The Role of Peroxidase in Tea Fermentation

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Enzymes and phenolics play an important role in tea processing. One of the enzymes, orthodihydroxy phenol specific polyphenol oxidase (PPO) contributes more to tea fermentation, which is essential for the development of aroma and colour of made tea. Extensive work has been carried out on PPO in relation to tea fermentation; however, earlier report indicated that PPO alone was not enough to characterize the tea clones. It is obvious that the collective response of more than one enzyme may decide the fermentation behaviour and hence the other enzymes involved in the process have to be investigated to understand the principles of tea fermentation. Such an information can be effectively used to improve tea quality.

In this context, another enzyme, peroxidase (PO) is of particular interest since PO is also involved in the oxidation of phenolics like PPO, but extremely specific for hydrogen peroxide (H_2O_2). The required H_2O_2 is also available in tea shoots and is formed during oxidation of certain flavanols by the activity of PPO. Although a few reports are available on PO, the exact role in tea fermentation is still obscure.

As such, the present investigation was undertaken to study the (i) existence of various fractions of peroxidase in different tea clones; (ii) the catalytic properties of tea shoot peroxidases using various substrates and (iii) the effect of exogenous addition of H_2O_2 on tea fermentation.

For the present investigation three clones namely, UPASI-3, UPASI-14 and SA-6 were selected based on the optimum fermentation time. Tender shoots comprising a terminal bud and three expanded leaves were used for analysis. Partially purified enzymes were separated on polyacrylamide gels (7.0%) and the activity staining was done using substrates such as benzidine, dianizidine, dihydroxyphenylalanine, catechol, pyrogallol, guaiacol, phloroglucinol, chlorogenic acid and gallic acid. The relative mobility (Rm) values were calculated against the bromophenol blue front.

It is evident from Table 1, that multiple molecular forms of isoperoxidase exist in the shoots of all the three clones studied. Tea shoot peroxidases catalysed all the nine different substrates and showed the capability of oxidizing various substrates such as phenolics, amine and aromatic diamines. Interestingly, a few slow migrating major fractions are having multicatalytic properties: different types of substrates can be catalysed by a single peroxidase fraction. These characteristics of tea shoot peroxidases add an advantage to its role in tea fermentation.

To get a further insight, various concentrations of H_2O_2 were added exogenously to the 'dhool' in order to enhance the level of endogenous PO activity. It was observed that H_2O_2 treated 'dhool' turned more brown than the untreated 'dhool'. Preliminary biochemical analysis of the quality parameters showed changes in the total liquor colour, theaflavins, thearubigins and high polymerized substances (Table 2), indicating that the changes are due to enzymic oxidation rather than non-enzymic oxidation.

It may be made out from the investigation that the existing multiple molecular forms of peroxidase are capable of oxidising various types of substrates. The slow migrating major isoperoxidases showed multicatalytic properties. Exogenously added H_2O_2 may enhance certain liquor characters of CTC black tea.

Table 1. Electrophoretic characteristics of tea shoot peroxidases on various substrates.

	Substrates	Number of isoperoxidases detected		
		UPASI-3	UPASI-14	SA-6
	Phenolic compounds			
1.	Catechol	4	4	3
2.	Pyragallol	3	4	3
3.	Guaiacol	3	4	3
4.	Phloroglucinol	2	3	2
5.	Chlorogenic acid	2	2	2
6.	Gallic acid	3	4	2
	Amine			
7.	Dihydroxyphenylalanine (DOPA)	3	4	3
	Aromatic diamines			
8.	Benzidine	5	8	4
9.	O-dianizidine	4	7	4

* Relative mobility (Rm) values may vary among the clones.

Table 2. Influence of hydrogen peroxide on tea fermentation

	Infusion characteristics *			
	Total liquor colour	Theaflavin %	Thearubigins %	High polymerized substances %
Control	3.52	0.75	6.34	11.23
0.05 % H_2O_2	3.53	0.72	6.67	11.13
0.10% H_2O_2	3.64	0.74	6.83	11.78
0.15% H_2O_2	3.52	0.77	6.78	11.46
0.20% H_2O_2	3.78	0.82	7.03	11.95

* Values are mean of two separate experiments