SPECIAL ARTICLES

CRYSTALLINE TURANOSE

THE trisaccharide melezitose can be hydrolyzed by weak acids to yield glucose and a disaccharide which was named turanose by its discoverer Alekhine. Turanose has in turn been hydrolyzed by G. Tanret, using stronger acids, to glucose and fructose. Turanose has never been obtained fully pure in the past because no one has succeeded in crystallizing it. In 1918 one of the authors (C. S. H.) found an abundant supply of the rare melezitose in a certain kind of honey and from it he prepared a small quantity of turanose in the hope of crystallizing it. Other samples of turanose sirup were prepared subsequently from this same stock of melezitose by other workers. Recently it was observed by Dr. D. H. Brauns that one of these sirups, the exact history of which is not now known, had crystallized after standing many years. By the use of these crystals to nucleate turanose sirups which we have lately prepared from melezitose it has been possible to obtain a rapid crystallization of turanose and the sugar has been recrystallized with ease from hot methyl alcohol in which it is moderately soluble. Crystalline turanose on solution in water shows a large and rapid mutarotation. At 20° the rotation of its freshly prepared aqueous solution three minutes after dissolving, was approximately $[\alpha]\frac{20}{D} = +43.5$ and the value became constant within 20 minutes at about $[\alpha]\frac{20}{D} = +75.6$. Crystalline turanose is thus a beta form of the sugar. The crystals are well-formed prisms with many faces developed. The sugar possesses a sweet flavor. Its melting point is 157°. A study of this interesting disaccharide, which can now be obtained in pure condition, has been undertaken.

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THE pH OF THE WHITE AS AN IMPORTANT FACTOR INFLUENCING THE KEEPING QUALITY OF HENS' EGGS[1]

HENS' eggs which are heavily infected with micro-organisms ordinarily have very poor keeping qualities. Several bacteriological investigations, the results of which appear in the literature, indicate that only a very small percentage of the freshly laid eggs contain bacteria. If a great number of eggs are shown to be heavily infected with bacteria after storage, improper treatment after the eggs were laid is indicated. However, eggs in the shell deteriorate in storage, but the presence of micro-organisms can not be demonstrated, therefore other factors besides micro-organisms must also be responsible for egg deterioration.

It is customary in grading eggs in the shell to consider the size of the air cell as one of the important factors indicating the quality of the egg, since the size of the air cell increases after the egg is stored, due to the evaporation of water from the egg. If the size of the air cell alone is used as the basis for estimating the interior quality of the egg, very erroneous conclusions may be drawn, since the size of the air cell is dependent upon the relative humidity of the atmosphere in which the egg is kept and upon the temperature. At the present time it is possible, by proper control of the temperature, circulation and humidity of the air in the storage room, to decrease the loss of water from the eggs to a very small amount.

Other changes take place in the eggs during storage which are more detrimental than the loss of water. Three of these changes which are probably the most important are: (1) The change of the thick jelly-like white which surrounds the yolk of the egg to a fluid condition, producing what is commonly called "watery whites." (2) The passage of water from the white to the yolk, producing a more fluid condition of the yolk contents. (3) The weakening of the yolk membrane, causing the yolk of the egg to flatten when the egg is broken, and, if this weakening has progressed far enough, to break.

Greenlee[2] has previously called attention to the passage of water from the white to the yolk as one of the changes taking place in eggs in storage.

[1] This is a preliminary note summarizing some of the results obtained in an investigation suggested by Dr. C. K. Powell, of the poultry department at Cornell University, and began with his cooperation. He was forced to drop out of the project about two years ago and since then the investigation has been continued. Drs. R. Whitaker, C. N. Stark and R. L. Bryant have helped with certain phases of the investigation, and valuable technical assistance has been rendered by Mr. A. E. Everhart, of the poultry department. Experimental evidence for the statements made will be published later.

This report will naturally raise the question as to the effect of the control of the pH of the white on the growth of the chicken embryo during incubation. Experimental work on this effect was begun in 1925 with the cooperation of Dr. C. K. Powell and is still being continued.

[2] A. D. Greenlee, "Deterioration of Eggs as Shown by Changes in Moisture Content," U. S. D. A. Bu. Chem. Circ. No. 83. 1911.

It has been found by Healy and Peter[3] and independently by Sharp and Powell[4] that as soon as the egg is laid the pH of the white begins to increase, due to the loss of carbon dioxide. Actually this change in pH may be from about 7.6, the pH of the white of a fresh egg, to 9.7. This means an increase in alkalinity of approximately one hundred times. The yolk of the fresh egg (1:1 dilution) has a pH of about 6.0. It also increases in pH during storage until a value of about 6.8 may be reached after long periods. The yolk, however, changes in pH more slowly than does the white. The increase in pH of the yolk is due to a loss of carbon dioxide from the yolk to the white which in turn loses it to the surrounding air.

Since it is known that the decomposition of other proteins is accelerated by high pH values, it seems only reasonable to suppose that the high pH of the white would be a factor in hastening the deterioration of the egg contents. Such was actually found to be the case. If eggs are stored in ordinary air, the pH of the egg contents rises and the three deterioration factors noted above are accelerated by this relatively high pH. This alkalinity can be easily and conveniently neutralized and controlled by placing the eggs in atmospheres containing small amounts of carbon dioxide. Carbon dioxide is taken up by the egg to establish an equilibrium between the concentration of carbon dioxide in the egg and in the air. Some of the carbon dioxide taken up by the egg, in the presence of the water, forms carbonic acid which neutralizes a proportionate amount of the alkalinity. By the use of proper concentrations of carbon dioxide the egg can be restored to and maintained at the same reaction it had when laid, that is, at more nearly the condition of the fresh egg. By so doing the deterioration of the egg is markedly retarded. The deterioration factor which is most readily correlated with the pH of the white is the "standing up" quality of the yolk as indicating the weakening and change in permeability of the yolk membrane. This factor may be readily expressed in numerical terms as the quotient of the height divided by the width, measured under controlled conditions. The keeping quality of the yolk of the egg improves as the carbon dioxide content of the atmosphere in which the egg is kept increases, except that in the higher ranges of carbon dioxide concentration relatively unrecognizable beneficial effects are obtained with increasing amount of carbon dioxide.

Watery white seems to be produced in two ways. As the pH is increased to relatively high values, the whole jelly-like white becomes increasingly more fluid until it can not be recognized as thick white at all. At relatively low pH values the thick white maintains its jelly-like properties but the amount of this jelly-like white decreases. This decrease in the amount of thick white at the low pH values may be caused by the precipitation of a protein in the thick white under such conditions. The largest amount of thick white of good quality is obtained at intermediate pH values and consequently at moderate concentrations of carbon dioxide.

At the relatively high concentrations of carbon dioxide the pH of the white is lowered to such an extent that a marked turbidity appears in the thick white due to the lowering of the pH to a point near the isoelectric point of one of the proteins of the thick white. This indicates the localization of the egg globulin in the thick white. The turbidity which is produced in the thick white by high concentrations of carbon dioxide can be made to disappear if some of the carbon dioxide is allowed to escape. This causes the pH to rise and the precipitated protein goes back into solution.

The concentration of carbon dioxide necessary in the atmosphere to hold the white at a pH corresponding approximately to that of a fresh egg lessens as the temperature decreases. At room temperature it takes approximately 10 to 12 per cent. of carbon dioxide in the atmosphere to hold the white at pH 7.6, while at near the freezing temperature about 3 per cent. of carbon dioxide in the atmosphere will accomplish the same purpose.

If the eggs, soon after they are laid, are placed in storage, in a carbon dioxide tight container in which the eggs occupy a relatively large part of the space, and if the humidity is properly controlled, the eggs will give up carbon dioxide to the atmosphere until the carbon dioxide in the atmosphere is in equilibrium with that in the eggs. This prevents the pH from increasing as much as it otherwise would and consequently exerts some preserving effect. Since the carbon dioxide escapes from the eggs so rapidly, the amount of carbon dioxide which the eggs contain by the time they reach the storage plant is sufficient to exert only a slight, but nevertheless recognizable preserving effect.

The importance of the alkalinity of the white as a factor in controlling egg deterioration has not pre-

[3] D. J. Healy, and A. M. Peter. "The Hydrogen-ion Concentration and Basicity of Egg Yolk and Egg White," *Amer. Jour. Physiol.*, 74: 363–368. 1925.

[4] P. F. Sharp, and C. K. Powell. "Physico-chemical Factors Influencing the Keeping Quality of Hens' Eggs in Storage," Proc. World's Poultry Congress. Ottawa, Canada. pp. 399–402. 1927.

viously been recognized, although the use of high concentrations of carbon dioxide (90 per cent. up to several atmospheres pressure) has been recommended for the preservation and sterilization (destroying micro-organisms) of eggs.

The lowering of the pH by using small amounts of carbon dioxide as recommended here would actually adjust the egg white to a pH more suitable for bacterial growth as shown by the experiments of Sharp and Whitaker,[5] Stark and Sharp[6] and Bryant and Sharp,[7] but if the eggs are properly cared for the deterioration due to bacteria is of minor importance and occurs in only a relatively few eggs.

The adjustment of the pH of the egg contents by means of carbon dioxide is of great practical importance since a relatively small amount of carbon dioxide in an air atmosphere will markedly retard deteriorative changes. The carbon dioxide produces markedly beneficial results at any temperature to which the eggs are subjected, proportionately greater amounts of carbon dioxide being required at the higher temperatures, and proportionately greater benefits being obtained. Carbon dioxide can be introduced into the air atmosphere of cold storage rooms in concentrations which greatly retard the destructive changes in the eggs, and yet the concentration will not be so high as to prevent workmen from entering the storage rooms.

The introduction of carbon dioxide into the air of shipping containers, refrigerator cars and the like can be used to retard deterioration.

Carbon dioxide tight containers, storage rooms and the like, although in some cases desirable, are not necessary since the carbon dioxide which escapes may be easily and cheaply replaced by the introduction of more. Carbon dioxide from any convenient source can be utilized, such as in the solid form or from cylinders. Solid carbon dioxide might be used in shipping eggs to serve the double purpose of keeping the eggs cold and holding the pH down, provided conditions were so regulated that the eggs did not freeze.

It has been estimated that if enough carbon dioxide were added to a storage room once each week for six months to give a concentration in the atmosphere of one per cent., the cost of the carbon dioxide would be less than one cent per case of eggs, or less than 0.03 cents per dozen. It would, however, be necessary to replenish the carbon dioxide more often, unless the walls of the storage room were so treated as to make them more impermeable to carbon dioxide.

The only method that approaches the one here described in cheapness and practicability is the oil dipping method, in which eggs are dipped for a short time in a suitable oil which very nearly seals the pores, and the eggs are then stored. The oil dipping largely prevents the loss of moisture and very materially slows down the rate of the loss of carbon dioxide. If the eggs are oil dipped as soon as they are laid, the preserving effect of a low pH is obtained, but the thick white becomes turbid during storage. If a considerable part of the carbon dioxide which was originally present in the eggs is allowed to escape before the eggs are dipped, then the turbidity of the white may be avoided, but the full preserving effect of the low pH is not obtained. Some carbon dioxide will diffuse from the yolk to the white after the eggs are dipped so that the pH of the white is somewhat lower than if the eggs were not oil dipped. If it is desired to oil dip eggs in which the pH has risen and obtain the full preserving effect of the low pH, all that is necessary is to place the eggs in an atmosphere containing sufficient carbon dioxide to lower the pH to the point desired, and then dip the eggs. If eggs which are not oil dipped are maintained in an atmosphere containing sufficient carbon dioxide to hold the pH down to near that of fresh egg white, a turbidity also appears in the white, but this turbidity disappears when the eggs are removed from the carbon dioxide atmosphere and some of the carbon dioxide is allowed to escape.

A number of household and commercial methods of preserving eggs in the shell have been studied with the introduction of many variables which might possibly influence the interior quality of the eggs at the end of the storage period. The results in all cases have been entirely consistent in indicating that the changes in the quality of the eggs during the storage period are dependent upon the effect of the following factors: (1) micro-organisms, (2) quality of the fresh eggs, (3) temperature, (4) passage of undesirable odors and flavors into the egg, (5) loss of water from the egg, and (6) the pH of the egg contents. The possibility of the control of the pH of the egg contents as a factor in egg preservation has apparently not previously been considered, and yet our experiments have shown that it ranks among the first in importance.

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[5] P. F. Sharp, and R. Whitaker. "The Relation of the Hydrogen-ion Concentration of Egg White to its Germicidal Action," *Jour. Bact.*, 14: 17–46. 1927.

[6] C. N. Stark, and P. F. Sharp. "The Relation Between the Hydrogen-ion Concentration of Egg White and the Growth of Anaerobes," *Jour. Bact.*, 13: 59–60. 1927.

[7] R. L. Bryant, and P. F. Sharp. Unpublished.