Results obtained at Alpha MOS Laboratory (Toulouse, France) with rice samples supplied by Panzani (France).

Introduction

Basmati rice is a naturally fragrant variety of long grain rice mainly cultivated in South Asia. Its typical flavor is due to the presence of 2-acetyl-1-pyrroline. Difficulty in differentiating genuine basmati from other types of rice and the significant price difference between them has led fraudulent traders to adulterate basmati rice with cheaper crossbred basmati varieties or long-grain non-basmati varieties.
This application note describes the analysis of several batches of so-called Basmati rice with an electronic nose. The objective is to quantify the concentration in 2-acetyl-1-pyrroline, which is an indicator of Basmati rice quality, and to characterize the overall aroma.

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

Ten batches of Basmati rice (labeled R01 to R10) sourced from different suppliers were evaluated with HERACLES electronic nose.

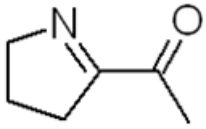
Sample	Indications
R01	Pure Basmati high quality
R02	Pure unflavored rice
R03	Unflavored rice
R04	Blend of Basmati & unflavored rice
R05	Flavored rice not from Asia
R06	Crossbred Basmati rice
R07	Basmati rice
R08	Basmati rice
R09	Flavored rice, but not Basmati
R10	Basmati rice

A fixed mass of rice (4g ± 0.04) is placed in a 20mL vial with an aqueous solution of saturated NaCl (4mL) and heated before headspace analysis. This preparation step corresponds to a cooking, and generates volatile compounds specific to each rice sample.

HERACLES analytical conditions

Parameter	Value
Headspace generation	20 min at 100°C
Injected volume	5000 µL
Injection speed	100 µL/s
Injector temperature	200°C
Injector carrier pressure	10 kPa
Injector vent	40 mL/min
Trap temperature	60°C
Trap pressure	60 kPa
Split	0 for 10s then 10 mL/min
Trapping time	60 s
Trap desorption	240°C
Columns temperature program	50°C (2s) to 130°C by 1°C/s then to 280°C (30s) by 4°C/s
FID temperature	290°C
Acquisition duration	150 s

Chromatograms



2-acetyl pyrroline

The chromatograms obtained with HERACLES instrument (fig. 2) clearly show the presence of 2-acetyl-1-pyrroline, at a retention time of around 71s on MXT-5 and around 76s on MXT-1701 column.

Quantification of 2-acetyl-1-pyrroline

Fragrant Basmati rice usually contains 2-acetyl-1-pyrroline at a concentration of about 600 µg/kg whereas unflavored rice contains very low amounts of this molecule (around 10 µg/kg).
There is no commercially available standard of 2-acetyl-1-pyrroline, which makes its quantification rather difficult. Nevertheless, it is possible to use the retention indices data of 2-acetyl-1-pyrroline included in AroChemBase database (table 1).

Type of column	Kovats Index
DB-1	894
DB-5	922
DB-1701	1014
DB-WAX	1331

Table 1: Kovats Indices of 2-acetyl-1-pyrroline on different columns (source: AroChemBase)

Since no visible co-elution was observed on MXT-5 column, the area of 2-acetyl-1-pyrroline peak is measured on this column. This allows to compare the relative proportion of this molecule in several samples (fig.3 & table 2).

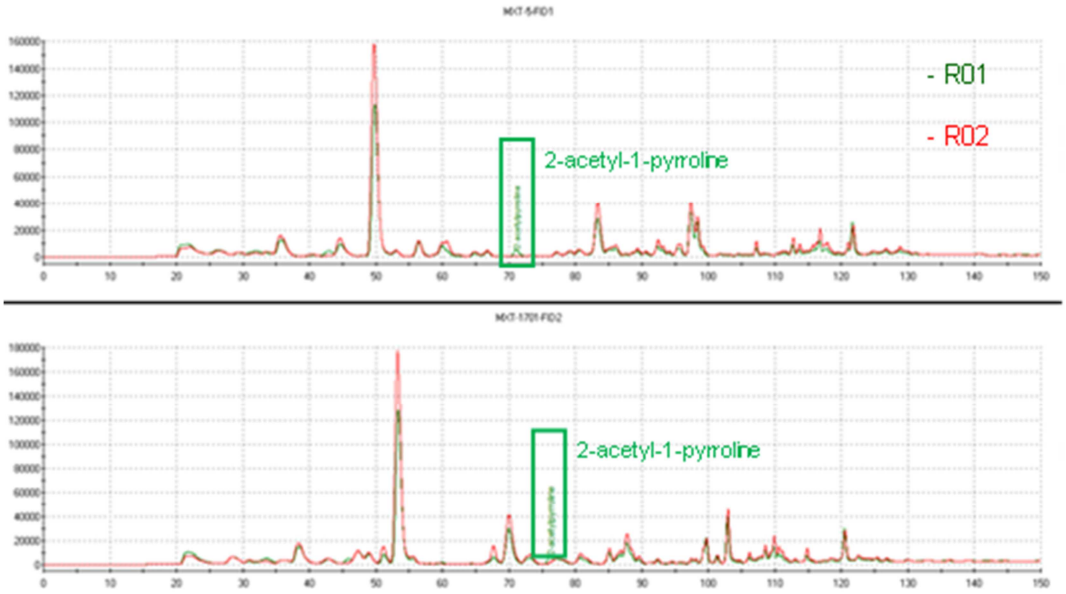


Figure 2: Chromatograms of 2 samples of Basmati rice on the 2 columns of HERACLES

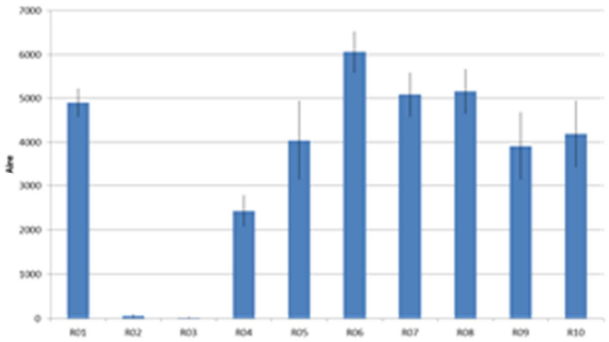


Figure 3: Peak area associated with 2-acetyl-1-pyrroline (the vertical line represents the standard deviation on 3 measurements)

Sample	Average area of 2-acetyl-1-pyrroline
R06	6054
R08	5161
R07	5091
R01	4908
R10	4195
R05	4043
R09	3914
R04	2430
R02	58
R03	19

Table 2: Area of 2-acetyl-1-pyrroline peak in the rice samples (average on 3 measurements), ranked by decreasing order

Unflavored rice samples R02 and R03 contain the lowest amounts of 2-acetyl-1-pyrroline (peak area near 0). Among the other samples of flavored rice, the Basmati ones (R01, R06, R07 & R08) show the highest amounts of 2-acetyl-1-pyrroline. The blend of Basmati & unflavored rice (R04) shows a concentration twice lower than pure Basmati rice (R01).

Chemical characterization

Using AroChemBase library, the volatile compounds contained in the headspace of rice samples can be characterized based on Kovats Index matching (table 3).
The method is first calibrated against a standard mixture of alkanes (from n-pentane to n-hexadecane) to be able to convert retention times into Kovats indices.
In all samples, the majority peak corresponds to hexanal, which is an indicator of the level of oxidation (fig. 4).

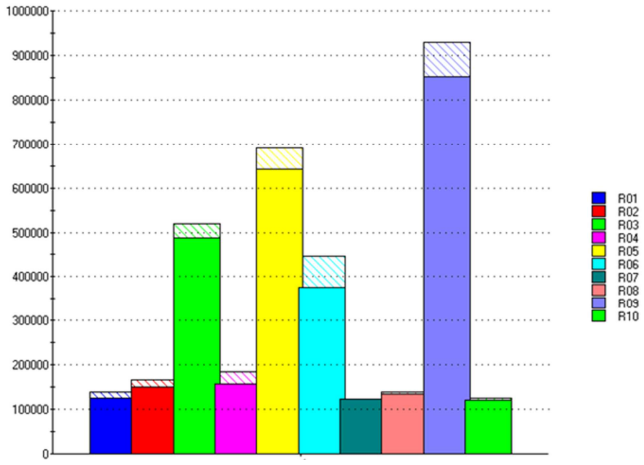


Figure 4: Area of hexanal peak in the rice samples

*RT MXT-5 (±0.1s)	*RT MXT-1701 (±0.1s)	**RI MXT-5 (±20)	**RI MXT-1701 (±20)	Possible compounds	Sensory attributes
21.6	21.9	509	599	Methylacetate	Fruity, blackcurrant
31.9	33.6	660	744	2-methylbutanal	Aldehyde, green, almond, burnt, malt
35.6	38.6	701	789	Pentanal	Aldehyde, acrid, almond, grass, green, malt
44.4	51.6	765	880	Pentanol	Sweet, anise, fruity, green, acrid, balsamic
45.7	43.9	775	827	2-methylthiophene	Sulfur
49.7	53.7	803	894	Hexanal	Aldehyde, green, fruity, tallow, fish, grass, leaf, fat
53.0	49.3	823	864	Butylacetate	Fruity, green, butter, banana, pear
60.6	68.3	870	983	3-hexen-1-ol / Hexan-1-ol	Green, fresh
66.7	70.4	906	995	Heptanal	Green, lemon, fat, dry fish, solvent, smoked, rancid, fruity
71.0	76.7	929	1035	2-acetyl-1-pyrroline	Grilled, overheated meat, ham, sweet, nut
80.4	85.5	983	1087	2-octanone	Fat, stew, green, fruity, cheese, apple, petrol, soap
83.3	70.4	999	995	Decane	Alkane
83.3	85.5	999	1087	Octanal	Aldehyde, lemon, stew, boiled meat, rancid, soap, green, flower, fruity
84.8	85.5	1010	1097	Trimethylpyrazine	Nut, peanut

Table 3: Possible volatile compounds identified by their Kovats indices in the headspace of rice
*Retention Time
**Retention Index (Kovats Index)

Odor map

Upon computing the chromatography data (retention times and peaks areas) with a Principal Components Analysis, an odor map comparing the volatile profile of all rice samples is obtained (fig.5).
The distribution of rice samples on this map is mainly linked with their hexanal content i.e. their level of oxidation. Samples R09, R05, R03 and R06 show an oxidation level significantly higher than that of other samples.

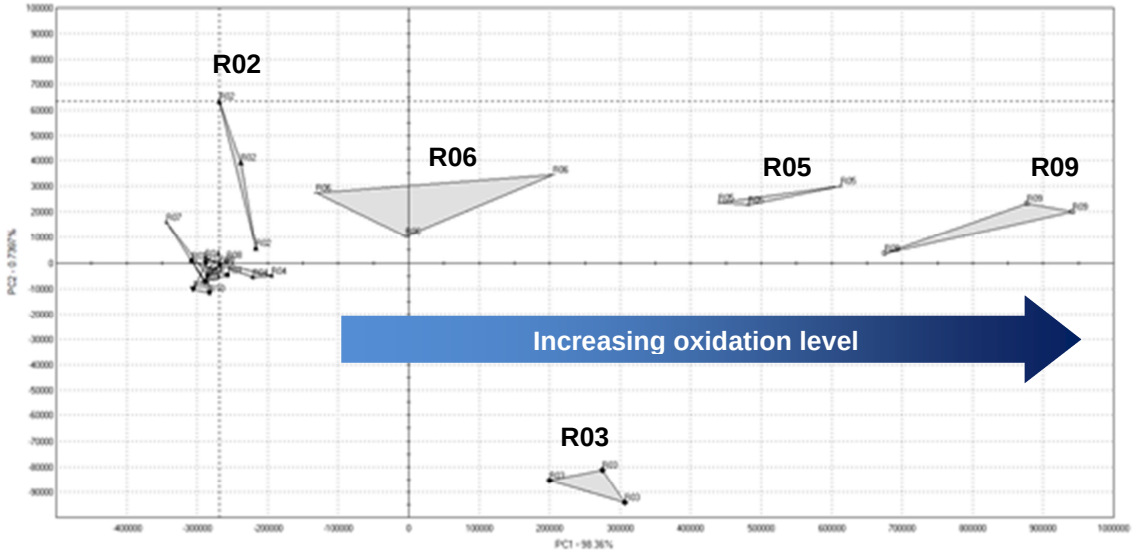


Figure 5: Principal Components Analysis of the volatile profile of rice samples obtained with HERACLES measurements

Conclusion

This application note shows that HERACLES electronic nose can be successfully employed to compare the amount in 2-acetyl-1-pyrroline in several rice samples.
Thus the e-nose can be a very powerful tool to rapidly assess the sensory quality of rice batches upon receipt and to select suppliers.