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Study of white truffle aging with SPME-GC-MS and the Pico2-electronic nose

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Abstract

We observe the change of aromatic compounds in the headspace of white truffles (Alba's Truffle) after storage at +4 °C over a period of a few days. Measurements have been performed using SPME-GC-MS technique and the Pico2-electronic nose (EN) developed at Sensor Lab. in Brescia. The EN shows a very high sensitivity towards the truffle's relevant molecules such as 2,4-dithiapentane and is able to detect gasses from truffle samples up to a mass of the order of 10 mg. As for truffle aging, results obtained with both techniques are strongly correlated and confirm that there is a variation of the truffle's headspace after circa 5 days.

Keywords: White truffle; Shelf-life; Electronic nose; Metal oxide sensors; Headspace analysis; SPME-GC-MS

1. Introduction

Electronic nose (EN) technology has been widely employed in the past in various fields: automotive, environmental monitoring, medical diagnostic, quality control of food and beverages. At present, it seems very difficult to create an all purpose instrument, so the trend is to develop systems for specific applications. Food quality assessment is one of the most promising applications, as demonstrated by the large amount of publications on this topic, see e.g. [1,2] for an overview.

From the methodological point of view, food analysis applications can be divided in two main groups: *static* classifications, e.g. different sample recognition, and *dynamic* classifications, e.g. monitoring the time evolution of a certain product throughout its lifetime [3]. In dynamic classification, usually the final task consists of distinguishing between fresh food (on shelf) and aged food (off shelf). EN has been applied to solve problems such as fruit and vegetables ripening control [4], fish and meat freshness monitoring [5], shelf-life evaluation of different preserved foods [6]. All these works report preliminary results which make the EN technology very interesting for a quick and objective evaluation of food aging.

In this work, we study the relative change of the white truffle's aroma (*Tuber magnatum* Pico) in the days following the harvesting, in order to determine the maximum preservation time for the white truffles (Alba's truffle). The flavour of the white truffle is mainly characterized by four parameters: the type, the origin, the ripening and the freshness (aging). The truffle freshness is extremely important for both consumer's safety and commercial points of view, i.e. determining the quality and price of the product. It would be therefore interesting to have a reliable system for truffle freshness evaluation.

It is well known that the truffle's aging implies several biochemical reactions, which cause the changing of flavour with time. Investigations about the time variation of truffle headspace composition have been performed on *Tuber aestivum* [7], *Tuber melanosporum* [8] and *Tuber borchii* [9]. Different storage conditions have also been explored. In all the cases, a strong flavour variation has been observed during a few days. Usually, in the fresh sample aroma, there are few molecules or even a single relevant molecule that play an important role. For *Tuber melanosporum*, two aldehydes (2- and 3-methylbutanal) and two alcohols (2- and 3-methylbutanol) are the relevant compounds, while 1-octen-3-ol is the main molecule in the headspace of fresh *Tuber borchii*. With time, an increase of alcohols and sulphur compounds have been observed and attributed to the fermentation process.

Tuber magnatum Pico is the most esteemed truffle type. DNA analysis is the main technique employed to investigate this white truffle [10]. Currently, few publications concerning headspace composition exist [11], while no work about

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headspace time variation of *Tuber magnatum* has been performed.

Here, two different techniques have been applied for the headspace analysis. The first one, SPME-GC-MS, allows us to obtain quantitative information about the compounds of the truffle headspace but is a complex and time consuming method. The second one, EN, is a promising technique to objectively investigate the aroma fingerprint of black and white truffles. The portable nose PEN-2 (Airsense, Germany) has been employed to certify the quality of *Tuber melanosporum* black truffles [12], while an EN equipped with semiconducting metal oxide (SMO) and metal oxide semiconducting field effect transistor (MOSFET) sensors have already been used to evaluate the white truffle quality, training the system with the sensorial analysis results [13]. Concerning quality assessment, MOSFET sensors provided better results in

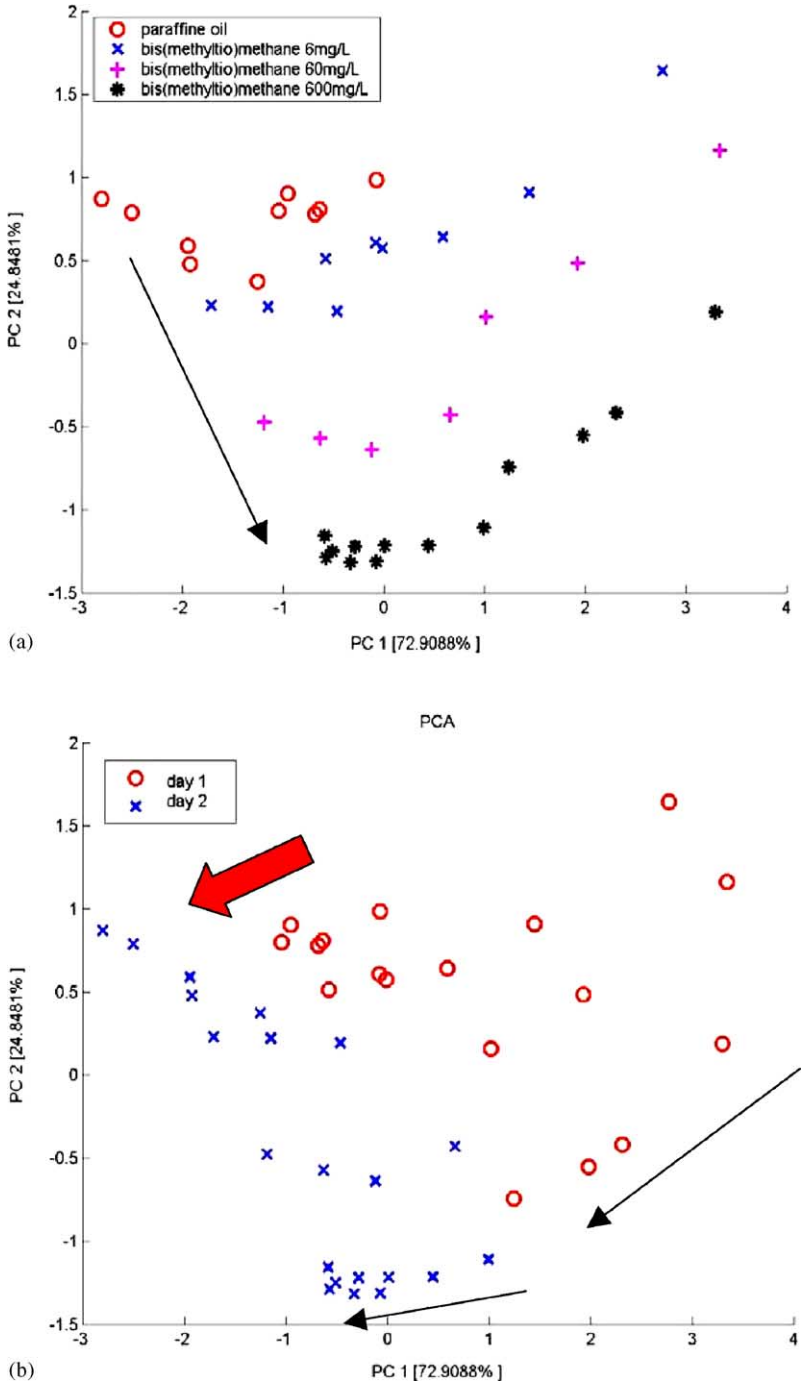

Fig. 1. (a) The PCA plot shows the response of Pico2-EN for increasing concentrations (as indicated by the black arrow) of bis(methylthio)methane diluted in paraffin oil. The response towards pure paraffin oil is also displayed. (b) The PCA displays the same data vs. the day of measurements. The thick arrow shows the variation due to measurements performed in different days, the two thin arrows indicate the order of subsequent extractions (from the first to the last) performed on the same vial.

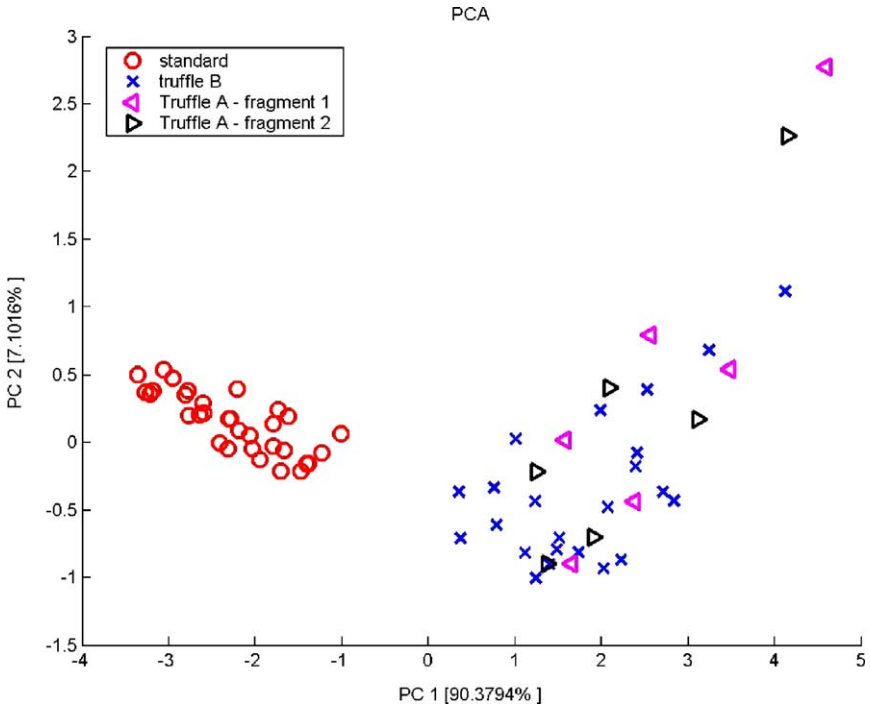

Fig. 2. PCA plot of measurements associated with the different truffle samples. Truffle A was divided in two fragments (1 and 2). The two fragments of sample A and the sample B are indistinguishable.

terms of sensitivity and selectivity. Actually, for the aging evaluation, the sensors stability is the most important parameter because the system variations could mask the sample variations. The Pico2-EN is based on high stability thin film nanostrucured SMO sensors specifically prepared for this application [14]. As shown in the following, high sensitivity towards truffle volatile compounds was also observed.

2. Experimental

2.1. Pico2-electronic nose

A detailed description of the Pico2-electronic nose can be found in previous contributions [15]. For these experiments, the system was equipped with six thin film SMO sensors based on different sensing materials: SnO_2, SnO_2–Au, SnO_2–Pd, WO_3, In_2O_3, SnO_2MoO_3 [16]. The sensors' heating temperatures were first optimized in order to improve the signal. Best results have been achieved with SnO_2 based sensors which show low drift with time, high responses to the truffle aroma and fast recovery of the baseline.

The white truffle aroma cannot be associated with a single molecule, although the most representative molecule is the 2,4-dithiapentane[1] (or bis(methylthio)methane). Therefore, as a first step, we measured bis(methylthio)methane at different concentrations in paraffin oil (6, 60, 600 mg/L) in order to determine the perception threshold of the EN for this compound. All the measurements were performed with dynamic headspace sampling using 500 mL vials held at constant temperature (30 °C).

With regard to white truffles, the measurements have been performed on two samples with the same harvesting time and an homogeneous ripening degree. Because the sample degradation with time is strongly related to the storage

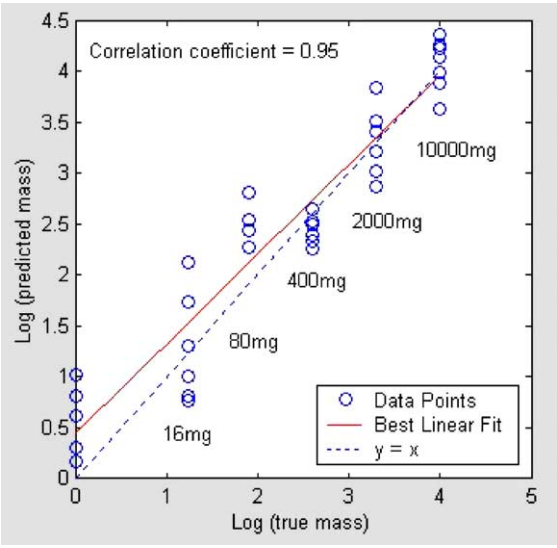

Fig. 3. Correlation curve between the value predicted by EN (*Y* axis) and the real truffle mass stored inside the vial (*X* axis). The log of mass has been actually reported because of the large range to be represented. Data show that the lowest value (16 mg) is correctly predicted by the EN.

[1] Other synonyms: bis(methylmercapto)methane; formaldehyde dimethyl dithioacetal; methylene bis(methyl sulfide).

conditions [8,9], the samples were preserved wrapped in blotting paper stored in the fridge at +4 °C (standard preservation conditions).

The second step was just to verify the homogeneity of two truffle samples (A and B). To this end, we made measurements on different truffle fragments with the same mass (2000 mg). Subsequently, we examined the detection limit of the EN towards the mass of the analyzed truffle samples (only the truffle B was considered), by performing tests with different truffle masses (16, 80, 400, 2000, 10 000 mg). In both cases we used 50 mL vials and dynamic headspace sampling. The headspace was generated at 30 °C for 10 min. The system was able to perform a single measurement in ∼10 min.

Last and most importantly, we examined the aging effects on the truffle headspace. The measurements on the sample B were replicated for 5 days and the variation with time was observed. The change has been correlated with the truffle aroma variation as measured by SPME-CG-MS. Bis(methylthio)methane diluted in paraffin (600 mg/L) has also been used as standard of reference in order to evaluate the system stability. Explorative analysis of data was

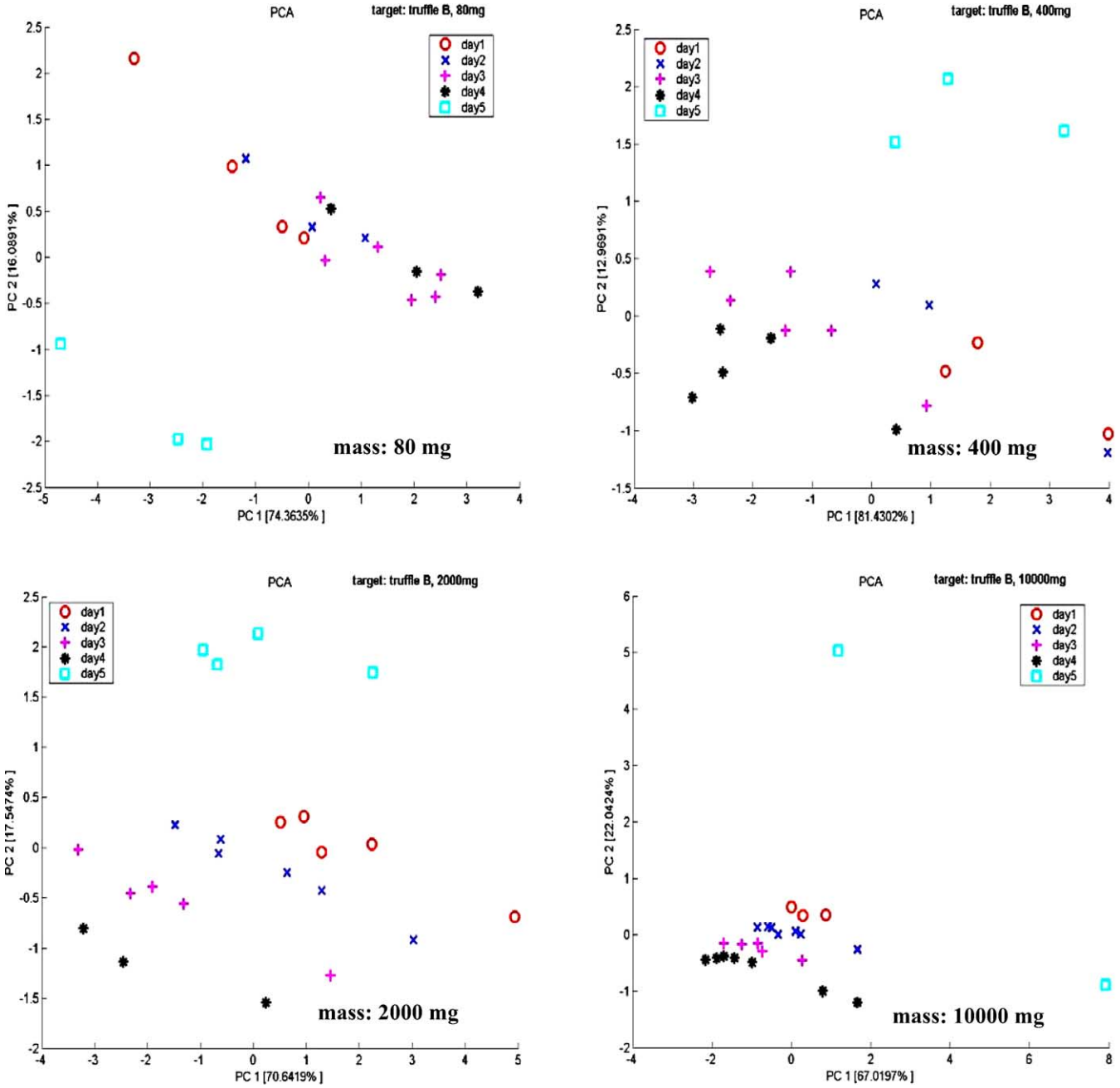

Fig. 4. PCA plot shows the variation of truffle aroma during the days of measurements for different truffle masses. The variation increases with increasing sample mass and the best results were obtained with 2000 and 10 000 mg samples.

performed with PCA by extracting the classical R/R0 feature.

2.2. SPME-GC-MS

For GC-MS analysis, the samples were measured within a period of 8 days. Static headspace (SH) analysis was carried out by SPME technique using DVB/Carboxen/PDMS adsorbing fibre (2 cm, 50/30 μm) held at temperature of 30 °C for 20 min. Volatile compounds were released into the injector of the GC (Varian 3400) at 250 °C. The carrier gas (helium) flow rate was 1.5 mL/min. Injection was set on splitless mode, with 60 s valve closed. A Column EC-WAX (Alltech) with 30 m × 0.25 mm internal diameter and 0.25 μm film thickness was used. The column temperature was programmed at 35 °C for 3 min, then it was increased by 4 °C/min until 190 °C. This temperature was held for 15 min. Mass spectrometry analysis was carried out using a Saturn ion trap (ITDMS) mass detector (Varian) coupled to the GC. Ionisation was performed by electronic impact (70 eV electron energy). Volatile compounds were identified by comparing their mass spectra with library patterns ("purity" method has been used), and the peak area was measured by total ion current of registered spectra.

3. Results and discussion

3.1. Pico2-electronic nose

As a first point, we determined the detection limit of Pico2 for 2,4-dithiapentane. The PCA plot (Fig. 1a) shows that the system is able to distinguish this compound from the pure paraffin oil up to a concentration of 6 mg/L in paraffin oil. The PC2, which explores ∼25% of the total variance, is clearly correlated with the concentration of 2,4-dithiapentane. The spread of the points along the PC1 (Fig. 1b) can be explained by taking into account two facts: the system variation between the two different days, the multiple extractions from the same vial which cause a variation of odour concentration in the headspace.

We then checked the homogeneity between the two truffle samples (A and B). We performed measurements with two fragments of truffle A (labeled 1 and 2) and with truffle B, taking equal masses of sample (2000 mg). All the samples are indistinguishable on the PCA plot (Fig. 2). For comparison, measurements of the standard of reference are also reported. This result confirms that the two truffles (A and B) actually are homogeneous so, for the shelf-life evaluation, only the sample B has been considered.

In order to estimate the detection limit for the truffle odour intensity, the sample B has been fragmented and measurements on different truffle masses have been performed, assuming that the odour intensity is related to the mass. We observed that the sensor response increase with the truffle mass, so the sensors are working well below the saturation threshold. In Fig. 3 we show the correlation between EN measurements and truffle mass obtained with a multilayer perceptron neural network [17]. The large variance observed for the lowest mass samples (i.e. 0 and 16 mg) is due to the log scale representation. The system was able to detect the truffle samples up to a mass of 16 mg, even if the 80 and 400 mg samples are not exactly predicted and a certain overlap between these two samples exists.

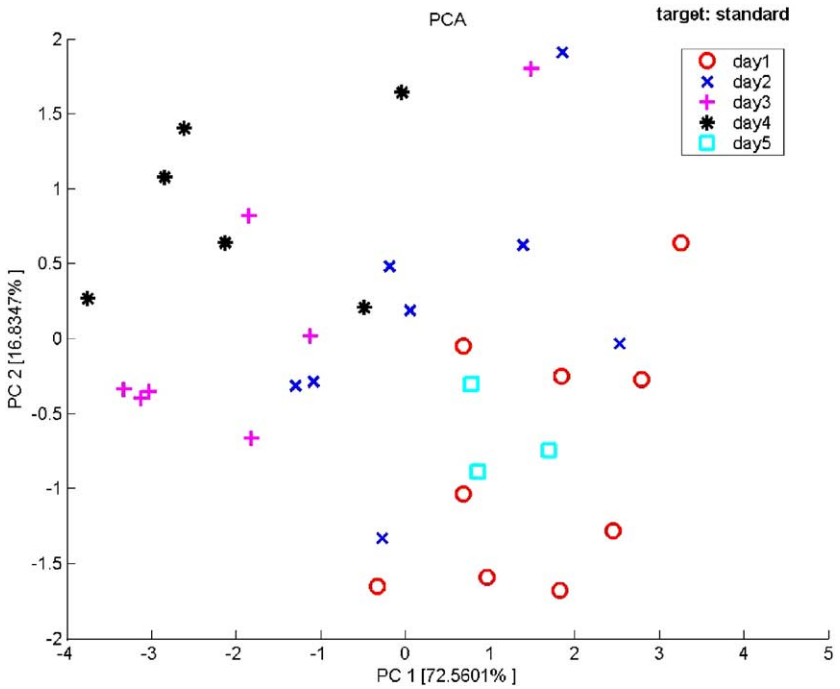

Fig. 5. The PCA shows the measurements of the reference standard (bismethyltiomethane diluted in paraffin oil at 600 mg/L) during different days.

Table 1
Identified compounds and their peak area in first and last (sixth) days of analysis ordered by retention time in the GC column

	Area (total ion current) × 1 000 000		Retention time
	First day	Last day	
Thiobismethane	324.3	137.4	1:56
Ethanol	2.9	146.6	4:03
Dimethyl disulphide	3.1	2.9	8:05
2-Methyl-1-propanol	0.7	25.4	9:15
1,2-Dimethylbenzene	0.2	0.9	12:46
Limonene	4.1	0.7	13:22
2-Methyl-1-butanol	5.9	206.7	14:15
2,4-Dithiapentane	2029.8	2055.7	17:50
3-Pentanol	0.5	1.4	20:14
2-Esanol	0.3	1.1	21:03
Dimethyltrisulphide	2.6	3.0	21:45
1-Metoxy-3-methylbenzene	4.5	3.9	24:53
Acetic acid	1.3	20.7	25:32
2-Acetyl-5-methylfuran	63.0	81.6	29:33
3-Ethyl-4-methyl-3-penten-2-one	12.1	4.9	31:05
2-Methyl-2-propen-1-olo	4.1	5.8	32:31
3,4-Dimethyl-3-esene-2-one	2.6	6.8	33:10
3-Methyl, butanoic acid	0.2	6.2	34:33
Tris(methylthio)methane	12.6	9.1	41:06
Benzeneethanol	0.4	5.5	43:26

As for the time variation of the truffle flavour, we found out that the headspace remains quite constant during the first 4 days, while a remarkable change appears the fifth day, as demonstrated by the PCA plot in Fig. 4. The variation increases with the sample mass. Assuming that the variation in headspace composition is the same for all the samples (independently of the mass), this fact could be explained considering that the EN resolution related to the sensor signal amplitude (which gets bigger with the truffle mass as previously observed). To prove that the change in the EN response is mainly due to the variation of truffle aroma only – and not to the predominantly variability of the EN system – we simultaneously measured the standard of reference. As shown in Fig. 5, a certain trend appears also in the measurements of the standard, but this is very small compared with that of truffle samples.

3.2. SPME-GC-MS

The result obtained with the Pico2 are in agreement with those of GC-MS analysis. Table 1 reports the most important compounds, identified by SPME-GC-MS, as measured in the first and the last day of analysis. Data show that 2,4-dithiapentane is the main compound in the white truffles headspace. Then, it was used to normalise the value of peak area for all the other compounds. It's noticeable that 2,4-dithiapentane and thiobismethane are the most representative compounds of the fresh product

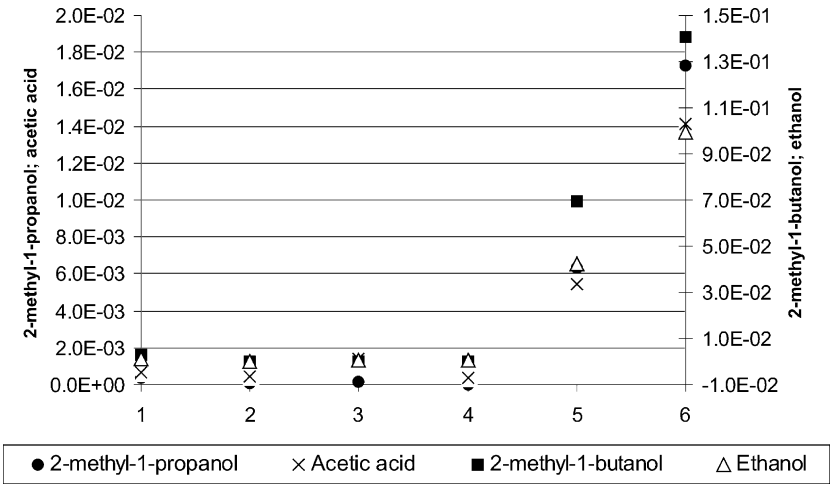

Fig. 6. Time variation of the most relevant compounds identified in the white truffles' headspace. Values are expressed as percentage of 2,4-dithiapentane (average value of different days).

(first day). With aging, we observe that the concentration of 2,4-dithiapentane remains quite constant while other compounds change their abundance in the head space. Within first 3 days, a great amount of the identified compounds was essentially the same, with a little variation for some of them (e.g. acetic acid). After 5 days, we notice a strong concentration increase for different compounds. Fig. 6 displays the time variation for ethanol, 2-methyl-1-butanol, 2-methyl-1-propanol and acetic acid. All these compounds may be due to wild fermentation affecting truffles, that can lead to the degradation of the product. At the same time, other compounds, as thiobismethane, tris(metylthio)methane and 3-ethyl-4-methyl-3-penten-2-one, displayed a reduction in the headspace while sulphur compounds like dimethyl disulphide and dimethyl trisulphide to remain quite constant.

4. Conclusions

The time variation of aromatic compounds in the headspace of white truffles (*Tuber magnatum*) has been investigated with CG-MS and Pico2-EN over a period of a few days. Preliminary, the detection limit of Pico2-EN versus the truffle aroma has been determined. The system was able to detect the most relevant molecule of fresh truffle headspace (2,4-dithiapentane) diluted in paraffin oil up to a concentration of 6 mg/L. With respect to the detection of white truffle a strong correlation between the Pico2-EN response and the truffle mass has been found. Very low truffle masses (of the order of 10 mg) are correctly predicted by the EN.

Concerning truffle aging, classical SPME-GC-MS and the Pico2-EN gave comparable results: a relevant change of truffle's aroma has been observed after 5 days from harvesting. This variation has been mainly attributed to an increase in the headspace of four compounds: acetic acid, ethanol, 2-methyl-1-butanol, 2-methyl-1-propanol. All these compounds are originated by truffle fermentation and can be considered as markers of the product degradation.

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