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THE QUANTATIVE DETERMINATION OF LIPASE IN MILK

Ruth Reder*
Stillwater, Oklahoma

The question of the presence of a natural true lipase in milk remains unanswered despite the numerous studies of the subject which have been made by different investigators. The contradictory evidence which has been presented may be attributed in part to the unsatisfactory methods of study of enzyme action which were in use at the time of the earlier investigations. In some instances no attempt was made to prevent bacterial action, the results of which might be ascribed to enzymatic action since acids would be produced thereby and in those cases where a preservative was used it is possible that it may either not have been sufficiently powerful to check bacterial growth or that it may have been so active as to inhibit the action of the enzyme.

More recent investigations have produced equally conflicting results. Palmer (1), in 1922, presented a series of carefully controlled experiments from the results of which he drew the conclusion that milk does not normally contain a true lipase. He found, however, that the bitter milk

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which is frequently obtained during advanced lactation is caused by an active lipase which hydrolyzes the milk fat quite readily. In the same year, Rice and Markley (2), reporting experiments in which they employed a new method for the detection of lipase, reached the conclusion that lipase does occur as a normal constituent of milk. Roadhouse (3), in 1926, inferred the presence of lipase in "rancid" milk samples when he obtained an increase of 2.4 cc. O.1 N acid after incubation of the rancid sample for 23 days. Bacterial action was prevented by the use of formaldehyde.

In this laboratory during a study of the cause of a certain off-flavor in milk it became necessary to determine whether an active lipase was responsible for the abnormality. The present paper describes the method adopted for the quantitative determination of lipase in milk.

The procedure adopted for the estimation of lipase was that of Wilstatter, Waldschmidt-Leitz and Memmen (4) as modified by McGillivray (5) and adapted for the present experiment. In this method calcium chloride was used to accelerate hydrolysis and an ammonium chloride - ammoniumhydroxide buffer, pH 8.9 was added to produce a favorable reaction. A commercial preparation of pancreatic steapsin was used as the source of fat-splitting enzyme. The degree of hydrolysis produced by increasing amounts of this preparation was first determined using water as the diluting agent.

The enzyme, suspended in water, was diluted with sufficient water to keep the total volume of the water solution 13 cc. 2.5 g. olive oil (acid-free), 2 cc. buffer solution, pH 8.9 and 1 cc. calcium chloride solution were added and the mixture shaken vigorously by hand for 3 minutes and then placed in a thermostat at 30° C. for 57 minutes. The contents of the reaction vessel were washed into an Erlenmeyer flask with 95 per cent alcohol so that the volume of the alcoholic liquid was 125 cc. 20 cc. ether were added and the acid titrated with 0.1 N alcoholic NaOH in the presence of thymolphthalein. The degree of hydrolysis was determined by subtracting the initial acidity of the reaction mixture, determined in controls, from the acidity of the reaction mixture following incubation.

To test the applicability of this method of the estimation of lipase in milk, a second experiment was run in which milk took the place of water as the diluting agent. The milk was heated to 70° C. to inactivate any lipase present. Formaldehyde was added to the reaction mixtures to prevent bacterial action. From the degree of hydrolysis produced by the different amounts of the lipase when added to sterile milk, it was evident that the emulsion of milk and olive oil provides a substrate which is more favorable to the action of lipase than is the emulsion of water and olive oil. Using a one hour incubation period, it is possible to detect the presence of 0.6 mg. lipase preparation in milk. By increasing the incubation period from one hour to 24 hours, it was possible to detect the presence of 0.05 mg. lipase preparation. Because of the small amount of lipase which can be detected by this method, it appears to be suitable for the determination of lipase in milk.

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