



Application Note 120916
Author: Karl-Heinz Maurer

Analysis of volatile food aroma compounds by Atmospheric Pressure Corona Discharge Ionization-Mass Spectrometry (APCDI-MS)

A) Introduction

Food aroma is considered to be an important issue for commercial food producers and consumers as well. Therefore, in this context analysis techniques for identification or confirmation of existence of relevant compounds in food play a significant role. Chromatographic and mass spectrometric methods (specifically PTR-MS) or combination of both (GC-MS) are involved in this field. Many scientific groups world-wide have made efforts by developing sophisticated sample preparations and optimization of analytical instruments for improvement of specificity and limits of detection.

We were interested to evaluate how far mass spectra of volatile food aroma compounds, produced in a simple and efficient way without specific sample preparation/enrichment and chromatographic compound separation techniques, can be used for substance classification by pattern recognition methods. In other words it was our aim to evaluate the capability of our miniaturized API-MS, the AMD Mini QuAS³AR mass spectrometer (www.amd-analysis.com) as an “electronic nose” as the method is usually described.

B) Summary

The described analytical results have been performed again with the experimental version of a new bench-top double focusing mass spectrometer. The dedicated and miniaturized API-MS system (AMD Mini QuAS³AR.pdf) is suitable for ESI-MS, APCDI-MS and GD-MS technologies (www.amd-analysis.com). The interface and the mass analyzer are integrated with a multi-stage turbo pumping system. The universal API Interface was used without any modifications for the volatile food aroma analyses in **APCDI mode**. The food samples were simply put into the closed API chamber at 25 °C and real-time head space analyses of the released VOC components by full scan techniques were performed. As reported before the water content in the ambient atmospheric air has been used as a reagent gas for the production of **protonated molecular ions of VOCs** under atmospheric pressure conditions.

Due to the orientating character of the analyses, emphasis was given to a rudimentary but very efficient methodology for evaluation of the system potential for future demanding analytical tasks. Therefore, it has been accepted that outstandingly low detection limits were not within the scope of this note. A small number of food examples have been chosen, exemplarily. The mass spectra of the released aroma compounds contained a majority of corresponding protonated molecular ions which could be assigned to volatile flavor components in accord with reliable publicly available information. More extended evaluations and additional supplementary investigations may result in the conclusion that the information content (“fingerprint”) of the obtained flavor mass spectra may be suitable for substance classification by pattern recognition methods. It has been demonstrated that the applied simple and robust **APCDI-MS** produces mass spectra of volatile compounds for authenticity determinations or quality changes during food storage processes. On-line control of fermentation processes could be another area of application. The use of an array detector for simultaneous ion detection will reduce the analysis time, significantly and make the AMD Mini QuAS³AR system suitable for high through-put automation.

C) Methodology

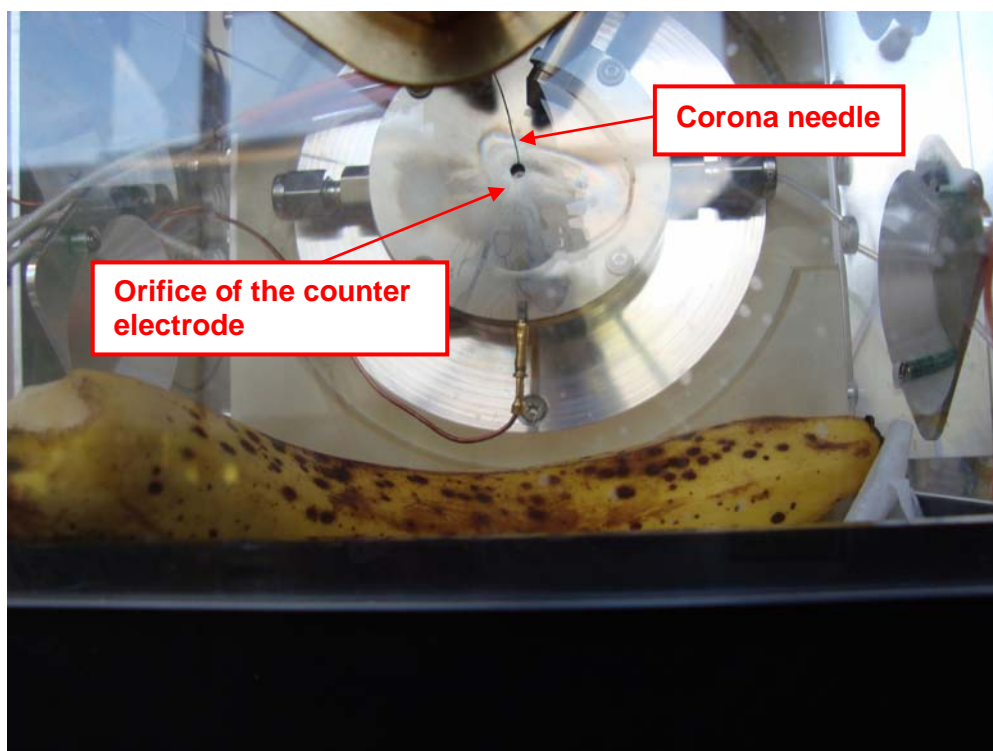


Fig. 1 Sample directly deposited in the API chamber for direct aroma release

The evaluation model of the API-MS mass analyzer ([AMD Mini QuAS³AR.pdf](#)) has already been described in various application notes, previously (www.amd-analysis.com). The experimental difference to the ESI, APCI, GD and APCDI applications before is indicated by above picture.

The sample for all experiments described here, in this case a Banana, is directly deposited in the API room of 2 l volume at atmospheric pressure and a temperature of about 25°C. The aroma compounds are released into the API room (head space) and the corona discharge is initiated for **production of protonated molecular ions** of the aroma compounds. The corona parameters have been described application notes before. The mass spectra have been recorded in full scan mode and raw data accumulation. The method is very simple and effective.

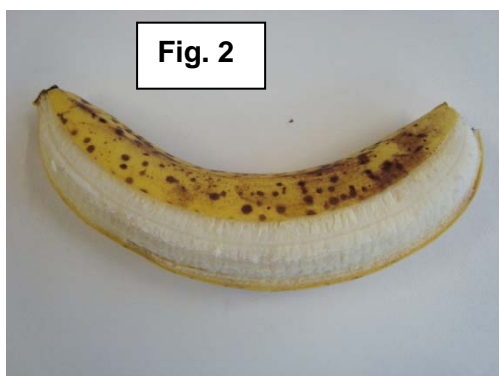


Fig. 2

The Banana deposited in the API room is shown in this picture beside.

The Banana is in a **full-ripe** status, recommended for immediate consumption. Therefore, release of typical banana aroma compounds is expected.

The analytical results, obtained within a few seconds or minutes depending on the intensity of the aroma compounds, are described below

D) Results

D1) Banana aroma release

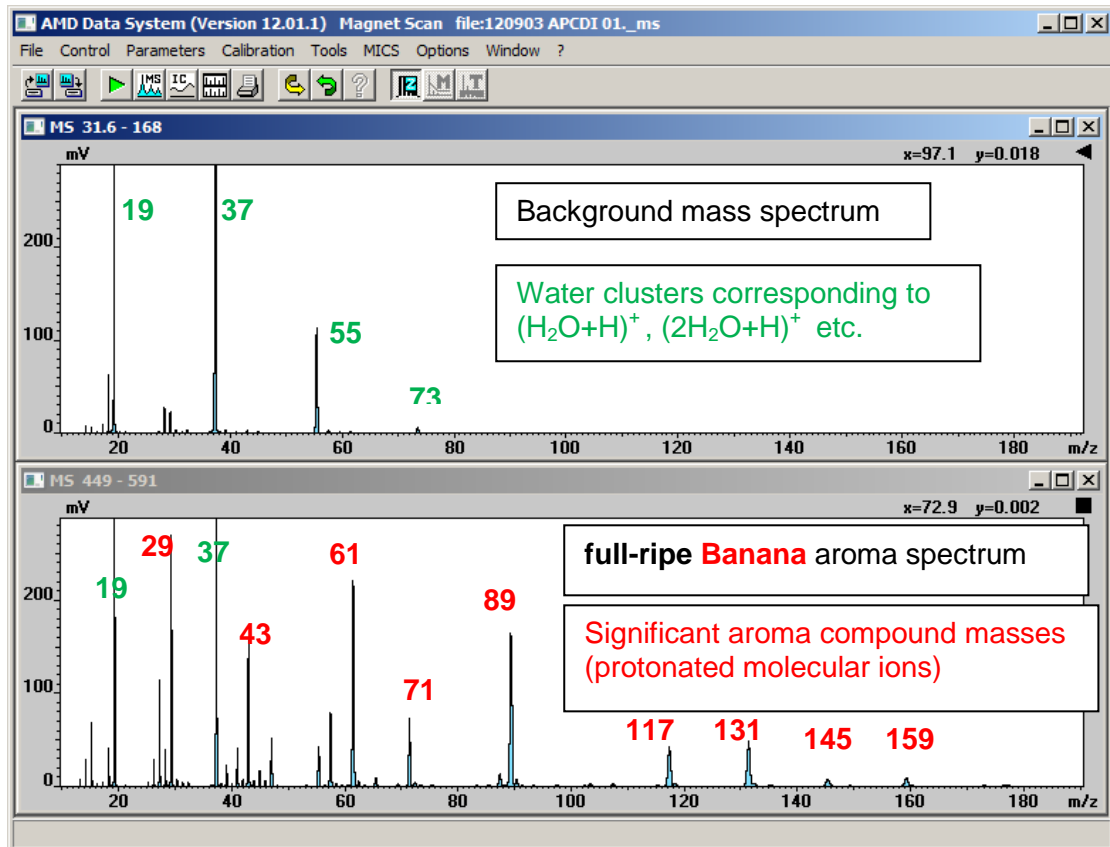


Fig. 3 APCI mass spectra of **full-ripe Banana** volatile aroma compounds

The **upper part** of the figure shows the ambient air background spectrum using the same intensity scale as for the banana aroma spectrum. The background spectrum is dominated by the water clusters and the lower intensity trace compounds of the ambient air are not detectable in this scale.

The **lower part** of the figure shows in this scale a number of significant aroma compounds of the banana. It has been proven in previous measurements using the APCI technique that the VOCs in the gas phase are represented by the M+H ions (proton transfer reaction).

The composition of significant aroma compounds in a banana is used from reliable commonly available sources. Therefore, major aroma components above can be identified with a high degree of confidence even if supplementary unequivocal compound identification has not been performed. Some components are summarized in the table below by the M+H ion, compound name and formula.

<p>159, Isopentyl butanoate ($C_9H_{18}O_2$); 145, Hexyl acetate ($C_8H_{16}O_2$); 131, Isoamyl acetate ($C_7H_{14}O_2$); 117, Isobutyl acetate, ($C_6H_{12}O_2$); 89, Ethyl acetate ($C_4H_{12}O$); Other ions occurring at lower masses like 71,61,43,29 have not been assigned to specific compounds.</p>



Fig. 4

Another Banana deposited in the API room is shown in this picture beside.

The Banana is in an **over-ripe** status, probably no more recommended for consumption. Therefore, release of additional compounds supplementary to the typical banana aroma compounds is expected.

The analytical results are described below and compared to the first banana.

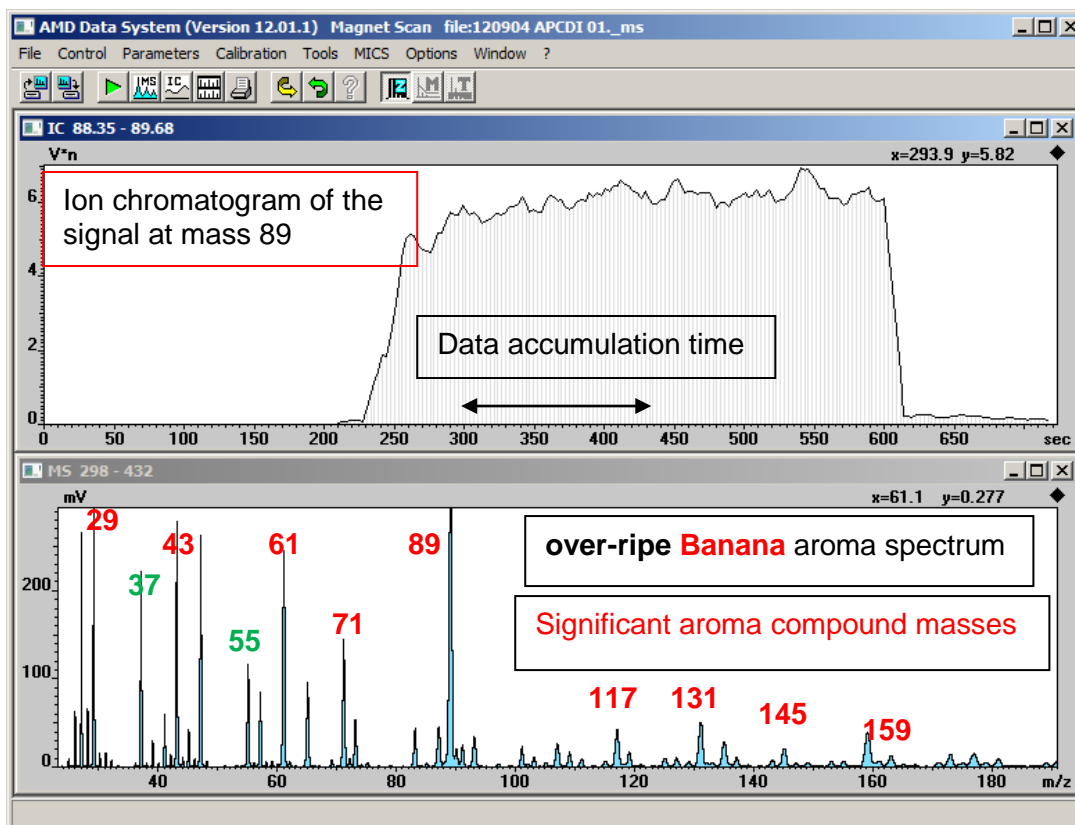


Fig. 6 APCDI mass spectra of **over-ripe Banana** volatile aroma compounds

All assigned aroma components of the full-ripe banana are existing in this spectrum. A number of additional ions appear in the higher mass range and it is obvious that low mass components as masses 29, 43, 61 and 89 are more intensive.

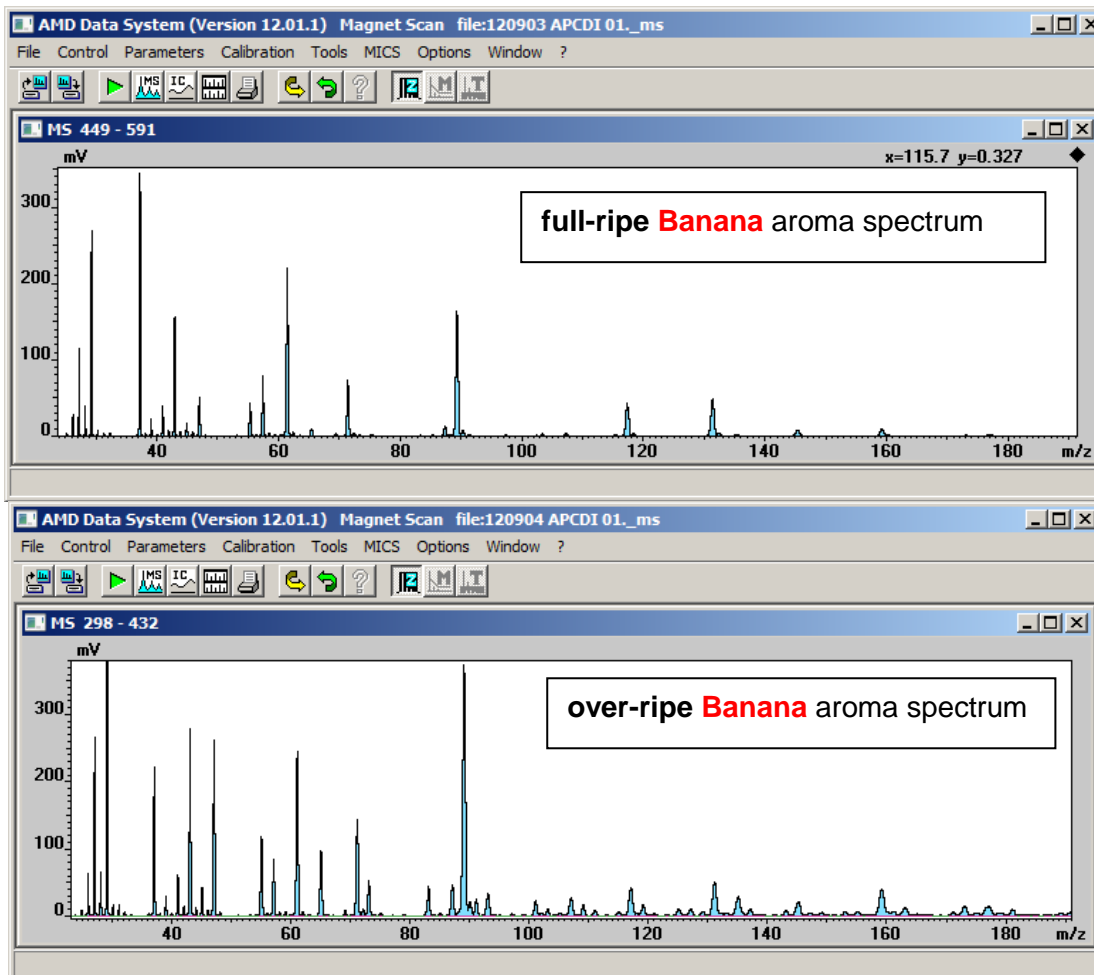


Fig. 7 APCI mass spectra of full-ripe and over-ripe Banana volatile aroma compounds in comparison

In this scale the changes in the low mass range (more and more intense peaks for the over-ripe banana) are well visible.

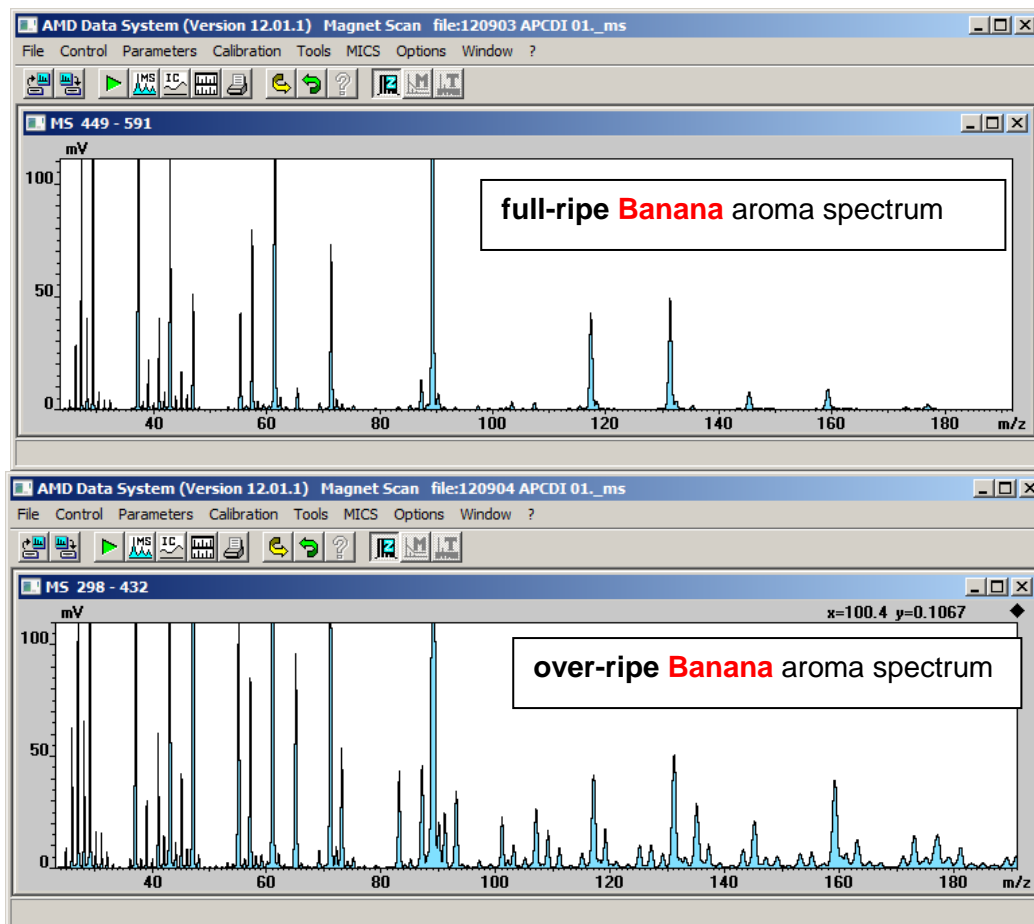
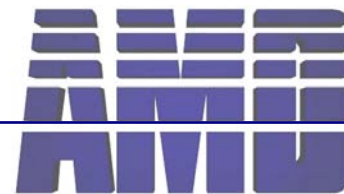
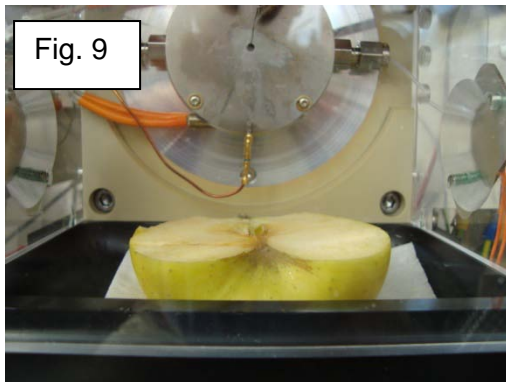


Fig. 8 APCDI mass spectra at extended scale of full-ripe versus over-ripe Banana

At extended scale the difference of the aroma compound mass spectra is very well documented. Quantification will require additional investigations regarding careful intensity calibration and normalization to independent relevant ions in the background spectra. Information for creating a so called **flavor "fingerprint"** and the application of **pattern recognition** methodologies may be contained in these spectra.

D2) Apple aroma release



The **Golden Delicious** apple is deposited in the API room as shown in this picture beside. Regarding API infrastructure compare Fig. 1 above.

The head space analytical results of the released aroma compounds, are described below.

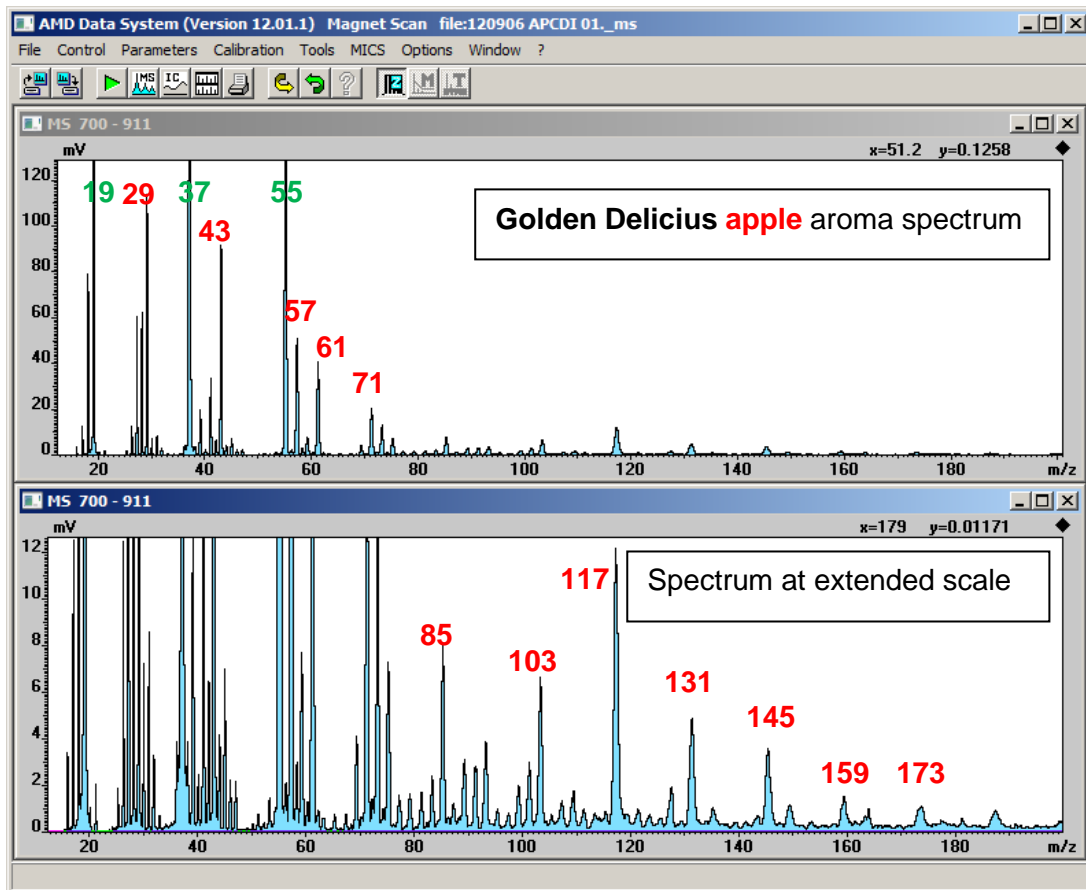


Fig. 10 APCDI mass spectrum of the **Golden Delicious** volatile apple aroma compounds

The apple aroma spectrum in the higher mass range shows quite some similarity to the banana spectra. Differences regarding masses and intensities occur mainly in the lower mass range. Some significant ions may be mentioned here as for example 103, 1-Hexanol ($C_6H_{14}O$) and others have not been assigned here.

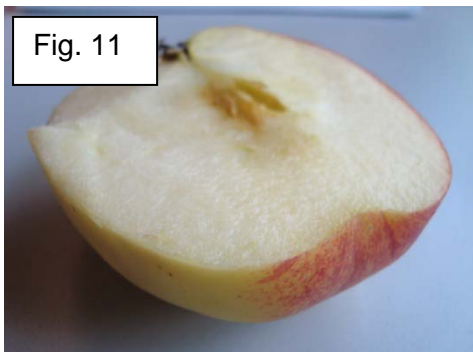


Fig. 11

The **Jonagored** apple (shown in this picture beside) is deposited in the API room in the same way as shown in fig. 9 before.

The head space analytical results of the aroma compounds, are described below

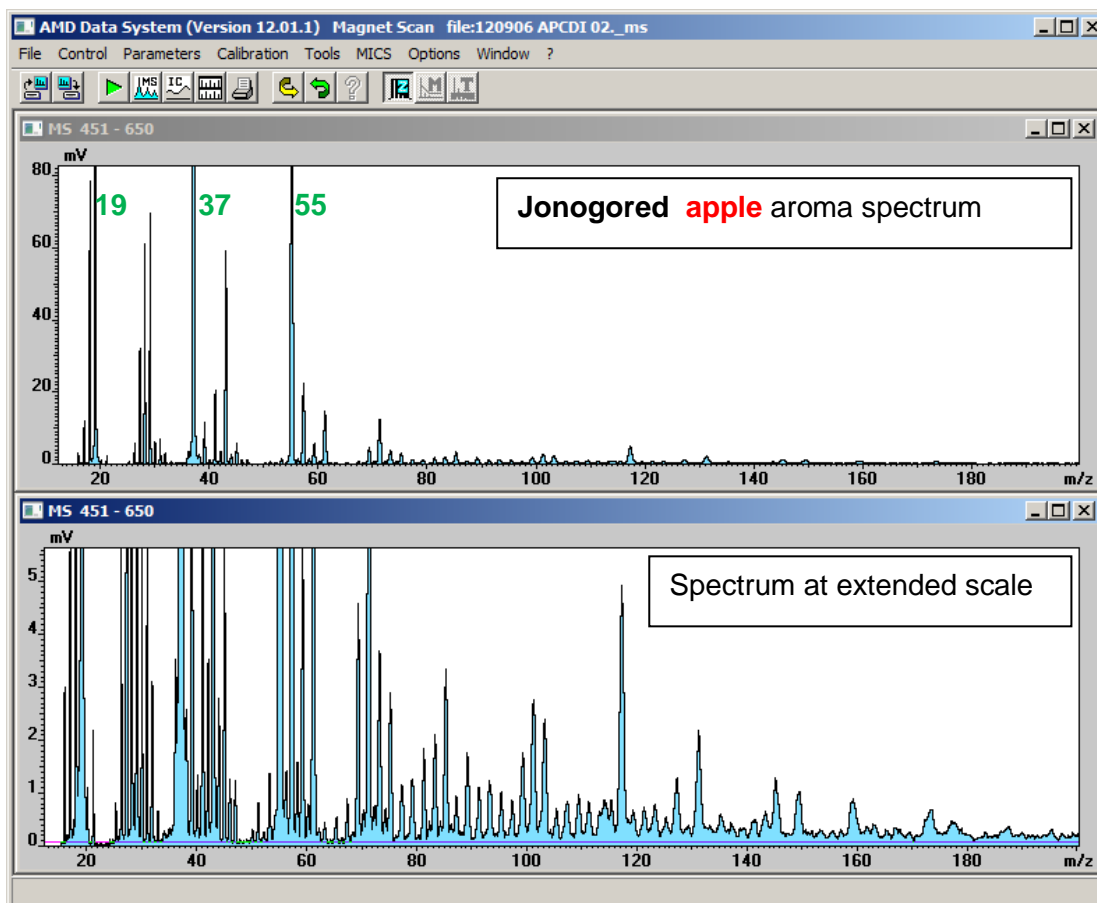


Fig. 12 APCI mass spectrum of the **Jonagored** apple volatile aroma compounds

At a first glance there is no much difference visible in this aroma spectrum compared to the Golden delicious. Therefore, the spectra of both apples components are shown below at extended intensity scale.

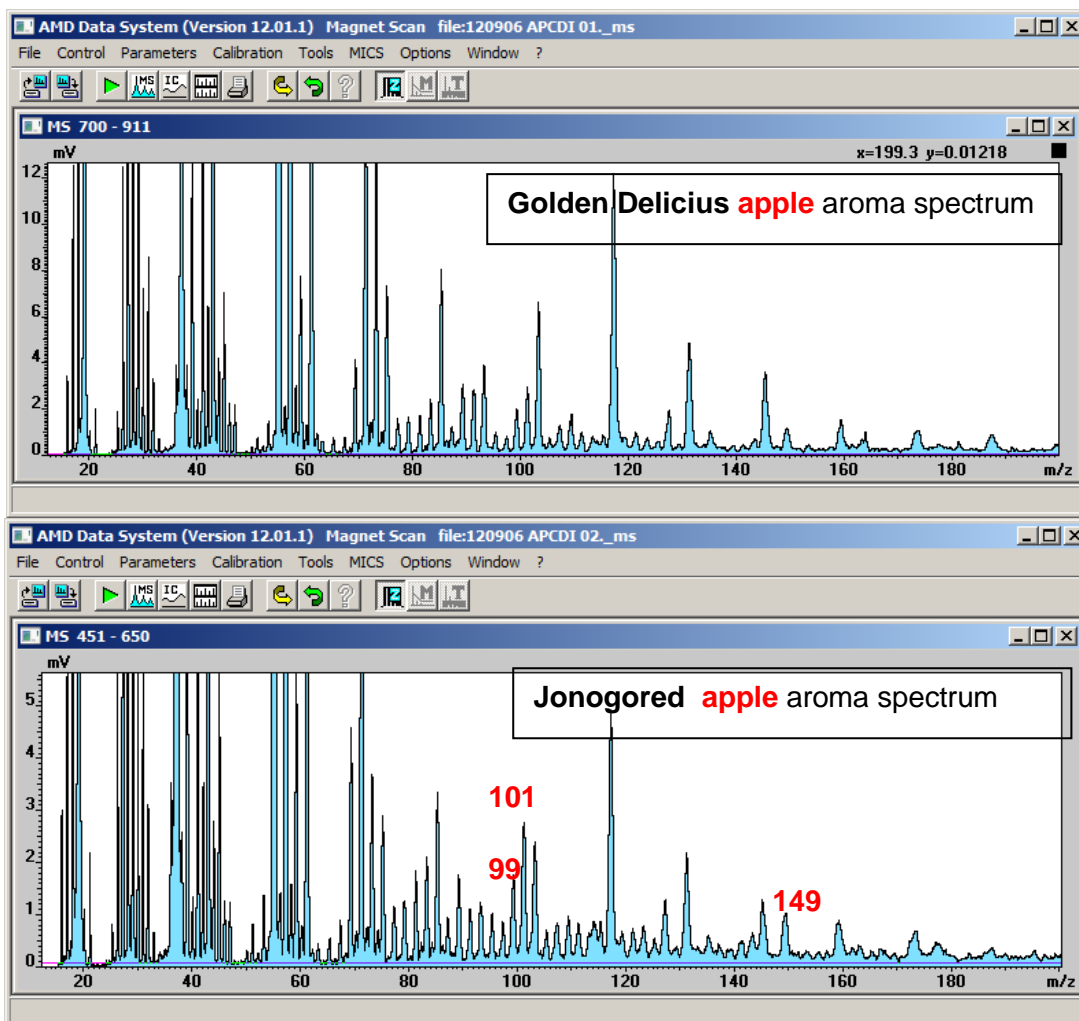


Fig. 13 APCDI volatile aroma compound spectra at extended scale of **Golden Delicious** versus **Jonagored** apples

Some differences regarding the pattern of the spectra are readily identifiable as indicated above. Quite a number of low intensity peaks are also concerned. However, a reliable difference could possibly be established only by the application of a sophisticated pattern recognition program. The protonated water signal and the protonated water clusters have to be eliminated, naturally.

D3) Coffee aroma release

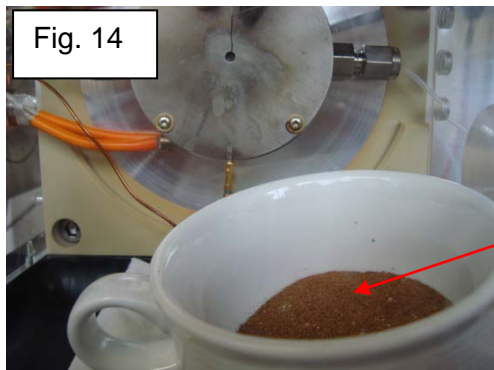


Fig. 14

A dry portion of **roast and ground coffee** in a coffee cup is deposited in the API room as shown in this picture beside. Regarding API infrastructure compare Fig. 1 above.

The head space analytical results of the released aroma compounds, are described below

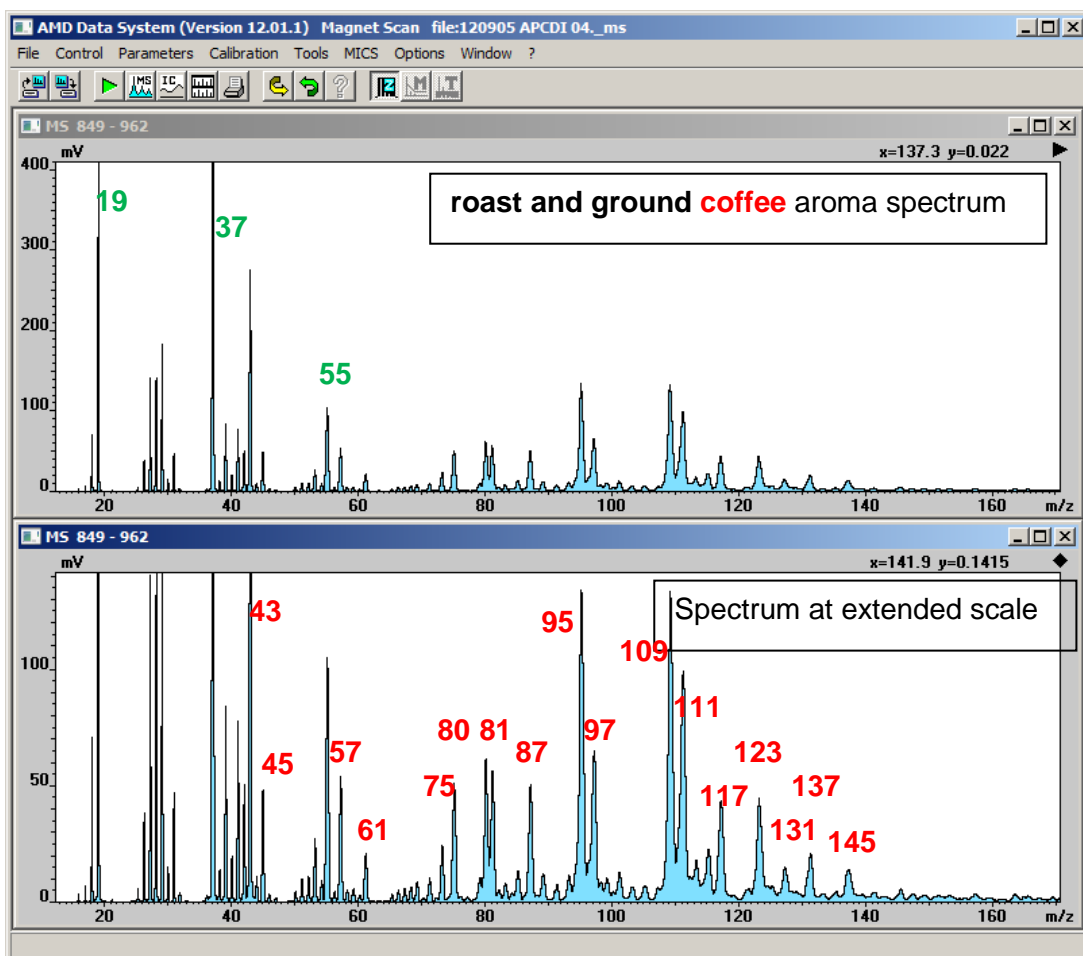


Fig. 15 APCDI volatile aroma compound spectra of roast and ground coffee

According to data available, generally there are several hundred volatile compounds in roasted coffee but probably 5% of those may form the so called coffee aroma. In the head space spectrum are a number of significant compounds marked and many other low intensity compounds are existing. Some compounds have been assigned below but a few only may play a role regarding the formation of the coffee aroma. However, the spectrum pattern may be specific for the sample.

Some assigned compounds described by: mass, compound name (formula)

- 137, Limonene (C₁₀H₁₆)/ 2-Ethyl-3,5-dimethylpyrazine (C₈H₁₂N₂);
- 123, 2-Ethyl-5-methylpyrazine (C₇H₁₀N₂);
- 97, Furfural (C₅H₄O₂);
- 87, 2-methylbutanal (C₅H₁₀O)/ Diacetyl (C₄H₆O₂);
- 81, Pyrazine (C₄H₄N₂);
- 75, Isobutylalcohol (C₄H₁₀O);
- 45, Acetaldehyd (C₂H₄O);

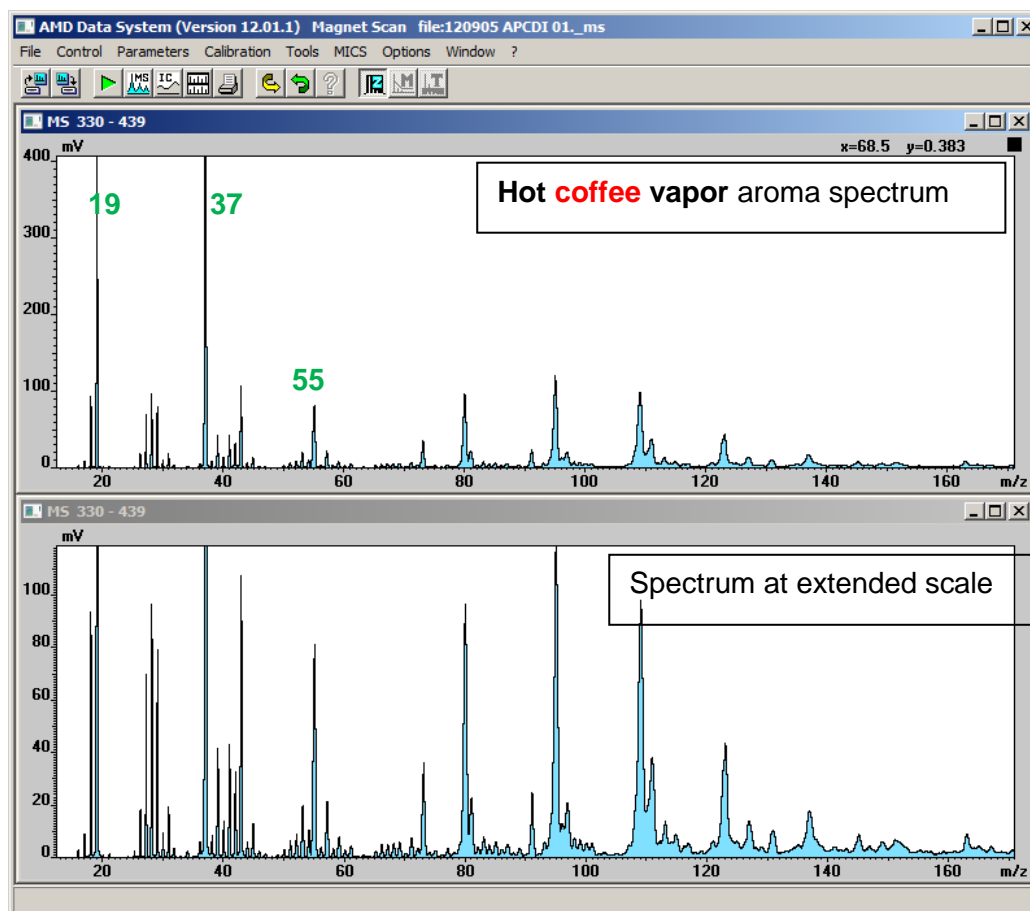


Fig. 16 APCDI volatile aroma compound spectra of hot coffee vapor

The hot coffee vapor mass spectrum is quite similar to the dry roast and ground coffee spectrum. There are some differences which are described in figure 18 below. It may be remarkable that obviously the much stronger water content of the head space does not influence the spectrum pattern, drastically.

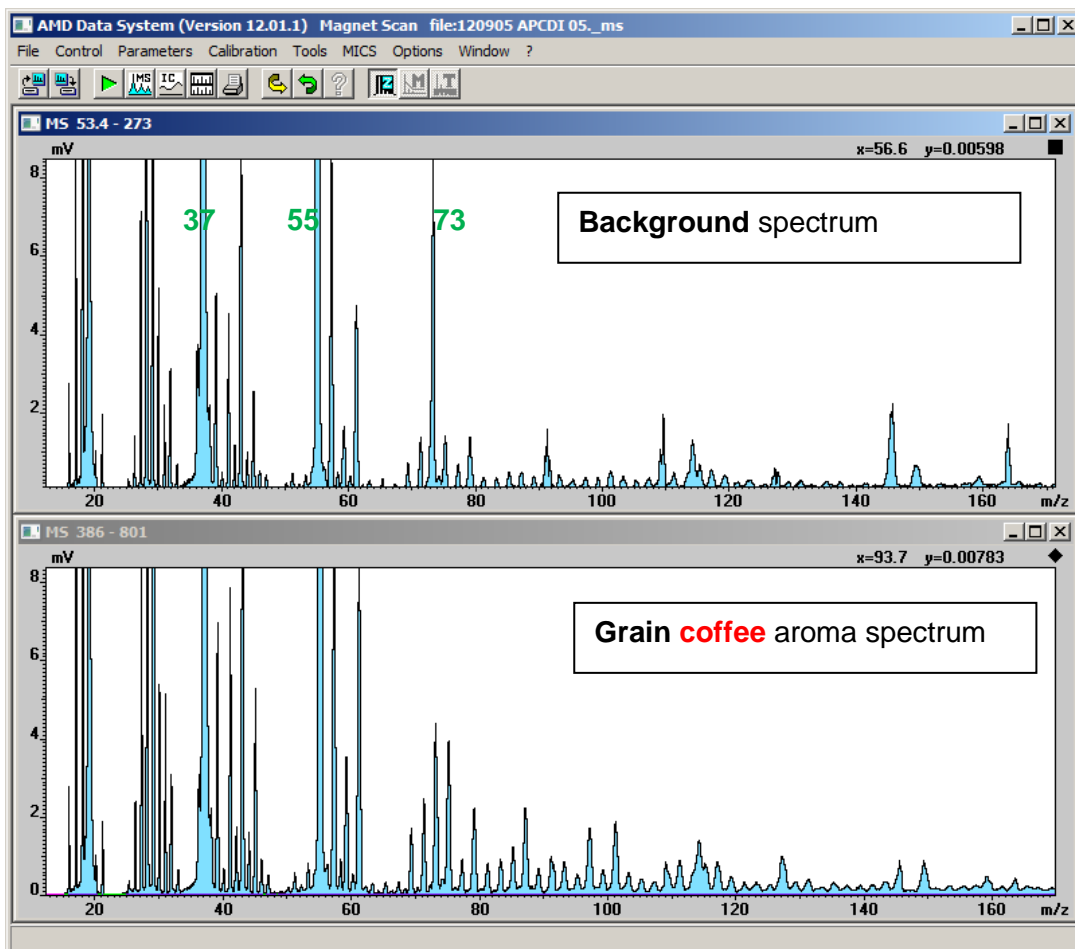


Fig. 17 APCDI volatile aroma compound spectra of **grain coffee**

As expected by a weak smell compared to the roasted coffee beans the intensities of the grain coffee aroma compounds in the head space spectrum are quite low. The background plays a significant role for possible pattern recognition purposes and will complicate the evaluation.

A more detailed comparison of the different coffee spectra is shown in figure 18 below.

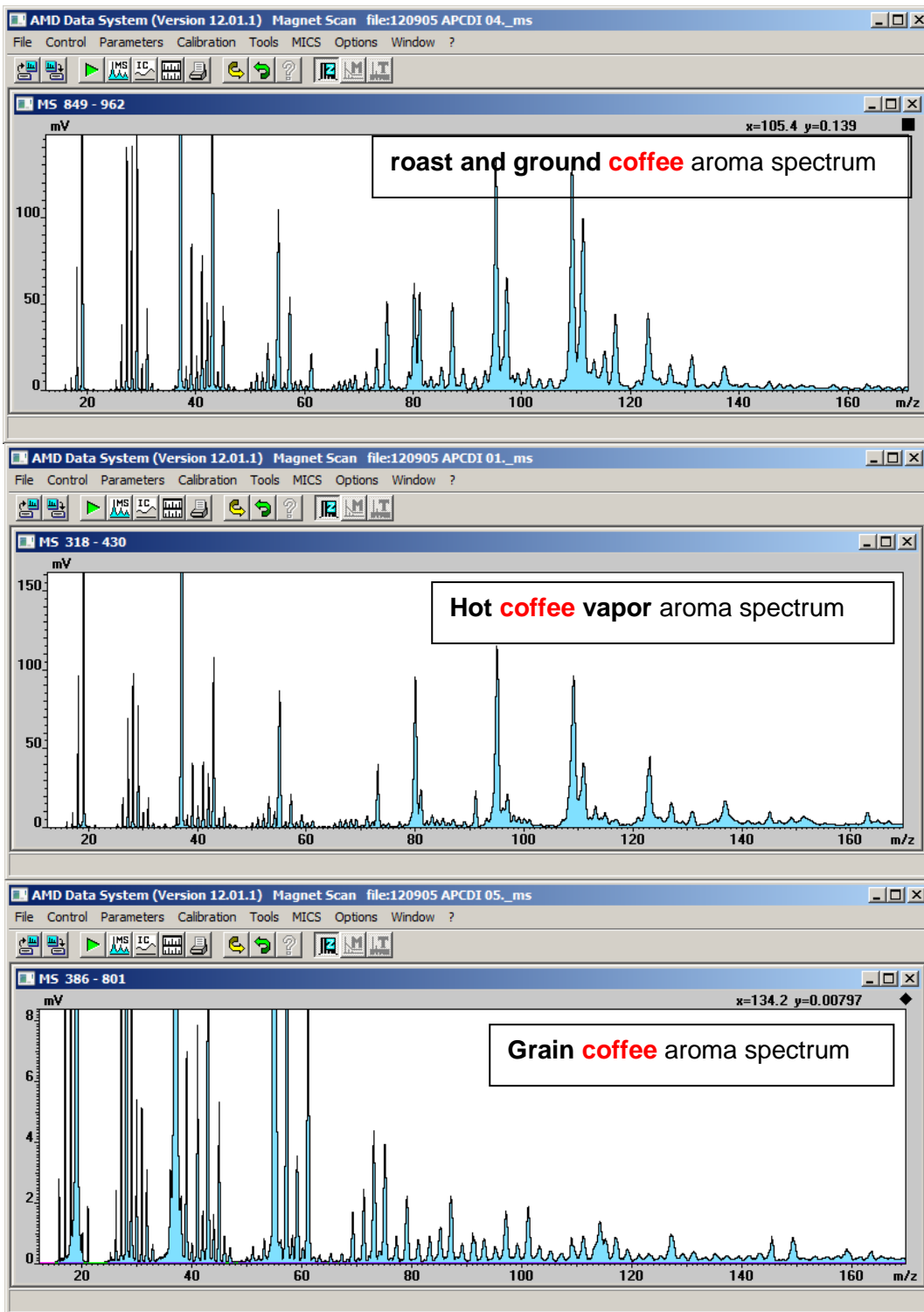


Fig. 18 APCDI volatile aroma compound spectra at extended scale of roast and ground coffee, hot coffee vapor and grain coffee in comparison



D4) Cheese aroma release

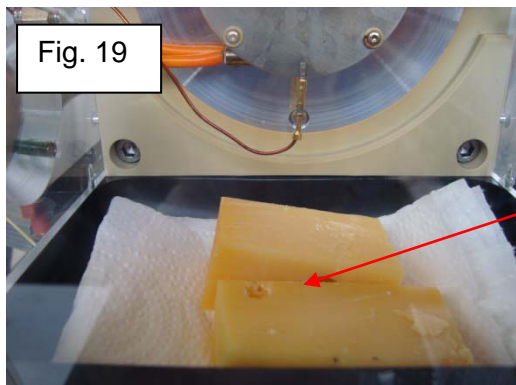


Fig. 19

Portions of **Young** and **Old Gouda** cheese are deposited in the API room, successively. An example is shown in this picture beside. Regarding API infrastructure compare Fig. 1 above.

The head space analytical results of the released aroma compounds, are described below

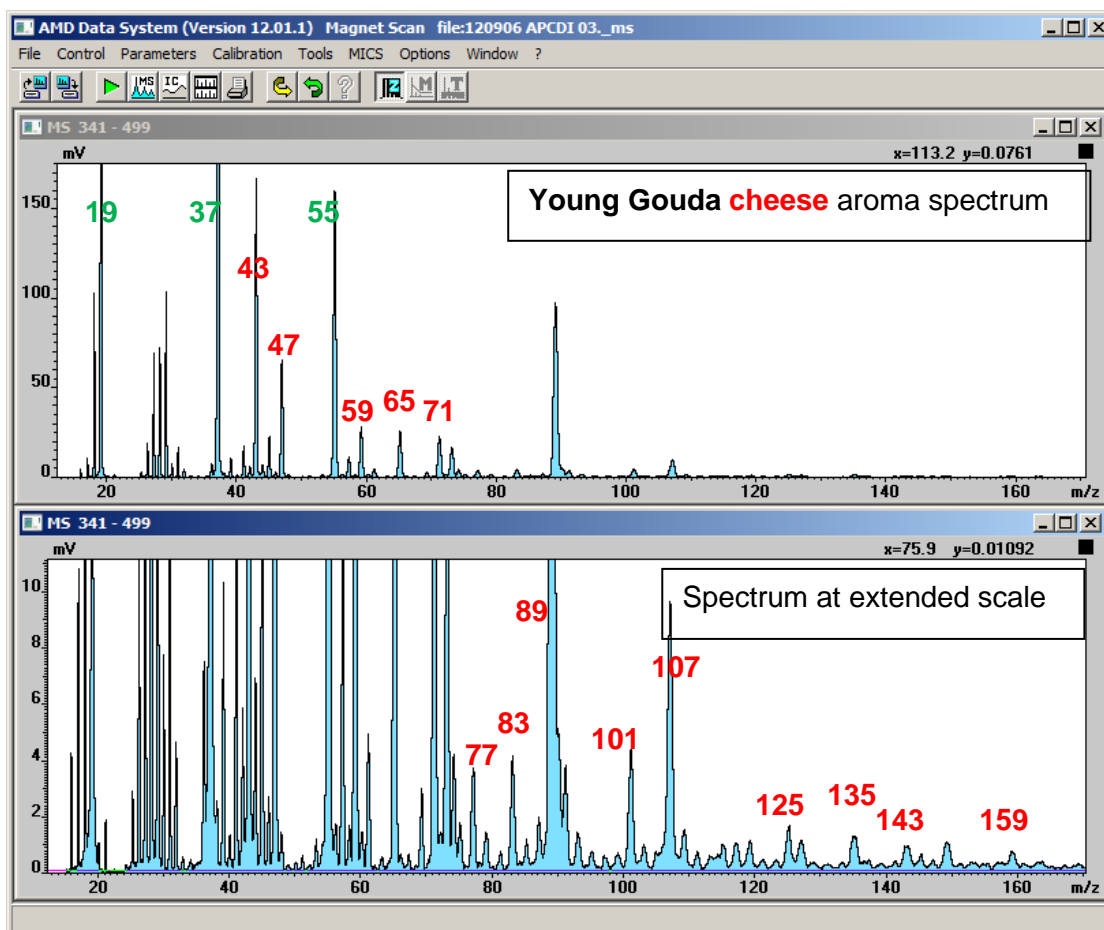


Fig. 20 APCI volatile aroma compound spectra of **Young Gouda** cheese

According to data available, generally, there are around one hundred identified volatile compounds in Gouda cheese. In the head space spectrum are a number of significant compounds marked and many other low intensity compounds are existing which may contribute to the smell of the cheese.

Only a few compounds have been assigned below since our interest was not the identification but the pattern of the sample: 107, Benzaldehyde (C_7H_6O); 89, 3-Methyl-1-butanol ($C_5H_{12}O$); 143, 2-Nonanone ($C_9H_{18}O$);

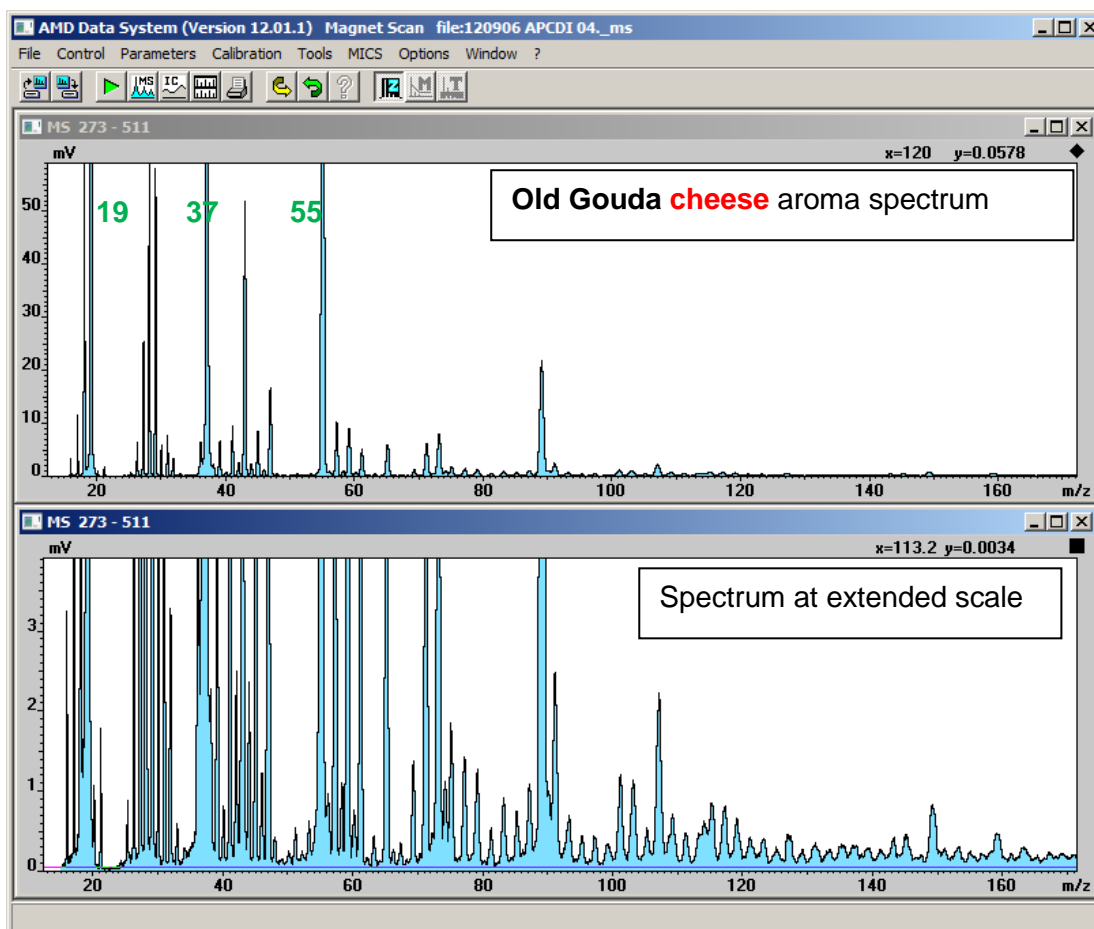


Fig. 21 APCI volatile aroma compound spectra of **Old Gouda cheese**

There are some obvious differences regarding the spectrum pattern between the young and the old Gouda cheese which are better noticeable in the figure 22 below.

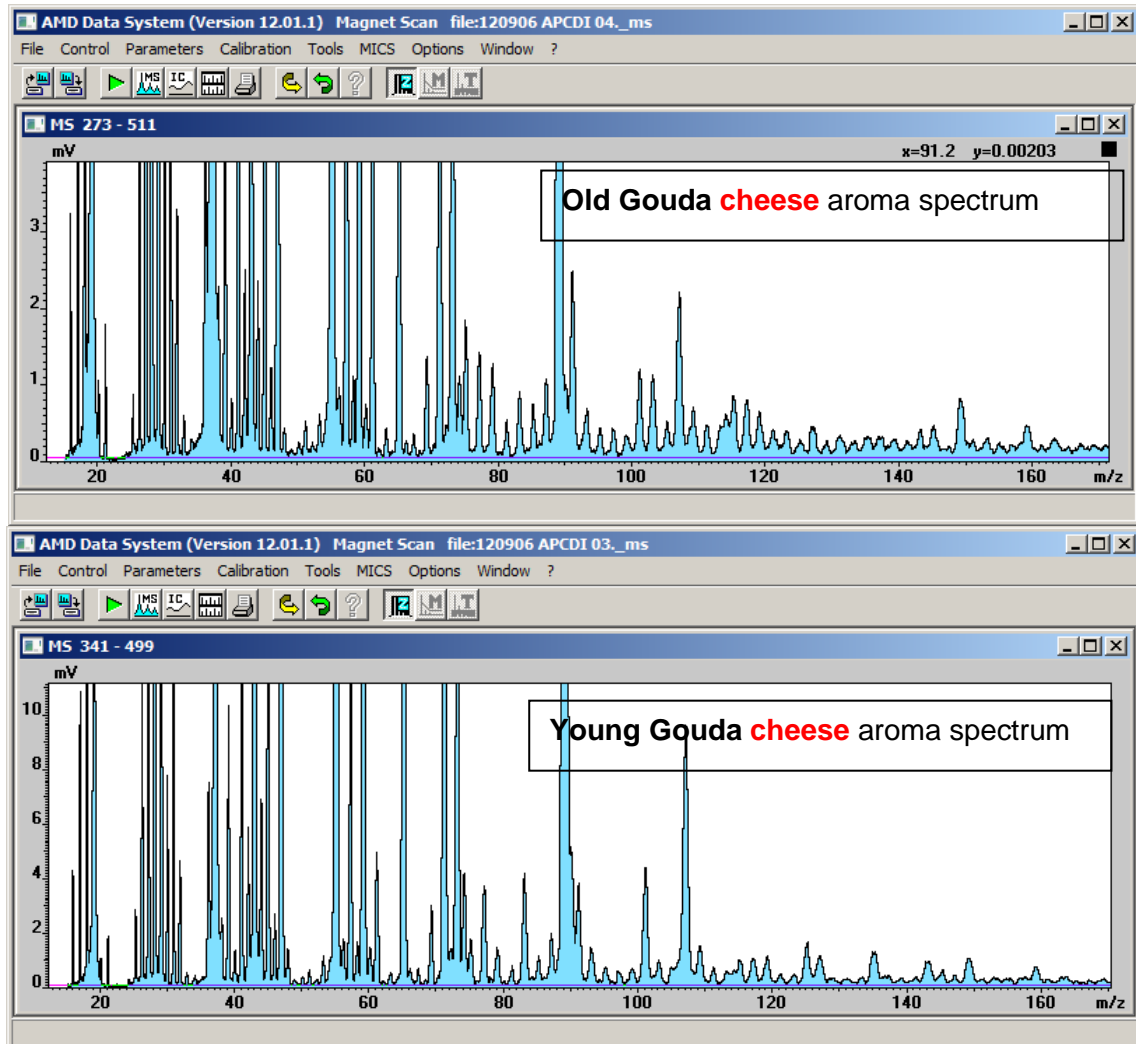


Fig.22 APCDI volatile aroma compound spectra of **Young Gouda cheese** versus **Old Gouda cheese**



D5) Considerations regarding reproducibility and sensitivity of the APCDI method

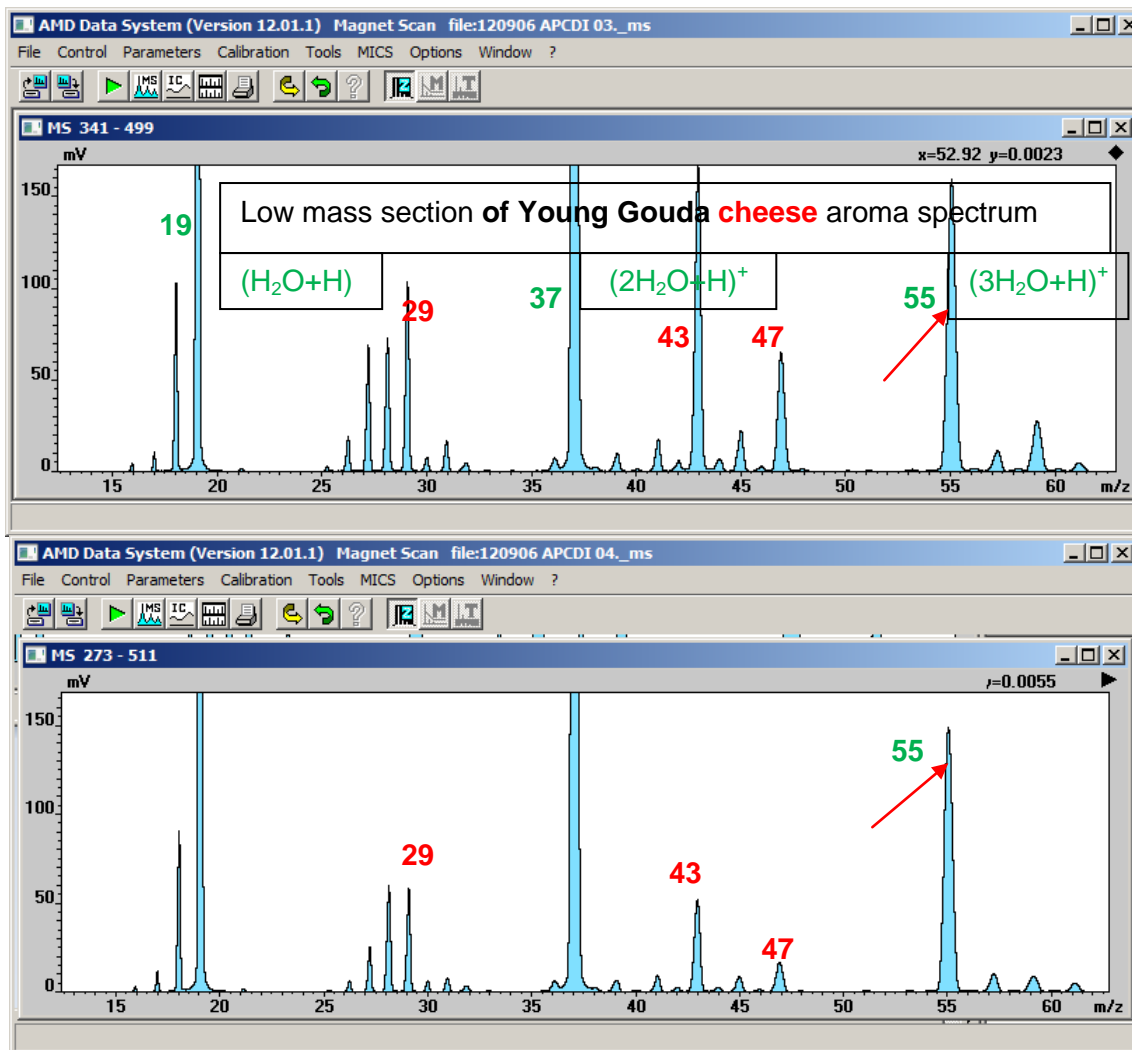


Fig.23 APCDI low mass section spectra of Young Gouda cheese and Old Gouda cheese in comparison

Major influence on reproducibility and sensitivity of the APCDI method is given by the corona discharge conditions and the water concentration in the gas phase to be analyzed. Since the protonated water acts as a proton donor to the VOC compounds producing $(M+H)^+$ ions in this process, the mechanism requires attention regarding the spectra evaluation. The control of the water cluster formation seems to be important for a reproducible production of the protonated aroma relevant component ions. Above figure shows that the water cluster at mass 55 (green) has been kept at the same intensity for the different measurement of the cheese samples. The intensities of ions (red) belonging to the aroma compounds reflect the differences in reality, at least semi-quantitatively.

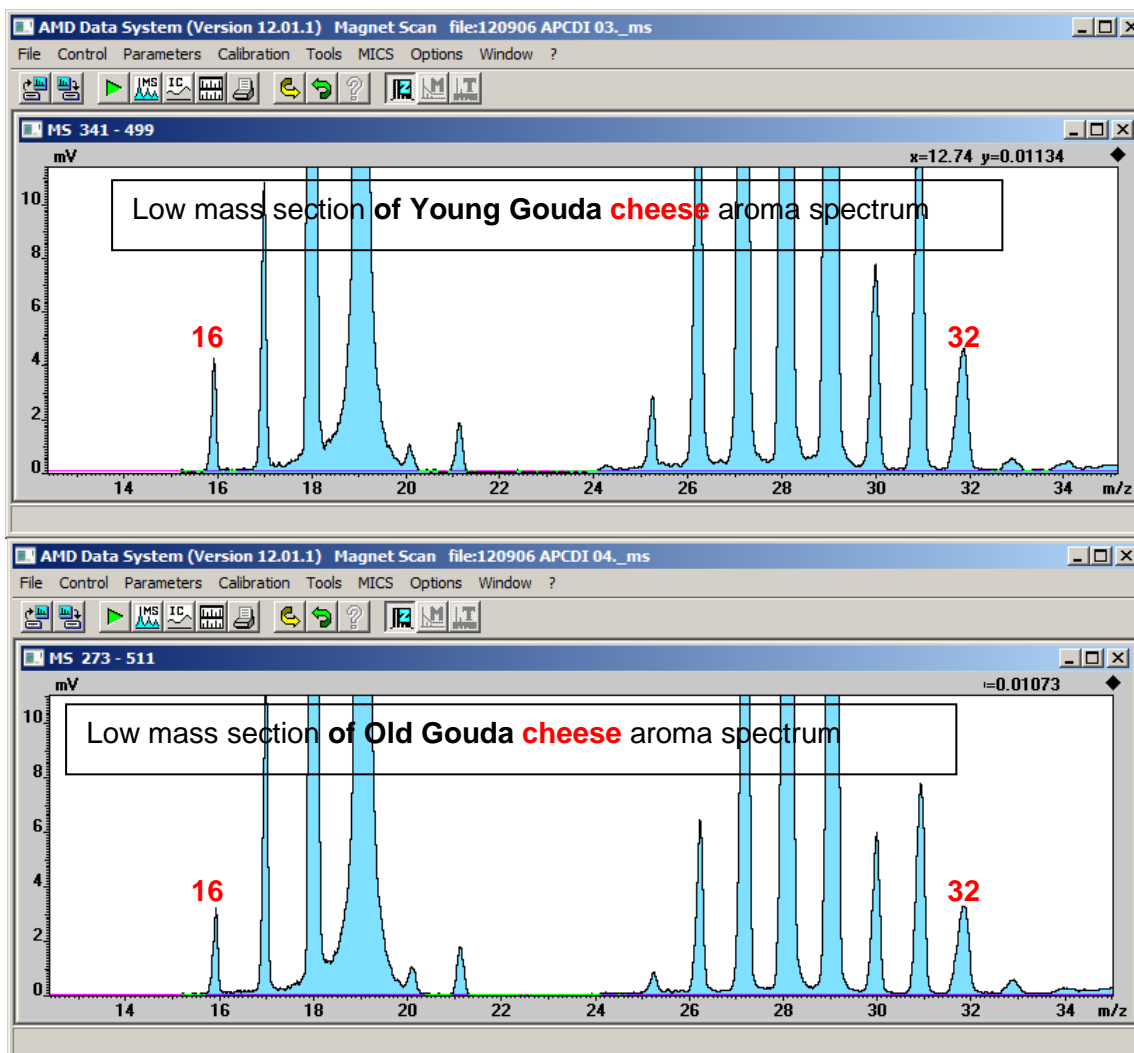


Fig. 24 APCI low mass section spectra of **Young Gouda** and **Old Gouda** cheese in comparison

The corona discharge conditions mainly depend on the position of the corona needle relative to the counter electrode and the applied voltage. Shown in this figure (same spectra as in figure 23 but smaller mass range) two ions seem to be of importance regarding reproducibility of the corona conditions. In all measurements it was observed that the ions at mass 16 and 32 (oxygen), produced in the corona discharge, are not influenced by proton transfer reactions. They may be used as markers for the corona conditions and give at least indications for sensitivity comparisons.

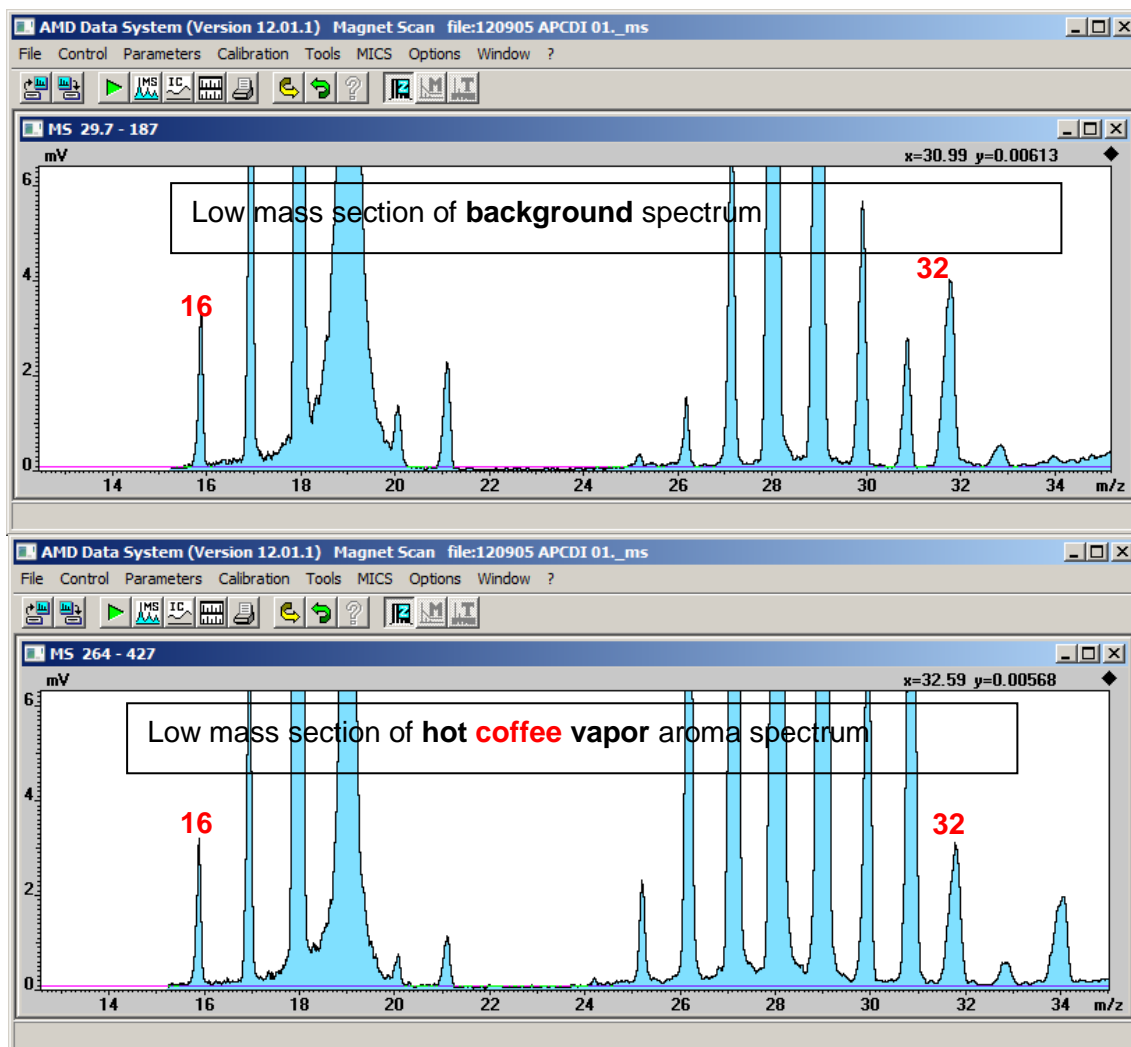
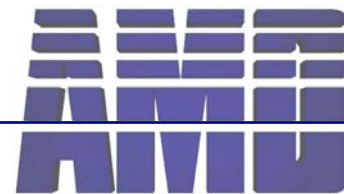


Fig.25 APCDI low mass section spectra of **background** and **hot coffee** in comparison

In this figure the background spectrum and the aroma spectrum taken indicate that the corona condition markers show relative deviations in the order of about 20%.

Above considerations give some indication that mass spectra produced by controlled APCDI conditions have the potential to be used for pattern recognition purposes.



D6) Considerations regarding applicability of pattern recognition methodologies

The application of pattern recognition programs in mass spectrometry is well established since a long time for library search methods based on electron impact (EI) mass spectra yielding significant substance relevant information. Also classification of PTR mass spectra, containing protonated molecular ions of VOC's is subject of research activities in the scientific community in various fields of application. The target for above described examples of APCDI-MS was the evaluation of the protonated ion spectra regarding the information content ("fingerprint"), possibly suitable for classification of a sample. The bar graph spectra below are shown, exemplarily,

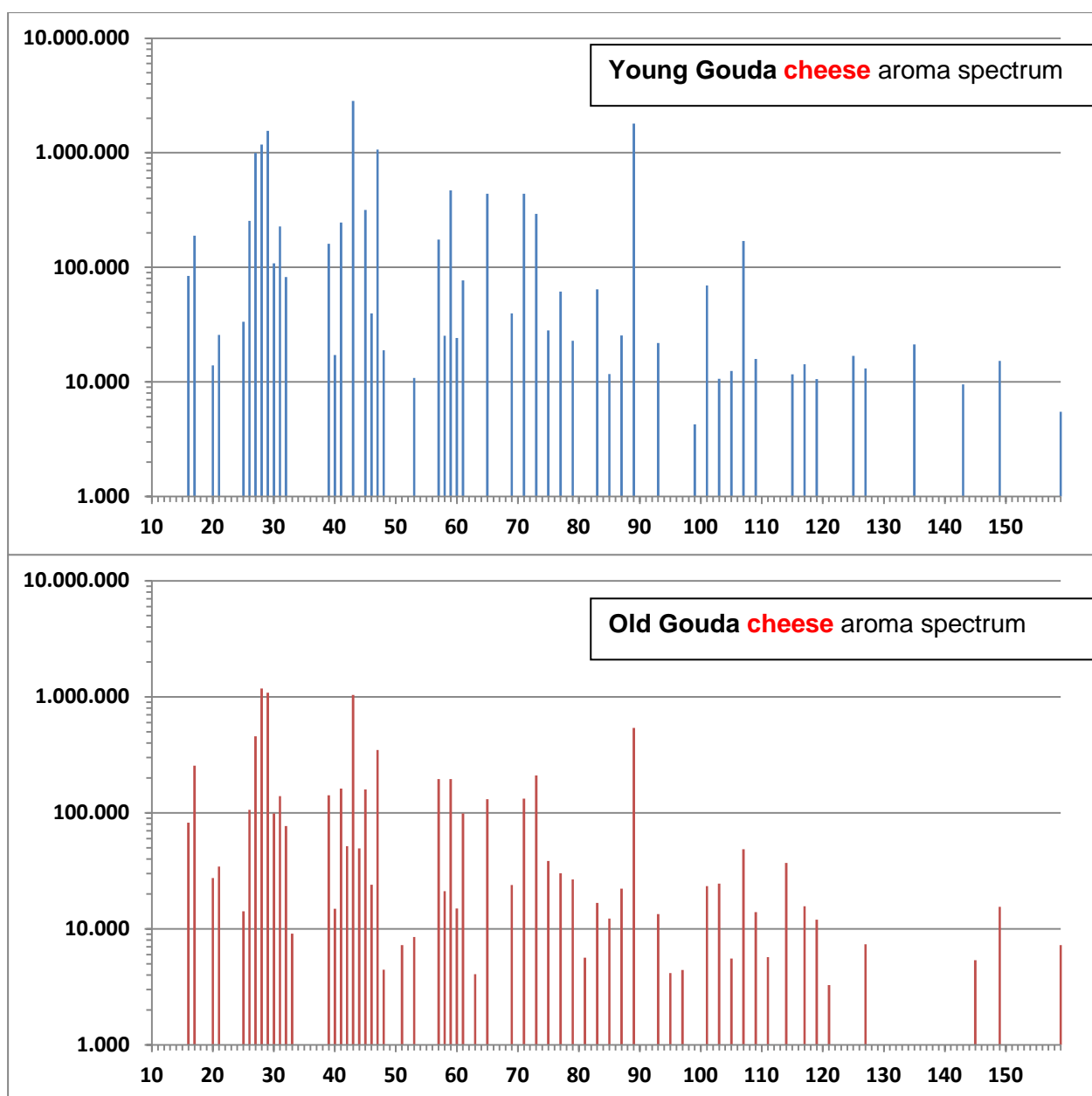


Fig.26 APCDI bar graph protonated ion mass spectra of Young Gouda versus Old Gouda cheese

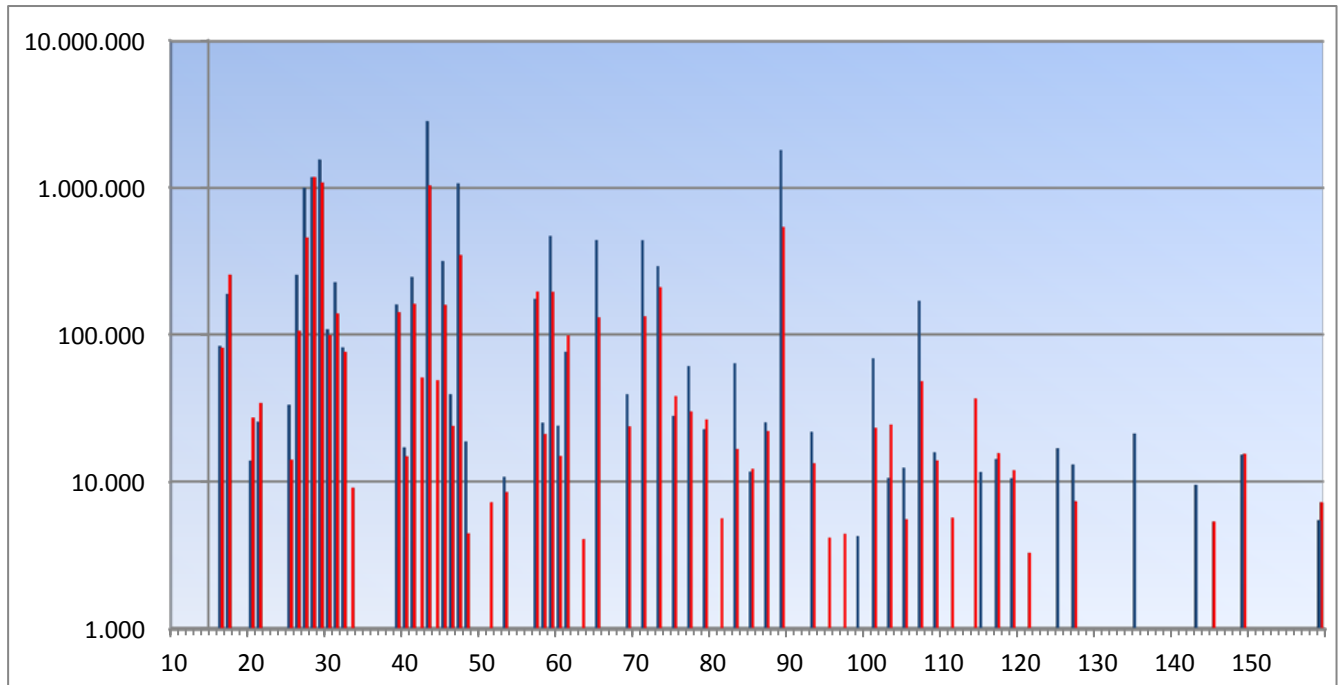


Fig.27 APCDI bar graph protonated ion mass spectra of Young Gouda (blue) versus Old Gouda cheese (red).

The raw data spectra of figure 22 have been evaluated at a certain threshold and displayed at logarithmic intensity scale for comparison. Very small peaks are not included and the water clusters at masses 19, 37 and 55 have been eliminated.

It can't be decided from this exemplary figure if the individual "fingerprint" of the different spectra would result in a reliable classification if a pattern recognition program would be applied and the data base for testing the reproducibility would be available.

Within the scope of this application note the principal potential of the APCDI-MS method using the headspace of a sample for analysis of the released VOC compounds has been demonstrated.