

Objective

p-cresol is a volatile compound that is responsible of a stale / barny off-odor in milk. This molecule can be produced by a bacterial contamination by *Bacillus circulans*¹.

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).

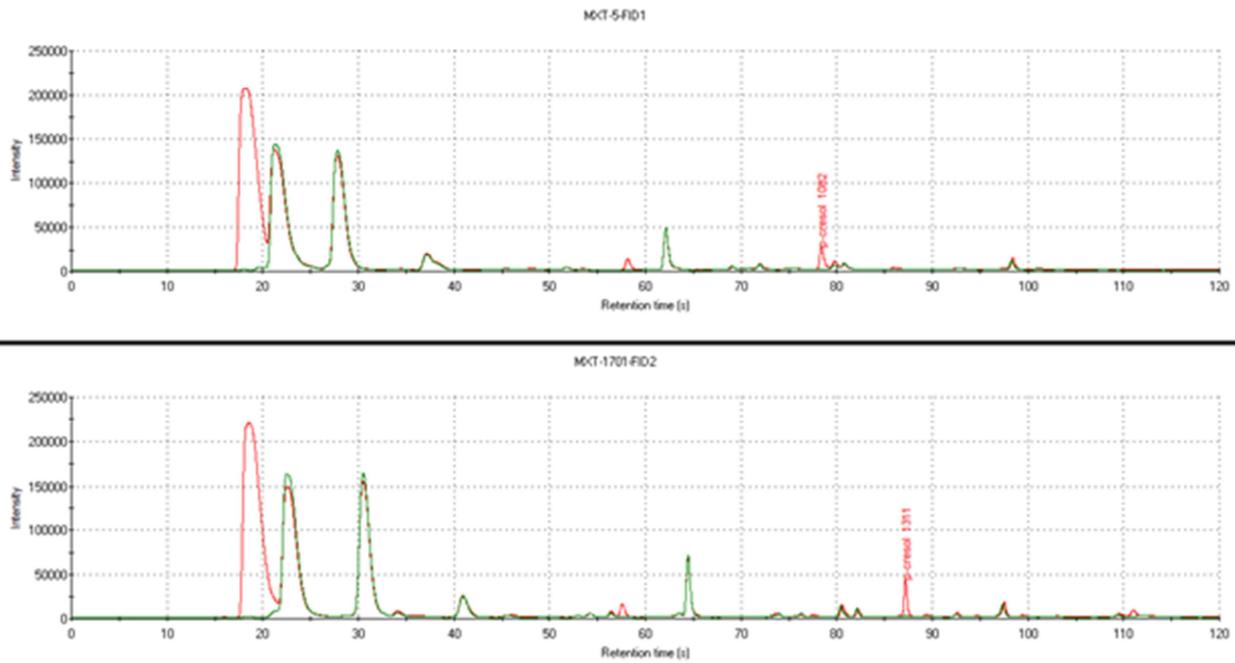


Fig. 2: Volatile profile of milk **without** and **with** p-cresol at 1 mg/L obtained on Heracles (with indication of retention index)

AroChemBase was 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).

Rank	Formula	CSI #1	Molecular	DB5-7	DB1701	DB1	DB19A	DB19F2
1	1,3-dioxolane	111 071	76	908	1176	1057	1096	1091
2	Acetophenone	181 862	128	1000	1209	1041	1050	1052
3	Benzoic acid	180 818	126	908	1044	1044	1029	1029
4	4-Hydroxy-2-ethyl-5-methyl-2-thiazole	3059 173	128	1008	1240	1038	2039	2037
5	acetal	188 394	108	978	1055	1055	2046	2042
6	Trimethylamine	139 180	140	1078	1202	1022	1424	1405
7	acetone	130 144	100	1078	1202	1022	2046	2037
8	Formic acid	130 041	108	1002	1144	1006	1444	1440
9	ethyl 3-benzyloxycarboxylate	130 012	122 123	114	1006	1048	1091	1091
10	acetone	130 011	108	1008	1207	1008	1059	1059
11	acetone	130 011	108	1008	1207	1008	1059	1059
12	acetone	130 011	108	1008	1207	1008	1059	1059
13	acetone	130 011	108	1008	1207	1008	1059	1059
14	acetone	130 011	108	1008	1207	1008	1059	1059
15	acetone	130 011	108	1008	1207	1008	1059	1059
16	acetone	130 011	108	1008	1207	1008	1059	1059
17	acetone	130 011	108	1008	1207	1008	1059	1059
18	acetone	130 011	108	1008	1207	1008	1059	1059
19	acetone	130 011	108	1008	1207	1008	1059	1059
20	acetone	130 011	108	1008	1207	1008	1059	1059
21	acetone	130 011	108	1008	1207	1008	1059	1059
22	acetone	130 011	108	1008	1207	1008	1059	1059
23	acetone	130 011	108	1008	1207	1008	1059	1059
24	acetone	130 011	108	1008	1207	1008	1059	1059
25	acetone	130 011	108	1008	1207	1008	1059	1059
26	acetone	130 011	108	1008	1207	1008	1059	1059
27	acetone	130 011	108	1008	1207	1008	1059	1059
28	acetone	130 011	108	1008	1207	1008	1059	1059
29	acetone	130 011	108	1008	1207	1008	1059	1059
30	acetone	130 011	108	1008	1207	1008	1059	1059
31	acetone	130 011	108	1008	1207	1008	1059	1059
32	acetone	130 011	108	1008	1207	1008	1059	1059
33	acetone	130 011	108	1008	1207	1008	1059	1059
34	acetone	130 011	108	1008	1207	1008	1059	1059
35	acetone	130 011	108	1008	1207	1008	1059	1059
36	acetone	130 011	108	1008	1207	1008	1059	1059
37	acetone	130 011	108	1008	1207	1008	1059	1059
38	acetone	130 011	108	1008	1207	1008	1059	1059
39	acetone	130 011	108	1008	1207	1008	1059	1059
40	acetone	130 011	108	1008	1207	1008	1059	1059
41	acetone	130 011	108	1008	1207	1008	1059	1059
42	acetone	130 011	108	1008	1207	1008	1059	1059
43	acetone	130 011	108	1008	1207	1008	1059	1059
44	acetone	130 011	108	1008	1207	1008	1059	1059
45	acetone	130 011	108	1008	1207	1008	1059	1059
46	acetone	130 011	108	1008	1207	1008	1059	1059
47	acetone	130 011	108	1008	1207	1008	1059	1059
48	acetone	130 011	108	1008	1207	1008	1059	1059
49	acetone	130 011	108	1008	1207	1008	1059	1059
50	acetone	130 011	108	1008	1207	1008	1059	1059

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).

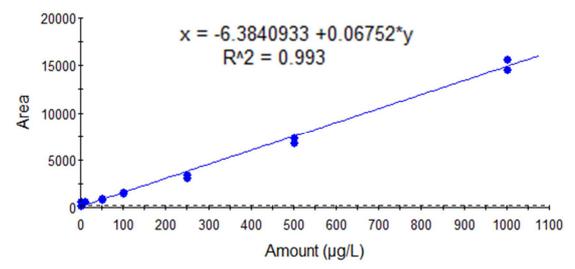


Fig. 4: Calibration curve of p-cresol in milk (MXT-5 column)

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