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# Estimation of theaflavin content in black tea using electronic tongue

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ABSTRACT

Biochemical components like theaflavins (TF) play very important role in the quality of finished CTC (cut, torn, and curled) variety of tea. TF are known to provide characteristic astringency to the taste of finished CTC tea. The quality indicators like brightness, briskness, strength, color and overall quality of tea liquor are also due to the amount of TF present. A positive correlation is normally observed between the amount of TF and the quality scores of finished tea. Biochemical tests that yield the percentage of TF are often time consuming, require meticulous effort of sample preparation, storage and measurement. This paper proposes an alternative approach of quality evaluation of CTC tea by predicting the amount of TF that may be present in a given tea sample, using a voltammetric electronic tongue.



## 1. Introduction

Quality of tea has a distinct effect on the human palate and sensory organs. The chemical basis of tea quality is the concentration and composition of different chemical constituents present in tea that produce the desired effect on these organs. Although individual chemical components have distinct taste and flavor, the flavor and aroma of food products are generally due to a complex mixture of chemical compounds present in them and tea is not an exception. The special characteristics that distinguish black tea from other beverages are a result of enzymic oxidation of polyphenols that takes place during oxidation (fermentation) stage of black tea processing. This oxidation is brought about by certain oxidase enzymes present in tea shoots that produces two major groups of pigments known as theaflavin and thearubigin (Roberts, 1962) and the most important taste attributes for tea liquor, "briskness" and "strength" are mainly due to these two classes of compounds. Table 1 lists some important chemicals in tea responsible for taste (Mahanta, 1988).

The presence of TF contributes to increased level of astringency and brightness in CTC tea, whereas the presence of increased amount of TR is known to increase mostly the ashy taste with slight improvement in astringency and reduction in brightness. Theaflavins normally constitute about 0.5–2% of dry weight depending on the processing parameters of tea while thearubigins constitute about 6–18% of dry weight. TF imparts briskness and brightness and TR contributes to the mouth feel (thickness) and color of the tea (Biswas et al., 1973). It may be noted that TR has an ashy taste and is only slightly astringent while TF is astringent enough to affect the overall astringency of liquor and contribute to differences among quality of various clones. Spectrophotometric and human sensory panel studies suggested that TF content correlated positively with liquor brightness (Roberts and Smith, 1963; Ngurea et al., 2009). The TR content was however found to relate negatively with liquor brightness. Though the presence of TF is much smaller than that of TR, but black tea quality is mainly influenced by total theaflavins (Wright et al., 2002). Hence, the estimation of TF has been the basis of quality evaluation for tea by using instrumental methods.

The qualitative gradation of tea quality is presently carried out by the organoleptic senses of taste, vision and olfaction of "Tasters Panel". However, the quality index assigned by the tea taster is subjective in nature and also depends on their mental state. Thus, the authenticity and reproducibility of the quality scores could not be guaranteed. The biochemical methods like gas chromatography, high performance liquid chromatography and spectrophotometry can yield valuable and quantitative information regarding tea quality, in terms of concentration of major quality determining

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**Table 1**
Bio-chemical compounds in tea responsible for taste.

| Compounds | Taste |
|---|---|
| Polyphenol | Astringent |
| Amino acids | Brothy |
| Caffeine | Bitter |
| Theaflavins | Astringent |
| Thearubigin | Ashy and slight astringent |

biochemical compounds. However, these methods are expensive in terms of time, money and also require expert handling. Thus the limitations of "Tasters Panel" and complexity of biochemical evaluation have been the motivation towards finding an instrumental, objective and inexpensive means for tea quality evaluation. Towards this direction, were proposed some electronic devices like electronic nose (Bhattacharyya et al., 2008) and electronic tongue (ET) (Palit et al., 2010) that respectively mimics the sense of smell and taste of human sensory panel. However, all of the rapid measurement instruments mentioned above are calibrated on quality scores given by the tea tasters. These quality scores are strongly affected by the mental conditions and sensory adaptation of the tea taster and hence their validity could not be guaranteed. Thus, the response of soft computing modules trained by the quality scores could not be free of uncertainties. In order to circumvent this problem, an alternate approach of quality evaluation is the estimation of biochemical constituents using rapid measurement techniques.

The methodology to estimate the content of theaflavins for an idea about tea quality is not new. Initial reports for estimation of TF involved spectrophotometric measurements (Roberts and Smith, 1961, 1963). Later various chemical methods for extraction of TF from tea liquor for better spectrophotometric measurements were also proposed (Hilton, 1973; Ullah, 1986). Improved methods of estimation for TF and their fractions were reported using HPLC instruments (Collier and Mallows, 1971; Robertson and Bendall, 1983). These instruments gave accurate indications regarding the amount of target chemical and are also able to separate their fractions. As a result, HPLC instruments are very popular for this purpose. HPLC coupled with Mass spectrometry (HPLC–MS) allowed more sensitive detection. However, these instruments are costly and require specialized effort of sample preparation. Studies on the detection of TF using HPLC with photo diode array (HPLC–PDA) were also reported (Bailey et al., 1990). The detection became simpler but the cost of the instruments remained high. NIR detection using commercial instrument has been used for TF estimation in Hall et al. (1988). Recently some new techniques for estimation of TF have been reported. High-speed countercurrent chromatography (HSCCC) has been applied for the separation of theaflavins and catechins in Wang et al. (2008). A new capillary electrophoresis (CE) method was developed to quantitate the four major theaflavins occurring in black tea (Wright et al., 2001). Use of micellar electrokinetic capillary electrophoresis (MEKC) method was reported for detection of theaflavins in tea (Hsiao et al., 2010). All the above methods used costly instruments with complex procedures for extraction of theaflavins from tea extracts and detection of the same. But it is expected that the estimation of TF using an electronic tongue shall be a novel, rapid and cost effective alternative. Use of electronic tongue was reported to estimate the concentrations of chemical components in a sample, based on their combined taste (Campos et al., 2009; Labrador et al., 2010; Natale et al., 1997; Winquist et al., 2011). It has been used to evaluate the levels of tea astringency (Hayashi et al., 2006; Scampicchio et al., 2006) and also employed to differentiate among various grades of tea (Hong and Wang, 2009; Ivarsson et al., 2001; Palit et al., 2010). This paper makes an attempt to study the correlation of only TF content as a first place with the electronic tongue responses to demonstrate the feasibility of such an instrument for rapid biochemical estimation. The major advantages of an electronic tongue compared to analytical methods are the cost and rapidity of operation.

The paper is organized as follows. The biochemical transformations during tea processing are presented in Section 2. The experimental component is presented in Section 3 where the procedure for estimation of theaflavins using spectrophotometry is described followed by the electronic tongue setup and the sample preparation technique adopted for the experimentation. In Section 4 the different data analysis methods are described and results and discussions are presented in Section 5.

## 2. Biochemical transformations during tea processing

The quality of finished tea is related to its physico-chemical characteristics that largely govern the quality indicators for taste, namely briskness, brightness, strength and color of a tea sample. Black tea processing is a complex conjugation of some biochemical reactions in addition to various processing techniques. Initiation of rapid and significant biochemical changes to a desired level is the objective of tea processing. In order to achieve this objective, leaf after plucking is subjected to physical conditioning in the very first step and then sequentially twisted, crushed, torn and curled (CTC). After that, the tea leaves undergo a series of different stages such as rolling, fermentation and drying to have the ultimate product.

Out of the various stages, fermentation (oxidation) is one of the most important stages of processing. Fermentation (oxidation) is a process which starts right at the time of plucking. However, the process is really accelerated just after rolling and gains its highest momentum in the fermentation stage. The continued and rapid breakdown of cellular components during rolling could be partly due to release of some enzymes. The process of fermentation is mainly enzymic oxidation reaction, the principal enzymes that govern the oxidation process are polyphenol oxidase (PPO) and peroxidase (PO). The mechanical injury of leaves during rolling results in the above enzymes to come in contact with vacuolar polyphenols to start the process of fermentation (Roberts, 1962; Sanderson, 1972). Reactions that proceed during fermentation are dependent on mechanical maceration, pH, temperature and humidity. The fermentation room is ideally maintained at a temperature of 80–85 °F with hygrometric difference of 2 °F. This enzymic oxidation leads to the formation of series of orthoquinones which further undergo a series of inter and intra molecular non-enzymic condensation and oxidation to form a class of dimeric and polymeric substances known as theaflavins and thearubigins (Roberts, 1962) that determine the liquor characteristics of tea.

## 3. Experimental

### 3.1. Sample collection

The tea samples have been produced under environmentally controlled atmosphere at Tea Research Association – Tocklai, Assam, India. In this experiment a total of 46 different tea samples have been procured over two seasons (June–July and September–October). The tea samples are processed in an environment controlled atmosphere to optimize their processing parameters, so as to get wide variations of TF among them. The TF content of samples has been determined using spectrophotometry and then presented to electronic tongue for collection of responses.

### 3.2. Biochemical estimation of theaflavins using spectrophotometry

Weighted sample of 6 g is added with boiling water in a thermo-flask and infused for 10 min. The infusion is then filtered into a 500 ml conical flask and is allowed to cool. Six milliliter of extract