NUTRITIONAL CHARACTERIZATION OF THE WHEAT-SOY UNLEAVENED FLAT BREAD BY RAT BIOASSAY

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ABSTRACT

Wheat flour was blended with enzyme active (full fat and defatted) and heat treated (full fat and defatted) soy flours @ 10%. Wheat-soy flour blends along with the control sample were analyzed for proximate composition, essential amino acids and mineral profile. Rat bioassay was conducted to evaluate the nutritional quality of unleavened flat bread (chapattis) prepared from wheat and soy flours blends. Soy flour blending significantly increase the crude protein, ash, crude fiber and fat contents while moisture contents and nitrogen free extract decreases significantly by the incorporation of soy flour. Micronutrient deficiency of wheat flour was compensated by the addition of soy flour. Essential amino acids composition of the wheat-soy flour blends was balance by meeting the deficiency of each other. Defatted soy flour samples (enzyme active full fat soy flour (T_1) and enzyme active defatted soy flour (T_2)) attain maximum score regarding the sensory parameter of the flat bread. All types of flour blends along with the control were fed to group of 4 rats for ten day. Heat treated soy flour samples (heat treated full fat soy flour (T_3) and heat treated defatted soy flour (T_4)) have higher feed intake, food efficiency ratio, and biological value. The parameters of biological assay were significantly higher than the control diet which interfered that these wheat-soy flour samples are nutritionally superior to the wheat flour.

Key words: Wheat-soy blends, chapattis, nutritional characterization, rats bioassay

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INTRODUCTION

Cereals are an important source of energy and protein in human diet. Wheat is the second most produced food among the cereal crops (USDA, 2003). Cereal grains provide an incredible 68 % of the world food supply. It is recognized as a major staple food and the cheapest source of protein and calories. In its refined state, its average consumption can supply 30 % energy and 49 % protein requirement of the population (Health and Welfare, 1990). Wheat is principally used for the production of unleavened flat bread locally known as chapattis and rotis in Indo-Pak. Chapattis and rotis are least expensive and utilize almost 90 % of the total wheat produced in the region (Sidddique, 1989). Cereals main dietary contribution are carbohydrates beside these they also provide protein and a smaller amount of lipids, fiber and vitamins. It is commonly known that the main nutritional drawback of cereal is their low protein contents and limited biological quality of their protein (highly deficient in lysine and tryptophan) (Mertz, 1970; Ortega et al., 1986; Waliszewski et al., 2000), when compared with animal protein. The protein quality in the given cereal can be improved by combining it with other sources of protein. Defatted wheat germ, casein and soy products are the best sources to improve the quantity and quality of protein found in the cereals. Soy flour addition improves the nutritional quality of the diet (Figueroa et al., 2003) as it improves the nutritional quality of the protein (Serna-Saldivar et al., 1988). Legumes proteins are major components of the diet of food-producing animals and are increasingly important in human nutrition. Soybean is the most important legume in relation to total world grain production and the most frequently used because of its high protein contents and relatively low prices (Gupta, 1987). Soybeans are rich in lysine and are, therefore, a good complement to wheat protein that is deficient in lysine contents. The availability of nutrient varies with the form of Soy food. Soybean is used in a variety of foods, and can be easily incorporated in the diet to promote desirable health effects (USDA, 1986; NB, 1989; Messina et al., 1994; Klein et al., 1996). Soybeans have been used in various products to mitigate the shortage of protein. One of such products is chapatti (unleavened flat bread) commonly used as staple food in Pakistan. It is well known fact that soybeans must be heat processed to destroy or partially inactivate antinutritional factors such trypsin inhibitor and phytates. Furthermore, proper heat treatment of soybean protein increases amino acid digestibility and decrease protein solubility (Anderson, 1992; Qin *et al.*, 1996). Soy flour incorporation to chapattis increases the protein quality of the product. Consumption of soy foods is increasing because of reported beneficial effect on the nutrition and health (Friedman and Brandon 2001). The aim of the study was to assess the nutritional status of wheat-soy based unleavened flat bread with different kinds of treated soy flours through biological assay.

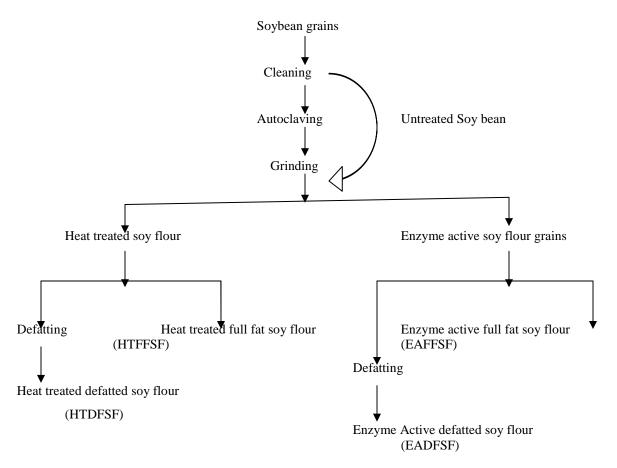
MATERIAL AND METHODS

Procurement of Raw Materials

Wheat (SH-2002) was purchased from Wheat Research Institute, Ayub Agricultural Institute, Faisalabad and grounded to whole wheat flour through china chakki (Small Scale Grinder used in Indo-Pak and is a type Pin mill). Soybean (95-I) was purchased from Oil Seed Research Institute, Ayub Agriculture Research Institute, Faisalabad.

Preparation of Soy Flour

Soy flour was prepared by following the schematic flow diagram



Defatted soy flour was prepared by removing the fat of full fat soy flour through solvent extraction method using food grade hexane as solvent (Wang *et al.*, 1999). Soy flours were mixed with wheat flour to prepare experimental treatments as shown in Table I.

Table I. Preparation of Wheat-soy flour blends

Treatments	Wheat flour	EAFFSF	EADFSF	HTFFSF	HTDFSF
T_0	100	-	-	-	-
T_1	90	10	-	-	-
T_2	90	-	10	-	-
T_3	90	-	-	10	-
T_4	90	-	-	-	10
EAFFSF	Enzyme active fu	ıll fat soy flour	EADFSF	Enzyme ac	tive defatted soy flour
HTFFSF	Heat treated full	fat soy flour	HTDFSF	Heat treate	d defatted soy flour

Chemical Assay

All types of flours were analyzed for their proximate composition according to their respective methods described in AACC (2000). Mineral contents of the different flour samples and chapattis prepared were determined by the method described in AOAC (1990).

Preparation of Chapattis

Chapattis were prepared from different flour blends and whole wheat flour according to the method described by Haridas *et al.* (1986). The dough was prepared by adding water @ 65-70% in the flour and mixing (kneading) was carried out for 3 minutes. The dough was allowed to rest for one hour at room temperature. A dough piece weighing 80g was rounded and turned into chapatti by using wooden roll. The chapattis were of uniform thickness. Baking of chapattis was done on thermo statistically controlled hot plat at 210°C for 1.5 min.

Sensory Evaluation of Chapattis

To assess the quality and acceptability, the chapattis were presented to a panel of judges and the sensory evaluation was carried out on 9 point hedonic scale for color, taste, aroma, chew ability, folding ability and overall acceptability characteristics according to methods as described by Land and Shepherd (1988). The highest score was given to the products like more while lower score was given to the product disliked by the panel of judges. The rejection level on 9 point hedonic scale was 5.

Efficacy Studies

Rats for biological studies were obtained from the National Institute of Health (NIH), Islamabad. To assess the protein quality of flours with different types of soy flours were fed to the group of 4 rats as depicted in Table II. Feed intake, water intake, body weight, digestibility, net protein evaluation (NPU), biological value (BV), net protein ratio (NPR), Protein efficiency ratio (PER) and feed efficiency ratio were determined during the experimental period according to the method of Miller and Bender (1953).

Table II. Composition of experimental diets fed to rats (g/100g)

Parameters	Flour	Corn oil	Mineral Mix	Vitamin Mix	Corn starch	Casein
T_0	75.53	10	5	3	6.47	-
T_1	65.40	10	5	3	16.6	-
\mathbf{T}_2	64.89	10	5	3	17.11	-
T_3	61.54	10	5	3	20.46	-
T_4	60.98	10	5	3	21.02	-
Casein	-	10	5	3	72.00	10
Protein Free	-	10	5	3	82	-

Statistical Analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance according to methods described by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Proximate Composition

The proximate composition of the material mixtures used for the preparation of chapattis (unleavened flat bread) is shown in Table III. The values obtained agreed with those reported by Iwe and Ngoddy (1998) and Khan et al. (2005).Addition of soy flours reduced significantly (p > 0.05) the moisture and carbohydrates in the whole wheat flour, but considerably increased the crude protein. Since the soy flour is low in sulphur containing amino acids but high in lysine and wheat flour is low in lysine but high in sulphur containing amino acids, the protein of soy flour complement the wheat flour and improve the nutritional quality of mixtures. There was no significant (p > 0.05) change in the oil contents of the mixtures following the addition of defatted soy flours but change was significant (p > 0.05) in case of adding full fat soy flours as whole wheat flour contain 1.87% oil which is increased significantly to a level of 3.55%. Robert and Conkerton, (1982) supplemented bakery item with high protein peanut flour and found that addition defatted peanut flour increased total solids, protein, moisture retention of bread after baking, and dietary fiber contents. Ash and energy contents of mixtures increased on addition of soy flour. Higher significant (p > 0.05) values of ash 2.07% were found for the defatted soy flour addition while full fat soy flour addition also result in increase ash content (1.95%) from the control value of 1.62%. Defatting result increase in the contents of protein, ash, crude fiber and carbohydrates but fat contents is decreased. Pearson (1976) and Lapedes (1977) made similar observations for defatted flour made from soybean.

Table III. Proximate composition of wheat soy flours mixtures

Composition	T_0	T ₁	T_2	T ₃	T ₄
Moisture	11.50 a	11.20 ab	10.89 b	11.20 ab	10.89 b
Protein	13.24 e	15.29 d	15.41 c	16.25 b	16.40 a
Fat	1.87 b	3.49 a	3.55 a	1.80 b	1.81 b
Fiber	2.16 c	2.44 b	2.46 b	2.57 a	2.59 a
Ash	1.62 c	1.93 b	1.95 b	2.05 a	2.07 a
NFE	69.61 a	65.64 e	65.74 d	66.11 c	66.22 b

Minerals Profile

Micro and macronutrients profile of the flour mixtures prepared are depicted in Table IV. Soy flour is rich in the minerals content especially iron, zinc, calcium and phosphorous and its blending with wheat flour increases the minerals contents of the mixtures significantly (p > 0.05). The results obtained are verified by Maqbool et al. (1987) who supplemented rotis with soy flour and revealed that Ca, P and Fe contents were as compare to those prepared from the whole wheat flour. It is further supported by Rawat et al. (1994) that soy blending increases the Ca and Fe contents of the chapattis. Defatted soy flour (EADFSF and HTDFSF) contain higher amount of minerals as compare to full fat soy flours (EAFFSF and HTFFSF) as defatting increase the mineral contents because proportion of nutrients change (Pearson, 1976). Fe, Zn and Cu contents increased significantly (p > 0.05) by soy flour blending as higher values of these minerals were found in T_3 (4.44, 2.87 and 0.79mg/100g respectively) and T_4 (4.37, 2.85 and 0.75mg/100g respectively) containing EADFSF and HTDFSF. T₁ (4.06, 2.95 and 0.62 mg/100g) and T₂ (4.01, 2.92 and 0.56 mg/100g) containing EAFFSF and HTFFSF also have higher amount of these minerals as compare to whole wheat flour (T_0) but their concentration is significantly (p > 0.05) lower in relation to T_3 and T_4 . Mn contents were higher in whole wheat flour as compare to wheat soy mixture as soybeans are low in Mn contents. Heat treatment of soybeans has non-significant effect on the minerals composition of full fat and defatted flour. Macronutrients (Ca, Mg, P, and K) increased significantly (p > 0.05) by blending soy flour with wheat flour. Similar to micronutrients higher values of these minerals are found in T₃ (52.79, 154.88, 365.40 and 581.80mg/100g respectively) and T₄ (51.04, 150.44, 360.51 and 564.52mg/100g respectively) containing defatted flour. Khan et al., (2005) prepared chapattis from the composite flours containing different levels of soy flours and found that minerals contents of these chapattis increased significantly with the increase in the level of soy flour supplementation. Anjum et al., (2006) conducted a study on the functional properties of soy hulls supplemented whole wheat flour which concluded that increasing the level of soy hulls in wheat flour boost the minerals level of whole wheat flour.

Table IV.	Minerals .	Profile o	f wheat soy	flours	mixtures

Minerals	T_0	T_1	T_2	T_3	T_4
Fe	3.92b	4.06b	4.01b	4.44a	4.37a
Zn	2.92	2.95	2.92	2.87	2.85
Cu	0.42e	0.62d	0.56c	0.79b	0.75a
Mn	3.83	3.64	3.63	3.75	3.73
Na	5.00c	5.59b	5.52b	6.50a	6.35a
Ca	34.00c	47.90b	46.60b	54.70a	52.89a
Mg	138.33c	153.50ab	151.32b	160.50a	155.90ab
P	345.83b	352.75b	351.75b	378.65a	373.59a
K	405.00c	575.80b	538.00a	602.90a	584.99a

Essential Amino Acids

The incorporation of soy flour to the wheat flour significantly (p > 0.05) improves the amino acids profile of the diet as depicted in Table V. Essential amino acids are of main concern as these amino acids are not synthesized in the body. Heat treatment (autoclaving) did not affect the amino acids composition of diet negatively as composite flour containing enzyme active soy flours (EAFFSF & EADFSF) and their respective heat treated flour sample did not vary significantly in their essential amino acids contents. Defatted soy flours samples (EADFSF & HTDFSF) result in significant increase in the amino acid contents of the wheat flour. Soy flour supplementation result in the nutritional quality improvement of the wheat flour due to balanced amino acids profile. Lysine deficiency of wheat flour is compensated by the soy flour addition and the scarcity of sulfur containing amino acids in the soy flour is balanced by their high concentration in the whole wheat flour and as result we get flour which have rate of protein absorption due to balance amino acids composition. Rawat, *et al.* (1994) who observed an increase in the protein and lysine contents of chapattis containing soy meal.

Table V. Essential amino acids of wheat soy flours mixtures (mg/100g of protein)

Amino acids	$\mathbf{T_0}$	T_1	$\mathbf{T_2}$	T_3	T_4
Threonine	467.23c	515.35b	559.7a	519.58ab	561.14a
Isoleucine	616.56b	618.89b	685.6a	622.35b	687.20a
Leucine	103.56c	369.44b	466.05a	376.68b	468.74a
Lysine	448.21c	572.29b	653.4a	579.38b	655.60a
Methionine	262.33a	233.06с	253.9ab	237.44bc	254.34ab
Cystine	387.95a	341.52b	360.7ab	347.76b	361.23ab
Phenylalanine	769.63b	762.41ab	826.55a	768.89ab	828.28a
Valine	736.18b	737.69ab	791.10a	743.26ab	792.75a
Histidine	376.89b	383.09ab	411.80a	388.76ab	412.69a
Alanine	592.87b	609.78ab	659.50a	616.88ab	661.06a

Sensory Evaluation

The blending of wheat flour with soy flour altered the organoleptic properties of different mixtures chapattis as evaluated by a panel of judges. Data on color, flavor, taste, texture, chewing ability and folding ability obtained is depicted in Table VI. Color of chapattis change significantly (p > 0.05) from creamy white to dull brown by blending soy flour. The color change was greater in chapattis prepared from full fat soy flours (EAFFSF and HTFFSF) than those prepared from defatted soy flours (EADFSF and HTDFSF). The dark color of chapatti may be because of greater amount of Maillard reaction between reducing sugars and proteins (Raidi and Klein, (1983). The change in flavor of chapattis was non significant (p > 0.05) for heat treated soy beans (HTFFSF and HTDFSF). However flavor of chapattis significantly change for the wheat soy mixtures containing enzymes active soybean flours (EAFFSF and EADFSF). Flavor of chapattis might be affected by beany flavor of soybeans. Sharma and Chauhan, (2000) noticed a similar decrease in the flavor of bread by supplementing fenugreek flour. Score for the taste of the unleavened flat bread (chapattis) decrease significantly (p > 0.05) by the addition of soybean however it was in acceptable range. Decrease in the scores was higher for untreated soy flour (EAFFSF and HTFFSF). Maqbool *et al.* (1987) supplemented rotis (unleavened flat bread) with soy flour and stated that soy addition up to

20% had no adverse effect on the sensory acceptability of chapattis. Khan *et al.* (2005) prepared chapattis by soy flour supplementation and soy flour addition up to 24% was acceptable by the panel of judges. Higher the levels of supplementation poor will the taste possibly because of the beany flavor of soybean (Rastogi and Singh, 1989). Texture score also by blending soy flour with wheat flour for the preparation of chapattis. Full fat soy flour (EAFFSF and HTFFSF) has higher effect on texture of flat bread as observed by the panel of judges. Rathna and Neelakantan (1995) supplemented bread with puffed Bengal gram and found similar deterioration in the texture of bread.

Table VI. Organoleptic properties of chapattis prepared from wheat soy mixtures

Characters	T_0	\mathbf{T}_1	T_2	T_3	T_4
Color	7.5a	6.5b	7.0ab	6.5b	7.0ab
Flavor	8.0a	7.0b	8.0a	7.0b	8.0a
Taste	8.0a	6.0d	7.0bc	6.5cd	7.5ab
Texture	8.0a	6.0b	7.0ab	6.5b	7.5ab
Chewing ability	7.5a	7.0ab	6.5bc	7.0ab	6.0c
Folding ability	7.5a	7.0ab	6.5bc	7.0ab	6.0c

Biological Assay

Diets based on chapattis prepared from all types of wheat-soy flour mixtures and control is shown in Table II. The mean body weight gain, food efficiency ratio (FER), protein efficiency ratio (PER) for control and mixture of wheat soy flour are shown in Table VII. Composite flour treatments significantly (p > 0.05) affect the food intake, protein intake, gain in body weight and PER values but have non-significant affect (p > 0.05) on the FER with respect to control diet (whole wheat flour). Wheat soy mixtures containing HTFFSF and HTDFSF showed significant (p > 0.05) higher values for all the parameter indicating the protein quality which are evident that heat treatment of soybean increase the quality of protein as compare to raw soybeans. Rackis (1981) stated low protein quality for the food supplemented with raw soybeans as compare to heat treated soybean. It is evident from the data depicted in Table 6 that soy supplementation of cereal foods increase the protein digestibility. Flour mixture containing soy flour has higher PER values as compare to wheat flour PER (2.30). This may be attributed to higher level of lysine content in soy flour which balances the amino acid profile wheat flour to improve its digestibility. These findings are in line with those of Lindell and Walker (1984) whom prepare chapattis from cereals blended with different levels of soy flour for nutrition improvement and found higher digestibility for cereal soy mixes as compare to cereals, Total Digestibility, net protein utilization and biological value for the protein of chapattis prepared from wheat-soy blends varied significantly (p > 0.05) as given in Table 7. Higher values of these parameters were fond for wheat-soy flour mixes containing heat treated full fat soy flour followed by heat treated defatted soy flour. Fat content of flour in case of full fat soy flour increase the protein digestibility, net protein utilization and biological value as apparent from the data. Maqbool et al. (1987) stated increased PER, NPU and BV for the rotis wheat flour supplemented with soy flour as compare to control rotis prepared from wheat flour. Parallel results were avowed by Begum and Kupputhai (1993) that soybean flour is an excellent source of protein and can be used to supplement cereal-based diets for the improvement in protein quantity and quality. Baskaran et al., (2004) prepared supplemented food based on cereals (wheat, ragi, bajra and sorghum) and Legumes (Soy and Bengal gram) and assessed their biological value in comparison with skim milk powder (SMP). It was sated that all supplemented foods were nutritionally and biologically as good as SMP with regard to proximate composition, PER and NPU.

Table VII. Biological evaluation of chapattis prepared from wheat soy mixtures

Parameters	T ₀	T ₁	T_2	T ₃	T ₄
Feed Intake	132.29e	137.51c	143.34a	136.87d	141.93b
Protein Intake	13.23d	13.76c	14.33a	13.69c	14.19b
Body weight	30.43e	34.88c	39.41a	32.84d	36.90b
FER	0.23e	0.25c	0.28a	0.24d	0.26b
PER	2.30d	2.50bc	2.75a	2.39cd	2.60b
TD	83.27e	84.91c	86.67a	84.21d	85.68b
NPU	53.55e	60.50c	68.38a	59.60d	67.34b
\mathbf{BV}	64.30e	71.25c	78.90a	70.77d	78.60b

CONCLUSION

In light of results obtained from the study it may be concluded that substitution of wheat flour with heat treated soy flour whether defatted or full fat up to an amount equivalent to 10% produced acceptable and nutritional superior chapattis than the traditional wheat flour chapattis

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