Ambient mass spectrometry

Direct analysis in real time (DART) is new ambient ionization technique for mass spectrometry (MS), which enables rapid analysis of liquid, solid or gaseous samples in the open atmosphere, without any separation of sample components. DART-MS has a high throughput and needs minimal sample preparation. When coupled to a mass detector it is an excellent tool for instant chemical characterization of various biological samples, including foods.
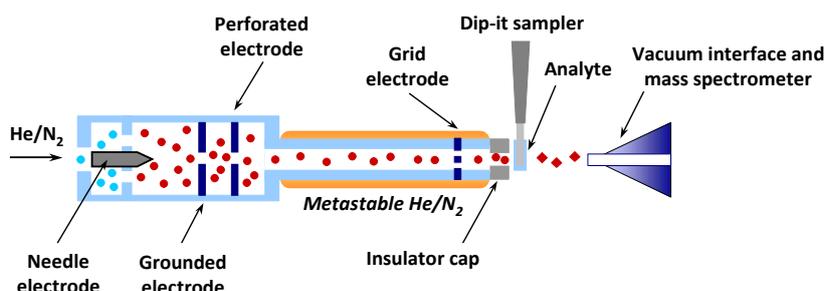

Figure 1. Layout of the DART ion source.

The scheme of DART ion source is provided in Figure 1. In DART, metastable excited-state atoms are formed via a glow discharge in a compartment separated from the sample desorption/ionization area. Charged particles are removed from the gas stream carrying metastable species through the perforated electrode. Then, the gas is heated to desorb the analytes from the sample. In the sampling region, metastable ions react with atmospheric components to form reactive species, ionizing the analytes. DART produces mass spectra, dominated by protonated molecules in positive-ion mode, or deprotonated molecules in negative-ion mode. Although DART represents a soft ionization technique, fragments can also be observed in DART mass spectra.

1 Methodology

The applicability of the DART technique for the purpose of the project was demonstrated with the use of four representative food matrices, supplied by the partners. The samples were as follows:

- Infant formula prepared without ohmic heating; samples prepared by heating at 90 to 140°C without a holding time and IF heated at 130 and 140°C for time corresponding to F0; and overheated samples.

- Biscuits prepared within atmospheric baking in laboratory scale oven under different conditions of temperature and time (180-200°C, 10-14 min).

- Baby food puree (carrots) prepared by 3 heating/freezing regimes) and sterilized at 2 different temperatures (117 and 127°C).

- Canned fish (tuna in brine; tuna in sunflower oil; sardines in sunflower oil) prepared by high pressure treatment under different conditions temperature and holding time 115°C, time 28 min or 121°C, time 7 min.(tuna in brine: temperature 115°C, time 28 min.

Two complementary sample preparation approaches were used to isolate and analyse consecutively both polar or non-polar compounds. For infant formula, dilution of the samples with methanol (1:5, v/v) was used to enhance the DART ionization of polar components . Non-polar compounds were simply extracted with toluene. For biscuits and baby food puree isolation of polar and non-polar compounds was performed via extraction by a methanol-water mixture (1:1, v/v) and toluene,

respectively. For canned fish isolation of both, the polar and non-polar compounds, was performed simultaneously in one step by homogenising in deionized water and cyclohexane and centrifuging.

For statistical evaluation of the data, group t-test (differences between contrast samples) and principal component analysis, PCA (visualization of multivariate fingerprint data) were used (Statistica, version 8.0; Statsoft, USA). The input data for PCA were the intensities of the ions pre-selected from DART fingerprints, normalized to the most abundant one.

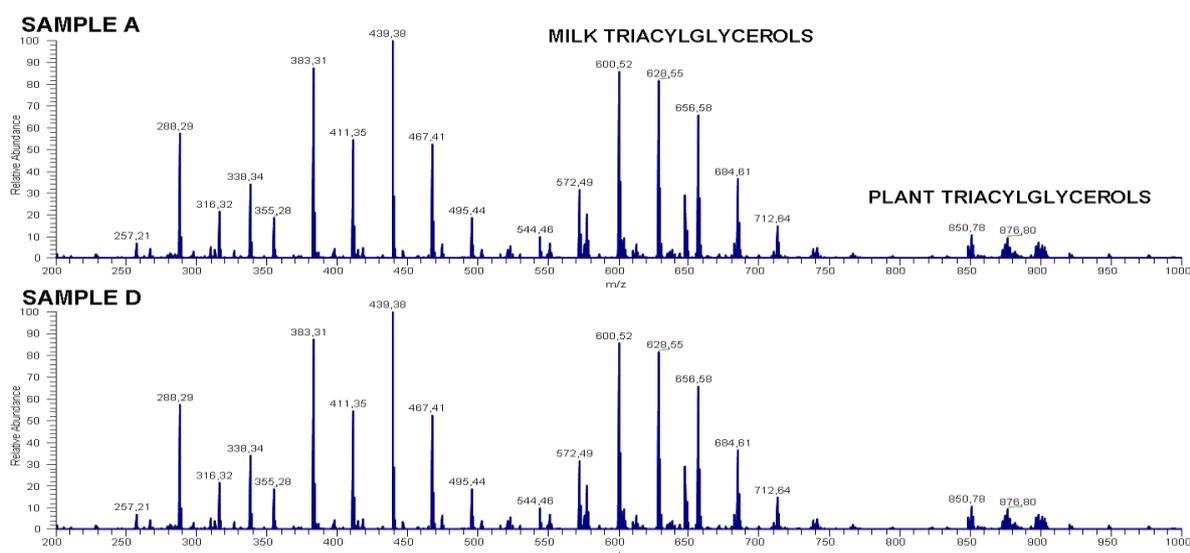## 2. Results

Infant formula

Figure 2. DART–MS spectra of infant formula toluene extracts of different samples.

Infant formula samples were analyzed in both the positive and negative DART ionization mode. The positive ionization mass spectra of the infant formula toluene extracts enabled monitoring and identification of milk and plant triacylglycerols (Figure 2). No statistically significant differences were observed, between non-treated and heat-treated samples, indicating that mild heating did not cause any changes (oxidation) of lipid In negative ionization mode, no abundant signals could be observed.

Principal components analysis (PCA) was used to show differences between the samples. It produced a visual plot of the data by transforming the input variables (relative intensities of 16 pre-selected ions) into principal components. A PCA plot constructed for the infant formula data is shown in Figure 3. As can be seen, the objects representing sample A and sample D are well resolved each other and from the objects in samples B and C. One of the marker signals was identified as HMF.
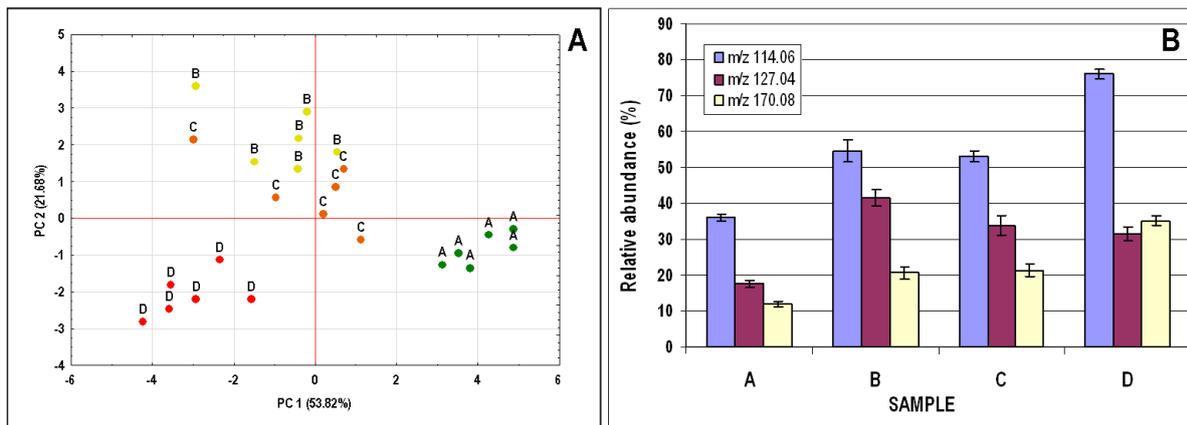
Figure 3. (A) PCA plot of positive ionization data obtained by analysis of methanolic-extracts of infant formulas, (B) relative intensities of selected marker signals in respective infant formula samples.

Biscuits

Analysis of toluene extracts of biscuits also showed differences between DART fingerprints of different samples. DART–MS spectra obtained in either positive or negative ionization mode exhibited much higher potential for monitoring of changes within experimental heating time/temperature combinations compared to the non-polar fraction fingerprints.

Numerous signals corresponding to various compounds are changing their relative intensities (both increase and decrease can be observed), some of them being formed or disappearing from the mass spectra. Such changes in chemical composition are clearly linked to a different extent of the Maillard reaction taking part in the samples during heat-treatment (baking). The extent of the alteration of the DART fingerprints during prolonged heating, and at increasing temperatures, is shown in Figure 4 and via PCA in Figure 5.
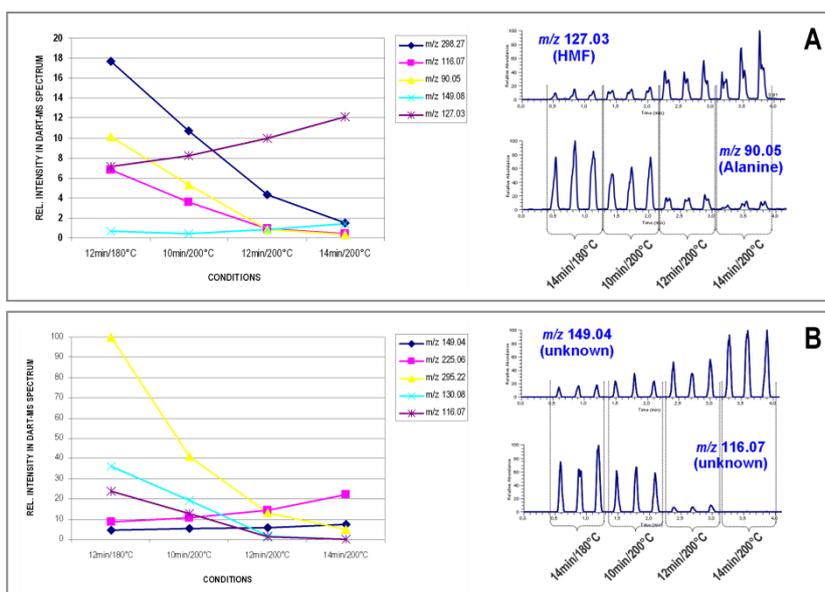

Figure 4. Changes in relative intensities of selected signals in biscuit samples (left) and example of extracted ion records (right). (A) Positive ionization mode data, (B) negative ionization mode data.
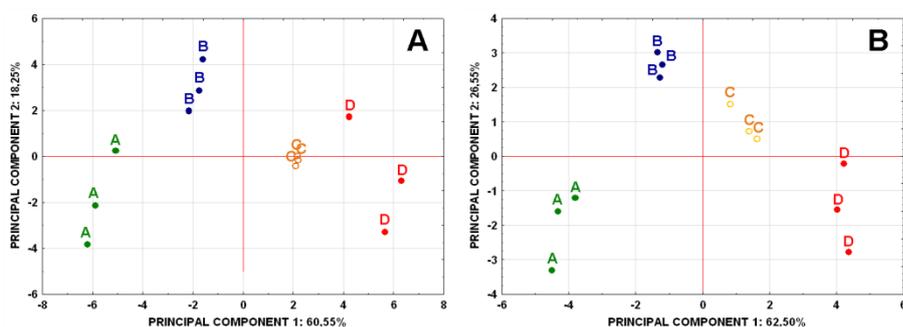
Figure 5. PCA graphs of DART fingerprints obtained by analysis of methanol-water extracts of biscuits samples. (A) Positive ionization mode data, (B) negative ionization mode data.

Statistical t-tests enabled the selection of the most discriminant ions. The intensities of some signals increased while others decreased, mimicking the dynamic changes in the concentrations of reactants, intermediates and end-products of Maillard reaction. The identification of some of these compounds was achieved by the accurate mass measurements by DART–MS.

Baby food puree and canned fish

The relative intensities of the ions present in baby food puree fingerprints (both polar and non-polar fractions) were not influenced neither by production process nor by the sterilization temperature. Similar results were obtained also for high pressure-treated canned fish. Differences related to the fish and/or oil type could be observed in cyclohexane extracts, however discrimination of the test samples according to the temperature-time combinations were not possible.

3. Discussion and conclusions

DART–MS-based fingerprinting of extracts prepared from four representative food matrices investigated within the project, enabled efficient fast monitoring of polar and non-polar compounds. The novel approach was able to monitor temperature-induced changes in chemical composition of infant formula (ohmic heating used for sterilization) and biscuits (baking).

Statistical analysis enabled selection of signals that changed during heating. These "marker" signals derived from precursors, intermediates and products of reactions in the food (mainly Maillard reaction). A tentative identification of "marker" substances could be made by estimation of elemental formulae, based on the knowledge of their accurate masses. The high-pressure treatment/sterilization and freezing/thawing/sterilization applied to baby food puree and canned fish, respectively, did not cause any notable changes in DART fingerprints.

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