The Fermentation Process in Tea Manufacture

10. THE CONDENSATION OF CATECHINS AND ITS RELATION TO THE CHEMICAL CHANGES IN FERMENTATION

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In an earlier communication in this series, Harrison & Roberts (1939) outlined what was known, at the time, of the chemistry of the so-called tea tannins. More recently Bradfield (1946) and Bradfield, Penney & Wright (1947) have examined the polyphenols in tea by the method of partition chromatography and have established that all the components investigated are derived either from epicatechin or gallocatechin. In view of this positive identification it seems advantageous to refer in future to the polyphenols in tea leaf as catechins.

The reasons for believing that catechins undergo condensation during fermentation after oxidation were summarized by Harrison & Roberts (1939), and further evidence for this belief was afforded by a comparison of the Löwenthal and alkaline iodine titration methods with the Stamm procedure, as applied to tea catechins by Barua & Roberts (1940). Reference to the work of Lamb & Sreerangachar (1940) and Bradfield & Penney (1944) will show that other workers share this view.

The present communication describes experiments, carried out mainly in 1940 and 1941, in which an attempt was made to devise some means of estimating the extent of condensation and to relate it to other chemical changes in fermented tea leaf. A brief outline of these findings has already been presented (Roberts, 1942).

Teas were fermented at temperatures of 60, 70, 80 and 90° F., varying the time of fermentation from 2 to 5.75 hr. Water-soluble solids, water-soluble catechins and alcohol-soluble (i.e. ethanol-soluble) solids were determined on these teas, and the changes in these figures, due to different extents of fermentation, related to the degree of condensation. Less extensive data relating the amount of extractable caffeine to the degree of fermentation are also presented.

Correlations were also found between the extent of condensation and the development of certain liquor characters recognized by the tea taster. These findings will be reported elsewhere.

METHODS

Manufacture of teas

Under normal conditions of manufacture the tea leaf undergoes mechanical rolling for some considerable time, during which period temperature control cannot be maintained. In order to carry out fermentation as far as possible under controlled conditions the period of rolling was reduced to 30 min., after which the leaf was rapidly passed through a McKercher C.T.C. machine. This machine is sometimes used in commercial factories to bring about further bruising of the coarser pieces of tea leaf after rolling is completed. In our experiments it was used to complete the damage necessary for fermentation within a reasonably short time. The remainder of the fermentation was carried out in air-conditioned cabinets at one of four temperatures, 60, 70, 80, or 90° F.; the relative humidity was 95–100%. After 2, 2.75, 3.5, 4.25, 5 and 5.75 hr. fermentation (taken from the time at which rolling was started) samples of leaf were dried at 180° F.

In this way teas which had received twenty-four different fermentation treatments were prepared. The series was repeated eight times in the manufacturing season of 1940. As only two cabinets were available, only two temperatures of fermentation could be dealt with in 1 day. All teas were manufactured from one block of tea, plucked at weekly intervals, so that 2 separate weeks' pluckings were required to cover all four temperatures of fermentation. Temperatures were randomized, so that in the complete series of manufactures, lasting 16 weeks, no one temperature could receive bias owing to seasonal trends.

In all, therefore, 192 different teas were available for investigation, but it was usual to obtain highly significant results with fewer samples.

Analytical procedures

The condensation index. The exact determination of the extent of condensation of catechins is hampered by lack of knowledge, and measurements must therefore be largely empirical. According to Harrison & Roberts (1939) the catechins precipitated from a tea infusion by 1% (v/v) H_2SO_4 probably represent the most highly condensed fraction. Advantage has been taken of this observation and the following procedure adopted.

A portion (20 ml.) of an infusion, obtained by refluxing 5 g. tea with 400 ml. water for 1 hr. and making up to

500 ml., is treated with 0.2 ml. conc. H_2SO_4 . After standing for 1 hr. the precipitate obtained is centrifuged down, washed once with 1% (v/v) H_2SO_4 and suspended in water. The suspension is then titrated according to Stamm's procedure (Barua & Roberts, 1940) and the result expressed as ml. 0.1M- KMnO_4 /g. of made tea (dry wt.). The titre is a measure of the catechins precipitated by 1% H_2SO_4 and is referred to subsequently as the condensation index or c.i. The method must be considered purely tentative, justified at present because it gives semi-quantitative and intelligible results.

Water-soluble catechins were determined by the method of Barua & Roberts (1940), which is applicable to the soluble catechins in green-leaf and fermented tea.

Water-insoluble catechins. The spent tea leaf, after extraction to make up the 1 hr. infusion, is extracted a second time for 1 hr. with 500 ml. water. The residue from the second extract is washed with boiling water, dried and weighed. The dried residue (50 mg.) is suspended in 2 ml. water in a Warburg vessel, and 1 ml. 0.2N-NaOH tipped in after attainment of temperature equilibrium. The total O_2 uptake, obtained after shaking for 2 hr., expressed as $\mu\text{l. O}_2$ /mg. dry wt. of the original tea, is a measure of the water-insoluble catechins. It may be compared with the figure obtained for the 1 hr. infusion similarly expressed as $\mu\text{l. O}_2$ /mg. dry wt. of made tea.

Caffeine. A 1 hr. or 5 min. infusion (200 ml.) is made alkaline with 10 ml. ammonia (sp.gr. 0.880). The solution is then extracted six times with 50 ml. portions of CHCl_3 , the solvent removed from the united extracts by distillation and the weight of the residue taken as caffeine.

Oxygen uptake during fermentation. To determine the O_2 uptake which has occurred at any stage of fermentation, the partly fermented leaf is very finely minced and its total uptake determined manometrically. Subtraction of this uptake from the total uptake of a sample of the unfermented green leaf gives the uptake up to the time of sampling (cf. Roberts, 1941).

RESULTS

Variation of condensation index with time and temperature of fermentation. The condensation index of all 192 teas was determined. On three occasions, however, as a result of high withers, in which the moisture content of the tea leaf fell in 18 hr. to about 50%, instead of the normal 65–70%, the teas fermented rather more slowly. These three sets have been left out in calculating the mean values for the whole season, which are recorded in Table 1. The increases in the condensation index with the time and temperature of fermentation are almost linear.

Table 1. *Effect of time and temperature of fermentation on the condensation index*

(Values are condensation indices.)

Temp. (° F.)	Fermentation time (hr.)					
	2	2.75	3.5	4.25	5	5.75
60	24.3	28.6	32.7	36.8	40.0	43.3
70	27.3	32.1	37.3	42.6	46.9	51.1
80	27.6	34.6	41.2	47.7	53.4	59.1
90	33.4	40.2	47.8	55.8	60.3	64.8

For comparison, the O_2 uptake at different stages of fermentation is recorded in Table 2. Each figure is a mean of three separate manufactures.

Table 2. *Oxygen consumption during fermentation*

Temp. (° F.)	Fermentation time (hr.)		
	2	4	5.75
	(Total $\mu\text{l. O}_2$ /mg. dry wt.)		
60	6.8	8.4	9.6
70	7.6	9.5	10.0
80	8.3	10.4	11.0
90	8.2	10.3	10.8

The results indicate a clear-cut distinction between oxidation and condensation of the catechins. Between 2 and 4.25 hr. fermentation at all temperatures the condensation index increases linearly, with some slight falling off from 4.25 to 5.75 hr., whereas the rate of O_2 uptake decreases as fermentation proceeds. The condensation index increases proportionately with temperature, but the increase in rate of O_2 uptake with temperature is not maintained beyond 80° F.

It would appear logical to conclude that although condensation follows oxidation, the subsequent rate of condensation is independent of the rate of oxidation, and is determined largely by time and temperature.

Water-soluble solids and catechins. It has long been known that the water-soluble solids decrease progressively as a result of fermentation. In Table 3 the mean values are given for water-soluble solids, water-soluble catechins and condensation indices for four times and four temperatures of fermentation. Values for five series of manufacture only are given, as the three high-withered teas were omitted.

As with the condensation index, variations in soluble solids and catechin with time and temperature of fermentation are very highly significant. Correlations between any two of these three variables are also found to be significant, but the eighty pairs of data cannot be considered homogeneous, owing to the systematic differences due to time, temperature and date of manufacture. It is therefore necessary to calculate the correlation coefficient (r) for each factor using the analysis of covariance.

The changes in soluble solids and catechins, due to varying time and temperature of fermentation, are significantly correlated with the condensation index, and with each other (Table 4). The only other significant effect is the residual correlation between soluble solids and catechins. As the catechins account for nearly half the total water-soluble solids, it is not unexpected that such a correlation should be found.

Table 3. *Effect of time and temperature of fermentation on soluble solids, soluble catechins and condensation index*

(C.I. = condensation index.)

Time (hr.)	...	2			3.5			4.25			5.75		
Temp. (° F.)		Soluble solids (%)	Catechin (%)	C.I.	Soluble solids (%)	Catechin (%)	C.I.	Soluble solids (%)	Catechin (%)	C.I.	Soluble solids (%)	Catechin (%)	C.I.
60	Mean	43.2	19.2	26.2	42.1	18.4	35.9	41.8	17.6	40.9	41.1	17.7	48.5
	S.E.	0.25	0.56	1.8	0.51	0.37	2.4	0.51	0.40	2.6	0.47	0.34	3.4
70	Mean	42.3	18.5	27.1	41.2	17.9	38.3	39.9	17.4	44.1	39.7	17.2	52.7
	S.E.	0.78	0.46	1.8	0.91	0.62	1.0	0.74	0.49	1.4	0.73	0.35	1.8
80	Mean	42.0	18.0	30.5	39.7	17.1	46.2	38.5	16.7	54.1	38.3	15.8	63.0
	S.E.	0.35	0.71	0.8	0.57	0.62	1.9	0.72	0.81	2.7	0.99	0.60	0.9
90	Mean	41.1	17.1	34.3	39.4	16.0	49.1	39.1	15.7	57.2	38.5	15.1	65.6
	S.E.	0.56	0.55	3.1	0.38	0.53	3.1	0.45	0.70	3.3	0.64	0.40	2.7

Table 4. *Analysis of covariance. Soluble solids, soluble catechins and condensation index (C.I.)*

	Degrees of freedom	Soluble solids and catechins	Soluble solids and C.I.	Catechins and C.I.
Temperature	3	0.903*	-0.906*	-0.978†
Time	3	0.993‡	-0.969†	-0.997‡
Date	4	-0.038	0.167	0.168
Temperature × time	9	0.143	-0.279	-0.176
Temperature × date	12	0.312	-0.254	-0.231
Time × date	12	0.304	0.516	0.298
Residual	35	0.392*	-0.139	-0.194

* $P=0.05\%$. † $P=0.01\%$. ‡ $P=0.001\%$.

The correlation coefficient (r) between all eighty pairs of data for soluble solids and catechins is 0.5649. If the date variance and the interactions with date are eliminated by averaging each set of five figures, we are left with sixteen comparisons, for which r now becomes 0.9093. A straight line relation may be calculated from these figures. (Catechin %) = $-9.6106 + 0.6624$ (soluble solids %.)

For a fall of 1.0 % in the water-soluble solids there is a corresponding decrease of 0.66 % in the catechins. Thus, although the decreasing solubility in water of the catechins is a major factor in the decrease in water-soluble solids as a result of fermentation, it is probable that other water-soluble substances are also concerned. This could, perhaps, be accounted for by an oxidation of respiratory substrates to CO_2 by the orthoquinones formed on oxidation of the catechins, or by a combination of oxidized and condensed products with water-soluble nitrogenous substances to form an insoluble product.

Water-insoluble catechins. Table 5 gives average figures of oxygen uptake determined in 1939 for water-soluble and insoluble catechins, obtained by the method of alkaline autoxidation, for teas fermented for 2.5 and 4.75 hr. at 60 and 90° F. The averages are of duplicate experiments in good agreement. The fall in water-soluble catechins is accompanied by a distinct increase in water-insoluble catechins.

Table 5. *Alkaline autoxidation of soluble and insoluble catechins in made tea*

	Temp. (° F.)	Fermentation time (hr.)	
		2.5 μl. O_2 /mg. dry wt. original tea	4.75
Soluble	60	42.7	41.0
	90	38.9	34.4
Insoluble	60	9.9	11.7
	90	12.2	13.0

Caffeine. Soluble solids and catechins in the 1 hr. infusion both decrease appreciably as a result of fermentation. The fall in soluble solids cannot entirely be accounted for in terms of loss of soluble catechins, and in the previous section it was pointed out that combination of oxidized catechins with soluble nitrogenous substances to form a water-insoluble product was a possibility.

The results in Table 6 show that with increase in the time or temperature of fermentation there is no detectable effect on the caffeine content in either the 1 hr. or 5 min. infusion. These results prove that during fermentation the water-insoluble products are not produced by combination with caffeine. It might also be expected that, as a result of the known combination of catechins with caffeine, there would be an appreciably lowered extractability of caffeine in the taster's 5 min. infusion, but the results in Table 6 disprove this expectation also.

Table 6. *Effect of fermentation on water-soluble caffeine*

Fermentation		Caffeine content (%)		Condensation index
Time (hr.)	Temp. (° F.)	5 min. infusion	1 hr. infusion	
2	60	2.97	4.30	21.0
2.75	60	3.17	4.40	24.0
4.25	60	2.82	4.50	26.5
4.25	60	3.02	4.50	32.0
5.75	60	3.00	4.35	39.0
4.25	70	2.97	4.50	36.5
4.25	80	2.94	4.35	38.0
2	90	2.97	4.30	32.2
2.75	90	2.92	4.40	37.5
4.25	90	2.94	4.55	58.2
4.25	90	3.25	4.60	47.2
5.75	90	2.90	4.30	66.8

Alcohol-soluble solids. As first established in this laboratory in 1930 (unpublished observations), and independently in Indo-China by Castagnol & Doan-ba-Phuong (1940), alcohol extracts much less material than water from fermented tea. According to the latter authors the percentage of alcohol-soluble solids decreased with increase in fermentation time and the amount of soluble matter, removed in a subsequent extraction of the residue with water, increased; estimation of the catechins by the hide-powder method (Association of Official Agricultural Chemists, 1945) showed that, with increasing time of fermentation, the proportion of catechins fell in the alcohol extract, and increased in the subsequent aqueous extract. The hide-powder method has been found in this laboratory (unpublished observations) to give uncertain results with made tea infusions.

Table 7. *Variation in alcohol and water-soluble solids*

(A.S.S. = alcohol-soluble solids (%); W.S.S. = water-soluble solids (%); C.I. = condensation index.)

Fermentation		A.S.S.	W.S.S.	W.S.S.-	
Time (hr.)	Temp. (° F.)			A.S.S.	C.I.
2	60	37.5	44.0	6.5	27.5
2	60	37.0	43.3	6.3	25.5
2	60	37.2	42.8	5.6	25.0
5.75	60	33.2	40.6	7.4	48.0
5.75	60	33.1	42.0	8.9	44.5
3.5	70	33.9	42.5	8.6	37.1
5.75	70	30.6	40.8	10.2	52.8
3.5	80	31.1	38.1	7.0	39.3
2	90	37.7	—	—	24.5
2	90	33.1	39.2	6.1	33.5
4.25	90	27.6	39.5	11.9	56.8
5.75	90	25.6	36.9	11.3	66.8
5.75	90	25.7	37.8	12.1	64.8

Table 7 records values obtained in this laboratory for alcohol-soluble solids (A.S.S.), water soluble products (W.S.S.) and condensation index (C.I.). The regression coefficients of A.S.S. on C.I., and (W.S.S.-A.S.S.) on C.I. are highly significant, the values for t_{11}

and t_{10} respectively amounting to 11.13 and 6.90, for both of which P is < 0.001 .

The observations of Castagnol & Doan-ba-Phuong (1940) relating to the extractability of catechins by alcohol are also confirmed, as the catechin content of the aqueous extract of the residue, after alcohol extraction, amounted to 3.79 % for a tea fermented 2 hr. at 60° F. and 6.81 % for a tea fermented 5.75 hr. at 90° F.

Scarcity of solvents made it impossible to extend these observations to caffeine at the time. Recently, however, it has been established that with increasing fermentation the extractability of caffeine in alcohol also decreases. In teas fermented for 2 and 5 hr. at 85° F., 0.41 and 0.60 % caffeine respectively (on dry wt.) has been found in the aqueous extracts of the residue left after extraction with alcohol.

DISCUSSION

Condensation of the catechins could account for changes in chemical composition and in liquor characters, which cannot be interpreted in terms of oxidation alone. An increase in the fermentation time from 3 to 4 hr. in one series of factory experiments, increased the oxidation of catechins from 77 to 80 %. The changes in water-soluble solids, in depth of colour of infusion and in the strength of the liquor, as estimated by a tea taster, were however quite incommensurate with this slight increase in oxidation. The effect of the extra fermentation would be to increase the condensation index from about 40 to 50. If this figure is related to the actual degree of condensation in a linear manner, this represents an increase of 25 % in the condensation, as compared with 3-4 % in the oxidation.

Apart from the obvious variations of liquor characters with time and temperature of fermentation, many of the chemical changes observed can be related to the condensation index. There is, in particular, a very close connexion between this value and the total amount of catechin in solution, increasing condensation resulting in decreased water-solubility of the catechins. This may in part be due to the formation of high polymers of catechins insoluble in water, but judging by the increased ability of the catechins to precipitate gelatine as fermentation proceeds, it appears that this decrease must also be partly due to an actual combination of catechin condensation products with leaf protein to form a water-insoluble product.

The presence of water-insoluble polyphenols in fermented tea has been demonstrated by the method of alkaline autoxidation. These insoluble substances increase with fermentation, but can only be estimated in an empirical way.

The existence of highly polymerized water-insoluble catechins, whether combined with protein

or not, accounts in part for the persistence of the brown colour of spent tea leaf after repeated extractions with water. Oxidation products of chlorophyll, according to Sreerangachar (1943), may also be responsible for the development of a brown pigment in the leaf on fermentation, but the suggestion of Popatov (1932) that these pigments are melanins obtained by oxidation of tyrosine cannot be accepted, as not only is the tyrosine content of tea leaf very small, but tyrosine is not oxidized by either tea oxidase or tea oxidase + catechins (unpublished observations).

The figures available for caffeine in the 1 hr. infusion suggest that there can be little or no combination between catechin condensation products and caffeine to form substances insoluble in boiling water. The values for alcohol-soluble solids suggest, however, that there is an increasing degree of combination between catechins and caffeine as the condensation index increases, with formation of products which are soluble in boiling water but not in boiling alcohol.

Infusions of tea in boiling water are initially clear, but on cooling frequently become cloudy owing to the separation of a finely divided precipitate containing both catechins and caffeine (cf. Bradfield & Penney, 1944). It has always been observed in this laboratory that this effect, known as creaming down, increases with both time and temperature of fermentation. This is a further indication that combination between catechins and caffeine increases with the extent of condensation of the former. Such combination does not decrease the extraction of soluble matter by

boiling water, but solubility in cold water and alcohol is appreciably reduced.

SUMMARY

1. An empirical method is described for determining the degree of condensation of catechins in fermented teas.

2. The condensation index increases in approximately linear fashion with time and temperature of fermentation; this increase continues at times and temperatures when the total oxygen uptake is no longer increasing.

3. Values for water-soluble solids and water-soluble catechins, in teas receiving varying fermentation, are significantly correlated with each other, and are correlated negatively with the condensation index. About two-thirds of the loss in soluble solids can be accounted for by an insolubilization of catechins, due to the formation of high polymers insoluble in water, and by combination of condensation products with leaf protein.

4. The much greater fall in alcohol-soluble solids with increase in the condensation index indicates a combination of condensation products with water-soluble nitrogenous compounds to form complexes soluble in boiling water, but insoluble in boiling alcohol.

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