Foods Stabilized by Glucose Conversion; Frozen Foods Nutrition Research Planned

FOOD STABILIZATION

Glucose Conversion in Preparation of Albumen Solids by Glucose Oxidase–Catalase System

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The glucose oxidase–catalase system is used to convert glucose to gluconic acid and thus render the glucose nonreactive insofar as the deteriorative Maillard reaction is concerned. Empirical relationships relating the glucose level, time, enzyme level, and hydrogen peroxide demand have been derived, permitting more efficient utilization of enzyme and peroxide. The amount of hydrogen peroxide required for albumen desugarization has been cut by 40%, foaming has been eliminated as a problem, and minor modifications in starting pH, have been made.

REMOVAL OF GLUCOSE FROM ALBUMEN by the use of the glucose oxidase–catalase system prior to drying has been shown to yield a stable odor-free product (*2*). Comparative evaluation indicated that there were no significant differences in flavor between angel food cakes baked with frozen albumen and rehydrated enzyme-stabilized albumen (*3*).

Glucose can act as the trouble maker by virtue of its ability to enter into the Maillard reaction with amino groups (*5*). The Maillard reaction was first reported in 1912, but only since World War II has its importance in foods been realized. At that time many processors were drying eggs and also vegetables for shipment overseas and for accumulation of a food reserve. When these products were to be consumed many were found to be inedible.

Maillard Reaction

The Maillard reaction occurs between an aldehyde group and an amino compound. The aldehyde groups encountered in foods are usually in the form of sugars; the amino groups in proteins. Thus when a proteinaceous material containing glucose or some other reducing sugar is subjected to conditions favorable to the Maillard reaction one notices a disappearance of glucose and of free amino groups and, after a short lag, the development of a brown color. The brown color development is accompanied by a decrease in solubility and changes in both the odor and flavor.

The occurrence of this Maillard reaction in foods is not always undesirable. It is believed that the color and flavor of the crusts of baked goods such as bread and cake are due to this reaction, as are the color and flavor of breakfast cereals, French fried potatoes, and the outer portion of roast meats.

When this reaction occurs in egg solids or dehydrated potatoes the reaction is definitely undesirable. Various means have been tried to prevent or retard the Maillard reaction. Most are based on making conditions unfavorable for the reaction. Thus, the military specifications for egg solids called for acidification to a pH of 5.5 and drying to a maximum moisture content of 2% (*6*). Another technique that retards the reaction is refrigeration. Yolk solids must be kept at refrigerator temperatures if they are to be stored more than a few weeks, and still retain their functional properties.

Of course yolk and whole egg contain phospholipides that are reported by the Western Regional Laboratory workers to combine with the natural glucose and play a major role in off-flavor development in yolk and whole egg solids (*4*). The ideal way, from both a theoretical and a practical standpoint, to prevent the Maillard reaction is to remove or render inactive one of the reactants. It has not yet been practical to remove the protein, but removal or conversion of the aldehyde group has been commercially achieved by the use of the glucose oxidase–catalase system.

Glucose Conversion

Glucose oxidase catalyzes the oxidation of glucose to gluconic acid and

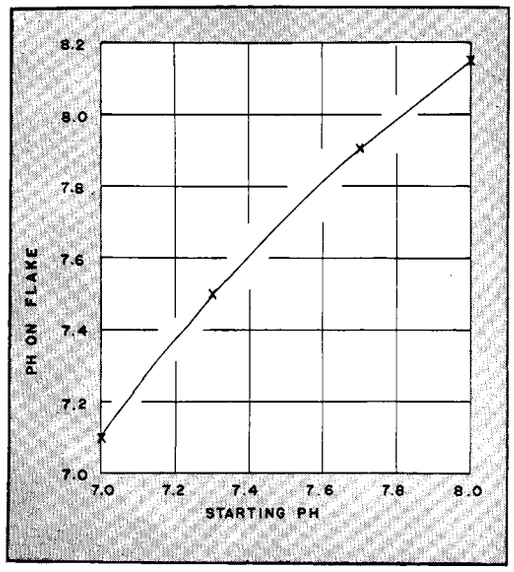

Figure 1. Effect of starting pH on end pH of flake albumen

hydrogen peroxide according to the following formula:

$$2\,H_2O + 2\,\text{glucose} + 2\,O_2 \xrightarrow{\text{glucose oxidase}} 2\,\text{gluconic acid} + 2\,H_2O_2$$

Catalase catalyzes the breakdown of the hydrogen peroxide

$$2\,H_2O + O_2 \xleftarrow{\text{catalase}} 2\,H_2O_2$$

Thus the net reaction for the enzyme system is:

$$2\,\text{glucose} + O_2 \xrightarrow[\text{catalase}]{\text{glucose oxidase}} 2\,\text{gluconic acid}$$

It has been found in practice that this enzyme system can be used to remove either glucose or oxygen depending on which is in excess. To remove glucose one adds an excess of oxygen; to remove oxygen one adds an excess of glucose. In beer, for example, there is usually sufficient glucose to react with the oxygen. In special cases a trace of amylase may be added to form the needed glucose from dextrins present in the beer.

The enzyme system was originally developed by Dwight L. Baker, then of this laboratory, for use in deoxygenation (*1*). Credit for recognizing the applicability of this enzyme system to food stabilization where glucose is a problem goes to Robert Baldwin of the General Foods Central Research Laboratory in Hoboken, N. J. It was he who applied the enzyme to the stabilization of egg solids (*2*).

Instead of relying on living organisms to carry out the desugarization, a mold is grown in the controlled climate of a fermenter, and a standard strength enzyme product free of cells is prepared from the mold mycelia. This product removes the process from the straight biological fermentation type of reaction and places it in the rigidly controllable chemical reaction group. The enzyme system converts the aldehyde group on the glucose to a carboxyl group, in which form it is nonreactive. The conditions of the desugarization can be rigidly controlled so that the desugarization can and does proceed on a fixed schedule, always in the same way, and always giving a stable odor-free product even when the product is pan dried, provided, of course, that the starting material is of good quality.

Albumen Stabilization

The albumen process, as normally practiced in industry, involves warming the albumen to 30° C., adjusting the pH with dilute acid, adding a measured quantity of enzyme, and adding hydrogen peroxide as a source of oxygen incrementally or continuously over the time required for the desugarization.

Acidification The initial acidification can be done with any acid acceptable for food use. Lactic, citric, tartaric, acetic, hydrochloric, and phosphoric acids have been compared, and no detectable difference in the rate or course of desugarization or in the flavor of the products after drying has been found. The use of citric is recommended because it can be obtained in a solid form, facilitating handling, and it is a natural food acid. It is cheap and can readily be obtained in pure form; it is not a skin irritant even in concentrated solutions (lactic acid is); and it gives off no vapors that cause difficulty for plant personnel (hydrochloric acid does).

The pH to which acidification is made is determined in part by the desired pH of the reconstituted albumen solids. Figure 1 relates starting pH to the pH of the albumen after pan drying in a commercial dryer where minimal agitation was used in processing. Agitation can be a very big factor in determining the pH. Table I gives the results of a comparative study on agitation. For controls 2-pound batches of albumen were placed in 2-liter stainless steel beakers in a water bath. For the agitated samples 2 pounds of albumen were placed in 4-liter beakers, forming a layer about 2 inches deep. Agitation was at 190 r.p.m. with a paddle-type stirrer measuring approximately 4 inches by 1 inch.

Under normal conditions the optimum starting pH for pan dried albumen for angel cakes is 7.0 to 7.3. In the preparation of specialty products the desugarization may be started at a pH of 5.5, for example. One has a wide range of pH to work in as the enzyme is most active between pH 4.0 and 7.0 but can also be used as low as 3.0 or as high as 9.0 for short periods of time.

The initial acidification was with 20% citric acid and was done prior to splitting the albumen into separate batches. Temperature was 30° C., and hydrogen peroxide was added according to the decreasing schedule presented below.

Glucose Determination Glucose was deproteinized by the Somogyi barium hydroxide–zinc sulfate method, and glucose was determined colorimetrically on the deproteinized filtrate by the arsenomolybdate method (*7*, *8*).

Glucose Oxidase Assay The glucose oxidase assay is measured in Sarett units. A Sarett unit of glucose oxidase is defined as that quantity of enzyme that will cause an oxygen uptake of 10 mm.^{3} per minute under the conditions and on the substrate given below in the presence of an excess of catalase.

Substrate. Dibasic sodium phosphate, 0.1 *M*

Sodium salt of dehydroacetic acid (Dow Chemical Co.'s DHAS), 0.4%

Concentrated phosphoric acid, to pH 5.9

Glucose monohydrate, 3.3%

Dissolve the calculated amount of dibasic sodium phosphate and DHAS in about three fourths of the total volume of water. Adjust to pH 5.9 with concentrated phosphoric acid. Then dissolve the glucose monohydrate and make up to volume. The substrate will keep indefinitely but should be protected against light and evaporation. Refrigeration is not necessary.

Assay. A suitable aliquot of enzyme-containing material (but not more than 0.5 gram or ml.) is placed in the bottom of a 100-ml. graduate or volumetric flask, substrate at about 25° C. is added rapidly to volume, and the contents are mixed. A portion of this diluted enzyme is placed in one or more Warburg flasks, and the flasks, with manometers attached, are placed in the 30° C. water bath and shaken at 120 oscillations per minute with a stroke of 4 cm. for 10 minutes with the stopcock on the reaction vessel side of the manometer open. After temperature equi-

Table I. Effect of Agitation on pH

Sample	A	B	C	D
Glucose oxidase, Sarett units/ 1000 lb. of albumen	0	0	75,000	75,000
Agitated	No	Yes	No	Yes
Initial pH	7.5	7.5	7.5	7.5
Final pH on liquid	7.8	8.2	6.9	7.5
pH of reconstituted flake albumen solids	...	...	7.7	8.2
% Glucose on dry basis	...	...	0.04	0.05

librium has been established, the stopcock is closed, and the flasks are allowed to shake for exactly 30 minutes. The change in pressure inside the reaction flask is then read after readjusting the manometer to give the initial volume for the reaction flask.

Calculation. The activity is calculated from the pressure drop, provided the flask constants are known. Commercial glucose oxidase contains catalase as well as glucose oxidase. If assaying a glucose oxidase devoid of catalase or assaying glucose oxidase with the catalase inhibited, an oxygen uptake double that found in the presence of catalase will be observed, due to the trapping of half of the oxygen taken up in the form of hydrogen peroxide. Catalase breaks down the peroxide and recycles the oxygen in the peroxide.

For a sample calculation assume that with an aliquot of 0.05-ml. of sample, and a flask constant of 1.5 to 2.2 ml. of fluid in the Warburg flask a pressure drop of 96 mm. was observed. Then oxygen uptake per 30 minutes = $96 \times 1.5 = 145$ mm.^{3} The oxygen uptake per minute = 4.83 mm.^{3}, and the oxygen uptake for the entire 0.05-ml. aliquot would have been $4.83 \times \frac{100}{2.2} = 220$ mm.^{3} per minute.

For a 1-ml. sample the uptake would have been $220 \times 1.00/0.05$ or 4400 mm.^{3} per minute. Since one unit is defined as taking up 10 mm.^{3} of oxygen in 1 minute under these conditions it is obvious that each milliliter of the sample contains 440 Sarett glucose oxidase units.

Check samples sent to another laboratory have given consistently good results checking ours within $\pm 1\%$.

Controlling Desugarization Plotting the log of the per cent glucose against time gives a straight line indicating that the conversion of glucose to gluconic acid, in the range of sugar concentrations normally encountered in albumen, and with excess peroxide, is a first order reaction. Figure 2 represents the change in glucose level with time for differing levels of the same enzyme preparation. A standard pound of glucose oxidase–catalase contains 75,000 Sarett glucose oxidase units. In Figure 2, 1.3 ml. is equivalent to 1.18; 1.1 to 1.0; 0.9 to 0.82; and 0.7 to 0.64 standard pounds per 1000 pounds of albumen.

Since the desugarization is a first order reaction it follows equation 1, where S_1 is the sugar concentration at time T_1, S_2 the sugar concentration at time T_2, and k is a constant for any given temperature and enzyme concentration in the presence of an excess of peroxide. Empirically the value of k for albumen at 30° C. has been found to be that represented in equation 2, where T is expressed in hours and E represents the number of Sarett glucose oxidase units per 1000 pounds of albumen. (The "eight-hour process" normally requires 75,000 Sarett units per 1000 lb. of albumen.)

$$k = \frac{1}{T_2 - T_1} \log \frac{S_1}{S_2} \qquad (1)$$

$$k = \frac{0.144E}{75{,}000} \qquad (2)$$

If one then solves equation 1 for $T_2 - T_1$ one can use it to predetermine the time to reach any given sugar level, provided the initial sugar level, the final desired sugar level, and the k values are known. One could also use equation 1 to determine how much enzyme to use to accomplish a given job in a specified time.

Hydrogen Peroxide Originally the hydrogen peroxide was added in increments of equal size at equal intervals throughout the process. This provided a rather large excess of peroxide toward the end of the process, which was not only wasteful but caused a loss of albumen in foam, as the excess oxygen simply bubbled off. To correct this, the peroxide requirements as a function of the sugar level were determined.

The hydrogen peroxide demand in any time interval is a function of the sugar level. The demand for any period at a given enzyme level and temperature can be ascertained by providing an excess of peroxide and determining how much sugar is oxidized to gluconic acid. Since, in the presence of excess peroxide, the log of the sugar concentrations versus time is a straight line, a straight line is obtained if the instantaneous hydrogen peroxide demand versus time is plotted. The slope of the line will depend on how efficiently peroxide is utilized by the system. If a certain "overhead" loss of peroxide is assumed at each addition, then the slope of this line will not correspond with the slope of the line for the removal of glucose from the same system, for the constant loss will become increasingly significant as the size of the dose decreases. The approach to this problem is an empirical one.

The slopes assigned to the lines were arbitrary rather than derived because of the unknown efficiency factors and a lack of knowledge as to what part of the loss was due to factors that varied with the size of the dosage of peroxide and what part was fixed. That a stoichiometric excess of hydrogen peroxide, on the basis of the glucose reacted, is required for the reaction to proceed at maximum rate is evident from the nature of the reaction and has been confirmed experimentally.

The empirical relationship is:

$$H_2 = \frac{440\, yS_2}{S_1} \qquad (3)$$

where H_2 is the ml. of 35% hydrogen peroxide to add per 1000 pounds of albumen at sugar level S_2 where y represents the time interval in hours between successive additions.

While from a theoretical standpoint it is best to base the hydrogen peroxide level on the sugar level it is not practical for plant operation. Since the sugar level can be calculated at any time, provided the amount of enzyme added is known, the peroxide demand can be equated against time instead of sugar level, assuming that the initial sugar level is essentially a constant for albumen.

Figure 2. Change in glucose level with time for differing levels of enzyme

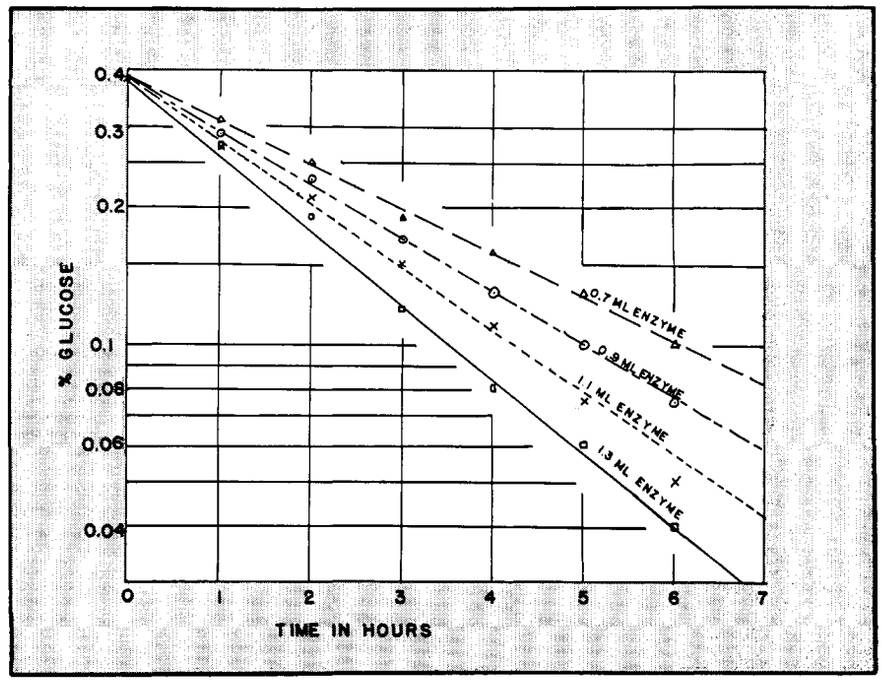

Table II. Schedule for Peroxide Addition

Time of Addition in Hours	Ml. of 35% H_2O_2 Per 1000 Lb. of Albumen	
	Decreasing	Regular
0.00	200	216
0.25	100	72
0.50	90	72
0.75	84	72
1.00	76	72
1.25	70	72
1.50	64	72
1.75	59	72
2.00	54	72
2.25	50	72
2.50	45	72
2.75	42	72
3.00	38	72
3.25	35	72
3.50	32	72
3.75	30	72
4.00	28	72
4.25	25	72
4.50	23	72
4.75	21	72
5.00	20	72
5.25	18	72
5.50	16	72
5.75	15	72
6.00	14	72
6.25	13	72
6.50	12	72
6.75	11	72
7.00	10	72
TOTAL	1295	2232

Thus, equation 4 relates peroxide demand with time elapsed.

$$H = \frac{440\,y}{\text{antilog}\,\frac{T}{Z}}, \quad Z = \frac{(6.75)\,E}{75{,}000} \qquad (4)$$

Equation 4 may be used to calculate all additions except the one at zero time. This addition is twice the addition after the first time interval.

Table II contrasts the peroxide additions for the old and the new procedures, based on 15-minute intervals between additions, and 75,000 units of glucose oxidase per 1000 pounds of albumen.

Compare the total amounts of peroxide used in both procedures. In addition to eliminating foaming problems the decreasing addition schedule saves 40% of the peroxide.

The quantities of hydrogen peroxide calculated by the use of equation 4 are based on the almost complete exclusion of atmospheric oxygen. Depending on the vessel used and the type of agitation used, it may be possible to reduce this level somewhat.

For plant operations where a continued step-wise decrease in peroxide additions is not convenient, the author recommends the addition of 200 ml. of 35% hydrogen peroxide at zero time followed by, for the "eight-hour process," the addition of 100 ml. after or during each succeeding 15-minute interval through 2 hours, 50 ml. through 4 hours, 25 ml. through 6 hours, and 10 to 15 ml. to completion of the process.

Yolk or Whole Egg Stabilization

Yolk and whole egg can be desugarized by means of the glucose oxidase–catalase enzyme system too.

Since the pH of yolks is approximately 6.6, no pH adjustment is required. The yolks are warmed to 35 to 38° C., and two standard pounds of glucose oxidase per 1000 pounds of yolk are added. Peroxide is added at an over-all level of about 3 parts per thousand. This will reduce the glucose level from the initial 1% down to 0.1% dry basis in $3\frac{1}{2}$ hours. Increasing the enzyme level to 3 pounds per 1000 pounds will reduce the time to 2 hours.

Whole eggs contain about 1.2% glucose, dry basis. The pH of whole eggs is about 7.5. The process may be started here or, if desired, the pH could be lowered to 7.0 to 7.3. On whole eggs the general practice is to use 3 pounds of standard enzyme mixture to each 1000 pounds of whole eggs. The glucose level will be reduced to 0.1% dry basis in about 4 hours, with about $3\frac{1}{2}$ pounds of 35% hydrogen peroxide being added per 1000 pounds of whole egg.

As with the albumen, the process is rather flexible and may be modified to produce specialty products. The most common modification of enzyme-stabilized whole egg solids is to incorporate about 10% sucrose in the liquid whole egg prior to drying. The sucrose, being nonreducing, does not exert a deleterious effect on the resultant egg solids.

The use of the glucose oxidase–catalase system for the stabilization of foods subject to nonenzymatic browning is being developed. Application will no doubt be made to other products such as dehydrated mashed potatoes.

Evaluation of Method

It is difficult to evaluate any new development so soon after it has come out. Certainly no single development can solve all of the problems in the food field. There is hardly a development that can be made to work economically wherever theory says it should work. But oxygen and glucose are such basic causes for difficulty to the food technologist that we can confidently expect the commercial realization of this enzyme system to solve some of the commonest difficulties confronting the food technologist today.

Literature Cited

(1) Baker, D. L. (to B. L. Sarett), U. S. Reissue Patent No. 23,523 (July 22, 1952).
(2) Baldwin, R. R., Campbell, H. A., Theissen, R., Jr., and Lorant, G. J., *Food Technol.*, **7**, 275–82 (1953).
(3) Carlin, A. F., and Ayres, J. C., *Ibid.*, **7**, 268–70 (1953).
(4) Kline, L., and Sonoda, T. T., *Ibid.*, **5**, 181–7 (1951).
(5) Maillard, L. C., *Compt. rend.*, **154**, 66 (1912).
(6) Military Specification, "Egg, Stabilized Dehydrated," MIL-E-**10006**(QMC), September 12, 1949.
(7) Nelson, N., *J. Biol. Chem.*, **153**, 375 (1944).
(8) Somogyi, M., *Ibid.*, **160**, 61 (1945).

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FOODS EVALUATION

Sampling Plan for Nutrition Research Program On Frozen Fruits, Juices, and Vegetables

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A LITERATURE SEARCH of *Chemical Abstracts* over the past three and one-half years revealed some 20 papers dealing with the occurrence of one or more nutrients in fruits, juices, and vegetables after preservation by freezing followed by subsequent storage and/or cooking studies. In consideration of the great scarcity of such data, the few tabulations on the composition of raw and processed foods that include frozen foods may be open to question, especially