Reliable assessment of aroma patterns in fruit and vegetables

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Abstract

For consumers, flavour is a very important fruit property. The measurement of aroma is necessary for breeding and quality control. A reliable method is shown for semi-quantification of volatile patterns. Exemplary results are discussed for strawberry.

Keywords: flavour, headspace SPME-GC, aroma-breeding, funnel effect

Introduction

The purchase decision of consumers of organic food is strongly influenced by flavour. The sensory quality (smell, aroma, taste and mouthfeel) is mainly based on aroma compounds which are volatile secondary metabolites in plants. Reliable methods for characterisation of aroma patterns are necessary for quality assessment, breeding purposes, cultivar characterisation as well as heritability studies.

Therefore, a reliable method for semi-quantification of volatile patterns was developed using headspace solid phase micro extraction, gas chromatography (headspace SPME-GC) and holistic data management. The sample preparation and handling are easy and low laborious. Advantageously, the sample can be prepared outside the analysis laboratory. Further, the samples can be shipped and stored at 4°C for several weeks. Due to the reliability and robustness of this method, it is applicable as selection tool in cultivar breeding and for general fruit quality assessment.

In the present research, this method described was used to semi-quantify the aroma pattern of old cultivars, wild types and breeding lines in comparison to high yielding standard cultivars in strawberry. The results demonstrate the gain and loss of volatile metabolites on the basis of up to 200 volatile GC peaks. They where obviously affected by the domestication and the selection process. This phenomena is intensively discussed as the so called “funnel effect” in plant breeding.

Materials and Methods

The patterns of volatile metabolites were determined by a rapid, non targeted analysis approach. This method is a combination of rapid sample preparation and non-targeted data processing. It consists of automated headspace solid phase micro extraction and data processing by pattern recognition.

Details of the sample preparation and GC separation: 10 ml fruit or vegetable homogenate saturated with NaCl; 20 ml-headspace vial; 100-µm-polydimethylsiloxane-SPME-fibre from Supelco, Bellefonte, PA, USA; equilibration time in the shaker: 10 min at 35°C and 300 rpm; extraction for 15 min at 35°C and 300 rpm; split/splitless injector at 250°C; desorption 2 min splitless and 3 min with split; GC Agilent Technologies 6890 equipped with MPS2 auto sampler from Gerstel, Mühlheim, Germany; column HP INNOWax, 0.25 mm ID, 30 m

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length and 0.5 µm film thickness; carrier gas hydrogen 1.1 ml/min; temperature programme 5 min at 45°C , from 45 to 210°C with 5 K/min and 15 min at 200°C; detector FID at 250 °C.

All samples were run in duplicate. For data processing, a non-targeted or holistic analysis approach (pattern recognition) was used. Data input for pattern recognition by the commercial software CHROMStatTM 2.6 was performed with percentage reports (retention time/peak area data pairs). This kind of data processing considers all detectable peaks of an analysis set without peak allocation or identification. Using CHROMSTAT, the chromatograms were divided into 174 time intervals. A modified principal component analysis (PCA) was performed to visualize the results and check the reproducibility of GC analyses (cases: genotypes, variables: peak areas). The volatiles were identified by parallel runs of mass spectrometric analysis (GC/MS) and by retention indices.

Results and Discussion

The developed method is a combination of an effective sample preparation and a non-targeted data processing (Olbricht et al. 2008). It consists of automated headspace solid phase microextraction (HS-SPME), gas chromatography (with FID or MS detector) and data processing by pattern recognition. SPME as sample preparation is a well established method for isolation of volatiles. This technique fulfils the requirements for rapid analyses of hundreds of samples also for small sample sizes. Instead of using a calibration table, the chromatograms are cut into time slices by the software Chromstat 2.6. Therefore and in principle, the area of all peaks of a chromatogram set are detectable above a given threshold. The commercial software Chromstat 2.6 by Analyt Mühlheim (Germany) was used for chemometrical data processing. Data input for pattern recognition are percent reports (retention time/peak area data pairs) performed with the software package Chemstation by Agilent. Using Chromstat, the chromatograms were divided in time intervals (maximum 200). Each of which represents a possible peak (substance) occurring in at least one chromatogram of the whole analysis set (up to several hundreds chromatograms). The peak areas of all detected peaks were processed by principal component analysis (PCA) to identify outlyers. The output of pattern recognition and data export is an Excel database comprising the areas of all peaks.

The method is fast and ensures that new or unexpected peaks which may occur as a result of diversity in aroma patterns are included in data processing. The results of data analysis by pattern recognition allow a fast and unbiased comparative multivariate analysis of the volatile metabolite composition.

Figure 1 shows the analysis of the aroma patterns of 7 strawberry genotypes, one high yielding, modern cultivar (Elsanta), one old cultivar (Mieze Schindler) and five breeding lines (p-clones). The p-clones derived from crossing low aromatic cultivars with a high aromatic *Fragaria chiloensis* accession. The location of the different samples in the PCA plot demonstrates that the aroma patterns of the p-clones are very diverse but differ from them of the two common cultivars. The PCA analysis was performed using all 174 peaks (time slices) without compound identification and peak allocation. The aroma content (sum of the detected compounds) is higher in the breeding clones (up to 3 times) in comparison to Elsanta. A subsequent MS identification of main components also shows higher concentrations of esters including methylanthranilate in most of the clones. These findings confirm the known process of genetic erosion occurring in breeding of high yielding cultivars and the creation of aroma diversity by crossing.

The method described was also successfully used in aroma research projects including apple, cherries, carrot and parsley (Ulrich 2008).

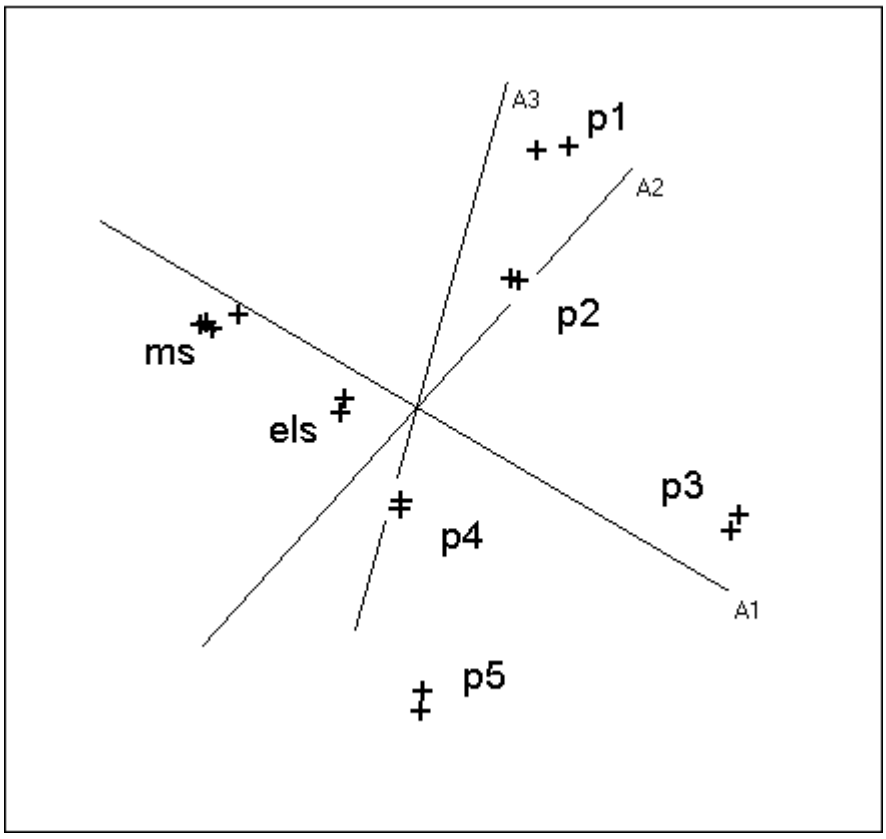

Figure 1: PCA analysis of the aroma pattern of seven genotypes (with repetitions, axis A1, 2 and 3: first three principal components). The explained variance is the following: A1 = 32 %, A2 = 20 % and A3 = 13 %). Nomenclature: ms - cultivar Mieze Schindler, els - cultivar Elsanta, p1 to p5 - breeding clones. Breeding clone p3 is applied for Plant Breeders Rights/EU under the denomination "Julia" (reference: P-5284).

References

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