HYDROTHERMAL TREATMENT – A METHOD TO REDUCE THE PHYTATE CONTENT OF THE RYE♦

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Abstract: The purpose of the research was to study the way the hydrothermal treatment influences the phytate content in the rye and to establish the optimal conditions for this treatment in order to obtain rye with reduced phytate content.

By applying a hydrothermal treatment under the controlled conditions of temperature and *p*H, 97.4% of the phytate contained in the rye was hydrolyzed. This is possible because the conditions under which the hydrothermal treatment is performed are the optimal conditions under which the phytate acts upon the phytine. The experimental results indicated as optimal conditions for the phytate degradation the temperature of 55 ºC and the acid solution concentration for the rye soaking of 1.2%.

Keywords: *rye, hydrotermal treatment, phytate, temperature, pH*

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INTRODUCTION

The phytic acid is myo-inositol for all the 6 groups of hydroxyl. A great part of the phosphorus in cereals is found under the form of phytine, calcium and magnesium salt of the phytic acid. Between 50% and 80% of the total phosphorus is found under the form of phytine. The content of phytic acid is influenced by more factors: the maturing degree of the seeds, the soil fertilization, the type of fertilizer (N/P/K), the fertilization period, and the content of the phosphorus in the soil.

In high concentrations, it can reduce the bioavailability of minerals, but also of proteins. Thus, one or two phosphate group in the phytic acid molecule can interact and therefore bind ions of Ca, Mg, Zn, Fe; at a low *p*H and *p*H = 5-10, they can interact and bind proteins [3]. They also inhibit the enzyme action as amylases, pepsine, trypsine and acid phospatase, unfavorably intervening in the digestion processes.

In small concentrations, the phytic acid can have beneficial effects, for example in reducing the cholesterol and triglycerides in the serum [1].

The phytase is an enzyme that hydrolyzes the phytate and is to be found in high quantity in rye and wheat. The phytase activity in the rye and wheat flour (ash content of 0.9%) is of 3.7 $U.g^{-1}$, respectively 2.6 $U.g^{-1}$, according to Fretzdorff and Weipert (1986), and in wheat of 3.27 $U.g^{-1}$ [4].

By applying the hydrothermal treatment there can be created the conditions that activate phytases in cereals. This enzyme, once activated, hydrolyzes the phytate (hexaphosphate myo-inositol) into myo-inositol, inorganic phosphorus and extremely small quantities of tri–pentaphosphate myo-inositol. Under optimal conditions of temperature and time, the phytate can be reduced to 46-99% [1].

By using hydrothemal treatments with lactic or citric acid, besides reducing the phytate content, the production of bacteria during the hydrothemal process is also prevented, providing corresponding hygienic qualities to products. The Ca, Mg, Zn, Fe ions bioavailability also takes place.

The cereals to which the hydrothermal treatment is applied can be used to obtain products based on cereals – flakes, muesli, with a reduced content of phytate.

The cereal phytase activity depends on humidity, *p*H and temperature. The purpose of the researches was to study the way the hydrothemal phytate influences the phytate content in the rye and to establish the optimal conditions for this treatment, in order to obtain rye with a reduced content of phytate.

MATERIAL AND METHODS

The Gloria rye was used – it is a semi-precocious variety, resistant to frost and wintering, cultivated for its grains.

The treatment applied to the rye was the following:

- maintaining the rye for 1 hour at temperatures ranging between 45 and 55 ºC in citric acid solutions of concentrations ranging between 0 and 1.5%, the ratio between the rye and the acid solution being of 1:2,
- drying for 5 h at temperatures of 55 ºC,

- maintaining the rye at temperatures ranging from 45 to 55 ºC in citric acid solutions of concentrations between 0 and 1.5%, the ratio between the rye and the acid solution was of 1:2,
- drying for 15 h at temperatures of 55 ºC
- drying for 8 h at 50 ºC, 2 h at 60 ºC, 6 h at 80 ºC.

The efficiency of the alternatives applied was measured function of the quantity of phytic acid remained unhydrolyzed after applying the hydrothermal treatment.

The phytic acid was determined through the method of Wheeler and Ferrel (1971). First it was extracted with a solution of trichloroacetic acid and precipitate as ferric salt, Fe_4Phy, using a solution of $FeCl_3$ in HCl. After the Fe_4Phy dissolution, the Fe^{3+} ions were precipitate as ferric hydroxide, which was dissolve with hot solution of HNO_3 3.2 N. The released iron was determined through the method indicated by Makower (1970), using *o-o*'-phenanthroline. The amount of phytic acid was estimated considering the ratio Fe:P as 4:6.

An experimental plan has been elaborated. It considered the simultaneous modification of the acid solution concentration and temperature, so that an adequate mathematical model could be obtained, where the response variables should be the content of phytic acid remained after the application of the treatment; the calculation of the regression coefficients of the model and the interpretation of the experiment data.

For the mathematical description of the hydrothermal treatment results, a polynomial of second order was used having the form of:

$$z = a + bx + cy + dx^2 + ey^2 + fxy \qquad (1)$$

where:

z is the response variable (the quantity of phytic acid remained after the application of the hydrothermal treatment),

x, *y* – the parameters of the hydrothermal treatment: the concentration of the citric acid, temperature,

a, b, c, d, e, f – parameters of the model (*a* – the free term, *b, c, d, e, f* – the regression coefficient).

In order to estimate the regression coefficients of such a model, each variable must take at least three levels. This suggests the use of a factorial experiment 3^2 where each factor *x* and *y* must take three coded levels: –1, 0, +1.

Under the circumstances, in order to evaluate the response function of the second order, we used an experimental plan, centrally compositional, of the second order, made up of:

- a complete factorial experiment, at 2 levels, 2^2 (2^k),
- 2·2 (2·k) experimental points situated at the α distance from the center; the values of the α parameter are established so that the ortogonality of the regression coefficients is ensured, for the factorial number $k = 2$,
- $k_0 = 4$ experimental points in the center of the plan.

The experimental program of the second order, centrally compositional, in coded variant and standard order [5, 8] is presented in Table 1.

The basic levels for *x* / temperature and *y* / concentration of citric acid solution were:

$$x^0 = \frac{x^{-1} + x^{+1}}{2} = 50^\circ C \;,\; y^0 = \frac{y^{-1} + y^{+1}}{2} = 0.75\% \qquad (2)$$

and the coded values:

$$x / coded = \frac{x - 50^0}{5} = \pm 1 \ , \ y / coded = \frac{y - 0,75}{0,75} = \pm 1 \qquad (3)$$

Determining the regression coefficient and the analysis of the experimental results dispersion, in order to evaluate the contribution of the elements of the Ist order (*a, b, c*) and of the IInd (*d, e, f*), but also to establish the degree of adequacy of the model, have been achieved through the program *Windows Statistics version 4.3*.

Table 1. *The experimental program used in studying the influence of the hydrothermal treatment on the phytate content*

Step	Experiment no.	Matrix of variables						Response
		x_0	x	y	xy	x^2	y^2	
Experiment 2^2	1	+1	+1	+1	+1	+1	+1	z_1
	2	+1	-1	+1	-1	+1	+1	z_2
	3	+1	+1	-1	-1	+1	+1	z_3
	4	+1	-1	-1	+1	+1	+1	z_4
Supplemental point on the axes	5	+1	+α	0	0	+1	0	z_5
	6	+1	- α	0	0	+1	0	z_6
	7	+1	0	+ α	0	0	+1	z_7
	8	+1	0	- α	0	0	+1	z_8
Point in the center	9	+1	0	0	0	0	0	z_9
	10	+1	0	0	0	0	0	z_{10}
	11	+1	0	0	0	0	0	z_{11}
	12	+1	0	0	0	0	0	z_{12}

The adequacy of the model, that is the hypothesis that the experimental data belong to the response surface generated by the regression equation, and the extent to which the correlation suggested by this equation is real, can be globally evaluated on the basis of the coefficient of this correlation. The closer to 1 it is, the better the model fits the experimental data, a very close relationship existing between the variables admitted in the study.

RESULTS AND DISCUSSIONS

We have analyzed 12 experimental samples according to the experimental plan.
Their statistic processing enabled the establishment of the significance level for each coefficient of the regression equation, but also the accomplishment of the model variance analysis, emphasizing the model lack of fit.
The correlation coefficient helped in establishing if there is a close relationship between the variables admitted in the study, and the determination report helped us in establishing if the variation of the response function – the phytate reduction, is due to the hydrothermal treatment parameters.
On the basis of the post-treatment reduced phytate, we achieved the optimization of the rye hydrothermal treatment according to the remarks made under *Methods*.

The regression equation of order II that models the evolution of the phytate content reduction was:

$$z = 27.4861 - 3.2680x + 142.3674y + 0.0676x^2 - 32.7948y^2 - 1.14xy \qquad (4)$$

the correlation coefficient being 0.9975.

We have noticed the existence of a significance level for a risk of 5% for the regression equation coefficient corresponding to the factor *p*H (y) of order I and for the coefficients of the factors *p*H (y^2) and of the interaction of factors *p*H and temperature (xy) of order II. These results show the importance of *p*H and of the interaction *p*H – temperature on the optimal conditions of the phytases action in the rye.

The data in Table 2 shows that the model lack of fit is not significant. The explained variance of the model is 99.87%. Under the circumstances we can say that the model fits the experimental data better.

***Table 2**. The analysis of the mathematical model variance based on the content of the phytic acid remained in the rye samples after the hydrothermal treatment*

Source of variation	Sum of square	Degrees of freedom	Mean square	F_{stat}	P > F
Regression	5063.1994	5	1012.6399	477.26	0.000
Error:	12.7306	6	2.1217	-	-
-lake fit	11.2606	3	3.7535	7.66	0.0642
-pure error	1.47	3	0.49	-	-
Total	5075.93	11	-	-	-

The graph corresponding to the response surface generated by the regression equation for the reduction of the phytate content is shown in Figure 1.

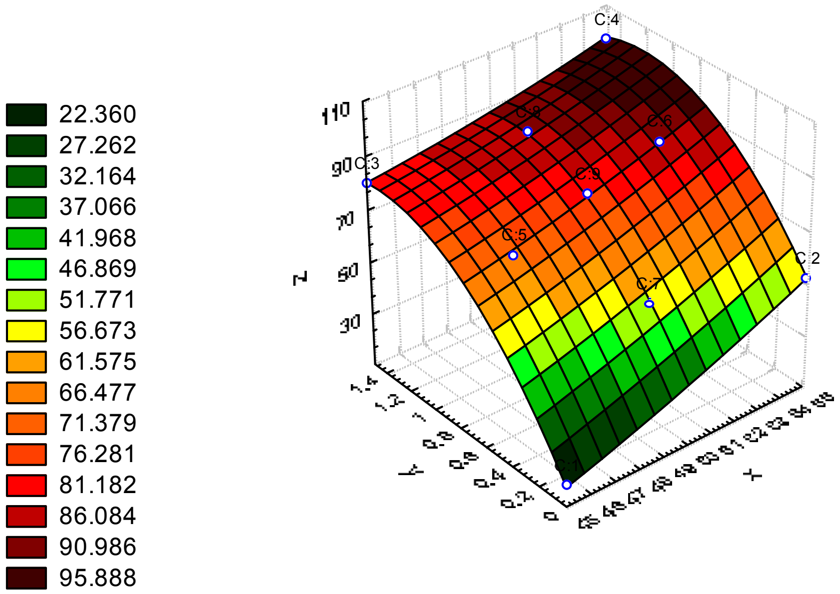

***Figure 1.** The graphical representation of the regression equation for the reduction of the phytate content*

By calculating and annulling the derived of order I for the reduction of the phytate content (z) in relation to the temperature (x) and pH (y), but also for the analysis of the iso-response curve, shown in Figure 2, we can obtain the optimal conditions for deploying the hydrothermal treatment.
The results showed that by applying a hydrothermal treatment, 97.4% of the phytate contained in the rye is hydrolyzed. The quantity remained is low enough to significantly negatively influence the Zn, Ca, Mg, Fe ions absorption.
The experimental results indicated as optimal conditions for the phytate degradation the temperature of 55 ºC and the concentration of the acid solution used in the rye soaking of 1.2%.
The cereals to which the hydrothermal treatment has been applied can be used in obtaining products based on cereals – flakes, muesli, with reduced phytate content.

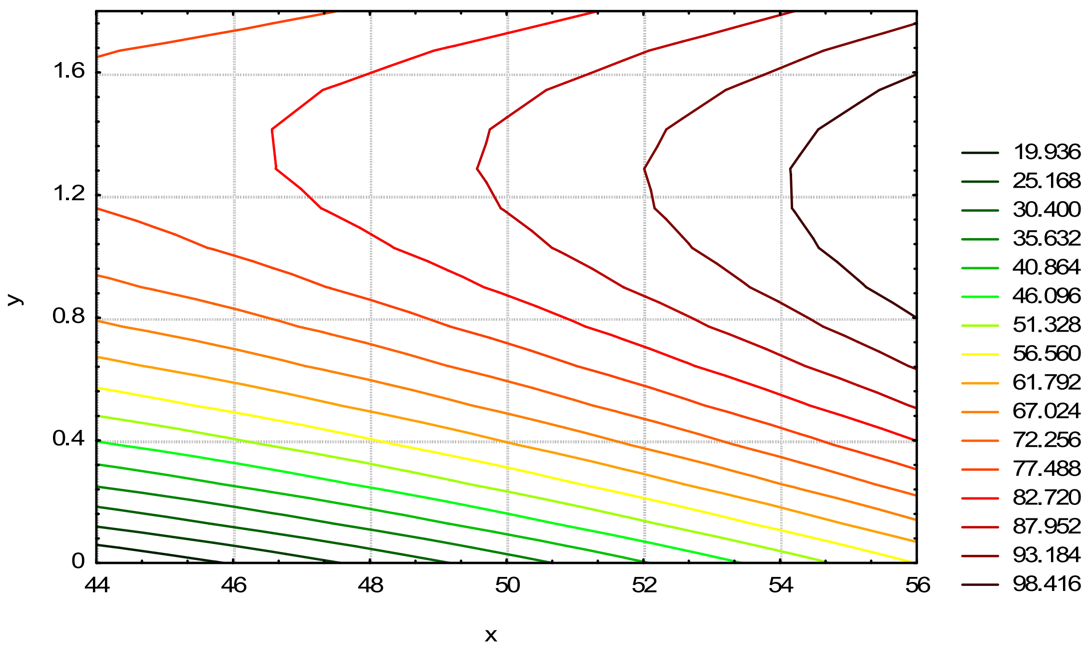

Figure 2. *The iso-response curve*

By applying a hydrothermal treatment under conditions of temperature and pH, 97.4% of the phytate in the rye is hydrolyzed. This is possible because the conditions for the hydrothermal treatment are the optimal conditions under which the phytases acts on the phytine. The experimental results indicated as optimal conditions for the phytate degradation are the temperature of 55 ºC and the concentration of the acid solution used in the rye soaking of 1.2%.

CONCLUSIONS

The purpose of the research was to study the way the hydrothermal treatment influences the phytate content in the rye and to establish the optimal conditions for this treatment in order to obtain rye with reduced phytate content.

By applying a hydrothermal treatment under the controlled conditions of temperature and *p*H, 97.4% of the phytate contained in the rye was hydrolyzed. This is possible because the conditions under which the hydrothermal treatment is performed are the optimal conditions under which the phytate acts upon the phytine. The experimental results indicated as optimal conditions for the phytate degradation the temperature of 55ºC and the acid solution concentration for the rye soaking of 1.2%.

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