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A Simple Chemical Method for the Determination of Optimum Fermentation Time in Black Tea Manufacture

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ABSTRACT. *Iodometric titrations were used to measure the total oxidisable matter to find its suitability as a fermentation tool in black tea manufacture. This method was tested for orthodox teas with colour and strength. The results indicated it could be used as a tool to determine the fermentation time more accurately than the conventional "nose" technique. The results were confirmed by TF, TR measurements & taste evaluation. The method is quite simple and does not involve any calculations. However, it could not be used during the quality (flavour) season.*

INTRODUCTION

The most important change occurring during tea manufacture is fermentation, *i.e.* the oxidation of polyphenols by the enzyme polyphenol oxidase. Fermentation effects the quality, colour & strength of the tea liquor. Apart from the thickness of dhool spread, temperature and humidity in the fermenting room, the widely used parameter to control the extent of fermentation is the period of fermentation which generally varies from 30 minutes (during quality season) to 4 hours (off quality season). It is known that the colour and strength increases at the expense of quality, beyond the optimum period of fermentation.

Shaw (1935) studied the total oxidisable substances in green tea leaf using the iodometric method. Lamb & Sreeranagachar (1940 a,b) and Sreerangachar (1943) carried out extensive studies on tea fermentation, especially the nature of the tea oxidase enzyme. The factors effecting quality of tea are well documented (Roberts and Smith, 1961; Ramaswamy, 1962; Sanderson, 1972). However, until to date there is no simple chemical test to determine the optimum fermentation time and is judged by the factory officer using the "nose technique". In this study therefore, a simple iodometric method based on initial work done by Shaw (1935) was tested with the objective of determining the optimum fermentation time.

MATERIALS AND METHODS

The trials were carried out at St. Coombs Estate, Talawakelle. The 1st and 2nd dhools for these trials were obtained from a modified mixed rotorvane - orthodox program (De Silva and Sanderson, 1964).

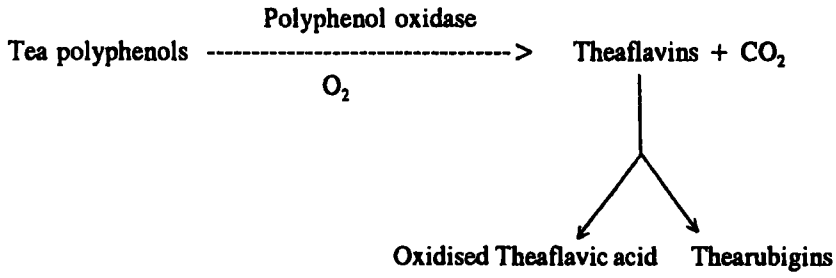
Samples of 6g wet weight of dhool obtained at various fermentation times were brewed in 375 ml boiling water in thermos flasks using a mechanical shaker for 10 min. and were filtered using a muslin cloth. Twenty ml of the tea brew was pipetted into a conical flask and 20 ml of 0.1 N I_2 in KI was added with 20 ml of 1N NaOH. This was allowed to stand for 90 min, followed by the addition of 0.66 M H_2SO_4. Liberated iodine was titrated with 0.05 N $Na_2S_2O_3$, with dilution to about 175 ml before the addition of starch as the indicator. The method followed is essentially that of Shaw (1935) except for the different extraction method used and the lengthened reaction time from 15 min. to 90 min.

In the mean time, 500 g (approx.) dhools obtained at various fermentation times were dried in a miniature experimental drier (Keegel, 1962) and the oxidisable matter present were determined, as above, using 2.5 g made tea from each sample.

The theaflavins (TF) and thearubigins (TR) values and colour of the liquor were determined by the method of Roberts and Smith (1961).

RESULTS AND DISCUSSION

The fermentation reaction during tea manufacture could be summarised as follows:



It is well documented that the theaflavins (TF) contribute to quality and the thearubigins (TR) contribute to colour and strength of the tea liquor. There are many methods in the literature to determine the amount of fermentation products, mainly TF & TR. All these methods either involve, tedious extraction procedures, expensive chemicals or colorimetry together with many calculations (Likoleche-Nkoma and Whitehead, 1988; Whitehead and Muhime, 1989).

The trials were carried out during the quality and off quality seasons. Figures 1 & 2 and Tables 1 & 2 represent the results obtained in 2 days, out of total 20 replicates, during the off quality season.

Table 1. Thiosulphate volume as an indicator of optimum fermentation time and some chemical parameters (TF, TR and Total Colour) on Day 1.

Ferm. time (min.)	Wet dhool $Na_2S_2O_3$ Vol(ml)	Fired dhool $Na_2S_2O_3$ Vol(ml)	TF μ.moles/g	TR mg/g	Colour
90	3.6	6.2			
105	3.7	6.5			
120	4.5	7.8	1.316	14.15	1.12
135	4.9	8.3	1.281	14.48	1.72
150	6.0	9.0	1.207	14.11	2.21
165	6.4	9.5	1.160	14.45	3.75
180*	6.8	9.5	1.228	17.42	3.91
195	6.8	9.5	1.306	16.33	3.92

* - fermentation time determined by this method

The figure underlined denotes the fermentation time decided by the factory officer.

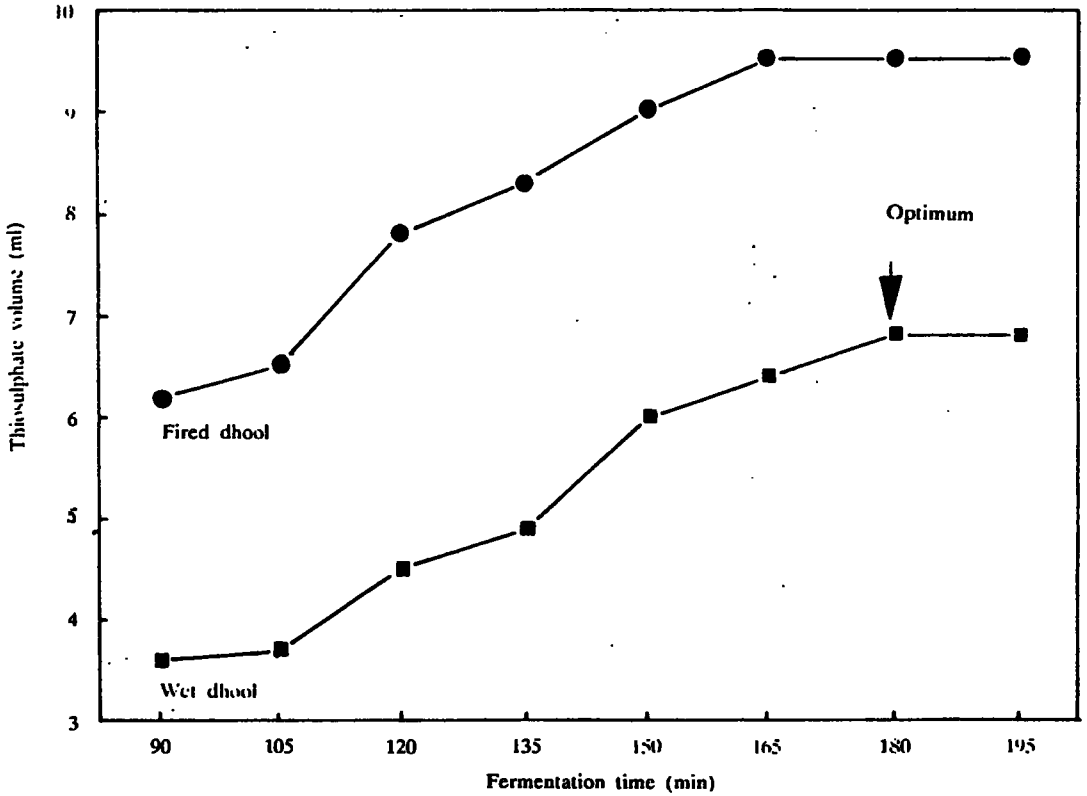


Figure 1. Thiosulphate volume against fermentation time (day 1).

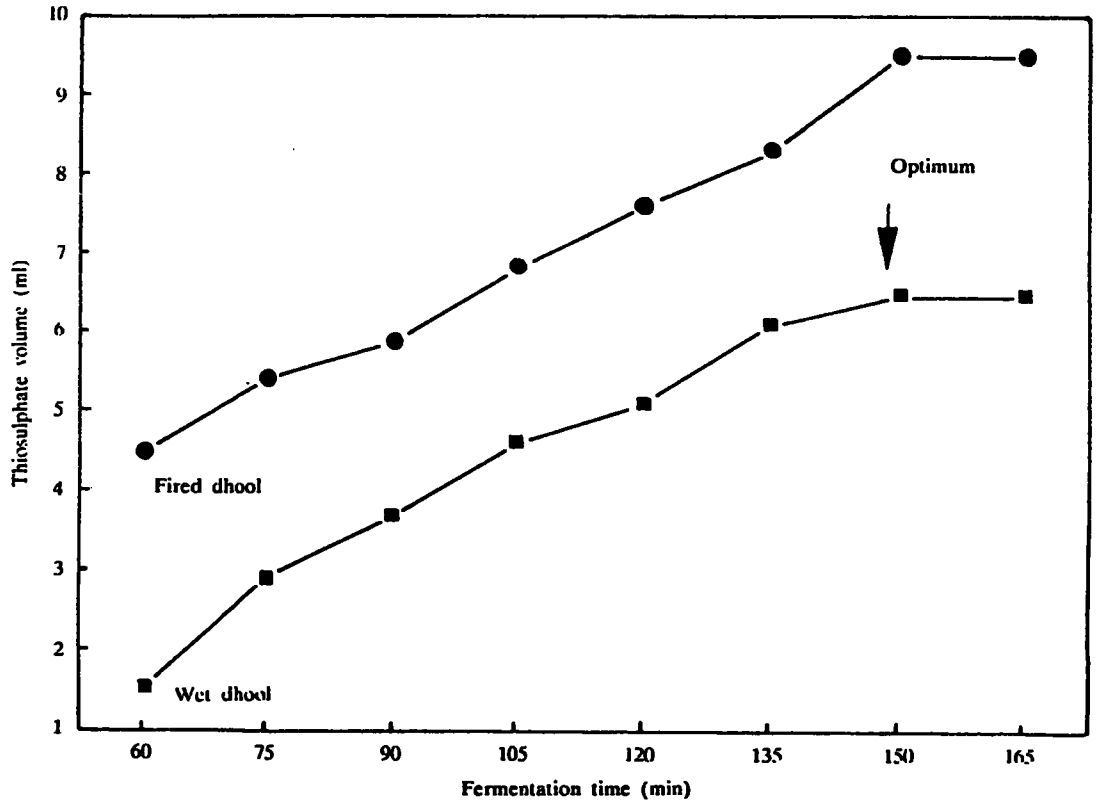


Figure 2. Thiosulphate volume against fermentation time (day 2).

Table 2. Thiosulphate volume as an indicator of optimum fermentation time and some chemical parameters (TF, TR and Total Colour) on Day 2.

Ferm. time (min.)	Wet dhool $Na_2S_2O_3$ Vol(ml)	Fired dhool $Na_2S_2O_3$ Vol(ml)	TF μ.moles/g	TR mg/g	Colour
60	1.5	4.5	0.478	7.81	1.16
75	2.9	5.4	0.629	7.99	1.95
90	3.7	5.9	0.856	8.68	2.05
105	4.6	6.8	0.965	9.07	2.38
120	5.1	7.6	1.103	8.99	2.47
135	6.1	8.3	1.177	10.16	2.75
150*	6.5	9.5	1.213	10.32	3.40
165	6.5	9.5	1.015	10.40	3.55

* - fermentation time determined by this method

The figure underlined denotes the fermentation time decided by the factory officer.

It is apparent from Figures 1 & 2 that the thiosulphate volume reaches the maximum at the optimum fermentation time. The TF, TR & colour values of made tea (Table 1 & 2) also confirm this. Valuation of the three tasters was also higher for teas corresponding to the maximum thiosulphate volume. In a few cases they could not detect much difference between the sample corresponding to maximum thiosulphate volume and the one before that.

However, during the quality season the thiosulphate volume reached its maximum value, much later than the optimum fermentation time determined by the factory officer. The tasters' evaluation also supported the judgement of the factory officers. Thus, this method could not be used to determine the optimum fermentation time during quality season.

Earlier authors (Sreerangachar, 1943) expressed reservations regarding the iodometric method it did not afford any indication of complex changes

such as condensation. Condensation can take place between 2 or more molecules of o-quinones or one molecule of o-quinone and one molecule of unoxidised polyphenol. Such condensed products are oxidised by iodine relatively slowly, extending the reaction time used in this study. However, in the same paper Sreerangachar (1943) recommended the iodine titre method for comparative study of enzyme activities based on the following explanation *i.e.* as condensation is a chemical reaction, it is reasonable to assume that the rate of and amount of condensation of oxidised polyphenols will bear a definite relation to the amount of oxidised polyphenols present, which in turn will be related to the activity of the enzyme. Ascorbic acid also undergoes oxidation in the presence of a suitable polyphenol. Thus Sreerangachar (1943) claimed that the ascorbic acid reaction would provide a more reliable method for measuring enzyme activity.

In ahother study, Lamb & Sreerangachar (1940 a) studied the catechol oxidation by oxidase and peroxidase using iodometry and found that oxidase activity produced very little change in iodine titre, where as peroxidase produced a marked fall in titre. It appeared that peroxidase activity resulted in the formation of more condensation products than does oxidase activity.

The reservation expressed by the earlier workers was overcome by the better extraction method and the extended time of reaction used in this study or there is the slight possibility that the peroxidase activity during this study was minimal. Further trials using this method along with peroxidase and oxidase activity at St. Coombs should give more weight to the adoption of this method. The advantages of this method are; the simplicity, absence of any calculations and the clear end point. Moreover, even the concentrations of the solutions used in this study need not be accurate.

It was also interesting to note that the St. Coombs factory officer's judgement of optimum fermentation time, by the "nose" technique was correct except on two occasions out of the 20 replicates tested.

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REFERENCES

De Silva, W.C.A. and Sanderson G.W. (1964). Rotorvane manufacture techniques. Tea Q 35:230-234.

Keegel (1962). Tea made from clones - Part 2 Tea Q. 33:183-188.

Lamb J. and Sreerangachar H.B. (1940a). Studies on the fermentation of Ceylon tea. Biochem. J. 34:1472

Lamb J. and Sreerangachar H.B. (1940b). Studies on the fermentation of Ceylon tea. Biochem.J. 34:1485

Lekloche-Nkoma J.W. and Whitehead, D.L. (1988). A new cheaper method for measuring the theaflavin content of made tea using aluminium chloride instead of flavognost. TRF, QNL 92:14-19.

Ramaswamy, S. (1962). The chemical basis of liquoring characteristics of Ceylon teas. Tea Q 33:156-165.

Roberts E.A.H. and Smith R.F. (1961). Spectrophotometric measurements of theaflavins and thearubigins in black tea liquors in assessments of quality in teas. Analyst (London) 96:94-98.

Sanderson G.W. (1972). Structural and functional aspect of phytochemistry. pp. 247-316. *In:* Runeckles V.C. and Tso T.C (Ed), New York & London: Academic Press.

Shaw W.S. (1935). Theotannin I. Theotannin in relation to green leaf Bull. Uni. Plant. Ass. South India, 4:42-46.

Sreerangachar H.B. (1943). Studies on the fermentation of Ceylon tea. Biochem.J. 37:653-655.

Whitehead, D.L. and Muhime, P.M. (1989). Black tea manufacture: A practical method to enable factory managers to control quality during fermentation. TRF, QNL 96:10-19.