Agricultural

Determination of Catalase in Agricultural Products. A. K. Balls and W. S. Hale. (*J. Assoc. Off. Agr. Chem.*, 1932, **15**, 483–490.)—The determination of catalase activity in agricultural products is beset by many difficulties, but the method described is satisfactory in so far that, with mixtures of clover and grain extracts, it yields additive results. Under the conditions specified the course of the catalytic reaction is unimolecular, and the constant is proportional to the amount of enzyme preparation used, but, owing to the fact that many factors seem to modify the results, this is not regarded as a proof that the true course of catalase reaction is unimolecular.

To prepare the enzyme material, 2 grms. of the substance are ground with sharp sand and 18 c.c. (20 c.c. if the substance is dry) of a mixture of 95 per cent. glycerol and 0·2 *M* phosphate buffer ($p_H = 7{\cdot}0$ to 7·2); it is best first to remove the air from the extraction fluid by boiling it for a short time in a vacuum. The grinding is continued for several minutes until the material is very finely divided, the resulting emulsion is centrifuged, and the supernatant liquid is again freed from air. A suitable quantity of the liquid is then allowed to stand for 5 minutes with one-tenth of its volume of boiled liver-juice (*vide infra*) and, if necessary, afterwards diluted with more glycerol and phosphate mixture prior to introducing a measured quantity (1 to 5 c.c.) into the hydrogen peroxide solution for the analysis. The amount of enzyme used should be sufficient to decompose at least one-half of the peroxide in 5 to 7 minutes. It is added from a measuring pipette into a stoppered cylinder containing 1 c.c. of 0·2 *N* hydrogen peroxide solution (*vide infra*), 4 to 6 c.c. of 0·2 *M* phosphate buffer ($p_H = 7{\cdot}0$), and approximately 1 grm. of freshly-dissolved dextrose in sufficient water to make, with the enzyme solution, a total volume of 50 c.c.; the cylinder and its contents must have been previously cooled in ice. The cylinder is replaced in the ice and, as soon as convenient, a 10 c.c. portion is rapidly pipetted into a flask containing 20 c.c. of 2 *N* sulphuric acid and 5 drops of saturated aqueous molybdic acid solution. Ten c.c. of 10 per cent. potassium iodide solution are added at once, and the liquid is left for 4 minutes, the iodine liberated being then titrated with 0·01 *N* thiosulphate solution.

Another 10-c.c. portion is removed after about 2 minutes, and a third after about 5 minutes, the moment when the delivery pipette is half empty being noted by a stop-watch in each case. The first titration gives the initial concentration

of the peroxide (a) and the subsequent ones the amounts of peroxide remaining ($a - x$) at the time of observation (t). Substitution in the formula $\frac{1}{t} \log \frac{a}{a-x} = k$ gives the constant, which varies with the amount of enzyme and serves as a direct measure of it. The value thus obtained may also be calculated back to the dry weight of the enzyme preparation (corresponding with the 10-c.c. portion), and thus to the catalase factor, which is the k units per grm. of dried material. Slight variations in the concentration of the hydrogen peroxide solution do not affect the results, and exact standardisation of the thiosulphate and peroxide is unnecessary. The method gives results agreeing to within about 10 per cent.

To prepare liver-juice, fresh liver is ground and mixed with approximately five times its weight of water, the liquid portion being removed and treated with about 1 per cent. of its volume of 0·1 N iodine; the mixture should not show free iodine. The liquid is heated rapidly to boiling and filtered, the filtered extract being quickly cooled. This extract retains its activity for several weeks if covered with toluene and kept cool. The hydrogen peroxide used must be free from stabilising preservatives and may be prepared by adding, gradually and with stirring, 7·8 grms. of the purest sodium peroxide to 1 litre of water containing 54·4 grms. of pure potassium dihydrogen phosphate and cooled to 0° to 2°. Practically no oxygen is evolved, and, after the sodium peroxide is dissolved, the solution should have p_H 7·0 to 7·1, and contains 0·2 N hydrogen peroxide and 0·2 M phosphate buffer. At a low temperature this stock solution keeps well.

T. H. P.