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Food Chemistry 91 (2005) 771–778
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

A reliable technique to identify superior quality clones from tea germplasm

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Received 7 February 2004; received in revised form 17 September 2004; accepted 18 October 2004

Abstract

Variations in the substrate level and enzyme activity of prominent south Indian tea germplasm were studied. The content of polyphenols, catechins (substrates) and polyphenol oxidase (PPO) showed variation, which influenced the final black tea quality. The enzyme PPO occurs in tea shoots and catalyzes the reactions between catechins to form theaflavins in the presence of oxygen. The catechins mainly epicatechin (EC), epigallo catechin gallate (EGCG), epicatechin gallate (ECG) and epigallo catechin (EGC) get mixed with PPO during the oxidation process to form quality constituents like theaflavins (TF), thearubigins (TR) and high polymerized substances (HPS). Theaflavins and their fractions such as simple theaflavin, theaflavin monogallate, (TFMG), theaflavin digallate (TFDG) in black tea are the essential quality constituents that are responsible for the liquor characteristics where as TR and high polymerized substances impart colour to the liquor. As oxidation of macerated leaves proceed through different stages of tea manufacture, a decline in PPO activity, polyphenol and catechin contents were observed. Data revealed that the oxidation reaction was faster during the initial stages of oxidation. During the period, oxygen consumption was higher and declined thereafter. Ratio between the enzyme (PPO) and its substrate (catechins) were used to characterize the quality potential of tea clones. An attempt has also been made to categorize prominent tea clones as high, moderate and average quality clones based on their enzyme substrate ratio. Theaflavin content (oxidation product) of different tea clones suggests that the ratio between PPO and catechins forms an important criterion which determines black tea quality. Results obtained were compared with standard clones of known high quality (CR-6017) and moderate quality (SA-6). The study reveals that the enzyme substrate ratio can be used to identify superior quality clones from the existing tea germplasm.

Keywords: Theaflavins; Catechins; Polyphenol oxidase; Oxidation; Enzyme substrate ratio

1. Introduction

Biochemical constituents such as polyphenols, catechins and polyphenol oxidase (PPO) of tea are intrinsically related to black tea quality. In recent years, knowledge on biochemical components and the changes that take place during oxidation (fermentation) has increased noticeably. Biochemical analysis has been generally constrained to the flush since it is the young crop shoots that are plucked and processed to produce black tea with distinctive aroma (Millin & Rustridge, 1967).

Cut, tear and curl (CTC) type of black tea manufacture involves withering, cutting, oxidation and drying. During withering, a decline in the moisture content and a slight augment in the substrate as well as enzyme levels are noticed. The over all quality and flavour of the final black tea depends on moisture content and the changes in the biochemical constituents that occurs

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doi:10.1016/j.foodchem.2004.10.005

during withering of the leaves. After withering, the leaves pass through "rotor vane", where the leaves are cut into pieces. Sliced leaves are then cut four times in ragged stainless steel rollers revolving oppositely. During oxidation major biochemical changes takes place, leading to the formation of quality constituents and the characteristic flavour of black tea. Thus a crucial stage is influenced by factors such as temperature, humidity, oxygen level, time and finally the biochemical constituents of macerated leaf (dhool). After optimal oxidation of leaves, it is subjected to firing at 120 °C for 10–20 min. During this stage, the enzyme activity is blocked and almost completes removal of moisture results.

During oxidation, PPO interacts with phenolic compounds mainly catechins and their fractions such as Epicatechin (EC), Epicatechin gallate (ECG), Epigallo catechin gallate (EGCG) and Epigallo catechin (EGC) in the presence of oxygen which results in the development of golden yellow theaflavins (theaflavin, theaflavin-3 gallate, theaflavin-3′ gallate and theaflavin-3-3′ gallate) a product of condensation reaction between two molecules of *o*-quinones (Owuor & Obanda, 1998; Madanhire, Whittle, & Khumalo, 1996). To comprehend the formation of quality constituents from potential fresh tea leaves, it is necessary that the enzyme completely converts the available substrates into products. PPO is generally found in association with chloroplast (Kato, Uritani, Saijo, & Takeo, 1976) and in the epidermal cells (Oparin & Shubert, 1950) due to its role in the initial oxidation of catechins to *o*-quinones which later, condenses into quality constituents (Wickramasinge, Roberts, & Perera, 1967). Polyphenols and catechins are mainly localized in the vacuoles and palisade cells of leaves (Mahanta, 1988). During withering, moisture content of the leaves is reduced to about 60%. During cutting and oxidation, the intact cells of tea leaves get ruptured and the substrates (polyphenols and catechins) come in contact with the vacuolar enzyme. This results in the formation of quality constituents of black tea (Selvendran & King, 1976). Since biochemical constituents influence the black tea quality, we have attempted to identify superior quality clones using the bioconstituents of tea leaves prior to black tea manufacture. We report that the enzyme (PPO):substrate (catechin) ratio plays an imperative role in determining the quality potential. We have employed this strategy to characterize the tea clones in terms of their quality potential, besides well known standard tea selections like SA-6 (moderate quality) and CR-6017 (good quality) were used for comparison. We believe that the technique developed will shorten tea breeding endeavors for faster and consistent screening of large seedling germplasm for superior quality traits.

2. Materials and methods

2.1. Materials

Two leaves and a bud were used for biochemical estimations and enzyme assay. Samples were collected for the study from the experimental farm of UPASI TRF, situated at 1050 M above MSL which have a diverse genetic background.

2.2. Biochemical estimations

The estimation of total polyphenols and catechins was carried out in green tea leaves. The ethanol extract of tea shoots was used for determination of total polyphenols and catechins. Total polyphenols were estimated by using Folin phenol Ciocalteu reagent in the presence of sodium carbonate. The absorbance of blue colour developed was measured at 700 nm (Dev Choudhury & Goswami, 1983). Total catechin was estimated using acidified vanillin reagent and the absorbance was measured at 500 nm. (+) Catechin was used as standard (Swain & Hillis, 1959). Total polyphenols and catechins were expressed as % on the basis of dry matter. Catechin fractions in green leaves was analysed by reverse phase HPLC system. Column used for analysis was Luna 5 μM Phenyl Hexyl. Authentic and certified flavanols procured from Sigma-Aldrich, USA were used as reference standards.

2.3. Assay of polyphenol oxidase

PPO was assayed by measuring oxygen uptake coupled to the oxidation of pyrocatechol using a Clark type oxygen electrode (Model 290A; ORION Inc., USA) by modified method of Molla (1992). Unless otherwise stated, the electrode chamber contained 4.5 ml of 100 mM sodium phosphate buffer pH 6.8 and 5 ml of 100 mM pyrocatechol and in a final volume of 10 ml. After the system had equilibrated, 500 μl aliquot of enzyme was injected through a small hole in the vessel cap.

2.4. Black tea manufacturing

Harvested tea shoots (two leaves and a bud) were immediately brought to miniature tea factory at UPASI TRF, spread out on wire trays and withered by passing cool air for 16 h to achieve a 25–30% decrease in fresh weight. Withered shoots were passed through a CTC machine four times. The macerated leaves were spread out in trays and placed in a cooling cabinet for a period of optimum oxidation time at 20 °C. The oxidized *dhool* was dried in a mini fluid bed drier at 107 °C for 20 min, packed in polythene bags and stored until further analysis.