



OptiPhos[®] outperforms Ronozyme[®] HiPhos on pH activity range, resistance to pepsin and speed of phytic acid hydrolysis

Trial description

1 Set-up

- **Location:** DIADEM, Russia
- **Trial period:** March/April 2014

2 Treatments

- OptiPhos[®] and Ronozyme[®] HiPhos *in vitro* research on pH profile, pepsin resistance and Michaelis-Menten kinetics.

3 Measured parameters

- **Activity assay:** phytase activity was measured using phytate as substrate in Glycine-HCl buffers at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 during 10 min (according to ISO 30024:2009).

- **Pepsin resistance:** pepsin (600 units/mg protein) was dissolved into 15 mM HCl (final pH 2). In a final volume of 100 ml, 10 mg of purified phytase was incubated with pepsin at different pepsin/phytase (w/w) ratios at 37°C for different time periods after which the remaining phytase activity was measured in a 0.2 M Glycine-HCl buffer (pH 3.5) at 39°C.
- **Kinetics (Michaelis Menten):** different kinetic parameters for phytase activity were determined in Glycine-HCl buffer at pH levels of 2, 2.5, 3, 3.5, 4 and 4.5 after which Vmax and Kcat were calculated. Vmax represents the maximum speed rate of P release from phytate, at maximum (saturating) substrate (phytate) concentrations. Kcat is the turnover number, which is the maximum number of substrate (phytate) molecules converted per enzyme molecule per second.

Results

- OptiPhos[®] showed a much better activity at different pH ranges compared to Ronozyme[®] HiPhos, which indicates that OptiPhos[®] is also performing better at acidic conditions (Fig. 1).
- OptiPhos[®] was by far more resistant to pepsin either when incubated with relative low doses of pepsin for a long time (2 h, Fig. 2) or when incubated with a higher pepsin/phytase ratio during a shorter time (40 min, Fig. 3).
- The Kcat value of OptiPhos[®] was also higher at all pH levels. On average, the Kcat value of OptiPhos[®] was 893 per sec. vs 668 per sec (+35 %, Fig. 4).
- The Vmax value of OptiPhos[®] was higher at most pH levels. On average, the Vmax value ($\mu\text{mol P/min per mg enzyme}$) of OptiPhos[®] was 1045 vs 806 (+23 %, Fig. 5).

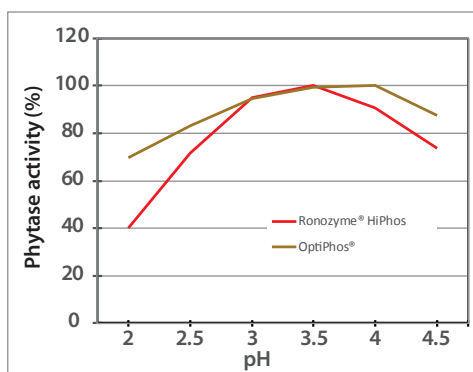


Fig. 1: relative phytase activity at different pH levels after incubation during 10 minutes

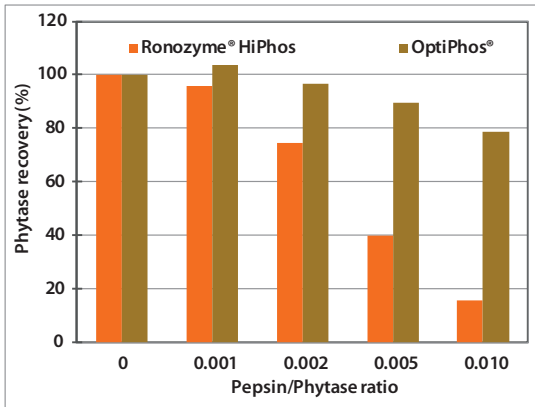


Fig. 2: phytase recovery after prolonged exposure of OptiPhos® and Ronozyme® HiPhos at different low pepsin levels during 2 h

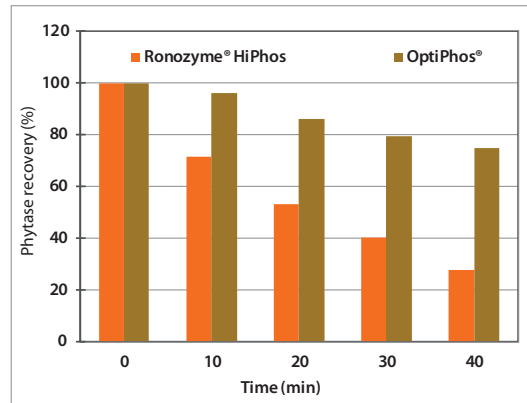


Fig. 3: phytase recovery after exposure of OptiPhos® and Ronozyme® HiPhos at an elevated pepsin/Phytase ratio (1/1) during 40 min

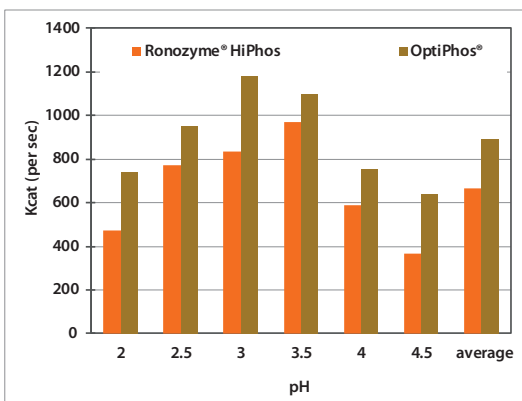


Fig. 4: the Kcat value of OptiPhos® and Ronozyme® HiPhos at different pH levels

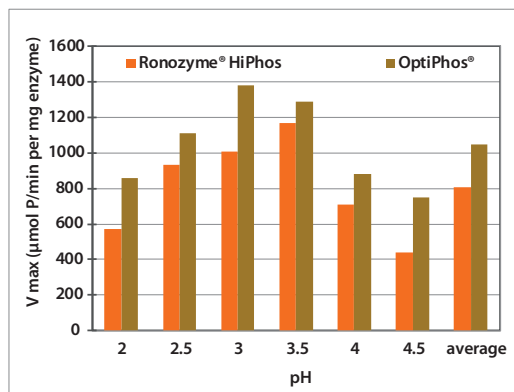


Fig. 5: the Vmax value of OptiPhos® and Ronozyme® HiPhos at different pH levels

Conclusions

- OptiPhos® has a much broader pH activity range with higher phytase activity than Ronozyme® HiPhos.
- OptiPhos® is far more resistant to pepsin breakdown.
- OptiPhos® works much faster in breaking down phytic acid at *in vivo* relevant pH levels between 2 and 4.5.