The Importance of the Measurement of Enzyme Activity in Food Chemistry

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The history of enzymes is closely linked to advances in the fields of food chemistry and food technology. It can be reasonably claimed that it was in processes of these two fields that the actions of biocatalysts were first observed, used and studied. Milestones in this evolution were the reports of *Th. Paracelsus* (about 1526) on fermentation products, the discovery of the proteolytic action of the juice of *Carica papaya* by *G. Hughes* (1750), the observation of *L. Spallanzani* (1783) that meat was liquefied by the gastric juice of birds, the work of *I. C. Irvine* (1785) on the saccharification of starch and of *K. G. S. Kirchhoff* (1785) on the diastase of wheat. Later *J. Liebig* and *F. Wöhler* (1837) described an enzyme from almonds, emulsin, and *H. Erlenmeyer* (1874) worked on the invertase in honey.

The assay of enzymes characteristic of a certain food is carried out to determine the degree of freshness of the food, to detect particular treatments (pasteurization, sterilization) or to see if decay has started. This type of investigation was carried out in food chemistry long before the introduction of enzymatic methods of analysis into other fields.

The measurement of enzyme activity in foodstuffs is mainly for two purposes:

1. The enzymes are measured as indicators of the state of foodstuffs. These are some of the most important measurements.
2. Many compounds (poisons, preservatives, antibiotics, insecticides, *etc.*) inhibit the action of certain enzymes. The extent of the inhibition gives information on the type and amount of the inhibitor. If the inhibition is specific it can serve to identify the enzyme.

The determination of inhibitors and activators (refer to p. 7) by measurement of their effect on the activity of enzymes is now an important branch of analytical food chemistry [1]. However, this category will not be dealt with in this survey.

The determination of enzymes as indicators of the state of foodstuffs is one of the oldest and most important procedures in food chemistry. These procedures give information about different aspects of the state of the foodstuffs:

a) The presence of certain enzymes allows deductions to be made about the constituents of some foodstuffs. The activity of one of the enzymes characteristic of the foodstuff in question is measured.

b) Decaying foodstuffs are attacked by micro-organisms. Measurement of the enzyme activity of these micro-organisms indicates the extent of the deterioration.

c) Enzymes are thermolabile. Measurements of enzyme activity serve to check whether heating has taken place (pasteurization, sterilization) or to find out whether undesirable enzymes, which affect the stability of the foodstuff, are destroyed to the required extent by preliminary treatment (blanching, steaming) (*e.g.* peroxidase test, p. 895).

At present, assays of the activity of the majority of enzymes give information on a) and more especially on c).

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[1] *W. Shive*, Int. Z. Vitaminforsch. *23*, 392 [1952]; Review: *J. Schormüller*, Z. Lebensm. Unters. Forsch. *106*, 372 [1957]; *107*, 40, 257, 352 [1958]; *Th. M. Devlin*, Analytic. Chem. *31*, 977 [1959].

Amylase (Diastase)

Honey

Tests for diastatic enzymes were first carried out on honey *). To determine the diastase activity (see p. 854) the decrease in the amount of substrate (starch[3]) or the amount of hydrolysis products formed (reducing sugar[4]) is estimated. In the determination of the "diastase number" according to *Gothe*[5] starch solution is added and after incubation the starch-iodine reaction is followed (negative with fresh honey which has not been heated).

Flour and malt

The diastatic activity of a flour is of special importance in determining the sugar formation during the preparation of dough. It is determined by estimating the maltose formed on incubation of flour[6]. Similar methods are used to determine the diastatic activity of malt flours and baking powders containing malt and malt extracts[7]. The determination of the starch liquefying activity ("dextrinogen amylase") has acquired a certain importance in the evaluation of malt preparations and malt extracts[8].

Milk

As the amylase of milk is especially sensitive to heat, the assay of its activity can be used as an indication of the degree of heating of the milk. In the assay, the hydrolysis of starch is followed by means of the starch-iodine reaction[9] (as in the case of honey).

Phosphatases

Phosphatases have only recently been used as indicators of changes occurring in foodstuffs.

Milk

Concomitant with the introduction of pasteurization of milk was the need for a quick, reliable method for distinguishing heated from raw milk. This was required to check that the pasteurization temperature had been adhered to, and to see whether raw milk had been added to that already pasteurized. The most important enzymes contained in milk are phosphatases, amylases, peroxidases and the Schardinger enzyme (xanthine or aldehyde oxidase). Measurements of peroxidase and phosphatase have generally proved more successful to assess pasteurization than measurements of other enzymes. The phosphatases of milk[10] are inactivated within the temperature range used for either method of

*) Consequently, "honey diastase"[2] was recognized by law, so that according to the honey order of March 21st, 1930 (Germany) a honey is considered to be definitely spoiled if the diastase has been destroyed by heating the honey.

2) *J. Valin*, Ann. Falsificat. Fraudes *51*, 269 [1958].

3) *A. Auzinger*, Z. Unters. Nahrungsm. *19*, 65, 353 [1910]; *F. Gothe*, Z. Unters. Lebensm. *28*, 286 [1914]; *H. Weishaar*, Z. Unters. Lebensm. *65*, 369 [1933].

4) *E. Moreau*, Ann. Falsificat. Fraudes *4*, 65, 145 [1911]; *K. Täufel*, *M. de Mingo*, and *H. Thaler*, Z. Unters. Lebensm. *71*, 190 [1936]; *G. Gorbach* and *K. Barle*, Z. Unters. Lebensm. *73*, 530 [1937].

5) See also *F. Kiermeier* and *W. Köberlein*, Z. Lebensm. Unters. Forsch. *98*, 329 [1954].

6) *K. Ritter*, Z. ges. Getreidewesen *15*, 13 [1928]; *D. W. Kent-Jones* and *J. Soxby*, Z. ges. Getreidewesen *16*, 171 [1929]; *I. A. LeClerc*, Cereal Chemistry *9*, 53 [1951].

7) *W. Windisch* and *P. Kolbach*, Wschr. Brauerei *42*, 139 [1925]; *E. Drews*, Getreide, Mehl, Brot *3*, 40 [1949]; *L. Pollack-Egloffstein*, Malzextrakte, J. Weichherz, Berlin 1928.

8) *I. C. Lintner* and *P. Sollied*, Z. ges. Brauwesen *26*, 329 [1903].

9) *W. Wedemann*, Z. Fleisch-, Milchhyg. *35*, 301 [1925]; *S. Rothenfusser*, Z. Lebensm. Unters. *60*, 103 [1930]; *B. S. Gould*, J. Dairy Sci. *15*, 230 [1932]; *H. Kluge*, Z. Lebensm. Unters. *65*, 71 [1933]; *A. Schloemer* and *B. Bleyer*, Z. Lebensm. Unters. *80*, 425 [1940]; *E. J. Guy* and *R. Jenness*, J. Dairy Sci. *41*, 13 [1958].

10) *W. Haab* and *L. M. Smith*, J. Dairy Sci. *39*, 1644 [1956]; *40*, 546 [1957]; *R. K. Morton*, Biochem. J. *55*, 786, 795 [1953]; *J. Jacquet* and *O. Vilette*, Bull. acad. vét. France *27*, 429 [1954]; *W. Haab*, Schweiz. Milchztg. *84*, [1958], wiss. Beilage No. 57.

pasteurization (short or long heating) of milk[11]. They are therefore suitable for the detection of milk which has been so treated.

The determination of phosphatase activity was later also used to test for pasteurization in milk products, such as cream and butter[12], iced cream, ice cream, chocolate milk[13] or cheese[14].

In the evaluation of the results of the tests it should be borne in mind that, occasionally, heat inactivated phosphatases are "regenerated" by means which are not yet clear[15].

The substrates for the determination of phosphatase activity are very numerous, and include suitable esters which on hydrolysis yield phosphoric acid or other constituents that can be estimated[16]. The first substrate used for the measurement of phosphatase activity in milk was the disodium salt of phenylphosphate[17], then later other substrates were employed: the phosphoric acid ester of phenolphthalein[18] (see p. 779) and *p*-nitrophenylphosphate[19] (see p. 783). A real advance in the routine control of milk pasteurization was the introduction of the reliable and rapid method of *Scharer*[20] for measuring phosphatase activity. In this method the free phenol formed on hydrolysis of phenylphosphate can be easily and rapidly determined with 2,6-dibromoquinone-chlorimide by a spectrophotometric procedure (see p. 785).

In order to be able to compare the values given by the different methods, an "International Phosphatase Unit" has been proposed by the "International Commission of the World Milk Organization for the Standardization of the Analytical Methods for Milk and Milk Products". It is defined as the amount of enzyme which yields the same blue colour with disodium nitrophenylphosphate as that with 1 μg. ml. phenol in 5 ml. of an aqueous solution of 2,6-dibromoquinone-chlorimide buffered with Na_2CO_3 and $NaHCO_3$. The phosphatase unit is related to 1 ml. of milk.

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11) *H. D. Kay* and *W. R. Graham*, J. Dairy Res. *6*, 191 [1935]; see also *S. A. Hansen*, *F. W. Wood*, and *H. R. Thornton*, Canad. J. Technol. *31*, 240 [1953].

12) *W. Ritter*, 13th Int. Dairy Congr., Den Haag, Vol. III, 1014 [1953]; *S. Dijkstra*, Off. Org. K. ned. Zuivelb. *45*, 686, 704 [1953]; *S. Zocchi*, Igiene mod. *47*, 441 [1954].

13) *F. V. Kosikowski*, *A. G. Wollin*, and *W. F. Witter*, J. Dairy Sci. *38*, 1096 [1955].

14) *M. Hietaranta* and *P. Jussila*, Karjantuotteesta *24*, 25 [1949]; *J. H. Mahon*, *C. Anglin*, and *R. A. Chapman*, J. Assoc. Off. Agric. Chem. *38*, 482 [1955]; *R. Blasi*, Il latte *30*, 99 [1956]; *J. Schormüller* and *E. Lahmann*, Z. Lebensm. Unters. Forsch. *100*, 114 [1955]; *103*, 211 [1956].

15) *R. C. Wright* and *J. Tramer*, J. Dairy Res. *20*, 177, 258 [1953]; *21*, 37 [1954]; *23*, 248 [1956]; *E. Siegenthaler*, Mitt. Geb. Lebensm. Unters. Hyg. *45*, 84 [1954]; *R. C. Wright*, 14th Int. Dairy Congr., Vol. III, 717 [1956]; *H. Fram*, J. Dairy Sci. *40*, 19 [1957]; *B. Paschke*, Milchwiss. *13*, 73 [1958]; *J. B. Mickle*, *R. K. Diab* and *H. C. Olson* 33rd. Annual Meeting of the Southern Div., Amer. Dairy Sci. Assoc., Birmingham, Alabama. Febr. 1960.

16) Reviews: *G. Schwarz* and *O. Fischer*, Milchwiss. *3*, 41 [1948]; *G. Schwarz* and *W. Lange*, Dtsch. Molkerei-Ztg. Kempten *1951*, No. 35 and 36; *F. W. Gilcreas*, Rep. of the New York State Assoc. of Milk Sanitarians *1952*, 25; *A. Scout*, Bull. de l'Institut Agron. et des Stat. de Rech. de Gembloux *30*, 314 [1952]; *W. Ritter*, Milchwiss. *7*, 301 [1952]; Schweiz. Milchztg., Wiss. Beilage No. 3 [1953]; *M. Mohazzeb*, Milchwirtsch. Forsch. Ber. Kiel, No. 6 [1953] and No. 1 [1954]; *G. Schwarz* and *J. Ludwig*, Dtsch. Molkerei-Ztg. Kempten *75*, 566 [1954]; *R. W. Henningson* and *F. V. Kosikowski*, J. Dairy Sci. *42*, 1294 [1959].

17) *H. D. Kay* and *W. R. Graham*[11]; *A. H. Webb* and *F. Humphries*, Dairy Industr. *19*, 305 [1954].

18) *C. Huggins* and *P. Talalay*, J. biol. Chemistry *159*, 399 [1945]; *W. J. Tulloch*, J. Dairy Res. *22*, 191 [1955]; *G. Schwarz* and *W. Lange*, Dtsch. Molkerei-Ztg. Kempten *73*, 1001 [1952]; *H. Janecke* and *W. Diemair*, Z. analyt. Chem. *130*, 56 [1949].

19) *R. Aschaffenburg* and *J. E. C. Mullen*, J. Dairy Res. *16*, 58 [1949]; *F. W. Marriner*, Austral. J. Dairy Technology *1955*, 55; *A. J. Sommer*, Med. Bull. St. Louis Univ. *4*, 165 [1952]; *E. Siegenthaler*, Mitt. Lebensm. Unters. Hyg. *47*, 1 [1956].

20) *H. Scharer*, J. Dairy Sci. *21*, 21 [1938]; *R. W. Henningson* and *F. V. Kosikowski*, Amer. Dairy Sci. Assoc., 22. 6. 1955, Michigan State Coll., East Lansing; *F. V. Kosikowski*, J. Dairy Sci. *34*, 1151 [1951]; *C. A. Zittle* and *E. W. Bingham*, Arch. Biochem. Biophysics *83*, 25 [1960].

Lipase

Determination of milk lipase as an indication of fat break-down in milk and milk products has been extensively investigated in recent years, especially by Anglo-Saxon authors[21]. Methods of assay are given by *Dunkley*[22] and *Stadhouders*[23].

Peroxidase*)

Peroxidases occur extensively in foodstuffs. Measurement of their activity plays a decisive role in the preparation, treatment, preservation and storage of individual products. Testing for the inactivation of peroxidases in the fruit and vegetable industry or in the preparation of oatmeal is essential, since the presence of peroxidases in foodstuffs often causes undesirable changes, for example, of colour, smell and taste[24]. Besides, as peroxidase is a constituent of fresh milk it is an important indicator for the detection of the heating of milk.

Cereals and flour

The "peroxidase test" for measuring the inactivation of this enzyme in blanching, steaming, drying processes or autoclaving is usually carried out with reduced 2,6-dichlorophenolindophenol. With material containing peroxidase a blue colour occurs in the presence of H_2O_2[25]. The inactivation of peroxidase is often relatively inefficient, especially with vegetable material, because of the enzyme's high resistance to heat and because of the protective action of large amounts of inert material[26].

Milk

Due to the relatively high temperature stability of peroxidase[27] only the "high heating" process of treating milk (81—83° C) can be detected with certainty. Therefore it is possible to distinguish between milk which has been flash heated and that which has been heated for a prolonged period.

The reactions used for the detection of peroxidase in milk are numerous. The oldest procedure is the guaiacum reaction (*Arnold's* reaction[28]), in which a mixture of a solution of guaiacum resin in ethanol or acetone, H_2O_2 and unheated milk (often only with raw milk) gives a blue colour. The method has been frequently criticised and for good reasons. To make the reaction more reliable the "New official guaiacum reagent" was introduced. *Storch*[29], in the reaction named after him, used *p*-phenylenediamine as oxygen acceptor. A variation of this method is the "pepper and salt test" described by *J. Tillmans*[30], in which solid *p*-phenylenediamine and powdered barium peroxide are sprinkled

*) Assay, see p. 895.

21) *E. N. Frankel* and *N. P. Tarass*, J. Dairy Sci. *39*, 1506, 1517, 1523 [1956]; *40*, 418 [1957]; *D. P. Schwartz*, *I. A. Gould* and *W. J. Harper*, J. Dairy Sci. *39*, 1364, 1375 [1956].

22) *W. L. Dunkley* and *L. M. Smith*, J. Dairy Sci. *34*, 935 [1951].

23) *J. Stadthouders* and *H. Mulder*, The Netherl. Milk Dairy J. *12*, 117 [1958].

24) *F. Kiermeier*, Angew. Chem. *60*, 175 [1958]; *B. Hottenroth*, Fette, Seifen, Anstrichmittel *57*, 528 [1955].

25) *A. Beythien* and *W. Diemair*, Laboratoriumsbuch für den Lebensmittelchemiker. 7th Edition Th. Steinkopff, Dresden-Leipzig 1957, p. 68; *A. Purr*, Biochem. Z. *321*, 1 [1950]; *R. Heiss*, Dtsch. Lebensm. Rdsch. *48*, 129 [1952]; *W. Diemair* and *H. Häusser*, Z. analyt. Chem. *122*, 12 [1941].

26) *F. Kiermeier*, Dtsch. Lebensm. Rdsch. *43*, 75 [1947]; Biochem. Z. *319*, 463 [1949]; *F. Herrlinger* and *F. Kiermeier*, Biochem. Z. *317*, 1 [1944]; *318*, 413 [1948].

27) *B. Bleyer*, Handbuch der Milchwirtschaft. Springer, Vienna 1930, Vol. 1/1, p. 57.

28) *K. Arnold*, Arch. Pharmazie *219*, 41 [1881]; *G. Schwarz* and *G. Sydow*, Milchwiss. *8*, 424 [1947]; *B. Paschke*, Milchwiss. *7*, 3 [1952].

29) *V. Storch*, Z. Unters. Lebensm. *2*, 239 [1899]; *K. Utz*, Angew. Chem. *16*, 869 [1903].

30) *J. Tillmans*, Z. Unters. Lebensm. *24*, 61 [1912].

on the milk. Raw milk gives a deep-blue colour. To increase the sensitivity of the test *Rothenfusser*[31] recommended the addition of *p*-phenylenediamine hydrochloride and guaiacol. Later, sodium bisulphite was added to increase the stability of this reagent mixture ("*Rothenfusser*'s paratetraol-sulphite"[32]). Benzidine is also used as a reagent[33,34]. Many other substances have been proposed for the detection of peroxidase (*e.g.* iodine-potassium iodide-starch[35], dithizone[36], organic bases[37], malachite green[38]), but none of these have attained any real importance in the testing for peroxidase in foodstuffs.

The peroxidase reaction must be evaluated with care[39]. Traces of heavy metals, especially copper, can simulate peroxidase activity. In addition, as is the case with phosphatases, a "regeneration" of the enzyme has recently been observed[40].

Catalase*)

Milk

The determination of catalase is of importance in milk analysis. Normal, satisfactory milk contains no significant amounts of catalase. If the enzyme is found in milk it indicates the presence of leucocytes (disorders of secretion or diseases of the udder), of colostrum or of bacterial contamination.

The amount of enzyme is defined by the "catalase number" (the amount of H_2O_2, which is decomposed in 2 hours by 100 g. milk). The oxygen liberated enzymatically is determined volumetrically[41] for example, in *Lobeck's* Katalaser[42]. Frequently the residual amount of H_2O_2 is determined iodometrically or manganometrically[43]. Manometric[44] and electrometric methods[45] are also used.

Flour

The measurement of catalase activity plays an important role in the analysis of flour[46].

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*) Assay, see p. 898.

31) *S. Rothenfusser*, Z. Unters. Lebensm. *16*, 68 [1908].

32) *S. Rothenfusser*, Z. Unters. Lebensm. *60*, 94 [1930].

33) *S. Rothenfusser*, Z. Unters. Lebensm. *16*, 74 [1908].

34) *K. Eble* and *H. Pfeiffer*, Z. Unters. Lebensm. *60*, 311 [1930]; *68*, 203 [1934]; see also: *F. Bengen*, Z. Unters. Lebensm. *66*, 126 [1933]; *F. Bengen* and *E. Bohm*, Z. Unters. Lebensm. *67*, 379 [1934].

35) *V. Storch*[29]; *M. Siegfeld*, Angew. Chem. *16*, 770 [1903].

36) *K. Eble* and *H. Pfeiffer*, Z. Unters. Lebensm. *68*, 307 [1934].

37) *A. Casolari*, Biochemica Terapia sper. *16*, 167 [1929].

38) *R. Willstätter* and *H. Weber*, Liebigs Ann. Chem. *449*, 156 [1926].

39) *M. E. Schulz* and *G. Sydow*, Milchwiss, *10*, 151 [1955].

40) *B. Paschke*, Milchwiss. *10*, 154 [1955]; *F. Kiermeier* and *F. Herrlinger*, Biochem. Z. *322*, 106 [1951].

41) See *e.g. B. J. Pritzker*, Z. Unters. Lebensm. *30*, 49 [1915]; *A. J. Burstein* and *F. S. Frum*, Z. Unters. Lebensm. *62*, 489 [1931]; *A. Zeilinger*, Milchwirtsch. Forsch. *14*, 342 [1932]; *W. Diemair*, Z. Unters. Lebensm. *88*, 58 [1948]; *G. Roeder:* Leitfaden der Milchuntersuchung. Heinrichs, Hildesheim 1948.

42) *A. Beythien* and *W. Diemair*, Laboratoriumsbuch für den Lebensmittelchemiker. Th. Steinkopff, Dresden and Leipzig 1957, p. 182.

43) *C. J. Koning*, Milchwirtsch. Zbl. *3*, 67 [1907]; *H. v. Euler* and *K. Josephson*, Liebigs Ann. Chem. *452*, 158 [1927]; *E. B. Anderson* and *R. J. McWalter*, J. Soc. chem. Ind. *56*, Trans. 270 [1939].

44) *D. Appleman*, Analytic. Chem. *23*, 1627 [1951].

45) *K. Damaschke* and *D. Winkelmann*, Z. Naturforsch. *11b*, 85 [1956]; *K. Damaschke* and *F. Tödt*, Z. Naturforsch. *11b*, 621 [1956].

46) *A. Beythien* and *W. Diemair*[42]; *H. Thaler* in *E. Bamann* and *K. Myrbäck:* Die Methoden der Fermentforschung. G. Thieme, Leipzig 1951, Vol. III, p. 2844.

Xanthine (Aldehyde) Oxidase

Milk

The enzyme which was originally called "Schardinger enzyme"[47] occurs in milk; it is a molybdenum-containing "yellow enzyme"[48]. The enzyme in raw (not boiled) milk reduces methylene blue anaerobically to the colorless leuco-methylene blue in the presence of formaldehyde. This "Schardinger reaction" was one of the first enzymatic methods used in food analysis. However, it has lost its importance as an aid to the differentiation of raw and boiled milk and has been superceded by the reductase test. In addition, according to[49] it is not suitable for cow's milk during the lactation period or for differentiating human milk from cow's milk[50]. It does, however, in certain cases allow distinction to be made between cooled and non-cooled milk.

Reductases*)

Milk

Normal milk contains only small amounts of reductases, while milk contaminated with bacteria contains increasing amounts. The reductases decolorize methylene blue solution anaerobically (layer of liquid paraffin) in the same way as xanthine oxidase, but without the need for an additional acceptor. Good, commercial milk decolorizes the redox indicator in about 3 hours, while a decolorization time of less than 1 hour indicates bacterial contamination; short decolorization times coupled with low acidity of the milk sample are of doubtful validity.

The "reductase test"[51] can also be carried out with other redox indicators. Recently triphenyltetrazolium chloride and related tetrazolium salts have proved most successful. They give strongly coloured formazans which are insoluble in water[52], and which can be determined colorimetrically without the necessity of excluding air[53]. To ease bacteriological control in dairies, paper strips impregnated with triphenyltetrazolium salts are manufactured (Bactostrip[54]). The oxazine dye, resazurin (blue at neutral pH, red in acid[55]) and the azo dye, brillant black[56] are also extensively used.

Other Enzymes

Seeds

The viability of seeds can be determined by means of the measurement of reductase activity (tetrazolium method)[57].

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*) Assay, see p. 898.

47) *F. Schardinger*, Z. Unters. Nahr. Genußmittel *5*, 22 [1902].

48) *D. E. Green* and *H. Beinert*, Biochim. biophysica Acta *11*, 597 [1953]; *D. A. Richert* and *W. W. Westerfeld*, J. biol. Chemistry *203*, 915 [1953].

49) *F. Kiermeier* and *K. Vogt*, Z. Lebensm. Unters. Forsch. *105*, 194 [1957].

50) *H. Pagenstecher*, Mschr. f. Kinderheilk. *97*, 321 [1949].

51) *A. Beythien* and *W. Diemair*[42]; *W. Ritter*, Schweiz. Milchztg. *77*, 369 [1951]; *S. M. Charlett*, Dairy Ind. *20*, 576, 662 [1955].

52) *R. Kuhn* and *D. Jerchel*, Ber. dtsch. chem. Ges. *74*, 941, 949 [1941].

53) *J. Schormüller* and *H. Gerth*, Z. Lebensm. Unters. Forsch. *106*, 13 [1957]; *109*, 154 [1959]; *110*, 183 [1959].

54) *F. J. Förg*, Mitt. Lebensm. Unters. Hyg. *47*, 191 [1956]; Bactostrip AG, Zollikon-Zurich (Switzerland).

55) *K. L. Pesch* and *H. Simmert*, Südd. Molkerei-Ztg. Kempten No. *38*, Sept. 20th, 1928.

56) *J. Eisenbrand* and *A. Klauck*, Dtsch. Lebensm. Rdsch. *55*, 175 [1959].

57) *G. Lakon*, C. rend. l'Association International d'Essais de Semences *1*, 1 [1940]; Ber. dtsch. bot. Ges. *57*, 191 [1937]; *60*, 299; 434 [1942].

Research on the use of other enzymes as indicators in food analysis is still in progress. Of interest is, for example, lipoxidase[58] which occurs in soya beans[59], other beans and in flour[60]. It destroys fat by oxidation of unsaturated fatty acids and at the same time it decomposes carotene ("carotene oxidase")[61]. Also worth mentioning is nitrate reductase, a metal containing flavoprotein[62] which is concerned in the ripening of raw sausage[63] and the ripening of cheese in the presence of nitrate[64]. The numerous enzyme systems in ripening cheese suggest many other possibilities.

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58) *E. Andre* and *K. W. Hou*, Compt. rend. Acad. Sci. Paris *194*, 727 [1932].

59) *J. B. Sumner* and *A. L. Dounce*, Enzymologia 7, 130 [1939].

60) *A. M. Siddiqui* and *A. L. Tappel*, J. Amer. Oil Chem. Soc. *34*, 529 [1957]; *M. Rohrlich*, *F. Tödt* and *G. Ziehmann*, Fette u. Seifen *58*, 1057 [1956].

61) *H. Tauber*, J. Amer. chem. Soc. *62*, 2251 [1940]; *A. K. Balls*, *B. Axelrod* and *M. W. Kies*, J. biol. Chemistry *149*, 491 [1943].

62) *F. Egami* and *E. Murakami*, J. Biochem. *37*, 73 [1950]; *S. P. Colowick* and *N. O. Kaplan*, Methods in Enzymology. Academic Press, New York 1955, Vol. II, p. 403.

63) *K. Coretti*, Fleischwirtsch. *10*, 218 [1958]; *M. Lerche*, Fleischwirtsch. *8*, 752 [1956]; *F. Niinivaara* and *M. S. Pohja*, Fleischwirtsch. *9*, 789 [1957]; Z. Lebensm. Unters. Forsch. *104*, 413 [1956]; *106*, 187, 298 [1957].

64) *F. Kiermeier* and *K. Dentler*, Z. Lebensm. Unters. Forsch. *105*, 390 [1957]; *O. M. Ystgard*, *G. Syrrist* and *E. Brandsäter*, Internat. Dairy. Congr. *2*, 893 [1959].