THE EFFECT OF STORAGE CONDITIONS ON THE STABILITY OF BOTH INVERTASE AND CATALASE OF ACTIVE DRY YEAST

By

MOHAMED I. MAHMOUD, Ph.D.

Women's College, Ain Shams University,

Heliopolis, Cairo, A.R.E.

Introduction :

Active dry yeast (ADY) is a dehydrated form of baker's yeast with a moisture content of about 8%. It has an advantage over fresh baker's yeast in the fact that it does not require refrigeration, and its sheif-life varies from 1-12 months depending on the storage conditions. Its stability is inversily proportional to the storage temperature, Theissen (1942), Morse and Fellers (1949) and Felsher et al. (1955). Also atmospheric oxygen has an adverse effect upon the storage stability of (ADY) Crane et al. (1955), and it has to be rehumified with water vapor before reconstitution to give satisfactory baking activity, Mitchell and Enright (1957), and Sant and Peterson (1958).

Peppler and Rudert (1953, Herrera et al. (1956), Ponte et al. (1960) and Chen et al. (1966) have thoroughly investigated the loss of viability of (ADY) on rehydration at different temperatures ranging from 4-50°C in relation to (ADY)'s original water content. Also they studied the stability of (ADY) on storage at wide ranges of temperature and relative humidities in the presence of atmospheric oxygen. They attributed the loss of yeast viabilty with leaching or extraction of vital cell constituents from dry cells on rehydration. This is due to the failure of the plasma membrane of the dry yeast cell to recover its original structure on rehydration.

The purpose of the present studies, however, is to investigate the effect of storing (ADY) under wide ranges of both temperature and relative humidity on the stability of some of its individual enzymes, namely invertase and catalase.

MATERIALS AND METHODS

Test Organism :

Commercial active dry yeast was used as the starting material for the present studies. It is available from the (Starch Products and Yeast Co.) as 750 g. capacity vacuum packed metal cans. The active dry yeast has a light tan colour with light odour, and a porous friable cylindrical structure of about 2 mm. diameter. It has to be mildly crushed to obtain small particles. After sieving, particle fractions of 0.7 — 1 mm. diameters were used. This fraction was found to have 6.5% water content, while cell viability was invariably above 90%.

Relative Humidity Control :

Moisture chambers were prepared using plastic containers of 12 cm. diameter and 8 cm. height. Each container has a plastic central well (3 cm. diameter and 5 cm. hight) fixed to its floor. About 40 gms. of (ADY) were transferred and equally dispersed over the floor of the plastic container, while the central well received specified saturated salt solutions in contact with its solid phase to give the desired relative humidity at the specified storage temperature. Relative humidity was controlled according to the tables presented by O'Brien (1948). The plastic containers were tightly sealed and stored at the desired temperature. Three levels of storage temperature were used, 20°C, 30°C and 37°C, while relative humidity ranged between 52 - 93%.

Acetone Drying of Humid (ADY) :

Storing (ADY) in atmospheres of high relative humidity increases its water content, so it was necessary to dry the cells without affecting its enzyme activity. This was done according to Umbreit et al. (1964), by shaking for 90 sec. about 2 g. of the stored (ADY) in a conical flask containing 150 ml. acetone at -10°C. Using a Buchner funnel, cells were filtered off the acetone, air dried and finally kept in a desiccator over $CaCl_2$ for at least three days.

Invertase Assay :

This was done according to Sumner and Howell (1935) with little modifications as follows :

250 mg. acetone dried (ADY) were transferred to 100 ml. capacity Erlenmeyer flask, to which one ml. distilled water was added. Cells were left to reconstitute for ten minutes at 30°C, then 40 ml. of 5%

sucrose solution in 0.01 M. acetate buffer pH 4.8 at 30°C were added. The incubation mixture was kept in a water bath at 30°C for 30 minutes and was shaken every 5 minutes. 0.5 g. solid sodium carbonate were then added to the incubation mixture to stop the reaction and let the sugar to mutarotate. Cells were centrifiged at 3000 r.p.m. for 15 min. The supernatant, however, was transferred to a polarimeter tube 150.3 mm long, and the optical rotation was determined using a Carl Zeiss Jena polarimeter with a sodium vapor lamp.

Catalase Assay :

This was done according to Maehly and Chance (1954) with little modifications : 150 mg. acetone dried (ADY) were transferred to 250 ml. capacity Erlenmeyer flask, and 36 ml. of 0.05 M. Phosphate buffer pH 7 were then added. Cells were left for 10 min. to reconstitute at 25°C. While the cell suspension was mechanically stirred at 25°C, 2 ml. of hydrogen peroxide solution (adjusted to give a final concentration approximately 0.03 M.) were added. After 30 sec. the reaction was stopped by adding 2 ml. of 50% sulfuric acid. Cells were then centrifuged for 15 min. at 3000 r.p.m. Residual hydrogen peroxide was determined by titrating 10 ml. portions of the supernatant with standard 0.03 N potassium permanganate solution. A blank was run without adding cells for determining the original concentration of hydrogen peroxide. Since on reconstitution (ADY) cells secrete some constituents with a reducing power, another blank was run without adding hydrogen peroxide. Catalase activity was expressed as the amount of consumed hydrogen peroxide expressed in ml. standard 0.03 N potassium permanganate solution used in titration, while invertase activity was expressed as the reduction in degrees of rotation. However, the stability of each enzyme was expressed as the percent of its original activity retained after storage.

Cell Viability :

Echigo et al. (1966) working on the viability of active dry yeast found that results obtained using the methylene blue staining method were in agreement with those obtained by the colony count method. In the present studies cell viability was determined using the mythylene blue staining method described as follows : 50 mg. (ADY) were hydrated with 1.5 ml. water at 30°C and stained with 0.01% methylene blue solution. Stained and unstained fractions were counted with a hemocytometer under the microscope. The percentage of the unstained fraction represents the viability of cells.

Water Vapor Adsorption Isotherm :

This was done according to Skujins and McLaren (1967). Ground (ADY) fractions with particle diameter of not more than 0.4 mm. were used. Samples containing 100-150 mg. of (ADY) were put in a desiccator over phosphorous pentoxide. After reaching a constant weight, they were placed in moisture chambers at 20°C, 30°C and 37°C, and the relative humidity ranged between 7 - 97%. Samples were weighed every 3 hours till maximum increase in weight was reached. This takes about 48 hours.

Cell Autolysis :

After reaching maximum increase in weight, samples were kept in the moist chambers and weighed every 24 hrs. for 7 days and the decrease in weight was recorded. Decrease in weight is due to cell autolysis which starts in cells after they reach a critical moisture content at which autolytic enzymes become active.

RESULTS AND DISCUSSIONS

Both invertase and catalase are surface enzymes fixed to the cell membrance, Kaplan (1965), and by virtue, they will be directly affected by the test conditions.

Fig. 1 represents the stability of both invertase and catalase after 4 week storage at 20°C, 30°C and 37°C, at relative humidities ranging between 52-93%. One notes that storage at 20°C showed some protection on catalase stability. Yet, relative humidities higher than 70% extered a deletorious effect on catalase stability irrespective of the storage temperature.

Fig. 2, however, presents invertase stability after both 10 and 16 weeks storage at 20°C, 30°C and 37°C. One notes that, although invertase showed relatively high stability over catalase, yet, prolonged storage at relatively high temperatures and relative humidities exerted a deletorious effect on its stability.

Rates of cell autolysis as a function of both temperature and relative humidity shown in Fig. 3, run parallel to the water adsorption isothern of whole yeast cells. Practically, no cell autolysis could be recorded for cells stored at relative humidities lower than 30%. Also, higher rates of cell autolysis were obtained at higher temperatures. This is in close agreement with the viable count of stored yeast cells presented in Table 1. So thermosensitivity of both enzymes at higher ranges of relative humidity could be attributed to cell

autolysis. This does not completely hold true for catalase, since after 4-weeks storage at about 93% R.H. (ADY)'s viable cell counts were 72%, 55% and 0% at 20°C, 30°C and 37°C respectively. However, catalase activities were almost negligible under the same conditions.

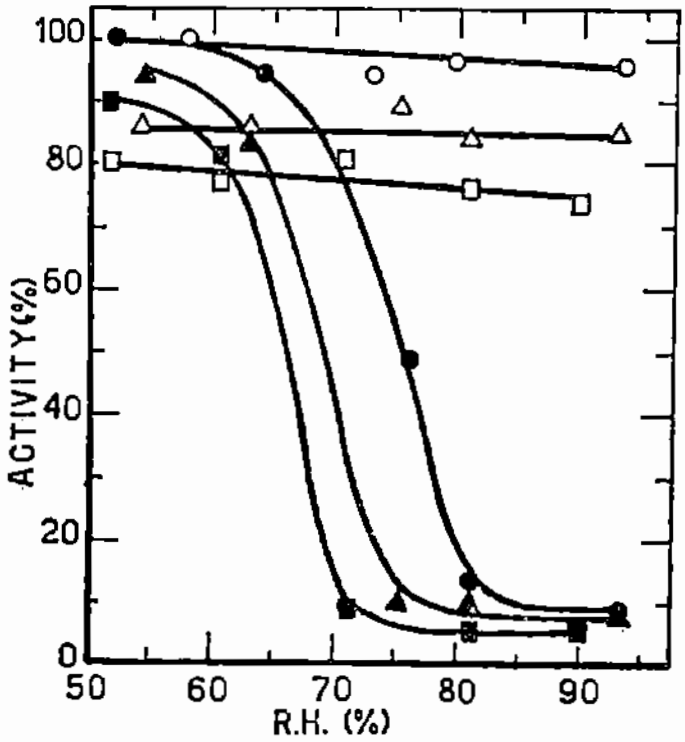

Fig. 1 — Stability of (ADY)'s invertase and catalase after four weeks storage as a function of relative humidity. Open circles, triangles, and squares are for invertase at 20°C, 30°C and 37°C, respectively. Solid circles, triangles and squares are for catalase at 20°C, 30°C and 37°C, respectively.

Koga et al. (1966) studied the physical properties of cell water in partially dried baker's yeast. They found that the physical states of the microbe can be classified into four regions in accordance with the states of cell water : the localized water region, the mobile adsorption region and the gel region, when cell possess water centent up to 5%, 10% and 20% respectively. Cells possessing water contents higher than 20% are said to be in the solution region.

From the water adsorption isotherm, one notes that cells stored at 60% R.H. possess 10% water content, while cells stored at 80% R.H. possess 20% water. It was found that storing (ADY) for four weeks at relative humidities higher than 80% had a deletorious effect over

catalase activity, while invertase was only slightly affected. So catalase stability is highly affected by its degree of hydration.

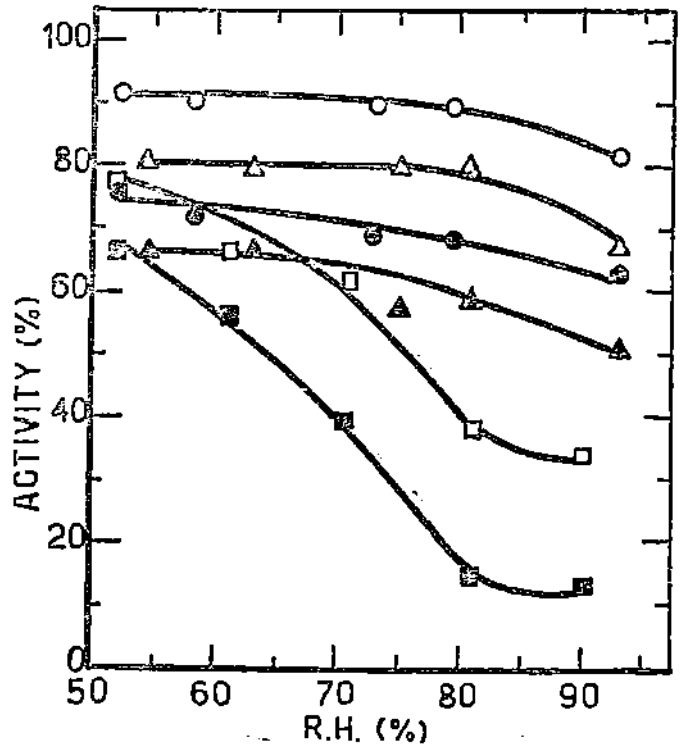

Fig. 2 — Stability of (ADY)'s invertase after ten and 16 weeks storage as a function of relative humidity. Open circles, triangles and squares are for ten weeks storage at 20°C, 30°C and 37°C respectively. Solid circles, triangles and squares are for 16 weeks storage at 20°C, 30°C and 37°C respectively.

TABLE 1

The effect of storage conditions on the viable count of active dry yeast cells.

Temperature	20°C			30°C			37°C	
Weeks	10	16		10	16		10	16
RH	%	%	RH	%	%	RH	%	%
52%	90	81	54%	90	74	52.4%	62	37
58%	90	69	63%	69	38	61.5%	59	5
73%	59	51	75%	27	0	71.5%	0	0
79%	45	41	81%	8	0	81.7%	0	0
93%	43	0	93%	0	0	90.0%	0	0

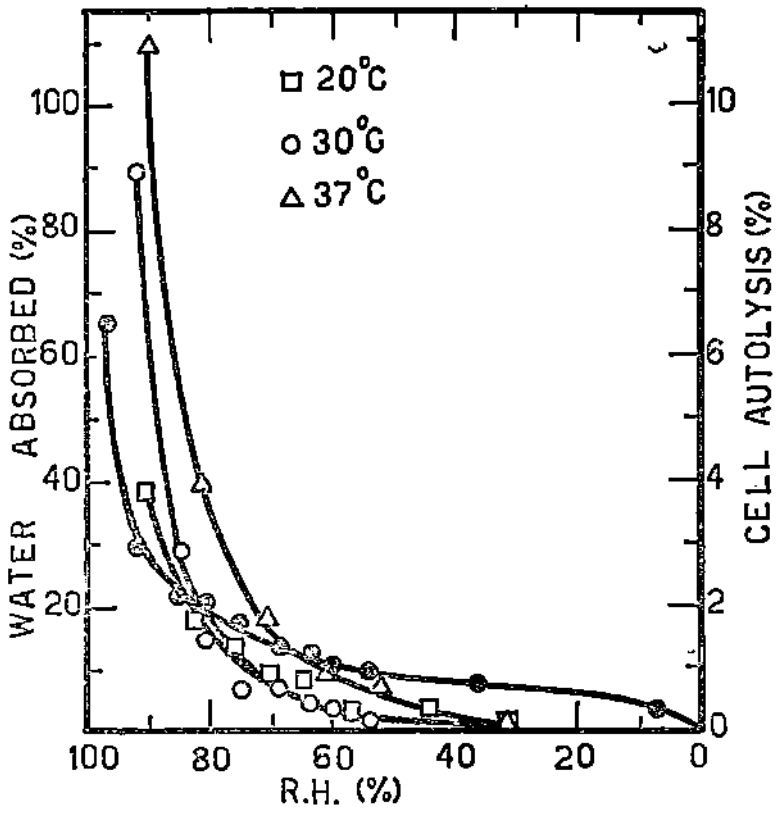

Fig. 3 — Solid circles are for water adsorption isotherm of whole yeast cells at 30°C. Open squares, circles and triangles are rates of cell autolysis per week at 20°C, 30°C and 37°C, respecively.

Sumner and Dounce (1937) have demonstrated that catalase is a conjugated protein with four iron atoms per molecule. Also Neumann and Lampen (1967) recently studied the chemical composition of invertase. The enzyme was shown to be a glycoprotein which contains about 50%, carbodydrate, predominently mannan with small percentage of glucan. This raises the question whether conjugation of mannan with the protein might play a role in the stability of invertase at higher degrees of hydration.

ABSTRACT

Temperature, relative humidity and atmospheric oxygen have already been shown to exert a deletorious effect on the storage stability of active dry yeast (ADY) cells. Stability of (ADY)'s individual

enzymes namely invertase and catalase, was investigated on storage at different temperatures and relative humidity in the presence of atmospheric oxygen.

Catalase showed a high sensitivity to the test conditions, while invertase was found to have higher stability over catalase.

Storage instability under the test conditions was partially attributed to cell autolysis which starts on storing (ADY) at both high temperature and relative humidity. The structure of both catalase and invertase may play a role in the noticed difference in the stability of both enzymes under the test conditions.

REFERENCES

1. Chen, S.L., E.J. Cooper and F. Gutmanis, 1966. Active Dry Yeast : Protection against oxidative deterioration during storage. Food Techol. 20, 1585.

2. Crane, J.C., H.K. Steels and S. Redfern, 1952. Techniques for estimating the stability of food products : Active dry yeast. Food Technol. 6, 220.

3. Echigo A., T. Fujita and S. Koga, 1966. Relationship between biological and physical properties of dry yeast cells. J. Gen. Appl. Microbiol. 12, 91.

4. Felsher A.R., R.B. Koch and R.A. Larson, 1955. The stability of vacuum packed dry yeast. Cereal Chem. 32, 117.

5. Herrera, T., W.H. Peterson, E.J. Cooper and H.J. Peppler, 1956. Loss of cell constituents on reconstitution of active dry yeast. Arch. Biochem. Biophys. 63, 131.

6. Kaplan, J.G. 1965. Action of non-penetarating heavy metals on the catalase activity of yeast cells. Nature, London, 205, 76.

7. Koga, S., A. Echigo and K. Nunomura, 1966. Physical properties of cell water in partially dried Saccharomyces cerevisiae. Biophys. J. 6, 665.

8. Maehly, A.C. and B. Chance, 1954. The assay of Catalases and peroxidases. Methods of Biochemical Analysis Vol. 1, 357. Interscience Publishers Inc., New York, U.S.A.

9. Mitchell, J.H. Jr. and J.J. Enright, 1957. Effect of low moisture levels on the thermostability of active dry yeast. Food Techol. 11, 359.

10. Morse R.E. and C.R. Fellers, 1949. Storage studies on active dry baker's yeast. Food Techol. 3, 234.

11. Neumann, N. P. and J. O. Lampen, 1967. Purification and prope.ties of yeast invertase. Biochemistry 6, 468.

12. O'Brien, F.E.M., 1948. The Control of Humidity by saturated salt solutions. J. Scientific Instruments, 25, 73.

13. Peppler, H.J. and F.J. Rudert, 1953. Comparative Evaluation of some methods for estimation of the quality of active dry yeast. Cereal Chem. 30, 1946.

14. Ponte, J.G., R.L. Glass and W.F. Geddes, 1960. Studies on the behaviour of active dry yeast in bread making. Cereal Chem. 37, 263.

15. Sant. R.K. and W.H. Peterson, 1958. Factors affecting loss of nitrogen and fermenting power of rehydrated active dry yeast. Food Technol. 12, 359.

16. Skujins, J.J. and A.D. McLaren, 1967. Enzyme reaction rates at limited water activites. Science, 158, 1569.

17. Sumner, J.B. and A.L. Dounce, 1937. Crystalline Catalase. J. Biol. Chem. 121, 417.

18. Sumner, J.B. and S.F. Howell, 1935. A method for determination of Saccharase activity. J. Biol. Chem 108, 51.

19. Theissen, E.J. 1942. The effect of temperature upon the viability and baking properties of dry and moist yeast stored for various periods. Cereal Chem. 19, 773.

20. Umbreit, W. W., R. H. Burris and J. F. Stauffer (1964). « Manometric Techniques », Fourth ed. (1964) p. 149. Burgess Publishing Co., U.S.A.