AN ELECTROPHORETIC STUDY OF CHICKEN EGG ALBUMEN

By

JOE PHILLIP HUDSPETH

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Thesis Approved:

George W. Newell

Thesis Adviser

John W. West

Head of the Department

Dean of the Graduate School

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INTRODUCTION

Egg quality breakdown is, and has been for many years, one of the most complex and intricate mechanisms which has been studied. As a consequence, considerable research on this subject has been accomplished in an attempt to determine the exact mechanism, on a molecular chemical basis, of this breakdown.

There have been many hypotheses advanced on this particular subject which deal with certain peculiar aspects of the unknown mechanism. However, to date, there has been no definite evidence as to what actually occurs during the breakdown of thick to thin egg albumen.

This study deals with this problem in a specific manner employing temperature, storage time, and oiling of shell eggs as variables and using electrophoretic migration as a research tool. This breakdown in egg quality was measured in relation to pH, Haugh units and individual protein composition of thick egg albumen. The data presented herein, it is hoped, will show the complexities of this problem and the practical need for a solution on a molecular chemical level.

If a way to correct this breakdown in egg quality can be found, it may be possible to develop more efficient marketing methods that would allow producers to deliver a higher quality product through the various marketing channels and thence to the consumer.

REVIEW OF LITERATURE

Due to the several factors being studied in this experiment the literature review will be divided into the following sections; Retention of Egg Quality and Methods of Measurement, and Protein and Chemical Composition of the Egg White.

Retention of Egg Quality and Methods of Measurement

In an attempt to find a basis for measuring the interior quality of eggs, many methods of measurement have been devised for this purpose. Wilgus and Van Wagenen (1936) suggested that the height of the thick egg albumen could possibly be used as a method of quality measurement. This work was later shown to be partially true with the advent of Haugh's method for measuring the interior quality of eggs (1937). Haugh was able to show that the change in thick egg albumen appeared to be a logarithmic function and formulated an equation to determine the interior quality of an egg using this method. Again, using the height of the thick egg albumen as a criterion for measurement, Evans (1943) indicated that the percentage loss of the albumen index was a satisfactory method to estimate deterioration. Even though many methods for the measurement of the interior quality of shell eggs have been used, Haugh units provide a uniform scale of measurement, whereas albumen height alone or albumen index does not (Brant et al., 1951). Although Eisen et al. (1962) suggested that a bias existed in the Haugh unit regression, which might prompt one to question the validity of this

technique, it has proven to be of tremendous value in this field of research.

Using Haugh units and pH values of the thick egg white, many studies have been designed to examine egg quality breakdown. Cotterill (1955) stated that the natural thinning of egg white, as measured in Haugh units, was retarded if the pH were maintained near the initial value. Also using Haugh units as a measuring device, Stadelman et al. (1954) showed that a decrease of 1.1548 Haugh units occurred for each ten degree increase in temperature. In further studies on this subject, Fry and Newell (1957) noted that high temperature resulted in great quality losses in terms of Haugh units, with the initial drop in quality being greatest for the first forty-eight hours of storage. It was also reported by Lorenz and Almquist (1936) that the percentage of firm white was definitely lowered by high air temperature immediately after laying. Other factors which have been studied and found to influence the percentage of thick albumen in the egg include: seasonal effect, Knox and Godfrey (1938); presence of ammonia in the atmosphere surrounding the egg, Cotterill and Nordskog (1954). In the latter study the presence of the ammonia affected the pH and hastened the deterioration of the egg, as measured in Haugh units.

In an attempt to lessen the breakdown which occurs in thick egg albumen, the coating of shell eggs with some type of sealing agent has been tried. Sharp (1937) indicated that the oiling of eggs retarded the escape of carbon dioxide and thus aided in maintaining egg quality. This work was supported by Evans and Carver (1942) when it was shown that the oil treating of shell eggs greatly increased keeping quality. In the same study it was further stated that time of oiling after the eggs are laid also tended to affect keeping quality (Evans and Carver, 1942).

Once established, the exact mechanisms through which the process of oiling works has been extensively studied. Stadelman and Wilson (1957) indicated that spray oiling of shell eggs was effective in egg quality maintenance. Work done by Homler and Stadelman (1963) showed that oiling resulted in a significant retention of egg quality. Even though it has become well established that oiling of eggs produces a high degree of keeping quality, the process through which quality is retained has not been shown. Bose and Stewart (1948) showed that with the spray oiling of eggs the pH of the albumen was held lower. This work was supported when Schwall et al. (1961) showed that the pH of untreated eggs increased significantly faster than the oil-treated eggs. In work done by Cotterill and Gardner (1957), it was shown that eggs held in a carbon dioxide atmosphere did not exhibit a market decrease in Haugh units or a significant increase in pH values.

Although oiling of individual shell eggs has been shown to be an effective means of maintaining egg quality, other methods have been devised to accomplish the same purpose. Romanoff and Yoshok (1948) were able to show that certain chemical agents aided somewhat in the retention of egg quality and pH when they were applied to the shell. In a later study Yoshok and Romanoff (1949) showed that the use of plastics is very effective in the maintenance of egg quality.

Even though much work has been done in an attempt to curb the egg quality breakdown, this phenomenon has been slowed somewhat but not completely stopped. The effect of time, the individual hen, and the age of the egg are all definite factors to consider (Spencer et al., 1956). It also appears that the action of the

thinning of thick egg albumen must be considered as being inherent in the white itself (Feeney et al., 1951).

Protein and Chemical Composition of the Egg White

The actual protein composition of egg white has been the subject of many experiments. Almquist and Lorenz (1932) found that the retention of thick albumen was aided through the use of a carbon dioxide atmosphere. These workers further stated that in the absence of carbon dioxide a change in the physical form but not in the chemical makeup occurred in egg albumen. The influence of pH has been discussed; however, it must be pointed out that Cunningham and Cotterill (1962) found that at a pH in excess of 11.9 egg white forms a translucent gel which, after a period of time, undergoes self liquefaction.

Several studies have been conducted on the individual amino acid content of egg white. Almquist and Lorenz (1933) were able to show that the solids concentration in egg white is highly variable, but that this variation is less from the same hen. Many of the analyses conducted on egg albumen have been based upon the procedure of precipitation to obtain purified protein samples. Haurowitz et al., (1946) stated that precipitation is due to the formation of salt-like bonds between the negatively charged groups of the azoproteins and the positively charged groups of the proteins.

In analyzing for differences in amino acid content between fresh and stored shell eggs, Evans et al., (1949b) found that arginine and tyrosine were present in lower amounts in stored eggs than in fresh eggs. These same workers also discovered that serine and threonine were present in lower amounts in stored eggs than in fresh eggs (Evans et al., 1949a). In work done by Evans et al. (1950),

it was shown also that feeding had very little influence on amino acid and thus the protein content of egg albumen. In further amino acid analyses by Evans et al. (1959), it was found that the content of arginine and phenylalanine of shell eggs increased while the proline content decreased in eggs stored for sixteen months.

All work done on the amino acid content of shell eggs must be related to the actual proteins present in the thick or the thin egg albumen. Charkey et al. (1947) stated that the type or composition of the protein complex varies between eggs of high and low albumen quality as measured in Haugh units. This being true, it was very essential that methods be devised to determine the protein content of egg albumen rather than the individual amino acid content.

Perhaps one of the most useful research tools adapted to this purpose has been the electrophoresis procedure. This process relies, to a great extent, upon the isoelectric points of the specific proteins under study, thus enabling proteins to be characterized in this manner (Sober et al., 1956). Work done by Longsworth (1939) also adds to the usefulness of this technique, in that it was shown that the relative amounts of each protein could be calculated. Some of the peculiar aspects of this technique were shown by Evans and Bandemer (1956). In their analysis of egg white proteins, using thick egg albumen, it was observed that as pH increased the positively mobile proteins became negative with the negatively migrating proteins becoming more negative. It was also shown in this study that the best separation of protein components was obtained by using a barbital buffer at a pH of 8.6 and an ionic strength of .05. In other studies of protein components by Steimer(1953), the serum albumen and lysosyme components were shown to interact at pH values intermediate to their isotonic points, with this interaction

being decreased by increasing the ionic strength of the buffer solution.

Several studies have been conducted using specific proteins as criteria to determine the molecular chemical effect of such factors as pH and enzymatic hydrolysis on the liquefaction of thick egg albumen. Csonka and Jones (1952) indicated that the percentages of the proteins in chicken egg white were influenced by the nature and quality of the dietary protein of the laying hen as well as by the genotype of the hen. Several attempts have been made to determine the genetic effects on electrophoretic patterns. Bain and Deutsch (1947) reported that it may be possible to differentiate each of the species in the Aves class using electrophoretic patterns as the criterion of measurement. Forsythe and Foster (1950) also demonstrated slight differences in the protein composition of egg white from six genetically different strains of chickens.

Other studies have been undertaken to determine the total protein components present in egg albumen. Evans and Davidson (1953) found that there were no losses of protein from eggs stored for 18 months at 0° C. Also Evans et al. (1949a) indicated that the percentage of water insoluble proteins increased up to nine months with a corresponding decrease occurring in the water soluble proteins. It appears then that total protein does not vary. This change was considered to be related to a single protein or combination of proteins. Cotterill and Winter (1954) showed that eggs of high and low quality did not vary appreciably in total solids, total proteins or lysosyme activity. It was also shown by Skala and Swanson (1962) that there were no differences in the protein complex in terms of major proteins as determined by electrophoresis. In other studies by Evans et al. (1958) the results indicated that no transfer of protein from yolk to white occurred

during twelve months of storage. Also, in this study, it was observed that the speed of migration increased slightly with length of storage.

In a study by Balls and Swenson (1934), it was indicated that the disappearance of thick white was due to a slow proteolysis catalyzed by an enzyme. The pH values obtained from thick egg albumen were found definitely to influence the viscosity of the thick egg white (McNally, 1943).

In an analysis of individual protein components present in egg albumen, Franpton and Romanoff (1947) observed ten protein components present in chicken egg white. In an attempt to analyze the lysozyme content in egg white it was found that lysozyme could be crystallized, giving a yield of sixty to eighty percent, under the conditions employed in the study (Alderton and Fevold, 1946). Wilcox and Cole (1954) devised an accurate method for determining the lysozyme concentration. In another study done by Wilcox (1956) it was shown that a substantial decrease in lysozyme concentration occurred with time. This study supported a previous experiment by Wilcox (1955), when he stated that there was no influence of lysozyme concentration on the decrease in albumen quality.

It has also been found that ovomucoid plays no important role in maintaining consistency of egg white (Cohen and Balls, 1955). In other studies conducted by Hill et al. (1949), it was found that the mucin protein components were four times as rich in tryptophan as the whole protein of egg white.

Forsythe and Bergquist (1951) found that blending causes an initial rapid decrease in fiber length, but that no change in ovomucin content resulted from the blending treatment. It was hypothesized by Balls and Hoover (1940) that if pH is held around a value of 8.0 liquefaction due to breaking of mucin fibers can be

retarded. Conrad and Scott (1939) stated that a change of the ovomucin during storage is not due to the enzymatic hydrolysis of the mucin but must be due to the effect of high pH values.

Much research has been conducted on the role that lysozyme and/or ovomucin plays in the breakdown of thick to thin egg albumen, with many theories being advanced on this subject. Hawthorne (1950) stated that the action of thinning of egg white may be due to a lysozyme-mucin interaction. Work done by Cotterill and Winter (1955) also indicated that the absence or reduction of the lysozyme-mucin interaction may be responsible for one stage of egg white thinning. It was also suggested in this study that this interaction may be responsible in part for the maintainance of the thick egg white in a firm state. In other work done on this subject, Feeney et al. (1952) indicated that no difference in lysozyme or ovomucin was found in thick versus thin egg white.

Very little work has actually been done concerning the other protein components present in chicken egg white. Newell and Odell (1960) reported, however, that as the percentage of ovalbumin decreases a corresponding increase in one or both of the other fractions occurred. In other work done by Evans et al. (1958), it was shown that twelve-month-old storage eggs contained more ovomucoid plus ovoglobulin fraction and less conalbumin and lysozyme than fresh eggs. The ovalbumin protein fraction did not change but there was less ovalbumin A_1 than ovalbumin A_2 and ovalbumin A_3 found in the older eggs than in the fresh eggs. It was further stated that the change of lysozyme to globulin may be associated with a loss of viscosity.

MacDonnell et al. (1951) suggested the possibility that the deterioration of shell eggs involves a reduction of the S-S bonds. It was further stated that the SH groups in denatured ovalbumin also plays a role in the deterioration of thick egg white. In another study by MacDonnell et al. (1954), differences were found in the sulfhydryl of cysteine content between thick and thin egg albumen. These studies and other work done in this field have prompted the following hypotheses, as stated by Feeney (1955):

1. The gel changes by salt or ion shifts from one part of the white to another.

2. The ovomucin denatures or chemically hydrolyzes.

3. The ovomucin slowly forms complexes with other protein constituents, possibly with lysozyme.

4. A complex already present in thick white, and containing ovomucin, depolymerizes.

5. The ovomucin reacts chemically with other constituents, such as sulfhydryl groups.

6. The ovomucin is split or hydrolyzed by unidentified enzymes.

Although much work has been done on the subject of the thinning of egg white, this problem is yet to be solved. The purpose of this paper is to offer one approach to the solution of this particular problem.

MATERIALS AND METHODS

The study reported in this thesis is involved with the proteins of egg albumen as related to length of storage time, hydrogen ion concentration as estimated by pH, and Haugh units.

Eggs used in this experiment were obtained between the hours of 1:00 and 2:00 in the afternoon during the period from May 20, 1963 to August 15, 1963. The eggs were laid by commercial strain White Leghorn hens that were fed a practical-type layer diet formulated by the Poultry Science Department of Oklahoma State University. All eggs were collected from eight individual hens housed in a windowless cage house located on the Oklahoma State University Poultry Farm. The environmental conditions of the house were partially controlled through the use of a water cooling system designed specifically for this house. Since this was a windowless cage house, the birds housed therein were supplied with artificial light during a fourteen-hour period each day.

Eggs were gathered each afternoon from these individual birds and were taken directly from the farm to the Poultry Industries Building where they were placed into one of four different treatments, each consisting of five different lengths of storage.

These four treatments consisted of the following combinations:

1. Refrigerated, oiled $34^{\circ}F.$
2. Refrigerated, unoiled $34^{\circ}F.$

3. Room temperature, oiled 70°F.

4. Room temperature, unoiled 70°F.

Eggs from each treatment were stored for periods of one, three, nine, twenty-seven, and eighty-one days. No attempt was made to control the relative humidity of the areas in which these eggs were stored. The oiled eggs were treated with a commercial oil by spraying until the shell was completely covered, prior to being placed into their respective storage areas.

At the time of removal from storage the weight of each egg was recorded to the nearest gram. The eggs were then broken and the albumen height was measured in millimeters using an Ames Tripod Micrometer for the purpose of Haugh unit determination. Only the thick albumen was saved for subsequent analysis. If there was no thick albumen present, a sample of the albumen relatively close to the yolk was taken. The pH of the albumen was determined using a Coleman Metrion II pH Meter, equipped with the standard-purpose glass electrodes.

As soon as the pH of the albumen was measured, hand homogenization was accomplished in order to facilitate easier handling during the pipetting phase of the electrophoresis procedure. After the albumen samples were prepared, a procedure similar to that described by Evans and Bandemer (1956) was followed with some modifications. A sample of .006 milliliters of the albumen was applied to the paper strip for separation. Duplicate albumen samples from each egg were placed on individual paper strips. A further modification of the system was made in that the electrophoresis cells were placed in a cooler in which the temperature was maintained at 34° F. The cells were then attached to a power supply unit that kept a constant seven milliamps current flow going into each electrophoresis unit.

After eighteen hours had elapsed, the power supply unit was disconnected and the paper strips were removed from contact with the wicks, in order to keep the resolution of the protein bands from being impaired. The paper strips were then removed from the cells and transferred to drying racks. At this time the strips were placed in a preheated oven at a temperature between 120° and 140° C. until completely dry.

When dry, the strips were transferred to staining and rinsing racks for the purpose of processing through five one-liter solutions, as follows:

1. A solution of 95 percent ethyl alcohol was used to wash the strips and rid them of any excess buffer or other particles which might have been on the paper strips. The strips remained in this solution for five minutes.
2. A second solution of 95 percent ethyl alcohol was used, to which was added one gram of bromphenol blue to impart color to the entire paper strip, including the protein bands. A period of fifteen minutes was allowed for complete staining.
3. A solution of 5 percent acetic acid was used for the purpose of decolorizing the strip, while leaving the dyed protein bands intact. The strips remained in this solution for ten minutes.
4. The same solution as outlined in 3 was used for further washing of strips.
5. A solution of 5 percent acetic acid was used, to which 5 percent sodium acetate, by weight, had been added. The strips were kept for 10 minutes in this solution, which aided in a further decolorization

of the strip, thus leaving the dyed protein bands more pronounced.

Immediately after removal from the fifth solution, the electrophoresis strips were transferred to the drying racks and placed in a preheated oven at a temperature of 120° to 140° C. After drying, the strips were then run through a photodensitometer integraph recording instrument. This instrument makes a chart of light transmission as well as a record of area which can be divided visually by protein fractions. Each chart was then examined and the percentage of each protein fraction present was calculated.

After obtaining a percentage value for each protein band in each electrophoresis strip, these percentages were converted to arcsine values. These values were analyzed using the facilities of the Statistics Department on the Oklahoma State University campus. The analysis of variance, means, and correlation tables presented in this thesis were obtained through the use of the 1410 IBM computer system presently employed by the Computing Center for the analysis of these types of data.

RESULTS AND DISCUSSION

General - The procedures followed in this study made possible the analysis of nine separate and distinct criteria which relate to egg quality changes. These criteria in order of presentation are: pH, Haugh Units, lysozyme, non-mobile protein, conalbumin, ovoglobulin and ovomucoid, ovalbumin A_3, ovalbumin A_2, and ovalbumin A_1. It was found necessary for the ovoglobulin and ovomucoid fractions to be considered as one fraction, since the procedures employed in this study made it impossible to analyze these proteins as separate components. The means, analyses of variance, and correlation coefficients are discussed in this order in an attempt to clarify the results obtained in this study.

pH - The results of pH determination are presented in Table 1. The values observed after one day of storage indicated only slight variations. These values ranged from 7.69 for the room temperature-oiled treatment to 7.89 for the refrigerated-oiled treatment, respectively.

The pH values for all treatments exhibited a tendency to increase throughout the entire study. Those eggs in the room temperature-unoiled treatment showed the greatest change and, as would be expected, the refrigerated-oiled eggs changed the least through the 81 days of storage. However, during the period between 27 and 81 days of storage, three of the four treatments presented a reversal of this tendency and did, in fact, show a decrease in pH during the

Table I

MEAN pH VALUES FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	7.89	8.48	8.13	9.17	8.71
Room temperature-oiled	7.69	7.73	8.10	8.13	8.21
Refrigerated-unoiled	7.93	7.89	8.53	9.18	8.59
Refrigerated-oiled	7.98	7.86	7.37	8.37	8.14

period. A partial explanation of this change may be that the greater number of days made possible an equilibrium to be established between the egg contents and the storage atmosphere.

A study of the data indicates that for all temperatures and at all storage times those groups which were oil treated maintained a lower pH than did the unoiled groups. Refrigeration did not have the universal effect of maintaining low pH, but did result in less change than that of the room temperature counterparts.

The greatest variation among treatments was found after nine days of storage, at which time refrigerated-oiled eggs had an average value of 7.37 and the refrigerated-unoiled eggs a value of 8.53. The pH values after 27 days of storage ranged from 8.13 to 9.18 for the room temperature-oiled and the refrigerated--unoiled groups, respectively. This trend toward smaller variations in pH among the

treatments continued through the 81 days of storage, at which time the difference was only 0.57 pH units.

The analysis of these data, using the analysis of variance technique, was performed on IBM equipment and the results are shown in Table II. It can be seen that the singular effects of number of days stored and shell treatment by oiling each had a significant effect at the .01 level of probability. The effects due to hen and to temperature of storage area did not exhibit any significant effect individually. None of the interactions in which the hen effect was a part showed significance at either the .01 or .05 level of probability. In fact, the only interactions that were significant were those which combined days (storage time) with oil treatment, days with temperature, and the three-way interaction among these variables.

Haugh Units - The means of Haugh units for all treatments and storage times are shown in Table III. The changes in Haugh units are striking in that a decrease was noted for all treatments. The greatest change occurred in the room temperature-unoiled group with a range of 90 to 43 for the one-day and 81-day storage periods, respectively. The effect of refrigeration is well exemplified in that egg quality, as measured by Haugh units, was maintained at a high level in both groups kept at the lower temperature. Likewise, oiling had the general effect of quality maintenance, although not to the same degree. Although a decrease in Haugh units was noted for both of the oiled groups, differences among the three treatments (refrigerated-oiled, refrigerated-unoiled, and room temperature-oiled) were not nearly as great as that between these three treatments and the room temperature-unoiled treatment, after 81 days of storage.

Table II

ANALYSIS OF VARIANCE FOR pH VALUES OBTAINED FROM EGGS USED IN THIS STUDY

Source of Variation	d.f.	Mean squares
Total	319	---
Hen (H)	7	.14714
Days (D)	4	7.81750**
Oil (O)	1	19.41000**
Temperature (T)	1	.13000
H x D	28	.05964
H x O	7	.06286
H x T	4	.02714
D x O	4	1.51250**
D x T	4	.50000**
T x O	1	.01000
H x D x O	28	.08357
H x D x T	28	.07964
D x O x T	4	1.92250**
H x O x T	7	.021429
H x D x O x T	28	.05714
Replicates	1	.02000
Error	159	.08446

**Significant at the .01 level of probability

Table III

MEAN HAUGH UNIT VALUES FOR ALL TREATMENTS

BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	90	84	72	58	43
Room temperature-oiled	91	91	87	82	66
Refrigerated-unoiled	92	90	89	83	78
Refrigerated-oiled	91	92	91	91	81

All of independent variables, i.e., hens, days, oil treatments, and temperature of storage, had a profound effect upon Haugh units. This is easily seen by a study of the analysis of variance data presented in Table IV. In spite of the apparent effect of each of these variable individually, interactions among some of them proved to be insignificant. For the most part, those interactions which involved storage time as one of the variables proved to be significant.

Lysosyme - The mean percentages of the total protein which were lysozyme is shown in Table V for all treatments and storage times. The percentages of lysozyme varied rather widely throughout this study. There were no clear relationships among the four treatments for any of the storage times tested in this study.

The inferences drawn in the preceding paragraph were further substantiated by a study of the data in Table VI.

Table IV

ANALYSIS OF VARIANCE FOR HAUGH UNIT VALUES OBTAINED FROM EGGS USED IN THIS STUDY

Source of variation	d.f.	Mean squares
Total	319	--
Hen (H)	7	937.4286**
Days (D)	4	6085.2500**
Oil (O)	1	5763.0000**
Temperature (T)	1	10440.0000**
H x D	28	40.9643**
H x O	7	11.1429
H x T	7	12.4286
D x O	4	638.2500**
D x T	4	1589.5000**
T x O	1	2565.0000**
H x D x O	28	22.8214
H x D x T	28	15.5000
D x O x T	4	243.2500**
H x O x T	7	9.5714
H x D x O x T	28	21.6429
Replicates	1	16.00
Error	159	14.36488

**Significant at the .01 level of probability

Table V

MEAN PERCENTAGE VALUES OF LYSOZYME FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	16.70	16.60	17.00	13.50	17.50
Room temperature-oiled	14.70	15.70	19.00	15.00	18.20
Refrigerated-unoiled	17.30	15.60	18.70	13.70	19.40
Refrigerated-oiled	16.00	16.30	15.50	14.40	19.70

These data indicate that the individual hen had a greater effect upon the percentage of lysozyme than any other individual effect studied except days (storage time). In the case of the latter effect the data in Table V do indicate a tendency for the amount of lysozyme to increase between the first and eighty-first days of storage. It should be pointed out, however, that the method of calculation used in this study was such that changes in protein composition are relative and not quantitative.

As might be expected the only interaction which proved highly significant (.01 level of probability) was that between hens and days. Other interactions were significant at the .05 level of probability only, and included those in which days were a part (Table VI).

Non-Mobile Protein - The amount of non-mobile protein in any egg albumen will vary but slightly under any conditions.

Table VI

ANALYSIS OF VARIANCE OF THE LYSOZYME FRACTION

Source of variation	d.f.	Mean squares
Total	319	--
Hens (H)	7	.39189**
Days (D)	4	1.12602**
Oil (O)	1	.0109
Temperature (T)	1	.02710
H x D	28	.45731**
H x O	7	.03522
H x T	7	.08114*
D x O	4	.10639*
D x T	4	.09420*
T x O	1	.07760
H x D x O	28	.05860
H x D x T	28	.05343
D x O x T	4	.16365*
H x O x T	7	.02782
H x D x O x T	28	.05879
Replicates	1	.00056
Error	159	.03437

**Significant at the .01 level of probability
*Significant at the .05 level of probability

This fact is well demonstrated by the data in Table VII.

Table VII

MEAN PERCENTAGE VALUES OF NON-MOBILE PROTEIN FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	3.60	4.20	3.90	4.60	4.90
Room temperature-oiled	3.90	4.10	3.90	3.70	4.30
Refrigerated-unoiled	3.30	3.90	3.60	4.20	4.50
Refrigerated-oiled	3.30	4.10	3.50	5.00	3.90

As can be seen this fraction is a small percentage of the total protein, and the largest variation among treatments at any storage period studied was from 3.70 to 5.00 percent for the twenty-seventh day of storage.

Information relative to the composition of non-mobile protein is virtually non-existent in literature. Whether it is true protein or some protein-like compound is not known. From this it can be concluded that the amount of this compound is relatively stable and any changes which are evident from the data in Table VII or from the analysis presented in Table VIII are relative. This is to say that the absolute amount probably remains the same, and because of the changes in other compounds the percentage of non-mobile protein appears to increase.

Table VIII

ANALYSIS OF VARIANCE OF THE NON-MOBILE PROTEIN FRACTION

Source of variation	d.f.	Mean squares
Total	319	--
Hen (H)	7	.45972**
Days (D)	4	2.00945**
Oil (O)	1	.21337
Temperature (T)	1	.69709*
H x D	28	.32062**
H x O	7	.07413
H x T	7	.05237
D x O	4	.27737
D x T	4	.39648*
T x O	1	.42067
H x D x O	28	.13192
H x D x T	28	.10732
D x O x T	4	.48402**
H x O x T	7	.31094*
H x D x O x T	28	.13405
Replicates	1	.0078
Error	159	.14033

**Significant at the .01 level of probability
*Significant at the .05 level of probability

There does appear to be a significant difference (.01 level of probability) in the percentage of non-mobile protein due to individual hens (Table VIII). Likewise, days (storage time) have an individual effect, but this is probably due to relative effect and not absolute effect. Interactions which exhibited significant effects were those which contained either hens or days.

Conalbumin - The average percentage of total protein which was conalbumin appeared to remain relatively stable throughout the entire study (Table IX).

Table IX

MEAN PERCENTAGE VALUES OF CONALBUMIN FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	17.90	15.70	17.50	15.80	17.10
Room temperature-oiled	13.60	15.60	16.80	16.70	15.40
Refrigerated-unoiled	16.60	17.60	11.90	15.20	14.20
Refrigerated-oiled	18.00	16.00	12.80	17.00	14.60

Wide flucuations were seen by treatments by days but no pattern is apparent from a study of these means.

Although the analysis of variance presented in Table X indicates that the days and storage temperatures have significant effects (.01 level of probability), the direction of change is not uniform within either of the

Table X

ANALYSIS OF VARIANCE OF THE CONALBUMIN PROTEIN FRACTION

Source of variation	d.f.	Mean squares
Total	319	--
Hen (H)	7	.06746*
Days (D)	4	.23149**
Oil (O)	1	.04439
Temperature (T)	1	.36147**
H x D	28	.08774**
H x O	7	.02782
H x T	7	.02871
D x O	4	.10907**
D x T	4	.69527**
T x O	1	.36646**
H x D x O	28	.03349
H x D x T	28	.02538
D x O x T	4	.16365**
H x O x T	7	.05652*
H x D x O x T	28	.05127
Replicates	1	.00537
Error	159	.02658

**Significant at the .01 level of probability
*Significant at the .05 level of probability

factors. Because of the wide variations shown within a particular treatment and among the storage times, interactions which contain days as one of the factors did prove to be highly significant (.01 level of probability).

Ovoglobulin and Ovomucoid - The results of ovoglobulin and ovomucoid determinations are presented in Table XI.

Table XI

MEAN PERCENTAGE VALUES OF OVOGLOBULIN AND OVOMUCOID FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	18.00	18.40	19.10	18.30	21.90
Room temperature-oiled	20.20	17.70	17.10	16.40	21.60
Refrigerated-unoiled	19.10	18.00	20.00	16.10	19.30
Refrigerated-oiled	18.50	19.10	22.20	17.00	20.10

Although these two protein fractions have different chemical and structural composition, the method of separation used in this study did not permit separate analysis of these two compounds. This combination undoubtedly contributed to the wide variation and lack of any definite pattern shown by any of the treatments. The range of percentages from 18.00 for the room temperature-unoiled treatment to 20.20 for the room temperature-oiled group after one day of storage was quite similar in magnitude and in level to the results after 81 days of storage. However, after 81 days of storage, the low and high groups were

the refrigerated unoiled and room temperature-unoiled, respectively. The widest range in percentages for these compounds was found after 9 days of storage, when the values were 17.10 and 22.20 for the room temperature-oiled eggs and refrigerated-oiled eggs, respectively.

A study of the analysis of variance data (Table XII) indicates that the independent variables of hens and days had highly significant (.01 level of probability) effects on these compounds. Interaction of these two independent variables or these in combination with storage temperature also exhibited a significant effect (.01 level of probability). As in the preceding two fractions, the wide variation in results by days undoubtedly contributed to this significance, but does limit the conclusion regarding any linear effect due to days of storage.

Ovalbumin A_3 - The mean percentages of ovalbumin A_3 of the total protein are shown in Table XIII. Although the effect is small, refrigeration did have an influence in maintaining the percentage of ovalbumin A_3 at a relatively high level. This can be concluded from the fact that the oiled and unoiled groups which were stored under refrigeration had the highest percentage of ovalbumin A_3 at all storage times, except at 27 days. Here the refrigerated--oiled group actually had the lowest percentage, but it will also be noted that all groups are quite low at the 27-day storage time.

The independent factor, hens, had a highly significant effect (.01 level of probability) on the percentage of ovalbumin A_3 as can be seen from the analysis of variance data in Table XIV. Likewise the factor days (storage time) exhibited a highly significant effect (.01 level of probability). However, the effect of temperature was significant at the .05 level of probability only.

Table XII

ANALYSIS OF VARIANCE OF THE OVOGLOBULIN AND OVOMUCOID PROTEIN FRACTIONS

Source of variance	d.f.	Mean squares
Total	319	---
Hen (H)	7	.50312**
Days (D)	4	.68098**
Oil (O)	1	.00946
Temperature (T)	1	.00168
H x D	28	.17247**
H x O	7	.03549
H x T	7	.03073
D x O	4	.01944
D x T	4	.29082**
T x O	1	.23231**
H x D x O	28	.02925
H x D x T	28	.05244**
D x O x T	4	.14586**
H x O x T	7	.02402
H x D x O x T	28	.02128
Replicates	1	.00009
Error	159	.02135

**Significant at the .01 level of probability

Table XIII

MEAN PERCENTAGE VALUES OF OVALBUMIN A_3 FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	9.20	10.10	11.30	8.90	9.30
Room temperature-oiled	10.20	10.30	10.10	9.90	8.90
Refrigerated-unoiled	10.20	9.80	11.40	9.50	11.10
Refrigerated-oiled	10.60	10.10	11.40	7.90	10.20

Table XIV

ANALYSIS OF VARIANCE OF THE OVALBUMIN A_3 PROTEIN FRACTION

Source of variation	d.f.	Mean squares
Total	319	--
Hen (H)	7	.23574**
Days (D)	4	.29566**
Oil (O)	1	.01011
Temperature (T)	1	.11261*
H x D	28	.23579**
H x O	7	.01771
H x T	7	.01802
D x O	4	.04992
D x T	4	.11609**
T x O	1	.04722
H x D x O	28	.02463
H x D x T	28	.04183
D x O x T	4	.07955**
H x O x T	7	.02734
H x D x O x T	28	.02874
Replicates	1	.01539
Error	159	.02283

**Significant at the .01 level of probability
*Significant at the .05 level of probability

Those interactions which contained hens or days were the only ones which were significant, and some of these did not exert a significant effect upon the results.

Ovalbumin A_2 - The average percentages of Ovalbumin A_2 are given in Table XV.

Table XV

MEAN PERCENTAGE VALUES OF OVALBUMIN A_2 FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	11.90	10.40	10.30	11.70	8.60
Room temperature-oiled	12.80	9.80	10.60	11.30	9.90
Refrigerated-unoiled	9.60	10.30	11.90	10.10	9.90
Refrigerated-oiled	11.70	10.60	11.10	10.10	9.30

An overall decrease in this protein fraction for all treatments may be noted, with the exception of refrigerated-unoiled eggs. This treatment exhibited an increase in percentage of ovalbumin A_2 from 9.60 for one day to 9.90 after eighty-one days of storage. Through examination of the data for the eggs stored at room temperature, it can be seen that the change in ovalbumin A_2 is quite abrupt between one and three days of storage. After this time and through the twenty-seventh day of storage, the percentage is rather constant, with a decrease occuring between this and the final storage period of the study.

In contrast it will be noted that for the eggs stored at refrigerator temperatures, the percentage of ovalbumin A_2 remained fairly constant through nine days of storage. Subsequent to this storage period, a rather abrupt drop in the percentage of this fraction occurred. It can be postulated from this that refrigeration has an effect on the percentage of ovalbumin A_2, and that this treatment has a greater effect than oil-treating of the shell.

The independent factors or hens and days show a highly significant effect when the analysis of variance technique is applied to the data (Table XVI). Although the foregoing discussion indicated a temperature effect on the percentage of ovalbumin A_2, this effect was not of sufficient magnitude to be significant at the .05 level of probability. It is suggested that the reason for this lack of significance may be the small number of degrees of freedom. This conslusion is substantiated by the observation that four of the seven interactions which are significant at either the .01 or .05 level of probability contain the factor of temperature.

Ovalbumin A_1 - The final individual fraction which is to be discussed is that of ovalbumin A_1, and the data on its distribution are shown in Table XVII. With the exception of the twenty-seventh day of storage, a marked decrease was found in this fraction for all treatments. There was no consistent treatment effect at any of the days of storage.

Again, the analysis of variance (Table XVIII) for this particular protein fraction indicated highly significant effects for the independent variables of hen and days of storage time (.01 level of probability). Also, two of the interactions which contained the variable of storage time showed a highly significant

Table XVI

ANALYSIS OF VARIANCE FOR THE OVALBUMIN A_2 PROTEIN FRACTION

Source of variation	d.f.	Mean squares
Total	319	--
Hen (H)	7	.15446**
Days (D)	4	.34123**
Oil (O)	1	.04908
Temperature (T)	1	.04993
H x D	28	.20964**
H x O	7	.01654
H x T	7	.06284**
D x O	4	.07332*
D x T	4	.19688**
T x O	1	.00198
H x D x O	28	.05009**
D x O x T	4	.06569*
H x O x T	7	.01207
H x D x O x T	28	.02130
Replicates	1	.00564
Error	159	.02138

**Significant at the .01 level of probability
*Significant at the .05 level of probability

Table XVII

MEAN PERCENTAGE VALUES OF OVALBUMIN A_1 FOR ALL TREATMENTS BY STORAGE TIMES

Treatment employed	Storage time in days				
	1	3	9	27	81
Room temperature-unoiled	23.30	22.40	20.10	26.90	19.20
Room temperature-oiled	24.90	22.90	27.70	26.80	21.00
Refrigerated-unoiled	23.30	23.50	22.10	29.70	19.70
Refrigerated-oiled	22.70	21.80	21.30	28.70	19.20

effect on the ovalbumin A_1 protein fraction. Although temperature and oil treatment effects were not discernible through examination of the means for this fraction (Table XVII), the interaction for these two variables was shown to be highly significant (Table XVIII).

pH-Haugh Units - The correlation coefficients for the pH and Haugh unit values obtained for this study are presented for room temperature-unoiled (Table XIX), room temperature-oil treated (Table XX), refrigerated-unoiled (Table XXI), and refrigerated-oil treated eggs (Table XXII) by each of the storage time.

The pH-Haugh unit correlations follow a consistently negative pattern for the room temperature-unoiled eggs (Table XIX) after one, three, nine, and twenty-seven days of storage. For these storage times, as the pH increased,

Table XVIII

ANALYSIS OF VARIANCE FOR THE OVALBUMIN A_1 PROTEIN FRACTION

Source of variation	d.f.	Mean squares
Total	319	--
Hen (H)	7	.32302**
Days (D)	4	2.81429**
Oil (O)	1	.00181
Temperature (T)	1	.02535
H x D	28	.40082**
H x O	7	.01424
H x T	7	.04060
D x O	4	.02810
D x T	4	.12373**
T x O	1	.36626**
H x D x O	28	.03056
H x D x T	28	.03958
D x O x T	4	.00896
H x O x T	7	.02246
H x D x O x T	28	.03995
Replicates	1	.07057
Error	159	.03164

**Significant at the .01 level of probability

Table XIX

CORRELATION COEFFICIENTS FOR ROOM TEMPERATURE - UNOILED EGGS BY STORAGE TIMES

	1 day		3 days		9 days		27 days		81 days	
	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units
Lysozyme	1.000	+.030	1.000	+.682	1.000	+.417	1.000	-.155	1.000	+.393
pH	-.043	-.373	-.118	-.202	-.341	-.034	-.006	-.097	-.371	+.017
Non-Mobile Protein	1.000	-.125	1.000	+.081	1.000	+.490	1.000	-.211	1.000	+.132
pH	+.400		+.012		-.202		+.233		+.105	
Conalbumin	1.000	+.321	1.000	-.640	1.000	-.336	1.000	+.371	1.000	+.297
pH	-.303		+.410		+.197		-.039		+.052	
Ovoglobulin & Ovomucoid	1.000	-.262	1.000	+.077	1.000	+.205	1.000	-.176	1.000	+.019
pH	+.202		-.192		+.290		+.190		+.362	
Ovalbumin A_3	1.000	-.152	1.000	+.608	1.000	-.542	1.000	+.136	1.000	-.097
pH	+.024		-.236		+.032		-.578		+.161	
Ovalbumin A_2	1.000	+.117	1.000	-.361	1.000	-.502	1.000	+.015	1.000	-.319
pH	-.240		-.035		+.432		+.316		-.074	
Ovalbumin A_1	1.000	-.047	1.000	-.579	1.000	-.095	1.000	+.217	1.000	-.359
pH	+.070		+.305		-.166		-.297		+.212	

Table XX

CORRELATION COEFFICIENTS FOR ROOM TEMPERATURE - OILED EGGS BY STORAGE TIMES

	1 day		3 days		9 days		27 days		81 days	
	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units
Lysozyme	1.000	-.335	1.000	+.533	1.000	+.210	1.000	+.255	1.000	+.221
pH	+.216	-.873	-.389	-.382	-.370	-.099	-.385	+.434	-.032	-.272
Non-Mobile Protein	1.000	-.224	1.000	+.379	1.000	-.262	1.000	+.246	1.000	+.320
pH	+.202		-.237		+.353		+.292		+.139	
Conalbumin	1.000	+.375	1.000	-.375	1.000	-.030	1.000	+.485	1.000	-.405
pH	-.263		+.381		-.377		+.319		+.093	
Ovoglobulin & Ovomucoid	1.000	-.109	1.000	-.203	1.000	-.146	1.000	-.423	1.000	+.253
pH	-.185		+.524		-.373		-.098		-.080	
Ovalbumin A_3	1.000	+.230	1.000	+.536	1.000	-.057	1.000	+.103	1.000	-.161
pH	-.325		-.439		-.248		-.446		-.033	
Ovalbumin A_2	1.000	-.138	1.000	+.226	1.000	-.090	1.000	+.056	1.000	-.204
pH	+.195		+.267		+.504		+.505		+.085	
Ovalbumin A_1	1.000	+.177	1.000	-.532	1.000	+.089	1.000	-.089	1.000	-.158
pH	+.036		+.191		+.506		+.230		-.147	

Table XXI

CORRELATION COEFFICIENTS FOR REFRIGERATED - UNOILED EGGS BY STORAGE TIMES

	1 day		3 days		9 days		27 days		81 days	
	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units
Lysotyme	1.000	-.108	1.000	+.595	1.000	+.100	1.000	+.333	1.000	+.639
pH	+.208	-.507	-.010	+.030	-.279	-.391	-.520	-.087	-.339	-.357
Non-Mobile Protein	1.000	-.102	1.000	+.391	1.000	+.521	1.000	+.301	1.000	+.085
pH	+.277		+.205		+.105		-.373		+.206	
Conalbumin	1.000	+.424	1.000	-.637	1.000	-.400	1.000	-.354	1.000	-.262
pH	-.369		+.132		+.322		+.653		-.521	
Ovoglobulin & Ovomucoid	1.000	-.210	1.000	+.252	1.000	-.229	1.000	-.375	1.000	-.075
pH	-.036		-.016		+.529		+.102		-.015	
Ovalbumin A_3	1.000	-.399	1.000	+.531	1.000	-.109	1.000	+.235	1.000	+.640
pH	+.112		+.005		-.375		-.205		+.178	
Ovalbumin A_2	1.000	-.084	1.000	-.682	1.000	+.050	1.000	-.197	1.000	-.399
pH	+.214		-.056		+.088		+.080		+.626	
Ovalbumin A_1	1.000	+.271	1.000	-.458	1.000	-.123	1.000	-.087	1.000	-.455
pH	-.119		-.039		+.199		+.317		+.594	

Table XXII

CORRELATION COEFFICIENTS FOR REFRIGERATED - OILED EGGS BY STORAGE TIMES

	1 day		3 days		9 days		27 days		81 days	
	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units	Fract.	Haugh Units
Lysotyme	1.000	-.137	1.000	+.668	1.000	+.390	1.000	-.194	1.000	-.426
pH	-.054	-.075	+.005	-.095	+.252	-.193	-.342	+.149	+.128	-.017
Non-Mobile Protein	1.000	-.162	1.000	+.007	1.000	+.158	1.000	+.353	1.000	-.068
pH	+.071		-.019		-.274		-.159		-.123	
Conalbumin	1.000	+.186	1.000	-.566	1.000	-.220	1.000	+.235	1.000	-.008
pH	-.445		+.055		+.450		-.471		+.202	
Ovoglobulin & Ovomucoid	1.000	-.012	1.000	-.195	1.000	-.200	1.000	-.315	1.000	+.053
pH	+.234		+.231		-.277		-.298		-.442	
Ovalbumin A_3	1.000	-.286	1.000	+.682	1.000	-.077	1.000	-.222	1.000	-.049
pH	+.261		-.020		-.184		-.254		+.153	
Ovalbumin A_2	1.000	+.289	1.000	-.651	1.000	-.363	1.000	-.055	1.000	-.352
pH	-.723		-.096		+.285		-.470		-.410	
Ovalbumin A_1	1.000	+.241	1.000	-.542	1.000	-.426	1.000	+.258	1.000	-.491
pH	-.003		+.049		-.011		+.674		+.278	

the Haugh units for this particular treatment decreased. At eighty-one days of storage time, the slight positive correlation of .017 indicated that, since Haugh unit measurements are based on albumen height, there may be a limit to which these units may drop. Conversely, the pH of the egg albumen may increase to a specific level and remain constant.

Very closely related to the above treatment are the pH-Haugh unit values for room temperature-oil treated eggs (Table XX). These values show an inverse relationship at one, three, nine, and eighty-one days of storage. These values are -.873, -.382, -.099, and -.272, respectively. For twenty-seven days of storage time, there existed a positive correlation of .434, which may have been due to biological and/or mechanical error.

For the pH-Haugh unit correlations under the refrigerated-unoiled conditions (Table XXI), it was noted that when pH increased, Haugh units decreased. This was indicated by the negative correlation values of .50% for one day, .391 for nine days, .087 for twenty-seven days, and .357 for eighty-one days of storage. Much the same type of relationship was present for refrigerated-oil treated eggs (Table XXII), with negative correlations being noted for one, three, nine, and eighty-one days of storage.

Lysozyme-pH; Lysozyme-Haugh unit Correlation Coefficients - The correlation coefficients for the lysozyme fraction under room temperature-unoiled conditions are presented in Table XIX. These values are presented for each separate storage period for the above conditions. It may be noted that a negative correlation existed between the lysozyme fraction and the pH value for the five storage periods, with one day of storage time being relatively minor in comparison

to the third, ninth and eighty-first days. Positive lysozyme-Haugh unit correlations of .030, .682, .417, and .393 for one, three, nine, and eighty-one days, respectively, were also observed. This would indicate that for room temperature--unoiled eggs, Haugh units and relative lysozyme content changed in the same direction, i.e., higher quality eggs contain a larger amount of lysozyme. However, it may also be observed that a negative correlation was present for lysozyme-pH and lysozyme-Haugh unit values at twenty-seven days of storage. These values cannot be explained at this particular time. However, it may be postulated that lysozyme was not influenced by pH or Haugh units at twenty-seven days of storage time under this treatment.

Much the same relationship may be noted for room temperature-oil treated eggs (Table XX) with a positive fraction-pH correlation of .216 at one day of storage and a negative Haugh unit-lysozyme correlation of .335 being observed. These values indicate that pH and lysozyme are quite closely related at this particular storage period. Conversely, for three, nine, twenty-seven, and eighty-one days of storage, negative lysozyme-pH correlations of .389, .370, .385, and .032, respectively, were obtained, while for the same time periods positive Haugh unit-fraction values of .533, .210, .255, and .221, respectively, were obtained. These correlations show that Haugh units and lysozyme appear to have much the same type of relationship, which was noted for pH and lysozyme for the one-day period of storage.

The refrigerated-unoiled eggs employed in this study differed somewhat in the type of relationship which was exhibited (Table XXI). A positive correlation of .208 was present for the lysozyme-pH value, with a negative Haugh

unit-fraction correlation of .108 for one day of storage, indicating that pH and lysozyme were again related to some extent at this storage period. For three days of storage there was observed a negative pH-fraction correlation of .010 with a positive Haugh unit-fraction correlation of .108, indicating again that Haugh units and lysozyme changed in the same direction. Also observed were negative pH-fraction correlations for nine days (.279), twenty-seven days (.520), and eighty-one days (.339) of storage, with positive Haugh unit-fraction values of .100, .333, and .639 for the same storage periods. The inference drawn from this indicates that Haugh units and lysozyme were interrelated to varying degrees at these storage times.

The correlations for the lysozyme fraction under refrigerated-oil treated conditions (Table XXII) showed that a negative pH-fraction value was observed for one and twenty-seven days of storage, with negative Haugh unit-lysozyme correlations for these same periods. These values, though possibly interrelated in some way, cannot be explained adequately in this study. This was also true for three- and nine-day storage times, i.e., positive correlations for lysozyme-pH of .005 and .252, respectively, for the three- and nine-day storage times. Positive Haugh unit-lysozyme correlations of .668 and .390 were also present for these storage periods. These values could possibly have been influenced by some variable that was not measured. In contrast to the other storage times, the eighty-one day period of storage exhibited a positive pH-lysozyme correlation of .128 and a negative Haugh unit-lysozyme value. This relationship showed that pH and lysozyme were positively related, to a small degree, for this storage period.

Non-Mobile Protein-pH; Non-Mobile Protein-Haugh unit Correlation Coefficients - Table XIX shows the correlation coefficients for the non-mobile protein fraction, pH, and Haugh units for room temperature-unoiled eggs. It may be noted that at one and twenty-seven days of storage there existed positive correlations of .400 and .233 for non-mobile protein-pH, and negative fraction--Haugh unit correlations of .125 and .211, respectively, for these storage periods. These values indicate that non-mobile protein and pH exhibited a marked relationship to one another during these storage times. For nine days of storage a negative fraction-pH value of .202 was observed, with a positive Haugh unit-fraction value of .490, indicating that non-mobile protein and Haugh units changed in the same direction for this storage time. The positive fraction-pH and fraction-Haugh unit correlations for three and eighty-one days of storage cannot be explained at this point.

The correlation coefficients for the non-mobile protein fraction for room temperature-oil treated eggs (Table XX) showed positive pH-fraction correlations of .202 for one day and .353 for nine days of storage time, and negative Haugh unit-fraction values for these same storage times. As was noted for room temperature-unoiled eggs (Table XIX), pH and non-mobile protein exhibited a definite relationship to one another at these particular periods of storage. For three days of storage time, there existed a negative pH-fraction value of .237 and a positive Haugh unit-fraction value of .379, indicating that at this level of storage the non-mobile protein and Haugh units exhibited a market relationship to one another. Positive pH-fraction correlations of .292 for twenty-seven days and .139 for eighty-one days, with positive Haugh unit-fraction values for these storage times,

indicate that for eggs stored under room temperature-oil treated conditions it is not possible to explain the above relationship when correlation coefficients were used as a research tool in this study.

In refrigerated-unoiled eggs it will be observed that, for one day of storage time, the non-mobile protein-pH and Haugh unit-fraction correlations (Table XXI) showed that non-mobile protein and pH may have changed in the same direction for this period. At three, nine and eighty-one days of storage, positive fraction-pH correlations of .205, .105 and .206 and positive Haugh unit-fraction correlations of .391, .521 and .301 were observed. The inferences which may be gained from these values are that the amount of non-mobile protein present at these levels of storage time neither increased with pH nor decreased with Haugh units. At twenty-seven days of storage a negative pH-fraction correlation of .373 and a positive Haugh unit-fraction correlation of .301 indicated that at this period of storage non-mobile protein and Haugh units exhibited a definite relationship.

The non-mobile protein, pH and Haugh unit correlations for refrigerated-oil treated eggs indicated that, at one day of storage, non-mobile protein and pH may have changed to some extent (Table XXII). This was shown by the positive pH-fraction correlation value of .071 and the negative value for fraction-Haugh units of .162. Conversely for three, nine, and twenty-seven days of storage negative pH-fraction correlations of .019, .274, and .159 and positive Haugh unit-fraction correlations of .007, .158, and .353, respectively, indicated that non-mobile protein and Haugh units exhibited a varied but definite relationship for these particular periods. For eighty-one days of storage, negative pH-fraction correlations of .123 and a negative correlation of .068 for non-mobile protein-Haugh units indicated that

the non-mobile protein had neither increased nor decreased with pH or Haugh units.

Conalbumin-pH; Conalbumin-Haugh unit Correlation Coefficients - The correlation coefficients for conalbumin in eggs (Table XIX) under room temerature-unoiled conditions exhibited a positive correlation for fraction-Haugh units of .321 and .371 and a negative correlation for fraction-pH of .303 and .039 at one and twenty-seven days of storage, respectively. The inferences gained from these values indicated that relative conalbumin content and Haugh units were highly correlated for the above storage times. For three and nine days of storage, the positive pH-fraction correlation values of .410 and .197 and negative fraction-Haugh unit values of .640 and .336 respectively, showed that the pH and conalbumin values were definitely related at these storage times. The positive fraction-pH and fraction-Haugh unit correlations obtained for eighty-one days of storage showed also that the conalbumin content did not increase with pH nor decrease with Haugh units for this particular period.

The correlation values for conalbumin under room temperature-oil treated conditions are given in Table XX. These values exhibited a positive Haugh unit-fraction value of .375 and a negative pH-fraction value of .263, indicating that Haugh units and relative percent of conalbumin changed in the same direction at one day of storage. On the other hand, the positive pH-fraction correlations of .381 and .093 for three days and eighty-one days, respectively, and the negative values for Haugh units of .375 and .405 for these storage times, showed that conalbumin and pH values were interrelated to some degree. The positive pH and Haugh unit-fraction values for nine days of storage

indicated that these variables were not related to any degree at this point. This was also true for the twenty-seven day period of storage, with negative pH and Haugh unit-fraction correlations exhibited.

The correlation coefficients for refrigerated-unoiled eggs (Table XXI) also showed that the Haugh unit and conalbumin values were definitely related for one day of storage time. Positive pH-fraction values of .132, .322, and .653 and negative Haugh unit-fraction correlations of .637, .400, and .354 for three, nine, and twenty-seven days of storage, respectively, were noted. These values indicated that pH and relative content of conalbumin were highly correlated for these storage periods. Unexplainable negative fraction-pH and fraction-Haugh unit values may be noted for eighty-one days of storage.

Correlation values for refrigerated-oil treated eggs are presented in Table XXII. The positive Haugh unit-fraction value of .186 and the negative pH-fraction value of .445 for one day of storage indicated that Haugh units and conalbumin may have decreased in eggs under this treatment. Conversely, the positive pH-fraction correlations of .055, .450, and .202 and the negative Haugh unit-fraction values of .566, .220, and .008 for three, nine and eighty-one days of storage showed that pH and conalbumin changed in the same direction when considering this treatment employed at the above storage times. Again unexplainable correlations were observed at twenty-seven days of storage.

Ovoglobulin and Ovomucoid-pH; Ovoglobulin and Ovomucoid-Haugh Unit Correlation Coefficients - The correlation coefficients for room temperature--unoiled eggs for the combined ovoglobulin and ovomucoid fractions exhibited positive correlations for the fraction-pH values of .202 and .190, and negative

Haugh unit-fraction correlations of .262 and .176 for one and twenty-seven days of storage, respectively. These values indicated that ovoglobulin and/or ovomucoid and pH were definitely related to one another at these storage times. Positive pH-fraction and Haugh unit-fraction values were observed for nine and eighty-one days of storage, indicating that ovoglobulin and/or ovomucoid neither decreased nor increased in relationship to pH or Haugh units for these storage times. A small negative correlation of .192 for fraction-pH and a small positive correlation of .077 for fraction-Haugh units existed at three days of storage time. This indicated a very small, but definite relationship existed in ovoglobulin and/or ovomucoid and Haugh units for this storage period.

The ovoglobulin and ovomucoid-pH and Haugh unit-fraction correlations for room temperature-oil treated eggs (Table XX) exhibited negative pH-fraction values of .185 for one day, .373 for nine days, and .098 for twenty-seven days of storage. Negative Haugh unit-fraction values of .109, .146, and .423, respectively, also occurred. These values infer that very little influence on ovoglobulin and/or ovomucoid by pH and Haugh units occurred. However, the positive pH-fraction correlation of .524 and the negative Haugh unit-fraction value of .203 for three days of storage indicated that pH and ovoglobulin and/or ovomucoid values exhibited a marked relation at this storage time. Conversely, at eighty-one days of storage, a positive Haugh unit-fraction correlation of .253 and a negative pH-fraction value of .080 showed that Haugh units and ovoglobulin and/or ovomucoid relationship existed but that it was of a much less magnitude than was pH and ovoglobulin and/or ovomucoid.

Refrigerated-unoiled eggs exhibited a small negative correlation (Table XXI) for ovoglobulin and ovomucoid-pH and Haugh units for one and eighty-one days of storage. These values indicate that there existed very little relationship between these variables during these storage times. For three days of storage, a negative pH-fraction correlation of .016 and a positive Haugh unit-fraction correlation of .252 inferred that Haugh units and ovoglobulin and/or ovomucoid changed in the same direction. Conversely, the positive pH-fraction correlations of .529 for nine days and .102 for twenty--seven days of storage with negative Haugh unit-fraction values of .229 and .375 showed that pH and relative ovoglobulin and/or ovomucoid content exhibited a definite relationship during these storage times.

The correlation coefficients for refrigerated-oil treated eggs (Table XXII) in the combined ovoglobulin and ovomucoid fractions exhibited a positive pH--fraction value of .234 for one, and .231 for three days of storage and negative Haugh unit-fraction values of .012 and .195, respectively. Those correlations indicate that pH and the combined fractions of ovoglobulin and ovomucoid changed in the same direction at these storage periods. Unexplainable negative pH and Haugh unit-fraction correlations for nine and twenty-seven days were also observed. The negative pH-fraction value of .442 and the small positive Haugh unit value of .053 at eighty-one days of storage indicated that Haugh units and the combined ovoglobulin and/or ovomucoid fraction were interrelated, though the relationship of these protein fractions to Haugh units is only slight.

Ovalbumin A_3-pH; Ovalbumin A_3-Haugh Unit Correlation Coefficients - The correlation coefficients for the ovalbumin A_3 protein fraction (Table XXII)

exhibited positive pH-fraction values of .024 for one, .032 for nine, and .161 for eighty-one days of storage. Negative Haugh unit-fraction values of .152, .542, and .097 were also present, thus indicating that pH and ovalbumin A_3 values were positively related in eggs under this treatment at these storage times. For three and twenty-seven days of storage, negative pH-fraction values of .236 and .578 and positive Haugh unit-fraction values of .608 and .136, respectively, for these days of storage were observed. In a converse relationship to the one-, nine- and eighty one-day values, the three- and twenty seven-day correlations indicated that Haugh units and ovalbumin A_3 decreased to some extent at these particular storage periods.

Under room temperature-oil treatment conditions, correlations for ovalbumin A_3, pH and Haugh units, presented in Table XX, exhibited negative pH-fraction values of .325 for one, .439 for three, and .446 for twenty--seven days of storage, and positive Haugh unit-fraction values of .230, .536, and .103, respectively, for these same storage periods. These values showed that the ovalbumin A_3 content and Haugh units exhibited a definite relationship to one another. Unexplainable negative pH-fraction values of .248 for nine, and .033 for eighty-one days of storage and negative Haugh unit-fraction correlations of .057 and .161 for these storage periods were also observed.

The ovalbumin A_3, pH and Haugh unit correlation coefficients for refrigerated-unoiled eggs (Table XXI) exhibited a positive pH-fraction value of .112 for one day and a negative Haugh unit-fraction value (.399), indicating that ovalbumin A_3 and pH had a small but definite relationship.

Positive three and eighty-one day pH and Haugh unit-fraction correlations also showed that ovalbumin A_3 is related very little to the pH and/or Haugh unit values obtained. The negative pH and Haugh unit-fraction values for nine days of storage also indicate the above. A negative pH-fraction value of .205 and a positive Haugh unit fraction value of .235 for twenty-seven days of storage indicated that ovalbumin A_3 and Haugh units exhibited a defined relationship for this particular period of storage.

The correlation values for ovalbumin A_3 under refrigerated oil treated conditions (Table XXII) showed positive pH-fraction values of .261 for one and .153 for eighty-one days of storage, respectively. Negative Haugh unit-fraction values of .286 and .049 for these storage times indicated that ovalbumin A_3 and pH changed in the same direction. Conversely, a small negative pH-fraction value of .020 and a positive Haugh unit-fraction value of .682 for three days of storage showed that ovalbumin A_3 and Haugh units exhibited a small but definite relationship at this point. Unexplainable negative pH and Haugh unit-fraction values for nine and twenty-seven days of storage were also observed.

Ovalbumin A_2-pH; Ovalbumin A_2-Haugh Unit Correlation Coefficients - The correlation coefficients for ovalbumin A_2 under room temperature-unoiled conditions (Table XIX) showed a negative pH-fraction value of .240 and a positive Haugh unit-fraction correlation of .117 for one day of storage. These values indicate that Haugh units and ovalbumin A_2 may have decreased for this storage period. A negative pH -fraction value of .035 for three days and .074 for eighty-one days accompanied by negative Haugh unit-fraction values of .361

and .319, respectively, indicated that very little change occurred in ovalbumin A_2 due to the influence of pH or Haugh units. This relationship is also true for twenty-seven days of storage, where positive pH and Haugh unit-fraction correlations were noted. For nine days of storage a positive pH-fraction correlation of .432 and a negative Haugh unit-fraction value of .502 showed that the volume of ovalbumin A_2 and pH values were closely related.

Room temperature-oil treated egg correlations for ovalbumin A_2 (Table XX) exhibited negative Haugh unit-fraction correlations of .138 for one, .090 for nine, and .204 for eighty-one days of storage, and positive pH-fraction values of .195, .504, and .085, respectively. These values indicated that pH and ovalbumin A_2 exhibited a very definite relationship at these storage periods. The positive pH-fraction correlations of .267 for three and .505 for twenty-seven days of storage and the positive Haugh unit-fraction values of .226 and .056 at these storage times indicated that little change in the ovalbumin A_2 protein fraction occurred due to pH and/or Haugh units.

Positive pH-fraction correlations for eggs under refrigerated-unoiled conditions (Table XXI) of .214 for one, .080 for twenty-seven, and .626 for eighty-one days of storage accompanied by negative Haugh unit-fraction correlations of .084, .197, and .399, respectively, for these storage times may be noted. These values indicate that ovalbumin A_2 and pH changed in the same direction. The negative pH-fraction value of .056 and the negative Haugh unit-fraction correlation of .682 exhibits that little change occurred in the ovalbumin A_2 protein-fraction due to pH or Haugh units. The small positive values for nine days of storage also indicated the above. This is, pH and

Haugh units did not influence the ovalbumin A_2 protein fraction to any great extent.

Ovalbumin A_2, pH and Haugh unit correlations for refrigerated-oil treated eggs are presented in Table XXII. The negative pH-fraction correlation of .723 for one day of storage and the positive Haugh unit-fraction value of .289 showed that ovalbumin A_2 and Haugh units are closely interrelated at this storage time. The negative pH-fraction values of .096 for three, .470 for twenty-seven, and .410 for eighty-one days of storage and the negative Haugh unit-fraction values of .651, .055, and .352, respectively, for these periods indicated that ovalbumin A_2 volume is influenced very little by pH and/or Haugh units at these storage times. The positive pH-fraction value of .285 and the negative Haugh unit-fraction correlation of .363 for nine days of storage would indicate that pH and ovalbumin A_2 are definitely interrelated at nine days of storage.

Ovalbumin A_1-pH; Ovalbumin A_1-Haugh Unit Correlation Coefficients - Room temperature-unoiled correlation coefficients for the ovalbumin A_1 protein-fraction (Table XIX) exhibited positive pH-fraction correlations of .070 for one, .305 for three, and .212 for eighty-one days of storage and negative Haugh unit-fraction values of .047, .579 and .359, respectively, for these storage times, indicating that a corresponding relationship between these variables existed. The negative pH-fraction and Haugh unit-fraction values, for nine days of storage, of .166 and .095, respectively, showed that ovalbumin A_1 volume is not influenced to any great extent by pH and/or Haugh unit values. At twenty-seven days of storage a negative pH-fraction value of .297 and a positive Haugh unit-fraction value of .217 exhibited that Haugh units and ovalbumin A_1 changed

in the same direction.

Room temperature-oil treated eggs (Table XX) gave positive pH-fraction correlations of .036 for one and .506 for nine days of storage along with positive Haugh unit-fraction values of .177 and .089, respectively, for these storage times. This indicated that very little change occurred in the ovalbumin A_1 fraction due to the influence of pH and/or Haugh unit values. Three and twenty-seven days of storage showed positive pH-fraction values of .191 and .230, respectively, and negative Haugh unit-fraction values of .532 and .089, indicating that pH and ovalbumin A_1 were interrelated to some extent at these storage periods. The negative fraction-pH correlation (.417) and fraction-Haugh unit value (.158) showed that little change in ovalbumin A_1 occurred for eighty-one days of storage due to pH and/or Haugh unit values, under conditions of this study.

The negative pH-fraction value of .119 and the positive Haugh unit--fraction correlation of .271, for one day of storage under refrigerated-unoiled conditions (Table XXI), indicated that Haugh units and ovalbumin A_1 exhibited a marked relationship at this storage time. For three days of storage, negative pH-fraction and Haugh unit-fraction values of .039 and .458, respectively, indicated that little change in the ovalbumin A_1 fraction occurred due to pH and/or Haugh units. Positive pH values of .199, .317, and .594 for nine, twenty-seven and eighty-one days, respectively, along with the negative Haugh unit-fraction correlations of .123, .087, and .455 for these storage periods, showed that overall pH and ovalbumin A_1 values changed in the same direction for these storage times.

A small negative correlation of .003 for the pH-fraction and a positive Haugh unit-fraction correlation of .241 for one day of storage under refrigerated--oiled conditions (Table XXII), indicated that Haugh units and ovalbumin A_1 exhibited a close relationship to one another. The positive correlation for fraction-pH of .049 for three, and .278 for eighty-one days of storage, along with the negative Haugh unit-fraction values of .542 and .491, respectively, exhibited that pH and ovalbumin A_1 were definitely interrelated at these storage times. The negative pH-fraction correlation of .011 for nine days, along with a negative Haugh unit-fraction value of .426 at this storage time, showed that little change occurred in ovalbumin A_1 at this storage level due to pH and/or Haugh units. The positive pH-fraction and Haugh unit-fraction correlations for twenty-seven days of storage also showed that little change occurred in ovalbumin A_1 due to the pH and Haugh unit values obtained.

SUMMARY AND CONCLUSIONS

Eggs from eight individual hens were stored under four treatment conditions, as per the following; room temperature-unoiled, room temperature-oiled, refrigerated-unoiled, and refrigerated-oiled for varying time periods of one, three, nine, twenty-seven, and eighty-one days of storage. These eggs were then broken out and by using the electrophoretic migration of protein as a research tool, data were collected on relative percentages of lysozyme, non-mobile protein, conalbumin, ovoglobulin and ovomucoid, ovalbumin A_3, ovalbumin A_2, and ovalbumin A_1. Haugh unit and pH measurements were also obtained in this study.

From the analysis of these data the following conclusions can be drawn:

1. In general, the average pH value increased throughout the period of the study for all treatments; however, some variations for the intermediate storage times were observed. Those groups of eggs which had been oil-treated exhibited a smaller pH change than the untreated eggs, regardless of temperature or time of storage.
2. Haugh units decreased for all treatments in relation to time in storage. Oil treatment of the shell resulted in a marked retention of egg quality as compared to the untreated groups. Temperature likewise had a definite effect on quality change with the refrigerated groups, maintaining higher quality than the non-refrigerated groups.

3. The percentage of lysozyme tended to increase between the first and the eighty-first day of storage, but no clear cut treatment effects could be discerned.
4. The percentages of non-mobile protein, conalbumin and ovoglobulin--ovomucoid appeared to flucuate to some extent throughout the entire study regardless of treatments.
5. There was a tendency for the percentage of ovalbumin A_3 to remain relatively high for those groups of eggs held under refrigerated conditions.
6. There was an overall tendency for the percentage of ovalbumin A_2 to decrease, with the exception of the refrigerated-unoiled group.
7. The overall percentage of ovalbumin A_1 decreased regardless of treatment.
8. The independent effect due to hens was significant at both the .05 and the .01 levels of probability for all of the criteria measured, except for pH.
9. The independent effect due to days of storage was significant at the .01 level of probability for all criteria.
10. The independent variable temperature (of storage location) had an effect upon Haugh units and conalbumin, (.01 level of probability) and upon the non-mobile protein and ovalbumin A_3 (.05 level of probability), but did not have an effect on the other criteria measured.

11. The fourth independent variable, that of oil treatment of the shell, had a significant effect (.01 level of probability) on pH and Haugh units, but no significant effect on any of the other criteria tested.

12. The day x temperature interaction proved to be significant in its effect on all criteria, but all other interactions varied in the magnitude of their individual effects.

13. For the most part the correlation coefficients between pH and Haugh units were negative.

14. Because of the method of calculation used in determining the correlation coefficients presented in this thesis, no conclusions can be drawn relative to the overall effect of pH and Haugh units on any of the criteria considered. The relationships evident from the values presented are valid only for the day or treatment for which the value is presented.

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VITA

Joe Phillip Hudspeth

Candidate for the Degree of

Master of Science

Thesis: AN ELECTROPHORETIC STUDY OF CHICKEN EGG ALBUMEN

Major Field: Poultry Products Technology

Biographical:

Personal Data: Born in Afton, Oklahoma, December 22, 1940, the son of Joe T. and Zetta L. Hudspeth.

Education: Graduated in 1958 from Afton High School; received the Bachelor of Science Degree in Agriculture from the Oklahoma State University with a major in Poultry Science, May, 1962; completed requirements for Master of Science Degree May, 1964.

Professional Experience: Research Assistant in Poultry Products Technology, Oklahoma State University, June, 1962 to present.

Organizations: Poultry Science Club, Poultry Science Association, Alpha Zeta.