1945

Dried egg albumen. I, Studies of the non-microbiological changes during storage

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UMI®
DRIED EGG ALBUMEN. I: STUDIES OF THE NON-MICROBIOLOGICAL CHANGES DURING STORAGE

by

Ralph W. Kline

A Thesis Submitted to the Graduate Faculty for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Poultry Products

Approved:

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1945
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INTRODUCTION

In the egg albumen industry, there are two kinds of products which are to some extent competitors: frozen and dried. They vie for use in two major fields: bakery products, where angel food cake is of paramount importance; and in candy products, creams, nougats, and divinity.

Of the factors which influence the use of the two products, dried albumen undoubtedly has the advantage in form of convenience of packaging, handling, shipping and storage. Frozen albumen, usually packed in 30-pound cans, must be kept frozen at all times until used and can be used in quantities of less than the unit package only with some inconvenience, such as refreezing and rethawing each time used, or incurring the danger of spoilage by holding unfrozen. Dried albumen can be packed in dry containers, held in non-freezing storage, and can be reconstituted for use in any amount.

From the standpoint of economy, there is some advantage in dried albumen. The shipping weight and storage space are about one-eighth that of the frozen, in addition to the lower cost of unrefrigerated storage room. The further apart the production and consumption points are, and the greater the time between the heavy-egg months and the date of use, the larger this margin should ordinarily be.

In spite of this, the factor which actually determines the use of these products is the relative ability of each to perform satisfactorily in the function to which it is put.
Of the frozen albumen, it can be said that it does perform satisfactorily in both of the uses mentioned above. It may in some cases be surpassed by special dried products, but its performance remains acceptable.

With dried albumens, the situation is different. There are a large number of types of product, but they can be classified in two groups: those produced directly from untreated liquid albumen, and those produced from liquid albumen which has first been fermented. The most important difference between these types is that the natural dried albumens are not stable in unrefrigerated storage, but deteriorate to the point of uselessness, one of the subjects of this thesis. The fermented albumen when dried, in general retains the properties it has on first being reduced to dryness, even though it is stored at elevated temperatures for extended periods of time. The problem of stability in the dry state can thus be solved in one way by fermenting the albumen.

Dried albumens prepared from fermented liquid are satisfactory for use in candy making, and other dried albumen products are now marketed which are of acceptable performance in angel food cake and other products. Thus, in general, dried albumen products are competing with frozen in these two important uses. A third major use of dried albumen is in making adhesives, and since the solution required for this must have four times the solid content of liquid albumen, the dry product only can be used here.
There are some characteristics of the albumen fermentation which are such that an alternative process which would stabilize the product would be most acceptable. The fermented dried product sometimes has an undesirable odor. The process requires 48 to 72 hours and requires equipment capable of handling several days' volume of production at one time. In order that the fermentation be properly controlled, continuous supervision by trained personnel is required. These and other reasons make it desirable that some process be developed which would be simple and quick in operation, adaptable to large scale production. To obtain information which might lead to the development of such a process is the prime objectives of this study.

The work is preliminary in nature. The results obtained are as yet only suggestive of a possible solution to the problem. No work was done on the actual performance of the product in any of its several uses, such as making angel food cakes, because simpler measures of the deterioration were available and these will serve the purpose until a process which shows promise of ultimate use is found. A further reason for not preparing angel food cakes in this study is that this property of dried albumen is influenced by the method of drying, which is not a part of this study but a separate problem of some magnitude.

In the course of the study, a quantitative record of some of the changes taking place has been made. These may be useful to others
investigating the problem. They have also been used to give an indication of the fundamental nature of the deterioration.
not in current use.

not the yeast fermentation have been patented (22, 62, 27, 74, 44) but the

products without the fermentation were found to be necessary to the

developed here and apparatus were made to prepare satisfactorily alcohols.

As the industry

concentrate resulting from the fermentation (44, 64), the Chinese product had been patented because of the odor and baterial

with the industry became re-sensitized here. Up to that time the

for making the dried product stable was not generally appreciated

allowing the liquor to ferment (7.7% of the necessity of the process

Aby Chinese alcohol was commonly known to be prepared by

1944.

(67) The volume has been on the increase ever since, reaching two

1927, when alcohol was introduced with the Chinese industry. The fourth

available in the orient. On domestic production is recorded until

these diseases in the country were concentrated to deep labor and ran importance

During about the time of World War I, this was due chiefly to the

the United States before 1900, the industry entered the fermentation to

although these products, introducing alcohols, were apparently dried in

REVIEW OF LITERATURE
1. Fermentation.

The fermentation of albumen has been described by a number of authors (7,49,64,70). Glaubau and Kepes were among the first to follow the change in acidity of the liquid during fermentation. They found that the pH fell steadily from about 7.8 to 5.9. Stewart and Kline (64) followed the course of the fermentation by determining pH and free glucose in the liquid. They found the liquid to remain standing for a few days without change. The pH then began to decrease from 8.9 and reached 5.9 in about 48 hours. The glucose decreased in a parallel fashion and reached 0.01% after 60 hours. During this period the albumen decreased in viscosity from a stringy consistency to a thin watery liquid, and the mucin of the thick white gathered on the surface.

Stuart and Goresline (65) studied the changes in pH, glucose, protein nitrogen, amide and amino nitrogen, and the bacterial content of fermenting albumen. Their results for pH and glucose were essentially the same as those of Stewart and Kline. They found no significant change in the nitrogen fractions. The numbers of bacteria increased to several billion per milliliter during the sharpest decrease in pH and glucose. In studying albumen from several commercial fermentations they found the organisms to be predominately Aerobacter and Escherichii. In a subsequent paper (66) these authors identified the organisms as Aerobacter aerogenes, Aerobacter cloacae and
**Escherichia freundii.** Cultures of these organisms produced a "normal" egg white fermentation in laboratory batches. Samples fermented with Serratia and Proteus species and with Pseudomonas aeruginosa, lost much less glucose and showed extensive proteolysis.

Hawthorne and Brooks (36) in a laboratory trial removed the glucose from egg white by adding yeast to the extent of 1%, and incubating the mixture at 37°C. for three hours. Glucose was reduced from 0.55% to 0.05% and 0.09%, respectively, in two trials by this method.

2. **Stability of dried albumen and the role of glucose.**

Balls and Swenson (5) developed a process wherein the proteolytic enzyme trypsin was used to reduce the viscosity of natural egg white to that of fermented albumen. The objective was to supplant the bacterial fermentation, which they believed to have as its chief function the "thinning" of the albumen and subsequent increase in its whipping property. They found, however, that the dried product prepared from enzyme treated albumen darkened and became insoluble on storage. Such a product is worthless (64).

Stewart and Kline (64) first demonstrated conclusively the role of glucose in the deterioration of dried egg white. They prepared glucose free albumen by fermentation and pan dried it to produce a flake product. The change in properties of this product and those of a flake albumen prepared from natural liquid albumen were compared.
When stored at 40°C, the latter showed a progressive change from pale yellow to dark brown, and became relatively insoluble within two weeks. The fermented sample on the other hand retained its original pale yellow color and underwent no observed change in solubility during 12 weeks of storage at 40°C.

To prove conclusively the role of glucose in the deterioration, they prepared dried samples from natural albumen, fermented albumen, and fermented albumen to which glucose had been added. The sample from the fermented albumen was the only one which did not develop color and become insoluble on storage. This sample contained 0.0% glucose, while the other two contained 0.42%. These authors further reported that partial fermentation increased the storage life of the product at room temperature and 40°C. Only the completely fermented sample retained its properties indefinitely.

Stewart, Best and Lowe (65) reported on the changes in solubility of a dried albumen sample stored at 50°C. The sample, containing 0.4% glucose (liquid basis) and 6% moisture, retained its solubility for 6 days, after which time it lost 22% of its original solubility in a steady decrease over 9 days' time. A fermented sample (0.00% glucose) under the same conditions retained its color and solubility completely for the same storage period.

Stuart and Goresline (65) dried samples of albumen which had been fermented to a low level of glucose with several different types of organisms. All retained their original color when stored for four
months at room temperature, while an unfermented sample darkened and became insoluble.

Hawthorne and Brooks (36) demonstrated the effect of glucose by fermenting a sample of albumen with yeast to a glucose content of 0.06%. When dried, this sample retained its color and solubility for 2 weeks at 46°C., while a dried sample of untreated albumen containing 0.55% of glucose lost 62% of its solubility.


According to Stewart and Kline, commercial producers have in the past observed sudden coloring and insolubilization of unfermented dried albumens in unrefrigerated warehouses during summer months.

Stewart and Kline (64) made qualitative notations of the solubility and color changes of flake albumen at room temperature (22°C. to 27°C.) and at 40°C. At the higher temperature, two weeks produced almost complete loss of solubility, while 10 weeks was required to produce the same change at room temperature. At the lower temperature, the first change was observed in 3 weeks; at 40°C. the first observation made was that of complete insolubility at 2 weeks. Stewart, Best and Lowe (65) observed the first change in solubility of an albumen stored 3 days at 50°C. Best (6) found albumen to show its first change in solubility in 5 hours at 60°C., and a complete insolubility at about 2 1/2 days. Although these various samples were not of identical characteristics they were all natural unfermented products, and the
various results indicate that as the temperature increases, the rate of deterioration increases very rapidly.

4. The effect of acid treatment on dried albumen.

The treatment of egg albumen with acid before drying has formed the basis for a number of patents (29, 37, 44). Usually a pH of 5.0 to 6.0 is used. The mucin is completely flocculated by such treatment and may either be filtered off (37, 43, 44) or allowed to remain and be reincorporated by agitation for the drying process (29, 48).

The function ascribed to acid treatment is usually that of increasing the foaming property or achieving stability of the dry product. Watts and Elliott (70) have shown that such a product gives a larger volume of foam in a shorter beating period than does liquid albumen or freshly dried untreated albumen.

Bumashnov (10) reported that the foaming property of dried albumen was enhanced by thinning with acid or with enzyme, but did not discuss the stability of the dry product.

Mulvany (49) describes an acid treatment process in which the pH was lowered to 5.6 and the mixture agitated at 135°F. in a vacuum during which time the pH rose to 7. This treatment was repeated a second time and the product dried. Such a dried albumen darkened in color "only slightly" after four years at room temperature and had an "excellent" whipping property. This is a much greater storage life than would be expected from an unfermented albumen, and at least
some of the effect is probably due to the treatment.

There is an indication in the results of Stewart and Kline that acid treatment has some effect on the stability of the dry product. Two samples of pH 6.3 showed a better retention of solubility and color for one week of storage at 40°C. than did corresponding samples dried at pH 8.5.

5. The effect of moisture content on the stability of dried albumen.

Bumazhkov (11) found that the moisture content of dried egg albumen affected the rate of denaturation at high temperatures. A sample containing "very little moisture" was found to lose only 6% of its solubility in 4 hours at 80°C. Contrasting values for higher moisture powders were not given, nor was it clear whether darkening accompanied the change in solubility.

Results indicative of the effect of moisture level in dried albumen on the rate of its insolubilization were obtained by Stewart and Kline (64). Samples of flake albumen were powdered and adjusted to 8.4% and 1.6% moisture. The difference in solubility between the two was negligible after 23 weeks of storage at 40°C., but at 61 weeks the high moisture sample was only two thirds as soluble as the other.

The experiment which Best (6) performed on dried albumen gave a clear cut demonstration of the effect of moisture. A 10.6% moisture sample decreased rapidly in solubility when stored at 60°C., reaching a minimum at 65 hours. In contrast a 2% moisture sample had 90% of
its original solubility at this time.

6. Fluorescence and dried albumen.

The use of fluorescent properties as an indicator of quality of dried egg products was first developed by Pearce and Thistle (53). They first defatted dried whole eggs with various solvents, then extracted the residue with salt solution and determined the fluorescence of the clear extract. This value was found to be related to the quality of the product in terms of flavor, the poorer powders showing the greater fluorescence.

Pearce (61) applied the test to dried egg albumen by eliminating the defatting step and found that a sample stored at 24°C. increased in reading from 10 to 52 in two months' time.

Stewart, Best and Lowe (63) studied the increase in fluorescence of dried albumen stored at 50°C. and found a progressive change from 34 to 104 in 15 days.

Best (6) found that in albumen (10% moisture) stored at 60°C., the fluorescence increased very sharply to 220 at 15 hours, then fell very abruptly to 68, and continued a slow decrease for the next 80 hours. At the time the fluorescence was reaching its peak, the solubility of the sample was 51%.
7. The amino acid-sugar reaction.

Because of the striking similarity between some of the properties exhibited by the deteriorating egg albumen and those of the reaction mixture of amino acids and reducing sugars under various conditions, it will be suggested that the nature of the two reactions is similar. That is, that the deterioration of dried egg albumen is the result of a reaction between the reducing sugar present and amino or other nitrogen groups on the protein molecules. To that end, a brief review of the properties of amino acid-sugar reactions will be offered here.

The literature pertaining to this group of reactions is voluminous, but in spite of this no clear picture of the reaction has been obtained since the multitude of products formed have not been completely characterized, even qualitatively.

In general, the reactions may be classified into two groups:

(a). Those taking place in relatively dilute solutions, often in the physiological pH range, at moderate temperatures, and not necessarily involving extensive changes in the physical properties of the reaction medium.

(b). Those taking place in concentrated solutions over the whole temperate range of 0 to 180°C., often at alkaline pH's and always involving changes, usually extensive, in the physical properties of the reaction mixture.
Undoubtedly, these are both manifestations of the same related series of reaction. The first group probably represents the initial step in the series of reactions, and is a necessary preliminary to the second. This is indicated by the fact that studies of the first often show evidences of the second. The conditions are, of course, overlapping, a change in any one accelerating the reaction beyond the first stage.

In dilute solutions at physiological pH's the reaction seems to proceed in some stoichiometric fashion between amino acids and aldoses, (41,61) the reaction taking place between the amino and aldehyde groups with a resulting tie up of Van Slyke nitrogen and sugar molecules (1,26) and a lowering of the pH of the reaction mixture (1,30). The course of this reaction has been followed and measured in terms of loss of Van Slyke N(1,8,59), formol titration (35), disappearance of reducing sugar (21), change in optical rotation (21), change in pH (30), decolorizing of methylene blue (8), and changes in freezing point depression. The reaction proceeds slowly at room temperature (21), more rapidly as the temperature is raised (8). The effect of pH is very pronounced; the reaction in this phase does not take place below pH 3.0 and becomes very extensive the higher the pH (1,59). The extent and speed of the reaction are less with dipeptides than with amino acids (8,59) and still slower with tripeptides, peptones and proteins (30).
Most of the amino acids have been reported taking part in such a reaction (30). It is generally reported that of a homologous series such as the aliphatic amino acids, the lower members are most reactive (21). Cysteine is a special case which will be mentioned separately. A wide variety of sugars have been reported undergoing the reaction. Generally, only aldehyde sugars are reported as reacting, including the pentoses arabinose and xylose, the hexoses glucose, galactose and mannose, and the disaccharides lactose and maltose (30,35). Sucrose and raffinose do not react. The literature on fructose is controversial, with its being reported both as reacting (21) and not (50). The pH may be the determining factor here with the reaction taking place primarily in alkaline solutions.

The reaction in this stage may or may not be reversible depending upon the time, temperature and pH of the medium (18). At higher temperatures and pH's some of the reaction product is not split to its components by acid (21). The reaction at this stage is not usually characterized by the development of color and the production of fluorescent substances has not been recorded.

More important are the reactions which take place, probably subsequent to the above, resulting in new, varied and complex reaction products as a result of more extensive reaction of the two compounds. These further reactions take place primarily in more concentrated solutions and at higher temperatures (47), and result in the production of complex, high molecular weight, highly colored, fluorescent compounds of pronounced odor and flavor, now generally given the name
melanoidins (17). At still higher concentrations and temperatures, the final product may be a charred mass (47). In both of these latter stages, the reaction or reactions have proceeded to the extent that they cannot be reversed, and the products already formed continue in the chain of reactions which ultimately result in the charred mass.

When a syrupy solution of glucose containing glycine is heated, the solution quickly turns yellow, and there follows a sequence of color changes from light tan through dark brown and black. If heating is continued, a violet evolution of gas will finally result, and the end product will be a porous charred mass (47).

During the earlier stages of this reaction there are produced substances; the aroma and flavor of which are characteristic of the amino acid employed. For example, the glycine-glucose reaction mixture has a caramel odor and flavor; the leucine products have a bread-like aroma and a flavor resembling that of honey; the valine complex is caramel like and that of phenylalanine has the aroma of withered roses (55). During the latter stages of evolution of gas, more pungent materials are given off of the reaction mixture (4).

The reaction mixture of glycine and glucose, when exposed to ultraviolet light, shows a very intense blue-green fluorescence (45, 17).

Another property of the reaction mixture is its ability to reduce the dyes, methylene blue and 2,6-dichlorophenolindophenol (8).
This has been reported as being important to the determination of vitamin C by titration with the latter, giving results which are higher than the true ascorbic acid content (18).

The temperature coefficient of this type of reaction is very high. It is observed to take place at 0°C. (as measured by change in pH of the solution), at 37°C. it is moderately rapid and at 100°C. very rapid, and at 150°C. the gases are evolved with explosive violence (47).

Representatives of various types of sugars also have been reported to react in this extensive fashion. These include pentoses, such as xylose and arabinose; the hexoses, glucose, mannose and galactose; and disaccharides such as lactose and maltose (47). It has been reported that only aldoses react, but exceptions to this are noted. Sucrose seems quite inert except under conditions which suggest its hydrolysis (47); raffinose and trehalose are also non-reacting; alkaline conditions which may lead to its fragmentation (47). Lower aldehydes such as methyl glyoxal likewise react (20).

Representatives of the several groups of amino acids are known to react (42).

Compounds reported to result from the reaction are many and varied. N-glycosides of glycine and lysine have been isolated from reaction mixtures (41, 61). Some of the dark brown reaction products called melanoidins have been isolated and partly analyzed (17, 71).
They have been reported to contain carboxyl groups, alcoholic and phenolic hydroxy groups, carbonyl and methoxy groups, and double bond carbon linkages (17). Extensive decomposition at high temperatures has led to the production of carbon dioxide, dehydration (47) production of aldehydes and isolation of hydroxymethyl furfural (4).

8. The amino acid-sugar reaction in food products.

The reaction between sugars and amino acids or proteins has been suggested as a factor in the preparation and storage of various food products. In the manufacture of molasses, vats of the product which are held at high temperatures sometimes begin to foam in much the same manner as the heated solutions of glucose and glycine, overflow the tank and on occasion have been reduced to the charred residue also characteristic of this reaction (38). Molasses have also been reported to deteriorate in the same manner, but much more slowly, when stored over a period of time at lower temperatures (9). There is the possibility that the melanoidins may be responsible in part for the color of molasses.

In the evaporation of water from milk for the preparation of concentrated product, the development of color is noticed. This is particularly true in the case of sweetened evaporated milk, in which glucose is added before the end of the heating period (54). These products are observed to further darken in color on storage. Dried milk and dried whey have been observed to darken and become
insoluble on dry storage (15). That the interaction of casein and lactose is responsible for the darkening of some milk products has been proven by Stewart (62).

Dried apricots develop an intense black color when stored at ordinary temperatures, unless the cut fruit is treated with sulfur dioxide before drying. This has been shown to be due to the reaction of amino acids (aspartic and glutamic acids) with glucose or fructose (71).

In the kilning of malt, a decrease in free amino acids is noted accompanied by the development of color and aroma. The changes have all the characteristics of an amino acid-sugar reaction and produce melanoidins which have been held as endowing the beer with color, flavor, and foaming power (19,42). Work which shows a correlation between the glucose content of potato slices and the darkness of potato chips prepared from them suggests the amino acid-sugar reaction may be involved in the production of an undesirable dark color (13). Fluorescing substances have been reported developing in dried milk, dried banana flakes, and soya flour on aging (62).

9. Cysteine sugar reactions.

The reaction of cysteine deserves some special attention. Because of the sulfhydryl group in its molecule, it forms with reducing sugars a type of stable compound which can be isolated.
Schubert (58) isolated the compounds formed by the reaction of cysteine with reducing sugars. In each case the reactants combined in equimolar ratios with the elimination of a molecule of water. The compounds were colorless, soluble in water, and gave no test for the sulfhydryl group as did free cysteine. These properties were considered suggestive of thiazolidine compounds such as are formed by cysteine and simple aldehydes (Schubert 57).

Agren (1) found that a cysteine and glucose containing solution buffered at pH 7.4 showed a decrease of 80% in its Van Slyke nitrogen over a period of 72 hours at room temperature. The pH decreased at the same time, but if it was kept at 7.4 by additions of alkali, the reaction took place in 10 hours. As the reaction was carried out in progressively more alkaline buffers, the speed of reaction increased. The compound formed gave a negative test for sulfhydryl groups and a negative test for Van Slyke nitrogen. He concluded that the compound formed was a thiazolidine carboxylic acid of the structure:

```
\begin{center}
\begin{array}{c}
\text{R} \\
\text{S} \\
\text{H} \\
\end{array}
\end{center}
```

\begin{center}
\begin{array}{c}
\text{C - COOH} \\
\text{H} \\
\end{array}
\end{center}

\begin{center}
\begin{array}{c}
\text{N H}^+ \\
\text{C} \\
\end{array}
\end{center}

where R is the sugar residue.

Comparing the rate of loss of Van Slyke nitrogen and sulfhydryl groups, he found that the initial reaction was between the amino group and the aldehyde of the sugar, with subsequent reaction of the sulfhydryl group to close the ring.
Agren (2) subsequently reported what appears to be another type of cysteine glucose compound. This compound gave a negative test for amino group, but a positive test for cysteine sulfur (original not seen, methods not given in abstract). The compound tended to dissociate in water solution. These properties are in contrast to those of the other compound he reports, and this is probably the simple aldehyde-amino combination, which was mentioned as the first step in the formation of the thiasolidine compound.

Schubert (58) found the reaction product of methylglyoxal and cysteine to be a brown amorphous gummy substance reminiscent of the melanoids, which methylglyoxal has been reported forming with other amino acids.
EXPERIMENTAL MATERIALS AND PROCEDURES

1. Egg albumen.

Shell eggs one day old were obtained from the college poultry farm and stored in a refrigerator at 40°F. until used, from one to ten days later. The eggs were broken and the albumen separated from the yolk and collected until a sufficient quantity was obtained. The liquid albumen was then treated in a Waring Blender until the structural quality of the thick white had been broken down and the liquid uniform in consistency.

2. Drying.

(a). Pan drying. In the earlier work the samples were prepared by this method, which gave a product similar to that obtained in the commercial pan-drying process. From 150 to 500 ml. of albumen was placed in a ten inch Pyrex glass pie plate and exposed to the radiation of several infra-red bulbs. An electric fan was used to keep a current of air flowing over the surface of the liquid, and resulting evaporation kept the temperature of the liquid below 45°C. As drying neared completion, the surface became a dry crust and the concentrated liquid beneath this adhered to it. This was then turned upside down in the pie plate, the lights were removed, and the drying completed by use of the fan only. The dry material was in translucent yellow flakes almost crystalline in appearance. It was ground in a mortar until it would pass a 30 mesh screen at which time it appeared almost white.
(b). Vacuum drying from the frozen state. Most of the samples used were prepared by this process because of its convenience of operation and the ease with which the material was reduced to a fine powder. The apparatus used was the same as that of Best (6) and he has described it and the process in detail. Figure 1 shows a diagram of the apparatus. The process may be summarized briefly as follows: the albumen to be dried was placed in a round bottom flask of one liter capacity (A) which had a standard taper ground glass mouth. The flask was stoppered, floated in a dry ice-alcohol mixture, and rotated continuously so that the albumen froze in a thin layer on the inner surface of the flask. The sample flask was then attached to the corresponding standard taper joint of the condenser flask (C), the latter connected to a Cenco Hyvac vacuum pump (D) and the system evacuated. The condenser flask was immersed in the dry ice-alcohol mixture which was used to freeze the sample, and which was held in a Dewar vacuum flask of one gallon capacity. The distillation of moisture from the sample was rapid enough, and the transfer of heat from the room air through the flask wall into the sample slow enough that the sample remained frozen during drying. The product obtained by this method is a porous white material of low density which crumbled to powder readily when subjected to slight pressure in a mortar. It was ground thus until it passed through a 30 mesh screen.
Fig. 1. Apparatus for vacuum drying in the frozen state.
3. Adjustment of moisture content of samples.

Best (6) reported that the moisture content of dried egg albumen depends upon the vapor pressure of the aqueous solution with which it has come to equilibrium in a closed chamber. He used a saturated solution of chromium trioxide to give an atmosphere which would produce 10% moisture albumen.

The samples were placed in the containers in which they were subsequently to be stored and these placed in a vacuum desiccator. A beaker of the solution used to obtain the desired moisture content was included. The chamber was evacuated until the solution showed a tendency to form bubbles and then the cock was closed. The samples were retained in this atmosphere until uniform moisture content in the samples was reached. This required from two to five days, depending upon the size and number of samples included.

For the production of moisture levels below 10%, concentrated sulfuric acid solutions were used. The concentrations of the solutions which produced 5.0%, 2.5% and 1.2% moisture levels were 72%, 77% and 90% by weight, respectively.

4. Containers.

In all of the work, the dried material of each treatment was subdivided into separate small samples for storage at the different lengths of time. Each sample was placed in a container of one of two types:
(a). Test tubes. Six inch soft glass test tubes were used, and after the sample had been brought to the appropriate moisture level, tightly fitting rubber stoppers were inserted. The stopper and upper one-half inch of the test tubes were dipped in a wax of melting range 160-165°F, to prevent loss in moisture vapor. This method was not used for samples stored above 50°C, or for samples of moisture content below 10%.

(b). Tin cans. Samples were placed in tin cans 2 11/16 inches in diameter and 2 inches deep. After the samples had reached the desired moisture content, the cans were removed from the desiccator and the lids double-crimp sealed on with a Dixie Automatic Can Sealer. This operation was performed as rapidly as possible to prevent any change in moisture content of the samples.

5. Fermentation of albumen.

The "spontaneous" fermentation of egg albumen has been described by Stewart and Kline (64), Goresline and Stuart (65). They pointed out a relationship between the decrease in pH of the fermenting liquid and the disappearance of glucose from it, indicating that when the pH has fallen below 6.0 and then rises again, the glucose has disappeared. Stuart and Goresline have shown that one of the predominating organisms in the fermentation is *Aerobacter aerogenes*.

A small sample of egg albumen was inoculated with a broth culture of *Aerobacter aerogenes* and allowed to develop an active fermentation.
as indicated by a "fruity" aroma. This egg white was then used to inoculate a larger volume of albumen to the extent of about 5%. The fermentation was allowed to proceed for several hours after pH 6.0 was reached, which was usually after 48 to 72 hours. As a test for completeness of fermentation a five ml. sample of the albumen in a small beaker was heated in an air oven at 120°C., for two hours and the color noted. Experience has indicated that when no color beyond the pale yellow shown by pan dried albumen develops under such conditions, the glucose has been completely removed.

The fermented liquid was then placed in the Waring Blender until uniform in consistency throughout.

6. **pH adjustments.**

Where adjustments of pH were made in the direction of greater acidity (liquid egg white was pH 8.5 to 9.0) normal hydrochloric acid solution was used and added dropwise with constant stirring until the desired pH was reached. Where alkali additions were required (hydrochloride addition products, neutralization of fermented albumen) normal sodium hydroxide was used. All pH measurements were made with a Leeds and Northrup Model 7663 A-1 pH assembly.

7. **Incorporation of added substances into liquid albumen.**

The amino acids, sugars, and other compounds which were soluble were dissolved in water (usually less than 10% of the volume of albumen to which addition was made) and the solution added to the albumen with
constant stirring. The amino acid hydrochlorides were dissolved in water, and in some cases the solution partly neutralized with alkali (cysteine hydrochloride) before addition. Where this caused precipitation of the insoluble amino acid, the acid solution was added and the albumen mixture subsequently neutralized.

The amino acid-albumen samples generally were allowed to stand overnight but the sugar-albumen samples were dried immediately upon preparation.

8. Analytical methods.

(a). Moisture. The method of the Association of Official Agricultural Chemists [50, p. 308] was followed except for two modifications. The sample size was reduced from two grams to one because of limitations in amount of material. The sifting of the sample was eliminated from the procedure because this was done in preparing the samples, and because the exposure to air involved in doing this may change the moisture content to a large extent, particularly in the low moisture samples.

A Weber electric vacuum oven was used. The temperature was 100°C, the pressure one centimeter of mercury, and the time five hours. Samples were cooled in a desiccator over fresh phosphorous pentoxide.

(b). Solubility. A 0.500 gram sample was weighed into a Pyrex test tube 22 x 175 mm. Buffer solution was added from a pipet which was calibrated to deliver that amount (about 24.5 ml.) which, added to
the sample, gave a total volume of 25.0 ml. The sample and buffer solution were then thoroughly mixed with a test tube homogenizer of the type described by Lundgren and Noble (46).

The test tube homogenizer, Figure 2, consisted of a rubber stopper ground with emery cloth until it formed a smooth cylinder about one inch long and of such a diameter that it fit snugly into a 22 x 175 mm. test tube (tubes which gave a close fit were selected from a larger group). The lower end was rounded with the emery cloth until it fit the bottom of the test tube. In the upper end a hole 3/16 inch in diameter and 1/2 inch deep was cut with a cork borer, and one end of a brass rod 1/4 inch in diameter was inserted. The other end of the rod was inserted in the chuck of a Cenco No. 18805 stirring motor.

The mouth of the tube containing buffer and sample was thrust over the rubber plunger, and the tube moved up until the plunger was completely immersed in the solution. The stirring motor was then turned on and, with the plunger rotating, the tube moved up and down several times, forcing the sample and solution through the narrow passage between the plunger and the tube wall. In this way, thorough dispersion of the solid material was accomplished.

The resulting solution or dispersion was allowed to stand one-half hour. At the end of that time one gram of the diatomaceous earth filter aid, Dicalite Speedex, was added and the mixture filtered using Watman No. 12 folded paper. A five ml. aliquot of the clear filtrate was pipetted into a dried, tared 50 ml. beaker. This was placed in
Fig. 2. Test Tube Homogenizer
the vacuum oven for five hours as in the moisture determination, and cooled over phosphorus pentoxide and reweighed.

The dry solids obtained from the 5 ml. aliquot of the filtrate represented the salt content of the buffer solution and the soluble material in one-fifth of the original sample. The weight of solids obtained in evaporating a 5 ml. portion of the buffer was therefore determined and subtracted from the total dry solid material obtained to give the solids arising from the 100 mg. of sample. Since the original sample contained moisture, this figure was never 100 mg., and was rarely that even when the moisture content was considered. For this reason, the "per cent solubility" for any series of samples was calculated using the amount of soluble material found in the original, unstored sample as 100%.

The use of water as the solvent was contemplated, but some difficulty was encountered in the filtration of the solution. It was found that the pH of the water solution varied directly with the age of the sample. For this reason, the solubility of a series of albumen samples in water and in two 0.1 molar phosphate buffers of pH 4.8 and 8.8 respectively was determined. Table 1 and Figure 3 show these results. Although water dissolved more material than either of the buffers, the effect of aging on the solubility of the samples was of the same nature for all three solvents. Because the pH 4.8 buffer was the only solvent which always gave solutions which filtered readily, it was selected for use in these studies.
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Fig. 3. Solubility of Egg Albumen in Buffer Solutions.
The buffer was prepared by mixing 700 ml. of 0.1 molar monobasic potassium phosphate and 10 ml. of 0.1 molar dibasic potassium phosphate.

(c). Fluorescence. A 0.250 gram sample of albumen was weighed into a test tube of the same size as that used for the solubility determination. A 25 ml. portion of the 0.1 molar phosphate buffer used for solubility determinations was added and the mixture blended with the test tube homogenizer. The solution was allowed to stand one-half hour, then filtered using Whatman No. 12 filter paper. A 15 ml. portion of the clear filtrate was used to determine fluorescence. A Model 12 Coleman Photo-fluorometer was used. The primary filter (B-1) transmitted incident light of 365 millimicrons, the secondary filter (PG-1), light of greater than 365 millimicrons. The instrument was calibrated to give a reading of 50 for a solution of quinine sulfate 0.2 milligram per liter in 0.1 normal sulfuric acid. When the full scale reading of 100 was exceeded, the extract was diluted with buffer solution and the reading multiplied accordingly.

(d). pH measurements. One gram of the dried albumen was added to 7 ml. of water, the mixture was stirred, and the pH thereof determined with the Leeds and Northrup Model 7665-A-1 pH assembly. This value is referred to as the pH of the reconstituted sample, as contrasted to the pH of the liquid albumen before it was dried.
EXPERIMENTAL RESULTS AND DISCUSSION

1. Characteristics of the deterioration.

The changes in several properties of vacuum dried egg albumen stored at two different temperatures are shown in Figures 4 and 5 and Tables 2 and 3.

The color of the dried albumen is white or faintly yellow before storage, but on aging turns first to a deep yellow, then orange, light brown, and finally a deep brown color, which is maintained for a very extensive storage period. This is similar to the color changes of pan dried flake albumen except that, because of its coarse granular nature, the latter shows its qualitative changes in apparent color more clearly, and often shows a definite red-brown phase in its color development.

The solubility of the product is not immediately affected, but shows a preliminary phase during which it retains its original solubility in a quantitative sense. However, the solution during this phase shows a tendency to be clearer than the sometimes turbid solution of unstored dried albumen. This is particularly true of unground flake (pan dried) albumen. After the preliminary period the solubility decreases rapidly until it has reached about 30 per cent of the original. After this it continues to decrease slowly until, in some cases, only 10 per cent of the originally soluble solids will go in solution. This quite possibly represents the inorganic material originally present, which constitutes
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* Vacuum dried, 10% moisture, pH 9.5, 70°C.
Fig. 4  Changes in Properties of Dried Egg Albumen.
Table 3

CHANGES IN PROPERTIES OF DRIED EGG ALBUMIN

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* Vacuum dried, 10% moisture, pH 9.6, 60°C.
Fig. 5  Changes in Properties of Dried Egg Albumen.
5 to 7 per cent of the solids of egg white.

The pH of the reconstituted albumen, like the solubility, undergoes little or no change in the early part of the storage period. At least the changes are of the magnitude of experimental error. At about the same time that the solubility begins to fall off, the decrease in pH becomes more pronounced and falls in a quite steady but unpronounced fashion during the rather sharp drop in solubility. After the solubility has slowed its fall in the range of its limiting value, the pH usually falls more sharply and reaches a value of one or more pH units below its original.

Of the several characteristics observed, the fluorescence shows the most unusual type of change. It begins an immediate and quite sharp increase in value, and reaches a maximum of 200 - 300 (depending on the particular sample) at about the same time that the solubility is showing its first slight changes in value. The fluorescence then falls again during the period of the sharp decline in solubility, but does not always show as sharp or as consistent a decline as does the latter characteristic. It does, however, continue to decrease until it eventually reaches values of 20 - 60. Sometimes there is a sharp drop in fluorescence from values of 150 - 200 to a range of 60 - 90 roughly coinciding with the end of the sharpest decrease in solubility.

Most interesting of the changes of the fluorescence value is the marked increase during the early storage period during which solubility shows no measurable change. There is almost always a change from an initial value of 15 - 25 to one of 100 - 125, in which range an increase
of 5 units is readily and accurately detected. This represents the most sensitive indicator of changes taking place during this period. A very cursory test of the angel food cake making properties of such samples indicates that changes in the performance of the albumen take place at about the time the fluorescence is around 100 and just before the solubility begins to show its measurable change. However, these tests are not yet extensive enough to warrant assuming a direct relationship. On the other hand, the desirability of a sample of albumen to be used for adhesive purposes is based to a large extent upon its solubility, and measurements indicating the decline of this characteristic do indicate its value. The results in most of the work following are therefore judged on the basis of solubility.

The effect of temperature, discussed more fully in the next section, can be briefly seen by comparing the scale of time in Figures 4 and 5. For graphs showing very similar curves for all properties, the scale at 60°C is three times that at 70°C, indicating roughly that the reaction proceeds three times as rapidly at the latter temperature.

2. Effect of temperature.

The changes in properties of dried albumen over a wide temperature range are shown in Tables 4 and 5 and Figures 6 and 7. For the temperatures from 40°C to 70°C the solubility curves are shown in Figure 6. It will be noted that the "S" type of curve shown in Figures 4 and 5 for solubility applies as well to these curves as far as they can be shown.
### Table 4

CHANGES IN SOLUBILITY OF DRIED EGG ALBUMEN* AT SEVERAL TEMPERATURES

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</tbody>
</table>

* Vacuum dried, 10% moisture, pH 9.5-9.7
Fig. 6 Changes in Solubility of Dried Egg Albumen at Several Temperatures

- 10% Moisture
- pH 9.5-9.7
Table 5

CHANGES IN FLUORESCENCE OF DRIED EGG ALBUMEN* AT SEVERAL TEMPERATURES

<table>
<thead>
<tr>
<th>Elapsed Time</th>
<th>50°C</th>
<th>40°C</th>
<th>30°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hours</td>
<td>14</td>
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<td>14</td>
<td>14</td>
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<tr>
<td>20</td>
<td>38</td>
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<td>70</td>
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<td>90</td>
<td>280</td>
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<td>100</td>
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</tr>
<tr>
<td>130</td>
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<td></td>
</tr>
<tr>
<td>6 days</td>
<td>110</td>
<td>150</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>160</td>
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<tr>
<td>13</td>
<td>265</td>
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<td></td>
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<tr>
<td>14</td>
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<td>16</td>
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<td>17</td>
<td>300</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>280</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 weeks</td>
<td>35</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>130</td>
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</tr>
<tr>
<td>7</td>
<td>175</td>
<td>48</td>
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<td></td>
</tr>
</tbody>
</table>

* Vacuum dried, 10% moisture, pH 9.5-9.7.
Fig. 7 Changes in Fluorescence of Dried Egg Albumen at Several Temperatures
To determine the time at which the fluorescence passes through 100 for this higher temperature, subtract 8.5, 5, 3, or 3.5 from the temperature.

It is necessary to consider the product at these temperatures. To do this, for the greatest period shown, in order to estimate the percentage, 100 per cent are recorded for 00 in solution after about one week.

It is desirable, however, that the temperature would be lower than 60 C. during the hot summer season, the situation would be just the opposite. The procedures of storage and measurement is that in a dry storage warehouse, with my readily reach a product is fine, the temperature for complete solubility for the present is in

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>115 hours</td>
<td>96</td>
</tr>
<tr>
<td>10-15 hours</td>
<td>90</td>
</tr>
<tr>
<td>9 hours</td>
<td>90</td>
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<tr>
<td>7 hours</td>
<td>99</td>
</tr>
<tr>
<td>5 hours</td>
<td>99</td>
</tr>
<tr>
<td>4 hours</td>
<td>99</td>
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<td>3 hours</td>
<td>99</td>
</tr>
<tr>
<td>2 hours</td>
<td>99</td>
</tr>
<tr>
<td>1 hour</td>
<td>99</td>
</tr>
</tbody>
</table>

When complete solubility is observed, the temperature for 60 C. or 60 C. and 90 C. respectively. If temperatures are reached by consideration the longest time for

If temperatures are reached by consideration the longest time for

get a very pronounced measure of the relative to the effect of temperatures on the determination is very pronounced. But to 60 C. and 90 C. is the temperature expected from the graph that the

order to have a same that produce different temperatures the curve for 70 C. or 90 C. or 90 C. is the temperature expected from the graph that the

If was necessary to estimate some of the positions for the 90 C. curve in
times very similar to those for the solubility data on the four higher temperatures. Again the ratios of "storage life", taking the lower values, are 1:5:20:100:500:2400, not far different from those derived from the solubility data above.

The implications of these data are that a dried albumen similar to that used here might be expected to retain its properties only 1 to 3 months in an unrefrigerated warehouse at cool temperatures. Considering that liquid egg products are produced to the extent of about 75 per cent of the yearly production during the four month period of March to June, part of the dried albumen produced during this season would be held in warehouses for a time greater than that necessary to bring about a change in its properties which will reduce or destroy its value, even under the best unrefrigerated storage conditions. An even greater possibility than this is the danger that a retail type package would be held on grocery and household shelves for even longer periods, almost ensuring that some product would prove of little use in the kitchen.

These data for the various temperatures may be used with reservation in making rapid estimates of the potential storage life of dried albumens. By storing them at a higher temperature, such as $60^\circ C$, and estimating the storage life at the more practical temperatures such as 20-40$^\circ C$, the elapsed time necessary to obtain some information on their stability is reduced. This method has been used in the following sections in which temperatures of 50 or 60$^\circ C$ only were utilized.
3. Effect of pH

It was suggested in the qualitative work of Stewart and Kline (64) that the pH of the liquid which was dried might affect the rate of deterioration. If true this would represent a commercially feasible process, since the addition of acid to egg white has been employed in commercial work (29, 37, 43, 44).

The results of such a test are shown in Table 6 and Figure 8.

The pH of the liquid albumen was adjusted downward from its original value of 8.5 with hydrochloric acid. Intervals of one pH unit were used down to pH 5.0, which was thought to represent about the lower practical limit. The vacuum dried material, on reconstitution, showed generally a much higher pH than the liquid, but the effect on the property of the dry material is clearly shown nevertheless.

Again using the longest time for which complete solubility was recorded, the "storage life" of the respective products is roughly:

<table>
<thead>
<tr>
<th>pH</th>
<th>Storage Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.6</td>
<td>6 hours</td>
</tr>
<tr>
<td>9.0</td>
<td>20 hours</td>
</tr>
<tr>
<td>7.5</td>
<td>36 hours</td>
</tr>
<tr>
<td>4.8</td>
<td>72 hours</td>
</tr>
</tbody>
</table>

or a ratio of 1:3:6:12. If this estimate is then compounded with that of section 2 and the estimated storage life for 20 and 30°C calculated, it appears that the products at pH 7.5 and 4.8 respectively might conceivably have a storage life of 6 - 18 months, and 12 - 24 months, respectively. These values seem rather large when compared with the result of Stewart and Kline (64) who found a decrease in solubility of albumen of pH 6.3 in one week at 40°C.
Table 6

CHANGES IN SOLUBILITY OF DRIED EGG ALBUMENS* OF DIFFERENT pH

<table>
<thead>
<tr>
<th>Elapsed Time</th>
<th>pH 9.5**</th>
<th>pH 9.0</th>
<th>pH 7.3</th>
<th>pH 4.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6 hours</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>93</td>
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<tr>
<td>20</td>
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<td>24</td>
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<td>93</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>58</td>
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<td>100</td>
</tr>
<tr>
<td>84</td>
<td></td>
<td></td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td>46</td>
<td>60</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>36</td>
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<td>6</td>
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</tr>
<tr>
<td>9</td>
<td>23</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Vacuum dried, 9.7% moisture, 60°C.
** The pH of the reconstituted dried albumen is shown in the column heads. The pH of the liquid from which each was dried is as follows: 8.6, 7.0, 6.0, and 5.0, respectively.
Fig. 8 Changes in Solubility of Dried Egg Albumens of Different pH's
It should be noted that the effect of adjusting the pH of the material downward is largely expressed in an increase of time during which the solubility remains high. After the period of steadily decreasing solubility is reached, the curves for the different samples show much the same slope, particularly, those for pH 4.8, 7.5 and 9.0. This is in decided contrast to the wide variation in the slopes of the curves shown in Figure 6 for the several temperatures.

This would seem to indicate that the effect of the lower pH is to retard the initial step in the reaction, which, when finally accomplished, permits the insolubilization to take place relatively unhindered.

4. **Effect of moisture content.**

The effect of redrying albumen to several different moisture levels on its stability during storage is shown in Table 7 and Figure 9. Using the longest recorded time for which complete solubility was observed as a measure, the relative storage lives of samples of 9.6, 5.2, 2.0 and 1.3 per cent moisture are 5 hours (from Table 6), 38 hours, 6 days, and 5 days, or a ratio of 1:7:30:25. The latter figure probably should be 50 or more, but no data were obtained for the period between 5 and 10 days on this sample. The effect of moisture, then, is quite striking, particularly so when reduced from about 10 per cent to the range of 1 to 2 per cent. If the temperature relationship of Part 2 would apply here as well, the suggested storage life at 30°C for an albumen of 1.3 per cent moisture would be in the neighborhood of 2 years, which would
<table>
<thead>
<tr>
<th>Percent</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
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<tr>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>66</td>
<td>1</td>
</tr>
<tr>
<td>86</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table 1**

**Differential Molar Conductance**

**Changes in Solubility of Dried Egg Albumen**

**Table 2**
Fig. 9  Changes in Solubility of Dried Egg Albumen of pH 9.5 at Four Moisture Contents
probably answer most of the demands of stability which would be put on such a product.

The effect of moisture is all the more interesting when considered in light of the fact that such moisture contents may be achieved in commercial practice. That is, a machine has been built by the Food Machinery Corporation which will reduce the moisture content of dried whole egg to 0.6 per cent. According to vapor pressure data obtained by Best (6), this corresponds to the moisture range of 1-2 per cent for albumen. The machine is capable of commercial scale production (600 to 750# per hour) on whole egg. Whether dried albumen powder, which is of a much finer consistency than dried whole egg, can be handled by this particular machine has not been determined yet.

The effect of moisture level on albumens adjusted to several lower pH's has also been explored. This was done to find out if some intermediate combination of adjusted pH and lowered moisture content might prove capable of prolonging the storage life to the extent that the extreme cases of moisture and pH do when used separately. These data are shown in Table 8 and Figures 10, 11 and 12. Unfortunately these do not cover the range of moisture levels as well as could be desired, but some conclusions are indicated.

In every case, the reduction to moisture levels of 1.5 to 1.0, and 0.6 per cent in one case, had the effect of retaining complete solubility for about the same length of time, i.e. between 5 and 10 days at 60°C (only data points shown). However, examination of the curves shows that the rate of decreasing solubility is definitely affected by the pH
Table 8

CHANGES IN SOLUBILITY OF DRIED EGG ALBUMIN* OF DIFFERENT MOISTURE CONTENTS AND DIFFERENT pH

<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
<th>pH 9.0**</th>
<th>pH 7.3</th>
<th>pH 4.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 100</td>
<td>100 100 100</td>
<td>100 100 100</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 1/2</td>
<td>97</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>97</td>
<td>100</td>
</tr>
<tr>
<td>2 1/2</td>
<td>85</td>
<td>93</td>
<td></td>
</tr>
<tr>
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<td>68</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>3 1/2</td>
<td></td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>4</td>
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<td>93</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>99</td>
<td>40 94 100</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>33</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td></td>
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<td>18</td>
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<tr>
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<td>94 93</td>
<td>97 99</td>
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<td>94 93</td>
<td>96 97</td>
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<td>89</td>
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<tr>
<td>40</td>
<td>77</td>
<td>70 95</td>
<td>88 -</td>
</tr>
</tbody>
</table>

* Vacuum dried, 80°C.

**The pH of the reconstituted dried albumen. The pH of the liquid from which each was prepared was 7.0, 6.0, and 5.0 respectively.
Fig. 10  Changes in Solubility of Dried Egg Albumen of pH 9.0 at Two Moisture Contents
Fig. 11  Changes in Solubility of Dried Egg Albumen of pH 7.3 at Three Moisture Contents
Fig. 12  Changes in Solubility of Dried Egg Albumen of pH 4.8 at Three Moisture Contents
level even at these low moisture contents, with lower pH slowing
the change in solubility. The differences are small, but may indicate
a possible difference in the length of time complete solubility is retained.
But the spread would only be between 5 and 10 days at most. That the
effect of pH is still operative at the lower moisture levels is also
shown in Figure 13. The three samples in the range 1.0 to 1.5 per cent
moisture have curves which show the effect of pH, i.e., the sample at
pH 9.4 decreases in solubility more rapidly than does the one at pH 9.0,
which in turn decreases more rapidly than the one at pH 7.3. The curve
for pH 4.8 and 0.6 per cent moisture falls in its expected place also, but
since it is of a lower moisture content, it cannot be said without question
that this is solely a pH effect. It should be pointed out that since
these four samples were all brought to equilibrium over a sulfuric acid
solution from the same stock, their vapor pressures should correspond,
and the samples be equivalent. The fact that their vapor pressures are
the same would indicate some change in the amount of water free enough
in a chemical sense to exert vapor pressure. Since reductions in moisture
at a single pH are effective in slowing the rate of deterioration, it must
be the reduction in that water "free" enough to exert vapor pressure
which is effective in changing the reaction. Thus it might be expected
that samples of the same vapor pressure would have the same "effective"
water content (insofar as it is required by the reaction), rather than
those which have the same moisture content by an even method.

That pH has an independent effect at the lower moistures is also
shown by the fact that the sample of 2.9 per cent moisture of pH 7.3
Fig. 13 Changes in Solubility of Dried Egg Albumen of Different pH and Different Moisture Contents.
deteriorated more slowly than the one of 2.0 per cent at pH 9.6, where the moisture content alone would have indicated the reverse.

5. Effect of glucose content.

It was decided to determine the extent to which the glucose need be removed from albumen to insure complete stability in the dry state. The data obtained have some rather interesting though unexpected implications.

Table 9 and Figure 14 show the curves for the development of insolubility of four samples of albumen. One is the unfermented albumen, the other three samples are ones from which the glucose was completely removed by fermentation, and to which amounts of glucose were added back and the pH returned to 9.6 = 9.7 before drying. Actually, all of the fermented material was neutralized together and then separated for the addition of sugars, so that they were dried at the same pH.

The data unfortunately did not catch the discrete points which permit an estimation of the storage "life" of complete solubility of the samples. However, they indicate that for safety from the standpoint of stability under all conditions of the dried product, fermentation should be complete enough to remove the glucose to a level lower than 0.02 per cent in the liquid. How much lower a value must be reached is not indicated by the data here, but since the 0.02 per cent of sugar represents only 5 per cent of the normal content of albumen, it would seem that only complete removal of the sugar would answer the need.
<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
<th>Solubility Concentration of glucose in liquid albumen</th>
<th>Fluorescence Concentration of glucose in liquid albumen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.45% 0.10% 0.05% 0.02%</td>
<td>0.45% 0.10% 0.05% 0.02%</td>
</tr>
<tr>
<td>0</td>
<td>100    100    100    100</td>
<td>16      51      29      20</td>
</tr>
<tr>
<td>1/4</td>
<td>99     89</td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>80     92</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50     81     100</td>
<td></td>
</tr>
<tr>
<td>1 1/2</td>
<td>56     73     98</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27     76     98</td>
<td></td>
</tr>
<tr>
<td>2 1/2</td>
<td>21     -</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>47     93</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>43     83</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>37     -       96</td>
<td></td>
</tr>
<tr>
<td>7 1/2</td>
<td>24     -</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>43     89</td>
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</tr>
<tr>
<td>15</td>
<td>35     66</td>
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<td>20</td>
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<td>35</td>
<td>14     -</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>-      16</td>
<td></td>
</tr>
</tbody>
</table>

* Vacuum dried, 10% moisture, pH 9.6-9.7
Fig. 14 Changes in Solubility of Dried Egg Albumen of Different Glucose Content
Sure present

 Fluorescent material in albumen is retarded in some way to the amount of
 depleted or prefered waste of sample of each sample

 It appears that in the case of the amount of
 fluorescence in the sample, the sample from the
 above range. This can be seen more readily in Figure 1, where the
 wide range that the potentials range of fluorescence are in that order over the
 been noted. By not employing more samples in that range, it will be noted
 while the potential should be considered that other values might have
 220 and 120 per cent. For 0.10 per cent choice
 three samples prepared by immersion and addition of sugar are 490.
 Finch equation for the higher ones. The highest value recorded for the
 indications do not reach as high a value for the lower sample.

 It should also be noted that the fluorescence (Table 8) these
 seems to be retarded to the amount of sugar present
 sufficient to produce marked changes in the protector, the color displayed
 that it is seen that, while very small amounts of choice are
 showed only a barely detectable change in apparent color
 at 0.05 per cent sample which had received such a low choice
 at 0.05 per cent sample which had received such a low choice
 normal concentration of dissolved albumen. The color of solubility
 color at 0.05 per cent of choice this was orange, a color which under
 0.1 per cent of choice this was reduced to a can of light brown
 at 0.5 per cent of choice (3 per cent choice) the sample showed the usual brown color.

 that series which had about 50 per cent solubility
 in the notation
 besides each curve in Figure 1 to show the color of a sample of

 -69-
Fig. 15  Effect of Concentration of Glucose in Dried Egg Albumen on Development of Fluorescence.
6. **Effect of type of sugar.**

It was believed inferential data might be obtained about the part of glucose in the reaction if the effect of various compounds incorporated into egg white in place of glucose could be studied. This was accomplished by fermenting the glucose from the egg white and then adding the various compounds (after adjusting the pH to about 9.5), drying and storing as usual. All samples were studied at 10 per cent moisture and 50°C. Figure 16 and Tables 10 and 11 show some of the results.

As might be expected, the order of reactivity of the sugars or the speed with which they insolubilize the protein of the albumen is, in descending order, pentoses, hexoses and disaccharides. In other words, the low molecular weight aldopentose sugars react more readily than the larger hexose molecules and very much more rapidly than the disaccharides molecules. Within each group, again, there is a difference in the rate at which the reaction is completed. Of the pentoses, xylose reacts more rapidly than does arabinose. Among the hexoses, the effect of the different sugars is most pronounced, galactose showing almost the same reactivity as arabinose, the less active pentose. Mannose shows a speed of reaction which is much slower than that of galactose and glucose and more nearly that of the disaccharides. It will be noted from Table 10 that in spite of the different rates of reaction, all of the sugars ultimately produced a thoroughly insoluble product (15-25 per cent of original solubility).
# Table 10

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<th>Arabinose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Mannose</th>
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<th>Maltose</th>
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</tbody>
</table>

* Vacuum dried, 10% moisture, pH 9.2-9.6, 50°C. Originally fermented to remove natural glucose

** Amounts added, on liquid basis, Pentoses 0.38%
Hexoses 0.45%
Disaccharides 0.35%
Table II

CHANGES IN FLUORESCENCE OF DRIED EGG ALBUMENS* CONTAINING DIFFERENT SUGARS

<table>
<thead>
<tr>
<th>Elapsed Time</th>
<th>None</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Mannose</th>
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</table>

* Vacuum dried, 10% moisture, pH 9.2-9.6
Fig. 16 Changes in Solubility of Dried Egg Albumens Containing Different Aldehyde Sugars.
Table 11 shows the fluorescence changes taking place in the sugar samples. It will be noted that the same general trend of a fairly sharp rise in fluorescence to a maximum value and a falling off again to a value of 20 to 50 is followed for all sugars. The exceptions to this are the two pentoses, which at zero time, showed very large values. With xylose, the most reactive, the original value was the highest fluorescence found while with arabinose, the value went up from 385 to 510 and then fell. This indicates that in the short time that the dry samples were being adjusted in moisture content (three days at 20°C) the reaction took place to the extent of producing this fluorescence. The two samples also developed a definite brown color during this period. By consulting Table 7 it can be seen that a sample of natural glucose containing albumen has not reached its fluorescence maximum yet at 7 weeks at 30°C, although there has been a measurable increase in 4 days at 20°C.

Included in these studies but not in the table were sugars of the non-reducing type. Samples containing sucrose, trehalose and raffinose were stored for 105 days without showing any change in solubility. No change in fluorescence beyond the same slight increase shown by the control, sugar-free sample was observed for the samples containing these sugars.

Two other substances which were tested at the same time were the hexahydrate alcohols obtained by the reduction of hexoses, sorbitol and mannitol. These differ from glucose and mannose respectively in that the aldehyde groups are replaced by alcohol groups. As in the case of the non-reducing disaccharides above, the solubility of these samples did not
change a bit in 103 days at 50°C, and the fluorescence showed no greater increase than that of the fermented control. This indicates at least that the hydroxyl group of the sugar is not sufficient to initiate the reaction which leads to insolubility. That the aldehyde group is necessary for the reaction seems clear.

The change which took place in two samples to which fructose had been added are shown also in Tables 10 and 11. Like the aldehyde sugars mentioned above, fructose produced complete insolubility. The two samples represent two different preparations of fructose. The preparation listed as I was dark in color, had a caramelized odor, and may have been impure. Sample II was colorless and supposedly chemically pure. Contrary to the suggestion of the previous paragraph that only aldehyde sugars react, fructose, a keto sugar, will also react to produce insoluble protein in dried egg albumen. This however is not in contradiction to the literature of reactions of amino acids with fructose (21, 24). It is known that fructose in dilute alkaline solution comes to equilibrium with several sugars and sugar products, among them glucose and mannose.

Whether the influence of a pH of 9.5 might be enough to accomplish this in the albumen medium cannot be said, but the possibility is offered.

7. Effect of amino acids.

a. General. It has been shown that peptides react with sugars less readily than do amino acids (30, 60). If the reaction of sugars with
albumen is of the same nature, involving amino groups, it would then be expected to be much slower than that of amino acids. Amino acids added to egg albumen containing its natural glucose might be expected to react more readily than the proteins and prevent insolubilization of the latter. The results of a series of such trials are shown in Table 12 and Figure 17.

Several of the amino acids had a remarkable effect on the course of the reaction. The sample containing glycine was completely soluble yet after 14 days of storage at 50°C, while the untreated control was half insoluble by this time and had previously reached 75% solubility at seven days. The glycine sample did decrease in solubility but at neither the rate nor to the extent of the control sample, being still 85% soluble at 72 days when the control had long since approached 25% solubility. At the same time that its solubility remained high, the color of the glycine sample became a very dark brown, much deeper in color than the control sample. The fluorescence (Table 13) also reached the unusually high value of 2600, several times the largest ever observed in an ordinary albumen sample.

Alamine, the next higher member of the homologous series of aliphatic \( \alpha \)-amino acids, showed a slighter effect than that of glycine in every way when used on an equimolar basis. The solubility had changed already at 7 days, although it was 94% in contrast to the 75% of the control sample, and its downward trend continued. The fluorescence of this sample reached a value of 2100, almost as great as that of the glycine sample, but the color developed was not as dark as that of the latter.
### Table 12

**Changes in Solubility of Dried Egg Albumens* Containing Added Amino Acids**

<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
<th>None</th>
<th>Glycine</th>
<th>Alanine</th>
<th>Lysine</th>
<th>Glutamic Acid</th>
<th>Cysteine</th>
<th>Arginine</th>
<th>Tyrosine</th>
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</tr>
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</table>

**pH of reconstituted sample**

| % of amino acid added liquid on basis** | 9.7 | 9.3 | 9.4 | 8.8 | 9.2 | 9.5 | 9.1 | 9.2 |

* Fan dried, 10% moisture, 50°C.

** The equimolecular equivalent of the 0.45% of glucose present in liquid egg white, plus 10 per cent in excess of this.

*** As the di-hydrochloride

**** As the hydrochloride
Table 13
CHANGES IN FLUORESCENCE OF DRIED EGG ALBUMENS* CONTAINING ADDED AMINO ACIDS

<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
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<th>Alanine</th>
<th>Lysine</th>
<th>Glutamic Acid</th>
<th>Cysteine</th>
<th>Arginine</th>
<th>Tyrosine</th>
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</table>

* Pan dried, 10% moisture, 50°C.
Fig. 17  Changes in Solubility of Dried Egg Albumens Containing Added Amino Acids.
Of all the amino acids tested here, lysine proved most effective. The solubility of this sample at 72 days was 97%. How long it retained complete solubility beyond 14 days is not shown in the data, but the period was probably a week or two, since between two and six weeks of storage it lost only 3% of its solubility. This sample showed about the same color development as the glycine sample and reached about the same high fluorescence, 2700. Lysine, it should be noted, contains two free amino groups, in the α and ε position respectively.

Arginine, like lysine a basic amino acid but containing instead of the second amino group a guanido group, had an effect similar to that of alanine. The solubility of this sample remained higher at the early storage period of 10 days, then fell rather more sharply than did that of the other samples. The sample did not develop the extreme fluorescence shown by the others already mentioned, 620 being the highest value recorded. The color developed was as deep as that of the glycine sample.

Glutamic acid was very similar to alanine in its effect on solubility and fluorescence.

Tyrosine, which was not in solution at the time the sample was dried, had no preservative effect at all. In fact, this sample decreased in solubility more quickly than the untreated albumen. No explanation is offered for this.

b. Cysteine. Cysteine, the only amino acid tested which contained a sulfhydryl group, was different in its effect than any of the others.
Although the solubility change in this sample was similar to that of the glycine sample, there was a noticeable absence of color developed in the dry product or in the solution thereof. The fluorescence of this sample resembled most closely that of untreated albumen. This amino acid is apparently capable of protecting the solubility of the albumen to a certain extent without any accompanying development of intense dark brown color. This is interesting because of its practical implications as well as because of Agren's statement that cysteine forms a colorless compound with sugars.

Two other samples containing cysteine were prepared and stored, and the results of these trials, together with those of the first sample and the untreated control from Table 12, are shown in Table 14 and Figure 13. In one of these additional samples, the same amount (0.48%) of cysteine was added as in the original trial. The pH of the liquid albumen and cysteine mixture, which stood overnight, was lower than before, 7.2 in contrast to 8.6, and the dry albumen was correspondingly lower in pH, 8.7 as against 9.5. The solubility fell off more rapidly than in the first trial, although more slowly than the control. Again the color development was much less than that of the other amino acid treated samples.

In the third sample, the possible effect of concentration of cysteine was considered, and over three times the molar equivalent of the glucose present was added. The solution of cysteine in liquid albumen stood overnight at a pH of 8.3, slightly above the 8.6 liquid pH of the first sample. The dry sample showed a pH of 8.6 similar to that of the
Table 14

CHANGES IN SOLUBILITY OF DRIED EGG ALBUMENS*
CONTAINING ADDED CYSTEINE

<table>
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<th>Elapsed Time</th>
<th>Control (none)</th>
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* Pan dried, 10% moisture, 50°C.

** Stored overnight in liquid albumen, at pH 7.2. pH of dry material 8.7

*** " " " " pH 8.6 pH " " " 9.5

**** " " " " pH 8.8 pH " " " 8.8
Fig. 18 Changes in Solubility of Dried Egg Albumens Containing Cysteine.
second trial. This sample had the best solubility retention of the three, being 90% soluble at 105 days. The sample showed a considerable development of color, however.

The results of these trials of cysteine are particularly interesting in the light of Agren's work on cysteine-glucose compounds. His work indicated the following: (1) The reaction which produces a colorless cysteine-glucose compound of the thiazolidine type is not instantaneous, requiring as much as 72 hours for completion. (2) The reaction product is very stable. (3) Increasing the pH of the reaction medium increases the speed of the reaction. (4) A second type of compound of the "classical" aldehyde-amino group reaction may be produced at the same time.

The use of higher pH in the liquid albumen cysteine mixture must have promoted the formation of the colorless stable compound of the thiazolidine type and resulted in less reaction between sugar and albumen in the dry sample. When the lower pH was used, the reaction of cysteine and glucose was not complete enough in 24 hours of holding the liquid to prevent subsequent reaction of glucose and dry albumen. The color development in the latter sample was not as great when it was finally insoluble as was that of the untreated control. This result is similar to that of the low concentrations of glucose in Part 5. Perhaps some combination of cysteine and glucose took place in this sample.

Some further tests run on cysteine samples at 60°C are shown in Table 15. The results were rather disappointing, and are probably a demonstration that the accelerated test at such a temperature cannot always
Table 15

CHANGES IN SOLUBILITY OF DRIED EGG ALBUMENS* CONTAINING
ADDED CYSTEINE AND ESTERS OF TYROSINE

<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
<th>Control</th>
<th>Cysteine** 0.46%</th>
<th>Cysteine*** 2%</th>
<th>Cysteine**** 2%</th>
<th>Ethyl tyrosinate 0.45%</th>
<th>Butyl tyrosinate 0.75%</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>49</td>
<td>100</td>
<td>62</td>
<td>92</td>
<td>92</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>100</td>
<td>42</td>
<td>68</td>
<td>59</td>
<td>19</td>
</tr>
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<td>3</td>
<td>98</td>
<td>100</td>
<td>36</td>
<td>67</td>
<td>46</td>
<td>18</td>
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<tr>
<td>4</td>
<td>85</td>
<td>100</td>
<td>29</td>
<td>55</td>
<td>39</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>82</td>
<td>100</td>
<td>29</td>
<td>71</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>100</td>
<td>29</td>
<td>55</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>100</td>
<td>29</td>
<td>67</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
<td>100</td>
<td>29</td>
<td>51</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
<td>100</td>
<td>51</td>
<td>75</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>41</td>
<td>100</td>
<td>52</td>
<td>75</td>
<td>22</td>
<td>18</td>
</tr>
</tbody>
</table>

* Vacuum dried, 10% moisture, 60°C.

** Stood overnight with liquid albumen at pH 6.9. pH of dry albumen 8.0

*** " " " " " " " pH 7.9 pH " " " " 8.5

***Dried without standing, liquid pH 8.8. pH of dry albumen 7.0
be used. The only sample which showed an encouraging result was one in which the molar equivalent of the glucose was used, and in which the liquid albumen stood at pH 6.9. This was similar in reaction to the sample of this pH which was stored at 50°C. However, this sample became darker brown than the control. The other two samples, in which high concentrations of cysteine were used, gave evidence of decomposition, with both ammonia and hydrogen sulfide in evidence when the cans were opened. These also were very dark brown.

c. Glycine. The effect of greater concentration of added glycine was tested. Table 16 shows fluorescence and solubility data for samples containing one mole equivalent (data of Table 12) and one in which over three times this amount was added. The solubility of the latter was better for the period of 72 days, but until this time was the same as that of the sample of lower glycine concentration. The fluorescence rose to higher values, but the color was approximately the same.

One more test was made of the effect of glycine on the keeping quality of dried albumen. An untreated sample and one containing 1% of added glycine were prepared and stored at 80°C. The properties are shown in Table 17. Although complete solubility was retained by the glycine sample while the control became quite insoluble, the solubility of the former might have fallen on longer storage.

Angel cakes were prepared from the four completely soluble glycine samples and from the first two control samples. As would be expected, the cakes of the glycine samples were brown in color, being progressively
Table 16

**EFFECT OF CONCENTRATION OF GLYCINE IN DRIED EGG ALBUMEN** on changes in solubility and fluorescence

<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
<th>Solubility Glycine content</th>
<th>Fluorescence Glycine content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0%</td>
<td>0.23%</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>54</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>37</td>
<td>98</td>
</tr>
<tr>
<td>72</td>
<td>25</td>
<td>85</td>
</tr>
</tbody>
</table>

*Pan dried, 10% moisture, 50°C.*
Table 17

EFFECT OF ADDED GLYCINE ON THE PROPERTIES OF DRIED EGG ALBUMEN*

<table>
<thead>
<tr>
<th>Elapsed Time (hours)</th>
<th>Solubility</th>
<th>Fluorescence</th>
<th>pH</th>
<th>Color**</th>
<th>Cake Volume co.</th>
<th>Volume of Foam 1 1/2 min. beating</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>33</td>
<td>9.80</td>
<td>yellow</td>
<td>667</td>
<td>450</td>
</tr>
<tr>
<td>5</td>
<td>98</td>
<td>115</td>
<td>9.66</td>
<td>dk.yellow</td>
<td>560</td>
<td>390</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>140</td>
<td>9.29</td>
<td>brown</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>74</td>
<td>9.11</td>
<td>brown</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

GLYCINE***

<table>
<thead>
<tr>
<th>Elapsed Time (hours)</th>
<th>Solubility</th>
<th>Fluorescence</th>
<th>pH</th>
<th>Color**</th>
<th>Cake Volume co.</th>
<th>Volume of Foam 1 1/2 min. beating</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>330</td>
<td>8.45</td>
<td>brown</td>
<td>651</td>
<td>540</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>910</td>
<td>8.29</td>
<td>dk.brown</td>
<td>611</td>
<td>475</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>2000</td>
<td>9.08</td>
<td>dk.brown</td>
<td>575</td>
<td>450</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>2500</td>
<td>8.10</td>
<td>almost blk.</td>
<td>515</td>
<td>420</td>
</tr>
</tbody>
</table>

* 10% moisture, 60°C. vacuum dried.
** Color of solution
*** 1% added, liquid basis.
darker for the more aged samples. They also had a toasted or coffee-like flavor which increased in intensity with age of the sample. In spite of the complete solubility of the albumen, the four samples showed a progressive decrease in cake volume and a proportionately more solid structure; apparently the leavening power of the albumen had disappeared.

The cakes from the first two untreated albumen samples were of normal white color and aroma, but of relatively poor volume. The second albumen sample, one of 98% solubility, gave a cake of volume about as small as the most aged of the glycine treated samples.

As suggestive information about the high fluorescences in the amino acid samples, a solution 3.2% glucose and 8.0% glycine was refluxed and its fluorescence measured at several intervals. The concentrations represent in rough measure the amount of these components in the glycine treated albumen shown above. The data are shown in Table 18.

The fluorescence rose steadily for 18 hours of refluxing, and reached a value of 98,000. At about the time it began to decrease again, black insoluble material began to separate from the solution. Neither glucose nor glycine solutions, when refluxed separately, showed any development of color or fluorescence. The values observed suggest that the fluorescence which develops in a dry albumen sample containing glycine may be due in part to a reaction of glycine with the glucose present.

8. Effect of miscellaneous compounds.

A number of other substances were added to egg albumen in the hope
<table>
<thead>
<tr>
<th>Time of Refluxing (hours)</th>
<th>Fluorescence</th>
<th>pH</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55**</td>
<td>6.08</td>
<td>none</td>
</tr>
<tr>
<td>1/4</td>
<td>390</td>
<td>5.89</td>
<td>pale brown</td>
</tr>
<tr>
<td>1/2</td>
<td>740</td>
<td>5.60</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>2400***</td>
<td>5.51</td>
<td>brown</td>
</tr>
<tr>
<td>1</td>
<td>5100</td>
<td>5.32</td>
<td>dark brown</td>
</tr>
<tr>
<td>1 1/2</td>
<td>4200</td>
<td>5.21</td>
<td>black</td>
</tr>
<tr>
<td>2</td>
<td>7200</td>
<td>5.11</td>
<td>black</td>
</tr>
<tr>
<td>3</td>
<td>9100</td>
<td>4.99</td>
<td>black</td>
</tr>
<tr>
<td>6</td>
<td>32,000</td>
<td>4.60</td>
<td>black</td>
</tr>
<tr>
<td>8</td>
<td>51,000</td>
<td>4.49</td>
<td>black</td>
</tr>
<tr>
<td>10</td>
<td>56,000</td>
<td>4.40</td>
<td>black</td>
</tr>
<tr>
<td>12</td>
<td>77,000</td>
<td>4.38</td>
<td>black</td>
</tr>
<tr>
<td>18</td>
<td>98,000</td>
<td>4.30</td>
<td>black</td>
</tr>
<tr>
<td>25</td>
<td>54,000</td>
<td>4.23</td>
<td>black</td>
</tr>
<tr>
<td>36</td>
<td>57,500</td>
<td>4.30</td>
<td>black</td>
</tr>
</tbody>
</table>

* Glucose 3.2%
  Glycine 6.0%
** Control on glycine solution, alone: fluorescence 55
  Control on glucose solution, alone: fluorescence 12
***control on glucose solution alone: fluorescence 15, color, none
that they might have a preservative effect on the solubility of the
dry material. The results are shown in Table 19 and Figure 19.

Agren and Taylor (3) reported that p-aminobenzoic acid formed
a stable compound with glucose. For this reason the acid and its
water soluble sodium salt were tested. Both showed a preservative
effect, the acid to a much greater extent than the salt which might
have been due in part to a difference in pH between the two samples,
9.0 and 9.8 respectively. The color developed was the same as that
of a control sample.

Thiourae, if it forms the structure suggested by Schubert (56),
would have both an amino and a sulfhydryl group. It might thus
conceivably react in the same manner as cysteine. This did not
prove to be the case, however, as the effect on albumen solubility
was slight.

Glucosamine contains an amino group which might react as that
of the amino acids, but its aldehyde group apparently outweighed this
effect, as this sample decreased in solubility even more rapidly than
the control.

Two samples containing an acid hydrolysate of egg albumen were
prepared. In the one in which the pH of the dried sample was 9.6,
the normal range, the effect was negligible. When the amount was
increased the pH was lowered to 6.8 and there was a definite preserv-
ative effect, but this was probably the effect of low pH alone.
Table 19

CHANGES IN SOLUBILITY OF DRIED EGG ALBUMEN* CONTAINING ADDED AMINO COMPOUNDS

<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
<th>None</th>
<th>Protein hydrolysate</th>
<th>p-aminobenzoic acid</th>
<th>Sodium p-aminobenzoate</th>
<th>Thio urea</th>
<th>Glucosamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
<td>98</td>
<td>100</td>
<td>100</td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td>14</td>
<td>54</td>
<td>65</td>
<td>100</td>
<td>97</td>
<td>79</td>
<td>70</td>
</tr>
<tr>
<td>25</td>
<td>37</td>
<td>47</td>
<td>94</td>
<td>88</td>
<td>60</td>
<td>54</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
<td>30</td>
<td>82</td>
<td>60</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>pH of reconstituted sample</td>
<td>9.7</td>
<td>9.6</td>
<td>6.8</td>
<td>9.0</td>
<td>9.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Amount added, liquid basis,%</td>
<td>0.42</td>
<td>0.48</td>
<td>0.42</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 10% moisture, 50°C., pan dried.
Fig. 19 Changes in Solubility of Dried Egg Albumens Containing Added Substances.
Agren and Taylor indicated that esterification of the carboxyl group of amino acids increased the reactivity of their amino groups toward aldehydes. The ethyl and butyl esters of tyrosine were available and were tried but with only slight effect in the case of the ethyl ester (Table 15).


The effect of $\text{SO}_2$ in retarding amino acid sugar reactions has been pointed out (45, 71). The reason for the effect is not understood, however. Weast and MacKinney found that more $\text{SO}_2$ was needed to suppress the amino acid sugar reaction than would be required by stoichiometric reaction with the aldehyde groups of the reducing sugar present in preventing blackening of apricots.

Because of the difficulty in incorporating into liquid albumen an amount of sodium bisulfite small enough that coagulation would not take place upon drying, it was thought that a minimum amount of sulfur dioxide might be incorporated by gas packing dry samples in it. Table 20 shows the result on two sets of samples, one in which the sample container was only part full of dry albumen, the other in which it was full. This gave two ratios of gas to powder, which differed by three to one.

Oddly enough, the samples of the lower ratio of gas to powder showed remarkable keeping qualities both in color and solubility,
Table 20

EFFECT OF STORAGE IN SULFUR DIOXIDE ON PROPERTIES OF DRIED EGG ALBUMEN STORED AT 60°C.

<table>
<thead>
<tr>
<th>Elapsed Time (days)</th>
<th>High ratio of SO₂ to sample*</th>
<th>Low ratio of SO₂ to sample**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solubility  Fluorescence  pH  Color***</td>
<td>Solubility  Fluorescence  pH  Color***</td>
</tr>
<tr>
<td>0</td>
<td>48        27      3.8 white</td>
<td>84        24      4.7 white</td>
</tr>
<tr>
<td>10</td>
<td>35        69      3.5 yellow</td>
<td>84        225     4.7 slight yellow</td>
</tr>
<tr>
<td>20</td>
<td>12        55      3.6 yellow</td>
<td>84        280     4.6 slight yellow</td>
</tr>
<tr>
<td>35</td>
<td>-         52      3.8 brown</td>
<td>84        340     4.8 slight yellow</td>
</tr>
</tbody>
</table>

* 11.0% moisture. Sample can one-fourth full of powder.
** 9.5% moisture. Sample can full of powder.
*** Color of dry powder
while those with the greater amount of gas became insoluble early and developed a brown color. It should be pointed out that in the latter case, the pH (3.5 or below) was low enough to cause coagulation. Even in the former (pH 4.6-4.8) the solubility of the material was reduced by presence of the gas, although it did not further decrease on extended storage in the gas. Nevertheless, the effect of retaining the solubility could scarcely be due to the pH alone, as comparison with Table 6 will show that a sample of pH 4.8 became completely insoluble in nine days at 60°C, while the sulfur dioxide sample had not changed in 35 days.

10. Disappearance of glucose.

A single experiment was run on the disappearance of glucose from dried albumen. The aged samples were thoroughly extracted by water using the test tube homogenizer. The protein was then coagulated by the method of Stewart and Kline (64) and further by phosphotungstic acid, to give a buiret free filtrate. Glucose was determined by the A.O.A.C. method (50, p. 500) using direct weighing of cuprous oxide. The results are shown in Table 21.

It will be noted first that the glucose content of the unstored product is lower than that usually found (0.40-0.45%) in liquid albumen. While it would be unwise to put too much emphasis on such preliminary data, the suggestion is that the glucose may have already in part reacted with the albumen during the drying process.
Table 21
FREE GLUCOSE CONTENT OF DRIED EGG ALBUMEN*

<table>
<thead>
<tr>
<th>Elapsed Time (hours)</th>
<th>Solubility</th>
<th>Fluorescence</th>
<th>pH</th>
<th>% Free glucose**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>18</td>
<td>9.62</td>
<td>0.31</td>
</tr>
<tr>
<td>12</td>
<td>82</td>
<td>230</td>
<td>9.45</td>
<td>0.09</td>
</tr>
<tr>
<td>17</td>
<td>61</td>
<td>510</td>
<td>9.38</td>
<td>0.08</td>
</tr>
<tr>
<td>48</td>
<td>29</td>
<td>100</td>
<td>8.97</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* 10% moisture, 60°C.
** Calculated to original liquid basis
exposure for a well buffered substance and indicate a substantial change.

Great as that observed for etidronate sodium reaction, but it is relatively

b. Change in pH, the decrease in pH of etidronate is not as

for heparin or sodium citrate solutions.

black (blank etidronate) is the same as needed by Malim and others

e. Color.

The development of etidronate to orange, brown and

As follows:

The suggested polystyrene may be summarized.

In etidronate etidronate is the same type as those tested.

In etidronate etidronate is the same type as the reactions responsible for the changes
detectable in etidronate and those of etidronate must be due to some other reagent.

Because of the striking similarity between the properties of


As no longer free, or has been decomposed seems clear.

Integrations in this study, however, that all of the etidronate eventually disappears.

Reasons for the differences in etidronate during the various stages of

Methane takes place, the data is not strong enough to support any of

The etidronate present has reached at the time the etidronate

of the etidronate present has reached at the time the etidronate

The amount of etidronate present. When the concentrations free was 0.01 M or

until that level was reached, the etidronate produced free was 0.01 M.

the formation of the etidronate complex, following the

or the etidronate disappeared, and little further change was noted while

During a decrease in solubility from 100 to 62, over two-thirds
in its properties. The effect could be due to either of two changes --
the destruction of some basic function of the buffer substances (protein),
or to the production of acid. If the former, the groups involved would
most likely be the free amino groups or even the amide groups of the
peptide linkage in the protein molecule. If the latter, the change
might result from the formation of acid degradation products of the
sugar molecule.

e. Change in solubility. If the aldehyde sugars do react with
the basic nitrogen groups of the protein, the resulting loss of sol-
ubility is precisely what would be expected from a decrease of these
lyophilic groups.

d. Fluorescence. The fluorescence which develops early in the
deterioration of albumen is qualitatively similar to that reported for
melanoidins (blue green) (19, 45). The decrease in this value observed
during the subsequent insolubilization of the albumen suggests that the
fluorescent property is associated with the reaction product of the
protein molecule and the glucose.

e. Effect of temperature. The albumen reaction has a temperature
coefficient well in excess of the classical doubling of rate with 10°C
rise in temperature. That amino acid sugar reactions display such a
large temperature effect has been shown by Maillard and others (47, 21).

f. Effect of pH. The effect of acid in retarding the progress
of albumen changes is similar to its effect in slowing the amino acid
sugar reaction. It is effective in the same pH range.
g. **Types of sugars.** There is almost perfect coincidence between the sugars which have been reported to react with amino acids and those which insolubilize egg albumen. This holds as well for those which do not react in either of these two cases.

h. **Amino acids in albumen.** That amino acids added to albumen react with the glucose present in the dry product is indicated by the amount of color and fluorescence developed and the resulting preservation of albumen solubility. Apparently the conditions in the dry albumen permit these reactants to make sufficient contact for the reaction to take place. The effect of low moisture in albumen may be due to removal of the reaction medium.

i. **Effect of sulfur dioxide.** Aside from the expectation that sulfur dioxide might react with sugar aldehyde groups, the mechanism of its preservative effect on dry albumen is unknown. However, the darkening of dried apricots, a change which has been shown to be due to an amino acid-sugar reaction (71), is completely prevented by the incorporation of sulfur dioxide into the cut fruit before it is dried.

12. **General discussion. Practical considerations.**

The results obtained on the moisture and pH trials indicate that about the practical limit of storage life that can be obtained by using even the most extreme combination (0.6% moisture, pH 4.8) is one to two years at 20°C or four to eight months at 30°C. This would probably
permit them to be kept commercially during most of the year. However, at 40°C, the expected storage life would be about one-fifth that at 30°C or one to two months. This severely limits the useful life of such a product held in dry storage through the summer months. The effect of pH alone, even at the lowest value (4.8) was much less than that of moisture and its use would be even less satisfactory for such conditions. In addition, it has been pointed out by Watts and Elliott (70) that acid treated albumens performed poorly in baked products.

The prospects for the amino acid treatment are debatable. Of those studied, cysteine shows the most promise. While both lysine and glycine were very successful in maintaining solubility, the dark color of these samples is an important objection to their use. Such a color would render the product unusable in an angel food cake, meringue or candy. The cases in which the color and aroma would be concealed by other ingredients would be few. Although the results of the later experiments with cysteine at 60°C were disappointing, there is indication that cysteine might be capable of maintaining solubility without color development. An exploration of the conditions required for glucose and cysteine to react in liquid albumen needs to be made. The possible use of cysteine is further made attractive because there should be no objection to its use on a public health basis.

The results of the sulfur dioxide treatment are most encouraging as the stabilization during storage was the greatest obtained for any sample studied. The effect on initial solubility might be overcome by the use of still lower concentrations of the gas. It was noted that in
the sample which showed this result, very little odor of residual sulfur dioxide was found when the sample can was opened. Sulfur dioxide would seem tentatively acceptable from a public health point of view, as it is used in the preparation of dried fruits, and a sulfite dip is used in blanching cut vegetables.

One other possibility which should probably be explored further is p-aminobenzoic acid. This compound and its ethyl ester was shown by Agren (3) to react with glucose to produce a colorless, water insoluble compound. The ester seems to represent the better possibility, as high temperatures (70°C) were necessary to prepare the compound of the free acid. The latter also showed a tendency to decompose to a colored compound.
CONCLUSIONS

On the basis of the data presented, the following conclusions are drawn:

1. The glucose present in egg albumen is the cause of the development of dark color and insolubility during storage of the dried product.

2. The glucose can be removed from albumen by bacterial fermentation. In order that the dry product have complete stability the glucose content of the liquid must be reduced to below 0.02%.

3. Increasing the temperature at which dry albumen is stored increases the rate of deterioration as measured by solubility. The increase in rate is approximately five-fold for an increase of 10°C in storage temperature.

4. The effective storage life of a dry albumen can be increased twelve times by adjustment of the pH of the liquid to 5.0 before drying.

5. The effective storage life of a dry albumen can be multiplied twenty times by a reduction in moisture content from 10% to 1%.

6. The incorporation of certain amino acids into the liquid albumen before drying will prevent or retard the loss of solubility of the dry product. Lysine, glycine and cysteine are most effective.
7. The packing of dry albumen samples in an atmosphere of sulfur dioxide will prevent solubility changes during subsequent storage.
SUMMARY

A study has been made of the characteristics of the non-microbiological changes which take place during storage of dried egg albumen. The original light yellow or white color of the dry product is replaced by orange then brown as the storage period is extended. The solubility of the dry product remains high for a short period, then falls rapidly to 30% of the original or less. The fluorescence increases sharply during the period in which the solubility remains high, then falls abruptly when the solubility does. The pH of the dry albumen, as determined by reconstituting it, falls steadily during the storage period.

The effect of temperature on the rate at which the changes take place was studied. Increasing the temperature of storage by 10°C generally increases the rate by five times. The length of time a normal dried albumen (pH 9.5, 10% moisture) retains complete solubility is 1 hour, 5 hours, 15 hours, and 115 hours for 70°C., 60°C., 50°C., and 40°C. respectively.

The effect of lowering the pH of the liquid albumen prior to drying is to increase the "storage life" (as measured by solubility) of the dry product. A sample of pH 4.6 (reconstituted) retains its solubility for 72 hours at 60°C. as contrasted to 6 hours for a sample of pH 9.6.

Redrying an albumen to low moisture contents increases its storage
life. An albumen of 1-2\% moisture will retain its solubility for five days at 60\^\textdegree{}C., while the sample albumen at 10\% moisture will lose solubility in 7 1/2 hours.

The glucose present in dried albumen has been shown to be responsible for the changes which take place during storage. The glucose can be removed from the liquid albumen by bacterial fermentation, but complete stability of the dry product can be obtained only if all of the sugar is removed.

Various types of sugars were added to glucose-free (fermented) albumen and the course of their reaction in the dry product studied. In general, all aldehyde sugars produced the changes typical of natural dried albumen. Pentoses produced the changes very rapidly, the hexoses less so, and the disaccharides quite slowly.

Amino acids added to liquid albumen in general retarded the change in solubility of the dry product in storage. Glycine and lysine were effective in maintaining almost complete solubility of the dry albumen over a period of 72 days at 50\^\textdegree{}C. They produced a much darker color than found in an ordinary stored albumen. Cysteine was almost as effective in retarding loss of solubility and rendered the dry product less colored than that of a control albumen. The effect of other amino acids and amino compounds was less pronounced.

The packing of dry albumen in an atmosphere of sulfur dioxide resulted in an immediate loss of solubility (15\%). However, on subsequent storage at 60\^\textdegree{}C., further changes in solubility and color were prevented for 35 days.
LITERATURE CITED


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