CALCIUM CONTENT OF FIRM AND WATERY WHITE OF EGGS

by

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INTRODUCTION

The poultry business suffers heavy losses every year due to deterioration of eggs in cold storage. This deterioration is not uniform in any sense. It varies yearly even though storage conditions remain the same. It varies in different seasons of the same year, in eggs produced in different parts of the country or in eggs from the same locality. The cause seems to lie within the egg itself since variation occurs when storage conditions are identical.

Eggs, which have undergone deterioration in storage, are spoken of as "watery white" eggs. The outstanding characteristics of watery white eggs are:

1. An increase in the air space in the egg.

2. A loss of considerable water.

3. The yolk membrane becomes brittle and is easily broken.

4. The contents of the egg "rattle" when shaken rapidly.

5. The yolk is apt to stick to the outside (shell) membrane.

6. The white frequently acquires a straw yellow color.

7. The outside (or shell) membrane becomes tough.

8. The egg shell becomes thinner; and finally

9. The egg white as a whole becomes more liquid (watery) and loses a great part of its original viscosity.

This last characteristic has given the name by which all these changes are called.

These changes take place gradually seemingly dependent upon time and temperature of storage. The question then arises, what chemical changes accompany these visible physical changes? The changes are not so apparent in the yolk, yet no doubt chemical and physical changes have occurred there also.

The white of the egg consists of at least three distinct portions or layers; first, a comparatively liquid portion just within the shell membrane and surrounding the remaining contents of the egg; second, a semi-solid portion just within the more liquid layer; and last, a viscous layer just outside of and surrounding the membrane of the yolk. The outer and more liquid portion is very similar to the other portions in many respects and very different in others. This portion has about the same moisture content as the other, notwithstanding the fact that it seems more liquid. It possesses no organized structure and has much less viscosity. The other two portions are very much alike, each possessing highly organized structures and great viscosity. This first portion is the substance referred to hereafter in the term watery white and the other two portions make up the firm white.

In studying the literature, I find very little work has been done on the percentage of firm and watery white in eggs or the properties of either. Rice and Young (1) made an effort to determine the cause of watery whites in storage eggs. They made a study of physical properties, especially osmotic activity in the eggs of four breeds of poultry. The differences they found were so slight for eggs of different breeds that they concluded the difference in appearance could not be attributed to osmotic activity.

Few investigators have made determinations upon the calcium in the white of eggs, presumably because it is present in such small and variable quantities. The question arose, when eggs were commonly preserved in lime water, concerning detection of preservation and cause of change in properties of contents. Kreis and Studinger (2) conducted an investigation to determine whether the calcium content of the egg white changed during storage in lime water. They found the calcium content of whites of fresh eggs to be a very variable factor. Eggs from the same pens showed great variation. The calcium content in storage they found to be also a variable factor and very little different from fresh eggs. The range for fresh eggs was .59% to 4.25% of ash as CaO. This converted into per cent calcium would range from .42% to 3.03%. The difference in fresh and storage eggs was well within the range of variability, therefore they concluded the calcium content was no index of age or condition of storage of eggs.

Winifred Mankin (3) found the average calcium content of unincubated eggs to be 23 mg. per egg and the calcium content of the chick just hatched is 105 mg. Thus there is a gain of 82 mg. Ca which must come from the shell which grows thinner during incubation. In experiments on this same topic, Buckner and his co-workers (4) showed that H_2O containing CO_2 when placed inside a carefully drained egg shell dissolved Ca from the shell as $Ca(HCO_3)_2$. This $Ca(HCO_3)_2$ can pass through the shell membrane and into the contents of the egg. The incubating egg produces H_2CO_3 which dissolves the $CaCO_3$ of the shell and forms a soluble diffusible salt which is available for metabolism.

Purpose

No reference was found concerning the change in calcium content of eggs during storage with the exception of the one article already mentioned for lime water storage. Since the shell gets thinner on storage as well as in incubation, perhaps it is possible that some of the loss is due to calcium dissolving and diffusing into the egg contents. In this investigation, it has been my purpose to try to determine in fresh eggs, market eggs, and cold storage eggs the calcium content of the firm and watery white of the egg. Then from these determinations to find the relationship between calcium content and different classes of eggs.

EXPERIMENTAL

In this investigation approximately eleven dozen eggs were used. They were of four different types, fresh eggs, market eggs, storage eggs and watery white eggs. The storage eggs had been in cold storage about one month. The firm and watery whites were separated by means of a pipette and moisture, ash and calcium determinations were run on each part of each individual egg.

Method

Two hundred cc. evaporating dishes were cleaned and heated in the ashing oven until they were red. They were then removed, cooled in a desiccator and weighed on analytical balances. Each egg was broken and the contents placed upon a watch glass. The watery white was separated by means of a pipette which was placed in the outer edge of the egg white. The watery white was then placed in a weighed evaporating dish and covered with a watch glass. The firm white was separated from the yolk in the same manner and placed in another weighed evaporating dish and covered. These dishes were then quickly weighed and placed in a thermostat drying oven to dry to constant weight. Rapid work was necessary because egg white loses moisture with great rapidity. The thermostat was set for 98°-108°. From 8 to 11 hours drying was necessary to get constant weight. Again rapid work was necessary since the dried egg white took up water almost as rapidly as the moist white lost it. After these reached constant weight, they were placed over an open Meeker flame and burned until the egg white was in at least a semi-ash like state. They were then placed in an ashing furnace and burned to white ash. The preliminary heating on an open flame was to eliminate the danger of the egg white puffing up and running over the side of the dish, due to escaping gases during burning. This could have been accomplished in the furnace with careful manipulation but was easier and more satisfactorily done over the open flame. This trouble has been observed by other investigators, therefore Alfend (5) determined to find a method for satisfactorily ashing eggs. He found that addition of a 60 mesh alundum powder reduces ashing time by 75%; that the addition of $Mg(CAc)_2$ solution prevents the material from creeping up the sides of the dish on charring but gives a higher ash content and corrodes SiO_2 dishes; that Al_2O_3 corrodes SiO_2 dishes but does not affect platinum or porcelain dishes at low red heat. After the contents were burned to a white ash, the dishes were removed, cooled in desiccators and weighed.

The calcium content of the ash was determined by volumetric methods. The ash was dissolved in about 20 c.c. dilute HCl. The HCl was diluted and added slowly to prevent loss by effervescence. Clear NH_4OH was added until the liquid was slightly basic to litmus. Twenty c.c. ammonium oxalate solution, prepared by heating to boiling 5 grams c.p. ammonium oxalate in 100 c.c. of distilled H_2O then cooling to form a saturated solution, was slowly added drop by drop from a pipette. The solution was stirred constantly during addition of ammonium oxalate to cause the precipitate of CaC_2O_4 to settle more rapidly. The solution was digested, below boiling, on the hot plate for 30 minutes. The solutions were then carefully poured on to filters and washed free from chlorides. The filtrate was tested for complete precipitation of calcium by the addition of a few drops of ammonium oxalate and for the absence of chlorides by addition of $AgNO_3$. The point of the filter was then pierced with a platinum wire and the contents carefully washed into a clean beaker with hot water. After thoroughly washing the paper with a small amount of hot water, the paper was washed with 15 c.c. of hot dilute H_2SO_4 in 5 c.c. portions to dissolve the precipitate which might remain on the filter. The paper was again washed with a few c.c. of hot water. The solution in the beaker was then heated, not boiled, for a few minutes to be sure all the precipitate had dissolved. The solution was titrated with a dilute $KMnO_4$ solution having a calcium factor of .0005511. The per cent of calcium was then calculated.

$$CaC_2O_4 + H_2SO_4 \rightarrow CaSO_4 + H_2C_2O_4$$

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O$$

The $KMnO_4$ solution was prepared by dissolving approximately the calculated amount of $KMnO_4$ in 1000 c.c. of distilled water. After standing for a few hours, it was filtered through an asbestos filter by suction and diluted to 2000 c.c. This solution was then titrated into a definite amount of $Fe(NH_4)_2(SO_4)_2$ dissolved in H_2O and H_2SO_4. The calcium factor for the $KMnO_4$ solution was then calculated.

The following calculations were then made for each egg: per cent calcium in watery white, whole white and firm white on moist, dry and ash basis, per cent firm and per cent watery white, per cent moisture in firm and in watery white, and in whole white, per cent ash in firm, watery and whole whites calculated on dry basis and on dry of whole white for a basis. These values were arranged in tables and averages calculated for all eggs analyzed, then for fresh eggs, market eggs, cold storage eggs and watery white eggs.

Results

For convenience in comparing results, a group of representative eggs was selected from each class to put in the following tables instead of including the entire list of eggs analyzed. These eggs were selected to show the wide variations in eggs rather than the average. The averages in these tables compare very closely with the averages of all the eggs of each class.

Table I (Fresh Eggs)

	Watery			Firm			Whole		
Egg	%Ca Ash	%Ca Dry	%Ca Orig.	%Ca Ash	%Ca Dry	%Ca Orig.	%Ca Ash	%Ca Dry	%Ca Orig.
21	8.72	.458	.048	8.37	.371	.046	8.47	.393	.046
23	2.84	.172	.017	3.34	.188	.022	3.19	.184	.020
30	3.98	.245	.028	3.65	.212	.025	3.84	.230	.026
47	2.93	.155	.018	2.82	.148	.017	2.89	.152	.017
56	2.77	.178	.019	2.18	.128	.014	2.48	.152	.017
60	1.98	.110	.012	1.96	.126	.014	1.97	.118	.013
63	1.37	.076	.009	2.39	.149	.018	1.72	.100	.012
66	1.34	.079	.008	1.66	.091	.009	1.44	.082	.009
92	1.71	.104	.013	2.55	.143	.018	2.30	.132	.017
97	3.04	.181	.020	2.13	.122	.014	2.54	.148	.017
Average	3.068	.1768	.0192	3.105	.1678	.0197	2.984	.1691	.0194

Table II (Market Eggs)

	Watery			Firm			Whole		
Egg	%Ca Ash	%Ca Dry	%Ca Orig.	%Ca Ash	%Ca Dry	%Ca Orig.	%Ca Ash	%Ca Dry	%Ca Orig.
26	6.73	.457	.045	5.90	.350	.039	6.21	.392	.041
32	1.82	.105	.013	3.65	.222	.028	2.07	.120	.015
36	2.48	.117	.015	3.52	.109	.014	2.82	.114	.015
43	3.42	.142	.017	4.17	.193	.024	3.85	.169	.021
46	3.06	.133	.016	2.77	.137	.016	2.88	.135	.016
52	1.36	.089	.099	2.20	.125	.014	1.66	.104	.012
59	2.17	.125	.014	1.90	.122	.014	2.01	.123	.014
68	3.47	.184	.022	3.65	.178	.022	3.55	.181	.022
82	2.19	.148	.017	2.21	.144	.016	2.19	.147	.016
90	1.80	.102	.012	2.35	.112	.014	2.04	.107	.013
Ave.	2.850	.1603	.027	3.232	.1692	.0201	2.928	.1592	.0185

Table III (Cold Storage Eggs)

	Watery			Firm			Whole		
Egg	%Ca Ash	%Ca Dry	%Ca Orig.	%Ca Ash	%Ca Dry	%Ca Orig.	%Ca Ash	%Ca Dry	%Ca Orig.
38				3.08	.165	.021			
42	3.51	.143	.018	3.47	.171	.022	3.49	.155	.020
44	1.86	.102	.012	3.21	.175	.020	2.63	.144	.017
53	2.00	.107	.013	1.69	.090	.011	1.85	.098	.012
64	2.53	.139	.017	1.47	.082	.010	1.98	.109	.014
74	1.62	.083	.010	3.61	.167	.020	2.01	.100	.012
80	3.92	.211	.025	5.99	.251	.033	4.55	.225	.027
85	3.38	.144	.019	2.16	.099	.013	2.55	.113	.015
99	2.24	.116	.014	2.34	.127	.016	2.28	.121	.015
100	1.55	.076	.009	1.66	.097	.012	1.60	.084	.010
Ave.	2.512	.1245	.0152	2.688	.1424	.0178	2.544	.1276	.0158

Table IV* (Watery White Eggs)

Egg	Watery %Ca Ash	Watery %Ca Dry	Watery %Ca Orig.	Firm %Ca Ash	Firm %Ca Dry	Firm %Ca Orig.	Whole %Ca Ash	Whole %Ca Dry	Whole %Ca Orig.
73	2.97	.111	.020	3.87	.157	.029	3.43	.133	.024
79	4.11	.111	.017	3.14	.114	.018	3.49	.112	.018
91	1.84	.099	.013	2.99	.155	.021	2.29	.121	.016
101	2.48	.128	.017	1.61	.081	.011	1.95	.099	.013
Ave.	2.85	.112	.0167	2.90	.127	.0197	2.79	.116	.0177

Table V
(Averages on all eggs analyzed)

	Watery %Ca Ash	Watery %Ca Dry	Watery %Ca Orig.	Firm %Ca Ash	Firm %Ca Dry	Firm %Ca Orig.	Whole %Ca Ash	Whole %Ca Dry	Whole %Ca Orig.
All eggs	2.60	.144	.0177	2.78	.149	.0176	2.587	.143	.01697

Table VI
(Averages on all eggs)

	%Ash Watery	%Ash Firm	%Ash Whole
Ash (dry basis)	5.60	5.47	5.51

Table VII
(Average on all eggs)

Eggs	%Moisture Watery	%Moisture Firm	%Moisture Whole egg
Fresh eggs	89.08	88.50	88.71
Market eggs	88.27	88.01	88.19
Storage eggs	87.49	87.13	87.36
Watery white eggs	84.73	85.47	84.62
Average	87.39	87.28	87.22

*The supply of watery white eggs was limited, thus accounting for the briefness of this table. This data is a trifle limited for reliable conclusions.

Table VIII
(Average on all eggs)

Eggs	% Watery	% Firm
Fresh eggs	47.79	52.21
Market eggs	54.41	45.59
Storage eggs	55.44	44.61
Watery white eggs	59.35	40.65
Average	54.25	45.76

DISCUSSION OF RESULTS

A wide variation was found in the calcium content of eggs whether they came from the same or widely different sources. The analysis of a large number of eggs was necessary therefore before any general tendency could be shown. There was wide variation in other properties of egg white besides calcium content. The moisture was the most nearly constant of the properties since its range of variation was about 4% on an 85 to 90% quantity. The ash had almost the same range of variation and it was never higher than 5%.

Per cent calcium on the ash basis shows a slight decrease from fresh eggs during the first month of storage then a slight increase. Further investigation by other workers shows a steady increase in calcium in the watery white with age either in cold storage or at room temperature. The per cent calcium in the firm white shows a slight average increase during the first few days, according to results on market eggs, then a decrease on storage. The tendency of the averages for the entire white follows closely that of the watery white. The fluctuation of the calcium in the two portions of the white in the first few days may be due to establishment of equilibrium. The decrease in calcium during the first month of storage may be explained in somewhat the same manner. It is possible that in the freshly laid egg chemical equilibrium between different elements has not been established. As the egg remains in storage this equilibrium is established by some slight amount of calcium being deposited upon the shell. Upon longer storage, CO_2 is given off, thus again changing the equilibrium and furnishing H_2CO_3 to dissolve $CaCO_3$, and form $Ca(HCO_3)_2$ which diffuses back into the egg contents to reëstablish equilibrium.

There is a slightly larger per cent of calcium in the firm white than in the watery white in all classes when figured on an ash basis. This is true for dry and moist basis with the exception of fresh eggs in the first and market eggs in the latter case. In these cases the relationship is reversed. The different percentage of moisture in the firm and watery white may account for the reverse condition between calcium in dry and original. The difference in percentage of ash may account for the other reverse order.

The different degree of viscosity of firm and watery white does not seem to be due to a change in moisture content. The slight difference shown in average moisture is well within the range of experimental error especially considering the nature of the material.

The total ash seems to average a trifle higher for watery white than for firm white. If we accept the physiologists' theory of the formation of the egg, we may be able to explain this seeming inconsistency. One theory (6) states that the yolk is developed first, then the albumin and globulin are formed surrounding the yolk, then the membrane is put on and the salts and like substances enter the egg by diffusion. If this is true, then the watery white, being nearest the membrane, would receive all of the inorganic salts first. The firm white would get its share after they passed through the watery white.

There is a larger percentage of watery than firm white in almost all eggs. This percentage gradually increases with the age of the egg. Individual eggs vary in amounts of the two divisions of white from all firm to all watery. This variation is found in eggs of all classes, therefore storage conditions do not seem to be wholly to blame for watery whites, yet there is a gradual increase in average percentage of watery white during storage at any temperature.

CONCLUSIONS

1. All properties of egg white in individual eggs are

extremely variable.

2. There is, on the average, a larger percentage of watery white than firm white in all classes of eggs. This percentage gradually increases with age.

3. The different degree of viscosity in firm and watery white is not due to different per cent water content.

4. There is a slight change in calcium content of white of egg on storage.

5. The gradual decrease in shell thickness is due to the dissolving of $CaCO_3$ into $Ca(HCO_3)_2$ which diffuses into the egg content.

6. Calcium content of eggs decreases for short time in storage, then gradually increases but at no time does this increase equal the increase during incubation.

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