Expression of phytase in yeast: phytate degradation and survival of yeast cells during *in vitro* digestion

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Objective. To investigate the potential of modified strains of baker's yeast, *Saccharomyces cerevisiae*, to degrade phytate in a meal during *in vitro* digestion at simulated physiological conditions.

Background. Phytate (*myo*-inositol hexa*kis*phosphate) is a naturally occurring compound in plants. Due to chelation of several dietary minerals, it acts as a potent anti-nutrient in foods. Degradation of phytate has previously been found to significantly improve absorption of nutritionally important minerals, such as iron and zinc, in the human intestine. By removing regulatory genes for phytase expression in *Saccharomyces cerevisiae*, high-phytase strains that constitutively degrade phytate have been constructed. A novel application is to use such strains for the degradation of phytate during gastrointestinal passage, and *S. cerevisiae* can constitute a suitable phytase carrier to the gastrointestinal tract since it has GRAS status (generally considered as safe).

Methodology. The phytate degrading capacity and survival of the yeast strains were tested during *in vitro* digestion. Yeast peptone dextrose growth medium (YPD) or commercial ready-made wheat gruel (~0.2 mM native phytate), with added phytate in known concentrations, were used as simple model meals. During the gastric phase, pepsin solution was added and the meal was submitted to a pH gradient (pH 5.5 to pH 2.0) for 90 minutes. Thereafter, pancreatin/bile solution was added and the pH was increased to 5.0 for 30 minutes, followed by an incubation at pH 6.5 for 60 minutes (intestinal phase). Phytate content and cell concentration was measured before, during, and after digestion.

Results and discussion. A degradation of 62 % (n=6) and 36 % (n=5) was found in YPD and gruel, respectively, when phytate (0.5 mM) and high-phytase yeast cells (corresponding to 1 mg dry weight per mL) was added, as compared to the wild-type yeast that did not show any significant degradation. The major part of the phytate degradation occurred during the gastric phase. A gradually descending gastric pH was however found crucial, since hardly any degradation could be observed when static pH 2 or pH 3 was tested in the gastric phase. No significant degradation was observed in the late intestinal phase, indicating that the very low solubility of phytate at pH 6.5 inhibited enzyme-phytate interactions. Furthermore, the strain was found to withstand acidic pH, bile salts, and digestive enzymes well, giving a good survival of the cells during digestion.

Conclusion. It was shown that the modified strain used in the study survived and can degrade phytate at digestive conditions. The level of phytate degradation seems however to be dependent on pH, incubation time and phytate solubility. Even though further studies must be done, our results show the potential of using high-phytase yeast to improve mineral absorption during the gastrointestinal passage.