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STUDY ON THE CHANGES OF POLYPHENOL OXIDASE AND PEROXIDASE ACTIVITY DURING DRYING AND STORAGE OF TEA

A. C. Liyanage, P. A. N. Punyasiri, P. S. F. Perera and
M. T. Ziyad Mohamed
(Tea Research Institute of Sri Lanka, Sri Lanka)

A method has been developed to assay the enzyme activity in the fermented dhool. Using this method polyphenol oxidase (PPO) and peroxidase (PO) activity in the dhool were assayed during different stages of drying both in Endless Chain Pressure (ECP) drier and in Fluid Bed Drier (FBD) and also during storage. Results indicate that when fermented dhool is fired in either ECP or FBD drier the enzymes PPO and PO are not irreversibly denatured. Both enzymes become re-activated with absorption of moisture during storage of black tea.

INTRODUCTION

Polyphenol oxidase (PPO) and peroxidase (PO) play a key role during black tea manufacture. These two enzymes act upon the catechins present in the tea flush to form TFs (Theaflavins) and TRs (Thearubigins) which are responsible for the taste, colour and strength of a tea liquor. However, prolonged activity of these two enzymes reduce the TF content of the made tea and increase the proportion of TR content. Excessive amounts of TRs could be detrimental to the quality of tea (Millin et al. 1969).

Although it has been hypothesised (Millin, 1987) that the enzymes get denatured completely during the conditions experienced in drying *i.e.* high temperature and low moisture, this has still not been demonstrated. This could be attributed to product inhibition which is caused by formation of the complexes of oxidised polyphenols and enzyme proteins which in turn makes it difficult to assay the enzyme activity in the dhool. Therefore, a method was developed to assay the enzyme activity in the fermented dhool. Using this method PPO and PO activity in the dhool was assayed during different stages of drying both in ECP and in FBD and also during storage.

MATERIALS AND METHODS

Manufacture

Teas manufactured using the FBD at st. Coombs Estate, Talawakelle and ECP drier (Samaraweera, 1986) at Mattekelle Estate, Talawakelle were used in this study. Manufacture was by a modified orthodox-rotorvane procedure (De Silva, 1964) carried out on six occasions on each drier.

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Sampling from the FBD was carried out from the various compartments of the FBD and for ECP, sampling was done from each tray. The temperature of each sample was recorded immediately and from each sample, 5gm was used to determine the moisture content and 10gm was frozen immediately at -20°C and subsequently used for extraction of the enzyme and for the assays. On both occasions samples of fermented unfired dhool and fully fired teas from the drier mouth were taken for analysis.

Storage

Made tea samples (500g) from each of the series of six manufactures from the FBD were stored in brown paper sacks for two months to monitor the changes in residual enzyme activity with the absorption of moisture during storage. All samples were assayed at weekly intervals for residual PPO and PO activity and were also analysed for TFs, TRs and total colour (TC).

Preparation of the extract

The extract was prepared as described by Wickremasinghe and Perera (1972).

Removal of Oxidised Polyphenols

5ml of the extract was shaken on a mechanical shaker for 2hrs in 10ml of pre-swollen Sephadex LH-20 (medium) in McIlvaine buffer pH 5.5 at 4°C. The suspension was centrifuged at 2000g for 5mins. and the supernatant was used for the enzyme assay.

Assay for PPO

Assay for PPO was carried out according to the procedure previously reported (Takeo and Baker, 1973)

Assay for PO

PO activity was carried out by following the change in absorbance at 460nm due to o-dianisidine oxidation in the presence of H_2O_2 and enzyme. (McEwen, 1971)

Determination of TF, TR and TC

The method used for the determination of TF, TR and TC was that determined by Roberts and Smith (1961).

Determination of moisture content

The moisture content was determined by the standard AOAC gravimetric method (1970).

RESULTS AND DISCUSSION

The changes in enzyme activity of PPO and PO, moisture content, TF and TR in the dhool in relation to temperature in the FBD are shown in Fig. 1. For each parameter the values were expressed as percentage of the initial levels. This approach allowed the quantitative changes in each of the four parameters to be compared directly. The results indicate that, at the commencement of drying, the enzymes were still active and the

fermentation reactions take place and enzyme activity declines steadily with loss of moisture and increase in temperature as the drying continues. At temperatures above 40°C in the dhool particles, enzyme activity combined with lower O_2 solubility, becomes a major issue and the fermentation process appears to be inhibited. However, it was seen that around 50% of the initial activity (activity in the fermented unfired dhool) of PPO is retained at around 52°C and at this stage the moisture content of the dhool was around 27.7%. In the case of peroxidase, around 58% of the initial activity was seen at around 60°C. In the fully fired teas, both enzymes appeared to be inactive. It was also seen that with increase in temperature, the TF content decreased to around 16% and the TR content increased by about 35%. It was seen that the activity of PPO ceased altogether when the moisture content had fallen to around 13%. The time taken for this point to be reached is critical. If the drying is too rapid the outer layer of the leaf particle or the agglomeration of the leaf particles will harden and prevent diffusion of moisture from within. This phenomenon is called case hardening. On the other hand if the drying is too slow the period of high temperature fermentation is too long and an unpleasant taste develops. In this instance the tea is said to be stewed.

Once the enzyme activity has been completely arrested the precise temperature/time regime is less important as far as the enzymatic reactions are concerned. However, is desirable that the leaf reaches the highest possible temperature consistent with the avoidance of a burnt taste. This is to ensure the maximum possible enzyme inactivation.

Similar experiments were carried out using an ECP drier. The results of these experiments (Fig. 2) indicate that for PPO around 47% of activity of the fermented unfired dhool was still retained at around 49°C. The dhool acquires this temperature when the dhool reaches one but the last tray in the ECP drier. In the case of peroxidase, enzyme activity was observed throughout the drying process. Even in made tea, 42% of the initial activity of peroxidase was retained. It was also observed that the TFs decreased by 11% and the TR content increased by 40% with the increase in temperature to around 58°C.

A major problem associated with the conventional drying process is that all biochemical and chemical reactions accelerate during the period of time taken to denature the heat stable PPO and other enzymes deleterious to tea quality and reduce water content to approx. 3%. Moreover, prolonged activity of the thermostable oxidase enzymes reduce the TF content of the made tea and increase the proportion of the TR fraction.

Experiments were carried out to check the residual enzyme activity during storage. Enzyme assays were carried out on four samples of made tea having a moisture content of 4.80%, 7.06%. 12.66% and 12.70%. Fig. 3 shows the enzyme activity of teas during storage. The results indicate that at a moisture content of 4.8%, PPO activity and PO activity was 8.5% and 10.5% respectively of the activity in the unfired dhool. The enzyme activity increased with increase in the moisture content upto about 12.66% and decreased with further increase in the moisture content. Fig. 4 shows the changes in TF, TR and TC during storage. It is seen that at a moisture content of 12.70% the TF levels decreased by 11% of that found in black tea from the drier mouth while the total colour decreased by 7% and the TR% increased to around 17%. The decline in enzyme activity could be attributed to the depletion of the available O_2. Under such O_2-limiting conditions the formation of TR pigments

predominates. The presence of excessive TR pigments is organoleptically undesirable. Therefore, these results indicate that the enzymes get re-activated under conditions experienced during storage *viz* high ambient temperature and high relative humidity.

In bulk tea immediately after drying the moisture content is around 3% but this residual water content may not be uniformly distributed and it is possible that localised regions within the tea particle contain considerably higher amounts of water. In conventional driers hot forced-air currents rapidly remove the superficial layers of water and in so doing may cool the interior of the tea particle to temperatures below those required for continued evaporation of moisture and for the thermal inactivation of the enzyme. Clearly, enzyme activity could continue in such niches within the tea particle after the formal drying operation has been completed. Furthermore, the water vapor absorbed from the atmosphere during the first few weeks of storage will extend the aqueous environment and enhance the enzyme activity *in situ*. Thus these results make it clear that the conventional drying systems do not irreversibly denature the enzymes.

As such modifications to the existing driers which may result in increased enzyme inactivation should be explored. Approaches such as the introduction of Infrared, Ultraviolet, Microwave or solar energy to the fermented dhool prior to drying may irreversibly denature the enzymes during drying.

Such improvements would result in complete and extremely rapid inactivation of PPO and Peroxidase, a situation which conventional drying systems are unable to achieve. This in return would prevent the changes during the 'come up' time in drying as well as the transformation of TF to TR due to prolonged activity of the enzymes. Moreover, the introduction of a two stage drying system would in the long run reduce the cost of drying.

Thus it could be concluded that both FBD and ECP driers do not irreversibly denature the enzymes PPO and peroxidase. Both enzymes become reactivated with the absorption of moisture during storage of black tea.

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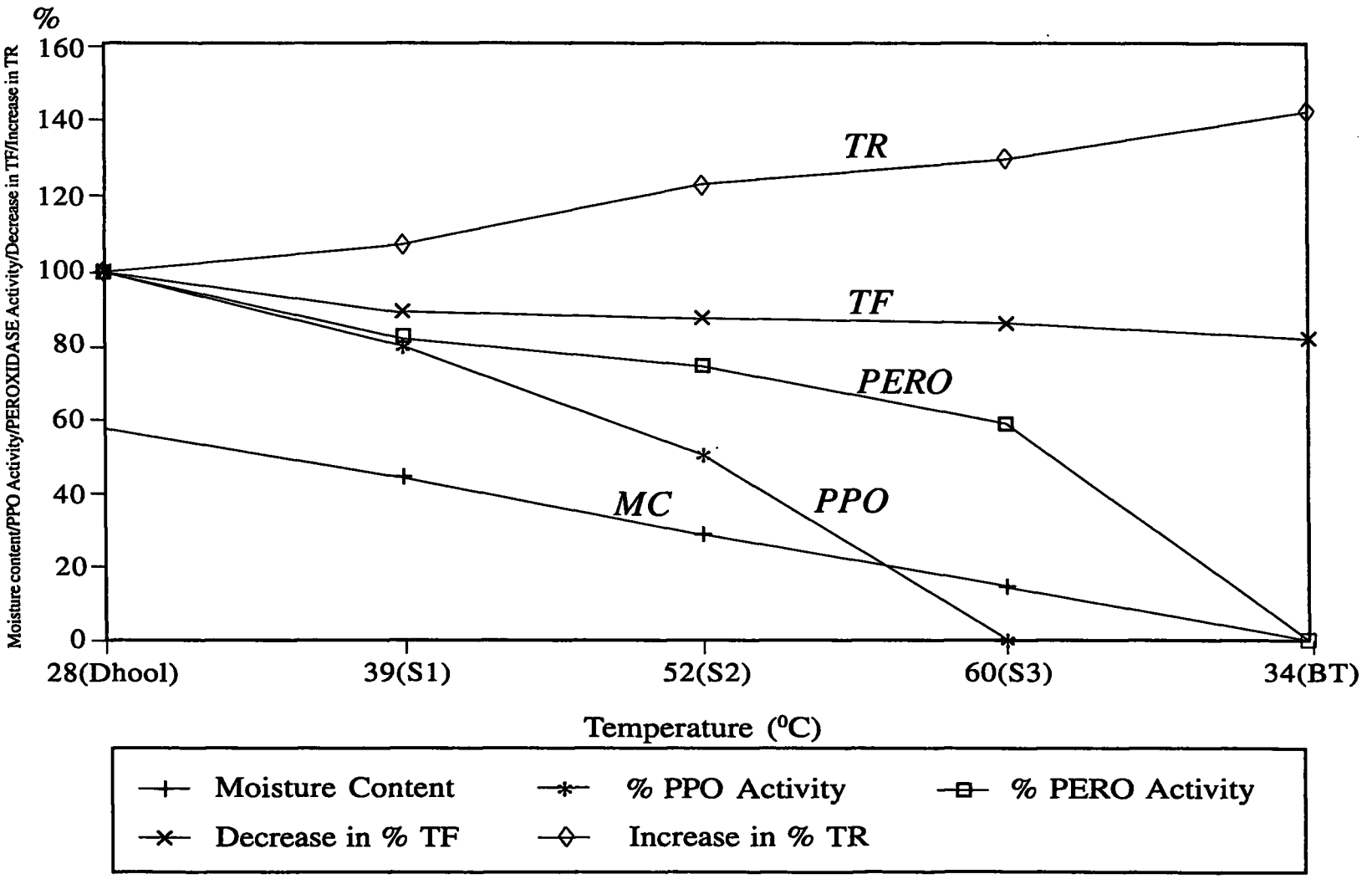
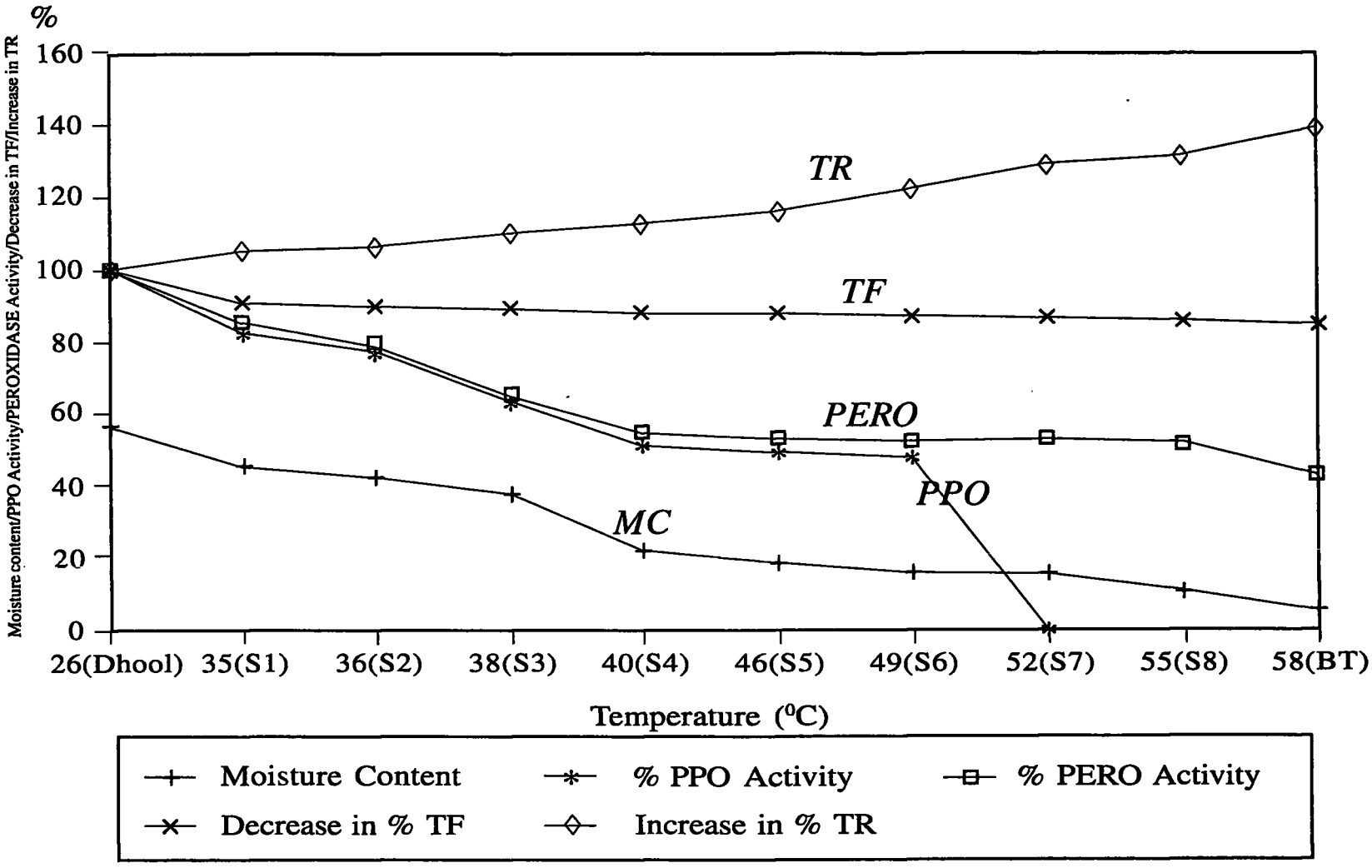


S1, S2, S3 – SAMPLES TAKEN FROM THE VARIOUS COMPARTMENTS OF THE FBD.
BT – BLACK TEA COMING OUT OF THE FBD.

Fig. 1 – *Changes in Enzyme Activity, Moisture Content, TF and TR in relation to temperature in the FBD*



S1 - S8 – SAMPLES TAKEN FROM THE VARIOUS COMPARTMENTS OF THE ECP.
BT – BLACK TEA TAKEN OUT OF THE ECP.

Fig. 2 – *Changes in Enzyme Activity, Moisture Content, TF and TR in relation to Temperature in the ECP*

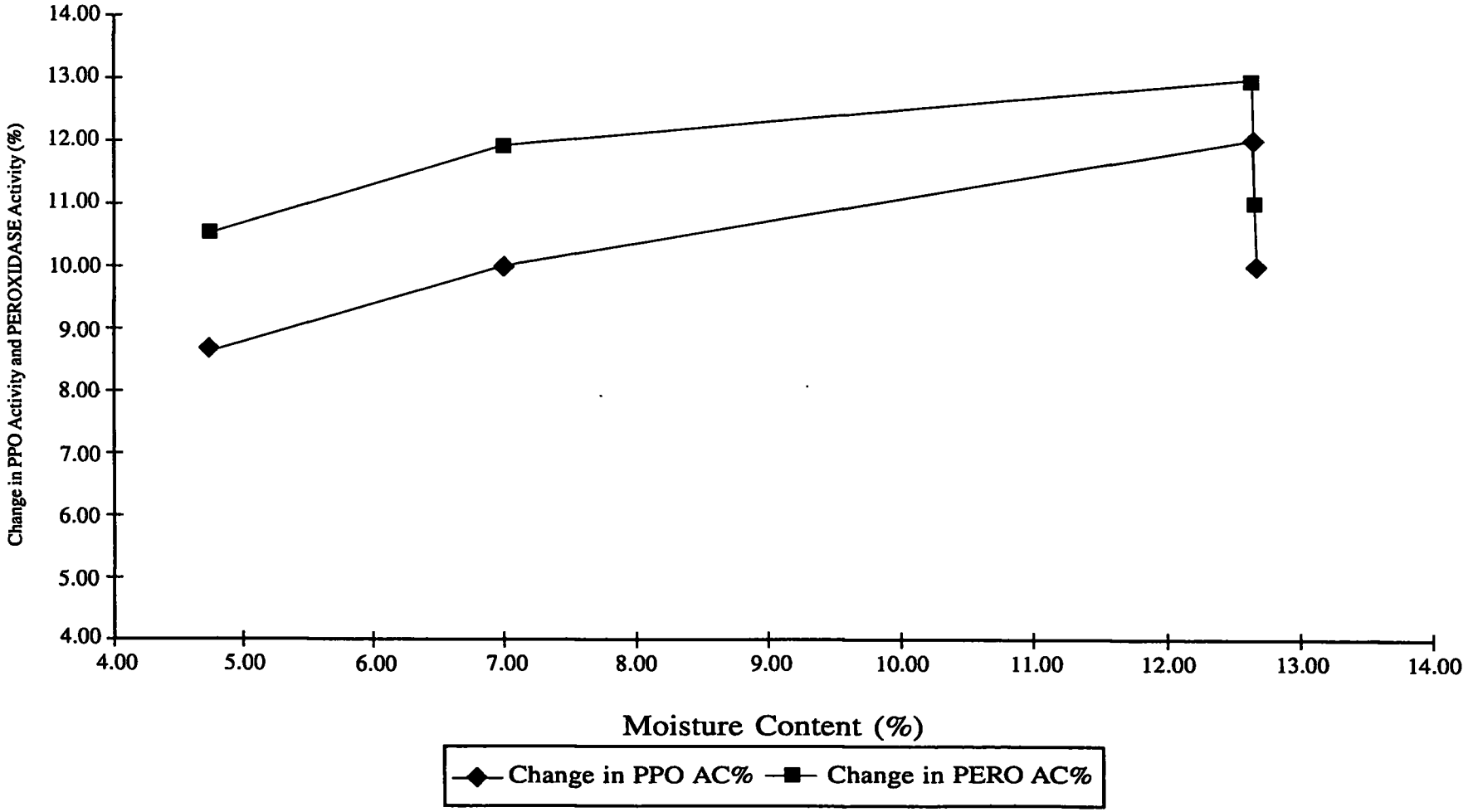


Fig. 3 – *Enzyme activity during storage of tea*

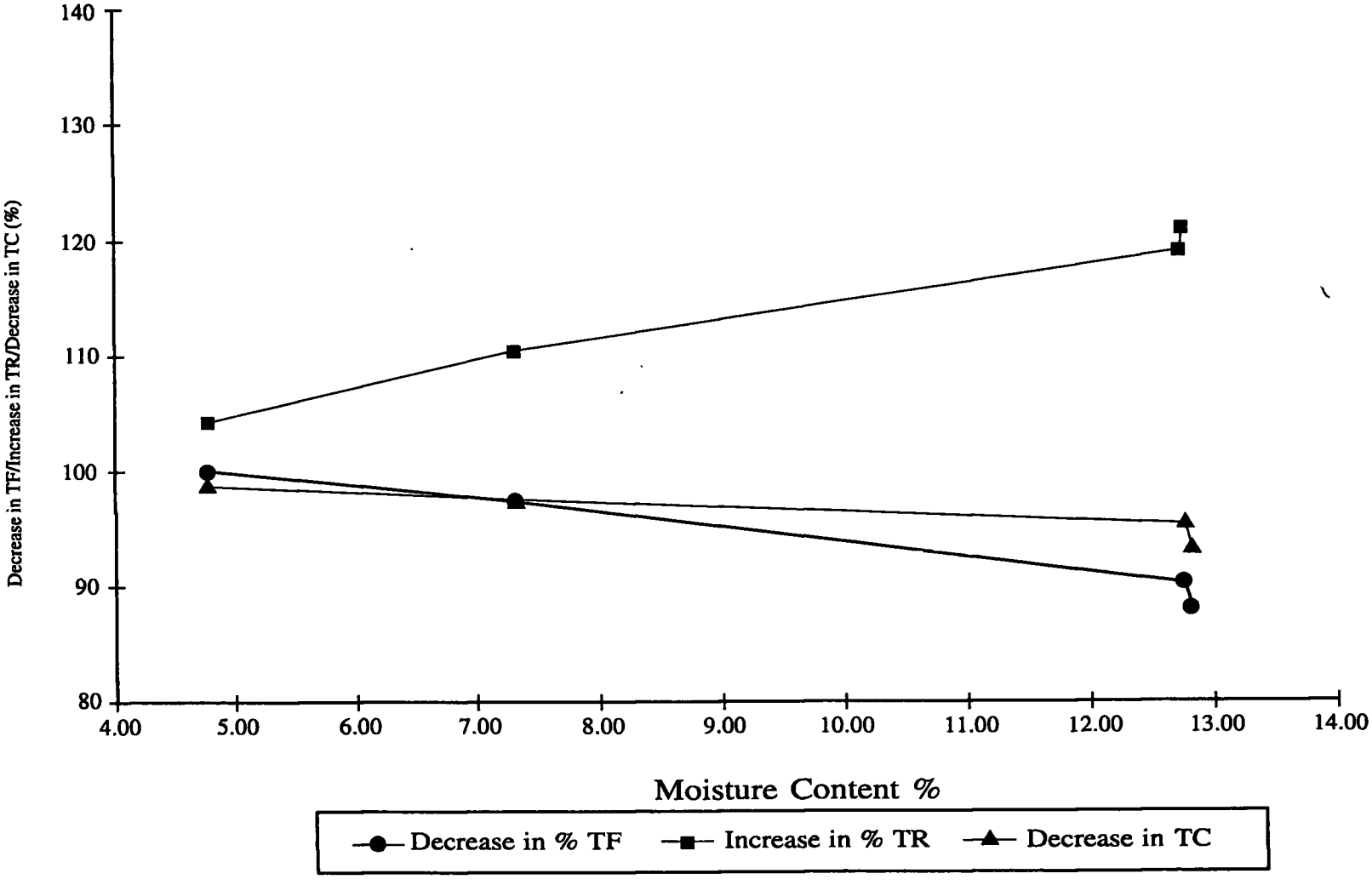


Fig. 4 – *Changes in TF, TR and TC during storage of tea*