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# Solid-State Fermentation Reduces Phytic Acid Level, Improves the Profile of *Myo*-Inositol Phosphates and Enhances the Availability of Selected Minerals in Flaxseed Oil Cake

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**Summary**

Flaxseed oil cake was subjected to fermentation with *Rhizopus oligosporus* (DSM 1964 and ATCC 64063), and the phytate ($InsP_6$) content, *myo*-inositol phosphate profile and *in vitro* bioavailability of essential minerals were studied. Flaxseed oil cake had a phytate mass fraction of 13.9 mg/g. A 96-hour fermentation of flaxseed oil cake by *R. oligosporus* DSM 1964 and *R. oligosporus* ATCC 64063 decreased the $InsP_6$ content by 48 and 33 %, respectively. The strains had different phytate-degrading activities: fermentation of flaxseed oil cake with *R. oligosporus* DSM 1964 was more advantageous, yielding $InsP_{3-5}$ as a predominating *myo*-inositol compound, while fermentation with *R. oligosporus* ATCC 64603 produced predominantly $InsP_{5-6}$. Solid-state fermentation of flaxseed oil cake enhanced *in vitro* bioavailability of calcium by 14, magnesium by 3.3 and phosphorus by 2–4 %.

*Key words*: flaxseed oil cake, solid-state fermentation, phytates, *myo*-inositol phosphates, mineral availability

## Introduction

Flaxseed oil cake is the solid remaining after pressing the flaxseeds to extract the oil. It is often used as a feed component due to its high nutritional value (*1*). However, flaxseed oil cake, especially after cold pressing, is high in soluble fibre, high-quality protein, plant lignans, minerals and polyunsaturated fatty acids and may offer benefits when used as a food additive (*2*). Protein isolates obtained from flaxseed oil cake have functional value (*3*) and pro-health and therapeutic properties (*4*). Flaxseed oil cake has also been used as a component of a food product obtained through solid-state fermentation (SSF) (*5*), as well as a valuable component of dough in baking bread (*6,7*).

Despite these advantages, flaxseed by-products also contain antinutrients. Depending on the variety and growth conditions of plants, flaxseed meal contains 2.3–3.3 % of phytic acid (*myo*-inositol-(1,2,3,4,5,6)-hexakisphosphate) (*8*). Phytates are the main storage forms of phosphorus in plants. A highly charged phytic acid molecule electrostatically binds minerals and proteins in complexes, decreasing their bioavailability (*9,10*). Phytate dephosphorylation may be performed by means of thermal hydrolysis, *e.g.*

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during cooking, or be the result of the action of phytases. Phytases are produced endogenously in plant seeds during soaking and sprouting (*11*), and are also secreted by various bacteria (*12*) and filamentous fungi (*13*). Feed supplementation with phytases is a commonly used practice in the poultry nutrition and can also be applied during dough processing in wholemeal bread manufacturing (*14,15*).

*Rhizopus oligosporus* has already been proven to reduce the phytate level during SSF of plant substrates (*16*). *Rhizopus* sp. are used in the production of tempeh, an Indonesian fermented food with interesting organoleptic characteristics and a high nutritional value. This kind of processing is an old and well-known treatment of soybeans, other legumes and legume-grain mixtures. It can also be applied as an alternative method for the utilisation of plant by-products. Our previous research showed that the application of *R. oligosporus* DSM 1964 and ATCC 64063 cultures in the processing of flaxseed oil cake may result in products with enhanced soluble phenolic content and increased antioxidant activity, suitable as functional food additives (*17*).

The aim of these investigations is to study the effects of fermentation of flaxseed oil cake with *R. oligosporus* DSM 1964 and ATCC 64063 strains on the content of phytates, profile of lower inositol phosphates, many of which are known for their active metabolic functions, and on the availability of essential minerals, assessed using multistep digestion *in vitro* procedure.

## Material and Methods

The substrate for the fermentation was a cold-pressed flaxseed oil cake, supplied by the Przedsiębiorstwo Nasienne Centrala Nasienna in Sanok (Poland).

### *Inoculum*

The spores of *Rhizopus oligosporus* DSM 1964 and ATCC 64063 strains (twelve-day cultures on potato extract agar slants) were harvested with sterile 0.85 % NaCl supplemented with 0.01 g/L of peptone and 0.1 mL/L of Tween 80. After filtration ($d$=11 μm, Nylon Net Filtres; Merck Millipore, Cork, Ireland), the spore density in the suspension was measured using Thoma chamber under an optical microscope (model NK4; PZO Sp. z o.o., Warsaw, Poland).

### *Fermentation of flaxseed oil cake*

Flaxseed oil cake (45 % moisture content, acidified to pH=4–5 with 5 %, by mass per volume, lactic acid) was autoclaved at 121 °C for 20 min (ELMI ESS-207; SMS Sp. z o.o., Warsaw, Poland). After sterilisation, the flaxseed oil cake was cooled to 30 °C, mixed with *R. oligosporus* inoculum ($3 \cdot 10^6$ spores added to 100 g of raw flaxseed oil cake) and packed into Petri dishes ($d$=11 cm, three replications in each fermentation stage). Inoculated material was incubated at 37 °C for 2 h (spore germination induction) followed by incubation at 30 °C. The plates were removed after 48 and 96 h of fermentation. The fermentation was stopped by steaming (10 min). The lyophilised products were stored at 4 °C for later analysis. A sample of flaxseed oil cake that was prepared for fermentation, but was not inoculated, was also kept for analysis.

### *In vitro digesting procedure*

The bioavailability of selected compounds was estimated by an *in vitro* method. Flaxseed oil cake samples prepared for inoculation and after 48 and 96 h of fermentation were treated as described by Stodolak *et al.* (*18*). Briefly, 0.5 g of material was mixed with 1.7 mg of pepsin (4750 U/mg; Sigma-Aldrich, Steinheim, Germany) dissolved in 0.1 mol/L of HCl and incubated at 37 °C, pH=2.0, for 2 h. Next, 2.5 mg of pancreatin (from porcine pancreas, 8×USP (United States Pharmacopeia); Sigma-Aldrich, St. Louis, MO, USA) and 31 mg of bile extract porcine (Sigma-Aldrich, St. Louis) dissolved in 0.1 mol/L of $NaHCO_3$ were added. The sample was put into a dialysis tube (cellulose membrane 25 mm×16 mm; Sigma-Aldrich, St. Louis) and incubated at 37 °C for 4 h in 50 mL of imidazole buffer (pH=7.0). The dialysates were collected and the levels of phytate, inositol phosphates, phosphorus, *myo*-inositol and minerals were determined (as described below). The amounts of phytate and minerals released into the dialysate divided by their total content expressed in percentages were used as a bioavailability indicator.

### *Determination of metal ions*

The contents of metals in the samples of fermented flaxseed oil cake and the dialysates from the *in vitro* procedure were determined by atomic absorption spectrometry with the flame atomisation technique (Varian AA 240FS; Agilent Technologies, Santa Clara, CA, USA), using an automatic dispensing sample system (SIPS–20; Agilent). The flows of gas (acetylene) and air were 3.5 and 14 L/min, respectively. Before analysis, the samples were subjected to a process of wet mineralisation, with the addition of 4 mL of concentrated $HNO_3$ in sealed pressure vessels using a microwave oven Mars Xpress (1200 W, 170 °C, 15 min; CEM Corp., Matthews, NC, USA). The elements were determined using a single sample aspiration *via* a rapid sequence mode (called fast sequential). Standard solutions of $Mg^{2+}$ (100 mg/L) and $Ca^{2+}$ (40 mg/L) were prepared from 1000 mg/L of stock solutions (Merck, Bilerica, MA, USA).

### *Phosphorus determination*

The Fiske-Subbarow method (*19*) was used to determine phosphorus content on dry mass basis (mg/g) in dialysates and in raw samples (total content) previously mineralised in the Hach Digesdahl® digestion aparatus at 280 °C (Hach Company, Loveland, CO, USA).

### *Determination of inositol phosphates*

Inositol phosphates were extracted from samples according to Duliński *et al.* (*20*). The profiles of the *myo*-inositol phosphates were determined by the analytical system using high-performance anion-exchange chromatography (HPAEC) with postcolumn derivatisation and UV/Vis detection (*21*). A reference sample was prepared by dissolving 2.3 g of sodium phytate in deionised water (50 mL) and adjusting the pH to 4.0 with 2 M HCl. Next, the solution was autoclaved for 40 min at 121 °C under 101 kPa

(autoclave ELMI ESS-207; SMS Sp. z o.o.). The elution sequence of $InsP_{6-2}$ isomers was established according to the work of Blaabjerg *et al.* (*21*) mentioned above, using appropriate standard solution, sodium phytate ($InsP_6$), Ins(1,2,4,5,6)$P_5$, Ins(1,4,5,6)$P_4$, Ins(1,3,4,5)$P_4$, Ins(1,4,5)$P_3$, Ins(1,3,4)$P_3$, and *myo*-inositol 2-monophosphate (all purchased from Sigma-Aldrich, Steinheim).

### *Phytate analysis*

Ion chromatography system (Dionex UltiMate 3000) coupled with a ED50a electrochemical detector and conductivity cell (Dionex, Sunnyvale, CA, USA) was used for the analysis. Briefly, samples extracted according to Duliński *et al.* (*20*) were separated on Omnipac Pax-100 anion exchange column (250 mm×4 mm i.d.) connected in series with Omnipac Pax-100 (8 mm×1 mm) guard column (Dionex). A mobile phase using a mixture of 200 mM sodium hydroxide (A), deionised water (B), and water-isopropanol (50:50, by volume) (C) was applied. An anion micromembrane suppressor AMMS 300 4-mm (Dionex) system was used to suppress the mobile phase conductivity before entering the conductivity cell (regenerant 0.25 M sulfuric acid) according to Dionex Application Note 65 (*22*).

### *Myo-inositol analysis*

The concentration of total and free *myo*-inositol in samples and in dialysates was measured by HPLC assay according to Duliński *et al.* (*20*), using Rezex™ RCM $Ca^{2+}$ column (375 mm×4 mm i.d., Phenomenex, Torrance, CA, USA).

### *Statistical analysis*

Experimental data were subjected to the one-way analysis of variance (ANOVA) to detect significant differences among mean values and expressed as a mean value±standard deviation (S.D.). Differences among mean values were checked by the Tukey's test at $p<0.05$ using Statistica for Windows, v. 12.5 (StatSoft Inc., Tulsa, OK, USA) statistical software.

## Results and Discussion

### *Profiles of phytate and inositol phosphates*

Phytate and inositol phosphates were analysed in flaxseed oil cake after 48 and 96 h of SSF with two different strains of *R. oligosporus*. The total phytate content on dry mass basis in flaxseed oil cake prepared for the inoculation was 13.9 mg/g (Table 1). *R. oligosporus* DSM 1964 decreased the phytate level to 7.8 mg/g in 48 h (a 44 % reduction). After 96 h, the phytate content was reduced to 7.2 mg/g, but this decrease was not statistically significant. *R. oligosporus* ATCC 64063, on the other hand, decreased the phytate level to 9.3 mg/g after 96 h, yielding 35 % reduction. The decrease in phytate levels was similar to the 32–42 % decrease reported during the fermentation of sorghum grains with lactic acid bacteria (*23*), but lower than the 74–89 % decrease reported during the fermentation of oat and barley grains with *R. oligosporus* (*24*).

The intermediate products of phytate degradation during the fermentation of flaxseed oil cake were: Ins(1,2,-4,5,6)$P_5$, with lower amounts of Ins(1,2,3,4,5)$P_5$, Ins(1,2,3,-4,6)$P_5$, Ins(1,4,5,6)$P_4$ and Ins(1,2,4,5)$P_4$ (B and C in Fig. 1). The profiles indicate that the phytases of *R. oligosporus* initially removed D-3 phosphate residue from the *myo*-inositol ring, suggesting that they were 3-phytases (EC 3.1.3.8). However, the two *R. oligosporus* strains differed significantly in phytase activity as proven by the differences in the magnitude of $InsP_6$ degradation and by the spectrum of lower inositol phosphates formed. *R. oligosporus* DSM 1964 produced more $InsP_3$ isomers, mainly Ins(1,2,6)/(1,4,5)$P_3$ and Ins(2,4,6)$P_3$ than *R. oligosporus* ATCC 64603 did (B and C in Fig. 1). The fermentation of flaxseed oil cake with *R. oligosporus* DSM 1964 produced a more advantageous profile of inositol phosphates in the products, with predominance of inositol phosphates having 3–5 phosphate moieties after 96 h, with levels of these isomers being reasonably similar (Table 1). In the case of *R. oligosporus* ATCC 64603 fermentation, $InsP_5$ (18–22 %) and $InsP_{1-2}$ (17–28 %) were the dominant inositol phosphates found in the product.

Inositol triphosphates and particularly those with conserved 1,2,3 or 2,4,5 conformation of phosphate moieties are known for their antioxidant and immunostimulating effects (*25*). The observed changes in the profiles of inositol phosphates could therefore be applied in value-added food products based on flaxseed oil cake, since the mixtures of phytate with lower inositol phosphates have proven anticancerogenic properties (*25*).

### *Mineral content*

The amount of magnesium in the initial flaxseed oil cake varied from 3.30 to 3.53 mg/g; these values are comparable to those reported previously of 4.91–5.85 mg/g (*6*) and 5.8–6 mg/g (*26*). Fermentation with both *R. oligosporus*

Table 1. Total phytate content, profile of residual lower inositol phosphates and total *myo*-inositol mass fraction in flaxseed oil cake subjected to solid-state fermentation with *Rhizopus oligosporus* DSM 1964 and ATCC 64063 strains

| Sample | *t*(fermentation) | *m*(phytate $InsP_6$) | Phytate reduction | Average relative peak area/% | | | | | *m*(*myo*-inositol $InsP_0$) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $InsP_6$ | $InsP_5$ | $InsP_4$ | $InsP_3$ | $InsP_{2-1}$ | |
| | h | mg/g | % | | | | | | mg/g |
| Flaxseed oil cake | 0 | (13.9±1.9)$^a$ | 0 | 74.5 | 17.2 | 1.3 | 1.1 | 5.9 | (6.98±0.04) |
| DSM 1964 | 48 | (7.8±0.2)$^c$ | –44 | 42.6 | 22.1 | 13.3 | 11.0 | 11.0 | (6.96±0.01) |
| | 96 | (7.2±0.5)$^c$ | –48 | 37.8 | 17.1 | 12.0 | 18.7 | 13.7 | (6.97±0.07) |
| ATCC 64063 | 48 | (9.1±0.1)$^b$ | –35 | 53.7 | 22.1 | 3.4 | 4.1 | 16.7 | (6.88±0.05) |
| | 96 | (9.3±0.5)$^b$ | –33 | 48.6 | 18.8 | 3.0 | 1.9 | 27.7 | (6.84±0.04) |

Values in columns are mean±S.D. of the sample ($N$=3), values with different superscripts within columns are significantly different ($p<0.05$)

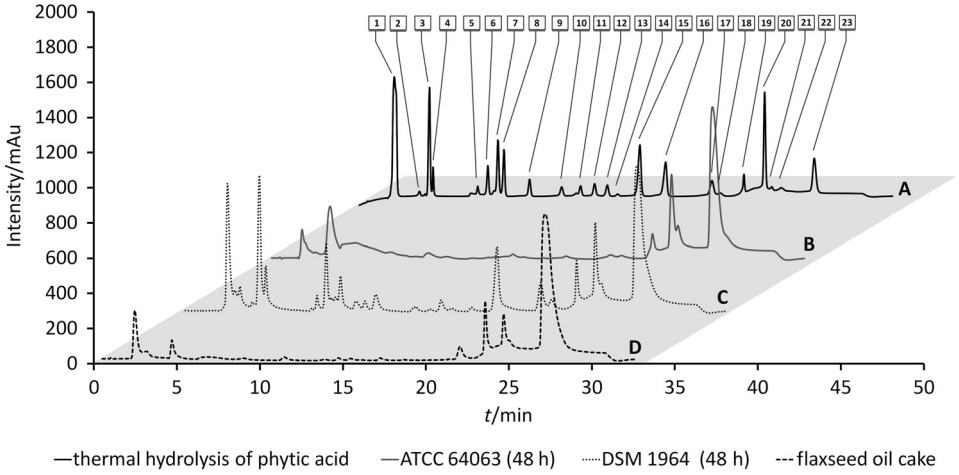


**Fig. 1.** Overlaid chromatograms of *myo*-inositol phosphate analysis in raw and fermented flaxseed oil cake after 48 h of fermentation. *Myo*-inositol phosphate in-house reference standard acquired after thermal hydrolysis of phytic acid (A), flaxseed oil cake fermented by *R. oligosporus* ATCC 64063 (B) and by *R. oligosporus* DSM 1964 (C), and raw flaxseed oil cake (D). Peaks: 1=inorganic phosphate, 2–4=$InsP_{1\text{-}2}$, 5=$Ins(1,3,5)P_3$, 6=$Ins(2,4,6)P_3$, 7=$Ins(1,2,6)P_3$, 8=$Ins(x,y,z)P_3$, 9=DL-$Ins(1,5,6)P_3$, 10=DL-$Ins(4,5,6)P_3$, 11=DL-$Ins(1,2,4,6)P_4$, 12=DL-$Ins(1,2,-\ 3,4)P_4$, 13=DL-$Ins(1,2,4,5)P_4$, 14=$InsP_4$, 15=DL-$Ins(1,2,5,6)P_4$, 16=$Ins(2,4,5,6)P_4$, 17=$Ins(1,4,5,6)P_4$, 18=$Ins(1,2,\ 3,4,6)P_5$, 19=$Ins(1,2,3,4,5)P_5$, 20=DL-$Ins(1,2,4,5,6)P_5$, 21=$Ins(v,w,x,y,z)P_5$, 22=$Ins(1,3,4,5,6)P_5$ and 23=$Ins(1,2,3,4,5,6)P_6$

strains caused a slight increase (7 % in 96 h) of the mass fraction of Mg (Table 2) but did not influence significantly the contents of Ca and P. The mass fraction of P in all tested samples was much higher (34–36 mg/g) than previously reported (6.43–8.24 mg/g) (*6*).

Table 2. The mass fractions of minerals in flaxseed oil cake before and after solid-state fermentation with *Rhizopus oligosporus* DSM 1964 and ATCC 64063 strains

| Sample | *t*(fermentation) | *w*/(mg/g) | | |
|---|---|---|---|---|
| | h | Ca | Mg | P |
| Flaxseed oil cake | 0 | (1.01±0.07) | $(3.30 \pm 0.07)^a$ | (35.8 ±2.4) |
| DSM 1964 | 48 | (0.96±0.04) | $(3.53 \pm 0.08)^b$ | (34.0 ±1.5) |
| | 96 | (1.02±0.07) | $(3.55 \pm 0.04)^b$ | (36.1 ±2.5) |
| ATCC 64063 | 48 | (1.01±0.03) | $(3.45 \pm 0.11)^{ab}$ | (36.0 ±1.2) |
| | 96 | (1.01±0.07) | $(3.55 \pm 0.04)^b$ | (35.9 ±2.6) |
| Mean value | | (1.00±0.06) | (3.47 ±0.07) | (35.5 ±2.0) |

Values in columns are mean±S.D. of the sample (*N*=3), values with different superscripts within columns are significantly different ($p<0.05$)

## *Inositol phosphates in the dialysates after in vitro digestions*

There was a high correlation between the phytate content in the flaxseed oil cake (unfermented and fermented) and the $InsP_6$ level detected in dialysates obtained from the *in vitro* digestion (R=0.91). The availability of phytate in the product of the 48–hour fermentation with *R. oligosporus* was higher (33 and 34 %) than that of the control sample (28 %) (Table 3). However, it was lower than the values reported for cooked buckwheat groats (39 %) and buckwheat tempeh (69–62 %) (*16*) as well as for rye bread (50 %) (*14*). The increase of the phytate bioavailability in fermented samples could result from the metabolic activity of the mould that loosened plant tissues, which, in turn, facilitated the release (leaching) of this low-molecular compound during the *in vitro* digestion.

Irrespective of the sample, *in vitro* digestions resulted in an increase in the percentage of $InsP_3$ (31–37 %) in the dialysates as compared to the control (26 %) and a de-

Table 3. Effects of solid-state fermentation of flaxseed oil cake with *Rhizopus oligosporus* DSM 1964 and ATCC 64063 strains and *in vitro* simulated gastrointestinal digestion on phytate bioavailability and the profile of residual lower inositol phosphates in dialysates

| Sample | *t*(fermentation) | *w*(phytate) | Phytate availability | Average relative peak area/% | | | | | *m*(*myo*-inositol $InsP_0$) |
|---|---|---|---|---|---|---|---|---|---|
| | h | mg/g | % | $InsP_6$ | $InsP_5$ | $InsP_4$ | $InsP_3$ | $InsP_{2\text{-}1}$ | mg/g |
| Flaxseed oil cake | 0 | $(3.9\pm0.4)^a$ | 28 | 45.4 | 16.6 | 10.7 | 25.8 | 1.5 | n.d. |
| DSM 1964 | 48 | $(2.6\pm0.4)^b$ | 34 | 30.1 | 18.8 | 13.3 | 32.9 | 9.6 | n.d. |
| | 96 | $(2.5\pm0.1)^b$ | 35 | 13.7 | 12.4 | 8.6 | 36.5 | 28.8 | n.d. |
| ATCC 64063 | 48 | $(3.0\pm0.2)^c$ | 33 | 29.8 | 26.9 | 7.0 | 35.1 | 2.2 | n.d. |
| | 96 | $(3.5\pm0.1)^a$ | 37 | 30.8 | 27.2 | 7.6 | 30.8 | 5.2 | n.d. |

Values in columns are mean±S.D. of the sample (*N*=3), values with different superscripts within columns are significantly different ($p<0.05$)

crease in the percentage of $InsP_{5-6}$. Moreover, in the dialysate obtained from the 96-hour culture of *R. oligosporus* DSM 1964, the percentage of the $InsP_{1-2}$ fraction was the highest (29 %), while that of the $InsP_{6-5}$ fraction was the lowest (26 %).

Changes in the profiles of inositol phosphates observed after *in vitro* digestion could result from the residual activity of plant and mould phytases and phosphatases that acted against higher phosphorylated forms of *myo*-inositol in previously fermented samples. Such phosphatases could be active in both the substrate and products of the fermentation despite the high temperature treatments during processing, like autoclaving before inoculation and steaming at the end of the fermentation. Affrifah *et al.* (*27*) showed that phytases of cowpea seeds retained 95–50 % activity after steaming for 2–32 min. High thermal stability of phytases from different fungal sources was described by Simon and Igbasan (*28*) and Azeke *et al.* (*29*). They found that activities of purified intracellular phytases isolated from *R. oligosporus* decreased by 20 and 80 % after treatment at 70 and 80 °C for 10 min, respectively. It has been proven that phytases present in the food matrix within the tempeh structure are more heat resistant than the purified enzymes (*30*).

In our previous study on rye bread (*20*), the supplementation of dough with an exogenous phytase resulted in almost complete dephosphorylation of phytic acid. As a consequence, a release of *myo*-inositol into dialysates was observed during *in vitro* digestion. This phenomenon was not observed in the present research. Free *myo*-inositol was not detected in the dialysates measured by an appropriate HPLC method (Table 3).

### *Mineral availability*

Significant differences were found in the *in vitro* bioavailability of Ca, Mg and P in fermented and unfermented flaxseed oil cake (Table 4). Fermentation of flaxseed oil cake by *R. oligosporus* DSM 1964 improved the bioavailability of Ca, on average by 15.5 %. This is consistent with earlier findings of many researchers concerning the preparation of dephytinised wheat (*31*), rye–wheat bread (*32*) and bread with the addition of pseudocereal grains (*33*), where the treatment of samples with microbial phytases increased the bioavailability of minerals, especially Ca, Zn and Fe. Recent paper by Abid *et al.* (*34*) demonstrates that transgenic expression of phytase in wheat endosperm increases Zn and Fe bioavailability, thus enhancing its nutritional value.

It is worth stressing out that in our study the fermentation decreased the content of $InsP_{6-5}$, the compounds that have a strong capacity for chelating minerals. This effect was most evident when *R. oligosporus* DSM 1964 strain was used (Table 1).

The *in vitro* bioavailability of Mg also slightly increased after fermentation of flaxseed oil cake with *R. oligosporus* DSM 1964, from 9.6 % in the control sample to 12.9 % in fermented flaxseed oil cake (Table 4).

The *in vitro* bioavailability of phosphorus increased in all processed samples, with the exception of flaxseed oil cake fermented with *R. oligosporus* ATCC 64063 for 48 h. Phosphorus bioavailability values obtained after SSF were 3.8 % higher after fermentation with *R. oligosporus* DSM 1964 and 1.6 % higher after fermentation with *R. oligosporus* ATCC 64063 than in control. The phosphorus level in dialysates from the *in vitro* digestion was strongly negatively correlated with total (R=–0.78) and dialysable (R=–0.7) phytate contents. Thus, it can be assumed that the increase in the phosphorus bioavailability was the consequence of the release of this mineral from the phytate. The efficient hydrolysis of $InsP_6$ (Table 1) observed in our study is the result of the action of fungal phytases and phosphatases (*35*). The increase in phosphorus bioavailability by 4.5 % was also reported for *Aspergillus niger* phytase added to corn-soy feed in a broiler diet (*36*).

Table 4. Effects of solid-state fermentation of flaxseed oil cake with *Rhizopus oligosporus* DSM 1964 and ATCC 64063 strains and *in vitro* simulated gastrointestinal digestion on mineral bioavailability

| Sample | *t*(fermentation) | *In vitro* bioavailability/% | | |
|---|---|---|---|---|
| | h | Ca | Mg | P |
| Flaxseed oil cake | 0 | (20.9±3.2)$^{a}$ | (9.6±0.2)$^{a}$ | (4.5±0.9)$^{a}$ |
| DSM 1964 | 48 | (35.02±5.1)$^{b}$ | (12.9±1.6)$^{b}$ | (8.4±0.8)$^{c}$ |
| | 96 | (37.80±6.1)$^{b}$ | (12.6±1.3)$^{b}$ | (8.2±0.2)$^{c}$ |
| ATCC 64063 | 48 | (28.6±7.3)$^{ab}$ | (11.5±0.5)$^{ab}$ | (4.2±0.3)$^{a}$ |
| | 96 | (34.0±2.2)$^{ab}$ | (11.8±0.4)$^{ab}$ | (6.1±0.3)$^{b}$ |
| Mean value | | (1.00±0.06) | (3.47±0.07) | (35.5±2.0) |

Values in columns are mean±S.D. of the sample (*N*=3), values with different superscripts within columns are significantly different ($p<0.05$)

## Conclusions

Solid-state fermentation of flaxseed oil cake with *Rhizopus oligosporus* DSM 1964 strongly reduced the content of antinutritional phytate and generated a favourable profile of lower inositol phosphates, with a significant amount of inositol triphosphates that have beneficial physiological activities. The changes in the phytate level and in the profile of inositol phosphates in the fermented flaxseed oil cake were correlated with the improvement in the *in vitro* bioavailability of calcium, magnesium and phosphorus. In conclusion, the fermentation of flaxseed by-product with *R. oligosporus* DSM 1964 can increase its potential application as a food additive.

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### *Conflict of interest*

There were no conflicts of interest expressed by the authors. The authors confirm their responsibility for the content and writing of the paper.

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