

Amendments of standards and specifications of Phytase

Ministry of Agriculture, Forestry and Fisheries (MAFF) will add standards and specifications to

“Ministerial Ordinance on the Specifications and Standards of Feeds and Feed Additives”
(Ordinance No. 35 of July 24th, 1976 of the Ministry of Agriculture and Forestry).

Major additions are as follows.

Phytase No.2 (6)

Specifications for method of manufacture of feed in general

Phytase No.2 (6) is allowed to be used as feed additives for pigs, broilers, layers, quails, fish and crustaceans only.

Specifications for feed additives

Active Substance

Compositional specifications

Unit $\geq 200,000$ phytic acid degradation/g

Physical and chemical properties

- (1) It comes in yellow-brown powder or particles.
- (2) pH of aqueous solution (1 part solute/100 parts solution) is 4.0 to 5.0.
- (3) It has the best enzyme activity when pH is between 2.5 and 5.0.

Purity test:

- (1) Lead: 0.1 g (0.95~1.04 g) of this product is weighed. When lead is tested using this sample by the lead test method (Method No. 1 of atomic absorption spectrophotometry test method), the concentration of the solution shall not exceed 5 $\mu\text{g/g}$.
- (2) Arsenic: Purity test No.2 for Active Substance of Phytase No.1.
- (3) Antibacterial activity: Purity test No.3 for Active Substance of Phytase No.1.

Loss on drying: $\leq 12.0\%$ (1 g, 105°C, 3 hours)

Ignition residue: $\leq 25.0\%$ (0.5 g)

Strength test of enzyme: Method No.3 of Strength test of decomposing phytic acid.

Preparation of Sample solution: 2.00 g (1.995 ~ 2.004 g) of this product is diluted with 0.2 mol/L citric acid/sodium citrate buffer (pH 5.5) to total 100mL. This solution is stirred at room temperature for 1 hour and centrifuged ($3,400 \times g$ for 10 minutes), and the supernatant is diluted with the same buffer to 0.1 phytic acid degradation/mL as sample solution.

Standard for method of manufacture

Genetically modified *Komagataella pastoris* should be cultured. The cultured solution should be

filtered. The bacteria in the cultured solution has to be removed by filtration, and the filtrate must be spray-dried.

Standard for method of storage

The products must be stored in light-shielding and airtight containers.

Standard for method of labelling

Containers or packaging of the products must be labeled that pH value (up to the first decimal place) showing the maximum enzyme activity.

Product No. 1**Compositional specifications**

This product comes in the form of liquid and is produced by adding aqueous solution containing sucrose to the Active Substance of Phytase No.2(6).

Unit This product contains the amount of Phytase corresponding to 85 to 170% of the phytic acid degradation unit of activity shown on the label.

Strength test of enzyme: Method No.3 of Strength test of decomposing phytic acid.

Preparation of Sample solution: 5 mL of this product is diluted with 0.2 mol/L citric acid/sodium citrate buffer (pH 5.5) to total 50mL. This solution is diluted with the same buffer to 0.1 phytic acid degradation /mL as sample solution.

Standard for method of storage

Same as the standard for method of Active Substance of Phytase No.2(6).

Standard for method of labelling

Containers or packages of the products must be labeled pH value (up to the first decimal place) that shows the maximum enzyme activity.

Product No. 2**Compositional specifications**

This product comes in the form of pieces, particles or powder and is produced by adding flour, mixing with aqueous solution containing α -starch to the Active Substance of Phytase No.2(6), granulating and drying. If necessary, it is mixed with excipient substances.

Unit This product contains the amount of Phytase corresponding to 85 to 170% of the phytic acid degradation unit of activity shown on the label.

Strength test of enzyme: Method No.3 of Strength test of decomposing phytic acid.

Preparation of Sample solution: Strength test of enzyme for Active Substance of Phytase No.2 (6).

Standard for method of storage

Same as the standard for method of Active Substance of Phytase No.2(6).

Standard for method of labelling

Containers or packages of the products must be labeled pH value (up to the first decimal place) that

shows the maximum enzyme activity.

Analytical method

Strength test of enzyme

Method No.3 of Strength test of decomposing phytic acid

(See Japanese attachment.)