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Phytic Acid Concentration and Phytic Acid: Zinc Molar Ratio in Wheat Cultivars and Bread Flours, Fars Province, Iran

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ABSTRACT

Consumption of whole-wheat breads prepared from high extraction flours is recommended because of their high content of fiber, vitamins, and minerals. Despite the beneficial effects of these breads, they contain high amounts of undesirable compounds like Phytic Acid (PA) which is believed to negatively interfere with the absorption of some such nutrients as zinc (Zn). Wheat genotypes are different in their PA and Zn concentrations; therefore, cereal-based foods may be prepared using grains of low PA and high Zn. Fars Province is ranked first in Iran in terms of wheat production. It is, therefore, important to evaluate the PA and Zn status of the wheat cultivars common in the province. Seventeen wheat cultivars obtained from Genotype Improvement Department of Fars Agricultural Research Center, Zarghan, Iran were employed in the study. These cultivars that are commonly used by local farmers were grown in test plots under identical conditions. A wheat grain sample from Parvardeh Wheat Milling Factory and 7 bread flour samples from Shiraz city's bakeries were also included in the study. The PA content and phytase activity of the grains either with or without bran as well as those of the flour samples were determined. Results showed that the method of grain debranning significantly affectted the concentration of PA and phytase activity. The least phytic acid was found in Pavarus and Niknejad cultivars. The PA to Zn molar ratios were highest in Falat, Niknejad and Shiraz cultivars. The highest concentration of Zn was observed in Estar, Falat, and Niknejad, while maximum phytase activity was found in cultivars Estar, S-78-11, S-79-10, and Niknejad.

Keywords: Bran, Bread, Phytase, Phytic acid, Wheat, Zinc.

INTRODUCTION

Wheat (*Triticum aestivum L.*) has been cultivated throughout the world especially in such countries as Iran, Greece, and Egypt, as early as 2500 B.C. (Araste, 1991). It is the most consumed cereal crop constituting approximately 30% of the total cereals grown, making it a major source of nutrition including minerals for many people (McKevith, 2004). Consumption of whole-wheat bread prepared from high extraction flours has been recommended because of their high content of fiber, vitamin, and minerals. Despite their beneficial effects, these breads contain high amounts of undesirable compounds like phytic acid (Malakouti, 2001; Faridi, 1980; *Reinhold et al.,* 1974). High extraction flour is the commonly used especially in the rural areas of Iran. On the other hand, because of population growth and the necessity to shorten the time of bread preparation, most bakeries do not use the proper yeast and skip fermentation. This leads to a production of breads with high PA content (Sheikh-ol-Eslami and Jamalian, 2003).

Malnutrition of iron (Fe), Zn, and calcium (Ca) observed in many parts of the Middle East, especially in Iran and Egypt, is

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believed to result from consumption of breads prepared from high extraction flours (Malakouti, 2001; Reinhold *et al.,* 1974; Hurrel, 2001). In cereals, approximately 1-2% w/w of the seed is PA and can reach even 3-6 % (Cheryan, 1980). Breads, despite supplying most of the calorie, protein, vitamins, minerals, and other important nutritional elements plays an important role in malnutrition caused by mineral deficiency (Reddy *et al.,* 1982). The level of mineral phosphate in grains and seeds at the time of maturation when phytate is the major chemical form of the phosphate is low. Phytic acid is low at early growth stages but it increases rapidly with starch synthesis (Kholdebarin and Esslamzadeh, 2001). Therefore PA content of seeds is affected by the level of phosphorus (P) application.

Phytic acid ($P_6C_6H_{18}O_{24}$) or myo-inositol hexaphophate is the storage form of P in grain which is comprised of to 75% of total P of grain (PA-P). More than 90% of PA is in aleuron layer of wheat and thus, whole-wheat flour contains a high amount of PA (Erdal *et al.,* 1998a; Pomeranz, 1990). Phytins are the calcium and magnesium salts of PA that are soluble. Bran constitutes about 10.5-16.5% of the seed in different wheat varieties, and on the average 7% of that belongs to aleurone layer, a large amount of PA being accumulated in this layer of wheat grain (Mahmudi *et al.,* 1999; Welch and Graham, 1999).

Phytic acid may have beneficial and/or deleterious effects on humans and/or animals through nutrition. Some benefits reported are that it has anticancerous properties and preventive effects against heart disease and diabetes (Janeb and Thompson, 2002). The typical negative effect known is the binding of such divalent cations as magnesium (Mg), Ca, Zn and Fe forming insoluble complexes, hence reducing their bioavailability. Phytic acid is also able to form complexes with proteins at high pH levels, and thus impair digestibility and bioavailability of seed proteins (Carnovale *et al.,* 1988).

Zinc deficiency is a global micronutrient deficiency in humans. It has been estimated that more than 2 billion people suffer from Zn deficiency (Madaiah *et al.,* 1964). Some *in vitro* studies have indicated that Zn forms the most stable (insoluble) complex with PA. Phytate forms complexes with not only dietary Zn but also with endogenous Zn (Flanagan, 1984).

Negative effects of PA can be alleviated by phytase. Humans and such monogastric animals as pigs and chickens have insufficient activity of phytase in their guts to have any substantial impact on phytate hydrolysis of food (Moses *et al.,* 2003), however, in the bread making processes, phytase hydrolyses PA, reducing it by up to 60% (Erdal *et al.,* 1998a).

Food enrichment process is the most reliable, cheapest, and best solution for a compensation of microelements' deficiency in calcareous soil conditions (Nagi, 1996). In fertilized areas, a large fraction of wheat grain micronutrients is accumulated in the bran that does not ordinarily enter the peoples' diets, therefore it is recommended that bread be prepared from whole wheat flour which has a low PA/Zn ratio. Erdal *et al.* (1998b) consider PA/Zn molar ratio, especially in cereals and legumes, to be a good criterion for assessment of Zn adsorption with PA/Zn of 25-30 as critical values in foods. Gibson *et al.* (1998) reported that PA/Zn molar ratio of 12 or higher caused a decrease in Zn absorption. According to WHO (1996) 55% of Zn content of foods is expected to be absorbed if PA/Zn ratio of foods is less than 5; whereas it would be 35% if the ratio is 5-15 and only 15% if it is higher than 15.

Great efforts have been made towards this purpose through agricultural approaches including application of fertilizers, conventional plant breeding, and genetic engineering techniques (Hurrel, 2001). Low phytate mutants, that are now available for such key staple food crops as maize, barley, and wheat, offer potential benefits for mineral aspect of nutrition of humans (Dorsch *et al.,* 2003; Guttieri *et al.,* 2004).

Malakouti (2001) reported that PA/Zn ratio of Sangak, Barbary, Lavash, Taftun, and Baget bread flours, i.e., the bread types popular in Iran, was higher than the standard limit. Studies conducted about the effect of grinding and debranning have revealed that while total P, PA-P, and phytase are accumulated in the outer layers of cereal grains, however variation in phytase activities is high within foodstuffs, depending on genetic and environmental factors (Cossa *et al.,* 2000). Absorption of Fe increases 3-5 folds in consumers through either an omission of PA, or decomposing it. Vitamin C and Ethylene Diamine Tetra Acetic acid (EDTA) lower the negative effects of PA on Fe absorption by preventing the formation of PA complexes with Fe (Hurrel, 2001; Hurrel *et al.,* 2000). Feil and Fossati (1997) observed a positive correlation between Total P (TP) and PA-P and as well reported PA-P to be 62.2 to 71.3% of TP (with an average of 66.7%) in Iranian wheat varieties. The level of PA declines rapidly once the germination process has started. Febles *et al.* (2001) reported PA concentrations in infant flour of cereals of <1 to>36 mg g^{-1} with most samples> 20 mg g^{-1}.

The objectives of the present study were to 1) evaluate PA, TP, and Zn status of common wheat varieties and wheat flours of Fars province, 2) determine PA-P, PA/Zn, and PA×Ca/Zn of the whole *vs.* debranned wheat flours, and 3) identify the promising variety with low PA and PA/Zn ratio aganist high Zn concentration.

MATERIALS AND METHODS

Seventeen wheat cultivars from Genotype Improvement Department of Fars Agricultural Research Center, Zarghan, Iran were taken to be used in this study. These cultivars which were harvested from plants grown under identical conditions during the cropping season of year 2006) are commonly used by local farmers. A wheat grain sample from Parvardeh Wheat Milling Factory and 7 bread flour samples from Shiraz city's bakeries were also included in the study (Table 1).

A sub sample of each seed was debranned through rubbing the moist grains between a set of two layers of rough cloth for 30 times, this being an imitation of the debranning device for bulk samples in the Wheat Milling Factories. The debranned portion, was used in the experiments along with other samples. Total N was determined through micro Kjeldhal method (Bremner, 1996). Nitrogen, used as an index for the protein content of flour is also an index of bread quality. One gram of ash was dissolved in 2M HCl and used for determination of Zn, Fe, copper (Cu), manganese (Mn), and Ca by means of a Schimatzo model A-670G atomic absorption spectrophotometer. For a determination of P the method described by Murphy and Riley

Table 1. The type of flours and wheat variety samples.

No	Grain / Flour	Variety
1	Seed	Hot weather aera
2	Seed	Pishtaz
3	Seed	S-78-11
4	Seed	Niknejad
5	Seed	Shiraz
6	Seed	S-79-10
7	Seed	Keras-Adl
8	Seed	Chamran
9	Seed	Estar
10	Seed	Shahriar
11	Seed	Falat
12	Seed	Kavir (Durum wheat)
13	Seed	Marvdasht
14	Seed	Pavarus (Durum wheat)
15	Seed	Azady
16	Seed	Darab2
17	Seed	Zarin
18	Seed	Alvand
19	Flour	Sandwichy
20	Flour	Bazary
21	Flour	Sweet cooking
22	Flour	Bazary
23	Flour	12 % flour
24	Flour	Sangak
25	Flour	Mashiny

(1962) was employed.

The PA content was determined by precipitation of ferric phytate and a determination of Fe remaining in the supernatant (Haug and Lantzsch, 1983). About 0.5 g of ground seed sample was used for extraction of PA in 25 mL of 0.2N HCl that lasted for 3 hours. The volume was made up to 50 mL with deionized water, centrifuged (Relative Centrifugal Force (RCF)= 4,000) for 30 minutes. Two mL of supernatant was treated with a ferric solution ($NH_4Fe(SO_4)_2.12H_2O$) in a boiling water bath for 30 minutes. Following cooling, samples were centrifuged and then 1 mL of the supernatant was treated with a bipyridine solution and finally Fe remaining in the supernatant was determined. Further details of the method are described by Haug and Lantzsch (1983).

Phytase activity was assessed as described by Kilmer *et al.* (1994). About 0.5 g of ground seed sample was incubated in 5 mL of 50 mM sodium citrate buffer (pH= 5.3) and 50 mM Na-phytate at room temperature for 2 hours. After incubation, 5 mL of 20% aqueous Trichloro acetic acid was added, shaken for 15 min, centrifuged (RCF= 4,000 for 40 minutes), supernatant volume being made to 25 mL, and analyzed for inorganic P. Data were analyzed through *F*-test, Duncan Multiple Range Test, and regression techniques, using Excel, MSTATC, and SPSS software packages.

RESULTS AND DISCUSSION

Samples significantly ($P< 0.05$) differed in PA concentration, PA/Zn molar ratio, PA×Ca/Zn, % N, phytase activity, and Zn, Ca, Fe, Cu, and Mn concentrations (Tables 2 and 3) in either one of whole or debranned samples. Phosphorus concentration was not significantly different among whole grain samples probably because all the wheat varieties studied were produced under the same agricultural practice and management.

Erdal *et al.* (2002) demonstrated that irrespective of Zn fertilization, seed PA concentrations were of large genotypic variation. Bassiri and Nahapetian (1979), Fiel and Fossati (1997), and also Singh and Reddy (1977) reported significant differences in PA content in different triticale cultivars. Steiner *et al.* (2006) observed that total P, PA-P contents and phytase activity differed significantly among wheat cultivars, but no effect of cultivar on total P or PA-P concentration in barley samples was observed. In wheat samples, total P and PA-P levels were not significantly different among cultivars (Kim *et al.,* 2002). Possible explanation for these conflicting findings might be the effects of harvest year and cultivar on phytase activity, total P and PA-P.

Mean PA concentration in whole grain was 8.2 g kg^{-1} (Table 4) equivalent to 2.3 g PA-P kg^{-1} similar to the range reported by Haug and Lantzsch (1983), Febles *et al.* (2002), and by Erdal *et al.* (2002). Phytic acid concentration in manually debranned flours was 7.6 g kg^{-1} (2.18 g PA-P kg^{-1}), whereas in mechanically debranned samples it was 2.6 g kg^{-1} (Table 4), representing a 7.4 to 69% decrease depending on debranning method. Febles *et al.* (2002) reported that a mean PA-P value of 3.77 g kg^{-1} for hand made refined flours, 2.96 g kg^{-1} for factory made refined flours, and 8.5 g kg^{-1} for the whole wheat flours. Significant differences in PA-P were observed among different types of flours with the refined ones containing the lowest.

PA/Zn ratios in whole wheat flour and hand made refined flours were higher than the critical limit (25, Erdal *et al.* 1998b) (Table 4), that may incite Zn deficiency in consumers. Hand debranninig increases the PA/Zn ratio significantly with PA×Ca/Zn ratio nearing its critical limit (0.2, Ellis et al 1987). The PA×Ca/Zn ratio and PA-P/P percentage did not significantly change through manual refining. The average phytase activity increased up to 4.8 % in hand refining whereas it decreased by 41% when refining accomplished through mechanical means. Debranning by hand increased the protein content of flour by 3%

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Table 2. Genotype differences in PA (g kg^{-1}), PA/Zn, Ca×PA/Zn (mol kg^{-1}), %PA-P/TP, and Zn (mg kg^{-1}) content in whole and debranned wheat flours.

Variety	PA		PA/Zn		Ca×PA/Zn		%PA-P/TP		Zn	
	Whole	Debranned	Whole	Debranned	Whole	Debranned	Whole	Debranned	Whole	Debranned
Hot weather aera	7.69efg*	6.97c	26.0def	25.7f	0.18de	0.15e-i	71.5bc	65.1d-i	29.33bcd	26.88bcd
Pishtaz	8.31bcd	7.88abc	29.5def	31.9def	0.17de	0.16de-h	72.7bc	73.4b-f	27.90cde	24.68c-f
S-78-11	8.43abcd	8.15abc	26.2def	43.0abc	0.15de	0.211b-e	70.0bcd	70.8b-f	33.03abc	18.85h-k
Niknejad	7.65fg	7.24bc	23.6f	25.8f	0.16de	0.14e-i	66.5bcd	69.9b-f	32.28a-d	27.83bc
Shiraz	8.09de	4.74d	25.5ef	16.2g	0.14e	0.08hij	68.3bcd	42.5ij	31.48a-d	28.85b
S-79-10	8.84a	8.65a	24.8ef	26.6ef	0.13e	0.11f-i	74.9bc	87.2ab	35.50ab	32.55a
Keras-Adl	8.55abc	8.12abc	45.6a	48.4a	0.29b	0.24abc	69.1bcd	75.0b-f	18.63g	16.63jkl
Chamran	8.31bcd	8.00abc	28.9def	34.7cde	0.16de	0.17c-g	72.1bc	74.8b-f	28.50b-e	22.88efg
Estar	8.18cd	7.76abc	25.1ef	34.5cde	0.17de	0.17c-g	66.8bcd	70.6b-f	32.25a-d	22.28e-h
Shahriar	8.59abc	8.51a	27.4def	39.1bcd	0.17de	0.21b-e	51.7d	84.9abc	31.15a-d	21.60fgh
Falat	8.69ab	8.28ab	23.4f	41.7abc	0.16de	0.224bcd	73.1bc	73.5b-f	36.85a	19.68g-j
Kavir(durum wheat)	8.79a	8.49a	26.5def	32.5def	0.154de	0.15e-i	75.4bc	79.4b-e	33.20abc	25.98b-e
Marvdasht	8.05def	6.99c	37/3bc	48.9a	0.22bcd	0.27ab	62.9cd	56.8f-i	21.53efg	14.18lm
Pavarus (durum wheat)	7.40g	7.13bc	26/9def	29.6ef	0.15de	0.141e-i	83.7ab	86.6ab	27.28cde	23.83def
Azady	8.16cd	7.64abc	41.4ab	41.2abc	0.26bc	0.21b-e	63.6cd	63.7efgh	19.53fg	18.40h-k
Darab2	8.16cd	7.08c	30.8de	28.9ef	0.16de	0.13f-i	72.9bc	65.9d-h	26.28cdef	24.40c-f
Zarin	8.09de	7.82abc	32.4cd	46.7ab	0.2cde	0.24abc	51.5d	83.3a-d	24.98d-g	16.68jkl
Alvand	8.04def	7.63abc	40.6ab	43.5ab	0.42a	0.3a	97.2a	98.4a	19.62fg	17.35i-l
flour										
Sandwichy	-	1.17g	-	16.1g	-	0.09ghi	-	28.7jk	-	7.13n
Bazary	-	3.05f	-	16.5g	-	0.08hij	-	48.3hi	-	18.40h-k
Sweet cooking	-	0.46g	-	3.0h	-	0.02j	-	14.3k	-	14.95klm
Bazary	-	3.04f	-	24.3f	-	0.14ef-i	-	47.7hi	-	12.38m
12 % flour	-	3.43ef	-	16.1g	-	0.11f-i	-	59.6f-i	-	21.10f-i
Sangak	-	2.47f	-	16.1g	-	0.08ij	-	50.3ghi	-	15.65j-m
Mashiny	-	4.38de	-	24.6cde	-	0.18c-f	-	67.4c-g	-	12.58m

* Means in a column followed by a different letter are significantly different (P< 0.05) by Duncan's Multiple Range test.

Table 3. Genotype differences in P, Fe, Cu, Ca, concentrations (mg kg^{-1}) and phytase activity (U g^{-1}) in whole and debranned wheat flours.

Variety	P		Fe		Cu		Ca		Phytase	
	Whole	Debranned	Whole	Debranned	Whole	Debranned	Whole	Debranned	Whole	Debranned
Hot weather area	3077	3098a-d	45.3def	41.8b	11.98abc	10.2b	271b	230bc	0.34fg	0.45f
Pishtaz	3273	3077a-d	49.4cde	42b	9.43c-f	8bcde	229bcd	200c-f	0.442bcd	0.41j
S-78-11	3447	3294abc	52.9b-e	41.6b	12.63a	4.38ghi	227bcd	197c-f	0.49ab	0.53b
Niknejad	3294	2964bcd	55.8bcd	40.6bc	11.3a-e	10.05b	261bc	221bcd	0.41b-e	0.37m
Shiraz	3389	3166a-d	45.5def	40.2bc	12.28ab	10.33b	222bcd	209c-f	0.41c-f	0.47d
S-79-10	3379	2890cd	43.2ef	37.5b-f	9.98b-f	8.98bc	201d	172f	0.46abc	0.4k
Keras-Adl	3540	3103a-d	51bcde	33.35b-g	5.35g	4.50ghi	250bcd	195c-f	0.37d-g	0.49c
Chamran	3332	3069a-d	53.75b-e	38.1b-e	9.03ef	7.08c-f	217bcd	194c-f	0.45bcd	0.44g
Estar	3508	3147a-d	62.1ab	36.3b-g	9.8b-f	7.05c-f	270b	196c-f	0.32g	0.329p
Shahriar	5196	2911cd	71.9a	42.4b	9.13ef	8.05b-e	247bcd	210c-f	0.52a	0.58a
Falat	3400	3226a-d	49.7cde	39.6bcd	11.73a-d	4.63ghi	270b	215cde	0.44bcd	0.46e
Kavir(Durum wheat)	3336	3060a-d	43.6ef	30.9d-h	12.13ab	8.43bcd	232bcd	180ef	0.43b-e	0.44g
Marvdasht	3661	3521a	45.1def	37.5b-f	9.08ef	5.78efg	237bcd	223bcd	0.43bcd	0.44gh
Pavarus (Durum wheat)	2529	2355ef	36.2f	31.3c-h	11.38a-e	7.15c-f	222bcd	191def	0.32g	0.3r
Azady	3676	3436ab	59.6bc	51.3a	6.50g	5.63fgh	249bcd	205cdef	0.36efg	0.44h
Darab2	3202	3081a-d	49.6cde	41.9b	7.73fg	6.35d-g	207cd	175f	0.44bcd	0.438i
Zarin	5047	2725de	47def	36.1b-g	9.33def	4.85f-i	245bcd	205c-f	0.45bcd	0.34o
Alvand	2366	2219fg	70.7a	29.3e-h	13.85a	12.83a	414a	277a	0.5c-f	0.38l
Sandwichy	-	1162ij	-	23.7hi	-	4.33ghi	-	230bc	-	0.17v
Bazary	-	1808gh	-	34.6b-g	-	6.98c-f	-	204c-f	-	0.25t
Sweet cooking	-	924j	-	18.4i	-	8.75bc	-	256ab	-	0.11w
Bazary	-	1817gh	-	35.6b-g	-	3.1i	-	229bcd	-	0.27s
12 % flour	-	1647h	-	27.9gh	-	9.95b	-	270a	-	0.31q
Sangak	-	1403hi	-	28.8fgh	-	8.08b-e	-	191def	-	0.21u
Mashiny		1859gh		30.1e-h		3.33hi		204c-f		0.35n

* Means in a column followed by a different letter are significantly different ($P \leq 0.05$) by Dunkan's Multiple Range test.

Table 4. Chemical composition of the wheat samples.

	PA[a] g kg^{-1}	PA/Zn[b]	Ca×PA/Zn mol kg^{-1}	PA-P/TP %	Phytase[c] U g-1	Protein %	P/Zn[d]	mg kg^{-1}						
								TP	Zn	Fe	Cu	Mn	Mg	Ca
Whole grain	8.2 a*	30.1 b	0.2 a	70.2 a	0.41 b	13.1 b	128b	3481a	28.3a	51.8 a	10.1a	44.6a	846a	248a
Hand-debranned grain	7.6 b	35.5 a	0.18 a	73.4 a	0.43 a	13.5 a	142a	3019b	22.4b	38.4b	7.7b	40.4b	407b	205c
Factory (Mechanically) made flour	2.6c	18.1 c	0.1 b	45.2 b	0.24 c	11.2 c	113c	1517c	14.6c	28.4 c	6.4c	19.9c	253c	226b

[a] Phytic acid concentration; [b] Phytic acid to zinc molar ratio; [c] Inorganic P released (μmol) from 1g dry flour seed, in 1 minute, pH 5.5, [d] Concentration ratio.
* Means within a column followed by a different letter are significantly different (P≤ 0.05).

whereas mechanical debranning decreased it by 14.5%. Phosphorus concentration decreased by 13.3 to a level of 56.4% through debranning. Debranning decreased Zn, Fe, Cu, Mn, Mg, and Ca concentrations by 21-48%, 26-45%, 24-37%, 9-55%, 52-70%, and 17-9%, respectively with mechanically made flours containing significantly lower concentrations than hand-debranned flours. This is probably due to the smaller portion of bran layer removed by hand. Liu *et al.* (2006b) analyzed the variances of phytase activity, PA, Fe, and Zn contents in 12 pearling fractions of 10 cultivars grown at two locations, and showed that pearling had the largest effect with a diminishing trend as pearling level increased from outer layer to the inner part of wheat grain.

S-79-10 showed the highest concentration of PA in whole flour and in extracted flour (8.8 and 8.6 g kg^{-1}, respectively, Table 2). The lowest concentration of PA was observed in whole wheat flour of Pavarus and in high extracted flour, a variety which is supposed to be used primarily in confectionary rather than bread bakeries. The highest (45) and lowest (23) PA/Zn ratios were detected in Kerase-Adl and Falat whole wheat flour, respectively (Figure 1 and Table 2).

The high extracted flour had the lowest ratio (3). Figure 1 shows that PA/Zn molar ratio in Niknejad, Estar, Falat, and S-79-10 varieties were less than the critical limit (25). These varieties are suitable for making whole wheat flour bread. The PA×Ca/Zn ratio was highest in Alvand (0.42 and 0.30 mol kg^{-1} in whole wheat and extracted flours, respectively). S-79-10 whole wheat (0.13) and confectionary flour had the lowest PA×Ca/Zn ratio (0.02) among the extracted flours, respectively (Figure 2 and Table 2).

The highest concentrations of Zn, P, Fe, and Cu in whole wheat flour were found in Falat for Zn (36.9 mg kg^{-1}), Shahriar for P and Fe (5,196 mg kg^{-1} and 71.9 mg kg^{-1}, respectively), and Alvand for Cu (13.9 mg kg^{-1}). The lowest concentrations of these minerals were recorded for Kerase-Adl (18.6 mg kg^{-1}), Alvand (2,366 mg kg^{-1}), Pavarus (36.2 mg kg^{-1}), and Kerase-Adl (5.3 mg kg^{-1}), respectively (Tables 2 and 3). The lowest concentrations of P and Fe were observed in confectionary flour while Zn and Cu in sandwich making bread flour and in Shabankare's Bazari bread flour, respectively (Tables 2 and 3). The highest and the lowest activity of phytase enzyme was recorded for whole wheat flour of Shahriar and Estar variety, respectively (Table 3). Alvand showed the highest PA-P/P percentage (97). Zarrin and Shahriar recorded the lowest ratios (51%, Table 2). In all, the varieties' P/Zn concentration ratios measured more than 100, none of them indicating P or Zn deficiency in their mother wheat plant (Figure 3).

The P/Zn ratio of 100 to 200 in wheat grain is indicative of very sufficient to sufficient Zn supply to the plants. The occurrence of P-induced Zn deficiency symptoms depends on wheat variety. Some varieties within a species are characterized by higher susceptibility to excess or deficiency of Zn, probably because of difference in the ability to take up P. Some studies report that the differences among varieties for the response to P are due to differences in Zn uptake (Stanislawska-Glubiak and Korzeniowska, 2005).

Phytic acid presented a positive correlation with phytase activity, Mn, and Mg concentrations, and as well with protein percentage (Table 5).

There was a significant ($P \leq 0.01$) positive correlation observed between PA/Zn molar ratio and P/Zn, while a negative one between PA/Zn molar ratio and Zn concentration. Among genotypes, Zn and Cu concentrations, total P and P/Zn ratio, Ca and Fe concentration, as well as Ca concentration and PA×Ca/Zn were positively correlated. On the contrary, total P and PA-P/TP % were negatively correlated ($r = -0.91$). Raboy *et al.* (1991) observed that variation in PA-P was highly and positively correlated with variation in grain total P, and as well with variation in

Table 5. Correlation coefficient between the measured factors in whole grain.

No.	Parameter [a]	Factor No. 1	2	3	4	5	6	7	8	9	10	11	12	13
1	PA													
2	PA/Zn	-0.004ns												
3	Ca×PA/Zn	-0.1ns	0.82**											
4	%PA-P/TP	-0.14ns	0.07ns	0.37ns										
5	Phytase	0.59*	-0.2ns	-0.24ns	-0.32ns									
6	%Protein	0.56*	-0.19ns	-0.36ns	-0.4ns	0.58*								
7	P/Zn	0.08ns	0.74**	0.44ns	-0.59**	0.09ns	0.14ns							
8	TP	0.33ns	-0.01ns	-0.2ns	-0.91**	0.5*	0.51*	0.65**						
9	Zn	0.3ns	-0.94**	-0.78**	-0.08ns	0.32ns	0.35ns	-0.7**	0.06ns					
10	Fe	0.14ns	0.26ns	0.51ns	-0.08ns	0.17ns	0.16ns	0.27ns	0.24ns	-0.2ns				
11	Cu	-0.19ns	-0.54*	-0.07ns	0.49*	0.04ns	-0.3ns	-0.7**	-0.4ns	0.49*	-0.05ns			
12	Mn	0.61**	-0.25ns	-0.16ns	-0.39ns	0.43ns	0.27ns	0.04ns	0.47*	0.44ns	0.2ns	0.11ns		
13	Mg	0.59*	0.01ns	-0.12ns	-0.1ns	0.26ns	0.45ns	0.08ns	0.2ns	0.22ns	-0.3ns	-0.2ns	0.35ns	
14	Ca	-0.19ns	0.36ns	0.82**	0.47ns	-0.24ns	-0.43ns	0.03ns	-0.3ns	-0.35ns	0.6*	0.39ns	0.01ns	-0.23ns

* and **; Significant at $P \leq 0.05$, and $P \leq 0.01$, respectively. ns: Not significant, [a] Unit of factors are the same as in Table 4. *Note*: For description of parameters, please refer to Table 4.

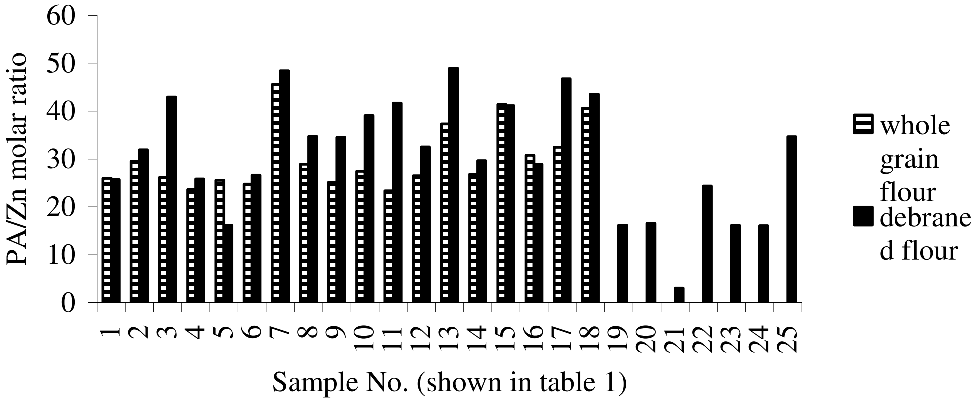

Figure 1.Means of PA/Zn molar ratio in different wheat flours.

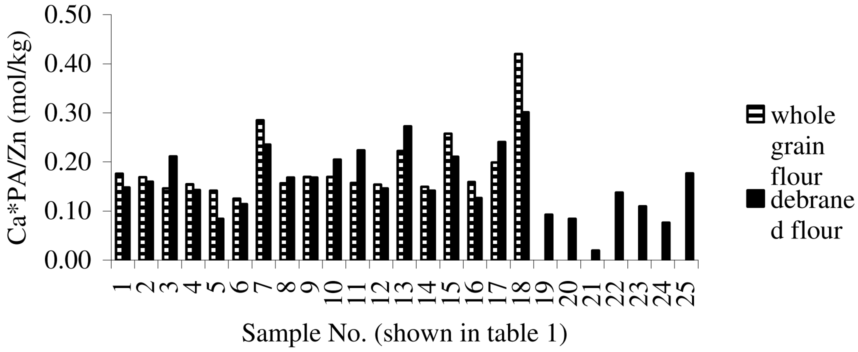

Figure 2. Means of PA×Ca/Zn in different wheat flours.

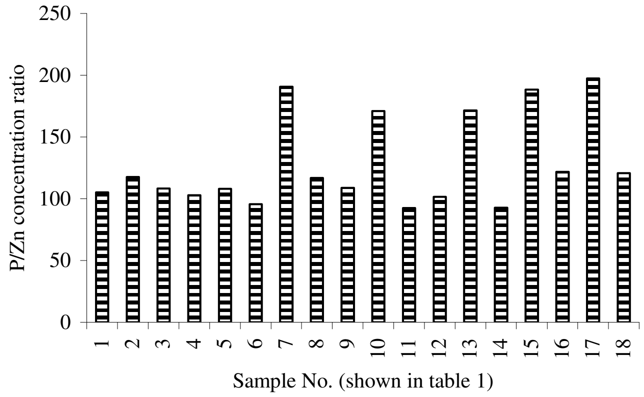

Figure 3. Means of P/Zn concentration ratio in different wheat grains.

grain protein, in winter wheat. The close correlation of PA-P with both total P and protein indicates that selection of varieties based on the least grain PA would lead to undesirable reductions in both grain total P and protein. Steiner *et al.* (2006) reported that total P and phytate-P concentrations were highly correlated in legume seeds ($r_= 0.95$), in cereal by-products ($r_= 0.96$) and, to a smaller extent, in cereals ($r_= 0.66$). Milling of cereal grains to bran and flour and their subsequent separate analysis revealed that phytase activity, total P, and phytate P are highly concentrated in the outer grain layers of cereals. Liu *et al.* (2006a) studying 186 wheat genotypes from China, reported that grain weight and phytate ($r_=$ -0.34), inorganic P ($r_=$ -0.23), and phytase activity ($r_=$ -0.43) were negatively correlated. In contrast, grain weight and iron ($r_=$ 0.20), and as well phytate and Zn ($r_=$ 0.37) were positively correlated. Results showed substantial differences in these chemical components among genotypes.

Regression equations were also developed to study the relationships between the studied characteristics (Table 6).

Up to 55 % of seed PA concentration could be explained by Mn concentration and protein percentage if used simultaneously in the equation but they predicted only 31 and 36% of PA concentration if used separately. Equations (2, 3, 4), by using Zn or Cu or both predict PA×Ca/Zn ratio, whereas simple equation using Zn predicts 61%, and Cu shows no significant simple regression with PA×Ca/Zn ratio. Using both Zn and Cu in the same equation increases the prediction to 73% (Equation (2), Table 6). Using Zn content in power model (Equation (2)), the prediction power for PA×Ca/Zn ratio increases from 61% (in simple equation) up to 75%. Using Cu concentration in a cubic model increases the prediction power for PA×Ca/Zn up to 78% (Equation (4)). As mentioned earlier, the simple regression for Cu was not significant (Table 5). Therefore, Equations (3) and (4) are recommended for an estimation of PA×Ca/Zn ratio using either Cu or Zn.

Exponential model (Equation (5)) estimates up to 76 % of PA×Ca/Zn ratio using PA/Zn molar ratio. As seen in Equation (6), using Zn concentration, one can estimate 97% of PA/Zn molar ratio whereas the simple equation only predicts 88% of the variation. Total P concentration in seed can lead to an estimation of 92% of PA-P/TP%, through the power model (Equation (7)).

CONCLUSIONS

According to this study the concentration of essential elements in manually-debranned flour is higher than in those made through mechanical means. Debranning method (i.e., how much of bran layer is refined from wheat grain) significantly affects the final composition of the flour.

The lowest concentration of PA was found in Pavarus (spaghetti flour) and Niknejad (bread flour). The lowest PA/Zn molar ratio was observed in Falat, followed by Niknejad, Shiraz, and S-79-10. The lowest PA×Ca/Zn ratio was observed in Shiraz and Niknejad. The highest activity of phytase enzyme was recorded for Estar, followed by S-78-11, S-79-10, Niknejad, and Shiraz. Estar, Falat Niknejad, and Shiraz, contained the lowest Zn concentration. Based on the

Table 6. Regression equations between the assessed parameters in whole wheat grain.

No.	Equation	Non-linear equation R^2	Simple linear equation R^2	Multiple linear equation R^2
1	$PA= 4953+(41.1\times Mn)+(682\times \%Protein)$	-	0.37, 0.31	0.55*
2	$Ca\times PA/Zn= 0.40 -(0.012\times Zn)+(0.013\times Cu)$	-	0.61,0.004	0.73***
3	$Ca\times PA/Zn= 9.982\times(Zn^{-1.207})$	0.75***	0.61***	-
4	$Ca\times PA/Zn= -0.97\ (0.51\times Cu) - (0.07\times Cu^2)+(0.003\times Cu^3)$	0.78***	0.004	-
5	$Ca\times PA/Zn= 0.057\times(e^{0.039\times PA/Zn})$	0.76***	0.67***	-
6	$PA/Zn= 108.6 - (4.71\times Zn)\ (0.07\times Zn^2)$	0.97***	0.88***	-
7	$\%PA\text{-}P/TP= 34096\times(TP^{-0.761})$	0.92***	0.83***	-

* and ***: Significant at $P\leq 0.05$, and $P\leq 0.001$, respectively.
Note: PA (mg kg^{-1}) and for other parameters and units please refer to Tables 2 and 3.

results of this study, Niknejad is recommended to be used as a suitable wheat variety for production of whole wheat flour for baking of bread. It is suggested that the effects of climate and management practices be also taken into consideration in the future similar studies.

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غلظت فیتیک اسید و نسبت فیتیک اسید به روی در چند رقم گندم و چند نوع آرد در استان فارس، ایران

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چکیده

در سالهای اخیراستفاده از آرد کامل (سبوس دار) به خاطر داشتن مقادیر زیاد فیبر، ویتامین و املاح معدنی مورد توجه قرار گرفته است. در آرد گندم کامل مقدار برخی از مواد نا مطلوب که مهمترین آن فیتیک اسید است، بالا می‌باشد. رقم های مختلف گندم از نظر غلظت فیتیک اسید تغییرات ژنوتیپی زیادی نشان می دهند. لذا می‌توان با توجه به این تغییرات، از ژنوتیپی که حاوی فیتیک اسید کمتر و روی بیشتری است در غذاهایی که پایه غلات دارند استفاده نمود. با توجه به این مسئله که استان فارس قطب تولید گندم در کشور است اطلاع از وضعیت فیتیک اسید ارقام مختلف کشت شده در این استان ضروری به نظر می رسد. به منظور بدست آوردن چنین اطلاعاتی تعداد ۱۷ رقم گندم رایج در استان که در شرایط یکسان در مرکز تحقیقات زرقان فارس کشت شده بود از بخش اصلاح ژنتیک این مرکز، یک نمونه گندم از کارخانه آرد پرورده (گندم منطقه گرمسیری)، و هفت نمونه آرد نان های مختلف از سطح نانوایی های شیراز جمع آوری و نمونه های با سبوس و بدون سبوس گندم ها بطور مجزا همراه با آردهای تهیه شده، از نظر غلظت فیتیک اسید و نسبت آن به روی و مقدار فعالیت آنزیم فیتاز مورد بررسی بررسی قرار گرفت. نتایج نشان می دهد روش جدا کردن سبوس (بر حسب اینکه چند درصد از پوسته دانه به عنوان سبوس جدا می شود) بر ویژگی های بررسی شده در این پژوهش اثر کاملا معنی داری دارد. کمترین غلظت فیتیک اسید در ارقام پاواروس و نیک نژاد مشاهده شد، بزرگ ترین نسبت مولی فیتیک اسید به روی در ارقام فلات، نیک نژاد و شیراز و بیشترین غلظت روی در ارقام استار، فلات و نیک نژاد، بیشترین فعالیت آنزیم فیتاز به ترتیب در استار، S-78-11، S-79-10، نیک نژاد، مشاهده گردید.

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