Myo-inositol phosphates profile and in vitro bioavailability of selected minerals from spelt: Effects of hydrothermal processing and solid-state fermentation with *Rhizopus oligosporus*

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Summary

The *myo*-inositol phosphates profile was analysed in spelt (*Triticum spelta* L.) and in green spelt (immature grains) subjected to thermal processing and solid-state fermentation with *Rhizopus oligosporus*. Raw mature grains were characterized by the highest level of phytate (13.09 g·kg^{-1}), with 41% share of inositol hexakisphosphate. Solid-state fermentation resulted in a decrease in both total phytate content by 10 % on average and the share of higher phosphorylated forms of *myo*-inositol in the profile. Fermentation generated high amounts of lower inositol phosphates, particularly in the case of immature spelt (72% share in the profile). Analysis of the dephosphorylation pattern indicates that the main enzymatic activity in this process could be attributed to 3-phytases from *R. oligosporous*. The fermentation of spelt did not enhance in vitro bioavailability of minerals (Ca, Mg, Fe, Mn, Zn, Cu). The results presented in this paper provide information on the impact of the proposed bioprocessing method on the level and profile of inositol phosphates in spelt grains. The conclusions can be helpful in designing fermented food products on the basis of cereals and pseudo-cereals, as the content of phytate is one of the factors that may limit the nutritional value of food.

Keywords

phytate; wheat; spelt; fermentation; mineral content; bioavailability

Spelt (*Triticum spelta* L.) is an ancient subspecies of common wheat (*T. aestivum* L.), characterized by a higher resistance to environmental factors and good tolerance to poor quality soil. The smaller yields per area and the necessity of dehulling make spelt cultivation more expensive than that of common wheat. On the other hand, spelt can be grown without pesticides in an eco-friendly manner, as a low-input plant. The nutritional composition of spelt grain is similar to common wheat, with a slightly higher level of proteins (up to 15 %), dietary fibre (10.5–15 %), lipids (approximately 4 %) and B group vitamins (approximately 0.6 mg of thiamine and 0.5 mg of riboflavin per 100 g). The proximate composition of spelt grains may vary between cultivars [1, 2].

Apart from mature grains, spelt can also be produced as 'green spelt' (German 'Grünkern'), when collected in the immature form (milk stage of ripening) and artificially dried. Grünkern originates from Southern Germany and has been used in traditional dishes – pastries, soups or sauces [1]. The proximate composition of raw and cooked grains of green spelt, as shown in a recent study of Starzyńska-Janiszewska et al. [3], is generally not very different from that of the mature form, with a slightly lower level of starch (by approximately 11 % in cooked grains), similar amount of total dietary fibre (11 %) and proteins (11.5–12 %), but up to 4-fold higher level of free amino acids.

In spite of the fact that spelt wheat is recently gaining more and more interest as an eco-friendly alternative to common wheat [2, 4], little is known about the level of phytates and profile of inositol phosphates of this cereal. Phytic acid (1,2,3,4,5,6–

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myo–inositol hexakisphosphate, $InsP_6$), being the main storage form of phosphorus in plants, is present in significant amounts in whole grain products, in particular in those made from cereals like wheat and rye [5, 6]. Phytate is scarcely digested in the human intestinal tract. $InsP_6$ and its lower phosphorylated derivative, inositol pentakisphosphate ($InsP_5$), form strong complexes with minerals, in particular divalent metal cations like iron and zinc, which negatively influences the absorption of these elements from the digestive tract [7, 8]. At the same time, staple foods such as wheat, rice and maize are an important source of these minerals in human diet. According to the National Diet and Nutrition Survey Program (NDNS) rolling program [9–11], cereals provide approximately 25 %, 31 % and 39 % of zinc, calcium and iron, respectively, in the United Kingdom consumers' diet. The level of phytate is widely accepted as one of the most important factors limiting bioavailability of these mineral components [9, 12, 13].

Dephosphorylation of phytates can occur, among others, at the stage of acid-thermal treatment of a product and also as a result of enzymatic hydrolysis [13, 14]. The latter concerns the activity of phytases and non-specific acid phosphatases produced by plants, bacteria and filamentous fungi [15–18]. Increasing bioavailability of iron and zinc, deposited mainly in the form of phytate complexes in the aleurone layer of kernels, by means of solid-state fermentation (SSF) of grains with *Rhizopus oligosporus* may be an alternative to fortification of wheat flour or other cereal products with iron and zinc, which were proven so far ineffective [9].

The purpose of the present study was to investigate the effects of cooking and subsequent solid-state fermentation with *R. oligosporus* on the content of phytates and the profile of lower inositol phosphates in immature and mature spelt grains. Another important objective was to provide data on bioavailability of selected mineral elements, which could be potentially affected by these compounds and could depend on their phosphorylation level.

MATERIAL AND METHODS

Rhizopus oligosporus ATCC 64063 was grown on potato dextrose agar (PDA) at 24 °C for 12 days. The spores were harvested with a sterile saline solution (8 $g \cdot l^{-1}$) supplemented with peptone (0.01 $g \cdot l^{-1}$) and Tween 80 (0.1 $ml \cdot l^{-1}$). The suspension was filtered three times through nylon net filters (mesh diameter 11 μm, serial code NY1104700; Millipore, Billerica, Massachusetts, USA) in order to remove mycelium fragments. The spore density was obtained by the spore counting method in a Thoma chamber. Spelt and immature spelt (produced by Natu, Sosnowiec, Poland) were obtained from a health-promoting food store in Krakow, Poland.

Cooking and fermentation procedure

The grains were boiled in distilled water (acidified with lactic acid to pH 4.5–5.0) for 20 min (immature spelt) or 25 min (mature spelt). After draining, they were dried on a surface with a sterile cloth (5 min), cooled (< 30 °C) and aseptically mixed with *R. oligosporus* spore suspension (10^4 spores per gram of dry grains). Then they were packed in sterile Petri dishes (4 replications for each spelt kind) and incubated at 31 °C for 30 h. Next, the material was steamed for 10 min in order to stop the fungal growth. The cooked and fermented grains were lyophilized and stored at 3 °C for a maximum of one week until analysed.

In vitro bioavailability determination

The in vitro bioavailability of mineral elements and *myo*-inositol phosphates was estimated according to the method described by Żyła et al. [19] modified in order to simulate human stomach and small intestine conditions. A sample of 0.5 g of the material was incubated with 1.7 mg of pepsin (E.C. 3.4.23.1, declared activity 4 750 $U \cdot mg^{-1}$; Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in 0.1 $mol \cdot l^{-1}$ HCl, at 37 °C, pH 2 for 2 h. Next, 2.5 mg of pancreatin (from porcine pancreas, 8×, United States Pharmacopeia; Sigma-Aldrich) dissolved in 0.1 $mol \cdot l^{-1}$ $NaHCO_3$ was added. The mixture was transferred to dialysis tubes (cellulose membrane, 25 mm × 90 mm, molecular weight cut off 12 000 Da) and incubated for 4 h at 37 °C in flasks containing 50 ml of imidazole buffer, pH 7.0. The dialysates obtained were used for high performance liquid chromatography (HPLC) analysis.

The term "in vitro bioavailability" was defined as the ratio between the level of compounds in the dialysate, which passed the pore barrier of the dialysis membrane and were found in the buffer solution after simulated in vitro digestion, and their total level in the material expressed in percent (w/w). The contents of metals in dialysates from the in vitro procedure were determined by atomic absorption spectrometry with the flame atomization technique described below in subsection Determination of metal ions.

Total phytate and inositol phosphates determination

Extraction of inositol phosphates from samples was conducted according to GAMBUŚ et al. [20]. A 2 g sample of the material was extracted by 20 ml of 0.5 mol·l^{-1} hydrochloric acid at 22 °C for 2 h. Extracts were centrifuged for 30 min at 2000 ×*g* and filtered. Low pressure ion exchange liquid chromatography was performed to separate *myo*-inositol phosphates from the extract. Chromatography columns were filled with 2 g of analytical grade, 8% crosslinked anion-exchanger (37–74 μm, chloride form; Bio-Rad, Hercules, California, USA) and conditioned with 10 ml of deionized water. Next, 15 ml of the extract was passed through the column and the column was rinsed with 10 ml of deionized water. Elution of *myo*-inositol phosphates was performed by 20 ml of 2 mol·l^{-1} hydrochloric acid. Eluates were evaporated in a water bath at 40 °C and re-dissolved in 5 ml of deionized water, frozen at –18 °C and stored for a maximum of one week until analysed by HPLC. The phytate analysed from these samples, expressed per 1 kg of dry matter (DM), was defined as "total phytate".

HPLC analysis

The profile of the isomers of *myo*-inositol phosphates was analysed by an analytical system using high pressure anion exchange chromatography (HPAEC) with post-column derivatization and spectrophotometric detection [6]. Before injection to a chromatographic column, samples were filtered through a syringe nylon filter (pore size 0.45 μm). A reference sample for identification of peaks was prepared by dissolving 2.3 g of sodium phytate in 50 ml of deionized water and adjusting pH to 4.0 by 2 mol·l^{-1} HCl. The solution was autoclaved for 40 min at 121 °C under pressure of 101.3 kPa. The elution sequence of individual isomers was established according to the work of BLAABJERG et al. [6] by using appropriate standard solutions, namely, sodium phytate – $InsP_6$, inositol-(1,2,4,5,6)-pentakisphosphate – $Ins(1,2,4,5,6)P_5$, inositol-(1,4,5,6)-tetrakisphosphate – $Ins(1,4,5,6)P_4$, inositol-(1,3,4,5)-tetrakisphosphate – $Ins(1,3,4,5)P_4$, inositol-(1,4,5)-trisphosphate – $Ins(1,4,5)P_3$, inositol-(1,3,4)-trisphosphate – $Ins(1,3,4)P_3$ and *myo*-inosistol 2-monophosphate (all from Sigma-Aldrich).

Determination of metal ions

The contents of metals in the samples and the dialysates from the in vitro procedure were determined by atomic absorption spectrometry with the flame atomization technique using AAvarian4289 (Agilent, Santa Clara, California, USA). Before analysis, the samples were subjected to a process of wet mineralization, with the addition of 4 ml of concentrated HNO_3 (Suprapur; Merck, Darmstadt, Germany) in sealed pressure vessels, using a microwave oven Mars Xpress (CEM, Matthews, North Carolina, USA) at 1200 W, 170 °C for 15 min. For assessment of calcium and magnesium contents, a buffer solution according to Schinkel (Merck) was applied in the amount of 10 ml per 50 ml. As part of the quality control of the method, Certified Reference Materials NCS ZC 73009 (China National Analysis Center for Iron and Steel, Beijing, China) were tested.

Statistical analysis

Experimental data were subjected to two-way analysis of variance (ANOVA) and expressed as mean ± standard deviation. Tukey' post-hoc test was applied ($p \leq 0.05$) to determine statistically significant differences. Data were processed using statistical software Statistica for Windows, ver. 13.1 (Statsoft; Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Phytate and inositol phosphate profile

The level of total phytate measured in raw grains of mature spelt (13.1 g·kg^{-1} DM, Tab. 1) was higher than values given by CENTENO et al. [21] for spring and winter wheat (7.5–8 g·kg^{-1}). On the contrary, immature spelt contained by approximately 40 % less phytate (4.33 g·kg^{-1} DM) than mature spelt or common wheat. This could be explained by the fact that maturing of grains is connected with mobilization of phosphorous, which is mainly stored in the mineral-$InsP_6$ complex. Boiling of grains did not significantly change the $InsP_6$ level, irrespective of the spelt kind (Tab. 1, factor: treatment kind). Subsequent fermentation slightly reduced total phytate in spelt grains, by 10 % on average, which contributed to the increase in the lower inositol phosphate and phosphorus fractions (Tab. 1).

The analysis of inositol phosphate profile showed that inositol hexakisphosphate was the main fraction representing 30–41% of total phytate in raw grains (Tab. 1). This is consistent with data presented by DENSTADLI et al. [22]. However, in the case of our study a much higher share of inositol di- and monophosphates ($InsP_{2\text{-}1}$) fraction was found, perhaps due to the use of a different analytical technique. Comparing the obtained data with the results presented by CENTENO et al. [21] for common wheat, where the

Tab. 1. Profile of inositol phosphates in spelt grains subjected to solid-state fermentation with *Rhizopus oligosporus*.

Sample		Total $InsP_6$ [g·kg^{-1}]	Relative peak area [%]				
			$InsP_6$	$InsP_5$	$InsP_4$	$InsP_3$	$InsP_{2-1}$
Grain kind x Treatment kind (interaction between factors)							
Spelt mature	Raw	13.09 ± 1.38^{c}	41.23	7.01	12.04	4.46	35.25
	Boiled	13.47 ± 1.18^{c}	28.10	16.49	11.81	6.59	37.04
	Fermented	11.69 ± 0.62^{b}	27.83	11.87	9.00	7.67	43.62
Spelt immature	Raw	4.33 ± 0.24^{a}	30.16	3.07	3.32	1.27	60.59
	Boiled	4.97 ± 0.20^{a}	23.86	2.41	5.18	1.62	66.94
	Fermented	4.05 ± 0.62^{a}	11.20	6.06	6.39	4.52	71.84
Treatment kind (factor 1)							
Raw		8.71 ± 4.67^{B}					
Boiled		9.22 ± 4.51^{B}					
Fermented		7.87 ± 4.01^{A}					
Grain kind (factor 2)							
Mature		$12.75 \pm 1.30^{\beta}$					
Immature		$4.45 \pm 0.44^{\alpha}$					

Two-way analysis of variance and Tukey's post-hoc test were applied to total InsP6 data. Values in columns are mean ± standard deviation ($n = 6$). Values with different superscripts within column are significantly different ($p < 0.05$).
InsP6 – inositol hexakisphosphate, InsP5 – inositol pentakisphosphate, InsP4 – inositol tetrakisphosphate, InsP3 – inositol triphosphate, InsP2-1 – inositol di- and monophosphates.

9 : 1 $InsP_6/InsP_5$ ratio was noted, it can be observed that spelt grains were characterized by a relatively diverse spectrum of inositol phosphates, i.a. inositol tertakisphosphates ($InsP_4$, 12 %), and lower fractions inositol di- and monophosphates ($InsP_{1-2}$, 35 %), without such a strong prevalence of the highest phosphorylated form. It is worth mentioning that the analytical method used by Centeno et al. [21], with the reversed-phase high-performance liquid chromatography (RP-HPLC) ion pair technique coupled to the refractive index (*RI*) detection, does not allow to estimate the isomers of the lower inositol phosphate (< inositol trisphosphates, $InsP_3$). The fermented spelt grains analysed in the present study had a relatively high share of lower inositol phosphates with two-to-one phosphate moieties ($InsP_{2-1}$), which amounted to 44 % in the case of spelt and as much as 72 % in immature spelt (Tab. 1).

The analysis of the phytate degradation pattern in processed grains indicated a higher share of intermediate products, namely, Ins(1,2,4,5,6)P_5, Ins(1,2,5,6)P_4 and Ins(1,4,5)P_3, as compared to the profile obtained from raw grains (Tab. 2). According to Nakano et al. [23], after the initial $InsP_6$ hydrolysis, the dephosphorylation pathway divides into two main routes. The pattern observed in the case of the present study is typical for fungal phytases, primarily removing orthophosphate from the D-3 position of *myo*-inositol ring, which are considered to be 3-phytases (EC 3.1.3.8). A relatively higher share of lower fractions in the inositol phosphate profile of fermented immature spelt (60–71 %), as compared to the mature grains (35–43 %, Tab. 1) was correlated with higher in vitro bioavailability (1.6–3.2 %) of *myo*-inositol in immature spelt (unpublished data). This may indicate simultaneous action of plant and microbiological phytases, as well as non-specific phosphatases, which accelerate hydrolysis not only towards monophosphates, but also to free *myo*-inositol. The activity of these enzymes in the thermally processed material could still remain significant. According to the findings of Hatzack et al. [24], 8–12 % activity of *Aspergillus fumigatus* phytases was retained after 20 min boiling of wheat flour. The differences in the phytate degradation pattern observed for hydrolysates from fermented immature and mature spelt could be not so much a result of specificity, as the main isomers are similar, but rather an activity of endogenic plant enzymes at particular stages of grain development. Such differences in the inositol phosphate profile were previously found, among others, in winter and spring wheat cultivars [21], and also in barley and its wild varieties, in the latter case amounting to 25 % [25].

Bioavailability of minerals

The average levels of minerals Ca (272 mg·kg^{-1}), Mg (1.245 g·kg^{-1}) and Mn (37.33 mg·kg^{-1}) (Tab. 3) in spelt were generally

not very different from values determined by CHOI et al. [26] in Korean wheat cultivars – Ca (313–463 mg·kg^{-1}), Mg (2.862–4.165 g·kg^{-1}) and Mn (16.5–44.8 mg·kg^{-1}). Similar data were also reported for various wheat cultivars by AKHTER et al. [27] – 251–535 mg·kg^{-1} Ca and 34.1–55.5 mg·kg^{-1} Fe. The possible discrepancies in the minerals level in grains can result from numerous factors, starting from genetic and phenotypic variation within individual varieties, agrochemical treatments, vegetation season and also analytical equipment used for testing [9]. Especially the level of zinc was relatively high compared to other studies [28, 29] and higher than that of Fe. However, as reported by SRINIVASA et al. [29], genetic diversity of spelt varieties is so wide that these differences could spread to almost 50 %.

The in vitro bioavailability of Ca determined for spelt grains was within the range of 24–43 %, while that of Mg was 44–67 %. The respective values determined for microminerals Fe, Mn, Cu and Zn were 49–63 %, 12–18 %, 72–89 % and 54–78 %, respectively (Tab. 4). With regard to the aforementioned parameters, in the paper by LEMMENS et. al. [7] concerning germinated wheat grains subjected to hydrothermal treatment, comparable bioavailability of Fe (36 %) but significantly lower bioavailability of Zn (24 %) were reported. Similarly, CHAWLA et al. [32] noted relatively low bioavailability of zinc (23–30 %) for pea seeds fermented with *Aspergillus oryzae* strain. In the study of VIGNOLA et al. [30] concerning wheat pasta, slightly lower (33–49 %) bioavailability of Mg was reported, as compared to values obtained in the present paper for fermented spelt grains (54 %) (Tab. 4).

The applied processing, boiling and fermentation differently influenced the in vitro bioavailability of minerals (Tab. 4). No significant differences were observed for Ca and microminerals Cu and Mn, regardless of the processing method. The fermentation of boiled spelt with *R. oligosporus* significantly decreased the in vitro bioavailability of Mg, Fe and Zn, on average by 6 %, 10 % and 13 %, respectively. Other studies recommended fermentation as an important factor affecting bioavailability of minerals in wheat [9, 31]. The positive effect of the said treatment on bioavailability of zinc and iron ions from pea seeds (Black pea), estimated with Caco–2 cultures, was also noted [32].

Phytate is considered an effective chelator of metal ions, hence, its decomposition can enhance the bioavailability of minerals in plant substrates. For example, bioavailability tests using a simulated gastrointestinal tract carried out by AKHTER et al. [27] for calcium, iron, zinc and copper ions in wheat flours dephytated with exogenous enzymes showed that bioavailability of these minerals increased from 16.3 % to 31.9 % in Sehar-2006 variety and from 14.9 % to 25.0 % for V–07096 variety. However, the level of phytate reduction in the cited study, in the range of 35.3–69.3 %, was much higher than values reported in our ex-

Tab. 2. Conformation of main inositol phosphates in spelt grains subjected to solid-state fermentation with *Rhizopus oligosporus*.

Sample		$InsP_5$	$InsP_4$	$InsP_3$
Spelt mature	Raw	I(1,2,3,4,6)P_5** I(1,2,4,5,6)P_5*** I(1,3,4,5,6)P_5*	I(1,2,4,5)P_4* I(1,4,5,6)P_4* I(1,2,3,4)P_4*	I(2,4,5)P_3* I(1,4,5)P_3* I(1,4,6)P_3*
	Boiled	I(1,2,3,4,6)P_5** I(1,2,4,5,6)P_5*** I(1,2,3,4,6)P_5*** I(1,3,4,5,6)P_5*	I(1,2,4,5)P_4* I(1,4,5,6)P_4** I(1,2,3,4)P_4* I(1,2,4,6)P_4*	I(4,5,6)P_3* I(1,5,6)P_3*
	Fermented	I(1,2,4,5,6)P_5*** I(1,2,3,4,6)P_5** I(1,3,4,5,6)P_5*	I(1,2,4,5)P_4**; I(1,4,5,6)P_4***; I(1,2,3,4)P_4*	I(4,5,6)P_3** I(1,5,6)P_3* I(1,4,5)P_3**
Spelt immature	Raw	I(1,2,3,4,6)P_5** IP_5*** n.i.	I(1,2,4,6)P_4*; I(1,4,5,6)P_4*;	I(2,4,5)P_3* I(1,4,5)P_3* I(4,5,6)P_3*
	Boiled	I(1,2,3,4,6)P_5* I(1,3,4,5,6)P_5**	I(1,2,4,6)P_4* I(1,4,5,6)P_4**	I(2,4,5)P_3* I(1,4,5)P_3* I(4,5,6)P_3*
	Fermented	I(1,2,3,4,6)P_5** I(1,2,3,4,6)P_5*	I(1,2,5,6)P_4** I(1,4,5,6)P_4** I(1,2,4,6)P_4*	I(1,4,5)P_3** I(4,5,6)P_3** I(1,5,6)P_3*

$InsP_5$ – inositol pentakisphosphate, $InsP_4$ – inositol tetrakisphosphate, $InsP_3$ – inositol triphosphate.
* – low share, ** – medium share, *** – high share, n.i. – conformation not identified.

Tab. 3. Total content of minerals in spelt grains subjected to solid-state fermentation with *Rhizopus oligosporus*.

Sample		Content [g·kg^{-1}]					
		Mg	Ca	Fe	Zn	Cu	Mn
Grain kind x Treatment kind (interaction between factors)							
Spelt mature	Raw	1 115.33 ± 4.54^{a}	298.08 ± 8.28^{a}	25.30 ± 0.23^{a}	39.55 ± 0.27^{d}	4.87 ± 0.10	23.23 ± 0.16^{a}
	Boiled	1 064.81 ± 18.20^{a}	275.11 ± 8.50^{bc}	35.93 ± 0.00^{d}	41.71 ± 0.09^{e}	5.06 ± 0.10	40.86 ± 0.02^{c}
	Fermented	1 360.18 ± 25.39^{d}	284.92 ± 0.44^{c}	30.74 ± 0.66^{b}	36.21 ± 0.47^{b}	4.67 ± 0.06	38.69 ± 0.19^{b}
Spelt immature	Raw	1 490.14 ± 9.63^{e}	340.86 ± 15.03^{d}	33.03 ± 0.70^{c}	32.34 ± 0.57^{a}	4.67 ± 0.06	39.53 ± 0.26^{b}
	Boiled	1 180.04 ± 6.38^{b}	290.72 ± 3.96^{c}	30.62 ± 0.37^{b}	36.39 ± 0.16^{b}	4.65 ± 0.01	42.06 ± 0.06^{d}
	Fermented	1 260.90 ± 10.31^{c}	247.37 ± 4.39^{b}	31.86 ± 0.11^{bc}	37.74 ± 0.02^{c}	5.09 ± 0.32	39.61 ± 0.53^{b}
Treatment kind (factor 1)							
Raw		1 302.74 ± 7.18^{A}	269.47 ± 83.03	29.16 ± 4.48^{A}	35.95 ± 4.18^{A}	4.77 ± 0.13	31.38 ± 9.41^{A}
Boiled		1 122.43 ± 67.46^{B}	282.92 ± 10.51	33.27 ± 3.07^{C}	39.05 ± 3.08^{C}	4.86 ± 0.24	41.46 ± 0.69^{C}
Fermented		1 310.54 ± 59.46^{A}	266.14 ± 21.83	31.30 ± 0.75^{B}	36.98 ± 0.93^{B}	4.88 ± 0.30	39.15 ± 0.62^{B}
Grain kind (factor 2)							
Mature		1 180.11 ± 142.01$^{\alpha}$	272.84 ± 42.87$^{\alpha}$	30.66 ± 4.77$^{\alpha}$	39.16 ± 2.49$^{\beta}$	4.87 ± 0.19	34.26 ± 8.60$^{\alpha}$
Immature		1 310.36 ± 144.04$^{\beta}$	292.98 ± 42,47$^{\beta}$	31.84 ± 1.14$^{\beta}$	35.49 ± 2.53$^{\alpha}$	4.80 ± 0.26	40.40 ± 1.32$^{\beta}$

Two-way analysis of variance and Tukey post-hoc test were applied. Values in columns are mean ± standard deviation (n = 3). Values with different superscripts within columns are significantly different ($p < 0.05$).

Tab. 4. Bioavailability of minerals in spelt grains subjected to solid-state fermentation with *Rhizopus oligosporus*.

Sample		Bioavailability [%]					
		Mg	Ca	Fe	Zn	Cu	Mn
Grain kind x Treatment kind (interaction between factors)							
Spelt mature	Raw	54.64 ± 0.40^{c}	42.50 ± 4.40^{c}	61.44 ± 2.92^{b}	57.29 ± 2.76^{ab}	73.23 ± 3.66	18.08 ± 1.80
	Boiled	67.42 ± 0.66^{e}	36.06 ± 0.76^{bc}	49.10 ± 0.41^{a}	77.89 ± 2.49^{c}	82.29 ± 5.06	12.98 ± 0.17
	Fermented	51.25 ± 0.56^{b}	31.97 ± 0.08^{ab}	50.24 ± 3.01^{a}	69.87 ± 0.14^{abc}	72.62 ± 3.66	12.63 ± 2.56
Spelt immature	Raw	44.52 ± 0.59^{a}	24.18 ± 0.24^{a}	63.09 ± 1.58^{b}	75.61 ± 1.13^{c}	89.64 ± 1.84	11.68 ± 2.18
	Boiled	61.30 ± 0.86^{d}	30.07 ± 0.03^{ab}	63.77 ± 2.62^{b}	72.74 ± 8.32^{bc}	84.00 ± 1.36	12.85 ± 1.57
	Fermented	56.49 ± 0.35^{c}	32.38 ± 1.93^{b}	50.75 ± 0.29^{a}	54.63 ± 4.58^{a}	83.46 ± 8.25	12.97 ± 1.70
Treatment kind (factor 1)							
Raw		49.58 ± 5.86^{A}	33.34 ± 10.88	62.27 ± 2.14^{C}	66.45 ± 10.71^{AB}	81.43 ± 9.77	14.88 ± 4.04
Boiled		64.36 ± 3.59^{C}	33.06 ± 3.49	56.43 ± 8.61^{B}	75.31 ± 5.83^{B}	83.14 ± 3.18	12.92 ± 0.92
Fermented		53.87 ± 3.05^{B}	32.18 ± 1.14	50.50 ± 1.77^{A}	62.25 ± 9.18^{A}	78.04 ± 7.88	12.80 ± 1.78
Grain kind (factor 2)							
Mature		57.77 ± 7.64$^{\beta}$	36.84 ± 5.15$^{\beta}$	53.59 ± 6.38$^{\alpha}$	68.35 ± 9.43	76.04 ± 5.60$^{\alpha}$	14.56 ± 3.07
Immature		54.10 ± 7.75$^{\alpha}$	28.87 ± 3.88$^{\alpha}$	59.20 ± 6.70$^{\beta}$	67.66 ± 11.04	85.70 ± 4.90$^{\beta}$	12.51 ± 1.56

Two-way analysis of variance and Tukey's post-hoc test were applied. Values in columns are means ± standard deviation of the sample (n = 3). Values with different superscripts within columns are significantly different ($p < 0.05$).

periment. It can be concluded that the degree of reduction in phytate in fermented spelt grains (10 %; Tab. 1) was probably insufficient to significantly influence the estimated in vitro bioavailability of minerals. It is also worth mentioning that bioavailability of minerals in phytase-treated cereal grains can be limited due to the interaction with these exogenous enzymes, which form high molecular weight complexes with minerals. Gabaza et al. [33] reported an increase in the total level of soluble zinc in maize and sorghum grains treated with a combination of phytase, laccase and tannase, from 20.2–59.4 % to 29.5–67.6%, as well as of iron – from 23.9–65.5 % to 48.7–87.3%. Despite these promising data, bioavailability of Zn and Fe ions, i.e. the ratio between the pool of minerals able to pass through the dialysis membrane (molecular weight cut-off of 12–14 kDa) and their total level in spelt grains, did not change.

CONCLUSIONS

Boiling of spelt grains and subsequent fermentation with *R. oligosporus* resulted in the reduction of phytate, by 10 %, and a change in the inositol phosphate profile in favour of lower phosphates. However, the observed partial dephosphorylation of the material did not cause improvement of in vitro bioavailability of macrominerals (Ca and Mg) and microminerals (Fe, Cu, Zn and Mn).

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