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OPTIMISATION OF SAMPLING METHODOLOGY FOR METABOLITE PROFILING OF SRI LANKAN TEA

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The quality of tea (*Camelia sinensis*) products depends on the chemical composition of fresh tea leaves, which is determined by the inherent genetic attributes of the cultivar and geo-climatic factors. Thus, metabolite profiling of the Sri Lankan tea germplasm by chemical analysis of tea flush would contribute immensely to the success of tea breeding programmes. However, the polyphenols, particularly catechins (flavan-3-ols), which are eventually responsible for the quality of tea, are readily oxidized by enzymes (polyphenol oxidase) and such enzymes are activated by light. The collection, handling and storage of a large number of leaf samples for metabolite profiling thus require a sampling procedure that would minimize the oxidation of polyphenols and formation of artefacts. We have compared two methods of sampling, fresh leaves (as in the conventional procedures) and freeze-dried leaves (a new procedure), for quantification of some critical metabolites, namely catechins and caffeine, by employing two cultivars, one (DT1) of which is known to yield high quality black tea and the other (TRI 2025) poor quality black tea.

Tea flush of cultivars DT1 and TRI 2025 in the *ex situ* field genebank of the Tea Research Institute, Talawakelle was separately collected, kept at 4 °C, immediately brought to the laboratory, stored at -80 °C for 6 hours, freeze-dried for 24 hours, ground to a fine powder, and sealed in triple laminated aluminium foil. In the conventional sampling procedure, tea flush was brought to the laboratory at room temperature and immediately subjected to solvent extraction. The tea samples were extracted into boiling 70% methanol in water and the extracts analysed for the total polyphenols using the Folin Ciocalteu's colorimetric method (ISO 14502-1) and for caffeine, (-)-epicatechin, (-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-epicatechin gallate, gallic acid and theobromine using a HPLC method (ISO/CD 14502-2).

The amounts of metabolites recorded in the new sampling procedure were significantly higher than those recorded in the conventional sampling procedure, indicating that the new method is suitable for metabolite profiling studies of the Sri Lankan tea germplasm.

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