

## STUDY OF FERMENTING RATES OF CLONES USING CHLOROFORM TEST

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The Chloroform test was adopted for determination of fermentation rates of clones and thereby it was possible to categorise clones into groups. Practical relevance of this categorisation has been explained.

### INTRODUCTION

Fermentation is probably the most critical stage in manufacture of black teas. Most important chemical changes which result in development of desirable liquor characteristics such as liquor quality, strength and colour occur during this stage. Thus for optimum results the fermentation period must be optimised. It is known that there is variation in fermenting rates between clones. Thus in order to achieve best results leaf harvested from a given clone must be given the appropriate optimum fermentation period of that clone.

The most obvious change occurring during fermentation, i.e. the oxidation of the tea flavanols are brought about by the enzyme polyphenol oxidase (Sreerangachar, 1943; Perera and Wickramasinghe, 1972). Sanderson (1964) has however found that the fermenting rate is not entirely dependent on the level of polyphenol oxidase activity; there were certain clones which are poor fermenters despite possessing high levels of polyphenol oxidase activity whereas there were other clones which are known good fermenters though they possessed rather low levels of the enzyme. Further he has also found that the level of flavanols themselves is not the deciding factor in determining fermenting rate. Though clone UH 9/3 had been found to have high enzyme activity and flavanol content it has been found to be a slow fermenter.

Thus it is clear that the flavanol content and/or the polyphenol oxidase activity cannot be used as parameters for the purpose of estimating fermenting periods or ranking of clones for fermentation rates. The Chloroform test has been described in detail by Sanderson (1963), who has explained that when leaf is subjected to an environment filled with chloroform vapour the cellular membrane permeability increases thus allowing contact between polyphenols and the enzyme polyphenol oxidase resulting in oxidation of the former and production of brown colour substances. In the method described by Sanderson, tests were done in glass stoppered test tubes and the material used were the first leaves from the harvested flush. The leaf colour after a period of time was taken to indicate the degree of fermentation. Employing this method it was possible to rank clones according to fermentation rates.

An investigation into the possibility of adoption of the Chloroform test for determination of fermenting periods of different clones was carried out with the object of grouping clones according to their fermentation properties.

## **MATERIALS AND METHODS**

### **Clonal and leaf variation**

The tests were carried out in a glass tank fitted with a lid with Chloroform soaked cotton wool kept at the bottom. The lid was kept closed for a period of about five minutes in order to saturate the atmosphere within the tank with Chloroform vapour. Leaves of clone TRI 777 (a known quick fermenter) and clone TRI 2142 (a very slow fermenter) selected for the experiment were hung on a wire and placed horizontally, so that the leaves were nearly equidistant from the soaked wool. After closing the lid the colour changes were monitored over a period of time. The fermentation was considered to be complete when the leaf turned to a brown colour after a period of exposure to the Chloroform vapour. The period of exposure was recorded. Further exposure led to darker shades of brown and ultimately to colours such as bahama brown or saddle.

### **Selection of leaf**

In order to arrive at the most representative leaf to be used in the Chloroform test, four clones, namely TRI 777, TRI 2023, TRI 2025 and DT 1, were screened and the average weights of different portions of the harvested flush were recorded. It was also noticed that the level of maturity of the harvested flush also influenced the above averages.

Therefore, flush at three levels of maturity, namely

- Level A — with bud about to open up, the next bud being almost visible,
- Level B — with adequately formed bud representing ideal shoot, and
- Level C — with very small bud, representing banji type flush, were screened. Two standards of plucking, namely 2 leaves and a bud and 3 leaves and a bud were also examined.

### **Fermenting rates of clones**

Fermenting periods of eight popular tea clones were studied by carrying out the Chloroform test. The second leaf from an ideal shoot is taken to be representative of the harvested shoots of the clone. The tests were done on five occasions.

In the preliminary tests all sections of the harvested flush namely first leaf, second leaf, third leaf and fourth leaf were used. The second leaf of a well formed shoot, i.e. one with an adequately formed bud, was used for subsequent tests.

## RESULTS AND DISCUSSION

### Clonal and leaf variation

Relative fermenting rates of different portions of the flush determined by the chloroform test on three occasions are presented in Table I,

TABLE I — *Fermenting rates of different portions of flush from clones TRI 777 and TRI 2142*

Period of fermentation (minutes)	1 st. leaf	2 nd. leaf	3 rd. leaf	4 th. leaf
<i>Clone TRI 777</i>				
Test 1	44	34	31	29
Test 2	45	34	31	29
Test 3	43	31	31	25
Mean	44	33	31	28
<i>Clone TRI 2142</i>				
Test 1	104	100	90	89
Test 2	101	98	85	82
Test 3	109	102	92	91
Mean	105	100	89	87

The following conclusions were arrived at,

- (i) There could be a large variation between fermenting rates of different clones.
- (ii) Even for a given clone, the fermenting rates of different portions of flush can be noticeably different. The tender portions of flush ferments slower than mature portions, when the permeability of cell membranes are increased by the action of Chloroform.
- (iii) The variation of fermentation rates of different portions of a given clone is small compared to the differences in fermenting rates of the two clones.
- (iv) As the fermenting rate depends on the maturity of leaf it can be hypothesised that the standard of harvested flush as one of the factors that will influence the fermenting rate; with a coarser standard of flush the fermentation period is reduced.

### Selection of leaf

The results are presented in Table 2.

TABLE 2.—Weights of different portions of flush expressed as percentages of the weight of shoot.

Components of flush	Percentage Weights					
	2 leaves and bud			3 leaves and bud		
	Level A	Level B	Level C	Level A	Level B	Level C
Bud	8.6	7.0	1.5	4.8	3.7	0.5
1st leaf	19.6	18.7	21.7	10.9	9.8	10.2
2nd leaf	43.9	45.9	53.5	23.7	22.1	26.0
3rd leaf	—	—	—	30.8	32.5	37.3
Stem	27.9	28.4	23.4	29.8	31.9	26.0

From data, given in Table 2 it is seen that if the standard of leaf is "2 leaves and a bud" then the 2nd leaf is the largest weight component whereas if the standard of leaf is "3 leaves and a bud" then the 3rd leaf is the largest weight component. In practice harvested flush may contain "2 leaves and bud", "3 leaves and bud", "banji single leaf" etc. The weight components of different portions when the harvested leaf is a mixture of "2 leaves and a bud" and "3 leaves and a bud" were also calculated (Table 3). It is assumed that the leaf is of the type B, i.e. the ideal shoot.

TABLE 3.—Percentage weights of different portions of flush when the harvested leaf is a mixture of "2 leaves and a bud" and "3 leaves and a bud".

2l+b	3l+b	Bud	1st. leaf	2nd. leaf	3rd. leaf	Stem
100%	0%	7.0	18.7	45.9	—	28.4
75%	25%	6.18	16.47	39.95	8.12	29.28
50%	50%	5.35	14.25	34.0	16.25	30.15
25%	75%	4.53	12.02	28.05	24.38	31.02
0%	100%	3.7	9.8	22.1	32.5	31.9

From the above it is seen that as long as the "2 leaves and a bud" component is more than 25% (this is almost always the case in commercial scale standards) the 2nd leaf has the largest weight component in the leaf portion of flush. It is known that important chemical constituents such as polyphenols, polyphenol oxidase, amino acids, etc., are in peak quantities in the 2nd leaf, at any rate more than that in third or other mature leaf or in the stem. The second leaf from an ideal shoot, i.e. one with an adequately formed bud, is therefore selected as the most desirable material for the purpose of the Chloroform test.

## Fermenting rates of clones

The results of the fermenting periods of clones are given in Table 4.

TABLE 4 - Fermenting periods (minutes) of some popular tea clones from the chloroform test.

Clone	TRI 777	DT I	TRI 2024	TRI 2025	TRI 2026	TRI 2027	TRI 2023	TRI 2142
Set 1	30	45	60	70	80	95	105	105
Set 2	25	35	60	60	70	105	100	110
Set 3	35	50	50	50	60	85	100	95
Set 4	25	45	45	60	80	85	95	110
Set 5	40	50	60	60	70	95	105	105
Mean	31	45	55	60	72	93	101	105
S.D.	6.52	6.12	7.07	7.07	8.37	8.37	4.18	6.12
Group	A	B	B,C	C,D	D	E	E	E

In the above results the reasons for set to set variation in fermentation may be the following :

- (i) Errors in selection of the ideal flush. If the shoot is more matured, then the measured period will be shorter than that from the ideal shoot and vice versa.
- (ii) Difficulties in observing the exact colour change. The tone of brown colour of the fully fermented leaf depends on the tone of the green colour of the fresh leaf which varies from clone to clone.
- (iii) Changes in the climatic conditions.

## Conclusions and practical relevance

While the method described by Sanderson (1964) is satisfactory for the purpose of ranking clones, the method adopted here allows, in addition, determination of the fermentation periods of different clones which has more practical meaning. It has been found that the estimated fermentation periods by the above method using the second leaf of an ideal flush which was proved to be representative of the harvested flush, nearly matches the optimum fermentation period of CTC dhools of the same clone. This has to be expected because in the CTC method maceration of leaf is so severe that it is nearly similar to the effect of chloroform on the leaf.

For convenience clones can also be grouped according to their fermentation rates, where the optimum period for each clone can be considered to be nearly equal to the group period and be within an acceptable margin of variation.

This point may be taken into account in planning harvesting and processing of tea in commercial scale to achieve best possible results from whatever available material. Further, this classification may be utilized as one of the factors in selection of clones for replanting and infilling purposes.

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