Extraction and Purification of Phytase from *Aspergillus phoenicis* URM 4924 by Aqueous Two-Phase Systems using PEG/citrate

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Phytase (EC 3.1.3.8 e EC 3.1.3.26) is a generic term used to describe an enzyme that hydrolyzes phosphomonoester bonds from fitic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), thereby liberating inorganic phosphorous, consequently increasing the availability of phosohorous for the absorption. It is presumed to be plant storage form of phosphate which also happens to have considerable antinutritive effects for most animals. Phytate, a salt of acid phitic, is the major storage form of phosphorus in typical animal feedstuffs. Phytases, derived from *Aspergillus*, are frequently used in animal feed to improve phosphorous and mineral availability. A possible way to extract phytase from fermented broths is to use aqueous two-phase (ATPS), which are made up of two aqueous solutions of two water-soluble polymers or a polymers and a salt. They have a recently been used to separate biomolecules such as enzymes, other proteins and antibiotics. Their technical simplicity, easiness of scaling-up and suitability for continuos operation make this process a promising alternative for a large-scale operation. Statistical design of experiment is widely used for optimization and control of ATPS. The purpose this work were study the partition of phytase produced by *Aspergillus phoenicis* URM 4924 in ATPS composed by Polyethylrne glycol (PEG) and sodium citrate using factorial design of experiments. The influence of variables, namely PEG molar mass (M_{PEG}), PEG concentration (C_{PEG}) and citrate concentration (C_C) on the partition coefficient (K), was evaluated from the results obtained with a 2^3 factorial design plus a central point, which was run quadruplicate to allow estimation of pure experimental error. All statistical and graphical analyses were carried out with the Statistica 8.0. The phases were assayed for protein concentration and phytase activity. The results of partition coefficient demonstrate that the positive M_{PEG} effect was the most significant, i.e. this parameter increased (2.22 to 6.93) with increasing M_{PEG} (400 to 8,000 g/mol). On the contrary, when using high M_{PEG}, a remarkable volume exclusion effect might have taken place, hence suggesting that there was no space enough for the enzyme in the top phase of the system. Thus, in these experiments it was verified that the volume exclusion effect did not influence the partition of phytase. The best result was partition coefficient (6.93) were obtained when using citrate concentration 20% (m/m), PEG molar mass of 8,000 g/mol, and PEG concentration of 20% at pH 6.0. According to these results, the ATPS seems to be an interesting alternative to extract fitase for use in animal feed.

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