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FAO JECFA Monographs 1

COMBINED COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

All specifications monographs from the
1st to the 65th meeting (1956–2005)

Volume 4

Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications

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Calculation

One α-amylase dextrinizing unit (DU) is defined as the quantity of α-amylase that will dextrinize soluble starch in the presence of an excess of β-amylase at the rate of 1 g/h at 30°.

Calculate the α-amylase dextrinizing units in the sample as follows:

$$DU\ (solution) = 24/(W \times T),\ and$$

$$DU\ (dry\ basis) = DU\ (solution) \times 100/(100 - M),$$

in which W is the weight, in grams, of the enzyme sample added to the incubation mixture in the 5 ml aliquot of the Sample Preparation used; T is the elapsed dextrinizing time, in minutes; 24 is the product of the weight of the starch substrate (0.4 g) and 60 min; and M is the percent moisture in the sample, determined by suitable means.

Antibacterial Activity

Scope

This procedure is designed for the determination of antibacterial activity in enzyme preparation derived from microbial sources.

Principle

The assay is based on the measurement of inhibition of bacterial growth under specific circumstances.

Culture Plates

Six organisms are tested: *Staphylococcus aureus* (ATCC 6538); *Escherichia coli* (ATCC 11229); *Bacillus cereus* (ATCC 2); *Bacillus circulans* (ATCC 4516); *Streptococcus pyrogenes* (ATCC 12344): and *Serratia marcescens* (ATCC 14041).

Make a test plate of each organism by preparing a 1:10 dilution of a 24 h Trypticase Soya Broth culture in Trypticase Agar (TSA) (for *Streptococcus pyrogenes* a 1:20 dilution).

Pour 15 ml of plain TSA into a Petri dish and allow the medium to harden. Overlay with 10 ml of seeded TSA and allow to solidify. Place a paper disk prepared according to Disk Preparation of the tested enzyme on each of the six inoculated plates.

Disk Preparation

Make a 10% solution of the enzyme by adding 1 g of enzyme to 9 ml of sterile, distilled water.

Mix thoroughly with a Vortex mixer to obtain a homogeneous suspension. Autoclave suitable paper disks (for instance, S & S Analytical Filter Papers No. 740-E, 12.7 mm in diameter), then saturate them with the enzyme by application of 0.1 ml (about 3 drops) of a 10% solution of the enzyme to the disk surface. Prepare six disks (one for each of the six organisms) for each enzyme: place one disk on the surface of the six inoculated agar plates.

Incubation

Keep the six plates in the refrigerator overnight to obtain proper diffusion. Incubate the plates at 37° for 24 h. Examine the plates for any inhibition zones that may have been caused by the enzyme preparation.