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Changes in enzyme activities (polyphenol oxidase and phenylalanine ammonia lyase) with type of tea leaf and during black tea manufacture and the effect of enzyme supplementation of dhool on black tea quality

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Variation in polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities with respect to different cultural and manufacturing processes and their effects on black tea quality were studied. There was a wide variation between enzyme activities of different clones, as well as variation due to seasonal changes and shoot maturity. Field practices such as plucking rounds and pruning had a great impact on enzyme activities. The enzyme activities positively correlated with tasters' scores. The extent of change in enzyme activities at different stages of manufacture differed widely. The loss of activity during withering could be restored by rehydration. Residual activity was observed in made tea. Supplementation of enzymes enhanced the black tea quality markedly in terms of cuppage and creaming properties. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The tea of commerce comes from plants belonging to *Camellia sinensis* (L) O Kuntze (Sanderson, 1972; Robertson, 1992). Only the tender shoots of the plant are processed, as they are a rich source of polyphenols and important tea enzymes (Bhatia and Ullah, 1968; Forrest and Bendall, 1969; Ota *et al.*, 1968; Jain and Takeo, 1984). Polyphenol oxidase (PPO) is the enzyme of most interest in the tea plant because of its key role in tea fermentation during black tea manufacture (Roberts and Myers, 1960; Takino and Imagawa, 1963; Bhatia and Ullah, 1968; Anderson *et al.*, 1992). It catalyses the oxidation of the catechins and leads to the formation of black tea pigments, namely, theaflavin, thearubigins, etc. (Robertson, 1992; Bailey *et al.*, 1992). Phenylalanine ammonia lyase (PAL) plays a vital role in the biosynthesis of flavanols (Sanderson, 1972), the prime substrate for PPO and is intimately involved in tea quality (Roberts and Fernando, 1981).

In the tea trade, the market value depends mainly on the quality of the brewed liquor and the texture or appearance of the made tea. Colour and cuppage attributes are of considerable importance in the tea industry. Pectinase and cellulase have a bearing on this, as they act on glucose polymers, which are important structural constituents of the cell wall and help by improving the infusion quantity and quality (Thomas, 1974, 1977; Novo Industri A/S, 1984). The phenomenon of creaming is also of considerable technological importance in connection with the evaluation of the quality of tea brews (Martin *et al.*, 1986; Roberts, 1963; Smith, 1968). Thus, the expediency of improving the commercial processing techniques to produce greater cuppage, through higher water solubles and better creaming properties, is one of the main objectives of tea manufacture.

The present experiment was designed to study the enzyme profile from cultivation to consumption, in order to assess the optimum processing conditions and to enhance the cuppage and quality of tea by enzyme supplementation.

MATERIALS AND METHODS

Tea leaves were harvested from the experimental farm of UPASI TRI, maintaining the three and a bud

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standard. The experimental plot was maintained according to recommendations of the tea scientific department (UPASI, 1996). UPASI-3, UPASI-9 and UPASI-17 represent three genetically diverse cultivars, of the Assam, China and Cambod varieties, respectively. SA-6 (China) is a poor quality cultivar and TRI-2024 (Cambod) and CR-6017 (Cambod) are two commercial cultivars.

The enzyme preparations were made by homogenizing the tissue in chilled acetone with acid-washed glass powder, using a pre-chilled glass pestle and mortar. The homogenate was rapidly filtered through a cotton wool plug in a funnel. The plug was washed free of phenolics, first with chilled acetone, then with cold aqueous acetone (80:20, acetone:water, v/v) and finally again with acetone. The white powder ('acetone powder') so obtained was dried under vacuum and used for preparation of the enzyme extracts.

The soluble components of the enzymes were extracted from the acetone powder by gentle grinding in a pestle and mortar with distilled water (1:10, w/v), followed by centrifugation at 5000 rpm for 15 min and separation of the supernatant ('soluble enzyme'). The residue was subsequently extracted by regrinding in 0.2 M Na_2SO_4, followed by centrifugation and collection of the supernatant ('bound enzyme') (Singh and Ravindranath, 1990).

PPO (EC. 1.10.3.1) was assayed spectrophotometrically at 380 nm in a Hitachi Model 150-20 spectrophotometer with 0.3 ml of 0.01 M D-(+)-catechin as subtrate. Sufficient enzyme preparation was added to achieve a linear increase in A with time. The temperature of the assay mixture was maintained at 20°C. pH was not adjusted as it did not vary much from the optimal pH value of 5. One unit of activity is defined as 1 μmol of catechin oxidized per minute by 1 g acetone powder. PAL (E.C.4.3.1.5) was assayed at 273 nm with phenylalanine as substrate. One unit of activity is defined as 1 μmol cinnamic acid formed per hour by 1 g acetone powder.

Addition of enzyme was carried out by mixing the commercially available enzymes with freshly cut dhool and carrying out manufacture as usual. It was found that 0.5% (w/w) PPO or a mixture of macerozyme (pectinase) and cellulase [0.45% (w/w) of each] gave the best results. The analysis of various quality parameters were according to the procedures reported elsewhere (Obanda and Owuor, 1995; Ravichandran and Parthiban, 1997). The organoleptic evaluation was performed by professional tea tasters, on a scale of 0 to 10 marks each for texture, brightness, quality and aroma.

RESULTS AND DISCUSSION

There have been many efforts to establish genotypical and process variables in enzyme contents and their substrate contents in many plants (Sanderson, 1972; Robertson, 1992). Although differences in content of the different enzymes could play an important role in the evaluation of cultivars, few studies have reported on tea. A wide variation in PPO and PAL activities with respect to different clones was observed (Table 1). The PPO and PAL activities of tea shoots in different clones ranged from 14 to 46 and 8 to 21 units, respectively. The PPO activity of the clone UPASI-3 was more than three times that of clone SA-6, which is in line with the fact that the former is known to be a fast-fermenting clone (UPASI, 1996). The same trend was again observed with PAL activity. Clone SA-6 had the least activity of 5 units, while clone UPASI-3 had four times this value. The black teas made from these clones were sent for organoleptic evaluation and the scores reported by professional tasters correlated well with the activities of PPO and PAL.

Table 1. Clonal variation of PPO and PAL activity (units) with sensory evaluation of black tea[a]

Clone	PPO[b]	PAL[c]	Taster's score
UPASI-3 (Assam)	46 ± 0.51	21 ± 0.39	39
UPASI-9 (China)	36 ± 0.44	16 ± 0.31	36
UPASI-17 (Cambod)	29 ± 0.19	8 ± 0.33	34
SA-6 (China)	14 ± 0.26	5 ± 0.33	29
TRI-2024 (Cambod)	39 ± 0.30	19 ± 0.37	37
CR-6017 (Cambod)	41 ± 0.31	20 ± 0.33	36

[a]Average of three trials in triplicate as mean with standard deviation.
[b]1 unit = 1 μmol of catechin oxidized/min/g acetone powder.
[c]1 unit = 1 μmol cinnamic acid formed/h/g acetone powder.

The seasonal variations in PPO and PAL activities were studied in UPASI-9 at regular intervals (Table 2). It can be seen that both PPO and PAL activities are lowest during the drought season (January–March), followed by July to September. However, they were greater during the high crop season (April–June), followed by October to December. Thus the climate, i.e. water availability, has a direct positive correlation with enzyme activity. Here also the tasters' score showed a positive relationship with enzyme activity. It can be visualized that high PPO and PAL activities will lead to the production of larger amounts of tea pigments and hence improved quality.

Table 3 gives the changes in PPO and PAL activities with shoot maturity. There is a marked decline in the

Table 2. Seasonal variation of PPO and PAL activity (units) with black tea sensory evaluations[a]

Season	PPO[b]	PAL[c]	Taster's score
January–March	29 ± 0.51	11 ± 0.22	32
April–June	38 ± 0.68	17 ± 0.15	38
July–September	33 ± 0.66	14 ± 0.19	35
October–December	35 ± 0.71	15 ± 0.28	36

[a]Average of three trials in triplicate as mean with standard deviation.
[b]1 unit = 1 μmol of catechin oxidized/min/g acetone powder.
[c]1 unit = 1 μmol cinnamic acid formed/h/g acetone powder.

Table 3. Changes in PPO and PAL activity (units) with shoot maturity and sensory evaluation of tea[a]

Shoot maturity	PPO[b]	PAL[c]	Taster's score
Shoot component			
Bud and 1st leaf	39 ± 0.39	18 ± 0.45	41
2nd leaf	34 ± 0.31	15 ± 0.41	36
3rd leaf	31 ± 0.33	14 ± 0.46	32
4th leaf	29 ± 0.37	11 ± 0.31	26
Stem	9 ± 0.20	5 ± 0.21	—
Plucking interval			
7 day	32 ± 0.36	13 ± 0.39	36
14 day	34 ± 0.31	15 + 0.31	36
21 day	31 ± 0.31	14 ± 0.37	35
Years from pruning			
1 year	33 ± 0.33	13 ± 0.22	33
2 year	31 ± 0.24	13 ± 0.29	34
3 year	29 ± 0.22	11 ± 0.31	33
4 year	28 ± 0.26	10 ± 0.33	34

[a]Average of three trials in triplicate as mean with standard deviation.
[b]1 unit = 1 μmol of catechin oxidized/min/g acetone powder.
[c]1 unit = 1 μmol cinnamic acid formed/h g acetone powder.

enzyme activity with the maturity of shoot and the stem contains the least. The taster's score increases with the tenderness of the shoot. Thus, the standard of plucking (number of leaves plucked) has a direct impact on quality and hence price of tea. However, due to the ever-increasing production costs, including wages, and the non-availability of labour, priority has to be given to yield (harvest) rather than quality.

The plucking interval (Table 3) should also be optimized, as variation in enzyme activities occurs in both of the cases studied. Pruning is important to maintain the plucking table height and it also leads to more growing than dormant buds. Hence it is considered to have a great effect on productivity. The PPO and PAL activities were found to be highest in recently pruned bushes and to decline slowly with the age of pruning. However, the tasters' scores remained steady.

The impact of processing conditions on PPO and PAL activities is shown in Table 4. Both the enzyme activities were found to decrease upon mechanical injury caused to the fresh leaf during haulage. This is due to the onset of fermentation at the site of injury. A reduction in activity was again noticed with the degree of wither. This reduction in activity is due to the loss in green leaf moisture content, as also reported elsewhere (Ullah and Roy, 1982). The original activity is restored upon rehydration. This emphasizes the need to control the moisture content of the green leaf in order to retain maximum PPO activity for better fermentation and hence better quality tea. The increase of 3 units in PPO activity (35 ± 0.29/32 ± 0.21) and 2 units in PAL activity (14 ± 0.11/12 ± −0.08) on rolling (maceration) is due to the gradual dissolution of the chloroplast membrane and release of PPO and cell sap (moisture) with the progress of cutting (Robertson, 1992). Thus, it leads to enhanced activity in the initial stage of fermentation, which is subsequently reduced due to the formation of brown tea pigments (product inhibition) (Marimuthu, 1996). Again, a rise in activity occurred in the initial stage of firing and this might be due to enzyme activation due to a sudden rise in temperature (heat-shock) (Marimuthu, 1996). However, a marked decline was noticed with drying time due to the denaturation of enzymes (proteins) at high temperature (150°C). Residual activity of PPO, but not of PAL, was noted in the dried black tea and this is expected to alter the quality of end-product on storage. This study shows very clearly how the enzyme activity, which has a direct correction with quality/price, changes as manufacture progresses.

Preliminary studies have also indicated that PPO activity can be enhanced by exposure of green leaf to red light (by 2 units), soil application of copper (by 4 units) and ethylene treatment of roots (by 2 units). Details are not given here.

Attempts were made to supplement the green leaf with enzymes to improve quality. The addition of PPO to cut dhool of the SA-6 clone markedly improved all the quality parameters of black tea (Table 5). It mainly improved the brown pigment content (TF, TR and HPS) and hence the colour index and total soluble solids. The aroma did not change much. The cream index increased. The tasters' scores and their price valuations were improved. The changes observed in

Table 4. Effect of manufacturing techniques on units of PPO and PAL activity[a]

Process	PPO[b]	PAL[c]
Fresh leaf (intact)	37	15
Injured leaf	33	12
Withered leaf		
3 h	35	13
6 h	34	14
9 h	34	13
12 h	32	12
Soft (70%)	33	14
Normal (65%)	30	12
Hard (60%)	28	10
Rehydrated	36	14
Rolled leaf	35	14
Fermented dhool		
15 min	39	11
30 min	39	11
45 min	34	9
60 min	21	6
Fired tea		
1 min	24	7
10 min	9	3
20 min	4	0
30 min	2	0
Made tea (6-months-old)	0	0

[a]Average of three trials in triplicate as mean with standard deviation less than 1.3%.
[b]1 unit = 1 μmol of catechin oxidized/min/g acetone powder.
[c]1 unit = 1 μmol cinnamic acid formed/h/g acetone powder.

Table 5. Effect of addition of PPO on black tea quality of clone SA-6[a]

Parameter	CTC process		Orthodox process	
	Control	Treated	Control	Treated
Theaflavin (%)	0.9	1.2	0.8	1.3
Thearubigin (%)	9.7	10.3	8.5	10.0
High poly. subs. (%)	6.7	8.0	6.0	7.7
Caffeine (%)	3.3	3.3	3.4	3.3
Colour index	5.2	6.6	5.5	7.3
Briskness index	21.4	26.7	19.0	28.3
Total liquor colour	2.9	3.1	2.6	2.8
Tea dregs (g)	6.2	5.6	6.5	6.0
Total soluble solids (%)	37.3	43.4	35.1	40.3
Cuppage	247	264	228	244
Cream index	7.8	9.9	6.9	8.2
Flavour index	1.4	1.5	1.7	1.8
Taster's score				
Texture	5.0	5.0	4.5	4.5
Briskness	4.5	5.5	5.0	5.5
Quality	4.0	4.5	5.0	5.5
Brightness	4.0	5.0	4.5	5.5
Aroma	2.5	3.0	3.5	4.0
Valuation (Rs/kg)	43.5	49.5	46.0	51.5

[a]Average of three trials in triplicate as mean with standard deviation less than 1.1%.

orthodox tea were similar, both teas being manufactured from the same leaf. Thus, addition of PPO helps oxidation and production of brown pigments, thus improving the value of black tea.

The presence of pectic substances (fibres) might restrict the infusion process and hence an experiment was conducted to study the effect of pectinase/cellulase on the liquor quality. Pectinase was added to cut dhool before fermentation and the tea was manufactured as usual. Tea made in this way showed a marked enhancement in liquor colour and water-soluble solids (Table 6). There were practically no changes in the other quality parameters. This is due to the action of pectinase on pectin which enhances the infusion rate. The tasters' scores and the price realizations also had positive trend. The liquoring ability was better in CTC tea than in orthodox tea. The results suggest that a combination of these two sets of enzymes might further enhance the quality and price realization, as their mode of action is totally different. PPO helps in pigment production and pectinase/cellulase helps with release. Both types of enzyme are present in the tea shoot (native) and so are not foreign materials.

Table 6. Effect of addition of macerozyme–cellulase mixture on black tea quality of clone SA-6[a]

Parameter	CTC process		Orthodox process	
	Control	Treated	Control	Treated
Theaflavin(%)	0.8	0.9	0.9	1.0
Thearubigin (%)	8.2	8.9	7.8	8.2
High poly. subs. (%)	6.5	6.9	6.1	6.3
Caffeine (%)	3.1	3.2	3.2	3.1
Colour index	5.4	5.7	6.5	6.9
Briskness index	20.5	22.0	22.0	24.4
Total liquor colour	3.2	3.5	3.0	3.3
Tea dregs (g)	5.9	4.2	6.1	4.8
Total soluble solids (%)	40.3	57.2	38.6	51.6
Cuppage	266	300	253	283
Cream index	7.6	8.8	6.5	7.6
Flavour index	1.4	1.4	1.6	1.6
Taster's score				
Texture	5.0	5.5	4.5	5.0
Briskness	4.5	5.0	5.0	5.5
Quality	4.0	4.5	5.0	6.0
Brightness	4.5	5.0	5.0	5.5
Aroma	2.5	2.5	3.5	3.5
Valuation (Rs/kg)	43.0	47.0	45.5	48.5

[a]Average of three trials in triplicate as mean with standard deviation less than 1.4%.

The present study outlines the significance of enzymes to black tea quality by demonstrating the changes they undergo during various cultural and manufacturing operations. The work has led to the technology of enzyme supplementation, which is considered vital in improving the tea quality and hence the price and profitability.

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