ANH24 – Quantification of p-cresol in milk with HERACLES Dual Fast GC E-Nose

Objective

p-cresol is a volatile compound that is responsible of a stale / barny off-odor in milk. This molecule can be produce by a bacterial contamination by Bacillus circulans1.
This application note describes the quantification of p-cresol in milk with a Fast GC electronic nose.

Equipment

HERACLES Flash GC Electronic Nose

The HERACLES Electronic Nose (Alpha MOS, France – Fig. 1) is based on the technology of ultra fast chromatography. It features two metal columns of different polarities (non polar RXT-5 and slightly polar RXT-1701, length = 10m, diameter = 180µm, Restek) mounted in parallel and coupled to 2 Flame Ionization Detectors (FID). Therefore, 2 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 8 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.

Samples & analytical conditions

A standard mixture of n-alkanes (n-hexane to n-hexadecane) was first analyzed to allow retention times conversion into Kovats indices.
Milk samples consisted of 5 mL of semi-skimmed milk spiked with a p-cresol solution at 0.5 g/L in methanol (addition of 0.1 to 10 µL of this solution) in order to give a final concentration of p-cresol ranking from 10 to 1000 µg/L.
Samples headspace was generated in septum capped 20-mL vials containing 5g of semi-skimmed milk mixed with 2g of sodium chloride.

Table 1: HERACLES analytical conditions for milk analysis

Parameters	Values
Sample mass	5 ml
NaCl	2 g
Headspace vial	20 ml
Heating temperature	90°C
Injection volume	5 ml
Trap concentrating temp.	60°C
Trap desorption temperature	240°C
Column pressure	60 kPa (0s) 0.25 kPa/s to 90 kPa
Initial isothermal temp.	40 (2 s) 1°C/s to 80°C, 3°C/s to 280°C (8 s)
Acquisition time	120 s
Time between two injections	8 min

Chromatographic profile

The milk samples can be analyzed and potential contaminant molecules can be separated in a chromatogram in less than 2 minutes (Figure 2).

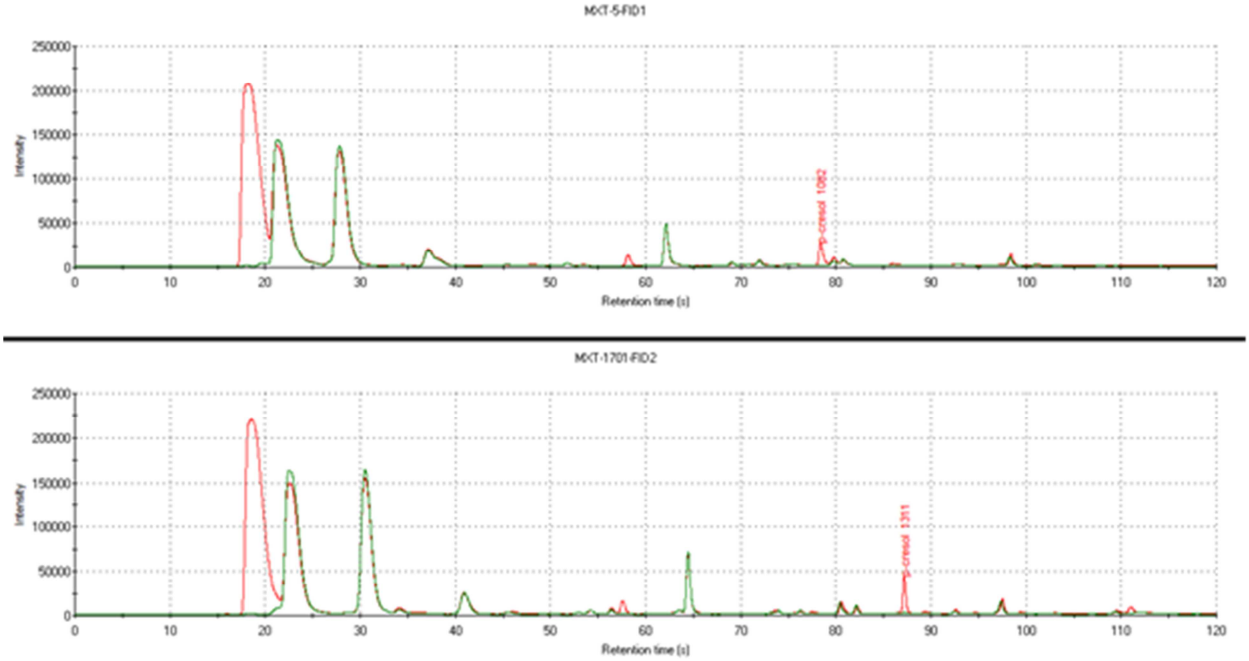

Fig. 2: Volatile profile of milk without and with p-cresol at 1 mg/L obtained on Heracles (with indication of retention index)

AroChemBase was be used to investigate the nature of unknown volatile compounds in milk based on the retention indices of the main peaks. Information on sensory features and odor threshold of several volatile compounds can be given (Figure 3).

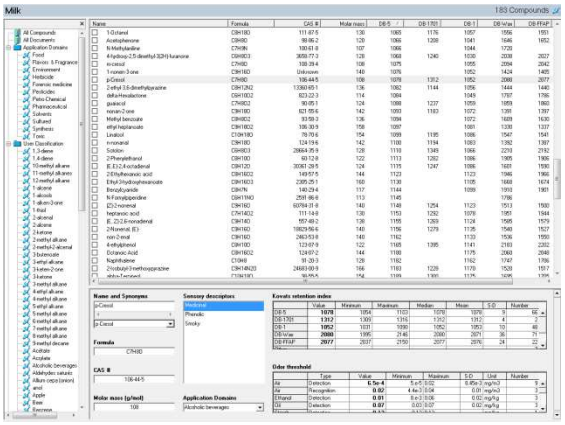

Fig. 3: Retention indices and odor threshold data of p-cresol in milk application domain of AroChemBase

Detection threshold

The concentration of p-cresol in semi-skimmed milk was calibrated and the curve obtained shows a good linearity (Figure 4).

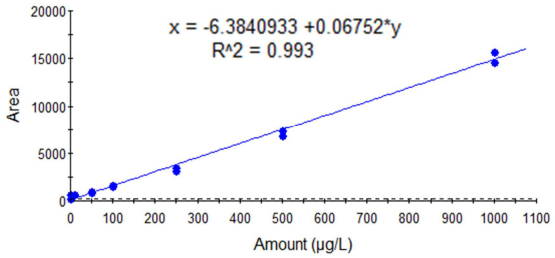

Fig. 4: Calibration curve of p-cresol in milk (MXT-5 column)

With this analytical method, the detection limit of p-cresol in milk was measured at 10 µg/L. This concentration is lower than the detection limit of 60 µg/L estimated in water.

Conclusion

The HERACLES instrument can be a very powerful tool to rapidly quantify p-cresol in milk.
The quantification of this compound in milk can be done in a few minutes with a sensitivity that is close to human detection threshold.

[1] Gürsoy & Kinik (2003) Off-flavours in milk and milk products. Journal of Engineering Sciences 9(1):79-88