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FOLLOWED BY THE USE OF ^{55}Fe AS A TRACER

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STUDIES ON THE BEHAVIOR OF METALS IN COLLOIDAL SOLUTION OF FOOD BY THE USE OF RADIOACTIVE TRACERS

II. BEHAVIOR OF IRON IN STARCH PASTE FOLLOWED BY THE USE OF ^{55}Fe AS A TRACER

By

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In Part I of this series (1), it was shown that iron in starch paste (concentration above 0.01%) and protein solution (concentration above 0.5%) remained at 100 per cent in the clear supernatant even after standing for 24 hours. In the present work, the amount of iron remaining in various kinds of starch paste after supercentrifugation was examined by the use of radioactive ^{55}Fe and the amount of starch remaining in the supernatant was also examined. Absorption curves of iron-containing starch paste are shown in the accompanying graphs.

Experimental Method

1. *Sample, Reagents, and Apparatus*

Suspension of Various Starches: Potato starch, corn starch, and soluble starch used were of Japanese Pharmacopoeia preparations (product of Toyo Seiyaku K.K.). Each starch was made into suspensions of 0.01 per cent, 0.1 per cent and 1 per cent concentrations.

Ferric Chloride Solution: ^{55}Fe and carrier FeCl_3 were added to make 0.1N hydrochloric acid solution containing 1 mg/ml of iron.

2 π -Gas Flow Counter (Q-gas used; product of Kobe Kogyo K.K.)

Supercentrifuge (Hitachi Model 40-P)

Beckman Model-DU Photoelectric Spectrophotometer

2. *Procedure*

2.1 *Preparation of Sample Solution:* To each of 0.01 per cent, 0.1 per cent and 1 per cent suspension of potato starch, corn starch, and soluble starch, 1 ml

of ^{55}Fe -containing ferric chloride solution was added and the whole volume run de up to 100 ml with water. These solutions were adjusted to pH 5.2 or pH 3.8 after standing at room temperature, or heating for two or 15 minutes at 80°C or 100°C, and after cooling.

2.2 Treatment of the Sample Solution: Each of the foregoing starch paste and suspensions of various concentrations containing radioactive iron was submitted to super-centrifugation at 40,000 rpm or 20,000 rpm for one hour and the residual amount of ^{55}Fe in the supernatant was measured by the 2π -gas flow counter, as described in Part I (1). At the same time, the residual amount of starch in the supernatant was calculated by multiplying the quantity of reducing sugar formed owing to hydrolysis by 0.9.

Experimental Results

1. Residual Rate of ^{55}Fe in Potato Starch Paste by Heating and Number of Centrifugal Revolution

Potato starch suspension of 0.01 per cent, 0.1 per cent, and 1 per cent concentrations was added with ^{55}Fe -containing ferric chloride solution to make an iron concentration of 1 mg, heated at 80°C or 100°C for two or 15 min, cooled, and adjusted to pH 5.2. Each of such sample solutions was centrifuged at 40,000 or 20,000 rpm for one hr and the residual radio activity of ^{55}Fe measured. At the same time, the residual amount of starch in the supernatant was examined. The amount of residual iron in the supernatant did not differ greatly in any concentration of starch and was around 5 per cent (error of radioactivity measurement was $\pm 3\%$), while the residual rate was 5 per cent to 55 per cent in starch paste. The residual rate was invariably greater in paste than in the suspension, especially with higher concentration of paste, when heated at 100°C

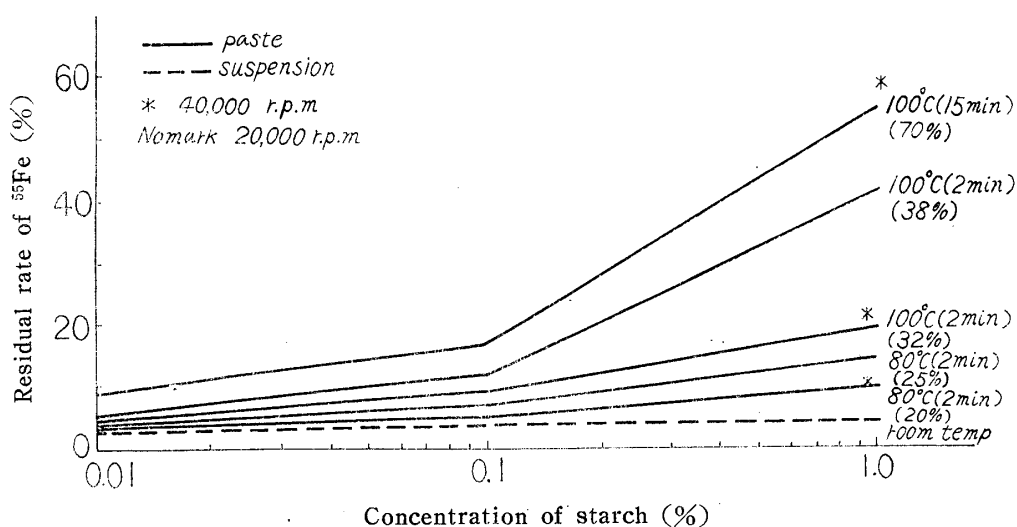


Fig. 1. Variation in residual rate of ^{55}Fe according to heating condition and centrifugal revolution.

Figures in parentheses indicate residual rate (%) of starch.

than at 80°C, when heated at 100°C for 15 min than for two min, and when centrifuged at 20,000 rpm than at 40,000 rpm if the paste was of the same concentration and heated at the same temperature for the same length of time. In the case of paste, starch remained in the supernatant of all samples after centrifugation and the residual rate was similar to that of residual ^{55}Fe . These results are illustrated in Fig. 1.

2. Residual rate of ^{55}Fe in Potato Starch Paste by the Addition of Iron.

In exactly the same way as in the foregoing experiment, ferric chloride solution containing ^{55}Fe was added to the potato starch suspension and heated at 100°C for 15 min or the suspension was first heated at 100°C for 15 min, cooled, and mixed with ferric chloride solution containing ^{55}Fe . Both were adjusted to pH 3.8 and the residual rate of ^{55}Fe in the supernatant was examined after centrifugation as in the foregoing experiment, using the same solution without heating and ferric chloride solution without the addition of starch as the controls. The residual rate of ^{55}Fe in the starch suspension did not differ greatly in any concentrations of starch from that in the iron solution alone. Starch was not found in the supernatant at all. The residual rate of ^{55}Fe was greater when ferric chloride solution was added to the suspension and heated

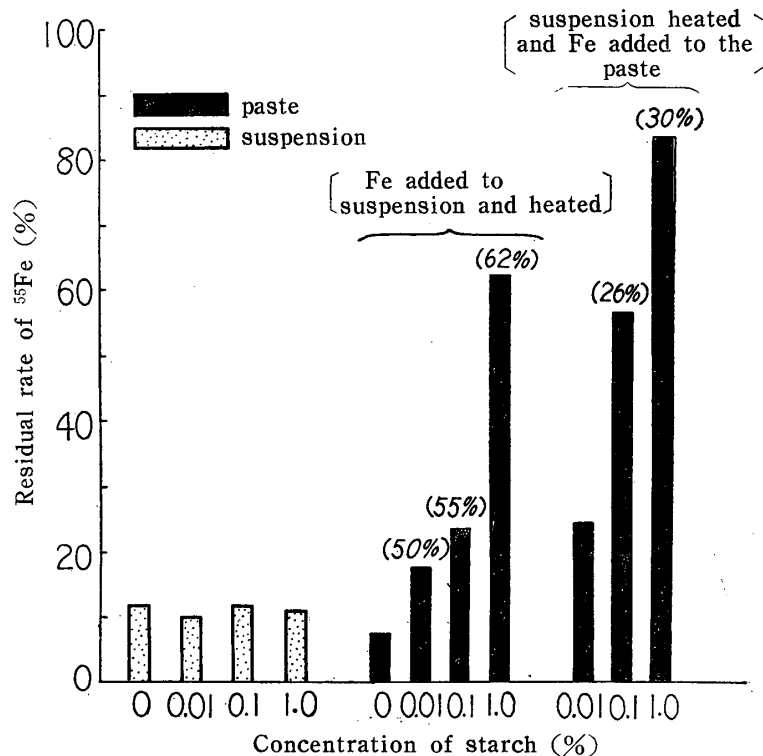


Fig. 2. Variation in residual rate of ^{55}Fe in potato starch paste according to the manner of the addition of iron.

Percentage in parentheses indicates amount of residual starch in the supernatant.

Note: Residual rate of starch in the supernatant of starch paste not containing iron was around 71 per cent at any concentration of starch.

to form a paste than when iron was added after heating. The residual rate increased with increasing concentration of starch. The residual rate of ^{55}Fe was somewhat smaller in heated iron solution than that left at room temperature. The residual rate of ^{55}Fe was greater when ferric chloride solution was added into starch paste than when added to the suspension and heated to make a paste, in all the concentrations of starch. The residual rate of starch in the supernatant from starch paste showed the same tendency as that of ^{55}Fe but the amount of starch in the supernatant was markedly smaller when iron solution was added to paste than in heated starch alone. The results of these experiments are illustrated in Fig. 2.

3. Residual Rate of ^{55}Fe in Corn Starch Paste and Soluble Starch Paste

3 · 1 Ferric chloride solution containing ^{55}Fe was added to corn starch suspension of 0.01 per cent, 0.1 per cent, and 1 per cent concentrations, as in the foregoing experiments, adjusted to pH 5.2, and heated at 100°C for two min. Measurement of residual rate of ^{55}Fe in their supernatant showed that the residual rate increased with increasing concentration, the maximum being around 5 per cent.

3 · 2 In exactly the same manner as in 3 · 1, the residual rate of ^{55}Fe in soluble starch paste was measured and the rate was found to be greater in both the paste and suspension than that in any of the other starches described above, at the same pH. Starch was found in the supernatant of soluble starch suspension. The residual rate of ^{55}Fe in the paste was 85 per cent. The fact that the residual rate of ^{55}Fe was smaller when the centrifugal revolution was faster is the same as in the case of the foregoing potato starch paste.

The results of these two experiments are illustrated in Fig. 3.

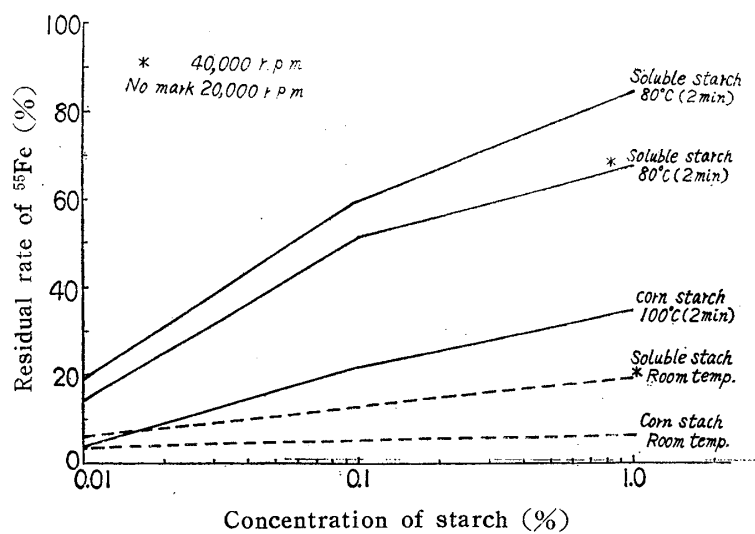


Fig. 3. Residual rate of ^{55}Fe in the supernatant of soluble starch and corn starch paste.

4. Absorption Curve of Iron-containing Starch Paste

A mixture of ferric chloride solution and potato starch suspension, containing 1 mg per cent of iron and 0.1 per cent of potato starch, was heated at 100°C

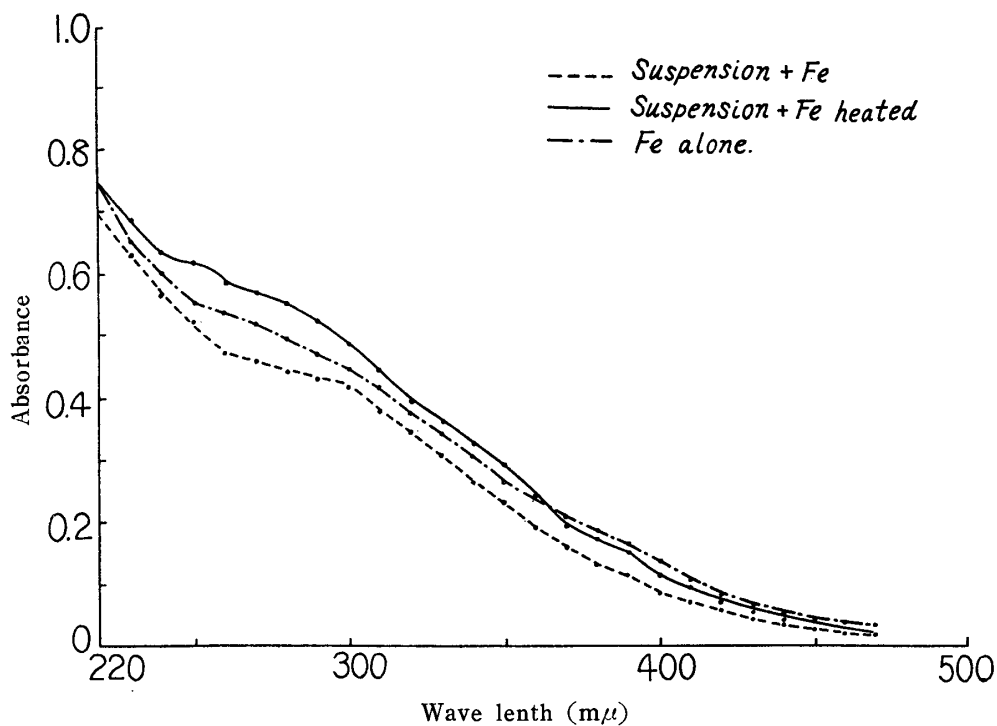


Fig. 4. Ultraviolet absorption curve of iron-containing starch paste (at pH 3.8).

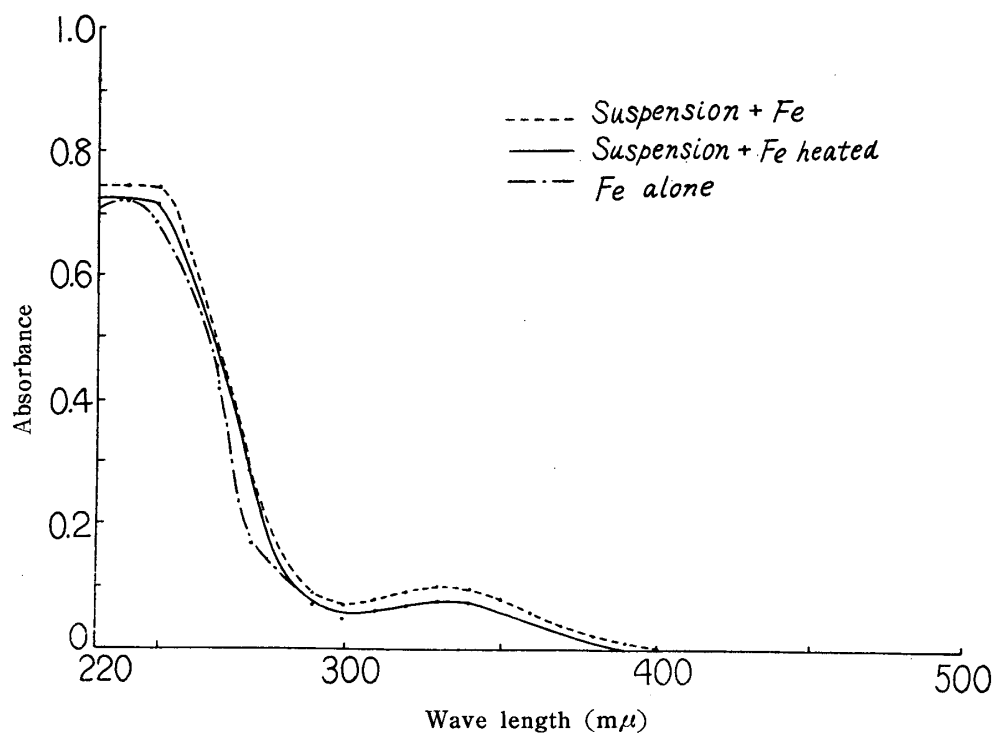


Fig. 5. Ultraviolet absorption curve of iron-containing starch paste (at pH 1.0).

for 15 minutes, cooled, and adjusted to pH 3.8 or pH 1.0. Each of these pastes was submitted to measurement of ultraviolet absorption spectrum, using the iron solution alone or the suspension without heating as the controls. The absorption spectrum of potato starch paste containing iron was approximately of the same shape as those of the control starch suspension containing iron and the iron solution alone. The absorption curves are shown in Figs. 4 and 5.

Conclusion and Considerations

Potato starch, corn starch, and soluble starch were made into suspensions of 0.01 per cent, 0.1 per cent, and 1 per cent concentrations, ferric chloride solution containing ^{55}Fe was added to make iron concentration of 1 mg per cent, heated at 80°C or 100°C for two or 15 minutes, cooled, and adjusted to pH 5.2 or pH 3.8. Each of these pastes was submitted to supercentrifugation at 20,000 rpm or 40,000 rpm for one hour and the residual rate of ^{55}Fe and starch in their supernatant were measured. In the case of potato starch, the residual rate of ^{55}Fe was compared when iron was added to the suspension and heated, and when iron was added after the suspension was heated. At the same time, the residual rate of ^{55}Fe and starch was measured in a solution of iron alone or in heated iron solution, as a control. Absorption spectrum of the foregoing iron-containing potato starch was examined. The results obtained from these experiments are summarized as follows:

(1) The residual rate of ^{55}Fe in the paste of potato starch, corn starch, and soluble starch was greater than that in their suspension used as the control, the residual rate being greater with increasing concentration of the paste, the higher the temperature of heating, the longer the time of heating, the smaller the number of centrifugal revolution, and when iron was added after the suspension was heated to form the paste. The residual rate of starch showed the same tendency as that of iron. The residual rate of ^{55}Fe was especially high in the paste of soluble starch.

There was no great difference in the residual rate of ^{55}Fe between the control iron solution alone and potato starch suspension. While the residual rate of ^{55}Fe in the heated iron solution was smaller than that in the solution left at room temperature, the rate was greater in starch suspension heated after addition of iron.

(2) Ultraviolet absorption was measured of iron-containing starch paste, with 1 mg per cent of iron and 0.1 per cent concentration of starch, at pH 3.8 and pH 1.0. In both these cases, the absorption curve was almost unchanged from that of the control iron solution at the same pH.

From the foregoing experimental evidences, the following conclusion is drawn.

Ferric chloride solution of pH 3.8 and pH 5.2 undergoes hydrolysis and this

hydrolysis is accelerated at higher pH value or by heating, and the residual rate of ^{55}Fe in the supernatant becomes smaller. If starch paste is present in this case, the residual rate of ^{55}Fe becomes greater, the tendency being greater with increasing concentration of the paste, with higher temperature of heating, and with smaller number of centrifugal revolution. The same tendency is observed in the case of the residual amount of starch in the supernatant. These facts clearly indicate binding of iron with starch in colloidal state. The fact that the residual rate of ^{55}Fe is smaller when iron is added to the suspension and then heated to form a paste than when iron is added to the paste is assumed to be due to the decreased viscosity of starch paste by a metal ion and hydrolysis of ferric chloride itself.

From the absorption curves of iron-containing starch paste, it is assumed that this bonding between iron and starch is not a chemical bond.

The difference in the absorption curves between pH 3.8 and pH 1.0 is thought to be due to the formation of a hydrated complex salts of iron ion with a different structure.

While the residual rate of ^{55}Fe in heated iron solution is smaller than that in the solution left at room temperature, the residual rate becomes greater if the starch suspension is heated after the addition of iron than in the suspension. Moreover, this residual rate of ^{55}Fe increases as the concentration of the paste increases, and this fact suggests that, even if the bonding between starch and iron is a mere adsorption, iron itself would change its shape since ferric chloride itself would undergo hydrolysis and starch coagulates into paste at the same time. It is hoped that further examinations will be made on the action of iron in such starch paste.

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Reference

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