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Effects of various phytase sources on phytic acid content, mineral extractability and protein digestibility of tarhana

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Abstract

Changes in phytic acid (PA), HCl-extractability (HCl-E) of some minerals and in vitro protein digestibility (IVPD) during the production of tarhana prepared with the addition of different phytase sources (bakers' yeast, barley malt flour and microbial phytase) were investigated. PA content of tarhana decreased significantly ($p < 0.01$) after addition of the yeast, malt and phytase. With respect to wheat flour used as raw material, PA content of tarhana decreased by 95.3%. After tarhana production, average values of HCl-E of Ca, Mg, Zn and K, and also IVPD of tarhana increased up to 80.2%, 86.4%, 73.9% and 92.6%, and 91.9%, respectively. Significant negative correlation coefficients were found between the PA and HCl-E of the minerals, and also IVPD. Tarhana production processes, including fermentation, drying and grinding, were able to remove the antinutritional effects of PA. Each one of the phytase sources used alone decreased the PA content to a limited extend. The results show that tarhana has good potential in the total amounts and bioavailability of the minerals and proteins.

Keywords: Tarhana; Protein digestibility; HCl-extractability; Phytic acid; Mineral

1. Introduction

Tarhana is often produced by lactic acid and yeast fermentation of a mixture of wheat products, yoghurt, tomato paste, onion and some spices, followed by drying and grinding (Ibanoğlu, Ainsworth, Wilson, & Hayes, 1995). Protein, carbohydrate and lipid components of tarhana mix are subjected to partial digestion and hydrolysis by lactic acid bacteria and yeast during fermentation, resulting in a product with improved digestive properties. (Pamir, 1977; Türker, 1995). Tarhana provides an increased nutritive value or a high protein food. The growth of pathogens and spoilage microorganisms are inhibited by the low pH and low moisture content of tarhana which increase the shelf-life of the product up to one year or more (Lazos, Aggelousis, & Bratakos, 1993).

Phytic acid (PA) has been termed as an "antinutrient" due to its ability to bind minerals and proteins, either directly or indirectly, and thus alter their solubility, functionality, digestibility and absorption (Rickard & Thompson, 1997). Most PA-mineral complexes are insoluble at physiological pH. This insolubility is considered to be the main cause of the poor bioavailability of the mineral complexes (Harland & Harland, 1980). Fermentation is one of the processes known to reduce PA. In general, lower pH, longer fermentation time and more yeast addition result in a more intensive degradation of PA (Lasztity & Lasztity, 1990). Phytase activity increases with the germination of cereals in malt production. Malt flour addition is a very effective method for degradation of PA (Greiner, Jany, & Alminger, 2000; Sripriya, Antony, & Chandra, 1997).

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The objectives of the present study were to investigate the changes in PA, HCl-E of Ca, Mg, Zn and K and in vitro protein digestibility (IVPD) during the tarhana production process with additions of different phytase sources and to determine the nutritional potential of tarhana.

2. Materials and methods

2.1. Materials

Commercial wheat flour (1.10% ash), concentrated full fat yoghurt (21.35% dry matter and 26.7% protein, N × 6.25 db), tomato paste (32 B×^{0}), chopped dry bulb onion, hot red pepper, and compressed bakers' yeast were purchased from local suppliers in Konya, Turkey. Malt flour (Anadolu Efes Biracílík ve Malt Co., Konya) and microbial phytase (Phynase P 10 000 FTU) (Röhm Co., Finlad) were used as phytase sources.

2.2. Experimental design and statistical analysis

Baker's yeast (0%, 2.5% and 5%), 0%, 2% and 4% barley malt flour and 0%, 0.05% and 0.5% microbial phytase preparate were added to tarhana dough mix on 100 g flour basis. The experimental design was completely randomised with 2 replications. The data were subjected to statistical analysis by three-way analysis of variance (ANOVA). The comparison of the means was done by Duncan's multiple range test (Steel & Torrie, 1960). Correlation coefficients and regression equations between the variables were analysed using the MINITAB statistical analysis programme.

2.3. Preparation of tarhana

The ingredients of tarhana, based on flour (100%) were: 40% yoghurt, 10% tomato paste, 5% onion, 2% pepper and 1% salt. Bakers' yeast, malt flour and microbial phytase were added at different levels, according to the experimental design. These ingredients were mixed for 5 min in a Hobart dough mixer. After mixing, each mixture was divided into two parts. One part was frozen (−18 °C) immediately and the other part was subjected to fermentation at 30 °C for 72 h. Fermented tarhana dough was cut into pieces of 2 cm in diameter, placed on trays as a single layer and dried at 55 °C in an air-convection oven (Ozkoseoglu PFS-9) to 9–12% moisture content. The dried tarhana pieces were ground in a hammer mill equipped with an 1-mm opening screen. The unfermented frozen tarhana doughs were also dried and ground by the same procedure as the fermented samples. The ground tarhana samples were stored in closed glass containers at room temperature.

2.4. Analytical methods

The AACC methods were used for the determination of moisture (method 44-12) and protein (method 46-12) contents of the ingredients and tarhana samples (AACC, 1990). pHs of the dough and tarhana samples were measured by a digital pH meter. The mineral amounts were determined by an ICP-AES (Vista series, Varian International AG, Switzerland) as explained by Bubert and Hagenah (1987). Phytic acid was determined by the method of Haugh and Lantzsch (1983). HCl-extractabilities of the minerals were carried out according to Saharan, Khetarpaul, and Bishnoi (2001). In vitro protein digestibility (IVPD) was determined by the methods given by Bookwalter, Kirleis, and Mertz (1987).

3. Results and discussion

3.1. Analytical results

Chemical compositions of the tarhana ingredients are listed in Table 1. High extraction wheat flour, the main ingredient of tarhana, had 487 mg/100 g of PA on a dry basis. Flours with higher extraction ratios contain larger amounts of PA (Pomeranz, 1988). Lasztity and Lasztity (1990) found 1230 mg/100 g of PA in whole wheat flour. Yoghurt, as the second main ingredient of tarhana, was very rich in total Ca (898 mg/100 g) and HCl-E of Ca (80.7%). Similar Ca values were obtained by Iyiopruk (2002). Protein digestibilities of wheat flour and yoghurt were 67.7% and 88%, respectively. These values were in accordance with those reported by Elgün and Ertugay (1995) and Türker (1995). The HCl-E of Zn (34.8%) of the flour with high phytic acid (487 mg/100 g) content was found to be lower than those of the other ingredients. The total K content of tomato paste, onion, yeast and red pepper were higher than those of the other ingredients of tarhana (Table 1).

3.2. The change in phytic acid content

Statistical data about the phytic acid (PA) and phytic acid loss (PAL) of tarhana dough and tarhana (end product) are given in Tables 2–5. The average PA contents of wheat flour, tarhana dough and tarhana were 487, 138 and 22 mg/100 g, respectively (Tables 1 and 2). With respect to wheat flour, PAL was 70.7% in dough and 95.3% in tarhana (Table 2). Toufeili, Melki, Shadarevian, and Robinson (1999) have reported 39.2–59.8% decrease in PA from dough to dried kishk samples. The differences between the values originated from the differences in dough formulations and processes. PA contents of dough and tarhana were reduced by yeast, malt and phytase additions, as shown

Table 1
Some analytical results of tarhana ingredients (based on dry matter)

Ingredients	Moisture (%)	Phytic acid (mg/100 g)	Ca		Mg		Zn		K		Protein (N × 6.25)	
			Total (mg/100 g)	HCl-E[a] (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	IVPD[b] (%)
Wheat flour	11.19	487.0	26	51.7	94.4	57.3	1.12	34.8	302	67.2	11.6	67.7
Malt flour	9.61	56.7	48	85.6	132	85.6	1.62	77.2	360	83.4	8.64	66.3
Yoghurt	78.65	–	898	80.7	102	96.7	1.02	70.6	1159	84.6	26.7	88.0
Tomato paste	71.09	–	146	56.6	208	81.6	1.75	58.9	5050	86.6	16.3	70.0
Onion	91.35	–	245	48.0	111	67.9	1.31	26.7	1766	81.6	7.85	76.9
Yeast	70.98	–	116	60.7	127	73.9	8.56	76.1	2557	81.1	47.5	88.3
Red pepper	5.75	18.1	120	48.8	254	73.6	1.20	50.8	3479	77.6	10.7	71.0

[a] HCl-E: HCl-extractability.
[b] IVPD: in vitro protein digestibility.

Table 2
Statistical summary of the data for the dough and tarhana samples (based on dry matter)

Sample	pH	Phytic acid		Ca		Mg		Zn		K		Protein (N × 6.25)	
		Total (mg/100 g)	Loss (%)	Total (mg/100 g)	HCl-E[a] (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	IVPD[b] (%)
Dough													
Mean	5.08 ± 0.05	138 ± 30.0	70.7 ± 6.4	146 ± 1.5	76.1 ± 1.1	156 ± 1.5	83.6 ± 1.2	1.0 ± 0.1	55.6 ± 6.9	651 ± 40.9	79.7 ± 2.6	13.8 ± 0.3	71.3 ± 0.8
min–max	4.98–5.16	90.2–174	62.9–80.9	143–147	73.1–77.9	153–156.6	81.1–85.0	0.9–1.1	44.1–61.3	637–669	74.0–83.0	13.5–14.7	70.0–72.5
Tarhana													
Mean	4.31 ± 0.08	22.1 ± 4.8	95.3 ± 1.0	155 ± 7.6	80.2 ± 1.0	164 ± 5.1	86.4 ± 1.2	1.2 ± 0.1	73.9 ± 5.5	734 ± 39.7	92.6 ± 3.7	16.1 ± 0.5	91.9 ± 1.3
min–max	4.17–4.41	18.5–32.2	93.2–96.0	147–160	77.3–81.9	152–168	84.0–88.8	1.0–1.4	64.4–79.7	673–779	85.4–96.9	14.5–16.8	87.8–94.8

[a] HCl-E: HCl-extractability.
[b] IVPD: in vitro protein digestibility.

Table 3
The effects of bakers' yeast on the changes of phytic acid and some minerals of the dough and tarhana samples as a result of Duncan's multiple range test[a]

Sample	Yeast level (%)	N	Phytic acid		Ca		Mg		Zn		K		Protein (N × 6.25)	
			Total (mg/100 g)	Loss (%)	Total (mg/100 g)	HCl-E[b] (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	IVPD[c] (%)
Dough	0	18	153a	67.7b	144b	74.9b	155a	82.7b	0.96c	46.3b	626b	76.9b	13.1a	70.7a
	2.5	18	137ab	71.1a	146a	76.5a	156a	84.2a	1.02b	59.8a	655ab	80.2a	13.6a	71.1a
	5	18	126b	73.4a	147a	76.9a	157a	85.2a	1.07a	60.7a	671a	81.4a	14.1a	71.2a
Tarhana	0	18	26.8a	94.3b	148b	79.1b	157c	85.2b	1.07c	66.7b	681c	91.2b	15.0c	89.0c
	2.5	18	20.6b	95.6a	157a	80.4a	166b	87.0a	1.24b	77.1a	744b	92.8a	16.3b	92.8b
	5	18	18.9b	96.0a	161a	81.0a	169a	86.9a	1.36a	77.9a	775a	93.8a	17.1a	93.9a

[a] The means with the same letter in column are not significantly different ($p < 0.05$).
[b] HCl-E: HCl-extractability.
[c] IVPD: in vitro protein digestibility.

Table 4
The effects of barley malt flour on the changes of phytic acid and some mineral values of the dough and tarhana samples as a result of Duncan's multiple range test[a]

Sample	Malt level (%)	N	Phytic acid		Ca		Mg		Zn		K		Protein (N × 25)	
			Total (mg/100 g)	Loss (%)	Total (mg/100 g)	HCl (%)[b]	Total (mg/100 g)	HCl (%)	Total (mg/100 g)	HCl c(%)	Total (mg/100 g)	HCl (%)	Total (mg/100 g)	IVPD[c] (%)
Dough	0	18	148a	68.8b	145a	75.7a	156a	83.1b	0.99b	55.0b	659a	79.5b	13.7a	71.2a
	2	18	132b	72.2a	146a	76.0a	155a	83.6ab	1.01ab	54.8b	640a	79.7ab	13.8a	71.0a
	4	18	136ab	71.2ab	146a	76.2a	156a	84.1a	1.03a	56.9a	654a	80.0a	13.5a	71.3a
Tarhana	0	18	23.7a	94.9b	155a	80.0a	164a	85.2b	1.21b	73.0b	730b	90.7c	15.9a	91.5a
	2	18	22.1ab	95.3ab	156a	79.9a	164a	87.0a	1.22ab	73.9ab	735a	92.2b	16.2a	91.9a
	4	18	20.5b	95.7a	155a	80.5a	164a	86.9a	1.23a	74.8a	736a	94.8a	16.3a	92.2a

[a] The means with the same letter in column are not significantly different ($p < 0.05$).
[b] HCl-E: HCl-extractability.
[c] IVPD: in vitro protein digestibility.

Table 5
The effects of microbial phytase on the changes of phytic acid and mineral values of the dough and tarhana samples as a result of Duncan's multiple range test[a]

Sample	Phytase level (%)	N	Phytic acid		Ca		Mg		Zn		K		Protein (N × 6.25)	
			Total (mg/100 g)	Loss (%)	Total (mg/100 g)	HCl-E[b] (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	HCl-E (%)	Total (mg/100 g)	IVPD[c] (%)
Dough	0	18	159a	66.3b	146a	75.8a	156a	83.6a	1.01a	54.7b	639a	78.6c	13.5a	70.9a
	0.05	18	151a	68.0b	146a	75.9a	156a	83.4a	1.01a	55.6ab	656a	79.2b	13.9a	71.2a
	0.5	18	105b	77.9a	145a	76.4a	156a	83.9a	1.02a	56.47a	656a	81.4a	13.6a	71.3a
Tarhana	0	18	23.8a	94.9b	155a	80.0a	163a	86.1a	1.22a	72.0b	733a	90.7c	16.0a	91.3b
	0.05	18	22.8ab	95.2ab	155a	80.0a	164a	86.3a	1.22a	74.1a	733a	92.2b	16.2a	91.9ab
	0.5	18	19.7b	95.8a	157a	80.3a	164a	86.7a	1.23a	75.6a	734a	94.8a	16.1a	92.5a

[a] The means with the same letter in column are not significantly different ($p < 0.05$).
[b] HCl-E: HCl-extractability.
[c] IVPD: in vitro protein digestibility.

in Tables 3–5. These phytase sources have high phytase activity (Reddy, Sathe, & Salunke, 1982). The PAL values of the 2.5% and 5% yeasted tarhana samples were not significantly different from each other ($p < 0.05$) and their losses were higher than that of the unyeasted one (Table 3). Tangkongchitr, Seib, and Hoseney (1981) found that doubling the yeast from 2% to 4% in a whole wheat bread formulation had little effect on the PAL. The loss of PA in the both yeasted and unyeasted tarhana samples occurred because of the phytase activity of bakers' yeast, and additionally, by the low acidity due to the lactic fermentation made by the bacteria sourced from yoghurt, wheat flour and the other ingredients. The PAL of unyeasted dough with natural and lactic acid fermentation, has been reported (Fredrikson, Larsson, Lemola, Laitala, & Sandberg, 1998; Lopez, Gordon, & Field, 1983; Sindhu & Khetarpaul, 2001). The pH of unyeasted tarhana was reported as lower than that of yeasted one by Bilgiçli (2004). Therfore, the PAL of unyeasted tarhana approached the yeasted one, due to the lower pH which contributes the degradation of PA (Fretzdorff & Brümmer, 1992).

The differences in PAL between 2% and 4% malted tarhanas were not significant. But the PALs of both malted tarhana samples were found to be higher than the unmalted one (Table 4). Faridi, Finney, and Rubenthaler (1983), and Harland and Harland (1980) have reported that, increasing the malt in wholemeal breads, reduced the PA content by 50%. High levels of PAL with the germination or malting of sorghum, oat, wheat, rye, finger millet, pearl millet and peas have been reported (Beal & Mehta, 1985; Elkhalil, El Tinay, Mohamed, & Elsheikh, 2001; Fredlund, Larrson, Marklinder, & Sandberg, 1997; Larrson & Sandberg, 1995; Mahgoub & Elhag, 1998; Saharan et al., 2001; Sripriya et al., 1997).

0.5% phytase preparation addition decreased the PA contents of tarhana doughs sharply. But, in tarhana samples, there is a marked decrease in PA and increase in PAL as a result of the all fermentation factors, together with phytase addition (Table 5). Phytase addition is one of the effective means to decrease the PA content and improve the availability of minerals (Lasztity & Lasztity, 1990). Sandberg and Svanberg (1991) have reported that, by phytase addition, all the PA content was hydrolysed within 2 hours.

A nonlinear equation between the PAL and the factors used, such as phytase sources, bakers' Yeast, barley malt and microbial phytase, is shown in Table 7. The coefficient of nonlinear regression equation (6) is very high (93.6%). It may be speculated that the marked increase in PAL is attributable to increase in phytase activity due to the additions of bakers' yeast, barley malt and microbial phytase together, not due to each one of them.

3.3. The change in mineral contents

3.3.1. Calcium

Average values of total Ca increased from 146 ± 1.5 mg/100 g in tarhana dough to 155 ± 7.6 mg/100 g in tarhana (Table 2) as a result of fermentation loss. The main source of this richness is yoghurt with 898 mg/100 g Ca content (Table 1). HCl-E of Ca indicates the bioavailability of Ca and increase as a result of PAL proportionally (Rickard & Thompson, 1997). Table 6 shows a significant and negative correlation coefficient ($r = -0.779$, $p < 0.01$) and regression equation (1) between HCl-E of Ca and PA content of tarhana. While the PA content decreases, HCl-E of Ca increases. So, the main reason was the fermentation, which includes indigenous phytases of the constituent ingredients which have natural fermenting microflora and supplements, such as baker's yeast, barley malt and microbial phytase in the present study. Similar results were obtained by Toufeili et al. (1999) on kishk.

Total Ca and HCl-E of Ca amounts are increased a little by the addition of the phytase sources in both dough and tarhana samples (Tables 3–5). High bakers' yeast addition is the best way to increase the total Ca amount, from the control to 5% yeast level, from 144 to 147 mg/100 g in the dough, and from 148 to 161 mg/100 g in tarhana ($p < 0.05$). Also in HCl-E of Ca in the dough and tarhana, changed from 74.9% to 76.9% and from 79.1% to 81.0%, respectively (Table 3). Marked increase was obtained with 2.5% bakers' yeast addition. Additional yeast (5%) did not affect the total amount and HCl-E of Ca (Table 3). The increases in total Ca due to fermentation loss (Bilgiçli, 2004), and in HCl-E of Ca due to the degradation PA during 72 h fermentation were caused by progressive fermentation, as a result of increasing bakers' yeast incorporation. Toufeili et al. (1999) reported similar results for whole-wheat kishk (92.8%) and bulgur kishk (89.1%) after a 96 h-fermentation period. Also the lower pH of the tarhana (4.31 ± 0.1) (Table 2) was a very dominant factor in PAL and HCl-E of Ca (Martin & Evans, 1986; Nolan, Duffin, & McWeeny, 1987) .

As shown in Table 7, the multiple regression equation (7) shows a nonlinear relationship at significant level ($r^2 = 80.3\%$, $p < 0.01$) between HCl-E of Ca and the increasing levels of phytase sources used as the most effective factor is bakers' yeast ($p < 0.01$).

3.3.2. Magnesium

As seen in Table 2, total Mg and HCl-E of Mg at the dough stage were 156 ± 1.5 mg/100 g and 83.6 ± 1.2%, respectively. These values increased after the fermentation procedure in tarhana 164 ± 5.1 mg/100 g and 86.4 ± 1.2%, respectively. Tomato paste (208 mg/100 g) and red pepper (254 mg/100 g) were the main sources of the Mg of the tarhana (Table 1). Compared to the

Table 6
Correlation coefficients and regression equations of the correlations between phytic acid and HCl-E values of the minerals (Ca, Mg, Zn and K), and also IVPD[a]

	HCL-E of Ca	HCL-E of Mg	HCl-E of Zn	HCl-Eof K	IVPD
Correlation coefficient (r)	−0.779[b]	−0.614[b]	−0.932[b]	−0.801[b]	−0.914[b]
Regression coefficient (r^2) (%)	60.7	27.0	86.9	64.2	83.6
Regression equation	$y = -2.95x + 259.42$ (1)	$y = -2.13x + 201.34$ (2)	$y = -0.74x - 77.69$ (3)	$y = -0.92x + 108.44$ (4)	$y = -1.69 + 177.96$ (5)

[a] HCl-E: HCl-extractability, IVPD: in vitro protein digestibility, Dependent variables: HCl-E of Ca, Mg, Zn, K and IVPD independent variable: phytic acid.
[b] Significant at $p < 0.01$ level.

Table 7
Nonlinear regression equations among phytic acid, HCl-E of minerals and the phytase sources[a]

Dependent variables	Nonlinear equations	
Phytic acid loss (y_1) ($r^2 = 93.6\%$)	$y_1 = 93.3 + 0.864x_1 + 0.381x_2 + 7.33x_3 - 0.0889x_1^2 - 0.0581x_2^2 - 10.5x_3^2 - 0.0027x_1x_2 - 0.248x_1x_3 - 0.225x_2x_3$	(6)
HCl-E[b] of Ca (y_2) ($r^2 = 80.3\%$)	$y_2 = 78.2 + 0.985x_1 - 0.207x_2 + 8.03x_3 - 0.0972x_1^2 + 0.122x_2^2 - 11.0x_3^2 - 0.0202x_1x_2 - 0.276x_1x_3 - 0.371x_2x_3$	(7)
HCl-E of Mg (y_3) ($r^2 = 71.1\%$)	$y_3 = 85.3 + 1.30x_1 - 0.455x_2 - 7.81x_3 - 0.166x_1^2 + 0.104x_2^2 + 19.5x_3^2 - 0.0208x_1x_2 - 0.308x_1x_3 - 0.052x_2x_3$	(8)
HCl-E of Zn (y_4) ($r^2 = 95.7\%$)	$y_4 = 63.0 + 6.31x_1 + 0.606x_2 + 51.2x_3 - 0.771x_1^2 + 0.082x_2^2 - -876x_3^2 - 0.174x_1x_2 + 0.069x_1x_3 - 0.526x_2x_3$	(9)
HCl-E of K (y_5) ($r^2 = 90.4\%$)	$y_5 = 84.2 + 3.62x_1 + 0.859x_2 + 33.3x_3 - 0.412x_1^2 + 0.016x_2^2 - -33.0x_3^2 - 0.0277x_1x_2 - 2.32x_1x_3 - 1.18x_2x_3$	(10)
IVPD[c] (y_6) ($r^2 = 95.4\%$)	$y_6 = 87.8 + 2.08x_1 + 0.383x_2 + 10.3x_3 - 0.210x_1^2 - -0.0396x_2^2 - 13.4x_3^2 - 0.0002x_1x_2 - 0.066x_1x_3 - 0.771x_2x_3$	(11)

[a] x_1 (bakers' yeast level), x_2 (barley malt level) and x_3 (microbial phytase level) were used as independent variables.
[b] HCl-E: HCl-extractability.
[c] IVPD: in vitro protein digestibility.

ingredients, the HCl-E of Mg of tarhana was the highest of the all except for yoghurt (96.7%) (Table 1). The increment of HCl-E of Mg during fermentation could be attributed to PAL as result of the negative relationship between PA and HCl-E of Mg ($r = -0.614$, $p < 0.01$), as seen in Table 6. Similar results were also reported by Toufeili et al. (1999) for kishk. These observations show that tarhana has a potentially superior nutritional value with its Mg content ranging between 152 and 168 mg/100 g (Table 2).

Bakers' yeast addition is the most effective factor, as a phytase source, for the total Mg and HCl-E of Mg values (Tables 3–5. Especially, in tarhana, the total Mg and HCl-E of Mg values were increased from the control to 5% yeast addition from 157 to 169 mg/100 g and from, 85.2 to 86.9%, respectively. The marked increments were obtained with 2.5% bakers' yeast addition, but not with 5% to the same degree in the total Mg and HCl-E of Mg (Table 3).

Table 7 shows that, there is a nonlinear relationship (8) at significant level ($r^2 = 71.1$ %, $p < 0.01$) between HCl-E of Mg and the phytase sources used. This relationship is rather more influenced by bakers' yeast than the others. All of these findings show that there was a significant negative correlation between PA and the bioavailability of Mg (Toufeili et al., 1999).

3.3.3. Zinc

The total amount and HCl-E of Zn were 1.0 ± 0.1 mg/100 g and $55.6 \pm 6.9\%$ in dough, respectively (Table 2). For tarhana samples, these parameters increased to the values of 1.2 ± 0.1 mg/100 g and $73.9 \pm 5.5\%$, respectively, as a result of fermentation. Bakers' yeast is the richest Zn source amoung the ingredients with 8.56 mg/100 g of total Zn and 76.1% HCl-E of Zn (Table 1). The increase in HCl-E of Zn in tarhana samples is related to PAL during fermentation (Murali & Kapoor, 2003). There is a negative and significant correlation coefficient ($r = -0.932$, $p < 0.01$) between HCl-E of Zn and PA (Table 6). The acidity of the medium and the phytase activities of the ingredients during fermentation led to increase in HCl-E of Zn (Toufeili et al., 1999).

The alterations of the total Zn and HCl-E of Zn contents of the dough and tarhana samples by the addition of the phytase sources are presented in Tables 3–5. Bakers' yeast, barley malt and microbial phytase showed an increasing significant effect on HCl-E of Zn ($p < 0.05$). The yeast addition was rather more effective than those of the barley malt and microbial phytase. Especially at tarhana samples, bekers' yeast addition, up to 5% versus control, caused increase in total Zn and HCl-E of Zn from 1.07 mg/100 g and 66.7% to 1.36 mg/100 g and 77.9%, respectively (Table 3).

By multiple regression calculations, a very high regression coefficient ($r^2 = 95.7$ %, $p < 0.01$) was obtained between HCl-E of Zn and the phytase sources

used. Bakers' yeast, barley malt and phytase enzyme were the effective factors in this nonlinear relationship (9) (Table 7).

3.3.4. Potassium

Average values of total K increased from 651 ± 40.9 mg/100 g in the dough to 734 ± 39.7 mg/100 g in tarhana due to the fermentation loss (Table 2). Also, HCl-E of K content increased from 79.7 ± 2.6% in the dough to 92.6 ± 3.7% in tarhana as a result of fermentation procedures. In tarhana formulation, the richest K source among the ingredients is tomato paste with 5050 mg/100 g total amount and 86.6% HCl-E of K (Table 1). Red pepper, bakers' yeast and onion were second in richness. A negative correlation coefficient ($r = -0.801$ $p < 0.01$) was found between HCl-E of K and PA (Table 6), likely due to the enhanced phytase activity during 72 h of fermentation and the acidic medium of the tarhana dough which cause the PAL and inhibit K-phytate complexes (Martin & Evans, 1986).

The parameters of tarhana are more affected by the phytase sources than those of the dough (Tables 3–5). The increasing levels of bakers' yeast, barley malt and phytase enzyme increased the total K due to their acceleration effects on fermentation loss. HCl-E of K values of tarhana were increased up to 93.8%, 94.8% and 94.8%, respectively, by the addition of bakers' yeast, barley malt and microbial phytase preparation.

The multiple regression equation (10) with its high coefficient ($r^2 = 90.4\%$, $p < 0.01$) of the correlation between HCl-E of K and the phytase sources used were shown in Table 7. All phytase sources were found to be effective on this parameter with a parabolic regression relationship. Both phytase sources, baker's yeast and barley malt, were found to be the best among the effective factors for enhancing HCL-E of K.

In our previous study, 72 h of fermentation time of tarhana and the acidic reaction of the dough, together with bakers' yeast, barley malt and microbial phytase supplementation at increased levels, resulted in an increase in the HCl-extractable ash and phosphorus amounts, which is in line with our finding about the availabilities of Ca, Mg, Zn and K as well as PAL during the fermentation of tarhana dough (Bilgiçli, 2004).

3.4. Total amount and in vitro digestibility of protein

The changes in the total protein amount (TPA) and the in vitro protein digestibility (IVPD) of the samples from the dough to tarhana were found to be changed from 13.8% to 16.1%, and from 71.3% to 91.9%, respectively (Table 2). Jandal (1989) reported that IVPD ranged from 92% to 92.6% on khisk samples. According to the results of our studies, there is a strong negative correlation (−0.914, $p < 0.01$) between the PA and IVPD of tarhana (Table 6).

By increasing the yeast level, TPA and IVPD of tarhana were also increased . IVPD of unyeasted tarhana was found to be 89.0% which increased to 93.9% on the addition of 5% yeast into tarhana formulation (Table 3). Türker (1995) found that the IVPD of unyeasted tarhana was 94.4% and, of yeasted tarhana, 98.7%. TPA of tarhana was increased by the yeast addition, due to the high protein content (47.5%) (Table 1). Bakers' yeast has high phytase activity and hydrolyses PA which binds protein and decreases its digestibility (Tangkongchitr et al., 1981). The other microorganisms, such as lactic acid bacteria naturally found in yoghurt, and the other ingredients, may also increase the IVPD. Fermentation is an effective process for increasing the IVPD (Temiz & Pirkul, 1991). Barley malt and microbial phytase additions did not change the total protein amount of tarhana at a statistically significant level ($p < 0.05$) (Tables 4 and 5).The IVPD values of tarhana, prepared with different malt levels, were found to be in the range 91.5–92.2% and were not statistically different from each other at the $p < 0.05$ level (Table 4). But, 0.5% phytase addition increased the IVPD from 91.3% to 92.5% (significantly $p < 0.05$) (Table 5).

As seen in Table 7, a nonlinear regression equation (11) with high regression coefficient ($r^2 = 95.4$, $p < 0.01$) may be suitable for prediction of protein digestibility, depending on bakers' yeast, barley malt flour and microbial phytase levels being the most effective one is bakers' yeast.

4. Conclusion

Tarhana is a good source of total minerals (Ca, Mg and K) with good (Ca, Mg, Zn and K) bioavailibilities. The fermentation medium with increasing acidity, in addition to the use of phytase sources, caused an increase in total amounts of minerals and proteins as a result of PA fermentation loss. Bakers' yeast, the favourite one, and also barley malt and microbial phytase additions helped to increase the availability of the nutrients and decreased the PA content from 138 mg/100 g in the dough to 22.1 mg/100 g in tarhana. PAL reached 95.3% (on average) in tarhana samples.

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