(11) Hodge, J. E., J. Agr. Food Chem. **1**, 928 (1953).
(12) Hulme, A. C., Wooltorton, L. S. C., *J. Sci. Food Agr.* **9**, 150 (1958).
(13) Ingles, D. L., Reynolds, T. M., *Australian J. Chem.* **12**, 483 (1959).
(14) Johnson, G., Schaal, L. A., *Science* **115**, 627 (1952).
(15) Kuc, J., Henze, R. E., Ullstrup, A. J., Quackenbush, F. W., *J. Am. Chem. Soc.* **78**, 3123 (1956).
(16) Macallum, A. D., *J. Soc. Chem. Ind. (London)* **42**, 468T (1923).
(17) Marvel, C. S., Rands, R. D., Jr., *J. Am. Chem. Soc.* **72**, 2642 (1950).
(18) Meyer, J., *Ber. deut. chem. Ges.* **36**, 3599 (1903).
(19) Palmer, J. K., *Conn. Agr. Expt. Sta. (New Haven), Bull. No.* **589**, February 1955.
(20) Porter, W. L., Buch, M. L., Willits, C. O., *Food Research* **16**, 338 (1951).
(21) Schwartz, J. H., *J. Chromatog.* **3**, 491 (1960).
(22) Schwimmer, S., Burr, H. K., in Talburt, W. F., Smith, O., "Potato Processing," p. 36, Avi Publishing Co., Inc., Westport, Conn., 1959.
(23) Talley, E. A., Carter, F. L., Porter, W. L., J. Agr. Food Chem. **6**, 608 (1958).
(24) Talley, E. A., Fitzpatrick, T. J., Porter, W. L., Murphy, H. J., *Food Technol.*, accepted for publication.
(25) Woodward, G. E., Reinhart, F. E., *J. Biol. Chem.* **145**, 471 (1942).

Received for review February 6, 1961. Accepted May 29, 1961. Work done at a laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

FOOD DISCOLORATIONS

Cottonseed Constituents and Discolorations in Stored Shell Eggs

VERNON L. FRAMPTON and FAIRIE LYN CARTER
Southern Regional Research Laboratory, New Orleans, La.
BIAGIO PICCOLO
National Cottonseed Products Association, New Orleans, La.
B. W. HEYWANG
Southwest Poultry Experiment Station, Glendale, Ariz.

The occurrence of pink whites in stored shell eggs resulting from feeding cottonseed meal to laying hens is directly correlated with the Halphen positive constituent of the meal. This constituent induces an increase in the pH of the yolks during storage of shell eggs. The development of the brown color in the yolks of stored shell eggs produced by hens fed cottonseed meal is enhanced by the presence of Halphen positive constituent, since the chromogen responsible for the brown color is pH-sensitive.

The two more obvious adverse quality factors appearing in stored shell eggs produced by hens fed cottonseed meal are the brown color in the yolks and pink color in the whites. The appearance of pink whites in such eggs has been attributed to the presence of a constituent of the cottonseed oil entrained in the meals (*4*). It was reported (*2*, *4*) that, because of the presence of a substance in the meal that gives a positive Halphen test, the permeability of the vitellin membrane of the egg is altered in such a manner that proteins diffuse across the membrane. Color effects in the yolks and whites occur only in the shell egg. If the whites and yolks are first separated and then placed in storage, there are no color changes (*5*) in either the whites or yolks. The pink color is attributed (*5*) to an iron-conalbumin complex that is formed because of the altered permeability.

More recently Shenstone and Vickery (*6*) reported a rapid convergence of the pH of whites and yolks in shell eggs produced by hens receiving oils from malvaceous plants, including cotton. This effect is attributed to the presence of cyclopropene - containing acid components of the oils.

The Halphen positive constituent present in cottonseed meals fed to laying hens contributes to an abnormally rapid increase during storage in the pH of the yolks of shell eggs produced. This increase in the pH of the yolks enhances the brown color in the yolks, because the chromogen in the yolks is a pH indicator (*3*). The intensity of the brown color increases with increasing pH in the range of 7 to 10. Thus, the intensity of the brown color in the intact yolks of stored shell eggs produced from cottonseed meals is a function of the Halphen-positive constituent of the meals fed.

The percentage of pink whites appearing in stored shell eggs is directly proportional to the oil and to the Halphen positive constituent of the meals fed. The data confirm the earlier reports that Halphen positive constituent causes the pink color to develop in the whites.

Experimental Procedures and Results

Cottonseed meals CM–52, –53, –54, –55, –56, –57, –66, –67, –69, and –70 from another investigation (*3*) were incorporated into rations and fed to laying hens as described for that investigation. The properties of the meals are also described (*3*).

The eggs produced by the hens fed the cottonseed meals were kept under cold storage conditions for six months before they were opened and examined.

Curves for the regression of percentage of stored shell eggs with pink whites on the oil contents of the meals fed and for the oil contents of the meals on the percentage of eggs with pink whites are plotted in Figure 1. The coefficient of correlation calculated from these data is 0.91, with 9 degrees of freedom. It is evident that the incidence of pink whites in stored shell eggs produced by hens fed cottonseed meals of commercial origin will increase as the oil content of the meals increases.

Curves for the regression of the percentage of stored shell eggs with pink whites on the average pH of the yolks of these same eggs and for the average pH of the yolks on the percentage of pink whites are plotted in Figure 2. The coefficient of correlation calculated from these data is 0.88, with 9 degrees of freedom. Thus there is a concomitant increase in the percentage of stored shell eggs that have pink whites with increase in the pH of the yolks. The modification in the permeability of the vitellin membrane that permits the diffusion of proteins into and from the yolk also per-

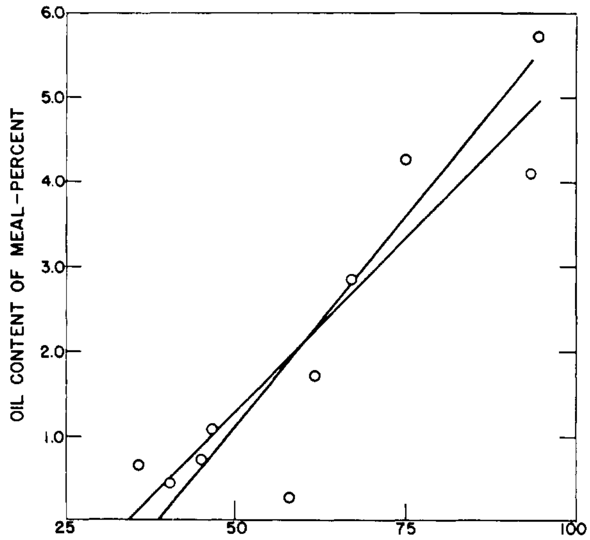

Figure 1. Occurrence of pink whites of stored shell eggs and oil content of meals fed in laying rations

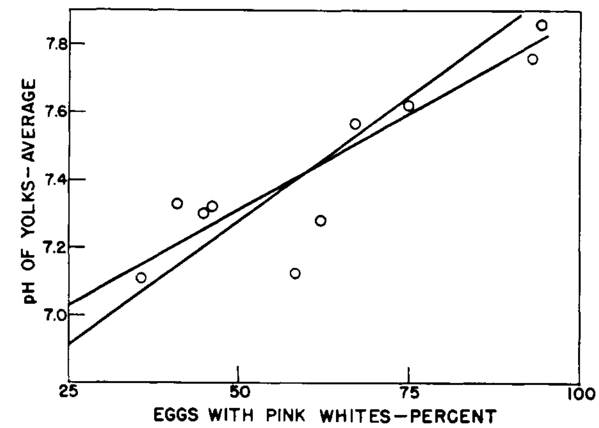

Figure 2. Occurrence of pink whites and pH of yolks of stored shell eggs

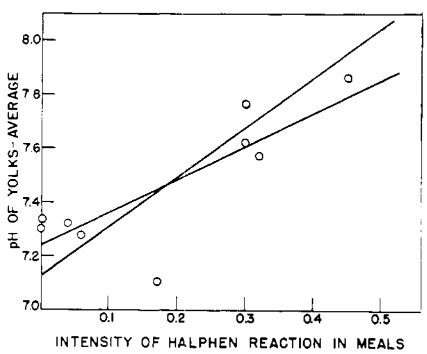

Figure 3. Intensity of the Halphen reaction in cottonseed meals fed in laying rations and pH of yolks of stored shell eggs

mits the diffusion of substances that cause the pH of the yolks to approach that of the whites.

The investigation was extended to determine the intensity of the Halphen test (*7*) for meals CM-52, -53, -54, -55, -56, -57, -66, -67, and -70. Ten grams of meal were thoroughly extracted with petroleum ether (boiling range, 68–71° C.) in a Butt extractor. The ether was removed from the extract by evaporation on a steam bath. All of the extract from each meal was used for each determination. The absorbance at 500 mμ was taken as a measure of the intensity of the Halphen test. There was a high level of background absorption in these determinations, and therefore the absorbance at 500 mμ is necessarily based upon an arbitrary base line. The results are recorded in Table I.

Curves for the regression of the intensity of the Halphen test for the meals on the pH of the yolks of stored shell eggs produced by hens fed the meals and the pH of the yolks on the intensity Halphen test are plotted in Figure 3. The coefficient of correlation calculated from these data is 0.94, with 7 degrees of freedom. It may be inferred that the Halphen positive constituent contributes to the increase in pH of the yolks while the eggs are in cold storage.

Table I. Intensity of Halphen Reaction for Cottonseed Meals

Meal	*Intensity of Halphen Reaction (Absorbance)*
CM-52	0.30
-53	0.32
-54	0.00
-55	0.00
-56	0.45
-57	0.30
-66	0.06
-67	0.04
-70	0.19

Curves for the regression of the intensity of the Halphen test content of the meals fed on the percentage of stored shell eggs produced from these meals which had pink whites and for percentage of pink whites on the intensity of the Halphen test content of the meals are plotted in Figure 4. The coefficient of correlation calculated from these data is 0.91, with 7 degrees of freedom.

It is apparent that the percentage of stored shell eggs with pink whites and the increase in pH of the yolks of the stored shell eggs are both directly correlated with the Halphen concentration of the positive constituent of the cottonseed meals used in the production of the eggs. It is apparent, also, that the Halphen positive constituent occurring in the meals is a component of the oil entrained in the meal, since the coefficient of correlation between the intensity of the test and oil contents of the meals is 0.92, with 7 degrees of freedom. It is expected, therefore, that the incidence of pink whites in stored shell eggs produced from cottonseed meal will be decreased if relatively oil-free meals are used in the laying rations.

A high speed screw press meal, CM-108 (3.83% oil), served as a source material for an additional series of experiments in which the effort was made to correlate cottonseed meal constituents further with the brown color of the yolk and the pink color of the whites of stored shell eggs obtained by feeding cottonseed meal.

A portion of CM-108 was extracted exhaustively with petroleum ether (boiling range, 68–71° C.). The extract was mixed with soybean meal and the mixture was incorporated into a laying ration at a rate equivalent to 20 pounds of CM-108 per 100 pounds of ration. A portion of the residue from the petroleum ether extraction of CM-108 was also incorporated into a laying ration at a rate equivalent to 20 pounds of CM-108 per 100 pounds of ration. A second portion of the residue was then extracted exhaustively with a mixture composed of 54 parts of petroleum ether (boiling range, 68–71°C.), 43 parts of acetone, and 3 parts of water. The extract was mixed with soybean meal and the mixture was also incorporated into a laying ration as described for the first extract. A portion of the residue from the extraction with the solvent mixture was also incorporated into a laying ration. This same pattern of exhaustive extraction and preparation of laying rations was continued successively with acetone, 99% isopropyl alcohol, and 91% aqueous isopropyl alcohol.

The detailed scheme for the fractionation of CM-108 is shown in Figure 5. Each extract and a portion of each meal residue were mixed into a ration in the manner indicated above. Each ration was fed to individually caged hens; six hens were used for each ration.

Eggs laid by these hens were kept under cold storage for six months before they were opened and examined (Table II). Pink whites appeared only when

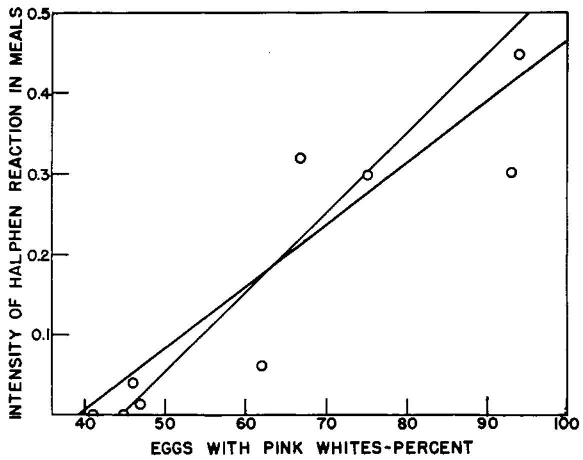

Figure 4. Occurrence of pink whites of stored shell eggs and intensity of Halphen test in cottonseed meals fed in laying rations

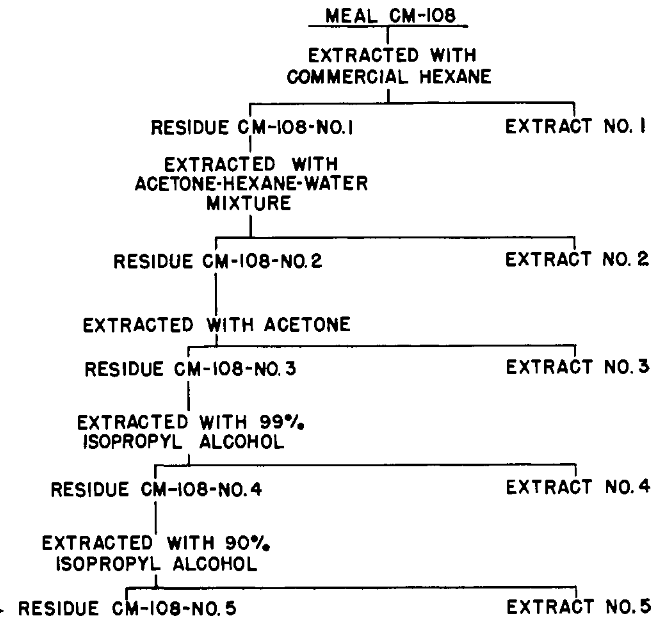

Figure 5. Scheme of successive extraction of a cottonseed meal with specified solvents ▶

Table II. Properties of Eggs Produced by Feeding Cottonseed Meal Fractions

Cottonseed Product Incorporated into Laying Ration	*pH*		*Color*			*Halphen Test*
	Whites	*Yolk*	*Whites*	*Intact yolk*	*Yolk at pH 10.4*	
Control	9.0–9.1	6.5–6.8	Normal	Normal	Normal	−
CM-108	8.0–8.1	7.9–8.5	Pink	Dark brown	Brown	+
Residue after extraction of CM-108						
1	8.8–9.1	6.5–7.3	Normal	Normal	Brown	−
2	9.0–9.1	6.4–7.0	Normal	Normal	Brown	−
3		6.3–6.9	Normal	Normal	Brown	−
4	6.5–6.9	6.5–6.8	Normal	Normal	Brown	−
5		6.4–6.8	Normal	Normal	Brown	−
Extracts from CM-108						
1	8.1–8.2	8.1–8.2	Pink	Translucent watery yolk, apricot color		+
2	9.1–9.2	6.0–6.5	Normal	Normal	Normal	−
3		6.6–7.0	Normal	Normal	Normal	−
4		6.5–7.0	Normal	Normal	Normal	−
5		6.3–7.2	Normal	Normal	Normal	−

CM-108 and the petroleum ether extract of CM-108 (extract 1) were fed, and it was only with these materials that abnormal yolk pH values were obtained. The intact yolks of CM-108-1, the petroleum ether–extracted meal, were normal, but the brown color appeared when the pH of the yolk contents was increased to pH 10.4 (Table II). The pH-sensitive chromogen was present in these yolks, but the brown color did not appear because of the low pH value (6.5 to 7.3).

None of the solvents used removed from CM-108 the constituent that is responsible for the chromogen in the yolks. Although it is easy to demonstrate that gossypol does produce a pH-sensitive pigment in the yolks of eggs when it is fed to laying hens, the constituent present in the residues used in this experiment is not gossypol or any gossypol derivative that was extractable from CM-108 with any of the solvents used. None of the yolks produced from the extracts developed the typical brown color when the pH of the yolk materials was increased to 10.4. The only extract that affected the quality of the eggs was that obtained with petroleum ether. In this case the pH of the yolks was abnormally high and the whites were pink.

Discussion

It is inferred that Halphen positive constituent of cottonseed has a marked effect on the organization of the stored shell egg. The chief effect seems to be on the permeability of the vitellin membrane. How the permeability is affected is not clear. Efforts to find Halphen positive constituent in the yolk fat and in the yolk phosphatides were not successful.

There is, according to Table I, a marked variation in the intensity of the Halphen reaction for cottonseed meals. A part is due to variation in the oil contents of the meals. However, the wide variation from meal to meal in intensity of the Halphen test content and correlation of the pH of the yolk with the Halphen-positive constituent should make egg yolk color data published in the past more meaningful. Obviously the brown color of the yolks of stored shell eggs produced by feeding cottonseed meal is enhanced when the concentration of the Halphen positive constituent is high and repressed when the concentration in the meal is low.

Literature Cited

(1) American Oil Chemists' Society, Chicago, "Official and Tentative Methods of Analysis," 2nd ed., 1959.
(2) Evans, R. J., Bandemer, S. L., Davidson, J. A., Bauer, D. H., Butts H. A., J. Agr. Food Chem. **2**, 1077 (1954).
(3) Frampton, V. L., Piccolo, B., Heywang, B. W., *Ibid.*, **9**, 519 (1961).
(4) Lorenz, W. F., *Poultry Sci.* **18**, 295 (1939).
(5) Schaible, P. T., Bandemer, S. L., Davidson, J. A., *Ibid.*, **25**, 440 (1946).
(6) Shenstone, F. S., Vickery, J. R., *Ibid.*, **38**, 1055 (1959).

Received for review December 5, 1960. Accepted May 8, 1961.