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Microwave-assisted acidic hydrolysis of phytate from rye bran – Experimental procedure and model prediction

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ABSTRACT

Phytate is an abundant phosphorus (P) compound in plant material, which cannot be taken up by monogastric animals but is to a significant part released into the environment with excreta. In order to make use of cereals' phytate content, phytate hydrolysis by microwave-assisted thermal treatment of an acidic rye bran extract into inorganic phosphate was investigated experimentally and by modelling for process application. A phytate conversion of 98.5% could be achieved at 200 °C and 15 min reaction time and showed to be highly dependent on the temperature by a sigmoidal correlation. Treatment time showed its main influence in the mid temperature range (between 150 and 180 °C), thus didn't play a role anymore at beginning hydrolysis and towards full conversion. A mathematical quartic model represented phytate conversion with an optimum at harshest examined conditions and allows for predictions of the hydrolysis within the parameter range investigated. A kinetic model gives parameters of reaction order 0.68 and an activation energy of 118.7 kJ/mol with the option to predict phytate hydrolysis beyond the investigated parameter range and for varying input concentration. Equal liberation of all six phosphate groups from phytate is mechanistically suggested. Both model approaches show comparable results.

1. Introduction

Phytate is the natural phosphorus (P) storage form within plants containing an inositol ring with six phosphate groups bound to it via phosphomonoester binding (Humer, Schwarz, & Schedle, 2015). Phytate is especially present in cereals and legumes for feedstuff, where it could serve as a supplier of P as an essential nutrient. However, phytate is hardly digestible and thus of little nutritional value for monogastric animals and even considered an anti-nutrient since complexation of e.g., calcium and iron can lead to major mineral deficiencies (Dersjant-Li, Awati, Schulze, & Partridge, 2015). Therefore and to prevent losses of the limited resource P, various approaches aim for a reduction of phytate content in feedstuff. Often, bacterial or fungal phytase is co-fed in mixed fodder to make use of their phytate-degrading enzyme activity in the intestinal tract of monogastric animals. This can, however, only make around half of the phytate available for digestion, which still leaves an enormous part of P being directly excreted with manure (Widderich et al., 2022).

On that account, chemical pre-processing of feedstuff before feeding is widely researched to provide free phosphate by extraction of phytate and subsequent hydrolysis to available P forms. The step of phytate hydrolysis as shown in equation (1) is hereby further investigated. Acidic hydrolysis has already been conducted by microwave treatment in acidic media with a phytate model solution and achieved over 99% phytate-P reduction (March, Grases & Salvador, 1998). Also, around 96% of barley phytate could be reduced by a stepwise soaking and drying method with lactic acid by slightly elevated temperature after a time span of several days (Bergman, Autio & Sandberg, 2000). So far, more effective methods in terms of reaction time, maximised conversion yield and applicability to other substrate yet seek implementation. Thus, in this work a novel pathway of hydrolysis of phytate from rye bran as one of the most used feedstuffs in Europe with very high phosphorus content will be presented (Federal Office for Agriculture and Food, 2020). The procedure itself might, however, be transferrable to other cereal types as well due to resemblance in structure of the substrate and binding of phytate (Mayer, Widderich, Scherzinger, Bubenheim, & Kaltschmitt, 2023). Hydrolysis will be conducted by microwave treatment at temperatures up to 200 °C and heating times of up to 15 min. Results of the experiments are first shown and discussed and then applied for both a mathematical and kinetic model of the according

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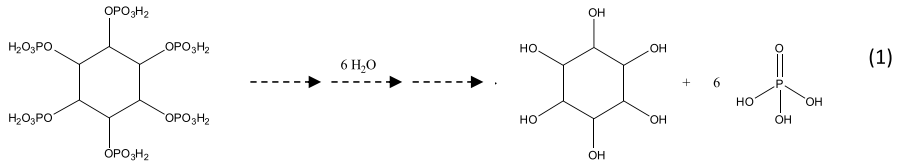
reaction taking place. Model results then lead to a mathematical optimum of the process conditions within the investigated range and to effective kinetic parameters for the hereby shown reaction set-up, which allow for more holistic conclusions on phytate hydrolysis.



(1)

2. Material and experimental methods

2.1. Experimental procedure

2.1.1. Sample preparation

Rye bran was provided by Aurora Mühlen GmbH in Hamburg/Germany containing finely ground hull and germs from a common mixture from different cultivation areas in Germany. Rye bran samples were dried at 105 °C for at least 4 h to ensure complete drying. Subsequently, rye bran was extracted in 0.66 mol/L hydrochloric acid with a solid-to-liquid ratio of 1 g_{bran}/20 mL_{HCl} for at least 3 h. Extraction was performed in an overhead shaker at room temperature according to the Megazyme K-PHYT instructions (Megazyme Ltd., 2017). The residual solid phase was separated by centrifugation (Hettich model Rotixa 50 RS) at 4500 1/min for 15 min. Within these experiments, the residual bran was discarded, however could be further used as a feedstuff to valorise the remaining nutrients. Liquid phase, filtered by Whatman Grade 589/3 filters, was taken for further treatment. The extract contains an average amount of 570 mg/L total P with around 10% ortho phosphate-P (determined by the analysis introduced in section 2.1.3) and has a pH value of around 0.22.

2.1.2. Thermal treatment of rye bran extract

10 mL from the acidic rye bran extract were each transferred into a reaction vessel fitting into the microwave system model "Multiwave Go" by Anton Paar. Microwave treatment was performed at varying temperatures for different reaction times according to the experimental procedure presented in Table 1. The heating time to reach target temperature was set to 10 min for all runs, cooling after the experiment was performed down to 60 °C in a time span of max. 15 min. The pressure during microwave treatment is given by the vapor pressure, which – approximated by pure water's vapor pressure – lies in the range of 0.2 to up to 1.5 MPa (Grigull, 2012). After thermal treatment, the liquid hydrolysate was filtered by Whatman Grade 589/3 filters. Volume was refilled to 10 mL with deionized water to compensate potential losses during heating as reactor vessels might open for venting. The hydrolysate was then used for P analysis.

Table 1
Experimental points of the Design of Experiments.

140–160 °C, 5–15 min			160–180 °C, 5–15 min			180–200 °C, 5–15 min		
No.	t[a]	T[a]	No.	t[a]	T[a]	No.	t[a]	T[a]
1	17	150	14	10	170	27	5	200
2	5	140	15	10	170	28	10	190
3	10	150	16	10	184	29	10	204
4	10	150	17	10	170	30	3	190
5	5	160	18	10	170	31	10	190
6	10	150	19	17	170	32	10	190
7	10	136	20	10	170	33	5	180
8	15	160	21	15	180	34	10	190
9	10	164	22	5	160	35	10	176
10	3	150	23	5	180	36	15	180
11	10	150	24	3	170	37	15	200
12	15	140	25	10	156	38	10	190
13	10	150	26	15	160	39	17	190

T = temperature in °C.
[a] t = holding time in min.

2.1.3. Phosphorus analysis

For determination of total, free and phytate-P content, sample extract was analysed by use of the Megazyme enzyme kit K-PHYT. The analysis gives the amount of inorganic P as well as the sum of all free P compounds after enzymatic treatment by phytase and alkaline phosphatase. These enzymes are supposed to cleave all inositol phosphates. Colorimetric reaction between free phosphate with ascorbic acid and a molybdate solution leads to colour change from yellow to blue, which is detected by UV/Vis spectrophotometry. Measurement was performed in the UV/Vis spectrophotometer Varian Cary 50 at 655 nm against a calibration with K_2HPO_4. Organic P content in the form of phytate is defined as the difference between the sum of all P compounds and the amount of inorganic P content, the latter determined without enzymatic treatment (Megazyme Ltd., 2017).

P analysis was carried out for the initial rye bran extract without microwave treatment as a reference and thermally treated extracts, respectively. All analysis for each experimental point was performed as a triplicate. Results are expressed as phytate conversion PhC, which is defined as the percentage of phytate reduction during thermal treatment as shown in equation (2). $c_{Ph\text{-}P}$ is the respective phytate-P concentration before and after thermal treatment, thus in the extract and the hydrolysate according to indices.

$$PhC_{exp} = \frac{c_{Ph-P,extract} - c_{Ph-P,hydrolysate}}{c_{Ph-P,extract}} \bullet 100\% \quad (2)$$

2.2. Design of experiments

An experimental procedure following combined Central Composite Designs (CCDs) was set up for the thermal treatment of the rye bran extract. CCD is a widely used type of design, which covers not only the parameter range to be investigated but also parameter combinations beyond that range, thus allowing for detailed examination of the range borders. These parameter points are cube points at the corners of the range, star points exceeding the range and a 5 time replicate of the same process conditions in the centre to determine statistical deviations. CCD is suitable for linear and quadratic correlations. The holding time t at operating temperature was investigated in a range of 5–15 min, the treatment temperature T was 140–200 °C. According to the CCD, randomized experimental runs were defined within three temperature ranges as shown in Table 1, thus combining three separate CCDs. For evaluation, all three CCDs were merged to a single model representing the complete examined range of temperature and time.

2.3. Modelling methods

The results achieved from experimental procedure were used for model fits of two different approaches:

- Firstly, a regression of time and temperature dependence of phytate conversion was performed by statistical evaluation of the CCDs. Being a combination of three CCDs, a quartic model could be fitted with a three-dimensional response plot as the result and least-square fitting being applied. Thus, purely mathematical criteria led to the fitting of theoretically 15 parameters by linear regression and an exact analytical solution is obtained. The result for the fit parameters, however, does not necessarily reflect a physical correlation.
- Following the principle of reaction kinetics, a second approach of modelling was executed by solving the differential equation of reaction rate. Thus, a physicochemical correlation is taken as a basis for the fitting. The solution, based on least-square-fitting, might thus not be the best mathematical fit, but results in only three mechanistic parameters, which are valid beyond the range of investigation. Also, kinetics is valid for any starting concentration, thus theoretically fitting three parameters: time, temperature and phytate concentration.

The implementation of these approaches is explained in detail below.

2.3.1. Quartic model fit

For the 3D model, statistical evaluation by use of the DesignExpert® software package was performed. A quartic model type for the three sets of CCD is suggested. Thus, the two variables are taken into account with up to quartic correlations (T, t, Tt, T^2, t^2, T^2t, Tt^2, T^3, t^3, T^2t^2, T^3t, Tt^3, T^4, t^4) for the fitting model as in equation (3). As all coefficients C are of linear type, an analytical solution can be determined.

Analysis of variance (ANOVA) was carried out resulting e.g., in measures for significance of the model itself (F-value and Lack of Fit), significance of the model parameters (p-value), values of the model coefficients and the degree of agreement between model and experimental data. The latter is given as the coefficient of determination (R^2) with the attempt to be close to 1 to minimize deviations between the model and experimental data. The average absolute deviation (AAD) as a more tangible value is defined by summing up all absolute values of deviation between modelled and experimental phytate conversion divided by the number of experiments. AAD was determined based on predicted and measured phytate conversion.

$$PhC_{quartic\ fit} = C_1 + C_2T + C_3t + C_4Tt + C_5T^2 + C_6t^2 + C_7T^2t + C_8Tt^2 + C_9T^3 + C_{10}t^3 + C_{11}T^2t^2 + C_{12}T^3t + C_{13}Tt^3 + C_{14}T^4 + C_{15}t^4 \quad (3)$$

2.3.2. Kinetic modelling

For the modelling of the phytate conversion via reaction kinetics, the differential equation of the reaction rate as displayed in equation (4) is used. $c_{Ph\text{-}P}$ is the phytate-P concentration, k_0 is the Arrhenius parameter representing maximum reaction rate at infinite thermal energy and E_A the activation energy needed to start phytate hydrolysis. R is the universal gas constant (Schrader, 2016).

Due to the experimental procedure that, firstly, does not allow for continuous sampling since being an enclosed system and, secondly, has varying temperature over time (including heating and cooling period), the differential equation cannot be solved by use of experimental data only. Thus, equation (4) was numerically solved by an Euler-type approach (equation (5)) based on the temperature profile and the known value of the initial and final phytate-P concentration for all experiments in Table 1. From the starting value, phytate-P concentration in the following time steps i and i + 1 were determined over time spans of Δt until the end of reaction time. Time span is defined by the resolution of the temperature profile in the microwave.

Random starting values for the variables of the Arrhenius parameter k_0, the activation energy E_A and the reaction order n were chosen and then fitted to minimize the Sum of Squared Residuals (SSR) between the model value for the final concentration and the measured value after reaction time. As a comparison to the other approaches, AAD was calculated additionally.

$$\frac{dc_{Ph-P}}{dt} = -k_0\, e^{-\frac{E_A}{R\,T}}\, c_{Ph-P}(t)^n \quad (4)$$

$$c_{Ph-P,i+1} = c_{Ph-P,i} + \frac{dc_{Ph-P}}{dt}\Delta t \quad (5)$$

3. Results and discussion

3.1. Phytate hydrolysis during thermal treatment

Degradation of the acidic rye bran extract in the microwave, before treatment being transparent and reddish in colour, could be visually observed by a change in colour towards a brownish appearance. The harsher the parameters time and temperature were, the darker the colour of the extract became. This can well be led back to condensation of reducing sugars from the cereals with residual amino groups, the so-called Maillard reaction being accompanied by an olfactorily notable roasted note (Martins, Jongen & van Boekel, 2001).

Fig. 1 presents analytic results for a set of different reaction temperatures and holding times at target temperature. The share of organic and inorganic P within the extract as a reference and the hydrolysates after different treatment conditions is shown. With both rising temperature and holding time at target temperature, phytate content decreased (hatched bars in Fig. 1) and, at the same time, free phosphate amounts increased (black bars in Fig. 1). Standard deviation of triplicate measurements is displayed as error bars in Fig. 1 and show reliable analysis. At mildest conditions less than 1% phytate conversion could be observed, stepwise increasing with temperature to higher yields of around 50% at 160 °C and over 90% at 180 °C after 15 min treatment. At highest operating temperature of 200 °C, the influence of reaction time is low and for both, 5 and 15 min, around 98% phytate was cleaved. During heat treatment, total P stayed constant within measurement uncertainties and biological variations due to the natural substrate. This implies no P losses within the sample processing and a successful conversion of phytate-P into free P.

Currently applied processes for phytate hydrolysis are mainly based on enzymes. However, most phytases only split off five phosphate groups with inositolmonophosphate as the product (Konietzny & Greiner, 2002), thus a maximum yield of 83% for liberation of free phosphate could theoretically be achieved. A maximum phosphate liberation of 98% in the experiments here shows that, in contrast to enzymatic phytate cleavage, all phosphate ester bonds were cleaved by the microwave-assisted thermal treatment presented here.

In literature, phytate and its lower inositol phosphates are reported to be quite stable at high temperature, particularly in its natural cell binding. Boiling in water, for instance, reduces phytate content only very little in cereals and legumes (Humer, Schwarz, & Schedle, 2015). Pressure cooking of brown beans at up to 140 °C increased the inositol phosphate reduction to around 40% (Schlemmer, Frølich, Prieto, & Grases, 2009). Taking aqueous phytate model solution, purely thermal degradation was observed from 150 °C onwards both visually by colour change and by quantitative thermogravimetric and IR spectroscopy (Daneluti & Matos, 2013). The same was proven by our experiments, however in cereal-based extract as a natural substrate. Also in acidic phytate model solution but at little higher pH than examined here, phytate reduction at lower temperature of 120 °C was reported, however in a time range of several hours to obtain around 80% phosphate liberation (Arnold, 1956). Most pure phytate salts in bindings that occur

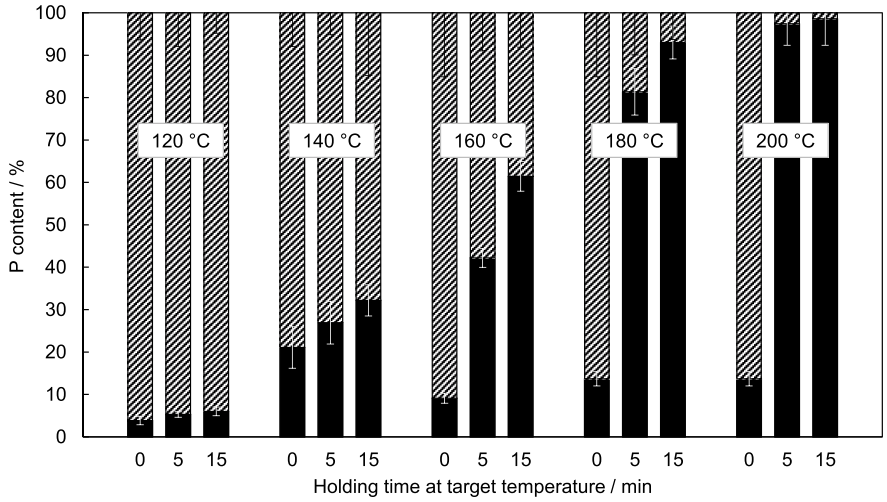


Fig. 1. Thermal treatment of rye bran extract at different times and temperatures; share of free P (filled columns) and phytate-P (striped columns) displayed in comparison to thermally untreated extract (0 min), error bars from triplicate measurements included.

with alkali and alkaline earth metals show to be both stable against rising temperature and against acidic hydrolysis separately. Particularly calcium phytate showed increasing degradation with longsome dissolution in acetic or hydrochloric solution (Sun, He & Jaisi, 2021). The microwave treatment of acidic extract hereby combines both the thermal and acidic effect and leads to significantly higher phytate degradation yields in, likewise, shorter times.

In the microwave set-up, additional experiments at 120 °C were performed, but did not show significant phytate hydrolysis. For 5–15 min treatment at 120 °C, only 2–5% phytate conversion was observed. Further rising of the heating period to a time span of hours might lead to higher conversion, but was not further investigated here. Thus, in comparison to yet published research, this article shows microwave-based phytate hydrolysis within cereal as a natural substrate with significantly reduced reaction time and outstandingly high yields of 98.5%, however at harsher conditions than reported before. Natural phytate might most probably occur as calcium, sodium or magnesium salts which is in good accordance with highest solubility of these salt forms and especially smallest degradation resistance of calcium phytate reported by literature in comparison to e.g., metal phytates.

Specific effects of the microwave radiation in comparison to conventional heating have been widely discussed especially due to the fact that only polar components will be heated by microwave treatment. In heterogeneous reactions, this can lead to non-homogeneous temperature distribution within the media and thus hot-spots, which then can have either supporting or adverse effect on the reaction. Hereby, microwave heating was primarily used for fast and homogeneous heating throughout the reaction volume as the rye bran extract is expected to present a homogeneous polar solution. However, non-polar solutes such as fatty acid or lipophile organic components might affect heat transfer. Also, non-thermal effects such as the electromagnetic field being induced by the microwave might influence reaction progress but have yet mostly been observed for photo-chemical reactions or reactions based on electron transfer (Díaz-Ortiz, Prieto P. & La Hoz, 2019). Experiments hereby don't indicate any specific microwave-induced effect.

3.2. Model approaches

The yet experimentally demonstrated phytate hydrolysis was performed by fractional factorial design to observe changes in phytate degradation with changes of both parameters, time and temperature. A whole parameter space can now be interpreted by statistical analysis to identify specific optima and allow for interpolation between the actual

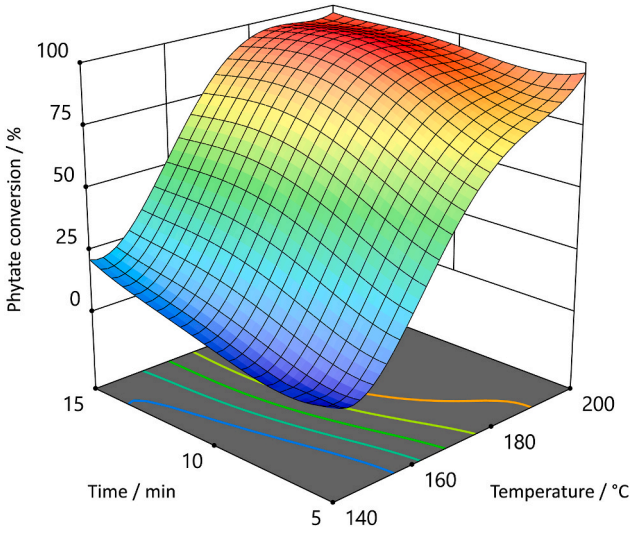


Fig. 2. Response Surface Plot of Phytate Conversion as a function of time (5–15 min) and temperature (140–200 °C) following a quartic model; colour range 0–100% conversion (from blue to red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

experimental data points. Kinetic models even go a step further by opening the interpretation for a more holistic model allowing for extrapolation beyond the experimental parameters. This might be helpful for further application of a phytate hydrolysis.

3.2.1. Quartic fit

The observations from the experiments were processed by statistical analysis and are displayed as a three-dimensional continuous plot showing the mathematical correlation between phytate degradation and time and temperature in Fig. 2. Phytate hydrolysis shows a sigmoidal increase with both, time and temperature, however has a significantly steeper slope in the direction of the investigated temperature. Especially from 150 °C onwards, a rapid increase of phytate conversion was observed, then flattening again due to being near full conversion at around 180–185 °C, depending on the reaction time. At high temperatures, phytate conversion is comparably high for all investigated holding times. Accordingly at low temperatures, phytate conversion is similarly

Table 2
Coefficients for quartic model fit equation, rounded to six decimals.

Coefficient (short)	Coefficient (value)	Variable (short)
C_1	40942.456170	–
C_2	−941.493833	T
C_3	−478.169448	t
C_4	5.207780	T*t
C_5	8.110614	T^2
C_6	26.182362	t^2
C_7	−0.015202	T^2*t
C_8	−0.232068	T*t^2
C_9	−0.031003	T^3
C_{10}	−0.420019	t^3
C_{11}	0.000673	T^2t^2
C_{12}	0.000040	T^4
C_{13}	0.009557	t^4

low for all investigated times; thus, reaction time at lowest and highest investigated temperatures doesn't have a significant influence on phytate conversion. Lowest phytate conversion (blue colouring) comprises the full-time range at 140 °C and lower reaction times up to 150 °C. At medium phytate conversion (green and yellow colouring) the steepest slope occurs, then flatting at conversions of above 90% at harshest conditions of 180–200 °C and longest reaction times (red colouring). The temperature range with steepest increase of phytate conversion decreases with rising reaction time.

The coefficient of determination (R^2) of 0.97 shows good correlation between the quartic model and the experimental points. The F-value of >70 implies significance of the model, thus valid approximation by the quartic coarse of phytate degradation over the parameter space. Within the quartic model, certain terms (T^3t, Tt^3) were dismissed due to insufficient p-values of >0.05 under consideration of model hierarchy. Thus, both parameters time and temperature during the reaction, significantly influence phytate hydrolysis separately, but also combined effects are of importance. Table 2 reveals the coefficients for the quartic model, which might be inserted into equation (3) to predict phytate conversion for any parameters within the regression range. The key parameters of the model are summarized in Table 3.

Thus, the hereby derived mathematic model following a quartic equation can be used for reliable prediction of phytate degradation within the range of investigated parameters i.e., for microwave treatment between 140 and 200 °C and holding times of 5–15 min. As a result, optimum process conditions in terms of maximum phytate cleavage were found to be the harshest hereby applied conditions. So, within the parameter range process conditions for a targeted phytate conversion can be derived from the model equation. However, a cost-benefit calculation for the invested energy to heat the system or similar considerations might lead to a different individual parameter optimum. In order to predict values beyond the investigated range e.g., at lower temperatures which might be interesting for a potential application of the hydrolysis reaction, a kinetic model approach is presented in the next chapter. The course of the kinetics might also allow for mechanistic correlations that can give an insight into the chemical pathway of thermally induced phytate degradation.

Table 3
Key parameters of the quartic model fit for phytate conversion in a temperature range of 140–200 °C and for times of 5–15 min.

Parameter	Value	Explanation
Model type	Quartic	All coefficients up to quartic correlations are taken into account
F-value	78.11	Model is significant
Lack of Fit	0.69	Lack of Fit is not significant
R^2	0.97	High model reliability

3.2.2. Kinetic modelling

As a comparison to the empirical model with purely mathematical fitting, reaction kinetics as a physicochemical approach was used to model phytate hydrolysis in the hereby described experimental set-up on the basis of the exact same experiments. Based on the classical equation for the reaction rate (equation (4)) of phytate conversion, the optima for the effective kinetic parameters (reaction order n, Arrhenius parameter k_0 and activation energy E_A) were determined by minimising the SSR between experimental and modelled final phytate-P concentration. For the set of experiments of Table 1, the effective kinetic parameters of microwave-assisted acidic phytate hydrolysis were determined to a reaction order of 0.68, an Arrhenius parameter of $9.5 \bullet 10^9$ L/mol·s and 112.7 kJ/mol activation energy. Within the kinetic observations and modelling, no intermediates (such as lower inositol phosphates) or side reactions, which might occur within the organic extract, are considered.

It is indeed unusual to find a non-integer number of <1 as a reaction order for a potential degradation reaction. Literature states first order kinetics for the hydrolysis of phytic acid model solution at pH of >3 (Arnold, 1956; Daneluti & Matos, 2013). The smaller hereby determined reaction order of 0.68 derives from modelling under consideration of the whole reaction time including heating and cooling time and the whole reaction without any intermediates or side reactions in a complex organic substrate. This might explain deviations, however gives valid parameters for the procedure described. Also, rye bran extract in acidic media of pH < 1 has been used as a substrate, where potential catalytic effects might occur. The activation energy of 112.7 kJ/mol determined here corresponds well to literature. Daneluti and Matos performed kinetic investigations by thermogravimetric measurements of phytate model solution according to the method of Ozawa and revealed a value of 118 kJ/mol (Daneluti & Matos, 2013; Ozawa, 1965).

The modelling of effective reaction kinetics resulted in a specific concentration profile for every experiment. Fig. 3 A exemplifies the decreasing phytate-P concentration over time for a 190 °C and 10 min experiment (continuous line). In the same diagram on a secondary axis on the right, phytate conversion, calculated from phytate-P concentration (dashed line), and the temperature profile of the experiment (spotted line) are shown for direct comparison. Hydrolysis in this modelled concentration profile starts at around 140 °C, then rising to the inflection point of the curve at maximum temperature of 190 °C and being completed after around 1000 s in total. This indicates an end of the reaction during treatment still at maximum temperature so that treatment time in practical application can be reduced without yield losses. Reaction within all experiments already starts during the heating period where temperature still rises, but for lower temperatures still pursues during cooling. Due to this, it is necessary to include both heating and cooling periods into the kinetic model.

According to the kinetic study, reduction of phytate-P concentration starts between 140 and 150 °C for all experiments and ends either when almost full conversion is reached or when temperature drops below 140 °C again. A comparison of the modelled reduction of phytate-P concentration at different temperatures is shown in Fig. 3 B for a reaction time of 10 min in the microwave. Fig. 3 C shows the concentration profile for holding times of 5 and 15 min at 180 °C. The initial concentration of all samples is within biological variations and final phytate-P concentration of the kinetic model decreases with rising temperature and higher holding times at target temperature, hence phytate conversion rises accordingly. The respective experimental data points of phytate-P concentration after reaction time are displayed as squares in Fig. 3. A satisfactory correlation is already visible and reflects in a minimum SSR of $1.5 \bullet 10^{-4}$ mol^2/L^2 as well. Thus, the model shows good correlation for all experiments and can be used for further interpretation. For practical implementation, kinetics suggest that treatment temperature should be above 140 °C for successful hydrolysis and, depending on target temperature, the exact time until a certain rate of

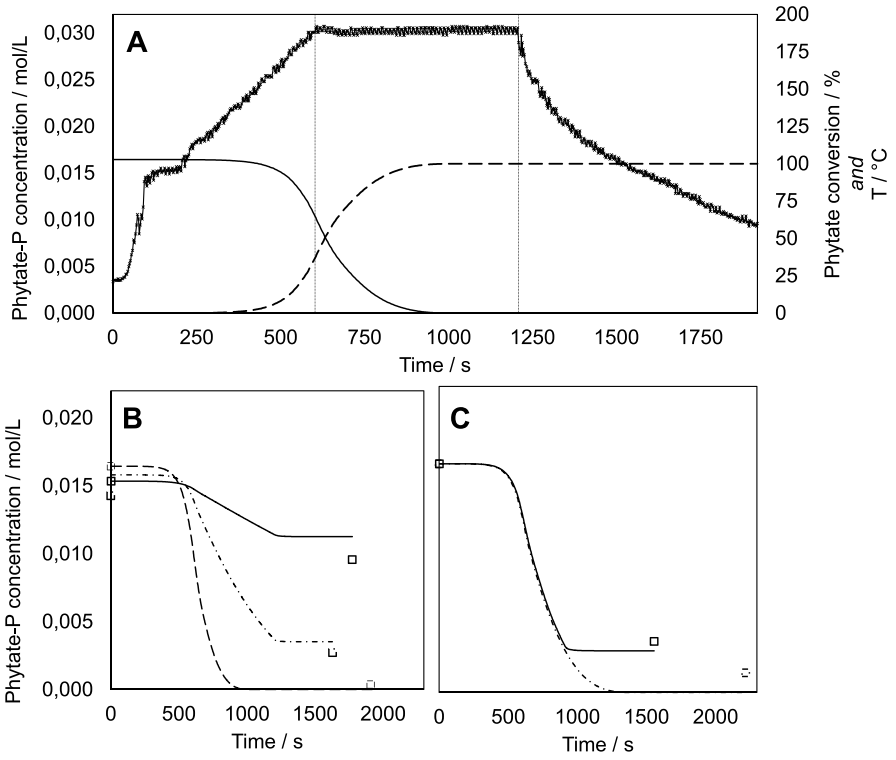


Fig. 3. (A) Course of model kinetics for 190 °C and 10 min (concentration: continuous line, conversion: broken line) in comparison to the temperature profile (connected data points) with heating and cooling period confined, (B) comparison of kinetic graphs at 150 (continuous line), 170 (dashed line) and 190 °C (broken line) with 10 min holding time at target temperature and (C) comparison of kinetic graphs at 5 (continuous line) and 15 min (dashed line) reaction time at 180 °C with experimental data marked as squares.

phytate conversion can be determined. A further comparison of the two models applied will be presented in the following.

3.3. Applicability of the models

The model approaches should give insight into how phytate hydrolysis might be applied, for the time being only under consideration of mechanistic aspects only. As being based on different assumptions and mathematical background, the two models have different informative value and different advantages and constraints in their application. Selected aspects playing a role for that are compared among the two model approaches to evaluate their suitability for practical application.

- **Number of fitted parameters.** Both models fit both the process variables (time and temperature). An addition of starting concentration as a variable is only feasible for kinetic modelling. The quartic model type was chosen specifically according to the input variables and must be evaluated again if adding more parameters such as starting concentration to be fitted.
- **Number of model parameters.** The kinetic fitting has an advantage in terms of practical implementation of the model due to the limited number of only 3 model parameters. Also, confidence of the model parameters usually increases with lower number of model parameters, thus showing superiority of kinetic fitting as opposed to the quartic model. In contrast, a large number of model parameters leads to higher accuracy of the quartic model.
- **Type of solution.** The kinetic model needed to be solved iteratively by a numerical approach, which for some application might require complex data analysis and processing power. As an analytical solution was feasible for the quartic model, this is not necessary here. Analytical solutions also potentially enhance model accuracy.

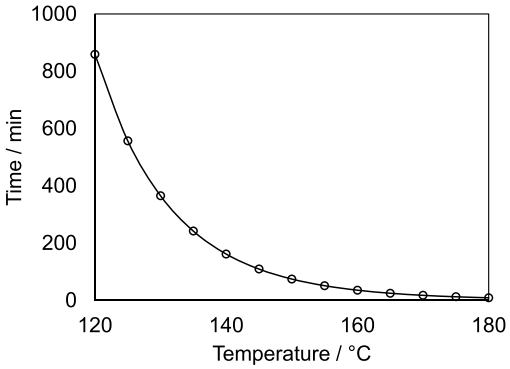


Fig. 4. Predicted reaction time for 95% phytate conversion yield at a starting concentration of 0,016 mol/L based on kinetic modelling.

- **Model correlation.** Good correlation might be the primary premise for an application of the models. The parity plot in Fig. 5 A, therefore, visualises the correlation between phytate conversion of the actual experiments and by model determination. The bisecting line is displayed as a dashed line. Model points of the quartic model (as circles in Fig. 5) and the kinetic modelling (as triangles in Fig. 5) all lie close to the bisectrix and thus show good model correlation with an AAD of 3.8 and 6.9%, respectively. As the model data is also close to each other and no data set stands out, the model results from both approaches seem to be alike as well. Thus, both models can be used for predictions of phytate conversion within the regression range of 140–200 °C and 5–15 min. Only the kinetic approach as a physicochemical model also allows for extrapolation of phytate conversion beyond the investigated time, temperature and concentration range.

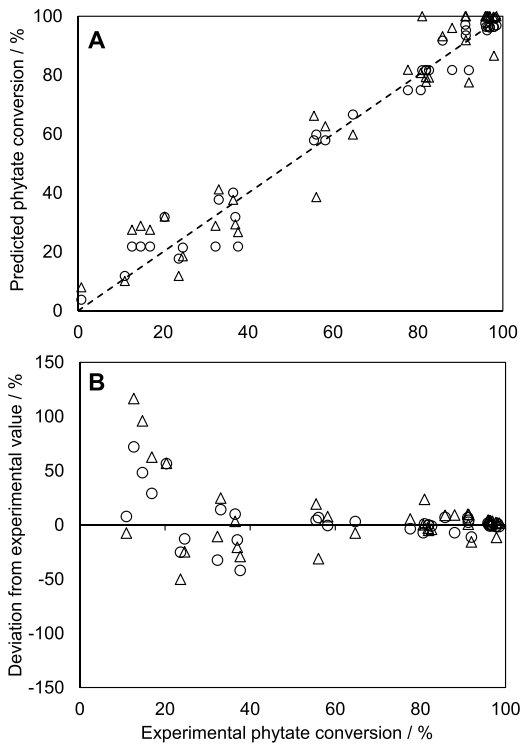


Fig. 5. (A) Parity plot of the quartic (circles) and kinetic model (triangles) with the bisecting line marked and (B) display of relative deviation from experimental values over experimental phytate conversion.

As an example, a kinetic-based prediction of reaction times within the microwave set-up for temperatures of below 180 °C and a target yield of 95% phytate conversion shows that at 120 °C reaction temperature, a holding time of several hours would be theoretically necessary to achieve the targeted yield of 95% phytate cleavage. As shown in Fig. 4, at increasing temperature, significantly lower reaction times are needed, reaching the investigated time range of under 15 min at slightly more than 170 °C.

Fig. 5 B additionally displays the relative deviation between experimental and model data over experimental phytate conversion. A clear trend of elevated deviations in the lower range of phytate conversion for both approaches is obvious. The deviation derives from the fact that similar absolute deviations divided by lower phytate conversion result in higher relative deviations. At higher conversion, thus within the desired reaction range, both models become significantly more precise. Particularly for the kinetic model, the uniform distribution of the data points around the bisecting line at higher conversion indicates that reaction rate does not significantly decrease throughout reaction progress and, thus, that the consideration of one reaction rate for the cleavage of all phosphate groups can sufficiently approximate the real reaction mechanism. This would implicate that all phosphate groups, also from lower inositol phosphates, are equally susceptible for acidic hydrolysis from the inositol ring.

Table 4
Model validation of the quartic and kinetic approach with independent experimental runs.

Reaction temperature/°C	Reaction time/min	Deviation to experimental data/ %	
		Quartic model	Kinetic model
120	5	≫ 100	2.7
120	10	≫ 100	0.75
140	5	59.1	3.4
140	10	5.8	0.1
170	5	5.9	1.8
180	10	8.9	12.2

- **Model validation.** Both models have additionally been validated with experiments that were not included in the according model set-up, but performed in the same way. An extract of some validation experiments being replicates but also some from outside the investigated range is summarized in Table 4. The measurements at 120 °C, which are outside the modelled range, can well be predicted by the kinetic model but, as expected, are not at all reflected within the quartic model as the borders of the data range bear uncertainties. Also, the other shown experiments correlate well to kinetic predictions, however with a little higher deviation towards full conversion where experimental deviations are higher as well. The quartic model predicts results less precisely in general, especially with outliers at the border of the investigated parameters, as reflected at 140 °C and 5 min.

For both modelling approaches, more data, thus additional experiments especially in regions with high measurement uncertainties and thus elevated risk of outliers, could improve the precision and confidence of the model parameters (Kemmer & Keller, 2010). Mathematic approaches can usually be more precise but kinetic modelling is the more generally valid option. Thus, depending on the intention of modelling, each approach can be more suitable for the respective application.

4. Summary and conclusion

In order to overcome losses of valuable phosphorus (P) from feedstuff, the process of acidic phytate hydrolysis in rye bran via thermal treatment was investigated in this work. Thermal treatment is a promising approach to achieve high yields of phosphate liberation from phytate as already shown for model solution in literature. Research in this article focuses on a natural substrate and a faster and/or higher yield application within the microwave than has been reported before. This might lay the basis for further development with the aim of a more efficient application of phytate hydrolysis in the feed-industrial context. Therefore, in this work, phytate was firstly extracted from the rye bran in acidic media. After separation of residual bran and the extract, the extract was treated by microwave-assisted heating to between 140 and 200 °C and for 5–15 min.

- A maximum yield of phytate hydrolysis of 98.5% was achieved, thus indicating that all six phosphate groups were cleaved from the inositol ring. The yield could be significantly increased and reaction time reduced compared to previous publications.
- The statistical evaluation fits a quartic model taking into account both time and temperature with an absolute average deviation (AAD) of 3.8%, thus good prediction quality within the investigated parameter range. The optimum parameter combination depends on individual requirements, but for maximum phytate conversion could be found to be 200 °C and 15 min treatment time.
- Kinetic modelling suggests a reaction order of 0.68, activation energy of 112.7 kJ/mol and an Arrhenius parameter of $9.5 \bullet 10^9$ L/mol·s for the experimental procedure as described. AAD of the kinetic model is 6.9%. As reaction rate doesn't obviously change throughout reaction progress, all six phosphate groups might be equally prone to be liberated. Kinetics here indicate that significant hydrolysis occurs from 140 °C on and reaction time at higher temperature can be well reduced without yield loss or must be significantly increased to achieve high yields at lower temperature.

Summarizing, to the best of our knowledge, a process of acidic

hydrolysis of phytate in rye bran into inorganic P has been described for the first time with almost full conversion. The resulting inorganic P from phytate hydrolysis can be recovered e.g., by precipitation techniques and serve as a substrate for food, feed or fertilizer applications, thus saving valuable mineral P resources. The potential impact of such a measure can only be roughly estimated but might well cover a significant part of up to 80 % of mineral P imports in Europe (Mayer & Kaltschmitt, 2022) thus supporting independency of European agriculture. However, for enhanced applicability of the process alternative reaction media at less corrosive conditions need to be investigated with regard to maximum solubilisation of phytate within the reaction media and subsequent high conversion yields of phytate. By determining an efficient and technically feasible way of phytate hydrolysis, the way for actual recovery of P from cereals can be paved on industrial scale.

CRediT authorship contribution statement

Natalie Mayer: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Martin Peter Dirauf:** Conceptualization, Methodology, Writing – review & editing. **Martin Kaltschmitt:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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