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Biochemical changes in tea, *Camellia sinensis* (L.) O. Kuntze. during different stages of leaf processing

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ABSTRACT

Biochemical analysis of tea, *Camellia sinensis* (L.) O. Kuntze, collected from Darjeeling was undertaken in the present study to determine the pattern of quantitative biochemical changes that take place during the different stages of tea manufacture *i. e.* withering, rolling, fermentation and firing. It has been found that the contents of soluble carbohydrate, insoluble carbohydrate etc. show a steady decline in each step of processing from the green tea leaf to the black tea stage. However, the free amino acids content and polyphenol oxidase activity showed a steady increase in their percentage as tea leaf was passed through successive manufacturing steps.

Biochemical studies of Tea at different stages of processing provides an insight into the relative chemical changes which are of immense importance for the characteristics which goes into making the brew 'tea' and also for improving its quality in the long run. Work on the biochemistry of tea is quite impressive, but sadly enough the work on the Darjeeling tea is quite negligible so far. Darjeeling tea is manufactured by orthodox technique which involves the processing of plucked green tea leaves through withering, rolling, fermentation and firing steps to produce black tea.

Tea contains proteins, carbohydrates, amino acids, lipids, vitamins and minerals. The wide variation in chemical components helps in determining the quality of different types of tea. Tea leaves also contain a large range of enzymes of which the specific activity of two principal enzymes viz. polyphenol oxidase (PPO) and peroxidase (PO) are responsible for the formation of theaflavins and thearubigins[8]. These two groups of pigments largely determine the liquor characters of black tea[11]. Caffeine (1, 3, 7 trimethylxanthine) which is responsible for the taste and briskness of tea is present in tea leaf. With other xanthines, caffeine acts as an important quality index of tea. Several amino acids are present in tea leaves plucked for manufacture of which theanine identified as the 5–N–ethyl glutamine accounted for more than 50% of the amino acid content. Sugar and starch are present in small quantities in tea leaves. Loss of sugar takes place during tea processing and those getting reduced include glucose–6–phosphate and glucose–1–phosphate. Total phenols, amino acids, chlorophylls as well as polyphenol oxidase activity of tea declined with increasing disease severity which corresponds to the magnitude of aroma component decline[9]. Biochemical estimation of different constituents of tea in its different processing steps provides an idea of the chemistry of tea and probably how it influences the tea quality.

The present experiment was carried out with tea (*Camellia sinensis* (L.) O. Kuntze.) leaves in different stages of processing *i.e.* green leaves (GL), withered leaves (WL.) rolled leaves (RL) fermented leaves (FL) and black tea (BT) that were collected from the Happy Valley Tea estate, Darjeeling. The materials were brought to the laboratory immediately after collection and proceeded with the biochemical analyses. To extract free amino acid 100 mg of leaf samples were homogenized with boiling 80 % ethanol and centrifuged at 6000 rpm for 10 minutes. The supernatant was taken in a watch glass and evaporated to dryness. Traces of chlorophyll if any, adhering on the surface of the watch glass was carefully removed using solvent ether. The remaining material was taken in a test tube by washing it several times with 80% ethanol and the volume was made up to 10 ml. This formed the source of free amino acids. The level of free amino acids was quantified following the method of Moore and Stein[7] using a standard curve prepared from glycine.

For extracting carbohydrates 1 gm. of each leaf sample was homogenized with 10 ml. of 80% ethanol, centrifuged at 6000 rpm for 10 minutes, the supernatant taken in a watch glass and evaporated to dryness. The watch glass was washed several times with 80% ethanol and the volume was made upto 5 ml. The solution served as the source of soluble carbohydrates. The residue obtained after centrifugation of the sample was digested with 5 ml. of 25% H_2SO_4 at 80°C for 30 minutes and this extract formed the source of insoluble carbohydrates. The carbohydrates were estimated by the method of Mc Cready *et al.*,[6].

For the estimation of caffeine 15 gms. of tea leaves (20 gms. for GL and 5 gms. for BT) was extracted with 200 ml. of chloroform and refluxed for 90 minutes. The content was filtered through Whatman no. 1 filter paper. The Chloroform was filtered out and the remaining crude caffeine was dissolved in boiling distilled water, filtered and made up to 250 ml. with distilled water. The extract was diluted 40 times and the absorbance measured at 275 nm with a UV-spectrophotometer (Beckman DU-64). The caffeine content was determined with the help of a calibration curve prepared from pure caffeine[4]. For extracting the polyphenol oxidase 100 mg. of leaf sample was homogenized with 5 ml. of 0.05 M sodium phosphate buffer (pH-6.8), which was centrifuged at 6000 rpm for 10 minutes, the supernatant was collected and the volume made up to 10 ml. with the same buffer. This formed the crude enzyme extract, the activity of which was as per Fick and Qualset[3]. The moisture content of the tea leaves in different stages of processing was calculated by keeping a weighed quantity of sample in a hot air oven at 70°C for 48 hrs.

The occurrence of caffeine is one of the important quality indices of tea. In the present experiment the caffeine content was found to be the lowest in GL and highest in

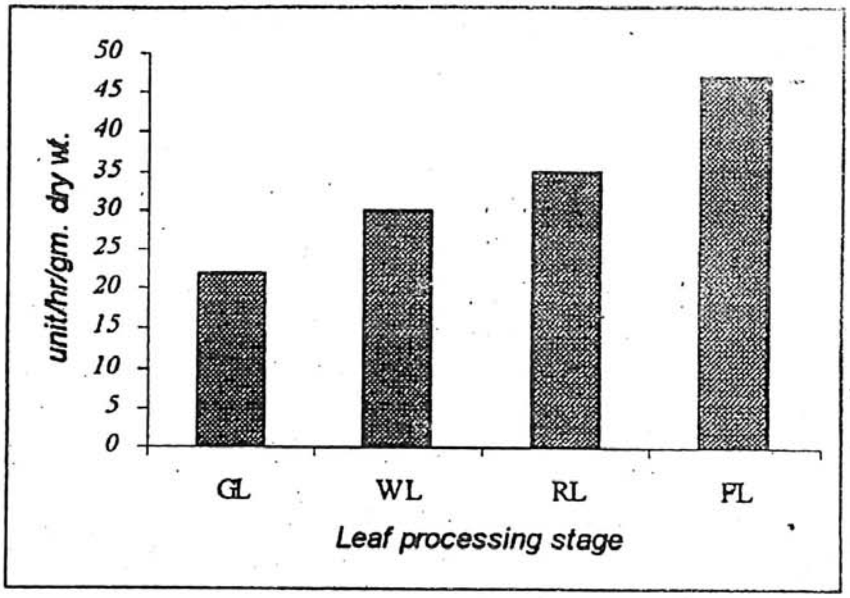

Fig. 1. Changes in polyphenol oxidase activity (unit / hr / gm dry wt.) during different stages of leaf processing (GL, WL, RL and FL) of tea, *Camellia sinensis* (L.) Kuntze.

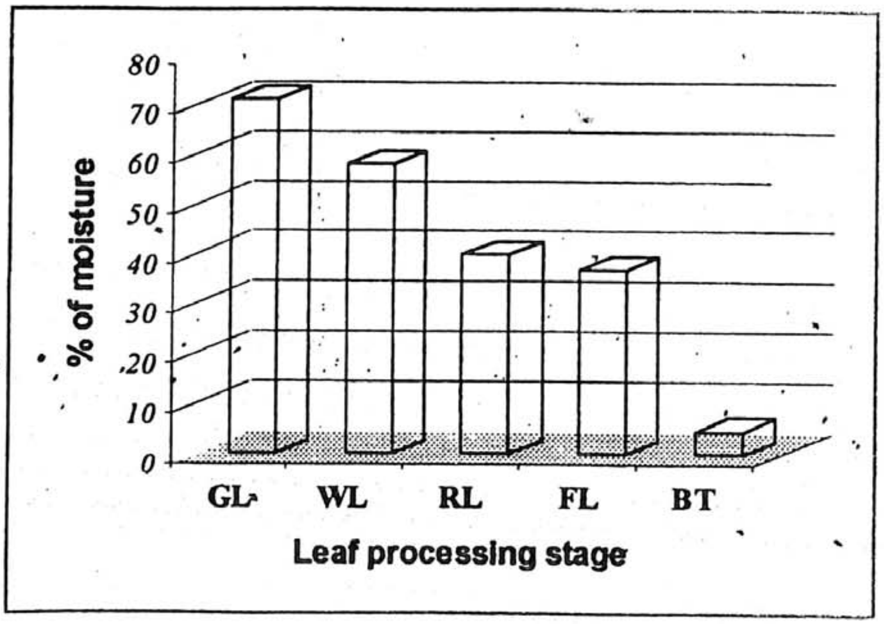

Fig. 2. Percentage of moisture in different stages of leaf processing (GL, WL, RL, FL and BT) of tea, *Camellia sinensis* (L.) Kuntze.

the WL (Table-1). The percentage of caffeine during different stages of manufacturing maintains the usual trend of increase in content during withering and gradual decrease during the subsequent steps[4]. During the processing of tea leaves the amino acid level shows a steady rise in content from GL to FL and a slight decline at the BT stage (Table-1). The steady increase in the amino acid level may be due to the presence of peptidase in the shoots which becomes active and breaks down proteins into amino acids[1]. In the final step of tea manufacturing some of the amino acids must have converted to thearubigins and theaflavins causing a decline in its level at the black tea stage.

Table-1. Changes in the contents of caffeine (mg/gm dry wt.) and free amino acids (mg/g dry wt.) during different leaf processing stages (GL, WL, RL, FL, and BT) of tea, *Camellia sinensis* (L.) Kuntze.

[values are mean ± SE of 3 replicates]

Leaf processing stage	Caffeine (mg/gm dry wt.)	Free amino acids (mg/gm dry wt.)
GL	17.38 ± 1.11	20.78 ± 0.23
WL	20.09 ± 1.35	28.01 ± 0.63
RL	19.01 ± 0 97	28.87 ± 4 52
FL	18.57 ± 0.23	30.31 ± 0.11
BT	18.43 ± 0.54	27.73 ± 2.62

Small quantities of soluble and insoluble sugars are present in the tea leaves. It has been found that maximum level of carbohydrates (both soluble and insoluble) occur in the green leaves while it decreases gradually in the processing steps reaching its lowest level in the black tea (Table 2). The decrease in the level of carohydrates may be also because of a part of sugar is metabolized into amino acids[2].

Table 2. Changes in the content of soluble and insoluble carbohydrates (mg/gm dry wt) during different leaf processing stages (GL, WL, RL, FL, and BT) of tea, *Camellia sinensis* (L.) Kuntze.

[values are mean ± SE of 3 replicates]

Leaf processing stage	Soluble carbohydrates (mg/gm dry wt.	Insoluble carbohydrates (mg/gm dry wt.
GL	80.05 ± 1.54	78.28 ± 1.12
WL	77.38 ± 0.47	73 78 ± 1.55
RL	77.30 ± 0.91	73.15 ± 1.07
FL	72.98 ± 1.62	68.50 ± 0.85
BT	72.01 ± 0.74	68 45 ± 1.10

Polyphenol oxidase (PPO) oxidizes polyphenols and plays a signigcant role for the preparation of black tea from green leaves. It is responsible for the formation of aromatic compounds[10]. In the present study PPO activity was found to increase starting from the green tea leaf stage till the fermentation step (Fig. 1). This increased PPO activity help in the fermenting of tea leaves. These oxidative changes are also supposed to be responsible for black tea aroma. The high temperature of firing completely jeopardizes the PPO activity and as such the activity was not measured for the black tea.

The determination of dry weignt clearly indicated that the moisture content of the tea leaf is highest in green leaf stage and remains same in the steps of rolled leaf and the fermented leaves. The slightly higher moisture content of 4.8 % in the black tea (fig. 2) may be due to the environmental factors like a high relative humidity and low temperature prevalent in Darjeeling.

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