1949

Removal of glucose from egg albumen by a controlled fermentation

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NOTE TO USERS

REMOVAL OF GLUCOSE FROM EGG ALBUMEN BY A CONTROLLED FERMENTATION

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Food Technology

Approved:

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In Charge of Major Work

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Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1949

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I. INTRODUCTION

A considerable quantity of dried egg albumen is produced for utilization in such products as cake frostings, meringue powders, marshmallow whips, cream candies and nougats. The product has also found use in cake mixes; however, it does not appear to have performed very satisfactorily in angel cake formulations.

The usual commercial procedure for the preparation of dried egg albumen consists of fermenting liquid albumen free of sugar, removing the mucin scum, then drying on trays. A minor fraction is spray dried and foam dried. It seems possible that the methods employed for fermentation and drying render the final product unsuitable for angel cakes. Evidence suggestive of this possibility is to be found in various publications and in the unpublished work done in the Poultry Products Laboratory at the Iowa State College. The primary objective of the work reported in this thesis was to develop a fermented egg white which, after drying, could be reconstituted and made into an angel cake comparable to that obtained from fresh egg white.

II. REVIEW OF LITERATURE

From the review of literature it was apparent that a number of factors underlie the successful fermentation and drying of egg white. They may be divided into the following: (a) the role of glucose in stabilizing dried egg albumen, (b) egg white fermentation methods and organisms responsible for same, (c) bacteriostatic properties of egg white, and (d) factors influencing the performance of egg white in foams and angel cakes.

A. Role of Glucose in Stability of Dried Albumen

During the 1930's many workers felt that the chief function of the fermentation of albumen was to 'thin' it and thereby improve the whipping properties of the dried product. Consequently workers attempted to improve on fermentation by substituting chemical thinning methods. For example, four patents (26, 35, 36, 37) were issued on methods of treating albumen with acid prior to drying. Balls and Swenson (7) developed a process in which trypsin was used to reduce the viscosity of natural egg white to a point similar to that found in fermented albumen. Their objective was to avoid the use of the bacterial fermentation. They found, however, that the dried product prepared from the enzyme-treated liquid

albumen darkened and became insoluble on storage.

Stewart and Kline (51) first demonstrated the role of glucose in the deterioration of dried egg white. They compared the storage properties of unfermented and fermented albumen. When stored at 40° C., unfermented dried albumen changed progressively from pale yellow to dark brown and became relatively insoluble within a two week period. On the other hand, fermented samples retained their original pale yellow color and solubility during the entire twelve week storage period. To prove the role of glucose in stability, Stewart and Kline (51) prepared dried samples from unfermented and fermented albumen, and fermented albumen to which glucose had been added. At 40° C. only the fermented albumen (glucose-free) retained its original qualities. In order to prevent this deterioration in unfermented dried albumen, a storage temperature (below 5° C.) was necessary. These workers concluded that fermentation of egg white was necessary to maintain color and solubility stability if the dried product were to be stored in unrefrigerated warehouses for extended periods of time.

Stewart, Best and Lowe (50) followed the changes in solubility of fermented and unfermented dried (6 per cent moisture) albumen stored at 50° C. The unfermented sample retained its solubility for six days, after which time there was a steady decrease in solubility over nine days' time. Under the same conditions fermented albumen retained its color

and solubility completely for the entire fifteen day storage period.

Hawthorne and Brooks (25) demonstrated that the removal of free glucose from egg white by yeast markedly retards deterioration during storage. Dried albumen containing 0.05 per cent glucose retained its color and solubility for two weeks at 45° C., while a dried sample of untreated albumen containing 0.5 per cent glucose lost more than half its solubility.

Stuart and Goresline (54) mention that their dried fermented egg white retained its original color during storage in the laboratory at room temperatures for four months whereas dried unfermented egg white turned a dark reddish brown.

In a later paper Stewart and Kline (52) more fully explored the effect of storage temperature, glucose and moisture content, and pH on the rate of deterioration of dried, unfermented albumen. They found that the rate of deterioration varied widely with storage temperature and that pH exerted a significant effect on this rate. Reducing the pH to acid levels decreased the deterioration rate. They also noted that the moisture content had a pronounced effect on deterioration, low levels being very beneficial. A study of the effect of glucose concentration revealed that it exerted a powerful effect on the deterioration rate in dried albumen. Concentrations of 0.02 per cent and higher caused appreciable

changes in fluorescence and color; concentrations of 0.05 per cent and above also gave rise to measurable changes in solubility.

Much evidence points to the fact that reactions between glucose and egg white proteins are responsible for changes in color, fluorescence and solubility of dried albumen during storage. Stewart and Kline (53) replaced glucose by other sugars and sugar derivatives. Their results substantiated the theory that the deterioration is of the Maillard type. Samples of fermented albumen containing the nonreducing sugars, sucrose, trehalose and raffinose showed no evidence of reaction, as indicated by changes in fluorescence, color and solubility. Similarly, no effects were noted when the sugar alcohol, sorbitol, replaced glucose. Only those sugars containing the free aldehyde group reacted with egg proteins to produce deteriorative changes.

B. Fermentation of Egg White

The spontaneous fermentation of albumen has been described by a number of workers (10, 41, 51, 58). Blomberg (10) in 1932 described a fermentation which has been used for many years by the Chinese. A spontaneous fermentation is allowed to take place in wooden casks. This worker did not identify the microorganisms which are responsible for the fermentation, but he did give a description of the physical changes which

occur. The process requires 36 to 60 hours at about 70° F. Blomberg states:

> As the fermentation continues, a scum rises to the top and is removed and discarded, and a sediment also settles to the bottom of the cask. While this is going on, samples are taken from the top by means of pipettes. When the sample shows that bubbling has practically ceased, and a sample that has been drawn off through a spigot located about three inches above the bottom of the cask also shows clear, the process is checked by the addition of about 2 ounces of aqua ammonia and about 3 ounces of alcohol per 100 pounds, stirred in as the albumen is drawn off into the wooden buckets.
>
> The liquid egg white is then thinner and not as sticky as it was before fermentation.

Glabau and Kepes (19) were among the first to follow the change in acidity of the liquid during spontaneous fermentation. They found that the pH fell steadily from about 7.9 to 5.8 over a period of four days. They did not identify the microorganisms responsible for their fermentation.

In a discussion of fresh, frozen and dried egg products, Le Clerc and Bailey (33) included a characterization of the albumen fermentation. They described the final fermented product as having a heavy, odorous, spongy foam or scum over the surface at the end of a four to six day fermentation period. The liquid white under the scum is stated to have a watery consistency, an odor comparable to that of alfalfa hay, and a slightly salty taste. It is acid in reaction (pH 5.5).

Stewart and Kline (51) followed the course of a spontaneous fermentation by determining pH and free glucose in the

liquid. Depending on the amount of bacterial contamination, they found the liquid to remain quiescent for several days. The pH then began to decrease from 8.9 and reached 5.9 in about 48 hours. As the acidity increased the mucin of the thick white contracted into a stringy mass which floated on the surface of the fermenting albumen. Analysis of this layer revealed that it contained 2 per cent mucin compared with 0.28 per cent for the remaining liquid portion. They noted that evolution of CO_2 ceased about the sixth or seventh day of fermentation. Isolation and identification of the bacteria responsible for the fermentation was not included in their study.

Stuart and Goresline (54) made bacteriological studies on eight commercial lots of fermenting egg white and found bacteria of the genera Aerobacter or Escherichia in such predominating numbers as practically to exclude other types of organisms. In addition to these genera, however, they did find Proteus, Serratia and Pseudomonas persisting in the fermenting egg white. Samples of fermented egg white in which Aerobacter and Escherichia types predominated during fermentation were found to yield a bright, crystalline, granular product on drying. On the other hand, fermented egg white in which such proteolytic bacteria as Proteus, Serratia and Pseudomonas persisted in large numbers throughout the fermentation period, yielded a dull, dingy and amorphous product upon drying.

Stuart and Goresline (54) also studied the changes in pH, glucose, protein nitrogen, amide and amino nitrogen and the bacterial count of fermenting albumen. They used as their inoculum a mixture of two samples of fermented egg white which contained predominantly Aerobacter-Escherichia-type organisms. The results relative to changes in glucose and pH correlated well with the results of Stewart and Kline (51). They found no appreciable change in the nitrogen fractions. By the time of complete glucose removal the number of bacteria had increased to several billion per milliliter. When the fermentation was allowed to continue beyond this point, there was an increase in the combined amide and amino nitrogen values; accompanying these changes was an abrupt increase in pH.

In a subsequent paper Stuart and Goresline (55) further identified the organisms they found in commercially fermenting egg white. Of the twenty isolations of the Aerobacter genus twelve were shown to be strains of Aerobacter aerogenes; the remaining eight strains more closely resembled Aerobacter cloacae. Thirteen other isolations previously identified as belonging to the genus Escherichia were shown to be strains of Escherichia freundii.

Stuart and Goresline (55) made other important discoveries. By using varying numbers of cells of a selected strain of Aerobacter aerogenes they found the rate of fermentation to be directly proportional to the size of inoculum. They also studied fermentations made with selected strains of Serratia

marcescens, Proteus sp. and Pseudomonas aeruginosa. These organisms did not produce changes in pH, sugar content and formol titration values corresponding to the ordinary spontaneous fermentations. Decreases in pH and sugar content were neither as great nor as rapid as with Aerobacter aerogenes and Escherichia freundii. The fermentations with all three of these organisms were characterized by rapid and marked increases in the amount of formol nitrogen, indicating strong proteolytic action by these species.

Kline (30) used a 5 per cent inoculum of an Aerobacter aerogenes culture to produce a fermentation of egg white in forty-eight to seventy-two hours. Slosberg (48) fermented albumen with 10 to 20 per cent inocula of Aerobacter aerogenes.

Several Streptococcus fermentations have been described in the literature. Stewart, Best and Lowe (50), using a culture of Streptococcus, studied the effect of fermentation on solubility and fluorescence during storage. They noted that changes in flavor resulted from this fermentation. Ayres (4) found that a controlled fermentation with a 20 per cent inoculum of Streptococcus sp. reached a minimum pH of 6.10 after 11 hours; no glucose was present after 12 hours. Ayres (4) isolated Streptococcus sp., from a highly acid fermentation of egg white and also from a commercially fermented dried albumen.

Hopkins, et. al. (27) claim that Streptococcus lactis, Streptococcus diacetilactic, Streptococcus thermophilus, Lactobacillus casei, and Lactobacillus bulgaricus completely ferment

liquid whole egg free of sugar within a few hours when one per cent inocula are used. However, they claim that, when these cultures are inoculated into egg white, there is relatively little decrease in the reducing sugar content even after twenty-four hours time.

Yeast has also been used to ferment glucose from egg white. Hawthorne and Brooks (25) reported a reduction in the glucose content of egg white from 0.5 to 0.05 per cent and 0.09 per cent respectively in two trials where they used an inoculation of a one per cent Saccharomyces apiculatis culture and incubated the material for three hours at 37° C. Ayres (4) also fermented egg white with yeast. By using a 20 per cent inoculum and incubating at 86° F. the fermentation reached a minimum pH of 6.7 in 15 hours; after 19 hours the albumen was glucose-free.

Ayres and Stewart (6) made a very thorough study of the yeast fermentation. Their study included determining the effect of size of inoculum, surface/volume ratio, initial pH, added growth factors and serial fermentations. Using an actively-growing yeast (25 per cent of a previous run as an inoculum) with added yeast extract as a growth factor, complete glucose removal was effected in two hours.

C. Bacteriostatic Properties of Albumen

The numbers and types of organisms able to attack carbohydrate in egg white are restricted in large measure by the protective agents resident in native albumen. As early as 1890 Wurtz (59) noted that fresh egg white is generally resistant to bacterial attack. Turro (56) observed that the white of fresh eggs was less germicidal than that of older eggs but it was not until twenty-five years later that Sharp and Whitaker (47) correlated the rapid rise of pH of egg white after laying with the increased bactericidal power of older eggs. Four major substances have been detected in hen's egg white to which specific microbiological activity may be ascribed. These active principles are: avidin, lysozyme, ovomucoid and conalbumin.

Avidin. The original report that rats fail to grow on a diet containing 30 per cent dried egg white is credited to Bond (12). Boas (11) later attributed the effect of certain foods to protect rats against this 'injury' to a 'protective factor', later identified with biotin. Eakin, Snell and Williams (14) isolated the constituent of raw egg white which is responsible for egg white 'injury'; they found that it is capable of combining with biotin in a stable stoichiometric complex rendering this vitamin unavailable to Saccharomyces cerevisiae. Application of heat released the biotin and made it available to the organism.

Gyorgy, Rose, Eakin, Snell and Williams (22) showed a direct correlation between the capacity of an egg white preparation to produce egg white 'injury' in the rat and its effectiveness in combining with biotin *in vitro*. The active constituent was accordingly named avidin (avid albumen), signifying its affinity for biotin (15).

Gyorgy and Rose (21) found the avidin content of hen's eggs is in excess of that required to combine with all of the biotin present.

Pennington, Snell and Eakin (43) prepared biologically active crystals of avidin and demonstrated that it is a protein with a large carbohydrate moiety.

Lysozyme. Laschtschenko (32) was apparently the first investigator to study the lysing power of egg white; he observed the lysis of *B. subtilis* and related organisms.

The lytic effect of some agent in egg white was first definitely demonstrated by Fleming (16), who appended the term 'lysozyme' to it. Fleming and Allison (17) and Nakamura (42) showed that hen's egg white is the best natural source of lysozyme.

The action of lysozyme on different types of microorganisms is rather unpredictable. However, of those bacteria tested, the ones susceptible to lysis have all been shown to be gram-positive. Accompanying the lysing action of lysozyme is the little-studied flocculating ability. Friedberger and Hoder (18) suggested such action of egg white on various bacteria to

be due to a principle different from lysozyme. However, Klemparskaya (29) reported the possibility of lysozyme's acting as both lytic and flocculating agent. He classified organisms as reacting to lysozyme by: (1) lysis, (2) lysis and flocculation, and (3) neither lysis nor flocculation. He concluded that flocculation depends on both pH and lysozyme concentration and suggested that such behavior depends on the difference in the colloidal structure of the bacteria involved.

Ovomucoid. While investigating antitryptic action Vernon (57) in 1904 found the activity to be more marked in egg white than with any other natural protein material. There is much controversy in the literature as to the action of this factor and also concerning its occurrence. However, in 1947 the picture was much clarified when Lineweaver and Murray (34) identified the trypsin inhibitor of egg white with the ovomucoid fraction. They showed that the active principle could be separated from the albumin, conalbumin, globulin and avidin fractions; however they failed to separate it from ovomucoid. Electrophoretically, the ovomucoid fraction could not be separated from antitrypsin; its electrophoretic character was identical with that given for ovomucoid by Longsworth (38).

Antitryptic activity is not restricted to raw egg white. Harte (24) showed that commercial dried egg albumen contains about as much active principle as fresh white.

Conalbumin. Schade and Caroline (44) reported that raw egg white inhibits the growth of Shigella dysenteriae,

Saccharomyces cerevisiae and certain other microorganisms. The effect was independent of the avidin-biotin phenomenon. Of ten vitamin factors and 31 elements tested, only iron overcame the inhibition. In cooperation with Schade and Caroline (44), Alderton, Ward and Fevold (1) concentrated the active principle and showed (electrophoretically) that it was essentially conalbumin. They came to the conclusion that the iron-binding protein and conalbumin were identical. Recently further substantiation of this conclusion has been provided by Schaible and Bandemer (45, 46).

D. Factors Influencing Culinary Properties of Egg White

1. Effect of acid on properties of liquid albumen.

Almquist and Lorenz (2) have shown that the thick white of albumen is composed of a fine network of ovomucin fibers entrapping thin white. McNally (40) reported that, at a pH less than 6.0-6.4 (depending on the salt concentration), ovomucin exists in a compact, precipitated form; from pH 6.4 to 8.5 it is in gel form.

It has been shown by Hanson (23) and Slosberg (48) that mucin plays an important role in the whipping quality of egg white and in its performance in angel cake. Slosberg (48) found that when the pH of liquid albumen was adjusted below about 6.6 (by direct addition of acid or by fermentation),

there was a definite decrease in beating quality. Furthermore, the work of Hanson (23) showed that egg white treated to remove or precipitate mucin did not make high quality angel cakes. Cakes from egg white so treated were characterized by low volume, a coarse, compact texture and decreased palatability. The results indicated that mucin in egg white must remain in dispersed form in order to permit the production of acceptable angel cakes.

2. Effect of acid on egg white foams and angel cakes.

The effect of cream of tartar as an ingredient of angel cakes has been studied by Grewe and Child (20), who summarize their results as follows:

> Angel cake made with acid potassium tartrate as a part of the ingredients is a fine-grained, white product, while without it the cake is yellow and coarse grained. Use of acid potassium tartrate causes an increase in the H-ion concentration of the cake.
>
> Citric, malic and tartaric acids used in place of acid potassium tartrate to change the H-ion concentration of the cake have the same effects on the color and grain of the cake as acid potassium tartrate.
>
> It is concluded that the change in color and grain of angel cake resulting from the use of acid potassium tartrate is due largely to acidity.

Barmore (8, 9) in studies on the influence of chemical and physical factors on egg white foams measured the stability of the foams produced under various conditions. He concluded that acids and acid salts increased foam stability considerably.

Barmore (9, p. 13) himself summarizes the study:

> It was found that for a pH of about 8.0 the foam stability was practically the same for the three kinds of acid used. . . . acid tartrate, acetic and citric. But at a pH of about 6.0 there was considerable difference in the foam stability, the acid tartrate producing the most stable foam, the acetic acid the least stable, and citric acid an intermediate degree of stability. From this finding it was predicted that the cakes would give equivalent texture with the three acids at a pH of 8.0, but that the texture would vary with the degree of stability when the pH was about 6.0. This was exactly the case. The cakes had an equal texture at a pH of 8.0, but at a pH of 6.0 the texture of the acetic acid cake was very coarse, and the potassium acid tartrate cake the least coarse of the three.
>
> On adding still more acid than was necessary to produce a pH of 6.0 in the finished cake, the relation of the cake texture to the foam stability was even more noticeable.

He also found the first portion of the beating period to be superior to the latter for adding acid to the egg white.

Cake volume depends not only on the amount of air beaten into the egg white, but also on maintaining this air in the batter during baking. In this connection Barmore (9) claims that there are two factors to be considered: first, the increased volume by including more air, second, the reduction in volume because of the instability of the foam. The point at which these two factors are in balance produces the largest cake.

Lowe (39, pages 367-381) has included in her text, "Experimental Cookery", an extensive review of work relating to angel cakes.

III. EXPERIMENTAL PROCEDURES

The investigations reported in this thesis fall into three general categories. The first phase of the work related to the selection of the most desirable organism for removing the glucose from egg white. Studies were then made to determine the optimal conditions for the fermentation of egg albumen by this organism. The final phase was concerned with studies designed to characterize a fermentation which would retain in the dried product those angel-cake making properties found in fresh egg white.

A. Preparation of Egg White Samples

During the course of the work, egg white from several sources was employed. In the studies relating to the selection of suitable organisms for fermentation, it was felt that albumen from fresh shell eggs, broken and separated under aseptic conditions, should be used in order to minimize the possibility of contamination. Later work with Aerobacter aerogenes indicated that such precautions are unnecessary to insure a satisfactory, pure culture fermentation with this organism. Therefore commercial frozen egg white was used in most of the studies involving this organism.

1. Fresh egg white.

Fresh eggs were obtained from the Iowa State College Poultry Farm and placed in a refrigerator at 18° C. They were usually about three days old when broken and separated into yolks and whites.

In the early studies where pure cultures were being used to determine their ability to ferment glucose from egg white, aseptic procedures were employed so as to obtain an albumen as free of microorganisms as possible. Shell eggs were soaked for five minutes in one per cent NaOH at 40° C., drained, and allowed to air dry. These eggs were broken and separated with the aid of a sterilized separator. Also, precautionary measures were taken in the use of the Waring Blendor and other pieces of equipment which came in contact with the egg white.

Ordinarily the eggs were broken and separated using commercial egg breaking equipment (so-called breaking tray and separator). After separation the egg whites were placed in a Waring Blendor of a size appropriate for the volume of egg white used and blended in a manner such that no air was incorporated. This was accomplished by repeatedly 'flicking' the switch on and off momentarily. In these experiments thirty 'flicks' were used to treat any one batch of albumen; the resultant liquid was thin, homogeneous and free-flowing.

The blended egg white was used immediately or stored over night at 10° C. A large number of the fermentations were

carried out at 37° C. and 40° C. In bringing the egg white up to these temperatures it was noted that, in spite of the blending, there was some separation of thick and thin white. Because of this the egg white was reblended (10 'flicks') just prior to use.

2. Commercial frozen egg white

Thirty pound cans of commercial frozen egg white were purchased from local sources. The albumen was allowed to thaw for twenty-four hours at room temperature. The contents were then blended, one liter at a time (30 'flicks'), placed in one quart cartons, refrozen, and stored at -15° C. until used. After rethawing, and just before using, it was again blended (10 'flicks').

B . Bacteriological Examination

The bacteriological examinations consisted of making dilution plate counts on tryptone glucose extract agar and dilution smear plate counts using eosin methylene blue agar. The technique employed in making the dilution smear plates consisted of pouring eosin methylene blue agar into petri dishes and allowing it to solidify. Then 0.1 ml. of appropriately diluted egg white was placed on the surface of the agar near the center of the plate. This was distributed uniformly (using a rotary motion) over the surface of the entire

plate with a sterile glass spreader. All plates were incubated at 30° C. for forty-eight hours. Readings were made after twenty-four and again after forty-eight hours.

Bacterial counts on dried albumen were made on some of the samples from the storage tests. In these cases a weighed sample of dried egg white was soaked in sterile, distilled water at 10° C. for five hours. Dilution smear plates were made on the reconstituted egg white, using eosin methylene blue agar.

C. Fermentation Methods

1. Source of cultures.

In the search for suitable organisms for the egg albumen fermentation, about fifty cultures were surveyed for their ability to remove glucose from egg white. Some of these organisms were isolated from fermenting and fermented egg white; some were from other food products. These organisms were not fully identified. Others were laboratory cultures which were made available by the Bacteriology and Dairy Industry Departments of the College and by Wilson and Co. The cultures surveyed included:

Aerobacter aerogenes
Aerobacter cloacae
Escherichia coli
Escherichia freundii
Intermediates
Pseudomonas saccharophila
Pseudomonas lindneri
Lactobacillus casei
Lactobacillus delbruckii
Lactobacillus arabinosus
Leuconostoc mesenteroides
Streptococcus lactis
Streptococcus fecalis

2. Inoculum.

In the early studies with Aerobacter aerogenes, washed suspensions of cells grown in tryptone glucose extract broth were added to egg white. A loop transfer of Aerobacter aerogenes was made into a tube containing ten ml. of tryptone glucose extract broth. The mixture was incubated at 30° C. for twenty hours. After incubation the culture was centrifuged and the supernatant liquid decanted. The cells were then washed with 0.9 per cent saline solution, centrifuged, and the supernatant again decanted. The cells were resuspended in 5 ml. of the saline solution and inoculated into egg white.

The volume of the culture was kept to a minimum to avoid excessive dilution of the egg white. The centrifuged cells from 10 ml. of tryptone glucose extract broth culture were considered a 10 per cent inoculum when added to 100 ml. of egg white. In later studies it was found possible to carry Aerobacter aerogenes directly in egg albumen. In this case 1 ml. was inoculated into 9 ml. of egg white containing 0.1 per cent Difco yeast extract; the cultures were incubated four and one half hours at 37° C. With this incubation period a very small amount of glucose remained in the egg white. This was done deliberately in order to avoid putrefaction. These cultures were passed in ordinary egg white, without trying to employ aseptic techniques. No difficulty was experienced in obtaining pure culture fermentations by this method. Between

passages these cultures were stored at -15° C.

3. Fermentation methods.

The fermentation procedure was adjusted as more suitable conditions for the organism were found. Study of a fermentation by specific organisms included determining the effects of the addition of yeast extract, surface/volume ratios, per cent inoculum, temperature and pH. The course of the fermentation was followed by p^H readings and qualitative glucose tests. Bacteriological examinations usually were made at the end of the fermentation.

4. Addition of yeast extract.

Yeast extract is well known for the accessory growth factors it contains. Since Ayres and Stewart (6) had shown that this material greatly accelerated the fermentation of egg white by yeast, studies were made to determine the effect of this material on the fermentation of albumen by Aerobacter aerogenes. A sterile concentrated solution of yeast extract was added to egg white in such a manner that the albumen used in fermentation always contained a definite percentage by weight of yeast extract.

5. Surface/volume ratio studies.

To determine the effect of surface/volume ratio on final pH of the fermenting albumen and on the time required

for glucose removal, fermentations were run using 100 ml. of egg white in beakers of 100, 250, 400, 600 and 1000 ml. capacity, respectively. In addition 100 ml. were placed in a 10 inch diameter pie plate and also 1000 ml. were placed in a one liter Erlenmeyer flask. In the latter case a paraffin-coated rubber stopper containing a glass tube 0.23 cm. in diameter was inserted to the level of the egg white. These latter two cases were studied to determine the effects of extremely large and extremely small ratios.

6. pH adjustments.

a. Before fermentation. In certain of the studies adjustments in the pH of liquid egg white were made to more acid levels. Normal hydrochloric acid was added dropwise with constant agitation (without incorporation of air) to the egg white until the desired pH was reached. All pH measurements were made using a Leeds and Northrup Model 7663A-1 glass electrode pH assembly. When it was necessary to adjust the pH of a liquid egg white for a particular test, a control sample, unadjusted, was tested at the same time. In order to compensate for the dilution of the egg white when acid was added, an equal amount of distilled water was added to the control sample.

b. During fermentation. In an effort to control pH during certain fermentations, two methods were used:

(1) By eliminating CO_2: e.g., during the fermentation

the egg white was agitated by means of a glass rod bent at 100° angle and rotated at a speed of 75-78 revolutions per minute.

(2) By neutralization: e.g., one per cent NaOH was added dropwise to the fermenting egg white with constant agitation (as described previously) as required to keep the pH of the fermenting albumen at 6.8-7.0.

c. After fermentation. In certain cases the fermented egg white was adjusted to alkaline levels by the use of one per cent NaOH or one per cent and 28 per cent NH_4OH. The actual manipulations were similar to those described above.

D. Glucose Tests

1. Two hour test.

Presence or absence of glucose was determined by means of a simple, rapid test devised by Stewart and Kline (51). Five ml. of the fermenting or fermented albumen were placed in a 50 ml. beaker which was then placed in a well-ventilated air oven at 120° C. for two hours. At the end of this time the color of the albumen was noted. Samples containing no glucose do not show color development (beyond the normal yellow color of dried albumen) during the drying and heating period.

2. Fifteen minute test.

Presence or absence of glucose was also determined by a modification of the two hour test. One-tenth ml. of egg white was placed on a preheated half of a petri dish and heated for fifteen minutes under a General Electric reflector-drying lamp placed four inches from the surface of the plate. At the end of that period, the color of the albumen was noted. Tests on samples containing varying amounts of glucose showed that, when no color developed during the heating period, the albumen was free of glucose. Results of the two hour and fifteen minute tests correlated very well.

Some precautions in the standardization of this procedure had to be observed. The area not in direct line with the reflection from the filament showed a lower intensity of heat; this rendered such areas unsuitable for use. This area approximated one half the petri dish for the lamp used in this laboratory. All samples were placed in the properly illuminated sector, equidistant from the center of the plate. It was found necessary to standardize the test for each bulb used.

E. Drying of Fermented Egg White

Between 250 and 300 ml. of egg white were placed on aluminum trays measuring approximately 27 cm. by 56 cm., giving

a liquid depth of 0.6 cm. The trays of egg white were then dried by placing under a battery of drying lights (General Electric Reflector Drying and G. E. Reflector Infra-red Heat lamps, 250 Watts, 105-120 Volts). The lights were placed about 85 cm. above the surface of the liquid. An electric fan operating at high speed served to keep an air current flowing over the liquid egg surface. The temperature of the liquid was kept below 40° C. by appropriate manipulation of lights and air flow. When drying was almost complete (about two hours), the lights were turned off and drying was finished without supplementary heat.

1. Moisture determination.

The moisture content of dried samples was determined by the A. O. A. C. method (3) modified by using a one gram sample, eliminating sifting, drying for exactly five hours, and cooling the dried samples over fresh P_2O_5 [Stewart and Kline (51)].

2. Reconstitution of dried egg white.

The dried egg white was reconstituted to its original moisture content with distilled water. Preliminary study indicated that a definite reconstituting period was necessary in order to secure satisfactory and uniform whipping tests. The procedure followed was to place the appropriate amount of dried white in a small beaker. After adding water the contents

were mixed so that all of the egg white particles came in contact with the water. This beaker was covered and the mixture allowed to stand at 10° C. for five hours. It was then stirred until homogeneous, at which time it was ready for further testing.

F. Beating Test

The procedure used has been described by Slosberg (48), Hanson (23) and their coworkers (49); for the convenience of the reader it will be repeated here. This whip test was devised to measure the beating rate of egg white in an angel cake meringue. The following formula is used:

Egg White	61.0 grams
Sugar	47.0 "
Cream of Tartar	0.9 "
Salt	0.3 "

This particular formula is the same as that described by Hanson (23). The egg white was brought to 21° C., placed in a mixing bowl and beaten at high (third) speed on a Hobart "KitchenAid" Model 4 electric mixer. The egg white was beaten continuously while the other ingredients were being incorporated. At the end of ten seconds the salt and cream of tartar were added. The sugar was added in four equal portions: after twenty, thirty, thirty-seven, and forty-five seconds of beating. Beating was continued for thirty additional seconds so that the total time of beating was 75 seconds.

After the beating period, one-fourth cup (60 ml.) of

meringue was weighed to the nearest one-tenth of a gram. Precautions were observed in placing the foam in the measuring cup so that no pockets of air were incorporated. Care was taken in handling the meringue in the cup in order that the foam was not compressed or broken. The meringue in the cup was leveled by running the blade of a spatula over it at right angles to the rim of the measuring cup.

Specific volume of the meringue was calculated by dividing the cup volume (60 ml.) by the foam weight per cup. Beating rate was determined by dividing the specific volume by the total time of beating (75 seconds). Thus, beating rate was measured in terms of increase in specific volume of foam per second of beating (ml./gm./sec.).

Previous workers (23, 48, 49) found that the beating rate results correlate reasonably well with angel cake volume; however, a few exceptions have been noted. Results indicate that egg white which beats rapidly always produces an angel cake of high volume; however, egg white which beats slowly does not necessarily produce a cake of poor volume.

G. Angel Cake Test

In many cases angel cakes were made. These cakes were prepared using essentially the method of Hanson (23) and Lowe (39).

1. Ingredients.

The angel cake formula contained:

Egg White	61.0 grams
Sugar	62.5 "
Cake Flour	22.5 "
Cream of Tartar	0.9 "
Salt	0.3 "

2. Mixing and baking.

Liquid egg white was placed in a mixing bowl (Hobart "KitchenAid" Model 4), brought to 21° C. and beating started [high (third) speed]. After five seconds the cream of tartar and salt were added. Forty-seven grams of sugar were added in three equal portions at ten, fifteen, and twenty seconds. Beating was continued to the soft peak stage. The total time of beating and the weight of a quarter cup of meringue were recorded at this point. The remaining sugar (15.5 grams) and flour were combined and sifted three times. This mixture was sifted over, and blended into, the meringue in four equal portions using ten strokes of a French balloon whip. The weight of a quarter cup of batter was then measured and recorded. One hundred and ten grams of batter were weighed into an ungreased baking pan measuring approximately 2.75 in. in height, 3.25 in. in width and 5.75 in. in length. A layer of waxed paper had been placed in the bottom of the pans just prior to use in order to facilitate removal of the finished cake. The cakes were baked at 170° C. for

twenty-five minutes in a Despatch (Rotary Hearth Oven Model 150) electric oven. After baking, the cakes were inverted, cooled and stored over night in a tin can at room temperature.

3. Objective and subjective measurements.

Cake volume was measured by the seed displacement method (23) before the cake was removed from the pan.

Palatability scores were determined by a panel of eight judges. Each judge was given a slice from the same relative position in each cake and was asked to score the piece for tenderness, texture, moistness, flavor and general palatability. In all except the first study they were also asked to comment on the odor of the cake. [See Appendix for palatability score sheet.]

IV. EXPERIMENTAL RESULTS AND DISCUSSION

The results of the various studies may be classified into three general categories: 1. The selection of a desirable organism for fermenting the egg white, 2. Optimal conditions for the fermentation of egg white by the selected organism, and 3. Studies concerned with producing a fermentation by the selected organism which would retain in the dried fermented product the foaming and angel cake making properties of fresh liquid egg white.

A. Choice of a Suitable Organism for Fermentation

The survey of about fifty species or strains of bacteria to determine their ability to grow in egg white and ferment the glucose revealed only three promising microorganisms: two strains of Aerobacter aerogenes and an Escherichia. One strain of the Aerobacter aerogenes group originally came from the Northern Regional Research Laboratory and was coded as N.R.R.L. #199. The other strain was isolated from a sample of commercial dried albumen. The Escherichia culture was isolated from a spontaneous fermentation of egg white.

The results of several studies indicated that N.R.R.L. #199 would ferment egg white in 16 hours less time than the

other Aerobacter culture, the fermentation time for which was 46 hours. The Escherichia culture was discarded after it was discovered that the fermentation with this organism produced a stringy, slimy product.

These studies indicated that the culture of Aerobacter aerogenes (N.R.R.L. #199) fermented glucose from egg white more rapidly than any other culture surveyed. As a result, further research was restricted to N.R.R.L. #199.

B. Surface/Volume Ratio

The fermentation of egg white in industry is generally accomplished in large vats. For example, one company uses a vat 10 feet in diameter and fills it with egg white to a depth of eight feet. This gives a surface/volume ratio of about 0.004 $cm.^2/cm.^3$. A study was undertaken of surface/volume to determine what the effect of changing the ratio would have on the rate of glucose removal, lowest pH attained and dispersion of the mucin.

The results of these surface/volume ratio studies are shown in Table 1.

Browning tests indicated that the minimum pH level was reached just as the glucose disappeared. At 30° C. the pH (at the time of glucose removal) appeared to be dependent upon the ratio of surface exposed to the total volume of egg white. However, at 40° C., the only outstanding difference was at a

Table 1

Effect of Surface/Volume Ratio on Fermentation of Egg White by Aerobacter aerogenes (N.R.R.L. #199)[a]

Surface/ Volume (cm.^{2}/cm.^{3})	30° C.[b]		40° C.[c]	
	Glucose Removal in hrs.	pH at time of complete glucose removal	Glucose Removal in hrs.	pH at time of complete glucose removal
3.08 (1 plate)	10	6.5	5	6.3
1.00 (200)	"	6.3	"	5.9
0.54 (600)	"	6.3	"	5.8
0.43 (400)	"	6.2	"	5.7
0.32 (250)	"	6.2	"	5.6
0.17 (100)	"	6.0	"	5.8
0.004[d]	--	---	"	5.8

[a]Conditions of fermentation:
10% inoculum (washed cells)
0.1% yeast extract
100 ml. egg white

[b]Data obtained at 30° C. are an average of 2 replications.

[c]Data obtained at 40° C. are an average of 3 replications.

[d]1000 ml. of egg white in a 1 liter Erlenmeyer flask

surface/volume ratio of 3.08.

Surface/volume ratio, within the limits used here, had no effect on the time required for complete glucose removal. On the other hand, a temperature increase of 10° C. (from 30° to 40° C.) reduced the fermentation time by half.

No mucin precipitation was observed in the present experiments even at the lowest pH levels (5.6). This is contrary to the work of McNally (40) who indicated that mucin is precipitated at a pH of 6.0 to 6.4. However, the egg white used in this surface/volume study was well blended and this may account for the difference. At the extremely low surface/volume ratio of 0.004 $cm.^2/cm.^3$, a very small amount of precipitated mucin appeared when the egg white reached pH 5.8.

The results of this study on the fermentation of egg white by Aerobacter aerogenes may be summarized as follows: Surface/volume ratio played a negligible role in controlling fermentation rate. It did not have any appreciable effect on mucin dispersion. Increasing the temperature from 30° to 40° C. reduced the fermentation rate by half, but had no effect on mucin dispersion. As a result of this study a uniform surface/volume ratio of 0.54 $cm.^2/cm.^3$ was adopted for further work.

C. Effect of Adjusting pH and Addition of Yeast Extract

Albumen used in this study ranged from pH 8.0 to 9.0. In general, bacteria grow slowly in this range. For example, Aerobacter aerogenes is reported by Cohen and Clark (13) to have an optimum pH range of 6.0 to 7.0 for growth in broth, with minimum and maximum limits of 4.4 and 9.0, respectively. It seemed desirable, therefore, to study the effect of adjusting the pH of the egg white prior to fermentation.

The effect of supplementing the egg white with yeast extract was investigated at the same time. Ayres and Stewart (6) had already demonstrated that yeast extract added to egg white induced more rapid fermentation by yeast.

Results of these studies are shown in Table 2 and Tables 27 and 28 (Appendix). It appears that starting the fermentation at the natural pH of egg white gave the most satisfactory results. When no yeast extract was added, pH adjustment did not have any appreciable effect on the rate of glucose removal. When yeast extract was added, acidifying the albumen prior to fermentation slightly decreased the rate of glucose removal. As was to be expected when the fermentation was run without prior pH adjustment, pH of the egg white at the end of the fermentation was least acid.

The addition of yeast extract reduced fermentation time appreciably. Apparently yeast extract supplied the necessary growth factors permitting the organism to grow rapidly and

Table 2

Effect of pH and Yeast Extract on the Fermentation of Egg White by N.R.R.L. #199[a]

pH at start	Glucose Removal in hrs.[b]	pH of Egg White when Glucose-free[c]
	Without Yeast Extract	
8.6 (Control)	39	6.2
8.0	39	6.0
7.0	40	6.0
6.0	38	5.5
5.0	38	4.8
	With 0.1% Yeast Extract	
8.6 (Control)	13	6.0
8.0	15	5.9
7.0	15	5.8
6.0	15	5.5
5.0	17	4.9

[a]Conditions of fermentation:
10% inoculum
100 ml. egg white
Surface/volume 0.54 $cm.^2/cm.^3$
Incubation temperature 30° C.
pH adjusted with 1 N HCl

[b]Average of four replications

[c]Average of three replications

thereby ferment the glucose in the egg white. In this connection it is of interest to recall that Aerobacter aerogenes is considered to have relatively simple growth requirements. Addition of yeast extract did not appreciably alter the pH of the egg white at the time of complete glucose removal compared to fermentations run without such supplementation.

From this study it is concluded that the time required to ferment glucose from egg white is considerably reduced by the addition of 0.1 per cent yeast extract and that the adjustment of pH prior to fermentation has no favorable effect. In all future fermentation studies 0.1 per cent yeast extract was added to egg white at its natural pH.

D. Effect of Temperature and Size of Inoculum

Two additional factors thought to affect the fermentation rate were size of inoculum and temperature. Jennison (28) studied the growth of Aerobacter aerogenes in nutrient broth at temperatures of 22°, 27°, 32°, 37°, and 42° C. Most rapid growth took place at 32° and 37° C.; growth at 42° C. was more rapid than growth at 27° C., but not as rapid as at 32° and 37° C.

Stuart and Goresline (55) found sterile egg white inoculated with various levels of a selected strain of Aerobacter aerogenes fermented (at 30° C.) in a manner similar to the commercial spontaneous fermentation. The rate was directly

proportional to the number of bacterial cells added. In their study, the course of fermentation was followed by pH measurements, glucose determinations, and formol titrations.

Figure 1 and Tables 29 and 30 (Appendix) illustrate the effect of temperature and inoculum level on the rate of glucose removal in egg white. The most rapid glucose removal rate occurred within the temperature range 34° and 45° C. Increased amounts of inoculum reduced the time required to ferment glucose.

These data indicate the ability of Aerobacter aerogenes to ferment glucose rapidly over a wide range of temperatures. By adjusting the size of inoculum and selecting the proper temperature it was found possible to ferment egg white on a widely variable time schedule. This is of considerable interest commercially since it permits the establishment of fermentation times which are adaptable to a variety of different industrial situations.

Conditions were selected from the data obtained in these experiments so as to give a short fermentation period. Those conditions included a 10 per cent inoculum and 40° C. incubation temperature; a four to six hour fermentation results. This period allowed for general observations, sampling and testing to be made throughout the fermentation cycle.

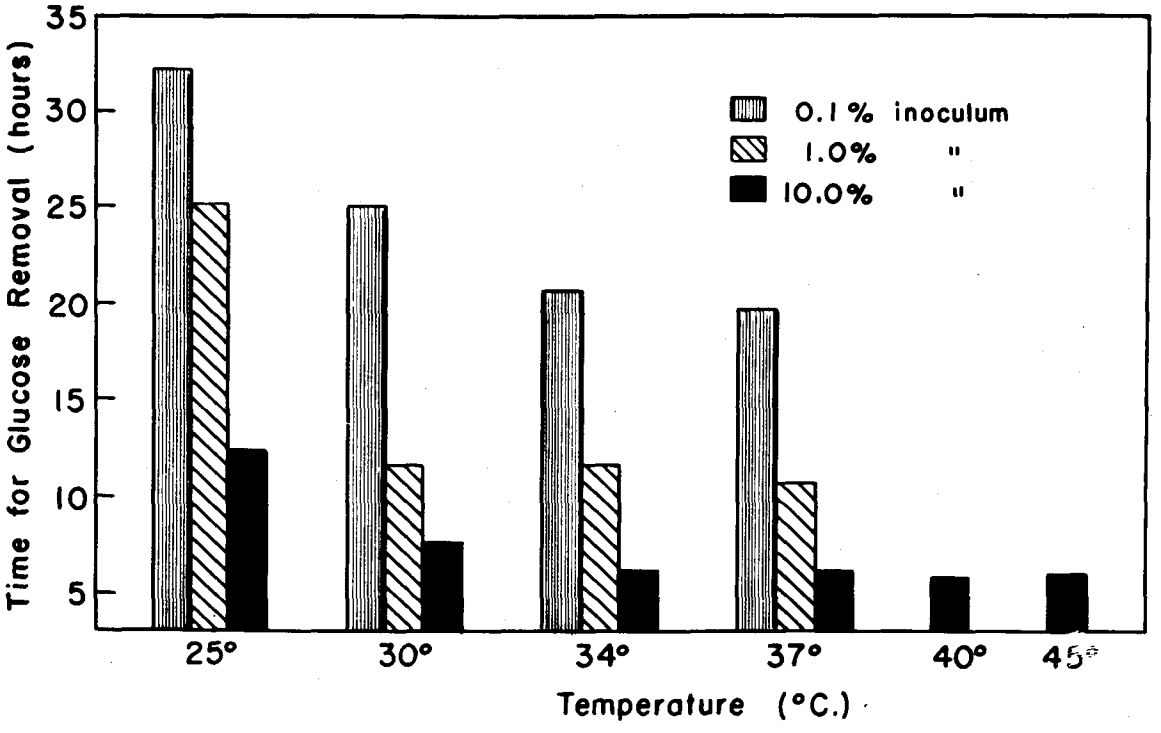

Fig. 1. Effect of Inoculum Level and Temperature on Time of Glucose Removal by N.R.R.L. #199.

E. Serial Passage of Organism in Egg White

In order to further simplify the fermentation procedure and to make it more convenient for industrial purposes, attempts were made to carry the selected organism (N.R.R.L. #199) directly in egg white (instead of using standard cultural medium or aseptic techniques). Serial passages were made in the following manner:

Passage 1

↓ *Aerobacter aerogenes* (N.R.R.L. #199) was inoculated in 10 ml. egg white containing 0.1 per cent yeast extract, incubated 4½ to 5 hours at 37° C.; then stored at -15° C. until needed for transfer.

Passage 2

↓ 1 ml. of previous culture was inoculated in 9 ml. egg white containing 0.1 per cent yeast extract, incubated 4½ to 5 hours at 37° C.; then stored at -15° C.

Passage 3

↓ ditto

Passage 160

The cultures were checked regularly for purity and constancy of count. As shown in Table 3, after 160 passages the culture still reproduced in egg white to a constant number of microorganisms. It also remained pure. Probably this can be

Table 3

The Effect of Carrying Aerobacter aerogenes (N.R.R.L. #199) in Egg White on Purity and Ability to Reproduce

Passage No.	Bacterial Count[a] (per ml.)	State of Culture[a]	Differential Tests[b] I M V C
0	--	Pure	- - + +
8	1×10^5	"	- - + +
12	14×10^8	"	
31	$14 \times$ "	"	
49	$13 \times$ "	"	
51	$15 \times$ "	"	
83	$14 \times$ "	"	
105	$14 \times$ "	"	
124	$24 \times$ "	"	
127	$14 \times$ "	"	
135	$17 \times$ "	"	
146	$15 \times$ "	"	
152	$13 \times$ "	"	
160	$14 \times$ "	"	- - + +

[a]Determined by plating fermented albumen on Bacto-eosin-methylene-blue agar.

[b]I - Indol; M - Methyl Red; V - Voges-Proskauer; C - Citrate.

attributed to four factors: 1. The 'anti-factors' naturally present in egg white minimized the development of contaminants. 2. The short incubation period. 3. Not all of the glucose was fermented from the egg white; and, 4. The large inoculum (10 per cent) eliminated, to a great extent, competition from other organisms which are present in the egg white.

At various intervals, the culture was checked for Salmonella using Bacto-SS agar. During the entire study, no Salmonella-type organisms were isolated from the egg white samples.

In addition to the convenience of carrying the culture in egg white, there was the possibility that the serial passage might acclimatize the microorganism to egg white, so that yeast extract could eventually be omitted, or, conversely, might acclimatize it to yeast extract, so that the bacterium could not grow in egg white at all without yeast extract being present. Table 4 shows that there is no appreciable change in the behavior of the culture after 125 passages through egg white.

F. Standardized Fermentation Procedure

Consideration of the results of the study thus far permitted the establishment of a standard fermentation for the additional studies. Somewhat larger fermentations were desired

Table 4

Fermentation Time as a Function of the Number of Serial Transfers of N.R.R.L. #199 in Egg Albumen[a]

No. of Transfers	Time of Glucose Removal (hours)	
	Egg White without Yeast Extract	Egg White, + 0.1% Yeast Extract
0	13	5-6
5	--	5-6
117	13	4
121	13	4-5
125	13	4-5

[a]Conditions of fermentation
10% inoculum N.R.R.L. #199
100 ml. egg white at natural pH
Incubation temperature 40° C.
Surface/volume 0.54 $cm.^2/cm.^3$

in order to study whipping quality. Accordingly the following conditions were adopted in subsequent work:

10% inoculum; N.R.R.L. #199 carried by serial transfer in egg white
0.1% yeast extract
1000 ml. blended egg white at natural pH
Incubation temperature 40° C.
Surface/volume ratio 0.40 $cm.^2/cm.^3$

Figure 2 illustrates a typical pH curve obtained during the fermentation of egg white under such conditions.

G. Control of pH During Fermentation

There was no serious precipitation of mucin in any of the fermentations even though the pH dropped to levels below 6.0. It seems conceivable that failure to reproduce the exact conditions of these experiments might lead to precipitation of mucin. With this in mind a series of fermentations was run in which the pH was controlled above that at which mucin has been reported to precipitate (pH 6.0-6.4). In addition, fermentation studies were made with a view to retaining, in the dried fermented product, those foaming and angel cake making properties which may be found in fresh liquid albumen. This necessitated some change in criteria. Beating rate was selected as the prime criterion of quality in the reconstituted product.

In an effort to control the pH of the egg white during fermentation two methods were used[1]: 1. Agitation of the

[1] A phosphate buffer was also used in an effort to keep the pH above 6.8 during fermentation. Unfortunately, the addition of the buffer interfered with the glucose test; consequently this method of controlling pH had to be discarded.

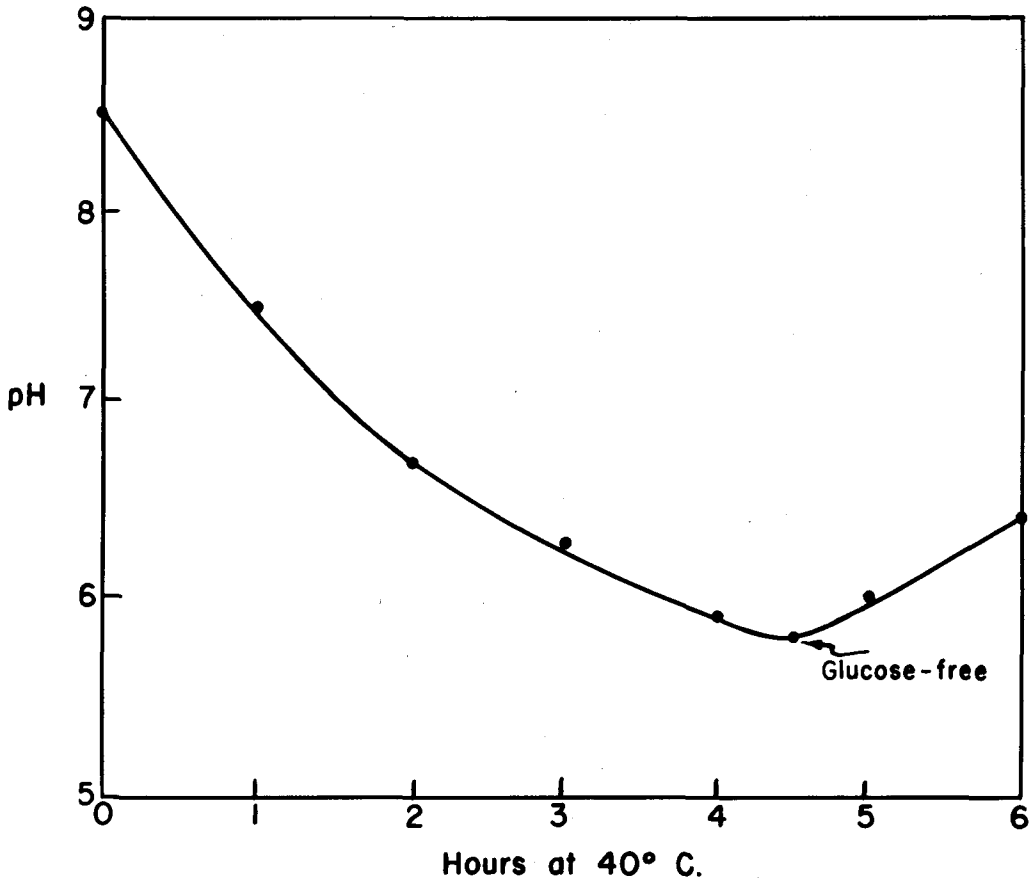

Fig. 2. Typical pH Curve for the Fermentation of Egg White by N.R.R.L. #199.

fermenting egg white in order to remove the CO_2 which was liberated. 2. Addition of dilute NaOH.

Results of the study are shown in Table 3 and Table 31 (Appendix). Mild agitation prevented the pH from dropping below 6.5; however, the mechanical action of the stirrer caused ever-increasing amounts of mucin to collect on it. Since the NaOH was added with agitation, the collection of mucin fibers was also apparent in cases where the liquid was so neutralized. The control sample was not agitated during fermentation and even though the pH dropped to 6.0, no mucin fibers accumulated.

Where the beating tests were made on the fermented albumen, it was reblended in the egg white before samples were drawn. The remaining egg white was pan dried; whip tests were run on the reconstituted samples. As shown in Table 5 there was considerable loss of whipping quality after drying those samples in which pH was controlled during fermentation. Very little loss of whipping quality occurred in the fermentation where the pH was permitted to change freely.

H. Adjusting pH prior to Drying

It is common practice in industry to add NH_4OH to egg white at the completion of fermentation to bring the pH to about 9.0, presumably in order to stop further bacterial action. The effect of adding alkali prior to drying on pH

Table 5

Effect of Agitation and pH Adjustment during Fermentation on Rate of Glucose Removal, pH and Beating Rate[a]

Treatment	Fermentation Time (in hours)	pH		Beating Rate[b]		
		After Fermentation	After Reconstitution	Before Fermentation	After Fermentation	After Drying and Reconstitution
Control[c]	4-5	6.0	6.9	.0739	.0760	.0702
Agitated[d]	4-5	6.5	7.2	.0752	.0820	.0638
Adjusted with 1% NaOH[e]	4-5	7.0	7.8	.0726	.0784	.0562

[a]Conditions of fermentation:
10% inoculum (N.R.R.L. #199)
0.1% yeast extract
1000 ml. egg white
Incubation temperature 40° C.
Surface/volume ratio 0.40 $cm.^2/cm.^3$

[b]Beating rate expressed as ml./gm./sec.

[c]Data are averages of 9 replications

[d]Data are averages of 5 replications

[e]Data are averages of 4 replications

and beating rate is shown in Table 6.

Adjustment of fermented egg white with NaOH to pH 9.0 prior to drying produced a product of the same pH on reconstitution. This treatment had an adverse effect on the beating rate of the reconstituted product. On the other hand, a similar adjustment with NH_4OH improved the whipping quality. It is of some interest to note that, in this case, the pH of the reconstituted sample was 7.2. Apparently not all of the NH_4OH was volatilized (compare data with that for the control). In the case of the control the difference in pH before and after drying was most likely due to loss of CO_2 during the process. The addition of various concentrations of NH_4OH showed no deleterious effect on beating rate even when concentrated NH_4OH was added dropwise (to pH 9.0).

Table 6

Effects of Adjusting pH Prior to Drying on Beating Rate and pH of the Reconstituted Albumen

pH of Egg White after Fermentation	Reconstituted Albumen pH	Beating Rate[a]
5.80 (Control)	6.7	.0708
9.00 (NaOH)	9.2	.0575
9.00 (NH_4OH)	7.2	.0808

[a]Beating Rate expressed as ml./gm./sec.

I. Blending Previous to Fermentation

Several reports in the literature make reference to the precipitation of mucin from the egg white during fermentation (10, 51). However, except for the few occasions already noted, mucin precipitation did not occur during the fermentations described herein. However, as has been mentioned previously, all of the egg white used in these fermentations was thoroughly blended just prior to inoculation. In view of these results fermentations were run using blended and unblended egg white.

After fermentation the unblended white showed a heavy, compact layer of denatured mucin floating on the surface; the blended white showed no sign of mucin precipitation. No satisfactory glucose tests could be made on the fermented unblended egg white. At the end of the two hour glucose test there always remained small flecks of brown color. Even when the fermentation in the unblended egg white was allowed to continue several hours after the control fermentation was completed, these flecks persisted. This suggests that the organisms were unable to penetrate the chunks of thick white and ferment out the glucose there.

Another interesting point in this study was the difference in final pH between blended and unblended albumen. The latter reached a minimum pH of 5.8; the unblended white registered a pH of 6.4 [Figure 3 and Table 32 (Appendix)]. It is conceivable that this difference in pH might be accounted for by the

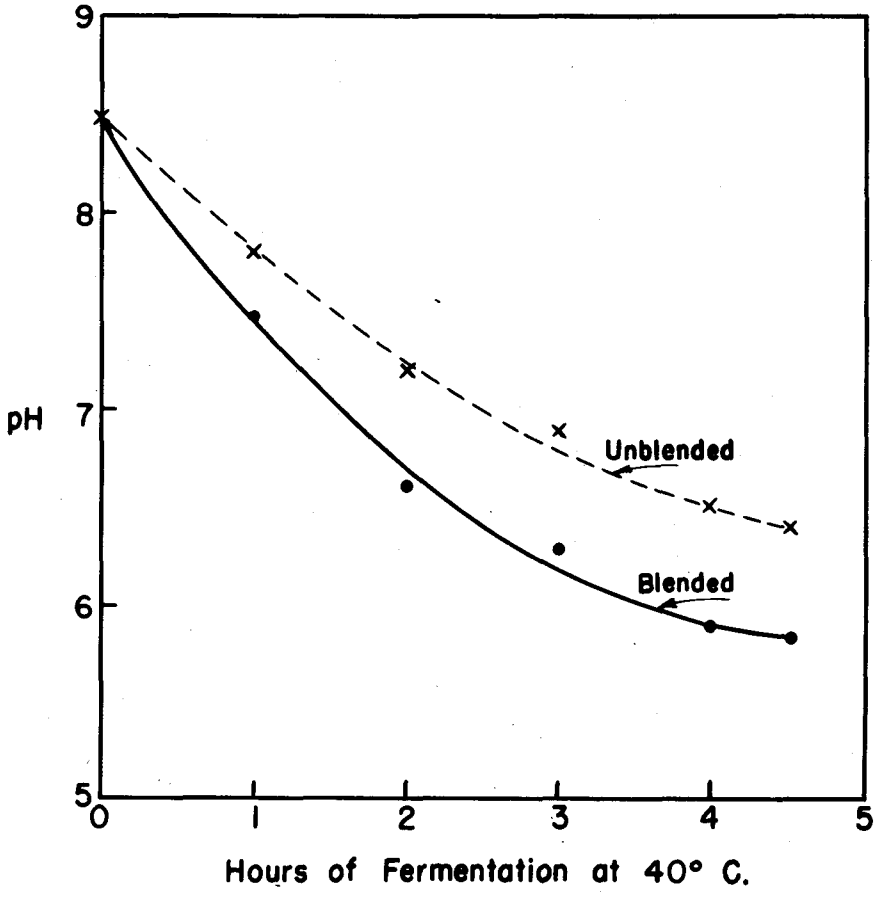

Fig. 3. Effect of Blending on pH of Fermenting Egg White.

more complete removal of CO_2 by being entrapped in the mucin as it is carried to the surface.

J. Angel Cake Studies

In order to determine the effect of the fermentation on the functional properties of egg white, three studies were undertaken in which the angel cake making properties of the fermented albumen were tested. The first study concerned determining the effects of fermenting and drying. The second study involved testing the effect of modifying the amount of cream of tartar in the cake formula on the angel cake making properties of fermented, dried egg white. The third study was an elaboration of the second: a flavoring agent, vanilla, was added to determine whether or not the judges could distinguish between cakes made from fresh vs. dried, fermented albumen.

1. Effect of fermentation, pH adjustment and drying on egg white used in preparing angel cakes.

Data concerning the fermentation are given in Table 33 (Appendix). Half the fermented albumen was divided into two lots. One portion was frozen; the other portion was pan dried. The remaining half of the fermented material was adjusted to pH 9.1 with NH_4OH. Part of this sample was frozen; the remainder was pan dried. From the original lot of unfermented control, samples were also frozen

and pan dried.

Five angel cakes were prepared for each of the six treatments. This enabled comparisons to be made on cakes prepared from the following: unfermented frozen; unfermented dried; fermented, unadjusted frozen; fermented, unadjusted dried; fermented, adjusted frozen; and fermented, adjusted dried egg white.

Results of subjective and objective tests on angel cakes are given in Tables 7, 8, 9, 10, 11 and Tables 34 and 35 (Appendix). In order to test the significance of the results, analyses of variance were made on the data for batter pH, volume, texture, tenderness, moisture, flavor and general palatability. These analyses are recorded in the Appendix (Tables 36 through 42).

A summary of the results of the statistical analyses which were significantly less than the control at the 5 per cent (designated significant) or 1 per cent (designated highly significant) levels are shown in Table 12. From this information it is evident that fermenting and freezing and fermenting and drying albumen produced angel cakes highly significantly different from those made from the control with respect to: volume, texture, flavor and general palatability. There was no significant difference in tenderness or moistness. In addition, there were highly significant differences in flavor when angel cakes were made from frozen and dried fermented egg white; the latter treatment also gave significant difference

Table 7

Effect of Adjusting pH of Fermented Albumen on pH of Cake Batter

Treatment	Replication I	II	III	IV	V	Ave.
Unfermented Frozen (Control A)	6.5	6.2	6.0	5.8	6.0	6.1
Unfermented Dried (Control B)	6.0	5.8	5.8	5.8	5.6	5.8**
Fermented-Unadjusted[a] Frozen	4.4	4.3	4.5	4.5	4.5	4.4**
Fermented-Unadjusted Dried	4.8	4.5	4.6	4.5	4.5	4.6**
Fermented-Adjusted[b] Frozen	5.7	5.7	5.7	5.9	5.9	5.8**
Fermented-Adjusted Dried	4.6	4.7	4.7	4.8	4.9	4.7**

[a] Fermented, then frozen or dried

[b] Fermented, neutralized to pH 9.1 before freezing or drying

**Significantly less than control at 1 per cent level

Table 8

Effect of Adjusting pH of Fermented Albumen on Volume of Angel Cake Made Therefrom[a]

Treatment	Replication I	II	III	IV	V	Ave.
Unfermented Frozen (Control A)	618	617	583	608	578	600.8
Unfermented Dried (Control B)	614	605	612	615	600	609.2
Fermented-Unadjusted[b] Frozen	571	573	566	568	527	561.0**
Fermented-Unadjusted Dried	595	565	577	576	586	579.8**
Fermented-Adjusted[c] Frozen	623	609	597	612	585	605.2
Fermented-Adjusted Dried	615	587	595	559	617	594.6

[a]Volume measured in ml. per 100 gm. batter

[b]Fermented, then frozen or dried

[c]Fermented, neutralized to pH 9.1 before freezing or drying

**Significantly less than control at 1 per cent level

Table 9

Effect of Adjusting pH of Fermented Albumen on Texture of Angel Cake Made Therefrom[a]

Treatment	Replication I	II	III	IV	V	Ave.
Unfermented Frozen (Control A)	7.6	8.0	8.5	8.3	7.2	7.9
Unfermented Dried (Control B)	7.5	8.0	7.9	7.5	7.4	7.7
Fermented-Unadjusted[b] Frozen	6.3	6.8	6.8	6.5	6.6	6.6**
Fermented-Unadjusted Dried	6.5	6.9	6.8	6.5	6.5	6.6**
Fermented-Adjusted[c] Frozen	8.1	7.3	6.8	7.3	7.8	7.5
Fermented-Adjusted Dried	7.3	7.1	6.5	7.3	7.1	7.1

[a]Average of eight judges' scores. Highest possible score 10; range 0-10

[b]Fermented, then frozen or dried

[c]Fermented, neutralized to pH 9.1 before freezing or drying

**Significantly less than control at 1 per cent level

Table 10

Effect of Adjusting pH of Fermented Albumen on Flavor of Angel Cake Made Therefrom[a]

Treatment	Replication I	II	III	IV	V	Ave.
Unfermented Frozen (Control A)	7.8	8.6	8.8	8.5	8.5	8.4
Unfermented Dried (Control B)	8.1	8.8	8.8	8.4	8.4	8.5
Fermented-Unadjusted[b] Frozen	6.8	6.5	6.5	6.6	7.1	6.7**
Fermented-Unadjusted Dried	7.2	6.0	6.0	6.4	7.0	6.5**
Fermented-Adjusted[c] Frozen	7.6	7.4	7.4	7.6	7.9	7.6**
Fermented-Adjusted Dried	7.8	7.0	6.6	7.6	8.2	7.4**

[a] Average of eight judges' scores. Highest possible score 10; range 0-10

[b] Fermented, then frozen or dried

[c] Fermented, neutralized to pH 9.1 before freezing or drying

**Significantly less than control at 1 per cent level

Table 11

Effect of Adjusting pH of Fermented Albumen on General Palatability of Angel Cake Made Therefrom[a]

Treatment	Replication I	II	III	IV	V	Ave.
Unfermented Frozen (Control A)	7.6	8.4	8.7	8.4	7.9	8.2
Unfermented Dried (Control B)	8.2	8.7	8.1	8.1	8.0	8.2
Fermented-Unadjusted[b] Frozen	7.0	6.7	6.6	6.4	7.6	6.8**
Fermented-Unadjusted Dried	7.1	6.4	6.7	6.4	7.4	6.8**
Fermented-Unadjusted[c] Frozen	8.1	7.7	7.0	7.6	7.7	7.6
Fermented-Adjusted Dried	7.8	7.4	6.4	7.3	8.3	7.4*

[a]Average of eight judges' scores. Highest possible score 10; range 0-10

[b]Fermented, then frozen or dried

[c]Fermented, neutralized to pH 9.1 before freezing or drying

*Significantly less than control at 5 per cent level

**Significantly less than control at 1 per cent level

Table 12

Summary of Mean Scores of Angel Cake Data

Treatment	Volume	Texture	Flavor	General Palatability	Tenderness	Moistness
Unfermented Frozen (Control A)	600.8	7.9	8.4	8.2	8.3	8.3
Unfermented Dried (Control B)	609.2	7.7	8.5	8.2	8.4	8.3
Fermented-Unadjusted Frozen	561.0**	6.6**	6.7**	6.8**	7.7	7.8
Fermented-Unadjusted Dried	579.8**	6.6**	6.5**	6.8**	8.0	7.8
Fermented-Adjusted Frozen	605.2	7.5	7.6**	7.6	8.2	8.1
Fermented-Adjusted Dried	594.6	7.1	7.4**	7.4*	8.3	7.8

*Significantly less than control at 5 per cent level
**Significantly less than control at 1 per cent level

in general palatability.

The treatments used also had a significant effect on the pH of the batters and on the volume of the cakes. The mean pH of the batters by treatments are highly correlated with both the mean flavor and volume by treatments (Tables 43 and 44, Appendix). Seventy-nine per cent of the variation in the flavor among treatments and seventy per cent of the variation in volume among treatments were associated with pH of the batters. An insignificant part of the experimental error for both flavor and volume are associated with pH of the batters.

Freezing or drying egg white produced no significant effect on angel cakes.

2. Effect of adjusting cream of tartar level in formula for angel cakes made from fermented albumen

From the previous study it seemed essential to explore further the effect of pH in angel cake batters. The cake batter prepared from reconstituted, fermented egg white was considerably more acid than the batter made from reconstituted, unfermented albumen. In view of this fact, a study was made which involved testing the effect of modifying the amount of cream of tartar in the cake formula on the angel cake making properties of fermented, dried egg white.

A preliminary experiment with cake meringues was run in which the amount of cream of tartar was varied . Figure 4 and Table 45 (Appendix) illustrate the effect of level of cream of

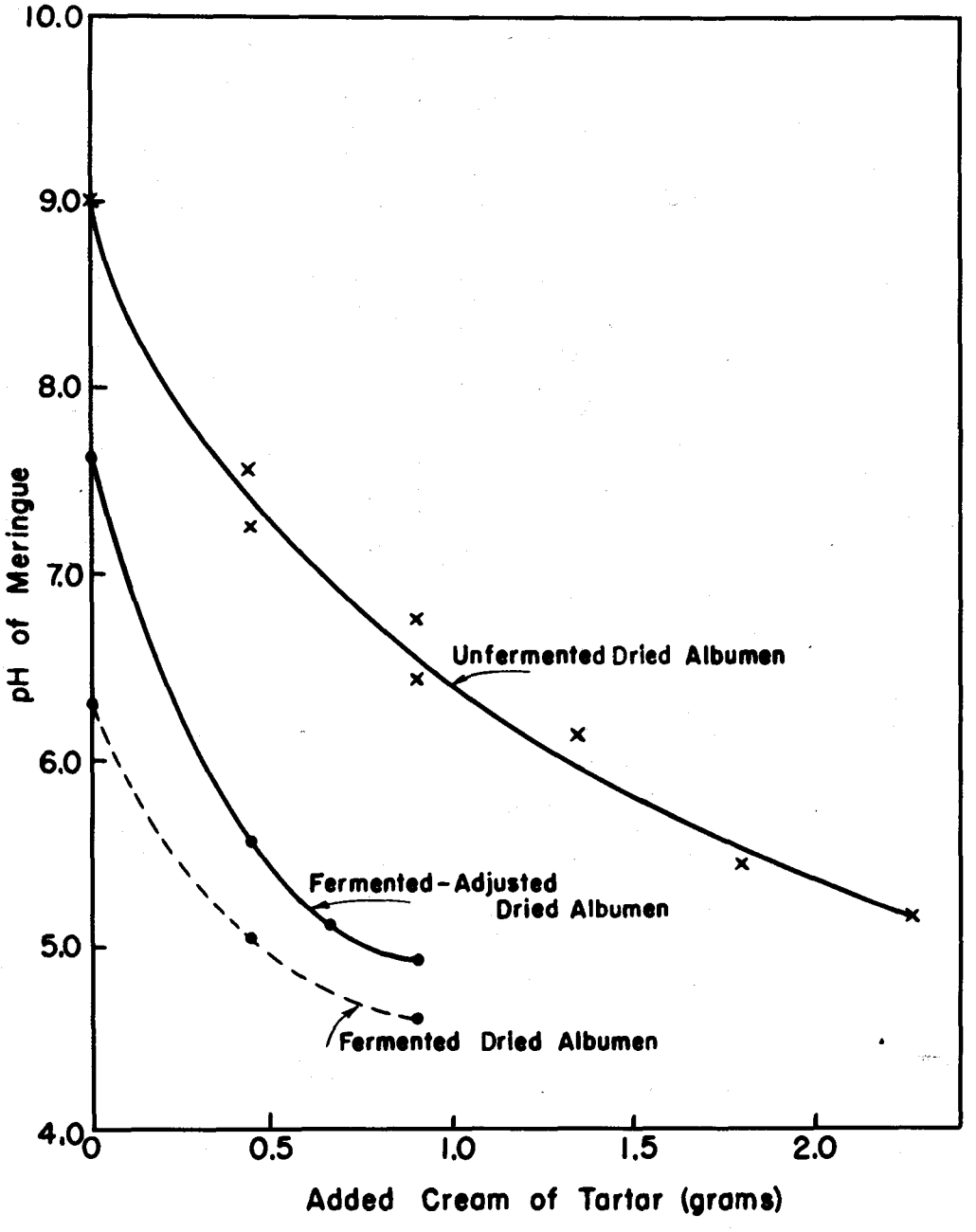

Fig. 4. Effect of Cream of Tartar Level on pH of Meringue made from Unfermented and Fermented Albumen

tartar on the pH of the meringue made from reconstituted egg white. It will be noted that even relatively large amounts of cream of tartar do not produce the acid meringue that fermented white does when the cream of tartar levels are as given in the angel cake formula used.

Figure 5 and Table 46 (Appendix) indicate the effect of cream of tartar level on cake volume. When using fresh egg white the highest volumes were obtained when cake batter had a pH range of 5.7 to 6.6.

Using the data in Figure 4 as a guide, the cream of tartar level for angel cakes was reduced in fermented, adjusted egg white to 0.3 grams; in the plain fermented egg white it was omitted entirely. Data concerning the fermentation of egg white used in this study are given in the Appendix (Table 47).

Results of subjective and objective tests on these angel cakes are given in Tables 13 through 17 (Tables 48 and 49, Appendix). Tests of significance were made by means of analysis of variance. (See Appendix, Tables 50 through 56.)

A summary of the results of the statistical analyses which were significantly less than the control at the 5 per cent (designated significant) or 1 per cent (designated highly significant) levels are shown in Table 18. Highly significant differences in volume, flavor and general palatability may be noted when fermented egg white is used to prepare angel cakes by the standard formula. However, when the cake formula was adjusted to the lower or zero levels of cream of tartar, there

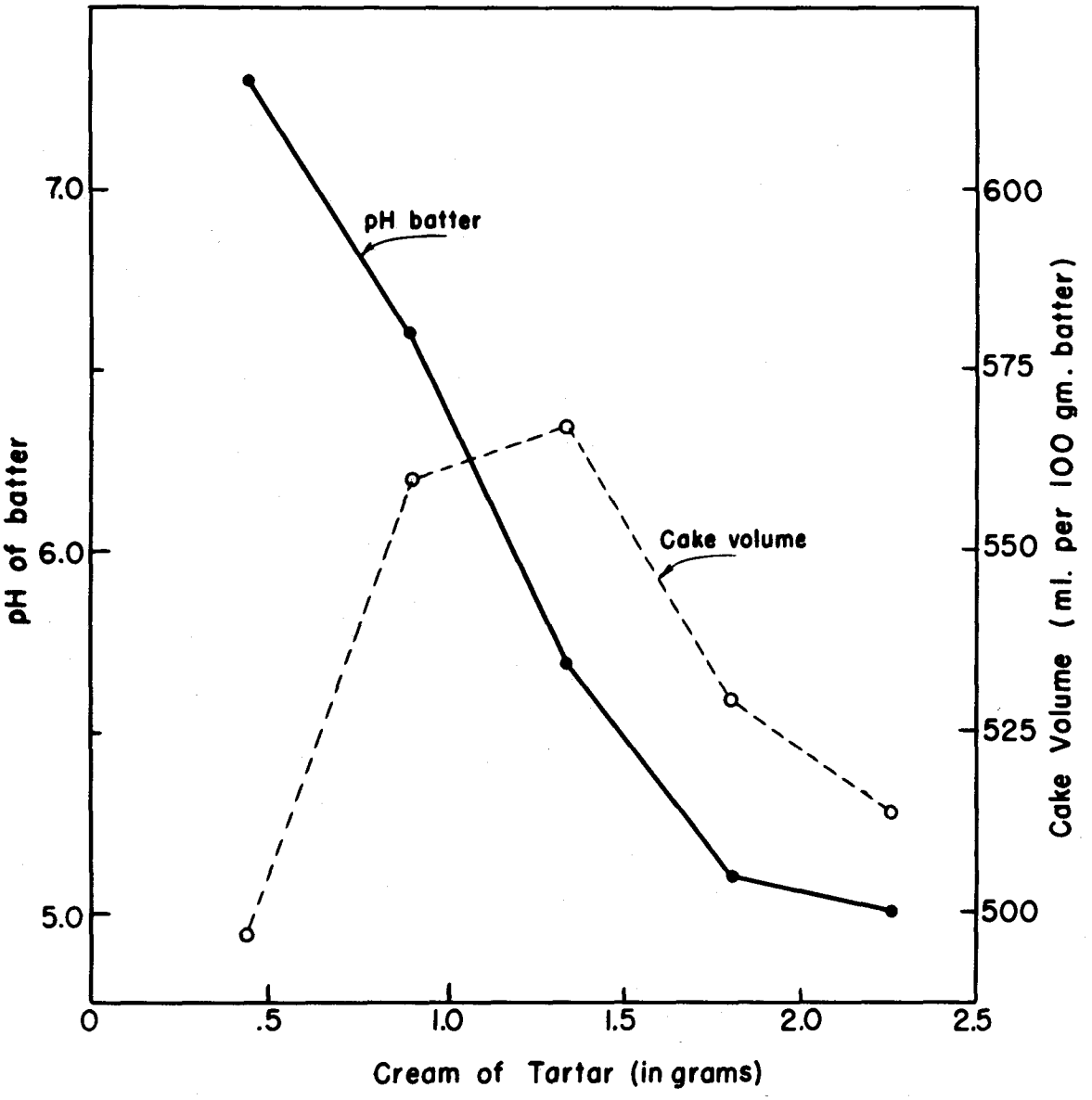

Fig. 5. Effect of Cream of Tartar Level on pH of Batter and Angel Cake Volume

Table 13

Effect of Cream of Tartar Level on pH of Angel Cake Batter Prepared from Dried Egg White

Treatment	Replications I	II	III	IV	V	Ave.
Unfermented 0.45 gm. c. t.[a]	6.5	6.7	6.8	6.8	6.7	6.7**
Unfermented 0.9 gm. c. t. (Control)	6.1	5.7	6.0	6.0	5.9	5.9
Fermented-Unadjusted 0.0 gm. c. t.	5.7	5.8	5.8	5.7	5.7	5.7**
Fermented-Unadjusted 0.9 gm. c. t.	4.7	4.6	4.5	4.6	4.6	4.6**
Fermented-Adjusted 0.3 gm. c. t.	5.4	5.3	5.5	5.6	5.6	5.5**
Fermented-Adjusted 0.9 gm. c. t.	4.8	4.8	4.9	4.9	4.9	4.9**

[a]Cream of Tartar
**Significantly less than control at 1 per cent level

Table 14

Effect of Cream of Tartar Level on Volume of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replications I	II	III	IV	V	Ave.
Unfermented 0.45 gm. c. t.[b]	571	560	509	544	524	540.6**
Unfermented 0.9 gm. c. t. (Control)	608	586	594	609	586	596.6
Fermented-Unadjusted 0.0 gm. c. t.	591	585	596	572	577	584.2
Fermented-Unadjusted 0.9 gm. c. t.	536	523	523	512	535	525.8**
Fermented-Adjusted 0.3 gm. c. t.	591	575	599	584	600	589.8
Fermented-Adjusted 0.9 gm. c. t.	566	556	562	543	545	554.4**

[a] Volume measured in ml. per 100 gm. batter

[b] Cream of Tartar

**Significantly less than control at 1 per cent level

Table 15

Effect of Cream of Tartar Level on Texture of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replications I	II	III	IV	V	Ave.
Unfermented 0.45 gm. c. t.[b]	8.1	8.4	7.0	7.6	8.0	7.8
Unfermented 0.9 gm. c. t. (Control)	7.9	8.4	7.5	7.6	7.4	7.7
Fermented-Unadjusted 0.0 gm. c. t.	8.0	8.1	8.6	8.8	7.7	8.2
Fermented-Unadjusted 0.9 gm. c. t.	7.4	6.8	6.9	7.0	7.3	7.1**
Fermented-Adjusted 0.3 gm. c. t.	8.6	7.7	8.2	7.4	8.8	8.1
Fermented-Adjusted 0.9 gm. c. t.	8.1	7.0	7.8	7.0	7.5	7.5

[a]Average of eight judges' scores. Highest possible score 10; range 0-10

[b]Cream of Tartar

**Significantly less than control at 1 per cent level

Table 16

Effect of Cream of Tartar Level on Flavor of Angel Cake Prepared From Dried Egg White[a]

Treatment	Replications I	II	III	IV	V	Ave.
Unfermented 0.45 gm. c. t.[b]	8.2	8.4	8.4	8.3	8.4	8.3
Unfermented 0.9 gm. c. t.	7.9	7.3	8.3	8.1	8.4	8.0
Fermented-Unadjusted 0.0 gm. c. t.	8.1	7.6	7.4	8.5	8.2	8.0
Fermented-Unadjusted 0.9 gm. c. t.	6.5	5.9	6.1	6.6	6.9	6.4**
Fermented-Adjusted 0.3 gm. c. t.	8.1	8.1	8.4	8.4	8.4	8.3
Fermented-Adjusted 0.9 gm. c. t.	6.5	6.6	6.1	6.1	6.6	6.4**

[a]Average of eight judges' scores. Highest possible score 10; range 0-10

[b]Cream of Tartar

**Significantly less than control at 1 per cent level

Table 17

Effect of Cream of Tartar Level on General Palatability of Angel Cake Prepared From Dried Egg White[a]

Treatment	Replications I	II	III	IV	V	Ave.
Unfermented 0.45 gm. c. t.[b]	8.3	8.4	7.8	7.9	8.4	8.1
Unfermented 0.9 gm. c. t. (Control)	8.5	7.7	7.0	7.9	7.9	8.0
Fermented-Unadjusted 0.0 gm. c. t.	8.2	7.6	7.7	8.6	8.1	8.0
Fermented-Unadjusted 0.9 gm. c. t.	7.0	6.0	6.7	6.7	7.1	6.7**
Fermented-Adjusted 0.3 gm. c. t.	8.7	8.0	8.2	8.0	8.1	8.2
Fermented-Adjusted 0.9 gm. c. t.	8.3	6.4	6.8	6.5	7.1	7.0**

[a] Average of eight judges' scores. Highest possible score 10; range 0-10

[b] Cream of Tartar

**Significantly less than control at 1 per cent level

Table 18

Summary of Mean Scores of Angel Cake Data

Treatment and pH of batter	Volume	Texture	Flavor	General Palatability	Tenderness	Moistness
Unfermented 0.45 gm. c. t.[a] pH 5.9	540.6**	7.8	8.3	8.1	8.6	8.4*
Unfermented 0.9 gm. c. t. pH 6.7 (Control)	596.6	7.7	8.0	8.0	8.6	8.1
Fermented-Unadjusted 0.0 gm. c. t. pH 5.5	584.2	8.2	8.0	8.0	8.3	8.3
Fermented-Unadjusted 0.9 gm. c. t. pH 4.9	525.8**	7.1**	6.4**	6.7**	8.2	8.0
Fermented-Adjusted 0.3 gm. c. t. pH 5.7	589.8	8.1	8.3	8.2	8.4	8.3
Fermented-Adjusted 0.9 gm. c. t. pH 4.6	554.4**	7.5	6.4**	7.0**	8.0	8.2

[a] Cream of Tartar
*Significantly less than control at 5 per cent level
**Significantly less than control at 1 per cent level

was no significant difference from the control angel cake made with the standard formula.

With the exception of fermented egg white containing 0.45 grams of cream of tartar, there was no significant difference by treatments for tenderness or moisture.

A covariance analysis was made on the data for pH and flavor. It indicated that, for this experiment, the mean pH of the batters by treatments are correlated with the mean flavor by treatments (Table 57, Appendix). Seventy-four per cent of the variation in flavor of treatment is associated with pH.

Judges' comments on odor were generally restricted to two of the twelve categories which were listed, sweet and acid. Those two treatments of cakes which had the lowest pH of batter were usually described as acid; otherwise judges considered the cakes sweet.

Photographs of cakes (magnification 1.75) are shown in Figures 6 to 10.

3. Effect of adding vanilla to cake formula

The final angel cake study was a duplication of that described in the previous section, except that vanilla was added as a flavoring agent. Also, the judging procedure was changed somewhat; e.g., when samples were presented to the judges, a cake prepared from fresh egg white was designated as a standard and was available to the judge for ready reference.

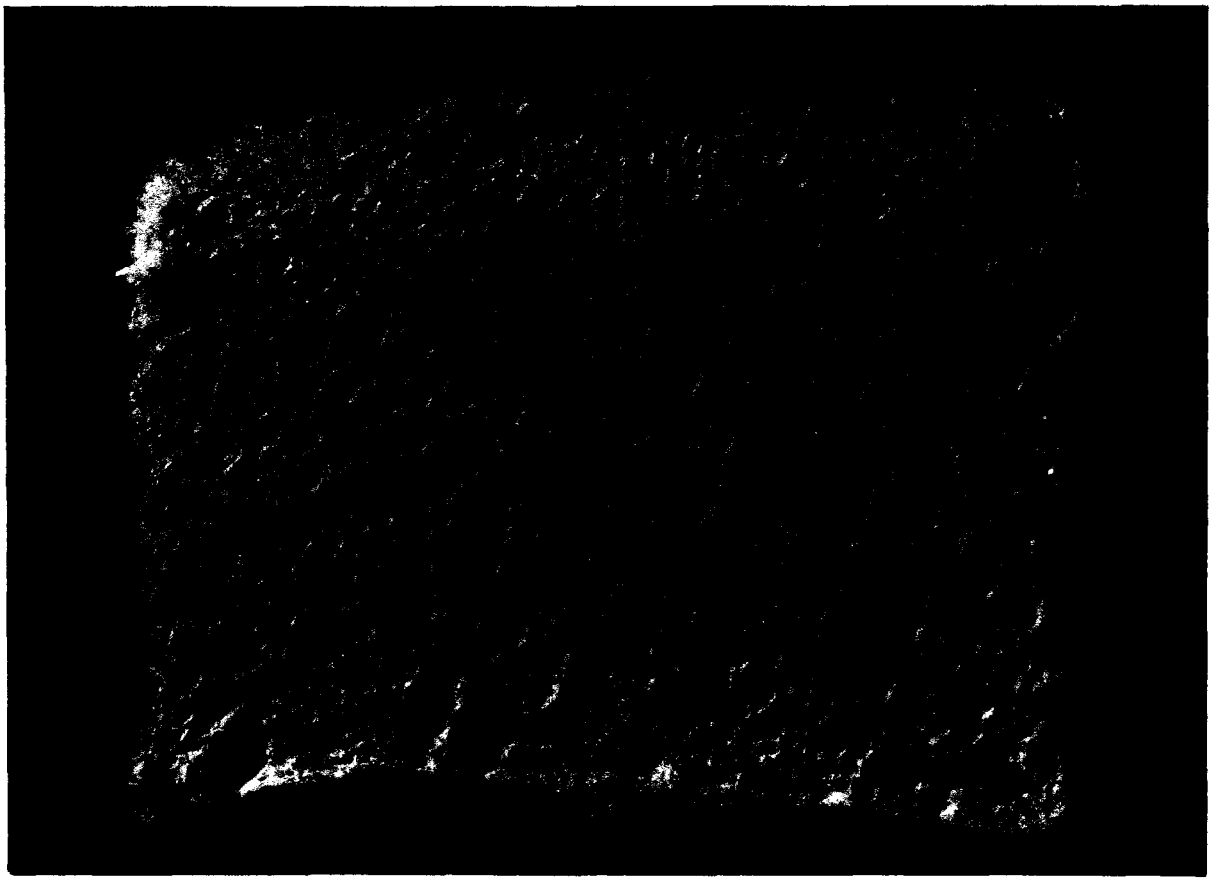

Fig. 6. Angel Cake Prepared from Unfermented, Dried Egg White (0.9 gm. of cream of tartar in formula).

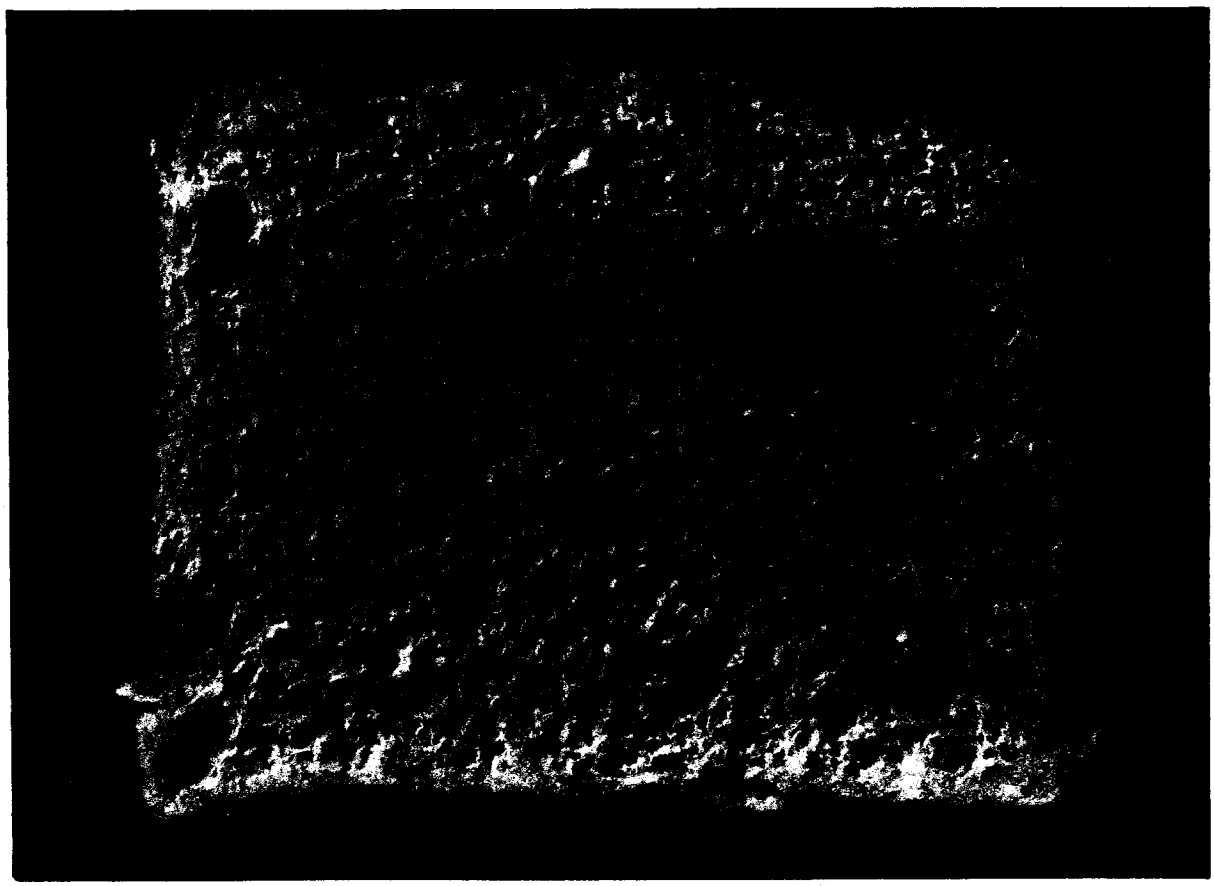

Fig. 7. Angel Cake Prepared from Fermented Dried Egg White (no cream of tartar in formula).

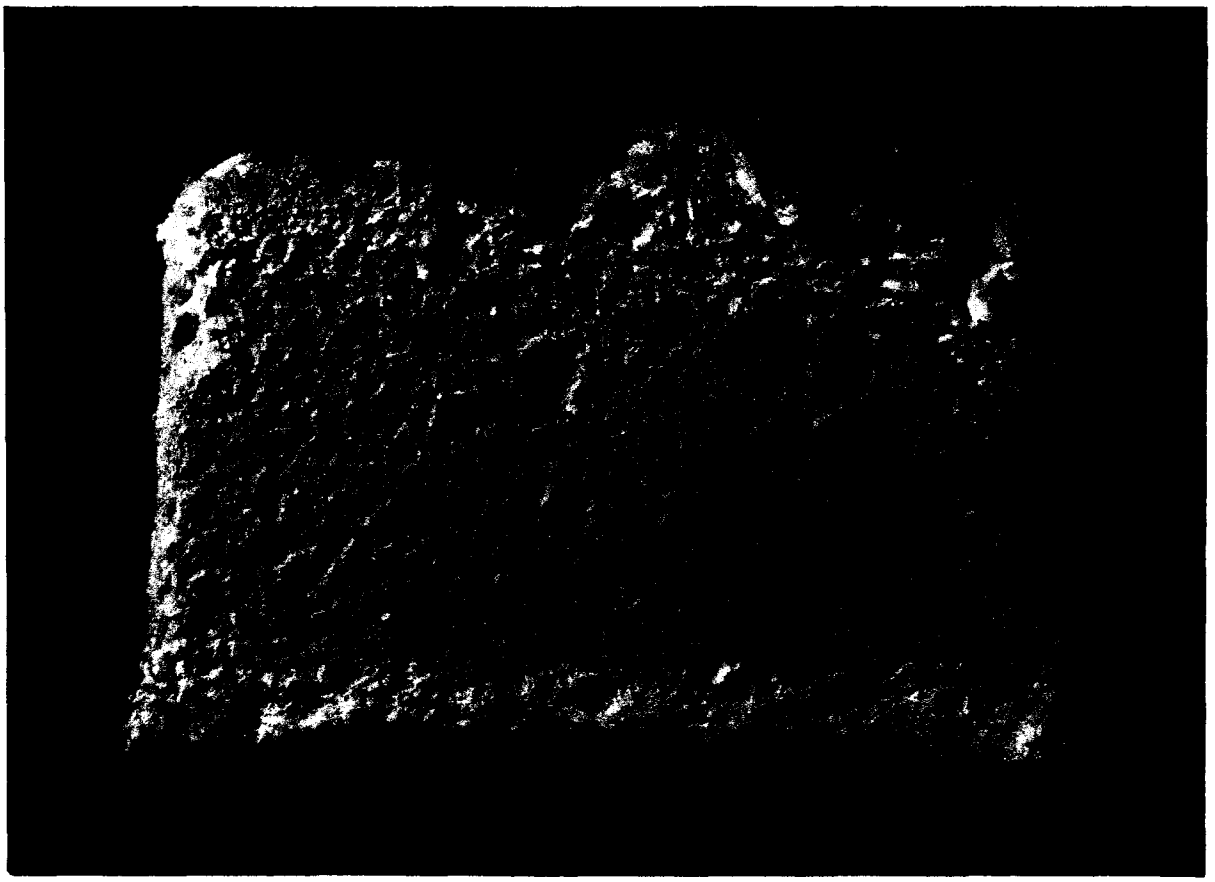

Fig. 8. Angel Cake Prepared from Fermented, Dried Egg White (0.9 grams of cream of tartar in formula).

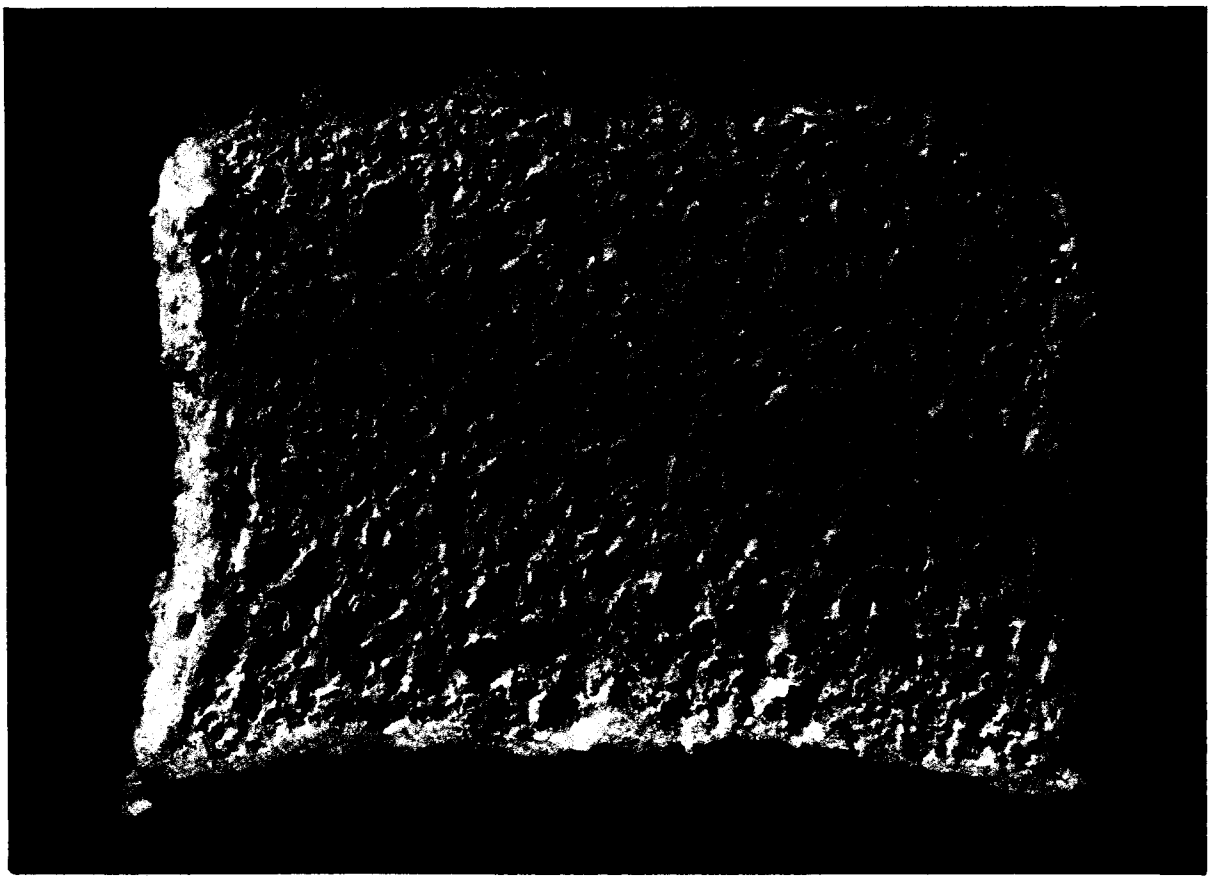

Fig. 9. Angel Cake Prepared from Fermented-adjusted Dried Egg White (0.3 grams of cream of tartar in formula).

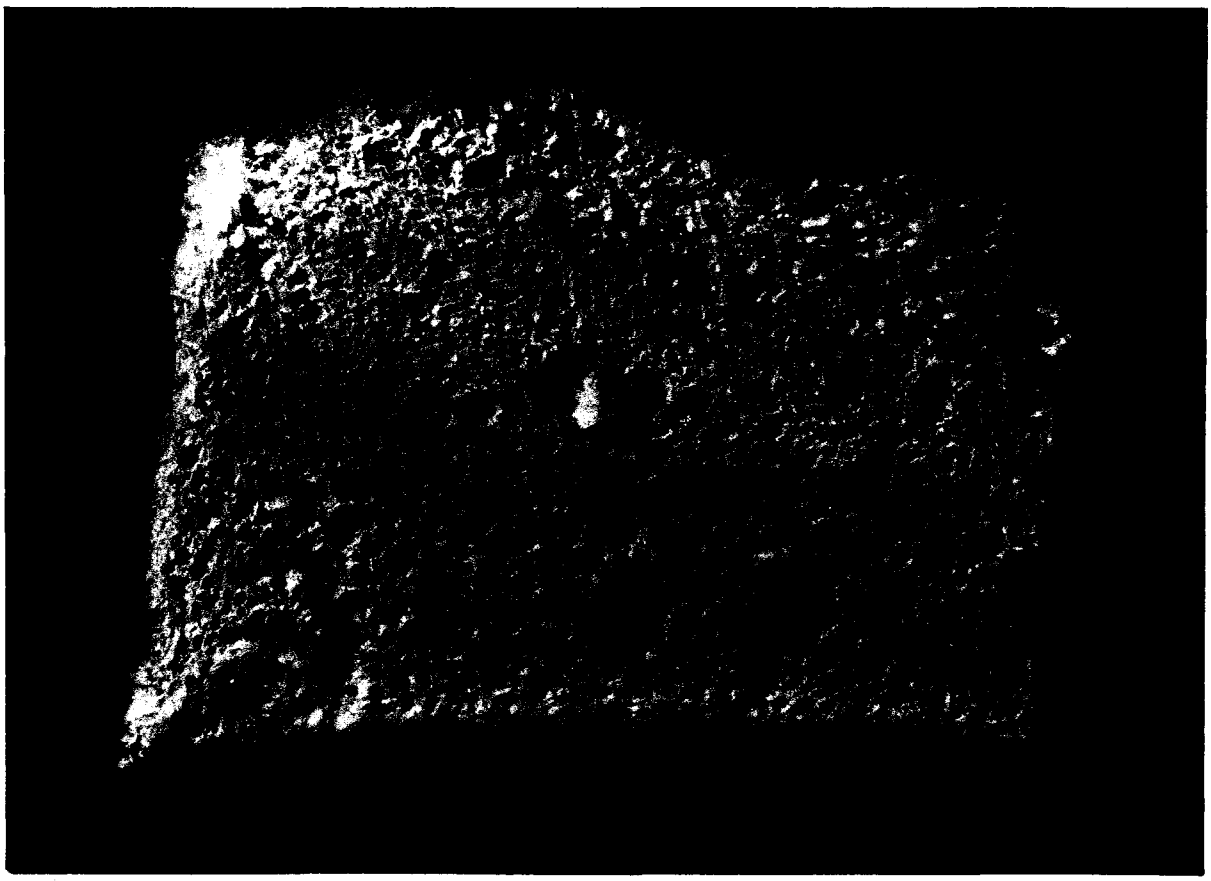

Fig. 10. Angel Cake prepared from Fermented-adjusted Dried Egg White (0.9 grams of cream of tartar in formula).

Data concerning the fermentation are shown in Table 58 (Appendix). Results of subjective and objective tests on the angel cakes are given in Tables 19 through 23 and in Tables 59 and 60 (Appendix). [See Tables 61 through 67 (Appendix) for statistical analyses.] With one exception the results of the statistical analysis are similar to those of the previous study (compare Tables 18 and 24). Where judges previously had found highly significant differences in flavor between two treatments, the differences were only significant. Apparently the addition of vanilla reduced the acid flavor present in the albumen. It should be stated that the judges were not aware of the addition of vanilla; they commented that the odor was aromatic, fragrant or sweet in cakes the batter pH of which was less acid than 5.0. In the two treatments where the pH of the batter was less than 5.0, judges generally commented that these cakes were acid. Occasionally a judge described the cakes as having a yeasty odor.

K. Effect of Storage on Bacterial Count in Dried Egg Whites

Ayres and Slosberg (5) found that storage of dried egg white at temperatures between 50° C. and 60° C. for four to eight days freed the products of suspect organisms. It was of interest to study the effect of a similar storage treatment on the number of Aerobacter aerogenes in dried, fermented albumen.

Table 19

Effect of Cream of Tartar Level on pH of Angel Cake Batter Prepared from Dried Egg White[a]

Treatment	Replication I	II	III	IV	Ave.
Unfermented 0.45 gm. c. t.[b]	7.4	6.9	7.0	7.4	7.2*
Unfermented 0.9 gm. c. t. (Control)	6.5	6.3	6.5	6.6	6.5
Fermented-unadjusted 0.00 gm. c. t.	5.8	5.6	5.8	5.8	5.8*
Fermented-unadjusted 0.9 gm. c. t.	4.9	4.7	4.6	4.9	4.8**
Fermented-adjusted 0.3 gm. c. t.	5.5	5.5	5.5	5.5	5.5**
Fermented-adjusted 0.9 gm. c. t.	4.9	4.9	4.9	4.9	4.9**

[a]Formula included vanilla

[b]Cream of tartar

*Significantly less than control at 5 per cent level

**Significantly less than control at 1 per cent level

Table 20

Effect of Cream of Tartar Level on Volume of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replication I	II	III	IV	Ave.
Unfermented 0.45 gm. c. t.[b]	559	555	543	548	551**
Unfermented 0.9 gm. c. t. (Control)	620	624	614	621	620
Fermented-unadjusted 0.0 gm. c. t.	597	606	613	610	607
Fermented-Unadjusted 0.9 gm. c. t.	546	539	562	525	543**
Fermented-adjusted 0.3 gm. c. t.	615	612	612	615	614
Fermented-adjusted 0.9 gm. c. t.	559	571	526	565	555**

[a]Formula contained vanilla; volume measured in ml. per 100 gm. batter

[b]Cream of tartar

**Significantly less than control at 1 per cent level

Table 21

Effect of Cream of Tartar on Texture of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replication I	II	III	IV	Ave.
Unfermented 0.45 gm. c. t.[b]	8.1	7.0	8.0	7.0	7.5
Unfermented 0.9 gm. c. t. (Control)	8.6	8.0	8.4	8.1	8.3
Fermented-unadjusted 0.0 gm. c. t.	7.4	7.9	8.4	8.4	8.0
Fermented-unadjusted 0.9 gm. c. t.	6.1	5.9	6.1	5.4	5.9**
Fermented-adjusted 0.3 gm. c. t.	8.0	7.4	8.1	8.0	7.5
Fermented-adjusted	5.9	6.0	7.1	6.7	6.4*

[a]Formula contained vanilla; average of seven judges' scores. Highest possible score 10; range 0-10

[b]Cream of tartar

*Significantly less than control at 5 per cent level

**Significantly less than control at 1 per cent level

Table 22

Effect of Cream of Tartar on Flavor of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replication I	II	III	IV	Ave.
Unfermented 0.45 gm. c. t.[b]	8.0	8.0	8.6	8.0	8.2
Unfermented 0.9 gm. c. t. (Control)	8.3	7.4	8.1	8.3	7.8
Fermented-unadjusted 0.0 gm. c. t.	8.1	8.1	8.1	7.9	8.1
Fermented-unadjusted 0.9 gm. c. t.	5.7	6.1	5.9	5.9	5.9*
Fermented-adjusted 0.3 gm. c. t.	7.4	8.3	8.7	8.6	8.1
Fermented-adjusted 0.9 gm. c. t.	5.9	6.4	6.3	6.6	6.3*

[a] Formula contained vanilla; average of seven judges' scores. Highest possible score 10; range 0-10.

[b] Cream of tartar

*Significantly less than control at 5 per cent level

Table 23

Effect of Cream of Tartar on General Palatability of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replication I	II	III	IV	AVE.
Unfermented 0.45 gm. c. t.[b]	8.3	7.9	8.6	7.4	8.0
Unfermented 0.9 gm. c. t. (Control)	8.6	8.1	8.7	8.4	8.5
Fermented-unadjusted 0.0 gm. c. t.	8.1	8.1	8.3	8.1	8.2
Fermented-unadjusted 0.9 gm. c. t.	6.1	6.1	5.9	5.7	6.0**
Fermented-adjusted 0.3 gm. c. t.	8.0	7.9	8.6	8.3	8.2
Fermented-adjusted 0.9 gm. c. t.	6.3	6.4	6.3	6.4	6.4**

[a] Formula contained vanilla; average of seven judges' scores. Highest possible score 10; range 0-10

[b] Cream of tartar

**Significantly less than control at 1 per cent level

Table 24

Summary of Mean Scores of Angel Cake Data

Treatment and pH of batter	Volume	Texture	Flavor	General Palatability	Tenderness	Moistness
Unfermented 0.45 gm. c. t.[a] pH 7.2	551**	7.5	8.2	8.0	8.3	8.5
Unfermented 0.9 gm. c. t. pH 6.5 (Control)	660	8.3	7.8	8.5	8.6	8.3
Fermented-unadjusted 0.0 gm. c. t. pH 5.8	607	8.0	8.1	8.2	8.5	8.4
Fermented-unadjusted 0.9 gm. c. t. pH 4.8	543**	5.9**	5.9*	6.0**	7.9	8.0
Fermented-adjusted 0.3 gm. c. t. pH 5.5	614	7.5	8.1	8.2	8.5	8.3
Fermented-adjusted 0.9 gm. c. t. pH 4.9	555**	6.4*	6.3*	6.4**	8.0	8.0

[a] Cream of tartar
*Significantly less than control at 5 per cent level
**Significantly less than control at 1 per cent level

Dried, fermented egg white was stored at 50° C. for one to five days and bacterial counts made at appropriate intervals. From the results of this storage study (Figure 11 and Table 68, Appendix) there appears to be approximately a ten thousand fold reduction in the number of bacteria.

L. Effect of Storage at 40° C. on Beating Rate and Cake Volume

It has been demonstrated numerous times that dried, unfermented albumen (mucin free) does not retain its functional properties on storage under commercial warehouse conditions. In an effort to study the effects of such conditions further, a storage study at 40° C. was made to determine the effect of such storage conditions on beating rate and angel cake making properties of unfermented and fermented dried white (both with natural mucin content). As a control, unfermented egg white was kept in the frozen state at -15° C.

The results are shown in Figure 12 and Table 69 (Appendix). Although the original quality of the dried, unfermented egg white was high, after two weeks storage at 40° C., there developed an orange-brown tinge; the whipping quality was very unsatisfactory. When the reconstituted liquid was beaten, there was a pronounced spattering on the sides of the bowl before a foam formed; even after prolonged beating periods relatively little foam was produced. Reconstituted, dried,

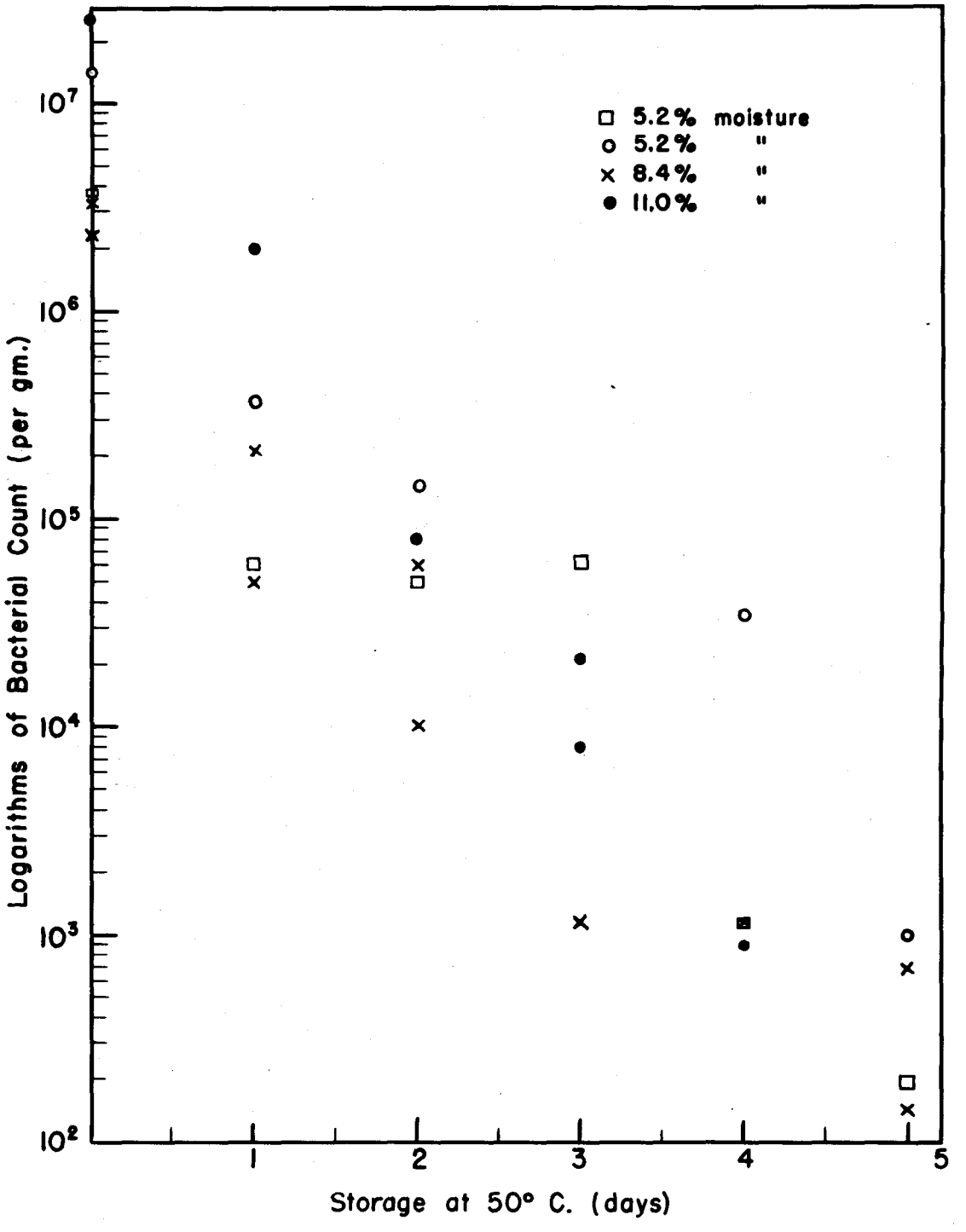

Fig. 11. Effect of Storing Fermented Dried Albumen on Bacterial Count.

fermented albumen showed no reduction in the beating rate after sixteen weeks storage. Naturally the frozen control also retained its whipping quality.

It can be concluded from this study that removing the glucose from egg white before drying considerably enhances keeping quality, as determined by beating rate.

In another storage study unfermented and fermented albumen samples were stored at 40° C. In this instance cakes were prepared from the stored samples. The results are given in Figure 13 and Table 70 (Appendix). With an unfermented product there was a serious loss in cake volume after four weeks storage; the loss in volume from the fermented samples was considerably less. After eight weeks storage, cakes prepared from the dried samples were all low in volume.

These results are not in agreement with those obtained in the previous study where beating rate was used as the criterion of quality. Since the beating rate of fermented albumen remained high (even after sixteen weeks storage at 40° C.), one would have expected the cake volume to have remained high also. This was not the case. The writer has no explanation for this difference but it is suggested that the storage treatment may have affected foam stability unfavorably. It should be remembered that cake volume depends not only on the amount of air beaten into the egg white, but also on maintaining the air in the batter during baking.

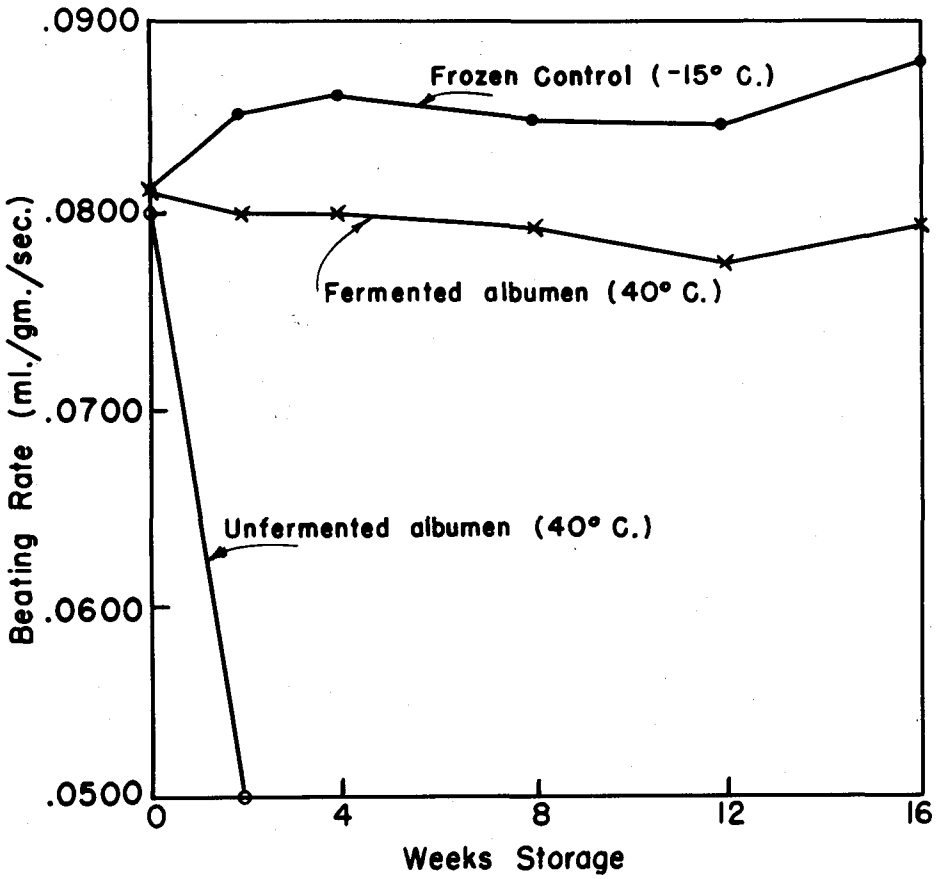

Fig. 12. Effect of Storing Dried Albumen on Beating Rate.

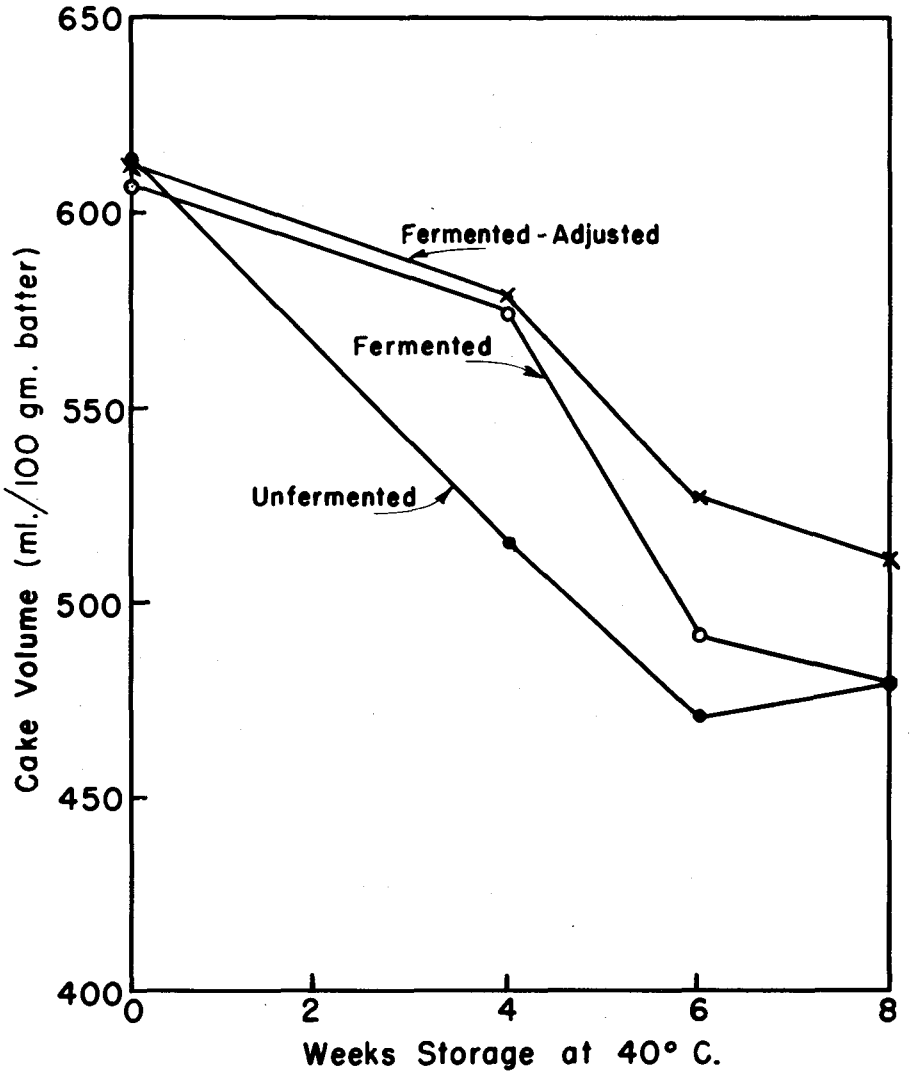

Fig. 13. Effect of Storing Albumen on Volume of Angel Cake Made Therefrom.

V. CONCLUSIONS

Results of the experiments described in this study made possible the following conclusions:

1. Aerobacter aerogenes (N.R.R.L. #199) ferments glucose from egg white rapidly when conditions are controlled.

2. Serial passage of Aerobacter aerogenes (N.R.R.L. #199) can be made in egg white containing 0.1 per cent yeast extract without evidence of contamination under certain conditions.

3. The addition of 0.1 per cent yeast extract accelerates the rate of glucose removal from albumen by Aerobacter aerogenes (N.R.R.L. #199).

4. Between the limits of 0.004 $cm.^2/cm.^3$ and 3.08 $cm.^2/cm.^3$ surface/volume ratio had no effect on the time required to ferment glucose in egg white with Aerobacter aerogenes (N.R.R.L. #199).

5. The adjustment of the albumen to pH levels of 5 through 8 before fermentation shows no positive effect on the glucose removal rate with N.R.R.L. #199.

6. By varying the temperature between 25° and 45° C. and adjusting the size of inoculum of N.R.R.L. #199 between 0.1 and 10 per cent, the time of fermentation of glucose from egg white containing 0.1 per cent yeast extract can be regulated from 5 to 32 hours.

7. The use of agitation and/or NaOH during the fermentation of albumen by N.R.R.L. #199 has a deleterious effect on the beating rate of the reconstituted product.

8. Adjustment of the pH of fermented albumen to pH 9 with NH_4OH just prior to drying improves the whipping quality of the reconstituted product; the use of NaOH decreases the whipping power of the reconstituted product.

9. Tray drying unfermented and fermented albumen at temperatures less than 40° C. has no significant effect on angel cakes prepared from reconstituted albumen.

10. Cakes similar to those made from fresh egg white can be made from albumen fermented under specified conditions with Aerobacter aerogenes (N.R.R.L. #199) if the cream of tartar level is adjusted in the cake formula.

11. The number of Aerobacter aerogenes organisms present in dried fermented albumen can be reduced to a great extent by storage for five days at 50° C.

12. Egg white must be blended prior to fermentation in order to prevent the precipitation of mucin during the course of the fermentation which is brought about by Aerobacter aerogenes (N.R.R.L. #199).

VI. SUMMARY

A study was made of methods for fermenting albumen for the purpose of removing glucose without impairing angel cake making properties. After a preliminary survey of a number of organisms, Aerobacter aerogenes (N.R.R.L. #199) was selected for further study. Conditions were determined for rapidly removing glucose which would allow the mucin to remain in the dispersed form.

Varying the surface/volume ratio had no appreciable effect on the time required for the removal of glucose; nor did adjustment of the pH to neutral or acid levels affect the rate favorably. Addition of 0.1 per cent yeast extract reduced the fermentation time by approximately half. By adjusting the temperature and size of inoculum it was possible to vary the time required for glucose removal from five to thirty-two hours; thus, the time of fermentation can be regulated to the convenience of almost any commercial contingency.

An adaptation of the two hour browning test for determining the presence of glucose in egg white was developed during the course of this study. One-tenth ml. of egg white was placed on half of a pre-heated petri plate and heated under a General Electric reflector-drying lamp for fifteen minutes. Under carefully standardized conditions it was possible to detect the

presence of glucose by the appearance of a yellow to brown color at the end of the fifteen minute period.

Serial passages of Aerobacter aerogenes (N.R.R.L. #199) were made in egg white containing 0.1 per cent yeast extract without benefit of aseptic techniques. After 160 passages the culture was still pure.

Adjustments in pH, both during and after fermentation, were also made and the effects studied. Agitation and/or addition of dilute alkali were used in an effort to control pH during fermentations. These treatments caused an accumulation of mucin on the stirrer and resulted in a dried product of inferior whipping quality. However, when fermented albumen was adjusted to pH 9.0 with NH_4OH just prior to drying, a dried product of improved whipping quality resulted.

Blending the egg white into a homogeneous liquid was essential to keep the mucin in a dispersed form at the lower pH levels reached during fermentation.

Angel cakes prepared from fermented egg white gave results significantly different from fresh liquid when the regular formula was used. However, when the amount of cream of tartar was reduced or omitted from the formula, the cakes prepared from the fermented albumen were not significantly different from those prepared from the fresh liquid.

VII. LITERATURE CITED

1. Alderton, G., Ward, W. H., and Fevold, H. L. Identification of the bacteria-inhibiting, iron-binding protein of egg white as conalbumin. Arch. Biochem. 11:9-13. 1946.

2. Almquist, H. J. and Lorenz, F. W. Liquification of egg whites. U. S. Egg and Poultry Magazine. 38 (no. 4):20-23. 1932.

3. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 6th ed. Washington, D. C., Association of Official Agricultural Chemists. 1945.

4. Ayres, J. C., Ames, Iowa. Microbiological fermentation of egg white. (Private communication) 1946.

5. _____ and Slosberg, H. Destruction of Salmonella in egg albumen. Food Tech. (in press) 1949.

6. _____ and Stewart, G. F. Removal of sugar from raw egg white by yeast before drying. Food Tech. 1:519-526. 1947.

7. Balls, A. K. and Swenson, T. L. Dried egg white. Food Res. 1:319-324. 1936.

8. Barmore, M. A. The influence of chemical and physical factors on egg-white foams. Colo. Agr. Exp. Sta. Tech. Bull. No. 9. 1934.

9. _____. The influence of various factors, including altitude, in the production of angel food cake. Colo. Agr. Exp. Sta. Tech. Bull. No. 15. 1936.

10. Blomberg, C. G. How eggs are dried. Food Ind. 4:100-102. 1932.

11. Boas, M. A. On observation on the value of egg white as the sole source of nitrogen for young growing rats. Biochem. J. 18:422-424. 1924.

12. Bond, M. A modification of basal diet for rat feeding experiments. Biochem. J. 16:479-481. 1922.

13. Cohen, B. and Clark, W. M. The growth of certain bacteria in media of different hydrogen ion concentrations. J. Bact. 4:409-427. 1919.

14. Eakin, R. E., Snell, E. E., and Williams, R. J. A constituent of raw egg white capable of inactivating biotin in vitro. J. Biol. Chem. 136:801-802. 1940.

15. _____, _____, and _____. The concentration and assay of avidin, the injury-producing protein in raw egg white. J. Biol. Chem. 140: 535-543. 1941.

16. Fleming, A. On a remarkable bacteriolytic element found in tissues and secretions. Proc. Roy. Soc. London. 93B:306. 1922.

17. _____ and Allison, V. D. A bacteriolytic substance ("lysozyme") found in secretions and tissues. Brit. J. Exptl. Pathol. 5:252-260. 1922.

18. Friedberger, E. and Hoder, F. Lysozym- und Flochungsphänomen des Hühnereiklars. Z. Immun. Forsch. 74:429-447. 1932.

19. Glabau, C. A. and Kepes, E. A study of the physical properties of dried egg albumen with the view of standardizing this substance for the official cake baking test. Cereal Chem. 12:108-120. 1935.

20. Grewe, E. and Child, A. M. The effect of acid potassium tartrate as an ingredient in angel cake. Cereal Chem. 7:245-250. 1930.

21. Gyorgy, P. and Rose, C. S. The liberation of biotin from the avidin-biotin complex. Proc. Soc. Exptl. Biol. Med. 53:55-57. 1943.

22. _____, _____, Eakin, R. E., Snell, E. E., and Williams, R. J. Egg-white injury as the result of non-absorption or inactivation of biotin. Science. 93:477-478. 1941.

23. Hanson, H. L. Effect of concentrating egg white on desirability of angel cake. Unpublished Ph.D. Thesis. Ames, Iowa, Iowa State College Library. 1945.

24. Harte, R. A. The in vitro proteolysis of egg white. Science. 102:563-564. 1945.

25. Hawthorne, J. R. and Brooks, J. Dried egg VIII. Removal of the sugar of egg pulp before drying--method of improving the storage life of spray-dried whole egg. J. Soc. Chem. Ind. 63:232-234. 1944.

26. Hess, L. E. Preparing dried egg whites. U. S. Patent 2,253,518. Aug. 26, 1941. Abstracted in Chem. Abstr. 35:8141. 1941.

27. Hopkins, E. W., Josh, G., and Harriman, L. A. Dried egg products. U. S. Patent 2,427,726. Sept. 23, 1947. Abstracted in Chem. Abst. 41:7579. 1947.

28. Jennison, M. W. Some quantitative relationships in bacterial population cycles. J. Bact. 30: 603-623. 1935.

29. Klemparskaya, N. N. Flockung der Bakterien durch Lysozym. Z. Microbiol., Epidemiol., und Immunitätsforschung (U. S. S. R.) No. 2/3:124. 1939. (Original not available for examination; abstracted in Chem. Zentr. 1939, II: 1686. 1939.)

30. Kline, R. W. Dried egg albumen I. Studies of the non-microbiological changes during storage. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1945.

31. Koser, S. A. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bact. 8:493-520. 1923.

32. Laschtschenko, P. Über die Keimtötende und entwicklungshemmende Wirkung von Hühnereiweiss. Z. Hyg. Infectkr. 64:419. 1909.

33. Le Clerc, J. A. and Bailey, L. H. Fresh frozen and dried eggs and egg products. Cereal Chem. 17:279-312. 1940.

34. Lineweaver, H. and Murray, C. W. Identification of the tryptic inhibitor of egg white with ovamucoid. J. Biol. Chem. 171:565-581. 1947.

35. Littlefield, V. D. Unfermented dried egg white product. U. S. Patent 2,191,257. Feb. 20, 1940. Abstracted in Chem. Abst. 34:4482. 1940.

36. Littlefield, V. D. and Fischer, N. C. Processing and drying egg whites. U. S. Patent 2,212,445. Aug. 20, 1940. Abstracted in Chem. Abst. 35: 531. 1941.

37. _____ and _____. Drying egg whites. U. S. Patent 2,237,087. April 1, 1941. Abstracted in Chem. Abst. 35:4514. 1941.

38. Longsworth, L. G., Cannon, R. K., and MacInnes, D. A. An electrophoretic study of the proteins of egg white. J. Am. Chem. Soc. 62:2580-2590. 1940.

39. Lowe, B. Experimental cookery. 3rd ed. New York, John Wiley and Sons. 1943.

40. McNally, E. H. Some characteristics of the ovamucin gel of egg white. Poultry Sci. 22:25-29. 1943.

41. Mulvaney, H. A. How eggs are dried--methods and standards. Food Ind. 13(no. 12):50-53. 1941.

42. Nakamura, O. Lysozyme action. Z. Immunitäts. 38:425-447. 1923.

43. Pennington, D., Snell, E. E., and Eakin, R. E. Crystalline avidin. J. Am. Chem. Soc. 64:469. 1942.

44. Schade, A. L. and Caroline, L. Raw hen egg white and the role of iron in growth inhibition of Shigella dysenteriae, Staphylococcus aureus, Escherichia coli, and Saccharomyces cerevisiae. Science. 100:14-15. 1944.

45. Schaible, P. J. and Bandemer, S. L. Composition of fresh and storage eggs from hens fed cottonseed and non-cottonseed rations. III. Iron content. Poultry Sci. 25:451-452. 1946.

46. _____ and _____. Composition of fresh and storage eggs from hens fed cottonseed and non-cottonseed rations. V. Cause of discoloration. Poultry Sci. 25:456-459. 1946.

47. Sharp, P. F. and Whitaker, R. The relation of the hydrogen ion concentration of egg white to its germicidal action. J. Bact. 14:17-46. 1927.

48. Slosberg, H. M. Some factors affecting the functional properties of liquid egg albumen. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1946.

49. Slosberg, H. M., Hanson, H. L., Stewart, G. F., and Lowe, B. Factors influencing the effects of heat treatment on the leavening power of egg white. Poultry Sci. 27:294-301. 1948.

50. Stewart, G. F., Best, L. R., and Lowe, B. A study of some factors affecting the storage changes in spray-dried egg products. Proc. Inst. Food. Tech. 1943:77-89. 1943.

51. _____ and Kline, R. W. Dried egg albumen. I. Solubility and color denaturation. Proc. Inst. Food Tech. 1941:48-56. 1941.

52. _____ and _____. Factors influencing rate of deterioration in dried egg albumen. Ind. Eng. Chem. 40:916-919. 1948.

53. _____ and _____. Glucose-protein reaction in dried egg albumen. Ind. Eng. Chem. 40:919-922. 1948.

54. Stuart, L. S. and Goresline, H. E. Bacteriological studies on the "natural" fermentation process of preparing egg white for drying. J. Bact. 44:541-549. 1942.

55. _____ and _____. Studies of bacteria from fermenting egg white and the production of pure culture fermentations. J. Bact. 44:625-632. 1942.

56. Turro, R. Zur Bakterienverdauung. Centr. Bakt. Abt. I. Orig. 32:105-110. 1902.

57. Vernon, H. M. The protective value of proteids and their decomposition products on trypsin. J. Physiol. 31:346. 1904.

58. Watts, B. W. and Elliot, C. Utilization of dried egg whites in bakery products. Cereal Chem. 18:1-9. 1941.

59. Wurtz, R. De l'action bactericide du blanc d'oeuf. Semaine Med., Paris. 3:21. 1890.

VIII. ACKNOWLEDGMENTS

To Dr. George F. Stewart the author wishes to express her sincere appreciation for his suggestion of the problem, his guidance and interest. The author also wishes to acknowledge the interest and cooperation of Dr. J. C. Ayres and Dr. Frances Carlin. She extends thanks to her judges: Dr. J. C. Ayres, Mr. Dwight Bergquist, the Misses Eloise Green, Frances Hettler, Harvye Lewis, Suzanne Jegard, Alice Morrison and Mrs. Grace Shugart. Appreciation is extended to Mr. Paul Homeyer for his assistance in making the statistical analyses. She also wishes to thank Mrs. Virginia Peart and Mrs. Luana Friesth for their invaluable technical assistance.

IX. APPENDIX

ANGEL CAKE PALATABILITY TEST

Judge _____ Date _____

Time _____ A.M. _____ P.M.

SCORE (Scoring range 10-0; 10 is the perfect score.)

Cake No.										
Texture[1]										
Tenderness										
Moistness[2]										
Flavor										
General Palatability										

[1] Desirable texture: fine and even cells, feathery, resilient crumb.
[2] Desirable moistness: not dry, yet not gummy in the mouth.

Odor: Place number of cake sample next to term which best describes odor.

1. Fragrant	_____	5. Acid	_____	9. Yeasty	_____
2. Sweet	_____	6. Burnt	_____	10. Repulsive	_____
3. Salty	_____	7. Fetid	_____	11. Goaty	_____
4. Bitter	_____	8. Aromatic	_____	12.	_____

Remarks: General impressions regarding the flavor or other characteristics of the cakes are important. Record them below.

Cake No.	Remarks

Table 25

Effect of Surface/Volume Ratio on Time of Glucose Removal from Egg White by Aerobacter aerogenes (N.R.R.L. #199)[a]

Surface-Volume $cm.^2/cm.^3$	Glucose Removal in Hours Replication I	II	III	IV	V
3.08	10	10	5	5	4.5
1.00	10	10	5	5	4.5
0.54	10	10	5	5	4.5
0.43	10	10	5	5	4.5
0.32	10	10	5	5	4.5
0.17	10	10	5	5	4.5

[a]Conditions of fermentation
10% inoculum
100 ml. egg white
0.1% Yeast Extract
Incubation Temperature 40° C., except for I and II which were run at 30° C.

Table 26

Effect of Surface/Volume Ratio on pH of Egg White After Fermentation of Egg White by *Aerobacter aerogenes* (N.R.R.L. #199)[a]

Surface-Volume Ratio ($cm.^2/cm.^3$)	pH when Glucose Free	
	30° C.	40° C.
3.08	6.5	6.4
	6.5	6.2
	---	6.3
1.00	6.3	5.8
	6.3	5.9
	---	5.9
0.54	6.5	5.8
	6.2	5.8
	---	5.8
0.43	6.3	5.6
	6.1	5.7
	---	5.8
0.32	6.3	5.6
	6.1	5.7
	---	5.6
0.17	6.0	5.8
	5.9	5.8
	---	5.8

[a]Conditions of fermentation
100 ml. egg white
10% inoculum
0.1% yeast extract

Table 27

Effect of pH and Yeast Extract on Time of Glucose Removal from Egg White by Aerobacter aerogenes (N.R.R.L. #199)[a]

Beginning pH	Time of Glucose Removal in Hours				
	I	II	III	IV	Ave.
	Without Yeast Extract				
8.6 (Control)	42	41	38	36	39
8.0	42	41	38	36	39
7.0	42	41	41	36	40
6.0	42	41	34	36	38
5.0	42	41	34	36	38
	With 0.1% Yeast Extract				
8.6 (Control)	16	9	12	11.5	13
8.0	16	12	11	19.5	15
7.0	16	12	13	19.5	15
6.0	16	12	13	17.5	15
5.0	16	15	17	17.5	17

[a]Conditions of fermentation:
10% inoculum
100 ml. egg white
Surface/volume ratio 0.54 $cm.^2/cm.^3$
Incubation temperature 30° C.
pH adjusted with 1N HCl

Table 28

Effect of pH and Yeast Extract on pH of Egg White After Fermentation by *Aerobacter aerogenes* (N.R.R.L. #199)[a]

Beginning pH	pH when Glucose-free			
	I	II	III	Ave.
	Without Yeast Extract			
8.6 (Control)	6.2	6.2	6.2	6.2
8.0	6.0	6.0	6.1	6.0
7.0	6.0	5.8	6.1	6.0
6.0	5.7	5.5	5.6	5.5
5.0	4.6	4.9	4.9	4.8
	With 0.1% Yeast Extract			
8.6	6.0	6.1	6.0	6.0
8.0	6.0	6.0	5.8	5.9
7.0	5.8	5.7	5.8	5.8
6.0	5.5	5.5	5.6	5.5
5.0	4.8	5.0	4.8	4.9

[a]Conditions of fermentation:
10% inoculum
100 ml. egg white
Surface/volume ratio 0.54 $cm.^2/cm.^3$
Incubation temperature 30° C.
pH adjusted with 1N HCl

Table 29

Effect of Inoculum Level and Temperature on Time of Glucose Removal by N.R.R.L. #199[a]

Temperature	% Inoculum	Glucose Removal in hours	No. Replications
25° c.	0.1	32	2
	1.0	25	2
	10.0	12.3	2
30° c.	0.1	25	2
	1.0	11.5	2
	10.0	7.4	5
	30.0	4.8	2
34° c.	0.1	20.5	2
	1.0	11.5	2
	10.0	6.0	2
37° c.	0.1	19.5	2
	1.0	10.7	3
	10.0	6.0	9
40° c.	0.1	--	-
	1.0	--	-
	10.0	5.7	6
	30.0	5.0	1
45° c.	10.0	6.0	7

[a]Conditions of fermentation
0.1% Yeast extract
100 ml. egg white, natural pH
Surface/volume 0.54 $cm.^2/cm.^3$

Table 30

Effect of Inoculum Level and Temperature on Time of Glucose Removal from Egg White by N.R.R.L. #199[a]

Temp. °C.	Inoculum %	Glucose Removal in Hours — REPLICATION									
		I	II	III	IV	V	VI	VII	VIII	IX	Ave.
25	0.1	31	33	—	—	—	—	—	—	—	32.0
	1.0	25	25	—	—	—	—	—	—	—	25.0
	10.0	12	12.5	—	—	—	—	—	—	—	12.3
30	0.1	25	25	—	—	—	—	—	—	—	25.0
	1.0	12	11	—	—	—	—	—	—	—	11.5
	10.0	7.5	8.5	—	—	—	7	—	7	7	7.4
	30.0	4.5	5	—	—	—	—	—	—	—	4.8
34	0.1	25	16	—	—	—	—	—	—	—	20.5
	1.0	12	11	—	—	—	—	—	—	—	11.5
	10.0	6	6	—	—	—	—	—	—	—	6.0
37	0.1	25	14	—	—	—	—	—	—	—	19.5
	1.0	10	11	11	—	—	—	—	—	—	10.7
	10.0	6	5	6.5	6.5	6	6	6	6	6	6.0
40	0.1	—	—	—	—	—	—	—	—	—	—
	1.0	—	—	—	—	—	—	—	—	—	—
	10.0	—	—	—	5.5	6	5	6	6	6	5.7
	30.0	—	—	—	5	—	—	—	—	—	5.0
45	0.1	—	—	—	—	—	—	—	—	—	—
	1.0	—	—	—	—	—	—	—	—	—	—
	10.0	—	—	6.5	6.5	6	5	7	6	6	6.0

[a]Conditions of fermentation:
0.1% Yeast Extract
100 ml. egg white at natural pH
Surface/Volume 0.54 cm.^{2}/cm.^{3}

Table 31

Effect of Agitation and pH Adjustment during Fermentation on Rate of Glucose Removal, pH and Beating Rate[a]

Treatment	Fermentation Time (in hours)	pH After Fermentation	pH After Reconstitution	Beating Rate[b] Before Fermentation	Beating Rate[b] After Fermentation	Beating Rate[b] After Reconstitution
Control	5	6.0	7.1	.0762	.0741	.0678
	4	6.0	7.2	.0741	.0720	.0646
	4	5.9	6.6	.0748	.0727	.0727
	4	6.2	7.3	.0755	.0784	.0696
	5	6.0	7.3	.0755	.0800	.0666
	5	5.9	—	.0734	.0762	.0616
	4	6.2	7.2	.0708	.0702	.0714
	4	6.0	6.8	.0741	.0808	.0741
	5	5.9	6.5	.0720	.0784	.0816
Agitated	5	6.6	6.8	.0762	.0851	.0631
	4	6.3	7.1	.0741	.0784	.0584
	4	6.3	6.8	.0748	.0800	.0661
	4	6.5	7.4	.0755	.0808	.0661
	5	6.8	7.9	.0755	.0860	.0651
Adjusted with 1% NaOH	5	7.3	8.0	.0734	.0808	.0516
	4	7.6	8.4	.0708	.0714	.0547
	4	6.5	7.8	.0741	.0816	.0512
	4	6.6	7.0	.0720	.0800	.0672

[a]Conditions of fermentation:
10% inoculum (N.R.R.L. #199)
0.1% yeast extract
1000 ml. egg white
Incubation temperature 40° C.
Surface/volume ratio 0.40 $cm.^2/cm.^3$

[b]Beating Rate expressed as ml./gm./sec.

Table 32

Effect of Blending on pH of Fermenting Egg White

Fermentation in hours	pH[a] Blended	Unblended
0	8.5	8.5
1	7.5	7.8
2	6.6	7.2
3	6.3	6.9
4	5.9	6.5
4½	5.8[b]	6.4

[a]Average of three replications

[b]Glucose-free

Table 33

Effect of Fermentation of Albumen on Beating Rate[a]

Treatment	pH	Beating Rate (ml./gm./sec.)
Before fermentation	8.5	0.0824
After fermentation	5.6	0.0816
After fermentation (adjusted with NH_4OH)	9.1	0.0888
Unfermented Frozen	---	0.0842
Unfermented Dried	---	0.0860
Fermented-Unadjusted[b] Frozen	---	0.0833
Fermented-Unadjusted Dried	---	0.0888
Fermented-Adjusted[c] Frozen	---	0.0833
Fermented-Adjusted Dried	---	0.0851

[a]Conditions of fermentation:
10% inoculum (N.R.R.L. #199)
0.1% yeast extract
Incubation temperature 40° C.
Surface/volume ratio 0.40 $cm.^2/cm.^3$
Bacterial count at start of fermentation
15×10^7 Aerobacter aerogenes per ml.
Bacterial count at completion of fermentation (5 hrs.)
15×10^8 Aerobacter aerogenes per ml.

[b]Fermented, then frozen or dried

[c]Fermented, neutralized to pH 9.1 before freezing or drying

Table 34

Effect of Adjusting pH of Fermented Albumen on Tenderness of Angel Cake Made Therefrom[a]

Treatment	Replication I	II	III	IV	V	Ave.
Unfermented Frozen (Control A)	8.2	8.5	8.6	8.4	8.0	8.3
Unfermented Dried (Control B)	8.6	8.1	8.2	8.2	8.9	8.4
Fermented-Unadjusted[b] Frozen	8.1	7.1	8.1	7.0	8.0	7.7
Fermented-Unadjusted Dried	8.2	7.5	7.8	7.8	8.8	8.0
Fermented-Adjusted[c] Frozen	8.6	8.0	8.4	8.2	7.6	8.2
Fermented-Adjusted Dried	8.1	7.4	8.9	8.4	8.5	8.3

[a]Average of eight judges' scores. Highest possible score 10; range 0-10

[b]Fermented, then frozen or dried

[c]Fermented, neutralized to pH 9.1 before freezing or drying

Table 35

Effect of Adjusting pH of Fermented Albumen on Moistness of Angel Cake Made Therefrom[a]

Treatment	Replication I	II	III	IV	V	Ave.
Unfermented Frozen (Control A)	7.6	8.4	8.6	8.8	7.9	8.3
Unfermented Dried (Control B)	8.1	8.4	8.4	8.4	8.1	8.3
Fermented-Unadjusted[b] Frozen	8.1	7.9	7.6	7.4	8.0	7.8
Fermented-Unadjusted Dried	7.8	7.9	7.8	8.0	7.6	7.8
Fermented-Adjusted[c] Frozen	8.2	8.1	8.5	8.1	7.8	8.1
Fermented-Adjusted Dried	8.1	7.7	7.0	8.1	8.2	7.8

[a]Average of eight judges' scores. Highest possible score 10; range 0-10.

[b]Fermented, then frozen or dried

[c]Fermented, neutralized to pH 9.1 before freezing or drying

Table 36

Analysis of Variance
An Analysis of the Effect of Adjusting pH of Fermented Albumen on the pH of Batter of Angel Cake
(Data of Table 7)

Source of Variation	Degrees of Freedom	Sum of squares	Mean Square
Total	29	13.9120	--
Replications	4	0.0687	0.0172
Treatments	5	13.3520	2.6704**
Error	20	0.4913	0.0246

Difference required for significance at $P_{.05} = 0.21$
$P_{.01} = 0.29$

**Significant at 1 per cent level

Table 37
Analysis of Variance
An Analysis of the Effect of Adjusting pH of Fermented Albumen on the Volume of Angel Cake Made Therefrom
(Data of Table 8)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	15,013.37	--
Replications	4	1,870.54	467.64
Treatments	5	8,318.97	1,663.79**
Error	20	4,823.86	241.19

Difference required for significance at $P_{.05} = 20.49$
$P_{.01} = 27.94$

**Significant at 1 per cent level

Table 38

Analysis of Variance
An Analysis of the Effect of Adjusting pH of Fermented Albumen on the Total Texture Scores given by Eight Judges to Angel Cakes Made Therefrom (Data of Table 9)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	239	428.6140	---
Replications	4	1.5644	.3911
Treatments	5	63.7675	12.7535**
Frozen x Dried	1	2.9927	
Unfermented x Fermented	1	42.1268	
Unadjusted x Adjusted	1	16.1290	
(Ferm. vs. Unferm.) x (Frozen vs. Dried)	1	0.3101	
(Frozen vs. Dried) x (Adj. vs. Unadj.)	1	2.2090	
Treatments x Replications	20	24.9346	1.2467
Judges	7	116.4420	16.6346**
Judges x Treatments	35	59.6525	1.7044**
Judges x Replications	28	44.1909	1.5782
Judges x Replications x Treatments	140	118.0621	.8433
Error for Treatments	--	---	2.1078

Difference required for significance at $P_{.05} = .69$
$P_{.01} = .94$

**Significant at 1 per cent level

Table 39

Analysis of Variance
An Analysis of the Effect of Adjusting pH of the Fermented Albumen on the Total Flavor Scores Given by Eight Judges to Angel Cakes Made Therefrom (Data of Table 10)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	239	453.1093	--
Replications	4	7.6622	1.9156
Treatments	5	140.1243	28.0249**
Frozen x Dried	1	0.4681	
Unfermented x Fermented	1	105.3750	
Unadjusted x Adjusted	1	33.5806	
(Ferm. vs. Unferm.) x (Frozen vs. Dried)	1	0.6825	
(Frozen vs. Dried) x (Adj. vs. Unadj.)	1	0.0181	
Treatments x Replications	20	26.5178	1.3259
Judges	7	83.1680	11.8811**
Judges x Treatments	35	73.3770	2.0965**
Judges x Replications	28	31.6091	1.1289
Judges x Replications x Treatments	140	90.6509	0.6475
Error for Treatments	--	---	1.7749

Difference required for significance at $P_{.05} = 0.63$
$P_{.01} = 0.87$

**Significant at 1 per cent level

Table 40

Analysis of Variance
An Analysis of the Effect of Adjusting pH of the Fermented Albumen on the Total General Palatability Scores Given by Eight Judges to Angel Cakes Made Therefrom (Date of Table 11)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	239	349.5066	---
Replications	4	3.9814	.9954
Treatments	5	83.4704	16.6941**
Frozen x Dried	1	.3604	
Unfermented x Fermented	1	58.8000	
Unadjusted x Adjusted	1	23.8702	
(Ferm. vs. Unferm.) x (Frozen vs. Dried)	1	.2708	
(Frozen vs. Dried) x (Adj. vs. Unadj.)	1	.1690	
Treatments x Replications	20	23.8686	1.1934
Judges	7	107.4103	15.3443*
Judges x Treatments	35	44.9859	1.2853*
Judges x Replications	28	15.4351	.5513
Judges x Replications x Treatments	140	70.3549	.5025
Error for Treatments	--	---	1.7749

Difference required for significance at $P_{.05} = 0.67$
$P_{.01} = 0.92$

*Significant at 5 per cent level
**Significant at 1 per cent level

Table 41

Analysis of Variance
An Analysis of the Effect of Adjusting pH of the Fermented Albumen on the Tenderness Scores Given by Eight Judges to Angel Cakes Made Therefrom (Data of Table 34)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	6.64	----
Replications	4	1.49	0.37
Treatments	5	1.84	0.37
Error	20	3.31	0.16

Table 42

Analysis of Variance
An Analysis of the Effect of Adjusting pH of the Fermented Albumen on the Moistness Scores Given by Eight Judges to Angel Cakes Made Therefrom (Data of Table 35)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	4.11	---
Replications	4	0.15	0.04
Treatments	5	1.34	0.27
Error	20	2.62	0.13

Table 43

Analysis of Covariance
An Analysis of the Relationship of Cake Flavor to the pH of Batter

Source of Variation	Degrees of Freedom	Sum of Squares and Products X^2	XY	Y^2	Correlation Coefficient
Total	29	13.9120	13.7540	22.2147	--
Replications	4	0.0687	0.0707	0.9314	--
Treatments	5	13.3520	13.7000	17.8707	0.8869**
Experimental Error	20	0.4913	-0.0167	3.4126	-0.0129

X = pH; Y = Flavor
**Significant at 1 per cent level

Table 44

Analysis of Covariance
An Analysis of the Relationship of Cake Volume to the pH of Cake Batter

Source of Variation	Degrees of Freedom	Sum of Squares and Products X^2	XY	Y^2	Correlation Coefficient
Total	29	13.9120	289,6800	15.013.37	--
Replications	4	0.0687	8.9133	1.870.54	--
Treatments	5	13.3520	279.4000	8.318.97	0.8383**
Experimental Error	20	0.4913	1.3667	4.823.86	0.0281

X = pH; Y = Volume
**Significant at 1 per cent level

Table 45

Effect of Cream of Tartar Level on the pH of Meringue

Treatment	Cream of Tartar (grams)	Meringue pH[a]
Unfermented dried	0.00	9.2, 9.3
	0.45	7.2, 7.5
	0.90	6.7, 6.4
	1.35	6.1
	1.80	5.4
	2.25	5.1
Fermented dried	0.00	6.3
	0.45	5.0
	0.90	4.6
Fermented adjusted dried	0.00	7.6
	0.45	5.5
	0.67	5.1
	0.90	4.9

[a]Egg white beaten 75 sec.

Table 46

Effect of Cream of Tartar on pH of Batter and Volume of Angel Cakes Prepared from Fresh Egg White

Amount of Cream of Tartar	pH Batter	Cake Volume ml./100 gm. Batter	Average Volume
0.45	7.3 7.3	500 496	498
0.90	6.6 6.6	548 572	560
1.35	5.8 5.6	562 575	568
1.80	5.1 5.1	523 535	529
2.25	5.0 5.0	505 522	514

Table 47

Effect of Fermentation and Cream of Tartar Level on the pH and Beating Rate of Albumen[a]

Treatment	pH	Beating Rate ml./gm./sec.
Before fermentation	8.7	0.0816
After fermentation	6.0	0.0816
After fermentation (Adjusted with NH_4OH)	9.1	0.0820
Unfermented Dried (0.45 gm. c. t.)	7.5	0.0816
Unfermented Dried (0.90 gm. c. t.)	6.4	0.0816
Fermented Dried (No c. t.)	6.3	0.0741
Fermented Dried (0.90 gm. c. t.)	4.6	0.0714
Fermented Adjusted Dried (0.3 gm. c. t.)	5.8	0.0816
Fermented Adjusted Dried (0.9 gm. c. t.)	4.9	0.0816

[a]Conditions of fermentation:
10% inoculum N.R.R.L. #199)
0.1% yeast extract
Incubation temperature 40° C.
Surface/volume ratio 0.40 $cm.^2/cm.^3$
Bacterial count at start of fermentation
13×10^7 Aerobacter aerogenes per ml.
Bacterial count at completion of fermentation (5 hrs.)
14×10^8 Aerobacter aerogenes per ml.

Table 48

Effect of Cream of Tartar Level on Moistness of Angel Cake Prepared From Dried Egg White[a]

Treatment	Replications I	II	III	IV	V	Ave.
Unfermented 0.45 gm. c. t.[b]	8.3	8.8	8.0	8.4	8.7	8.4*
Unfermented 0.9 gm. c. t. (Control)	8.1	8.0	8.0	8.0	8.4	8.1
Fermented Unadjusted 0.0 gm. c. t.	8.5	8.0	8.0	8.5	8.3	8.3
Fermented Unadjusted 0.9 gm. c. t.	8.8	7.4	7.7	8.1	8.2	8.0
Fermented Adjusted 0.3 gm. c. t.	8.2	8.1	8.2	8.5	8.5	8.3
Fermented Adjusted 0.9 gm. c. t.	7.9	7.9	8.8	8.2	8.0	8.2

[a]Average of eight judges' scores. Highest possible score 10; range 0-10

[b]Cream of Tartar

*Significantly less than control at 5 per cent level

Table 49

Effect of Cream of Tartar Level on Tenderness of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replications I	II	III	IV	V	Ave.
Unfermented 0.45 gm. c. t.[b]	8.1	7.9	6.8	7.7	8.8	8.6
Unfermented 0.9 gm. c. t. (Control)	9.0	9.0	8.0	9.1	8.1	8.6
Fermented Unadjusted 0.0 gm. c. t.	8.5	7.9	7.9	8.6	8.5	8.3
Fermented Unadjusted 0.9 gm. c. t.	8.8	7.4	7.8	8.1	8.8	8.2
Fermented Adjusted 0.3 gm. c. t.	8.4	7.9	8.1	8.4	9.1	8.4
Fermented Adjusted 0.9 gm. c. t.	8.6	7.6	8.0	7.8	7.9	8.0

[a]Average of eight judges' scores. Highest possible score 10; range 0-10

[b]Cream of Tartar

Table 50

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the pH of Angel Cake Batter (Data of Table 13)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	14.5537	--
Replications	4	0.0529	0.0132
Treatments	5	14.2817	2.8563**
Error	20	.2191	0.0110

Difference required for significance at $P_{.05}$ = 0.14
$P_{.01}$ = 0.19

**Significant at 1 per cent level

Table 51

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the Volume of Angel Cakes Prepared from Fermented Albumen (Data of Table 14)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	25.623.2	--
Replications	4	1.096.5	274.12
Treatments	5	20.889.2	4,177.84**
Error	20	3.637.5	181.88

Difference required for significance at $P_{.05}$ = 17.79
$P_{.01}$ = 24.27

**Significant at 1 per cent level

Table 52

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on Texture Scores Given by Eight Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 15)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	669.4	--
Replications	4	54.0	13.5
Treatments	5	318.6	63.72**
Error	20	296.8	14.84

Difference required for significance at $P_{.05} = 1.04$
$P_{.01} = 1.43$

**Significant at 1 per cent level

Table 53

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on Total Flavor Scores Given by Eight Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 16)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	239	466.6156	--
Replications	4	6.7666	1.6916
Treatments	5	162.5594	32.5119**
Treatments x Replications	20	15.1958	.7598
Judges	7	103.8739	14.8391**
Judges x Treatments	35	59.8823	1.7109*
Judges x Replications	28	34.6001	1.2357
Judges x Replications x Treatments	140	83.7375	.5981
Error for Treatments			1.8726

Difference required for significance at $P_{.05} = 0.65$
$P_{.01} = 0.89$

*Significant at 5 per cent level
**Significant at 1 per cent level

Table 54

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on Total General Palatability Scores Given by Eight Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 17)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	239	309.9166	---
Replications	4	7.8026	1.9506
Treatments	5	92.9174	18.5835**
Treatments x Replications	20	9.3778	.4689
Judges	7	107.2069	15.3153**
Judges x Treatments	35	22.3483	.6385
Judges x Replications	28	17.4121	.6219
Judges x Replications x Treatments	140	52.8515	.3775
Error for Treatments			0.7299

Difference required for significance at $P_{.05} = 0.40$
$P_{.01} = 0.55$

**Significant at 1 per cent level

Table 55

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on Moistness Scores Given by Eight Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 48, Appendix)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	173.0	--
Replications	4	54.5	13.6**
Treatments	5	82.6	15.5**
Error	20	36.0	1.8

Difference required for significance at $P_{.05} = 0.27$
$P_{.01} = 0.37$

**Significant at 1 per cent level

Table 56

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on Tenderness Scores Given by Eight Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 49, Appendix)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	713.7	--
Replications	4	194.7	48.7*
Treatments	5	196.5	39.3
Error	20	322.5	16.12

Difference required for significance at $P_{.05} = 2.4$
$P_{.01} = 3.3$

*Significant at 5 per cent level

Table 57

Analysis of Covariance
An Analysis of the Relationship of Cake Flavor to the pH of Batter

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Correlation Coefficient
		X^2	XY	Y^2	
Total	29	14.5537	14.7187	23.0337	--
Replications	4	0.0529	0.1129	0.8487	--
Treatments	5	14.2817	14.5897	20.0777	0.8616*
Experimental Error	20	0.2191	0.0161	2.1073	0.0237

X = pH; Y = Flavor
*Significant at 5 per cent level

Table 58

Effect of Fermentation on the pH and Beating Rate of Albumen[a]

Treatment	pH	Beating Rate ml./gm./sec.
Before fermentation	8.3	0.0833
After fermentation	5.7	0.0860
After fermentation (Adjusted with NH_4OH)	9.0	---
Unfermented dried	--	0.0860
Fermented dried	--	0.0800
Fermented adjusted dried	--	0.0824

[a]Conditions of fermentation:
10% inoculum (N.R.R.L. #199)
0.1% yeast extract
Incubation temperature 40° C.
Surface/volume ratio 0.40 $cm.^2/cm.^3$
Fermentation completed in 5 hrs.

Table 59

Effect of Cream of Tartar on Moistness of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replication I	II	III	IV	Ave.
Unfermented 0.45 gm. c. t.[b]	8.1	8.3	8.7	8.6	8.5
Unfermented 0.9 gm. c. t. (Control)	8.6	7.9	8.3	8.7	8.3
Fermented-Unadjusted 0.0 gm. c. t.	8.3	8.3	8.7	8.3	8.4
Fermented-Unadjusted 0.9 gm. c. t.	8.1	7.4	8.4	8.0	8.0
Fermented-adjusted 0.3 gm. c. t.	8.1	8.1	8.6	8.3	8.3
Fermented-adjusted 0.9 gm. c. t.	8.0	7.4	8.7	7.9	8.0

[a]Formula contained vanilla; average of seven judges' scores. Highest possible score 10; range 0-10.

[b]Cream of tartar

Table 60

Effect of Cream of Tartar on Tenderness of Angel Cake Prepared from Dried Egg White[a]

Treatment	Replication I	II	III	IV	Ave.
Unfermented 0.45 gm. c. t.[b]	8.6	7.9	8.8	8.4	8.3
Unfermented 0.9 gm. c. t. (Control)	8.6	8.4	8.6	8.9	8.6
Fermented-unadjusted 0.0 gm. c. t.	8.6	8.3	8.7	8.3	8.5
Fermented-unadjusted 0.9 gm. c. t.	8.1	7.6	9.1	7.9	7.9
Fermented-adjusted 0.3 gm. c. t.	8.1	8.0	9.1	8.6	8.5
Fermented-adjusted 0.9 gm. c. t.	8.0	7.7	8.6	7.7	8.0

[a]Formula contained vanilla; average of seven judges' scores. Highest possible score 10; range 0-10.

[b]Cream of Tartar.

Table 61

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the pH of Angel Cake Batter (Data of Table 19)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	21.52	--
Replications	3	.15	.05
Treatments	5	17.15	3.43**
Error	15	4.22	.281

Difference required for significance at $P_{.05}$ = 0.625
$P_{.01}$ = 0.86

**Significant at 1 per cent level

Table 62

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the Volume of Angel Cakes Prepared from Fermented Albumen (Data of Table 20)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	27,079.0	--
Replications	3	129.0	43.0
Treatments	5	25,706.0	5,141.2**
Error	15	1,244.0	82.66

Difference required for significance at $P_{.05}$ = 46.8
$P_{.01}$ = 64.7

**Significant at 1 per cent level

Table 63

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on Texture Scores Given by Seven Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 21)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	1059.83	--
Replications	3	33.50	11.16
Treatments	5	906.33	181.26**
Error	15	153.5	10.2

Difference required for significance at $P_{.05} = 1.8$
$P_{.01} = 2.49$

**Significant at 1 per cent level

Table 64

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the Flavor Scores Given by Seven Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 22)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	1175.333	---
Replications	3	22.33	7.44
Treatments	5	1055.83	211.166**
Error	15	97.16	6.477

Difference required for significance at $P_{.05} = 1.44$
$P_{.01} = 1.99$

**Significant at 1 per cent level

Table 65

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the General Palatability Scores Given by Seven Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 23)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	1201.33	---
Replications	3	19.66	6.55
Treatments	5	1119.33	223.86**
Error	15	62.33	4.162

Difference required for significance at $P_{.05}$ = 1.16
$P_{.01}$ = 1.59

**Significant at 1 per cent level

Table 66

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the Moistness Scores Given by Seven Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 59, Appendix)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	148.96	---
Replications	3	66.12	22.04**
Treatments	5	38.21	7.642
Error	15	44.63	2.975

Difference required for significance at $P_{.05}$ = 0.977
$P_{.01}$ = 1.35

**Significant at 1 per cent level

Table 67

Analysis of Variance
An Analysis of the Effect of Cream of Tartar Level on the Tenderness Scores Given by Seven Judges to Angel Cakes Prepared from Fermented Albumen (Data of Table 60, Appendix)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	188.62	---
Replications	3	71.89	27.293**
Treatments	5	74.38	14.876**
Error	15	42.36	2.824

Difference required for significance at $P_{.05} = 0.952$
$P_{.01} = 1.31$

**Significant at 1 per cent level

Table 68

Effect of Storage of Dried Fermented Albumen on Bacterial Count

Storage at 50° C. (in days)	Bacterial Count per gram of Dried Fermented Egg White[a] 11% moisture	8.4% moisture	5.2% moisture	5.2% moisture[b]
0	26×10^7	31×10^6 24×10^6	13×10^7	36×10^6
1	20×10^6	21×10^5 50×10^4	37×10^5	57×10^4
2	7×10^4	6×10^4 10×10^4	15×10^5	49×10^4
3	8×10^3	11×10^3	22×10^4	64×10^4
4	$< 1 \times 10^3$	12×10^3	34×10^4	11×10^3
5	—	7×10^2 15×10^2	10×10^3	20×10^2

[a]Bacterial Count determined by Smear Plate Technique on Bacto-eosin-methylene-blue agar.

[b]Fermented albumen adjusted with NH_4OH to pH 9 prior to drying.

Table 69

Effect of Storage of Fermented and Unfermented Dried Albumen on Beating Rate

Storage (in weeks)	Beating Rate[a] Control Unfermented (stored - 15° C.)	Unfermented (stored - 40° C.)	Fermented (stored - 40° C.)
0	.0816	.0800	.0816
2	.0851	<.0500[b]	.0800
4	.0860	<.0500[b]	.0800
8	.0851	<.0500[b]	.0792
12	.0842	<.0500[b]	.0776
16	.0878	<.0500[b]	.0792

[a]Beating Rate expressed in ml./gm./sec.

[b]Orange-brown tinge to albumen

Table 70

Effect of Storage of Fermented and Unfermented Dried Albumen on Angel Cake Volume

Storage at 40° C. in weeks	Cake Volume (ml. per 100 gm. batter) Unfermented	Fermented	Fermented Adjusted
0	620, 624 614, 621	597, 606 613, 610	615, 612 615, 612
4	517, 518	557, 594	581, 576
6	472, 474	500, 485	517, 540
8	489, 470	491, 467	507, 516