Topics on "Libra" electronic nose for fruit quality

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1. Introduction

Quartz crystal microbalances (QCM) are piezoelectric devices. To turn a quartz crystal into a chemical sensor it is necessary to coat it with a layer of a material capable of capturing molecules from the environment. When a mass is absorbed or placed onto the quartz crystal surface, the oscillation frequency changes decreasing in proportion to the amount of mass (Di Natale et al. 1997).

The ability to control a QCM´s selectivity by applying different coatings is an important feature, and makes this sensor type extremely versatile. However, the coating of QCM is, ironically, their greatest drawback. Batch-to-batch variability in the manufacturing leads to inadequate reproducibility(Sarig 2000). Indeed, the response of sensors depends on numerous factors that may be difficult to control, such as the temperature and the humidity of the carrier gas. All these factors cause changes in the selectivity of sensors affecting the reproducibility of measurements.

The detection threshold of the human nose is typically between 1000 ppm (10^{-6})and <1ppt (10^{-12}). Therefore, the absolute detection threshold of a gas sensor should be very low. For a QCM e-nose, only a few molecules are required to react with the sensitive elements leading to sensitivities close to the ppm or tenth of a ppm range as measured in the vapour phase (Mielle 1996) .

Despite previous studies using electronic nose, no QCM calibration statement has been established in relation to the sensitivity needed in the sensors for organoleptic quality measurement in pear or in other fruit. Present work will show the possibilities of using this equipment as a non destructive method to evaluate fruit quality. The work will be developed in different steps or topics to get a better understanding.

2. General Procedure

The *"Rome Tor Vergata"* electronic nose has eight Quartz Microbalance Sensors coated with different pyrrolic macrocycle solid-state films, combined for each sensor with different metals. The sensors are housed in a test chamber having a volume of about 20ml. Each sensor has a fundamental frequency of 20MHz and it is part of an oscillator circuit. The measurement of frequency is performed on-board by dedicated electronics. The instrument works connected to a personal computer via a serial link. Dedicated software runs on the PC .The signal unit is Hz.

The users program of "Libra nose" electronic nose establishes as internal reference the frequency in the starting point (around 20 MHz), that is to say, it obtains a value of relative frequencies subtracting to each measurement its first value of oscillation frequency.

Figure 1. Example of signal for one QCM sensor
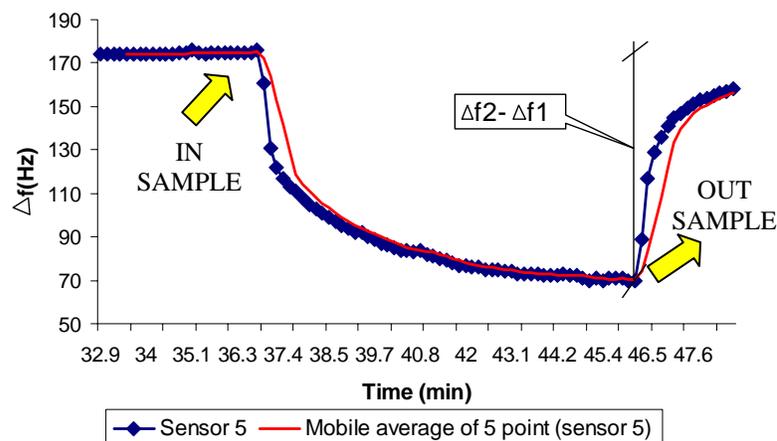
The variable used as sensor response is the increment between the stabilised relative signals, before and after exposure to the gas sample. During sampling the oscillation frequency decreases (Figure 1) and the cleaning system must assess the recuperation of signal to previous level. One of the problems to evaluate the increment (Δf) is the noise in the base line, to correct it, a software has been developed to smooth and to integrate the signal.

As I stated in the introduction, this work will be presented in five topics summarising the results obtained working with this equipment during one year and a half . In each one of them different experimental design will be described. Let us begin with common characteristics:

QCM sensors
During the first year of experiments (99-00), single layer-QCM sensors (S) were used. In this case, the pyrrolic macrocicles films were combined with the following metals:
Batch 1; Mn (S1), Cop-OCH3 (S2), Cop-NO2 (S3), Ru (S4), Sn (S5), Rh (S6), Cr (S7) and Co (S8)
Since the manufacturer (Torvergata Rome University-Technobychip) recommended the substitution after 400-500 h. work, a new batch of sensors was used for the period 00-01. These new ones were double faced. This fact showed a dramatic effect on the sensors response. Thus our reference (1-propanol) leads to a 66,4 Hz response in the single face sensors while obtaining 300Hz with the latter ones.
The films used to cover this new batch of sensors were exactly the same as before. However, for further comparison the single face S5 (S5A) was not changed and the old S6 was substituted by a new double face S5 (S5B):
Batch 2; Mn (S1), Cop-OCH3 (S2), Cop-NO2 (S3), Ru (S4), Sn (S5A), Sn (S5B), Cr (S7) and Co (S8)

Headspace generation
Two different systems were used:
Type 1: used to sample fruit. One single and entire fruit is placed inside an hermetic bottle of 0.75 l. In its cover two total-flow cock valves are installed, to make easier and more efficient the transference of headspace to the nose. One hour period for headspace generation is used.

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Type 2: to sample liquid pure chemical product (metrology experiments), hermetic bottles of 50 ml were used filled to a 10%. These bottles were closed with septum cover and a system of needle plus tubes was used to transfer headspace. The time of headspace generation is 20 minutes.

To keep constant the conditions of headspace generation, the hermetic bottles are introduced in a thermostatic bath to 22.5 ºC. On the other hand the ambient air is removed from inside the bottle to the outside medium pushed by a flow of synthetic dry air to remove the effect of relative humidity.

Headspace transfer

When the equilibrium between the gas and liquid phase is achieved, a controlled synthetic dry air flow of 0.2 l/min, generated by suction with a micro-pump placed inside the nose, carries the effluent towards the sensors. To avoid overpressure, the synthetic dry C50 (Carburos Metálicos SA, Madrid) air is stored in a Teflon bag connected by tubes to one of the valves (needles) of the hermetic bottles (Figure 2), the synthetic dry air enters the bottle pushing the headspace to the nose through the other valve (needle).

Figure 2. Scheme of fluent transference to electronic nose
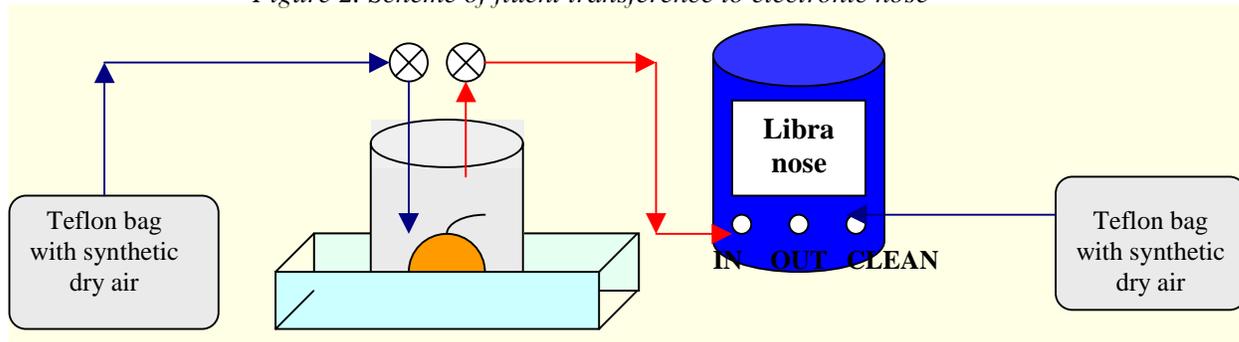
Cleaning system

Once the sample achieves the gas sensors changing the oscillation frequency, the sensors chamber must be cleaned. The carrier gas used is also synthetic dry air C50 (Carburos Metálicos SA, Madrid) stored in a Teflon bag to avoid overpressures. Again is the micro-pump placed inside the nose which generates a suction flux of 0.2 l/min (Figure 2), taking the clean air from the bag to the nose.

Reference system

Similarly to other techniques like the Gas Cromatography (GC), it is necessary to use patterns or references to control the state of the equipment and to allow data merging from different sessions or days of work, as a calibration tool. Based on preliminary studies, we have select several pure chemical compounds relevant for aromatic quality in pear. Thus several mixtures were created and labelled and the 1-propanol was chosen as our reference in order to achieve a better repeatability.

The headspace generation for 1- propanol is *Type 2* and it is measured at the end of a day´s work to avoid miss working alterations of the sensors´ response, since this headspace is much too concentrated when compared to a fruit headspace.

3. Approximation to the problem or topics

Five topics have been considered:

3.1.   First topic

> ***Can we detect any difference between different volatiles, typically present in fruit?***
> *Yes we can, but for high concentrations as we can see in the following experiments with patterns.*

3.1.1. Experiment description

Two different experiments were carried out:

1. **Differentiation between four liquid samples with strong differences among them**.
    Artificial mixtures (Table 1) were generated as to obtain smell like effect of pear, rotten pear (pear plus ethanol) and peach. The fourth sample was apple juice, stabilised with cisteine to avoid the oxidation process.
    Three repetitions per sample were made and the total number of samples analysed was equal to 12.

Table 1. Composition of liquid expressed in ppm for artificial fruit patterns

Product	Pear	Pear + ethanol "rotten pear"	Peach
Ethyl acetate	159	159	
Propyl acetate	157	157	
Butyl acetate	155	155	
Pentyl acetate	155	155	
Hexyl acetate	463	463	
1-Propanol	284	284	
Butanol	286	286	
1-Hexanol	289	289	
Ethanol		279	
Linalool			17
Acetaldehyde	278	278	
Benzaldehyde			21
Gamma-Undecalactone			188
Gamma-Decalactone			381
Delta-Decalactone			190

2. **Differentiation between four liquid samples with slight differences among them.**
    Two of them mimicking pear and peach aroma already showed on step 1 (one measurement per pattern). The other two samples were obtained mixing the artificial samples of pear and peach in two different percentage (60% pear plus 40% peach and 60% peach plus 40% pear) and were measured three times. The total number of samples analysed was equal to 9 including 1-propanol as the reference at the en of the experiment.

In both experiments, a volume of 10 ml for each sample was placed inside the hermetic bottle, with *Type 1* generation of headspace, samples were kept in a thermostatic bath to 40 ºC during 10 minutes. First bath of sensors (*batch 1)* was used.

The headspace concentration (calculated as is indicated in the point 5.3.1) at equilibrium is around 500 ppm

These studies were performed within a collaborative experiments (KU Leuven – Belgium, February 2000) in the framework of the European Concerted Action ASTEQ.

3.1.2. Results

PCA analysis (Figure 3) shows that all sensors are very correlated (see Factor 1) except for sensor 3, this one defines the second factor of PCA analysis. The PCA scores indicate (Figure3) for this experiment that it possible to use the electronic nose to differentiate between the four standards considered also with respect to the reference (1-propanol). Most sensors show their ability to promote different signal when the differences in concentration are very high. Thus along Factor 1 it is possible to segregate the 1-propanol (the reference 150 times more concentrate than standards) from the rest of samples. However sensor 3 gives finer information because along Factor 2 it is possible to differentiate between the standard of pear, peach and apple juice. This fact indicates that there are possibilities to perform qualitative analysis.

Figure 3. PCA analysis for artificial fruit patterns, experiment 1. Left: representation of sensors, and right: samples projection.
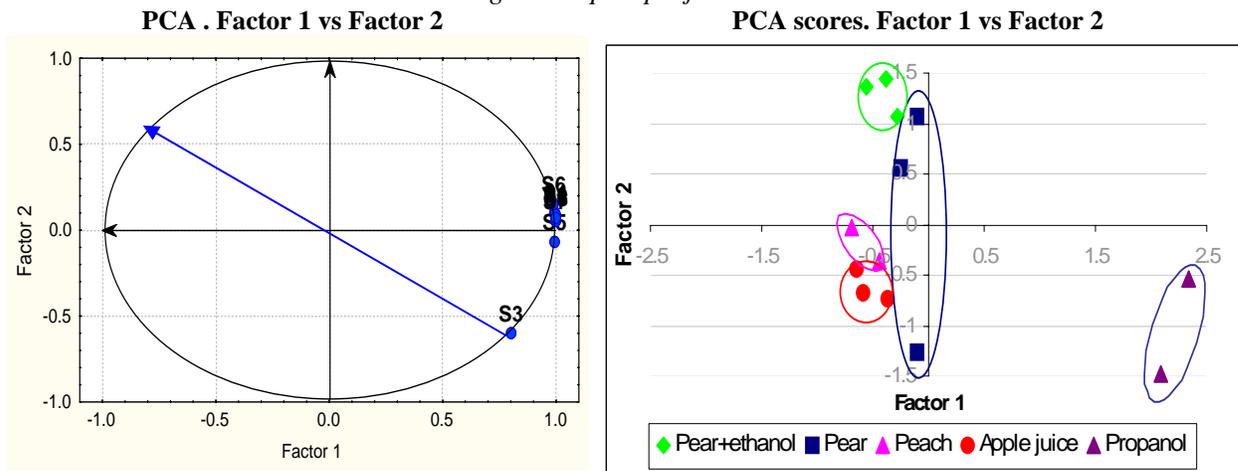

A One Way Analysis of Variance (ANOVA) carried out on experiment 1 (see Figure 4) indicates that the standards of *pear* and *rotten pear* produce a sensor response with significant differences between them, and from the standard of *peach* and *apple juice* ($F_{S6} = 12.7$).

In experiment 2 (see Figure 4), the effect of concentration is not so clear but the mixtures of two patterns give an intermediate signal indicating:

- a coherent tendency of results
- the difficulty to perform quantitative analysis

Figure 4. ANOVA analysis of sensor 6 (S6) for artificial fruit patterns

Experiment 1 (F_{S6} = 12.7) **Experiment 2** (F_{S6} = ns)
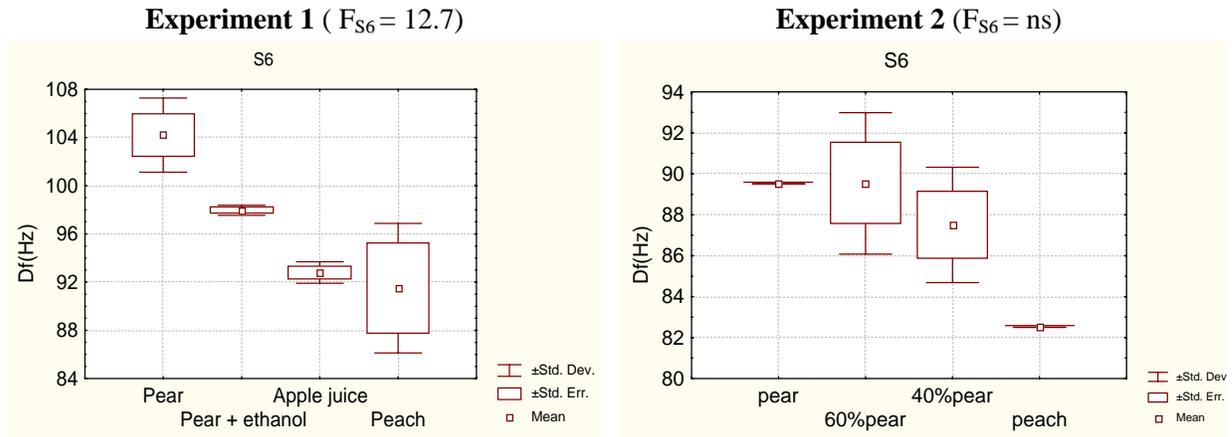

3.2.   Second topic

> ***Can we detect differential response when skin is opened or punched?***
> *Yes we can, as signal is consistently higher for punched apples as we can see in the following experiment with damaged apples.*

3.2.1 *Experiment description*

Differentiation between apples with three different levels of *open skin* damage. A cylindrical probe of 1.7 cm of diameter size is used. 1 and 3 cylindrical holes of 2-3 mm deep were performed on 3 apple fruits. The experiment is summarised in Figure 5.

Figure 5. Experiment design used to test the effect of skin breakage on the headspace and the response of the e-nose
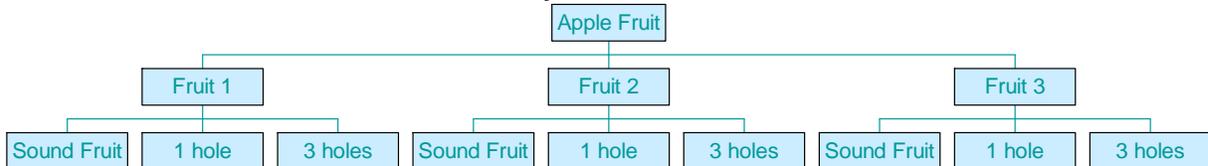
Each fruit was measured without any damage, with one hole and finally with three holes, being the total number of samples measured equal to 9.

As for Topic 1, this experiment was carried out along the same collaborative work in the framework of ASTEQ (KU Leuven – Belgium, February 2000). The reduced work time available (1 week) explains the low amount of material used for this case.

The headspace generation was *Type 1* and the sensors used corresponded to *batch 1*

3.2.2. *Results*

The ANOVA analysis (see Figure 6) shows that there is a linear increase of sensor signal with the increase of the damaged surface of apple, with significant differences (F_{S2} = 17.5) among the three levels of damage.

The PCA analysis (Figure 7) confirms for all sensors the results obtained for sensor 5 (Figure 6). The score of Factor 1 gathers the response of all sensors of the electronic nose and yet it is possible to distinguish between the three cases considered (sound, one hole and three holes).

Figure 6. ANOVA analysis of the response of sensor 2 (S2) for headspaces generated by apples with increasing skin damage.
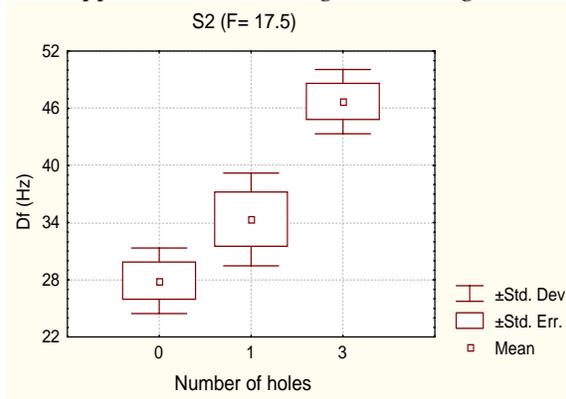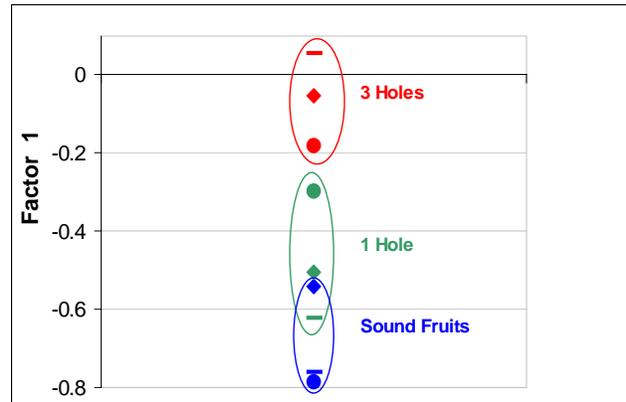

Figure 7. Representation of Factor 1from PCA (linear combination of the eight sensors) for apples with increasing skin damage.

3.2.3 Discussion

The conclusion is that the presence of some damage on apples´ skin will seriously affect our measurements and thus we must choose carefully the piece of fruit to sample to avoid this source of variation. However another question remains unclear: is this result due to moisture alone? or are there more volatiles in the headspace due to punched skin? Further experiments must be planned to give a proper answer.

3.3. Third topic

> *Using 1-propanol as reference, are the sensor´s signal stable among sequences of tests?*
> *No, the nose data need to be corrected for each testing date with regard to the signal of the reference specie*

3.3.1. Experiment description

It consist of the evaluation of the signal drift test for a selected reference (1-propanol) throughout a year of measurements (September 99- May 2000, 400 work hours). This experiment was carried out during the first period of use of the electronic nose (*bath 1* sensors) where the headspace generation (*Type 2*) and headspace transfer were made using ambient air passing through cartridges of ClCa as to remove the air humidity. These cartridges were connected to the electronic nose and to the hermetic bottle in the same way as explained for the Teflon bags (see Figure 2).

This study was held simultaneously with a large scale experiment on pears which will be mentioned in paragraph 3.3.4. The reference (1-propanol) was always tested at the end of the day as indicated in paragraph 2.

3.3.2. Results

A cycle of variation is found in the level of response which corresponds to changes in the relative humidity of the ambient (Correa et al. 2000) even though the use of ClCa cartridge, see Figure 8. An additive correction, see Figure 9, is proposed to improve the reproducibility level, estimated as standard deviation (STD) of daily average, from 11.23 Hz to 3.26 Hz.

Figure 8. Average response of all wight sensors of the electronic nose for 1-Propanol. Raw data.
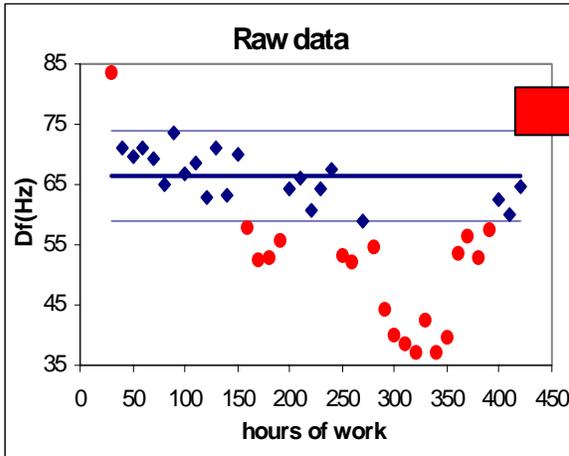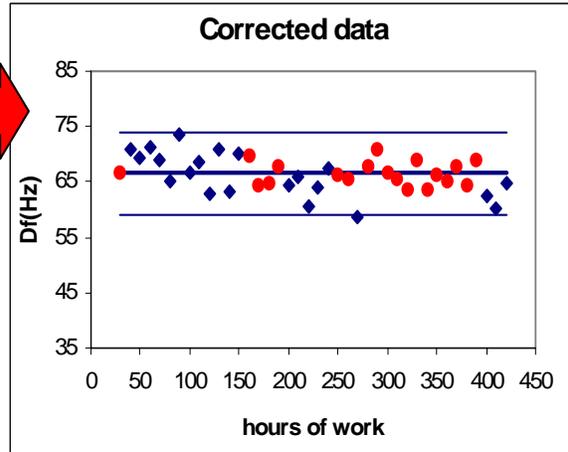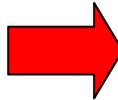
Figure 9. Average values after additive correction.

	Raw data
Mean (Hz)	58.798
STD (Hz)	11.229

	Corrected data
Mean (Hz)	66.371
STD (Hz)	3.260

3.3.3. *Discussion*

A lot of effects, external (relative humidity and temperature mainly) and internal of the electronic nose, introduce sources of variation in the response of sensors´ signal, some of them not isolated at the present stage.

With the objective of using a carrier gas absolutely standard to avoid known sources of variation as the relative humidity and unknown ones, the ambient air and the cartridges of ClCa, were substituted in latter experiments by synthetic dry air and Teflon bags. Using synthetic dry air the reproducibility of the measurements (estimated as STD) improves with regard to ambient air from 74.8 to 49.9 Hz working with *bath 2* sensors (Correa et al. 2001).

3.3.4. *Application of third topic*

Does storage period affect aromatic quality of "Doyenne du Comice" pears?
Yes as detected with the nose, when using corrected data against 1-propanol, as we can see in the following experiment.

3.3.4.1 Experiment description

Determination of the influence of storage period in cold storage rooms, and of shelf life on the aromatic quality of "Doyenne du Comice" pears (September 99- May 2000). Harvest date and three different times of cold storage (5, 7 and 8 months) were considered. Three periods of shelf life at room temperature (1, 4 and 7 days) after cold storage were used. The size of batches of fruits was equal to 60, except at harvest , where the batch contained 15 fruits, being the total number of samples analysed equal to 195 fruits.

The sensors *batch 1*, headspace generation *Type 1* was used. Headspace transfer as described the 3.1.1. section.

3.3.4.2.Results

As PCA analysis shows (Figure 10), the headspace generated by the fruit induces a sensors´ response less homogeneous than for the first topic (artificial mixtures), and therefore more interesting. Factor 1 of PCA is clearly correlated with sensor five, and Factor 2 is defined mainly by sensor 7. Due to previous studies (Correa et al. 2000) we know that the sensors 1, 6 and 8 present the lowest sensitivity, being exactly these ones the most affected for the correction applied when compared to sensors 3, 4 and 7.

Figure 10. PCA plot of variables for the experiment on pear fruits (n=195). Cold storage and shelf life evolution are evaluated in this experiment (September 99-May 00). Additive correction has been applied as explained.
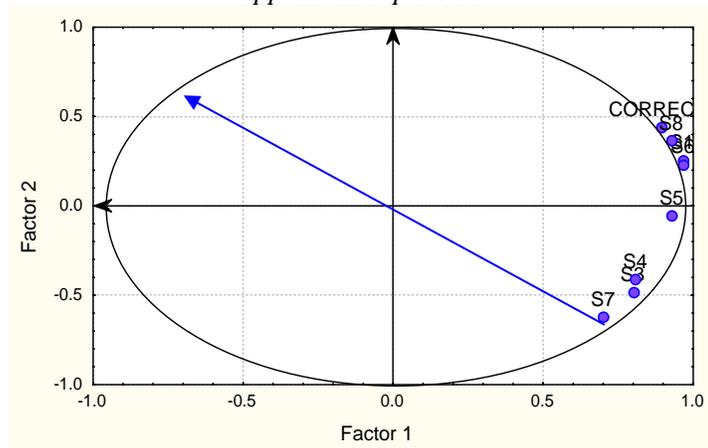

Figure 11 shows (left part) that thanks to the correction of data of fruit with respect to 1-propanol (the reference) it possible to distinguish between the four groups of fruits, each one corresponding to a different time of storage.

Factor 2 (sensor 7) allows the segregation of data from harvest with regard to the data from stored fruits. This is to say that sensor 7 is reacting to some volatile or volatiles that disappear during cold storage. Along Factor 1 we can distinguish the three times of storage (5, 7 and 8 months), thus sensor 5 is reacting to some volatiles which evolve during cold storage.

Figure 11. A PCA scores plots (n=195) for the pear experiment (September 99-May 00)

Cold Storage Period   **Cold Storage Period plus Days of Shelf Life**
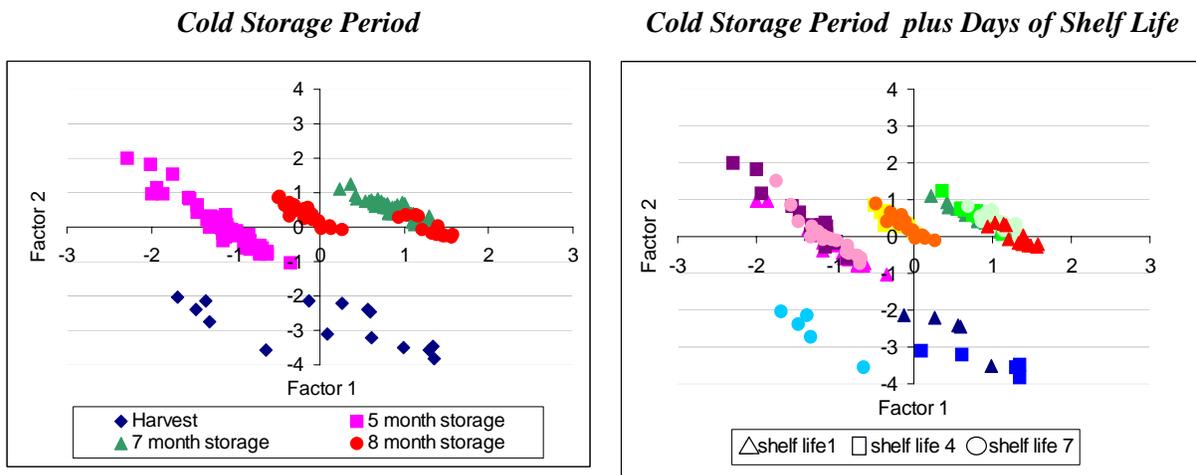
In Figure 11 both storage time (left) and shelf life (right) effects are presented. Only at harvest there is a significant evolution during shelf life, as sensor 5 indicates.

The shelf life does not explain the dispersion of data along the axis defined by sensor 7, in this case this sensor is reacting to some effect unknown and not controlled which is only presents at harvest time.

3.4. Fourth topic

> *Are the sensors stable enough within a test sequence?*
> *No, there appears a constant drift during the same day of testing, it is necessary to apply further correction.*

3.4.1. Experiment description

The experiment planned was the evaluation of signal drift when measuring the same fruit three times during a day of work: at the beginning, at the middle and at the end of each sequence. This experiment was made using "Fuji" apples and it was repeated during six non consecutive days. This study was held simultaneously within a large scale experiment which will be described at paragraph 3.4.5. The apples used in this experiments were held at constant temperature storage, no physiological changes would occur. It was decided not use the reference (1-propanol) since it would have affected the measurement within the large scale experiments.

The headspace generation used to sample fruit was *Type 1* and the headspace transference equal to the method explained in the General Procedure. *Batch 2* sensors was used.

3.4.2. Results

The plot (Figure 12) shows an average increase of sensors´ signal, Δf from the beginning of the sequence (time 0 min), along the time (time 180 min), reaching the maximum response at time 400.

Figure 12 . Average evolution (n=6) of Δf for some sensors, with respect to time, for same fruit and day of work.
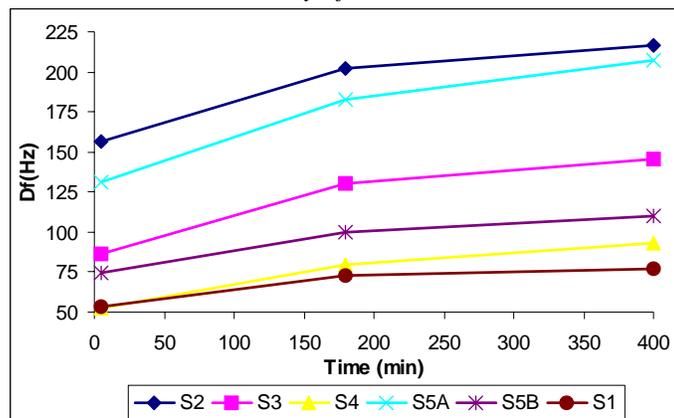
It can be observed that the increase of Δf is not linear from 0 to 400 minutes. It is possible to distinguish two section defined by two different slopes or drifts. The average and standard error (SE, n=6) of the drifts have been computed as follows:

$$drift_{0-400}(Hz/min) = \frac{\Delta f_{400} - \Delta f_0}{400}$$

$$drift_{0-180}(Hz/min) = \frac{\Delta f_{180} - \Delta f_0}{180}$$

$$drift_{180-400}(Hz/min) = \frac{\Delta f_{400} - \Delta f_{180}}{220}$$

To be sure that these drifts are significant there is a need for applying some steps analysis (see Table 2):

first step: when the global drift (0-400 min) minus 1.96 SE is positive, it is significantly different from 0 and therefore there is a need for correction,

second step: drift 0-180 and drift 180-400 can be compared by means of ANOVA analysis; when the *p* value is below 0.05 both drifts are significantly different a therefore different correction has to be applied for each period, and

third step: it consists of comparing each of the partial drifts (0-180, 180-400) with respect to no drift as mentioned for step 1.

Table 2. Description of the step analysis used to characterise the drifts present in the sensors response along work sessions.

		First step	Second step	Third Step	
		Total slope vs. horizontal From 0 to 400 min	1°slope vs. 2°slope	1° slope vs. horizontal From 0 to 180 min	2° slope vs. horizontal From 180 to 400 min
TEST	Mean - 1.96 SE	> 0		> 0	≤ 0
	ANOVA		$F_{s3} = 28.6$		
Significantly different		YES	YES	YES	NO
Correction necessary		YES	YES	**YES**	NO

The conclusion that we can extract from this table is that a multiplicative correction of drift is necessary only for the first period (from 0 to 180 min) of a day work (400 min). The multiplicative correction has to be applied in such a way that it does not alter previous additive correction based on 1-propanol data.

Applying multiplicative correction the average variability of data (estimated as STD) decreases from 24.1 Hz to 6.2 Hz.

3.4.5. Application of fourth topic

Does shelf life affect "Fuji" apples aromatic quality?
Yes, as detected with the nose, using corrected data, between sequences against 1-propanol and within sequence.

3.4.5.1. Experiment description

Determination of the influence of shelf life in the aromatic quality of "Fuji" apples. Three times of shelf life (1, 5 and 10 days) were considered after 3 months of cold storage and two different harvest date (early and late). 60 fruits by harvest date were considered, testing each

shelf life time 20 fruits. Taking to account that each day of work one fruit was measured three times as explained paragraph 3.4.1. the total number of samples analysed was equal to 132. Headspace generation *Type 1* and sensors *batch 2* sensors were used. Transference of headspace was made as it was described in the General Procedure.

3.4.5.2. Results

The PCA analysis (Figure 13) shows that all sensors are giving a very similar information, defining the Factor1 of the PCA, just the contribution of sensor 3 makes a little bit of difference defining a second factor in the PCA.

Along Factor 1 of PCA scores plots (Figure 14), it is possible to distinguish between the three times of shelf life, mainly for early harvest. Thus, the amount of volatiles would increase during the first four days of shelf life, decreasing afterwards not so much to reach the initial state in the case of early harvest. Under a physiological point of view this could be assigned to the beginning of senescence of fruits.

Along the axis defined by sensor 3 exists a variability inside each group that at this stage we can not explain. Sensor 3 is reacting to an effect unknown for us and thus not controlled.

Figure 13. PCA plot: Factor 1 vs Factor 2 of sensors response for the experiment on Fuji apples. Shelf life evolution for two different harvest dates (early and late). Additive and drift correction is used.
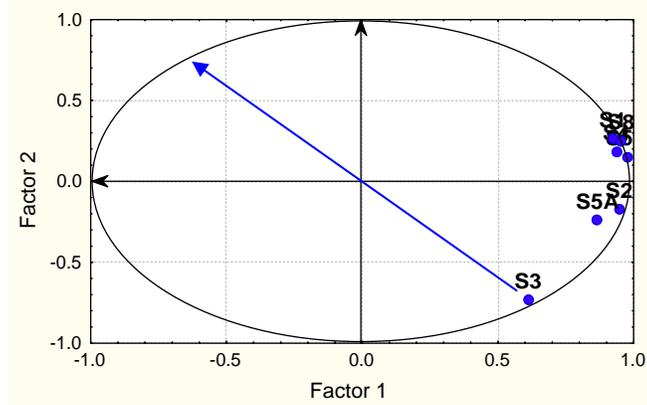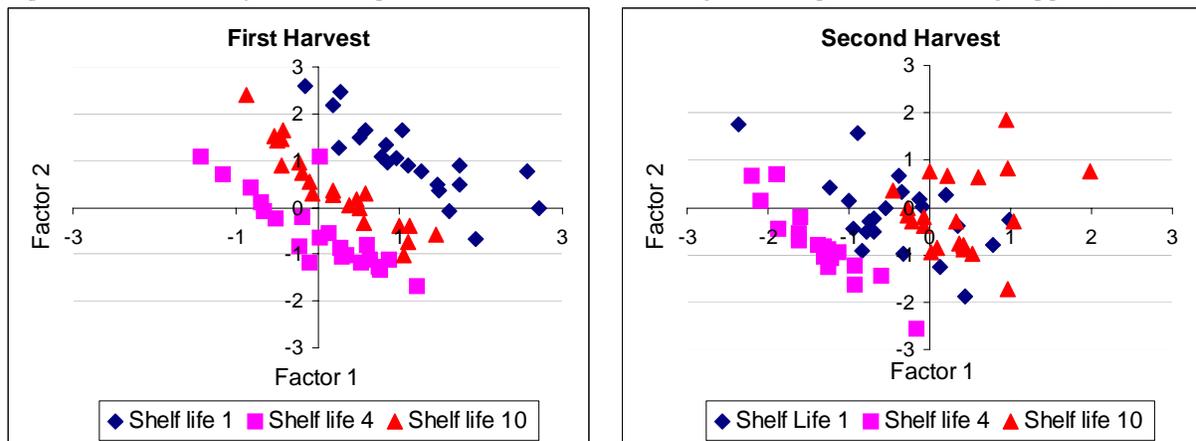

Figure 14. PCA analysis scores plots: Factor 1 vs. Factor 2 for the experiment on Fuji apples (n=132).

3.5. Fifth topic

Are metrology analysis necessary?
Yes, the lack of stability of sensors´ signal implicates that it is necessary to carry out metrology test, using well controlled chemical references and headspaces.

3.5.1. *Experiment description*

The experiment (November 2000) consisted of metrology analysis of QCM electronic nose for some chemical compounds present in the highest proportions in the aroma of pear fruits. Determination of precision, sensitivity and specificity of the electronic nose in relation to these chemical products were carried out.

The compounds chosen were an alcohol (1-propanol) and two esters (propyl acetate and ethyl acetate) and four concentrations of headspace (5, 20, 100 and 1000 ppm) for each one of those compounds. The different concentrations of headspace were obtained closing in an hermetic bottle dilutions of each pure compound in distillate water. It is indispensable to use analytes which form homogenous dissolution with the solvent. The calculations of the final concentration of each analyte in the air were made according to the following equation derived from the *Dalton* and *Raoult* laws, where *Pm* is the molecular weight, $P_{Vanalyte}$ the partial vapour pressure of pure compound and P_T the total pressure (Grenier 1998):

$$C_{analyte}(ppm) = \frac{G_{analyte}(g)}{G_{water}(g)} \cdot \frac{Pm_{water}}{Pm_{air}} \cdot \frac{P^o_{Vanalyte}(mmHg)}{P_T(mmHg)} \cdot 10^6$$

Type 2 of headspace generation and *batch 2* sensors were used and headspace transference was made as described in the General Procedure.

3.5.2. *Results*

3.5.2.1. Precision

The precision is calculated for each sensor (i:1-8) as the STD of the measurements (Δf) obtained inside the same day or sequence, for a same product to a same concentration (three repetitions), averaged for 6 days of work. The level of average precision for all the sensors was equal to 16.44 Hz.

Table 3. Precision in Hz of QCM sensors evaluated in a metrology experiment.

$STD_{n=6}$, p(Hz)	S7	S2	S3	S4	S5A	S5B	S1	S8
STD average	21.04	23.71	15.81	9.46	13.06	22.28	19.26	6.88

3.5.2.2. Sensitivity

This parameter was calculated for three different chemical compounds (propyl acetate, ethyl acetate, 1-propanol) and for 8 sensors (i: 1-8).

The change in the oscillation frequency in each sensor, due to the interaction between the molecules present in the gaseous phase and its reactive coating, can be explained adding two independent contributions due to the adsorption of those molecules in different positions. A first adsorption on preferential (specific) sites exists and once these are complete, it begins the adsorption on non-preferential (unspecific) positions (Paolesse et al. 1999; Bodenhöfer et al. 1997):

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$$\Delta f_{total} = \Delta f_{spec} + \Delta f_{unspec}$$

Bodenhöfer et al., 1997, defined the analytical curve of response of each sensor versus different concentration of an analyte (Figure 15), assuming a combination of the two types of interactions, specific interactions, which are carried out with low concentrations of headspace, described in the *Langmuir* law (logarithmic component) and unspecific interactions corresponding to a high concentration of headspace and defined by the *Henry* law (linear component):

$$\Delta f_{Total}(Hz) = K_L(Hz) \cdot \frac{k(ppm^{-1}) \cdot c(ppm)}{1 + k(ppm^{-1}) \cdot c(ppm)} + K_H(Hz/ppm) \cdot c(ppm)$$

K_L is the constant corresponding to the sensors´ signal when every specific position is saturated, that is to say, when the curve described for *Langmuir* law reaches the saturation. K_H, constant corresponding to the slope of the linear part of curve. k is the ratio between the kinetic constants of the adsorption – desorption processes of the analyte on the recognition points of the sensors. c is the concentration of the analyte in the headspace.

Figure 15. Experimental data and curve for a headspace generated with different dillutions of 1-propanol in water. It shows the two components of the curve; the Langmuir component (logarithmic) reaches the saturation of the response at 175 Hz.
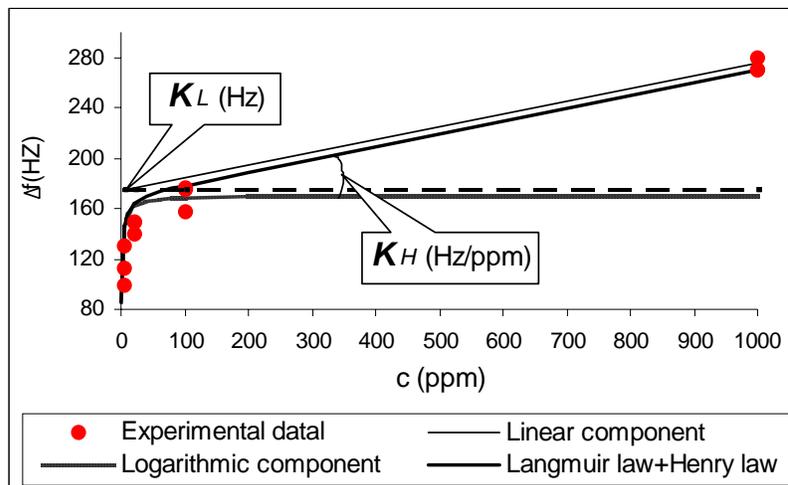
The sensitivity (Stv) is defined as the variation of the frequency with respect to concentration. Thus, the Δf_{Total} derivative versus c gives, for each sensor, concentration and analyte, the expression of the sensitivity:

$$Stv(Hz/ppm) = \frac{K_L \cdot k}{(1 + k \cdot c)^2} + K_H$$

The average rate of concentration (ppm/h) reached in the headspace of our system for "Doyenne du Comice" pears under average maturity and ripeness conditions is around to 9.6 ppm/h (data derived from Miro et al. 2000). Therefore, the sensors´ sensitivity for any concentration is not relevant to evaluate the quality in fruits. The area of interest for the sensitivity curve corresponds to headspace concentrations between 0 to 20 ppm. This section can be approached to a straight line and it corresponds with the highest sensitivity of sensors´ response to concentration.

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The maximum sensitivity was detected for propyl acetate (see Table 4), with respect to the other two chemical compounds, and for sensors 2 and 5B, compared with the rest of sensors, being the average sensitivity of sensor 2 equal to 5.18 Hz/ppm.

Table 4. Sensitivity in Hz/ppm of QCM sensors for three analytes evaluated in a metrology experiment.

Sensitivity, Stv(Hz/ppm)	S7	S2	S3	S4	S5A	S5B	S1	S8
Ethyl acetate	2.49	**4.30**	4.94	0.99	2.87	3.27	2.25	1.70
Propyl acetate	3.73	**9.24**	3.64	1.64	2.98	5.30	5.33	1.64
1-Propanol	2.54	**2.01**	0.04	0.45	1.64	2.13	2.21	0.74

3.5.2.3. Specificity

It was defined as the capacity of each sensor (in our case from i=1 to i=8) to react in a preferent way to a single analyte when other ones are present. Thus, it was used as a parameter to evaluate the specificity $Spe_{i,j} = \dfrac{\Delta f_{i,j}}{\sum_{j=1}^{j=3} \Delta f_{i,j}}$, for three analytes (see Table 5).

Table 5. Specificity (Spe) of QCM sensors for three analytes and two concentrations 5 and 20 ppm

$Spe_{i,j} = \dfrac{\Delta f_{i,j}}{\sum_{j=1}^{j=3} \Delta f_{i,j}}$	**c (ppm)**	S7	S2	S3	S4	S5A	S5B	S1	S8
Ethyl Acetate	**5**	0.38	0.39	0.39	0.4	0.4	0.38	0.37	0.39
	20	0.36	0.37	0.42	0.39	0.4	0.36	0.33	0.39
Propyl Acetate	**5**	0.4	**0.42**	0.39	0.4	0.41	**0.4**	**0.4**	0.39
	20	0.4	**0.45**	0.39	0.42	0.41	**0.42**	**0.44**	0.4
1-Propanol	**5**	0.22	0.19	0.23	0.2	0.19	0.22	0.24	0.22
	20	0.24	0.18	0.19	0.19	0.2	0.22	0.23	0.21

As expected, the specificity found is poor, only a little bit different from the value of unspecificity ($1/j_{max}$= 0.33) for three compounds; in order to avoid the problems of sensors´ reversibility, non selective or partially selective sensors are used to make the electronic noses. For our case, the highest specificity was detected to esters for all sensors and sensors. On the other hand sensors: S2 (0.42, 0.45), S5B (0.4, 0.42) and S1 (0.4, 0.44) showed a special specificity to propyl acetate.

Figure 16. Fingerprint for two different analytes in the same headspace concentration (1000 ppm). The specificity to propyl acetate (1000 ppm) is 0.58, 0.56 and 0.77 for the sensors 2, 5B and 1 respectively.
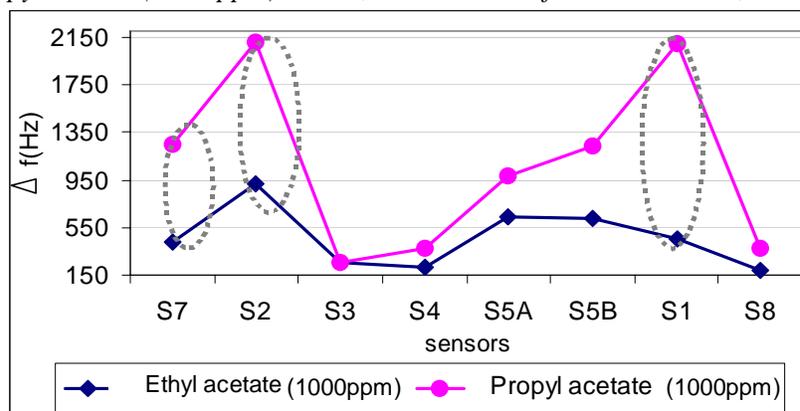
The differences in specificity (Figure 16), of some sensors with respect to some chemicals, produce small variations on the fingerprint or *spectrum*, curve formed by the signal of all sensors against an analyte. But the change in the fingerprint is obvious only for too high concentrations (1000 ppm), in relation to the volatiles emitted by the pear fruit (maximum value around 20 ppm), giving moderate perspectives to carry out qualitative analysis of data.

3.5.2.4. Specifications of an *e-nose for volatile evaluation of pears*

Miró et al., 2000 quantified the emission (µg/kg$_{fruit}$·h) of a wide number of aroma volatiles produced by "Doyenne du Comice" pears, harvested with different maturity stage and storage under distinct cold conditions in commercial cold rooms (Miró et al. 2000). To extract, identify and quantify these volatiles, a non destructive method of dynamic headspace generation was used, capturing the volatiles in a solid phase of Tenax and latter GC-mass spectrometry analysis (López et al. 1999). In the case of electronic nose, *Type 1* headspace generation was used and knowing the volume of hermetic bottle and the fruit mass, it is possible to determine the concentration rate (*E*) in ppm/h, where *e* (intrinsic fruit aroma emission) depends on maturity stage of fruit (µg/kg·h):

$$E(ppm/h) = \frac{e(\mu g / kg_{fruit} \cdot h) \cdot 0.25(kg_{fruit})}{V_{air}(l) \cdot \delta_{air}(g/l)}$$

Figure 17 summarises the maximum differences between pears batches, in total emission of volatiles (ethyl acetate, propyl acetate, butyl acetate, 2-metylbutyl acetate, pentyl acetate, hexyl acetate, ethanol, 1-propanol, 2-methylpropanol, 1-butanol, 2-methylbutanol and 1-hexanol). The maximum concentration rate (*E$_{max}$*= 24.4 ppm/h) in the headspace is reached for the highest maturity and highest ripeness stage, while the minimum concentration rate (*E$_{min}$*=1.7 ppm/h) in the headspace corresponds to the lowest maturity and lowest ripeness stage.

From metrology experiments the detection threshold of sensors of the electronic nose can be estimated as $2p/Stv$, equal to the minimum concentration of headspace that there must be in an hermetic bottle to be sure that the sensors´ response is due to the volatiles emitted by the fruit.

At this stage we know how to create the adequate headspace, depending on the objective, it means the number (n) of maturity classes to distinguish in pears:

$$n = \frac{\left(E_{max}(ppm/h) - E_{min}(ppm/h)\right) \cdot t(h) \cdot Stv(Hz/ppm)}{2p(Hz)}$$

Figure 17 shows the increase of concentration in the headspace with respect to time, and expected time needed to distinguish between 2 (33 minutes) and 3 (50 minutes) maturity stages.

To obtain quantitative prediction models the ratio error/range should be lower than 0.1 according to chemometrics studies performed by us (data not shown). In our case the error would be the detection threshold (6.34 ppm) and the maximum range of the measurements *(E$_{max}$ – E$_{min}$)·t.* With these data and with our system of headspace generation for the electronic nose, we would need a minimum time of headspace generation of 2.79 h. Thus, the restrictions to carry out continuous predictions are much too high compared to those needed to carry out a discreet prediction in four categories (67.04 minutes) of aromatic quality as shows in Table 6.

Figure 17. Evolution of headspace concentration, generated by pears in their maximum and minimum stage of emission, along time.
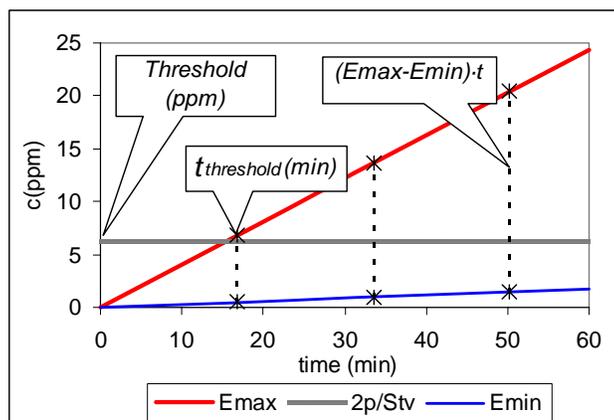
Table 6. Time of headspace generation necessary to differentiate between n maturity stages.

Time of headspace generation (min) for:			
Detection Threshold	**nº of categories (n)**		
	2	**3**	**4**
16.75	33.51	50.27	67.04

4. Conclusions

- This work shows that the stability and the reproducibility of sensors´ response is low, due to external and internal factors. The majority of them are unknown and thus not controlled, introducing sources of variation, forcing to carry out additive corrections between sequences and multiplicative corrections inside sequences.

- Some important metrology parameters that define the QCM sensors characteristics were calculated:

  Average sensitivity : 5.18 Hz/ppm
  Average precision: 16.44 Hz
  Detection Threshold: 6.34 ppm

- For high concentrations of headspace (for each compound starting from saturation of *Langmuir* curve), changes in the fingerprint would permit to carry out qualitative analysis. However in the case of fruits as pears or apples, the headspace concentration is very low (around 20 ppm) and the possibility of using qualitative analysis is limited. To analyse fruit quantitative analysis must be used.

- The type of relevant sensor is very much dependent on the application (topics in our study) which means that a proper selection of the pool of sensors to be mounted in the electronic nose is a major issue.

- The knowledge from:
  - metrology analysis: metrology parameters of QCM sensors have been calculated, and
  - GC analysis: the maximum and minimun emission of volatiles ($\mu g/kg_{fruit} \cdot h$) produced by "Doyenne du Comice" pears have been determined,
  allows to establish the specifications of an e-nose for volatile evaluation on pears, and to calculate the minimum time of headspace generation to be able to distinguish between 1, 2, 3 and 4 volatile quality stage of pear fruit.

5. Discussion

To improve the previous results it is possible to act in two different ways:

- ▪ <u>Modifying sensors characteristics</u> to improve, on one hand, their properties of stability and reproducibility in order that internal and external factors have a minimum effect on sensors´ signal, and on the other hand, to adapt the metrology properties to measure fruit samples, mainly lowing the lack of precision and increasing the sensitivity of sensors.
  About this aspect, only the manufacturer can do the job and he must know the users requirements. Therefore the maintenance of active contact between user-maker and vice versa is critical.
- ▪ <u>Controlling the sampling</u>, that is to say, headspace generation and headspace transference. In this point, the knowledge and care of users are fundamental: to control the temperature of headspace generation, to select the kind of carrier gas, the flux and so on; all these factors can improve enormously the stability and reproducibility of measurements.

At the time being, we do not present the electronic nose data crossed and validated with G-C data. However in all fruit experiments parallel measurements with the electronic nose and with the gas chromatograph were made by another research team (Post Harvest Department of Lleida University-Udl IRTA, Spain). The results obtained will be presented in later reports.

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7. Acknowledgements