Plate 2: Whole and sliced bread loaves prepared from wheat flour and full fat soy supplemented flours; where (1) control (100% wheat flour); (2) W+SF (95:05); (3) W+SF (90:10)

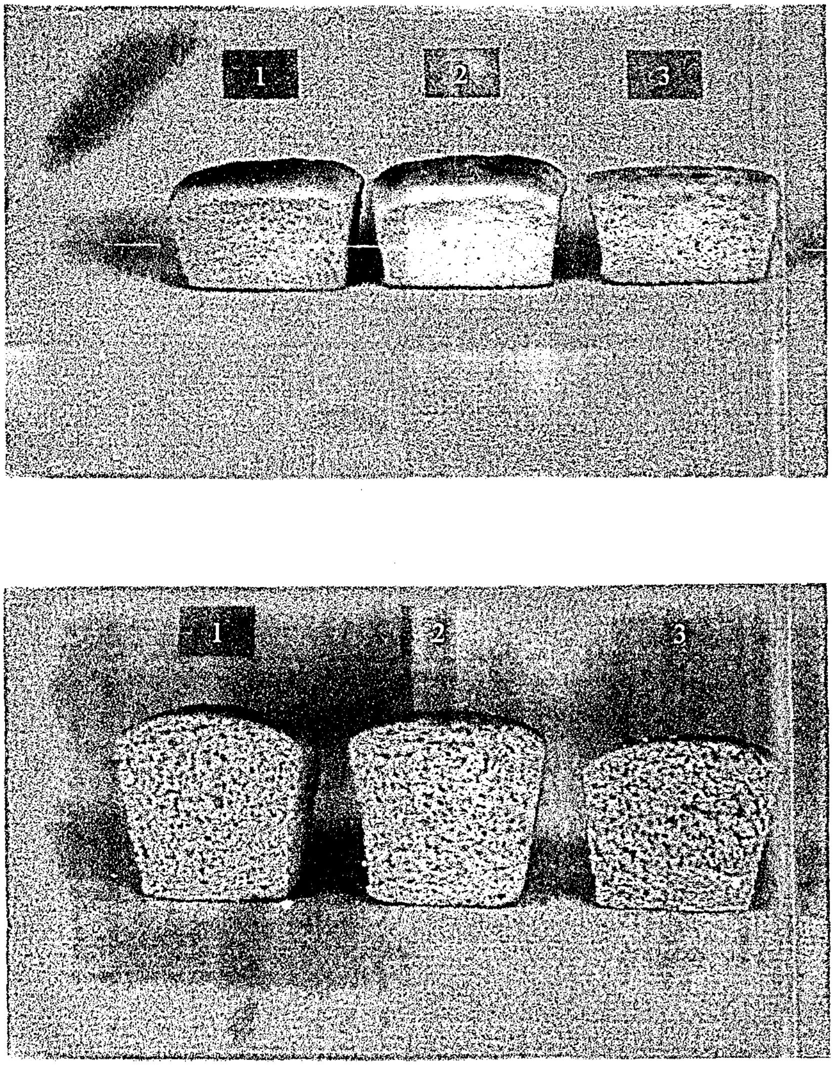


Plate 3: Whole and sliced bread loaves prepared from wheat flour and defatted soy supplemented flours; where (1) control (100% wheat flour); (2) W+DSF (95:05); (3) W+DSF (90:10)

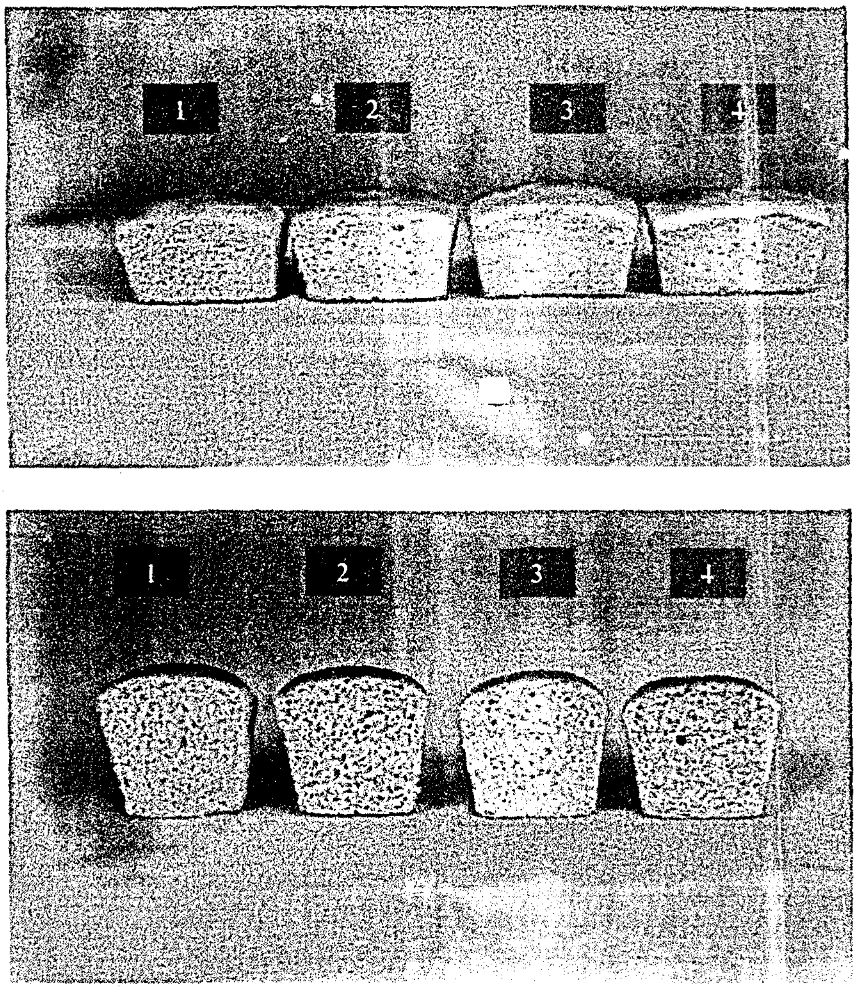


Plate 4: Whole and sliced bread loaves prepared from wheat flour and both barley and full fat soy supplemented flours; where (1) control (100% wheat flour); (2) W+B+SF (95:05); (3) W+B+SF (90:10); (4) W+B+SF (85:15)

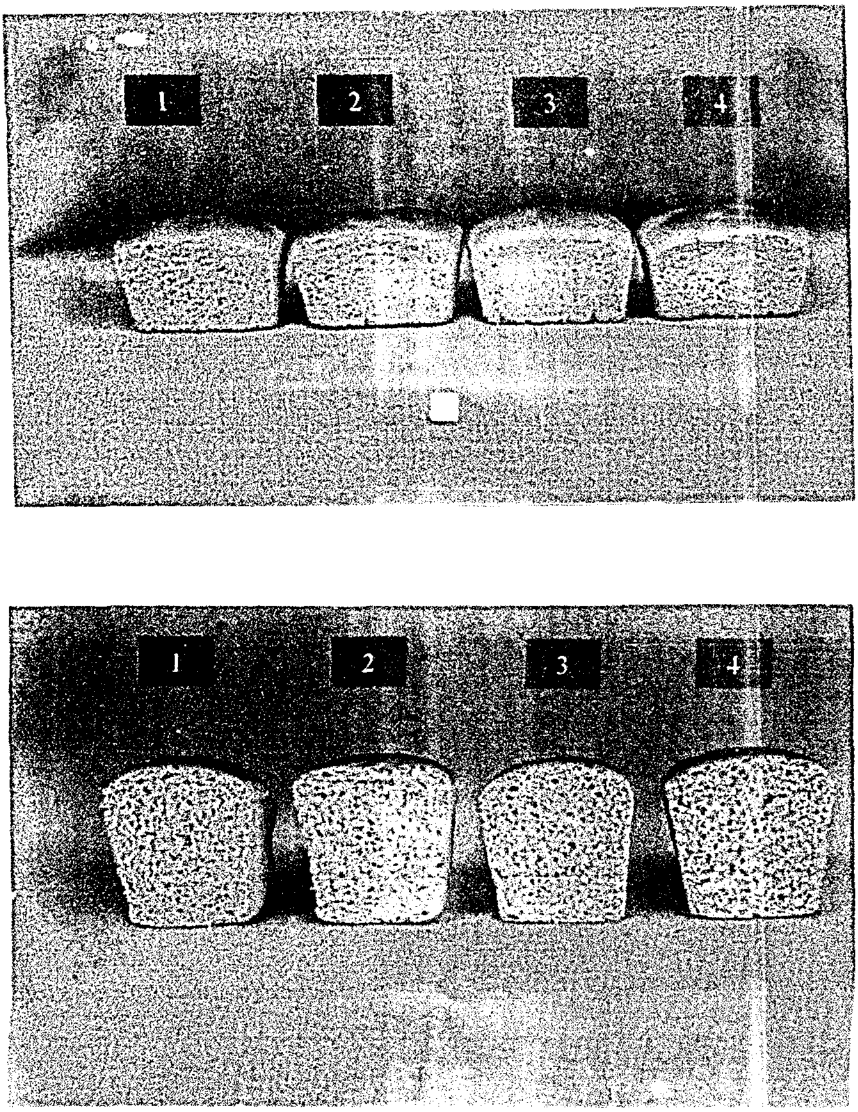


Plate 5: Whole and sliced bread loaves prepared from wheat flour and both barley and defatted soy supplemented flours; where (1) control (100% wheat flour); (2) W+B+DSF (95:05); (3) W+B+DSF (90:10); (4) W+B+DSF (85:15)

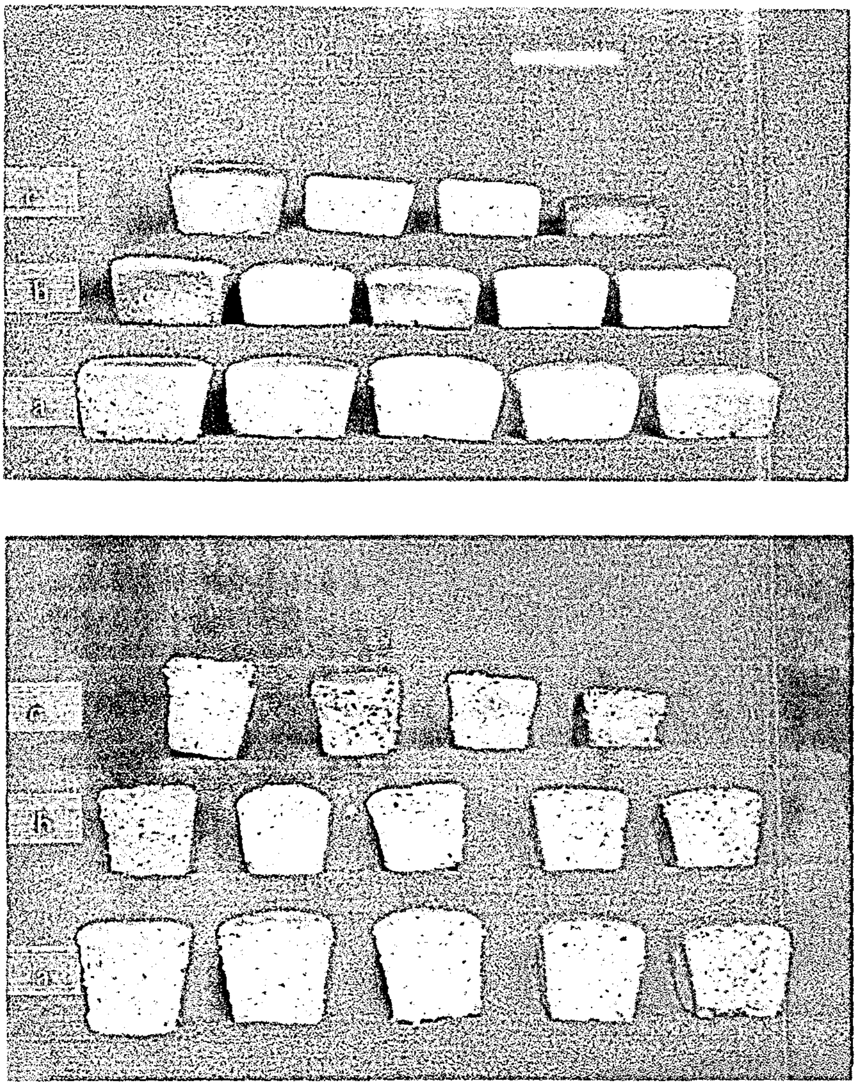


Plate 6:Whole and sliced bread loaves prepared from wheat flour and various supplemented flours; where

(a) (95:05): W+B; W+DSF; W+SF; W+B+DSF; W+B+SF

(b) (90:10):W+B; W+DSF; W+SF; W+B+DSF; W+B+SF

(c) (85:15): Control; W+B; W+B+DSF; W+B+SF

There was a non-significant difference in fat content of breads made from barley, defatted soy and both barley and defatted soy supplemented flours as compared to control. The breads containing barley, both barley and full fat soy and both barley and defatted soy flour at 15% level had 5.58, 6.27 and 5.51% fat contents, respectively. Sharma and Chauhan (2000) observed an increase in fat content from 0.90 to 1.50% in breads supplemented with fenugreek flour (0.5 to 9% level).

Ash

The ash content of control bread was 2.10% and it remained almost same in various supplemented breads at all the levels (Table 8).

Protein

The control bread exhibited 11.47% protein which decreased 10.56% at 15% blending with barley flour (Table 8). There was a significant increase in protein content in the full fat and defatted soy supplemented breads at 10% level and was found to be 13.66 and 13.81%, respectively. In breads containing both barley and soy (full fat and defatted) flours at 15% level, the protein content increased to 12.53 and 12.80%, respectively. The use of composite flours to improve the nutritional value of breads has received considerable attention and several workers have also reported increase in protein content of breads supplemented with different non-wheat flours particularly pulse flours (Rathna and Neelkantan, 1995; Sharma and Chauhan, 2000).

Total lysine

Data pertaining to total lysine content is presented in Table 8. The quality of protein is evaluated by lysine content. The total lysine content of control bread was 2.36 g/100g 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

Table 8. Chemical composition and *in vitro* protein digestibility of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Fat (g/ 100 g)	Ash (g/100 g)	Protein (g/100 g)	Total lysine (g/100 g protein)	Protein digestibility (%)
Control (Wheat)	5.44±0.45	2.10±0.44	11.47±1.95	2.36±0.22	74.00±0.61
W + B					
95:05	5.49±0.50	2.14±0.37	11.40±0.88	2.52±0.44	74.14±0.60
90:10	5.55±0.45	2.21±0.21	10.98±0.98	2.71±0.60	74.20±0.06
85:15	5.58±0.52	2.33±0.41	10.56±1.23	2.80±0.47	74.32±1.47
W+SF					
95:05	6.32±0.41	2.28±0.36	12.05±0.95	2.74±0.50	70.35±0.59
90:10	6.83±0.58	2.40±0.33	13.66±0.81	3.02±0.09	69.05±0.29
W+DSF					
95:05	5.43±0.23	2.23±0.59	12.44±0.44	2.76±0.28	72.10±1.59
90:10	5.38±0.43	2.35±0.29	13.81±1.02	3.05±0.52	70.69±0.69
W+B+SF					
95:05	5.78±0.78	2.17±0.62	11.69±1.15	2.68±0.50	72.67±0.61
90:10	6.10±0.61	2.31±0.12	12.32±0.32	2.83±0.68	71.25±1.74
85:15	6.27±0.55	2.44±0.38	12.53±1.33	3.02±0.46	69.83±0.65
W+B+DSF					
95:05	5.44±0.30	2.13±0.37	11.97±0.77	2.68±0.53	72.98±0.27
90:10	5.47±C.39	2.26±0.15	12.39±0.48	2.85±0.52	71.88±1.00
85:15	5.51±0.22	2.42±0.31	12.80±0.80	3.05±0.46	70.72±0.92
SE (m)	0.27	0.32	0.30	0.14	0.49
CD ($P<0.05$)	0.78	NS	0.89	0.46	1.43

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

increased significantly to 3.02 and 3.05 g/100g protein, respectively. The increase was found to be 28 and 29%, respectively. Similarly, in breads made from both barley and soy (full fat and defatted) supplemented flours at 15% level, total lysine content increased by 28 and 29%, respectively. It was due to high content of total lysine in soy flour. Gayle *et al.* (1986) also reported an increase in lysine content of breads substituted with pigeonpea flour at 25% substitution.

Protein digestibility

The data on protein digestibility (Table 8) revealed that control bread had 74.00% 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) the *in vitro* protein digestibility decreased significantly. This decrease could be due to high content of trypsin inhibitors and other anti-nutrients in soybean flour. 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 *et al.*, 1991). At 10% level of substitution, *in vitro* protein digestibility was 69.05, 70.69, 71.25 and 71.88%, respectively in breads made from barley, full fat soy, defatted soy, both barley and full fat soy and both barley and defatted soy blended flours. Grewal (1992) also reported low protein digestibility of soy flours.

Correlation studies (Table 9) also indicated a significant ($P<0.05$) negative correlation between *in vitro* protein digestibility and trypsin inhibitor activity, phytic acid, polyphenols and amylase inhibitor activity.

Sugar content

Total, reducing and non-reducing sugar contents of control bread were 20.74, 8.33 and 12.41%, respectively (Table 10). Total sugar contents did not

Table 9. Correlation coefficient of *in vitro* digestibilities of protein and starch with phytic acid, polyphenols, amylase inhibitor activity and trypsin inhibitor activity

Antinutrients	Protein digestibility	Starch digestibility
Phytic acid	-0.581*	-0.492*
Polyphenols	-0.487*	-0.454*
Amylase inhibitor activity	-0.440*	-0.483*
Trypsin inhibitor activity	-0.834**	-0.563*

* Significant at 5% level.
** Significant at 1% level.

change significantly upto 5% blending level of barley and soy flours. Whereas in full fat and defatted soy supplemented breads at 10% level, total sugar contents were 21.50 and 21.67%, respectively. In barley and both barley and full fat soy supplemented breads upto 10% level, the total sugar contents did not vary significantly from control. At 15% blending level, total sugar contents were 21.84, 21.88 and 21.91%, respectively in barley and both barley and soy (full fat and defatted) supplemented breads. There was also a non-significant variation in reducing sugar contents of various supplemented breads as compared to control bread and the values ranged from 8.16 to 8.33%, respectively. A significant ($P<0.05$) increase in non-reducing sugars was found in barley and both barley and soy (full fat and defatted) supplemented breads at 15% level and values were 13.68, 13.69 and 13.72%, respectively. However, non-reducing sugar contents in soy (full fat and defatted) supplemented breads were statistically similar to that of control (12.41%).

The increase in total soluble sugars and non-reducing sugars may be partially due to addition of sugar in flours and partially due to hydrolysis of starch during fermentation which is also indicated by higher values of reducing sugars obtained in present study as compared to normal values of wheat and other cereal pulse flours. Rastogi and Singh (1989) also showed increase in reducing and non-reducing sugars in blends containing 20% full fat soy flour.

Starch digestibility

The *in vitro* starch digestibility of whole wheat (control) bread and other cereal-pulse blended breads have been discussed in Table 10. The *in vitro* starch digestibility decreased significantly ($P<0.05$) at 15% level in the breads made from barley supplemented flour (40.38 mg maltose released/g meal) as compared to control (42.83 mg maltose released/g meal). Poor starch digestibility of barley supplemented breads was due to binding property of inhibitors with amylase

Table 10. 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)	20.74±0.36	8.33±0.38	12.41±0.74	42.83±1.62
W+B				
95:05	21.08±0.70	8.33±0.50	12.75±1.20	41.79±0.51
90:10	21.38±0.55	8.26±0.53	13.12±0.02	41.06±0.30
85:15	21.84±0.26	8.16±0.21	13.68±0.47	40.38±1.58
W+SF				
95:05	20.99±0.54	8.29±0.30	12.70±0.24	43.00±0.34
90:10	21.50±0.38	8.24±0.58	13.26±0.91	43.20±1.00
W+DSF				
95:05	21.25±0.58	8.31±0.37	12.94±0.21	43.15±0.36
90:10	21.67±0.46	8.27±0.67	13.40±1.10	43.40±0.54
W+B+SF				
95:05	21.00±0.49	8.31±0.64	12.69±1.13	42.29±0.66
90:10	21.41±0.31	8.25±0.33	13.16±0.02	42.09±0.47
85:15	21.88±0.14	8.19±0.63	13.69±0.49	41.90±1.00
W+B+DSF				
95:05	21.12±0.54	8.31±0.38	12.81±0.92	42.37±1.11
90:10	21.48±0.39	8.26±0.52	13.22±0.13	42.18±0.72
85:15	21.91±0.48	8.19±1.00	13.72±0.52	42.00±0.91
SE (m)	0.25	0.28	0.40	0.60
CD ($P<0.05$)	0.72	NS	1.18	1.75

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

enzymes (Kalra, 1996). The starch digestibility of breads prepared from full fat soy (43.20 mg maltose released/g meal)) and defatted soy (43.40 mg maltose released/g meal) flours at 10% substitution level were at par with that of control. In both barley and soy (full fat and defatted) supplemented breads at 15% level, the starch digestibility was 41.90 and 42.00 mg maltose released/g meal, respectively.

Correlation studies (Table 9) also showed a significant ($P<0.05$) negative correlation between starch digestibility and amylase inhibitor activity, phytic acid, polyphenols and trypsin inhibitor activity.

Antinutrients

The results regarding the anti-nutritional factors viz., phytic acid, polyphenols, trypsin inhibitors and amylase inhibitors in control and various supplemented breads are presented in Table 11.

Phytic acid:

The control (whole wheat flour) bread exhibited 225.56 mg/100g phytic acid and it increased significantly with the rise in the levels of barley and soy flours. The maximum increase of 20% in phytic acid over control was found in bread containing both barley and full fat soy flour at 15% level (270.19 mg/100 g) while minimum increase of 2% was found in barley supplemented bread at 5% level (230.18 mg/100 g) over the control. The phytic acid contents of full fat and defatted soy supplemented breads at 10% level were 259.57 and 253.24 mg/100 g, respectively. In the breads containing both barley and soy flours (full fat and defatted), the phytic acid contents were 253.28 and 249.63 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 flour which have high contents of phytic acid. Gayle *et al.* (1986) suggested similar increase

Table 11. **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)	225.56±0.82	314.84±0.59	184.52±0.76	68.25±0.75
W+B				
95:05	230.18±0.51	327.52±0.62	184.00±0.84	71.44±0.71
90:10	244.88±0.69	338.69±0.58	184.00±0.16	72.30±0.36
85:15	267.96±0.58	346.20±0.52	183.92±1.08	73.16±0.52
W+SF				
95:05	238.00±0.45	323.68±0.44	197.40±1.56	68.02±0.73
90:10	259.57±0.55	331.32±0.22	204.32±0.22	66.88±0.74
W+DSF				
95:05	233.00±0.75	320.97±0.47	192.51±0.37	67.92±0.49
90:10	253.24±0.65	329.13±0.48	198.23±1.00	66.45±0.44
W+B+SF				
95:05	235.50±0.55	324.76±0.62	188.61±0.40	69.33±0.33
90:10	253.28±0.69	338.08±0.47	195.32±0.46	69.71±0.47
85:15	270.19±0.66	343.81±0.22	199.73±0.85	70.23±1.00
W+B+DSF				
95:05	231.87±0.57	323.16±0.32	185.01±1.00	69.68±0.80
90:10	249.63±0.57	336.60±0.48	189.13±0.71	69.89±0.54
85:15	269.28±0.52	341.02±0.76	191.64±0.85	70.52±0.53
SE (m)	0.38	0.29	0.37	0.46
CD ($P<0.05$)	1.10	0.89	1.08	1.33

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

in phytate concentration from 160 to 250 mg/100 g in pigeonpea flour supplemented breads at 5, 10, 15 and 25% levels. Similarly, Sharma and Chauhan (2000) also observed the increase in phytic acid from 1.95 in control to 2.15 mg/g in 9% fenugreek supplemented bread.

Polyphenol

The polyphenolic content of control bread was 314.84 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/100g and it increased to 346.20 mg/100 g at 15% level. Whereas at 5% level in defatted soy supplemented bread and in both barley and defatted soy supplemented bread, the increase in polyphenol contents were found only 2% and 3%, respectively as compared to control. The results suggested that there was little increase in polyphenol content of barley supplemented breads as compared to soybean supplemented breads and the reason could be dehulling of soybean which reduces the activity of tannins (Reddy *et al.*, 1985).

Trypsin inhibitors

Control bread manifested trypsin inhibitor activity 184.52 T1U/g which did not change significantly in barley supplemented breads at all the levels. Maximum trypsin inhibitor activity (204.32 T1U/g) was found in breads containing full fat soy flour at 10% level. Significant variations in the level of trypsin inhibitor activity was also noticed in both barley and soy (full fat and defatted) supplemented breads at all the levels. At 15% level, there was a increase of 8% and 4% in the trypsin inhibitor activity as compared to control. Grewal (1992) and Saxena *et al.* (1994) also reported high content of trypsin inhibitors in raw soybean flour.

Amylase inhibitors

Control bread had low levels of amylase inhibitor activity (68.25 A1U/g). In barley supplemented bread at 5% level; the value was 71.44 A1U/g which increased to 73.16 A1U/g at 15% supplementation level. In soy supplemented breads (full fat and defatted) at 10% level, the amylase inhibitor activity decreased to 66.88 A1U/g and 66.45 A1U/g, respectively. The contents of amylase inhibitor activity in breads made from both barley and soy (full fat and defatted) supplemented flours at all the lèvels were intermediate between that of barley and soy supplemented breads. Results showed that amylase inhibitor activity in barley supplemented breads was highest and it might be due to high activity of amylase inhibitors in barley flour (Henry *et al.*, 1992; Jood and Kalra, 2001).

Total and available minerals

Data regarding total and available minerals in control as well as supplemented breads are presented in Table 12.

Total minerals

Control bread had 8.52 mg/100g total iron contents. A significant ($P<0.05$) decrease was observed in barley supplemented breads (7.51 mg/100 g) at 15% lèvel. However, in soy (full fat and defatted) as well as both barley and soy (full fat and defatted) supplemented breads, non-significant difference was observed in total iron content as compared to control. Total calcium content was found to be 57.10 mg/100 g in control bread and it decreased significantly ($P<0.05$) to 52.63 mg/100 g in barley supplemented bread at 15% substitution level whereas it increased significantly in breads supplemented with full fat soy (81.36 mg/100 g), defatted soy (81.79 mg/100 g), both barley and full fat soy (67.0 mg/100 g) and both barley and defatted soy (68.12 mg/100 g) at 10% supplementation level. High calcium content of breads made from soy supplemented flours might be due to high calcium content of soybean flour (Ologhoba, 1989, Suibaba, 1990 and Misra

Table 12 Total and percent availability of minerals of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Total Fe (mg/100 g)	Fe availability (%)	Total Ca (mg/100 g)	Ca availability (%)	Total Zn (mg/100 g)	Zn availability (%)
Control (Wheat)	8.52±0.41	50.23±1.00	57.10±0.22	48.26±0.23	3.75±0.35	52.00±0.30
W+B						
95:05	8.17±0.28	48.83±0.63	55.20±0.44	47.11±0.51	3.62±0.21	50.82±0.53
90:10	7.82±0.27	48.08±0.29	53.88±0.19	46.62±0.29	3.50±0.39	49.14±1.22
85:15	7.51±0.46	47.40±0.43	52.63±0.42	46.38±0.26	3.45±0.34	48.40±1.41
W+SF						
95:05	8.70±0.29	47.94±0.43	70.20±0.21	46.76±0.38	3.75±0.22	49.12±0.41
90:10	8.89±0.26	47.80±0.47	81.36±0.13	45.68±0.23	3.80±0.32	47.36±0.85
W+DSF						
95:05	8.78±0.16	48.17±0.12	71.88±0.32	46.86±0.28	3.75±0.15	49.20±0.80
90:10	8.92±0.68	47.86±0.53	81.79±0.58	45.78±0.43	3.83±0.29	47.78±0.85
W+B+SF						
95:05	8.42±1.00	48.20±0.56	61.55±0.55	46.88±0.45	3.70±0.33	49.72±0.25
90:10	8.30±0.65	47.83±0.35	67.20±1.00	46.20±0.20	3.68±0.11	48.36±0.53
85:15	8.23±0.53	47.02±0.65	72.81±0.48	45.70±0.24	3.63±0.22	46.83±0.30
W+B+DSF						
95:05	8.45±0.42	48.40±0.51	63.25±0.73	46.97±0.34	3.74±0.45	50.00±0.27
90:10	8.32±0.86	47.95±0.27	68.12±0.73	46.27±0.60	3.70±0.23	48.37±0.24
85:15	8.29±0.57	47.28±0.36	73.10±0.46	45.77±0.33	3.76±0.26	47.39±0.52
SE (m)	0.49	0.63	0.45	0.31	0.17	0.36
CD ($P<0.05$)	0.85	1.08	0.77	0.55	NS	1.05

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

et al., 1991). Control bread exhibited zinc content of 3.75 mg/100g. However, there was no significant variation in total zinc contents of breads made from all the supplemented flours at all levels as compared to control.

Available minerals

Control bread exhibited 50.23% *in vitro* iron availability but there was a significant ($P<0.05$) decrease in per cent availability of iron in various supplemented breads. The per cent iron availability decreased by about 6% in barley supplemented bread at 15% level as compared to control. Maximum decrease was found in barley and full fat soy supplemented bread (47.02%) at 15% level. *In vitro* calcium availability of control bread was 48.26% which decreased significantly ($P<0.05$) upon increasing the levels of barley and soy flours in bread. The maximum decrease in per cent availability was found in the full fat soy (45.68%) as well as both barley and full fat soy (45.70%) supplemented breads at 10 and 15% level, respectively. In barley supplemented breads at 15% level, the calcium availability decreased by about 4% as compared to control. Similar trend was also reported in zinc availability. Control bread had 52.00% zinc availability which decreased to 48.40, 46.83 and 47.39% in barley and both barley and soy (full fat and defatted) supplemented breads at 15% level.

Results showed a decrease in the availability of various minerals which could be attributed to presence of anti-nutrients specially phytic acid in barley and soy flours which are known to form insoluble phytate-mineral complexes and hence decrease the bio-availability of minerals (Cheryan, 1980; Haug and Lantzsch, 1983).

Correlation studies (Table 13) also revealed a significantly negative correlation between *in vitro* availability of iron, calcium and zinc with phytic acid polyphenols and trypsin inhibitors whereas non-significant negative correlation . was observed with amylase inhibitor activity.

Table 13. Correlation coefficient of *in vitro* availabilities of iron, calcium and zinc with phytic acid, polyphenols, amylase inhibitor activity and trypsin inhibitor activity

	***In vitro* availability**		
Antinutrients	**Iron**	**Calcium**	**Zinc**
Phytic acid	-0.777**	-0.846**	-0.878**
Polyphenols	-0.793**	-0.812**	-0.816**
Amylase inhibitor activity	-0.269	-0.223	-0.155
Trypsin inhibitor activity	-0.555*	-0.709**	-0.730**

* Significant at 5% level.
** Significant at 1% level.

Dietary fibre:

The results regarding total, soluble and insoluble dietary fibre are given in Table 14. 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 in breads. 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) manifested 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, respectively. It might be due to that 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 a significant change was found in soluble dietary fibre contents of wheat bread supplemented with both barley and soy (full fat and defatted) flours.

β-glucan

Results regarding the total, soluble and insoluble β-glucan contents of control and various supplemented breads are presented in Table 14. Wheat bread exhibited 0.41% total, 0.22% soluble and 0.19% insoluble β-glucan contents. Breads containing barley flour at different levels manifested significantly ($P<0.05$) higher contents of β-glucan. At 5, 10 and 15% supplementation level, there was an increase in total (2, 4 and 5 folds), soluble (4, 5 and 6 folds) and insoluble (1, 2 and 3 folds) β-glucan contents as compared to control bread which could be

Table 14. 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

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

attributed to high β-glucan in hull-less barley variety (Kalra, 1996). It has also been reported that β-glucan, specially soluble β-glucan has hypoglycemic (Knuckles *et al.*, 1997) and hypocholesterolemic (Kalra and Jood, 2000) effects.

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 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-glucan contents. At 15% supplementation level, increase was observed in total (3 times), soluble (4 times) and insoluble (2 times) β-glucan contents of both barley and soy (full fat and defatted) supplemented breads. The increase was more in soluble β-glucan as compared to insoluble β-glucan in all the supplemented breads. The soluble β-glucans are present primarily in the endospermic cell walls of barley grain (Jood and Kalra, 2001) which has been reported to be useful in regulating cholesterol level and blood glucose levels (Knuckles *et al.*, 1997; Kalra and Jood, 2000). 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 β-glucan rich barley fraction at different levels as compared to control bread.

4.5 Shelf life

The data regarding sensory evaluation, fat acidity and peroxide value is discussed below:

Organoleptic evaluation

A significant ($P<0.05$) difference was found in the overall acceptability score of various breads as compared to control on 0, 2 and 4 days of storage (Table 15). On 0-day, control bread had an overall acceptability score of

Table 15. Effect of storage on overall acceptability of breads prepared from wheat and various cereal pulse blends[a]

	Storage (days)						
	Room temp.			Refrigeration temp.			
Breads	0	2	4	2	4	SE (m)	CD (P<0.05)
Control (Wheat)	7.70±0.33	7.00±0.41	6.86±0.24	7.16±0.51	6.96±0.26	0.15	0.45
W+B							
95:05	7.65±0.84	7.56±0.40	6.90±0.30	7.66±0.39	7.13±0.27	0.14	0.42
90:10	7.57±0.66	7.20±0.35	6.60±0.12	7.36±0.29	6.83±0.23	0.10	0.32
85:15	7.15±0.58	6.90±0.30	6.33±0.10	6.96±0.26	6.46±0.10	0.08	0.25
W+SF							
95:05	7.67±0.53	6.96±0.34	5.30±0.76	7.33±0.45	6.80±0.17	0.19	0.58
90:10	7.40±0.65	6.56±0.23	5.30±0.43	7.00±0.40	6.43±0.08	0.12	0.38
W+DSF							
95:05	7.67±0.48	6.96±0.26	6.80±0.35	7.13±0.41	6.76±0.26	0.13	0.39
90:10	7.42±0.60	6.73±0.32	6.56±0.34	6.76±0.48	6.76±0.19	0.14	0.42
W+B+SF							
95:05	7.77±0.71	6.90±0.20	5.56±0.51	7.16±0.23	6.23±0.23	0.12	0.38
90:10	7.22±0.47	6.53±0.35	5.30±0.32	6.90±0.27	6.13±0.32	0.12	0.38
85:15	6.75±0.56	6.53±0.30	5.06±0.30	6.50±0.45	6.03±0.26	0.13	0.40
W+B+DSF							
95:05	7.72±0.46	6.96±0.29	6.66±0.30	7.23±0.19	6.90±0.16	0.09	0.29
90:10	7.27±0.82	6.56±0.15	6.63±0.15	6.96±0.45	6.73±0.16	0.10	0.31
85:15	6.87±0.57	6.60±0.40	6.53±0.16	6.50±0.41	6.53±0.24	0.10	0.30
SE (m)	0.13	0.13	0.13	0.16	0.08		
CD (P<0.05)	0.38	0.37	0.38	0.47	0.25		

[a]Values are mean ± SD of ten penalists. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

7.70 ± 0.33 which decreased significantly ($P<0.05$) to 6.86 ± 0.24 and 6.96 ± 0.26 on 4^{th} day of storage at room and refrigeration temperature, respectively but still found in the category of 'like moderately'. However, the breads made from both barley and full fat soy supplemented flour at 15% level, exhibited maximum decrease in overall acceptability score i.e. 5.06 and 6.03 on 4^{th} day at room and refrigeration temperature, respectively. In full fat soy supplemented bread at 10% level, overall acceptability score on 0-day was 7.40 ± 0.65 which also decreased significantly to 5.30 ± 0.43 and 6.43 ± 0.08 on 4^{th} day at room and refrigeration temperatures, respectively. Whereas barley substituted bread (15%) had score of 7.15 on 0-day which decreased significantly ($P<0.05$) to 6.33 and 6.46 on 4^{th} day at both room and refrigeration temperatures. It was interpreted that there was a decrease in overall acceptability score on increasing the days of storage and also the decrease was more at room temperature because of high room temperature during storage.

Fat acidity

The changes in lipids during storage were followed by determining the fat acidity. The fat acidity of various supplemented breads varied significantly ($P<0.05$) from that of control (Table 16). The fat acidity contents of all the supplemented breads on the 2^{nd} day at room temperature did not vary significantly as compared to 0 day of storage. Similarly, on 2^{nd} and 4^{th} day of storage at refrigeration temperature, there was no significant change observed in fat acidity contents as compared to 0 day of storage. The control bread had 18.80 mg KOH/100 g fat acidity on 0 day which increased significantly to 20.71 mg KOH/100 g on 4^{th} day at room temperature. In barley supplemented breads at 15% level, fat acidity was 19.05 mg KOH/100 g on 0 day but it increased to 20.86 mg KOH/100 g on 4^{th} day of storage at room temperature. In breads containing defatted soy flour as well as both barley and defatted soy flour at 5% level, the fat

Table 16. Effect of storage on the fat acidity content (mg KOH/100 g of sample) of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Storage (days)						
	Room temperature			Refrigeration temperature			
	0	2	4	2	4	SE (m)	CD (P<0.05)
Control (Wheat)	18.80±0.54	19.37±0.80	20.71±0.61	19.02±0.81	19.41±1.08	0.41	1.30
W+B							
95:05	18.85±0.71	19.46±0.58	20.75±0.36	19.10±0.77	19.52±1.31	0.34	1.09
90:10	18.97±0.45	19.54±0.53)	20.83±0.83	19.18±1.72	19.55±1.14	0.39	1.24
85:15	19.05±0.40	19.60±0.62	20.86±0.34	19.22±0.99	19.61±1.02	0.29	0.93
W+SF							
95:05	20.12±0.82	20.76±0.63	21.90±0.79	20.40±1.52	20.87±1.02	0.47	1.50
90:10	20.36±1.53	21.06±0.43	22.40±0.60	20.63±1.41	21.58±1.07	0.62	1.96
W+DSF							
95:05	18.88±1.67	19.41±0.46	20.77±0.89	19.06±1.21	19.45±1.45	0.71	NS
90:10	18.93±1.02	19.47±0.49	20.88±0.56	19.15±0.67	19.51±0.51	0.46	1.45
W+B+SF							
95:05	19.60±1.75	20.16±0.71	21.54±1.18	19.82±1.04	20.12±0.85	0.49	1.56
90:10	19.79±1.79	20.25±1.00	21.90±1.01	19.97±1.10	20.48±0.62	0.53	1.67
85:15	19.86±0.71	20.34±0.59	22.29±0.83	20.10±1.00	20.69±1.13	0.45	1.43
W+B+DSF							
95:05	18.90±1.15	19.43±1.15	20.76±1.00	19.08±1.08	19.47±0.51	0.61	NS
90:10	18.94±0.07	19.50±0.45	20.85±0.89	19.16±0.25	19.53±1.54	0.36	1.15
85:15	19.03±0.35	19.55±1.00	21.05±0.92	19.19±0.63 0.8	19.61±1.15	0.51	1.62
	1.2	0.9	1.6		1.0		
SE (m)	0.45	0.42	0.41	0.47	0.50		
CD (P<0.05)	1.30	1.20	1.18	1.36	1.43		

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

Table 17. Effect of storage on peroxide value (meq/kg of fat) of breads prepared from wheat and various cereal-pulse blends (on dry matter basis)[a]

Breads	Storage (days)						
	Room temperature			Refrigeration temperature			
	0	2	4	2	4	SE (m)	CD ($P<0.05$)
Control (Wheat)	2.20±0.20	2.33±0.17	2.42±0.60	2.27±0.17	2.35±0.24	0.23	NS
W+B							
95:05	2.27±0.17	2.39±0.02	2.46±0.40	2.32±0.45	2.43±0.25	0.17	NS
90:10	2.35±0.21	2.47±0.06	2.57±0.59	2.41±0.48	2.49±0.61	0.23	NS
85:15	2.41±0.23	2.58±0.14	2.63±0.26	2.5±0.17	2.56±0.44	0.13	NS
W+SF							
95:05	2.78±0.27	2.91±0.08	3.21±0.59	2.88±0.59	2.94±0.09	0.12	0.40
90:10	3.02±0.02	3.08±0.08	3.47±0.45	3.05±0.49	3.18±0.04	0.13	0.41
W+DSF							
95:05	224±0.09	2.41±0.05	2.48±0.48	2.35±0.35	2.42±0.59	0.17	NS
90:10	2.31±0.08	2.46±0.05	2.51±0.46	2.43±0.06	2.48±0.33	0.17	NS
W+B+SF							
95:05	2.54±0.16	2.67±0.05	3.12±0.42	2.61±0.64	2.63±1.00	0.16	0.52
90:10	2.69±0.22	2.84±0.05	3.17±0.26	2.77±1.00	2.85±0.63	0.12	0.39
85:15	2.89±0.23	2.99±0.11	3.25±0.07	2.92±0.92	3.00±0.00	0.09	0.30
W+B+DSF							
95:05	2.25±0.25	2.36±0.06	2.46±0.36	2.31±0.35	2.39±0.36	0.16	NS
90:10	2.33±0.12	2.50±0.05	2.53±0.33	2.43±0.29	2.50±0.35	0.12	NS
85:15	2.38±0.08	2.53±0.06	2.63±0.64	2.49±0.42	2.56±0.24	0.23	NS
SE (m)	0.11	0.06	0.26	0.28	0.19		
CD ($P<0.05$)	0.31	0.17	0.74	NS	0.56		

[a]Values are mean ± SD of three independent determinations. W – Wheat flour; B – Barley flour; SF – Soy flour; DSF – Defatted soy flour.

acidity contents did not change with increase in storage period. Maximum increase was found in full fat soy supplemented breads at 10% level where fat acidity increased from 20.36 mg KOH/100g (0 day) to 22.40 mg KOH/100 g (4^{th} day) at room temperature. This could be attributed to hydrolysis of triglycerides resulting in formation of free fatty acids which increase the fat acidity. Gahlawat and Sehgal (1994) and Gupta (1994) also reported increase in fat acidity with increase in storage period.

Peroxide value

The primary products of lipid oxidation are hydroperoxides which are generally present as peroxides. Thus, it seems reasonable to determine the concentration of peroxides as a measure of extent of oxidation and thus of rancidity. The peroxide value of control bread on 0 day was 2.20 meq/kg fat which did not change significantly with increase in storage period (Table 17). In full fat soy supplemented bread at 10% level, the peroxide value was 3.02 meq/kg on 0 day which increased significantly ($P<0.05$) to 3.47 meq/kg fat on 4^{th} day of storage at room temperature, whereas at refrigeration temperature, there was no significant variation in peroxide value as compared to 0 day . The breads made from barley, defatted soy, both barley and defatted soy supplemented flours at all levels, the peroxide value did not change significantly with increase in storage period both at room and refrigeration temperature. Among all the supplemented breads, the breads made from full fat soy flour (10%) exhibited maximum peroxide value might be due to more fat content which upon auto-oxidation forms peroxides during storage. Sehgal (1988) also found that peroxide value in weaning mixtures increased with increase in storage period.

5. SUMMARY AND CONCLUSIONS

Cereals have an important place in our dietary pattern. Among cereals, wheat is a less expensive source of calories and other nutrients. Traditionally, it is consumed in the form of home made products like *chapattis* but in recent years, it has find use for the preparation of value added bakery products like breads in urban and semi-urban areas of the country.

Apart from good source of nutrients, wheat is deficient in certain essential amino acids. So whole wheat flour can be successfully blended with other cereal and pulse flours to improve the nutritional quality of wheat products. These days, bakery products made from composite flours based on cereals and pulses have gained wide importance for improving the nutritional status of population.

In the present study, an attempt has been made to supplement whole wheat flour with fibre rich barley and protein rich soy flours and their effect on bread making and nutritional quality.

Among the various physical characteristics wheat, barley and soybean grain had 8.35, 10.54 and 6.58% moisture and 40.84, 30.92 and 71.70 g 1000-grain weight. Barley had maximum grain hardness (17.28 kg/grain) followed by soybean (11.27 kg/grain) and wheat (8.23 kg/grain). The hydration capacity ranged from 0.02 to 0.05 ml/seed. The grain colour varied between light yellow to light golden yellow in soybean and wheat.

The moisture content of wheat flour was 10.12% and it did not change upon blending with barley and both barley and soy (full fat and defatted) flours. The blend containing full fat soy flour exhibited minimum moisture content (9.59%) at 20% level. Wheat flour had 30.60 and 10.30% wet and dry gluten and it decreased upon increasing the levels of barley and soy (full fat and defatted) flours. At 20% level, the wet and dry gluten content decreased significantly in blends containing barley (23.00 and 8.00%), full fat soy (22.81 and 7.88%), defatted soy (22.57 and 7.68%) both barley and full fat soy (22.87 and 7.91%) and both barley and defatted soy (22.72 and 7.80%) flours, respectively. Wheat flour had 34.25 ml sedimentation value and it showed a decreasing trend on increasing the levels of barley and soy flours. Maximum decrease in sedimentation value was observed in defatted soy blended flour (26.25 ml) at 20% level. The water absorption capacity increased significantly with each increment of non-wheat flour and the blend containing full fat soy flour showed maximum increase (81.85%) at 20% level as compared to control (70.00%).

The wheat, barley and soy flours (full fat and defatted) had 13.11, 11.15, 40.80 and 44.60% protein, respectively. Among various protein solubility fractions, wheat and barley flours had highest concentration of prolamins (3.08 and 2.71%) whereas soy flours (full fat and defatted) had maximum content of glutelins (12.29 and 13.89%). The protein content of various blends ranged from 12.54 to 17.29% and blend containing defatted soy at 20% level had maximum protein (17.29%), albumin (2.13%), globulin (2.19%) and glutelin (3.92%) contents, respectively. Wheat flour dough had highest mixing time (3.85 min) and it decreased to 2.87, 2.39, 2.61, 2.13 and 2.24 min in dough containing barley, soy (full fat and defatted) and both barley and soy (full fat and defatted) flours at 20% level, respectively.

As far as various baking characteristics are concerned, the control bread had 160.0 g loaf weight and it increased with increase in level of non-wheat flours. Whereas whole wheat flour bread manifested maximum loaf volume (520 ml) and specific loaf volume (3.24 ml/g). Among the supplemented breads, full fat soy (20%) bread exhibited maximum loaf weight (186.0 g). However, the loaf volume and specific volume decreased upon increasing the level of barley and soy (full fat and defatted) flours as compared to control bread. The breads containing full fat soy flour (20% level) had minimum loaf volume (444.7 ml) compared to other supplemented breads.

The whole wheat bread had overall acceptability score of 7.70 (like moderately). Similarly, the breads made from barley (5, 10 and 15%), soy (full fat and defatted, 5 and 10%) and both barley and soy (full fat and defatted, 5, 10 and 15%) flours were found acceptable. But significant decrease was found in the overall acceptability score of breads containing barley as well as both barley and soy (full fat and defatted) flours at 20% blending level. Whereas, the soy (full fat and defatted) supplemented breads at 15 and 20% level, exhibited the poorest overall acceptability score. Hence, these were considered to be unacceptable breads.

Whole wheat flour (control) bread had 5.44, 2.10 and 11.47% fat, ash and protein contents. The ash content in various supplemented breads did not vary significantly from that of control. The fat and protein content did not vary significantly in barley blended breads at all the levels as compared to control. The maximum fat content was found in full fat soy supplemented bread (6.83%) whereas protein content was maximum in defatted soy supplemented bread (13.81%) at 10% level. In both barley and soy (full fat and defatted) supplemented breads at 15% level, the fat and protein contents were 6.27 and 5.51% and 12.53 and 12.80%, respectively. The total lysine content was maximum in defatted soy (3.02 g/100g protein) and both barley and defatted soy (3.05 g/100 g protein) supplemented breads at 10 and 15% level. Maximum protein digestibility was found in control bread (74.0%) and minimum in full fat soy supplemented bread (69.05%) at 10% supplementation level. The breads containing both barley and soy (full fat and defatted) at 15% level had 69.83 and 70.72% protein digestibility, respectively.

The control bread had 20.74, 8.33 and 12.41% total, reducing and non-reducing sugars. There was a non-significant difference in the reducing sugar content of various supplemented breads as compared to control. The highest concentration of total and non-reducing sugars was observed in breads containing both barley and defatted soy flour (21.91 and 13.72%) at 15% blending level. However, in soy (full fat and defatted) supplemented breads (10%), the non-reducing sugar content was at par with that of control. The starch digestibility of control bread was 42.83 mg maltose released/g meal, whereas among the supplemented breads, starch digestibility was found in defatted soy supplemented bread (43.40 mg maltose released/g meal) at 10% level and minimum was observed in barley blended bread (40.38 mg maltose released/g meal) at 15% level.

The breads containing both barley and full fat soy flour at 15% level had maximum phytic acid (270.19 mg/100 g) content as compared to control bread (225.56 mg/100 g). The control bread had 314.84 mg/100 g polyphenol content and it increased significantly on increasing the level of barley and soy (full fat and defatted) flours in wheat bread. The trypsin and amylase inhibitor activity was 184.52 TIU/g and 68.25 AIU/g in control bread. The breads containing full fat soy flour (10%) had maximum trypsin inhibitor activity whereas it did not change in barley supplemented breads at all the levels. The amylase inhibitor activity was maximum in barley blended bread (73.16 AIU/g) at 15% level whereas soy (full fat and defatted) containing breads showed decrease in amylase inhibitor activity.

The whole wheat bread had 8.52, 57.10 and 3.75 mg/100 g total iron, calcium and zinc contents which increased by about 5, 43 and 2% in defatted soy supplemented bread at 10% level. The control bread had 50.23, 48.26 and 52.00% iron, calcium and zinc availability, respectively, which showed a decreasing trend in various supplemented breads. The breads containing both barley and full fat soy flour (15%) showed maximum decrease in per cent availability of iron, calcium and zinc.

The total, soluble and insoluble dietary fibre contents increased to 9.80, 4.48 and 5.32%, respectively in barley supplemented bread at 15% level as compared to control (8.90, 3.95 and 4.95%). In both barley and soy (full fat and defatted) supplemented breads at 10% level, the total, soluble and insoluble dietary fibre were 8.52, 4.02, 4.50 and 8.60, 4.05, 4.55%, respectively. The control bread had 0.41, 0.22 and 0.19% total, soluble and insoluble β-glucan which increased to 2.20, 1.38 and 0.81% in barley supplemented bread at 15% level. Also the breads containing both barley and soy (full fat and defatted) flours at 15% level manifested many folds increase in total, soluble and insoluble β-glucan as compared to control bread.

Storage studies also indicated that control bread had overall acceptability score of 7.70 on 0-day and it decreased to 6.86 on 4th day of storage at room temperature. The maximum decrease in overall acceptability was observed in breads containing both barley and full fat soy (15% level) flour as well as full fat soy supplemented bread (10% level) on the 4th day of storage at room temperature. Fat acidity and peroxide value of the control bread were 18.80 mg KOH/100 g and 2.20 meq/kg fat, respectively. On the 4th day of storage at room temperature the breads containing full fat soy flour at 10% level exhibited maximum (22.40 mg KOH/100 g) fat acidity and peroxide value (3.47 meq/kg fat). Results showed that there was non-significant change in the overall acceptability score as well as fat acidity and peroxide value of various supplemented breads stored at refrigeration temperature for 4 days as compared to fresh breads.

It may be inferred from the present study that the breads containing both barley and defatted soy flour had high protein and total lysine content. It also contained appreciable amount of dietary fibre and β-glucan and had high mineral profile. Storage studies also indicated that these breads could be successfully stored at both room as well as refrigeration temperature upto 4 days. So the breads made from both barley and defatted soy flours upto 15% level were considered as most acceptable which can improve the nutritional status of general population.

In recent years, there is also increased interest in the role of soy fibre and barley β-glucan in human health. These are useful in bridging the fibre gap for consumers unable to ingest the recommended level of dietary fibre. The efforts can be made to popularize the fibre and protein rich products like breads among population. Development and consumption of such functional foods claiming health benefits can go a long way in improving the nutritional status of the population especially those suffering from degenerative diseases associated with today's changing life styles and environment.

LITERATURE CITED

AACC (1984). Approved methods of analysis. The American Association of Cereal Chemists, St. Paul., Minnesota.

Adsule, R.N., Lawande, K.M., Dhotre, V.A. and Kadam, S.S. (1985). Studies on some promising cultivars of wheat. I. Quality of maida, bread and cookies. Bull. Grain Technol. 23: 7-15.

Akeson, W.E. and Stahmann, M.A. (1964). A pepsin pancreatin digestibility index of protein quality evaluation. J. Nutr. 83: 257-259.

Akingbala, J.O., Oguntimein, G.B. and Sobande, A.O. (1995). Physico-chemical properties and acceptability of yam flour substituted with soy flour. Plant Fd. Human Nutr. 48: 73-80, 1995.

Alauddin Chowdhary, A.K.M., Howlader, M.A.A., Kabir, K.A. and Hossain, M.A. (1999). Biochemical and functional properties of some released wheat varieties of Bangladesh. Bangladesh J. Agril. Res. 24: 211-218.

Anderson, M.A.; Cook, J.A. and Stone, B.A. (1978). Enzymatic determination of 1,3 : 1,4-beta-glucans in barley grain and other cereals. J. Inst. Brew. 84: 223-226.

Anjum, F.M. and Walker, C.E. (2000). Grain, flour and bread-making properties of eight Pakistani hard white spring wheat cultivars grown at three different locations for 2 years. Int. J. Fd. Sci. Technol. 35: 407-416.

Anonymous (1973). Nutritional improvement of food legumes by breeding. Ed. Max Milner PAG, Italy, pp. 61-68.

AOAC (1995). Official methods of Analysis. Association of Official Analytical Chemists, Washington, D.C.

Bakshi, A.K. and Bains, G.S. (1987). Study of the physico-chemical, rheological, baking and noodle quality of improved durum and bread wheat cultivars. J. Fd. Sci. Technol. 24: 217-221.

Belderok, B. (2000). Developments in bread making processes. Plant Fd. Human Nutr., 55: 3-86.

Berglund, P.T.; Fastnaught, C.E. and Holm, E.T. (1994). Physicochemical and sensory evaluation of extruded high-fibre barley cereals. Cereal Chem., 717: 91-95.

Bernfeld, P. (1955). Amylases alpha and Beta. In: Methods in Enzymology. Ist edn. (Eds.) S.P. Colowick and N.O. Kaplan, Acad. Press, N.Y., p. 149-159.

Bhatty, R.S. (1986). Physicochemical and functional (Bread making) properties of hull-less barley fractions. Cereal Chem., 63(1): 31-35.

Bhatty, R.S. (1993). Physicochemical properties of roller-milled barley bran and flour. Cereal Chem., 70: 397-402.

Boyacioglu, M.H. and D'Appolonia, B.L. (1994). Characterization and utilization of durum wheat for bread making. I. Comparison of chemical, rheological and baking properties between bread wheat flours and durum wheat flours. Cereal Chem. 71: 21-28.

Carson, L., Sitser, C. and Sun, X.S. (2000). Sensory characteristics of sorghum composite bread. Int. J. Fd. Sci. Technol., 35: 465-471.

Chaudhary, V.K. and Weber, F.E. (1990). Barley bran flour evaluated as dietary fibre ingredient in wheat bread. Cereal Fds. World 35(6): 560-563.

Chawla, V.K. and Kapoor, A.C. (1982). Chemical composition and protein quality of wheat-triticale chapattis. J. Fd. Sci. 47: 2015-2017.

Chen, C.H. and Bushuk, W. (1970). Nature of proteins in triticale and its parental species. I. Solubility characteristic and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50: 9-14.

Cheryan, M. (1980). Phytic acid interaction in food systems. CRC Crit. Rev. Food Sci. Nutr. 13: 297-335.

Chowdhury, S. (1993). Preparation and nutritional evaluation of some traditional foods from pearl millet. M.Sc. Thesis, CCS HAU, Hisar, India.

Dahiya, S. and Kapoor, A.C. (1994). Development, nutritive content and shelf life of home processed supplementary foods. Plant Fd. Human Nutr. 45: 334-342.

Dreese, P.C. and Hoseney, R.C. (1982). Baking properties of bran fractions from brewer's spent grains. Cereal Chem. 59: 89-91.

Duhan, A.; Chauhan, B.M. and Bhat, C.M. (1986). Preliminary studies in nutritive value of some new strains of wheat. Haryana Agric. Univ. J. Res., 16: 308-313.

Ereifej, K.I. and Shibli, R. (1993). Rheological properties and baking quality of flour from a landrace and durum wheat cultivars grown in Jordan. Cereal Chem. 70: 486-488.

FAO (1998). Quarterly Bulletin of Statistics. Vol. 11, Food and Agriculture Organization of United Nations, Rome, Italy.

Feng, G.H., Chen, M., Kramer, K.J. and Reeck, G.R. (1991). Alpha-amylase inhibitors from rice: Fractionation and selectivity toward insect, mammalian and bacterial alpha-amylases. Cereal Chem., 68: 516-521.

Forrest and Wainwright (1977). The mode of binding of β-glucans and pentosans in barley endosperm cell walls. J. Inst. Brew., 83: 279-286.

Frolich, W. and Nyman, M. (1988). Minerals, phytate and dietary fibre in different fractions of oat grain.

Furda, I. (1981). Simultaneous analysis of soluble and insoluble dietary fibre. The Analysis of Dietary Fibre in Food. W.P.T. James and O.Theander (Eds.). Marcel Dekker, Inc., New York, 163-172.

Gahlawat, P. and Sehgal, S. (1994). Shelf life of weaning foods developed from locally available food stuffs. Plant Fd. Human Nutr. 45: 349-355.

Gallardo, F., Araya, H., Pak, N. and Togle, M.A. (1974). Toxic activity in Chilean legumes. II. Trypsin inhibitor activity. Arch. Latinoamer. Nutr. 24: 183.

Gayle, P.E.; Knight, E.M.; Adkins, J.S. and Harland, B.F. (1986). Nutritional and organoleptic evaluation of wheat breads supplemented with pigeon pea flour. Cereal Chem. 63(2): 136-138.

Gopaldas, T.; Inamdar, F. and Patel, J. (1982). Malted versus roasted young child mixes, viscosity, storage and acceptability trials. Indian J. Nutr. Dietet. 19: 327-334.

Grewal, R.B. (1992). Nutritional improvement of soybean through fennentation and its utilization in traditional foods of India. Ph.D. Thesis, CCS HAU, Hisar, India.

Gupta, H.O. (1994). Protein quality evaluation of sprouted maize. Plant Fd. Human Nutr., 46: 85-91.

Gupta, M. (1994). Studies on nutritional improvement of traditional supplementary foods for lactating women. Ph.D. Thesis, CCSHAU, Hisar, India.

Hanslas, V.K. (1997). Wheat flour-Basic raw material for baked products and its quality requirements, In: Bakery industry in 21st century. 59-67.

Haug, W. and Lantzsch, H.J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Fd. Agric. 34:1423-1426.

Henry, R.J.; Battershell, V.G.; Brennan, P.S. and Oono, K. (1992). Control of wheat alpha-amylase using inhibitors from cereals. J. Sci. Fd. Agri. 58: 281-284.

Hira, K., Kochhar, A., Kaur, B. and Sharma, S.K. (1991). Protein, lysine, mineral and phenol contents of some Indian wheat varieties. J. Fd. Sci. Technol. 28: 112-113.

Hofer, P.J. (1986). The composition and nutritional value of high lysine, high sugar barley (*Hordeum vulgare* L.) Master's Thesis, Montana State University, Bozeman, MT.

Hoseney, R.C. (1994). Principles of Cereal Science and Technology. American Association of Cereal Chemists. Inc. St. Paul, Minnesota, USA.

Hulme, A.C. and Narain, R. (1931). The ferricyanide method for the determination of reducing sugars. A modification Hagedom Jensen-Hones Technique. Biochem. J. 25: 1051-1061.

India (1994). A reference Annual, Research and Reference Division, Ministry of Information and Broadcasting, Govt. of India, New Delhi.

Indrani, D. and Rao, G.V. (1992). Effect of processing methods on the quality of whole wheat flour bread. J. Fd. Sci. Technol. 29(5): 293-295.

Irwin, G.N. and McMullan, M.E. (1960). The 'Remix' baking test. Cereal Chem., 37: 603-613.

Jood, S. and Kalra, S. (2001). Chemical composition and nutritional characteristics of some hull-less and hulled barley cultivars grown in India. Die Nahrung/Food (In press).

Jood, S. and Kapoor, A.C. (1992). Effect of storage and insect infestation on protein and starch digestibility of cereal grains. Food Chem. 44: 209-219.

Jood, S., Bishnoi, S. and Sharma, A. (1998). Chemical analysis and physico-chemical properties of chickpea and lentil cultivars. Nahrung 42: 71-74.

Jood, S.; Chauhan, B.M. and Kapoor , A.C. (1989). Protein digestibility (*in vitro*) of chickpea and black gram as affected by domestic processing and cooking. Plant Fd. Human Nutr., 39: 268-271.

Kahlon, T.S., Chow, F.I., Knuckles, B.E. and Chin, M.M. (1993). Cholesterol lowering effects in hamsters of β-glucan enriched barley fraction, dehulled white barley, rice bran and oat bran and their combinations. Cereal Chem., 70: 435-440.

Kakker, S. (1992). Bioavailability of minerals in chickpea: Effect of domestic processing and levels of antinutrients. Ph.D. Thesis, CCS HAU, Hisar, India.

Kalra, S. (1996). Studies on the nutritional and anticholestremic properties of barley. M.Sc. Thesis, CCS HAU, Hisar, India.

Kalra, S. and Jood, S. (2000). Effect of dietary barley β-glucan on cholesterol and lipoprotein fractions in rats. J. Cereal Sci., 31: 141-145.

Katiyar, S.K., Kumari, K. and Bhatia, A.K. (1989). Nutrient composition of "Nikku muth", a traditional soybean cultivar of Kashmir Valley, with particular reference of lipids and proteins.

Kim, H. and Zemel, M.R. (1986). *In vitro* estimation of the potential bio-availability of calcium from sea mustard, milk and spinach under stimulated, normal and reduced gastric acid conditions. J. Fd. Sci., 51: 957-963.

Knizhko, P.O., Kochetova, A.A.; Tsysar, N.V. and Tkachenko, T.Z. (1974). Contents of some trace elements in cereal components of concentrate feeds. Khimiya V Sel'skom Khozyeistne, 12: 617-618.

Knuckles, B.E., Hudson, M.M. and Sayre, R.N. (1997). Effect of β-glucan barley fraction in high fibre bread and pasta. Cereal Fds. World 42(2): 94-100.

Knuckles, B.E., Kuzmicky, D.D. and Betschart, A.A. (1985). Effect of phytates and partially hydrolyzed phytates on *in vitro* protein digestibility. J. Fd. Sci. 50: 1080-1082.

Kulshreshtha, K. and Usha, M.S. (1992). Biochemical composition and nutritional quality of triticale. J. Fd. Sci. Technol. 29: 109-110.

Lindsey, W.L. and Norwell, M.A. (1969). A new DPTA-TEA soil test for zinc and iron. Agron. Abstr. 61:84.

Liu, K. and Markakis, P. (1987). Effect of maturity and processing on the trypsin inhibitors and oligosaccharides of soybeans. J. Fd. Sci. 52(1): 222-224.

Lockhart, H.B. (1978). Nutritional quality of the cereal grains. Cereals: Better nutrition for the world's million ed. Y. Pomeranz, pp. 201-221.

Lolas, G.M., Palamidis, N. and Markakis, P. (1976). The phytic acid-total phosphorus relationship in barley, oats, soybean and wheat. Cereal Chem., 53: 867-871.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randail, R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Maria, L. San Buenaventura, Faye M. DONG, and Barbara, A. Rasco (1987). The total dietary fibre content of Wheat, Corn, Barley, Sorghum and Distillers' dried grains with solubles. Am. Assoc. Cereal Chemists, 64: 135-136.

Marlett, J.A. (1991). Dietary fibre content and effect of processing on two barley cultivars. Cereal Fds. World, 36: 576-578.

McCleary, B.V. and Glennie-Holmes, M. (1985). Enzymatic quantification of (1-3) (1-4) β-D-glucans in barley and malt. J. Inst. Brew., 91: 285-295.

Misra, B.K., Gupta, R.K. and Ram, S. (1998). Protocol for evaluation of wheat quality. Directorate of Wheat Research, Karnal.

Misra, P., Usha, M.S. and Surjain Singh (1991). Soy-wheat flour blends : Chemical, rheological and baking characteristics. J. Fd. Sci. Technol. 28(2): 89-91.

Miyahara, S. and Jikoo, K. (1967). Examination of the method of calorimetric determination of lysine. J. Jap. Soc. Fd. Sci. Tech., 14: 512-513.

Mouliswar, P., Kurien, S., Depiel, V.A., Malleshi, M.G. and Rao, V.S. (1993). *In vitro* digestibility of protein and starch of energy food and its bulk reduction. J. Fd. Sci. Technol. 30: 36-39.

NABIM (1996). Flour Incorporation. National Association of British and Irish Millers Ltd., London.

Naik, M.S. (1968). Lysine and tryptophan in protein fractions of sorghum. Indian J. Genet., 28: 142-146.

Nemeth, L.J., Williams, P.C. and Bushuk, W. (1994). A comparative study of the quality of soft wheat from Canada, Australia and the United States. Cereal Fds. World 39: 691-700.

Newman, R.K. and Newman, C.W. (1991). Barley as a food grain. Cereal Fds. World. 36(9) : 800-805.

Ologhobo, A.D. (1989). Improving the nutritive value of soybean through processing: Biochemical and protein quality studies. Trop. Agric., 66: 290.

Osborne, T.B. (1907). The protein of wheat kernel Carnegie. Institution of Washington, Pub. No. 84.

Osborne, T.B. and Mendell, L.B. (1914). Nutritive properties of proteins of maize kernels. J. Biol. Chem. 18: 1-16.

Panse, Y.G. and Sukhatme, P.V. (1961). Statistical methods for agricultural workers. 2nd Edn. Indian Council of Agricultural Research, New Delhi, pp. 12-87.

Paredes-Lopez, O., Barba-Rosa, A.P. and Gonzalez-Castaneda, J. (1987). Physicochemical and functional properties of Mexican wheat flours for bread making. Cereal Fds. World. 32: 602-608.

Pawar, V.D. and Parlikar, G.S. (1990). Reducing polyphenols and phytates and improving the protein quality of pearlmillet by dehulling and soaking. J. Fd. Sci. Technol. 27: 140-143.

Pena, R.J. and Ballance, G.M. (1987). Comparison of gluten quality in triticale: A fractionation-Reconstitution study. Cereal Chem. 64: 128-132.

Pomeranz, Y., Czuchajowska, Z., Shorgen, M.D., Rubenthaler, G.L., Botte, L.C., Jeffers, H.C. and Mattern, P.J. (1988). Hardness and functional (bread and cookie making) properties of U.S. wheats. Cereal Fds. World. 33: 297-304.

Popli, S. and Dhindsa, K.S. (1980). Quality characteristics of improved wheat varieties of Haryana and Punjab. Bull. Grain Technol. 18: 10-16.

Quinn, J.R. and Paton, D. (1979). Cereal Chem., 56: 38-44.

Raidi, M.A. and Klein, B.P. (1983). Effect of soy or field pea flour substitution on physical and sensory characteristics of chemically leavened quick breads. Cereal Chem. 60(5): 367-370.

Ranhotra, G.S. (1991). Nutritional quality of cereal and cereal based foods. In : Handbook of Cereal Science and Technology. Loranze and Kulp (Ed.) Yarcel Dekker, Inc. pp. 845-861.

Rao, B.S.N. and Prabhawathi, T. (1978). An *in vitro* method for predicting the bioavailability of iron from foods. Am. J. Clin. Nutr. 31:169.

Rao, H. P. and Hemamalini, R. (1991). Effect of incorporating wheat bran on rheological characteristics and bread making quality of flour. J. Fd. Sci. Technol., 28: 92-97.

Rao, J.S. and Rao, V.G. (1997). Effect of incorporation of sorghum flour to wheat flour on chemical, rheological and bread characteristics. J. Fd. Sci. Technol. 34: 251-254.

Rastogi, A. and Singh, G. (1989). Effect of addition of full fat soy flour of different varieties on quality characteristics and bread making quality of white flour. Bull. Grain Technol. 27: 26-34.

Rathna, K. and Neelakantan, S. (1995). Effect of incorporation of puffed bengal gram flour on the quality of bread. J. Fd. Sci. Technol. 32: 169-171.

Reddy, M. M., Nirmala, B.Y., Rao, M., Srinivasan, C.N. and Hanchinal, R.R. (1998). Grain and gluten quality of some cultivars of wheat species and their suitability for preparation of traditional South Indian sweet products. J. Fd. Sci. Technol. 35(5): 438-441.

Reddy, N.R., Pierson, M.D., Sathe, S.K. and Salunkhe, D.K. (1985). Dry bean tannins: A review on nutritional implications. J. Am. Oil Chemists Soc., 62: 541.

Reddy, N.R., Sathe, S.K. and Salunkhe, D.K. (1982). Phytates in legumes and cereals. Adv. Food Res. 28: 1-9.

Riaz, M.N. (1999). Healthy baking with soy ingredients. Cereal Fds. World, 44(3): 136-139.

Roy, D.N. and Rao, P.S. (1971). Evidence isolation, purification and some properties of *Lathyrus sativus*. J. Agric. Food Chem. 19: 257-259.

Ryan, C.A. and Santarins, K. (1976). Immunological similarities of proteinase inhibitors from potatoes. Plant Physiol., 58: 683-685.

Savitri, A. and Desikarhar, H.S.R. (1990). Effect of germination of kalatur and tur on carbohydrate profile and its relation to flatus formation. Legume Res., 13: 1.

Saxena, S.; Singh, G. and Mital, B.K. (1994). Physical characteristics and composition of certain new varieties of soybeans. 31(2): 145-147.

Sehgal, S. (1988). Studies on the development and nutritional evaluation of weaning foods. Ph.D. Thesis, CCS HAU, Hisar.

Sekhon, K.S.; Saxena, A.K.; Randhawa, S.K.; Gill, K.S. (1980). Use of triticale for bread, cookie and chapatti making. J. Fd. Sci. Technol. 17: 233-235.

Selvaraj, A. and Shurpalekar, S.R. (1982). On improving the quality of soy fortified bread. J. Fd. Sci. Technol. 19: 242-246.

Sharma, H.R. and Chauhan, G.S. (2000). Physicochemical and rheological quality characteristics of fenugreek (*Trigonella foenum graecum* L) supplemented wheat flour. J. Fd. Sci. Technol. 37:87-90.

Sharma, K. (2000). Rheological and baking characteristics of some wheat varieties grown in Haryana. M.Sc. Thesis, CCS HAU, Hisar, India.

Sharma, S., Sekhon, K.S. and Nagi, H.P.S. (1999). Suitability of durum wheat for flat bread production. J. Fd. Sci. Technol. 36: 61-62.

Shewry, P.R. and Tatham, A.S. (1994). Wheat endosperm proteins and their impact on human mankind. In "Wheat Kernel Proteins : Molecular and Functional Aspects", Univ. of Tuscia : 19-26.

Shivraj, B. and Pattabiraman, T.N. (1980). Natural plant enzyme inhibitors. Part VIII – Purification and properties of two alpha-amylase inhibitors from ragi (*Eleusine coracana*) grains. Indian J. Biochem. Biophys. 17: 181-185.

Shivraj, B.; Sharma, K.K. and Pattabiraman, T.N. (1979). Natural plant enzyme inhibitors: Part VII – Alpha amylase inhibitors and amylase in plant tubers. Indian J. Biochem. Biophys. 16: 52-55.

Shurpalekar, S.R., Haridas Rao, P. and Prabhavati, C. (1976). Bread and chapatti making quality of Indian durum wheats. J. Fd. Sci. Technol. 13: 313-316.

Singh, B. and Reddy, N.R. (1977). Phytic acid and mineral composition of triticale. J. Fd. Sci. 42: 1077-1083.

Singh, G. and Paliwal, S.C. (1986). Physicochemical, milling and bread making quality of two varieties of durum wheats. Bull. Grain Technol. 24: 73-76.

Singh, N. Usha, M.S. and Chauhan, G.S. (1990). Evaluation of some U.P. hill wheats for their physical, chemical, rheological and bread making characteristics. J. Fd. Sci. Technol. 27: 198-201.

Singh, U. (1984). The inhibition of digestive enzymes by polyphenols of chickpea and pigeon pea. Nutr. Rep. Int. 29: 745.

Singh, U. and Jambunathan, R. (1981). Studies on desi and kabuli chickpea (*Cicer arietinum*) cultivars. Level of protease inhibitors, levels of polyphenolic compounds and *in vitro* protein digestibility. J. Fd. Sci. 46: 1364-1367.

Singh, U., Khedekar, M.S. and Jambunathan, R. (1982). Studies on desi and kabuli chickpea cultivars. The level of amylase inhibitors, levels of oligosaccharides and *in vitro* starch digestibility. J. Fd. Sci. 47: 510.

Slavin, J.L. (1987). Dietary Fibre: Classification, chemical analysis and food sources. J. Am. Diet. Assoc., 87: 1164-71.

Srivastav, P.P., Das, H. and Parsad, S. (1990). Effect of roasting process variables on *in vitro* protein digestibility of bengal gram, maize and soybean. Food Chem., 35: 31.

Srivastava, S. (1994). Study on the effects of processing on nutrient and non-nutrient composition of *Lathyrus sativus*. M.Sc. Thesis, CCS HAU, Hisar, India.

Suibaba, A. (1990). Barley. Nutrition, 28: 3-7.

Sundberg, B. and Amarl, P. (1994). Fractionation of different types of barley by roller milling and sieving. J. Cereal Sci. 19: 179-184.

Swain, J. and Hills, W.E. (1959). The phenolic constituents *Pramus domestical*. The qualitative analysis of phenolic constituents. J. Sci. Fd. Agric., 10: 63-68.

Tabekhia, M.M. and Luh, B.S. (1980). Effect of germination, cooking and canning on phosphorus and phytate retention in dry beans. J. Fd. Sci., 45: 406-408.

Trowell, H. (1974). Definition of dietary fibre and hypothesis that it is a protective factor in certain diseases. Am. J. Clinical Nutr., 29: 417-427.

Vaidehi, M.P. (1984). Nutritional and rheological properties of triticale varieties. Mysore J. Agric. Sci., 18: 70-74.

Vander Riet, W.B., Wight, A.W., Cilliers, J.J.L. and Datel, J.M. (1987). Food chemical analysis of tempeh prepared from South African grown soybean. Food Chem., 25: 197.

Williams, P.C.; Nakoul, H. and Singh, K.B. (1983). J. Sci. Food Agric., 34: 492-496.

Yadav, S. and Khetarpaul, N. (1994). Indigenous legume fermentation: Effect on some antinutrients and in vitro digestibility of starch and protein. Food Chem. 50: 403-406.

Yamamoto, H., Worthington, S.T., Hou, G. and Ng, P.K.W. (1996). Rheological properties and baking qualities of selected soft wheat grown in the United States. Cereal Chem. 73: 215-221.

Yee, Y.B. (1995). Update on health benefits of soy fibre, American Soybean Association, Singapore.

Yunus, M. (2000). Proc. Conf. Plant Breeding (Perspective in third millennium) (Jan. 13-14, 2000), CCS HAU, Hisar.

APPENDIX - I

Score sheet for taste panel/data under hedonic scale:

Name............

Product................. Date............

Test these samples and check how much you like or dislike each one. Use appropriate scale to show your attitude by assigning points that best describe your feeling about the sample. An honest expression of your feeling will help us.

Code No.	**Crust colour**	**Appearance**	**Flavour**	**Crust texture**	**Taste**	**Overall acceptability**	**Total remarks**

Rate	**Organoleptic score**
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Note: Please rinse your mouth before and after tasting each product.