CAPÍTULO 4

CATALYTIC PROPERTIES OF PHYTASES FOR INCLUSION IN POULTRY AND PIG DIETS

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ABSTRACT: Cost reduction in animal production has generated wide discussion, especially because of the frequent use of foods containing anti-nutritional factors such as phytate, which impair the bioavailability of nutrients in non-ruminant animals. Addressing this challenge is crucial to ensure efficient and sustainable production. In this context, phytase enzymes stand out as essential tools, catalyzing the breakdown of phytate and increasing the availability of crucial minerals, such as phosphorus, thus improving the absorption of these nutrients by animals. This approach not only benefits the health of animals but also contributes to the economic efficiency of production operations by promoting more sustainable practices. This study aims to highlight the importance of phytase enzymes, covering their production, purification, biochemical characterization, and catalytic properties, providing valuable insights for implementing these enzymes in animal diets and, thus, promoting more sustainable and economically efficient production practices.
KEYWORDS: Exogenous enzymes, Animal production, Phytate.

PROPRIEDADES CATALÍTICAS DE FITASES PARA INCLUSÃO EM DIETAS DE AVES E SUÍNOS

RESUMO: A redução de custos na produção animal tem gerado ampla discussão, especialmente devido ao uso frequente de alimentos contendo fatores antinutricionais como o fitato, prejudicando a biodisponibilidade de nutrientes em animais não-ruminantes. Enfrentar esse desafio é crucial para garantir uma produção eficiente e sustentável. Nesse contexto, as enzimas fitases destacam-se como ferramentas essenciais, catalisando a quebra do fitato e aumentando a disponibilidade de minerais cruciais, como o fósforo, melhorando a absorção desses nutrientes pelos animais. Essa abordagem não apenas beneficia a saúde dos animais, mas também contribui para a eficiência econômica das operações de produção, promovendo práticas mais sustentáveis. Este estudo visa ressaltar a importância das enzimas fitases, abrangendo sua produção, purificação, caracterização bioquímica e propriedades catalíticas, proporcionando entendimentos valiosos para a implementação dessas enzimas em dietas animais e, assim, promovendo práticas de produção mais sustentáveis e economicamente eficientes.
PALAVRAS-CHAVE: Enzimas exógenas, Produção animal, Fitato.

INTRODUCTION

Reducing production costs has been a widely discussed issue in the context of animal production. Diet formulations often use foods that contain antinutritional factors, such as phytate, which reduce the bioavailability of nutrients and make digestion difficult for non-ruminant animals (Nascimento et al., 2023).

The importance of facing this challenge becomes even more evident when we consider the need to guarantee efficient and sustainable production (Nascimento et al., 2023). In this context, phytase enzymes have emerged as essential tools to mitigate the negative effects of phytate. By catalyzing the breakdown of this compound, phytases increase the availability of crucial minerals, such as phosphorus, thus improving the absorption and use

of these nutrients by animals (Nezhad et al., 2020). This approach not only favors animal health but also contributes to the economic efficiency of production operations (Rodrigues et al., 2023), promoting more sustainable practices aligned with contemporary demands.

Based on the above, this study aimed to address the relevance of phytase enzymes, covering the production, purification, and biochemical characterization of these enzymes, as well as the catalytic properties associated with them.

PHYTASE

Phytase was discovered by Suzuki et al. (1908) and further researched in 1960. The first commercial phytase was produced by *Aspergillus niger* with the ability to release phosphorus linked to phytate and reduce its excretion (Gourley et al., 2018). This enzyme was commercialized in 1991, known as Natuphos®, produced and introduced to the market by BASF® (Engelen et al., 1994; Selle and Ravindran, 2007).

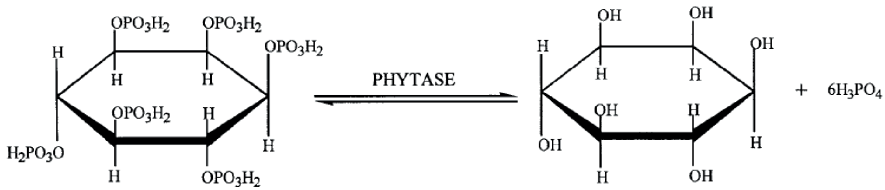
Phytases form a group of enzymes, generically called *myo-inositol hexaphosphate phosphohydrolase*, and are classified as specific monoester phosphatases that can be differentiated according to the position of hydrolysis in the phytic acid molecule (Selle and Ravindran, 2007).

According to the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB), two categories of phytases are recognized according to the position where phytate hydrolysis is initiated: 3-phytase (EC 3.1.3.8), which initiates the removal of an orthophosphate group from carbon 3, however 6-phytase (EC 3.1.3.26) carries out the reaction at carbon 6 of the *myo-inositol hexaphosphate* molecule, and 3-phytase is mainly of microbial origin (bacterial or fungal) and 6-phytase is derived from vegetables, associated with breaking dormancy in plant seeds, releasing orthophosphate groups from phytate for plant growth (Pandey et al., 2001; Konietzny and Greiner, 2002).

3-phytases (EC 3.1.3.8) are systematically called *myo-inositol hexakis*phosphate-3-phosphohydrolases that act on the hydrolysis of the ester bond in the third position of IP6 in IP5 and free orthophosphate (Vats and Banerjee, 2004). However, 6-phytases (EC 3.1.3.26) are chemically designated as myo-inositol hexakisphosphate-6-phosphohydrolase, which promotes the hydrolytic catalysis of the ester bond in the sixth position of IP6. Recently, this was reported for phytase produced by *A. niger*, which shows 3-phytase activity, whereas *Peniophora lycii* and *Escherichia coli* show 6-phytase activity (Selle et al., 2003).

The dephosphorylation reaction catalyzed by phytases is illustrated in Figure 1. In this reaction, phytic acid is transformed into phosphoric acid, inositol, and intermediate compounds (inositol phosphatides) including mono-, bi-, tri-, tetra-, inositol esters, and pentaphosphate (IP1-IP5) depending on the degree of dephosphorylation catalyzed by the enzyme (Konietzny and Greiner, 2002).

The catabolism pathway for the degradation of phytic acid follows a model of gradual dephosphorylation: IP6 → IP5 → IP4 → IP3 → IP2 → IP1 → inositol. Phytate has six groups that can be released, depending on the phytase used, at different speeds and in different orders. Phytases synthesized by filamentous fungi and the bacterium *Escherichia coli* can release only five of the six orthophosphate groups, with the final product being *myo-inositol* 2-monophosphate (Dasgupta et al., 1996).



$C_6H_{18}O_{24}P_6$ MW = 659.86

Figure 1. Enzymatic hydrolysis is catalyzed by phytase in the phytic acid molecule (Reproduced from Krishna and Nokes, 2001).

The determination of enzymatic activity using phytic acid salts, such as sodium phytate, allows the separation of phytases from other acid phosphatases, which are inefficient in hydrolyzing phytic acid. However, substrate specificity for phytase may vary because of differences in the molecular characteristics of enzymes purified from different sources (Ries, 2010). The released inorganic phosphate is measured by a colorimetric method, and reversed-phase high-performance liquid chromatography methodology has been well developed for the separation and determination of phytic acid and minor inositol phosphatides resulting from reactions catalyzed by phytases (Ries, 2010).

Colorimetric methods for determining inorganic orthophosphates are mainly based on the reduction of a phosphomolybdate complex by ferrous sulfate or ascorbic acid, which generates “blue molybdenum” (Engelen, et al., 1994). The colorimetric method for determining phosphorus, known as the ascorbic acid method of Fiske and Subbarow (1925), as well as adaptations arising from it, has been used for decades by several authors to determine the activity of phytases (Bindu et al., 1998).

Phytase activity can also be measured through the activity of acid phosphatases using synthetic substrates, such as *p*-nitrophenyl phosphate, as described by Ullah and Gibson (1986) and is widely used by several authors, according to Ries (2010).

Phytase activity is measured by the amount of inorganic phosphate released by the catalytic action of the enzyme. One unit of phytase enzyme activity can be defined as the amount of enzyme required to release one (1.0) µmol of inorganic phosphate per minute of reaction under conditions of pH, temperature, and phytate concentration in which the experiments were carried out (Selle and Ravindran, 2007).

Phytases have already been found in several plant sources, such as wheat, corn, some herbs, shrubs, lettuce, rye, and oilseeds, with the highest activities being found in wheat (*Triticum aestivum*), rye (*Secale cereale*) and in barley (*Hordeum vulgare*). Phytases obtained from germinating seeds and pollen were purified and characterized. Alkaline phytases have also been identified in the pollen of *Lilium longiflorum* and *Typha latifolia* and in legume seeds (Ramachandran et al., 2005). Vegetables are generally attributed to low values of phytase activity; however, Mroz et al. (1994) demonstrated that wheat phytase can improve phosphorus digestibility from 27 to 50%. Furthermore, Kornegay (1996) demonstrated that wheat phytase acts at a much lower pH limit than fungal phytase (Figure 2) and highlighted some advantages, such as greater substrate specificity and therefore a lower affinity constant (*K*m) of fungal phytase when compared with plant origin (Fireman and Fireman, 1998).

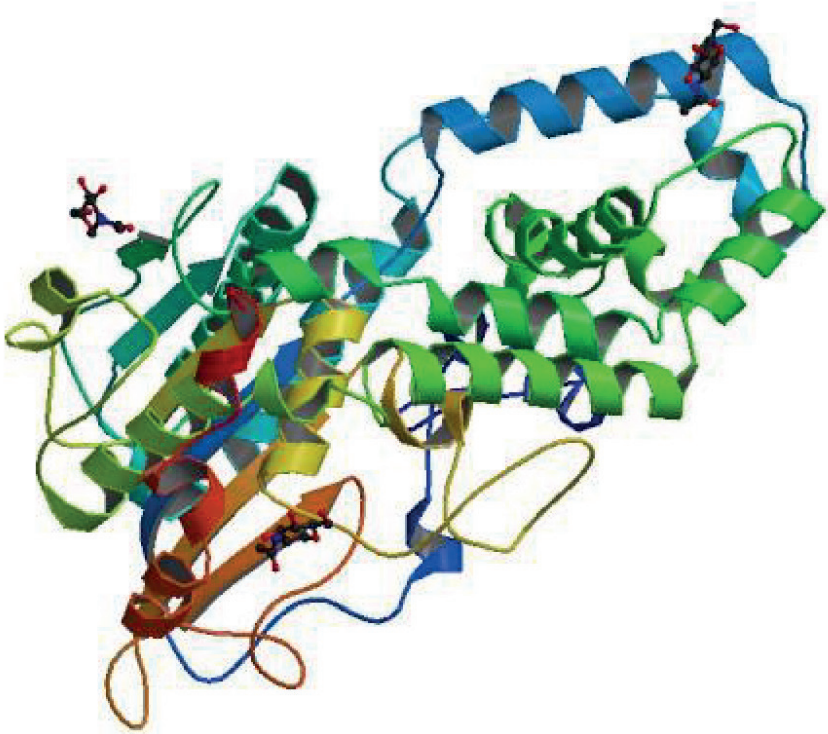


Figure 2. Molecular structure of phytase produced by Aspergillus niger (Oakley, 2010).

Although animal and plant sources represent important scientific advances, their practical applications in phytase production are limited. On the other hand, microbial sources have more desirable characteristics, which allow high yield and scale expansion and are thus widely and effectively used in the animal feed industry. Several species of fungi, bacteria, and yeast have been used in the production of phytases, genetically modified or not (Joudaki et al. 2023).

Some wild species of bacteria are used to produce phytase, mainly because of their enzyme production values and because they have an optimal pH range that is not suitable for the digestive system of non-ruminant animals. The activity of phytases can be intracellular or extracellular. When studied in *Enterobacter sp.*, 81.7% of activity was detected in the extracellular fraction, 4.4% in the periplasmic fraction, and the remainder in the intracellular portion (Roopesh et al., 2006).

PRODUCTION, PURIFICATION, AND BIOCHEMICAL CHARACTERIZATION OF PHYTASES

Fermentation is greatly influenced by several physicochemical variables in the biomolecule production medium. Cultivation conditions, microbial species and strain, nature of the substrate, and nutrient availability are important factors that affect the production and secretion of phytases, which are obtained from the addition of sodium phytate to the medium as a source of inorganic phosphorus (Ries, 2010).

The production of phytase has already been well cited in liquid media, i.e., through submerged fermentation; however, the use of agro-industrial residues for the production of phytase by fungi of the genus *Aspergillus* has been reported (Meta et al., 2009). Some studies have shown the use of alternative cultivation media in the production of phytases: oats in the production of phytase by *Kodamaea ohmeri* (Li et al., 2008), olive residues in the production of phytase by *Aspergillus niger* (Vassilev et al., 2007), and sugarcane molasses in the production of phytase by *Sporotrichum thermophile* (Singh and Satyanarayana, 2008) and by *Pichia anomala* (Vohra and Satyanarayana, 2004). Table 1 lists the main commercial phytases available on the world market.

Product	Company	Microorganism	Fermentation
Natuphos ®	BASF ®	*A. niger*	Liquid
Allzyme phytase ®	Alltech ®	*A. niger*	Solid
Phyzame ®	Femic ®	*A. oryzae*	Liquid
Ronozyme ®	Novozyme ®	*A. oryzae*	Liquid
Finase ®	AB Enzyme ®	*A. awamori*	Liquid
Amaferm ®	Biozyme ®	*A. oryzae*	Liquid
Bio-Feed Phytase ®	DSM ®	*Peniophora lycii*	Liquid

Table 1. Examples of commercial phytases available on the world market

The next stage in production is purification, which involves a set of processes that remove contaminants to obtain a product with a high degree of purity. Enzymes are purified by successive physicochemical methods, and the purification of phytases normally involves ultrafiltration, centrifugation, gel filtration, separation by chromatographic column, or even partial purification by ammonium sulfate precipitation. Fractionation according to solubility in ammonium sulfate solutions is widely used in protein isolation because it provides preparations with a lower degree of contamination and is more concentrated in proteins of interest (Ries, 2010).

The recovery of microbial enzymes, whether from plants or animals, is a complex step in the process of obtaining a biotechnological product because purification techniques depend on the specific molecular characteristics of each enzyme. During the purification process, enzymatic stability, purification efficiency, activity yield, and degree of purity must be considered. However, there are industrial enzymes whose application does not require a high degree of purity; therefore, chromatographic operations are not necessary because a simple concentration of the fermentation medium is sufficient for the commercialization of the bioproduct (Salmon, 2011).

Several methods for purifying phytases have been described in the literature, such as Spier et al. (2011), who used ion exchange chromatography followed by chromatofocalization. Greiner et al. (2009) used the following phytase purification methods: ammonium sulfate precipitation, molecular exclusion chromatography, ion exchange chromatography, and gel filtration chromatography. Guo et al. (2007) performed microfiltration and ion exchange chromatography.

Described in the literature are studies on the purification and characterization of phytases from different sources of origin, such as *Aspergillus ficcum* NRRL 3135 (Ullah and Gibson, 1986); *Schwanniomyces castelli* (Segueilha et al., 1992); *Bacillus subtilis* (Shimizu,

1993); *Bacillus sp*. DS11 (Kim et al., 1998); *Arxula adeninivorans* (Sano et al., 1999); *Peniophora lycci, Agrocybe pediades, Ceriporia sp.* and *Trametes pubescens* (Lassen, et al., 2001); *Pichia anomala* (Vohra and Satyanarayana, 2002); *Rhyzopus oligosporus* (Casey and Walsh, 2003); *Lilium longiflorum* (Garchow et al., 2006); *Aspergillus niger* (Casey and Walsh, 2003; Vats and Banerjee, 2006); and *Yersinia intermedia* (Huang et al., 2006).

Phytases generally have a molecular mass between 40 and 100 KDa and an optimal activity temperature between 45° - 60°C (Pandey et al., 2001). Most plant enzymes are denatured at temperatures above 70°C, whereas enzymes of microbial origin remain with significant activity after incubation periods of more than 3 hours (Konietzny and Greiner, 2002). Phytases isolated from *A. fumigatus* (Pasamontes et al., 1997) and *S. castellii* (Segueilha et al., 1992) have been reported to be resistant to high temperatures, i.e., they are thermotolerant (Ries, 2010). Table 2 describes the in vitro properties of some phytases of microbial origin.

Origin	Commercial name	pH stability (% activity)	optimal pH	Optimum temperature (°C)	Reference
-	Natuphos ®	2.5 - 6 (50 – 100%)	5.5	50	Igbasan et al., 2000
-	Ronozyme ®	3.5 – 5.0 (80%)	4.5	50	Igbasan et al., 2000
-	Ronozyme ®	4.0 – 4.5 (100%)	-	-	Brejnholt et al., 2011
-	Ronozyme ®	3.0 (35%)	-	-	Brejnholt et al., 2011
-	HiPhos ®	3.0 – 5.0 (70%)	3.5	-	Brejnholt et al., 2011
-	HiPhos ®	3.0 – 4.5 (90%)	3.5	-	Brejnholt et al., 2011
E.coli	-	3.5 – 5.5 (60%)	4.5	60	Igbasan et al., 2000
Bacillus sp.	-	5.5 – 7.5 (70%)	7.0	60	Igbasan et al., 2000

Table 2. *In vitro* properties and biochemical characteristics of commercial phytases of microbial origin

Among the phytases, most are active within the pH range 4.5–6.0 and can be classified according to the optimum pH for enzymatic activity, and act at pH around 5.0 (acidic) or 8.0 (alkaline). The stability of plant phytases decreases at pH values below 4 or above 7.5, whereas most phytases of microbial origin maintain activity between pH values below 3.0 and above 8.0. This large difference in their optimum pH values can be partially or fully reflected in the variation of the molecular structure or stereospecificity of the enzyme depending on the source of origin (Konietzny and Greiner, 2002).

Despite the availability of different commercial phytases, work must be developed to investigate the existence of new microbial sources that produce phytases, without the need for gene alteration, becoming frequent in the search for characteristics such as thermostability and acid stability.

CATALYTIC PROPERTIES OF PHYTASES

Phytases have been isolated and characterized from plants and various microorganisms, and depending on the optimum pH of the enzyme, they can be classified into acidic phytases and alkaline (basic) phytases. Owing to the acidic environment in the GIT of non-ruminant animals, there is a greater interest in phytases with an acidic profile. Acidic phytases are subdivided into 3 (three) structurally different groups: acidic histidine phosphatases, β-propeller phytases, and purple acidic phosphatases (Lei et al., 2007).

Phytases can recognize phytic acid as a substrate, which coincides with the non-hydrolyzing properties of non-specific phosphatases. In general, most enzymes belong to the histidine acid phosphatase (HAP) family, which is characterized by a conserved active site RHGXRXP and a catalytically active dipeptide HD (Ries, 2010). These phosphatase enzymes rarely have a similar structure, and the cleavage of phosphate groups is not performed by the same mechanism. This group of enzymes catalyzes the hydrolysis of phytic acid in two steps: nucleophilic attack of histidine from the active site of the enzyme on the phosphoester bond of phytic acid, which is easier to hydrolyze, and protonation of the remaining group by the aspartic acid residue of HD (Ostanin et al., 1992).

Xiang et al. (2004) determined the three-dimensional structure of phytase produced by Aspergillus fumigatus by crystallography. The enzyme has a small α domain and a large α/β domain in its molecular structure. The small α-helical domain consists of a central α-helical structure surrounded by seven α-helices. Relative to the large α/β domain, the β-sandwich filament forms the core structure, with two long α-helices on the opposite side. The conserved amino acid residues, the catalytic motif 58RHGARXP64, and the substrate binding motif 338HD339 are located between the large α/β domain and the small α domain.

It is worth highlighting another group of acid phytases, the β-propeller phytases, which have a three-dimensional conformation called β-propeller, which are dependent on calcium for their catalytic activity, such as the phytases produced by *Bacillus subtillis* and *B. amyloliquefaciens*. These phytases have two binding sites with the substrate, and hydrolysis is performed in the catalytic site itself (Monteiro, 2011).

Purple acid phosphatases are phytases classified as metalloenzymes with an iron or zinc atom in their catalytic site. There are examples of phytases from this group, including the phytases isolated from soybean (*Glycine max*) and the phytase produced by *A. niger* NRRL 3135. This group of phytases has lower catalytic activity than the group of acidic histidine phytases (Monteiro, 2011).

CONCLUSION

In short, the potential of phytase enzymes for application in the animal nutrition industry, especially in poultry and pig diets, is remarkable. The results presented in this literature review point to the feasibility of producing phytases in various industrial processes using microorganisms to improve nutritional efficiency, especially in the use of phytic phosphorus for non-ruminant animals.

REFERENCES

BINDU, S.; SOMASHEKAR, D.; JOSEPH, R. **A comparative study on permeabilization treatments for *in situ* determination of phytase of *Rhodotorula gracilis***. Letters Applied Microbiology 27(6): 336-340, 1998. doi: https://doi.org/10.1046/j.1472-765X.1998.00451.x

CASEY, A.; WALSH, G. **Purification and characterization of extracellular phytase from *Aspergillus niger* ATCC 9142**. Bioresource Technology 86(2): 183-188, 2003. doi: https://doi.org/10.1016/S0960-8524(02)00145-1

DASGUPTA, S.; DASGUPTA, D.; SEN, M.; BISWAS, S.; BISWAS, BB. **Interaction of myoinositoltriphosphate-phytase complex with the intracellular Ca2+ mobilization in plants**. Biochemistry 35(15): 4994-5001, 1996. doi: https://doi.org/10.1021/bi9525233

ENGELEN, A.J.; VAN DER HEEFT, F.C.; RANDSDORP, P.H.G.; SMTT, L.C. **Simple and Rapid Determination of Phytase Activity**. Journal of AOAC International 77(1): 760-764, 1994. doi: https://doi.org/10.1093/jaoac/77.3.760

Fireman, A.K.B.A.T.; Fireman, F.A.T. **Fitase na alimentação de poedeiras**. Revista Ciência Rural 28(3): 529-534, 1998. doi: https://doi.org/10.1590/S0103-84781998000300029

FISKE, C.H.; SUBBAROW, Y. **The colorimetric determination of phosphorus**. Journal of Biological Chemistry 66(2): 375-400, 1925. doi: https://doi.org/10.1016/S0021-9258(18)84756-1

GARCHOW, B.G.; JOG, S.P.; MEHTA, B.D.; MONOSSO, J.M.; MURTHY, P.P.N. **Alkaline phytase from *Lilium longiflorum:* Purification and structural characterization**. Protein Expression and Purification 46(2): 221-232, 2006. doi: https://doi.org/10.1016/j.pep.2005.07.031

GOURLEY, K M.; WOODWORTH, J.C.; DEROUCHEY, J.M.; DRITZ, S.S.; TOKACH, M.D.; GOODBAND, R.D. **Determining the available phosphorus release of Natuphos E 5,000 G phytase for nursery pigs.** Journal of Animal Science, 96(3): 1101–1107, 2018. doi: https://doi.org/10.1093/jas/sky006

GREINER, R.; SILVA, L.G.; COURI, S. **Purification and characterization of an extracellular phytase from *Aspergillus niger* 11T53A9**. Brazilian Journal of Microbiology 40(4): 795-807, 2009. doi: https://doi.org/10.1590/S1517-83822009000400010

GUO, M.J.; ZHUANG, Y.P.; CHU, J.; ZHANG, S.L.; XIONG, A.S.; PENG, R.H.; YAO, Q.H. **Production and purification of a novel thermostable phytase by *Pichia pastoris* FPHY34**. Process Biochemistry 42(12): 1660-1665, 2007. doi: https://doi.org/10.1016/j.procbio.2007.09.003

HUANG, H.; HUIYING, L.; YANG, P.; MENG, K.; WANG, Y.; YUAN, T.; BAI, Y.; YAO, B. **A novel phytase with preferable characteristics from *Yersinia intermedia***. Biochemical and Biophysical Research Communications 350(4): 884-889, 2006. doi: https://doi.org/10.1016/j.bbrc.2006.09.118

JOUDAKI, H.; ARIA, N.; MORAVEJ, R.; YAZDI, M.R.; EMAMI-KARVANI, Z.; HAMBLIN, M.R. **Microbial Phytases: Properties and Applications in the Food Industry.** Current Microbiology 80(12): 374, 2023. doi: https://doi.org/10.1007/s00284-023-03471-1

KIM, Y.O.; KIM, H.K.; BAE, K.S.; YU, J.H.; OH, T.K. **Purification and properties of a thermostable phytase from Bacillus sp. DS11.** Enzyme and Microbial Technology 22(1): 2–7, 1998. doi: https://doi.org/10.1016/S0141-0229(97)00096-3

KONIETZNY, U.; GREINER, R. **Molecular and catalytic properties of phytate-degrading enzymes (phytases).** International Journal of Food Science & Technology 37(1): 791-812, 2002. doi: https://doi.org/10.1046/j.1365-2621.2002.00617.x

KORNEGAY, E.T. **Effect of phytase on the bioavailability of phosphorus, calcium, amino acids, and trace minerals in broilers and turkeys**. BASF Technical Symposium World Congress Center, Atlanta, Georgia. January 23, 1996, p. 39- 70,1996.

KRISHNA, C.; NOKES, S.E. **Influence of inoculum size on phytase production and growth in solid-state fermentation by Aspergillus niger.** Trans. ASABE 44(4): 1031-1036, 2001. Available at: https://uknowledge.uky.edu/cgi/viewcontent.cgi?article=1106&context=bae_facpub

LASSEN, S.F.; BREINHOLT, J.; OSTERGAARD, P.R.; BRUGGER, R.; BISCHOFF, A.; WYSS, M.; FUGLSANG, C.C. **Expression, gene cloning, and characterization of five novel phytases from four basidiomycete fungi: *Peniophora lycci, Agrocybe pediades, Ceriporia* sp., and *Trametes pubescens*.** Applied and Environmental Microbiology 67(10): 4701- 4707, 2001. doi: https://doi.org/10.1128/AEM.67.10.4701-4707.2001

LEI, X.G.; PORRES, J.M.; MULLANEY, E.J.; BRINCH-PEDERSEN, H. **Phytase: Source, Structure and Application.** In: Polaina, J., MacCabe, A.P. (eds) Industrial Enzymes. Springer, Dordrecht. 505-529, 2007. doi: https://doi.org/10.1007/1-4020-5377-0_29

LI, X.Y.; LIU, Z.Q.; CHI, Z.M. **Production of phytase by a marine yeast Kodamaea ohmeri BG3 in an oats medium: Optimization by response surface methodology**. Bioresource Technology 99(14): 6386-6390, 2008. doi: https://doi.org/10.1016/j.biortech.2007.11.065

META, A.; NAKATAKE, H.; IMAMURA, T.; NOZAKI, C.; SUGIMURA, K. **High-yield production and characterization of biologically active recombinant aprotinin expressed in *Saccharomyces cerevisiae*.** Protein Expression and Purification 66(1): 22-27, 2009. doi: https://doi.org/10.1016/j.pep.2009.02.005

MONTEIRO, P.S. **Produção e Caracterização Bioquímica de Fitases de *Rhizopus stolonifer* e *Aspergillus niger* UFV-1 e suas aplicações em ração animal.** Tese (Doctor Scientiae). Universidade Federal de Viçosa, Minas Gerais, 2011. Available at: https://www.locus.ufv.br/bitstream/123456789/305/1/texto%20completo.pdf

MROZ, Z.; JONGBLOED, A.W., KEMME, P.A. **Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs.** Journal of Animal Science 72 (1): 126-132, 1994. doi: https://doi.org/10.2527/1994.721126x

NASCIMENTO, J.C.S.; RIBEIRO, A.G.; SILVA, D.A.; TEIXEIRA, J.A.C.; PORTO, T.S.; PORTO, A.L.F. U**tilização do sistema de duas fases aquosas para extração e purificação de fitases aplicada à nutrição animal.** Avances científicos y tecnológicos en ciencias agrícolas. 3ed. Ponta Grossa: Atena Editora 1: 7-50, 2023. doi: https://doi.org/10.22533/at.ed.11523101016

NEZHAD, N.G.; RAJA ABD RAHMAN, R.N.Z.; NORMI, Y.M.; OSLAN, S.N.; SHARIFF, F.M.; LEOW, T.C. **Integrative Structural and Computational Biology of Phytases for the Animal Feed Industry.** Catalysts 10(8): 844, 2020. doi: https://doi.org/10.3390/catal10080844

OAKLEY, A.J. **The structure of Aspergillus niger phytase PhyA in complex with a phytate mimetic.** Biochemical and Biophysical Research Communications 397(4): 745-749, 2010. doi: https://doi.org/10.1016/j.bbrc.2010.06.024

OSTANIN, K.; HARMS, E.H.; STEVIS, P.E.; ZHOU, M.; VAN ETTEN, R.L. **Overexpression, site-directed mutagenesis and mechanism of Escherichia coli acid phosphatase.** Journal of Biological Chemistry 267(32): 22830-22836, 1992. doi: https://doi.org/10.1016/S0021-9258(18)50022-3

PANDEY, A.; SZKACKS, G.; SOCCOL, C.R.; RODRIGUEZ-LEON, J.A; SOCCOL, V.T. **Production, purification and properties of microbial phytases**. Bioresource Technol 77(3): 203-214, 2001. doi: https://doi.org/10.1016/S0960-8524(00)00139-5

PASAMONTES, L.; HAIKER, M.; WYSS, M.; TESSIER, M.; LOON, A.P.G.M. **Gene cloning, purification, and characterization of a heat-stable phytase from the fungus *Aspergillus fumigatus*.** Applied and Environmental Microbiology 63(5): 1696-1700, 1997. doi: https://doi.org/10.1128/aem.63.5.1696-1700.1997

RAMACHANDRAN, S.; ROOPESH, K.; NAMPOOTHIRI, K.M.; SZAKACS, G.; PANDEY, A. **Mixed substrate fermentation for the production of phytase by *Rhizopus* spp. using oilcake as substrates.** Process Biochem 40(5): 1749-1754, 2005. doi: https://doi.org/10.1016/j.procbio.2004.06.040

RIES, E.F. **Estudo da produção, caracterização e aplicação de nova fitase de *Saccharomyces cerevisiae*.** Tese (Doutorado em Ciência de Alimentos) Universidade Estadual de Campinas, Campinas, São Paulo, 2010. Available at: https://repositorio.unicamp.br/acervo/detalhe/773707

RODRIGUES, E.J.D.; ITO, P.I.; RIBEIRO, L.F.M.; CARVALHO, P.L.P.F.; XAVIER, W.S.; GUIMARÃES, M.G.; FERNANDES JUNIOR, A.C.; PEZZATO, L.E.; BARROS, M.M. **Phytase Supplementation under Commercially Intensive Rearing Conditions: Impacts on Nile Tilapia Growth Performance and Nutrient Digestibility.** Animals 13(1), 136. 2023. doi: https://doi.org/10.3390/ani13010136

ROOPESH, K.; RAMACHANDRAN, S.; NAMPOOTHIRI, M.; SZAKACS, G.; PANDEY, A. **Comparison of phytase production on wheat bran and oilcake in solid-state fermentation by *Mucor racemosus*.** Bioresource Technology 97(3): 506-511, 2006. doi: https://doi.org/10.1016/j.biortech.2005.02.046

SALMON, D.N.X. **Desenvolvimento de um bioprocesso para a produção, caracterização e recuperação da fitase de *Schizophyllum commune* obtida por fermentação em estado sólido.** Dissertação (Mestrado em Processos Biotecnológicos). Universidade Federal do Paraná, Curitiba, 2011. Available at: https://acervodigital.ufpr.br/handle/1884/26974

SANO, K.; FUKUHARA, H.; NAKAMURA, Y. **Phytase of the yeast *Arxula adeninivorans*.** Biotechnology Letters 21: 33-38, 1999. doi: https://doi.org/10.1023/A:1005438121763

SEGUEILHA, L.; LAMBRECHTS, C.; BOZE, H.; MOULIN, G.; GALZY, P. **Purification and properties of the phytase from *Schwanniomyces Castelli*.** Journal of Fermentation and Bioengineering 74(1): 7-11, 1992. doi: https://doi.org/10.1016/0922-338X(92)90259-W

SELLE, P.H.; RAVINDRAN, V. **Microbial phytase in poultry nutrition**. Animal Feed Science and Technology 135(1): 1-41, 2007. doi: https://doi.org/10.1016/j.anifeedsci.2006.06.010

SELLE, P.H.; WALKER, A.R.; BRYDEN, W.L. **Total and phytate-phosphorus contents and phytase activity of Australian-sourced feed ingredients for pigs and poultry.** Animal Production Science 43: 475-479, 2003. doi: https://doi.org/10.1071/EA02155

SHIMIZU, M. **Purification and characterization of phytase and acid phosphatase produced by *Aspergillus oryzae* K1**. Bioscience, Biotechnology, and Biochemistry 57(8): 1354-1365, 1993. doi: https://doi.org/10.1271/bbb.57.1364

SINGH, B.; SATYANARAYANA, T. **Phytase production by a thermophilic mold Sporotrichum thermophile in solid-state fermentation and its potential applications**. Bioresource Technology 99(8): 2824-2830, 2008. doi: https://doi.org/10.1016/j.biortech.2007.06.010

SPIER, M.R.; SCHEIDT, G.N.; PORTELLA, A.C., RODRÍGUEZ-LEÓN, J.A., WOICIECHOWSKI, A.L.; GREINER, R.; SOCCOL, C.R. **Increase in Phytase Synthesis During Citric Pulp Fermentation**. Chemical Engineering Communications 198: 286-297, 2011. doi: https://doi.org/10.1080/00986445.2010.493115

SUZUKI, U.; YOSHIMURA, K.; TAKASHI, M. **Ueber ein Enzym, "Phytase" das "Anhydro-Oxymwthylen diphosphorsaure" Spaltet**. Tokyo Imper. Univ. Coll. Bull., v.7, p.503-512, 1908. Available at: https://publikationen.ub.uni-frankfurt.de/opus4/frontdoor/deliver/index/docId/9548/file/E001647381_2.pdf

ULLAH, A.H.L.; GIBSON, D.M. **Purification and characterization of acid phosphatase from cotyledons of germinating soybean seeds.** Arch Biochem Biophys 260(2): 514-520, 1986. doi: https://doi.org/10.1016/0003-9861(88)90476-6

VASSILEV, N.; VASSILEVA, M.; BRAVO, V.; FERNANDEZ-SERRANO, M.; NIKOLAEVA, I. **Simultaneous phytase production and rock phosphate solubilization by *Aspergillus niger* grown on dry olive wastes**. Industrial Crops and Products 26(3): 332-336, 2007. doi: https://doi.org/10.1016/j.indcrop.2007.04.003

VATS, P.; BANERJEE, U.C. **Catalytic characterization of phytase (myo-inositol hexakisphosphate phosphohydrolase) from *Aspergillus niger* van Teighem: Glycosylation pattern, kinetics and molecular properties**. Enzyme and Microbial Technology 39: 596-600, 2006.

VATS, P.; BANERJEE, U.C. **Production studies and catalytic properties of phytases (myo-inositol hexakisphosphate phosphohydrolases): an overview**. Enzyme and Microbial Technology 35(1): 3-14, 2004. doi: https://doi.org/10.1016/j.enzmictec.2004.03.010

VOHRA, A.; SATYANARAYANA, T. **A cost-effective cane molasses medium for enhanced cell-bound phytase production by Pichia anomala**. Journal of Applied Microbiology 97(3): 471-476, 2004. doi: https://doi.org/10.1111/j.1365-2672.2004.02327.x

VOHRA, A.; SATYANARAYANA, T. **Phytase production by the yeast *Pichia anomala*.** Biotechnology Letters 13: 551-554, 2002. Available at: https://link.springer.com/article/10.1023/A:1010314114053

XIANG, T.; LIU, Q.; DEACON, A.M.; KOSHY, M.K.; LEI, X.G.; HAO, Q.; THIEL, D.J. **Crystal structure of a heat-resilient phytase from *Aspergillus fumigatus*, carrying a phosphorylated histidine**. Journal of Molecular Biology 339(2): 437-445, 2004. doi: https://doi.org/10.1016/j.jmb.2004.03.057