A NOTE ON THE PREPARATION OF STARCH SUBSTRATES FOR AMYLASE DETERMINATIONS

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In a recent communication (1) a sensitive precision method of estimation of amylolytic activity applicable to human serum has been reported. In order to avoid delay in preliminary preparation of the starch substrate, particularly where a series of observations in close succession is to be made, a modification was developed which consisted essentially of storage at 25° (after autoclaving as usual) of the solution of 3 gm. of soluble starch in 15 ml. of M sodium acetate and 45 ml. of water in the muslin-capped flask regularly used, followed by heating just to the boiling point before proceeding with the preparation as usual. Evaporation up to this point is unimportant quantitatively, as dilution to a final volume of 100 ml. follows (with certain other inclusions as described previously (1)). It is by no means obvious, however, that an equivalent substrate is obtained in this way; which is definitely not the case if instead the solution were stored in a refrigerator at 0–5°.

In the previous work (1) it was found convenient to take 1/ω as the index of activity of digestion mixtures, where ω is the time (in hours since mixing) such that a 7.5 per cent change in viscosity has occurred in the last three-fourths of this time. Relative values of ω with similar mixtures, with the same enzyme solution (pancreat-in), were obtained by division of ω found for a given mixture by the corresponding value for a mixture with regularly prepared substrate. Thus preparations from starch solutions stored 2 days at 25° were compared with the standard preparation, two in each of two experiments, giving respective values of relative ω, 1.002, 1.032, 0.963, and 1.008 (mean = 1.001, and average deviation = 0.019, approximately). Similarly, with storage for 5 to 6 days, the mean of four such evaluations was 1.010 with a.d. ≦ 0.030.
In contrast with this, two preparations with starch solution stored 2 days in a refrigerator (0–5°) gave relative $\omega$ values of 0.888 and 0.873, respectively.

In conjunction with a simple method of estimating $\omega$, which has been described (1) (a modified pantograph being used), there is automatically indicated a quantity, $\bar{y}_1$, defined as the relative viscosity of the mixture as estimated from the digestion curve at the time, $\omega/4$. In the first experiments described above the mean and a.d. of $\bar{y}_1$ for the standard preparation were 2.499 and 0.009, while for 2 days storage at 25°, $\bar{y}_1 = 2.548$ with a.d. = 0.009, and for 5 to 6 days storage, $\bar{y}_1 = 2.542$ with a.d. = 0.021. In contrast again the preparations after 2 days storage in the refrigerator gave $\bar{y}_1 = 2.646$ and 2.712, respectively. All digestions were followed in triplicate.

It appears, accordingly, that storage, for 5 days or less at 25°, of the preparation after autoclaving, followed by heating just to the boiling point before continuing as in the standard technique, is an admissible substitute. However, the proper value of $\bar{y}_1$ should be used if correction for viscosity elevation is required, as described (1) for the case where human serum$^1$ is used in the digestion mixture.

BIBLIOGRAPHY


$^1$ A unit of pancreatic amylase concentration based on a reproducible standard has been described (2) previously, against which the present system is standardized. It is worthy of note that this seems to be very nearly 10 times that used by Elman and his coworkers (3). Thirty-six combined normal values given by them for human serum (3) have a mean of 5.43 and relative standard deviation of 13.4 per cent, in comparison with which our first nine observations (1) gave a mean of 0.534 with relative standard deviation of 14.4 per cent.
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