CLXXIII. THE FERMENTATION PROCESS IN TEA MANUFACTURE

IV. TEA TANNIN AND ITS FERMENTATION PRODUCTS

BY C. J. HARRISON AND E. A. HOUGHTON ROBERTS

*From the Tocklai Experimental Station, Indian Tea Association, Cinnamara,
Assam, India*

(Received 17 July 1939)

INVESTIGATIONS into the nature and structure of tea tannin in both green leaf and made tea have occupied workers at Tea Experimental Stations in Ceylon, the Dutch East Indies, Assam, South India, Japan and Formosa for many years. Much of this work has remained unpublished until now, while the results which have appeared in the various journals are either mutually contradictory or must be taken to indicate the existence of a group of tannin bodies in the leaf, the relative proportions of which may vary considerably.

Working in Java, Nanninga [summarized by Deuss, 1914] obtained a crystalline product, $C_{20}H_{16}O_9$. Later Deuss [1923] reported the isolation of an amorphous product to which he ascribed the mol. wt. 404 and molecular formula $C_{20}H_{20}O_9$. Shaw [1930] and Shaw & Jones [1932] described a tea tannin preparation which was obtained by a method very similar to that previously elaborated at Tocklai and which will be described more fully below. Shaw's tannin seems very similar to that obtained by Deuss, and both preparations gave no gallic acid on hydrolysis with acids. Tsujimura [1930, 1, 2; 1931; 1935] obtained a crystalline tea tannin to which she assigned the structure

Later Deys & Dijkman [1937] obtained a similar crystalline product which liberated gallic acid on enzymic hydrolysis with a tannase preparation obtained from *Aspergillus niger*.

Other tea tannins have been reported, notably that of Oshima [1936] who considered an amorphous product he obtained to be bis-(5:7:3:4':5'-penta-hydroxy) flavpinacol. It is doubtful whether this claim can be substantiated as Russell & Todd [1934] have synthesized the corresponding 5:7:3:4'-tetra-hydroxy compound, which latter substance differs, principally in the colour of its aqueous solutions, from Oshima's tea tannin preparation.

Preparation and properties of tea tannin from green leaf

The standard procedure for the preparation of tea tannin at Tocklai is as follows.

500 g. freshly plucked leaf are boiled with $1\frac{1}{2}$ –2 l. distilled water for 1 hr. All damaged and diseased leaf is rejected; otherwise tannin oxidation and condensation products will be found in the final product. After cooling, the infusion is

filtered through a cotton wool plug. The filtrate is subjected to two extractions each with benzene and ether, and is then rapidly extracted two or three times with ethyl acetate. The united ethyl acetate extracts are dried over anhydrous Na_2SO_4 and then evaporated to 150–200 ml. on a water bath. The extract is then cooled and the tannin precipitated by adding 300–400 ml. CHCl_3 . The precipitate is filtered at the pump and dried *in vacuo*. The purity of this product as estimated by the Lowenthal and Hide powder methods usually varies from 80 to 90 %. The yield is only about 20–25 % of the total amount of tannin found in the leaf by analysis.

So prepared tea tannin cannot be classed as a true tannin. It is precipitated by nitrogenous bases such as quinine, and by Pb acetate, but it is not completely precipitated by gelatin except in the presence of acid and salt. Strong solutions are precipitated by FeCl_3 , and very dilute solutions give an intense colour reaction. This colour varies from green to an intense blue indicating that tea tannin may be either a catechol or a pyrogallol derivative or probably a mixture of both.

Prolonged acid hydrolysis never yields the slightest trace of glucose but in some cases gallic acid can be detected in the hydrolysate. As tea tannin is precipitated by Br water, gives the pine-shaving reaction for phloroglucinol and gives a rose pink precipitate (tannoform) on warming with formaldehyde and conc. HCl, it must be considered a condensed tannin and therefore derived from a catechin. The isolation in certain cases of gallic acid but not sugars from hydrolysates does not indicate the presence of gallotannins or hydrolysable tannins, and is explained satisfactorily by the presence of Tsujimura's product, the galloyl ester of epicatechin, in the tea tannin complex.

The nature of the tea tannin complex

From a consideration of the above properties of tea tannin preparations it follows that in all probability they are condensed tannins derived from both catechin and gallo catechin (3:5:7:3':4':5'-hexahydroxyflavan). The galloyl ester of epicatechin may or may not occur in this complex.

It is however a moot point whether the tannins of the green leaf are identical with the tannin preparation just described. According to Freudenberg [1933] the catechins undergo condensation on boiling in aqueous solution and it seems likely that this treatment, in the course of preparation, will modify the tannins originally present in the leaf. This belief is strengthened by the finding that the properties of the final product are to some extent affected by the method of extraction from the leaf.

The tea tannins obtained from cold acid extracts of leaf, using 5 % oxalic acid or 1 % HCl, show higher rotations than preparations obtained from the 1 hr. infusion with boiling water, as shown in Table I.

Table I

Extraction agent	$[\alpha]_D^{26}$
Boiling water	– 76.5°
1 % HCl	– 123.2°
5 % Oxalic acid	– 132.5°

Further, after extracting leaf with 5 % oxalic acid and salting out the extract, about 75 % of the tannin remained in the filtrate. On the other hand the greater part of the tannin is salted out from an aqueous infusion which has been boiled for 1 hr. These observations would accord with a greater degree of condensation of the tannin preparation from the boiled aqueous extract.

This latter preparation goes readily into aqueous solution, and is pure white in colour when freshly prepared. It has not the properties of a highly condensed substance. It would therefore appear likely that the tannins of the green leaf are not far removed from the parent catechins themselves. Small amounts of both *l*-epicatechin and galocatechin have been detected in the tea leaf, the former by Tsujimura [1929] and the latter by Oshima & Goma [1933]. These observations have been confirmed by several workers and a substance with all the properties of *l*-epicatechin was isolated by one of us [C. J. H., unpublished] as early as 1925.

It will therefore be adopted as a working hypothesis that the tannins in green leaf consist of a mixture of these two catechins, and probably of their simpler condensation products, together with the galloyl ester of *l*-epicatechin.

Analyses of amorphous preparations correspond closely with those expected for mixtures of the two catechins, whilst Nanninga's crystalline tea tannin approximates in its molecular formula to Tsujimura's product as the following table shows:

Table II

Tannin preparations	C	H	O
Nanninga crystalline	54.60	4.80	41.60
Deuss amorphous	59.97	4.96	35.07
Tsujimura crystalline	55.49	5.29	39.22
Epicatechin crystalline	62.07	4.83	33.10
Galocatechin crystalline	58.82	4.57	36.61

Condensations of tea tannin

According to Freudenberg [1933] catechins are converted into true tannins by condensation. This condensation is stated to take place between the secondary carbinol group in the pyran ring of one molecule and the phloroglucinol nucleus of another. As the condensation product still possesses the two necessary groups for further condensation the degree of complexity of possible condensation products is theoretically limitless. Such condensations of catechin according to Freudenberg take place on warming its aqueous solution, on treatment with acid or alkali, as a result of enzymic action or as a consequence of oxidation. The tannin bodies in green tea leaf undergo very similar changes to those of catechin.

If an aqueous solution of a tea tannin preparation is evaporated down to dryness a reddish-brown glassy product is obtained consisting of higher condensation products. Further, on heating a green tea leaf infusion under pressure at 100° the solution reddens and its Lowenthal tannin titre falls.

Condensations in acid solution. Acidification of a green leaf infusion to pH 1.2 produces no precipitate but decreases the Lowenthal titre of the solution. On boiling with acids however a tea tannin solution reddens and a red-brown precipitate is eventually formed. Deposition of this insoluble product increases with acidity and with the time of boiling, and after 24 hr. boiling with 5% H₂SO₄ no tannin is left in solution. At the same time gallic acid can be detected in the filtrate. Shaw [1930] found no gallic acid under the same conditions, which finding may indicate that Tsujimura's galloyl ester does not occur in the particular samples of South Indian leaf used by him. The tannin products precipitated in this way represent a whole range of substances whose solubilities in water, dilute acid and organic solvents vary considerably with the time of boiling. The presence of air does not seem to be necessary for the production of these products and consequently they are not considered to be the same as the tannin bodies in fermented tea in the formation of which oxygen is essential.

Effect of alkalis on tea tannin. In alkaline solution tea tannin behaves very similarly to other polyphenols with adjacent hydroxyl groups. In dilute alkaline

media a yellow coloration first develops deepening to light brown and finally to a dark brownish-black. In more strongly alkaline solutions the deepening of the colour is much more rapid.

The darkening in colour, with decrease in Lowenthal titre, will take place in slightly acid solutions; in fact tea tannin solutions are unstable at any pH greater than 5.0. At a higher pH the colour steadily darkens and the $KMnO_4$ titre using indigo carmine as an indicator decreases, as is shown in Table III where figures are given for the $KMnO_4$ titres of 10 ml. portions of tea tannin solutions at varying pH , kept at 25° .

Table III

pH	0 hr.	2 hr.	4 hr.	6 hr.	24 hr.
4.0	—	23.0	22.9	23.1	22.3
5.0	—	23.1	23.1	23.2	22.5
6.0	22.9	23.0	22.3	21.6	21.4
7.0	—	22.0	21.0	20.2	17.6
8.0 (phosphate)	—	22.0	21.7	—	17.0
8.0 (borate)	—	22.7	22.2	21.8	21.6

Titres are in ml. 0.04 N $KMnO_4$.

Browning of the solution and decrease in oxidizability are significant even at pH 6.0. A borate buffer was first used for the pH 8 value but here the change in 4 hr. was very slight. With a phosphate buffer at this pH browning was rapid. Borates were also found to inhibit the autoxidation of catechol and pyrogallol in slightly alkaline solutions. These phenomena are probably related to the enhancement of the acidity of boric acid by compounds, particularly sugars, containing the glycol group, and in that case are due to the formation of a boron-containing ring.

The tea tannin in alkaline solution at first retains its tanning powers and can precipitate gelatin from acid salt solution. Eventually however it loses its tanning properties. After 3 hr. in $N/10$ alkali at a temperature of about 25° the amount of oxidizable matter precipitated by gelatin in acid-salt solution is negligible.

The changes in tea tannin and other polyphenols in alkaline solution are associated with a considerable uptake of O_2 . At first both the rate and extent of O_2 uptake increase with increasing alkalinity, but in strongly alkaline solutions the rate only is affected by the strength of alkali. In $2N$ $NaOH$ O_2 uptake comes almost to a standstill within 10 min.

Assuming that the tannins in green leaf are a mixture of epicatechin, galocatechin and their simpler condensation products, the average mol. wt. of each tannin unit will be about 300. This figure was adopted in calculating the average O_2 uptake per mol. of tannin.

The O_2 taken up during alkaline autoxidation of various polyphenols was determined manometrically. 2 ml. portions of $M/100$ polyphenol solution were introduced into the main compartments of Warburg vessels and 1 ml. of approximately N $NaOH$ into the side bulbs. After attainment of temperature equilibrium the $NaOH$ was tipped into the polyphenol solution and uptakes recorded until they came to a standstill. The results expressed in atoms O per mol. of polyphenol are recorded in Table IV.

Each of the figures quoted is a mean of either three or four determinations.

The tea tannin contents of both the green leaf infusion and the tannin preparation were determined by Lowenthal's method. The discrepancy between the amounts of O_2 taken up under these varying conditions cannot at the moment be explained.

Table IV

Polyphenol	Atoms O taken up per mol.
Tea tannin (green leaf infusion)	6.6, 6.4, 7.1, 6.9, 6.4
Tea tannin (87% pure preparation)	4.8, 4.7, 4.7
Catechol	4.7
Pyrogallol	3.1, 3.5
Gallic acid	4.7, 4.8
Phloroglucinol	0.2

The negligible degree of autoxidation of phloroglucinol and the close agreement between the total uptakes of other polyphenols and tea tannin preparations suggests that the alkaline autoxidation of tea tannin is a property of its catechol or pyrogallol nucleus alone.

Condensation during fermentation. In tea fermentation the tea tannin molecule is oxidized, and then as shown in the previous communication [Roberts, 1939, 2] undergoes further irreversible changes. Oxidized catechins are known to undergo condensation very readily. The properties of these oxidized and condensed products of tea tannin will be discussed after the changes undergone by tea tannin in the fermentation process have been described.

Fermentation changes in tea tannin

It was previously calculated [Roberts, 1939, 2] that each tannin molecule took up approximately 1 atom of O during the fermentation process. This calculation assumed the mol. wt. of tea tannin to be 442 and that about 60% of the total O₂ uptake was accounted for by tannin oxidation. The figure of 1.08 ± 0.08 was the mean of six experiments only and with many more results available the average value became 1.4. If this is expressed in terms of an average mol. wt. of 300 per tannin unit the O₂ uptake comes very close to 1 atom of O per catechin molecule. Subtraction of the CO₂ outputs from the total O₂ uptakes to give the oxygen consumed in tannin oxidation is not completely satisfactory. Theoretically on the reaction scheme put forward for tea fermentation more and not less than 50% of the total O₂ consumed should be used in carbohydrate oxidation. However we are dealing with a very much more complicated system than one of pure substrates and enzymes alone. The proteins, amino-acids and lipins are not likely to be entirely unaffected by the chain of reactions advanced to explain the fermentation process and, until these possibilities have been explored further, the above method of estimating tannin oxidation will be retained.

Oxidations of polyphenols by tea-enzymes. Two manometric methods are available for studying the oxidation of polyphenols by the enzyme system of the tea leaf. The minced tea leaf may be suspended in a solution of the polyphenol and the increase determined in total O₂ uptake over that shown by controls suspended in water alone. Alternatively the polyphenol may be added to the system when tannin oxidation has come almost to a standstill. In the latter case it is better to work with 100 mg. portions of minced tea leaf as with these smaller quantities of leaf, oxidation of tannin is relatively faster, and amongst other advantages enzymic inactivation by the products of reaction is likely to be less marked.

The total increase in O₂ uptake is not entirely due to polyphenol oxidation. The polyphenol may take the place of the tea tannin as an O₂ carrier and carbohydrate oxidation is thereby also increased. The ratio of CO₂ evolved to O₂ taken up is not very much altered by the addition of catechol or pyrogallol as is

shown in Fig. 1 which gives curves for O_2 uptake and CO_2 output for normal fermentation and in the presence of pyrogallol. (These curves do not represent the same sample of leaf.)

Fig. 1. Normal fermentation: I, O_2 uptake; II, CO_2 output; ditto + added pyrogallol; III, O_2 uptake; IV, CO_2 output.

Approximately, therefore, about 60 % of the increase in total O_2 uptake may be ascribed to the added polyphenol. Shaking must usually be prolonged to $2\frac{1}{2}$ hr. or more before the added polyphenols are nearly completely oxidized. Table V gives total uptakes with and without added polyphenol, and the calculated proportion of the increase due to polyphenol oxidation alone. In all cases with the exception of gallic acid the increase is almost equivalent to an uptake of 1 atom O per mol. polyphenol.

Table V

Substrate	mg. added	Total uptake control	Total uptake with polyphenol	Increase in uptake due to polyphenol oxidation	Theoretical uptake 1 atom O per mol. polyphenol
Catechol	1.10	543	689	88	112
		269	413	86	112
		152	288	82	112
Pyrogallol	1.26	269	455	111	112
		151	302	91	112
Gallic acid	1.70	383	497	68	112
Tea tannin (80 % pure)	3.50	156	304	89	104

From the similarity in uptakes between catechol and pyrogallol on the one hand, and tea tannin on the other, it may be concluded that the oxidation of the tea tannin molecule is restricted to the catechol or pyrogallol nucleus only.

The simpler polyphenols however are less rapidly oxidized by peroxidase than is tea tannin. It was shown in the previous communication in this series

[Roberts, 1939, 2] that addition of tea tannin to the system before oxidation commences prolongs the period where the rate of O_2 uptake is linear. The linear rate is not maintained any longer than for the normal period when either catechol or pyrogallol is added at the commencement of fermentation as is shown in Fig. 2.

Fig. 2. I, normal fermentation; II, ditto + 1 ml. $M/100$ catechol; III, ditto + 1 ml. $M/100$ pyrogallol.

This effect is shown much better when the polyphenol is added when tannin oxidation is almost complete (Fig. 3). With tea tannin the initial rate of O_2 uptake is almost regained but the uptakes with pyrogallol and catechol are much slower.

Fig. 3. I, normal fermentation; II, 1 ml. $M/100$ catechol added; III, 1 ml. $M/100$ pyrogallol added; IV, 1 ml. $M/100$ tea tannin added.

Gallic acid is oxidized even more slowly than catechol; in fact on occasions the increase in total O_2 uptake in the presence of gallic acid has not been significant. Using only 100 mg. of tea leaf tissue in each Warburg vessel the uptakes given in Table VI were recorded at 35° .

The influence of substitution in the catechol or pyrogallol ring on its efficiency as a peroxidase substrate finds a ready explanation in terms of the electron theory.

Table VI

	10	20	30	45	60	75	90	120 min.
Control	102	198	264	310	333	350	361	383 μ l. O ₂
1.70 mg. gallic acid added	98	181	244	312	362	401	433	497 μ l. O ₂

The O₂-ring in tea tannin is strongly cationoid and acts therefore as a proton repeller. Consequently the loss of 2H atoms to form an *o*-quinone is much easier in a catechin or other flavan derivative than with the simpler polyphenols. Conversely in gallic acid the carboxyl group is strongly anionoid and *o*-quinone formation is correspondingly more difficult.

As flavans such as catechin and, probably, quercetin are more rapidly oxidized by peroxidase than the simpler polyphenols such as catechol, it is not surprising that the former are the more generally found in plant tissues where they function as O₂ carriers in the respiratory process.

The condensation changes in tea tannin in fermentation

Two methods have been used in the past to study the changes taking place during the fermentation of tea. In the manometric method O₂ uptakes and CO₂ outputs were determined, while in the titrimetric method, as described in an earlier paper [Roberts, 1939, 1], the falls in the KMnO₄ titres of both water-soluble tannins and non-tan oxidizable matter were followed. By a comparison of these two methods it is immediately obvious that the fall in the total KMnO₄ titre (tannins + non-tans) is greater than would be expected if this fall were due to oxidation alone. In Table VII are recorded total O₂ uptakes at about 25° for fermenting leaf in μ l. per mg. dry wt. of tissue compared with the decrease in KMnO₄ titre for tannins and non-tans also expressed in terms of μ l. of O₂ per mg. dry wt. The uptake of O₂ by leaf crushed in the rollers is slower than in the Warburg vessel. When the bruised leaf is spread about 1 in. thick on the fermenting surface the rate of diffusion of O₂ into the tissues is the limiting factor which decides the rate at which fermentation proceeds. The rate at which O₂ is taken up in this process may be measured by manometric determinations, at intervals, of the total amount of O₂ taken up by finely-minced samples of partly fermented leaf, and subtracting these from the total uptake of fresh leaf after mincing. O₂ is taken up by leaf fermenting under factory conditions at rather less than half the rate of similar leaf finely minced and suspended in Warburg vessels. This is conclusively shown by comparing the manometric figures below with those plotted in Fig. 6 where the uptake exceeds 8 μ l. per mg. dry wt. after 30 min. shaking at 25°.

Table VII

	Rolled	Fermented			
		1 hr.	2 hr.	3 hr.	25 hr.
Manometric	1.53	6.24	9.49	9.95	11.55
Lowenthal: total KMnO ₄	3.4	8.6	14.5	17.9	24.7
non-tan	0.6	2.4	3.6	5.7	6.0
tannin	2.8	6.2	10.9	12.2	18.7

The figures represent μ l. O₂ per mg. dry wt.

Throughout the whole fermentation process (usually interrupted after 2-3 hr.) the decrease in oxidizability as measured by KMnO₄ titres is greater than would be expected from the amount of O₂ taken up. The decrease in non-tan oxidizable matter is just about equivalent to the normal CO₂ output so that

it is the tannin whose oxidizability decreases so much more than would be expected from the amount of O_2 consumed in its oxidation.

This decrease in tannin titre cannot be entirely accounted for by a less complete extraction of the tannins from the fermented leaf. As will be shown later part of the tannins of fermented leaf seem to combine with leaf-proteins and are no longer soluble in hot water, but the proportion of water-insoluble tannin is not large enough to account for the discrepancy under consideration. It was shown by Roberts & Sarma [1938] that in the oxidation of tea tannin in aqueous solution by peroxidase and H_2O_2 , the decrease in tannin titre was greater than the equivalent amount of H_2O_2 used up in the oxidation. In this case the whole of the tannin after oxidation remains in solution.

In Part II of this series [Roberts, 1939, 1] it was shown that no O_2 uptake was recorded in the oxidation of tea tannin by H_2O_2 and a peroxidase preparation, which agrees with the findings in this communication that 1 atom O only is consumed by each mol. of tea tannin. Some change, which lowers the $KMnO_4$ titre of tea tannin, must therefore occur in addition to ordinary oxidation, and it seems almost certain that this change must be a condensation.

It has always been observed that the curve obtained for the decrease in the tannin titre during fermentation is not continuous (see Fig. 2 in Part II and Fig. 4 in Part III of this series) but shows a point of inflexion.

Such a curve usually indicates that the changes measured are the summation of two or more consecutive changes. The general shape of the O_2 uptake curve is known and one would expect the rate of condensation to follow a sigmoid curve. The summation of both can give a curve similar in character to that normally observed.

Fig. 4. I, normal O_2 uptake curve; II, decrease in tannin titre during fermentation; III, rate of condensation by difference.

Influence of temperature on fermentation

At higher temperatures of fermentation there is a greater decrease in both tannin and non-tan $KMnO_4$ titres as shown in Fig. 5 where curves for the decreases in these values at 38° (100° F.) and 21° (70° F.) are shown.

An increase in temperature results in accelerated O_2 uptake and a greater total uptake as shown in Fig. 6. The greater total uptake however is due to increased carbohydrate oxidation as the carbohydrate in the leaf, unlike the tannin, is not all oxidized in the fermentation process. Although the initial rate of tannin oxidation (O_2 uptake- CO_2 output) is accelerated by increasing the temperature, the total amount of tannin oxidized remains the same, as shown in Fig. 7.

Although at higher temperatures oxidation cannot proceed beyond an uptake of 1 atom O per molecule of tannin, the condensation process is not limited in any such way. Presumably the higher the temperature of fermentation the more highly condensed are the products of reaction, and the greater the decrease in the $KMnO_4$ titre.

From 25 to 40° the rate of the enzymic reactions in the fermentation process increases by only about 20 %. This very low temperature coefficient is an indication that the enzymes are working at very nearly their maximum temperature and that inactivation would follow any further increase in the temperature. On the other hand the temperature coefficient of condensation is likely to be normal, so that an increase in temperature from 25 to 40° while increasing the rate of

Fig. 5. Tannin and non-tan titres during fermentation at different temperatures. I and III, 21°. II and IV, 38°.

Fig. 6.

Fig. 7.

Fig. 6. Normal O_2 uptake in fermentation: I, 25°; II, 40°.

Fig. 7. Variation of tannin and carbohydrate oxidation with temperature. I, tannin oxidation at 40°; II, tannin oxidation at 25°; III, carbohydrate oxidation at 40°; IV, carbohydrate oxidation at 25°.

tannin oxidation by only 20 % increases the rate of condensation by about 150 %. A marked difference in the characters of infusions of teas fermented at these two temperatures would therefore be expected. In confirmation of this Benton [1939] has shown that there is an inverse correlation between quality, and temperature of fermentation, at temperatures of 70° F. and over.

The tannins of made tea

The tannins of green tea leaf are themselves a fairly complex mixture but the tannin bodies of made tea are very much more so. Under normal manufacturing conditions oxidation of the tannins is about 80–90% complete and the oxidized tannins are present as a huge range of condensation products. It seems unlikely at present that these products will be such well-defined substances as the proteins or carbohydrates, and in the general formula

$$n \left[\begin{smallmatrix} \text{C}_{15}\text{H}_{14}\text{O}_6 \\ \text{C}_{15}\text{H}_{14}\text{O}_7 \end{smallmatrix} \right] - (n-1)\text{H}_2\text{O}$$

for condensed tea tannins, n may apparently have any value.

The isolation of definite compounds, to which various characters of the tea infusion may be attributed, does not therefore seem likely to be very successful. All that can be hoped for is a separation of the tannin bodies into fractions of greater or less pungency, colour and other such qualities. This has to a certain extent already been achieved. Tannin bodies in made tea are precipitated by acid and by salt, and apparently the ease of precipitation increases with the extent of condensation. Solutions of white tea tannin or green leaf infusions are not precipitated by dilute acids whereas an infusion of made tea gives a precipitate when acidified to 1% by H_2SO_4 . After acidification, saturation with salt precipitates a further quantity of tannin bodies. The filtrate after the two precipitations is a clear golden colour and some unchanged white tannin can be obtained from it by extraction with ethyl acetate.

The precipitates obtained are still oxidizable by the Lowenthal method but not to the same extent as is the tannin in a green leaf infusion.

Precipitated from	g. tannin oxidized by 1 ml. <i>N</i> KMnO_4
White tannin	0.0416
Acid precipitate	0.1365
Acid-salt precipitate	0.1092

The reducing powers of the precipitated red tannin substances are much lower than that of white tannin, which is to be expected as they are formed from the tannin of the green leaf by processes of oxidation and condensation.

Judging by the high Lowenthal factor of the acid-precipitated tannin, the increase in the acid-precipitable matter as fermentation proceeds [Evans, 1930] and the light colour of the filtrate it is likely that this fraction represents some of the more highly condensed tannins which are responsible for the dark colour of the infusion. Too great a proportion of this fraction might be expected to produce a liquor remarkable for its depth of colour alone and lacking in the astringent character due to the presence of less highly condensed products. This expectation is borne out by the correlation found by Evans [1929] in Ceylon between valuation and acid-soluble tannin on teas from any one estate. If quality of leaf and conditions of manufacture are reasonably constant the acid-soluble tannin content seems largely to determine the value of the made tea.

According to Freudenberg [1933] the condensation products of catechin have quite marked tanning powers. In the case of tea it was established by Carpenter & Harler [1932] that whereas the unoxidized tea tannin of a green leaf infusion gave only a cloudiness with gelatin, a considerable proportion of the tannin in a made tea infusion is precipitated by gelatin alone.

One would expect from the above considerations that tea tannin would have a greater tendency to combine with the leaf proteins after fermentation. Green

leaf after repeated extractions with hot water leaves a residue which on treatment with alkali gives a light brown extract while at the same time there is a small O_2 uptake. With made tea three successive extractions for 1 hr. with boiling water remove all the water-soluble tannin but the spent leaf still has a brownish colour. The brown pigment may be dissolved out by alkali. There is a considerable darkening in colour of the alkali extract and manometrically a large O_2 uptake may be demonstrated. If the uptake of O_2 be assumed to give an approximate measurement of the tannins present a comparison of this uptake with those of the various extracts will give an idea of the proportion of the tannin insoluble in water. The results of one such experiment are recorded below.

Table VIII

	Total O_2 uptake per mg. dry wt. of original tea μ l.
First aqueous extract	34.0
Second and third aqueous extract combined	4.9
Residue	10.0

Some 20% of the total tannins are apparently insoluble in hot water and it seems quite likely that this amount is in chemical combination with the proteins of the leaf. Alternatively the water-insoluble tannin in this case may be too highly condensed to be readily soluble in water.

As fermentation proceeds there is a decrease not only in the Lowenthal titre but also in the proportion of water-soluble solids in the made tea. A highly significant correlation exists between these two values. The greater part of the decrease in water-soluble matter can be attributed to a diminished solubility in water of the tannin bodies.

SUMMARY

The preparation and properties of tea tannin are described. Tea tannin in green leaf probably consists of a mixture of *l*-epicatechin, gallo catechin and their simpler condensation products. The galloyl ester of epicatechin is present in some types of leaf only. Tannins in made tea are much more complex.

In the fermentation process the catechol or pyrogallol nucleus only becomes oxidized. The oxidized tannin then undergoes extensive condensations. At higher temperatures of fermentation (40°) the temperature coefficients of the enzymic processes are lower than that of the condensation process; consequently teas manufactured under such conditions contain more highly condensed tannins.

Strongly cationoid polyphenols such as tea tannin are readily oxidized by peroxidase while the anionoid gallic acid is oxidized slowly.

The authors are grateful to Mr P. H. Carpenter, Chief Scientific Officer, for his continued interest in the work, and to the Indian Tea Association for permission to publish these results.

Acknowledgement must also be made of the high standard of technical assistance throughout the course of this work rendered by Mr P. B. Sen Gupta and Mr S. N. Sarma.

REFERENCES

- Benton (1939). *Proc. of the 3rd Annual Conference*, Tocklai (in the Press).
- Carpenter & Harler (1932). *Quart. J. Indian Tea Ass.* p. 9.
- Deuss (1914). *Med. v.h. Proefstation voor Thee*, 31.
- (1923). *Rec. trav. chim. Pays-Bas*, 42, 496.
- Deys & Dijkman (1937). *Proc. K. Akad. Wet. Amst.* 40, 518.
- Evans (1929). *Tea Quart.* 2, 111.
- (1930). *Tea Quart.* 3, 3.
- Freudenberg (1933). *Tannin, Cellulose, Lignin*. Berlin.
- Oshima (1936). *Proc. Imp. Acad. Japan*, 12, 189.
- & Goma (1933). *J. agric. chem. Soc. Japan*, 9, 948.
- Roberts (1939, 1). *Biochem. J.* 33, 836.
- (1939, 2). *Biochem. J.* 33, 842.
- & Sarma (1938). *Biochem. J.* 32, 1819.
- Russell & Todd (1934). *J. chem. Soc.* p. 1066.
- Shaw (1930). *Tannin Principles of Tea*, United Planters' Association of Southern India. Madras.
- & Jones (1932). *Theotannin*. United Planters' Association of Southern India. Madras.
- Tsujimura (1929). *Sci. Pap. Inst. phys. chem. Res., Tokyo*, 10, 252.
- (1930, 1). *Sci. Pap. Inst. phys. chem. Res., Tokyo*, 14, 63.
- (1930, 2). *Bull. Agric. chem. Soc., Japan*, 6, 70.
- (1931). *Bull. Agric. chem. Soc., Japan*, 7, 23.
- (1935). *Sci. Papers Inst. phys. chem. Res., Tokyo*, 26, 186.