PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 12 (5): 441-447, 2013
ISSN 1680-5194

Variation in the Levels of B-Vitamins and Protein Content in Wheat Flours

Madiha Rohi, Imran Pasha, Masood Sadiq Butt and Haq Nawaz
National Institute of Food Science and Technology, Institute of Animal Nutrition and Feed Technology,
University of Agriculture, Faisalabad, Pakistan

Abstract: Wheat is a vital crop having imperative nutrients for human diet e.g., protein and certain B-vitamins (thiamin, riboflavin, niacin and pyridoxine), well-intentioned for body metabolic processes. These vitamins are concentrated in grain outer layer thus milling process affects these vitamins to a larger extent and may reduce their level. The aim of the present study is to determine the level of protein and B-vitamins in whole wheat and straight grade flours of different spring wheats. Ten spring wheat varieties were procured from different parts of the country, milled and quantified. The protein content was determined by Kjeldhal's method and B-vitamins were analyzed through High Performance Liquid Chromatography (HPLC) using UV-VIS detector. Results revealed that the level of protein as well as the B-vitamins was high in whole wheat flours of spring wheats as compared to straight grade flour. Only 3, 15, 6 and 6% of thiamin, riboflavin, niacin and pyridoxine of whole wheat flour was detected in straight grade flour. Simple Pearson's correlation has been studied among wheat B-vitamins and its protein content which exhibited that in whole wheat flour a positive correlation for protein content with thiamin (r = 0.54), riboflavin (r = 0.07) and niacin (r = 0.22) whereas in straight grade flour protein concentration was examined to be positively correlated with riboflavin concentration (r = 0.19) only. Conclusively, the results of the current study are useful for different stake holders for their intended uses.

Key words: B-vitamins, thiamin, riboflavin, niacin, pyridoxine, HPLC

INTRODUCTION

An important part of human diet is composed of cereal grains products. Thus human being obtain ample nutrients from these products like carbohydrates, dietary fibers, fats, proteins, B-vitamins and a variety of minerals (Beaugrand *et al*., 2004). All these nutrients are being included in dietetic formulations for the purpose of prevention and treatment of various ailments. These food commodities are highly essential for modern food industry for producing a variety of functional food ingredients (Jenkins *et al*., 2008). B-vitamins rich food commodities like whole grain cereals and whole grain breads, vegetables and fruits should be consumed in diet. Cereals especially wheat, are one of the essential food produces having nutrients e.g., proteins, together with B-vitamins (Lebiedzinska and Szefer, 2006).

Wheat (*Triticum aestivum* L.) is an imperative food crop cultivated worldwide for human consumption. It provides energy and vital nutrients to people. It is staple diet providing more than 60% dietary nutrients for daily requirements. Wheat occupies largest area and contributes maximum production as compared to other food grain crops worldwide. In Pakistan, the area under wheat cultivation is 8, 666 thousand hectare with an estimation of 23, 517 thousand tonns production and average wheat grain yield is 2, 714 kg per hectare (Government of Pakistan (GOP), 2011-12).

Wheat is considered as one of the best protein sources but it is deficient of some essential amino acids exclusively methionine, lysine and tryptophan (Singh *et al*., 2000; Sliwinski *et al*., 2004). There are certain factors which are responsible for the amount of protein content in wheat grain, these factors include wheat cultivar types and various environmental conditions (OECD, 2003). Wheat kernels have 8 to 15% of protein contents and protein represents to be present as second major component of wheat grain. On the basis of protein the wheat can be easily characterized into its basic classes; the soft wheat or hard wheat (Slaughter *et al*., 1992). The protein content is not simply a feature which assesses the end use property but it can also affect the baking characteristics of hard wheat flour (Orth and Bushuk, 1972) and soft wheat flour (Kaldy and Rubenthaler, 1987). Wheat nutritional value as well as dough rheological properties can be determined by the protein contents of wheat flour (Payne *et al*., 1987).

Wheat based products are major source of nutrients for people of many regions of world (Hussain *et al*., 2012). Wheat is generally acknowledged to be a fine source of numerous B-vitamins especially thiamine, niacin, pyridoxine and folate. B-vitamins are categorized as water soluble molecules as these molecules are vital components for metabolism of carbohydrates, proteins and fats. Thiamin, riboflavin and pyridoxine play

Corresponding Author: Madiha Rohi, National Institute of Food Science and Technology, Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan

important role in breakdown and synthesis of carbohydrates, proteins and fat respectively as these are co-enzymes of many metabolic pathways (WHO (World Health Organization), 2000; Batifoulier *et al.*, 2005).

The concentration of B vitamin in wheat grains are affected by certain parameters i.e., wheat variety, growing locality, soil type, use of fertilizers and herbicides and year crop production (Pomeranz, 1988). In addition, milling process is also an important parameter that affects B-vitamin concentrations of wheat grain (Adrian and Petit, 1970). Wheat grains constitute about 2.2-6.3 µg/g thiamine, 0.8-2.2 µg/g Riboflavin, 19-64 µg/g niacin and 1.3-7.5 µg/g riboflavin (Serna-Saldiver, 2005; Piironen *et al.*, 2006).

On removal of wheat bran more refining of the grains are attained. Consumption of these refined products is coupled with the appearance of micronutrients marginal deficiencies e.g., thiamine deficiency called beri beri (Hercberg *et al.*, 1994). Attenuation in cereal utilization in daily diet is another main cause of these deficiencies (Bertrais *et al.*, 2000). A change in lifestyle cause marginal vitamin B deficiencies along with others disorders i.e., the increased incidences of obesity, cancers and cardio-vascular diseases (Adachi *et al.*, 2000). One of the interesting methods to avoid these deficiencies is the selection and utilization of wheat cultivars with a high nutritional quality, e.g., whole grain products (Indrani *et al.*, 2011).

B-vitamins in wheat grains are correlated with proteins and ash contents of grains (Keagy *et al.*, 1980). Riboflavin level of the wheat flour has no correlation with protein but at the same time thiamin and pyridoxine have strong correlation with flour protein content. Level of B-vitamins in wheat grain, its flour and breads can be determined by various methodologies. Microbiological assay is one of the extensively used techniques for the quantification of water soluble vitamin, e.g., pyridoxine, cyanocobalamin, biotin, niacin, folic acid and pantothenic acid in foodstuffs and some other biological samples (Tsukatani *et al.*, 2011). The levels of B-vitamin have been quantified conventionally by thiochrome method and microbiological assay but HPLC (High Performance Liquid Chromatography) methods permit a highly precise and correct analysis of B-vitamin for quantitative and qualitative determination (Batifoulier *et al.*, 2005).

A lot of research work has already been performed with respect to the characterization and chemical profiling of spring wheats. But there is no data available regarding B-vitamin quantification of spring wheats. This piece of research work is expanded to the B-vitamin and protein quantification in whole wheat flour and wheat fine flour (straight grade flour). Further the interaction of protein content with the B-vitamins has also been determined and the B-vitamin losses from whole flour to fine flour has been quantified.

MATERIALS AND METHODS

Raw material procurement: Ten commercially available varieties of wheat were procured, seven varieties (AARI-11, Millat-11, Punjab-11, Fsd-08, Lasaani-08, Sahar-06 and Shafaq-06) from Wheat Research Center, Ayub Agricultural Research Institute, Faisalabad and three varieties (AAS-11, Mairaj-08, Fareed-06) from Regional Agriculture Research Institute (RARI), Bahawalpur. All standards and chemicals were procured from Sigma Aldrich and commercial local market.

Milling of grains: Wheat grains were cleaned manually to remove damaged seeds, dust particles, seeds of other crops and all impurities. Then grains of each wheat variety free from dockage and foreign matter were subjected to tempering at 14.5% moisture level. The tempering of wheat was carried out in plastic containers at room temperature for 24 hours in order to equilibrate the moisture within the grains. The amount of water required for tempering was calculated as per procedure given in (AACC, 2000) method No. 26-95.

$$AWR = \left[\frac{100 - OM\ (\%)}{100 - DM\ (\%)}\right] - 1\ X\ \text{weight of sample}$$

Where:
AWR = Amount of water (ml) required
OM = Original moisture
DM = Desired moisture

The tempered wheat was milled through Brabender Quadrumate Senior Mill (C.W. Brabender Instruments, Inc.) to obtain straight grade flour i.e., combination of break and reduction flour, according to AACC (2000) method No. 26-21A. All wheat varieties were milled separately to prepare whole wheat flour by using China chakki (grinder).

Protein quantification: Whole wheat and straight grade flours were analyzed for their protein content according to AACC (2000) method No 46-10.

Determination of B vitamins: Wheat milling fractions and WWF from different varieties was analyzed for thiamin, riboflavin, niacin and pyridoxine. For determination of these B vitamins five steps were performed.

First of all standards were prepared and for each B vitamin standard preparation was carried out separately according to the method of (Aslam *et al.*, 2008).

Thiamin standard solution:

- Stock solution-Dissolve 26.7 mg of thiamine hydrochloride in 25 ml of distilled water
- Working standard-1.068 mg/ml and 562 µl was taken in 10 ml volumetric flask

Riboflavin standard solution:

- Stock solution-Dissolve 6.9 mg of riboflavin in 100 ml of extraction solution
- Working standard-0.069 mg/ml and 5.1 ml was taken in 10 ml volumetric flask

Niacin standard solution:

- Stock solution-Dissolve 41.5 mg of nicotinamide in 25 ml of distilled water
- Working standard-1.66 mg/ml and 60.5 µl was taken in 10 ml volumetric flask

Pyridoxine standard solution:

- Stock solution-Dissolve 20.8 mg of pyridoxine hydrochloride in 25 ml of distilled water
- Working standard-0.832 mg/ml and 72.2 µl was taken in 10 ml volumetric flask

After preparation of standards buffer was prepared by dissolving 1.08 g of hexane sulphonic acid sodium salt and 1.36 g of potassium dihydrogen phosphate in 940 ml of HPLC water. Then 5 ml of triethylamine was added to it and the pH was adjusted to 3.0 with orthophosphoric acid. For the Preparation of mobile phase Buffer and methanol were mixed with a ratio of 96:4 and filtered through 0.45 µ membrane filter and degassed by using helium gas. Then the extraction solution was prepared by mixing 50 ml of acetonitrile with 10 ml of glacial acetic acid and its volume was made up to 1000 ml with double distilled water.

Sample preparation: Weigh 10 g of homogenized sample and transferred into conical flasks. Add 25ml of extraction solution in the sample. Kept samples along with extraction solution on shaking water bath at 70°C for 40 min. Allow sample to cool down and then filter this solution. Finally make volume up to 50 ml with extraction solution (Aslam *et al.*, 2008).

HPLC conditions for determination of B vitamins: Column used for HPLC quantification was C18 (25cm x 4.5mm, 5 µm). A linear gradient of Buffer: methanol (96:4) was used at a constant flow rate of 1 ml/min. A UV (210nm) detector for detection of peaks was used.

Statistical analysis: The data obtained for all parameters were analyzed by using statistical tools. Analysis of variance technique was applied and results were interpreted according to Duncan´s Multiple Range Test at 5% probability level. Pearson simple correlation was applied among different quality parameters (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Concentrations of B-vitamin and protein in Pakistani spring wheats: Concentration of protein examined in ten different spring wheats ranged from 9.84±0.62 to 10.77±0.78% in straight grade flour while in whole wheat flour the protein content was examined to be ranged from 9.50±0.41 to 12.53±0.74% as presented in Table 1

Table 1: B-vitamin and protein concentrations in straight grade flour

	Concentration				
Wheat varieties	Thiamin	Riboflavin	Niacin	Pyridoxine	Protein
AARI-11	0.11±0.04	0.28±0.05	3.83±0.70	0.36±0.06	10.36±0.63
AAS-11	0.14±0.01	0.27±0.06	4.06±0.32	0.31±0.08	9.90±0.59
Millat-11	0.15±0.09	0.29±0.02	3.14±1.01	0.35±0.01	10.77±0.78
Punjab-11	0.11±0.02	0.28±0.06	4.05±0.96	0.27±0.13	11.15±0.81
FSD-08	0.21±0.02	0.27±0.05	4.22±0.38	0.29±0.07	10.52±0.71
Lasani-08	0.19±0.06	0.21±0.03	3.03±0.63	0.30±0.04	10.52±0.88
Mairaj-08	0.17±0.07	0.30±0.01	3.70±0.60	0.29±0.06	9.88±0.91
Fareed-06	0.14±0.06	0.25±0.02	3.72±0.28	0.30±0.08	10.51±0.51
Sahar-06	0.19±0.01	0.22±0.01	3.54±0.57	0.30±0.15	9.84±0.62
Shafaq-06	0.22±0.01	0.32±0.14	2.71±0.58	0.23±0.07	10.55±0.14

Table 2: B-vitamin and protein concentrations in whole wheat flour

	Concentration				
Wheat varieties	Thiamin	Riboflavin	Niacin	Pyridoxine	Protein
AARI-11	6.09±1.09	1.92±0.14	59.38±0.70	6.84±0.26	11.59±0.88
AAS-11	6.23±0.70	2.12±0.04	60.71±1.32	6.72±0.47	12.53±0.74
Millat-11	6.33±0.65	2.06±0.15	60.88±0.61	7.25±0.47	12.33±0.62
Punjab-11	5.57±0.13	1.92±0.18	58.10±5.29	7.54±0.74	9.50±0.41
FSD-08	6.61±0.56	1.84±0.13	58.09±4.43	7.18±0.52	12.99±0.99
Lasani-08	5.92±0.44	2.09±0.19	63.10±2.86	6.02±0.92	11.61±0.71
Mairaj-08	6.56±0.52	2.14±0.17	58.39±4.04	6.88±0.59	12.00±0.66
Fareed-06	4.63±0.98	2.39±0.02	60.36±1.96	6.49±0.56	11.46±0.52
Sahar-06	6.21±0.57	1.85±0.15	60.34±0.81	6.64±0.39	12.17±0.91
Shafaq-06	6.04±0.09	2.23±0.35	60.81±2.93	7.27±0.36	12.72±1.02

and 2. Similarly regarding the level of B-vitamins the data has also been presented in Table 1 and 2 for the B-vitamin levels in straight grade and whole wheat flour respectively. Thiamin concentration detected in straight grade flour of different spring wheats ranged from 0.11±0.02 to 0.22±0.01 µg/g whereas the level of this B-vitamin detected in whole wheat flour was ranged from 4.63±0.98 to 6.61±0.56 µg/g. Riboflavin concentration present in straight grade flour ranged from 0.21±0.03 to 0.32±0.14 µg/g whereas 1.84±0.13 to 2.39±0.02 µg/g ranged has been observed in whole wheat flour of spring wheats. Niacin concentration observed in straight grade flour and whole wheat flour were 2.71±0.58 to 4.22±0.38 µg/g and 58.09±4.43 to 63.10±2.86 µg/g, respectively. On the other hand concentration of pyridoxine present in straight grade of spring wheats ranged from 0.23±0.07 to 0.36±0.06 µg/g whereas 6.02±0.92 to 7.54±0.74 µg/g of pyridoxine has been detected in whole wheat flours.

Different Pakistani wheat varieties has been investigated for the protein content and results exhibited that the protein content was in range of 9.50 to 12.83% (Saeed, 2012). The protein content in whole flours of various wheat varieties ranged between 11.94-16.07% and in bran protein content ranged between 14.29-17.22% (Butt *et al*., 1997; Ahmad, 2001; Akhtar *et al*., 2005; Ikhtiar and Alam, 2007). All these previous results support the present investigations regarding protein concentration in straight grade and whole wheat flour.

Wheat grain endosperm is 80-85% of the grain mass but it contains only a little portion of total grain thiamin (3%). More than 80% of the thiamin as well as pyridoxine found to be distributed in grain outer layers (bran) (MacMasters *et al*., 1971). These investigations also support present results that the whole wheat flour contains an interesting level of B-vitamins but unfortunately this level does not reach people because of its loss during milling (in straight grade flour). As the people eager to consume products prepared from white flours (e.g., breads, cakes, muffins, biscuits, etc.) so they get less amount of these vitamins (Pederson *et al*., 1989). It has been suggested that the accumulation of thiamin and pyridoxine in various grain layers is controlled genetically this is the reason cultivar type, wheat variety as well as the year of growing crop significantly affect the level of thiamin.

A protein is present in wheat grain which binds thiamin inside grain, it suggests that thiamin is synthesized and accumulated in the aleurone layer (Adachi *et al*., 2000; Watanabe *et al*., 2003, 2004). Not only the high amount of thiamin is present in whole wheat and other cereal products but the vitamin from these sources are able to utilized and absorbed well by different organisms. An admirably excellent level of precaecal digestibility of thiamin was observed when thiamin comes from the cereals e.g. wheat bran (92%), barley (94%) and rye (84%) in pigs (Roth-Maier *et al*., 1999).

Different wheat cultivars have been examined for B-vitamin concentration and found that the thiamin content in whole flour of wheat varieties ranged from 2.59 to 6.13 µg/g, whereas white flours of different wheat varieties contain 1.25 to 2.20 µg/g of thiamin content (Batifoulier *et al*., 2006). All these investigations strongly support the present results.

MacMasters *et al*. (1971) investigated that the level of riboflavin present in wheat grain is quite different from the level of thiamin, e.g., 32% of riboflavin is present in wheat endosperm, 26% in the germ and 37% in the aleurone layer. Similar results have been observed in the present investigations. Dairy products are the basic source of riboflavin on the other hand bread prepared from the whole wheat flour can fulfill 20% of the daily riboflavin needs (Rivlin and Pinto, 2001). Cereals B-vitamins are also fascinating due to their high bioavailability. The results of riboflavin concentration in whole wheat flour are supported by the findings of Batifoulier *et al*. (2006) who found that riboflavin content in different wheat whole flours ranged from 0.63 to 1.07 µg/g. Range of riboflavin content observed in white flour of different wheat varieties was 0.43 to 0.58 µg/g (Batifoulier *et al*., 2006). All these results are in line with the present investigations. The present data is strongly supported by the results of these researchers.

Most of the pyridoxine (80%) being found in the external layers of wheat grain. The external layers represent a very interesting fraction due their nutritional density but it is lost in the white flour (Pederson *et al*., 1989). Above findings are in accordance with the investigations of Batifoulier *et al*. (2006) who observed that the wheat whole flours contain pyridoxine content in range of 1.44 to 3.16 µg/g whereas the pyridoxine content in white flours of different cultivars was ranged from 0.27 to 0.54 µg/g.

Correlation of wheat B-vitamins with its protein content: Simple Pearson's correlation has been studied among wheat B-vitamins and its protein content (Table 3 and 4). With respect to whole wheat flour a positive correlation has been detected for protein content with thiamin ($r = 0.54$), riboflavin ($r = 0.07$) and niacin ($r = 0.22$) whereas in straight grade flour protein concentration was examined to be positively correlated with riboflavin concentration ($r = 0.19$).

Independently of the milling yield, a positive correlation has been studied between grain thiamin and pyridoxine concentrations (Batifoulier *et al*., 2006). This previous study strongly supports present correlation results which showed that grain thiamin and pyridoxine contents are positively correlated with each other. In contrast, the lack of correlation between grain and reconstituted wheat flour riboflavin concentrations could be explained by the homogenous repartition of riboflavin in wheat grain: 32% in the endosperm, 26% in the germ and 37% in the aleurone layer (MacMasters *et al*., 1971).

Table 3: Correlation among B-vitamins and protein in straight grade flour

	Thiamin	Riboflavin	Niacin	Pyridoxine
Riboflavin	-0.06			
Niacin	-0.46	-0.06		
Pyridoxine	-0.54	-0.22	0.22	
Protein	-0.23	0.19	-0.11	-0.13

Table 4: Correlation among B-vitamins and protein in whole wheat flour

	Thiamin	Riboflavin	Niacin	Pyridoxine
Riboflavin	-0.57			
Niacin	-0.18	0.39		
Pyridoxine	0.24	-0.28	-0.66	
Protein	0.54	0.07	0.22	-0.13

The accumulation of thiamin and pyridoxine in the different grain layers could be under genetic control (Batifoulier *et al*., 2006). Positive association of thiamin and grain protein content in the present study can be supported by the reason that there is a thiamin binding protein which was recently isolated from wheat grain and the results suggest that it is synthesized and accumulate in the aleurone layer as thiamin (Watanabe *et al*., 2004; Adachi *et al*., 2000). So the presence of thiamin binding protein makes thiamin presence definite. It has been studied that the grain B-vitamin concentrations are not only dependent on grain size but also on some genetic factors controlling their concentration and repartition in external layers of the grain (Batifoulier *et al*., 2006). More coarse layers in milling fractions, more will be the B-vitamins.

B-vitamin losses after milling: Comparison of whole wheat and straight grade flours exhibit that the upto 97% loss of thiamin was detected in straight grade flour after milling. Which make it clear that only 3% of whole wheat flour thiamin content was detected in straight grade flour. On the other hand after milling upto 90% losses in riboflavin content was observed and 15% of whole wheat flour riboflavin was detected in straight grade flour. For niacin losses it has been examined that upto 95% of niacin content was observed to be lost in straight grade flours of after milling and approximately 6% of niacin content of wheat was present in straight grade flour. Losses of pyridoxine content detected in straight grade flours of spring wheats were upto 96% which exhibited that milling process has drastic affect on pyridoxine content of wheat and only 6% of pyridoxine content was detected in white flour after refining (milling).

Straight grade flour after flour milling is usually proceeded for the production of certain products (bread, cakes and biscuit, etc.). When compared with whole wheat flour this white flour (straight grade flour) has a minute level of B-vitamins as it has only a little percentage of course bran layes e.g., alueron and scutellum layers. Increasing evidence suggests that the reduction in cereals consumption and the increase of more refined products consumption are associated with the appearance of marginal deficiencies in micronutrients such as thiamine (Hercberg *et al*., 1994; Bertrais *et al*., 2000).

Whole wheat flour contains 5.5 mg/g dry matter of thiamin i.e., more than twice the amount in white flour (Batifoulier *et al*., 2006). These investigations are in line with the present study. Batifoulier *et al*. (2006) explored that after milling, 43, 67 and 20% of thiamine, riboflavin and pyridoxine were recovered in white flour, compared to 80, 100 and 95% in reconstituted whole wheat flour, respectively.

Conclusion: A lot of research work has already been performed with respect to the characterization, chemical profiling and biochemical mapping of spring wheats. Still data is lacking regarding B-vitamin contents of spring wheats. This piece of research work was designed to estimate B-vitamins in wheat varieties (in whole wheat and straight grade flour). Protein and B-vitamin losses were examined to be high in fine wheat flour (straight grade flour). Protein contents were observed to be positively correlated withal B-vitamins especially thiamin and riboflavin. This investigation will be useful for breeders, growers, traders, millers and bakers. Keeping in view the results obtained from the study the future wheat grain improvement programs can be designed with more ease.

REFERENCES

AACC, 2000. Approved methods of American Association of Cereal Chemists. 10th Edn., Am. Assoc. Cereal Chem. Inc., St. Paul. Minnesota, USA.

Adachi, T., K. Watanabe and T. Mitsunaga, 2000. Characterization of thiamine binding protein from wheat germ. Cereal Chem., 77: 578-581.

Adrian, J. and L. Petit, 1970. Vitamins of cereals and their evolution during technologic treatment. Annales de Nutr. Aliment., 24: 131-168.

Ahmad, I., 2001. Varietal difference in amino acid composition, milling and baking properties of spring wheats. Ph.D. Thesis, National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan.

Akhtar, S., F.M. Anjum and S.U. Rehman, 2005. Effect of iron and zinc fortification on chemical composition of whole wheat flour. J. Res. Sci., 16: 95-103.

Aslam, J., M.S. Mohajir, S.A. Khan and A.Q. Khan, 2008. HPLC analysis of water-soluble vitamins (B_1, B_2, B_3, B_5, B_6) in *in vitro* and *ex vitro* germinated chickpea (*Cicer arietinum* L.). Afric. J. Biotechnol., 7: 2310-2314.

Batifoulier, F., M.A. Verny, E. Chanliaud, C. Remesy and C. Demigne, 2005. Effect of different bread making methods on thiamine, riboflavin and pyridoxine contents of wheat bread. J. Cereal Sci., 42: 101-108.

Batifoulier, F., M.A. Verny, E. Chanliaud, C. Remesy and C. Demigne, 2006. Variability of B vitamin concentrations in wheat grain, milling fractions and bread products. Europ. J. Agron., 25: 163-169.

Beaugrand, J., D. Cronier, P.L. Thiebeau, P. Schreiber, P. Debeire and B. Chabbert, 2004. Structure, chemical composition and xylanase degradation of external layers isolated from developing wheat grains. J. Agric. Food Chem., 52: 7108-7117.

Bertrais, S., M.L. Polo-Luque, P. Preziosi, B. Fieux, M. Torra-De-Flot, P. Galan and S. Hercberg, 2000. Contribution of ready-to-eat cereals to nutrition intakes in French adults and relations with corpulence. Annals Nutr. Metab., 44: 249-255.

Butt, M.S., F.M. Anjum, A. Rehman and A. Ali, 1997. Physico-chemical characteristics of spring wheats. J. Agric. Res., 35: 413-422.

GOP (Government of Pakistan), 2011-12. Economic Survey of Pakistan. Economic Affairs Division, Ministry of Finance, Islamabad, Pakistan.

Hercberg, S., P. Preziosi, P. Galan, M. Devanlay, H. Keller, C. Bourgeois, G. Potier-de-Courcy and F. Cherouvrier, 1994. Vitamin status of a healthy French population: dietary intakes and biochemical markers. Int. J. Vitamin Nutr. Res., 64: 220-232.

Hussain, A., H. Larsson, M.E. Olsson, R. Kuktaite, H. Grausgruber and E. Johansson, 2012. Is organically produced wheat a source of tocopherols and tocotrienols for health food? Food Chem., 132: 1789-1795.

Ikhtiar, K. and Z. Alam, 2007. Nutritional composition of Pakistani wheat varieties. J. Zhejiang Univ. Sci. B., 8: 555-559.

Indrani, D., P. Swetha, C. Soumya, J. Rajiv and G.V. Rao, 2011. Effect of multigrains on rheological, microstructural and quality characteristics of north Indian parottas-An Indian flat bread. Food Sci. Technol., 44: 719-724.

Jenkins, D.J., C.W. Kendall, T.H. Nguyen, A. Marchie, D.A. Faulkner, C. Ireland, A.R. Josse, E. Vidgen, E.A. Trautwein, K.G. Lapsley, C. Holmes, R.G. Josse, L.A. Leiter, P.W. Connelly and W. Singer, 2008. Effect of plant sterols in combination with other cholesterol-lowering foods. Metab., 57: 130-139.

Kaldy, M.S. and G.L. Rubenthaler, 1987. Milling, baking and physical-chemical properties of selected soft white winter and spring wheat. Cereal Chem., 64: 302-307.

Keagy, P.M., B. Borenstein, P. Ranum, M.A. Connor, K. Lorenz, W.E. Hobbs, G. Hill, A.L. Bachman, W.A. Boyd and K. Kulp, 1980. Natural levels of nutrients in commercially milled wheat ours. II. Vitamin analysis. Cereal Chem., 57: 59-65.

Lebiedzinska, A. and P. Szefer, 2006. Vitamins B in grain and cereal-grain food, soy-products and seeds. Food Chem., 95: 116-122.

MacMasters, M.M., J.J.C. Hinton and D. Bradbyru, 1971. Microscopic structure and composition of wheat kernel. In: Pomeranz, Y.E. (Ed.), Wheat: Chemistry and Technology. American Association of Cereal Chem., St. Paul. Minnesota, USA, pp: 51-113.

OECD, 2003. Consensus document on compositional considerations for new varieties of bread wheat (*Triticum aestivum*): key food and feed nutrients, anti-nutrients and toxicants. Environment directorate joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology. 2 rue André-Pascal, 75775 Paris Cedex 16, France.

Orth, R.A. and W. Bushuk, 1972. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Che., 49: 268-275.

Payne, P.I., M.A. Nightingale, A.F. Krttiger and L.M. Holt, 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British grown wheat varieties. J. Sci. Food Agric., 40: 51-56.

Pederson, B., K.E.B. Knudsen and E.O. Eggum, 1989. Nutritional value of cereal products, beans and starches. In: Bourne, G.H.B. (Ed.), World Review of Nutrition and Dietetics. Karger, Basel, pp: 2-76.

Piironen, V., A.M. Lampi and P. Ekholm, 2006. Micronutrients and phytochemicals in wheat grain. In: Khan, K. and P.R. Shewry (Eds.), Wheat Chemistry and Technology (4th Edn.), Am. Assoc. Cereal Chem., Inc. St., Paul. Minnesota, USA, pp: 179-185.

Pomeranz, Y., 1988. Chemical composition of kernel structures. In: Pomeranz, Y.E. (Ed.), Wheat: Chemistry and Technology. Am. Assoc. Cereal Chem., Inc. St., Paul. Minnesota, USA, pp: 97-158.

Rivlin, R.S. and J.T. Pinto, 2001. Riboflavin. In: Ruker, R.B. and J.W. Suttie (Eds.), The Handbook of Vitamins (3rd Edn.), Dekker, New York, pp: 255-273.

Roth-Maier, D.A., S.I. Wild, W. Erhardt, J. Henke and M. Kirchgessner, 1999. Investigations on the intestinal availability of native thiamin in selected foods and feedstuffs. Europ. J. Clinical Nutr., 38: 241-246.

Saeed, F., 2012. Biochemical characterization of non-starch polysaccharides in relation to end-use quality of spring wheats. Ph.D Thesis, National Institute of Food Science and Technology. University of Agriculture, Faisalabad, Pakistan.

Serna-Saldiver, S.O., 2005. Chemical composition of cereals grains. In: Barbosa-Canovas, G.V. (Ed.), Cereal grains properties, processing and nutritional distribution. CRC press Taylor and French group. Boca Raton, London, pp: 81-90.

Singh, J., P.J. Sharp and J.H. Skerritt, 2000. A new candidate protein for high lysine content in wheat grain. J. Sci. Food Agric., 81: 216-226.

Slaughter, C.D., H.N. Kari and R.H. William, 1992. Quality and classification of hard red wheat. Cereal Chem., 69: 428-432.

Sliwinski, E.L., P. Kolster, A. Prins and T. Van-Vliet, 2004. On the relationship between gluten protein composition of wheat flours and large-deformation properties of their doughs. J. Cereal Sci., 39: 247-264.

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. Principles and Procedures of Statistics: A biometrical Approach (3rd Edn.), McGraw Hill Book Co. Inc., New York.

Tsukatani, T., H. Suenaga, M. Ishiyama, T. Ezoe and K. Matsumoto, 2011. Determination of water-soluble vitamins using a colorimetric microbial viability assay based on the reduction of water-soluble tetrazolium salts. Food chem., 127: 711-715.

Watanabe, K., M. Ampo, T. Ohtsuka, H. Takahashi and T. Mitsunaga, 2003. Synthesis of thiamine-binding protein during seed development and its accumulation in sesame seed. Plant Biotechnol., 20: 221-224.

Watanabe, K., N. Nishida, D. Adachi and M. Ueda, 2004. Accumulation and degradation of thiamine-binding protein and level of thiamine in wheat seeds during seed maturation and germination. Bioscience, Biotechnol, Biochem., 68: 1243-1248.

WHO (World Health Organization), 2000. Human vitamins and mineral requirements: Report of a joint FAO/WHO expert consultation. Food and nutrition division, Bangkok, Thailand, pp: 27-32.