EFFECTS OF SOME ACIDIC AND ALKALINE ATMOSPHERES ON pH AND EGG QUALITY

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INTRODUCTION

Egg quality deterioration is a problem which has been of vital concern to the egg industry for many years. Efforts to find better methods for maintaining egg quality have been the subject of research for considerable time.

It has been shown by research workers that egg quality deterioration will occur more rapidly by increasing the pH of an egg. It has been shown also that quality deterioration can be retarded by preventing any pH increase which may occur in eggs under normal storage conditions.

In this study some volatile acids and bases have been used which provided a range of pH levels so that comparisons could be made among those substances which provided different pH levels in the atmospheres in which the eggs were stored. Further study has been made on the relationship of pH to Haugh units. It is hoped that the information gained in this study will be helpful in understanding the relationship between pH and egg quality.

REVIEW OF LITERATURE

There are many factors which affect the quality of an egg as measured by Haugh units. Sharp (1937) in a survey of work done on egg quality deterioration reported that time, temperature, pH, and carbon dioxide were factors affecting the deterioration of egg albumen.

Spencer et al. (1956) observed that albumen quality declined linearly with the logarithm of elapsed time after breakout. In this study it was found that individual hens as well as the age of an egg also influenced the rate of quality loss. Cotterill et al. (1958) reported that as temperature was increased the amount of carbon dioxide lost from an egg likewise increased. Stadelman et al. (1954) observed a linear regression coefficient of -1.1548 for every 10 degrees C increase in temperature for eggs held in normal atmosphere.

The consistency of the thick white of an egg has been shown to be related to its pH. Schaible et al. (1935) observed that the thick white of an egg consisted of many laminated layers containing mucin fibers. Feeney (1955) reported that a high content of ovamucin was present in the thick white of an egg and that it probably existed in a fibrous form. In a study by McNally (1943) on ovamucin, varying conditions of this protein were shown to be present at different pH levels. This worker suggested that ovamucin exists as a salt in the thick white of a fresh egg. It was further stated that ovamucin exists in a precipitated condition at any pH less than 6.0 to 6.4, depending on the salt concentration, while at any pH between 6.0-6.4 to 8.3-8.5 the ovamucin exists in the form of a gel, and at

higher pH values it exists as a viscous solution. Healy and Peters (1925) observed a pH change in egg albumen from 8.2 for fresh eggs to a pH of 9.5 at 96 hours. This constituted a rapid pH change over time. In this same study it was reported that a carbon dioxide environment increased the hydrogen ion concentration of an egg.

Sharp (1929) reported that the pH of eggs stored under normal conditions will increase from 7.6 to 9.7 over long periods of storage due to carbon dioxide loss. It was also shown that varying amounts of carbon dioxide were necessary to hold pH at a constant level. It was suggested by this study that watery whites were produced in two different ways. The first of these ways was that as pH increases the thick white assumes a more fluid condition, and the other was that as pH decreases the amount of thick white decreases, even though it maintains its jelly-like properties. In a later study, Sharp and Stewart (1931) reported also that carbon dioxide retarded egg quality deterioration. This study also confirmed the hypothesis that eggs treated with an excess of carbon dioxide showed an increase in thin white and a decrease in thick white, although the thick white which remained did maintain its jelly-like properties and did stand up higher than the albumen of eggs stored under normal conditions.

Bose and Stewart (1948) reported that retention of carbon dioxide in an egg retarded both pH increase and egg quality deterioration. Cotterill (1955) observed that deterioration of the thick white of an egg was retarded by placing eggs in a carbon dioxide environment or by preventing loss of carbon dioxide from the egg contents. Cotterill and Gardner (1957) further reported that eggs treated with carbon dioxide or held in sealed containers showed less deterioration than eggs not

receiving these treatments. It was reported in this study that carbon dioxide retarded deterioration at all treatment temperatures.

Almquist and Lorenz (1932) reported losses in egg quality when eggs were placed in a concentrated carbon dioxide atmosphere. It was their feeling that the thin white and thick white of the egg differed in physical form. Wilhelm (1942) observed losses in quality of eggs when eggs were treated with an excess of carbon dioxide. Cloudiness of albumen in these eggs appeared to be an indication of a low pH, according to Wilhelm.

Evans and Carver (1942) observed that the oiling of eggs soon after they were laid retarded quality deterioration, but after several weeks of storage the thick white of these eggs showed considerable thinning. They reported that egg quality could be best maintained between a pH of 7.65 and 8.46, with a pH of 8.2 giving the best results. They indicated that eggs oiled the day after they were laid showed the least drop in quality. Results of work done by Swenson (1938) showed that the oiling of eggs using a vaccumn method improved the keeping quality of eggs even more than oiling methods used prior to that time. Homler and Stadelman (1963) confirmed that the oiling of eggs retarded quality deterioration. In other studies on the oiling of eggs to prevent quality losses, Stadelman and Wilson (1957) and Schwall et al. (1961) showed that spray oiling was an acceptable method of oiling eggs to preserve quality. Yoshok and Romanoff (1949) reported the successful use of plastics to retard deterioration of egg quality. These studies on the relationship of carbon dioxide and pH to egg quality indicated that quality is best maintained by holding carbon dioxide in an egg at a certain level and maintaining the pH within a particular range.

Romanoff and Yushok (1948) reported that the adding of acids to mineral oil as an oiling agent decreased shell permeability, kept pH low and retarded quality losses. Shell permeability was decreased seven to eleven percent more by mineral oil and acid mixtures than by plain mineral oil. Acids used in this study were lactic acid, stearic acid and acetic acid. Acetic acid was used by McNally (1943) as a precipitant for ovamucin. In this work as well as other isolated reports, hydrochloric acid has been used in egg albumen analysis, but no reports were found in which hydrochloric acid or acetic acid had been used as agents which might have an effect on egg quality deterioration.

The influence of ammonia on egg quality was shown to be a profound one by Cotterill and Nordskog (1954). A rapid increase in the deterioration of thick albumen was observed when eggs were treated with ammonium hydroxide. The yolks of these eggs became more translucent and acquired a deep orange hue. Similar results were found by Newell et al. (1966). Cunningham and Cotterill (1962), in a study involving the alkaline coagulation of egg white, reported that the viscosity of egg white gradually increased as pH increased up to a level of 11.5. Above a pH of 11.5 the viscosity of the egg white increased very rapidly until a gel was formed at pH 11.9. It was reported also that not only did pure ovamucin form a similar gel but also that albumen with the ovamucin fraction removed likewise formed a gel, thus the reason for alkaline albumen gel formation could not be tied entirely to ovamucin. It was stated in this study that the time required for alkaline coagulation of egg white was dependent on pH, rate of addition of alkali, temperature, volume of reagents and strength of alkali.

One conclusion reached after consideration of the reports in literature was that the physical state of thick albumen is dependent on pH and can be altered by substances which affect the pH. The study reported herein involved the effects of some volatile acids and bases on pH and egg quality when the eggs were stored in atmospheres created through the use of these acids and bases.

MATERIALS AND METHODS

Eggs used in this experiment were laid by 22 commercial strain Leghorn hens that were housed in individual cages in an environmentally controlled cage laboratory located on the Oklahoma State University Poultry Farm. The birds were provided with artificial light for a period of fourteen hours each day. A practical ration for laying hens, formulated by the Poultry Science Department at Oklahoma State University, was fed to the layers.

Eggs were gathered each afternoon at 4:30 p.m. and stored overnight in a cooler at 45 degrees to 50 degrees F. The following morning weights for each egg were taken and recorded in grams. Each egg was then broken and placed in the atmospheric treatment to which it had previously been randomly assigned. The eggs were exposed to atmospheres creased by the volatilization of the following six solutions;

1. 90 percent hydrochloric acid (HCL) solution
2. 25 percent acetic acid (CH_3COOH) solution
3. Concentrated ammonium hydroxide (NH_4OH) solution
4. 33 percent methylamine (CH_3NH_2) solution
5. Carbon dioxide (CO_2) gas
6. Normal air (control treatment)

After being broken into a 20 x 150 mm Petri dish, each egg was placed in an airtight plastic bag supported by a wire frame and sealed with a rubber band. Approximately five milliliters of one of the acids or bases in a separate container

was placed in each bag with the egg. A CO_2 atmosphere was produced by placing dry ice in a flask with a side arm for attaching a piece of rubber tubing and extending the tubing into the plastic bags. Those eggs placed in an environment of normal air were simply placed in a bag which was then sealed. While under treatment, the eggs were kept in a cooler at 48 degrees F.

Haugh unit measurements were made with an Ames tripod micrometer and pH readings were made using a Coleman metrion II pH meter, equipped with the standard-purpose glass electrodes. Haugh unit and pH readings were recorded at the following time periods: 1.5, 3, 6, 12, 24, 48 and 96 hours.

Analysis of the data obtained in this experiment was done with the aid and guidance of the Statistical Laboratory at Oklahoma State University. Overall means for each treatment and for all treatments combined were determined. The analysis of variance and correlation phase of the results and discussion were obtained through the use of the 1410 IBM computer system. The regression phase of the results and discussion was done with a desk calculator, using the means obtained for Haugh units in each treatment at each time period.

RESULTS AND DISCUSSION

General

The purpose of this study was to observe the effect of some previously untried chemical reagents on egg quality and to gain more information on the relationship between pH and egg quality. The results of this experiment will be discussed under the four main headings listed below:

1. Effect of treatments on pH,
2. Effect of treatments on the physical appearance of the eggs,
3. Effect of treatments on Haugh units, and
4. Relationship of pH and time to Haugh units.

Effect of Treatments on pH

A wide range of pH levels was established in eggs used in this experiment so that the relationship which may exist between pH and egg quality could be investigated more fully. The overall means for the pH of the egg albumen in each treatment are listed in Table I. It can be seen that the pH of the eggs used in this study was decreased by the two acidic reagents and by carbon dioxide. The opposite is true for those eggs stored in the atmospheres with the two alkaline reagents and with air. By comparing the effects of the two acidic reagents and carbon dioxide, it can be seen that carbon dioxide maintained the pH at the lowest level throughout the entire treatment period except at 96 hours. Acetic acid decreased the pH to 7.389 at 96 hours, which was the lowest pH reading attained by any

TABLE I

THE AVERAGE pH VALUES FOR ALL TREATMENTS FOR EACH TIME PERIOD

Atmospheric Treatment	Time periods in Hours						
	1.5	3	6	12	24	48	96
Hydrochloric Acid	8.577	8.543	8.461	8.343	8.411	8.255	8.130
Acetic Acid	8.582	8.605	8.520	8.218	8.205	7.875	7.389
Ammonium Hydroxide	11.041	11.084	11.189	11.277	11.182	11.093	11.070
Methyl Amine	10.509	10.770	10.905	11.073	11.027	10.998	10.989
Carbon Dioxide	8.100	8.052	8.018	7.548	7.741	7.611	7.432
Normal Air	8.925	9.011	9.066	9.000	9.020	9.123	9.145

treatment. Although hydrochloric acid volatilizes rapidly at room temperature, when it was placed inside the plastic containers used in this experiment and placed in the storage temperature at 48 degrees F., the rate of volatilization was reduced markedly. This is possibly a reason why the higher pH values were observed for eggs stored in the hydrochloric acid atmosphere compared to those values obtained with acetic acid and carbon dioxide atmospheres. The pH of the eggs stored with hydrochloric acid was maintained within the most narrow limits of all the treatments. The pH range for these eggs was maintained between 8.577 and 8.130. From six hours to 96 hours, the pH for these eggs was maintained within the range of 7.65 to 8.46 recommended by Evans and Carver (1942) for best maintenance of

egg quality. Increases in pH were noted for the hydrochloric acid and carbon dioxide treatments between the 12th and 24th hours of treatment. An increase was observed between the one and one-half hour and the three-hour time periods of treatment for eggs stored in acetic acid. A discussion of the possible significance of these fluctuations will be given when Haugh units are discussed.

The pH of those eggs treated with either ammonium hydroxide or methyl amine increased to 11.014 and 10.509, respectively, at 1.5 hours from time of breakout. Eggs in both atmospheres continued to increase in pH up to 12 hours, after which both declined slightly. The decline of pH in these two treatments after 12 hours may have been partially influenced by the opening of the treatment containers to make later observations on the eggs under treatment. The mean values of pH for eggs treated with methyl amine from 24 hours through 96 hours are misleading and are the result of continuous use of the reagent after most of its strength was lost. Eggs treated with previously unused methyl amine gave pH readings ranging between 11.5 and 12.0 from 24 hours through 96 hours of treatment. The pH values at earlier time periods are more representative of the treatment and are probably due to the inability of methyl amine to penetrate the egg albumen as rapdily as ammonium hydroxide.

Although the values are not listed in Table I, pH readings were made on the eggs used in this experiment immediately after breakout. The overall means for these initial readings ranged from about 8.4 to 8.6. These readings were taken the day after the eggs were laid, which may account for the slightly higher pH values than those reported by other workers for newly laid eggs. Only a slight increase in pH was observed from 1.5 to 96 hours for eggs held in normal air. The mean pH value of 9.145 listed at 96 hours for eggs in normal air is .355

units less than the pH value of 9.5 at 96 hours that Healy and Peters (1925) reported for eggs held at room temperature. This difference can be attributed to the lower temperature used in the present experiment. A part of this difference can also be attributed to the fact that the eggs used in this experiment were in a sealed container except when observations were being made, so that the rate of carbon dioxide loss may have been retarded.

The results of statistical analysis using the analysis of variance technique of Snedecor (1956) with the mean values for pH are shown in Table II. The F values obtained in this analysis for hens, treatments, time, and hen X treatment, and treatment X time were found to be highly significant. The effect due to the hen X time interaction was found to be insignificant. The results of this analysis indicated that the treatments, hen and the amount of elapsed time had effects on the rate of pH increase in eggs under treatment. Hens had an effect on the rate of pH increase in the eggs used in this experiment. The conclusion drawn from this analysis is that pH increased with time at the same rate, regardless of the hen producing the eggs within each treatment.

Effect of Treatments on the Physical Appearance of the Eggs

Changes in the physical appearance of eggs used in this study which were due to treatment effect varied widely among treatments. Eggs treated with carbon dioxide became cloudy after 12 hours of treatment. The pH after 12 hours of treatment was 7.548 which indicates that as the pH dropped below 8.0, the proteins in the albumen began to precipitate, creating the cloudy appearance. The same condition appeared in eggs treated with acetic acid at 48 hours when the pH had dropped to 7.875.

TABLE II

THE RESULTS OF STATISTICAL ANALYSIS USING THE ANALYSIS OF VARIANCE TECHNIQUE WITH THE AVERAGE pH VALUES

Source	D.F.	M.S.	F value
Hen (R)	21	1.4662	6.08**
(RC + RAC + RBC + RABC)[1]	882	.2413	
Treatment (A)	5	632.9426	603.03**
(RA + AC + RAC)[1]	215	1.0496	
RA	105	1.0107	4.15**
(RAC + RABC)[1]	735	.2435	
Time (B)	6	3.5321	31.12**
(RB + BC + RBC + RAB + RABC)[1]	1518	.1135	
AB	30	2.3486	20.97**
(RAB + ABC + RABC)[1]	1290	.1120	
RB	126	.1244	1.11**
(RBC + RABC)[1]	756	.1117	
RAB	630	.1140	
Egg (C)	1	.2252	
BC	6	.0731	
AC	5	1.8088	
ABC	30	.1402	
RC	21	.8525	
RBC	126	.1265	
RAC	105	1.0523	
RABC	630	.1087	

**Significant at .01 level of probability

[1]The sums of squares for these interactions were pooled to use as an error term for testing the significance of the effect immediately above.

Layers of thick albumen appeared to break away from the main mass of thick albumen leaving very little thick albumen remaining around the egg yolk after 96 hours under the influence of both of these atmospheres. The remaining thick albumen in the eggs treated with carbon dioxide and acetic acid remained as a thick gel. The results reported here are in agreement with results reported by Sharp (1929) and Sharp and Stewart (1931), which indicated that eggs treated with an excess of carbon dioxide showed a decrease in the amount of thick albumen and an increase in the amount of thin albumen. The atmosphere of hydrochloric acid generally failed to cause any general precipitation of the egg albumen proteins but did cause complete precipitation in localized areas of some eggs. It is suggested that these localized areas of precipitation were the result of condensed drops of moisture from the wall of the treatment containers, which had absorbed hydrochloric acid from the atmosphere, and not a characteristic of the acid. The pH of eggs treated with hydrochloric acid generally failed to fall below a pH of 8.0, which probably accounts for the lack of generalized precipitation of the proteins in these eggs. In preliminary work done with this acid it was found that eggs treated with a strong solution of acid were dry cooked in a very short period of time. No apparent effects on the yolks of the eggs used in this experiment were created by any of these acetic reagents.

The albumen of eggs treated with ammonium hydroxide appeared to be a viscous homogeneous solution after 96 hours of treatment. The yolks of these eggs became very enlarged and flattened. This effect was apparent after only three hours of treatment, but became progressively more evident during the later time periods. The yolk membrane was also weakened by this treatment and the color of the yolks turned to a very deep orange. The results are in accordance with those reported by Cotterill and

Norkskog (1954).

Methyl amine produced similar results to those produced by ammonium hydroxide, in that the albumen of eggs treated with both reagents became more viscous with the passage of time; however, the similarity of the two treatments ended at this point. When the pH of individual eggs treated with the former reagent rose above 11.5, the whole egg turned to a rubber-like gel. In preliminary work, use was made of some amines bearing larger alkyl radicals than the methyl group of the compound, methyl amine. It was found that diethyl amine and triethyl amine caused the whole egg to gel at pH values under 11.0. This pH level of 11.5, although necessary for the formation of a gel in eggs due to the influence of methyl amine, was lower than the 11.9 reading reported by Cunningham and Cotterill (1962). However, these workers introduced sodium hydroxide directly into a homogenized solution of egg albumen to cause the formation of a gel. It was suggested by these workers that salt bonds were formed between sodium hydroxide and acidic portions of the protein molecules. Formation of these bonds caused breakage of salt bonds and hydrogen bonds which were important to the structure of the native proteins and subsequently caused denaturization of the proteins. The action caused by methyl amine on the proteins of egg albumen probably was the same as that caused by sodium hydroxide, but the type of linkage formed between the amine and protein would have to be something other than a salt bond. It is possible that an amide linkage could have been formed between the amine molecules and the carboxyl group of the different amino acids making up the proteins in egg albumen. A reaction such as this might have been catalyzed by the lysozyme fraction of egg albumen. Many research workers feel that this protein has catalytic properties. It is doubtful that the type of bond formation

between egg albumen proteins and these basic reagents are directly related to the formation of alkaline gels from egg albumen. It is important, however, that the amine was incorporated into the proteins of egg albumen and caused the formation of a gel at a lower pH than sodium hydroxide. The reason for this difference can be attributed to the methyl group of methyl amine. It is suggested, therefore, that the time required for egg albumen to form an alkali gel is dependent on the size of the molecule incorporated into it. The fact that both diethyl amine and triethyl amine produce gels at even lower pH values should confirm this hypothesis. The time required for alkaline coagulation then is dependent on pH, rate of addition of the alkali, temperature, volume of reagent, strength of the alkali suggested by Cunningham and Cotterill (1962), and additionally the size of the molecule incorporated into the egg albumen as suggested in this study. It should be stated further that the strength of the alkali necessary to produce alkaline coagulation is dependent on the size of the molecules making up the alkaline reagent. Self-liquefaction also failed to occur in these eggs when they were allowed to set out after treatment. The yolk membrane of the eggs treated with methyl amine weakened very rapidly and was ruptured in almost every case after only 12 hours of treatment. The yolk color changed from a normal color to a very pale yellow.

Those eggs held in an atmosphere of normal air showed no physical changes except for an increase in thin white and a decrease in thick white. The yolks appeared to be only slightly enlarged.

Effect of Treatments on Haugh Units

During the analysis of data from this study it was determined by plotting the

Haugh Unit means shown in Table III on semi-log paper that these units declined linearly with the logarithm of elapsed time. This same relationship between egg quality deterioration and elapsed time was reported by Spencer et al. (1956). The means for Haugh unit readings taken immediately after breakout of eggs used in this experiment are not included in the data presented in Table III, but the readings ranged from about 74 to 78 Haugh units. By observing the means for Haugh units at 1.5 hours and for each succeeding time period, it can be seen that the initial drop in quality from time of breakout to 1.5 hours is the largest quality drop between any two time periods, which coincides with the results published by previous authors.

In Figure 1 is illustrated the regression lines fitted to the means presented in Table III. The regression coefficients and estimated Y-values for fitting these regression lines are listed in Table IV. As can be seen by observation of the regression lines in Figure 1, quality deterioration was retarded most by the hydrochloric acid treatment. This can also be seen from the b_1 values as shown in Table IV. The rate of regression in Haugh units between time periods was 2.2134 for this acid. The means listed in Table I for eggs treated with hydrochloric acid indicate that a gradual decline in Haugh units occurs up to 48 hours of treatment. However, a drop from 57.189 to 52.473 was observed between the 48th and 96th hour of treatment. It is suggested that egg quality was retained best by the hydrochloric acid treatment because it maintained the pH within a desirable range for quality retention, as indicated under the discussion of the effect on pH. It was pointed out in the discussion that the pH of eggs stored in the hydrochloric acid atmosphere increased between the 12th and 24th hour of treatment. This pH increase had no unusual effect on quality change during this period, as indicated by the means for this treatment as shown in Table III.

TABLE III

THE AVERAGE HAUGH UNIT VALUES FOR EACH TREATMENT FOR EACH TIME PERIOD

Atmospheric Treatments	Time Periods in Hours						
	1.5	3	6	12	24	48	96
Hydrochloric Acid	66.657	64.598	62.739	60.275	58.139	57.189	52.473
Acetic Acid	69.352	65.109	63.500	61.055	58.368	56.502	52.423
Ammonium Hydroxide	59.923	53.466	48.473	42.725	36.582	32.491	25.357
Methyl Amine	62.736	55.295	47.643	52.009	33.714	28.666	25.530
Carbon Dioxide	66.723	63.584	61.130	58.486	57.816	53.784	48.714
Normal Air	67.686	63.609	61.136	57.564	55.752	53.848	50.125
Overall	65.513	60.944	57.453	53.686	50.062	47.080	42.437

A b_1 value of -8.6893 was determined for eggs treated with acetic acid. These eggs showed a regression rate of 2.5157 between time periods, which was the second lowest rate of decline in egg quality deterioration among the treatments in this study. The corresponding b_1 balues for carbon dioxide and normal air were -9.1292 and -9.2061, respectively. Regression rates for these two treatments between time periods were 2.7418 for carbon dioxide and 2.7713 for normal air. By comparing these b_1 values and regression rates, it can be seen that the rate of egg quality deterioration was quite similar for all three treatments. The same conclusion can be drawn from the regression lines for these three treatments as shown in Figure 1. These lines are almost parallel indicating that the rate of quality decline for eggs held in each of

TABLE IV

REGRESSION COEFFICIENTS AND ESTIMATED Y VALUES CALCULATED

FROM HAUGH UNIT VALUES BY TREATMENTS

AND BY TIME PERIODS

Trt.	Regression Coefficients		Log-time						
	b	b_1	.18	.48	.78	1.08	1.38	1.68	1.98
			Estimated Y Values						
1	68.2308	- 7.3528	66.9360	64.7226	62.5092	60.2958	58.0824	55.8690	53.6556
2	70.2931	- 8.6893	68.7630	66.1473	63.5315	60.9158	58.3000	55.6843	53.0686
3	62.8894	-18.6923	59.5979	53.9709	48.3440	42.7170	37.0901	31.4632	25.8362
4	65.1235	-21.2156	61.3876	55.0011	48.6146	42.2280	35.8415	29.4550	23.0685
5	68.4575	- 9.1292	66.8499	64.1018	61.3536	58.6054	55.8573	53.1091	50.3610
6	68.4666	- 9.2061	66.8455	64.0742	61.3029	58.5316	55.7602	52.9889	50.2176

1. Hydrochloric Acid
2. Acetic Acid
3. Ammonium Hydroxide
4. Methyl Amine
5. Carbon Dioxide
6. Normal Air

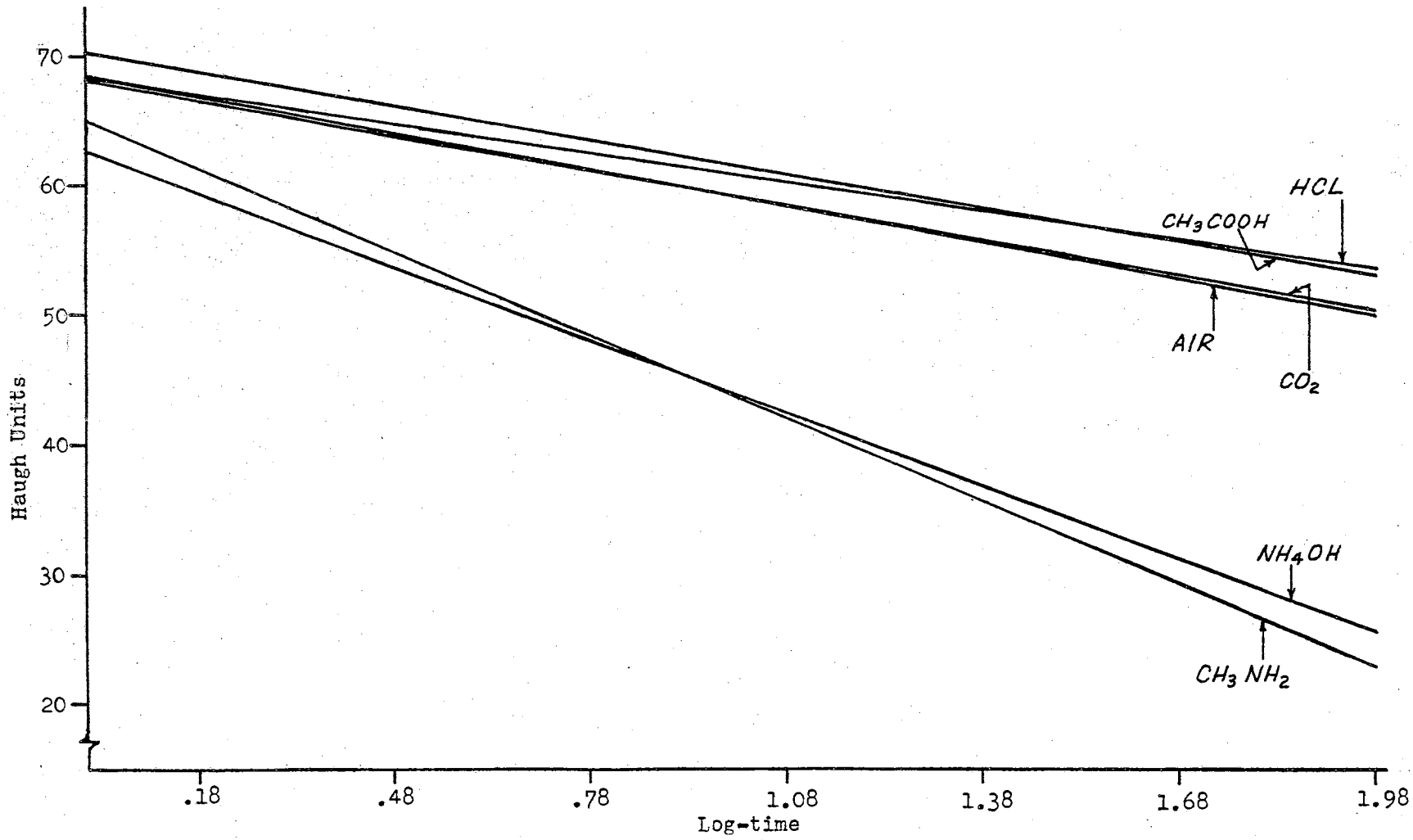

Figure 1. Haugh Unit Regression Lines for Each Treatment

these atmospheres was at almost the same rate. By referring to the overall means for Haugh units in Table III, it can be seen that a large decrease in Haugh units occurred between 1.5 and three hours for eggs treated with acetic acid. The time at which this change in Haugh units occurred corresponded quite closely with the time at which a pH increase was noted for the eggs treated with this acid. A large decrease in Haugh units was also observed between the 48th and 96th hour of treatment for eggs treated with this acid. Eggs treated with carbon dioxide showed a sharp decrease in Haugh units between the 24th and 48th hour of treatment and also between the 48th and 96th hour. The pH of these eggs also had decreased to a value of 7.611 after 48 hours of treatment. This is somewhat lower than the 7.65 limit recommended by Evans and Carver (1942) for best maintainence of egg quality. The pH dropped even lower, to 7.432, after 96 hours for eggs treated with carbon dioxide. This decrease in pH may be quite closely correlated with the sharp drop in quality of the eggs in this treatment during the last 72 hours of storage. The pH of the carbon dioxide treated eggs dropped to 7.548 after 12 hours of treatment but rose again to 7.741 after 24 hours of treatment. This increase in pH corresponds in magnitude to the decrease in Haugh units from 58.486 to 57.816, a change of less than one Haugh unit. Egg quality deterioration for eggs held in air seems to be relatively constant between time periods. A change in pH from 9.066 to 9.000 for eggs which had been held in normal air occurred between the sixth and 12th hours of treatment, but no change in rate of decline was noted.

In the discussion of these three treatments it has probably been noticed that egg quality decreased within all three treatments, while a similar trend by the pH values was not found. In the case of acetic acid and carbon dioxide treatments, pH decreases were noted, while an increase in pH was noted for those eggs held in normal air. A

conclusion which may be drawn from these data is that egg quality can be best maintained by holding the pH of the eggs within certain relatively narrow limits. Results of this study indicate that egg quality is best maintained when pH is held close to 8.2, which is in agreement with the results reported by Evans and Carver (1942). The limiting points of 7.65 to 8.46 would seem to be a good estimate for the range necessary for control of egg quality. As the pH is decreased from the most desirable level for quality maintenance, the fibrous proteins of the thick albumen begin to precipitate. The protein fibers, which appear as this pH decrease occurs, break away from the main mass of thick albumen and become incorporated into the thin albumen. Eggs treated with carbon dioxide and acetic acid deteriorated in this manner. They manifested a decrease in thick albumen and an increase in thin albumen. Haugh units also decreased more in eggs held in these atmospheres than in those held in the hydrochloric acid atmosphere, where the pH was maintained at a higher level. Contrary to results reported by Sharp and Stewart (1931), the albumen of eggs treated with carbon dioxide in this study did not stand as high as the albumen of eggs held in normal air. The thick albumen of these eggs actually appeared to float on top of the thin albumen. Therefore, the Haugh unit readings observed for these eggs may be misleading. In the eggs held in normal air, the thick white simply assumed a more fluid condition as pH increased and Haugh units decreased.

As expected, ammonium hydroxide and methyl amine produced the most rapid rate of decline in egg quality. A more fluid condition of the thick albumen of eggs treated with these reagents developed just as it did in normal air. However, the effects of these two reagents were more rapid and adverse than the effects of untreated air. In Table IV the b_1 values of -18.6923 and -21.2156 are recorded for ammonium hydroxide

and methyl amine, respectively. Regression rates for eggs treated with these two alkaline reagents were 5.6270 for ammonium hydroxide and 6.3865 for methyl amine. These regression rates indicate the rapid rate at which egg quality, as measured by Haugh units, decreased in eggs held in these two atmospheres. These figures also show that methyl amine caused the most rapid decline in egg quality for all treatments. It can be seen by observing the regression lines in Figure 1 for these treatments that the two lines intersect during the course of their descent. Examination of the means presented in Table III reveals that this change in the albumen height of eggs held in these two atmospheres did not occur until the 24th hour of storage. It is postulated that the albumen height of eggs treated with methyl amine remained above that of eggs treated with ammonium hydroxide because of the slower entrance of methyl amine into the egg albumen. This could also account for the difference in pH levels of the two treatments during this 24 hour period, as indicated in earlier discussions. It is also true that the means for the pH of eggs held in an atmosphere of methyl amine remained below the pH of those treated with ammonium hydroxide during the last 72 hours of treatment, but the difference is not as great for the last three time periods. As indicated in the discussion relative to pH, the means for pH of eggs treated with methyl amine are misleading in that a fresh solution of methyl amine increased pH to a higher level after 24 hours of treatment than did a similar solution of ammonium hydroxide. However, since the pH of eggs treated with methyl amine generally remained below the pH of eggs held in an ammonium hydroxide atmosphere, it is assumed that the individual characteristics of this reagent had some effect on the rate of egg quality deterioration. Conclusions drawn from these two treatments are that the strength and the individual characteristics of the reagents used had an

effect on the deterioration of egg quality.

A test was designed to check the parallelism of the regression lines in Figure 1. This test was made to determine if any significant differences existed among the effects of the treatments used in this study on egg quality deterioration. The results are shown in Table V.

TABLE V

THE RESULTS OF THE APPLICATION OF ANALYSIS OF VARIANCE TECHNIQUES ON HAUGH UNIT MEANS FOR PARALLELISM DETERMINATION

Source of Error	df	SS	MS	F-ratio
Hydrochloric acid	1	6,034		
Acetic Acid	1	8,027		
Ammonium Hydroxide	1	39,000		
Methyl Amine	1	50,240		
Carbon Dioxide	1	9,460		
Normal Air	1	9,302		
Total	6	122,466		
Common	1	102,611		
Parallelism	5	19,855	3,971	53.5877**
Error	215	15,933	74	

**Significant at the 0.01 level of probability.

An F-ratio of 53.58377 was determined for the parallelism of these regression lines, which was significant at the .01 level of probability. This indicates that the regression lines are not parallel and that significant differences do exist among the effects of

some of the treatments on egg quality as measured by Haugh units. The error term for making this test was obtained by pooling the sums of squares of the linear effects of the following interactions; RAB, ACB, and RACB.

Duncan's Multiple Range test (Steel and Torrie, 1960) was used to determine which treatments had significant effects on egg quality as measured by Haugh units. A brief summation of this test is given in Table VI.

TABLE VI

DUNCAN'S MULTIPLE RANGE TEST APPLIED TO REGRESSION COEFFICIENTS*

Hydrochloric Acid	Acetic Acid	Carbon Dioxide	Normal Air	Ammonium Hydroxide	Methyl Amine
-7.3528	-8.6893	-9.1292	-9.2061	-18.6923	-21.2156

*Any two numbers underscored by the same line do not differ significantly (P=.01).

It was found that the only significant differences among the effects of treatments existed between the two alkaline reagents and the other four treatments. No significant differences were found to exist between eggs treated with hydrochloric acid, acetic acid, carbon dioxide, or normal air. Also no significant difference was found to exist between eggs held in an ammonium hydroxide or methyl amine atmosphere. The results of this test indicate that none of the six treatments tested had any significantly beneficial effect on the retention of egg quality as measured by Haugh units. Even though quality was maintained by hydrochloric acid at a higher level than by any other treatment, it did not retard quality deterioration enough to be significantly better than acetic acid, carbon dioxide or normal air. The two alkaline reagents used in this study, however, did have a significantly detrimental effect on egg quality. The difference between the regressions rates for these two alkaline reagents

was of no real importance, however.

Analysis of variance for Haugh units is shown in Table VII. The F ratios determined for the effects of treatments and time in this analysis were found to be significant at the .01 level of probability. The interaction between these two effects was also found to be highly significant. These three variables likewise had a significant effect on the pH changes within the egg. However, in the analysis of Haugh units it is also seen that the effects due to hens, hen X treatment, and hen X time interaction exerted a highly significant effect on egg quality deterioration. The effect produced by hens is thought to be due to the initial quality of eggs laid by the individual hen. Since eggs laid by a particular hen tend to be of about the same quality, those eggs which were low in quality at time of breakout tended to deteriorate more rapidly, regardless of treatment, than did eggs of higher quality. This is suggested as the reason for the significant effect shown by hens on egg quality deterioration. It is concluded that hens had an effect on the way that either treatments or time affected any one particular egg. Because of the highly significant F ratios determined for the effects of treatments, time, and the interaction between the two, there is every indication that all three effects were very important in causing changes in egg quality.

Relationship of pH and Time to Haugh Units

In previous studies concerning the relation of pH and Haugh units, indications have been given that a causal relationship exists between the pH of egg albumen and the deterioration of egg quality as measured by Haugh units. Results from this study indicated that there was a decrease in egg quality as the pH of the albumen was increased. However, a decrease in egg quality was also shown by

TABLE VII

THE RESULTS OF APPLICATION OF THE ANALYSIS OF VARIANCE TECHNIQUES TO HAUGH UNIT DATA

Source	D.F.	M.S.	F value
Hen (R)	21	2,274.3305	55.98**
(RC + RAC + RBC + RABC)[1]	882	40.6254	
Treatment (A)	5	24,333.3708	162.83**
(RA + AC + RAC)[1]	215	149.4396	
RA	105	144.2323	3.48**
(RAC + RABC)[1]	735	41.4092	
Time (B)	6	17,150.8936	631.93**
(RB + BC + RBC + RAB + RABC)[1]	1518	27.1405	
AB	30	698.8330	25.83**
(RAB + ABC + RABC)[1]	1290	27.0535	
RB	126	38.1505	1.72**
(RBC + RABC)[1]	756	22.1914	
RAB	630	30.9329	
Egg (C)	1	25.3638	
BC	6	21.3114	
AC	5	162.7139	
ABC	30	38.2340	
RC	21	137.2994	
RBC	126	19.9406	
RAC	105	154.0147	
RABC	630	22.6416	

**Significant at the .01 level of probability

[1]The sums of squares for these interactions were pooled to be used to determine the error term for testing the significance of the effect immediately above.

those eggs in which the pH decreased. Even though the results reported in this thesis indicated that egg quality can be degraded by either increasing or decreasing pH, it is an established fact that, under normal storage conditions, the pH of eggs will always increase. An effort has been made in this study to show that an increase in pH is negatively correlated to egg quality deterioration. Also an effort will be made to show how a decrease in pH can occur and still produce a decrease in Haugh units.

Simple correlation coefficients calculated to show the correlation between Haugh units and the three variables in this study related to Haugh units are -.495868 for pH, -.525876 for time, and -.669863 for the interaction between pH and time. These coefficients indicate that there is a negative correlation of approximately 50% or more between all three variables and Haugh units. The greatest amount of correlation was found between Haugh units and the interaction between pH and time. This indicates that a greater decrease in Haugh units should be observed as a result of this interaction than for either of the two variables considered independently. In Figure 2 it is shown that egg quality does deteriorate more as a result of this interaction than for either of the independent variables alone. The result of this interaction can be observed by following an imaginary line from the left upper corner of this surface diagonally across the surface. Figure 2 is a three-dimensional illustration of the means for the pH and Haugh units given in Tables I and III, respectively. In this illustration, Haugh units are shown as a response of time and pH. The points of intersection between the treatment lines and the lines representing time on the top surface of this three-dimensional illustration are the mean Haugh unit readings listed in Table III. It can be seen by following the lines representing treatment (across the surface of the illustration from left to right) that Haugh units decrease with time due to treatment. A

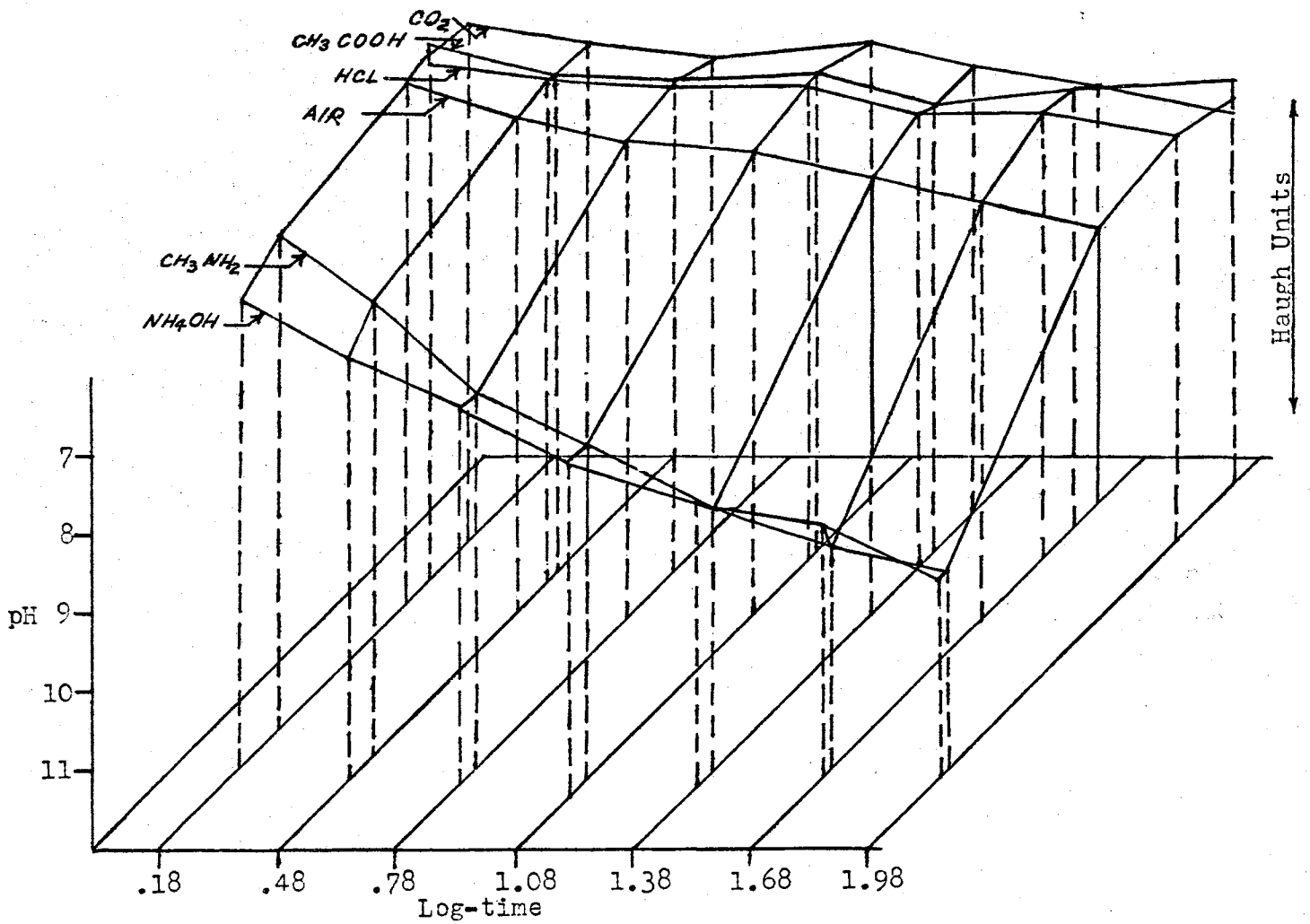

Figure 2. Observed Mean Haugh Unit Values Plotted as a Response of Log-time and pH Means

decrease in Haugh units will also be noticed by following the line for any time period across the surface of this illustration. This decrease in Haugh units is also due to treatment effect and is represented by changes in pH. This illustration is not meant to show comparison of treatment effects and should not be used to do so. The reason for this is the variation of pH among treatments.

By using the statistical formula given below, a model to represent the decrease of Haugh units due to pH and time was constructed;

$$HU = b + b_1 \times pH + b_2 \times pH \times \text{Log-time}.$$

A model expressing this formula is shown in Figure 3. In this illustration a decrease in Haugh units is observed by following either pH or time down their respective scales at any particular time or pH level. An even greater decrease will be noticed by observing the differences in Haugh unit levels, where both an increase in pH and time are observed, suggesting that an interaction between these two measurements exists. It can easily be seen in this model that a decrease in Haugh units also occurs in eggs in which the pH has been decreased. If it is assumed that the pH of a fresh egg has a pH of 8.0, this model shows a decrease in Haugh units from 70 at 1.5 hours to 56 at 96 hours, if the pH is decreased to seven. This, in general, is representative of the regression rates shown by eggs treated with acetic acid and carbon dioxide in this experiment. However, the model implies that the loss is due only to time, and that quality deterioration is retarded by decreasing the pH of eggs. In this study it was observed that a decrease in pH below a certain point had adverse effects on egg quality. Therefore, it is thought that decreasing pH to near neutrality caused detrimental changes in egg quality as measured by Haugh units. Because of the precipitation of egg albumen proteins, any pH below about 7.65 is thought to

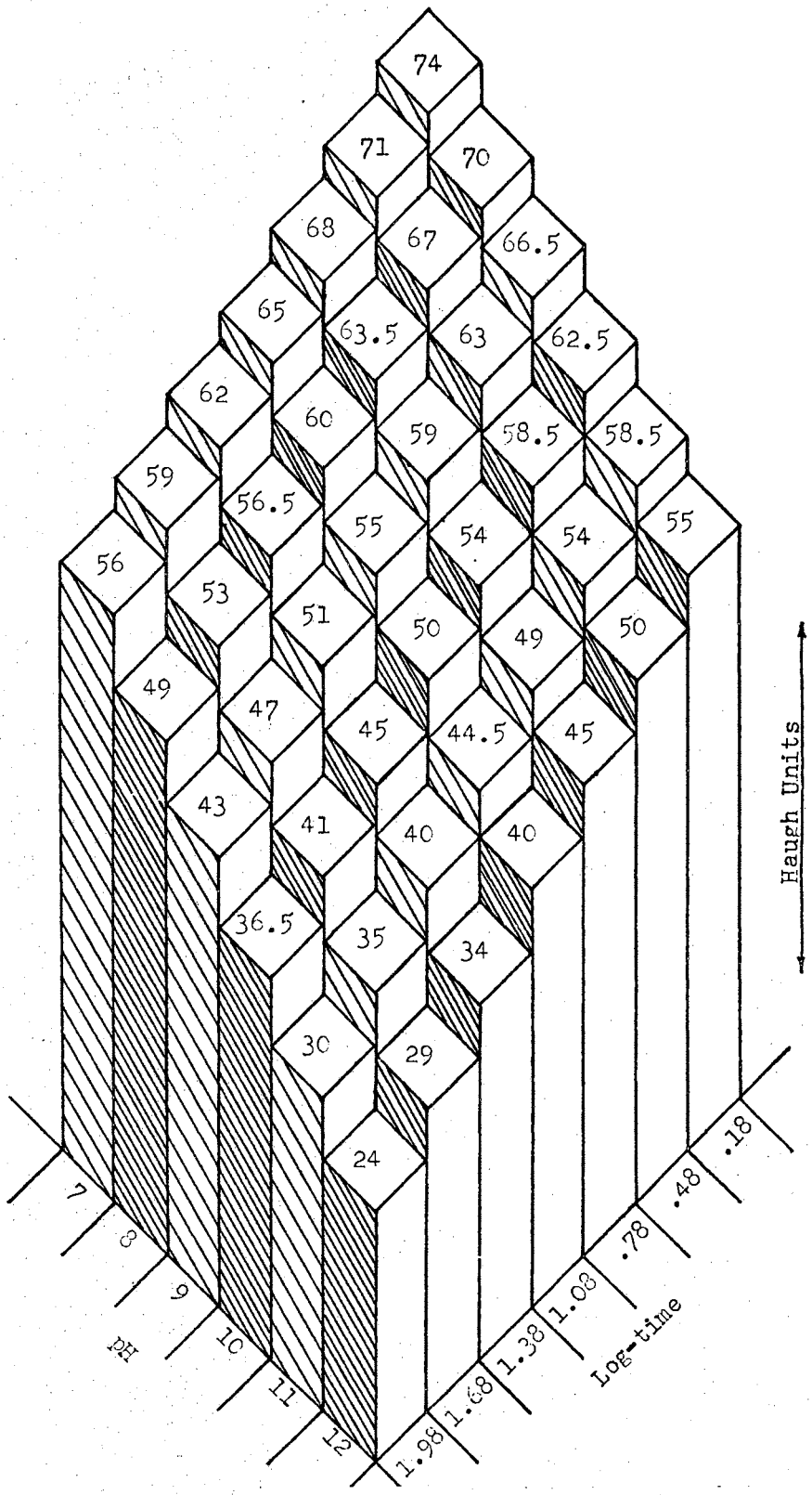

Figure 3. Derived Haugh Unit Values Shown as a Response to Selected pH and Log-time Values

have adverse effects on egg quality. If the pH is decreased to about 6.5, the proteins are completely precipitated and no true measurement of quality can be made. Since, under normal storage conditions, the pH of eggs increases, the effect of a pH decrease becomes of importance only if methods of storage involving control of pH are devised.

A multiple correlation coefficient obtained between the two variables pH and pH x log-time and Haugh units was -0.7534. The square of this coefficient represents the amount of variation explained by the variables involved in its computation; therefore, the amount of variation in egg quality due to pH and the pH x log-time interaction in this study is 56.76 percent. Time is not included in the computation of this multiple correlation coefficient, but it is definitely related to changes in egg quality. Temperature also is a factor responsible for part of the remaining 43.24 percent of the variation in egg quality.

SUMMARY AND CONCLUSIONS

In this study eggs were held in the following atmospheres; hydrochloric acid, acetic acid, carbon dioxide, ammonium hydroxide, methyl amine and normal air. Temperature was maintained at 48 degrees F. with Haugh unit and pH readings being made after 1.5, 3, 6, 12, 24, 48 and 96 hours of storage. The data were analyzed and the following conclusions are suggested by these analyses:

1. The pH of eggs decreased during storage in acidic atmosphere.
2. A gradual increase in pH occurred in eggs held in an atmosphere of normal air, while the pH of eggs held in an alkaline atmosphere increased very rapdily throughout the period of this study.
3. The individual effects of both treatment and time on the pH of the stored eggs were highly significant. These factors were independent of the effect due to hen.
4. Egg quality can be best maintained when the pH of eggs is held between 7.65 and 8.46. Fluctuations of pH within this range are the least harmful to egg quality.
5. None of the treatments used in this study had any significantly beneficial effects on egg quality.
6. The quality of eggs held in an alkaline atmosphere deteriorated rapidly.
7. When the pH was reduced to near neutrality, changes occurred in the thick albumen which caused a decrease in quality as measured

by Haugh units.

8. Deterioration of egg quality occurred in two ways:
   a. The thick white assumed a more fluid condition as pH increased.
   b. The amount of thin white increased while the amount of thick white decreased, even though it maintained its jelly-like properties.
9. The pH at which egg albumen formed an alkaline gel was dependent on the strength of the alkali and the size of alkali molecules.
10. Treatments and time were highly significant in their effect on egg quality deterioration, as measured by Haugh units.
11. Hens had a highly significant effect on egg quality, which was probably due to the variation in initial quality.
12. Egg quality deterioration was negatively correlated with pH increase and with time.
13. Egg quality declined linearly with the logarithm of elapsed time between 1.5 and 96 hours.
14. The greatest decrease in egg quality occurred between the time of breakout and 1.5 hours of storage time.

SELECTED BIBLIOGRAPHY

Almquist, H. J., and F. W. Lorenz, 1932. Liquefaction of egg whites, U. S. Egg and Poultry Mag. 38:20-23.

Bose, S., and G.F.Stewart, 1948. Comparative and complimentary effects of heat treating and oiling shell eggs on their keeping quality. Poultry Sci.27:228-234.

Cotterill, O. J., 1955. The effect of carbon dioxide, mechanical shaking, and thioglycol on the thinning of egg white. Poultry Sci. 34:1189.

Cotterill, O.J., and F.A.Gardner, 1957. Retarding thick white deterioration by holding shell eggs in sealed containers. Poultry Sci. 36:196-206.

Cotterill, O.J., F.A.Gardner, E.M.Funk and F.E.Cunningham, 1958. Relationship between temperature and CO_2 loss from shell eggs. Poultry Sci. 37:479-483.

Cotterill, O. J., and A. W. Norkskog, 1954. Influence of ammonia on egg white quality. Poultry Sci. 33:432-434.

Cunningham, F. E., and O. J. Cotterill, 1962. Factors affecting alkaline coagulation of egg white. Poultry Sci. 41:1453-1461.

Evans, R. J., and J. S. Carver, 1942. Shell treatment of eggs by oiling. 1. Effect of time between production. 2. Oiling on interior quality of stored eggs. U.S. Egg and Poultry Mag. 48:546-549.

Feeney, R. E., 1955. Factors affecting albumen. Poultry Sci. 34:1181-1182.

Healy, D. J., and A. M. Peters, 1925. The hydrogen ion concentration and basicity of egg yolk and egg white. American J. Physiol. 74:363-368.

Homler, B.E., and W.J.Stadelman, 1963. The effect of oiling before and after cleaning in maintaining the albumen condition of shell eggs. Poultry Sci. 42:190-194.

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

Newell, G. W., R.G.McKerley, J.G.Berry, G.W.Foreman and G.V.Odell, 1966. The effect of pH changes on protein patterns in chicken eggs. Poultry Sci. 45: in press.

Romanoff, A. L., and W. D. Yushok, 1948. Preservation of intact eggs by sealing with chemical agents. Food Res. 13:331-335.

Schaible, P. J., J. M. Moore and J. A. Davidson, 1935. A note on the structure of egg white. U. S. Egg and Poultry Mag. 41:38-39.

Schwall, D. V., F. A. Gardner and E. D. Parnell, 1961. Effects of oil treating on shell egg quality during short term refrigerated storage. Poultry Sci. 40:583-588.

Sharp, P. F., 1929. The pH of the whites as an important factor influencing the keeping quality of hen's eggs. Science 69:278-280.

Sharp, P. F., 1937. Preservation and storage of hen's eggs. Food Res. 2:447-498. Also in U. S. Egg and Poultry Mag. 44:148-155.

Sharp, P. F., and G. F. Stewart, 1931. CO_2 and the keeping quality of eggs. U. S. Egg and Poultry Mag. 37:30, 63, 65, 66, 68.

Snedecor, G. W., 1956. Statistical Methods, 5th Edition. Iowa State College Press, Ames, Iowa.

Spencer, J. W., W. J. Stadelman, E. A. Sauter and J. G. Darrock, 1956. Measured albumen quality as affected by time after breakout. Poultry Sci. 35:319-322.

Stadelman, W. J., and M. L. Wilson, 1957. Spray oiling of eggs. Poultry Sci. 36:1159.

Stadelman, W. J., T. Zeigler and J. G. Darrock, 1954. The effect of egg temperature on its broken out albumen quality evaluation. Poultry Sci. 33:1082-1083.

Steel, R. G. D., and J. H. Torrie, 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc. N.Y. New York.

Swenson, T. L., 1938. Storage of shell eggs. Food Res. 3:599-608.

Wilhelm, L. A., 1940. The pH and cloudiness of egg albumen in relation to albumen quality. U. S. Egg and Poultry Mag. 46:397-401.

Yoshok, W. D., and A. L. Romanoff, 1949. Studies on preservation of shell eggs with plastics. Food Res. 14:113-122.

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