XV. THE FORMATION OF FERROUS SULPHIDE IN EGGS DURING COOKING.

BY CHARLES KENNETH TINKLER AND MARION CROSSLAND SOAR.

From the Chemical Laboratory, Household and Social Science Department, King's College for Women.

(*Received February 27th, 1920.*)

IT is a matter of common experience that on prolonged cooking of an egg in its shell (*e.g.* when it is immersed in boiling water for 15 minutes or longer) a greenish black coloration is produced on the surface of the yolk.

It is also well known that if a so-called "hard-boiled egg" be immersed in cold water immediately after cooking, the green colour is either not apparent at all, or is much less marked than is the case when the egg is allowed to cool slowly.

In view of the fact that this coloration is only on the *surface* of the yolk, it would appear that in its production some chemical action takes place, either between some constituent of the membrane which surrounds the yolk and some constituent of the yolk itself, or that some substance which is produced from the egg white, and which can penetrate this membrane, interacts with some constituent of the yolk.

The Nature of the Coloured Substance.

It appeared probable at the outset of the investigation that the greenish black coloration was due to ferrous sulphide in a fine state of division, and the various experiments which have been made seem to confirm this view.

Thus the coloured substance is readily soluble in dilute acids, the solution so obtained giving positive reactions for iron and evolving hydrogen sulphide on warming, whereas cooked egg yolk showing no greenish colour does not give these reactions. Moreover, the coloured egg yolk is decolorised on prolonged exposure to air or on treatment with hydrogen peroxide. This is also the case with the greenish coloured precipitate of ferrous sulphide when, for example, this substance is precipitated in a fine state of division on a piece of filter paper by the interaction of a solution of ferrous sulphate and ammonium sulphide.

It is well known that egg white on prolonged heating evolves considerable quantities of hydrogen sulphide and that the yolk of egg contains iron, probably in combination with proteins. The amount of iron in egg yolk, according to Sherman [1914], is 0·0085 % by weight. A solution of ferrous ammonium sulphate containing iron equivalent to that in egg yolk was made up and portions of the solution poured on to filter papers. On the addition of colourless ammonium sulphide to these filter papers, colorations due to ferrous sulphide were obtained, which were almost identical with those obtained on the yolks of eggs when unbroken eggs are immersed for 15 minutes or more in boiling water.

Pollacci [1904] has investigated the losses of sulphur which take place on heating yolk and white of egg separately, and also the effect of keeping uncooked whole eggs for 24 hours in an atmosphere of hydrogen sulphide. The chocolate brown colour produced in the yolk of the uncooked egg in this case is said to be due to the formation of iron sulphide.

The Effect of Prolonged Heating on Yolk and White of Egg separately.

Evidence that the green coloration was due to interaction between constituents of both the yolk and the white was obtained in the following way. Two yolks in their membranes, separated completely from egg white and washed with distilled water, were heated in boiling water for 20 minutes. One was then cooled quickly by washing in a stream of cold water, and the other was allowed to cool slowly. In neither case was the slightest green colour observed, but on placing portions of these yolks in a gas jar containing hydrogen sulphide to which a few drops of ammonia had been added, green colours were obtained both in the portions still covered with membrane and on those taken from the inside of the yolk.

It was found that the green colour was produced only very slowly by the action of hydrogen sulphide alone, but was immediately formed on the addition of ammonia.

Yolk of egg, separated from egg white, was also heated alone in a test tube for 90 minutes in boiling water, and even in this case no green coloration was apparent. Cooked egg white treated in the same way with hydrogen sulphide and ammonia gives no coloration until a considerable time has elapsed, and even then the colour produced is very faint. It should be noted in this connection that the iron content of egg white (0·0001 %) is only about one-eighty-fifth that of egg yolk [Sherman, 1914]. According to Pollacci [1904], egg white is free from iron.

From the results of these experiments it may be concluded that some constituent of the white of egg plays a part in the formation of the green layer of ferrous sulphide. Additional evidence that the yolk alone does not give rise to both constituents necessary for the formation of the ferrous sulphide is afforded by the fact that the latter is only produced as a thin layer on the surface of the yolk. If both the iron and sulphur were provided by the yolk, the green coloration would probably extend through the whole of the yolk, diminishing in intensity on approaching the centre, owing to the fact that the temperature at the centre of the egg does not rise as rapidly on cooking as that of the outside of the yolk.

It can also be shown that whereas neither white nor yolk of egg on heating alone shows any green coloration, when heated in contact with one another the characteristic green coloration is produced (see below).

Experiments relating to the Membrane round the Yolk.

In order to determine whether or not any constituent of the membrane round the yolk takes any part in the reaction, an experiment was made as follows. Egg white was heated in a test tube until coagulated, and egg yolk, free from membrane, was then poured into the test tube to form a layer above the white. On heating the test tube for about 15 minutes in boiling water, a green ring was obtained at the junction of the yolk and white.

On examining a "hard-boiled" egg, the colourless yolk membrane can be seen adhering to the inside of the white of the egg, and does not become absorbed by the yolk as appears at first.

It would seem, therefore, that the membrane round the yolk does not play any part in the formation of the green coloration. It must, however, be permeable to hydrogen sulphide.

The Effect of Time of Heating on the Coloration obtained.

Two eggs were heated in boiling water for 15 minutes and two for 30 minutes. In both cases one of the eggs was cooled down quickly by removing the shell and placing in a stream of running water, while the other was allowed to cool with the water in which it had been heated.

In the case of the eggs heated for 15 minutes practically no green colour was obtained on the yolk of the egg which was cooled quickly, whereas a slight green colour was apparent on the other.

In the case of the eggs heated for 30 minutes, very little difference was apparent in the amount of green colour developed, whether the egg was cooled quickly or allowed to cool with the water; but a much more intense coloration was produced round the yolks of these eggs than round the yolks of those heated only for 15 minutes.

It appears, therefore, as would be expected, that the amount of green coloration depends upon the length of time the egg is heated, and that the effect of the cold water in preventing the formation of the green colour is simply that, on cooling the egg quickly, the preliminary decomposition necessary for the formation of ferrous sulphide is checked. The act of placing a hard-boiled egg in cold water does not therefore lead to the *removal* of the green coloration already formed, but prevents its formation.

This view is supported by the following observation. Two eggs were heated in boiling water for 15 minutes, the shell of one was removed at once and the white of the egg removed. No green colour was observed. The white was then replaced round the yolk and secured with cotton to prevent the access of air. The egg was allowed to cool slowly, and when cold, the white of the egg was again removed. The green coloration was then apparent.

The other egg was allowed to cool in the water in which it had been cooked and, on examination when cold, was found to show the usual green coloration round the yolk. In addition to the effect of the cold water in checking the decomposition of the egg white, the rapid cooling on the outside of the egg will lead to the diffusion of the hydrogen sulphide from the white away from the yolk, and in this way also diminish the amount of ferrous sulphide formed.

In order to investigate further the effect of prolonged heating on the formation of the colour, two eggs were heated in boiling water, one for two hours, when still only a thin film of green round the yolk was apparent, and the other for seven hours. In the latter case the colour of the white after cooking was buff and the green layer was thicker than in the previous case, especially on one side where its thickness was about one millimetre.

Subsequent experiments showed that if the yolk of an egg after cooking is not surrounded by a layer of white of uniform thickness, the thickness of the layer of ferrous sulphide is greater where the yolk has been surrounded by a greater thickness of white.

The object of heating one of the eggs for seven hours was also to ascertain, if possible, whether the hydrogen sulphide from the white could penetrate the thin film of ferrous sulphide at first formed, and produce in the yolk layers of ferrous sulphide as in the Liesegang phenomenon. No such layers were, however, apparent, either in the yolk of this egg cooked in boiling water for seven hours, or in an egg yolk which, completely surrounded by its membrane, washed free from adhering white, and cooked in boiling water for 20 minutes, was then placed in a gas jar with hydrogen sulphide and ammonia.

It was found that if an uncooked egg is kept for a day in an atmosphere of hydrogen sulphide, as in Pollacci's experiments, and then heated in boiling water for 20 minutes, the greenish black coloration due to ferrous sulphide extends throughout the whole of the yolk.

The Effect of the Age of the Egg upon the Amount of Green Coloration.

It was thought that the age of the egg might determine to a considerable extent the amount of green coloration formed on prolonged cooking. To ascertain if this were so, three eggs, (1) an egg laid one day previously, (2) an egg laid five weeks previously, (3) an egg laid six weeks previously, were immersed in boiling water for 20 minutes and allowed to cool in the water. The yolks were then separated from the whites and treated in exactly the same way, each being immersed in dilute iron-free sulphuric acid. After ten minutes the acid liquid was filtered and the solutions diluted to the same volume in each case. There was certainly a difference in the amount of iron contained in the solutions; the extract from the yolk of the fresh egg containing least iron, and that from the six week old egg most, but the difference was not very great. This was in accordance with the appearance of the yolks before treatment with the acid. Only slightly more green coloration was apparent in the case of the egg laid six weeks previously, than in the case of the one day egg.

The Liberation of Hydrogen Sulphide from Egg White and Yolk.

Although the sulphur content of the yolk of an egg—0·157 %—is not very much less than that of the white—0·196 % [Sherman 1914]—this element is evidently in less stable combination in the latter than in the former. On heating white and yolk of egg separately in a test tube immersed in boiling water, hydrogen sulphide is evolved (shown by its action upon lead acetate paper) from the white after about three and a half minutes, while no trace of the gas is obtained from the yolk in this time.

An approximate comparison of the amounts of hydrogen sulphide obtained on distilling equal weights of egg white and yolk (1 g.) with water (400 cc.) was obtained by the addition of standard lead acetate solution to the distillates, and comparing the colours obtained with those formed with known amounts of the lead acetate solution to which hydrogen sulphide solution had been added.

In the case of the egg white the first 100 cc. of the distillate was matched by 2·5 cc. of standard lead acetate solution (1 cc. = 0·0001 g. Pb), whereas a similar volume of the distillate from the egg yolk was free from hydrogen sulphide.

On continuing the distillation 2 cc. of the standard lead solution were required for the next 100 cc. of the distillate from the egg white and 0·7 cc. from a similar volume of distillate from the yolk.

Sulphuretted hydrogen is thus more readily obtained from egg white than from egg yolk. [See also results obtained by Pollacci, 1904.]

The Reactions of Egg White and Yolk towards Litmus.

Uncooked egg white has an alkaline reaction, and uncooked egg yolk an acid reaction, towards litmus. It was at first thought possible that ammonia, or a substituted ammonia, might be produced by the decomposition of the egg white. With the hydrogen sulphide this ammonia or amine might pass through the membrane, and by neutralising the acid of the yolk facilitate the production of ferrous sulphide. Small quantities of ammonia and substituted ammonia are produced both from the white and yolk on distillation with water, but this ammonia is perhaps not necessary for the production of the ferrous sulphide, as it was noticed in the course of the experiments that the yolk of egg, which is originally acid to litmus, becomes alkaline on heating.

On heating a mixture of egg yolk and water containing blue litmus the indicator is seen to change colour at approximately 70°.

When an egg is heated in boiling water in the ordinary process of "boiling" for three to five minutes, the temperature of the yolk probably does not reach 70°, so that if hydrogen sulphide were present it might possibly not react immediately with the yolk to form ferrous sulphide, owing to the acid reaction of the yolk at a temperature below 70°. On prolonged heating, however, the yolk will become alkaline and the conditions for the formation of ferrous sulphide will be favourable.

The Formation of Ferrous Sulphide in preserved and dried Eggs.

Eggs which have been preserved in water glass appear to behave normally with regard to the formation of ferrous sulphide on cooking. Thus two eggs which had been preserved for ten months in water glass were boiled for 20 minutes. One was cooled quickly and the other allowed to cool slowly. In the case of the former no formation of ferrous sulphide was apparent, but in the case of the latter the usual appearance of the substance on the surface of the yolk was noted.

Eggs which have been dried appear to be altered in some way which largely prevents the formation of iron sulphide on prolonged cooking. Thus if the white and yolk of an egg be beaten together and some of the mixture heated in a test tube in boiling water for 20 minutes, a slight green colour is apparent, whereas if the beaten egg is dried in a vacuum desiccator and then mixed with water immediately after drying, only a very faint coloration is observed on cooking. If the dried egg is kept for some time, the formation of ferrous sulphide on cooking does not take place. Some commercial preparations of dried eggs also failed to show the formation of ferrous sulphide on cooking.

SUMMARY.

The greenish black coloration observed on the surface of the yolk of a "hard-boiled" egg appears to be due to the formation of ferrous sulphide, the hydrogen sulphide necessary for the formation of the substance being produced by the decomposition of a sulphur compound of the egg white.

The non-formation of the coloration when a "hard-boiled" egg is placed in cold water immediately after cooking is due to the checking of the decomposition owing to cooling.

REFERENCES.

Pollacci (1904). *Gazzetta*, **34** (i), 278.
Sherman (1914). *Food Products* (Macmillan).