Food and Environmental Protection Laboratory, Seibersdorf

Development and Application of UPLC-QTof/MS Method for the Differentiation between Tea Varieties

Technical Officer: Zora Jandrić

Tea (*Camellia sinensis* L.) is one of the most popularly consumed beverages worldwide. It has been used as a natural medicine for thousands of years, containing many compounds beneficial to health. The two most popular varieties are green (favoured in Asia) and black tea (favoured in the western countries). The different growing season, geographical regions, processing and fermentation methods create many varieties of tea, some of which have premium value compared to the others. The expansion of the consumer market, which has increased demand for "manufactured" food as well as transported "pure" food such as tea, has encouraged adulteration simply because of the prospects for increased profit. The adulteration of tea has become a common problem. Mixing exhausted tea-leaves with leaves of some other plants (e.g. elder, hawthorn, sloe), addition of the dust of the tea leaves and sand, chemical enhancement of green tea (with Prussian blue and sulphate of lime or gypsum) and simply redried and resold tea-leaves, are some of the main examples of tea adulteration. To help address these issues, the Food and Environmental Protection Laboratory (FEPL) applied an untargeted metabolomics approach previously developed for some other commodities (e.g. honey, fruit juices) to investigate the possibility of distinguishing teas from different origins, and detecting varieties that had been adulterated.

Tea samples were obtained from the market (black (China, India, Nepal, Sri Lanka), green (Japan, Kenya), oolong (Taiwan) and rooibos (South Africa)), infused in water and analysed by ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry (UPLC-QToF MS).

Using an untargeted metabolite profiling approach and multivariate statistical data analysis, reliable discrimination was obtained between various tea types (black, green, oolong and rooibos), as well as between black and green teas produced in different countries (Figure. 1A). Some of the metabolites that contribute to discrimination of the sample groups were tentatively identified using a loadings plot (Figure. 1B) and database search.

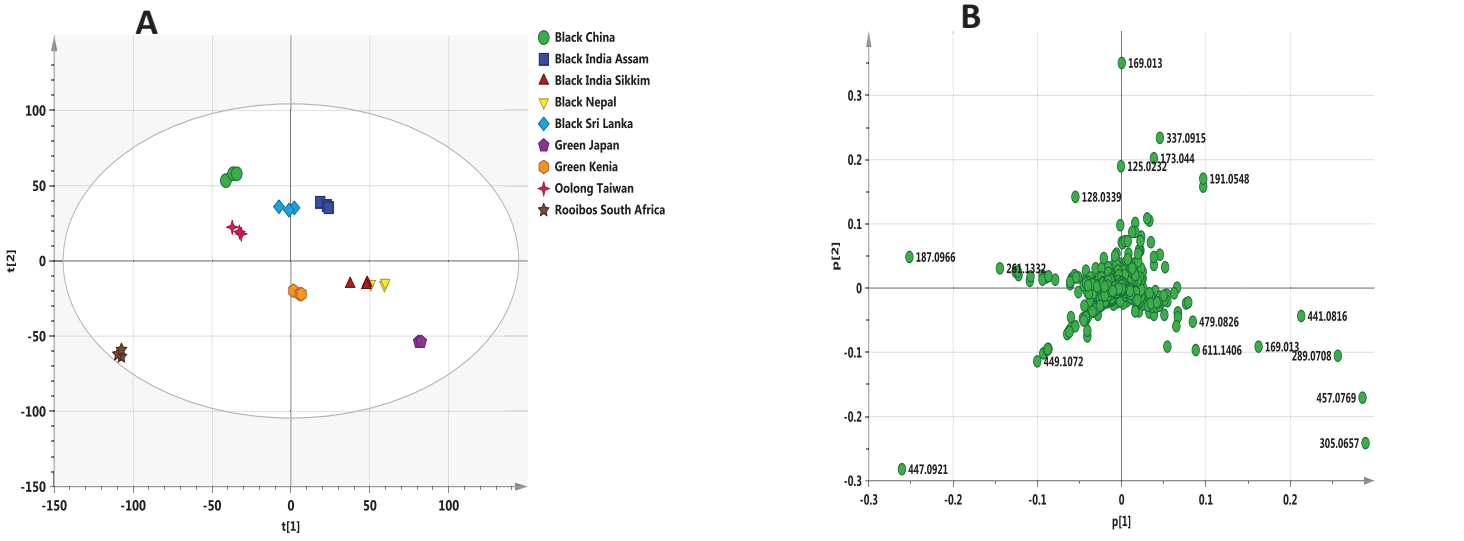

Figure 1. Principal component analysis performed on tea samples: (A) PCA-X plot of various tea samples; (B) loadings plot.

The ability of this methodology to differentiate between different tea varieties and types suggests possible applications of untargeted metabolomics for authentication testing of tea samples. In the near future, the methodology will be applied in FEPL to test tea samples from Sri Lanka as a part of Technical Cooperation Project. Sri Lanka is the world's fourth-largest producer of tea; tea is one of the country's major agricultural products.

We have previously reported the application of this untargeted metabolomics approach for the detection of orange juice adulteration with cheaper citrus juices, with the further development of a cheaper and less complex detection method through the identification of selected markers that can be used to differentiate the authentic and adulterated samples using targeted analysis. We will also attempt to identify chemical markers that would enable the

differentiation of various tea varieties and points of origin using a cheaper, more convenient targeted analytical method. Both untargeted and targeted metabolomics are included in the suite of methods, with other techniques such as stable isotope analysis, spectroscopic and trace element profiling, that are being developed in FEPL to support authenticity testing and food traceability systems.

A Comparison of Two Approaches for the Ruggedness Testing of an Analytical Method

Technical Officer: Britt Maestroni

As part of an initiative under the "Red Analitica de Latino America y el Caribe" (RALACA) network the FAO/IAEA Food and Environmental Protection Laboratory validated a multi-residue method for pesticides in potato. One of the parameters to be assessed was the intra laboratory robustness or ruggedness. The objective of this work was to implement a worked example for RALACA laboratories to test for the robustness (ruggedness) of an analytical method.

There is currently no harmonisation in the definitions of the terms robustness and ruggedness. A review of current international guidelines (see Table 1) shows that the terms are either used as synonyms or as complementary terms. According to the "Proposed draft guidance on performance criteria for methods of analysis for the determination of pesticides residues" by Codex Alimentarius (2015), the ruggedness of an analytical method is the resistance to change in the results produced by an analytical method when minor deviations are made from the experimental conditions described in the procedure. In this study the goal was to test the ruggedness, as defined by Codex, by assessing the degree of intra-laboratory reproducibility of the method under small variations in the conditions of the test. Figure 1 describes the analytical method and the factors (marked 'X' in the figure) that were chosen for the test.

Among the possible statistical experimental designs that are available, the Plackett-Burman design (PBD) and the Definitive Screening design (DSD) were chosen because they are relatively cheap to implement and give substantial information to the analytical chemist on the sources of variability of an analytical procedure. In general the PBD can identify main effects and some two-factor interactions, and was used to study 7 factors using 8 experimental runs; the DSD can estimate main effects, some two-factor interactions, and also some quadratic effects, and was used to study the same 7 factors using 34 experimental runs. Knowing the type of effect caused by a variation in conditions is very important to be able to control the analytical procedure. Linear effects are easier to take into account or compensate for in the method. Quadratic effects are problematic as one cannot know in which direction the change caused by the factor will be and therefore it will be difficult to account for. The analysis of the results using both designs showed that the method was robust (rugged).

In general the choice of the experimental design is a compromise between the statistical significance and the resources one can put into the study. Table 2 provides a summary comparison of the PBD versus the DSD. It is important to note that "ruggedness" of the method should be checked on an ongoing basis, as part of the analytical quality control applied in the laboratory. If additional ruggedness testing is required then the laboratory may opt for a PBD or DSD depending on the available budget. It is important to highlight that the suggested methodology can be very useful when applied in the early stages of method adaptation and development to identify critical steps in the method and possible sources of uncertainty.

As a conclusion to this study, it is evident that there is a need for harmonization of the definition of the terms robustness/ruggedness, the limits, the methodology and the statistical treatment of the generated data. A worked example for RALACA laboratories to test for the robustness (ruggedness) of an analytical method will soon be posted on the RALACA website (www.red-ralaca.net). This study was carried out with collaborators from LVA (Austria), University of Antwerp (Belgium), University of Leuwen (The Netherlands), Universidad de la Republica (Uruguay) and Agilent technologies.