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Changes in Polyphenols, Amino Acids and Volatile Compounds during Fermentation and Firing in Orthodox Processing of Tea

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Abstract : In the orthodox manufacture of tea, prolongation of fermentation leads to a reduction in the solubility of the tea polyphenols due to increased binding with other tea leaf cell components ; the period of fermentation has no effect on total amino acid content. On firing, the bound polyphenol and the total amino acid contents increase and there is also a considerable loss of volatile compounds.

1. Introduction

Polyphenols, amino acids and volatile compounds play an important role in determining the character of tea[2,4] but detailed studies of the quantitative changes in these classes of compounds which occur at various stages of fermentation and on firing have received insufficient attention. As was first pointed out by Roberts,[1] polyphenols undergo dramatic transformations during fermentation, and the amino acids are converted to compounds which are partly responsible for the aroma of tea. It is also to be expected that some of the essential oils, which contribute in a significant way to tea flavour will be lost at the high temperatures employed in the firing stage of processing, but the extent of this loss is unknown. The present investigation presents the results of the first systematic study of the progressive oxidation of polyphenols and changes in the amino acid content, after various periods of fermentation, and the extent of loss of essential oils during firing.

2. Materials and Methods

Flush of clone TRI 2024 collected in dry weather during early February 1977, was tat-withered for 12 hours and rolled in orthodox mini-rollers having a capacity of 14 kg withered leaf. The leaf was first preconditioned for 10 min and then rolled under pressure for 15 min. First dhool* samples were removed after periods of fermentation of 30, 60, 90, 120 and 150 min. Part of each of these samples was fired, and each of the five samples of dhool and black tea respectively, analyzed for contents of total polyphenols, vanillin-reacting polyphenols, amino acids, and essential oils.

*Dhools = fermenting leaf mass

2.1. Estimation of polyphenols and amino acids

Samples of dhool (10g) and fired tea (5g) respectively were extracted with 50 ml of boiling absolute alcohol for 10 min. The extract was decanted and the residue re-extracted twice more in a similar manner, after which the three successive extracts were pooled and made up to 250 ml. The residue after extraction was then extracted with boiling aqueous (80% v/v) alcohol three times successively, the successive extracts pooled, and made up to 250 ml. Each pooled extract was analyzed for total polyphenols and vanillin-reacting polyphenols according to the methods described by Swain and Hillis[3] and for total amino acids by the method of Yemm and Cocking.[6]

2.2. Estimation of essential oils

Essential oils were collected by steam distilling 200g samples of fermented dhool and 50g of the corresponding fired dhool. One thousand ml of steam distillate were collected, after which it was saturated with sodium chloride and the essential oils extracted with peroxide-free diethyl ether (3 × 100 ml). The ethereal extracts were dried with anhydrous sodium sulphate and the weight of essential oil determined after distilling off the ether at 38°C. Loss of essential oils was calculated on the dry weight basis of dhool and black tea.

3. Results

3.1. Total polyphenols

Analysis of total polyphenols extractable by absolute and aqueous alcohol, respectively, from dhools after periods of fermentation varying from 30 min to 150 min together with the results of analysis of the corresponding fired tea, are shown in Table 1.

TABLE 1. Changes with time of fermentation of total polyphenols in dhools and black tea (mg/g dry weight)

Time of fermentation (min)	Absolute alcohol extract		Aqueous alcohol extract		Total	
	Dhool	Black Tea	Dhool	Black Tea	Dhool	Black Tea
30	242	111	85	239	327	350
60	229	79	102	258	331	337
90	199	59	124	253	323	312
120	198	33	112	276	310	299
150	180	28	122	265	302	293

Total polyphenols estimated by the method of Swain and Hillis[3], using Folin-Ciocalteau reagent.

The results indicate that in dhools a greater proportion of polyphenols were extractable by absolute than by aqueous alcohol and that this pattern was reversed on firing. It is also seen that periods of fermentaion greater than 1 h lead to a decrease in the amount of extractable polyphenols and this trend is more marked in black tea than in dhools.

3.2. Vanillin-reacting polyphenols

Table 2 depicts the results obtained with vanillin-reacting polyphenols corresponding to those shown in Table 1 for total polyphenols.

TABLE 2. Changes with time of fermentation of vanillin-reacting polyphenols in dhools and black tea (mg/g dry weight)

Time of fermentation (min)	Absolute alcohol extract		Aqueous alcohol extract		Total	
	Dhool	Black Tea	Dhool	Black Tea	Dhool	Black Tea
30	125	50	41	120	166	170
60	106	39	44	121	150	160
90	98	28	49	113	147	141
120	88	18	48	121	136	139
150	77	14	49	116	126	130

Vanillin- reacting polyphenols estimated by the method described by Swain and Hillis.[3]

The results show the same trend as in Table 1 with regard to the differential extraction with absolute and aqueous alcohol. The decrease of extractability with increasing time of fermentation is also evident, but to a much greater degree than with total polyphenols.

3.3. Total amino acids

Table 3 shows the results of analyses for total amino acids in aliquots of the extracts previously examined for content of polyphenols.

TABLE 3. Changes with time of fermentation of total amino acids in dhools and black tea (mg/g dry weight)

Time of fermentation (min)	Absolute alcohol extract		Aqueous alcohol extract		Total	
	Dhool	Black Tea	Dhool	Black Tea	Dhool	Black Tea
30	8	5	5	16	13	21
60	7	4	6	16	13	20
90	6	3	6	17	12	20
120	6	3	7	17	13	20
150	7	3	6	16	13	19

Total amino acids estimated by the ninhydrin method of Yemm and Cocking,[6] by reference to a standard curve prepared with α—alanine.

The results show that firing leads to a decrease in total amino acids extractable by absolute alcohol, while the amount extracted by aqueous alcohol is significantly increased. It is also seen that firing leads to an increase in total amino acids, which is independent of the period of fermentation.

3.4. Essential Oils

Table 4 depicts the weight of steam distillable essential oils in samples of dhool before and after firing to black tea.

TABLE 4. Changes with time of fermentation of essential oils in dhools and black tea

Time of fermentation (min)	Dhool (mg/g dry wt)	Black Tea (mg/g dry wt)	% Loss
30	0.13	0.12	8
60	0.20	0.08	68
90	0.19	0.12	36
120	0.24	0.08	65
150	0.26	0.19	28

The results indicate that there is a considerable loss of volatile compounds on firing.

Discussion

The polyphenols, and particularly the vanillin-reacting polyphenols, are responsible for the brightness and quality of tea liquors and their progressive decrease as the period of fermentation exceeds 1 h confirms the undesirability of unnecessary extension of fermentation. The decrease is particularly relevant in the production of instant tea where a decrease in extractability would seriously impair the economic viability of the process technology.

The results of differential extraction with absolute and aqueous alcohol indicate that firing leads to binding of the polyphenols to other components of the tea leaf. Such binding is indicated in the finding that aqueous, but not absolute alcohol, extracts the greater part of the polyphenolic compounds. This interaction is the primary cause for the reduction of astringency in black tea as compared to dhools, and suggests that differential extraction could provide a laboratory method for monitoring changes of astringency with fermentation time.

The increase of amino acids on firing is due to chemical and enzymic hydrolysis of protein at the elevated temperatures used, and their easy solubility in aqueous alcohol suggests that these amino acids are, for the greater part, bound in complexes containing polyphenols, proteins, and other compounds. Such complexes are likely to make an important contribution to the colour of tea liquors, and their formation is one of the useful reactions which occur during firing.

Essential oils are the class of compounds which are mainly responsible for the aroma of tea, and their considerable loss during firing would, at first sight, appear to be a disadvantage. However, tea aroma is due to a very complex mixture of compounds, some of which detract from flavour,[4] and the loss of these detrimental compounds may be expected to result in improved flavour. Recent work[5] has provided a means of recovering the essential oils lost during firing, and it has been found that these include compounds which could be used to enhance natural tea flavour.

References

1. ROBERTS, E. A. H. (1962). *Economic importance of flavonoid subtances : Tea fermentation In Chemistry of Flavonoid Compounds* T.A. Geissmann, ed. pp. 468-512. Pergamon Press, London.
2. SANDERSON, G. W. (1972). *The chemistry of tea and tea manufacturing. In Structural and Functional Aspects of Phytochemistry* V. C. Runeckles, ed. pp. 247-316, Academic Press, New York.
3. SWAIN, T. & HILLIS, W. E. (1959). *J. Sci. Fd. Agric.*, **10**, 63-68.
4. WICKREMASINGHE, R. L. (1978). *Tea In Adv. Fd. Res.* C. O. Chichester and E. Mrak eds **24**, 229-286. Academic Press, New York.
5. WICKREMASINGHE, R. L. & EKANAYAKE, A. (1978) Unpublished.
6. YEMM, E. W. & COCKING, E. C. (1955). *Analyst*, **80**, 209-213.