AVIAN PEPSIN

Prepared at the 20th JECFA (1976), published in FNS 1B (1977) and in FNP 52 (1992). An ADI 'not specified' was established at the 20th JECFA (1976)

SYNONYMS None

SOURCES Commercial preparations of Avian Pepsin contain proteolytic enzymes obtained from the forestomach (proventriculum) of chicken or turkey.

Active principles: Pepsin (aspartic proteinase)

Systematic names and numbers: None (EC 3.4.23.1)

Reactions catalyzed: The enzyme preparations hydrolyzes polypeptides yielding peptides of lower molecular weight. It clots milk.

Assay: Activity not less than 85% and not more than 115% of the declared

DESCRIPTION Clear amber solution, tan suspension, or light tan powder

FUNCTIONAL USES Enzyme preparation
Used in clotting of milk in cheese making

GENERAL SPECIFICATION Must conform to the General Specifications for Enzyme Preparations used in Food Processing (See Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Pepsin activity: The sample shows milk clotting activity
See description in the Method of Assay

PURITY

Aflatoxin: Not more than 5 µg/kg
Determine as directed in Journal of the AOAC 49, 544 (1966); Pons et al., Determination of Aflatoxins in Agricultural Products: Use of Aqueous Acetone for Extraction.

Microbiological criteria: *Salmonella* spp.
Negative
Determine as directed in Section 10, Bacteriological Analytical Manual, 2nd. Edition (1969), U.S. Department of Health, Education, and Welfare, or Microbial Limit Tests, U.S.P. XIX, page 588.

Pseudomonas aeruginosa
Negative
Determine as directed under Microbial Limit Tests, U.S.P. XVIII, page 850, or U.S.P. XIX, page 588.

Coliforms
Not more than 30 per g
Determine as directed in Section 41.009, Official Methods of Analysis of the A.O.A.C., 11th Edition (1970), page 841.

Antibiotic activity — Negative when examined by suitable methods

METHOD OF ASSAY

Principle
Reconstituted milk is coagulated with an avian pepsin solution. The time required to form visible clot is compared with that obtained with a reference standard.

Definition of activity
The time of clotting T is related to the concentration C of active enzyme by the relation $T = t_o + kC$ where k is a proportionality factor which is constant for the same batch of milk, under constant conditions of pH, temperature and calcium ion concentration.

Procedure
To prepare the substrate solution, disperse 12 g of low-heat non-fat dry milk powder in 94 ml of 0.01 M calcium chloride solution contained in a 100-ml short-neck volumetric flask, stir magnetically 20-30 min then make up to mark with 0.01 M calcium chloride solution. Distribute 10-ml portions in separate test tubes and keep them at 30±0.2° for at least 10 min, but not more than 1 h. To prepare the enzyme dilutions, introduce into 50-ml or 100-ml volumetric flasks 2 ml and 4 ml respectively of 1.25 M sodium acetate buffer pH 5.7, add distilled water to about two-thirds of the volume of the flask and pipet accurately measured aliquots of the enzyme sample (dissolve, if powdered), and the reference enzyme, dilute to volume. To obtain the desired dilutions (about 1:5,000 strength, arrived at by diluting original sample solution between 25 and 200 fold), distribute 1-ml aliquots of each dilute enzyme solution in test tubes at (18x250 mm) and incubate at 30±0.2°.
To start the reaction pour rapidly the substrate solution contained in one test tube into that containing the dilute enzyme and start concomitantly a stop watch and mix the test solution twice by rapid inversion of the test tube and keep in a slanted position in the water bath kept at 30±0.2°. Rotate the test tube slowly by hand until the first clots are observed whereupon the watch is stopped and the time is recorded to the nearest 0.1 sec.

Calculation
Plot the clotting times obtained with enzyme dilutions being assayed and that with the reference standard against the dilution factors employed. Straight lines are obtained for clotting times between 50 and 300 sec. The ratio of the slopes of the lines are inversely proportional to the ratio between the concentrations of enzyme.