Update on characteristics of commercial phytases

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Abstract

Phytase is present in about 75% of all diets for simple-stomach animals and its market volume exceeds US$350 million annually. The current global phytase market was estimated to account for more than 60% of the total enzyme market. The rapid growth of the phytase market resulted in a huge increase in competition and an increase in the number of commercialized phytase products. The phytases used for animal feed application differ in their enzymatic properties such as pH profile, stability under stomach conditions, temperature stability, kinetic constants, and substrate specificity. Their biochemical characterization is therefore essential, but enzymatic properties could only be guidelines on potential functionality in animal feeds and digestive systems. There is no unique property responsible for a better performance *in vivo* but a conjunction of all properties. Biological efficacy can only be fully determined by direct animal trials.

Introduction

Phytases [*myo*-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolases] are phosphor-hydrolytic enzymes that initiate the stepwise dephosphorylation of phytate [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate], the major form of phosphate present in plant seeds and pollen (Konietzny and Greiner, 2002). They have been detected in plants, microorganisms, and in some animal tissues. The ability of phytases to hydrolyze phytate is well understood from *in vitro* assays but their activity *in vivo* remains largely unknown. Thus, some of the enzymes classified as phytases today may not be involved in phytate degradation *in vivo* but may have completely different functions (Konietzny and Greiner, 2004).

Phytases were originally proposed as an animal feed additive to enhance the value of plant material in animal feed by liberating phosphate (Mitchell *et al.*, 1997).

Due to the lack of significant phytate-degrading activity in the gastrointestinal tract, simple-stomach animals such as swine, poultry and fish require extrinsic phytase to make phytate phosphate available for growth and development. The increase in economic pressure and increased concern over the environmental impact of life-stock production paved the way for the economic success of phytases as an animal feed additive. Natuphos, the first globally commercialized phytase, was launched into the market in 1991. Meanwhile phytase is present in about 75% of all diets for simple-stomach animals and its market volume exceeds US$350 million annually (Shivange *et al.*, 2012). The current global phytase market was estimated to account

for more than 60% of the total enzyme market. The rapid growth of the phytase market resulted in a huge increase in competition and an increase in the number of commercialized phytase products (Table 1). The phytases from *Buttiauxella* sp. (DuPont) and *Yersinia* sp. (BASF) are to our knowledge not marketed so far, but their enzymatic properties suggest that they might be highly suitable for feed application.

Table 1. Commercially available phytase products (histidine acid phytases)

Product	Donor organism	Production organism	Company
Natuphos	*Aspergillus niger*	*Aspergillus niger*	BASF
Finase P/L	*Aspergillus niger*	*Trichooderma reesei*	AB Vista
Rovabio	*Penicillium funiculosum*	*Penicillium funiculosum*	Adisseo
Finase EC	*Escherichia coli*	*Trichooderma reesei*	AB Vista
OptiPhos	*Escherichia coli*	*Pichia pastoris*	Enzyvia
Phyzyme XP	*Escherichia coli*	*Schizosaccharomyces pombe*	DuPont
Quantum	*Escherichia coli*	*Pichia pastoris*	AB Vista
Ronozyme P	*Peniophora lycii*	*Aspergillus oryzae*	Novozymes/DSM
Ronozyme HiPhos	*Citrobacter braakii*	*Aspergillus oryzae*	Novozymes/DSM
-	*Buttiauxella sp.*	*Trichoderma reesei*	DuPont
-	*Yersinia sp.*	*Aspergillus niger*	BASF

Classification of phytases

Phytases are a diverse group of enzymes that encompass a range of sizes, structures and catalytic mechanisms. Based on the catalytic mechanism, phytases can be referred to as histidine acid, ß-propeller, cysteine/tyrosine or purple acid phytases (Greiner and Bedford, 2010). Very recently an enzyme acting on phytate that does not fit in any of the four mentioned classes was identified in *Pseudomonas putida* (Sarikhani *et al.*, 2012). Its amino acid sequence showed maximum identity (80%) to the major facilitator superfamily from P. *putida*. Thus, additional chemical means to accomplish phytate degradation might be realized by nature. Today, all phytases used for animal feed application belong to the class of histidine acid phytases. All cysteine/tyrosine phytases and the majority of the histidine acid phytases release five of the six phosphate residues of phytate and the final degradation product was identified as *myo*-inositol(2)phosphate (Puhl *et al.*, 2007; 2008a;b; 2009). Dephosphorylation of *myo*-inositol(2)phosphate occurs only in the presence of high enzyme concentration during prolonged incubation. After removal of the first phosphate group from phytate these phytases continue hydrolysis adjacent

to a free hydroxyl group. A subgroup of histidine acid phytases identified in members of the *Enterobacteriaceae* family such as *Escherichia coli, Enterobacter cloacae* and *Pantoea agglomerans* were reported to degrade phytate in a stereospecific way to D-*myo*-inositol(1,2,4,5,6)pentakisphosphate as the sole degradation product (Cottrill *et al*., 2002; Greiner, 2004; Herter *et al*., 2006). These enzymes preferably degrade glucose-1-phosphate and are therefore glucose-1-phosphatases rather than phytases. The alkaline phytase from lily pollen possesses the conserved active site motifs characteristic for histidine acid phytases and prefers removal of adjacent phosphate groups generating *myo*-inositol(1,2,3)trisphosphate as the end product of phytate hydrolysis (Mehta *et al*., 2006). In addition, all ß-propeller phytase studied in respect to their phytate degradation pathway (*Shewanella oneidensis, Bacillus subtilis, Bacillus amyloliquefaciens*) remove three phosphate residues from the *myo*-inositol ring to yield *myo*-inositol (2,4,6)trisphosphate as the final phytate dephosphorylation product (Greiner *et al*., 2007b). Thus, these phytases seem to prefer hydrolysis of every second phosphate over that of adjacent ones.

Depending on their pH optimum, phytases have been divided into acid and alkaline phytases. Histidine acid phytases, cysteine phytases and purple acid phytases exhibit maximum enzymatic activity at pH values below 6 (acid phytases), whereas ß-propeller phytases show maximum activity between pH 7 and 8 (alkaline phytases). Up to now only one alkaline phytase was reported to contain the amino acid motifs characteristic for histidine acid phytases (Mehta *et al*., 2006). This enzyme was identified in lily pollen and requires Ca^{2+} for full catalytic activity. Plant alkaline phytases whose activity is enhanced in the presence of Ca^{2+} were also found in cattail (*Typha latifolia* L.) pollen (Hara *et al*., 1985) and a number of legumes (Konietzny and Greiner, 2002; Scott, 1991). Unfortunately, none of the corresponding genes have been cloned and no sequence data exist to confirm the presence of the signature motifs of histidine acid phytases.

Based on the carbon in the *myo*-inositol ring of phytate at which hydrolysis is initiated the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB) recognize three classes of phytases: 1/3-phytases [E.C. 3.1.3.8] initially remove the phosphate residue from the D-3 (L-1) or D-1 (L-3) position of phytate, 4/6-phytases [E.C. 3.1.3.26] preferentially initiate phytate dephosphorylation at the D-4 (L-6) or D-6 (L-4) position and 5-phytases [E.C. 3.1.3.72] at the 5 position in the *myo*-inositol ring. It is very likely that nature realized all six possibilities of initiating phytate hydrolysis, even if phytases initially removing the phosphate residue from the D-1 and the 2 position of phytate have not been identified so far. Due to the fact that phytate turnover in animal cells is high and all intracellular occurring partially phosphorylated *myo*-inositol phosphates are dephosphorylated at position 2, a 2-phytase must exist. 3-phytases have been identified in fungi (*Aspergillus niger*), yeast (*Saccharomyce*

cerevisiae), bacteria (*Klebsiella terrigena, Pantoea agglomerans, Selenomonas ruminatium*) and plants (lupine, soybean) (Greiner, 2002; 2004; Greiner and Carlsson, 2006; Greiner *et al.*, 2001; 2009; Puhl *et al.*, 2007, 4-phytases in cereals (spelt, rye, barley, wheat) and legumes (lupine, faba bean) (Greiner and Larsson Alminger, 2001; Greiner *et al.*, 2002), 5-phytase in plants (lily pollen) and bacteria (*Selenomonas ruminantium subsp. lactilytica*) (Mehta *et al.*, 2006; Puhl *et al.*, 2008a), and 6-phytase in bacteria (*Buttiauxella* sp., *Citrobacter braaki, Escherichia albertii, Escherichia coli, Peniophora lycii, Yersinia* sp.) (Greiner *et al.*, 2000; 2007b; Lassen *et al.*, 2001; Pontoppidan *et al.*, 2012). The preference to initiate phytate dephosphorylation at a certain position in the *myo*-inositol ring is not absolute. Even at optimal pH for phytate hydrolysis, 3-phytases exhibit a low (in general < 10%) 6-phytase activity and 6-phytases a low 3-phytase activity (Puhl *et al.*, 2007a; Pontoppidan *et al.*, 2012). At other pH values, the phytases seem to be even less specific in respect to their preferred initiation site (Greiner, unpublished observation). Last but not least, it is worth mentioning that independent from the classification system used for phytases, phytases of different classes can be found in a single organism.

Phytases as animal feed additives

'Ideal' phytases for animal feed applications should fulfil a series of quality criteria. They should be effective in releasing phytate phosphate in the digestive tract, stable to resist inactivation by heat from feed processing and storage as well as cheap to produce. Commercially available phytase products can be classified according to their origin (fungal or bacterial), the carbon in the *myo*-inositol ring of phytate at which dephosphorylation is initiated (3- or 6-phytases) or on the presence of any protection against the high temperatures present in feed production (coated or uncoated). Phytases from five different sources can be found in the marketplace today (Table 1). Three are of fungal (*Aspergillus niger, Penicillium funiculosum, Peniophora lycii*) and two of bacterial origin (*Escherichia coli, Citrobacter braakii*). Furthermore, variants of the above mentioned phytases with significant improvements over the parent enzymes through selective molecular modifications have found their way to the marketplace. There is no indication so far, that fungal or bacterial phytases are more efficient in monogastric animals. This statement is even truer with improvements of enzyme properties through molecular modifications. Besides the above mentioned phytases two further phytases of bacterial origin are in the pipeline (*Buttiauxella, Yersinia*). As already mentioned, the commercial phytase products consist either of a 3-phytase (*A. niger, P. funiculosum*) or a 6 phytase (*E. coli*, *P. lycii*, C. *braakii, Buttiauxella, Yersinia*) (EFSA, 2007; Greiner *et al.*, 2000; 2007; 2009; Lassen *et al.*, 2001; Pontoppidan *et al.*, 2012). However, the preference to initiate phytate dephosphorylation at the third or sixth phosphate group in the *myo*-inositol ring is not known to affect phytase efficacy in animals. Stahl *et al.*, (2004), for example, did not observe any synergistic effect on plasma phosphate level and

growth performance when supplementing a weanling pig diet with phytases from *P. lycii*/*E. coli* and *A. niger*/*E. coli*, respectively. In addition, no synergistic effect of a combination of a 6- and a 3-phytase (*A. niger*/*P. lycii*, *A. niger*/*E. coli*) on phosphate release or *myo*-inositol phosphate concentrations (InsP6-InsP3) were found in an *in vitro* digestion model that simulates the conditions of the animal gastrointestinal tract (poultry, pig) (Greiner, unpublished observation). It was concluded from these studies that, in general, a combination of 6- and 3-phytase exhibit linear additivity in the response on phosphate release in the *in vitro* digestion model. This result implies that both types of phytases degrade phytate independently from each other. In rare cases even an antagonistic effect on phosphate release was identified. This observation might be due to the fact that a *myo*-inositol phosphate intermediate generated by one of the phytases used in combination acts as a competitive inhibitor for the other phytase.

Below, the properties of commercial phytases relevant to their animal feed application are discussed. The focus is on the properties of the three major commercial phytases (*A. niger*, *E. coli*, *P. lycii*). The properties for these three enzymes were taken from the scientific literature (Greiner *et al.*, 1993; Golovan *et al.*, 1999; Garrett *et al.*, 2004; Elkhalil *et al.*, 2007; Igbasan *et al.*, 2000; Lassen *et al.*, 2001; Ullah, 1988a,b; Ullah and Gibson, 1987; Ullah and Sethumadhavan, 2003; Wyss *et al.*, 1999) and from own studies with the purified phytases and commercial phytase products. The properties of the other phytases (P. funiculosum, *C. braakii*, *Buttiauxella*, *Yersinia*) are presented when available.

Properties of commercial phytase enzymes

To guarantee an efficient phytate hydrolysis in the crop and stomach, high phytate dephosphorylation activity under acidic conditions, stability in an acid environment and resistance to pepsin are properties that are reported to be highly desirable for supplementary phytases. The pH profiles of the phytases from *E. coli*, *P. lycii* and *A. niger* are remarkably different. The phytases from *E. coli* and *P. lycii* show a single pH optimum between pH 3.5 and 4.5 and pH 4.0 and 5.0, respectively. The *A. niger* phytase, however, shows a bi-hump pH optimum at pH 5.5 and 2.2. The pH profile of the *P. lycii* phytase is rather narrow and the enzyme does not show significant activity below pH 2.5. The pH optimum of the phytases from *C. braakii* (Kim *et al.*, 2003) was reported to be pH 4.0 and for the phytases from *Buttiauxella* sp. (Shi *et al.*, 2008) and *Yersinia* sp. (Huang *et al.*, 2006; 2008; Shivange *et al.*, 2012) a pH optimum of 4.5 was found. In respect to animal feed application it is worth to mention that the pH optimum of the phytases in the digestive tract of the animal might be different to that determined with highly soluble sodium phytate. In general, phytases act only on soluble phytate and under physiological conditions a pH dependent equilibrium of soluble and insoluble phytate exists. It was recently shown that phytase activity at pH 3.0 is a relatively good predictor for the biological efficacy of the enzyme used as a feed supplement (Greiner and Bedford,

2010). In this respect, the *E. coli* phytase has an advantage over the other two phytases, because at pH 3.0 the enzyme still exhibits 86-149% of its activity at pH 5.5 (the pH specified by the current standard AOAC assay methodology), whereas at pH 3.0 the phytases from *A. niger* and *P. lycii* show only 38-50% and 18-25% of their activity at pH 5.5, respectively. At pH 3.0, the phytases from *C. braakii, Buttiauxella* sp. and *Yersinia* sp. exhibit 80-120% of their activity at pH 5.5 (Kim *et al.*, 2003, Huang *et al.*, 2008; Shi *et al.*, 2008) which is comparable to the *E. coli* phytase. The phytate accessible to the phytase in the gastrointestinal tract of an animal is among others dependent on the composition of the animal's diet and of the quality of the ingredients used. To keep a high phytate degrading activity even in low phytate concentrations, a high affinity (low km) of the enzyme for phytate is desirable. The km values at pH 3.0 and 37°C of the phytases from *P. lycii*, *A. niger* and *E. coli* were reported to be 73-81 µM, 113-135 µM and 173-386 µM, respectively. The km value is however only one parameter affecting phytase efficacy on low phytate concentrations. Feed components for example could modulate phytase activity. However, these effects are not in all phytases in a parallel and synchronous manner. For example, the activity of the *E. coli* phytase is reduced in the presence of citrate and succinate and improved in the presence of BSA bovine serum albumin and triton, whereas these compounds do not have any effect on the activity of the phytases from *A. niger* and *P. lycii*.

Stability of the phytase under the pH conditions of the stomach or crop and its susceptibility to pepsin degradation are further factors discussed in respect to the biological efficacy of phytases. *In vitro* studies mimicking the conditions found in the monogastric upper digestive tract revealed that exposure to full simulated gastric digestion resulted in a significant decrease in the activity of the phytases from *P. lycii* and *A. niger* (Table 2).

Table 2. pH stability and pepsin tolerance of commercial phytases

Characteristics	*P. lycii*	*A. niger*	*E. coli*
pH stability			
pH 2.0, 37°C, 1 hour + BSA (1 mg/ml)	42 - 49% 45 - 50%	69 - 83% 74 - 84%	88 - 93% 94 - 100%
Protease resistance			
pH 2.0, 37°C, 1 hour, 2000 U	14 - 25%	40 - 50%	92 - 98%
Pepsin			
pH 2.0, 37°C, 1 hour 37°C, 1 hour + stomach digesta + crop digesta	15 - 25% 42 - 49% 45 - 50%	39 - 51% 69 - 83% 74 - 84%	93 - 97% 88 - 93% 94 - 100%

Presence of BSA during incubation with pepsin had no effect on proteolytic digestion. However, a much higher proteolytic stability of the phytases from *A. niger* and *P. lycii* in the digesta supernatants of the stomach and crop was observed (Table 2). The cause for the increase in stability is not known. The greater tolerance seems not to be due to the presence of additional proteins serving as substrates for the proteases. However, it can be speculated that the presence of the substrate phytate is capable of stabilizing the phytases. In contrast to the phytases from *P. lycii* and *A. niger*, the *E. coli* phytase retained almost its complete activity under the previously mentioned conditions (Table 2). In addition, the phytases from *P. lycii* and *A. niger* showed a higher instability at low pH compared to the *E. coli* phytase (Table 2). In conclusion, the data available on the intrinsic proteolytic resistance of a phytase point to a minor importance of this property for its *in vivo* performance. However, by far the major contributing factor to activity loss of the phytases was instability to low pH. The phytases from *C. braakii* (Kim *et al.*, 2003), *Buttiauxella* sp. (Shi *et al.*, 2008) and *Yersinia* sp. (Huang *et al.*, 2006; 2008) exhibited a high stability at pH 3.0 (>80% residual activity) and the phytases from *C. braakii* and *Yersinia* sp. were also reported to be pepsin tolerant (>80% residual activity), whereas no data on pepsin tolerance of the *Buttiauxella* phytase was available. The pH stability and the pepsin tolerance of the phytases from *C. braakii*, *Buttiauxella* sp. and *Yersinia* sp. are comparable to these of the *E. coli* phytase. In addition, Pontopiddan *et al.* (2012) observed that the *C. braakii* phytase survives the conditions of the stomach during the first two hours after feeding.

In vitro feed experiments with microbial phytases suggest that enzymes with broad substrate specificity are better suited for animal nutrition purposes than enzymes with narrow substrate specificity (Wyss *et al.*, 1999). In general, phytases accept a variety of phosphorylated compounds as substrates (Konietzny and Greiner, 2002). The phytases from *E. coli* and *C. braakii* (Kim *et al.*, 2003) show narrow substrate specificity, whereas the phytases from *A. niger* and *P. lycii* are less specific for phytate and accept a wider variety of phosphorylated compounds as substrates. The enzymatic hydrolysis of phytate is a stepwise process in which each generated partially phosphorylated *myo*-inositol phosphate may become a substrate for further hydrolysis. The phytases from *C. braakii* (Pontopiddan *et al.*, 2012), *E. coli*, *P. lycii* and *A. niger* do not have the capacity to dephosphorylate phytate completely. The phosphate residue at position C-2 in the *myo*-inositol ring was shown to be resistant to dephosphorylation and therefore *myo*-inositol(2)monophosphate is the final product of enzymatic phytate dephosphorylation. Furthermore, a marked decrease in hydrolysis rate was observed during phytate dephosphorylation by these phytases *in vitro*. The decrease in the rate of phosphate release is due to product inhibition by phosphate and a lower hydrolysis rate of the partially phosphorylated *myo*-inositol phosphates (Greiner, unpublished observation). During enzymatic phytate dephosphorylation, an accumulation of *myo*-inositol tetrakisphosphate and –trisphosphate by the phytases from *C. braakii* (Pontopiddan *et al.*,

2012) and *E. coli* was observed, whereas the phytases from *P. lycii* and *A. niger* did not accumulate *myo*-inositol tetrakisphosphate, but only *myo*-inositol trisphosphate. Thermal stability is a particularly important issue since feed pelleting is commonly performed at temperatures between 60 and 95°C. Although phytase inclusion using an after-spray apparatus for pelleted diets and/or chemical coating of phytase may help bypass or overcome the heat destruction of the enzyme, thermostable phytases will no doubt be better candidates for feed supplements. Even though the phytases from *P. lycii*, *A. niger* and *E. coli* show optimal activity at about 55°C their susceptibility to high temperature at 80°C is quite different (Table 3).

Table 3. Temperature optima and stability of commercial phytases

Characteristics	*P. lycii*	*A. niger*	*E. coli*
pH 2.0, 37°C, 1 hour	50 - 55	50 - 58	55 - 60
Refolding capacity (after heat denaturation)	high	high	low
80°C, 10 min, 0.1 Naac pH 5.5, [residual activity]	32 - 38%	52 - 60%	72 - 85%
Feed pelleting [residual activity]: 70°C 80°C 90°C	 100% 86 - 90% 78 - 82%	 100% 68 - 85% 52 - 69%	 100% 86 - 90% 78 - 82%

The temperature optimum of the phytases from *Buttiauxella* sp. (Shi *et al.*, 2008) and *Yersinia* sp. (Huang *et al.*, 2006) was also determined to be 55°C, whereas the temperature optimum of the *C. braakii* phytase was reported to be 50°C (Kim *et al.*, 2003). After ten minutes at 80°C, the phytases of *Buttiauxella* sp. and *C. braakii* retained about 10% of their initial activity, whereas the residual activity of the *Yersinia* phytase was determined to be about 40%. Thus, the *E. coli* enzyme was the most thermostable among these phytases. Furthermore, the stability of phytases to pelleting was tested. Plant-based diets supplemented with phytase were conditioned for 30 seconds at 70, 80 and 90°C and pelleted. The percentages of recovery of the initial enzymatic activities are shown in Table 3. The *E. coli* and *P. lycii* phytases are more stable during pelleting than the phytase from *A. niger*. Recently the stability of the *C. braakii* phytase during pelleting at 80 and 90°C was determined in a feed for chickens for fattening. Recovery after pelleting was reported to be 106% and 94%, respectively (EFSA, 2012). Likewise, an enzyme that can tolerate long-term storage or transport at ambient temperatures is undisputedly attractive. Sulabo *et al.*, (2011) concluded from their studies, that the stability of commercially available phytases during storage is affected by numerous factors, such as storage time, temperature, product form, coating, and source (Table 4). Pure phytase products stored at 23°C or lower were the most stable. This suggests that storing phytases in pure forms may

have advantages in retaining the original phytase activity compared with including it in premixes when stored at 23°C or lower. In premixes, longer storage times and higher temperatures reduced phytase activity, but coating mitigated some of these negative effects. Among the phytase products studied, OptiPhos was the most stable in premixes (Table 4). Data of other commercial phytase products are available, but the conditions to obtain these data are different to those used by Sulabo *et al.* (2011). The pure Rovabio phytase *(P. funiculosum)* retained >90% of its initial activity after storage at 20°C for 90 days and in premixes after 30 days storage time (EFSA, 2007). The residual activity of the pure Quantum phytase (*E. coli*) was determined to be 86% and 52% after storage at 21 or 37°C for 18 months (EFSA, 2008). In vitamin-mineral premixes, no loss of activity was observed during storage for 84 days at 22°C. The residual activity of the pure Finase phytase (*E. coli*) was reported to be 95% after storage at 25°C for 12 months (EFSA, 2009). In premixes, recoveries were at least 73% after three months at 23°C. The pure Natuphos phytase *(A. niger)* retained >90% of its initial activity after 18 months at 20°C and 84% after 120 days at 35°C (EFSA, 2010). In premixes, the enzyme was stable (>95% residual activity) for six months. The mean recovery of the pure Ronozyme HiPhos GT phytase (*C. braakii*) activity after 18 months at 25°C was at least 90% (EFSA, 2012). Its mean recovery in vitamin-mineral premixes after six months at 25°C was reported to be >80%.

Table 4. Storage stability of commercial phytase products

Characteristics	OptiPhos	Phyzyme	Ronozyme
Feed pelleting [residual activity]:			
30 days	> 91%	> 91%	> 91%
60 days	> 85%	> 85%	> 85%
90 days	> 78%	> 78%	> 78%
120 days	> 71%	> 71%	> 71%
180 days	> 85%	> 85%	> 85% (uncoated only 50%)
Storage stability, pure enzyme, 37°C, 30 days			
coated	191 - 93%	69 - 74%	69%
uncoated	91 - 93%	69 - 74%	36%
Storage stability, 23°C, 180 days			
pure enzyme	> 85%	> 85%	> 85% (uncoated only 50%)
in mineral premixes	> 73%	> 73% (uncoated only 67%)	> 73%
in vitamin-mineral premixes	> 60% (uncoated only 67%)	> 60%	> 60%
Storage stability, 23°C, 360 days			
in mineral premixes	> 83%	> 75%	> 63%
in vitamin-mineral premixes	> 83%	> 75%	> 63%

Source: Sulabo et al., (2011)

Finally, a phytase will not be competitive if it cannot be produced in high yield and purity by a relatively inexpensive system. An advantage in respect to their heterologous production is the fact that all commercially available phytases are monomeric proteins. Furthermore, a high specific activity would be favorable in respect to an economic production of a phytase. The specific activities reported for the commercial phytases are 3457 U/mg (*C. braakii*) (Kim *et al.*, 2003), 1080 U/mg (*P. lycii*), 751-811 U/mg (*E. coli*) and 103 U/mg (*A. niger*). The phytases of interest for a future commercialization from *Buttiauxella* sp. and *Yersinia* sp. were determined to be 1180 U/mg (Shi *et al.*, 2008) and 1073-3960 U/mg (Huang *et al.*, 2008; 2008; Shivange *et al.*, 2012).

Last but not least, the expression system (*Aspergillus niger, Aspergillus oryzae, Pichia pastoris, Penicillium funiculosum, Schizosaccharomyces pombe, Trichooderma reesei*) used for phytase production has an effect on the enzymatic character of the phytase. All phytases, including the bacterial ones, are produced in eukaryotic hosts. Therefore, all phytases are glycosylated, which they are not in their native bacterial organisms. Thus, the choice of the expression system for phytases is not incidental. Expression of the phytase-encoding gene from *P. lycii* in different systems *(Aspergillus oryzae, Pichia pastoris)* for example resulted in differences in optimal pH, optimal temperature and thermal stability of the produced phytases. At least five *E. coli* phytases produced in three expression systems *(Pichia pastoris, Schizosaccharomyces pombe, Trichooderma reesei)* exist in the marketplace. All five are engineered uniquely, and three share the same expression system (Pichia pastoris). Due to the different molecular manipulations and the different production systems used, these phytases are expected to perform differently. Finally, post-fermentation activities such as product formulation and optimization can influence the properties of the final phytase preparation. Especially efficacy and storage stability of the phytase are dependent on product formulation.

Conclusion

Commercial phytases differ in their enzymatic properties such as optimum pH, pH profile, stability under stomach conditions (pH, pepsin), temperature stability, kinetic constants (vmax, km, kcat), and substrate specificity. Biochemical characterization of phytases is therefore essential, but enzymatic properties could only be guidelines on potential functionality in animal feeds and digestive systems. There is no unique point responsible for a better performance *in vivo*, but it is a conjunction of all factors. Biological efficacy can therefore only be fully determined by direct animal trials.

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