

Black Discoloration in Canned Asparagus: Interrelations of

Iron, Tin, Oxygen, and Rutin

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The dark discoloration sometimes developing in canned all-green asparagus after the can is opened is attributed to a black, water insoluble coordination compound of iron and rutin. The iron is derived from the can; the rutin is extracted from the asparagus during the sterilization process. The iron must undergo some atmospheric oxidation from the ferrous to the ferric state before discoloration becomes apparent. The iron-rutin complex is sensitive to

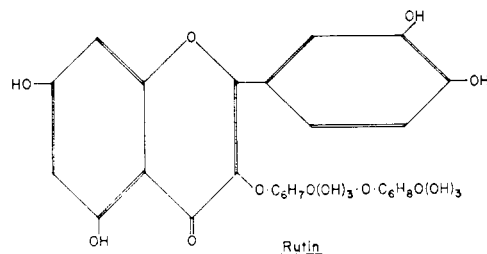
hydrogen ion concentration. Tin forms a bright yellow, water soluble complex with rutin, unobjectionable in appearance. Whether or not objectionable black discoloration develops after the can is opened is dependent on the relative amounts of tin and iron derived from the can. Both are in competition for the ligand present in rutin. The mol ratio of stannous ion to dissolved rutin must exceed 1:1.

For many years canners of all-green asparagus (that which is harvested after the spear has emerged from soil and been exposed to sunlight) have been plagued with a greenish-black discoloration of the product packed in tin cans. Its most obvious symptom is that, while the product appears perfectly normal when the can is first opened, the objectionable discoloration begins to develop on exposure to air, becoming readily apparent within a few minutes to 3 hr. This is most apparent if the can contents are transferred to a porcelain dish immediately after opening. The discoloration does not develop in white asparagus that is harvested before the spear emerges from the soil.

Historically, canning technologists have regarded the problem as comprising a chemical combination of iron derived from the can with some tannin-like substance in the asparagus. It has been assumed that with the iron in the ferrous state, as it always is in the sealed container, the combination is colorless. Contact with the air, serving to oxidize some of the iron to the ferric condition, has been thought essential to the development of the discoloration.

In recent years can manufacturers have reduced the frequency of occurrence by so fabricating the container as to promote the corrosion of tin and minimize the dissolution of iron. It has been observed repeatedly that the iron content of canned asparagus with a tendency to discolor is high relative to that of tin.

As investigation has proceeded the flavonol, rutin, the 3-rutinoside of quercetin, 2-phenyl 3,5,7,3',4'-pentahydroxy benzopyrone, has been identified as the critical substance in the asparagus.



Nakaoki (1932) was the first to direct attention to the presence of a glycoside of the flavonol series in asparagus. Camp-

bell (1939), examining a yellow deposit in glass jars of home-canned asparagus, confirmed the observations of Nakaoki. In addition, he described the solubility of the material and its color reactions with basic lead acetate, ferric chloride, and uranium acetate; also, he described the results of hydrolysis. De Eds and Couch (1947), also studying the yellow deposit observed by Campbell, were the first to identify it as rutin. Stevenson (1950), investigating causes for the black discoloration in asparagus, described the preparation of crude rutin from fresh asparagus and determined a relationship between quantity of rutin present and the maturity at time of harvest. Dame *et al.* (1957), employing a spectrophotometric method of analysis, based on a yellow color complex formed with $AlCl_3$, determined the distribution of rutin in the spear and the influence of maturity, spear length, and diameter on the amount of rutin present. They found it to be concentrated in the tips and cladophylls. Davis *et al.* (1961) attributed the discoloration to a chemical combination of rutin with iron, and ascribed its gradual development on exposure to air to the oxidation of Fe(II) to Fe(III). From observations on the canned product, these authors concluded that the discoloration will not develop where the *weight* ratio, Sn to Fe, in the product is 15:1 or more and that the tin functions to retain the iron in the ferrous state. They also observed the effect of pH value, demonstrating that when 0.05% citric acid was added in the canning operation to increase the hydrogen ion concentration, the probabilities of discoloration were diminished. Hernandez and Vosti (1963), working in a model system buffered to pH 5.5, observed that the characteristic discoloration can be reproduced by adding either ferrous or ferric ion to a solution of rutin. These authors state, "These results throw doubt on any simple explanation that the dark streaks of discoloration occur when ferrous ion changes to ferric ion in the presence of atmospheric air."

The study reported herewith was undertaken to clarify some of the contradictions in the literature and elucidate further the separate roles of iron, tin, and oxygen in the discoloration.

GENERAL

All experimental work was performed in model systems comprising rutin dissolved in 0.2 M acetate buffer with either iron or tin as the chlorides. The rutin was high purity obtained from Dr. F. De Eds, Western Regional Laboratory, USDA. One percent NaCl was added, this being a normal

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constituent of canned asparagus. In working with model systems it is vital that the pH level be controlled within the limits common to canned asparagus, namely, 5.2 to 5.7. Like many metal-organo coordination complexes, that of rutin with either tin or iron is sensitive to hydrogen ion concentration.

If to a solution of rutin containing 200 mg per l. and buffered to pH 5.2 to 5.5 is added a freshly prepared FeCl_3 solution in quantity to bring the Fe to Ru mol ratio to 1:1, a greenish-black discoloration, identical with that in canned asparagus, develops immediately. On standing for a few hours, evidences of agglomeration are apparent. Within 48 hr a voluminous black precipitate has formed and, after filtration, the filtrate contains only traces of rutin. If the FeCl_3 solution is allowed to age before use to permit hydrolysis or if it is boiled to produce the familiar ferric oxide sol, no coloration develops when added to a rutin solution. Obviously, the iron must be in the ionic state to produce the coloration.

If to the buffered rutin solution containing 200 mg per l. is added a freshly prepared SnCl_2 solution in quantity sufficient to bring the Sn to Ru mol ratio to 1:1, a daffodil-yellow coloration develops immediately and the solution remains clear indefinitely. The rutin content as measured by the modified AlCl_3 method described by Dame *et al.* (1958), remains close to the initial level. If the amount of SnCl_2 is increased to bring the Sn to Ru mol ratio to 7:1, a dense yellow cloud develops immediately. On long standing (a week or more) a fine-grained yellow precipitate is formed and the solution loses its color. After centrifugation plus filtration through Whatman No. 42 filter paper, the filtrate is essentially devoid of rutin. The precipitate formation is enhanced markedly by the presence of the 1.0% NaCl. Without it the filtrate is rarely clear.

Similar results are obtained with SnCl_4 solutions, although the precipitate formed tends to be gelatinous. This suggests that the function of tin in minimizing the discoloration in canned asparagus is other than that postulated by Davis *et al.* (1961).

OXYGEN

Mild Oxidation. The fact that the discoloration does not develop in the sealed can but becomes apparent only after the contents are exposed to the air suggests that atmospheric oxygen is an important factor.

A closed system was devised wherein a rutin solution could be freed of all traces of oxygen by boiling under a reflux condenser in a stream of water-pumped nitrogen freed of oxygen in a suitable train. Ferrous ion with no traces of ferric ion was prepared in the closed system by H_2S reduction of a ferric chloride solution containing 130 mg HCl per l. to minimize hydrolysis. Excess H_2S was removed by boiling under a reflux condenser in a stream of nitrogen. The colloidal sulfur formed during reduction of the FeCl_3 was coagulated and precipitated during removal of the excess H_2S and did not interfere with transfer of the clear FeCl_2 solution to a burette incorporated in the system. By a suitable arrangement of stopcocks, it was possible to transfer pneumatically, measure, and mix the respective solutions, avoiding any contact with the air. The entire system was maintained under nitrogen pressure 50 mm Hg in excess of atmospheric pressure to insure against oxygen contamination at possible points of leakage.

When 150 ml of rutin solution, pH 5.45, containing 30 mg rutin was admixed with 20.7 ml of FeCl_2 solution containing 2.07 mg Fe(II) (mol ratio Fe to Rutin is 0.75:1) no coloration was observed. This is in marked contrast to results obtained

when an equivalent quantity of Fe(III) is added to a rutin solution.

The admixture of rutin and ferrous ion was allowed to stand in the closed system for 22 hr with no indication of color. The system was opened at this point and the mixture transferred to a porcelain casserole. Within 10 min some evidence of color was visible and within 3 hr the characteristic greenish-black discoloration had developed. On standing until 48 hr had elapsed, the characteristic black precipitate had formed. The final pH was 5.35.

From these results it can be concluded that atmospheric oxygen is essential to the development of the discoloration in asparagus after the can is opened and that the postulate of the early canning technologists is correct insofar as the role of oxygen is concerned.

Why Hernandez and Vosti (1963) obtained the discoloration with ferrous ion is not clear, since these investigators did not report the conditions under which ferrous ion was admixed with rutin. If, however, the solution of ferrous ion was added to the rutin in contact with the air or if the rutin solution had not been freed of air completely before mixing, their observations are understandable. Ferrous ion solutions at pH 5.1 to 5.5 are extremely sensitive to oxidation and even minute traces of ferric ion are sufficient to produce discoloration, since the rutin-Fe(III) complex possesses high tinctorial power.

Whether ferrous ion fails to complex with rutin or whether the ferrous complex is colorless and soluble is not known at this time. The latter is suspected.

Strong Oxidation. To 80 ml of 0.2 M acetate buffer solution incorporating 1.0% NaCl and containing 20 mg of rutin was added a FeCl_3 solution, 200 mg Fe(III) per l., sufficient to bring the Fe to Ru ratio to 1:1. The mixture was made up to 90 ml with buffer solution. The characteristic greenish-black coloration developed immediately and after 3 hr a quantity of the black precipitate had formed. Ten ml of 15% H_2O_2 were added. Within 1 hr the precipitate had disappeared, although a pale sherry color remained in the solution. After a lapse of 23 days all color had been dissipated. A minute amount of hydrated ferric oxide was apparent on the bottom of the vessel.

Reversing the order of hydrogen peroxide addition, 10 ml of 15% H_2O_2 were added to 80 ml of buffer solution containing 20 mg of rutin. After 8 days sufficient FeCl_3 solution was added to bring the Fe to Ru mol ratio to 1:1. Sufficient buffer solution was added to bring the volume to 100 ml. A dark amber coloration developed. There was no evidence of the black precipitate. Within 4 hr the color had bleached to a pale sherry and after 23 days the appearance was identical with that described in the preceding paragraph.

Parallel results were obtained with H_2O_2 on the yellow complex formed when SnCl_2 was added to rutin in the mol ratio 5 Sn to 1 Ru. Here, however, the precipitate did not dissolve but bleached to a white, presumably the aquo-complexes of tin; only the yellow color disappeared in both precipitate and solution.

It is evident that degradative oxidation induced by H_2O_2 interferes with the formation of the colored complexes of rutin with either iron or tin. Here the observations of Paul Karrer and coworkers referred to by Link (1944) are of interest. These workers have reported on the H_2O_2 oxidation of malvin chloride, an anthocyanin related in structure to rutin. Cleavage of the pyran ring occurred between carbon atoms 2 and 3. It appears, therefore, that the O-heterocyclic ring in rutin must be intact for color to develop with either iron or tin.

SOLUBILITY OF RUTIN

Krewson and Naghski (1952) have reported the solubility of rutin in water to be 130 mg per l. at room temperature and 5000–5500 mg per l. at the boiling point. As noted below, the present author has not been able to confirm their solubility at room temperature, at least in the 0.2 M acetate buffer, pH 5.44. When cooled from the boiling point, rutin has a strong tendency to supersaturate, and solutions up to 200 mg per l. can be worked with for several hours after cooling to room temperature with no loss in concentration. Dame *et al.* (1958) observed this tendency to supersaturate; a solution with an initial rutin content of 150 mg per l. showing little loss in concentration after 5 weeks, whereas an appreciable drop to below the 150 mg per l. level occurred in solutions initially at 200–350 mg per l.

A solution of rutin in 0.2 M acetate buffer was prepared at an initial concentration of 199 mg per l. Solution was attained by heating to the boiling point. This was permitted to cool and stand at room temperature for stated periods. At the end of each period a portion was filtered off for rutin determination and also to provide another sample, initially at a lower level. The process was repeated over a total period of 1848 hr. Dissolved rutin was determined by the modified spectrophotometric AlCl_3 method described by Dame *et al.* (1958). The results are shown in Table I.

It is apparent that equilibrium with precipitated rutin crystals is reached, but slowly, and that the equilibrium concentration is in the neighborhood of 34 mg per l. As the initial concentration is reduced the rate of crystallization decreases significantly. At the higher initial concentrations where the nucleation pressure (tendency to nucleate) is great, a seed crystal will form in relatively short time. Thereupon crystallization and reduction in the concentration of dissolved material proceeds more rapidly than where the initial concentration is at lower levels.

There is evidence that the equilibrium concentration indicated above is high. An excess of rutin was added to 250 ml of 0.2 M acetate buffer at room temperature and allowed to stand. Within 24 hr the concentration of dissolved rutin was 23 mg per l. Repeated observations over a period of 113 days produced readings of 22–25 mg per l.

The unique characteristics of rutin disclosed in Table I have an influence on its performance in the presence of iron and tin ions as described later.

IRON

Krewson and Couch (1952) have reported that rutin, even in high concentrations (4667 mg per l.) is solubilized by FeCl_3 where the Fe to Ru mol ratio is 1:1 or greater. However, the high hydrogen-ion concentration of their solution after the FeCl_3 addition, pH 2.1, is such that their results are not relevant to canned asparagus. We have reduced the pH of a rutin-acetate buffer solution to 2.02 with HCl and added FeCl_3 in excess of the 1:1 ratio. A green discoloration was produced, but there was no evidence of the black precipitate previously noted. Even at pH 3.3 there is but slight evidence of the black precipitate.

To explore the nature of the iron-rutin complex, varying quantities of a $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (156.25 mg Fe per l.) were added to 80 ml volumes of three series of rutin solutions in 0.2 M acetate buffer. Each 80 ml volume contained 20 mg of rutin. After the Fe(III) addition, the volumes in each instance were brought to 100 ml with buffer solution, bringing the measured initial rutin concentration in each case to 199 mg per l. The rutin solutions contained 1.0% NaCl; the

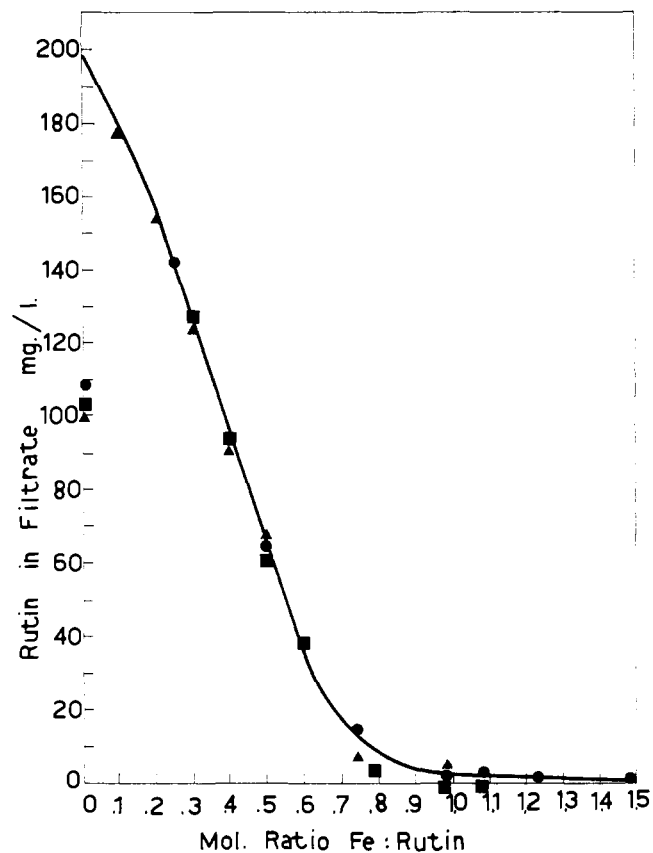


Figure 1. The influence of ferrous ion on the solubility of rutin in 0.2 M acetate buffer—pH 5.4

- Series 1
- Series 2
- ▲ Series 3

Table I. Solubility of Rutin in 0.2 M Acetate Buffer—pH 5.44

Elapsed Time	Original mg. per l.	Filtrates, mg per l.					
		24 hr	72 hr	168 hr	336 hr	768 hr	1176 hr
0 hrs	199						
24	186	186					
72	146	181	146				
168	94	102	142	94			
336	72	66	107	97	72		
768	41	43	52	93	63	41	
1176	40	36	47	89	65	38	40
1848	34	34	42	76	60	35	32

pH was 5.4. The FeCl_3 additions were such as to produce Fe to Ru mol ratios between 0:1 and 1.5:1 in each series. The mixtures were allowed to stand at room temperature for 7 to 12 days to allow any precipitate to settle, after which they were filtered and rutin determined on the filtrate, employing the AlCl_3 spectrophotometric method described by Dame *et al.* (1958). The results are shown in Figure 1.

In all instances the volume of black precipitate formed was proportional to the amount of Fe(III) added up to a 1:1 mol ratio Fe to Ru. No precipitate was formed when the FeCl_3 was added to the acetate buffer with no rutin present. It will be noted that the quantity of rutin remaining in solution varies inversely as the mol ratio of Fe(III) to Ru increases, essentially no rutin remaining in solution once the Fe(III) to Ru ratio exceeds 1:1.

Where no Fe(III) was added, the rutin dropped to values

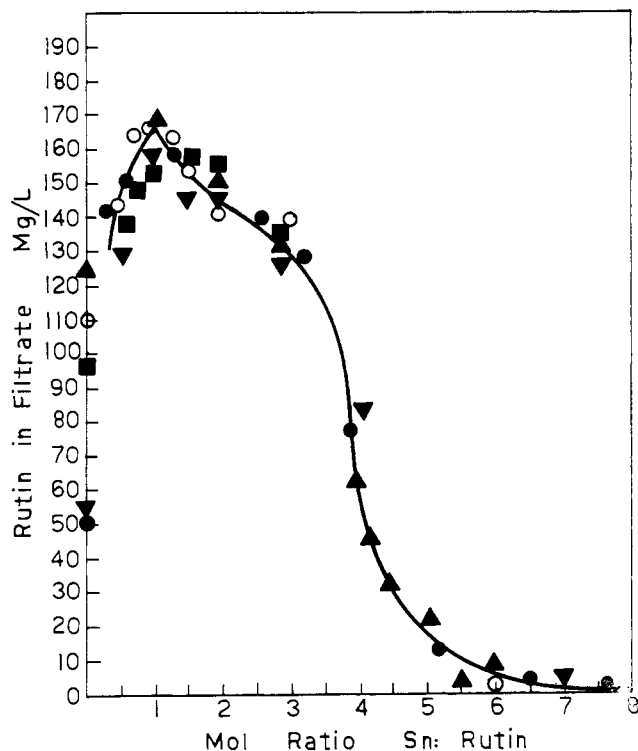


Figure 2. The influence of stannous ion on the solubility of rutin in 0.2 M acetate buffer—pH 5.2

- Series 1
- Series 2
- ▲ Series 3
- ▼ Series 4
- Series 5

between 101 and 111 mg per l. during the 7 to 12 days elapsed between preparation of the solution and determination of rutin remaining in solution. As shown in a preceding section, entitled "Solubility of Rutin," the tendency of unreacted rutin to crystallize out of a supersaturated solution decreases as the initial concentration decreases. It is likely, therefore, that all points on the curve above the 101 to 111 range represent a compromise between two opposing factors: one being the reduced tendency of unreacted rutin to drop out of solution as its concentration is decreased by reaction of a portion with iron. This would tend to lift the value. Opposing this is the loss of some rutin in the insoluble iron complex removed by filtration.

It appears from these results that the Fe to Ru mol ratio in the complex is 1 Fe to 1 Ru, and that the complex is insoluble at the pH of canned asparagus.

To further validate the 1:1 mol ratio a quantity of the black precipitate was obtained by addition of an excess of Fe(III) to a rutin solution containing 200 mg per l. and 1.0% NaCl. After thorough washing on a Gooch crucible to remove all trace of chloride and acetate ion, followed by drying for 8 hr at 120° C and 4 hr at 160° C, the residue was assayed for iron employing the *o*-phenanthroline method. The determined value was 9.20% iron; in good agreement with a calculated value of 9.18% for a 1:1 mol ratio.

TIN

Dame *et al.* (1958) in an experiment designed to determine the distribution of rutin between brine and spear in canned asparagus observed that the greater the tin content of the product, the lower the rutin content of the brine after 26 days' storage. There was a corresponding increase in the

rutin content of the spear as the tin content increased. From this observation one might postulate that the tin-rutin complex is insoluble at the normal pH of asparagus and is deposited *in situ* in the spear as it is formed. However, from other observations, these authors concluded that the complex is more soluble than rutin itself and that tin has the effect of solubilizing rutin. They observed that when 100 ppm of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ was added to varying concentrations of rutin in 0.1 M acetate buffer, the final concentration of rutin after 4 weeks' storage was in direct proportion to the initial concentration; whereas, in controls with no tin added, the final concentration decreased as the initial concentration increased. It should be noted here that if their data is plotted to show the relationship of final rutin concentration to the mol ratio, tin to rutin, the higher the ratio the lower the final rutin concentration.

To gain further insight into the character of the tin-rutin complex, varying quantities of a $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ solution (1950.8 mg Sn per l.) were added to 80 ml volumes of five series of rutin solutions in 0.2 M acetate buffer. Each 80 ml volume contained between 19 and 20 mg of rutin. After the Sn(II) addition, the volumes in each instance were brought to 100 ml with buffer solution, bringing the measured initial rutin concentration in the controls with no added Sn to 190–199 mg per l. Care was taken to maintain the initial rutin content constant in any one series. The SnCl_2 additions were such as to produce Sn to Ru mol ratios between 0:1 and 7:1. Final pH values fell between 5.17 and 5.27. The mixtures were allowed to stand for 7 to 12 days to permit any precipitate to settle; then centrifuged and filtered through Whatman No. 42 paper and rutin determined in the filtrate, employing the AlCl_3 spectrophotometric method as modified by Dame *et al.* (1958). It should be noted here that this method measures all the rutin in solution, that which is combined with tin as well as the free rutin. In all series 1.0% NaCl was added to the rutin solution. Without the added salt the precipitates would not centrifuge or filter cleanly, much remaining in colloidal suspension which passed through the filter. Consistent results were unobtainable in the absence of NaCl.

The results are shown graphically in Figure 2. It is evident that, with no tin present, the values for rutin remaining in solution fall to levels far below the initial concentration. Here a straw yellow precipitate of rutin readily soluble in hot water can be observed. As the amount of tin added is increased to the 1 Sn to 1 Ru mol level, the quantity of rutin remaining in solution increases to a maximum. This can be either rutin or the tin-rutin complex, since both are converted to the yellow aluminum complex in the AlCl_3 shift method of analysis. As the ratio of tin to rutin is further increased, the amount of rutin remaining in solution falls and becomes essentially zero at a mol ratio of 7 Sn to 1 Ru. Such mixtures are characterized by a daffodil-yellow precipitate, the amount increasing as the Sn to Ru ratio is increased.

One might construe the above pattern as indicating the tin-rutin complex is insoluble and that the mol ratio is 7 Sn to 1 Ru. However, there are considerations that cast doubt on any such conclusion. Among these are: the increase in the quantity of rutin remaining in the filtrate as the Sn to Ru mol ratio is increased from 0:1 to 1:1; and a ratio, 7 Sn to 1 Ru, is improbable in a coordination complex involving a metal ion.

A more plausible explanation stems from the observation that when SnCl_2 is added to a 0.2 M acetate buffer at pH 5.4, a dense white cloud is formed which, on standing, results in a white precipitate of the aquo-complexes of tin. Such ex-

planation further assumes that the tin-rutin complex is soluble in accordance with the conclusions of Dame *et al.* (1958) and the mol ratio, Sn to Ru, in the complex is 1:1. Addition of Sn(II) beyond this ratio then results in the formation of the aquo-complexes which adsorb the yellow tin-rutin complex and carry it down in the precipitate.

To test the validity of this postulate, four 100 ml solutions were prepared, each containing 20 mg of rutin. To the first three SnCl₂ was added in the mol ratio 1 Sn to 1 Ru. To the fourth, sufficient SnCl₂ was added to bring the mol ratio to 7 Sn to 1 Ru. Quantities of the aquo-complexes of tin were prepared by adding SnCl₂ to 0.2 M acetate buffer solution, pH 5.4, containing 1.0% NaCl. After thorough washing to remove traces of tin ion, the precipitate was added to Nos. 2 and 3 in the quantities shown in Table II. After standing for 9 days the solutions were centrifuged and filtered and rutin determined in the filtrates.

It is evident that the addition of the aquo-complexes has reduced the concentration of the soluble tin-rutin complex. The aquo-complexes prepared separately are not as effective adsorbents as those formed in the presence of the tin-rutin complex as in No. 4. It is likely that in those prepared separately, there was considerable agglomeration and reduction of surface area during the sedimentation and washing procedures.

The bleaching action of H₂O₂ on the yellow precipitate formed when SnCl₂ is added to rutin solutions was alluded to under the section "Strong Oxidation." This is further evidence that the precipitate is composed principally of the aquo-complexes of tin.

Mantell (1949) has directed attention to the power of the tin aquo-complexes to adsorb and extract soluble material from aqueous solutions.

STABILITY CONSTANTS OF THE METAL COMPLEXES

The stability constants of the metal complexes were not measured directly, but a semi-quantitative idea of their relative values, adequate for determination of what proceeds in canned asparagus, can be obtained by observing their reactions with ethylenediaminetetraacetic acid (EDTA).

To 80 ml of a solution containing 20 mg rutin in 0.2 M acetate buffer were added 12 ml of a solution of SnCl₂·2H₂O containing 23.41 mg Sn(II). This represents a Sn to Ru mol ratio of 6:1. The characteristic yellow cloud developed immediately. Then were added 8 ml of an aqueous solution containing 84.3 mg Na₂H₂EDTA, sufficient to complex all the tin. Final pH was 5.02. Within 1 hr the cloud was considerably reduced, and after 8 days there was no evidence of cloud or precipitate. However, the yellow color of the Sn-Ru complex persisted in the solution.

When the same procedure was repeated, omitting only the rutin, the white precipitate of the aquo-complexes of tin, formed when tin solution is added to acetate buffer, was completely dissipated within 1 hr after the EDTA addition. No color was apparent.

The same procedure was followed in studying the reactions with the iron complex, except that 9.18 ml of a solution of FeCl₃·6H₂O containing 1.836 mg Fe(III) was substituted for the tin. This gave a Fe to Ru mol ratio of 1:1. The Na₂H₂EDTA addition was 13.8 mg dissolved in 10.8 ml of water, sufficient to complex all the iron present. The characteristic greenish-black discoloration and black precipitate, formed on addition of the iron, disappeared within 1 hr after the EDTA addition. After 8 days a pale yellow precipitate of rutin crystals, readily soluble in hot water, was apparent at the

Table II. Adsorption of Tin-Rutin Complex on Aquo-Complexes of Tin

No.	Sn(II)	Rutin	Mol Ratio Sn to Ru	Aquo-Complex Added—Sn Equiv.	Rutin in Filtrate
1	3.90 mg	20 mg	1:1	None mg	15.7 mg
2	3.90	20	1:1	26.61	7.2
3	3.90	20	1:1	53.22	2.6
4	26.61	20	7:1	None	0

bottom of the vessel. The supernatant solution was clear and water white.

Identical end results were obtained when the procedure described above was varied so as to mix the EDTA with the rutin solutions prior to the additions of the iron and tin salts.

The results provide evidence that the stability constant of the iron-rutin complex is lower than that of the iron-EDTA complex. So also the stability of the aquo-complexes of tin is lower than that of the tin-EDTA complex. However, the action of EDTA on the tin-rutin complex leads to the conclusion that the stability constant of the tin-rutin complex is higher than that of the tin-EDTA complex, as well as that of the iron-rutin complex and the aquo-complexes of tin. The yellow color of the tin-rutin complex persists after addition of EDTA, even though the insoluble aquo-complexes are dissolved.

The results suggests that the objectionable black discoloration might be avoided by the incorporation of a little EDTA in the brine used in canning asparagus. The CaNa₂EDTA salt is a permitted food additive in many food products.

EFFECT OF SN(II) TO FE(III) RATIO ON DISCOLORATION

Since the stability constant of the Sn(II)-rutin complex exceeds that of the Fe(III)-rutin complex, the presence of tin should prevent or minimize the formation of the undesirable iron complex unless the iron is present in such excess as to shift the equilibrium significantly.

To test this postulate, 2 ml of a solution of SnCl₂·2H₂O containing 3.90 mg Sn(II) were added to each in a series of rutin solutions containing 20 mg rutin in 80 ml. This produced a Sn to Ru mol ratio of 1:1. At this level only minor amounts of the insoluble aquo-complexes are formed, but the distinct clear yellow color of the Sn-Ru complex is apparent. To this series Fe(III) was added at six levels, varying between 0.2165 and 0.6503 mg. The Fe(III) was added as a FeCl₃·6H₂O solution containing 32.514 mg Fe(III) per l. All mixtures were made up to 100 ml with acetate buffer, pH 5.45. At the 0.6503 level the *weight* ratio Sn to Fe was 6:1; at the 0.2165 level it was 18.02:1.

Under the above conditions the characteristic greenish-black discoloration does not develop on the addition of Fe(III); instead a reddish-brown coloration is apparent. No black precipitate forms on long standing. A slight tan colored precipitate was apparent at the 6 Sn to 1 Fe level, but none could be observed at higher tin levels. If the iron and tin solutions are premixed before adding to the rutin, the greenish-black discoloration is apparent momentarily. However, it quickly fades, being replaced by the reddish-brown coloration previously noted. Identical Fe(III) additions to the rutin solutions with no tin present produced black precipitates at all levels, the volume increasing with increase in Fe(III) addition.

In our model systems prevention of the characteristic black discoloration was obtained at lower Sn to Fe *weight* ratios

than those reported by Davis *et al.* (1961) from their observations on canned asparagus. The presence of the asparagus spears may have an influence on the ratio required.

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