



Comparison of Phosphorus and Phytase Activity Distribution in Wheat, Rye, Barley and Oats and Their Impact on a Potential Phytate Separation

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Abstract

When facing today's scarcity of mineral phosphorus (P) resources and the environmental issues following enhanced P losses especially from agriculture, new solutions need to be implemented. In this framework, the potential for a mechanical separation of a P rich grain fraction from wheat, rye, barley and oats is investigated in order to provide animal feed with reduced organic P content. Thus, P accumulation in manure and soils should be prevented. Also, the subsequent utilization of the separated organic P, which occurs in the form of inositol P, for a sustainable P management via activation of intrinsic enzymes is evaluated. It was shown that in grain layers at 7.0, 5.5, 6.4 and 2.5% cross section of wheat, rye, barley and oats, respectively, maximum inositol P occurs with 1.6, 0.8, 1.4 and 1.2 g/100 g. Phytase activity is also highest in the outer layers of the grains with maxima of 9300, 12,000, 8400 and 2400 U/kg, respectively. A removal of the specific layers where inositol P is accumulated could possibly achieve a 24, 31, 60 and 27% organic P reduction for wheat, rye, barley and oats with 7, 14, 25 or 7% grain elimination. A debranning, eliminating all the outer grain layers to a certain extent, in contrast, leads to significantly higher mass losses. Within the P enriched layer determined from inositol P distribution, phytase activity is calculated to be around 285, 831, 777 and 42 U/kg for wheat, rye, barley and oats, respectively.

Keywords Phytate separation · Grain processing · Phytase activation · Phosphorus recovery

Introduction

On the today's food and feed market, grains contribute to a great part not only as a source of carbohydrates but also as a valuable supplier of proteins, vitamins and minerals (O'Dell et al., 1972; Shewry, 2007). Every year, around $7 \cdot 10^8$ ha of grains are harvested globally, making up a grain mass of $2.6 \cdot 10^9$ t (McGaughey, 2019; Wrigley, 2017). Among them, the most important types are corn, rice and wheat with 40, 31 and 24% of grains' world market, respectively (Raubold, 2018). Other widely processed cereal species are rye, barley, oats, triticale or sorghum (Table 1) (Knight, 2019; Wrigley,

2017). They all belong to the grass family *Poaceae* building a one-seeded fruit, the grain. The grain is the part of the plant being rich in energy (Klingler, 1995). Applications are e.g. in the baking industry, as animal fodder, fermentation to produce alcoholic beverages and — in the course of renewable energy sources — the provision of biofuels. Especially in the EU, around half the mass of all produced grains is used as protein fodder for livestock as local alternative to, for example, soy beans (Raubold, 2018). However, when used as a fodder for non-ruminant animals such as pigs or poultry, complex problems occur regarding the high phosphorus (P) content of cereals (Lott et al., 2011). Though P is an essential nutrient for every kind of life — particularly during growth stage — only specific forms of P can be metabolized in the digestive tract of monogastric animals, among them mainly mineral forms (Ingelmann et al., 2019; Schlemmer et al., 2009; Smil, 2000). In contrast, most of the P in grains is stored in the organic compound phytic acid being hardly digestible by non-ruminants due to the lack of suitable enzymes (Bi et al., 2013; Yamaji et al., 2017). The grain itself contains phytases for internal liberation of inorganic phosphate from phytic

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Table 1 Phosphorus content and phytase activity in different types of cereal grains

Grain type	Total P in g/100 g	Inositol P in g/100 g	Phytase activity in U/kg _{DM} [*]
Wheat^a	0.29–0.35 (King et al., 2017; Humer et al., 2015)	0.23 (Humer et al., 2015)	1700–3000 (Rodehutscheid et al., 2016; Steiner et al., 2007; Viveros et al., 2000)
Wheat bran	1.01–1.16 (King et al., 2017; Humer et al., 2015)	0.88 (Humer et al., 2015)	ca. 10,000 (Steiner et al., 2007)
Rye^b	0.33 (King et al., 2017)	0.20 (Humer et al., 2015)	4000–5000 (Rodehutscheid et al., 2016; Viveros et al., 2000)
Rye bran	0.96 (Humer et al., 2015)	0.73 (Humer et al., 2015)	ca. 9000 (Steiner et al., 2007)
Barley^c	0.25–0.31 (King et al., 2017; Humer et al., 2015)	0.19 (Humer et al., 2015)	960–2000 (Rodehutscheid et al., 2016; Steiner et al., 2007; Viveros et al., 2000)
Barley bran	-	-	-
Oats^d	0.29–0.41 (King et al., 2017; Humer et al., 2015)	0.17 (Humer et al., 2015)	< 100 (Viveros et al., 2000)
Oat bran	0.73–0.83 (King et al., 2017; Humer et al., 2015)	0.68 (Humer et al., 2015)	-

Approx. production volume in Europe (EU-27): ^a116 10⁶ t/a, ^b6 10⁶ t/a, ^c50 10⁶ t/a, ^d7 10⁶ t/a (European Commission, 2021)

*Determination of phytase activity by liberation of inorganic phosphate from 1.5 µM (Viveros) or 100 µM (Steiner, Rodehutscheid) sodium phytate model solution at pH 5.5 (Viveros) or 5.0 (Steiner, Rodehutscheid) and 37 °C (Viveros, Steiner) or 45 °C (Rodehutscheid) in a time span up to 60 min

acid during germination. However, at digestive conditions with very low pH, the grains' native phytases are inactivated (Greiner et al., 1998). As a result, excess P from animal fodder in the form of phytate, the salt form of phytic acid, is excreted by the animal, deployed to soils and then promotes P wash-out to surface waters. Thus, the risk of eutrophication particularly in standing water bodies by high amounts of excess P is strongly increased (Mayer & Kaltschmitt, 2022).

To prevent this series of problems, this article aims to evaluate the potential for a mechanical separation of P in grains. Therefore, P distribution across the grain layers of wheat, rye, barley and oats — as the most important grain types used as animal feed within the EU — is determined both in inorganic and organic form. Based on that, a separation of the P rich grain layers by a stepwise milling process is theoretically evaluated. A closer look is also taken on the distribution of phytase activity throughout the grain layers in order to evaluate measures for a further processing of the P enriched, separated grain fraction via activation of the grains' intrinsic enzymes. The hereby gained P in inorganic form could serve as a sustainable P source for feed, food or fertilizer industry and, thus, substitute mineral P. Phytase activity distribution has already been described quite detailed for wheat in reference to the various hull layers, germ and endosperm (Peers, 1953), however not as a specific weight-related distribution. Phytate content, also only of wheat grains, was determined for 12 different pearling fractions, thus over layers of 4 to 10 wt.-% wheat fractions (Liu et al., 2007). Optical methods reveal the P content in the cross section of wheat grains, however not specified to its chemical bond (de Brier et al., 2016). Therefore, we complement the yet available literature by the detailed P and phytase activity distribution of other grain types with the according graphical illustration and the subsequent evaluation and comparison of a mechanical

phytate separation. The latter is not available in published literature so far.

Background

All grains consist of three biological parts: endosperm, germ and the hull with several different cell layers (Fig. 1). From the milling perspective, all components that are not pure endosperm and therefore do not serve as white flour are summarized as bran and make up around 13 to 25 wt.-% of the whole grain (Prückler et al., 2014; Sramkova, 2009). Especially wheat and rye are debranned during the milling

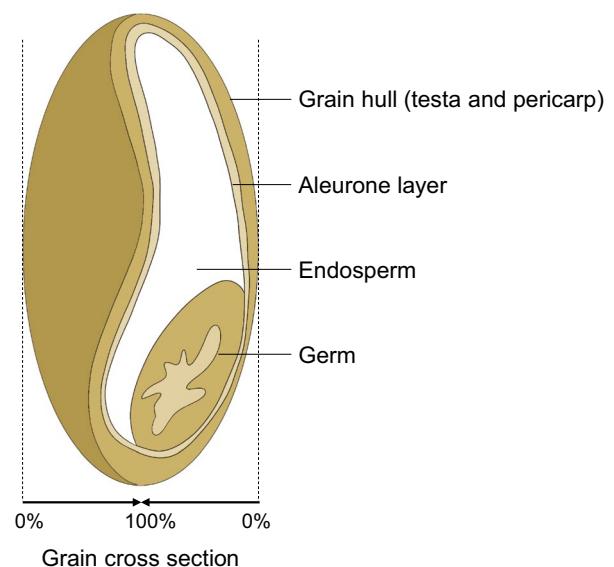


Fig. 1 Grain structure with the main parts: endosperm, germ and the hull

process to supply the starchy endosperm for the baking industry (75 to 87 wt.-%) (Caballero, 2003); bran as a by-product is mostly used for animal feed because of its high amount of proteins and dietary fibre (13 to 25 wt.-%) (Alam et al., 2014; Andersson et al., 2017). Barley is used as whole grains, mostly in animal feeding (55 to 60 wt.-%) and for malting in breweries and whiskey distilleries (30 to 40 wt.-%); only very little barley is directly used for human food (2 to 3 wt.-%) (Gupta et al., 2010; Ullrich, 2011). Also, whole grain oats are mostly fed to livestock (70 wt.-%) or used as breakfast cereal (30 wt.-%) (Webster & Wood, 2011).

The endosperm mainly comprises starch; the residual parts show a significantly higher mineral and fibre content (Awika, 2012; Klingler, 1995). Grains contain 0.22 to 0.70 g P per 100 g dry matter (King et al., 2017) and are therefore a major source of P as essential mineral nutrient. Around 50 to 85% of total P in grains are in the form of phytic acid (inositol hexakisphosphate) and lower inositol P or their respective salt forms (Bi et al., 2013; Steiner et al., 2007; Yamaji et al., 2017). Phytic acid is accumulated in protein globoids in a layer in between the endosperm and the actual hull of the grain, the so-called aleurone layer being part of the cereal bran. This was qualitatively determined by use of toluidin-blue colouring (Bergman et al., 2000) and autoradiography by different research groups (Lott & Spitzer, 1980; Tanaka et al., 1974). With 80 to 88 wt.-%, the P share dominates within the cereal hull. Germ and endosperm contain 6 to 13 wt.-% and 1 to 3 wt.-% of total P amount, respectively (excluding rice and maize with a significantly different P distribution) (Canan et al., 2011; Reddy & Sathe, 2002). Specific values for the P contents of wheat, rye, barley and oats are summarized in Table 1. The distribution of phytase activity within the grain parts shows similar results. Almost 40% of phytase is reported to be located in the aleurone, and the residual part is mostly within the endosperm (Peers, 1953). Native phytase activity in whole grains strongly varies from 80 to 6000 U/kg dry matter (DM) (Rodehutscord et al., 2016; Steiner et al., 2007).

Currently, the industrial milling process, particularly relevant for wheat and rye, is set up to separate the endosperm for milling industry from the bran fraction being used as feedstuff. Thus, the major P content is separated with the bran into animal feed. As P in the inositol form is not used up by monogastric animals, significant environmental issues, such as eutrophication as mentioned before, are the result. Barley is increasingly investigated to be fed hull-less to improve salubriousness of the feed by reduced fibre content, which would also be accompanied by decreased phytate amounts (Caballero, 2003). Industrial approaches for a mechanical P separation within the bran or whole grain meal are not established or published yet. All other investigations on P reduction in grains focus on wet-phase processes by either activation of intrinsic grain enzymes or hydrolysis through external enzymes (Herrmann et al., 2020;

March et al., 1998; Widderich et al., 2022). The treatment of a pre-separated P rich layer can significantly reduce energy costs and the amount of process media needed compared to a direct enzyme treatment of whole grains as less mass is used for the wet-phase process.

Material and Methods

Raw Material

Wheat and rye grains were provided by *Aurora Mühlen GmbH* in Hamburg/Germany containing a common mixture from different cultivation areas in Germany. Grains were superficially cleaned before use as typically applied for the milling process. Barley and oats were purchased in a pre-cleaned state from *demeter/Donath Mühle* as germinable grains. All grains were used as provided without further preparation steps.

Debranning

The grains were debranned to different extents of 1 to ca. 50 wt.-% of initially applied grain mass. The hull was then analysed with regard to P content and phytase activity in the respective grain layer being removed. The P content is determined both in inorganic and organic form to specify the accumulation of the organic P share. For each experimental run, a new batch of whole grains was taken. Parameters for all experimental runs are given in Table 2.

For debranning, a stepwise removal of the outer layers of wheat, rye, barley and oat kernels was performed according to a simplified pearlizing process via friction and abrasion. A debranning apparatus as shown in Fig. 2 was set up by use of a drill machine (a, *Optimum Opti B 16 H*) with grinding disks (b) of grain size 100 made of corundum with negligible P content. Upward mixing of the grains (c) ensured homogeneous grinding. A lid (d) prevented loss of substrate and abrasion. During grinding, temperature did not exceed 40 °C to prevent enzyme deactivation. After the debranning, residual grains were separated from the abrasion by a shaking separation unit (*Fritsch Analysette Pro 3*) with a sieve size of 1 mm. Amplitude was set to 1.5 mm for 4 min. Further analysis was then performed with the abrasion as a part of the grain hull. Whole grain flour as a reference was obtained by grinding whole grains to less than 1 mm particle size in a cutting mill.

Analytical Methods

Total Phosphorus

Samples of 1 g were taken as a triplicate and thoroughly dried at 105 °C for at least 4 h. For analysis of total P content, the samples were completely incinerated according to

Table 2 Overview of the debranning experimental runs

Wheat		Rye		Barley		Oats	
Run no	Abrasion/wt.-%						
W1	1.82	R1	2.82	B1	1.02	O1	1.39
W2	2.92	R2	4.10	B2	1.85	O2	3.47
W3	5.96	R3	6.83	B3	2.07	O3	4.91
W4	8.21	R4	11.84	B4	3.88	O4	6.08
W5	11.26	R5	21.41	B5	5.32	O5	10.19
W6	13.60	R6	38.00	B6	7.49	O6	12.65
W7	27.00	R7	49.48	B7	9.15	O7	20.74
W8	47.93	R _{whole}	100	B8	17.36	O8	37.26
W _{whole}	100			B9	26.98	O _{whole}	100
				B10	33.00		
				B11	47.05		
				B _{whole}	100		

DIN EN ISO 18122 at a temperature of 550 °C and then extracted in 10 mL of 6 M HCl heated to boiling point. The extracts were then analysed colorimetrically by use of the molybdenum blue method according to AOAC method 995.11 (Association of Official Agricultural Chemists, 2000). P content was determined against a calibration with KH₂PO₄ by use of the UV/vis spectrophotometer *Varian Cary 50* at 823 nm.

Inositol Phosphorus

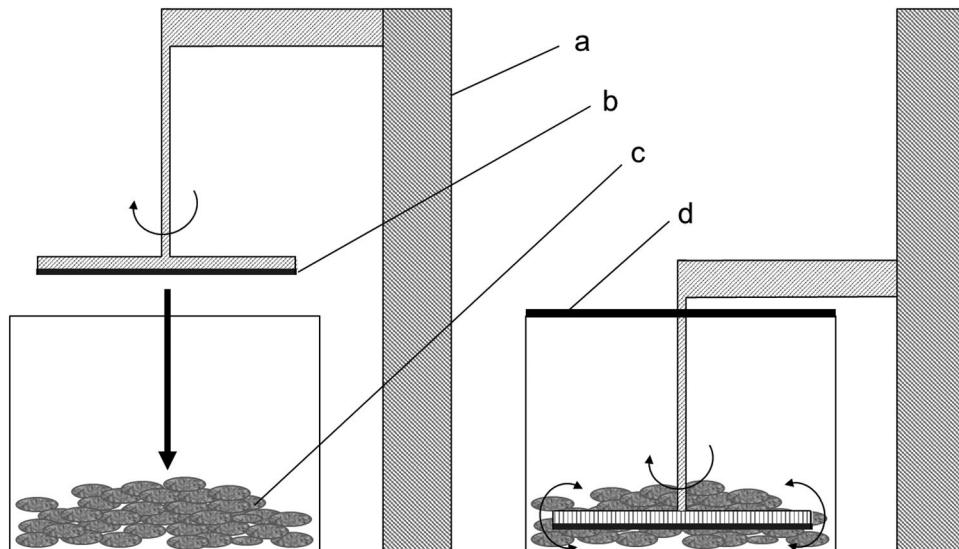
For determination of the phytate P content, dried samples (ca. 0.5 g) were used in a triplicate and extracted in 10 mL 0.66 M HCl overnight. The supernatant was then analysed by use of the Megazyme enzyme kit K-PHYT providing the amount of inorganic P as well as the sum of all inositol Ps

via colorimetric reaction (Megazyme Ltd., 2017). The measurement was performed in the UV/vis spectrophotometer *Varian Cary 50* at 655 nm.

Phytase Activity

Intrinsic phytase activity was determined following a modified protocol of Eeckhout and DePaepe (1994). A total of 0.1 to 0.2 g of samples were incubated in 50 mL preheated 1.5 mM potassium phytate reaction solution at 120 min⁻¹ and 37 °C using a water bath. The pH of the solution was adjusted to 5.5 with concentrated H₂SO₄. During incubation, 2 mL samples were taken after 10 and 70 min and filtered. Immediately after sample collection, 50 µL of the filtrate was mixed with 450 µL of 10% trichloroacetic acid solution and 500 µL of the colour reagent. The reagent consisted of four

Fig. 2 Dehulling set-up made up of **a** a drill machine with **b** a circulating grinding disk over **c** the substrate operated under closure of **d** the lid; filling level, heights and contact pressure manually kept constant for all runs; left: filling mode, right: operation mode



parts acidic ammonium heptamolybdate tetrahydrate solution and one part of iron(II) sulfate heptahydrate solution. After 5 min, the absorbance of the colour complex formed was measured at 700 nm by the UV/vis spectrophotometer *Shimadzu UV-1280* and P concentration was determined against a calibration series using KH_2PO_4 . Intrinsic phytase activity was calculated according to Eq. (1). The *activity* is expressed in units (in $\mu\text{mol}\cdot\text{min}^{-1}$ per kg of biological material as dry matter). P represents the micromoles of orthophosphate liberated by intrinsic enzymes in a time span of 60 min, m is the sample mass (in g) and 60 is the incubation time taking into account P measured in the 10 min incubate as a blank. Small sample volumes and phytase activity can hereby lead to rather high deviations within the measurement.

$$\text{activity} = \frac{P \cdot 1000}{m \cdot 60} \quad (1)$$

Microscopy

Grains were firstly cut by a trim knife in the Ultramicrotome *Leica EM UC7* to reveal the inner cross section of the grain and smooth the surface. All cuts were done within the upper third of the grain (referring to the opposite side of the germ) and cut slices discarded. Then, photos of the cross section were taken by *Leica DFC 495*, 10-times magnified.

Data Analysis

From the analysis after dehulling, accumulated values for the respective parameters are determined because no distinct grain layers were analysed. To derive values for local layers, consecutive measurement points n and $n-1$ were processed following Eqs. (2) and (3).

$$PA_n = \frac{PC_n/100}{m_n} \quad (2)$$

$$PC_{l,n} = \frac{PA_n - PA_{n-1}}{m_n - m_{n-1}} \quad (3)$$

$$s_{n,l} = \sqrt{s_n^2 + s_{n-1}^2} \quad (4)$$

Hereby, the P content PC (given as g/100 g) was firstly transferred to an absolute P amount PA (in g) taking into account the sample mass m . P amounts of the two measurement points were subtracted and then referred to the difference in sample mass to obtain the local P content PC_l . The respective grain layer was defined as the mean abrasion of the measurement points n and $n-1$.

The uncertainty of the values at distinct layers $s_{n,l}$ was determined by error propagation of the single standard deviations s_n and s_{n-1} according to Eq. (4). Display of the results is done as part of the grain cross section (in %) representing the amount of total abrasion as indicated in Fig. 1. The cumulative distribution of P content was achieved through numerical integration of local P contents PC_l by the trapezoidal rule within the investigated range of the cross section. Standardization was performed by referring all values to the P content at maximum cross section of 100%, thus in the middle of the grain. The same integration method was applied to determine cumulative P contents and phytase activity within a certain range of grain cross section from the absolute P and phytase activity distribution, respectively.

Results and Discussion

All results in the following are referred to the grain cross section between 0% at the outermost layers and 100% in the grain centre as visualized in Fig. 1. Grain cross section is hereby defined equally from all sides of the grain towards the centre so that whole grains are represented by 100% grain cross section.

P Distribution in the Grain Layers

Figure 3 shows the quantitative distribution of inositol and inorganic as well as total P in wheat, rye, barley and oat grains plotted above the respective mass-related grain cross section. The trends of the graphs are comparable between all grain types and P forms. The P distribution shows low P amounts in the outermost layers, then increasing to a peak in the cell layers up to 20% and decreasing again towards the P amount in the whole grain. The last data point at 100% cross section is extrapolated from the trend of the graphs. Inositol P makes up around 75 to 80% of total P and therefore shows a distinct peak whereas inorganic P is present in low amounts and thus only slightly elevates in the named range.

The share of organic P among total P in whole grains correlates well to literature (Humer et al., 2015). Total P contents in whole grains were determined to 0.58, 0.48, 0.26 and 0.23 g/100 g for barley, oats, rye and wheat, respectively. Values for barley and oats are slightly above the reported range. The rye P content is marginally lower than the range given in Table 1; however, all values are within natural biological variations.

Due to very low sample mass and measurement uncertainties especially for wheat at 37.5% cross section and oats at 4.2%, inositol P exceeds total P values. As uncertainties of total P analysis are higher than for inositol P, the specific total P contents are therefore excluded from

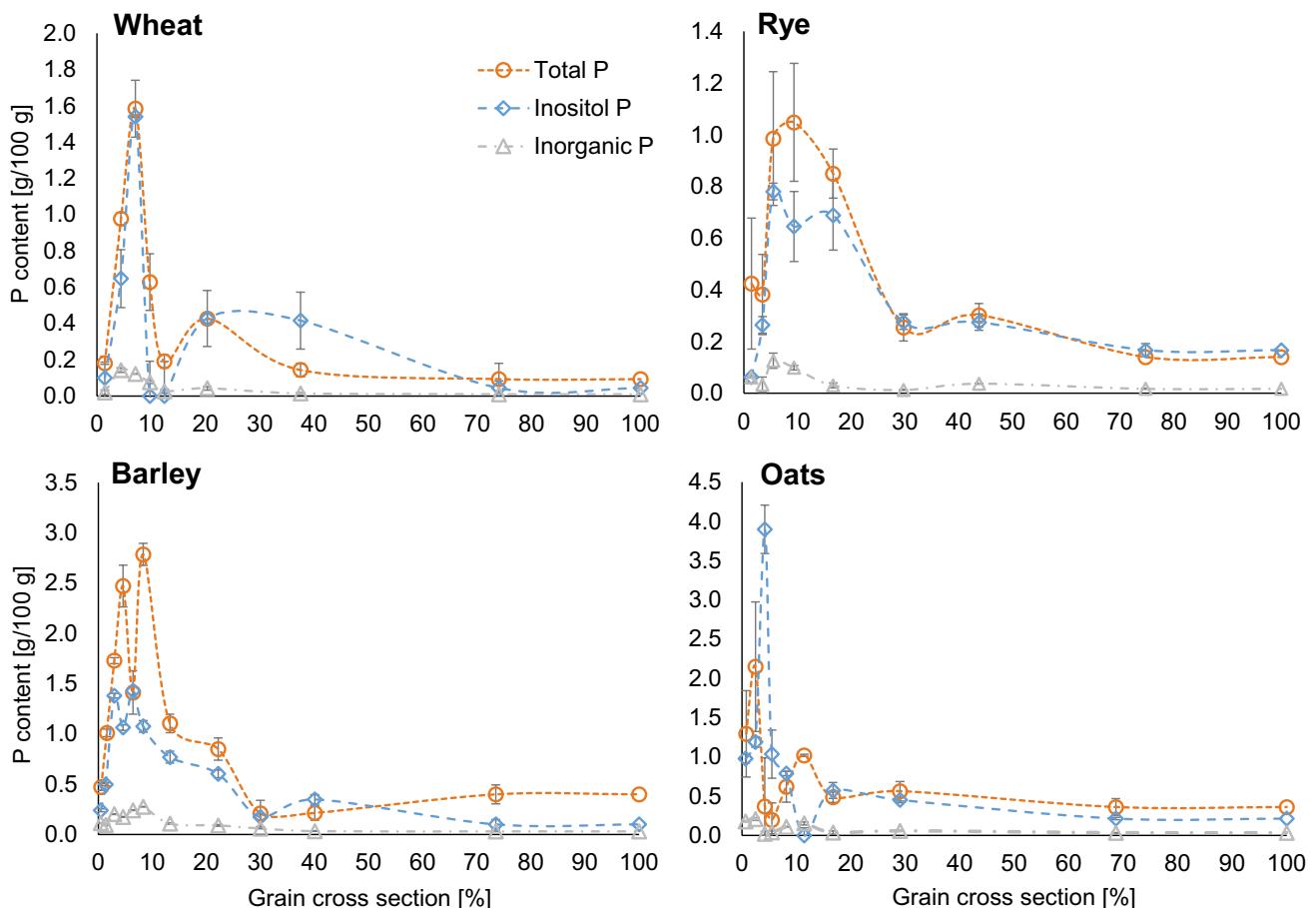


Fig. 3 Distribution of total, inositol and inorganic P over grain cross section of wheat (upper left), rye (upper right), barley (lower left) and oats (lower right)

quantitative comparison. However, the overall trends outlined above remain valid. Comparing the cereal types among each other, differences in P amounts as well as the location and width of the P maxima occur. The direct comparison of inositol P content from wheat, rye, barley and oats is additionally visualized in Fig. 6.

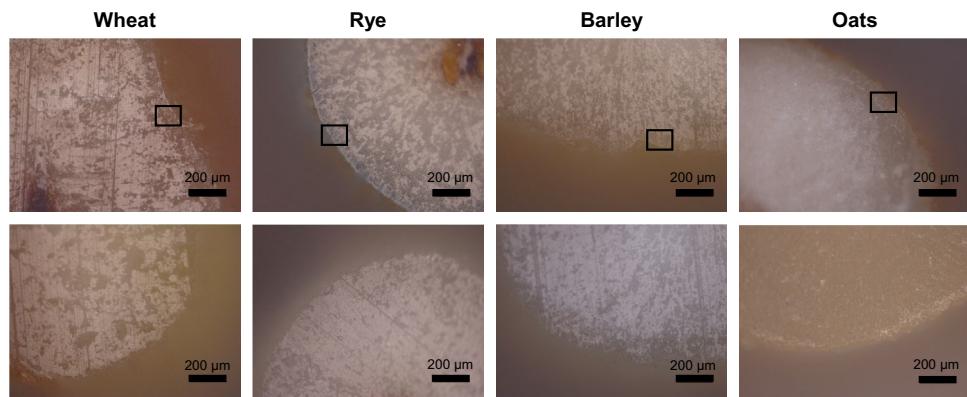
- Barley shows highest total (2.5 to 2.8 g/100 g) and inositol P (ca. 1.4 g/100 g) with a maximum at 5 to 8% cross section.
- Wheat follows with max. 1.5 to 1.6 g/100 g total and inositol P at 7%. Wheat (and oats) show very slim peaks, hence the most specific accumulation of inositol P within the experiments.
- Oats contain 2.1 g/100 g total P and 1.2 g/100 g inositol P at a maximum of 2.5%. So, the outermost peak of total P occurs in oats.
- Rye contains 1.0 and 0.8 g/100 g total and inositol P, respectively. The inositol P maximum for rye lies at

5.5% and the total P maximum at 9.3%. Rye shows the broadest peak among the investigated cereal types.

Thus, a mechanical removal of the grain layers with high inositol P content could lead to P-reduced animal feed with valorization of the separated P rich fraction. A closer look on that potential is taken in the “[Potential for a Mechanical P Separation Within the Grains](#)” section with an according evaluation of applicability for the different grain types.

To determine whether the measured P maxima correlate to the aleurone layer where literature reports a particular accumulation of organic P, microscopic pictures of grain cross sections were taken. Figure 4 displays these cross sections of all grain types in comparison of two different dehulling states. The upper row shows cross sections of the whole grain before dehulling, and the photos below represent cross sections right after removal of the layer with the maximum inositol P according to Fig. 3.

Fig. 4 Ten-times magnified photos of wheat, rye, barley and oat grain cross sections before (upper row) and after (lower row) removal of max. inositol P layer; the black bar indicates a length of 200 µm, and aleurone cells are marked in a square



- In the upper row, all aleurone cells are well visible as a large cell structure between the endosperm and the hull (see marked squares in Fig. 4).
- In the second dehulling state, aleurone is completely removed at the outer edges of the grains; only near the grain crease, where grinding disks could not reach, aleurone cells are still intact. This confirms that inositol P maxima correlate to the removal of the aleurone layer for all examined grain types.

Intrinsic Phytase Activity Distribution in Grain Layers

Phytases are known to be present in grains for a liberation of P from phytate during the germination process (Greiner et al., 1998). However, they can also be externally activated. Phytate degradation in grains can be enforced by treatment under specific conditions such as temperature, pH and water content (Humer & Zebeli, 2015; Lemmens et al., 2018). Now, additionally to the P distribution, also the distribution of intrinsic phytase activity throughout the grain was investigated especially with regard to a further enzymatic hydrolysis of the organic P in the layer with accumulated P amounts.

- As visible from the error bars, measurement uncertainties from triplicates of analysis must be considered in the interpretation of all results. The values should be seen as a base for comparison between the cereal types and the different layers and reflect the actual order of magnitude.
- According to Fig. 5, intrinsic phytase activity is highest in outer layers of the grains with the maximum mostly corresponding to the region of high P content. Phytase activity in oats appears slightly shifted compared to maximum inositol P due to variations in abrasion mass during the grinding process and thus slightly differing measurement points.
- Maximum absolute phytase activity was measured in rye with roughly 12,000 U/kg at 16.6%, followed by wheat (9300 U/kg at 4.4%) and barley (8400 U/kg

at 8.3%). Oats, as indicated in literature (Bartnik & Szafranka, 1987), have lowest phytase activity with a maximum around 2400 U/kg. The values for wheat and rye approximate literature data for corresponding bran fractions (Table 1).

- For rye and oats, additional peaks in phytase activity were measured at 44 and 30% cross section, respectively. Wheat and barley show elevated phytase activity within the whole grain fraction. According to literature, indeed endosperm contains up to a third of total phytase amount, as being the largest mass fraction (Peers, 1953).
- A comparison of the phytase activity among the cereal types is shown in Fig. 6. The graph reveals that the height of phytase activity peaks strongly varies between the four grain types. Phytase activity distribution over the grain layers differs significantly more among the cereal types than the P distribution, and measurement uncertainty is relatively higher.

Exploitation of the native phytase activity for a liberation of inorganic P from phytate in the separated P enriched grain layer will be discussed and evaluated in the “[Potential for a Phytate Reduction Within a P Enriched Grain Layer](#)” section.

Potential for a Mechanical P Separation Within the Grains

An elimination of the organic P from the cereal grains could be obtained by mechanical removal of the grain layer with the highest P content according to the P distribution presented. This can either be achieved by dehulling of the whole outer layers until the P content sufficiently decreased or by a specific exclusion of the grain layers within the distinct peaks in Fig. 3. Both the methods are theoretically evaluated below.

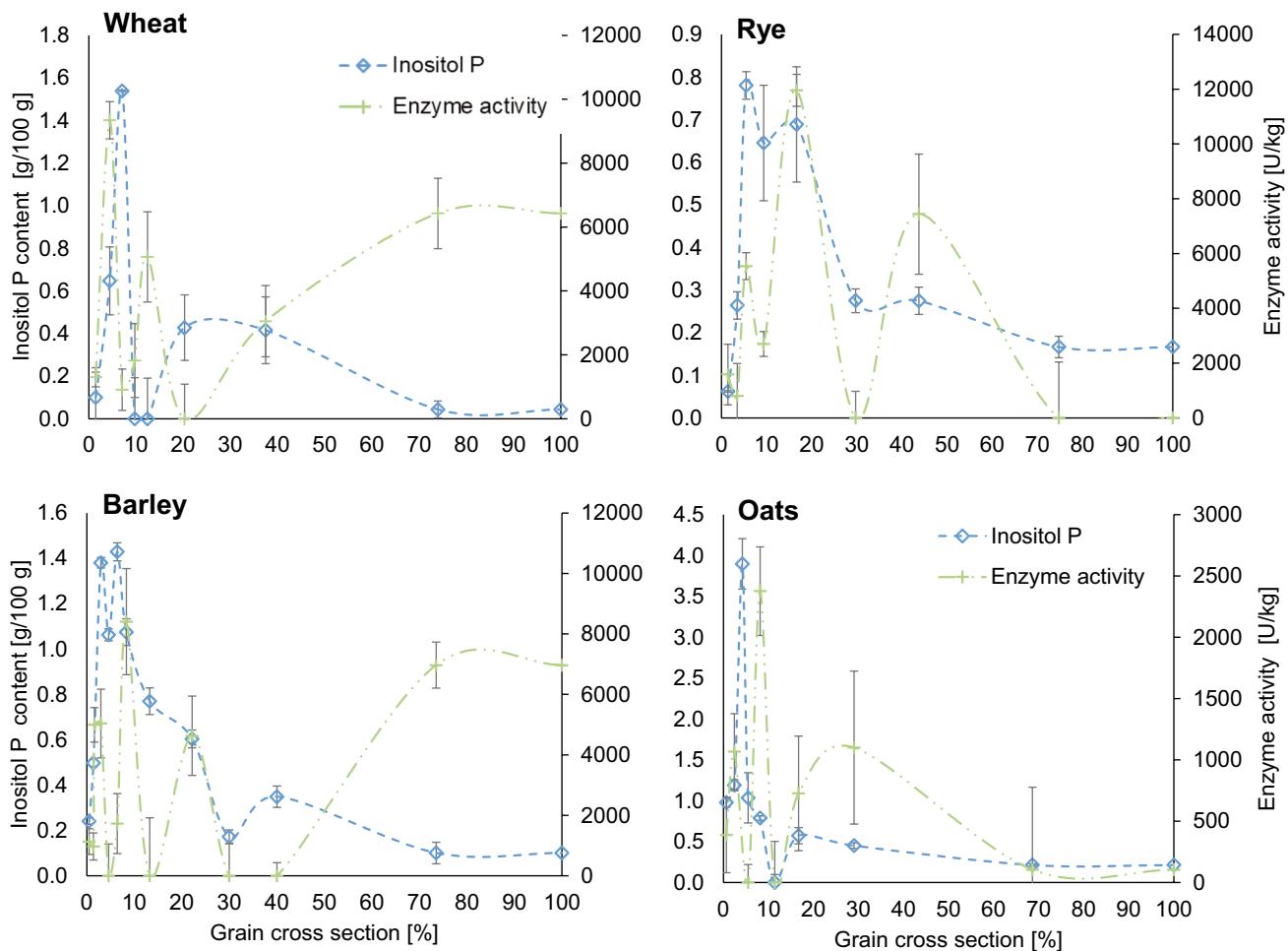


Fig. 5 Intrinsic phytase activity throughout grain cross section of wheat (upper left), rye (upper right), barley (lower left) and oats (lower right) in relation to inositol P content

Removal of the Outer Grain Layers (Debranning)

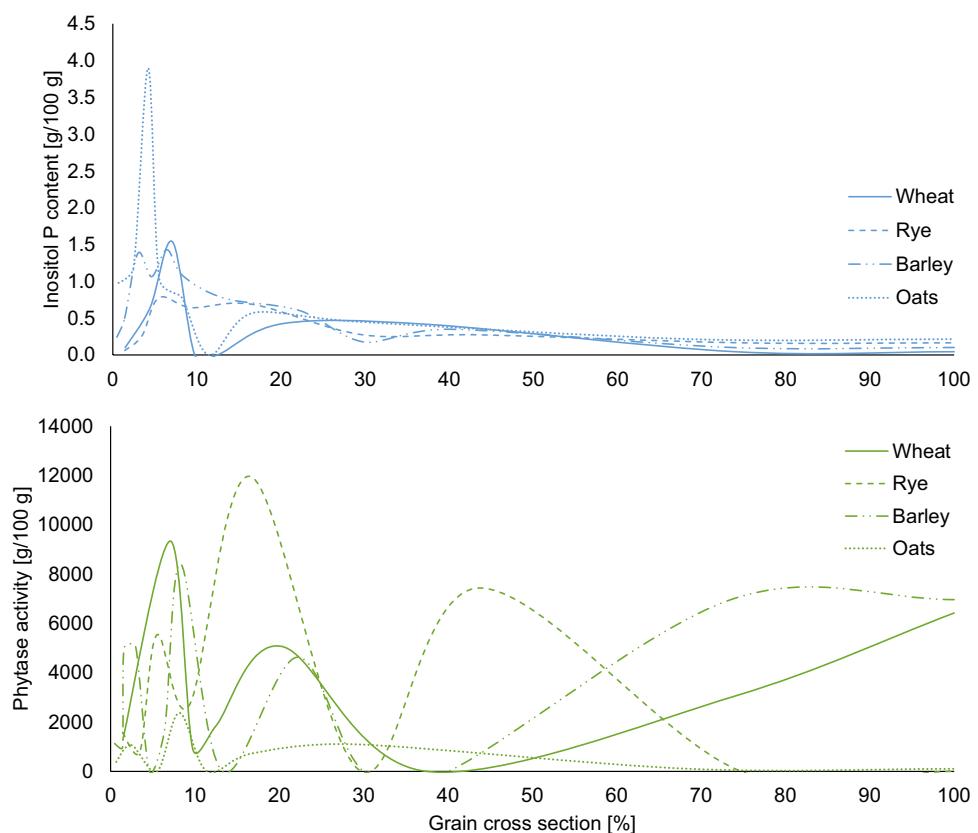
The cumulative inositol P distribution of the investigated grain types (Fig. 7) displays the share of inositol P being separated by the removal of the outer layers until the grain cross section indicated at the abscissa. Thus, the graph directly displays the extent of debranning needed for a certain separation rate of organic P. The percentage of outer grain layers that need to be removed for a separation of 50 and 75 wt.-% of inositol P, respectively, are marked in the graphs in Fig. 7 and additionally noted in Table 3. For a separation of half the inositol P, around 28 to 31% grain cross section of the outer layers of wheat, rye and oats needs to be removed; only barley needs to be dehulled less to an extent of 20% to achieve a halving of the inositol P content. Also, for a separation of three-quarters of the inositol P content, barley needs to be dehulled the least with 47% of the outer layers. All other grain types require around 52 to 60% hull removal

to achieve the 75 wt.-% inositol P separation. As shown, P can over-proportionally be eliminated by debranning as a technically easily feasible process. Thus, a high share of organic P from the grains could be removed, however at high mass losses. This would significantly decrease P emissions by animal excretion, but in parallel considerably reduce the fraction of grains being available for animal feeding. Thus, such a process is not applicable in a sustainable way.

Removal of the P Rich Grain Layer (Specific Layer Exclusion)

A more precise method than debranning to a certain extent is the removal of the specific P rich grain layer, i.e. the separation of the layer with the biggest inositol P peak(s) from Fig. 3. Thereby, a larger grain fraction remains for direct use as animal feed corresponding to a higher share of valuable nutrients from the outer hull being available within the animal feed fraction. Also, considering a further processing

Fig. 6 Comparison of inositol P content (upper graph) and phytate-cleaving enzyme activity (lower graph) of the cereal types wheat, rye, barley and oats



of the P enriched layers, less grain mass would need to be processed for a utilization of the organic P (Andersson et al., 2017).

The amount of cumulated inositol P within the maxima from Fig. 3 was determined by integration within the peak borders. The lower limits for integration were set where at least 25% of the maximum inositol P content (as described in the “P Distribution in the Grain Layers” section) was reached, and the upper limits are defined as the value where still at least 4 times the inositol P content of the last fraction investigated was contained. Values for the integration limits are given in Table 4. The integration led to a theoretically possible inositol P removal within these limits of 24, 31, 60 and 27% for wheat, rye, barley and oats, respectively. As also summarized in Table 4, this P removal is accompanied by a respective 6.7, 14.5, 24.9 and 7.2% elimination of the grain. This amount would not further directly be available as animal feed. Oats showed highest separation rates by a dehulling of all the outer layer, so similar to the debranning, only to a lesser extent. Thus, for oats as well as for barley, the amount of grain mass eliminated via specific layer exclusion is about the same as for the total debranning. For wheat and rye, this mass fraction is significantly lower than shown in the section before. Thus, for wheat and rye, specific layer exclusion is the favourable method in terms of preservation of animal feed mass. However, a layer exclusion would

require higher precision in layer removal, thus more precise instruments for grinding with the particular challenge of handling natural biological variations within the grains.

An alternative method to a layer exclusion by mechanical techniques could be a separation of the aleurone cells by triboelectric separation via electrostatic forces. This process was developed for an aleurone separation from wheat bran. It achieved aleurone concentration in the separated stream of 95%, however only low yield, thus only separated a low share of total aleurone (Brouns et al., 2012; Chen et al., 2014). With the literature-based assumptions that 85% of inositol P is accumulated in the aleurone cells (Caballero, 2003) and around 30% of aleurone can be clearly separated (Cochrane, 1994), the organic P content of the animal feed fraction could decrease by about 25%. Thus, the process is rather unsuitable for an aleurone elimination from the bran. Additionally, the process of triboelectric separation is quite complex and not yet investigated for other substrates than wheat bran (Brouns et al., 2012; Stone & Minifie, 1988).

Potential for a Phytate Reduction Within a P Enriched Grain Layer

Analogous to the method to determine the theoretical accumulation of inositol P within the peaks, the cumulated phytase activity within the P enriched layer was calculated

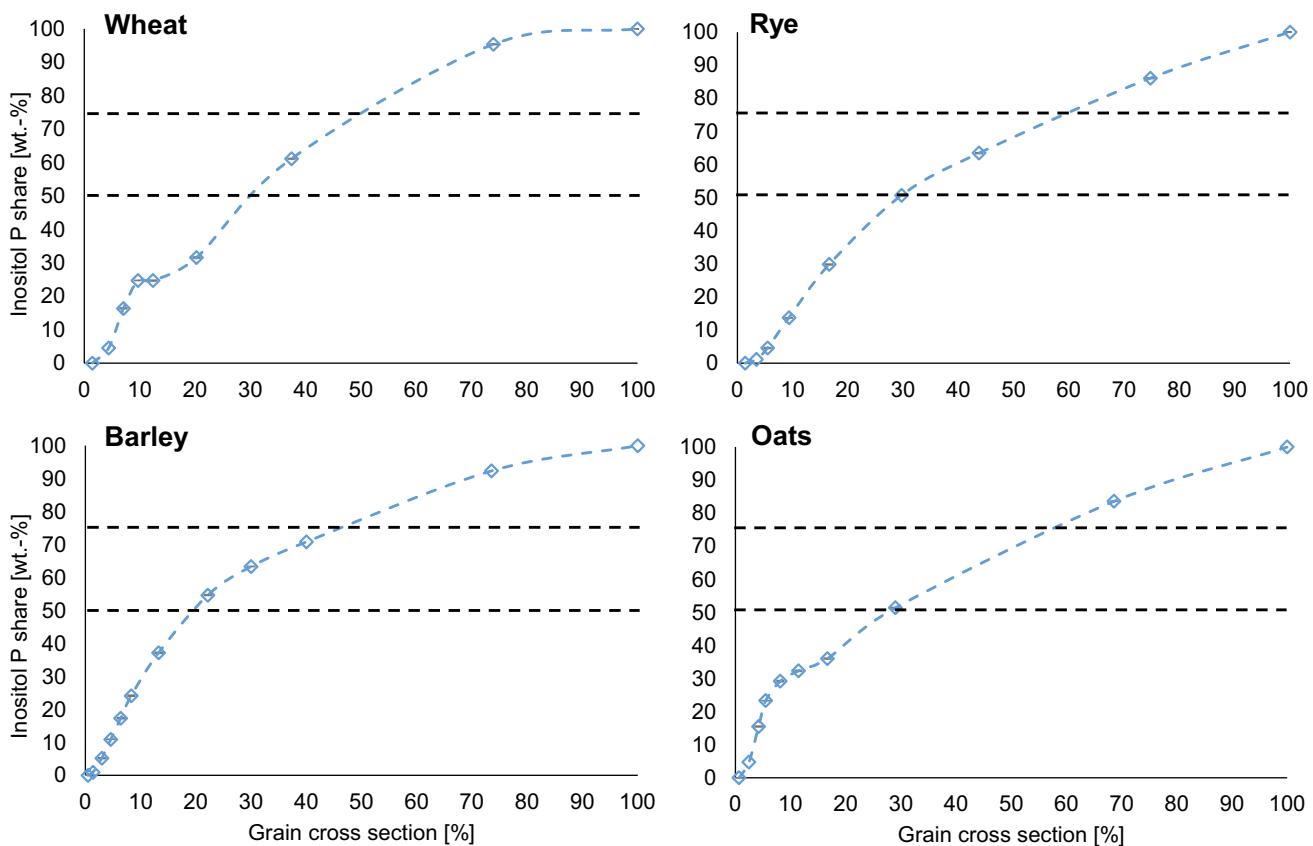


Fig. 7 Cumulative and standardized distribution of inositol phosphate above the grain cross section of wheat (upper left), rye (upper right), barley (lower left) and oats (lower right); 50 and 75% inositol P marked by horizontal lines

Table 3 Extent of debranning of wheat, rye, barley and oat grains for a removal of 50 and 75 wt.-% inositol P content

Inositol P removal	Extent of debranning			
	Wheat	Rye	Barley	Oats
50 wt.-%	31.0%	29.3%	19.8%	27.9%
75 wt.-%	52.2%	59.6%	46.5%	58.0%

by integration. The last row in Table 4 shows the results for the phytase activity within the same integrational limits as for the specific layer exclusion. For rye, the highest phytase activity of 831 U/kg within the theoretical P enriched layer is predicted, followed by barley with 777 U/kg. According

to literature, both activities would be sufficient for an efficient phytate degradation process by enzyme activation. Barley, for instance, has already been successfully treated with a native phytase activity of 600 U/kg and 90% inositol reduction. Germination treatment was performed at 21 °C for 12 days after soaking in water for 4 h (Bouajila et al., 2020). Wheat phytase activity within the integration limits was determined to a significantly lower value of 285 U/kg, but would still have the potential for enzyme activation at optimum conditions. Especially oats' phytase activity, in contrast, is very low within the potentially separated fraction and is thus hardly applicable for efficient enzyme activation. Literature reports enhanced phytate degradation in oats by long germination times, investigated up to more than

Table 4 Integration limits of the grain cross section and resulting inositol P and grain mass removal for specific layer exclusion of grains; cumulated phytase activity of the P enriched grain layer within the limits of cross section

	Wheat	Rye	Barley	Oats
Inositol P at lower integration limit in g/100 g	0.38	0.20	0.35	0.30
Inositol P at upper integration limit in g/100 g	0.16	0.66	0.40	0.86
Inositol P removal in %	23.57	30.89	59.58	27.33
Grain mass removal in wt.-%	6.74	14.48	24.92	7.20
Phytase activity in U/kg	285.27	830.95	777.26	42.37

4 days at 10 to 20 °C (Hübner et al., 2010), and the addition of other grains with high intrinsic phytase activity. Larsson and Sandberg, for instance, achieved 85% phytate reduction in oat flour and increased that value to 93% by addition of sprouted rye and barley flour and germination at 37 °C for 4 h. Samples of 1 g were soaked in 10 mL distilled water for treatment (Larsson & Sandberg, 1992). Thus, conditioning of mixed oats fodder containing grains with high intrinsic phytase activity, e.g. rye, could support phytate degradation by enzyme activation under suitable conditions.

Conclusion

Against the background of depleting natural resources of the valuable nutrient phosphorus (P), various methods of P recovery and savings are investigated in literature, based on different industrial and biological waste and side streams. High losses occur in feeding industry as a major share of feed P is present in the form of phytic acid, which cannot be digested by monogastric animals and is, thus, excreted without further utilization. A mechanical separation of a phytate-rich grain layer from cereal-based feed could firstly separate and thus reduce P content within feedstuff and therefore, secondly enhance efficient P utilization. Specific processing of the P enriched layer, e.g. via phytate-cleaving enzymes before being fed, could liberate significant amounts of the otherwise unused phytate in available inorganic form. The goal of this paper is, therefore, to show the P and phytase activity distribution in grains and, based on that, to evaluate the potential for a mechanical P separation from cereal grains in combination with a subsequent enzyme activation for a liberation of P in inorganic form. Like that, lower P contents in cereal-based animal feed should be achieved and, thus, P accumulation in manure/soils and eutrophication as a resulting problem be prevented. In contrast to a direct enzyme activation of the whole grains, less mass needs to be soaked for enzyme activation and thus energy for subsequent drying and process reactants can be saved.

Major findings can be summarized as follows:

- An investigation on the P distribution showed the quantitative accumulation of inositol P in outer grain fractions at 7.0, 5.5, 6.4 and 2.5% grain cross section for wheat, rye, barley and oats with 0.8 to 1.5 g/100 g organic P. These layers correspond to the aleurone cells as being visible in the microscope at tenfold magnification.
- Phytase activity is also highest in the outer layers. The measurements showed 9300, 12,000, 8400 and 2400 U/kg maximum intrinsic phytase activity in wheat, rye, barley and oats at 4.4, 16.6, 8.3 and 8.1% grain cross

section, respectively. However, measurement uncertainties, partly exceeding common ranges, need to be considered in the absolute values.

- The evaluation of a potential separation of the inositol P rich layer by debranning showed either a removal of around 20 to 30% grain cross section to achieve 50 wt.-% inositol P reduction or 45 to 60% to separate 75 wt.-% of inositol P. By a separation of specific P enriched layers, 25 to 60 wt.-% of inositol P could be removed with a loss of 6 to 25% mass of the grain cross section. Thus, a specific layer exclusion should generally be the preferred technique.
- Especially rye and barley have shown a similarly high native phytase activity of around 800 U/kg within this theoretical separated grain fraction and thus show promising potential for an enzyme activation process. Enzyme treatment at optimum conditions could achieve phytate cleavage and a recovery of phosphate in a usable form for food, feed or fertilizer application.

As a final evaluation, especially barley would be well suited as a substrate for the presented process of mechanical P separation with subsequent enzyme activation as.

- (a) Whole grains of barley are fed, thus grain mass losses are not as significant for feed industry as for wheat and rye,
- (b) Highest inositol P removal was achieved for barley within the defined limits and
- (c) A considerable intrinsic phytase activity can be assumed within the separated P rich grain layer of barley.

The first two points are accordingly valid for oats. So, a mechanical P removal might achieve good results. However, phytase activity might not be sufficient for further processing as stated before. Although the P distribution of rye grains shows a broad inositol P peak, a quite high separation rate of around one-third could be achieved under the conditions given before. Also, phytase activity in the separated rye grain fraction was determined to be rather high. However, as usually only outer hulls of rye are used in feed industry, the removal of 14% grain cross section would make up a considerable part of the animal feed stream. The same accounts for wheat. This makes rye and wheat less suitable substrates for the process evaluated here.

Author Contribution NM: experimental design and procedure, writing of the main manuscript text and preparation of all figures; NW: experimental procedure, revision of the manuscript; MS: experimental design, revision of the manuscript; PB: revision of the manuscript and supervision; MK: structuring and revision of the manuscript, supervision.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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