

## COFFEE CHEMOMETRICS AS A NEW CONCEPT: UNTARGETED METABOLIC PROFILING OF COFFEE

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### Abstract

Considerable work has been devoted in the last decades to the identification and quantification of key aroma-active compounds in coffee as well as their precursors. The aim of this work was to demonstrate the applicability of a data-driven holistic method rather than a targeted chemical study. As an illustrative example, coffees at different roast degrees were analysed with a range of instrumental techniques (LC-MS, GC-MS, PTR-MS) and evaluated by a sensory panel. This allowed identifying correlations between chemical markers and sensory qualities and developing a deeper understanding on reaction mechanisms involved in coffee aroma formation.

### Introduction

Already in the early 1970s, chemometrics led to the development of statistical methods to treat multivariate data sets obtained by chemical analysis (1,2), in parallel to the design of optimized measurement strategies. Most of the theories developed at that time are still used when dealing with multiple and multivariate datasets, even though today's computers allow the treatment of much larger volumes of data. The application of "omics" approaches to monitor metabolites in the human body related to various diseases accelerated the development of statistical and instrumental techniques. Minimalistic approaches, such as principal component analysis (PCA) and partial least squares (PLS) and their extensions to orthogonal-PLS (OPLS), hierarchical PCA, PLS and OPLS, with the aim to reduce a multidimensional space to a lower dimensional planes, are regularly used to investigate complex problems. A main advantage of "data driven" methods is that they are not based on fundamental chemical theories and can therefore be applied to reproducible unbiased data.

The application of chemometrics to coffee is interesting because of its complexity, e.g. the formation of coffee flavour during roasting, but also due to the success of chemometrics in linking quality differences to aroma compounds and precursors. The range of datasets that can be included in such studies is large and may encompass genetic fingerprints, agricultural information, meteorological data during bean maturation, chemical fingerprints and sensory profiles. Some of these data can directly be compared between samples (e.g. the number of days of sunshine or the growing region) while others need to be pre-processed. In particular, GC and LC data need to be pre-processed in such a way that peaks are recognized

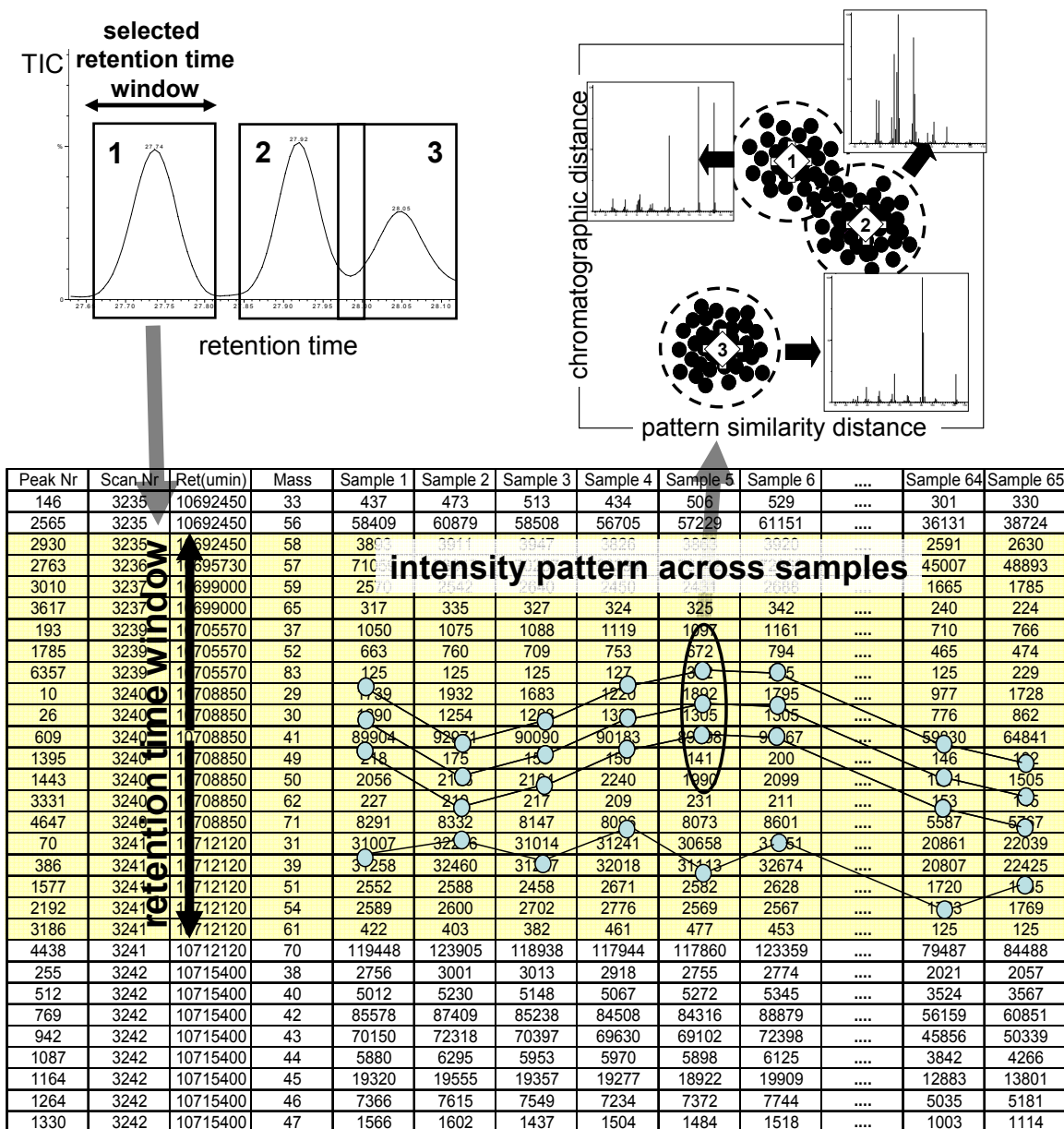
and aligned to compensate for shifts in retention time. A fully targeted approach requires that all compounds be identified before applying multivariate analysis. An untargeted approach overcomes these limitations but requires a more sophisticated pre-processing of the data including baseline correction, peak picking, alignment and centrotyping of raw data sets. As a consequence, time consuming identification can be focused on characteristic markers selected by multivariate statistics.

## Experimental

To relate differences in chemical composition to cup quality, 65 coffee varieties grown in well defined conditions were evaluated by ten trained coffee panellists. Chemical data of volatile compounds was obtained by GC-TOF-MS/PTR-MS (Tenax desorption) and online PTR-MS measurements of roast and ground (R&G) coffee, prepared with an espresso machine. Volatiles released from coffee extracts within a sampling cell were analysed by online PTR-MS and trapped during two minutes on a Tenax trap for desorption on column using an automatic thermal desorption unit (4). Online PTR-MS data were interpreted by combining the GC-PTR-MS datasets with GC-MS (3). Thus, the various molecular contributions to single PTR-MS ion signals can be quantified and traced over time. Non-volatile compounds of extracts of green, slightly roasted and dark roasted coffees were analysed with LC-MS and GC-TOF-MS (after MSTFA derivatization) (6). For LC-MS measurements, 20 mg of powder from beans were weighed in 10 ml glass tubes and dissolved in 3 ml pure water and 75% methanol (containing 0.1% formic acid), respectively, by vortexing and sonication for 15 min. After centrifugation, the extracts were filtered through 0.2  $\mu\text{m}$  PTFE filters, and directly used for LC-PDA-QTOF-MS analyses in ESI positive mode (6). For GC-TOF-MS measurements, 20 mg were weighed and extracted with pure water (80°C) in addition of an internal standard (Ribitol; Sigma, cat. no. 488-81-3). After stirring (10 min at 70°C in a thermomixer at 950 r.p.m.) the sample was centrifuged (10 min at 11000 g) and 750  $\mu\text{L}$  chloroform (-20°C) added to the supernatant. The upper clear water phase (15  $\mu\text{L}$ ) was taken, dried in a vacuum concentrator and used for on-line MSTFA derivatization and GC-TOF MS analysis.

LC-MS and GC-TOF-MS raw data were processed by using the Metalign software ([www.metalign.nl](http://www.metalign.nl)). The software includes base line correction, peak picking respecting a limitation in signal to noise ratio and alignment of the detected peaks through all samples by an algorithm which compensates for slight shifts in retention time. Due to the ionization induced fragmentation by electron impact in GC-MS, single compounds are represented by an average of 10 mass signals. To reduce the data volume and eliminate redundant information a “centrotyping” program, similar to that reported in Ref. (5), was applied. This program correlates the intensity profiles of individual mass signals across all samples within a predefined retention time window which can be adjusted according to the shifts in retention time caused by the limitation of instrumental accuracy. Mass signals that correlate are clustered and expressed as single centrotypes since they are expected to belong to one and the same compound (Figure 1). This reduces the data volume without loss of information, since the fragmentation pattern of each centrotypes is stored. Hence, this information can be used for identification via comparison with databases.

Cluster analysis and correlation maps were obtained to visualize correlations between sensory data, volatile compounds and non volatile compounds. Correlation maps help identifying groups of related centrotypes and show their interrelation.



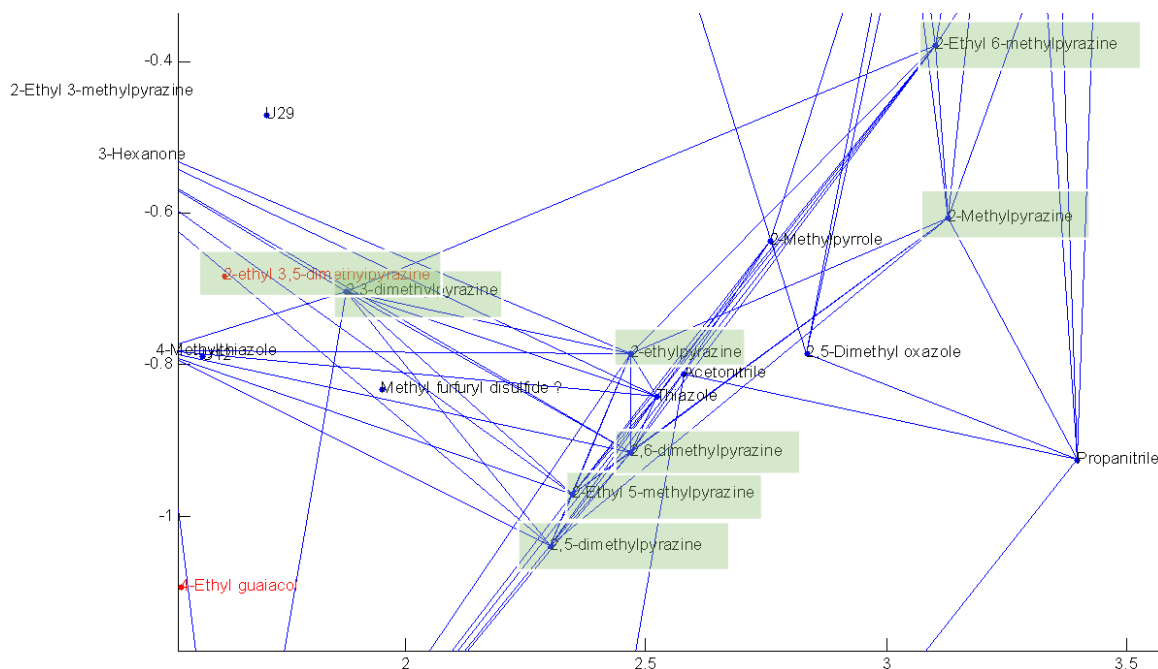
**Figure 1.** Example of eluted peaks selected within a defined retention time window. Three mass peaks correlate strongly through all samples and therefore most probably belong to the same compound. A fourth mass peak intensity shows a different pattern and therefore belongs to another compound.

**Results and Discussion**

A large set of markers were aligned through all samples applying the Metalign program to GC and LC data. Further reduction of the data using a centrotyping program helped remove redundant information, improving the readability and accuracy of correlation maps and multivariate analysis. This approach allowed comparing the differences in concentration of more than 200 volatile and 500 non-volatile compounds. The fragmentation patterns of individual compounds allowed identifying more than 150 volatile compounds. The identification of non-volatile compounds was focused on key correlations, where cluster analysis and correlation maps showed to be useful when investigating correlations between volatile

compounds their precursors and sensory profiles. The obtained datasets allowed developing a sensory predictive model which goes beyond the one already published (4), mainly by increasing the number of compounds included in the model (data not shown).

While the approach works for most of the compounds, volatile pyrazines were challenging to be differentiated by the automatic pre-processing and needed frequent manual intervention to avoid misalignment. However, the network of pyrazines was highly correlated (Figure 2) when analyzing the corresponding GC-TOF-MS correlation map.



**Figure 2.** Correlation map: This network shows a series of pyrazines that are highly correlated (blue lines indicate a correlation higher 0.9).

By investigating changes in the chemical composition of volatiles and non-volatiles and testing their impact on predicted or evaluated sensory profiles, a robust method was developed to identify coffee varieties with the highest potential of in-cup quality.

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