

EFFECT ON POLYPHENOL OXIDASE ACTIVITY IN EXTRACTS OF TEA LEAF PREPARED BY MACERATION AT DIFFERENT TEMPERATURES

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Development of alluring bright-red or blood-red colour by polyphenol oxidase is the most important factor in Bangladesh Black Tea. During processing, the temperature for optimum enzyme activity has been identified. On the basis of this, an equation and graph have been derived to describe the enzyme activity that would produce the best quality black tea, during the industrial processing of tea leaf.

INTRODUCTION

The quality of tea is greatly influenced by the enzyme, polyphenol oxidase. Temperature plays an important role in controlling its activity. In orthodox manufacture Harley (1963) observed that after every 30 minutes rolling in Sirocco or Jackson Rapid Roller, there was a simultaneous cooling effect on tea, when the leaves were taken out to sieve the small fractions.

Vickreasinghe *et al.* (1963) conducted a comparative study of the proportions of oxidised to unoxidised and partially oxidised polyphenols in black tea manufactured by the orthodox, Rotorvane, Cutting, Tearling and Curling (CTC) and Clivemere methods. They found by quantitative measurement that the proportion of oxidised and partially oxidised polyphenols was low in teas manufactured by CTC and Clivemere with a relatively high concentration of partially oxidised compounds in CTC teas. It was observed by Roberts (1961) that the fermentation of black tea was essentially enzymic oxidation of polyphenolic substances to theaflavins and thearubigins that contribute to characteristic colour, briskness and strength to tea liquor.

According to Wickreasinghe (1967), the manufacture of tea is a biochemical process, brought about by enzyme systems which are responsible for the development of an important part of the quality and colour of tea liquors. Heath (1958) observed that the cooling of macerated leaf (Dhool) improved the quality. Dev and Choudhury (1969) found that different enzyme systems were responsible for the characteristic colour, taste and flavour of tea liquor.

Sam, Molla and Millin (1985) found that higher average temperature (42.6°C) at CTC cutting point caused deterioration of the quality of tea over that cut at 31°C. This is evident from measurements of theaflavin content in black tea samples, which was found to bear a negative correlation to temperature.

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Based, on these findings and observations, a simulated experiment was set up in the laboratory to study the effect of temperature during maceration on polyphenol oxidase activity.

MATERIALS AND METHODS

- a) Controlled temperature circulating water bath, Gallenkamp Thermostirrer - 85
- b) Rangs oxygen electrode, fitted with magnetic stirrer and Phillips Recorder MP B215.
- c) Oxygen meter, MSE spectroplus, MSE Scientific Instruments.
- d) Beat Beater Leaf homogeniser Bartenesnell okla 74005 fitted with jacket for ice or hot water.
- e) 10 mM citrate buffer, pH 5.6.
- f) Pyrocatechol 100 mM solution in citrate buffer.
- g) Enzyme from Bangladesh Tea 1 (BT1) clonal leaf at two leaves and a bud stage.

The method described by Islam, Molla and Millin (1985) was followed. The results presented are the means obtained from 3 to 4 readings. Two grams of green leaf were macerated in the Beat Beater homogeniser with cooled citrate buffer at pH 5.6 at maximum speed (14000 rpm) for 5 minutes. The outer jacket of the homogeniser containing the leaf under investigation was filled with ice, ice-water, cold water (20°C) or hot water at varying temperatures ranging from 20 to 100°C. The temperature of the macerate was measured after each maceration and 0.3 ml of the macerate was injected into the reaction chamber containing 3 ml of 100 mM pyrocatechol which had been saturated with air for 5 minutes at 25°C. The rise or fall of oxygen concentration in the substrate was measured by attaching the reaction chamber to a digital oxygen meter.

After injecting 0.3 ml of the macerate, the concentration of oxygen in the reaction chamber was recorded in the chart recorder. The flow of chart was maintained at 60 mm per minute. Two grammes of the samples were also dried to calculate the results on a dry weight basis.

RESULTS AND DISCUSSION

The results on enzymic activity equivalent to micromole of oxygen utilised by polyphenol oxidase present in one gramme dry leaf are presented in Table 1.

TABLE 1 – *The effect of temperature on enzymic activity*

Temperature (°C) of the macerate	Enzymic activity equivalent to micromole of O ₂ utilized/g dry leaf
10	98.6
28	96.6
32	94.9
35	91.7
44	69.7
45	69.7
51	64.4
53	59.0
56	59.0
57	48.3
63	18.8
66	17.4
69	17.4
70	18.1
71	16.1
72	17.4

The results showed that during maceration the temperature of the homogenates could be maintained at values of 10, 28, 32, 35, 44, 45, 51, 53, 56, 57, 63, 66, 69, 70, 71 and 72°C.

The results also showed that enzymic activity decreased with the rise of temperature from 10 to 57°C after which it remained almost static. But there was no appreciable loss in polyphenol oxidase activity in the range of temperature from 10 - 32°C. The loss of polyphenol oxidase activity during the temperature of 32°C over that at 10°C was 3.8%. The findings are in conformity with the observations made by Islam, Molla and Millin (1985). The relationship between temperature and enzyme activity is shown in Fig. 1.

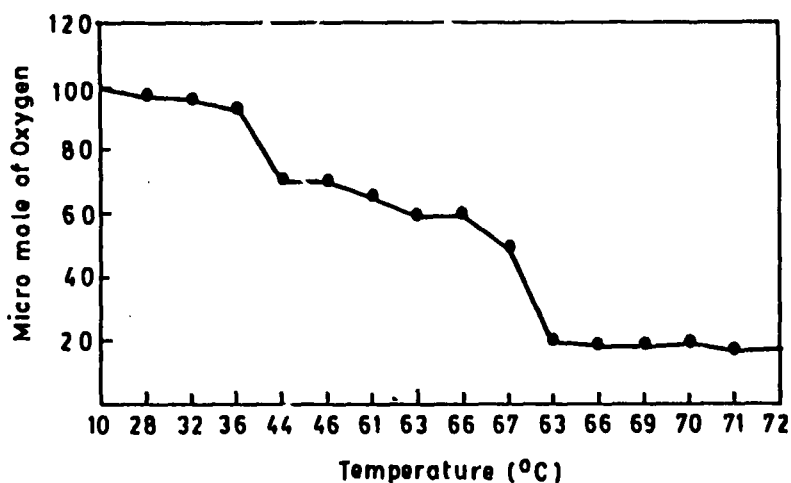


Fig. 1 – Relationship between temperature and enzymic (polyphenol oxidase) activity

Utilizing the above relationship an equation for enzyme activity in tea leaf macerated at different temperatures could be derived as given below:

$$Y = 17.56 + 0.5912 \times t - 0.02528 \times t^2$$

Where Y = enzymic activity
t = temperature (°C)

On the basis of the above equation, values of polyphenol oxidase activity in macerated tea leaf under different temperatures are presented in Fig. 2.

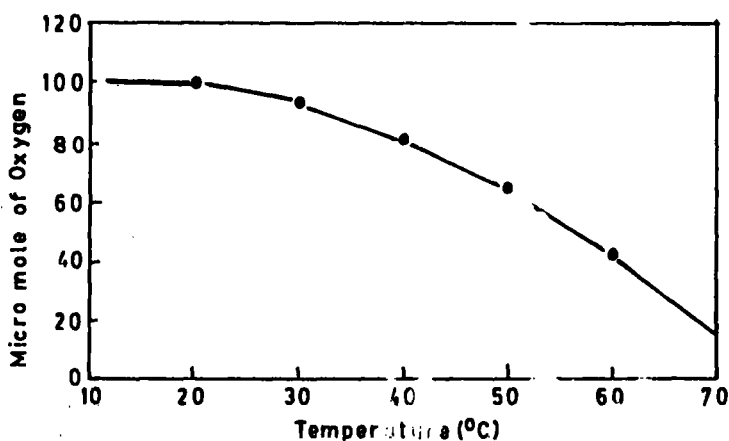


Fig. 2 – Expected relationship between temperature and enzymic (polyphenol oxidase) activity

Expected values of the enzyme activity at any particular stage of processing can be calculated by using the equation or the graph in Fig. 2. These would serve as useful guidelines for production of quality teas.

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