Objective

Diacetyl (2,3-butanedione) is an important flavour active, oxidative compound. It has a negative impact on cellular health and has been associated with neurodegenerative diseases and general cell aging. It also impacts sensory features. At low levels, diacetyl is characterized by a sweet flavor that even makes the mouth feeling of fermented drinks such as beer more pleasant. As the concentration increases, diacetyl results in an unpleasant buttery or butterscotch flavor. In soft drinks it can be an indicator of an undesirable fermentation. Therefore it is very important to be able to reliably detect diacetyl in final beverage products.
This application note describes the quantitative analysis of diacetyl in orange juice using a Flash Gas Chromatography electronic nose, the aim being to detect the compound at a concentration as low as 0.1mg/L (100 ppb).

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 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

Several samples (table 1) consisting of commercial orange juice (9g) with water (1g) and different quantities of added diacetyl were analyzed with the HERACLES electronic nose.
The objectives were to set up a calibration model for quantifying the compound concentration in other samples and to determine the detection threshold.

Sample label	Orange juice	Water	Diacetyl solution*	[Diacetyl] (mg/L)
S0	9 g	1 mL	0 mL	0
S0.25		0.75 mL	0.25 mL	0.25
S0.5		0.5 mL	0.5 mL	0.5
S0.75		0.25 mL	0.75 mL	0.75
S1		0 mL	1 mL	1

*Solution of diacetyl at 10 mg/L

Table 1: Samples description

Chromatographic conditions were set to obtain high sensitivity and peak resolution in a short time range (Table 2).

Parameters	Values
Vial volume	50 mL
Incubation	20 min on a heated tray at 50°C
Injection volume	5 mL
Trap concentrating temp.	40°C
Trap desorption temp.	240°C
Column pressure	60 kPa (10s) 0.5 kPa/s to 120 kPa
Initial isothermal temp.	40 (10 s) 3°C/s to 280°C (30 s)
Acquisition time	120 s
Time between 2 injections	6 min

Table 2. Heracles e-nose parameters

Chromatograms and repeatability

All peaks of orange juice samples with added diacetyl are eluted in less than 2 minutes (fig.2). Thanks to AroChemBase library, the peak of diacetyl can be easily detected and identified on MXT-5 column of HERACLES system. On the second column (MXT-1701), interference with other compound peaks does not allow a precise quantification. Therefore, the calibration model will be established with the measurements on MXT-5 column.

The repeatability of the analysis was determined over 4 replicates of orange juice containing 500 µg/L of diacetyl. The average concentration was determined at 0.53 mg/L with a standard deviation of 0.02 mg/L which represents a relative standard deviation of 4%.

Diacetyl calibration

The calibration of diacetyl was performed in orange juice on the concentration range 0 to 1 mg/L (fig. 3). This calibration curve shows a high linearity (correlation coefficient R2 = 0.999). From this quantification model it can also be deduced that the orange juice used for the calibration contains 0.06 mg/L (60 ppb) of diacetyl.

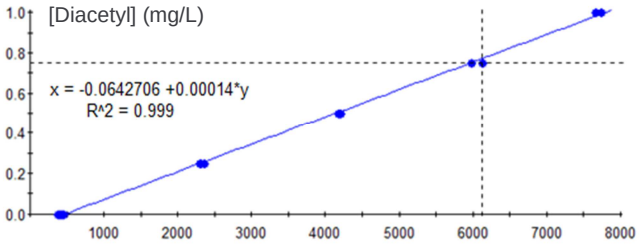

Figure 3. Calibration curves of diacetyl in orange juice on Heracles II e-nose on MXT-5 column

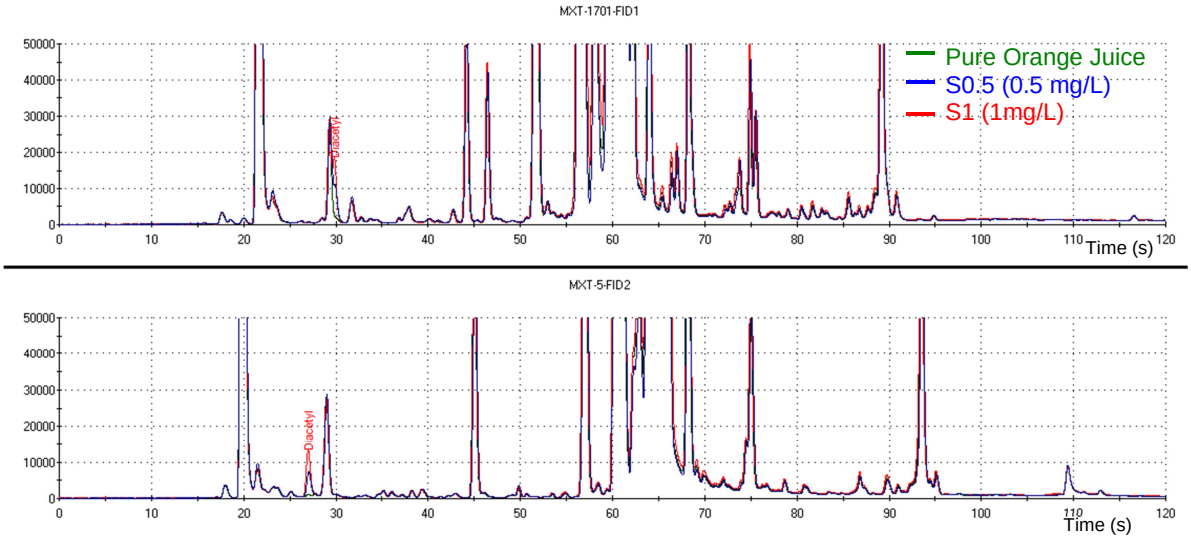

Figure 2. Chromatograms of orange juice with and without addition of diacetyl obtained on the 2 columns of HERACLES e-nose

Diacetyl detection threshold

From the calibration step, it could be observed that diacetyl was reliably and clearly detected on the range of concentration 0 - 1 mg/L.

With an aim to go down to the detection threshold, orange juice containing concentrations of diacetyl lower than 0.25mg/L were analyzed.

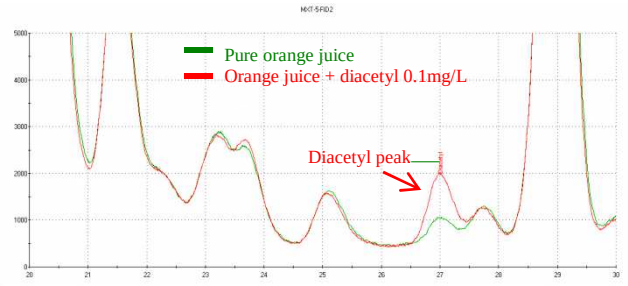

Figure 4: Comparison of the chromatograms of pure orange juice and orange juice with addition of 0.1 mg/L (100 ppb m/v) of diacetyl obtained on column MXT-5 of HERACLES

The chromatogram on Figure 4 shows that the peak of diacetyl at a concentration of 0.1 mg/L is clearly detected. This indicates that the detection threshold for diacetyl is lower than 0.1 mg/L.

Conclusion

This application shows that HERACLES Electronic Nose can be successfully employed to quantify diacetyl in beverage with high repeatability and a very good precision on the range 0 - 1 mg/L.
A high sensitivity of detection is achieved, with diacetyl detection threshold found below 0.1 mg/L.
Thus, HERACLES e-nose is able to rapidly and reliably control the diacetyl content in drinks, and additionally assess their sensory profile and properties.