

Phytases Improve *Myo*-Inositol Bioaccessibility in Rye Bread: A Study Using an *In Vitro* Method of Digestion and a Caco-2 Cell Culture Model

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Summary

Preparations of 6-phytase A (EC 3.1.3.26) and phytase B (acid phosphatase, EC 3.1.3.2) were applied alone and combined in the preparation of dough to estimate their catalytic potential for *myo*-inositol liberation from rye flour in the breadmaking technology. The experimental bread samples were ground after baking and subjected to determination of *myo*-inositol bioavailability by an *in vitro* method that simulated digestion in a human alimentary tract, followed by measurements of *myo*-inositol transport through enterocyte-like differentiated Caco-2 cells to determine its bioaccessibility. *Myo*-inositol content was measured by a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) technique. The concentration of *myo*-inositol in the dialysates of control bread was 25.3 µg/mL, whereas in the dialysates of bread sample baked with 6-phytase A, the concentration increased to 35.4 µg/mL, and in the bread baked with phytase B to 64.98 µg/mL. Simultaneous application of both enzymes resulted in *myo*-inositol release of 64.04 µg/mL. The highest bioaccessibility of *myo*-inositol, assessed by the measurement of the passage through the Caco-2 monolayer was determined in the bread baked with the addition of 6-phytase A. Enzymatically modified rye bread, particularly by the addition of 6-phytase A, may be therefore a rich source of a highly bioaccessible *myo*-inositol.

Key words: phytic acid, *myo*-inositol, enzymatically modified bread, Caco-2 cells

Introduction

Phytic acid (1,2,3,4,5,6-*myo*-inositol hexakisphosphate) is difficult to digest in the gastrointestinal tract of monogastric animals and humans, and is therefore considered as an antinutritional factor because it reduces bioavailability of minerals, proteins and starch (1). Enzymatic methods of phytate degradation in foods and feeds have gained considerable interest, regarding their numerous advantages over physical or chemical methods (2). Phytases, phosphohydrolases of *myo*-inositol hexakisphosphate, hydrolyse phytate to a pool of lower *myo*-inositol phosphates (3,4). In breadmaking, phosphorolytic activity ori-

ginates from wheat or rye flour enzymes as well as from the yeast phytase (5-7). The joint action of these enzymes determines the final profile of inositol phosphates and may also lead to the formation of free *myo*-inositol (8). There are 3-phytases A (*myo*-inositol hexakisphosphate 3-phosphohydrolase, EC 3.1.3.8), which start catalytic action at C3 atom of the *myo*-inositol ring, 6-phytases A (*myo*-inositol hexaphosphate 6-phosphohydrolase, EC 3.1.3.26), which initiate the hydrolysis at the C6, and phytases B (EC 3.1.3.2), non-specific acid phosphomonoesterases (3). Generally, phytases A are not able to hydrolyse phosphate residue at the C2 of the *myo*-inositol ring. Phytase B, on the other hand, may liberate phosphorus from

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any position and therefore concurrent action of phytase A and phytase B may lead to almost complete dephosphorylation of phytate, and generation of substantial amounts of *myo*-inositol (4). *Myo*-inositol is hexahydroxylic cyclohexane derivative present in all types of living cells, in concentrations varying from 0.03 mM in human plasma to 3 mM in brain tissue, cerebrospinal fluid, foetal serum and human milk (9). The content in human diet oscillates between 0.8 and 1 g per day, more than half of this compound in the form of inositol phospholipids. Decreased levels of *myo*-inositol are associated with the risk of cataracts, retinopathy, and neuropathy development (10). Studies on rodents and humans demonstrated that *myo*-inositol was effective in treating diabetes and may be a suitable marker for mass screening of diabetes, insulin resistance and glucose intolerance (11,12). Larner *et al.* (13) proposed a new model of insulin signalling with inositol glycans as putative second messengers. Kim *et al.* (14) found this substance to be effective in inducing the response of macrophages to bacterial infections and other inflammatory conditions. Okazaki and Katayama (15) found that *myo*-inositol prevented accumulation of fat in the liver of rats intoxicated with DDT. This compound also regulates fat metabolism in the myocardium of rats with chemically induced diabetes (16). *Myo*-inositol is also known to exert positive effects in the treatment of psychiatric disorders (17), and is also involved in several aspects of human reproduction (18). Remarkably, some reports indicate that mixtures of *myo*-inositol and phytate are more effective in cancer cell elimination than these substances administered separately (19,20). In the literature, there are reports that clearly indicate bioactive properties of this compound, which suggests that supplementation of foods with *myo*-inositol might be a proper way to modulate sugar and lipid metabolism in humans. The strategy presented here is based on the concept of enzymatic conversion of endogenous phytates of rye flour into *myo*-inositol by means of commercial microbial phytases added to the dough. Although phytase application in the breadmaking has been studied by a few researchers (5–7,21), there is no evidence to suggest that this can be utilized as a method for producing bread with enhanced *myo*-inositol content.

The objectives of the current study are therefore to assess the impact of the addition of 6-phytase A and phytase B, alone or in combination, to the dough on the bioavailability of *myo*-inositol from rye bread by an *in vitro* procedure simulating digestion in human alimentary tract. Also, its bioaccessibility is determined by the measurement of an uptake by the Caco-2 cell monolayer.

Materials and Methods

Enzymes

The preparation of 3-phytase A (Natuphos®) was obtained from BASF, Ludwigshafen, Germany, 6-phytase A (Ronozyme® NP) was a product of Novozymes, Bagsvaerd, Denmark, and phytase B (Finase® AP) of AB Enzymes, Rajamäki, Finland. Pepsin and pancreatin were purchased from Sigma Chemical Co (St. Louis, MO, USA). Acid phosphatase activity was determined at 40 °C by incubating the enzyme with 5.5 mM solution of sodium *p*-nitrophenylphosphate at pH=4.5 for 15 min. The

unit of acid phosphatase activity (APAU) was defined as the amount of enzyme which, under the reaction conditions, liberates 1 μmol of *p*-nitrophenol during 1 min. One unit of phytase A activity (PAU) was defined as the amount of enzyme that liberates 1 μM of inorganic phosphorous from 2 mM of sodium phytate in 1 min at 40 °C, pH=4.5.

Breadmaking procedure

Wholemeal rye bread was baked using the following recipe for dough preparation: 1000 g of wholemeal rye flour type 2000 from Podlaskie Młyny Wodne (Tokary, Poland), 80 g of an acidifier from Bionat S.A. (Kraków, Poland), 50 g of lyophilized baker's yeast from Lesaffre P.S. (Łódź, Poland) and 30 g of table salt from POCh (Gliwice, Poland) were mixed with 720 mL of tap water. Experimental dough consisted of the above components, but 670 mL of tap water and 50 mL of 0.05 M acetate buffer (pH=4.5) was used. Appropriate amounts of enzyme preparations were diluted in the acetate buffer to obtain 30 APAU or 5000 PAU per kg of flour. The dough ingredients were mixed for 9 min and then dough pieces of 250 g were formed, put in metal forms and placed in a fermentation chamber at 35 °C and constant moisture. After 150 min, fermented dough samples were transferred to an oven preheated to 260 °C. After 30 min, the loaves were removed from the oven and left for 2 h to cool. Baking procedures were performed in triplicates and included a control bread in each run.

In vitro procedure

Bioavailability of *myo*-inositol was estimated by an *in vitro* technique according to the method developed by Zyla *et al.* (22) and subsequently modified by Starzyńska-Janiszewska *et al.* (23) to simulate the conditions of human gastrointestinal tract. Half a gram of samples of bread was incubated with 1.7 mg of pepsin (Sigma-Aldrich, Steinheim, Germany; the declared activity was 4750 U/mg) dissolved in 0.1 mol/L HCl at 37 °C, pH=2.0, for 2 h. Next, 2.5 mg of pancreatin (Sigma-Aldrich, from porcine pancreas, 8× United States Pharmacopeia) dissolved in 0.1 mol/L of NaHCO₃ were added and the whole samples were incubated in dialysis tubes (Sigma-Aldrich, cellulose membrane 25 mm×16 mm) at 37 °C, pH=7.0, for 4 h. These incubations simulated the digestion in the stomach and small intestine, respectively.

Cell culture

The human colorectal carcinoma cell line, Caco-2, was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % of foetal bovine serum (FBS Gold, PAA Laboratories GmbH, Cölbe, Germany) with antibiotics and antimycotics (PAA Laboratories GmbH). Cultures were passed when they had reached 50 % confluence and 5·10⁵ of cells were seeded on the porous membranes of cell culture inserts (BD Falcon transparent inserts for a 12-well plate, pore diameter 0.4 μm, 1.6·10⁶ pores/cm², BD (Becton, Dickinson and Company), Franklin Lakes, NJ, USA). The full differentiation to enterocyte-like cells was achieved after subsequent two weeks of culture. The quality of epithelial layers was regularly monitored by measuring their transepithelial elec-

trical resistance (TEER) with epithelial voltohmmeter (EVOM, World Precision Instruments, Sarasota, FL, USA). One day before the experiment, the media were changed to serum-free DMEM (SF-DMEM) supplemented with 0.2 % of bovine serum albumin (BSA) in all wells. Cells were serum-starved for 24 h, then 250 μ L of bread dialysates were added to the apical chambers along with 250 μ L of SF-DMEM. In the *in vitro* control treatment, blanks were used instead of a bread dialysate in the *in vitro* procedure. Basolateral chambers were filled with 1 mL of *myo*-inositol-free SF-DMEM (Institute of Immunology and Experimental Therapy, Wrocław, Poland). After 48 h, the media were collected from the basolateral and apical chambers, centrifuged at 1000 \times g for 5 min, and frozen at -80 °C for future analysis.

Chromatographic system and detection

Analysis of *myo*-inositol phosphates in bread samples

Extraction of inositol phosphates from bread samples was conducted according to Gambuś *et al.* (24). Profiles of the isomers of *myo*-inositol phosphates were analyzed on the Ultimate 3000 system (Dionex Corporation, Sunnyvale, CA, USA) using high-performance anion exchange chromatography (HPAEC) with postcolumn derivatization and spectrophotometric detection, as described earlier (25).

Analysis of *myo*-inositol in dialysates

Chromatograms were generated using HPLC column Rezex™ RCM Ca²⁺ (375 mm \times 4 mm i.d., Phenomenex, Torrance, CA, USA) for comprehensive analysis of monosaccharides and sugar alcohols filled with sulphonated styrene-divinylbenzene beads in cross-linked polymer (8 %) in cationic form (Ca²⁺). The detection was performed using the pulsed amperometric detector (PAD). Analyses were carried out at 25 °C with flow rates of 0.6 mL/min using deionized water as an eluent after postcolumn modification with sodium hydroxide (200 mM, flow rate of 0.15 mL/min) (26). The HPLC experiments were done in triplicate.

Analysis of *myo*-inositol in conditioned cell culture media

Chromatograms were generated using Dionex CarboPac™ MA1 HPLC column (250 mm \times 4 mm i.d., Dionex Corporation) and the PAD detection system. Analyses were carried out at 25 °C with flow rates of 1 mL/min using linear gradient of deionized water and 1 M NaOH as the eluent according to the Dionex AN67 brochure. HPLC experiments were done in triplicates. Two different methods were used in the analysis. The Rezex™ RCM column (Phenomenex) allowed for a better separation of stereoisomers, *chiro*- from *myo*-inositol in the dialysates from the *in vitro* method, whereas direct detection of *myo*-inositol on the CarboPac MA1 column by the HPAEC-PAD procedure was more sensitive in the case of *myo*-inositol determination in the cellular fluids.

Statistical analysis

Experimental data were subjected to the one-way analysis of variance (ANOVA) to detect significant differences among the results and expressed as a mean value \pm

standard error of mean (SEM). The significance of differences was checked by the Tukey's test using STATISTICA for Windows, v. 10 (StatSoft Inc., Tulsa, OK, USA) statistical software.

Results and Discussion

Loaves of rye bread baked from dough samples with the addition of either 6-phytase A (5000 PAU per kg of flour) or phytase B (30 APAU per kg of flour), or a combination of 6-phytase A with phytase B were analyzed by the *in vitro* procedure that simulated gastric and intestinal digestion in humans to assess bioavailability of *myo*-inositol. Significant differences among concentrations of dialysable *myo*-inositol released from bread samples in the *in vitro* simulation of multiple digestions were observed (Fig. 1). The generation of *myo*-inositol in the control bread samples was probably caused by the activity of endogenous phytases from rye and yeast phytases. In samples of control bread baked without enzymes the concentration of dialysable *myo*-inositol was 25.3 μ g/mL, in the dialysates of bread baked with 6-phytase A 35.49 μ g/mL, whereas in bread supplemented with phytase B, *myo*-inositol concentration was almost 2.56-fold higher (64.98 μ g/mL). However, simultaneous application of both phytases during breadmaking (6-phytase A at 5000 PAU per kg of flour and phytase B at 30 APAU per kg of flour) did not enhance dialysable *myo*-inositol (64.04 μ g/mL) above the level obtained with phytase B used as a sole supplemental enzyme. Phytase B as unspecific acid phosphatase most probably releases phosphates in a random way, proportionally decreasing the level of existing inositol phosphates in rye bread and liberating free *myo*-inositol. In the presence of phytase A, however, the levels of *myo*-inositol were not higher and the final products were detected as inositol mono- (IP1) and diphosphates (IP2).

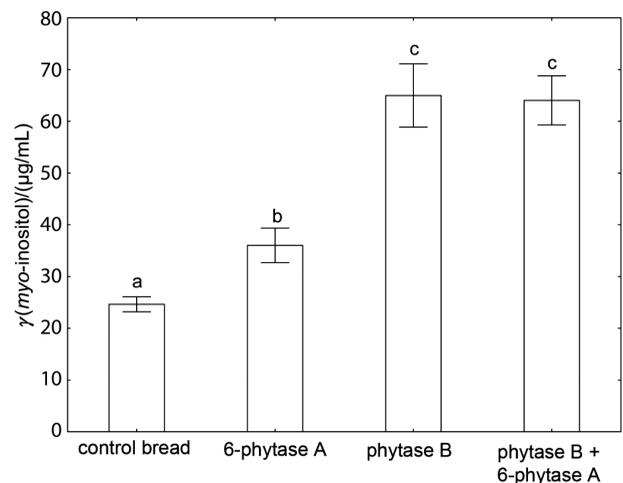


Fig. 1. Concentrations of *myo*-inositol in the dialysates of enzymatically modified loaves of bread subjected to the *in vitro* procedure of multiple digestions measured by the HPLC-PAD method. Control bread=rye bread made without enzyme addition, 6-phytase A=bread supplemented with 6-phytase A at 5000 PAU/kg flour, phytase B=bread made from dough supplemented with phytase B at 30 APAU/kg flour, phytase B+6-phytase A=rye bread supplemented with both phytases. PAU=phytase A activity units, APAU=phytase B activity units. Different letters denote statistical difference at $p \leq 0.05$

In the next step, bioaccessibility of *myo*-inositol was assessed by measuring the transport of this compound through the monolayer of the Caco-2 cells. Schematic diagram of the cell culture procedure is depicted in Fig. 2. This cell line derived from colonic adenocarcinoma surprisingly well mimics intestinal cell behaviour upon long culture. The fortnight culture of these cells on porous membranes of inserts leads to the creation of an impervious epithelial layer of polarized cells which resemble mature enterocytes (27). Tissue culture tests provide a relatively quick and cost-effective tool for predicting the absorption of bioactive components by assessing the probability of their transport through epithelial layer (28).

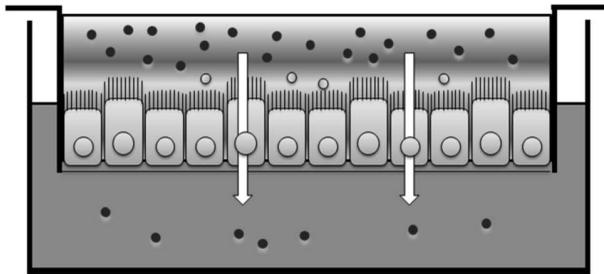


Fig. 2. Schematic diagram of the cell culture. Caco-2 cells were cultured for two weeks on the porous membrane which separates apical and basolateral chambers of the insert to obtain continuous monolayer of differentiated cells. *Myo*-inositol or dialysates of bread were added from the apical side diluted (1:1) in 0.5 mL of *myo*-inositol-free SF-DMEM medium. A volume of 1 mL of *myo*-inositol-free DMEM medium was poured into the basolateral part. After 48 h the media were collected from both chambers to estimate the paracellular flow of *myo*-inositol. The consistence of monolayers was monitored by measuring the TEER values at the beginning and the end of incubation period. TEER=transepithelial electrical resistance

These tests are often a reasonable alternative to *in vivo* studies on laboratory animals (*e.g.* rats) in the analysis of the phytate conversion; however, it should be taken into account that phytase activity in rodent small intestine is 30-fold higher than in human tissue. In the control experiment three concentrations of *myo*-inositol were used (0, 25 and 50 µg/mL) to demonstrate a relationship between the *myo*-inositol transport rate and its concentration (Fig. 3). The uptake of *myo*-inositol by Caco-2 cell line was found to depend on the *myo*-inositol concentration, which is in agreement with the literature data describing two main sodium/*myo*-inositol cotransporters (29). The fluid from basolateral chambers of inserts from Caco-2 monolayers exposed for 48 h to the digest of bread supplemented with phytase B and the fluid from the same chamber before the addition of the digest were analyzed. Caco-2 cells exposed to the dialysates from digests of bread absorbed *myo*-inositol from the apical chambers and then secreted it to the basolateral chambers of the inserts.

To our knowledge, this is the first evidence of *myo*-inositol transport across Caco-2 monolayers. The amounts of *myo*-inositol secreted to the basolateral chambers from the inserts not exposed (control *in vitro*) and exposed to the bread digests for 48 h, also expressed as a percentage of the initial amounts of *myo*-inositol in apical chambers, are given in Table 1. Total amounts of *myo*-inositol determined in both the apical and the basolateral chambers at

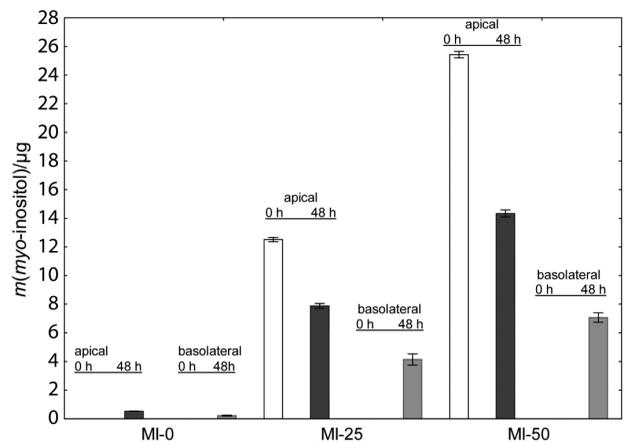


Fig. 3. Control experiment: transport of the *myo*-inositol through Caco-2 monolayer after 48 h. Total amounts of *myo*-inositol determined by HPAEC-PAD in both the apical and the basolateral chambers at the beginning of the experiment compared to the total amounts of this compound determined after 48 h of the Caco-2 cell culture. MI-0, MI-25 and MI-50=initial concentrations of *myo*-inositol in the solutions of 0, 25, 50 µg/mL, respectively, were diluted at the ratio of 1:1 with *myo*-inositol-free SF-DMEM medium

Table 1. Amounts of *myo*-inositol in the basolateral chambers of the inserts after 48 h of Caco-2 culture and as a percentage of the initial concentration in the apical chamber

Sample	$m(\text{myo-inositol})_{\text{BC}}$ µg	$\frac{\gamma(\text{myo-inositol})_{\text{BC}}}{\gamma(\text{myo-inositol})_{\text{AC}}}$ %
control <i>in vitro</i>	(5.2±0.40) ^a	(130.5±14.0) ^c
control bread	(9.3±0.4) ^a	(149.1±1.2) ^c
6-phytase A	(14.7±0.4) ^c	(123.3±2.8) ^{bc}
phytase B	(11.9±0.3) ^b	(61.3±0.4) ^a
phytase B + 6-phytase A	(12.40±0.09) ^b	(65.9±1.7) ^a

Mean values followed by different letters in superscript are significantly different ($p < 0.05$)

BC=basolateral chamber, AC=apical chamber

the beginning of the experiment were subtracted from the total amounts of this compound determined after 48 h of the Caco-2 cell culture.

The initial *myo*-inositol amounts in the apical chambers differed among treatments because a standardized volume of 0.25 mL of the bread digests or of the reagents used in the *in vitro* procedure (the control *in vitro* treatment), mixed with 0.25 mL of the DMEM solution, was applied on the inserts. Generally, no close relationship was found between *myo*-inositol amounts in the apical and basolateral chambers of the inserts, which may suggest that specific transport mechanisms rather than passive diffusion phenomena were active in the *myo*-inositol monolayer metabolism. The highest amounts of *myo*-inositol were released from the Caco-2 cells immersed in the digests of bread modified with the addition of 6-phytase A, whereas significantly lower amounts of this compound were determined in the basolateral chambers of inserts filled with the dialysate of bread made with phytase B. The amounts of *myo*-inositol determined in the basolater-

al chambers after 48 h of Caco-2 culture accounted for 123 %, and for 61 % of its initial amounts in the apical chambers filled with the digests of bread made with the addition of 6-phytase A, and phytase B, respectively.

It seems possible that Caco-2 cells dephosphorylated *myo*-inositol phosphate intermediates generated by 6-phytase A, but were unable to degrade lower *myo*-inositol phosphates produced by the catalytic action of phytase B. Such hypothesis should be considered because these enzymes are known to release two distinct pools of *myo*-inositol phosphates during their action on *myo*-inositol hexakisphosphates (phytates). The profiles of *myo*-inositol phosphates analyzed by HPAEC in the enzyme-modified bread shown in Table 2 may serve as a confirmation of the hypothesis mentioned above. In bread supplemented with phytase B the main fraction is inositol phosphate with 3 to 4 phosphorus moieties, but in the bread made with the addition of 6-phytase A, these were inositol di- and triphosphates. The application of both phytases resulted in bread samples whose digests influenced Caco-2 metabolism similarly as in the case of bread baked with phytase B alone. The explanation of this phenomenon is similar to the situation observed in determination of *myo*-inositol in bread subjected to *in vitro* procedure.

Table 2. Profile of *myo*-inositol phosphates in rye bread supplemented with phytases analysed by HPAEC

Sample	Inositol phosphate fraction/%				
	IP2	IP3	IP4	IP5	IP6
6-phytase A	35.43	30.69	3.44	4.33	24.63
phytase B	9.44	21.99	42.97	4.83	20.77
phytase B + 6-phytase A	27.14	33.99	26.78	2.95	9.14
control bread	16.22	27.97	38.09	8.47	9.25

IP2=*myo*-inositol diphosphate, IP3=*myo*-inositol triphosphate, IP4=*myo*-inositol tetraphosphate, IP5=*myo*-inositol pentaphosphate, IP6=*myo*-inositol hexaphosphate

An additional evidence of a substantial difference in *myo*-inositol metabolism in Caco-2 cells subjected to digests from the bread supplemented with 6-phytase A and that supplemented with phytase B is provided in Fig. 4, where the net changes in *myo*-inositol mass in both apical and basolateral chambers of the inserts in the course of 48-hour culture are shown. In the *in vitro* control, control bread and 6-phytase A treatments, *myo*-inositol was synthesised by the Caco-2 cells most probably due to the action of cellular phosphatases on lower *myo*-inositol phosphates, but in the treatments where the digest contained the products of phytase B, *myo*-inositol consumption or degradation was evidenced. It can be postulated, therefore, that in contrast to 6-phytase A, the action of phytase B on the dough components of rye bread must have produced compounds which negatively affected *myo*-inositol bioaccessibility.

While phytase B seems to be the key activity that determines *myo*-inositol bioavailability from rye bread, 6-phytase A caused the highest enhancement in its bioaccessibility. Suzuki *et al.* (30) observed that phytate hydrolysates, especially *myo*-inositol triphosphates, were able

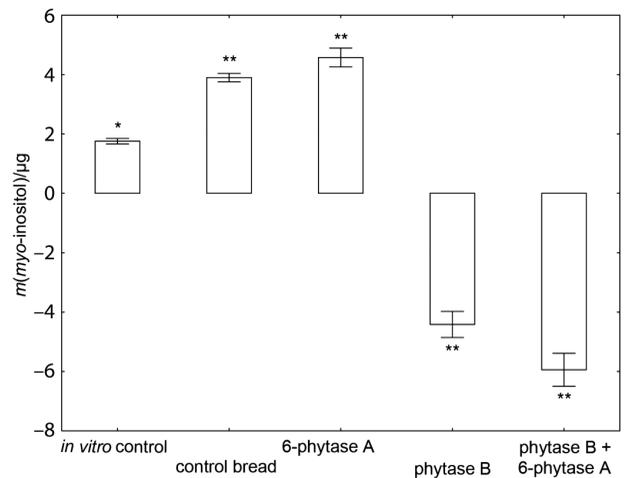


Fig. 4. *Myo*-inositol balance after 48 h of culture of the Caco-2 monolayers. Total amounts of *myo*-inositol determined in both the apical and the basolateral chambers at the beginning of the experiment were subtracted from the total amounts of this compound determined after 48 h of the Caco-2 cell culture. *In vitro* control=culture medium without bread sample addition, control bread=rye bread made without enzyme addition, 6-phytase A=bread supplemented with 6-phytase A at 5000 PAU per kg of flour, phytase B=bread supplemented with phytase B at 30 APAU per kg of flour, phytase B+6 phytase A=rye bread supplemented with both phytases. Significant differences between the final and initial *myo*-inositol mass at * $p < 0.01$ and ** $p < 0.001$

to induce intracellular signalling in the Caco-2 cell line, and *myo*-inositol phosphate synthase (MIPS) is known to be regulated by phosphorylation (31). It could not be excluded that physiologically beneficial distribution of *myo*-inositol triphosphates generated by 6-phytase A stimulated Caco-2 cells to synthesize and effectively secrete *myo*-inositol, thus enhancing its bioaccessibility. Shockravi *et al.* (21) studied iron and zinc bioaccessibility from breads made from flour with different extraction rates and dephytinized by *Aspergillus niger* 3-phytase A during *in vitro* digestion. As a result of phytase application, they reported substantial improvements in bioaccessibility of iron and zinc determined in the Caco-2 cell line.

The current study confirms that *myo*-inositol is released during hydrolysis of flour phytates in this individual bread-making process more efficiently by phytase B than by 6-phytase A. The 6-phytase A, however, generates *myo*-inositol which is significantly more bioaccessible. *Myo*-inositol is known to exert multiple physiological effects that are manifested by antioxidant, immunostimulating and antidepressant properties (9,32). Many important questions regarding dephosphorylation of phytates in cereals have not been addressed yet. This study contributes to the development of an entirely new and innovative approach in breadmaking, involving the enzymatic conversion of phytates, which have antinutritional properties, into *myo*-inositol – a biostimulating and health-promoting compound.

Conclusions

The application of proper phytate-degrading enzymes in the technology of rye breadmaking leads to gen-

eration of *myo*-inositol – a component with bioactive, health-promoting effects. Its actions include insulin sensitivity modulation, regulation of cholesterol metabolism, central nervous and reproduction system development and maintenance, as well as regulation of the bone calcification processes. Simultaneous application of phytase A and phytase B or phytase B alone to the dough leads to the release of 2.4-fold higher amounts of bioavailable *myo*-inositol, as assessed by an *in vitro* procedure which simulates digestion in the human intestine. Determination of bioaccessibility of this compound by the measurement of an uptake by Caco-2 cell monolayer revealed that 6-phytase A was the most effective enzyme in enhancing the amount of bioaccessible *myo*-inositol in the experimental bread. Enzymatically modified rye bread may therefore be a rich source of bioavailable and bioaccessible *myo*-inositol. Enzymatic generation of *myo*-inositol seems to be a promising alternative to a strategy of food supplementation with health-promoting components.

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