Experimental Evidence that the Stem-end Blackening Pigment of Potatoes is a Compound of Iron

By H. G. WAGER, *Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge, and Department of Scientific and Industrial Research*

(Received 13 January 1948)

In studies of the environmental conditions of growth which lead to the production of potatoes liable to show stem-end blackening, the quantity of pigment present was estimated by visual judgement (Wager, 1946, 1947). In the course of experiments to develop an alternative, physical method for the estimation of the stem-end blackening pigment, it was found that the intensity of the colour of the extracts was increased by the addition of small amounts of iron salts, and further work was directed to this point.

Robison (1941) suggested that the pigment might be a compound of iron. The evidence given was concerned with the relationship between the iron content of the tubers and the amount of stem-end blackening shown by them, and was conflicting. She also suggested that the blue-black compound was an oxide of iron. These views were criticized by Cowie (1941). Nutting (1942), on the basis of analogy with other pigments, suggested the possibility that the blue-black pigment was a metallic complex, but offered no supporting evidence.

METHODS

The potato extracts were prepared from median longitudinal slices, 3 mm. thick, of peeled tubers: 40 or 50 potatoes were sliced to obtain a composite sample. The slices were washed free of surface starch, cooked in glass-distilled water, reduced to a sludge and left to stand for about 1 hr. to allow time for equilibration of soluble material between the potato cells and the liquid. The potato cells were then filtered off and the cloudy filtrate left to stand with chloroform for several days, during which time precipitation occurred. One or two successive filtrations gave an extract with only a trace of opalescence.

The stem-end blackening pigment is sensitive to change in acidity, being colourless or nearly so at an acidity of low pH (3 or less), and attaining a maximal intensity of colour at one of pH 8-9 (Wager, 1945). This change in colour was the basis of the method of estimating the pigment. The absorption of light by the solution was measured at pH 7-40 and also at about pH 3, and the difference between these two values was used as an estimate of the content of stem-end blackening pigment. This estimation was fairly specific, but in some cases there may have been a slight change in the degree of opalescence of the solution on acidification, and it seems probable that in all extracts there were small amounts of compounds which were pale yellow in alkaline solution and colourless in acid solution; such compounds

could be seen in some of the extracts containing little stem-end blackening pigment.

Iron was estimated by $\alpha\alpha'$ -dipyridyl using the potato extract prepared as above (i.e. without ashing). The development of colour was slow; 3-4 hr. were required for maximal intensity, whereas in pure solutions of iron 15 min. only. The average recovery of iron added to the solution was within about 2% of the value shown by the calibration curve, but individual values showed some experimental error. The complete recovery of amounts of iron as low as 1 $\mu\text{g.}/\text{ml.}$ from solution with 3-4 times this amount of iron, as determined by the $\alpha\alpha'$ -dipyridyl reagent, suggests that all the ionizable iron present was estimated, and this has been assumed.

pH was determined with a glass electrode, and light absorption by a photometer (Hilger Spekker) using a violet filter, Ilford no. 601.

The samples of potatoes used in this work were all commercially grown in East Anglia. They came from a variety of soil types and consisted of five samples of King Edward, five of Doon Star and eleven of Majestic potatoes. (The author's thanks are due to Mr B. S. Smith of the Potato and Carrot Division of the Ministry of Food, Peterborough, who arranged for the collection of these samples.)

RESULTS

Extracts prepared from tubers liable to stem-end blackening are grey in colour at pH 6 and become somewhat browner as the pH is adjusted to 7.5. The addition of a solution of an iron salt, which increases the iron concentration by a few $\mu\text{g.}/\text{ml.}$, leads to a marked increase in intensity of colour. This increase in intensity of colour may be explained by the assumption that the stem-end blackening pigment is a compound of a colourless precursor and iron, and that there is normally an excess of the colourless precursor. On the other hand, there might be present two pigments, the stem-end blackening pigment and another which was affected by iron, e.g. tannin. To test whether tannin was present an extract with a high intensity of colour was shaken at intervals with droplets of gelatin for 2 hr. The gelatin was filtered off and melted, and then to the diluted and melted gelatin and to the filtrate was added an excess of iron (10 $\mu\text{g.}/\text{ml.}$). The change in intensity of colour with pH was determined for both solutions. The gelatin contained slightly less material that increased in colour with added iron than did the

filtrate, i.e. there was no evidence of concentration in the gelatin. The same technique when applied to a solution of tannic acid of similar concentration resulted in a marked concentration of tannic acid in the gelatin. As a further test for the presence of tannin an attempt was made to absorb the pigment on goldbeater's skin. This was also negative. There is, therefore, a *prima facie* case for assuming that the increase in colour with iron is not due to the presence of tannins.

Extracts were prepared from different samples of potatoes and their pH was adjusted to 7.40. To 10 ml. portions of these extracts 1–15 $\mu\text{g./ml.}$ of iron was added as ferrous sulphate. The concentration of iron and also the amount of stem-end blackening pigment were then determined, with the results shown in Fig. 1. The lowest points on the curves are for the extracts with no added iron.

Fig. 1. The relationship between the estimated content of iron in potato extracts and the concentration of stem-end blackening pigment, expressed as the optical density of the extract.

If all these curves, when extrapolated backwards, had passed through the origin it would have been clear evidence that the stem-end blackening pigment was a compound of iron. Some of the curves, however, when extrapolated, do not appear to pass through the origin, and it seems reasonable to assume that in these cases there were present compounds, sensitive to the change in pH, other than the stem-end blackening pigment: a faint yellow colour at pH 7.4, which was lost in acid solution, was noticed in some extracts which contained very little stem-end blackening pigment. The curves in Fig. 1, therefore, offer fair evidence for the combination of a colourless precursor with iron to give the stem-end blackening pigment.

The colour of any given extract is clearly dependent on the concentration both of iron and of the colourless precursor of the stem-end blackening pigment. The maximum intensity of pigment obtained with a high concentration of iron may be taken as a measure of the total amount of stem-end blackening pigment present. This has varied in different extracts from 6 to 31 units, whilst the concentration of iron in the extract merely varied from 1.4 to 3.5 $\mu\text{g./ml.}$ and could not be correlated with the variation in stem-end blackening pigment. It follows, therefore, that the major factor in determining the amount of blackening that develops in potatoes after cooking is the content of the stem-end blackening pigment precursor and not the content of iron (cf. Robison, 1941). The relationships between the content of iron, the content of stem-end blackening pigment, and the variety and conditions of growth of the tuber will be discussed in a subsequent paper.

Fig. 2. The concentration of stem-end blackening pigment, expressed as optical density, in potato extracts at a low concentration of iron, 2 $\mu\text{g./ml.}$, plotted against the concentration of pigment in the same extract at a high concentration of iron, 18 $\mu\text{g./ml.}$

If these extracts contained coloured stem-end blackening pigment and a different colourless compound, liable to darken with iron, it would be very unlikely that the concentration of both compounds would always be in the same ratio. Unless this were so the intensity of colour developed at a high concentration of iron would bear no constant ratio to that at a low concentration. These two values have been plotted against each other in Fig. 2 for all extracts prepared, and a sensibly constant ratio between them is clearly shown. Some small divergencies from a constant ratio must be expected in view of the experimental errors and of the presence of other light-absorbing compounds in some of the

extracts. The view that the extracts contained only one main compound that combines with iron, and that this combination gives rise to the stem-end blackening pigment receives support, therefore, from Fig. 2.

A simple but very conclusive visual demonstration of the combination of the stem-end blackening pigment with iron may be made by immersing one half of cooked tubers, showing stem-end blackening, in a solution of 10 $\mu\text{g./ml.}$ of iron for about 1 hr. and immersing the opposite halves in distilled water as a control. The iron causes a great intensification of colour in regions previously blackened, but a

negligible discoloration occurs in the rest of the tuber.

SUMMARY

Evidence is offered to support the view that the stem-end blackening pigment of potato is a compound of iron with a colourless precursor. The concentrations of iron and of the precursor vary in different samples of potatoes. The range of concentration of the precursor is greater than that of iron.

The work described above was carried out as part of the programme of the Food Investigation Board of the Department of Scientific and Industrial Research.

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