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Biochemical and Nutritional Evaluation of Unleavened Flat Breads Fortified with Healthy Flaxseed

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ABSTRACT

Flaxseed flours were used to fortify the unleavened flat breads to improve their nutritional and biological properties. Replacement of whole wheat flour (WWF) with varying levels (4-20%) of full fat (FFF) and partially defatted flaxseed flour (PDF) was done. The resultant flours showed higher contents of crude protein and fibers as the level of flaxseed substitution was increased. The sensory evaluation of unleavened flat breads revealed that addition of up to 12% FFF and 16% PDF resulted in acceptable products. The unleavened flat breads with maximum levels of flaxseed acceptability (containing 12% FFF & 16% PDF) were analyzed for their dietary fiber and essential amino acid contents. The level of soluble, insoluble and total dietary fiber and essential amino acids were higher in unleavened flat breads with added flaxseed when compared with control (prepared from 100% WWF). The protein quality parameters of the selected unleavened flat breads were assessed using Albino rats. It was noticed that the net protein utilization (NPU), biological value (BV), protein efficiency ratio (PER) and true digestibility (TD) improved significantly ($p \leq 0.05$) when diets containing flaxseed supplemented unleavened flat breads were fed to rats. The diet prepared from unleavened flat bread containing 16% PDF was biologically superior among other experimental diets due to 84.6±1.97% TD, 54.65±1.85% NPU, 1.87±0.0.08 PER and 64.6±2.06% biological value. Thus, addition of flaxseed in unleavened flat breads improved their nutritional and biological value. © 2012 Friends Science Publishers

Key Words: Flaxseed; Unleavened flat bread; Amino acid profile; Protein quality; Sensory evaluation

INTRODUCTION

The maintenance of good health is primarily dependent upon the utilization of good quality proteins. The proteins from animal sources are thought to be superior in quality than the proteins obtained from vegetable sources (Salcedo-Chávez *et al.,* 2002). Protein malnutrition is a common problem in the major population segments of the developing countries due to consumption of more plant proteins which are imbalanced in their amino acid profile (Rangel *et al.,* 2003). Cereal based diets are responsible to divest the indispensable amino acids (Seena *et al.,* 2006). A survey of the world wheat collection has revealed that total lysine variation in common wheat protein was no more than 0.5%, which was less than needed for nutritional balance (Johnson *et al.,* 1985). Among different amino acids, lysine is considered to be the first limiting amino acid in cereal products. The gap between the protein requirement and supply can be abridged by amino acid fortification, uses of protein mixtures (protein supplementation & complementation), genetic modification of food crops and identification and evaluation of underexploited sources (Egbe & Akinyele, 1990). Several factors affect the definitive quality of protein *in vivo*. The quality of proteins is best judged by their biological evaluation (Sogi *et al.,* 2004). The amino acid content and profile is a critical determinant of protein quality and most of the methods which measure protein quality are directly or indirectly related to the efficacy with which they can satisfy amino acid requirements (Srikantia, 1981). The technology of composite flour preparation is initially referred to the process of blending of wheat flour with legume and cereal flours for making biscuits and bread. However, this declaration can also be used with respect to blending of other non wheat flours like roots, tubers or other raw materials (Dendy, 1992). The legume proteins have been used widely for many years in the food industry as a food product constituent to improve the nutritional value of foods because of their better amino acids profile (Horax *et al.,* 2004). The bakery products can be improved in their nutritional profile by using the composite flour technology. Shehata and Fryer (1970) concluded that rats fed on diets

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with 10 and 20% chickpea flour showed protein efficiency ratios of 1.46 and 1.56, which were better than that of diets with 0% chickpea flour.

The flax (*Linum usitatissimum*) is a blue flowering rabi crop and is a member of family Linaceae, commonly known as "*Alsi*" (Gujrati, Hindi & Punjabi). The proximate composition of flaxseed indicates that it contains 30% protein, 35% lipids and 35% fiber; though it varies depending upon the seed variety, cropping year, cropping location and environmental conditions (Bajpai, 1985). Due to health promoting properties and excellent nutrient profile of flaxseed, it has become a popular candidate for incorporation in human diet. Flaxseed is being used extensively for the development of functional foods. The components of flaxseed, identified to exhibit the health benefits are fiber, lignans and linolenic acid (Omega-3 fatty acid). Moreover, flaxseed is a good source of high quality protein, soluble fibers and phenolic compounds (Oomah, 2001). Lysine is the first limiting amino acid in wheat flour; whereas, in flaxseed flour it has been reported as 6.8 mg/100 g of protein (Dev & Quensel, 1986), which is higher than that in soy flour, i.e., 5.8 mg/100 g of protein (Friedman & Levin, 1989). Wheat is the cheapest source of protein and calories for the inhabitants of IndoPak sub continent and also used worldwide. The quality of wheat based products can be improved by supplementing them with other cheap and healthy plant based food materials. The addition of flaxseed flour in unleavened flat breads can be helpful for improving the biological parameters. This paper describes the nutritional importance of flaxseed in unleavened flat breads with special reference to the improvement in the quality attributes and biological parameters.

MATERIALS AND METHODS

Collection and preparation of raw material: Two types of food grains were used in the study. The whole wheat flour was used as a base material for the preparation of composite flours with flaxseed. The wheat variety SH 2002 was purchased from Wheat Research Institute, Faisalabad and Chandni variety of flaxseed was purchased from Oil Seeds Research Institute, Faisalabad, Pakistan. The cleaning of flaxseed and wheat grains was performed manually to remove damaged seeds, dust particles, seeds of other grains/crops and other impurities such as metals and weeds. The flaxseed grains were roasted in the household microwave oven for 2.5 min with 480 W output under the operating frequency of 2450 MHz by following the method described by Yang *et al.* (2004). Flaxseed subjected to roasting through microwave processing was divided into two parts. Half of the roasted flaxseed grains were partially defatted by using the screw type mechanical press. The whole wheat flour and flaxseed flours were prepared by milling through pin mill.

Preparation of composite flour blends: The whole wheat flour (WWF) was mixed with partially defatted flaxseed flour (PDF) and full fat (FFF) flaxseed flour @ 4, 8, 12, 16 and 20% to get the different types of composite flours. The whole wheat flour was used as a control in the different experiments. All types of flours were tightly packed and stored below 5◦C.

Chemical composition of composite flour blends: The moisture, ash, crude fat, crude fiber and crude protein contents in composite flours were determined according to the methods of AACC (2000). The peroxide value of samples was determined according to the method described by Kirk and Sawyer (1991).

Preparation and sensory evaluation of unleavened flat breads: The unleavened flat breads were prepared from whole wheat composite flours following Haridas *et al.* (1986). The dough for unleavened flat bread was made by mixing 200 g of whole wheat flour with predetermined quantity of water for 3 min and allowed to rest for 30 min. A dough piece weighing 80 g was rolled on a sheet of 2 mm thickness with a wooden roller pin on a specially designed wooden platform and cut into circle of 17 cm diameter. The unleavened flat breads were baked on thermostatically controlled hot plate at a temperature of 210°C for 1.5 minutes. The unleavened flat breads were presented to a trained panel of six judges and the evaluation for sensory parameters such as color, taste, aroma, chewing ability, foldability and overall acceptability characteristics were carried out using a 9 point hedonic scale following Land and Shepherd (1988).

Analysis of unleavened flat breads (Dietary fiber & amino acid profile): The selected unleavened flat breads (containing 12% FFF & 16%PDF) were analyzed for total, soluble and insoluble dietary fiber contents by gravimetric kits method (AACC, 2000) i.e., Methods No. 32-05, 32-07 and 32-20, respectively by employing the Megazyme Assay Kit (Megazyme International, Ireland Ltd; Wicklow, Ireland). The profile of essential amino acids in each selected product was determined by using ion-exchange chromatography with automatic Amino Acid Analyzer, (Hitachi L8500, Tokyo, Japan) following (Adeyeye & Afolabi, 2004). Two grams of ground product samples were defatted through soxhlet extraction methods. The defatted sample were re-dried and milled into fine powder. The 30 mg ground sample was weighed into glass ampoules and 5 mL of 6 M HCl and 5 μmol norleucine were added. The ampoules were evacuated with liquid nitrogen and sealed with burner flame and hydrolyzed in an oven at 110°C for 24 h. The ampoules were cooled, broken at the tip and the contents were passed through filter. The filtrates were dried in rotary evaporator at 40°C under vacuum. The residues were dissolved to 5 μL (for acid & neutral amino acids) or 10 μL (for basic amino acids) with acetate buffer, pH 2.2 and the solutions were dispensed into the cartridge of amino acid analyzer. The quantification was performed by comparing the peak area of each amino acid in the sample to the area of the corresponding standard amino acid of the protein hydrolysate.

Biological assay of selected unleavened flat breads: Three experimental diets were prepared for biological assay. All the diets were kept isonitrogenous and isocaloric. All the diets contained 10% protein on dry weight basis. The casein diet (10%) was used as a reference diet. The protein free diet was also introduced to measure the metabolic faecal nitrogen. The detailed composition of the diets is presented in Table I. Twenty weanling albino rats (21 days old) were used for the biological assay of experimental diets. The rats were fed to a stock diet for one week so that they attain 50-58 g weight prior to experiment and then randomly divided into five groups i.e., four rats in each group. The rats were kept under 12 h light-dark cycle. The diets were randomly assigned to the experimental rat groups and were fed *ad libitum* for a period of ten days. The temperature of the animal room was maintained at 25±3°C. Each group of 4 rats was separately kept in wire screen mesh bottom; underneath each cage metal tray covered with a sheet of filter paper was placed. The composite weight of each group of the rats was recorded daily with top loading balance. At the end of 10 days i.e., expiry of the experiment, the faecal material from each group was collected and dried to a constant weight in an oven at temperature of 105°C and stored in polyethylene bags for further studies. The spilt food collected from each cage was also dried and then weight was recorded. At the end of the experiment, the rats were sacrificed by chloroform anesthesia. The skull and abdominal cavities were opened and whole body was dried in an oven at a temperature of 105°C till to a constant weight. The dried carcass was passed through electric grinder and stored for further studies. The nitrogen content in dried carcass was determined. The calculations for true digestibility (TD), net protein utilization (NPU), protein efficiency ratio (PER) and biological value (BV) were made by using the formulae of Miller and Bender (1955).

Statistical analyses: The data obtained for each parameter were subjected to statistical analyses using Minitab statistical package (Minitab Quality Companion, 2003). The level of significance ($p \leq 0.05$) at 0.05 alpha was determined by applying analysis of variance technique (one factor & two factor factorial CRD). Significant ranges were further postulated using DMR mean comparison test (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Chemical composition of composite flours: The chemical composition of composite flours differed significantly ($p \leq 0.05$) due to the supplementation of PDF or FFF in WWF. The contents of crude fiber, crude fat and crude protein increased as the level of supplementation of PDF or FFF was increased in the composite flours (Table II). The supplementation of flaxseed in wheat flour significantly ($p \leq 0.05$) improved the nutrient profile of the composite flours. Results of the present study supported the findings of Hussain *et al.* (2006) who have reported significant improvement in the proximate composition (ash, fat, crude protein & crude fiber) of full fat flaxseed flour supplemented whole wheat flours. According to Gambus *et al.* (2004), a 3% increase in linseed in the recipe for flax hermit cookies and a 5% rise in flax muffins resulted in an increase in the amount of proteins and dietary fiber contents (Frank & Sarah, 2006). In the present study, flaxseed flour supplementation into whole wheat flour improved the chemical constituents of the composite flours. This reflects the potential for use of flaxseed into wheat flour for enhancement of this nutrients/chemical constituent. The improvement in the chemical constituents such as protein, fat, fiber and was due to the fact that flaxseed flour had higher content (21.27% protein, 38.53% fat, 3.48% ash, 8.12% crude fiber) of these constituents.

Sensory evaluation of unleavened flat breads: The most important parameters of unleavened flat bread quality are colour, texture and flavor, which are evaluated as greater pliability, soft texture, and light creamish brown colour with small brown spots, fully puffed, slight chewiness and baked wheatish aroma (Haridas *et al.,* 1986; Shaikh *et al.,* 2007). The sensory parameters of unleavened flat breads supplemented with full fat and partially defatted flaxseed flours in the present study were affected significantly ($p \leq 0.05$) due to supplementation levels of flaxseed flour into whole wheat flour. Data presented in the Table III indicate that increase in supplementation level of FFF and PDF in the whole wheat flour significantly ($p \leq 0.05$) decreased the scores assigned to the taste, colour, aroma, chewability, foldability and overall acceptability of unleavened flat breads. The scores given to overall acceptability of unleavened flat breads declined significantly as the level of supplementation of PDF and FFF in whole wheat flour increased. The highest scores (8.08) for over all acceptability were given to unleavened flat breads prepared from 100% WWF (control). The overall acceptability scores of unleavened flat breads prepared from PDF supplemented WWF significantly ($p \leq 0.05$) decreased from 8.06 (control) to 4.61 (unleavened flat breads supplemented with 20% PDF). The unleavened flat breads supplemented below 20% level of PDF i.e., 4, 8, 12 and 16%, were found acceptable by the judges with respect to their overall acceptability scores. The results of the present study are supported by the earlier findings of Maheshwari and Devi (2005) who reported that addition of flaxseed in unleavened flat breads caused unacceptable changes in flavor, taste, and texture ultimately decreased overall acceptability scores of unleavened flat breads. Reduction in sensory score was also noticed due to addition of dietary fiber like guargum and pectin by Butt *et al.* (2007). The colour scores of unleavened flat breads decreased as the level of flaxseed supplementation increased. The darker brown colour of flaxseed flours may be one of the main reasons to impart dark colour of flaxseed supplemented unleavened flat breads. The judges disliked the darker colour unleavened flat breads and assigned lower scores. The chew ability and

Table I: Composition of experimental diets

Diets	Unleavened flat breads (g)	Corn starch (g)	Glucose (g)	Corn oil (g)	Mineral mix (g)	Vitamin mix (g)	Casein (g)	Total (g)	C.P (g)
D_1	82.30	4.7	4	4	3	2		100	10
D_2	66.23	20.77	4	4	3	2		100	10
D_3	76.63	10.37	4	4	3	2		100	10
Casein	-	76	4	4	3	2	10	100	10
No protein	-	87	4	4	3	2		100	0.05

D_1= Diet prepared from Unleavened flat bread (100% whole-wheat flour); D_2= Diet prepared from Unleavened flat bread (16% partially defatted flaxseed flour); D_3= Diet prepared from Unleavened flat bread (12% full fat flaxseed flour); Casein= Reference diet; No protein= Protein free diet

Table II: Chemical composition of flaxseed supplemented whole wheat flours

% Flaxseed flour	Moisture %		Ash %		Crude fat %		Crude fiber %		Crude protein %		POV (mEq/Kg)	
	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF
0	8.63± 0.37a	8.63± 0.37a	1.53± 0.07f	1.53± 0.07f	1.81± 0.05d	1.81± 0.05f	2.15 ± 0.08f	2.15± 0.08f	12.21± 0.61f	12.21± 0.61c	0.64± 0.03a	0.64± 0.03a
4	8.50± 0.25a	8.28± 0.25a	1.68± 0.07e	1.60± 0.06e	1.94± 0.03d	3.42± 0.14e	2.53± 0.05e	2.41± 0.05e	13.10 ± e	12.53± 0.5c	0.65± 0.07a	0.69± 0.06a
8	8.42± 0.24a	8.27± 0.39a	1.84± 0.08d	1.69± 0.09d	2.09± 0.06c	4.69± 0.06d	2.89 ± 0.05d	2.64± 0.06d	13.98± 0.48d	13.03± 0.34bc	0.66± 0.03a	0.72± 0.05a
12	8.43± 0.35a	8.25± 0.28a	1.97± 0.06c	1.75± 0.02c	2.21± 0.05 bc	6.17± 0.33c	3.28 ± 0.09c	2.86± 0.07c	14.82± 1.03c	13.42± 0.38ab	0.69± 0.02a	0.74± 0.02a
16	8.27± 0.36a	8.20± .30a	2.14± 0.07b	1.84± 0.06b	2.33± 0.08ab	7.63± 0.50b	3.64 ± 0.04b	3.18± 0.10b	15.74± 0.97b	13.74± 0.45ab	0.72± 0.06a	0.75± 0.04a
20	8.21± 0.28a	8.18± 0.35a	2.30± 0.08a	1.93± 0.08a	2.44± 0.10a	9.06± 0.52a	4.08± 0.06a	3.33± 0.09a	16.64± 1.04a	14.16± 0.84a	0.73± 0.04a	0.78± 0.04a

Values with different alphabets in the columns differ significantly ($p \leq 0.05$); WWF= Whole wheat flour; FFF= Full fat flaxseed flour; PDF= Partially defatted flaxseed flour

Table III: Sensory characteristics of unleavened flat breads prepared from flaxseed supplemented whole wheat flours

% Flaxseed flour	Colour		Taste		Aroma		Chewability		Foladbaility		Overall acceptability	
	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF	WWF+ PDF	WWF+ FFF
0	7.94± 0.14a	7.94± 0.14a	7.72± 0.13a	7.72± 0.13 a	8.22± 0.12 a	8.22± 0.12 a	7.89± 0.16a	7.89± 0.16a	8.06± 0.18a	8.06± 0.18a	8.06± 0.11a	8.06± 0.11a
4	7.11± 0.10b	6.78± 0.13b	6.89± 0.14b	6.67± 0.12b	7.39± 0.14b	6.83± 0.12b	7.33± 0.12ab	7.17± 0.13ab	7.50± 0.16a	7.33± 0.14ab	7.17± 0.09b	7.17± 0.10b
8	6.78± 0.13bc	6.50± 0.10b	6.22± 0.16c	5.89± 0.09c	6.78± 0.12c	6.28± 0.14c	6.61± 0.14bc	6.83± 0.10bc	6.72± 0.18b	6.67± 0.15bc	6.72± 0.17c	6.61± 0.14c
12	6.33± 0.08cd	5.78± 0.12c	6.00± 0.14c	5.61± 0.15c	6.06± 0.10c	6.06± 0.13c	6.17± 0.13bc	6.17± 0.16cd	6.28± 0.16bc	6.11± 0.13cd	6.11± 0.10d	5.78± 0.13d
16	6.00± 0.11d	5.22± 0.09cd	5.44± 0.08d	5.06± 0.10d	5.33± 0.15d	5.22± 0.16d	5.78± 0.10c	5.50± 0.18 de	5.72± 0.18cd	5.39± 010de	5.33± 0.13e	4.89± 0.13e
20	5.22± 0.13e	4.89± 0.11d	5.11± 0.08 d	4..56± 0.12e	5.00± 0.13e	4.56± 0.17e	5.50± 0.15c	5.11± 0.16e	5.28± 0.17d	4.94± 0.16e	4.61± 0.14f	4.39± 0.15f

Values with different alphabets in the columns differ significantly ($p \leq 0.05$); WWF= Whole wheat flour; FFF= Full fat flaxseed flour; PDF= Partially defatted flaxseed flour

foldability scores of unleavened flat breads also decreased due to higher levels of flaxseed supplementation in WWF that might be due to coarser texture of flaxseed flour and reduction in the gluten contents of unleavened flat breads. The taste, aroma and overall acceptability scores of unleavened flat breads also declined by the addition of higher levels of PDF and FFF in WWF. The FFF supplemented unleavened flat breads got less scores as compared to PDF supplemented unleavened flat breads that might be due to slight increase in the moisture content and development of rancid flavour in flours due to presence of oil.

Dietary fiber contents of unleavened flat breads: The results presented in Table IV indicate that total dietary fiber (TDF), soluble dietary fiber (SDF) and non soluble dietary fiber (NSDF) varied significantly ($p \leq 0.05$) among different unleavened flat breads due to differences of flaxseed supplementation levels in wheat flours. The total dietary fiber content was found to be significantly ($p \leq 0.05$) the highest (17.45%) in unleavened flat breads supplemented with 16% PDF as compared to other unleavened flat breads. The unleavened flat breads prepared from 100% WWF possessed significantly ($p \leq 0.05$) the lowest (12.64%) content of TDF. It is obvious from the results that

Table IV: Dietary fiber and fatty acid contents (g/100 g) of selected unleavened flat breads

Unleavened flat breads	TDF	SDF	NSDF
C_0	12.64±0.57c	1.74±0.10c	10.90±0.36b
C_1	17.45±0.46a	3.98±0.14a	13.47±0.39a
C_2	14.58±0.51b	2.68±0.12b	11.90±0.43b

Values with different alphabets in the columns differ significantly ($p \leq 0.05$); C_0= Control unleavened flat bread (100% WWF); C_1=Selected unleavened flat bread 1 (16% PDFF+ 84%WWF); C_2= Selected unleavened flat bread 2 (12% FFFF+ 88%WWF); TDF= Total dietary fiber; SDF= Soluble dietary fiber; NSDF= Non soluble dietary fiber

Table V: Essential amino acid contents (g/100 g) of selected unleavened flat breads

Unleavened flat breads	Lysine	Isoleucine	Leucine	Threonine	Valine	Histidine	Methionine	Arginine
C_0	0.46±0.03c	0.61±0.05c	1.11±0.10c	0.49±0.09d	0.75±0.05c	0.39±0.01c	0.26±0.0c	0.78±0.05c
C_1	1.03±0.04a	1.24±0.03a	1.92±0.10a	1.13±0.10a	1.45±0.08a	0.78±0.2a	0.58±0.01a	2.36±0.10a
C_2	0.93±0.06ab	1.15±0.06ab	1.82±0.08ab	1.04±0.03b	1.36±0.06ab	0.73±0.02ab	0.53±0.02b	2.14±0.08b

Values with different alphabets in the columns differ significantly ($p \leq 0.05$); WWF= Whole wheat flour; FFF= Full fat flaxseed flour; PDF= Partially defatted flaxseed flour; C_0= Control unleavened flat bread (100% WWF)
C_1: Selected unleavened flat bread 1 (16% PDFF+ 84%WWF)
C_2: Selected unleavened flat bread 2 (12% FFFF+ 88%WWF)

Table VI: Protein quality parameters of unleavened flat breads based diets fed to albino rats

Diets	Feed intake g (10 days/group)	Weight gain g (10 days/group)	TD	NPU	PER	BV
D_1	170	48.54	79.4±2.03c	44.3±1.92d	1.51±0.10d	55.79±1.75d
D_2	201.6	61.43	84.6±1.97b	54.65±1.85b	1.87±0.08b	64.6±2.06b
D_3	215.5	58.84	81.45±2.04bc	49.4±1.32c	1.8±0.0.13c	60.65±1.51c
Casein	200.5	63.4	90.8±2.31a	76.87±2.05a	2.76±0.11a	84.66±1.62a

Values with different alphabets in the columns differ significantly ($p \leq 0.05$); D_1= Diet prepared from unleavened flat bread (100% whole wheat flour); D_2= Diet prepared from unleavened flat bread (16% partially defatted flaxseed flour); D_3= Diet prepared from unleavened flat bread (12% full fat flaxseed flour); TD= True digestibility; NPU= Net protein utilization; PER= Protein efficiency ratio; BV = Biological value

significantly ($p \leq 0.05$) the highest (3.98%) soluble dietary fiber (SDF) and non soluble dietary fiber (13.47%) was recorded in unleavened flat breads containing 16% partially defatted flaxseed flour. The unleavened flat breads from both PDF and FFF supplemented composite flours yielded significantly ($p \leq 0.05$) higher contents of soluble, non soluble and total dietary fibers. Flax Council of Canada reported that dietary fiber is a major form of non digestible carbohydrates in flaxseed. Institute of Medicine (2002) has reported that one-third of the flaxseed fiber is soluble, while remaining two-thirds is insoluble which aids digestion. The addition of defatted rice bran, flaxseed oilseed cake improved the dietary fiber contents of breads (Ajmal *et al.,* 2006; Ogunronbi, 2007). The results of the present study are in line with the earlier studies conducted by Gambus *et al.* (2004) who reported that dietary fiber contents of breads, muffins and cookies improved due to the supplementation of flaxseed in wheat flours. The findings of the above mentioned researchers indicated that flaxseed is rich in soluble, non soluble and total dietary fibers, and the addition of flaxseed resulted in the improvement of dietary fiber contents of products in which it is present. Thus, improvement in the dietary fibers contents (TDF, SDF & NSDF) of unleavened flat breads observed during the present study may be attributed to the higher contents of dietary fibers of flaxseed.

Amino acid profile of unleavened flat breads: The unleavened flat breads prepared from 16% PDF supplemented WWF yielded significantly ($p \leq 0.05$) the highest content of lysine (1.03 g/100 g flour) followed by the unleavened flat breads prepared form 12% FFF supplemented WWF i.e., 0.93 g/100 g flour. The unleavened flat breads prepared from 16% PDF supplemented WWF possessed significantly ($p \leq 0.05$) the highest content of isoleucine (1.16 g/100 g flour). The threonine, valine, histidine, methionine and arginine contents were found significantly ($p \leq 0.05$) higher which were 1.13 g/100 g, 1.45 g/100 g, 0.78 g/100 g, 0.58 g/100 g and 2.36 g/100 g, respectively in unleavened flat breads prepared from 16% PDF supplemented WWF. The results in Table V indicated that contents of all the tested essential amino acids were improved substantially by the supplementation of PDF and FFF in the wheat flours. It is also evident that flaxseed supplemented unleavened flat breads possessed higher contents of all the essential amino acids than the control unleavened flat breads. The cereal based diets are responsible to divest the indispensable amino acids (Seena *et al.,* 2006). Total lysine variation in common wheat protein has not been found more than 0.5%, which is less than needed for nutritional balance (Johnson *et al.,* 1985). The role of essential amino acids is very important in the diet as these cannot be synthesized in the body but must be provided by the diet. Nine essential amino acids: histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are required by humans (Sinclair, 2005). The profile of essential amino acids of flaxseed is similar to the amino acid profile of soy, which is balanced as compared to most of other plants proteins (Youle & Huang, 1981). The essential amino acids components of flaxseed has been found as lysine 4.0 mg, valine 4.6 mg, histidine 2.2 mg, methionine 1.5 mg, arginine 9.2 mg, leucine 5.8 mg and isoleucine 4.0 mg/100 g of protein. The composition of same amino acids of soy as compared to flaxseed have been reported as lysine 5.8 mg, valine 5.2 mg, histidine 2.5 mg, methionine 1.2 mg, arginine 7.3 mg, leucine 7.7 mg and isoleucine 4.7 mg/100 g of protein (2003). Madhusudhan and Singh (1985) reported that flaxseed is good in lysine, cystein, glutamic acid and glycine. The present study showed that supplementation of PDF and FFF in unleavened flat breads improved substantially the level of essential amino acids especially lysine which is first limiting amino acid in cereals including wheat as observed in case of control unleavened flat breads. It is obvious that supplementation of PDF in unleavened flat breads resulted in more improvement of essential amino acids as compared to FFF supplementation though in both the cases, improvement was evident. The PDF containing more contents of essential amino acids contributed more towards increasing the level of amino acids as compared to FFF. The results of the present study suggested that use of flaxseed either partially defatted or full fat in the wheat flour enhanced the level of all the eight determined essential amino acids.

Biological assay of protein quality: The results regarding rats feeding trials to evaluate the protein quality of flaxseed supplemented unleavened flat breads compared to reference diet (casein) are presented in Table VI. The results in general revealed that feed intake was higher in those groups of rats with added unleavened flat breads containing flaxseed flours in their diet plan. Similarly, the weight gain was more in those groups fed 61.43 g/10 days/group and 58.84 g/10 days/group as compared to 48.54 g/10 days/group i.e., control diet. Significant differences were observed for TD, NPU, PER and BV among different groups of rats fed on experimental and reference diets when compared to control. True protein digestibility is measured through rats balance assay by measuring nitrogen in food and feces (McDonough *et al*., 1990). The results of the present study provided useful information that there is a significant ($p \leq 0.05$) improvement in the nutritive value of unleavened flat breads as a result of flaxseed flour supplementation (either full fat or partially defatted) in whole wheat flours. The true digestibility increased significantly ($p \leq 0.05$) with the increase of flaxseed flours in composite flours and this increase can be due to the excellent profile of essential amino acids possessed by flaxseed flours as compared to wheat flours. Youle and Haung *et al.* (1981) observed flaxseed protein is rich in lysine than wheat proteins. The results of the present study demonstrated that increase in flaxseed flour supplementation in the diets improved substantially the NPU value. This increase in NPU may be due to the presence of higher amounts of essential amino acids, particularly lysine and threonine, which are limiting in the wheat flour. The improvement in NPU with lysine has been documented by Estevez *et al.* (1987), who reported increase in NPU from 52.35% to 67.50% by the incorporation of 15% chickpea flour in the cookies formulation. The protein efficiency ratio is dependent on essential amino acids as well as the ability of human or animal to digest and utilize these amino acids. It has been reported by Cheeke (2005) that flaxseed incorporation enhanced the protein efficiency ratio when added at different levels into the wheat flour and fed to the rats. The results of the present study support the findings of Ogunronbi (2007) who described that protein efficiency ratio of diet improved with the incorporation of flaxseed flour. The biological value is a measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism's body. The improvement in biological value of diets containing flaxseed flour is because of the higher content of essential amino acids. The increase in BV of diets by the incorporation of flaxseed flours is well supported by Ogunronbi (2007) who reported higher biological value of flaxseed flour, which supports the results of present study. The improvement in biological value supports the facts that the pattern of limiting amino acids in wheat flour improved as a result of supplementation of flaxseed in wheat flour.

CONCLUSION

The true digestibility, net protein utilization, concentration of essential amino acids and dietary fiber were significantly improved due to inclusion of flaxseed in the diets. Thus, acceptable unleavened flat breads can be prepared from 12% FFF supplemented WWF and 16% PDF supplemented WWF, which can provide more nutrition and health benefits. The findings may help improving the malnutrition problem especially due to protein quantity and quality in the diets of developing countries.

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