

Development of Predictive Models for Optimization of Phytate Degradation in Wheat and Rye During Hydrothermal Processing

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ABSTRACT

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Whole kernels of wheat (*Triticum aestivum* 'Kosack'), rye (*Secale cereale* 'Motto') and rice (*Oryza sativa*) were hydrothermally treated to reduce the phytate content. The hydrothermal processes used in this study included alternating wet and dry steeping periods. Two hydrothermal processes were used for wheat and rye with two and three wet and dry steeping steps, respectively. The process using two wet and dry steeping steps (process 1) was optimized for phytate reduction with respect to temperature during the process and lactic acid concentration used in the wet steeping steps. Optimal conditions for phytate breakdown in wheat

and rye in process 1 were 55°C during the whole process and 1.3–1.5% lactic acid solution as the soaking agent in the wet steeping periods for wheat and 1.3% for rye. The hydrothermal process with three wet and dry steeping periods for wheat and rye (process 2) showed that the temperature used in the third wet and dry steepings did not significantly influence ($P > 0.05$) the phytate reduction in wheat and rye during these steps. The maximal phytate reduction was 94.4–95.6% in wheat and 99.0–99.5% in rye during hydrothermal processing. In rice, hydrothermal processing resulted, at best, in a 99.8% phytate reduction.

Dietary guidelines for the Nordic countries (Sandström et al 1996) and for other industrialized countries recommend increased intake of carbohydrates and dietary fiber and a decreased intake of fat, especially saturated fat. Cereals are in good agreement with these recommendations as they have high contents of starch, dietary fiber, vitamins and minerals and low contents of fat and sucrose (Sundberg 1995). However, cereals also contain antinutrients, for example phytic acid (*myo*-inositol hexaphosphoric acid). Phytic acid impairs the bioavailability of divalent ions such as zinc (Lönnerdal et al 1988; Sandström and Sandberg 1992), calcium (Heaney et al 1991), and iron (Brune et al 1992) by forming complexes (phytates) with these minerals. By means of the naturally occurring enzyme phytase in cereals, it is possible to degrade phytate to lower *myo*-inositol phosphates, inorganic phosphate and, in some cases, free *myo*-inositol (Cosgrove 1980) and thus achieve cereal products with a higher bioavailability of minerals. The activity of cereal phytases is dependent on moisture, pH level, and temperature. In the literature, the optimum for phytase activity in wheat, rye and rice is pH 5.1 (Peers 1953), pH 5.0–5.4 (Hoff-Jørgensen and Porsdal 1946, Bartnik and Szafranska 1987), and pH 4.2 (Yoshida et al 1975), respectively. The temperature optimum for phytase activity in wheat, rye and rice is 55°C (Peers 1953), 50–55°C (Hoff-Jørgensen and Porsdal 1946; Bartnik and Szafranska 1987), and 45°C (Yoshida et al 1975), respectively.

The focus of interest in phytic acid in the field of food science is on antinutritional effects. However, some potentially beneficial effects of phytic acid have also been suggested in the last few years. Dietary protective effect of phytic acid on induced carcinogenesis in mice and rats has been seen (Nelson et al 1989; Shamsuddin and Ullah 1989). Phytic acid or inositol phosphate intermediates have been suggested to have a negative effect on the rate of starch digestion and blood glucose response in humans and can thus be beneficial in the management of diabetes and hyperlipidemia (Thompson 1986). A lowering effect of phytic acid on serum cholesterol and triglycerides in rats has also been seen, which could be positive in prevention of coronary heart diseases (Jariwalla et al 1990).

Modern dry-milling generally removes the bran (i.e., pericarp, the seed coat, the nucellar epidermis, and the aleurone layer) and the

germ (Hoseney 1986) of the cereal grain. Both the bran and the germ are relatively rich in protein, B vitamins, minerals, and fat and, hence, the milled product is lower in these nutrients than the original grain. Before the advent of the modern milling industry, people prepared cereals by moistening or soaking the whole grains, drying them, and then lightly roasting them (Meyer-Renschhausen 1991). This made it easier to dehull the grains with a domestic pestle and the hulls were separated from the grains by the wind or by sifting. The process of moistening, drying, pounding, and separating had to be repeated several times but afterwards the cereals were almost ready to eat. This milling technique dehulled the grains in a much gentler way than modern dry-milling does, and the aleurone layer was kept intact. The nutrients were thus better preserved and the shelf life of the grains was very long (Meyer-Renschhausen 1991). Our previous studies (Fredlund et al 1997; Bergman et al 1999) also show that phytate in wheat, rye, barley, and oats is degraded during hydrothermal processing.

In this study, we hydrothermally processed wheat, rye, and rice on a pilot scale in a way similar to that used before the milling industry was modernized. The aim was to study how hydrothermal processing of cereals influences the phytate content and to create response surface plots that describe the phytate content in the experimental domain. The aim was also to optimize the hydrothermal process to achieve cereal products with a low level of phytate.

MATERIALS AND METHODS

Cereals

Whole kernels of wheat (cv. Kosack) of 1995 year's crop and rye (cv. Motto) of 1995 year's crop were provided by Skånska Lantmännen (Malmö, Sweden). Whole kernels of rice (regular long grain brown rice from Guyana) were provided by Boost distribution.

Hydrothermal Processes

Hydrothermal processes involve elevation of temperature and increase of moisture. Here we use the term hydrothermal processing for processes consisting of alternating wet and dry steeping. Flow charts of the processes used are shown in Figs. 1 and 2. One of the processes used for wheat and rye (process 1) has previously been optimized for phytate degradation and increase of free *myo*-inositol in barley (Bergman et al 1999). The cereals were processed in a pilot plant at Oy Lahden Polttimo AB (Lahti, Finland). Samples taken out during the process were freeze-dried before they were sent to us for analysis. These samples and the dry end products were frozen and stored below -18°C until analyzed.

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Wheat and Rye

Flow charts of the hydrothermal processes used for wheat and rye are shown in Fig. 1. The whole kernels were soaked in 1.5 vol of lactic acid solution (0–1.5%, v/w) (Table I) for 1 hr. Superfluous lactic acid solution was drained, and the seeds were dry-steeped for 5 hr. The seeds were then steeped in 1.5 vol of lactic acid solution for 1 hr, after which the superfluous lactic acid solution was drained off and the seeds underwent dry steeping for 15 hr (process 1) or 13 hr (process 2). After the 13-hr dry steep, the kernels were wet-steeped for 1 hr in 1.5 vol of deionized water followed by 1 hr of dry steeping. The cereals from both processes were dried at 50°C for 8 hr. There followed a progressive increase of temperature to 80°C during 2 hr, and drying was continued at 80°C for 6 hr. The dried end-products were analyzed for contents of *myo*-inositol tri- to hexaphosphates (IP₃ – IP₆). The product from process 1 is called DP1 and the product from process 2 is called DP2. Wheat samples from experiments 5–8 and 10–11 (Table I) were also collected after the first wet steep (AWI), after the first dry steep (ADI), after the second wet steep (AWII), after the second dry steep (ADII), and after the third dry steep (ADIII) and analyzed for contents of IP₃ – IP₆. Samples were removed from the process after each wet and dry steep to measure the pH level of the grains.

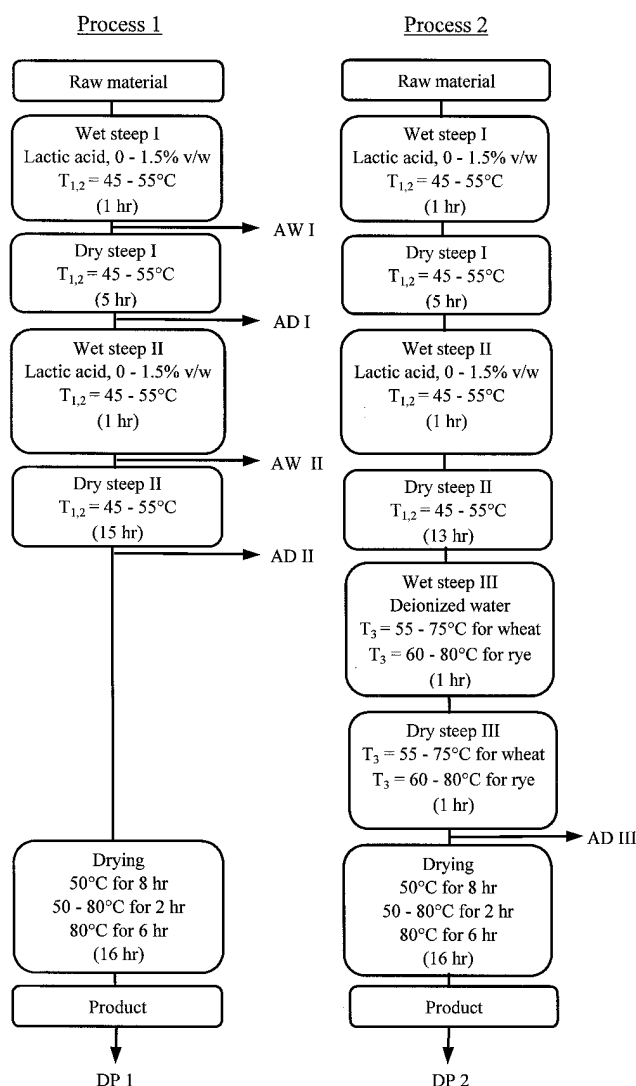


Fig. 1. Flow charts of hydrothermal processes used for wheat and rye. In process 1, samples were taken out after the first wet steep (AWI), after the first dry steep (ADI), after the second wet steep (AWII), after the second dry steep (ADII) and as dried end product (DP1). In process 2, samples were taken out after dry steep III (ADIII) and as dried end product (DP2).

Rice

Three hydrothermal processes were used for rice. The temperature during all three processes was 45°C. Flow charts over the processes are shown in Fig. 2. The whole kernels were soaked in 1.5 vol of 0.8% (v/w) lactic acid solution for 2 hr. Superfluous lactic acid solution was drained, and the seeds were dry-steeped for 2 hr. The seeds were then steeped in 1.5 vol of lactic acid solution or deionized water for 2 hr, and the superfluous lactic acid solution or water was drained off. Dry steeping for 19.5 hr and 11.5 hr followed in processes 1 and 3, respectively, and, in process 2, dry steeping for 2 hr and wet steeping in deionized water for 2 hr were repeated two more times before a dry steeping for 11.5 hr followed. The processed cereals were dried at 50°C for 8 hr, after which the temperature was progressively increased to 80°C for 2 hr, and drying was continued at 80°C for 6 hr. The dried end-products were analyzed for contents of *myo*-inositol tri- to hexaphosphates (IP₃ – IP₆). Samples were removed from the process after each wet and dry steep to measure the pH level of the grains.

Experimental Design

A variation of a central composite inscribed design (CCI) was used. The hydrothermal processes used for processing wheat and rye were optimized with respect to two variables for process 1. The variables used for optimizing process 1 were: temperature (°C) used in the first and second wet and dry steeps (T_{1,2}), and lactic acid solution concentration (% v/w) used in the first and second wet steeps (C). Three variables were used in process 2: temperature

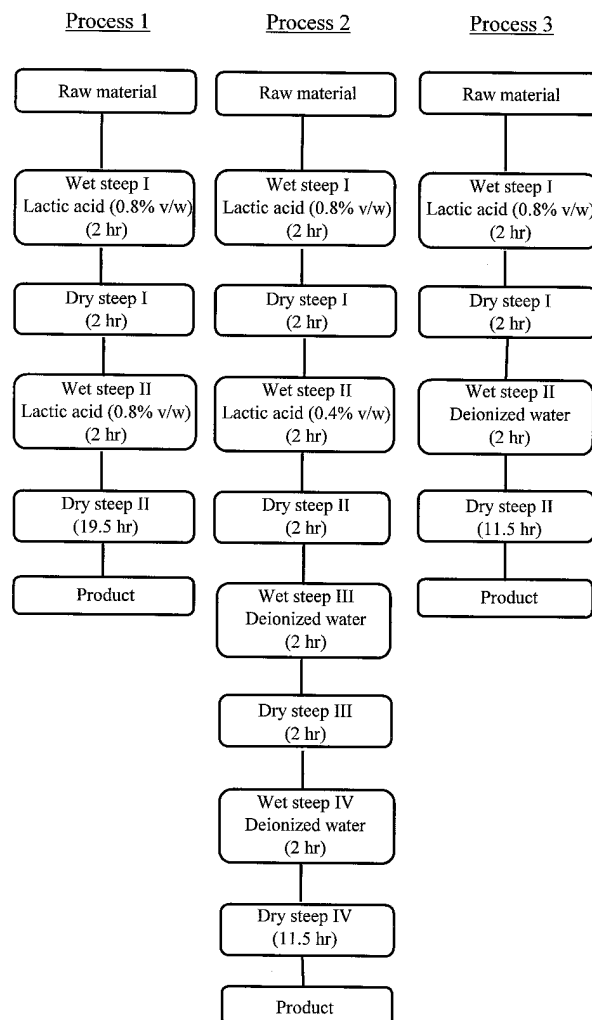


Fig. 2. Flow charts for hydrothermal processing of rice. Temperature during all three processes was 45°C.

(°C) used in the first and second wet and dry steeps ($T_{1,2}$), lactic acid solution concentration (% v/w) used in the first and second wet steeps (C), and temperature used in the third wet and dry steeps (T_3). Real and scaled values of the variables are given in Table I. The experimental conditions at the center point of the design were $T_{1,2} = 50^\circ\text{C}$, $C = 0.75\%$, and $T_3 = 65^\circ\text{C}$ for wheat, and $T_{1,2} = 50^\circ\text{C}$, $C = 0.75\%$, and $T_3 = 70^\circ\text{C}$ for rye. The scaled values were $x_1 = (T_{1,2} - 50)/5$, where $T_{1,2}$ was varied between 45 and 55°C for both wheat and rye; $x_2 = (C - 0.75)/0.75$, where C was varied between 0 and 1.5% (v/w) for both wheat and rye; and $x_3 = (T_3 - 65) / 10$, where T_3 was varied between 55 and 75°C for wheat, and $x_3 = (T_3 - 70)/10$, where T_3 was varied between 60 and 80°C for rye. Five screening experiments (Experiments 1–4 and 9, Table I) were conducted to ensure that the factors were sufficiently varied to give significantly different results. The results were satisfying, and six further experiments (Experiments 5–8 and 10–11, Table I) were conducted.

We did not optimize the hydrothermal process for rice. Three experiments were conducted with rice.

Data Analysis

Mathematical models were fitted to the results from hydrothermal processing according to process 1 of wheat and rye by multiple linear regression (MLR) (Carlsson 1992) using the computer program Modde version 4.0 (Umetri AB, Umeå, Sweden). The observed values of *myo*-inositol hexaphosphate in product 1 (y_{obs} , Eq. 1) were fitted to mathematical models in two independent processing variables expressed in scaled values:

$$y_{\text{obs}} = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1x_1 + b_{22}x_2x_2 + b_{12}x_1x_2 + e \quad (1)$$

$$e = y_{\text{obs}} - y_{\text{calc}} \quad (2)$$

In these equations, b_0 is a constant; b_1 and b_2 express the main effect of each process variable; b_{11} and b_{22} are the square coefficients; b_{12} shows the interaction between the variables. The difference between the measured values (y_{obs}) and the calculated values (y_{calc}) gives the residual in Equation 2 (Lindgren et al 1995). Both the real values and the scaled values of the experimental variables are shown in Table I. The mathematical models were used to create

TABLE I
Process Conditions in Real^a and Scaled Values^b for Hydrothermal Treatment of Wheat and Rye

Experiment	Real Values				Scaled Values		
	$T_{1,2}$ (°C)	C (% v/w)	T_3 (°C) Wheat	T_3 (°C) Rye	x_1	x_2	x_3
1	45	0.00	55	60	-1.0	-1.0	-1.0
2	55	0.00	55	60	1.0	-1.0	-1.0
3	45	1.50	75	80	-1.0	1.0	1.0
4	55	1.50	75	80	1.0	1.0	1.0
5	47	0.75	65	70	-0.6	0.0	0.0
6	53	0.75	65	70	0.6	0.0	0.0
7	50	0.30	59	64	0.0	-0.6	-0.6
8	50	1.20	71	76	0.0	0.6	0.6
9	50	0.75	65	70	0.0	0.0	0.0
10	50	0.75	65	70	0.0	0.0	0.0
11	50	0.75	65	70	0.0	0.0	0.0

^a $T_{1,2}$ = temperature used in wet and dry steeps I and II; C = lactic acid concentration used in wet steeps I and II; T_3 = temperature used in wet and dry steeps III.
^b $x_1 = (T_{1,2} - 50)/5$, where $T_{1,2}$ range is 45–55°C; $x_2 = (C - 0.75)/0.75$, where C range is 0.0–1.5% (v/w); $x_3 = (T_3 - 65)/10$ for wheat, where T_3 range is 55–75°C; $x_3 = (T_3 - 70)/10$ for rye, where T_3 range is 60–80°C.

TABLE II
Myo-Inositol Tri- to Hexaphosphates in Products 1 (DP 1) and 2 (DP 2) of Wheat and Rye Processed Hydrothermally^a

Experiment/ Product	Wheat					Rye				
	IP ₃ (µmol/g db)	IP ₄ (µmol/g db)	IP ₅ (µmol/g db)	IP ₆ (µmol/g db)	Reduced IP ₆ (%)	IP ₃ (µmol/g db)	IP ₄ (µmol/g db)	IP ₅ (µmol/g db)	IP ₆ (µmol/g db)	Reduced IP ₆ (%)
Raw material	0.11	0.08	0.30	10.59	...	0.00	0.00	0.59	12.93	...
1 DP1	0.22	0.15	1.05	11.31	-6.8	0.11	0.14	1.14	10.46	19.1
1 DP2	0.00	0.11	0.84	10.28	2.9	0.35	0.19	0.75	8.55	33.9
2 DP1	0.15	0.15	0.66	7.97	24.8	0.13	0.08	0.46	6.22	51.9
2 DP2	0.36	0.14	0.75	7.88	25.7	0.20	0.10	0.49	5.69	56.0
3 DP1	0.18	0.18	0.40	4.35	59.0	0.25	0.12	0.21	2.09	83.8
3 DP2	0.52	0.54	0.57	3.76	64.5	0.37	0.25	0.25	1.85	85.7
4 DP1	0.44	0.18	0.09	0.59	94.4	0.00	0.09	0.00	0.06	99.5
4 DP2	0.36	0.17	0.08	0.47	95.6	0.00	0.11	0.00	0.13	99.0
5 DP1	0.30	0.25	0.84	7.28	31.3	0.18	0.10	0.40	3.36	74.0
5 DP2	0.49	0.22	0.34	3.85	63.7	0.31	0.32	0.31	2.67	79.4
6 DP1	0.33	0.24	0.39	3.56	66.4	0.10	0.10	0.12	1.20	90.7
6 DP2	0.42	0.28	0.24	2.32	78.1	0.04	0.15	0.16	1.31	89.8
7 DP1	0.35	0.19	1.02	9.56	9.8	0.00	0.11	0.56	5.54	57.1
7 DP2	0.38	0.20	0.72	7.10	33.0	0.35	0.17	0.37	3.97	69.3
8 DP1	0.29	0.22	0.33	3.26	69.2	0.04	0.12	0.16	1.22	90.5
8 DP2	0.54	0.44	0.39	2.34	77.9	0.02	0.17	0.14	0.92	92.9
9 DP1	0.20	0.17	0.47	4.91	53.7	0.24	0.13	0.20	2.15	83.4
9 DP2	0.59	0.25	0.25	3.20	69.8	0.36	0.17	0.19	2.09	83.8
10 DP1	0.32	0.22	0.48	5.17	51.2	0.24	0.12	0.34	3.02	76.6
10 DP2	0.62	0.31	0.34	3.22	69.6	0.47	0.29	0.31	2.51	80.6
11 DP1	0.22	0.22	0.50	4.72	55.5	0.04	0.12	0.26	2.27	82.4
11 DP2	0.58	0.28	0.32	3.56	66.4	0.29	0.16	0.25	2.32	82.1

^a See Fig. 1 for flow charts of hydrothermal processes. IP₃ – IP₆ are *myo*-inositol tri- to hexaphosphates. Values are means of $n = 2$.

response surface plots to see how the content of *myo*-inositol hexaphosphate varied with the experimental variables and to predict optimal conditions for degradation of *myo*-inositol hexaphosphate. The explained variation (R^2) and the predictive capacity (Q^2) were calculated for the models to estimate the reliability of the models. R^2 is the variation of the response explained by the model, and Q^2 is the fraction of the variation of the response that can be predicted by the model. Q^2 must exceed 0.5 if any conclusions are to be drawn from the model, and the model is generally considered excellent if both R^2 and Q^2 exceed 0.9 (Lindgren et al 1995).

As can be seen in Table I, the lactic acid concentration (C) and the temperature used in the third wet and dry steeps (T_3) was varied in the same way in scaled variables. This made it impossible to evaluate the results of process 2 mathematically because the effect of C and T_3 can not be separated when they are exactly correlated. Instead, the differences between the contents of *myo*-inositol hexaphosphate in product 1 and product 2 were calculated and compared using one-way ANOVA (Motulsky 1995).

Determination of Myo-Inositol Phosphates

Approximately 30 g of whole cereal kernels were thawed and ground in a grinder (Braun AG type: 4041, Kronberg/Ts., Germany)

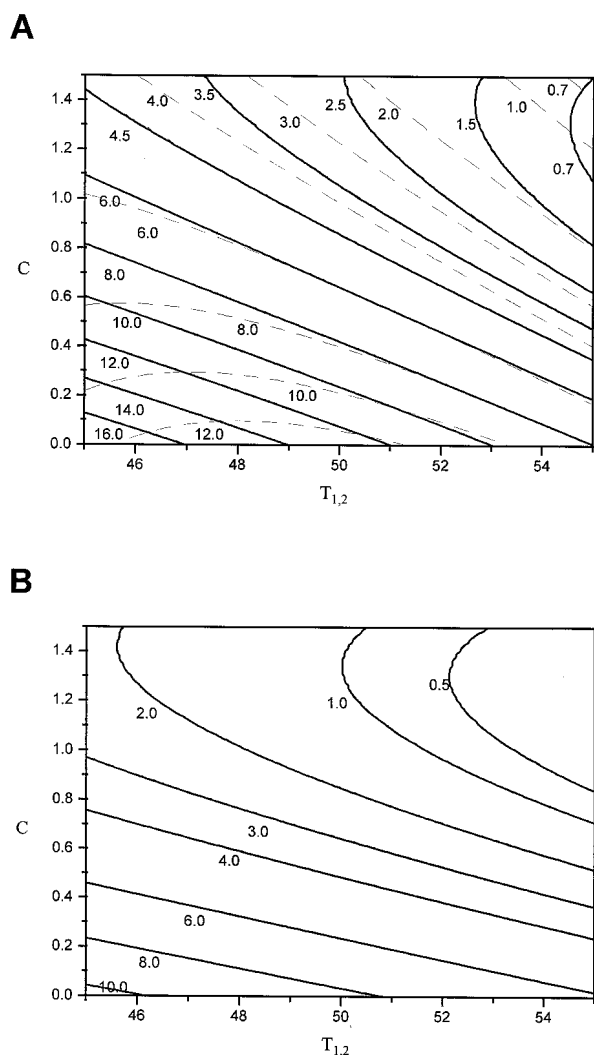


Fig. 3. Response surface plots based on the mathematical models for the contents of *myo*-inositol hexaphosphate (IP_6) in dried end product 1 (DP 1) in wheat (A) and rye (A). Values are μmol of $IP_6/\text{g db}$. $T_{1,2}$ ($^{\circ}\text{C}$) is the temperature used in wet and dry steeps I and II; C (% v/v) is the lactic acid concentration used in wet steeps I and II. For wheat, mathematical evaluation was done without (—) or with (----) logarithmical transformation of the content of IP_6 .

for 30 sec, after which 0.4 g was analyzed in duplicate for dry matter with a moisture balance (Precisa HA 300, Zürich, Switzerland). Duplicate samples (0.5 g, db) of ground seeds were extracted with 0.5M HCl (20 mL) for 3 hr. The extracts were centrifuged, and the supernatants were decanted, frozen overnight, thawed and centrifuged again. After the second centrifugation, an aliquot (15 mL) of the supernatant was evaporated to dryness under a flow of air at 40°C and then redissolved in 0.025M HCl (15 mL). The *myo*-inositol phosphates were separated from the crude extract by ion exchange chromatography according to Sandberg and Ahderinne (1986). *Myo*-inositol tri-, tetra-, penta- and hexaphosphates were determined by ion-pair C18 reverse-phase HPLC using formic acid and methanol and tetrabutylammoniumhydroxide in the mobile phase (Sandberg and Ahderinne 1986; Sandberg et al 1989). The HPLC included an HPLC pump (model 510, Waters Associates); a C18 Kromasil (5 μm) column, 2 mm i.d.; and a refractive index detector (ERC-7510 RI-detector Erma Optical Works Ltd., Japan). The flow rate was 0.4 mL/min. Retention times and peak areas were measured by the laboratory data system HP 3350 (Hewlett Packard, Palo Alto, CA). Injections were made with a 20- μL loop.

pH Measurements

The pH level was measured in samples removed during the processes. The cereal kernels were ground in distilled water (grains to water, 1:0.5, v/v). The pH was then measured from the suspension by using a pH meter (Orion model 720A, Boston, MA).

RESULTS

Wheat

The contents of *myo*-inositol tri- to hexaphosphates ($IP_3 - IP_6$) in products 1 and 2 (DP1 and DP2) of the 11 experiments from processes 1 and 2, respectively, are shown in Table II. The IP_6 values of product 1 were evaluated statistically by MLR. We eval-

TABLE III
Analysis of Variance^a for the Mathematical Models Based on Content of *Myo*-Inositol Hexaphosphate in Hydrothermally Treated Products (DP 1) of Wheat and Rye

	Wheat		Rye	
	DF ^{b,c}	SS ^{c,d}	DF ^b	SS ^d
Total	10 (11)	323.60 (6.10)	11	216.277
Constant	1 (1)	263.89 (4.91)	1	128.455
Total corrected	9 (10)	58.71 (1.18)	10	87.822
Regression	4 (4)	59.06 ^e (1.18) ^e	4	87.82 ^e
Residual	5 (6)	0.66 (0.007)	6	0.56
Lack of fit (model error)	3 (4)	0.55 ^f (0.006) ^f	4	0.12 ^f
Pure error (replicate error)	2 (2)	0.10 (0.001)	2	0.45

^a Calculated using computer program MODDE 4.0 (Umetri AB, Umeå, Sweden).

^b DF = degrees of freedom.

^c Figures in brackets were values achieved when the response was logarithmically transformed.

^d SS = sum of squares.

^e $P < 0.001$.

^f $P > 0.05$.

TABLE IV
Myo-Inositol Tri- to Hexaphosphates in Raw Materials and Products after Hydrothermal Processing and Drying of Rice^a

Rice	IP_3 ($\mu\text{mol/g db}$)	IP_4 ($\mu\text{mol/g db}$)	IP_5 ($\mu\text{mol/g db}$)	IP_6 ($\mu\text{mol/g db}$)	Reduced IP_6 (%)
Raw material	0.00	0.11	0.87	9.34	...
Product 1	0.49	0.19	0.04	0.04	99.6
Product 2	0.23	0.10	0.04	0.06	99.4
Product 3	0.06	0.04	0.02	0.02	99.8

^a See Fig. 2 for flow charts of processes. Products 1, 2 and 3 achieved by treating rice according to processes 1, 2 and 3, respectively. $IP_3 - IP_6$ are *myo*-inositol tri- to hexaphosphates. Values are means of $n = 2$.

uated the results in two ways. First, the normal probability plot showed experiment 1 to be an outlier, and that experiment was thus excluded from the calculations. Because the square term of the temperature during the first and second wet and dry steeps (x_1x_1) was not significant ($P > 0.05$), this term was removed from the model. The mathematical model achieved, expressed in scaled variables, was:

$$y_{\text{calc}} (\mu\text{mol of IP}_6/\text{g db}) = 5.2 - 3.4x_1 - 5.2x_2 + 2.6x_2x_2 + 1.6x_1x_2 \quad (3)$$

The temperature during the first and second wet and dry steeps (x_1), the lactic acid concentration during the first and second wet steeps (x_2), the square term of the lactic acid concentration (x_2x_2), and the interaction term of the temperature and the lactic acid concentration (x_1x_2) significantly influenced the content of *myo*-inositol hexaphosphate in product 1 ($P < 0.01$). The model showed no significant lack-of-fit (Table III), and the explained variation (R^2) was 0.99 and the predictive capacity of the model (Q^2) was 0.86; thus, conclusions can be drawn from the model. A response surface plot based on Equation 3 is shown in Fig. 3A. The model predicted optimal conditions for phytate degradation in the experimental domain to be $T_{1,2} = 55^\circ\text{C}$ and $C = 1.3\%$. At these conditions, the model predicted the phytate content in product 1 to be 0.49 ± 0.72 .

We also evaluated the results from process 1 by logarithmically transforming the response (concentration of *myo*-inositol hexaphosphate) because the data was unevenly distributed. As the square term of lactic acid concentration (x_2x_2) was not significant ($P > 0.05$), this term was removed from the model. The mathematical model achieved, expressed in scaled variables, was:

$$y_{\text{calc}} ({}^{10}\log \text{ of } \mu\text{mol IP}_6/\text{g db}) = 0.72 - 0.26 x_1 - 0.39x_2 - 0.13 x_1x_1 - 0.18 x_1x_2 \quad (4)$$

The temperature during the first and second wet and dry steeps (x_1), the lactic acid concentration during the first and second wet steeps (x_2), the square term of the temperature during the first and second wet and dry steeps (x_1x_1) and the interaction term of the temperature and the lactic acid concentration (x_1x_2) significantly influenced the content of *myo*-inositol hexaphosphate in product 1 ($P < 0.01$). The model showed no significant lack-of-fit (Table III), and the explained variation (R^2) was 0.99 and the predictive capacity of the model (Q^2) was 0.98. Thus, the model was excellent, and conclusions can be drawn from it. A response surface plot based on Equation 4 is shown in Fig. 3A. The model predicted optimal conditions for phytate degradation in the experimental domain to be $T_{1,2} = 55^\circ\text{C}$ and $C = 1.5\%$. At these conditions, the model predicted the phytate content in product 1 to be $0.60 \pm 0.10 \mu\text{mol/g db}$. Experiment 4 was conducted at these conditions, and the phytate content of product 1 was $0.59 \mu\text{mol/g db}$.

It is difficult to tell which of these two models gives the best picture of reality but, as the second model had a better predictive capacity (Q^2), this model may perhaps be the best. A disadvantage of transforming the response logarithmically is that the response can then never have a value of zero, which in this case, is our aim. However, both models show that a high temperature and a high lactic acid concentration during the hydrothermal process is most favorable for phytate degradation in wheat.

One can assume that the temperature during the first and second wet and dry steeps and the lactic acid concentration during the first and second wet steeps influence the phytate degradation during process 2 in about the same manner as in process 1. To evaluate the effect on phytate degradation of the temperature used in the third wet and dry steeps, the difference of the *myo*-inositol hexaphosphate in product 1 and product 2 was calculated for various settings of T_3 and compared using one-way ANOVA. There were no significant differences between how much phytate was degraded during

TABLE V
Myo-Inositol Tri- to Hexaphosphates in Samples Removed During Hydrothermal Processing of Wheat^a

Experiment/ Sample	IP ₃ ($\mu\text{mol/g db}$)	IP ₄ ($\mu\text{mol/g db}$)	IP ₅ ($\mu\text{mol/g db}$)	IP ₆ ($\mu\text{mol/g db}$)	Reduced IP ₆ (%)	pH
Raw material	0.11	0.08	0.30	10.59	...	
5 AW I	0.21	0.14	0.81	11.60	-9.5	5.1
AD I	0.18	0.18	0.72	10.53	0.6	5.2
AW II	0.38	0.36	0.76	10.11	4.5	4.8
AD II	0.25	0.36	0.64	7.64	27.9	5.0
AD III	0.46	0.39	0.32	4.10	61.3	5.4
6 AW I	0.32	0.32	0.80	10.66	-0.7	5.1
AD I	0.21	0.28	0.70	9.22	12.9	5.1
AW II	0.40	0.54	0.71	7.36	30.5	4.6
AD II	0.26	0.38	0.42	4.16	60.7	5.0
AD III	0.24	0.32	0.27	2.35	77.8	5.1
7 AW I	0.16	0.13	0.80	11.45	-8.1	5.6
AD I	0.18	0.18	0.75	11.22	-5.9	5.6
AW II	0.20	0.16	0.78	11.02	-4.1	5.3
AD II	0.34	0.30	0.78	10.80	-2.0	5.6
AD III	0.55	0.63	0.76	8.56	19.2	5.7
8 AW I	0.36	0.34	0.83	11.40	-7.6	4.9
AD I	0.22	0.33	0.66	8.62	18.6	5.0
AW II	0.48	0.58	0.60	6.79	35.9	4.5
AD II	0.35	0.36	0.33	3.54	66.6	4.7
AD III	0.52	0.51	0.40	2.80	73.6	4.9
10 AW I	0.22	0.20	0.86	11.85	-11.9	5.1
AD I	0.26	0.28	0.76	10.95	-3.4	5.4
AW II	0.45	0.46	0.69	7.90	25.4	4.7
AD II	0.28	0.46	0.57	5.69	46.3	5.0
AD III	0.49	0.36	0.32	3.42	67.7	5.1
11 AW I	0.40	0.28	0.84	11.08	-4.6	5.0
AD I	0.16	0.21	0.60	8.40	20.7	5.1
AW II	0.38	0.39	0.71	8.20	22.6	4.7
AD II	0.30	0.32	0.48	5.34	49.6	5.0
AD III	0.58	0.36	0.34	3.88	63.4	5.2

^a See Fig. 1 for flow charts of the hydrothermal process. AW, after wet steep I; ADI, after dry steep I; AWII, after wet steep II; ADII, after dry steep II; and ADIII, after dry steep III. IP₃ - IP₆ are *myo*-inositol tri- to hexaphosphates. Values are means of $n = 2$.

the third wet and dry steeps when using 55, 65 or 75°C ($P > 0.05$). Thus, temperature during the third wet and dry steeps did not significantly influence phytate degradation in wheat during these steps.

Rye

The contents of *myo*-inositol tri- to hexaphosphates (IP₃ – IP₆) in products 1 and 2 (DP1 and DP 2) of the 11 experiments are shown in Table II. The results for product 1 were evaluated statistically by MLR. Since the square term of lactic acid concentration ($x_1 x_1$) was not significant ($P > 0.05$), this term was removed from the model. The mathematical model achieved, expressed in scaled variables, was:

$$y_{\text{calc}} (\mu\text{mol of IP}_6/\text{g db}) = 2.4 - 1.6 x_1 - 3.6 x_2 + 2.3 x_2 x_2 + 0.6 x_1 x_2 \quad (5)$$

The temperature during the first and second wet and dry steeps (x_1), the lactic acid concentration during the first and second wet steeps (x_2), the square term of the lactic acid concentration ($x_2 x_2$) and the interaction term of the temperature and the lactic acid concentration ($x_1 x_2$) influenced the content of *myo*-inositol hexaphosphate in product 1 significantly ($P < 0.01$). The model in Equation 5 showed no significant lack-of-fit (Table III), and the explained variation (R^2) was 0.99 and the predictive capacity of the model (Q^2) was 0.98. Thus, the model was excellent, and conclusions can be drawn from it. A response surface plot based on Equation 5 is shown in Fig. 3B. The model predicted optimal conditions for phytate degradation in the experimental domain to be $T_{1,2} = 55^\circ\text{C}$ and $C = 1.3\%$. At these conditions, the model predicted the phytate content in product 1 to be $-0.19 \pm 0.56 \mu\text{mol/g db}$. Experiment 4 was conducted at $T_{1,2} = 55^\circ\text{C}$ and $C = 1.5\%$, and the phytate content of product 1 was $0.06 \mu\text{mol/g db}$.

One can assume that the temperature during the first and second wet and dry steeps and the lactic acid concentration during the first and second wet steeps influence the phytate degradation during process 2 in about the same manner as in process 1. To evaluate the effect on phytate degradation of the temperature used in the third wet and dry steeps, the difference in the *myo*-inositol hexaphosphate in products 1 and 2 (DP1 and DP2) was calculated for various settings of T_3 and compared using one-way ANOVA. As in the processing of wheat, there were no significant differences between how much phytate was degraded during the third wet and dry steeps when using 55, 65, or 75°C ($P > 0.05$). Thus, the temperature during the third wet and dry steeps did not significantly influence the phytate degradation in rye during these steps.

Rice

The results of hydrothermal processing of rice are shown in Table IV; the phytate content in rice was reduced by 99.4–99.8%.

DISCUSSION

The results show that it is possible to degrade the phytate content by 95.6, 99.5, and 99.8% in wheat, rye, and rice, respectively. The remaining contents of *myo*-inositol tri- to hexaphosphates were low enough not to be considered to significantly affect zinc absorption (Rossander et al 1992) and low enough not to have a strong negative effect on iron absorption (Brune et al 1992).

The response surface plots based on Equations 3–5 are shown in Fig. 3. The response surface plots show the calculated content of *myo*-inositol hexaphosphate in wheat and rye at various combinations of the experimental variables and can thus be used to find optimal conditions for phytate degradation and to obtain an estimation of phytate degradation at various settings of the experimental variables within the experimental domain. The latter is very important because low phytate content is, of course, not the only criterion that must be met to achieve a good cereal product. Other crucial criteria are oxidative and microbiological stability, desired rheological properties, and a high sensory quality. Some of these parameters will be studied in wheat and rye and the results fitted to mathe-

tical models that can be illustrated by response surface plots for each parameter. The various response surface plots can be compared and superimposed to find experimental conditions at which acceptable values of each parameter can be achieved.

Phytase activity and phytate degradation in wheat have been extensively studied in earlier investigations (Peers 1953; Dagher et al 1987; Frölich et al 1988; Türk et al 1996; Fredlund et al 1997). Peers showed as early as 1953 that the optimal conditions for phytase activity in wheat are 55°C and pH 5.15. Our results show that 55°C during the whole process and 1.3–1.5% (v/w) lactic acid solution as soaking agent in the two wet steeping periods are the best conditions for phytate degradation in wheat during hydrothermal process 1. The temperature during the third wet and dry steeps did not significantly affect the phytate degradation in wheat during process 2 ($P > 0.05$). However, after process 2, the products had a significantly ($P < 0.001$) lower phytate level than the products resulting from process 1. The pH in mixtures of crushed wheat grains and distilled water of samples removed during the processes was 4.5–5.0 when 1.2% lactic acid solution was used (Experiment 8, Table V). Table V shows that the first wet steep did not result in any phytate reduction, the first dry steep resulted in a quite rapid phytate reduction, the second wet steep resulted in an even more rapid phytate reduction, and there was a slow phytate reduction during the second dry steep. Because the second dry steep was the longest step in this process (15 hr), the highest phytate breakdown took place during this step.

Phytase activity and phytate degradation in rye have also been studied previously (Hoff-Jørgensen and Porsdal 1946; Bartnik and Szafranska 1987; Sandberg and Svanberg 1991; Larsson and Sandberg 1992; Fredlund et al 1997), and optimal conditions for phytase in rye are 50–55°C and pH 5.0–5.4 (Hoff-Jørgensen and Porsdal 1946; Bartnik and Szafranska 1987). The phytase activity in rye at these conditions is higher than in wheat, barley and oats (Bartnik and Szafranska 1987). Table II and Equations 3 and 5 show that the phytate in rye was also more easily degraded during the hydrothermal processes than phytate in wheat. According to the response surface plot in Fig. 3B, the best conditions for phytate degradation in rye during hydrothermal process 1 are 55°C during the whole process and 1.3% (v/w) lactic acid solution as soaking agent in the two wet steeping periods. The temperature during the third wet and dry steeps did not significantly influence ($P > 0.05$) the phytate content of product 2. However, the products resulting from process 2 had a significantly ($P < 0.05$) lower phytate level than the products resulting from process 1.

Rice has not been studied as much as wheat and rye with respect to phytase activity and phytate degradation, but some studies exist (Yoshida et al 1975; Yamagata et al 1980; Marfo et al 1990; Kikunaga et al 1991; Marero et al 1991). The phytase in rice aleurone particles has an optimal activity at 45°C and pH 4.2 (Yoshida et al 1975). The shortest and most economically favorable hydrothermal process tested for processing rice gives the most extensive phytate breakdown (process 3, Fig. 2, Table IV). The temperature during all three processes was 45°C, but the pH varied because various lactic acid solution concentrations were used. The pH during the process, measured in mixtures of crushed grains and distilled water, was 4.6 and 4.5 after the first wet and dry steeps, respectively, in all three processes. The pH was lowered during processing and was 3.7, 4.0, and 4.3 after process 1, process 2, and process 3, respectively. Thus, the pH during process 3 was closest to the optimal pH reported for phytase activity in rice, and this process also resulted in the greatest phytate reduction.

Although phytase activity in wheat, rye and rice has been studied earlier, the results of the findings have not been used to industrially produce cereal products with a low level of phytate. The hydrothermal process used in this study was conducted in a pilot plant that can process 25–50 kg grains. Our aim is to enable the process up to industrial scale to produce cereal-based infant formulas, gruels, flakes, and muesli products with a low phytate level.

CONCLUSIONS

This study shows that it is possible to produce wheat, rye and rice products with a very low content of phytate and hence a good mineral bioavailability by hydrothermal processing which is a process very well suited to be used industrially. Estimations of the phytate contents in wheat and rye after hydrothermal processing at temperatures and lactic acid concentrations within the experimental domain ($T_{1,2}$ between 45 and 55°C for both wheat and rye, a lactic acid concentration between 0 and 1.5%, v/w, for both wheat and rye, and T_3 between 55 and 75°C for wheat, T_3 between 60 and 80°C for rye) are shown in response surface plots. The response surface plots can be used to find optimal conditions for phytate degradation but they are also valuable tools when other criterion must be met to achieve a good cereal product.

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