CXLIII. A SIMPLIFIED METHOD FOR THE ISOLATION OF GLUTATHIONE FROM YEAST

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(*Received 25 May 1939*)

One of the difficulties in isolating glutathione from yeast is the extremely slow filtration encountered in removing the yeast residues after extraction. For example, in Pirie's [1930] method, which is probably the simplest thus far proposed, the filtration requires 4–6 hr. It has now been found that this time can be reduced to 20 min. by liberating the glutathione with acetone, extracting the yeast residue with water, and filtering in the presence of a large quantity of infusorial earth. The details of the method are as follows.

Experimental

Two lb. (900 g.) of pressed baker's yeast are crumbled coarsely by hand, dropped into 2500 ml. of acetone and stirred for 10 min. The yeast residue is filtered on an 8 in. Büchner funnel and washed once with a small quantity of acetone, suspended in about 1350 ml. of water and stirred for a few minutes, until homogeneous, with a strong electric stirrer; the larger clumps may be broken up by hand if necessary; 200 g. of infusorial earth are added, and the stirring is continued for a few minutes longer; the mixture is then filtered through a 12 in. Büchner funnel, about 1150 ml. of clear, light-yellow filtrate being obtained in 20 min.

The remainder of the procedure is essentially that described by Hopkins [1929]. The filtrate is made approximately 0·5*N* with respect to H_2SO_4 by addition of $\frac{1}{9}$ vol. 5*N* acid, and warmed to 50°. A suspension of 0·25–0·30 g. of Cu_2O in about 20 ml. of water is added in 2 ml. portions, with stirring.[1] The white cuprous glutathione formed is allowed to settle for 30 min. and, after decantation of the supernatant liquid, is washed by centrifuging until sulphate-free (usually 5 washings with 100 ml. portions of O_2-free water are sufficient). It is suspended in 35–40 ml. of water and treated with H_2S for 5–10 min. The cuprous sulphide formed is removed by centrifuging and filtering. After expulsion of the excess H_2S with a stream of nitrogen, the filtrate is transferred to a 3 in. crystallizing dish and evaporated to dryness by placing it overnight in a vacuum desiccator containing H_2SO_4. The clear glassy residue thus obtained is dissolved by slight warming, in about 7 ml. of water, and mixed with 3 or

[1] If desired, the cuprous oxide may be prepared immediately before use by heating 50–60 ml. of Benedict's qualitative sugar reagent (173 g. sodium citrate, 100 g. anhydrous Na_2CO_3 and 17·3 g. $CuSO_4$, $5H_2O$, made up to 1 l. with water) with excess glucose for a few minutes at 80–90° until the scarlet-coloured oxide appears. The hot suspension thus obtained is added directly, in small portions, to the yeast extract; 1 ml. of the reagent yields approximately 5 mg. of cuprous oxide. When this method is used, it is advisable first to add 20 or 25 ml. extra 5 *N* H_2SO_4 to the yeast extract to neutralize the alkali in the Benedict's reagent.

4 ml. of alcohol, a little more alcohol being poured over the surface of the solution. After seeding, the dish is placed over NaOH in a vacuum desiccator containing also a small beaker of alcohol. Crystallization begins in 2 or 3 hr., and is allowed to proceed overnight. The dry crystalline residue is transferred to a small funnel by means of 70 % alcohol, and washed first with 70 % and finally with absolute alcohol. It is dried over H_2SO_4.

The yields have ranged from 0·5 to 0·75 g., depending upon the original glutathione content of the yeast. Pirie [1930] reported somewhat higher yields (0·9 g. from 2 lb. of yeast); he, however, extracted his yeast residues three times instead of once. The purity of the product obtained as just described is sufficiently high for most purposes (98 %); found, for two different preparations, 10·68 and 10·27 % sulphur; theory, 10·42 %. Samples of three different preparations, titrated by the method of Woodward & Fry [1935], required per mg. 3·28, 3·26 and 3·32 ml. of 0·001 *N* iodate; theory, 3·26 ml. After one recrystallization from dilute alcohol the sulphur content was 10·43 %.

The cuprous glutathione precipitated in this procedure is impure, probably containing substances derived from the infusorial earth. The sulphate-free salt, after being washed with alcohol and dried to constant weight at 50° *in vacuo*, contains 15–16 % Cu (electrolytic determination) instead of the 17·2 % required by theory. This, however, causes no difficulty, since the impurities are removed in the subsequent H_2S treatment.

By this new method it is possible to complete precipitation of the cuprous glutathione within 1 hr., starting from the original yeast. When larger amounts of glutathione are needed, it is advantageous to carry several 2 lb. lots of yeast to this stage, the precipitates being combined before washing.

The yield of final product obtained represents about 50 % of the glutathione present in the original yeast, as shown by the specific glyoxalase method of Woodward [1935]. For example, a 2 lb. lot of pressed yeast contained 1·37 g. of reduced glutathione. The filtrate obtained on extraction (after acetone treatment, which liberates all the glutathione but removes none) contained 1·09 g., or 80 % of the total. About 80 % of this was precipitated as the cuprous salt, and the yield of crystalline glutathione obtained from the cuprous salt was also 80 %.

The acetone may be recovered by simple distillation. Extraction of yeast with aqueous acetone (60 %), as was done by Kozlowski [1931], yielded filtrates from which cuprous glutathione could not be precipitated directly in satisfactory form. Extracts prepared by first liquefying the yeast with a small quantity of acetone (100 ml./lb.), then extracting with water, contained little glutathione.

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