Phytate Degradation during Breadmaking: The Influence of Flour Type and Breadmaking Procedures

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Abstract

Požrl T., Kopjar M., Kurent I., Hribar J., Janeš A., Simčič M. (2009): **Phytate degradation during breadmaking: The influence of flour type and breadmaking procedures**. Czech J. Food Sci., **27**: 29–38.

Phytic acid has been considered to be an antinutrient due to its ability to bind minerals and proteins, either directly or indirectly, thus changing their solubility, functionality, absorption, and digestibility. In this study, the influence of the flour type (type 500, type 850, and whole meal flour) and three different breadmaking procedures (direct, indirect, and with sourdough addition) on phytic acid was investigated. The results showed that the flour type influenced the phytic acid content. The phytic acid contents of flour type 500, type 850, and whole meal flour was 0.4380, 0.5756, and 0.9460 g/100 g dm, respectively. The dough and bread prepared from flour with a higher phytic acid content also contained higher amount of phytic acid. During fermentation and baking, degradation of phytic acid occurred. Phytic acid was also influenced by pH. Samples of lower pH had a lower phytic acid content. Dough prepared from flour type 500 and type 850 with 10% addition of sourdough had especially low phytic acid contents, and the bread prepared from the respective dough contained no phytic acid at all.

Keywords: phytic acid; flour type; breadmaking procedure

Phytic acid (*myo*-inositol hexaphosphoric acid) is present in substantial quantities in a variety of plant foodstuffs. It is the predominant form of phosphorus in cereals, oil-seeds, and seeds of leguminous plants and as such is the major natural phosphorus source in animal feed (Ravindran *et al.* 1995; Zhou & Erdman 1995; Rickard & Thompson 1997). Phytic acid has often been considered as an antinutrient due to its ability to bind minerals and proteins, either directly or indirectly, and thus change their solubility, functionality, absorption, and digestibility (Rickard & Thompson 1997; Bilgiçli *et al.* 2006; Dewettinck *et al.* 2008; Frontela *et al.* 2008; Palacios *et al.* 2008a, b).

The cations of interest in this regard include zinc, iron, calcium, and copper (Harland & Harland 1980; Weaver & Kannan 2001; Abebe *et al.* 2007). Most phytic acid-mineral complexes are insoluble at physiological pH, which is the main cause of the poor bioavailability of the mineral complexes (Tamim & Angel 2003). The bioavailability of proteins, vitamins, and some minerals may be restricted when complexed with phytic acid. Tamim & Angel (2003) found that the order of stability of mineral-phytate complexes was $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$. The stability of the mineral complexes depends on the number of phosphate groups on the inositol ring. Weaker complexes are formed with lower inositol phosphates (Harland & Harland 1980). Phytases are enzymes that catalyse the degradation of phytate to lower inositol phosphates and free inorganic phosphorus, depending on the extent of the enzyme activity (Pandey *et al.* 2001).

Some practical and relatively inexpensive procedures such as soaking, germination, and fermentation are reported to reduce the phytic acid content of legumes (Cheryan 1980). In general, lower pH, a longer fermentation time, and a higher yeast addition result in a more intensive degradation of phytic acid (Hesseltine 1979; Lasztity & Lasztity 1990). During baking of bread the degradation of phytic acid occurs due to the activation of the phytase present in flour and the high temperatures. Breadmaking is a multiphase procedure, and the most important phases are fermentation and baking. A high water content in the dough increases the hydrolysis of phytic acid (Türk & Sandberg 1992). The most important factor is the acidity of the dough. The optimal pH for phytase activity in wheat dough is 4.5, and the optimal temperature is 55°C (Fretzdorff & Brummer 1992). The rate of hydrolysis and the consequent decrease of the phytic acid content depend on the phytase activity, temperature, pH, water content, fermentation time, added enzymes, and other parameters (Türk & Sandberg 1992; Penella *et al.* 2008). The amount of hydrolysed phytic acid in different bread types varies between 13–100% (Lopez *et al.* 2001). The addition of yeast may increase the amount of hydrolysed phytic acid (Türk *et al.* 1996). Phytic acid hydrolysis is a consequence of the activity of phytase which is present in wheat and yeasts, and probably due to the presence of the microorganisms that are involved in dough fermentation (Giovanelli & Polo 1994).

A reduction of the phytate content can be achieved by adding exogenous phytate-degrading enzymes (Türk & Sandberg 1992; Palacios *et al.* 2008a, b). Phytases are produced by a wide range of plants, bacteria, fungi, and yeasts (Pandey *et al.* 2001). Phytases from microbial sources are the most promising ones for the production on a commercial level. Many of them were proven to have good properties for their use as starters in the breadmaking process (Palacios *et al.* 2008b).

Many authors report that the addition of sourdough and lactic acid bacteria with high phytate degrading activity during the breadmaking process can reduce the phytic acid content in bread (Katina *et al.* 2005; Corsetti & Settanni 2007; Palacios *et al.* 2008a, b).

The objective of this study was to investigate the influence of different flour types (500, 850, and whole meal) and different breadmaking procedures (direct, indirect, and with the addition of sourdough) on phytic acid degradation during the bread preparation.

MATERIALS AND METHODS

Material. The basic material was soft wheat (hectolitre weight 82.02 kg/hl, 14% water content, 13,6% protein content, 34 ml Zeleny sedimentation value), which was milled to flour type 500, type 850, and whole meal flour. The soft wheat milling process took place on a Bühler AG (Uzwil, Switzerland), commercial mill with the combination of five-and eight roller mills after 18 h of conditioning (water content 15.5%). The yield of whole meal flour was 99% and that of flours 78% (55% flour type 500, 23% flour type 850). All three types of flour were prepared from the same wheat to avoid the influence of biodiversity on the wheat properties. From these three types of flour, bread was prepared by three different technological procedures. The lyophilised yeast Saf Instant (composition: *Saccharomyces cerevisiae*, rehydrating agent) from S. I. Lesaffre (Marcq en Baroeul, France) and the lyophilised starter for sourdough La1 (composition: *Pediococcus acidilacti*, lactose support, *Saccharomyces cerevisiae*) from Lallemand S. A. (Blagnac Cedex, France) were used. Ash content, protein content, and falling number are presented in Table 1.

Breadmaking procedure. The ingredients for all bread samples were mixed in an Diosna SP 12 spiral mixer (Diosna Dierks & Söhne, GmbH, Osnabrück, Germany) for 6 min in 15 rpm bowl and a 105 rpm spiral velocity, and then for 3 min in 30 rpm bowl and at 210 rpm spiral velocity. After mixing, the dough rested in the mixer bowl for 20 minutes. It was then divided (500 g) and put in moulds for fermentation and baking. Fermentation took place in Gostol-Gopan fermentation chamber FK (Gostol-Gopan, Nova Gorica, Slovenia) at 76% relative humidity. For baking, a Miwe aero CS oven (Miwe Michael Wenz GmbH, Arnstein, Germany) was programmed as follows: 5 min at 230°C with steaming, than 20 min at 190°C, and 4 min at 200°C.

Table 1. Ash and protein contents, Falling Number of flour types 500, 850 and whole meal flour

	Type 500	Type 800	Whole flour
Ash/(dm) (%)	0.535	0.869	1.828
Protein content/(dm) (%)	12.4	12.8	13.6
Falling Number (s)	366	352	385

Sourdough was prepared from all three types of flour as follows: 1000 g of flour, 1500 g of water, and 1 g of lyophilised starter for sourdough were mixed and fermented for 20 h at a temperature of 32°C. The pH value of the sourdough was 3.8.

Direct breadmaking procedure. In the direct breadmaking procedure, the dough rested after mixing in the mixer bowl for 20 min and then fermented for 30 min in the fermentation chamber. In one experiment, the fermentation was conducted at 20°C, and in another at 30°C. The dough formula was the following: flour (1000 g), water (680 g), yeast (20 g), salt (25 g), and sugar (20 g). The dough temperature in the first experiment was 20°C and in the second 30°C. After mixing, fermentation and baking, samples for analyses were taken.

Indirect breadmaking procedure. The indirect breadmaking procedure was a procedure with prolonged fermentation, where – after mixing – the dough rested in the mixer bowl for 20 min and fermented for 30 min in the fermentation chamber at 20°C. After mixing and a short fermentation, samples for analysis were taken. The prolonged fermentation in the first experiment lasted for 3 h and in the second for 6 h at 20°C. Every hour the dough was kneaded and samples for analysis were taken. The dough formula was the following: flour (2000 g), water (1340 g), yeast (40 g), salt (50 g), and sugar (40 g). The dough temperature was 20°C. After baking, samples for analysis were taken again.

Procedure with sourdough addition. The third breadmaking procedure involved the addition of 5% or 10% of sourdough to the dough. The dough formula was the following: flour (900 g if 10% of sourdough was added and 950 g if 5% of sourdough was added), sourdough (100 g if 10% of sourdough was added and 50 g if 5% of sourdough was added), water (680 g), yeast (20 g), salt (25 g), and sugar (20 g). After mixing, the dough rested in the mixer for 20 min and fermented for 30 min in the fermentation chamber at 20°C. The dough temperature was 20°C. After mixing, fermentation and baking, samples for analysis were taken.

Sampling. All samples for phytic acid determination were frozen by submerging in liquid nitrogen, packed in PE bags, and stored in a freezer at –25°C for a week until analysis. The determination of dry matter and pH was conducted immediately after the baking and cooling of the bread. Dry matter, pH, and phytic acid content were determined in all samples (flour, dough, fermented dough, and baked bread, bread crust and crumb). Bread crust and crumb were taken as separate samples. Bread crust and crumb were separated with a sharp knife approximately 3 mm from the upper part of the mould type of bread on the border of the intensive brown colour change.

Determination of dry matter. Dry matter (dm) was determined indirectly according to Amtlichen Sammlung von Untersuchungsverfahren, Methode L 17.00–1 (1982). Most results are expressed on the dry matter basis.

Determination of pH. pH was determined by a potentiometric method, according to AOAC Official Method 943.02 (1995).

Determination of phytic acid content. Phytic acid was measured on an HP 8453 spectrophotometer using the colorimetric method according to Haugh and Lantzsch (1983) at 519 nm with slight modifications. The phytic acid in samples was extracted with a solution of HCl (0.4M) and precipitated with a solution of Fe^{III} (ammonium iron (III) sulphate $\cdot 12\ H_2O$).The results are expressed as g phytic acid/100 g dry matter.

Statistical analyses. The data obtained in determination of the dry matter content, pH, and phytic acid content were statistically analysed by means of the program package SAS/STAT (SAS Software. Vers. 8.01, 1999). The results were expresed as mean values of four replicates ± standard deviation. A multivariate analysis of variance (MANOVA) with the interaction by the GLM procedure was used. The means for the experimental groups were obtained using the Duncan procedure and were compared at 5% probability level. The relationships between pH values and PA contents were assessed by Pearson correlation coefficients, using the CORR procedure.

RESULTS AND DISCUSSION

The phytic acid contents of flour types 500, 850, and whole meal flour were 0.4380 g/100 g dm, 0.5756 g/100 g dm, and 0.9460 g/100 g dm, respectively. Comparing the results for the phytic acid content of the flours with those of dough and bread prepared from the respective flours, it can be seen that dough and bread (Tables 2–4) prepared from flour with a higher phytic acid content also contained higher amounts of phytic acid, regardless of the breadmaking procedure.

Direct breadmaking procedure

The direct breadmaking procedure was conducted at two fermentation temperatures, 20°C and 30°C. Table 2 presents the results obtained for dry matter content, pH, and phytic acid content in all samples (dough, dough after fermentation, bread crumb and crust) prepared from all three types of flour (type 500, 850, and whole meal flour) by the direct breadmaking procedure. The dry matter content of the samples investigated was the lowest in dough regardless of the flour type used at both fermentation temperatures. The highest dry matter content was found in bread crust, also regardless of the flour type and fermentation temperature. Fermentation temperature did not have so pronounced an influence on the dry matter content of dough, fermented dough and bread crumb as it had on bread crust. There was a significant difference in the dry matter content between bread crust prepared with flour type 850 and whole meal flour at 20°C and 30°C. Bread crust prepared from flour type 500 had the same dry matter content (69.9%) regardless of the fermentation temperature, 20°C or 30°C.

In the case of pH, the results it show that pH values were the highest in dough, then followed by dough after fermentation, the crumb of bread, and the lowest values were found in the crust regardless of the flour type used. Also, it can be seen that the type of flour had an influence on the pH of samples. The lowest pH values were in the samples prepared with flour type 500 while the highest values were in the samples prepared with whole meal flour when fermentation was conducted at 20°C. When fermentation was conducted at 30°C, the same tendency was observed. The lowest pH values were found in the samples prepared with flour type 500 and the highest values in the samples prepared with whole meal flour. The temperature of fermentation had an influence on pH, and from the results it can be seen that the samples fermented at 20°C had a higher pH than those fermented at 30°C, regardless of the flour type.

The results presented in Table 2 reveal that the fermentation temperature influenced the phytic acid content since all samples prepared at 20°C had a higher phytic acid content than those prepared at 30°C. The type of flour used for the sample preparation had a very high influence on the phytic acid content. All samples prepared from flour type 500 had the lowest phytic acid content, while the samples prepared from whole meal flour had the highest phytic acid content. The highest phytic acid content was found in dough regardless of the fermentation temperature and flour type, followed by fermented dough, bread crumb, while the lowest phytic acid content was found in bread crust.

Indirect breadmaking procedure

The indirect breadmaking procedure is a process with prolonged fermentation. Fermentation was conducted at 20°C for 6 hours. The results for the dry matter content, pH, and phytic acid content in the samples made from all three types of flour (type 500, 850 and whole meal flour) prepared by the indirect breadmaking procedure at the fermentation temperature 20°C are presented in Table 3. The lowest dry matter content was found in dough, followed by fermented dough and bread crumb, and the highest dry matter content was found in bread crust regardless of the flour type used. The difference in the dry matter content during fermentation was also determined every hour for 6 hours. At the beginning (fermented dough after 1 h), the lowest dry matter content was found but during fermentation it increased, regardless of the flour type used, so it can be concluded that the fermentation time had an influence on the dry matter content of fermented dough. The fermentation time of 3 or 6 h, did not influence the dry matter content of bread crumb, while it did influence the dry matter content of bread crust.

As to pH, dough had the highest pH value; during fermentation the pH decreased, and it also decreased after the baking of bread. The type of flour had a significant influence on the pH of

Table 2. Dry matter contents, pH, and phytic acid (PA) contents in samples made from three types of flour by the direct breadmaking procedure

Parameters	Fermentation temperature (°C)	Sample	Flour			P_F
			type 500	type 850	whole meal	
Dry matter (%)	20	dough	46.5 ± 0.093^{d,y}	46.8 ± 0.102^{c,x}	46.8 ± 0.004^{d,x}	0.0003
		fermented dough	46.7 ± 0.078^{c,z}	47.1 ± 0.050^{c,y}	47.2 ± 0.009^{c,x}	< 0.0001
		bread crumb	47.6 ± 0.135^{b,y}	47.9 ± 0.122^{b,x}	47.9 ± 0.011^{b,x}	0.0003
		bread crust	69.9 ± 0.293^{a,x}	70.7 ± 0.943^{a,x}	69.9 ± 0.308^{a,x}	0.1725
		P_S	< 0.0001	< 0.0001	< 0.0001	
	30	dough	46.3 ± 0.096^{c,z}	46.6 ± 0.136^{d,y}	46.8 ± 0.131^{d,x}	0.0013
		fermented dough	46.6 ± 0.091^{c,z}	46.9 ± 0.091^{c,y}	47.2 ± 0.043^{c,x}	<0.0001
		bread crumb	47.5 ± 0.101^{b,y}	47.7 ± 0.050^{b,x}	47.9 ± 0.186^{b,x}	0.0050
		bread crust	69.9 ± 0.406^{a,x}	69.0 ± 0.307^{a,y}	69.1 ± 0.098^{a,y}	0.0079
		P_S	< 0.0001	< 0.0001	< 0.0001	
pH	20	dough	5.96 ± 0.013^{a,z}	6.06 ± 0.005^{a,y}	6.35 ± 0.005^{a,x}	< 0.0001
		fermented dough	5.90 ± 0.008^{b,z}	6.01 ± 0.006^{b,y}	6.28 ± 0.005^{b,x}	< 0.0001
		bread crumb	5.73 ± 0.005^{c,z}	5.89 ± 0.006^{c,y}	6.18 ± 0.006^{c,x}	< 0.0001
		bread crust	5.67 ± 0.014^{d,z}	5.82 ± 0.008^{d,y}	6.14 ± 0.006^{d,x}	< 0.0001
		p_S	< 0.0001	< 0.0001	< 0.0001	
	30	dough	5.94 ± 0.005^{a,z}	6.03 ± 0.005^{a,y}	6.31 ± 0.005^{a,x}	< 0.0001
		fermented dough	5.88 ± 0.016^{b,z}	6.01 ± 0.010^{b,y}	6.26 ± 0.008^{b,x}	< 0.0001
		bread crumb	5.73 ± 0.006^{c,z}	5.87 ± 0.008^{c,y}	6.17 ± 0.006^{c,x}	< 0.0001
		bread crust	5.65 ± 0.012^{d,z}	5.82 ± 0.008^{d,y}	6.13 ± 0.013^{d,x}	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	
PA (g/100 g dm)	20	dough	0.44 ± 0.008^{a,z}	0.58 ± 0.006^{a,y}	0.95 ± 0.004^{a,x}	< 0.0001
		fermented dough	0.37 ± 0.003^{b,z}	0.51 ± 0.010^{b,y}	0.88 ± 0.008^{b,x}	< 0.0001
		bread crumb	0.21 ± 0.004^{c,z}	0.35 ± 0.007^{c,y}	0.72 ± 0.002^{c,x}	< 0.0001
		bread crust	0.07 ± 0.002^{d,z}	0.14 ± 0.004^{d,y}	0.30 ± 0.001^{d,x}	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	
	30	dough	0.41 ± 0.001^{a,z}	0.55 ± 0.005^{a,y}	0.91 ± 0.002^{a,x}	< 0.0001
		fermented dough	0.36 ± 0.005^{b,z}	0.50 ± 0.008^{b,y}	0.85 ± 0.002^{b,x}	< 0.0001
		bread crumb	0.19 ± 0.003^{c,z}	0.34 ± 0.006^{c,y}	0.69 ± 0.001^{c,x}	< 0.0001
		bread crust	0.06 ± 0.002^{d,z}	0.13 ± 0.005^{d,y}	0.28 ± 0.006^{d,x}	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	

F – effect of flour type, S – effect of sampling; levels of significance: $P \leq 0.05$ statistically significant, $P \leq 0.001$ highly statistically significant, $P > 0.05$ statistically not significant; $^{x, y, z}$means with a different superscript within rows (effect of flour type) differ significantly ($P \leq 0.05$); $^{a, b, c, d}$means with a different superscript within columns (effect of sampling) differ significantly ($P \leq 0.05$)

Table 3. Dry matter contents, pH, and phytic acid (PA) contents in samples made from three types of flour by the indirect breadmaking procedure

Parameters	Sample	Flour type 500	Flour type 850	Whole meal flour	P_F
Dry matter (%)	dough	46.5 ± 0.009^{f,z}	46.6 ± 0.069^{h,y}	4.7 ± 0.046^{g,x}	< 0.0001
	fermented dough – 1 h	46.8 ± 0.095^{e,y}	46.9 ± 0.076^{g,x}	47.0 ± 0.005^{f,x}	0.0037
	– 2 h	46.9 ± 0.092^{e,y}	47.0 ± 0.062^{fg,x}	47.1 ± 0.078^{f,x}	0.0027
	– 3 h	47.0 ± 0.008^{e,z}	47.1 ± 0.078^{f,y}	47.3 ± 0.055^{f,x}	0.0002
	– 4 h	47.2 ± 0.001^{d,z}	47.3 ± 0.074^{e,y}	47.7 ± 0.050^{e,x}	< 0.0001
	– 5 h	47.5 ± 0.165^{c,x}	47.9 ± 0,016^{c,x}	47.7 ± 0.502^{de,x}	0.3489
	– 6 h	47.3 ± 0.201^{d,y}	47.6 ± 0.050^{d,x}	47.8 ± 0.146^{de,x}	0.0055
	bread crumb – 3 h	47.6 ± 0.229^{c,y}	47.9 ± 0.003^{c,x}	48.60 ± 0.002^{cd,x}	0.002
	– 6 h	47.8 ± 0.264^{c,y}	47.9 ± 0.007^{c,xy}	48.1 ± 0.094^{c,x}	0.0354
	– 3 h	70.1 ± 0.151^{b,y}	70.8 ± 0.183^{b,x}	70.9 ± 0.175^{b,x}	< 0.0001
	– 6 h	72.2 ± 0.209^{a,y}	72.5 ± 0.134^{a,xy}	72.5 ± 0.318^{a,x}	0.0705
	P_S	< 0.0001	< 0.0001	< 0.0001	
pH	dough	5.96 ± 0,005^{a,z}	6.05 ± 0.008^{a,y}	6.35 ± 0.006^{a,x}	< 0.0001
	fermented dough – 1 h	5.90 ± 0,005^{b,z}	6.01 ± 0.005^{b,y}	6.29 ± 0.005^{b,x}	< 0.0001
	– 2 h	5.82 ± 0,013^{c,z}	5.97 ± 0.005^{c,y}	6.22 ± 0.005^{c,x}	< 0.0001
	– 3 h	5.75 ± 0,000^{d,z}	5.94 ± 0.005^{d,y}	6.18 ± 0.006^{d,x}	< 0.0001
	– 4 h	5.71 ± 0,008^{e,z}	5.86 ± 0.005^{e,y}	6.12 ± 0.005^{e,x}	< 0.0001
	– 5 h	5.68 ± 0,008^{f,z}	5.83 ± 0.005^{f,y}	6.10 ± 0.005^{f,x}	< 0.0001
	– 6 h	5.62 ± 0,005^{h,z}	5.78 ± 0.008^{h,y}	6.08 ± 0.006^{g,x}	< 0.0001
	bread crumb – 3 h	5.66 ± 0,008^{g,z}	5.80 ± 0.006^{g,y}	6.07 ± 0.005^{g,a}	< 0.0001
	– 6 h	5.60 ± 0,000^{i,z}	5.74 ± 0.005^{i,y}	6.01 ± 0.006^{i,x}	< 0.0001
	– 3 h	5.61 ± 0,005^{i,z}	5.78 ± 0.008^{h,y}	6.02 ± 0.008^{h,x}	< 0.0001
	– 6 h	5.58 ± 0,005^{j,z}	5.71 ± 0.008^{j,y}	5.98 ± 0.008^{j,x}	< 0.0001
	P_S	< 0.0001	< 0.0001	< 0.0001	
PA (g/100 g dm)	dough	0.43 ± 0.005^{a,z}	0.57 ± 0.005^{a,y}	0.94 ± 0.001^{a,x}	< 0.0001
	fermented dough – 1 h	0.37 ± 0.004^{b,z}	0.52 ± 0.005^{b,y}	0.88 ± 0.002^{b,x}	< 0.0001
	– 2 h	0.30 ± 0.007^{c,z}	0.44 ± 0.011^{c,y}	0.81 ± 0.001^{c,x}	< 0.0001
	– 3 h	0.25 ± 0.005^{d,z}	0.40 ± 0.009^{d,y}	0.75 ± 0.003^{d,x}	< 0.0001
	– 4 h	0.18 ± 0.001^{e,z}	0.35 ± 0.004^{e,y}	0.69 ± 0.011^{e,x}	< 0.0001
	– 5 h	0.14 ± 0.001^{f,z}	0.32 ± 0.003^{f,y}	0.65 ± 0.003^{f,x}	< 0.0001
	– 6 h	0.10 ± 0.001^{g,z}	0.27 ± 0.007^{g,y}	0.60 ± 0.001^{h,x}	< 0.0001
	bread crumb – 3 h	0.10 ± 0.004^{g,z}	0.27 ± 0.007^{g,y}	0.61 ± 0.001^{g,x}	< 0.0001
	– 6 h	0.07 ± 0.002^{h,z}	0.20 ± 0.004^{h,y}	0.53 ± 0.001^{i,x}	< 0.0001
	– 3 h	0.02 ± 0.001^{i,z}	0.07 ± 0.001^{i,y}	0.21 ± 0.002^{j,x}	< 0.0001
	– 6 h	0.01 ± 0.001^{i,z}	0.07 ± 0.001^{i,y}	0.18 ± 0.003^{k,x}	< 0.0001
	P_S	< 0.0001	< 0.0001	< 0.0001	

F – effect of flour type, S – effect of sampling; levels of significance: $P \leq 0.05$ statistically significant, $P \leq 0.001$ highly statistically significant, $P > 0.05$ statistically not significant; ^{x,y,z}means with a different superscript within rows (effect of flour type) differ significantly ($P \leq 0.05$); ^{a,b,c,d,e,f,g,h,i,j,k}means with a different superscript within columns (effect of sampling) differ significantly ($P \leq 0.05$)

samples. The samples prepared from whole meal flour had the highest pH, while the sample prepared from flour type 500 had the lowest pH.

Dough had the highest phytic acid content. During 6-h fermentation, the phytic acid content decreased in dough made from all types of flour. Bread crumb and especially bread crust had low phytic acid contents. The flour type had a very strong influence on the phytic acid content in all samples, from dough to bread. Flour type 500 samples had the lowest phytic acid contents, while those prepared from whole meal flour had the highest contents of phytic acid.

Breadmaking procedure with sourdough addition

Table 4 presents the results obtained for the third breadmaking procedure where sourdough (5% and 10%) was added. The fermentation temperature was 20°C and the samples were made of all three types of flour. The dry matter content was the lowest in the dough, followed by the fermented dough and bread crumb, and the highest dry content was in bread crust, regardless of sourdough addition. All samples with 10% of sourdough addition, except bread crust, had a higher dry matter content than those prepared with 5% sourdough addition. The bread crust made with 10% sourdough addition had a 2% lower dry matter content than that prepared with 5% sourdough addition. The flour type also influenced the dry matter content. With the samples prepared at 5% sourdough addition, the highest dry matter content was found in those prepared from whole meal flour, while the samples prepared with flour type 500 had the lowest dry matter content. The samples prepared with 10% sourdough addition did not have the same tendency. In this case, the samples prepared from whole meal flour had the lowest dry matter content, while those prepared from flour type 500 had the highest one. The only exception was bread crust.

pH was the highest in the dough and decreased with fermentation and baking, regardless of the sourdough addition. The samples with 10% of sourdough addition had a lower pH than those with 5% of sourdough addition. The flour type also influenced pH. Type 500 flour samples had the lowest pH, while those prepared from whole meal flour samples had the highest pH.

The highest content of phytic acid was found in dough, regardless of the bread type and sourdough addition. As was the case with the samples prepared by the direct and indirect breadmaking procedures, the same tendency could be observed with those prepared with sourdough addition. The samples prepared with flour type 500 had the lowest phytic acid content, while the highest phytic acid content was found in the samples prepared with whole meal flour. The addition of 10% of sourdough caused a very high decrease of phytic acid content in all samples. Interestingly, in some samples (fermented dough prepared from flour type 500, bread crumb and bread crust prepared from type 500 and type 850 flour) phytic acid was not detected at all.

Comparison of breadmaking procedures

Comparing the dry matter contents of dough, fermented dough, and bread crumb prepared by all three breadmaking procedures investigated (direct, indirect, and with sourdough addition), it is obvious that the samples prepared with the addition of sourdough had a higher dry matter content than those prepared by the direct and indirect procedures. Interestingly, the bread crust prepared with the addition of sourdough had a much lower dry matter content than the bread crust of the samples prepared by the direct and indirect procedures.

pH in all samples prepared with the use of sourdough addition had lower values than in the samples prepared by the direct and indirect procedures. pH was especially low when a higher amount of sourdough was added.

As to the phytic acid content, the samples prepared with the addition of sourdough had a lower content of it than the other samples. With 10% sourdough addition, phytic acid content significantly decreased. The degradation of phytate during wheat bread making has been intensively investigated (Fretzdorff & Brummer 1992; Türk & Sandberg 1992). During the transformation of flour into dough and finally into bread, the phytate content decreases as a consequence of the activity of native phytase. The reduction of the phytate content during bread making depends on the phytase action, which in turn is influenced by several other factors, such as the degree of flour extraction, proofing time and temperature, acidity of the dough, yeast, enzymes added to the dough, and the presence of calcium salts (Türk & Sandberg 1992).

Table 4. Dry matter contents, pH, and phytic acid (PA) contents in samples made from three types of flour by the breadmaking procedure with the addition of sourdough

Parameters	Addition of sourdough (%)	Sample	Flour			P_F
			type 500	type 850	whole meal	
Dry matter (%)	5	dough	$48.0 \pm 0.099^{d,y}$	$48.5 \pm 0.081^{d,x}$	$48.6 \pm 0.007^{d,x}$	< 0.0001
		fermented dough	$48.3 \pm 0.113^{c,y}$	$48.8 \pm 0.002^{c,x}$	$48.9 \pm 0.004^{c,x}$	< 0.0001
		bread crumb	$49.1 \pm 0.091^{b,y}$	$49.7 \pm 0.005^{b,x}$	$49.7 \pm 0.053^{b,x}$	< 0.0001
		bread crust	$66.3 \pm 0.037^{a,z}$	$66.3 \pm 0.054^{a,y}$	$66.7 \pm 0.005^{a,x}$	< 0.0001
		P_S	< 0.0001	<0.0001	< 0.0001	
	10	dough	$49.9 \pm 0.021^{d,x}$	$49.7 \pm 0.004^{d,y}$	$49.4 \pm 0.004^{d,z}$	< 0.0001
		fermented dough	$50.1 \pm 0.003^{c,x}$	$49.9 \pm 0.002^{c,y}$	$49.7 \pm 0.049^{c,z}$	< 0.0001
		bread crumb	$50.9 \pm 0.025^{b,x}$	$50.7 \pm 0.089^{b,y}$	$50.4 \pm 0.003^{b,z}$	< 0.0001
		bread crust	$64.2 \pm 0.035^{a,y}$	$64.2 \pm 0.028^{a,y}$	$64.6 \pm 0.003^{a,x}$	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	
pH	5	dough	$5.86 \pm 0.008^{a,z}$	$5.98 \pm 0.013^{a,y}$	$6.26 \pm 0.005^{a,x}$	< 0.0001
		fermented dough	$5.80 \pm 0.005^{b,z}$	$5.92 \pm 0.010^{b,y}$	$6.20 \pm 0.006^{b,x}$	< 0.0001
		bread crumb	$5.63 \pm 0.010^{c,z}$	$5.75 \pm 0.008^{c,y}$	$6.03 \pm 0.006^{c,x}$	< 0.0001
		bread crust	$5.57 \pm 0.008^{d,z}$	$5.69 \pm 0.006^{d,y}$	$5.97 \pm 0.008^{d,x}$	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	
	10	dough	$5.56 \pm 0.010^{a,z}$	$5.69 \pm 0.005^{a,y}$	$5.97 \pm 0.005^{a,x}$	< 0.0001
		fermented dough	$5.50 \pm 0.016^{b,z}$	$5.62 \pm 0.008^{b,y}$	$5.90 \pm 0.000^{b,x}$	< 0.0001
		bread crumb	$5.35 \pm 0.013^{c,z}$	$5.47 \pm 0.005^{c,y}$	$5.76 \pm 0.005^{c,x}$	< 0.0001
		bread crust	$5.31 \pm 0.006^{d,z}$	$5.42 \pm 0.013^{d,y}$	$5.69 \pm 0.005^{d,x}$	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	
PA (g/100 g dm)	5	dough	$0.34 \pm 0.008^{a,z}$	$0.47 \pm 0.015^{a,y}$	$0.83 \pm 0.007^{a,x}$	< 0.0001
		fermented dough	$0.28 \pm 0.005^{b,z}$	$0.41 \pm 0.011^{b,y}$	$0.75 \pm 0.008^{b,x}$	< 0.0001
		bread crumb	$0.12 \pm 0.009^{c,z}$	$0.21 \pm 0.009^{c,y}$	$0.51 \pm 0.008^{c,x}$	< 0.0001
		bread crust	$0.03 \pm 0.004^{d,z}$	$0.07 \pm 0.004^{d,y}$	$0.22 \pm 0.007^{d,x}$	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	
	10	dough	$0.05 \pm 0.009^{a,z}$	$0.14 \pm 0.006^{a,y}$	$0.44 \pm 0.007^{a,x}$	< 0.0001
		fermented dough	$0.00 \pm 0.000^{b,z}$	$0.07 \pm 0.009^{b,y}$	$0.34 \pm 0.000^{b,x}$	< 0.0001
		bread crumb	$0.00 \pm 0.000^{b,y}$	$0.00 \pm 0.000^{c,y}$	$0.15 \pm 0.007^{c,x}$	< 0.0001
		bread crust	$0.00 \pm 0.000^{b,y}$	$0.00 \pm 0.000^{c,y}$	$0.03 \pm 0.002^{d,x}$	< 0.0001
		P_S	< 0.0001	< 0.0001	< 0.0001	

F – effect of flour type, S – effect of sampling; levels of significance: $P \leq 0.05$ statistically significant, $P \leq 0.001$ highly statistically significant, $P > 0.05$ statistically not significant; $^{x, y, z}$means with a different superscript within rows (effect of flour type) differ significantly ($P \leq 0.05$); $^{a, b, c, d}$means with a different superscript within columns (effect of sampling) differ significantly ($P \leq 0.05$)

Fretzdorff and Brummer (1992) found that pH was the most important factor in reducing the content of phytic acid during bread making as phytic acid in doughs with pH 4.3 to 4.6 was more effectively reduced than in doughs with higher pH.

The correlation between pH and phytic acid content can be made. The results showed that the samples with higher pH had higher phytic acid contents, so it can be concluded that phytic acid content depends on the pH of the sample. A very high correlation was found between the pH value and phytic acid content (R = 0.93, P < 0.0001). In all samples with a lower pH, phytic acid content was also lower. The influence of lower pH could be observed especially when sourdough was added. With a low (5%) addition of sourdough, the decrease of phytic acid content in dough was up to 0.10 g/100 g dm, and with a higher (10%) addition of sourdough even a higher decrease occurred of phytic acid content in dough, up to 0.50 g/100 g dm according to the flour type tested. Bread making with the addition of sourdough may result in more suitable pH conditions for the degradation of phytate by endogenous phytases, and the sourdough may also be a source of microbial phytases. Lopez *et al.* (2001) found that the phytate content was more efficiently reduced in wheat sourdough bread (62%) as compared to yeast fermented bread (38%). Furthermore, prolonged fermentation with sourdough increased acidification and led to an improved solubility of magnesium and phosphorus. Reale *et al.* (2004) also found an increased degradation of phytic acid in sourdough wheat bread made with the use of a long fermentation time as compared to yeast fermented bread using a short fermentation time.

Leenhardt *et al.* (2005) also showed that a slight acidification to pH 5.5 of bread dough by either sourdough or lactic acid addition promoted a significant phytate breakdown.

CONCLUSION

In this study, phytic acid degradation was investigated in the dependence on the flour type (500, 850, whole meal flour) and different bread making procedures (direct, indirect, and with sourdough addition). During breadmaking, the degradation of phytic acid occurred, its level depending on the initial phytic acid content. The flour type influenced the phytic acid content. The highest phytic acid content was found in the samples prepared from whole meal flour, while those prepared from flour type 500 had the lowest content of it. pH had a significant influence on phytic acid degradation. The samples with lower pH had lower phytic acid contents, which was especially noticeable in the samples prepared with sourdough addition. The fermentation temperature in the direct breadmaking procedure also influenced the phytic acid content. When a higher (30°C) fermentation temperature was used, the phytic acid content was lower. The amount of the added sourdough also influenced the phytic acid content. The higher addition of sourdough resulted in a lower phytic acid content.

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Received for publication July 28, 2008
Accepted after corrections January 8, 2008

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