Introduction

Food products such as spices are strictly controlled to guarantee that they are safe for consumption, free of any possible contamination and also that their sensory quality is preserved and consistent.
In this study, it is proposed to use HERACLES fast gas chromatography Electronic Nose to detect possible contamination of black pepper by mineral oil and to assess the overall sensory quality of the spices.

Equipment

HERACLES Flash GC Electronic Nose

HERACLES Electronic Nose (Alpha MOS, France – Fig. 1) is based on ultra fast chromatography. It features 2 metal columns of different polarities (non polar RXT-5 and slightly polar RXT-1701, length = 10m, diameter = 180µm, *Restek*) in parallel and coupled to 2 Flame Ionization Detectors (FID). Two chromatograms are obtained simultaneously, allowing a sharper identification of the chemical compounds. It allows headspace or liquid injection modes.
The integrated solid adsorbent trap thermo-regulated by Peltier cooler (0-260°C) achieves an efficient pre-concentration of light volatiles and shows a great sensitivity (in the pg range).
With fast column heating rates (up to 600°C/min), results are delivered within seconds and the analysis cycle time is around 5 to 9 minutes.

Fig. 1: Ultra Fast GC based HERACLES Electronic Nose

The electronic nose is coupled to an autosampler (HS 100, CTC Analytics) to automate sampling and injection.
The instrument is operated through Alpha Soft software. In addition to classical chromatography functionalities, it provides chemometrics data processing tools such as sample fingerprint analysis and comparison, qualitative and quantitative models, quality control charts.

AroChembase: Kovats Index library for chemical & sensory characterization

HERACLES e-nose was additionally equipped with AroChembase module (Alpha MOS, France) that can be used within AlphaSoft E-Nose software. It consists of a library of chemical compounds with name, formula, CAS number, molecular weight, Kovats retention Index, sensory attributes and related bibliography. It allows pre-screening the chemical compounds and giving sensory features by directly clicking on the chromatograms' peaks.

Samples & Analytical Conditions

First, a standard mixture of n-alkanes (n-butane to n-hexadecane) was analyzed to allow retention time conversion into Kovats indices.
Then, several batches of pepper, contaminated and non-contaminated ones (Tables 1.1 & 1.2), were analyzed with HERACLES instrument (Table 2) for a qualitative and a quantitative analysis.

Table 1.1: Pepper samples for qualitative study

Sample	Quality	Comments
Q1T2	Conform	No mineral oil contamination
Q1T3		
Q1T4		
Q1T5		
Q2T2	Not conform	Mineral oil contamination
Q2T3		
Q2T4		
Q2T5		
BL1	Blind sample	Quality unknown
BL2		
BL3		
BL4		
BL5		

Table 1.2: Pepper samples for quantitative study

Sample	Comments
CA01	Conform pepper + 0.1% of mineral oil
CA05	Conform pepper + 0.5% of mineral oil
CA1	Conform pepper + 1% of mineral oil
CA2	Conform pepper + 2% of mineral oil
CA3	Conform pepper + 3% of mineral oil
BN1	Blind samples. Mineral oil content unknown
BN2	
BN3	
BN4	
BN5	

Table 2: HERACLES e-nose parameters for pepper analysis

Parameters	Values
Sample mass	1 g ± 0.1g
Headspace vial	20 mL
Headspace generation	20 min at 60°C
Injection volume	2 mL
Initial isothermal temp.	50°C (2s) to 80°C by 1°C/s then to 250°C (21s) by 3°C/s
FID temperature	270°C
Acquisition time	110 s
Time between two injections	8 min

Chromatograms

The comparison of the volatile profiles of contaminated and non contaminated pepper shows significant differences (Figure 2). Contaminated pepper contains much more volatile compounds than non-contaminated pepper.

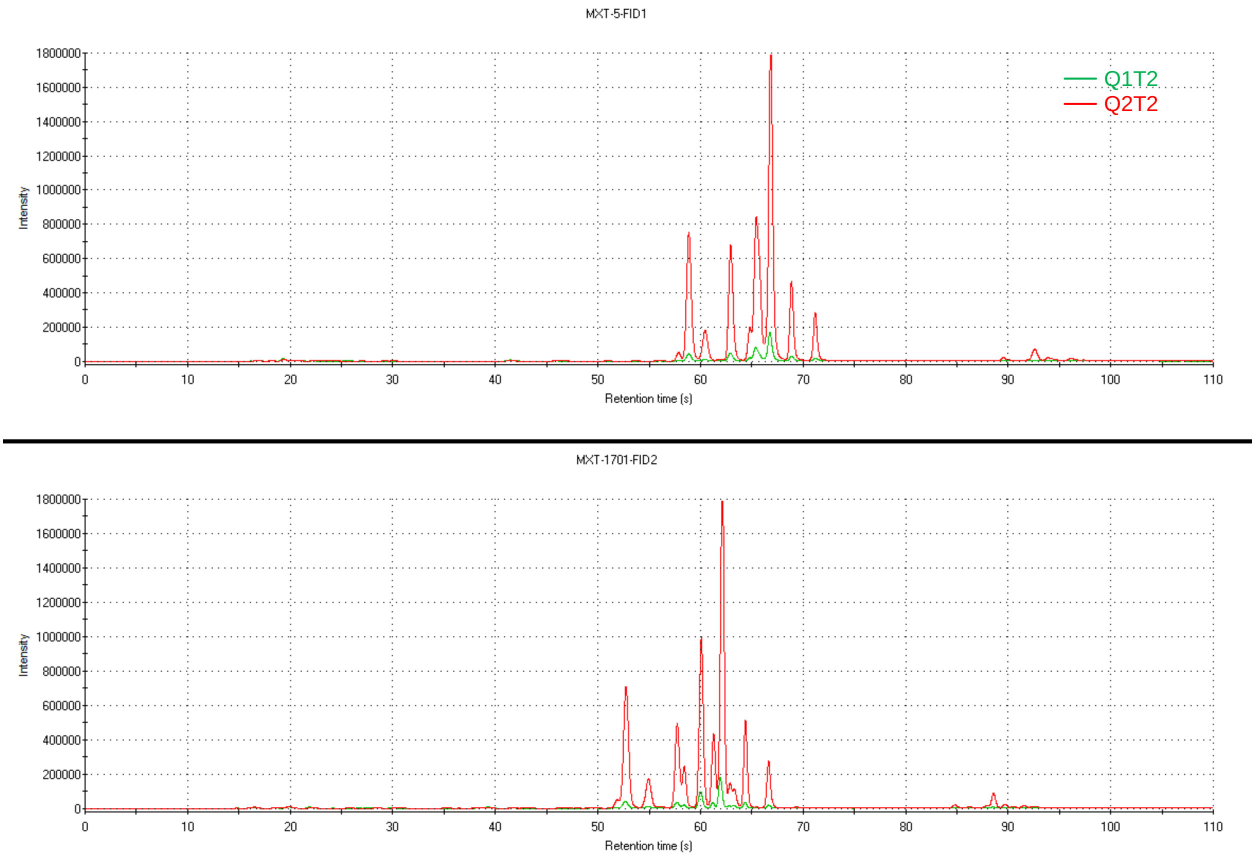

Fig.2: Chromatograms of a contamination-free pepper (Q1T2) and a pepper contaminated with mineral oil (Q2T2) obtained with HERACLES e-nose

Qualitative Analysis of Pepper Odor

To rapidly and easily compare the odor profiles of the different peppers (Table 1.1), an odor map based on Principal Components Analysis (PCA) was set up (Figure 3).

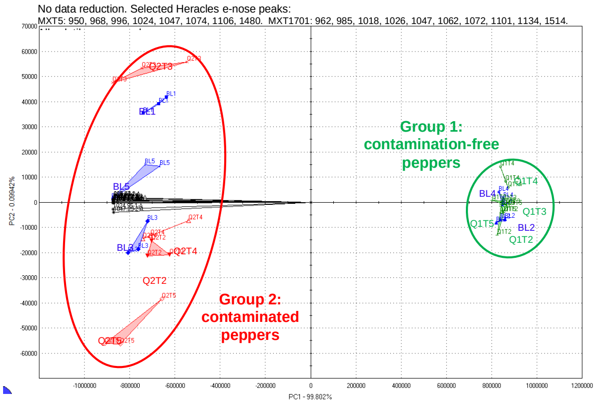

Fig. 3: Odor map of peppers obtained from HERACLES measurements

The statistical processing confirmed the differences observed between the two qualities of pepper. Samples BL4 and BL2 are the closest to group 1 which contains contamination-free peppers. Samples BL1, BL5 and BL3 are clearly discriminated from other blind samples and are closer to group 2 which is composed of peppers contaminated with mineral oil. The projection of the main volatile compounds shows the compounds responsible for the differences between samples.

The nature of the most discriminant volatile compounds involved in black pepper odor differences was investigated using their Kovats index and the AroChemBase database (Table 3). The retention indices in bold corresponds to the most discriminant molecules. Contaminated and contamination-free peppers contain the same volatile compounds, but in different proportions (Figure 4). Contaminated peppers contain higher amounts of volatile compounds.

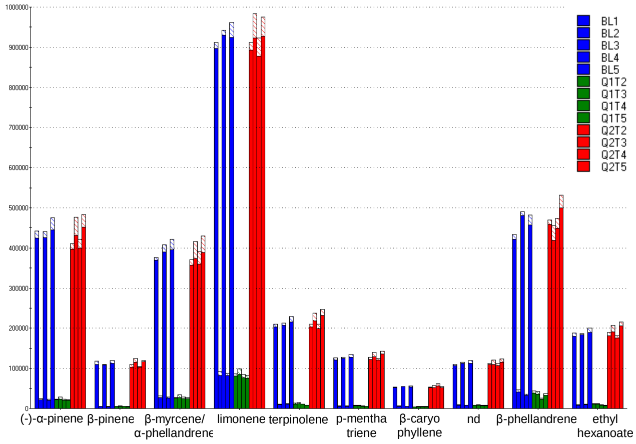

Fig. 4: Peak intensity of the main volatile compounds in black pepper samples

A quality control model based on Statistical Quality Control processing was established, by taking the 4 contamination-free peppers as the reference quality (Figure 5). The 3 blind batches BL1, BL3 and BL5 (in blue) as well as the 4 contaminated peppers (Q2T2 to Q2T5) are projected out of the conformity area (green band), indicating that they do not comply with the desired quality.

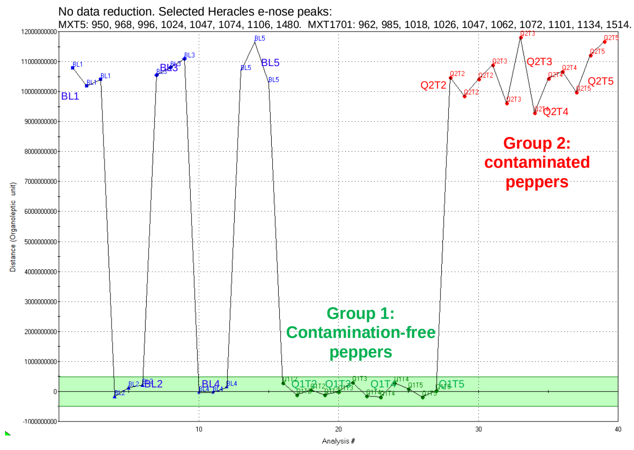

Fig. 5: Quality Control Card of black pepper

RT MXT-5 (± 0.1s)	RT MXT-1701 (±0. 1s)	KI MXT-5 (± 20)	KI MXT-1701 (± 20)	Possible identification	Descriptor
16.8	16.6	448	501	methanol	pungent
19.4	20.0	501	600	2-propanol	alcoholic, etheral
41.5	39.3	777	829	toluene	caramelized, paint, solvent
59.0	52.9	**950**	**962**	**(-)-α-pinene**	fruity, green, terpenic
60.5	55.0	**968**	**985**	**β-pinene**	pine, sweet
63.0	57.9	**996**	**1018**	**β-myrcene/α-phellandrene**	balsamic, fruity/spicy, terpenic
64.9	58.6	1020	**1026**	**nd**	-
65.6	60.2	1029	**1047**	**β-phellandrene**	herbaceous, minty
-	61.3	-	**1062**	**ethyl hexanoate**	anise, sweet
66.9	62.2	**1047**	**1072**	**limonene**	citrus, minty, woody
69.0	64.5	**1074**	**1101**	**terpinolene**	fruity, pine, sweet
71.2	66.8	**1106**	**1134**	**p-menthatriene**	turpentine
92.6	88.6	**1480**	**1514**	**β-caryophyllene**	woody, spicy

Table 3. Main volatile compounds identified in the headspace of ethanol samples using AroChemBase

Quantitative Analysis of Pepper Contamination

To assess the proportion of mineral oil contamination in pepper, a correlation model based on Partial Least Square (PLS – Figure 6) processing can be calculated from the samples containing known amounts of oil contamination (samples CA01 to CA3 from Table 1.2).

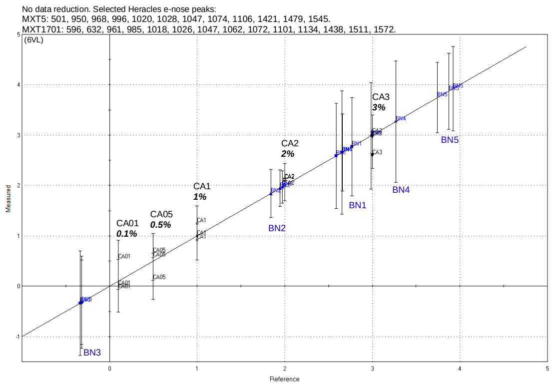

Fig. 6: Partial Least Square (PLS) model correlating the mineral oil proportion (%) with HERACLES measurements

Since the PLS shows a good correlation, it is possible to use this model to determine the proportion of mineral oil contamination in blind samples BN1 to BN5 (Table 4).

Sample	Calculated proportion of oil contamination (%)
BN1	2.7
BN2	1.9
BN3	-0.3
BN4	3.0
BN5	3.9

Table 4: Estimated proportion of mineral oil contamination in blind samples of pepper after projection on PLS model

It seems that BN3 sample is not contaminated by mineral oil (proportion close to CA01 oil content), whereas BN5 is the most contaminated (proportion close to 4% contamination).

Conclusion

This application note shows that the quality of black pepper can be monitored using HERACLES e-nose. The analysis highlighted significant differences of odor profiles depending on the quality, related to the presence of oil contamination.
The proportion of oil contamination was evaluated according to a quantitative model previously set up with samples of known amount of contaminant.

Thanks to AroChemBase database, it was also possible to find several molecules involved in the odor profile variations between black pepper qualities. These molecules are mainly solvents (2-propanol, acetonitrile), ester (ethyl hexanoate) and terpenes ((-)-α-pinene, β-pinene, β-myrcene/α-phellandrene, β-phellandrene, limonene, terpinolene, p-menthatriene, β-caryophyllene).