1944

Effect of sugar or salt upon denaturation produced by beating and upon the ease of formation and the stability of egg white foams

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NOTE TO USERS

EFFECT OF SUGAR OR SALT UPON DENATURATION PRODUCED BY BEATING AND UPON THE EASE OF FORMATION AND THE STABILITY OF EGG WHITE FOAMS

by

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Foods

Approved:

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1944

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INTRODUCTION

The beating of egg white into a foam is accompanied by very marked physical changes. The foam increases greatly in volume due to the inclusion of a large quantity of air; it changes in color from a pale greenish yellow translucency to whiteness, the opacity of which increases with the amount of beating; it loses its shine and becomes dull in appearance and correspondingly appears drier and even powdery with long beating. As the beating is continued, the size of the air bubbles steadily decreases and a tremendous area of thinner and thinner film is produced. Coincident with the increase in the area of film is a loss in its elasticity.

Egg white is essentially a solution of protein, the other constituents being present in much smaller proportions. The egg white proteins are coagulable - a change which in beating a foam is induced by the exposure of such a large surface area and by the mechanical impacts of the whisk. The opacity and loss of elasticity of the films are thought to be due to a partial coagulation of the protein concentrated at the air/egg white interface. The film thus is visualised as made up of some coagulated and some fluid egg white. As the beating and thus the coagulation is increased, the film becomes increasingly less fluid and, thus assumes a more rigid character until a point is reached where the stability of the film with its included liquid phase

is at a maximum. With further beating, the film becoming more rigid and thin, reaches a point of such lessened tensile strength that a breaking of bubbles occurs and the fluid phase is released. Thus, with increased beating an egg white foam becomes, at first, more stable and, then, less stable.

Such marked physical changes are known to be influenced by the addition of certain substances. In cookery, sugar is added to egg whites in large amounts. It is the purpose of these experiments to study the effect of the addition of sugar or of salt to the expansion in volume, to the stability of the foam, to its degree of stiffness and to the proportion of coagulated and liquid components of the film. Also, since coagulation of albumin, the chief protein in egg white, is always preceded by denaturation, this study includes certain chemical determinations in an attempt to assess the degree of denaturation at different stages of beating and the effect of sugar and salt upon it. Thus, the variables in this study are the lengths of the beating periods and the additions of sugar or salt to egg white foams.

In this study the terms denaturation and coagulation are not considered synonomous. Denaturation is the intra-molecular change in the protein which prepares it for flocculation. Coagulation is the subsequent flocculation and formation of solid. The sum total denaturation produced in beating egg white foam is made up of true surface denaturation and of the denaturation produced mechanically by the whisk and by whatever denaturing agents may be added.

There are definite changes in viscosity and in solubility due to denaturation. Although the change in viscosity of a protein dispersed in liquid is a very accurate measure of its degree of denaturation, this criterion is not applicable to a heterogeneous system such as an egg white foam which contains bubbles of air and films made up of solid and liquid. Since a denatured protein is less soluble in the dispersing medium than a native one, the amount of protein coagulated at its isoelectric point often is taken as a measure of its denaturation. In an egg white foam, the difficulty of separating the insoluble from the soluble portions is very great. The various proteins of egg white are not at their respective isoelectric points. Any dilution in an attempt to wash the solids produces some further precipitation. Thus, some other criterion of the degree of denaturation than the tendency toward insolubility is desirable.

Since certain groups in the molecule are more reactive in a denatured than in a native protein and since chemical tests for them have been suggested, this study includes determinations of this nature. The reducing value of sulfhydryl groups rendered more reactive by denaturation has been a favorite measure of evaluating its extent. Two methods for such determinations are applied in this study to the denaturation produced in the beating of an egg white foam.

This problem has been planned from the standpoint of research work in foods. If a choice between the strict controlled conditions and purified materials of a chemistry laboratory and the condition of

natural foods was necessary, strict control was sacrificed. It seems to the author that in order to learn the reactions of natural food materials in cookery, study must be made of them under such conditions.

REVIEW OF LITERATURE

Foams of Egg White and Egg Albumin Solutions

In an early paper, St. John and Flor (53) showed that beating egg white at icebox temperatures gave a smaller volume and required longer to beat than at 21 or 30° C. Using a hand beater of a slitted disc type, they found that eggs which had been stored at 30-35° C. gave as good or better volume than the control fresh eggs. They noted, however, that the foam from the fresh eggs gave less separation of liquid upon standing. As the storage period increased, the firmness of the whites decreased, the foam volume obtained increased and the time required for beating decreased. Thus, in any controlled experiment on egg white foams, the age and quality of the eggs and the temperature of beating need to be considered.

Barmore (16) has studied extensively "the influence of chemical and physical factors on egg white foams" measuring the amount of liquid draining from the foam produced under various conditions, the viscosity of the drained liquid, the pH and surface tension of egg whites as well as the specific gravity of the foam formed. It was found that the stability of the foam, as measured by the amount of liquid draining from it, was inversely proportional to the viscosity of that liquid. The relation of the amount of drainage in grams per minute, the slope, and

viscosity derived from their data was expressed.

Slope = $-0.39\eta + 1.94$ where η, the viscosity, was measured in centipoises.

Slope was defined as the amount of drainage per minute. The relationship of the specific gravity of the foam to the viscosity of the drained liquid also was a linear one, expressed as

Specific gravity = $+ 0.0195\eta + 0.043$

The amount of insoluble protein formed in the beating of the foam was another measurement which Barmore made. This was found to increase as the specific gravity of the foam decreased and to be roughly proportional to the drainage.

The time of beating was found by Barmore to be a very important factor in determining the stability of the foam. The greatest stability was shown by a foam beaten 1 minute; the instability increased with each additional minute of beating. The beater used by Barmore was a mechanical food mixer of the Dover beater type giving 925 revolutions per minute. It appears to the author that Barmore's data illustrate instability due to overbeating only. A linear relationship was presented for foam stability in terms of drainage per minute of standing and specific gravity, but the specific gravity ranged from 0.14 to 0.07, that is, within the range of well-beaten foams only. Another factor studied on foam stability was temperature of beating. Within the range of 20 to 34° C., there was no difference noted. Foam stability was also influenced by the age of the eggs. At the one minute period of beating, older eggs produced less stable foams. As the beating

period was increased, there was less difference between the foams from different aged eggs, all giving a high degree of instability. The data indicate also that there was markedly lower specific gravity at 1 and 2 minutes beating with the aging of the eggs while at 3 and 4 minutes there was less difference.

Bailey (13) compared two types of motor driven beaters, the one a Dover type which rotates about a stationary axis and the other a Hobart C-10, having a beater which follows a hypocycloid path through the egg white. She recommended the latter for all experimental work. Foaming power was calculated as

$$F = \frac{(1.04\ V\ 100)}{W} - 100$$ and the leakage as

$$L = \frac{(1.04\ l)}{W}\ 100$$ where

l = ml. liquid draining in 1 hour

1.04 = specific gravity of egg white

w = weight of foam

v = volume of crystallizing dish in which it was weighed

Thawed frozen egg whites and an aliquot not frozen were beaten for periods of 3, 6, 9, 12, 15 and 18 minutes. The fresh egg white had definite points of minimum leakage as related to the time of beating. Thus, there were examples of instability due to underbeating and to overbeating. The optimum time of whipping for minimum leakage was shown to be less than that for maximum foaming. "Therefore, if stability of

foam is desired, the egg white should not be whipped to its maximum degree of foaming" (13).

Both Barmore (16) and Bailey (13) had used mixtures of whites for their samples, the mixes being prepared by using the low speed of the mechanical beaters. Bailey (13) compared samples churned and not churned, and found them to be essentially the same in foaming power. Barmore (16) noted a rise in temperature of the foam with the beating. The maximum rise obtained was 5° C. which he attributed to the friction developed by beating.

In addition to these reports on egg white itself, the foaming of solutions of egg albumin has been studied. A group of Russian workers report (29) that the maximum foam volume is obtained for albumin just below the coagulation temperature. Mannitol and sugars increased the amount of the foam and rendered it more highly dispersed and more stable (28).

Clark and Ross (26) described dynamic and static foam meters in which they had studied albumin solutions. Air and carbon dioxide were the gases used to produce the foams. The unit of foaminess was designated E and interpreted to be a measure of the average life of a bubble in the foam. It was thus a measure of stability. For beer, the dynamic and static methods gave essentially the same value, which values followed a logarithmic relation. Albumin solutions, however, did not. Estimations of E for albumin solutions by a dynamic foam meter using CO_2 gave higher values than with air, whereas with a static foam meter

just the reverse was true. The authors postulated a reaction of CO_2 and albumin and discussed the interference due to denaturation and coagulation of the bubble films, that a stiffening of the film prolonged the life of bubbles. Foaminess as measured by a static foam meter is based on the rate of decomposition of a foam already formed (much like a drainage test used by Barmore, etc.) while as measured by a dynamic foam meter, it is a measure of the equilibrium between rates of formation and decomposition. The authors concluded that a static foam method is the only one which can be used for egg albumin solutions and that comparative data can be obtained by its use.

Denaturation and Coagulation Involved in Egg White Foams

Only one report was found in the literature which reported any actual experimentation on the denaturation of proteins in egg white foams. A report by Harris (32) contained the statement that egg white, whipped and dried, gave a very feeble nitroprusside reaction. He found that mechanical shaking caused egg white to react with nitroprusside as did the passing of a vigorous stream of nitrogen through the solution to cause frothing.

In an earlier report, Ramsden had stated:

> Bubbles of solutions of egg-albumin, caseinogen and saponin exhibit remarkable phenomena, which show that the bubble film as a whole is very imperfectly elastic and is covered with solid membranes. Egg albumin bubbles are deformed on collapse by the formation of persistent folds of solid protein in the bubble film (50).

> Every solution capable of forming moderately persistent bubbles which has hitherto been examined has yielded solid or highly viscous mechanical surface aggregates. This very remarkable fact indicates that the power of forming such bubbles is due to the presence of matter which has accumulated at the free surfaces in a solid or highly viscous condition (50).

Further understanding of the changes occurring in egg white foams may be obtained from a brief review of the theory of protein structure and of surface denaturation produced by other means.

As early as 1931, Wu, in his studies on denaturation of proteins, had stated a theory of denaturation (56).

> Evidence is adduced in support of the hypothesis that the molecule of natural soluble proteins is not a flexible open chain of polypeptide but has a compact structure. The force of attraction between the polar groups (of which there are 200-300) in a single molecule of protein holds them together in an orderly way, just as the force of attraction between different molecules holds many molecules together in a crystal.
>
> Denaturation is disorganization of the natural protein molecule, the change from the regular arrangement of a rigid structure to the irregular diffuse arrangement of the flexible open chain. If denaturation occurs in acid or alkali or in urea solution, the individual molecules are disrupted but they remain separate (because they carry the same charges). ... When the charge is removed, they attract each other by virtue of secondary valences. This is flocculation. Coagulation is the interpenetration of many protein molecules. The deeper the penetration, the more compact is the resulting coagulum. Deep penetration is possible only when the individual molecules carry no charge and collide with a large amount of kinetic energy. This is why the most coagulum is formed by heating at the isoelectric point.

Thus, Wu really described three steps: denaturation, which involves an intra-molecular change in protein, flocculation and coagulation.

Since that time, the greatest contribution made to our knowledge of protein structure has come through the X-ray diffraction studies of Astbury and his co-workers, recently reviewed by Astbury (9, 10) and by Neurath and others (46). Astbury divided proteins into two classes on the basis of their molecular configuration, globular which showed a compact structure as judged by X-ray diffraction patterns, and fibrous which showed a more extended configuration, the beta configuration. In their studies on keratin and myosin, Astbury and Dickinson (12) postulated a third structure, a supercontracted form which is characterized by a pattern of disoriented fibres of the beta configuration. For a globular protein such as albumin, denaturation is characterized by an extension of the native or compact structure. Neurath et. al. (46) stated: "In general, one may conclude that denaturation of globular proteins results in a structure similar to that revealed by keratin when it 'supercontracts', i.e., a bundle of disoriented polypeptide chains." In the denaturing process, the globular proteins undergo a rupture of weak bonds to allow the polypeptide chains to extend. Then, during coagulation, there is a formation of bonds in the "interpenetration" of these chains to form coagulum. Thus the X-ray diffractive studies have supplied information concerning denaturation and the changes in the structure of proteins which are in accord with Wu's theory expressed earlier (56).

The types of bonds or cross linkages which hold the polypeptide chains of the native protein together have been listed by Astbury (8)

as van der Waal forces and hydrogen bonds in addition to stronger bonds such as cystine and peptide linkages. In addition, Heafield, Hopkins and Hunter (34) have shown that bonds of the type -S-H-O- and -S-H-N- can be formed in simpler compounds. Compounds having -C-S-NH_2 and -CS-N-H- possess the property of molecular association which suggests that such sulfur bonds may be present in proteins and take part in hydrogen bonding. The change from native to denatured albumin is known to release sulfur linkages to form active groups giving a sulfhydryl test (46).

Among the agents known to produce denaturation are the shaking of protein solutions and the spreading of proteins in monolayers. Surface denaturation and coagulation occur in each case.

Ramsden, in 1894, (49) was able to coagulate a dilute acid solution of albumin by shaking. He showed that it was not a reaction with air by obtaining coagulation of 94 per cent of the albumin when shaken with sand for 60 hours in an evacuated flask.

In 1927, Wu and Ling (56) reported studies of the factors controlling the coagulation of proteins by shaking. They used dilute (1 and 5%) solutions of egg albumin and filtered off and weighed the solid formed by shaking. Since there was no denatured albumin left in solution, they concluded that surface denaturation consisted of only one step - coagulation. They found maximum coagulation at the isoelectric point and that the absolute amount of coagulation, within the ranges used, was independent of the concentration of the albumin but that on a percentage basis, there was an inverse relationship between

concentration and the amount of protein denatured. The amount of protein coagulated was proportional to the shaking. They reported the temperature coefficient between 25 and 38° C. as being 1.09 for 10° rise.

In 1936, Neurath and Bull (45) compared heat and surface denaturation on the basis of the densities of native and denatured egg albumin. They considered the mechanism for the two types of denaturation to be different. Later, this was retracted (21). It is now generally accepted that the mechanism for all types of denaturation is the same, an extension of the globular form of the native protein to a structure having a longer axis.

In a series of articles, (22, 58, 23, 55), there raged a controversy on two points, whether surface denaturation and coagulation were two processes or only one and whether the protein concentration affected the rate of denaturation. Both groups were agreed that at the isoelectric point, the amount of surface coagulated protein formed is a true measure of the amount of denaturation. According to Bull and Neurath's work, the shaking of a dilute solution of albumin at pH 2.55 or 3.05 resulted in 52 and 57 per cent respectively of the total denatured albumin being soluble, which showed that denatured albumin may exist without being coagulated. The Chinese workers are not agreed on this point. On the second point, it is generally accepted that in shaking dilute solutions, the reaction rate is independent of the concentration, i.e., it is a zero-order reaction.

Mirsky, in 1941 (42), reported the application of his technique of estimation of the sulfhydryl group to the study of albumin denatured by shaking. He found that the same number of sulfhydryl groups reduced ferricyanide in surface films of egg albumin as if denatured by urea, guanidine, duponol or heat, provided the reaction with ferricyanide occurred while the films were still at the surface. In order to accomplish this, the oxidizing agent was added to the albumin solution before shaking and the test completed on the protein free filtrate. If the oxidizing agent was added after clumping had occurred, the reaction was slow and incomplete as was also true for cooled heat-denatured or for urea-denatured albumin if the urea was not present. There is, thus, reason to believe that in film formation, sulfhydryl groups are made accessible to ferricyanide molecules, but in clumping they are again covered and rendered less accessible. Thus, Mirsky suggests that the mechanism is the same in surface denaturation as when produced by other agents.

Surface denaturation which occurs in spreading of monomolecular films is of interest as a possible source of clues as to what occurs in the films of egg white foams. That the formation of protein monolayers involves a change in molecular structure is shown by the following quotation from a review by Langmuir and Schaefer: (39)

> ... the globular proteins have a symmmetrical, highly organized cage-like structure which breaks down or unfolds during this spreading process, permitting hydrophobic groups to come into contact with the air-water interface. ... Protein monolayers thus consist mainly of polypeptide chains which are anchored to the air-water interface at intervals along their length by hydrophobic groups contained in the side chain.

Gorter, Ormondt and Meijer (31) reviewed their earlier work and considered complex protein films. Within the pH range of .6, the maximum spreading occurred near the isoelectric point with minima on either side of this point. At such a minimum, the spreading could be increased by the addition of electrolytes, the effect of the ions varying with the valence. Approximately the same effect was produced by about 10 millimols of a univalent ion, 1 millimol of a divalent ion, or 1/40 millimol of a trivalent ion. A lyotropic series was suggested: K>Li and CNS>Cl. If the most effective ion was positive, it increased the spreading at the minimum on the alkaline side; if negative, it affected the minimum on the acid side. Gorter et al. stated that egg albumin spread better as the undissociated protein or as a non-ionized salt than in ionic form. It was thought the effect of electrolyte ions was to discharge the protein ions, thus shifting them into a form more easily spread. Complex proteins containing albumin may or may not affect the spreading properties.

Bull (20, 21) was able to spread urea- or heat-denatured egg albumin. Very dilute solutions of albumin in strong acid could be spread and coagulation followed in the film. Such solutions could also be coagulated by shaking. These experiments on solutions of egg albumin lend credence to the theory that all denaturation is alike and is the unrolling of the globular structure of the native protein to the extended form and that coagulation is the precipitation of the denatured protein due to the predominant hydrophobic properties. Coagulation is the

process of the orientation and packing of the extended forms into closer, heavier aggregates. Evidence of the parallel arrangement of polypeptide chains in spread films has been added by Astbury and his coworkers (11).

Development of Quantitative Chemical Methods for the Study of Denaturation

The nitroprusside test was used by Arnold (7) and, later, by Harris (32) in a study of the coagulation of proteins. This reagent was shown to react with coagulated proteins as it did with mercaptan groups. Consequently, the development of quantitative chemical methods for denaturation have made use of various reagents to measure sulfhydryl groups. One procedure used was to oxidize these groups and to measure the amount of oxidant necessary for the denatured protein to give a negative nitroprusside test (4). An oxidizing agent used extensively by Anson and Mirsky is potassium ferricyanide (3, 4, 5, 6, 41, 43, 44). In this series of articles, they showed that oxidation of sulfhydryl groups by ferricyanide was inhibited by an acid solution and the presence of cyanide and that it was accelerated by high temperature or by the presence of copper. The reaction was shown to be affected by the concentration of ferricyanide and the time during which it acted. They also used detergents, urea and the guanidine hydrochloride as solvents for the protein in the development of the color of ferri-ferrocyanide which was measured on a spectrophotometer.

The conditions under which various other oxidizing agents, porphorindin, indephenol dyes, iodoacetic acid, iodine and iodosobenzoate may be used have been studied by a number of other workers (38, 19, 54, 51, 5, 35, 36). It has been found that the specificity of the reaction depends upon the strength of the oxidizing agent and upon the pH of the solution. At a pH higher than 7, tyrosine and tryptophane groups are thought to react also but more slowly than sulfhydryl.

The Effect of Sugar upon Denaturation and Coagulation of Egg White or Egg Albumin

That sucrose inhibited the coagulation of egg albumin was first reported by Beilinnson (18) who showed that, with an increase in the concentration of sugar present in a 5 per cent albumin solution heated for one hour, there was an increase in the nitrogen content left uncoagulated.

In 1931, Bancroft and Rutzler (15) reported the peptization of heat coagulated egg white by grinding with sucrose. Clayton (27) in a lecture on the application of colloid chemistry to foods stated: "It is interesting to note that in my laboratory we show that high concentration of sugar in solution in egg white will prevent such surface denaturation," i.e. the coagulation of protein in a solution by shaking. Barmore (17) noted that, when a larger proportion of sugar was used, a more desirable cake resulted with a higher baking temperature. He described an experiment which showed that the higher the concentration of sugar in egg albumin solutions, the higher the temperature had to be raised to

induce coagulation. Thus sucrose retarded the coagulation of albumin.

In a recent report, Ball, Hardt and Duddles (14) confirmed the inhibitory effect of sucrose upon the coagulation of albumin by heat. The solutions of egg albumin, with or without sugar buffered to pH of 4.8, were heated at 70° C. for 15 minutes and the soluble nitrogen determined in the filtrate. Monosaccharides had an effect greater than sucrose, the descending order of effect being l-arabinose, d-xylose, d-glucose and sucrose. All were compared on an equimolar basis. They found that allowing egg albumin solution to remain in contact with glucose or fructose for 96 hours did not increase the inhibitory effect. These workers determined the sulfhydryl activation produced by heating egg albumin solutions in the absence or presence of various sugars. Sucrose was not included but the monosaccharides showed a definite inhibitory influence on the release of such groups in heat coagulation. This is the only paper on the effect of sugars on denaturation of albumin by any denaturing agent in which sulfhydryl determinations were made. Thus, there is ample evidence that sucrose exerts an anti-coagulating effect but none on the question as to whether the effect is on denaturation proper or on the later flocculation and coagulation.

The Influence of Sodium Chloride on Denaturation and Coagulation

There has been found no report in the literature on the effect of sodium chloride on the rate of surface denaturation. One paper, however,

has dealt with its effect on heat coagulation. Chick and Martin (25) found that although a concentration of 0.1 N sodium chloride inhibited coagulation to a slight extent, the effect increased with increasing amounts. A concentration of 3 N sodium chloride allowed very little coagulation when heated at 70.9° C. Heating periods from 10 to 25 minutes were used. The velocity rate was reduced for a normal solution of sodium chloride to 1/16th and for 0.2 N solution to about 1/34 that of the control. These authors stated that this effect of salts was not due to any influence on the second phase of coagulation, i.e., the separation of the solid. In part, the effect of sodium chloride was due to a reduction in hydrogen ion concentration but this accounted for only a small part of the inhibitory effect of sodium chloride. There was, in addition to sodium chloride, some ammonium sulfate which had not been dialized out of the egg albumen.

Wu and Yang (59) either adjusted the pH or controlled the pH of the albumen solutions by a buffer and measured the amount of denaturation induced by urea in the presence of 0.0, 0.5, 1.0 or 2.0 M sodium chloride. The results showed a decrease in denaturation with an increase in sodium chloride concentration. The criterion of denaturation was the amount of heat-coagulable protein left in the filtrate.

In a more recent article, Burk (24) studied the effect of electrolytes on the liberation of sulfhydryl groups in albumin solutions denatured by urea or by calcium chloride. His criterion was the minimum concentration of salt added to a definite amount of albumin

and denaturant which would just permit a positive nitroprusside reaction. The concentrations of albumin and denaturant were also varied. In all concentrations used, sodium chloride was shown to retard the liberation of sulfhydryl groups. Thus, whereas none of the denaturing agents were of the type effective in producing an egg white foam, the three studies do agree that denaturation is retarded by the presence of sodium chloride.

EXPERIMENTAL PROCEDURE

Preparation of the Egg White Mix

The eggs used were obtained from the Poultry Department of Iowa State College and were taken from eggs laid the previous day. They were judged for quality in terms of Haugh units as follows: The shell eggs, brought to room temperature, were weighed to the nearest 0.1 gm. and broken onto a level glass surface. The height in millimeters was measured at the point equidistant from the yolk and the edge of the firm white on the axis where the firm white was widest. A micrometer mounted on a tripod was used for these measurements. The Haugh units were read from a nomograph developed for this purpose (1, 33). Only those eggs having Haugh units of 75 to 90 were included in the mix.

After the removal of the yolks and chalaza, the whites were mixed in a Waring blendor having variable speeds. Very little foam was formed since the blendor was filled 1/3 to 1/2 full and the low speed used for 20 to 40 seconds. The blendor was stopped when the mixture began to draw into the center a spiral of air bubbles. Each mix required 40 to 58 egg whites, hence several runs of the blendor were made and the portions combined for the mix.

The egg white mix was divided into two portions. One of these was stored in the refrigerator in a closed glass jar for use the following day; the other was used on the same day the mix was prepared. The

experiments performed the first day were designated by letters A, B, whereas those which were completed on the second day were designated correspondingly A^1, B^1 and so on.

Determinations of the pH of the mix were made each day with a glass electrode pH meter, Coleman Style 200 manufactured by Coleman Electric Company, Maywood, Illinois.

Method of Beating the Foams

For each foam, 80 gm. of the egg white mix were placed in the 5 quart bowl of a kitchen size mechanical beater, Kitchen-Aid Model #4, manufactured by the Hobart Manufacturing Company, Troy, Ohio. The French whip used for all foams was 4-3/4 inches tall and 3-1/4 inches at widest portion. It had 12 wires each 1/16 inch in diameter and reached to within 1/16 inch of the bottom of the bowl, giving very efficient beating. This beater had both a rotational and a revolutionary motion and described a planetary path in the egg white. The beater rotated twice on each revolution. From 135 to 150 complete revolutions per minute was the rate of the beater at second speed which was used for all foams. The period of beating was varied. The intervals were timed with a stopwatch which was started on the second round of the beater. The sugar or salt was added after 5 seconds of beating and during a 5 second interval.

All egg white aliquots were brought to a temperature of 25° C. before beating. The room temperature was not under strict control and

varied from 24 to 28.7° C. However, of the 192 foams whose data are reported, 152 were beaten when the room temperature was between 24 and 26° C.

It was considered important to avoid metallic contamination; hence equipment of glass or plastics was used whenever possible. The beater and bowl were new and their surfaces were kept covered with lacquer as was the steel blade of the spatula. The lacquer coat was replaced at frequent intervals to prevent any exposure of the metal surface.

Physical Measurements on the Foams

In order to study the stability of the egg white foams, 20 gm. were weighed into a 6 inch glass funnel supported in a tared 100 ml. graduate cylinder and covered with a watch glass during the drainage period. The weight of the drained liquid was taken after one hour. After standing overnight, the weight of the drained foam was determined. Small brass screen plugs were fitted into the angles of the funnels. These were useful in separating liquid and bubbles of a foam which had been only slightly beaten and was thus too fluid to support itself in the funnel as a stiffly beaten foam can do. These plugs were made of a brass ring, shaped like a cross section of a cone fitting into the angle of the funnel and covered with a platform of brass screen, 60 meshes to the inch. A larger mesh screen would have been easier to clean. Soaking in a concentrated Dreft solution with brushing was the most effective method of cleaning.

A portion of each foam was carefully piled into a one-third cup measure and weighed. Weights of this measure full of unbeaten egg whites or of egg white in which 50 per cent sugar had been dissolved were also determined. The expansion factor was calculated by dividing the weight of the unbeaten egg white by the weight of the same measure of foam. A quotient of 10 gave an expansion factor of 10, i.e., the weight of that cup of foam was 1/10 that of the unbeaten egg white or the volume had expanded 10 times. This allowed for the comparison of the expansion of foams with or without the addition of some added substance such as sugar. For the expansion factors of the foams containing sugar, the weight of the measure full of unbeaten egg white in which 50 per cent sugar had been dissolved was used. It was difficult to get a representative sample from a foam which was underbeaten or which easily separated into liquid and foam layers. Inaccuracies also occurred in measuring the very stiff foams since pockets of air would often be enclosed with the egg white foam and, in addition, it was difficult to fill the cup without crushing and compressing the stiff foam. The foam was leveled by running the blade of the spatula over it at right angles to the rim of the measuring cup. The same cup was used for all measures.

The temperatures of the room and of the foam were recorded at the end of the beating period. The foams were rated for stiffness and other characteristics were observed. This characterization of the stage to which the foam was beaten was made by one observer only and, hence, cannot be considered as precise information. The stiffness scores ranged

from zero to three. The stiffness was rated from zero to one according to the proportion of liquid to foam, one being the stage where all liquid was incorporated in the foam, at least temporarily and lifting the whip from the foam did not pull the foam with it; the range of one to two characterized the formation of peaks when the whip was lifted, two being the formation of peaks which stood straight and stiff; the range of two to three included all foams beaten stiff and dry.

The periods of beating chosen were from 3 to 16 minutes except for foams to which sugar was added. Stiffness was slower to develop in foams containing sugar so the beating periods used were 8 to 32 minutes. In some cases, the control was omitted because it was too stiff and dry, and in other cases, the foam with sugar was omitted because the beating time was insufficient to incorporate the liquid into foam. Measurements would be inaccurate in either case.

The amount of sugar was 40 gm. or 50 per cent of the weight of egg white for all foams to which it was added. This amount is about half the proportion usually added in such food products as hard meringues and angel cakes. When salt was added, 2 gm. were used for an 80 gm. portion of egg white. This is approximately twice the amount usually used for a mild flavor.

Chemical Determinations

Two methods were applied in an attempt to assess the degree of denaturation produced by the various periods of beating. The foams of Series I were subjected to an oxidation by o-iodosobenzoate and the excess oxidant determined iodometrically, a method used by Hellerman *et. al.* (35, 36).

Those of Series II were oxidized with ferricyanide and the blue color of ferriferrocyanide measured in a spectrophotometer (3, 5, 6).

o-Iodosobenzoate method for active reducing groups

Materials and solutions needed:

Potassium iodide, reagent quality

Hydrochloric acid, C.P. approximately 0.1 N solution

Sodium thiosulfate, analytical reagent approximately 0.1 N solution, standardized against potassium dichromate

Potassium dichromate, analytical reagent, approximately 0.1 N solution, factor determined accurately by weight

o-Iodosobenzoic acid, Eastman Kodak Company #2289

Potassium hydroxide, C.P. pellets

For the foams of Series I, 2 ml. of an iodosobenzoate solution were accurately measured and added to 80 gm. of egg white immediately before the beating was started. The oxidizing agent thus was present to react with reducing groups as they were activated by the beating process and to prevent the escape, without measuring, of volatile sulfur groups which may have been formed. The iosodobenzoate solution was made by dissolving 15 gm. of iodosobenzoic acid in 75 ml. 2 N potassium hydroxide and then diluted to 200 ml. volume. It was filtered just before using.

Samples of 10 gm. of egg white or an equivalent amount of foam were weighed into 250 ml. Erlenmeyer flasks. In order to wash down the sides of the flask, 25 ml. of redistilled water was added to each flask

and incorporated by swirling. Then a freshly prepared solution of 2 gm. potassium iodide in 25 ml. 0.1 N hydrochloric acid was added with swirling to prevent the formation of clumps of coagulated protein. Starch indicator, 1 ml., was added and the sample titrated with 0.01 N sodium thiosulfate until both foam and the underlying liquid were white.

The thiosulfate solution was made up each day by diluting a 0.1 N sodium thiosulfate solution exactly ten times, thus giving 0.01 N solution. The 0.1 N solution was standardized at two week intervals against a standard 0.1 N potassium dichromate solution. The normality factor of the dichromate was computed from its weight.

The equivalent for the iodosobenzoate was determined each day by blank determinations with the standard thiosulfate solution. The blanks used for the samples from foams containing sugar were on the average 0.2 ml. larger, due to the effect of the 5 gm. sugar added to the blank. This was the amount of sugar contained in the aliquot of foam titrated.

The calculations were as follows:

Reducing equivalent of sample = (ml. thiosulfate used for blank − ml. of thiosulfate used for sample) x correction factor of 0.01 N thiosulfate

$$\frac{\text{Reducing equivalent of sample} \times \text{equivalent of cysteine}}{10} = \text{reducing value calculated as cysteine per gm. egg white}$$

Equivalent weight of cysteine is 1.2115 mg. per ml. of the 0.01 N solution.

The method as used differed in several important respects from the conditions outlined by Hellerman (35, 36) for use as a specific

determination of sulfhydryl groups. A buffer was not used since it involved much greater dilution of the egg white than was desirable for foam formation and because additional ions might change the course of the reaction markedly. The oxidizing reaction thus was carried out in an alkaline medium, a medium 0.3 to 0.5 pH units higher than the pH of the egg white mix, which gave approximately 8.5 to 9.0 in the mixture of foam and reagent. Thus, the beating occurred at a pH normal to egg whites. A further loss of specificity may have been due to the rather large excess of oxidizing agent present.

Ferricyanide method for active sulfhydryl groups

Materials and solutions needed:

Sodium tungstate, crystalline, Baker's Analyzed

Sulfuric acid, C.P. approximately 1 N solution

Potassium phosphate, dibasic, crystalline, reagent quality

Potassium monophosphate, crystalline, reagent quality

Potassium ferricyanide, reagent quality, crystalline

Ferric sulfate, Merck, reagent quality

Gumghatti, soluble, City Chemical Corporation, New York. #6709.

Samples containing 5 gm. of egg white or of foam were weighed into 50 ml. beakers, 2 ml. redistilled water were added and covered with a watch glass until all foams for the day had been beaten. Then 2 ml. phosphate buffer (pH = 6.8) and 0.1 ml. of 0.2 M potassium ferricyanide (freshly diluted from stock solution of 1 M strength) were added and the

foam thoroughly stirred with short, heavy stirring rods. The oxidising reaction was allowed to progress for 1 hour at room temperature during which the stirring was repeated several times. The protein was precipitated by the addition, first of 2 ml. of 50 per cent sodium tungstate and then 10 ml. 1 N sulfuric acid solution and the mixture stirred. This procedure had been found to give a clearer filtrate for this large amount of protein than to mix the reagents first and add as one solution. A Whatman folded filter #12 was used to remove the precipitate which was carefully stirred on the filter paper several times with the short stirring rods in order to facilitate filtering and washing. Three small portions of wash water were used for the beakers and precipitate. This was sufficient to remove all yellow color of excess ferricyanide from the filtrate as it flowed down the funnel stem.

The filtrate was made up to a 15 ml. volume in the 25 ml. graduate cylinder into which it had been filtered. The color was developed by the addition of 2 ml. of ferric sulfate solution containing gum ghatti prepared by the method of Folin and Malmros (30) and read after 15 minutes against a reference standard. This blank was made up of the ferricyanide, 0.5 ml. 10 per cent sodium tungstate, 0.5 ml. normal sulfuric acid and the ferric sulfate solution, all diluted to 17 ml. volume. No buffer could be used in the reference standard for it gave a precipitate as did excess tungstic acid. The amount of acid added gave approximately the same pH in the reference standard as in the filtrates from the egg white samples.

The color readings were made in terms of per cent transmission on the Coleman universal spectrophotometer, model #11, using the null reading taken directly from the drum. A red filter, P.C. = 5, and the wave length of 710 mmu. were used since that wave length had been found in preliminary work to be in the region of minimum transmission. More uniform readings had also been found to be obtained with a more constant voltage so a rheostat and voltmeter were connected in series with the storage battery and the spectrophotometer; readings were made at 7.5 volts.

The values obtained for per cent transmission were read in terms of milliliters of 0.0002 N potassium ferrocyanide against a reference curve which had been determined as follows: The dilutions and amounts of reagents were the same as the blank reference standards above, that is, 0.5 ml. 10 per cent sodium tungstate, 0.5 ml. 1 N sulfuric acid, 0.1 ml. of 0.2 M potassium ferricyanide and 2 ml. ferric sulfate solution. To some, potassium ferrocyanide was added in amounts of 0.5, 1.0, 1.5, 2.0, 2.5, or 3.0 ml. of a 0.0002 M solution. The ferrocyanide had been weighed to the sixth decimal place on a microbalance and made up accurately to 0.1 M concentration. Four such stock solutions were made. The second dilution of 1 to 500 to give the 0.0002 M concentration was prepared immediately before using. Portions of the dilute ferrocyanide solution were measured accurately by the use of certified Pregl micropipettes into the reference samples. A period of 15 minutes, the same as for egg white samples, was used for color development, the final volume being 17 ml.

This method was more sensitive and specific than the iodosobenzoate procedure. However, since the oxidant was not present in the egg white as the sulfhydryl groups were made available, not the total activation but relative values of it were measured. In the preliminary work on this method, attempts had been made to make use of the solvent action of duponol or urea. Since the denaturing action of these substances would interfere with the measurement of the denaturation produced in the foams by beating, they were added after the ferricyanide action had been stopped by acid in order to give a clear solution for color development without resort to the tungstic acid precipitation of the proteins. With duponol, so much was needed to put the foam into solution that the color was too dilute to read accurately. With the addition of urea, the concentrated solution was opalescent instead of being clear.

RESULTS

Egg Quality

Since Haugh units of the eggs used were determined, data of egg quality were available and have been summarized in Table 1 with a graphic presentation in Figure 1. The average Haugh units of all eggs examined, varied from 74.5 to 83.5. The percentages of eggs examined which were discarded because of poor quality have been included in Table 1 and, at times, this amounted to nearly one-third of the total but, on one occasion, only two eggs or one-thirtieth of the total number had to be discarded. Thus irregularities in egg quality were considerable.

In contrast, the average Haugh units for the eggs used in the mixes ranged from 80.4 to 83.5 with only one value, the last one, falling below 81.5. Thus, the plan of discarding eggs whose Haugh units were below 75 or above 90 gave mixes of fairly constant quality.

The pH determinations made upon the egg white mixes and recorded in Table 1 showed some but no extreme variation. Usually there was a slight increase in the pH on the second day. The method of preparation involved considerable exposure to air and thus continual release of carbon dioxide so the alkalinity might be expected to be increased above that in the intact day-old eggs. The values presented in the table are the pH values at which all of the foams were beaten except those which had an addition of iodosobenzoate.

Table 1. Quality of Eggs

Date	Average Haugh units		% of eggs discarded	pH of egg white mix	
	Of eggs for mix	Of all eggs examined	because of low quality	First day	Second day
3-13	82.8	81.9	15.2	8.50	-
3-16	83.4	83.5	11.3	8.47	-
3-20	82.6	83.4	6.4	8.48	8.55
3-23	82.6	81.5	11.1	8.25	8.45
3-28	83.5	82.8	5.4	8.40	8.50
3-30	81.5	80.5	14.3	8.45	8.47
4-3	83.2	78.5	15.0	8.42	8.48
4-12	82.2	74.5	21.8	8.36	-
4-17	82.2	78.7	29.4	8.40	8.45
4-20	82.2	81.8	8.1	8.46	8.47
4-27	83.2	82.1	14.1	8.43	8.40
5-1	82.5	80.0	20.3	8.40	8.39
5-4	82.0	78.5	24.1	8.48	8.43
5-11	81.7	79.4	19.4	8.34	8.38
5-15	81.9	78.4	28.2	8.38	8.47
5-18	82.9	82.6	3.3	8.47	8.48
5-22	81.9	77.6	31.3	8.46	8.48
5-29	80.4	75.8	32.5	8.55	8.50

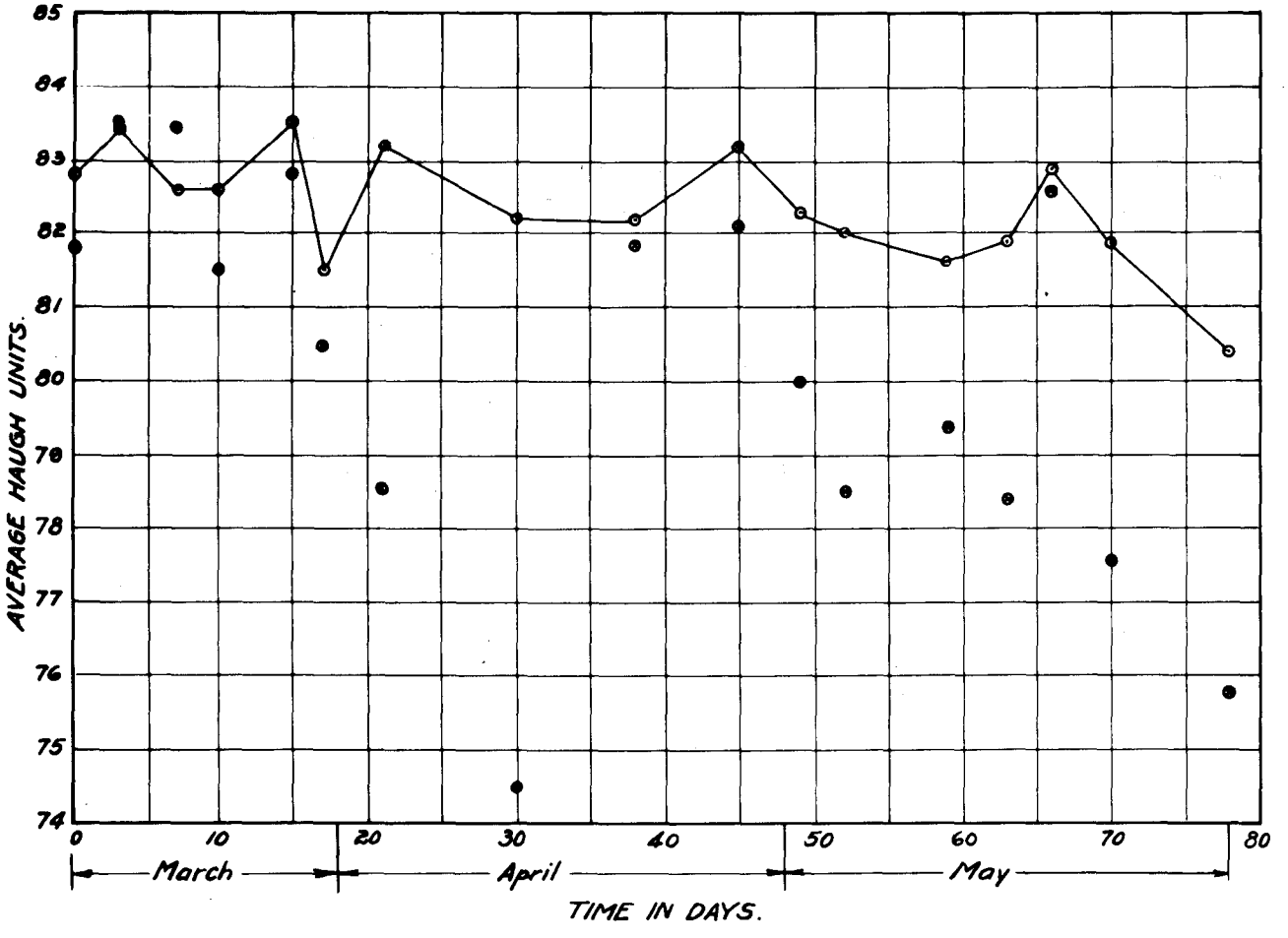

Figure 1. Egg Quality in Terms of Average Haugh Units. The points connected by the solid line designate the average Haugh units of the eggs used in each of the egg white mixes. The isolated points represent the average Haugh units of all eggs examined on each day.

Physical Characteristics of the Egg White Foams with or without Sugar

Texture and appearance

The foams presented a contrast in texture and appearance. The foams beaten from egg white alone formed very quickly but were not smooth and plastic. The foam did not follow the beater into tall peaks as the beater was removed, but formed short abrupt peaks. They tended to "set" and stiffen after beating was stopped; were friable in texture; and began draining quickly. With longer beating, flecks formed and the texture was not uniform. These coagulated flecks formed after about 8 to 10 minutes of beating. After 10 to 12 minutes of beating, the foam was dry and stiff enough to break away from the beater and accumulate on the sides of the bowl as it was flung from the whip. It was difficult to get representative samples, at times, due to the quick drainage and, at other times, to the stiffness and friability of the longer beaten foams.

In contrast, the egg white foams to which either sugar or iodosobenzoate or both had been added were much more plastic and followed the beater as it was removed from the foam into long slender peaks. These soft pliable foams were easy to handle and "set" less readily than the others. The flecking of foams containing sugar was not noticeable with

less than 24 minutes beating and foam did not fly off the beater until 25 to 30 minutes beating. These foams, containing either sugar or iodosobenzoate or both, once formed, did not separate into foam and liquid layers during the time of taking samples as the others had done.

Change in temperature of the foams

The data on the change in temperature of the foams have been presented graphically in Figure 2. The details of these data have been presented in Tables A to D. All tables designated by letters have been included in the appendix. The beating of the foams resulted in a decrease in temperature of 1.2 to 2° C. If sugar had been added, a decline also occurred but, with longer beating, the foam temperature approached that of the room. It cannot be inferred from these data whether the foams containing no sugar would evidence a rise in temperature if beaten longer as had these foams with sugar. All foams were 25° C. in temperature at the beginning of the beating; the room temperature was, on the average, higher. Yet, each foam exhibited a drop in temperature in the early stages of beating.

Ease of foam formation

Two methods were used to assess the ease with which foams were produced from egg white alone or with sugar added, the subjective scoring of stiffness and the amount of expansion obtained with a definite period of beating.

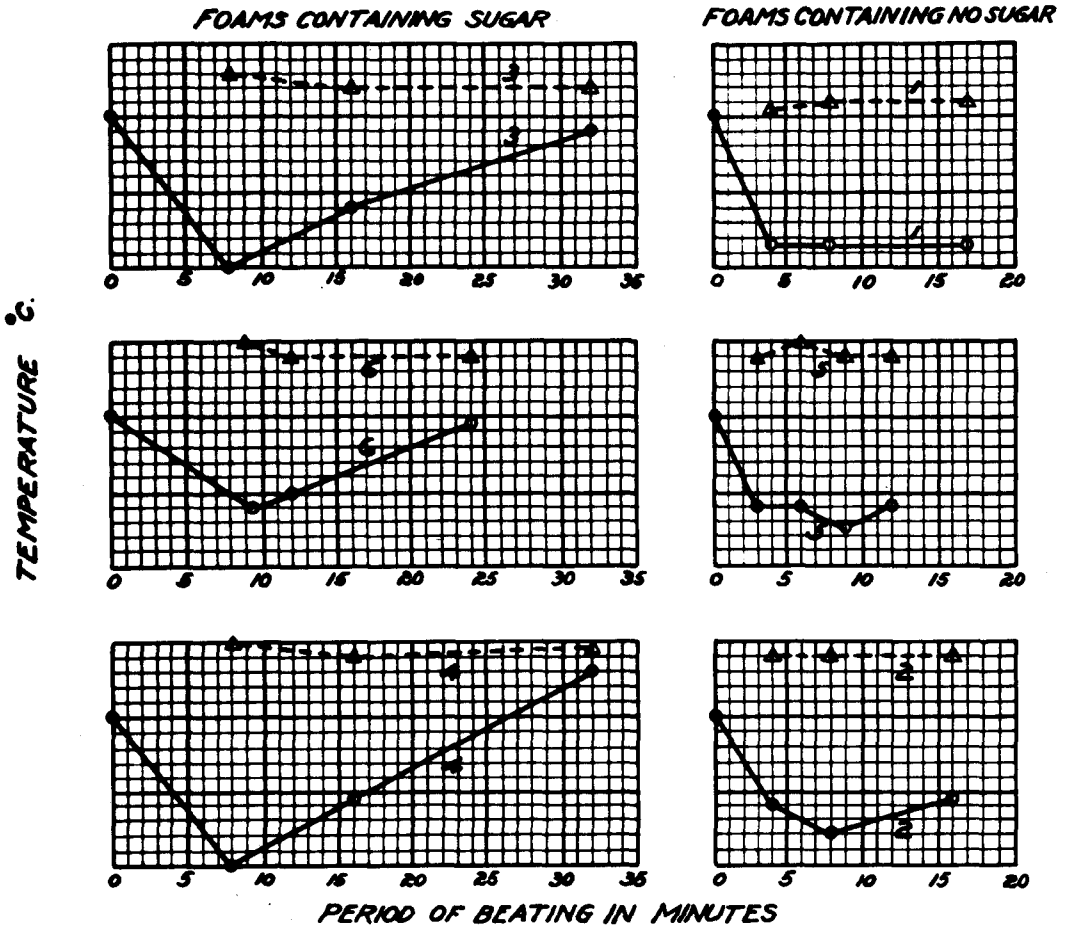

Figure 2. Variations in Temperature of the Room and of the Egg White Foams. Each point represents the average of seven or eight foams. The dotted lines are room temperatures; the solid line, foam temperatures. Foams of curves 1 and 5 contain egg white alone; curves 3 and 6 egg white and sugar; curve 2, egg white and iodosobenzoate; curve 4, egg white, sugar and iodosobenzoate.

The stiffness scores for Series I and II were tabulated in Tables E, F, and 2 and, also, presented in graphic form in Figure 3. The addition of either sugar or iodosobenzoate reduced the stiffness attained at every period of beating. The effect of sugar appeared to have been exerted in the early part of the beating period for on Figure 3, curves 2, 4 and 6 all show a lag in foam formation. It required more than 9 minutes to incorporate all the liquid egg white into foam when 50 per cent sugar was present whereas egg white alone required about 3 to 4 minutes. The scores obtained in Series I at 8 minutes beating illustrate the reduction in stiffness: the score for egg white alone was 2.5; for egg white plus iodosobenzoate, 1.6; for egg white plus sugar, 0.8; and for egg white with both added, 0.6. Approximately equal scores were given for the foams of egg white alone beaten 16 minutes as for the ones with sugar beaten 32 minutes.

In Series II, the same relation was obtained. Equal scores were given to egg white beaten 3 minutes and to egg white with 50 per cent sugar beaten 9 minutes: an approximately equal degree of stiffness was produced by 12 minutes beating of the control as by 24 minutes when sugar was added. When compared at the same beating time, there was no case in which a smaller stiffness score was given to a foam of egg white alone than to the foams containing an additional substance.

The amount of expansion attained by beating gave a less subjective measure of the ease of foam formation. These data have been presented in Tables G, H and 3 and in Figure 4. The expansion factor represents the multiple by which the initial volume increased, i.e., a factor of 3

Table 2. Summary of Stiffness Scores of Egg White Foams

Addition	None		50% sugar		Iodosobenzoate		Iodosobenzoate and 50% sugar	
Period of beating in minutes	Range	Average	Range	Average	Range	Average	Range	Average
Series I								
4	1.1-2.4	1.9	-	-	0.8-1.7	1.2	-	-
8	2.2-2.8	2.5	0.3-1.0	0.8	1.2-2.2	1.6	0.3-1.3	0.6
16	2.8-3.0	2.9	1.7-2.6	2.3	1.6-2.5	2.1	1.2-1.8	1.5
32	-	-	2.9-3.0	3.0	-	-	1.8-2.7	2.2
Series II								
3	0.8-1.0	0.9	-	-				
6	1.3-2.2	1.8	-	-				
9	2.2-2.5	2.3	0.5-1.1	0.9				
12	2.5-2.8	2.7	1.1-1.7	1.2				
24	-	-	2.4-2.9	2.8				

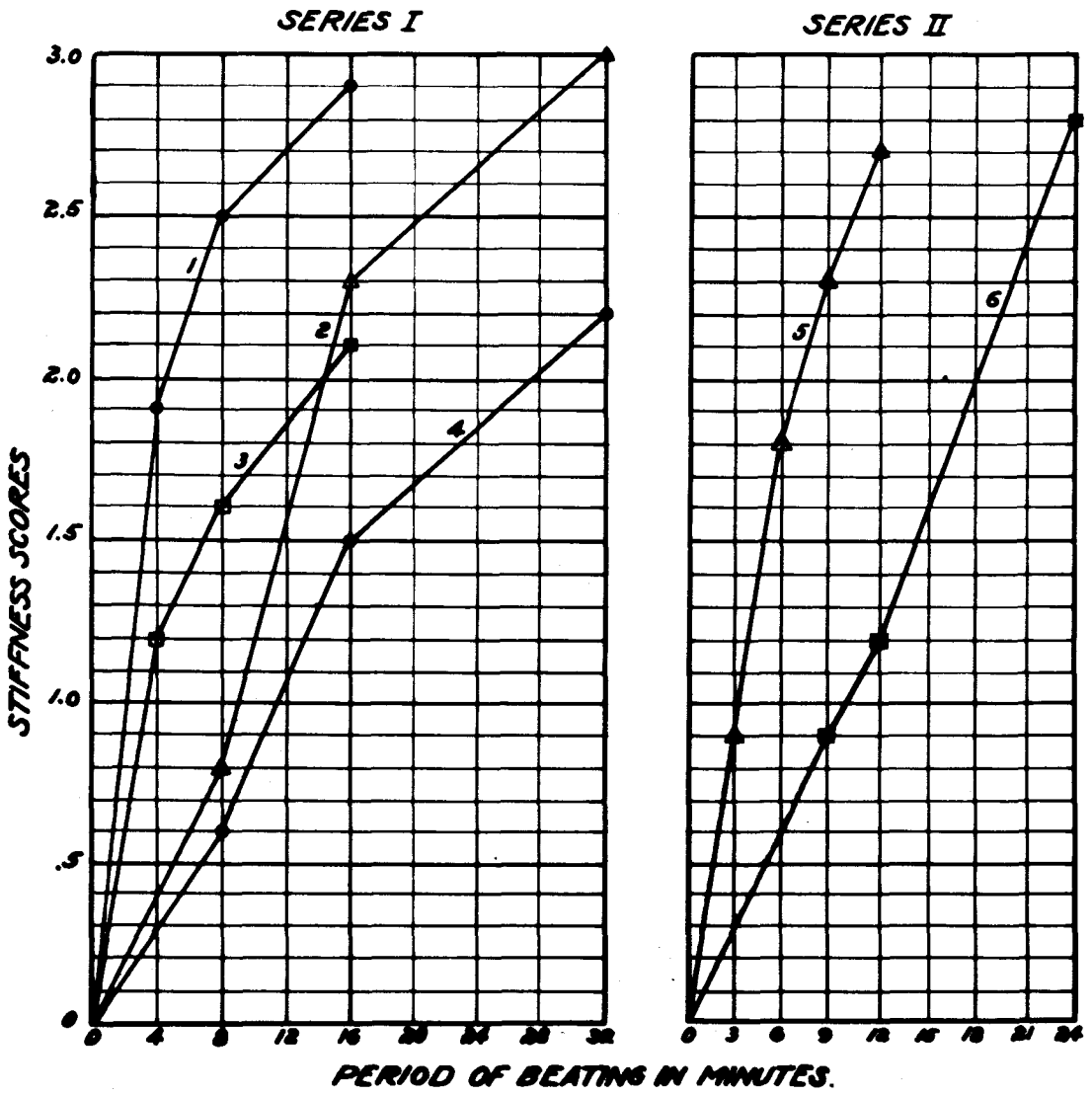

Figure 3. The Development of Stiffness in Egg White Foam. Each point represents the average of eight foams. Foams of curves 1 and 5 contain egg white alone; curves 2 and 6, egg white and sugar; curve 3, egg white and iodosobenzoate; curve 4, egg white, sugar and iodosobenzoate.

Table 3. Summary of Expansion Factors

Addition	None		50% sugar		Iodosobenzoate		Iodosobenzoate and 50% sugar	
Period of beating in minutes	Range	Average	Range	Average	Range	Average	Range	Average
Series I								
4	6.8-8.2	7.3	-	-	5.6-7.4	7.0	-	-
8	7.0-8.9	7.6	2.1-4.4	3.1	6.8-8.4	7.8	2.0-2.5	2.2
16	9.7-11.9	10.6	6.3-7.5	6.8	7.8-8.7	8.2	6.5-7.0	6.8
32	-	-	7.6-11.4	9.7	-	-	7.9-9.6	8.9
Series II								
3	5.3-6.0	5.5	-	-				
6	6.0-7.2	6.7	-	-				
9	7.0-7.5	7.3	2.5-6.4	4.7				
12	7.5-8.1	7.8	5.8-6.4	6.2				
24	-	-	8.4-9.6	9.0				

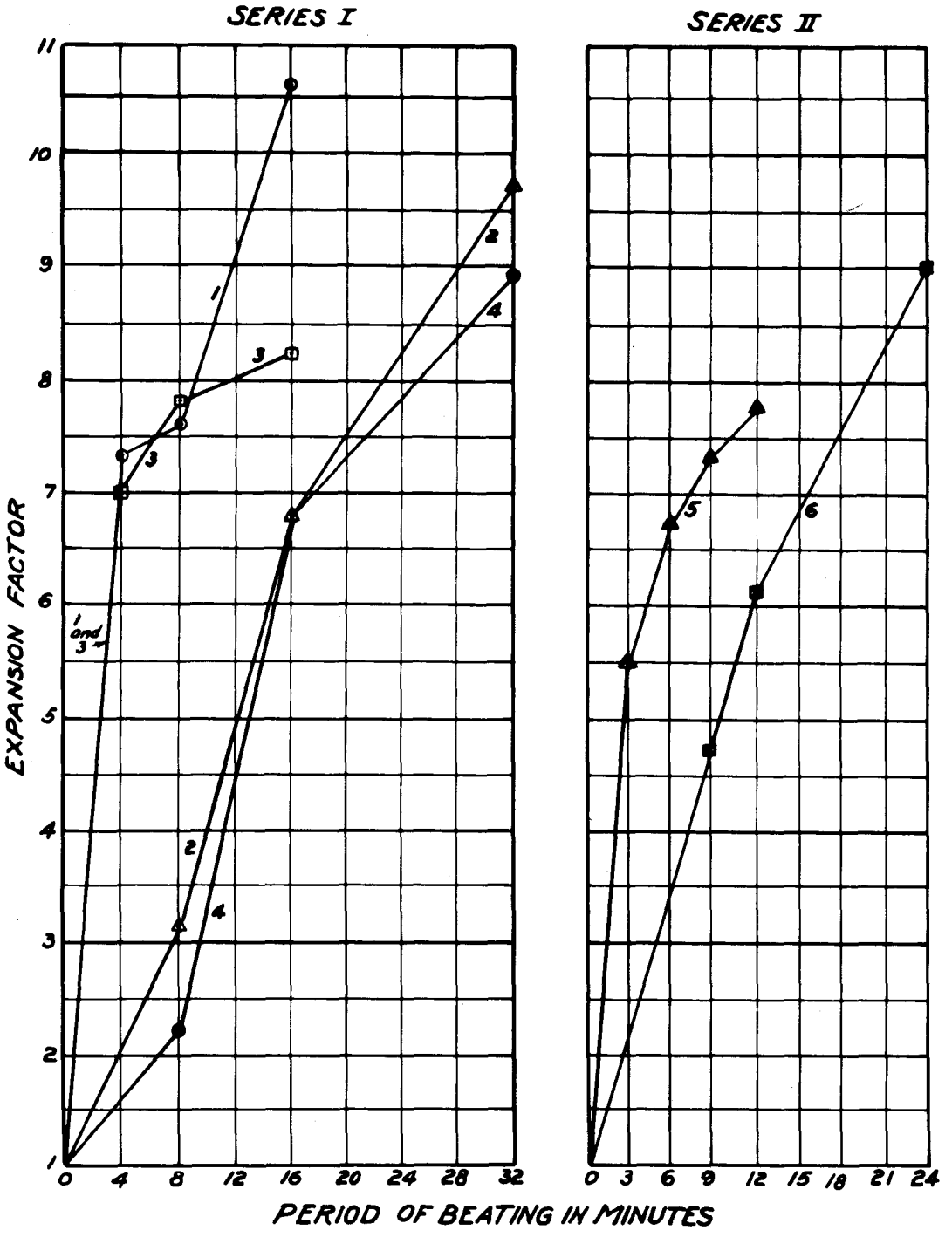

Figure 4. The Expansion of Egg White Foams. Each point represents the average of eight foams. Foams, of curves 1 and 5 contain egg white alone; curves 2 and 6, egg white and sugar; curve 3, egg white and iodosobenzoate; curve 4, egg white, sugar and iodosobenzoate.

means that the final volume was three times as large as the initial. Here again, the lag in the early stages of foam formation due to the presence of sugar was illustrated as shown by curves 2, 4 and 6 in comparison with curves 1, 3 and 5 respectively. Also, in the summary, Table 3, it may be seen that at every comparable beating period, not only the average but also the entire range of individual foams were higher in expansion factors for the foams without sugar than for those with sugar added. This method of measuring ease of foam formation confirmed the conclusion obtained by the more subjective method of scoring stiffness that sugar delayed the early stages of foam formation. The high value on Curve 1 was thought to be in error since curve 5 in Series II showed no such trend. The foam beaten 16 minutes was stiff and dry and did not pack into the measure satisfactorily. The effect of iodosobenzoate was not as marked when judged by expansion factors as by the stiffness scores.

The shape of the curves in Figure 4 suggested that expansion of the foams with beating was an exponential relationship of the form, $y = a + b \log x$. Accordingly, using the expansion factors and the logarithm of the periods of beating, the data were plotted in Figure 5. As in Figure 4, curve 1 gave evidence of the inaccuracy of the data for the foams which were beaten 16 minutes with no addition. These foams were too stiff and dry to pack into the measure without including large air spaces. The other data were seemingly well-defined by the linear relationship of expansion factors and the logarithms of the beating periods. In all cases, the slopes of the lines for foams containing sugar appeared to be uniform

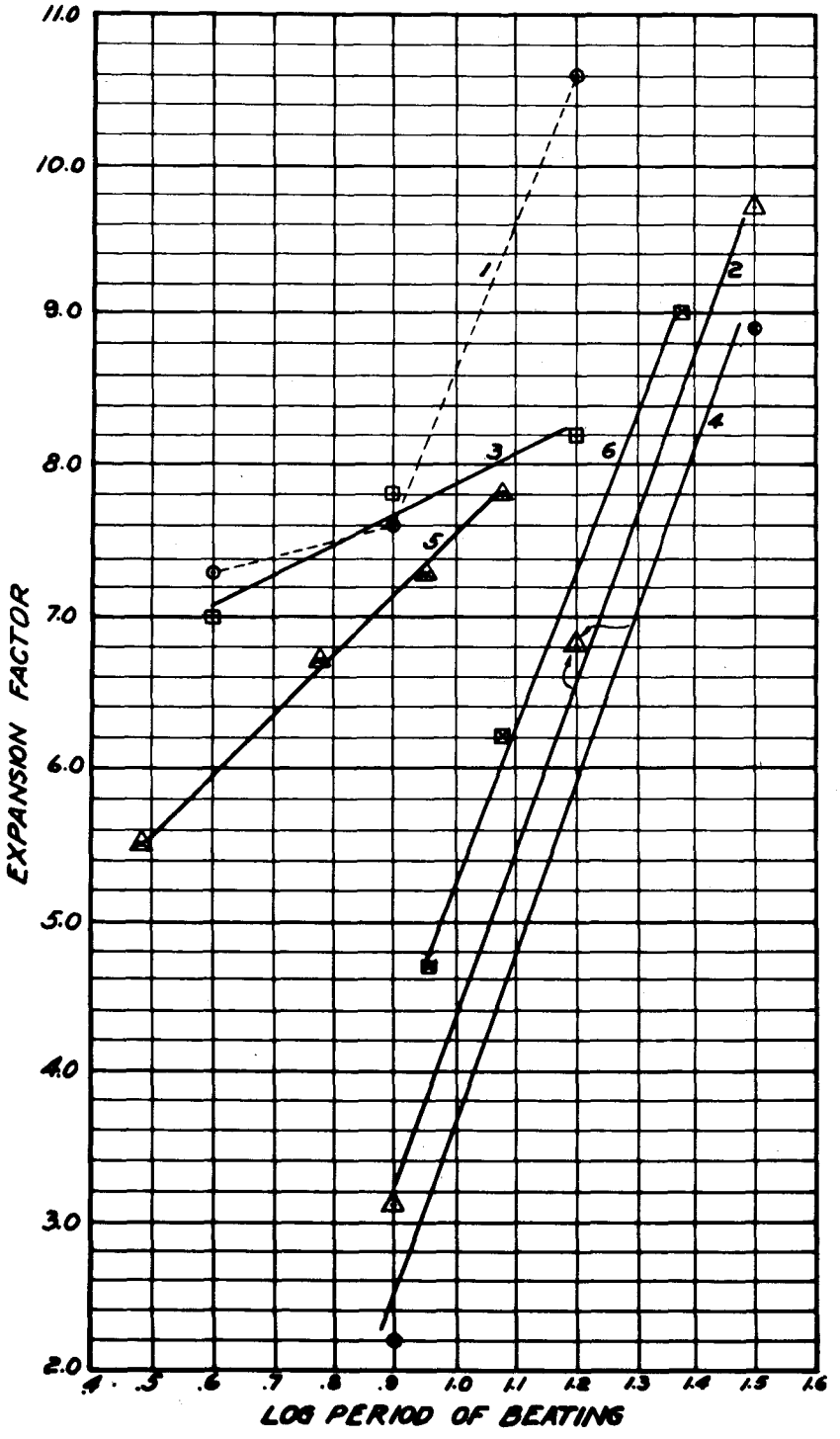

Figure 5. The Relation of Expansion Factors of Foams to Logarithms of Periods of Beating.
Each point represents the average of eight foams. The foams of curves 1 and 5 contain egg white alone; curves 2 and 6, egg white and sugar; curve 3, egg white and iodosobenzoate; curve 4, egg white, sugar and iodosobenzoate.

and greater than the slopes of the lines defining foams of egg white alone or with iodosobenzoate added.

Stability of the foams

A foam may have been unstable and hence have given a greater amount of liquid draining because it had not been beaten sufficiently to incorporate thoroughly the liquid or because it had been beaten too much. In the latter case, the bubbles would break faster and liquid be released. The data obtained on drainage of liquid from egg white foams and presented in Figure 6 and Tables I, J and 4 give several examples of each type. Curves 2, 3 and 5 showed values of higher drainage from both causes with a lower value at a period of beating between them. Curves 4 and 6 showed examples of instability due to underbeating only. It is evident from the curves in Figure 6 that the addition of either sugar or iodosobenzoate prolonged the period before the point of low drainage was reached but reduced the total amount of liquid drained markedly. Sugar produced a remarkable effect as is evident in the following comparisons.

		Period of beating to reach maximum stability minutes	Amount of liquid in gm.
Series I	Egg white + sugar	16	2.4
	Egg white alone	4	7.4
Series II	Egg white + sugar	24	2.1
	Egg white alone	6	8.4

Thus, in each series, it took four times as long to reach a point of maximum stability when sugar was present. At these points, the amount of liquid draining in one hour was 1/3 to 1/4 as much.

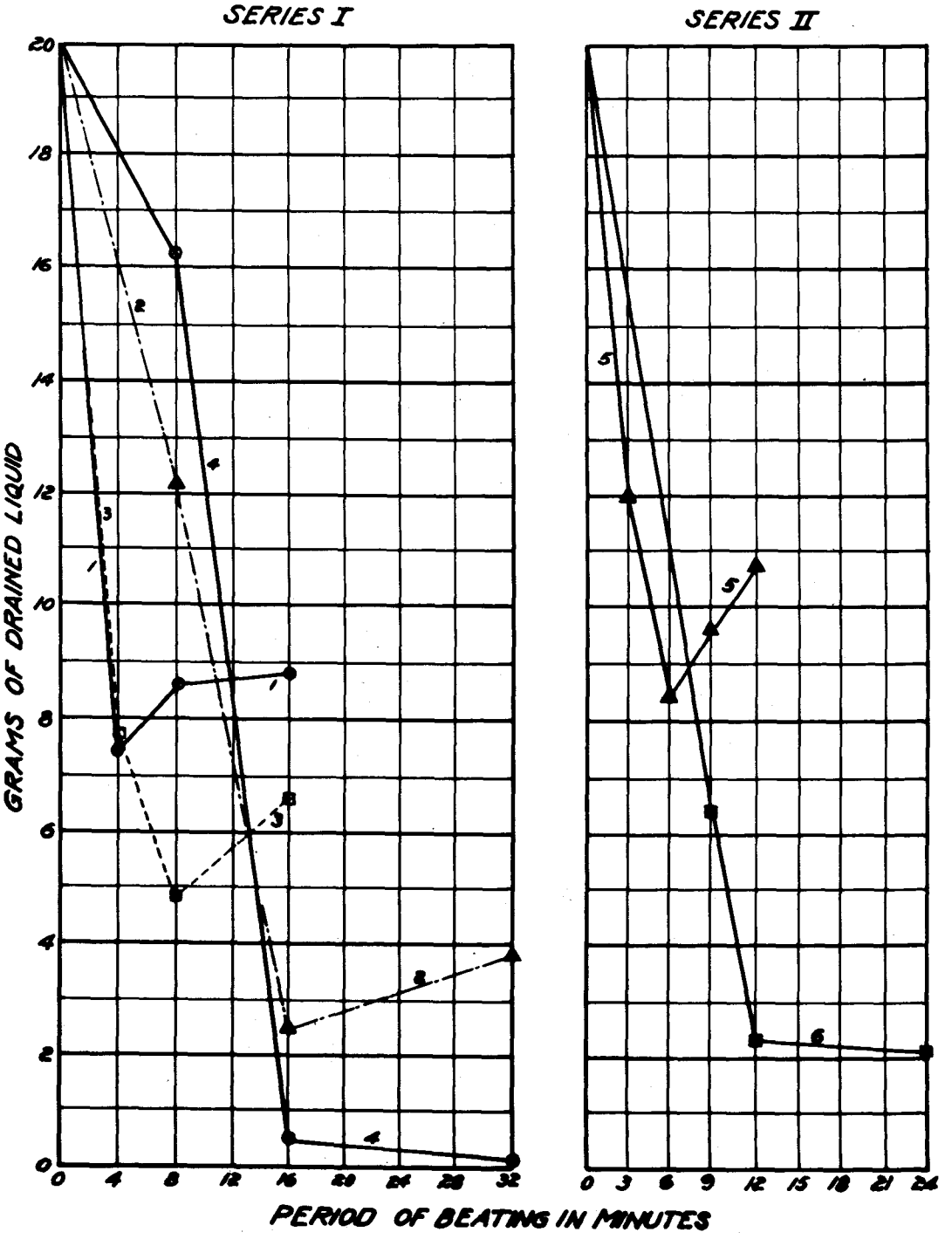

Figure 6. The Amount of Drainage from Egg White Foams. Each point represents the average of eight foams. The foams of curves 1 and 5 contain egg white alone; curves 2 and 6, egg white and sugar; curve 3, egg white and iodosobenzoate; curve 4, egg white, sugar and iodosobenzoate.

Table 4. Summary of Amount of Drainage from Egg White Foams

Addition	None		50% sugar		Iodosobenzoate		Iodosobenzoate and 50% sugar	
Period of beating in minutes	Range	Average	Range	Average	Range	Average	Range	Average
Series I								
4	5.7- 9.3	7.4	-	-	5.0-10.5	7.8	-	-
8	7.1-10.1	8.6	4.4-16.4	12.1	2.2- 7.1	4.8	14.7-17.3	16.2
16	6.9- 9.5	8.8	1.8- 3.0	2.4	5.3- 8.1	6.6	0.1- 0.8	0.5
32	-	-	3.3- 4.7	3.9	-	-	0.0- 0.3	0.1
Series II								
3	10.0-13.9	12.0	-	-				
6	6.6-10.0	8.4	-	-				
9	7.8-11.5	9.6	2.0-14.8	6.4				
12	10.0-11.5	10.7	1.1- 4.1	2.3				
24	-	-	0.3- 2.7	2.1				

A second measure of foam stability employed in this study was to take the weight of the foam remaining after overnight drainage. These data are to be found in Table K and summarized in Table 5 as follows:

Table 5. Summary of Weights of Drained Foam

Addition Period of beating in minutes	None Range	None Average	50% sugar Range	50% sugar Average
Series I				
4	1.3-3.0	2.1	-	-
6	1.9-3.5	2.5	1.4-4.7	1.8
16	2.1-3.3	2.5	2.6-3.9	3.3
32	-	-	2.7-3.8	3.2
Series II				
3	0.9-1.5	1.1	-	-
6	1.7-2.7	2.2	-	-
9	1.9-2.9	2.2	1.2-4.6	2.7
12	1.1-2.9	2.0	2.6-4.7	3.7
24	-	-	2.6-3.3	3.1

Thus, even in the foams which were most stable during the hour drainage test, there was little material left after overnight drainage. At least 80 to 90 per cent of a foam, therefore, is in the liquid phase even when beaten stiff and dry. Again the foams with sugar evidenced a slower formation of coagulated protein since it required 12 to 16 minutes of beating to attain the maximum quantity of drained foam. No attempt was made to determine the proportion of sugar in the drained foam when that substance

had been added during the beating. In general, the points of minimum drainage occurred at about the same period at which the foams first showed maximum weight of drained foam, 4 to 8 minutes for the foams without sugar, 12 to 24 minutes for the foams with sugar. In the foams with sugar of Series I and the foams without sugar of Series II, the points were exactly the same.

Evidence has been presented of a retardation of coagulation in egg white foams in the presence of sugar. It has been shown that foams with sugar differ in the longer beating time required for their formation and in greater stability than foams without sugar. The stability of an egg white foam in the baking of a food, however, is affected by certain other conditions which were not measured by these tests, the factors of elasticity and expansion with rise in temperature. This fact explains why, in cookery, the foams of greatest stability in these tests are not the most desirable. Nevertheless, the influence of sugar in stabilizing the foam can be studied by this method and later confirmed in cooking tests, for the plastic and less "set" nature of the foams with sugar suggests a greater elasticity.

Physical Characteristics of Egg White Foams Containing Salt

Less extensive data were obtained on the foams containing salt than sugar. The foams to which salt was added were much like those without it in their characteristics of friability, fast drainage and "set"

texture. There seemed to be, in fact, a greater speed in separating a liquid layer. There was no evidence of increased softness or plasticity as was true of the foams containing sugar or iodosobenzoate. In fact, the changes in the general texture and appearance due to the addition of salt were opposite to those produced by sugar.

Only two periods of beating were used for these comparisons for which the following data were obtained. Each value is the average of seven or eight foams. The detailed results were tabulated in Table L.

Table 6. Summary of Physical Characteristics of Foams Containing Salt

Period of beating in minutes	Addition	Stiffness score		Expansion factor		Amount of drainage in grams	
		Average	Range	Average	Range	Average	Range
6	None	1.8	1.3-2.2	6.7	6.0-7.2	8.4	6.6-10.0
6	2 gm. salt	1.7	1.2-2.0	6.4	5.8-7.1	11.2	9.0-13.0
9	None	2.4	2.2-2.7	7.3	6.7-7.9	10.1	9.4-10.8
9	2 gm. salt	2.3	2.0-2.5	6.4	5.7-7.1	11.2	10.2-11.7

While the expansion factors showed a tendency for less ease of foam formation, the data are not sufficiently extensive for a significant difference. The drainage at 6 minutes beating was considerably higher than for foams containing no salt; at 9 minutes, this difference was smaller.

Thus, the foams with and without salt were much alike, differing only in minor degree for all characteristics except that of stability. The addition of salt produced none of the effects which sugar had been shown to produce.

Chemical Determinations to Assess the Degree of Denaturation

Iodosobenzoate method

As was stated in the procedure, this method as used was not controlled carefully enough to warrant the assumption that the values obtained are specific and precise measurements of the sulphydryl groups liberated in the beating of egg white foams. The results have been presented in detail in Table M and in a summary as follows:

Table 7. Reducing Value Calculated as Mg. Cysteine for 1 Gm. Egg White

Beating period in minutes	Addition	Reducing value mg/1	Increase above 0.74 mg.
0	None	0.74	0.0
4	None	0.86	0.12
8	None	0.90	0.16
16	None	0.90	0.16
0	50% sugar	0.86	0.12
8	50% sugar	0.84	0.10
16	50% sugar	0.90	0.16
32	50% sugar	0.94	0.20

Two comparisons, the difference between 0 and 4 minutes of beating with no sugar and between samples of unbeaten egg white with and without sugar, were tested for statistical significance. The value of t for the significance of differences between pairs was computed (52). The t value for the differences between the period of beating, 0 and 4 minutes, with no sugar was 2.04; for the unbeaten egg white, with or without sugar, 1.38. A value of 2.36 for t is necessary before the difference can be stated as

statistically significant (5 per cent level) on the basis of eight determinations. Neither comparison, thus, was statistically significant.

These data, however, suggest the possibility that the major portion of the denaturation produced in beating the egg white may occur in the early stage of beating and that longer beating would give no further increase in reducing value. The high value of the unbeaten egg white to which sugar was added, if confirmed by a more specific and accurate method, would raise the question whether sugar itself might be a denaturant.

Ferricyanide method

This method had several disadvantages for its use in this study. The ferricyanide could not be added to the egg white before it was beaten because of the instability of the ferrocyanide formed to such great exposure to air as necessarily occurred. The dilution at which ferricyanide reacted best was far too great to permit the formation of a good egg white foam. Another disadvantage was that in the acid solution required for tungstic acid precipitation of the proteins, the sugar gave such high reducing value as to overwhelm the very small effect of the activated sulfhydryl groups in the protein components. The ferricyanide method, therefore, was employed for a series of foams with or without salt but with no sugar additions.

The determinations from which reference curve of Figure 7 was computed have been given in Table N in terms of milliliters of 0.0002 N ferrocyanide or cysteine. In Table O has been included the data from Series II, exclusive of the foams containing sugar. The averages for the eight foams for each period of beating in Series II have been summarized as follows:

Period of beating in minutes	Ml. equivalent of 0.0002 N cysteine per gm. egg white	Mg. cysteine per gm. egg white
0	0.18	.0044
3	0.31	.0075
6	0.32	.0076
9	0.31	.0076
12	0.35	.0084

These data indicate a relative increase in sulfhydryl groups activated in the initial stage of beating as had been suggested by the iodosobenzoate method. The total quantity measured was low and the data varying, yet the difference was great enough to give statistical significance (at 5 per cent) when the t test for differences between pairs was applied (52).

The addition of salt gave lower values for active sulfhydryl groups than the controls without it. The data have been included in Table N. A further comparison was made on foams beaten 9 minutes, all the egg white being taken from one mix. The differences due to the addition of salt to foams beaten 6 minutes were not statistically significant while those beaten 9 minutes were statistically significant. These results are confusing. Since the method as applied under these conditions has

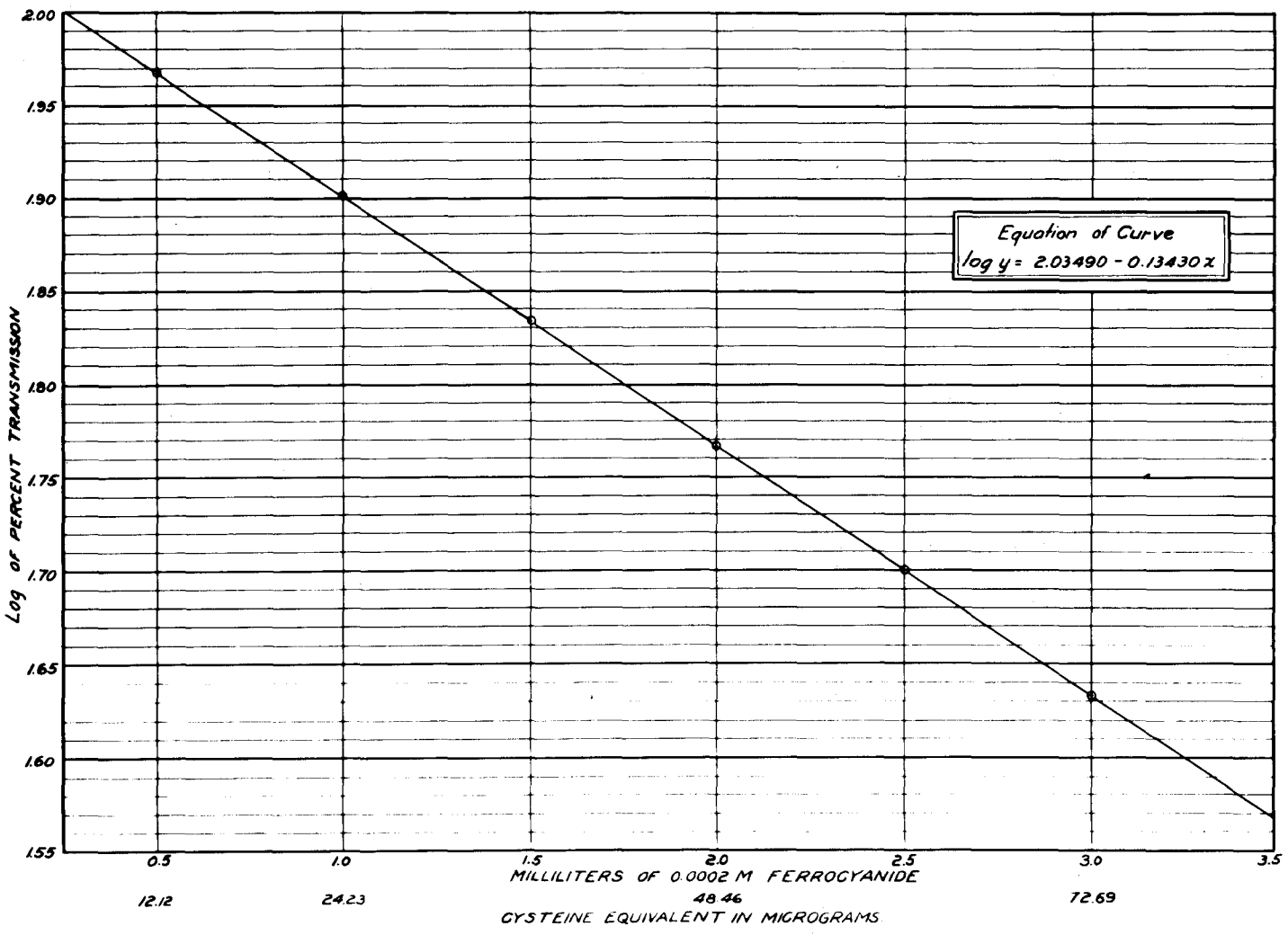

Figure 7. Reference Curve for Ferricyanide Method. The data and the calculation of the equation are given in Table N in the appendix. The points represented were calculated from this equation. The points actually obtained varied from these by 0, 0.003, 0.01, 0.008 and 0.002 respectively on the y axis.

given such low values with so much variation in the other tests, it is questionable how much confidence can be placed in the significant difference due to the addition of salt. It may be recalled, however, that Burk (24) had shown a retarding effect on sulfhydryl liberation due to the addition of salt.

Neither method employed to assess the degree of denaturation has given satisfactory results. The experience obtained in the trial of these two methods has indicated that a satisfactory method must be more sensitive and more specific than the iodosobenzoate method and the oxidant must be present during the beating process.

DISCUSSION

One fact is indicated in every comparison of physical measurements made on the egg white foams. There is a retardation in the formation of coagulated protein when sugar is present. This was shown in the greater amount of beating required to produce any measurable weight of drained foam. While this weight is not an accurate measure of the amount of protein coagulated, since it still contains liquid egg white, a lack of drained foam shows the absence of coagulation. The retardation of coagulation in the presence of sugar is the explanation for the slow stiffening of the foam and the delayed development of stability. Expansion, too, lagged at first in the foams containing sugar because there was insufficient coagulated film to hold the bubbles of air. The presence of sugar, therefore, retards the coagulation of egg white when beating is the coagulating agent employed, as had been reported for heat coagulation (15, 17, 18).

For albumin, the production of a coagulum must be preceded by denaturation. Therefore, the action of sugar may be to retard the denaturation of the protein, that is, to delay the extension of the compact structure of the native albumin into the rod-shaped configuration of the denatured form. Or, sugar may act to obstruct the aggregation of the extended form of the denatured protein into a coagulated film. Among sugars, sucrose is most inert chemically. It has no free aldehyde group

available for oxidation or condensation reactions nor will it hydrolyze, in the alkaline medium of egg white, to more active sugars. The predominant characteristic which might explain the anti-coagulating or peptizing action of sucrose is the large number of polar groups in the molecule. The proportion of eight hydroxyl groups to twelve carbon atoms gives sucrose great solubility and the well-known characteristic of forming aggregates with water. The number of polar groups available on the soluble native protein and on the sucrose molecule would give ample sites for hydrogen bonding, which suggests that sucrose and the native protein would tend to form aggregates. If an aggregate formed between the denatured protein and sucrose, it might be expected to be more soluble than the denatured protein itself and to explain the retarded development of a coagulum. Aggregates of both native and denatured albumin with detergents are known to be formed and to vary in solubilities, depending upon the proportions of each present, the pH and temperature (47, 40). These workers postulate that van der Waal forces between non-polar groups of the detergent and native protein play a part in complex formation between those two substances.

The plasticity of foams containing sugar or iodosobenzoate, in contrast to those with neither or to those containing salt, raises a question of interest in cookery. Increased ease of blending in the preparation of angel or sponge cakes or souffles is a characteristic greatly desired. Iodosobenzoate is an active oxidant, sugar a rather inert substance, chemically, in the alkaline range yet each induces greater plasticity in the egg white foam. A trial was made of beating

egg white to which iodine in concentrated potassium iodide solution was added. Again, a plastic foam was obtained. The effect was not due to the presence of the potassium iodide nor, in the case of iodosobenzoate solution, to the potassium hydroxide for controls with those electrolytes gave foams of the same texture and appearance as with no addition or with salt added. The presence of duponol in egg white (1 gm. solid technical duponol or 5 ml. of 10 per cent solution per 80 gm. egg white) also gives a plastic foam but one which is not stable. A plastic foam is probably one in which the film is less stiff, perhaps one in which fewer or weaker linkages are present in the coagulated protein. Whether iodosobenzoate or iodine forms some substituted sulfur derivative or induces the formation of some complex, or whether sugar does, must be left to further research.

The chemical methods employed have given little insight into the mechanism by which sugar retards coagulation. In the ferricyanide method, low values for active sulfhydryl groups were obtained. Some reducing groups were shown to be present in the unbeaten egg white. The significant increase due to the 3 minutes of beating, 0.003 mg. per gm. egg white, represents a denaturation of 3 per cent of the protein present, based on the assumption that the protein, on the average, would give about 1 per cent sulfhydryl calculated as cysteine if completely denatured. The conditions under which the determinations were made may not have given maximum color development for the active sulfhydryl groups present. The color in tungstic acid solution is known to vary with

temperature, time of reaction, pH and concentration of protein and ferricyanide. Within the limits of experimental error of the volumetric measurements and weighing involved, these were kept constant, except for room temperature. In any one replicate, however, all foams were tested at the same room temperature so comparable results should have been obtained.

The high reducing value obtained in the iodosobenzoate method on unbeaten egg white needs explanation. The assumption had been made that while there would be some reduction due to certain constituents of unbeaten egg white, the increase in reducing value might be assigned to the effect produced by beating. Calculation has been made from the analyses given by Howe and Titus (37) of the amount of the various proteins, the kind and content of sugar in them and the free glucose in egg white, that egg white would contain about 0.6 per cent reducing sugars. These might be expected to react with iodosobenzoate to some extent even at room temperature. That the addition of hydrochloric acid may produce some denaturation is suggested by the work of Burk (24) while iodine in the presence of potassium iodide can oxidize native egg albumin, according to Anson (3, 5). In addition, at pH = 8.5 or 9.0, other amino acids as cystine, tryptophane or tyrosine, may react. Nothing is known about the reactions of the reducing groups of proteins except albumin. These other proteins constitute 30 per cent of the protein content of egg white. The sum total of the various reductants was high as judged by the value obtained by the iodosobenzoate method on

unbeaten egg white. A possible overstepping of the true endpoint in the titration may have contributed to this high value.

This exploratory study has given evidence of an effect of sugar in retarding coagulation of the proteins in egg white foams, but no evidence on the mechanism by which this is done. The addition of salt gives a different appearance and texture to the foams than sugar does. The lower value for active sulfhydryl groups suggested by the ferricyanide tests, if confirmed, would indicate that the salt effect occurred in denaturation proper, that is, in the extension of the compact structure into a rod-shaped form. The greater instability of the foams with salt denotes a lessened tendency to bind liquid in the film.

SUMMARY

The effect of the addition of sugar or of salt on certain physical characteristics of egg white foams has been determined and an exploratory study made upon the degree of denaturation induced by beating for various periods of time. A uniform mix was prepared of the whites of 40 to 58 eggs which ranged in quality from 75 to 90 Haugh units and was used for two consecutive days' tests. In Series I, the beating period was varied from 0 to 32 minutes; in Series II, from 0 to 24 minutes. Only one amount of each substance was added, 50 per cent sugar or 2 gm. table salt to the 80 gm. of egg white used for each foam. The average of seven or eight foams was used in each case.

The ease of formation of the foam was judged by a subjective scoring of stiffness and by a comparison of the expansion of the foam after various periods of beating. The addition of sugar required a longer period of beating to form the structure of the foam as judged by each of these methods. The effect of sugar seemed to be more marked in the early part of the beating period. It required 3 to 4 minutes of beating to incorporate all the liquid into foam for egg white alone, whereas, with 50 per cent sugar, more than 9 minutes beating was required. The scores were assigned by tenths from zero to three, a score of one being given when all the liquid was incorporated into foam, two when the peaks were straight and stiff,and three when the foam was stiff and dry. The

reduction in stiffness due to sugar may be noted from the foams beaten 8 minutes; for egg white alone, the average score was 2.5; for egg white plus sugar, 0.8. Approximately equal scores were given for foams without sugar beaten 16 minutes as for the ones with sugar beaten 32 minutes; for foams of egg white alone beaten 3 minutes as for those with sugar beaten 9 minutes; or, for foams beaten 12 minutes with no sugar as for those beaten 24 minutes with sugar.

The expansion factors which designate the multiple by which the initial volume had increased during the beating, also show the retardation due to the presence of sugar. Volumes had increased from six to nine times for foams of the stiffness used in cookery. At every period of beating the foams containing sugar had expanded appreciably less than those without it. A linear relationship was apparent when the expansion factors were plotted against the logarithms of the beating periods.

The stability of the egg white foams was determined by weighing the liquid draining in one hour from 20 gm. of the foam. Examples were obtained of foams which had a high drainage at a short beating period and thus were unstable due to underbeating and of foams which were beaten long and were unstable because of overbeating. There appeared, thus, to be a region of minimum drainage or maximum stability. For foams containing sugar, the data show only one example of lessened stability due to overbeating. It required about four times as long a beating period to reach maximum stability when sugar was present but the amount of liquid draining in one hour was about 1/4 to 1/3 as much as from the control foams with no sugar.

The weight of foam remaining after overnight drainage also was less for the shorter periods of beating of foams containing sugar. Thus, one can conclude that the presence of sugar retarded coagulation of the egg white proteins since it required longer beating to produce a measurable amount of drained foam. When the amount of drained foam showed no further increase with beating, there was still 80 to 90 per cent or more liquid incorporated with the coagulated protein in the foam.

The effect of the addition of salt was compared at two periods of beating only, 6 and 9 minutes. The only appreciable difference in the physical measurements due to the addition of salt was a lessened stability of the foam beaten 6 minutes.

The texture and plasticity of the foams with sugar or salt were quite different. The foams containing sugar were much more plastic and less friable than those with salt or with no addition. The presence of the oxidizing agent, iodosobenzoate, also, was correlated with a more plastic foam.

Two chemical methods were employed in an attempt to assess the degree of denaturation produced by the beating. In the first method, a concentrated solution of iodosobenzoate was added to the egg white before beating, in order to oxidize the reducing groups activated during the production of the foam. Specificity for the sulfhydryl groups required that the reaction occur at pH 7 but it was sacrificed in favor of working with the egg white at its natural alkaline medium. The excess oxidant in the foam was determined by titrating the iodine it

released from potassium iodide with 0.01 N thiosulfate. The data suggest that the major portion of the denaturation occurs in the early part of the beating period but the difference was not statistically significant. The statistical method used in each case was the calculation of the *t* value for differences between pairs. The iodosobenzoate method yielded a very high reducing value for unbeaten egg white. A discussion is given of the various constituents which may have contributed to the reducing value. Unbeaten egg white to which sugar was added gave a still higher value; the difference from the control without sugar, however, was not statistically significant. The effect of sugar on unbeaten egg white suggested by these data is a question which needs careful study by more specific and precise methods. The iodosobenzoate method was not satisfactorily specific or precise.

The second method employed was the ferricyanide oxidation of sulfhydryl groups at pH 7 and determination made spectrophotometrically after the removal of the protein by tungstic acid precipitation. Since the oxidant was not present during the beating of the foams, relative rather than total values were obtained of the amount of denaturation produced by the beating. This method could not be applied to the foams which contained sugar because of the hydrolysis of sugar to reducing sugars produced by the tungstic acid. The data showed the greatest increase in sulfhydryl groups with a beating period of the first three minutes. This difference was found to be statistically significant and represented a denaturation of about 3 per cent of the protein present if it is assumed that all the proteins in egg white react as does albumin.

Since the conditions of this method allow only an incomplete measure of the total amount of sulfhydryl activated, the amount of denaturation produced by three minutes of beating is somewhat greater than 3 per cent. The addition of salt produced a significantly smaller active sulfhydryl content in the egg white foams of one series while in another series, the result was lower but not statistically significant.

That the effect of sugar is to delay coagulation of egg white foams is demonstrated in this study. The inhibition of coagulation by sugar has been shown previously by other workers for heat denaturation. The retardation of coagulation has a definite measure in the longer period of beating required to produce any measurable amount of drained foam. The delay in coagulation of protein is the suggested explanation for the slow development of stiffness, for the retarded expansion of the foam in the early stages, for the longer beating period required to reach maximum stability and for the less friable but more plastic texture of the foams containing sugar. A discussion is given in terms of the structure of albumin molecules of the question as to the step in which the sugar effect is exerted. No conclusion can be stated, as yet, as to whether sugar retards the extension of the globular structure of the native protein into the denatured form or whether sugar blocks the intermingling and clumping of the denatured protein structures into a coagulum.

CONCLUSIONS

1. There was considerable variation in egg quality measured in terms of Haugh units. The procedure of discarding eggs with Haugh units below 75 or above 90, gave egg white mixes of more uniform quality.
2. Sugar affects the stiffness, expansion and stability of egg white foams. Both the expansion and the development of stiffness showed a lag in the early stages of foam formation when sugar was present.
3. The expansion of the egg white foam with beating may be represented by a linear relation when expansion factors are plotted against the logarithms of the period of beating.
4. A foam may be unstable because of too little or too much beating. A longer beating period was required to reach maximum stability when sugar was present in the egg white foam as when absent, but such foams were much more stable.
5. In contrast, the addition of salt increased instability. In other respects, the physical characteristics were alike for foams, with or without salt.

6. The appearance and texture of egg white foams was modified by the presence of sugar or iodosobenzoate, giving in each case a more plastic and less friable foam.

7. The addition of sugar had the effect of retarding the coagulation of proteins during the beating of egg white foams, as was shown by the lessened amount of drained foam produced in the early stages of beating. The slow coagulation of protein in the presence of sugar may explain the reduced stiffness and expansion in the early stages of beating.

8. An egg white foam contains at least 80 to 90 per cent of liquid free to drain.

9. Neither of the chemical methods were satisfactory in measuring the degree of denaturation produced by beating. The iodosobenzoate method, as used, was lacking in specificity and precision. The data suggested a high reducing value for unbeaten egg white and an increase with four minutes of beating.

10. On the basis of the values obtained by the ferricyanide method, some active sulfhydryl groups were present in unbeaten egg white and a significantly greater amount were present in the foam beaten 3 minutes. Calculation of the amount of

protein corresponding to this increase indicated that more than 3 per cent of the protein was involved in the denaturation.

11. This study gave no indication of whether the effect of sugar was exerted in the denaturation of the protein or in the subsequent flocculation or coagulation.

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ACKNOWLEDGMENTS

The author wishes to express her appreciation to her major professor, Miss Belle Lowe, for her suggestions and guidance throughout this study; to Dr. Julia Southard Lee and Dr. George W. Stewart for their courtesy in sharing laboratory facilities for a portion of this work.

APPENDIX

Table A. Foam Temperatures - Series I

Addition	None				Sugar 50%			
Period of beating in minutes	0	4	8	16	0	8	16	32
A^1	25.0	23.5	22.5	23.3	25.0	23.0	24.5	26.0
B^1	25.0	22.8	23.0	25.0	25.0	23.2	23.5	25.5
C_1	25.0	23.8	22.8	22.7	25.0	22.0	23.5	24.5
D^1	25.0	22.5	23.6	23.0	25.0	23.5	24.0	24.0
E^1	25.0	24.0	24.0	23.5	25.0	22.5	24.0	24.0
F	25.0	22.0	22.5	22.0	25.0	22.4	22.0	23.0
G	25.0	22.8	-	22.3	25.0	22.0	22.0	23.3
P	25.0	25.2	24.6	26.6	25.0	25.2	26.5	28.0
Average	25.0	23.3	23.3	23.3	25.0	23.0	23.8	24.8

Addition	Iodosobenzoate				Iodosobenzoate and 50% sugar			
A	25.0	24.0	22.0	24.0	25.0	22.0	23.0	25.0
B_1	25.0	22.6	23.3	24.0	25.0	22.4	22.5	25.0
C^1	25.0	24.0	24.2	24.0	25.0	22.8	24.5	25.6
D	25.0	22.7	22.7	21.3	25.0	22.7	23.0	24.0
E_1	25.0	23.8	23.5	24.0	25.0	22.5	24.5	25.0
F^1	25.0	24.2	23.5	24.0	25.0	23.6	23.5	26.2
G^1	25.0	23.0	22.0	22.5	25.0	22.0	22.6	25.0
P^1	25.0	26.5	25.6	27.5	25.0	25.6	27.3	28.6
Average	25.0	23.8	23.4	23.9	25.0	23.0	23.9	25.6

Table B. Foam Temperatures - Series II

Addition	None					50% sugar				
Period of beating in minutes	3	6	9	12	24	3	6	9	12	24
K_1	22.4	23.0	22.8	22.2	-	-	-	22.6	23.3	23.5
K^1	23.5	22.0	22.0	22.3	-	-	-	22.0	23.0	23.7
L_1	23.4	24.0	22.0	22.7	-	-	-	23.0	23.0	24.3
L^1	23.2	23.6	23.6	24.3	-	-	-	24.5	24.0	25.0
M_1	22.5	23.0	21.5	22.8	-	-	-	23.0	22.3	23.8
M^1	24.0	23.5	23.2	24.2	-	-	-	22.8	23.0	24.0
N_1	24.5	23.6	24.3	24.3	-	-	-	24.0	24.4	25.5
N^1	24.5	24.6	25.2	24.0	-	-	-	25.0	24.8	25.2
O_1	24.8	25.3	25.4	25.8	-	-	-	25.0	25.8	26.7
O^1	25.5	25.4	25.2	25.0	-	-	-	26.0	26.0	27.7
Average	23.8	23.8	23.5	23.8	-	-	-	23.8	24.0	24.9

Table C. Room Temperatures - Series I

Addition	None			50% sugar		
Period of beating in minutes	4	8	16	8	16	32
A_1	25.3	24.5	25.5	25.8	25.8	26.0
B_1	25.5	25.3	25.0	26.0	26.0	26.2
C_1	25.0	25.4	25.3	25.0	25.0	25.2
D_1	24.0	25.3	24.0	25.6	25.0	24.0
E_1	25.0	25.3	25.5	25.8	25.5	25.0
F	24.0	24.5	24.0	24.4	24.0	24.0
G	24.0	24.0	24.3	24.0	24.0	24.0
P	28.3	27.7	28.3	28.2	28.0	28.4
Average	25.1	25.2	25.2	25.6	25.4	25.4

Addition	Iodosobenzoate			Iodosobenzoate and 50% sugar		
A	25.5	26.2	25.5	26.0	26.0	26.0
B_1	25.3	26.0	26.0	25.5	25.6	25.4
C_1	26.2	25.6	25.5	25.5	26.0	25.8
D	24.0	24.2	24.0	24.0	24.0	24.0
E_1	25.0	25.0	26.2	27.0	26.3	25.0
F_1	26.8	26.6	26.0	26.8	25.8	26.8
G_1	25.0	25.0	24.8	25.5	24.2	25.4
P_1	28.4	27.7	28.5	28.0	28.2	28.5
Average	25.8	25.8	25.8	26.0	25.8	25.9

Table D. Room Temperatures - Series II

Addition	None					50% sugar				
Period of beating in minutes	3	6	9	12	24	3	6	9	12	24
K_1	24.2	24.5	24.3	24.0	-	-	-	24.0	24.0	24.2
K^1	24.8	25.0	24.5	25.0	-	-	-	25.0	25.0	25.0
L_1	25.0	25.2	24.6	24.8	-	-	-	25.0	24.8	25.0
L^1	24.5	25.0	24.7	25.3	-	-	-	25.5	25.2	25.0
M_1	25.0	25.6	25.0	24.4	-	-	-	25.2	24.8	25.0
M^1	25.8	26.0	26.0	26.4	-	-	-	25.5	26.2	26.2
N_1	26.0	26.0	26.0	26.2	-	-	-	26.0	25.6	26.0
N^1	26.0	26.6	27.0	26.0	-	-	-	27.0	26.4	26.0
O_1	28.0	28.5	28.0	28.4	-	-	-	28.6	28.5	27.2
O^1	28.2	28.0	28.2	27.7	-	-	-	28.2	28.2	28.7
Average	25.8	26.0	25.8	25.8	-	-	-	25.8	25.8	25.8

Table E. Stiffness Scores of Egg White Foams - Series I

Addition	None				50% sugar			
Period of beating in minutes	0	4	8	16	0	8	16	32
A_1	0	2.1	2.5	2.9	0	2.2	2.4	3.0
B_1	0	2.2	2.3	2.8	0	0.9	2.3	2.9
C_1	0	2.1	2.4	3.0	0	0.3	2.1	2.9
D_1	0	2.1	2.6	2.9	0	0.8	2.5	2.9
E_1	0	2.4	2.5	3.0	0	1.0	2.6	3.0
F	0	1.6	2.8	3.0	0	0.4	1.7	3.0
G	0	1.7	2.7	3.0	0	0.4	2.3	3.0
P	0	1.1	2.2	2.8	0	0.4	2.2	3.0
Average	0	1.9	2.5	2.9	0	0.8	2.3	3.0

Addition	Iodosobenzoate				Iodosobenzoate and 50% sugar			
A	0	1.3	2.2	2.4	0	0.6	1.7	2.3
B_1	0	1.4	2.0	2.3	0	1.3	1.7	2.3
C_1	0	1.7	2.0	2.5	0	0.6	1.6	2.7
D	0	0.8	1.4	1.7	0	0.4	1.8	2.1
E_1	0	1.2	1.4	2.2	0	0.7	1.4	2.5
F_1	0	1.1	1.2	1.6	0	0.4	1.3	1.8
G_1	0	1.2	1.4	1.9	0	0.8	1.2	1.8
P_1	0	1.1	1.2	1.9	0	0.3	1.2	1.8
Average	0	1.2	1.6	2.1	0	0.6	1.5	2.2

Table F. Stiffness Scores of Egg White Foams - Series II

Addition	None						50% sugar					
Period of beating in minutes	0	3	6	9	12	24	0	3	6	9	12	24
K_1	0	0.9	2.0	2.5	2.6	-	0	-	-	0.5	1.1	2.4
K^1	0	0.8	2.2	2.3	2.5	-	0	-	-	1.1	1.2	2.7
L_1	0	0.8	2.2	2.3	2.7	-	0	-	-	0.9	1.1	2.9
L^1	0	0.8	1.3	2.5	2.7	-	0	-	-	1.1	1.7	2.8
M_1	0	0.9	1.3	2.3	2.7	-	0	-	-	0.6	1.1	2.9
M^1	0	1.0	1.8	2.2	2.7	-	0	-	-	1.0	1.1	2.9
N_1	0	0.8	2.0	2.3	2.8	-	0	-	-	0.9	1.2	2.7
N^1	0	0.9	2.0	2.3	2.7	-	0	-	-	1.1	1.4	2.8
Average	0	0.9	1.8	2.3	2.7	-	0	-	-	0.9	1.2	2.8

Table G. Expansion Factors of Foams - Series I

Addition	None				50% sugar			
Period of beating in minutes	0	4	8	16	0	8	16	32
A_1	1.0	7.1	7.5	11.4	1.0	2.6	7.0	11.2
B_1	1.0	7.8	8.9	11.9	1.0	4.4	6.7	11.4
C	1.0	7.3	7.4	10.2	1.0	2.5	6.4	10.0
D_1	1.0	7.0	7.6	10.5	1.0	3.8	7.0	9.8
E_1	1.0	8.2	7.0	10.9	1.0	4.3	7.5	7.6
F	1.0	7.0	7.1	9.8	1.0	2.1	6.4	8.9
G	1.0	7.1	7.6	9.7	1.0	2.4	8.3	9.2
P	1.0	6.8	7.3	10.4	1.0	2.5	7.4	9.2
Average	1.0	7.3	7.6	10.6	1.0	3.1	6.8	9.7

Addition	Iodosobenzoate				Iodosobenzoate and 50% sugar			
A	1.0	7.5	8.4	8.7	1.0	2.2	7.0	9.6
B_1	1.0	7.0	7.7	8.5	1.0	-	6.6	8.7
C_1	1.0	6.4	7.6	7.9	1.0	2.1	6.7	8.9
D	1.0	5.6	6.8	7.8	1.0	2.0	6.5	7.9
E_1	1.0	7.7	8.1	8.7	1.0	2.4	6.9	9.3
F_1	1.0	7.0	8.0	7.9	1.0	2.2	6.5	9.5
G_1	1.0	7.4	7.7	8.2	1.0	2.5	6.8	8.8
P_1	1.0	7.5	8.2	8.1	1.0	2.2	7.0	8.7
Average	1.0	7.0	7.8	8.2	1.0	2.2	6.8	8.9

Table N. Expansion Factors of Foams - Series II

Addition	None						50% sugar					
Period of beating in minutes	0	3	6	9	12	24	0	3	6	9	12	24
K_1	1.0	5.5	7.0	7.3	7.9	-	1.0	-	-	2.5	6.4	8.4
K^1	1.0	5.3	7.1	7.5	8.1	-	1.0	-	-	5.5	6.4	9.0
L_1	1.0	5.5	6.5	7.2	7.5	-	1.0	-	-	4.8	6.1	9.1
L^1	1.0	5.6	6.0	7.0	7.6	-	1.0	-	-	5.5	6.4	9.6
M_1	1.0	5.3	6.5	7.0	7.5	-	1.0	-	-	2.5	5.8	8.9
M^1	1.0	5.5	6.7	7.2	8.1	-	1.0	-	-	5.9	6.4	9.1
N_1	1.0	6.0	6.9	7.5	7.7	-	1.0	-	-	4.7	6.4	9.1
N^1	1.0	5.6	7.2	7.5	7.9	-	1.0	-	-	6.4	6.1	8.8
Average	1.0	5.6	6.7	7.3	7.8	-	1.0	-	-	4.7	6.2	9.0

Table I. Amount of Drainage from Egg White Foams - Series I

Addition	None				50% sugar			
Period of beating in minutes	0	4	8	16	0	8	16	32
A_1	20.0	7.3	8.9	8.7	20.0	14.6	2.9	4.3
B_1	20.0	7.0	10.1	8.1	20.0	4.4	2.1	3.3
C_1	20.0	5.7	7.1	9.4	20.0	16.4	1.8	3.5
D_1	20.0	7.1	7.4	9.0	20.0	9.3	2.7	4.3
E_1	20.0	9.3	10.0	9.3	20.0	7.2	2.5	4.3
F	20.0	6.8	7.4	9.3	20.0	14.6	2.2	3.7
G	20.0	8.8	8.6	6.9	20.0	15.9	2.0	3.3
P	20.0	7.6	9.5	9.5	20.0	14.2	3.0	4.7
Average	20.0	7.4	8.6	8.8	20.0	12.1	2.4	3.9

Addition	Iodosobenzoate				Iodosobenzoate and 50% sugar			
A	20.0	6.8	3.8	5.3	20.0	17.3	0.5	0.2
B_1	20.0	7.3	2.2	6.5	20.0	0.7*	0.1	0.0
C_1	20.0	6.0	4.6	7.2	20.0	17.0	0.2	0.0
D	20.0	8.8	7.1	6.1	20.0	16.6	0.6	0.3
E_1	20.0	5.0	4.2	6.9	20.0	15.3	0.7	0.0
F_1	20.0	9.1	3.7	7.1	20.0	16.4	0.5	0.0
G_1	20.0	8.5	6.2	5.8	20.0	15.8	0.6	0.0
P_1	20.0	10.5	6.3	8.1	20.0	14.7	0.8	0.0
Average	20.0	7.8	4.8	6.6	20.0	16.2	0.5	0.1

*Omitted from average and range.

Table J. The Amount of Drainage from Egg White Foams - Series II

Addition	None						50% sugar					
Period of beating in minutes	0	3	6	9	12	24	0	3	6	9	12	24
K_1	20.0	10.0	8.0	9.1	10.6	-	20.0	-	-	13.7	1.4	1.1
K^1	20.0	13.9	7.6	9.9	10.8	-	20.0	-	-	3.4	2.5	2.7
L_1	20.0	12.4	8.3	9.4	10.8	-	20.0	-	-	4.1	1.6	2.5
L^1	20.0	11.9	10.0	11.5	10.2	-	20.0	-	-	4.4	3.4	2.3
M_1	20.0	10.4	6.6	8.3	10.0	-	20.0	-	-	14.8	1.1	2.8
M^1	20.0	11.8	9.3	7.8	10.3	-	20.0	-	-	2.0	2.6	0.8
N_1	20.0	12.6	8.3	9.9	11.5	-	20.0	-	-	5.5	1.7	2.4
N^1	20.0	12.0	8.4	11.2	11.3	-	20.0	-	-	3.1	4.1	1.9
Average	20.0	12.0	8.4	9.6	10.7	-	20.0	-	-	6.4	2.3	2.1

Table K. Weight of Drained Foam

Series I

Addition	None			50% sugar		
Period of beating in minutes	4	8	16	8	16	32
A_1^1	3.0	2.4	2.7	2.0	2.8	2.7
B	2.5	2.0	2.4	4.7	3.8	3.8
C_1	2.7	3.4	2.8	1.4	3.4	2.9
D_1^1	1.7	2.2	2.4	2.7	2.6	2.7
E^1	1.5	1.9	2.1	2.5	3.8	3.4
F	2.1	3.5	2.1	1.8	3.9	3.2
G	1.3	2.3	3.3	1.6	2.9	3.4
Average	2.1	2.5	2.5	1.8	3.3	3.2

Series II

Addition	None					50% sugar				
Period of beating in minutes	3	6	9	12	24	3	6	9	12	24
K_1	1.5	1.7	-	2.1	-	-	-	1.6	4.7	3.3
K^1	1.3	2.7	2.0	2.0	-	-	-	2.9	3.7	3.2
L_1	1.0	2.2	1.9	2.1	-	-	-	3.8	4.4	3.2
L^1	1.1	2.3	2.0	2.2	-	-	-	3.3	3.5	2.6
M_1	1.2	2.0	2.9	2.0	-	-	-	1.2	4.4	3.2
M^1	0.9	2.2	2.1	1.1	-	-	-	4.6	2.7	-
N_1	1.2	2.4	2.3	1.6	-	-	-	2.8	3.9	3.2
N^1	0.9	1.8	2.4	2.9	-	-	-	3.3	2.6	2.9
Average	1.1	2.2	2.2	2.0	-	-	-	2.7	3.7	3.1

Table L. Physical Measurements on Egg White Foams with and without Salt

Period of beating in minutes	6	6	9	9
Addition	None	2 gm. salt	None	2 gm. salt
		Stiffness scores		
	2.0	2.0	2.3	2.0
	2.2	1.8	2.4	2.2
	2.2	2.0	2.4	2.5
	1.3	1.2	2.2	2.3
	1.3	1.7	2.4	2.2
	1.8	2.1	2.5	2.3
	2.0	1.9	2.5	2.3
	2.0	1.2	2.7	2.5
Average	1.8	1.7	2.4	2.3
Range	1.3-2.2	1.2-2.0	2.2-2.7	2.0-2.5
		Expansion factors		
	7.0	6.9	6.7	6.2
	7.1	5.9	7.1	6.9
	6.5	6.6	7.7	6.3
	6.0	6.8	7.5	5.7
	6.5	7.1	6.8	6.5
	6.7	6.3	7.1	6.8
	6.9	5.8	7.8	6.0
	7.2	6.1	7.9	7.1
Average	6.7	6.4	7.3	6.4
Range	6.0-7.2	5.8-7.1	6.7-7.9	5.7-7.1
		Amount of drainage in one hour		
	8.0	9.4	10.0	11.0
	7.6	12.2	10.1	11.0
	8.3	12.1	10.3	11.6
	10.0	11.5	10.6	10.2
	6.6	9.1	9.4	10.6
	9.3	11.3	9.5	12.6
	8.3	11.1	10.4	11.2
	8.9	13.0	10.8	11.7
Average	8.4	11.2	10.1	11.2
Range	6.6-10.0	9.1-13.0	9.4-10.8	10.2-11.7

Table M. Reducing Value per Gram Egg White Calculated as Milligrams Cysteine Iodosobenzoate Method

Addition	None				
Period of beating in minutes	0	4	8	16	32
A	0.63	0.89	1.02	0.88	-
B_1	0.87	1.00	1.02	1.07	-
C^1	0.84	0.85	0.93	0.93	-
D	0.72	0.91	0.92	0.93	-
E_1	1.06	0.83	0.74	0.77	-
F^1	0.47	0.71	0.77	0.77	-
H_1	0.58	0.85	0.88	0.98	-
P^1	0.72	0.89	0.95	0.92	-
Average	0.74	0.86	0.90	0.90	-
Addition	50% sugar				
A	0.62	-	0.75	0.93	0.77
B_1	0.94	-	0.88	0.98	1.01
C^1	0.77	-	0.92	0.95	0.98
D	1.08	-	0.90	0.90	1.37
E_1	0.78	-	0.79	0.76	0.78
F^1	0.96	-	0.80	0.88	0.80
H_1	0.96	-	0.77	0.94	0.82
P^1	0.86	-	0.88	0.87	0.97
Average	0.87	-	0.84	0.90	0.94

Table N. Readings of Per cent Transmission Used for Reference Curve[1] Ferricyanide Method

Milliliters of ferrocyanide	0.5	1.0	1.5	2.0	2.5	3.0
Solution 1	94.5	82.4	68.2	58.3	48.4	41.9
	94.2	83.2	68.3	58.3	48.2	42.2
	-	81.2	69.3	60.5	48.7	42.2
Average	94.4	82.3	68.6	59.0	48.4	42.1
Solution 2	93.7	77.0	69.4	58.4	48.9	44.8
	90.5	76.0	66.8	58.4	49.5	44.8
	93.5	75.4	64.5	56.7	48.6	44.0
Average	92.6	76.1	66.9	58.4	49.0	44.5
Solution 3	91.0	78.3	66.3	61.2	48.3	42.0
	95.0	78.8	67.5	59.8	48.2	42.0
	88.5	78.1	68.2	60.6	48.7	42.5
Average	91.5	78.4	67.3	60.5	48.4	42.2
Solution 4	95.8	81.7	69.6	60.8	50.4	43.5
	94.1	80.4	68.7	60.5	50.3	43.5
	90.4	77.1	69.2	-	50.6	-
Average	93.4	79.7	69.2	60.6	50.4	43.5
General Average:	92.96	79.14	68.00	59.65	49.06	43.05

[1] Calculation of the equation for the curve was made by the least squares method, using the averages of the readings for each point and for each solution.

Substituting in the two normal equations:

$Na + b\Sigma x = \Sigma (\log y)$

$24a + 42b = 43.19729$

$a\Sigma x + b\Sigma x^2 = \Sigma (\log y \cdot x)$

$42a + 91b = 73.24509$

Solving simultaneously for b, $b = -0.13430$

Substituting above and solving for a, $a = 2.03490$

Equation for curve $\log y = 2.03490 - 0.13430x$

Table O. Sulfhydryl or Cysteine Equivalents in Milliliters of 0.0002 N Solution per Gram Egg White
Ferricyanide Method

Addition	None					2 gm. salt
Period of beating in minutes	0	3	6	9	12	6
K_1	0.10	0.10	0.14	0.25	0.27	0.16
K^1	0.07	0.15	0.10	0.20	0.11	0.08
L^1	0.32	0.45	0.67	0.31	0.61	0.25
M_1	0.19	0.44	0.32	0.30	0.47	0.27
M^1	0.07	0.09	0.09	0.08	0.14	0.08
N_1	0.11	0.15	0.14	0.14	0.17	0.13
N^1	0.18	0.32	0.32	0.63	0.46	0.32
O_1	0.17	0.57	0.54	0.64	0.53	0.38
O^1	0.39	0.51	0.48	0.22	0.41	0.22

Addition	None	2 gm. salt
Period of beating in minutes	9	9
1	0.48	0.22
2	0.48	0.17
3	0.53	0.18
4_1	0.65	0.32
1^1	0.64	0.14
2^1	0.55	0.28
3^1	0.66	0.33
4^1	0.64	0.36