# Classification and characterisation of apple cultivars and clones by rapid non-invasive PTR-ToF-MS analysis

Luca Cappellin[1,2], Christos Soukoulis[1], Eugenio Aprea[1], Pablo Granitto[3], Nicola Dallabetta[4], Fabrizio Costa[1], Roberto Viola[1], Tilmann D. Maerk[2], Flavia Gasperi[1], Franco Biasioli[1]

[1] IASMA Research and Innovation Centre, Fondazione Edmund Mach,
Via E. Mach, 1 – 38010, San Michele all'Adige (TN), Italy
[2] Institut für Ionenphysik und Angewandte Physik, Leopold-Franzens Universität Innsbruck,
Technikerstr. 25, A-6020, Innsbruck, Austria
[3] CIFASIS, French Argentina International Center for Information and Systems Sciences, UPCAM (France)
/ UNR-CONICET (Argentina), Bv 27 de Febrero 210 Bis, 2000, Rosario, Argentina
[4] IASMA Consulting and Services Centre, Fondazione Edmund Mach,
Via E. Mach, 1 – 38010, San Michele all'Adige (TN), Italy

*Summary: Clone characterization is an urgent problem in technical management and royalty application. We show that Proton Transfer Reaction-Mass Spectrometry may be successfully employed to obtain accurate varietal and clonal physical fingerprints. In particular, we studied the VOCs emission profile of five different clones belonging to Fuji, Golden Delicious and Gala.*

*Keywords: apple (malus domestica), clones, proton transfer reaction-mass spectrometry.*

## 1 Introduction

Proton Transfer Reaction-Mass Spectrometry, in its recently developed implementation based on a time-of-flight mass spectrometer (PTR-ToF-MS) has been evaluated as a possible tool for rapid non-destructive investigation of the volatile compounds present in the metabolome of apple cultivars and clones. We show that PTR-ToF-MS coupled with multivariate and data mining methods may be successfully employed to obtain accurate varietal and clonal physical fingerprinting. In particular, we studied the VOCs emission profile of five different clones belonging to three well known apple cultivars, such as Fuji, Golden Delicious and Gala. In all three cases we found classification models able to distinguish all cultivars and some of the clones considered in this study. Furthermore, in the case of Gala we also identified a set of compounds contributing to such clone characterization.

Beside its applied relevance, no data on the volatile profiling of apple clones are available so far.

## 2 Experimental

We considered five clones of three different cultivars (Table 1). For each sample (thought as a clone) 20 fruits from three plants of the same clone were harvested. For the analysis of volatile compounds, each single fruit was placed in glass jars (1000 ml, 30°C) provided with two teflon/silicone septa on opposite sides. VOCs were then measured by direct injection of the head space mixture into the PTR-ToF- MS drift tube via a heated (110°C) peek inlet. Measurements were carried out following the procedure described in previous works for simlar food samples[1] using a commercial PTR-ToF-MS 8000 apparatus. Peak identification and area extraction then followed the procedure described in details by by Cappellin *et al.*[2], which allows to retrive VOCs headspace concentations with a remarkable accuracy[2]. Supervised classification methods were employed to actually assess the separability of the apple cultivars and clones. Data analysis follows the procedure explained in previous studies[2].

*Table 1. List of considered clones.*

|  | GALA | FUJI | GOLDEN DELICIOUS |
|---|---|---|---|
| 1. | Brookfield | Fujiko | Clone B |
| 2. | Cherry | Fubrox | Golden 2000 |
| 3. | Galaxy | Kiku 8 | Smoothee 764 |
| 4. | Schniga | Aztec | Quemoni |
| 5. | Venus | Spike | Leratess |

## 3 Results

The three cultivars are well separated by discriminant analysis (RF method), with a very marked discrimination between Gala and the

other two cultivars (Fig. 1 left). This result confirms that PTR-ToF-MS is capable of distinguishing the different VOCs profile in the headspace of different apple cultivars as it was already pointed out[2]. The graphical representation of the Gala clones classification by Random Forest (Fig. 1 right) highlights the separation of Gala Venus from the other clones. Moreover, it suggests the presence of roughly three groups, represented by Venus, Schniga and Galaxy. The mass peaks that are especially relevant to separate the Gala clone classes include two peaks, corresponding to estragole and hexyl 2-methyl butanoate. Similarly, we found that three Fuji clones (Aztec, Fubrox and Fujiko) and two Golden Delicious clones (Golden 2000 and Quemoni) can be discriminated with good accuracy.
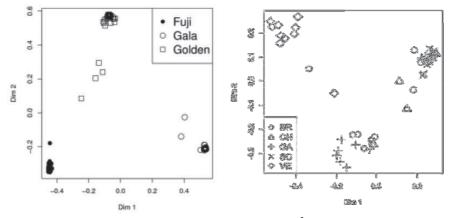


Fig. 1. Discriminant analysis (Random Forest graphical output[2]) of the PTR-ToF-MS spectral data of all apple cultivars (left) and of Gala clones only (right).

## 4 Conclusions

Our methodology can easily differentiate apple cultivars. Relevant differences were also found for the considered clones of Gala, Golden Delicious and Fuji. In the case of Gala, the clone Venus remarkably showed a clear separation from the other four Gala clones. Compounds responsible for such separation were identified; for instance, estragole, which is an important constituent of apple flavour profile.

## References

[1]    L. Cappellin, F. Biasioli, A. Fabris, et al.; *International Journal of Mass Spectrometry* 290 (2010), pp 60-63.
[2]    L. Cappellin, F. Biasioli, P. Granitto, et al.; *Sensors and Actuators, B* 155 (2011), pp 183-190.