Monitoring the freshness of meat: new possibilities

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Abstract

This paper investigates the use of the DART ionisation technique for meat freshness evaluation. Top round beef steak was cut into 50 g chunks and packed in a modified atmosphere. Five different extraction reagents were used for the extraction of low-molecule substances. The extracts were subsequently measured using the DART technique in combination with TOF-MS high-definition spectrometry. Data from the mass spectra were modified and subjected to an analysis of the main components with the result of a dispersion diagram of the component score. Based on the results, we can assert that DART in combination with TOF-MS is usable for meat freshness evaluation if suitable extraction reagents and analysis conditions are applied.

DART TOF-MS, freshness, metabolome profile

Introduction

Meat is an important component of human nutrition, primarily for its high content of proteins, vitamins and minerals. However, demands for quality and safety are increasing with people's growing standard of living. This is why the requirements for methods evaluating meat freshness are also increasing, specifically in terms of speed and accuracy, while non-destructive methods are preferred.

The most commonly used methods today are sensory evaluation and objective measurement. Sensory evaluation is performed by a panel of trained evaluators by using their sight, touch and smells, though the results obtained are subjective (Alimelli et al. 2007). Analytical methods such as gas chromatography, high-performance liquid chromatography and spectrophotometry and determination of the total number of microorganisms (Song et al. 2012) define meat freshness indirectly by measuring freshness markers such as the amount of degradation products of adenosine triphosphate (ATP) (Mora et al. 2010), the content of trimethylamine (TMA) (Béné et al. 2001), the content of substances reacting with 2-thiobarbituric acid (TBARS) (Xiong et al. 2015) and biogenic amines (Vinci and Antonelli 2002). These methods are more reliable than sensory evaluation, though their disadvantages include their laboriousness, destruction of the sample and the impossibility of online evaluation which is necessary due to the increasing demands for freshness evaluation.

Recent research has suggested that it is possible to use some non-destructive methods to evaluate the freshness of meat, such as image analysis (Dowlati et al. 2013), spectroscopic techniques (VIS, near and mid-infrared spectroscopy) (Sinelli et al. 2010; Alamprese et al. 2013; Khojastehnazhand et al. 2014), the electronic nose (Huang et al. 2014) and the electronic tongue (Zhang et al. 2012). However, despite certain characteristic advantages of these methods they do have some drawbacks. For example, image analysis is not able to determine the chemical composition of the sample due to the lack of spectral data, while spectroscopy cannot provide the spatial distribution of freshness markers, etc.

The development of analytical methods offers new ionisation techniques that are non-destructive and enable online analysis. One of these is DART in combination with

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TOF-MS which enables analysis of solid samples without prior treatment. The DART technique was patented in 2005 (Curtis et al. 2009), since when it has been used for various applications both in the food industry (Cody et al. 2012) and in other areas. The output of this method is a mass spectrum which can serve as "metabolomic fingerprinting" and enables the tracking of deviations from the "standard" in a weight diagram. It is also possible to identify meat freshness markers, if their mass is known.

The aim of this paper was to determine whether it is possible to use the DART technique in combination with TOF-MS to evaluate meat freshness based on the metabolome profile which is evaluated by statistical methods.

Materials and Methods

Samples of beef were used for the analyses (leg – round steak, young bull) which were obtained from a meat processing plant 4 days post mortem. The round steak was cut into slices (50 g) and packed in a modified atmosphere (80% oxygen, 20% carbon dioxide). Measurement was performed on the day of sampling and then after 1, 2, 3, 4, 7, 11 and 14 days of storage.

Sample preparation

Totally 2.5 g of the sample was weighed out in a 50 ml PP cuvette. An extraction reagent (25 ml) was added to the sample and the mixture was homogenised for 1 minute using Ultra Turrax at a speed of 8 000 rpm. The selection of extraction reagents - methanol, distilled water, toluene, chloroform-methanol (2:1) and hexane-isopropanol (2:1) and sample preparation were performed on the basis of data published in the literature.

In the case of extraction with methanol, the sample was kept at 60 °C for 15 minutes following homogenisation. The mixture was then centrifuged for 10 minutes at 20 °C and 7 380 rpm. The supernatant was transferred to a vial and analysed by DART TOF-MS in the positive mode.

DART TOF-MS analysis

The DART measurement conditions: speed of sticks container was 1 mm·sec^{-1}, helium was used as the ionisation gas, the flow rate was 3 l·min^{-1} and the temperature 300 °C. The voltage on the fragmentor was 175 V and the voltage on the skimmer 65 V. The measuring range m/z on TOF-MS was 100 to 1 500, and the scan speed was one spectrum per second.

Statistical analysis

The chemometric analysis included principal component analysis (PCA) and the modification of data from the mass spectra which consisted of the unification of m/z values for individual samples using macros in MS-Excel 2007. The data was then standardised in the programme Statistica 10.0 and subjected to multivariate analysis of principal components.

Results and Discussion

Five different extraction reagents were tested in the investigation of the best measurement conditions on the basis of several studies (Folch et al. 1957; Ferraz et al. 2004; Custódio et al. 2007; Václavík et al. 2011 and 2013) in which the authors looked into the isolation of individual groups of compounds serving as freshness markers or extracted various foodstuffs for the DART technique.

The mass spectra changed during meat storage for various lengths of time (Plate III, Fig. 1 and 2). The main differences are in mass abundance where the most significant change can be seen in triacylglycerols (m/z 1013.00) where disintegration into diacylglycerol and monoacylglycerol occurs due to post mortem changes and spoilage. Other significant changes in abundance were found among masses 675.67; 355.37; 338.34; 274.27 and 270.17 where it is not exactly clear what kind of substances they were; in addition, some mass is observed to appear or disappear.

Since it was impossible to identify all significant masses, we have subjected the data from the mass spectra to a multivariate statistical method – analysis of the main components (Plate III, Fig. 3). Based on chemometric analysis, we differentiated samples of different ages depending on the sample metabolome profile with relatively high classification efficiency. Clusters of points for fresh samples and samples stored

for a long period of time are in separate groups and do not intersect. These results were obtained using toluene and methanol as extraction reagents. The remaining 3 extraction reagents did not yield these results; the values for fresh meat and meat stored for a long period of time were dispersed in the diagram between them.

Conclusions

The new DART technique in combination with high-definition mass spectrometry (TOF-MS) was applied to evaluate the freshness of beef. Simple, fast and efficient procedures were designed for the treatment of samples. Several extraction reagents were used, of which methanol and toluene proved to be the most suitable. The DART measurement parameters (temperature, mode) were optimised prior to testing. The data obtained show that the DART technique can be used to distinguish samples based on the period of storage.

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Plate III
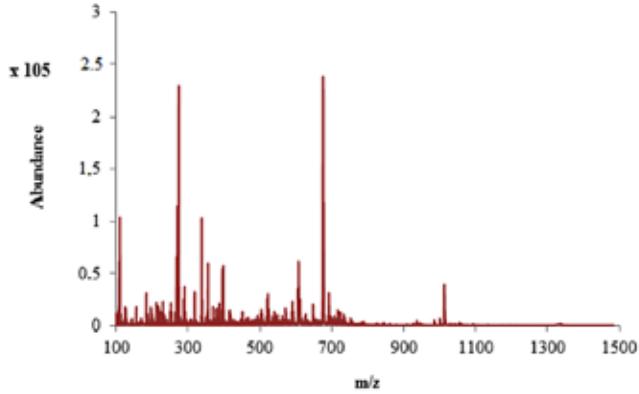Škorpilová T. et al.: Monitoring... pp. 15-18

Fig. 1. Mass spectra (DART TOF-MS) of beef metabolome, positive mode of ionisation; analysis of toluene extract; red spectrum – fresh meat
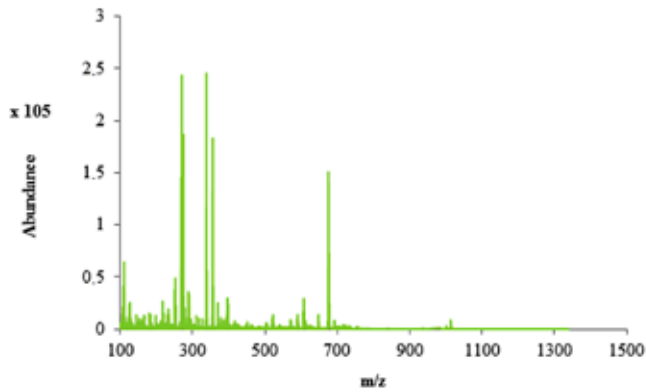

Fig. 2. Mass spectra (DART TOF-MS) of beef metabolome, positive mode of ionisation; analysis of toluene extract; green spectrum – meat stored for 14 days)
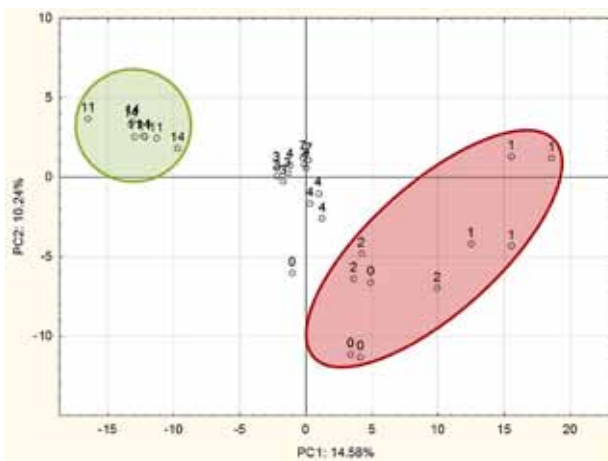

Fig. 3. The PCA analysis of mass spectra of toluene extracts; red – fresh meat, green – old/spoiled meat; values of points indicate the length of storage (days)