LIPASE FROM ANIMAL

Prepared at the 15th JECFA (1971), published in NMRS 50B (1972) and in FNP 52 (1992). An ADI 'not limited' was established at the 15th JECFA (1971)

SYNONYMS Lipase, triglycerin lipase, tributyrase; INS No.1104

SOURCES Obtained from two primary sources, 1) edible forestomach of calves, kids and lambs and 2) animal pancreatic tissue. These preparations may be partially purified edible tissue preparations or they may be aqueous extracts.

Active principles: Triacylglycerol lipase

Systematic names and numbers: Triacylglycerol acylhydrolase (EC 3.1.1.3)

Reactions catalyzed: The enzyme preparations hydrolyze triglycerides or simple fatty acid esters yielding di- or monoglycerides plus free fatty acids.

DESCRIPTION Dispersible in water and insoluble in ethanol

FUNCTIONAL USES Enzyme preparation
Used in cheese making and modifications of lipids

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume 1, Introduction)

CHARACTERISTICS

IDENTIFICATION

Pregastric esterase activity: The sample shows pregastric esterase activity
See description under TESTS

Esterase activity: The sample shows esterase activity
See description under TESTS

TESTS

IDENTIFICATION TESTS

Pregastric esterase activity: According to Richardson, G.H. and J.H. Nelson, Assay and Characterization of Pregastric Esterase, J. Dairy Sci., 50, 1061-1065, (1967).

Principle
The action of an enzyme inoculum on the assay substrate is measured by a recording pH stat.
One lipase unit is defined as 1/250th of chart width or 0.01 ml of base.

Instrument

Equip a sargent recording pH stat (E.H. Sargent & Co., Chicago, Ill) with a 2.5 ml burette and 7 ml reaction vessels. Set the stat at v/5 vol with stirrer at 8 throughout the work. Adjust temperature and pH indicated. Fill the burette with 0.025 N NaOH. Use 1 ml of a 1:100 dilution of the sample as the inoculum.

Preparation of Enzyme Inoculum

Prepare the enzyme dilution by adding 0.200-1,000 g powder into a dry 100 ml volumetric flask. Add approximately 75-80 ml 0.5 M NaCl solution, and place the flask on a rotary shaker (120 rpm) for 19.0 min. Remove the flask, make up to 100 ml with 0.5 M NaCl and mix well. Remove the sample and inject so that inoculation occurs after a rehydration time of exactly 20 min.

Preparation of Substrate

Place 95 ml distilled water in a half-pint glass freezer jar suitable for attachment to an osterizer; add 2,600 g casein (Sheftone C 2 Soluble Casein Flour, Sheffield Chemical Co., Norwich, N.Y.), 0.5 ml 10% lecithin solution and 5.0 ml n-tributyrin. Tighten an osterizer head atop the jar. Homogenize the substrate mixture at low speed for 1 min. Cover spare substrate and temper to 42^{o} in a water bath until used.

Assay Procedure

Charge the small pH stat reaction vessel with 5 ml substrate and a magnetic stirring bar. To begin an assay, transfer a reaction vessel to the pH stat, and turn on the temperature control and stirring control. Turn the pH stat function control to Run. The unit adjusts to the control pH of 6.20. Then inoculate 1 ml of enzyme dilution.

Use the slope developed over the first 5 min following instrument pH adjustment to calculate activity. Prepare standard curves and compare with colorimetric assay for standardization of esterase activity.

Relative Precision

A comparison of the precision of the colorimetric vs. the pH stat method favoured the latter. Between substrates, as prepared from one day to the next and using the same source of enzyme powder, the pH stat method had a relative standard deviation of 8.4% compared with 17.2% for the colorimetric assay.

Esterase activity

According to Ramsay, H.A., Photometric Procedure for Determining Esterase Activity, J. Clin. Chem., 3, 185 (1957).

Principle

Fatty acid esters of 2-naphthol are employed as substrates. The 2-naphthol liberated by hydrolysis is coupled with a diazonium salt to form an azo dye, the concentration of which is estimated photometrically.

Reagents

- Stock solution of 2-naphthyl ester, 2.96×10^{-2} M: To a 10 ml volumetric flask add successively 3.75 g of melted Brij 35 (Brij 35, a polyoxyethylene lauryl alcohol, is a colourless, nonionic surfactant

having a melting range of approximately 40-44^{o} and is manufactured by Atlas Powder Company, Wilmington 99, Del), 0.296 M of a 2-naphthylester and approximately 5 ml of redistilled 1,4-dioxane, reagent grade. Mix the contents of the flask, warming slightly if necessary to obtain solution, and dilute to volume with additional dioxane. The ester should contain no free 2-naphthol or, at the most, only traces. Stock solutions of the caprylate and the palmitate esters are stable for at least several months if refrigerated.
- Phosphate or citrate buffer, 0.067 M. The choice of buffer will depend upon the pH optimum of the esterase.
- Phosphate buffer, 1.0 M, pH 6.8
- 4-Sulfamoylbenzenediazonium chloride solution: Prepare this reagent by mixing equal volumes of a solution of sulfanilamide, 0.035M in 0.48 N hydrochloric acid, and a solution of sodium nitrite, 0.042 M. Let this mixture stand for several min at room temperature, and then place it in an ice bath. When kept ice-cold, this reagent is stable for at least 6 to 8 h.
- Hydrochloric acid, 1.6 N
- Sodium hydroxide, 3.3 N

Note: 2-naphthyl caprylate and 2-naphthyl palmitate may be synthesized according to the general procedure of Nachlas and Seligman (Nachlas, M.M. and A.M. Seligman, J. Biol. Chem., 181, 343, (1949)) or of Gomori (Gomori, G., J. Lab. and Clin. Med., 42, 445, (1953)); they possess melting points at 43.0-44.0^{o} and 69.0-70.5^{o}, respectively.

Assay Procedure

Prepare a buffered solution of substrate in the following manner immediately prior to its use. All reagents should be at room temperature. Into a gently agitated mixture of 40 ml of 0.067 M phosphate or citrate buffer and approximately 50 ml of water, slowly add 1 ml of the stock solution of ester with a pipette, the tip of which is held beneath the surface of the mixture. Dilute to 100 ml with additional water.
Transfer 5 ml of the buffered solution of substrate to a 25 x 200 mm Pyrex test tube calibrated to contain 25 ml. Place the tube in a constant temperature water bath. After equalization of temperature, add 1 ml of an esterase preparation, and mix the contents of the tube thoroughly. Following incubation, the length of which is determined primarily by the rate at which the substrate is hydrolyzed, add 5 ml of 1.0 M phosphate buffer, pH 6.8. Immediately add 0.5 ml of 4-sulfamoylbenzenediazonium chloride solution. Wait exactly 1 min for the development of colour, and add 5 ml of 1.6 N hydrochloric acid. Place the tube in water which is kept at a rolling boil for 20 min. Cool the contents of the tube to room temperature. Add 5 ml of 3.3 N sodium hydroxide, letting it flow down the wall of the tube. Without undue agitation, dilute the contents of the tube to 25 ml with water and mix thoroughly. Measure the optical density of this solution at 460 nm using as a reference blank the solution from a concomitant control in which the esterase was inactivated prior to incubation by heating.
Many esterase preparations may be assayed simultaneously by staggering the addition of enzyme to the buffered solution of substrate at 2 min intervals. In order that all samples will be incubated for the same length of time, the sequential addition of 1.0 M phosphate buffer, 4-

sulfamoylbenzenediazonium chloride solution and 1.6 N hydrochloric acid, all of which requires approximately 1.5 min for a single tube, is staggered also at 2 min intervals. At this stage, i.e., after addition of the hydrochloric acid, subsequent steps do not need be performed immediately because the colour is stable if the mixture is not exposed to sunlight.

Standard Curve

From stock solutions of 2-naphthol in 1,4-dioxane and Brij 35, prepare buffered solutions of this compound in the same way that the buffered solution of substrate is prepared.

Take 5 ml samples of these buffered solutions, and, except for incubating them with esterase, subject them to the same procedure outlined in the assay technique. When measuring the optical densities of the final solutions, use as a reference blank the solution of a concomitant control without the 2-naphthol. The stock solutions of 2-naphthol are somewhat less stable under refrigeration than are those of the 2-naphthyl esters and, therefore, should be prepared immediately prior to their use. The standard curve is reproducible, thus obviating the preparation of standards for every series of assays.