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Following butter flavour deterioration with an acoustic wave sensor

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ABSTRACT

Off-flavours develop naturally in butter and the process is accelerated by heat. An acoustic wave sensor was used to detect the aroma compounds evolved from heated butter and the results have shown that registered marked changes were coincident to odour changes detected by sensory analysis. The flavour compounds have also been analysed by GC/MS for identification. The response of the sensor was fully characterized in terms of the sensitivity to each of the identified compounds, and sensitivities of the system SPME/sensor were compared with the sensitivities of the system SPME/GC/MS. It was found that the sensor analytical system was more sensitive to methylketones than to fatty acids. The SPME/GC/MS system also showed the highest sensitivity to 2-heptanone, followed by 2-nonanone, but third place was occupied by undecanone and butanoic acid, to which the sensor showed moderate sensitivity. 2-heptanone was found to be an appropriate model compound to follow odour changes till the 500 h, and the lower sensitivity of the sensor to butanoic acid showed to be a positive characteristic, as saturation was prevented, and other more subtle changes in the flavour could be perceived.

1. Introduction

During storage, off-odours develop in butter. Although a low degree of rancidity, necessarily low, is desired by a few markets, its intensification is associated to spoilage. Oxydation, lypolysis and heat induced breakdown of flavour precursors are the main processes responsible for flavour deterioration [1]. Although lypolysis can be minimized by sterilisation, good packaging and cold storage, the oxidation process is impossible to stop by lowering the temperature, due to its low activation energy. Factors that accelerate oxidation are high temperature, the presence of metals, oxygen and light [1,2].

Chemical tests for lipid oxidation [3,4] are either predictive, case accelerated conditions are used to measure the stability of fat, or indicator tests, based on the quantitative analysis of some degradation compound or family of compounds [3,4]. It is generally not enough to run a single chemical test to characterize rancidity of a product and the selection of the appropriate chemical tests to measure lipid oxidation must be made in straight correlation with sensory testing [5].

Electronic noses based on several transducers have already been applied to dairy products, a few of them based on piezoelectric quartz crystals [6,7]. They are based on an array of sensors, and the responses must be treated mathematically in order to extract useful information.

A unique reliable sensor, capable of detecting a series of compounds responsible for off-flavours would be useful, especially if the response of the sensor could be kept simple enough to be translated by a single figure. This simplicity, allied to an effective correlation with sensory analysis would be highly appreciated when compared to the complexity of results obtained by the array of sensors or GC/MS. GC/MS has the advantage of a more complete characterization of the sample, but useful meaning, in terms of butter oxidation degree, can only be extracted by trained analysts. Besides, GC/MS instrumentation is expensive.

In this work we used a single bulk acoustic wave sensor. Acoustic wave sensors are known by their simplicity, sensitivity and low cost. They are based on the fact that the frequency of oscillation of a piezoelectric quartz crystal decreases proportionally to the mass attached to the crystal electrodes [8]. This is the principle of gravimetric sensors, and although other factors can influence the frequency of oscillation, and be useful for some applications [9], there is no need to complicate this simple explanation, as gravimetric is the most important factor in the present application.

A chemical sensor is composed by a recognition layer and a transducer that translates the chemical signal into a measurable electrical signal. The recognition layer must be carefully chosen, as sensitivity and selectivity depend almost exclusively on this choice. This recognition layer must coat the sensitive area of the quartz crystal: the gold electrodes [9].

Short chain compounds, most of them free fatty acids, aldehydes and ketones are responsible for the rancid smell, and thiols can interact with them. Besides, a thiol group is also known to attach covalently to gold, producing a stable coating for the piezoelectric quartz crystal. As the thiol group attached to gold

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cannot be available to interact with the target compounds, a dithiol was chosen.

Butter flavour varies and the first source of this diversity is the milk itself. Feeding of the animals plays here an important role. As feeding is highly dependent on soil and clima, it is not surprising that it differs with the geographic location as well as seasonally along the year. The degradation of a particular brand of butter was followed and the efforts were focused in developing a sensor capable of giving a response that could be correlated with rancidity and sensory evaluation. At the same time, analyses of the flavour of butter were conducted by GC/MS, in an attempt to characterize the response of the sensor and to identify the compounds evolved along the degradation studies.

This paper presents a new sensor designed to follow butter degradation. A complete study showing the response of the sensor along time, the results of the analysis of replicates by GC/MS and sensory evaluation by common persons are also presented. The interpretation of data from the three methodologies allows the understanding of the chemical meaning of sensor responses, as well as to evaluate the correlation between those responses and sensorial data.

2. Materials and methods

2.1. Apparatus

Analyses of butter headspace were conducted by solid phase microextraction (SPME). The SPME fibre was 75 mm Carboxen/Polydimethylsiloxane (CAR/PDMS) from Supelco.

The fibre, after being in the butter vials headspace, was introduced into the injection port of the gas chromatograph coupled to a quadrupole mass spectrometer 6890 N Network System from Agilent Technologies) or into a home-made oven where a flow of nitrogen carried the compounds to a piezoelectric quartz crystal AT cut of 9 MHz HC6U (ICM-International Crystal Manufacturing Co, Inc.). The gas flow was divided in two streams and each of them was directed to the centre of one of the faces of the quartz crystal, by entering inside the crystal box from two holes opened in the original crystal can. Those holes were drilled on the centre of two opposite sides of the crystal box, and the inlet gas tubes were connected to the metal inlets welded to the borders of the holes. The coated gold electrodes of the piezoelectric quartz crystal were located right underneath the holes. The gas leaved through a third hole at the bottom of the box. The crystal was driven by an oscillator (ICM), and the frequency of oscillation was monitored by a frequency meter (Leader LF827) interfaced to a computer through a GPIB.

Fig. 1 shown the quartz crystal experimental set-up.

2.2. Reagents

All reagents were used as bought, without any purification step.

For the crystal cleaning process, piranha solution was prepared from hydrogen peroxide (Fluka 95321) and sulphuric acid (Fluka 84720). Potassium chloride (Merck 1.04936) was used for the electrochemical stripping of the oxide layer from the Au surfaces.

To coat the crystal, 1.10-decanedithiol (TCI D0015) dissolved in absolute ethanol (Merck 1.11727) was used.

Standards were prepared from butanoic acid (Fluka 19210), 2-heptanone (Fluka 68950), 1-hexanol (Fluka 73117), 2-nonanone (Aldrich 10.873-1), 2-pentanone (Fluka 68950), 2-undecanone (Fluka 68160), 2-propanone (Merk 1.00014.2511), acetic acid (Panreac 131008), propanoic acid (800821), hexanoic acid (Aldrich 15.374-5), 2-butanone (José M. Vaz Pereira), and methanol (Fluka 65543).

Nitrogen and helium were Alphagaz from ArLíquido.

Butter, in 1 kg package, was bought from a bakery, short after being delivered, and was from Milagaia-Soc. Prod. Lácteos, Lda.

2.3. Procedure

2.3.1. Coating of the quartz crystal

The electrodes of the quartz crystal were cleaned by immersing in piranha solution for 10 s. The electrodes were then subjected to several electrochemical potential cycles, between 0 and −0.8 V in 1 M KCl to strip the oxide layer [10], rinsed in Milli *Q* water and dried in a stream of nitrogen. The crystal was then immersed in a solution of 1.10-decanedithiol in ethanol for 55 h. It was rinsed with ethanol to eliminate the excess of dithiol. The frequency decrease due to coating was determined after drying and a value of 5.0 kHz was found.

2.3.2. Analysis of butter

2.3.2.1. SPME adsorption. Butter volatile compounds were extracted by SPME. Butter was divided in portions of 30.0 g and each portion was stored in a 100 mL vial within a magnetic bar. The vial was then encapsulated with a silicone rubber septa coated with Teflon and a removable center crimp seal. Three vials were used in immediate analyses, while the others were kept in an oven at 45.0 °C where they waited to be analysed.

According to the analysis schedule, the vial selected for analysis was immersed in a thermostated water bath set at 45.0 °C, over a magnetic stirrer set for a constant speed of 30 rpm. The SPME fibre

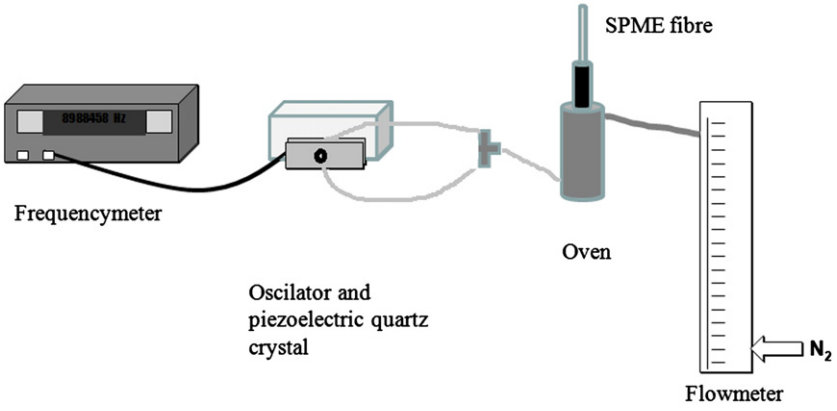

Fig. 1. Experimental layout.

was introduced through the septa into the butter headspace for precisely 1 h, after which the fibre was inserted either into the GC/MS injector or the oven connected to the sensor.

2.3.2.2. GC/MS analysis. For the analysis by GC/MS, the injector was at 250 °C, working in the splitless mode, and the carrier gas was helium at a flow rate of 1.7 mL min^{-1}. The fibre was within the injector for 15 min. The compounds were separated by a fused silica capillary column DBFFAP, 30 × 0.25 mm. The interface GC–MS was kept at 280 °C and the ionization was carried out by electron impact (EI) with energy of 70 eV. The detector was kept at 250 °C, while the temperature of the column was maintained at 25 °C for 3 min, after which it was increased at constant rate of 1.25 °C min^{-1}, til 40 °C, after which the heating rate changed to 5 °C min^{-1}, til a temperature of 120 °C, when the heating rate changed to 20 °C min^{-1} til the maximum temperature of 220 °C was reached, which was kept for 2 min.

The complete recover of the fibre was always checked by performing a blank analysis, after which it was inserted into an encapsulated empty vial, to avoid adsorption of volatile compounds during the transport between the GC/MS apparatus room and the laboratory.

Identification of the compounds separated by the GC was done by performing similar experiments with standard solution of the pure compound in methanol, and comparing not only the retention times but also the mass spectra corresponding to the selected chromatographic peak.

Sensitivity of the SPME/GC/MS system to each compound was evaluated integrating the peak areas of the total ionic current vs. retention time of the chromatograms obtained with several standard solutions of the pure compounds.

2.3.2.3. Acoustic wave sensor analysis. Baseline frequency was read under a constant flow of nitrogen, (flowmeter set at 30 mL min^{-1}). The SPME fibre was then introduced into the oven and when the volatile compounds desorbed from the fibre reached the coated quartz crystal, frequency started to decrease. The lowest frequency was registered. Then, frequency started to increase as compounds were being desorbed from the crystal coating, and baseline frequency was reached, indicating that both sensor and fibre were ready for the next analysis.

2.3.2.4. Sensory analysis. For sensory analysis two portions of 30.0 g of butter were reserved, which were kept closed in two vials, one in the refrigerator, and another one in the oven at 45 °C.

The panel of analysts was composed of six persons, all non specialists.

According to a schedule, each person took the vial from the oven, described the smell, closed the vial again and stored it in the oven. The sample kept in the refrigerator was only used if anyone would need to fell the smell of fresh butter, for comparison.

3. Results and discussion

Fig. 2 shows two signals of the sensor, one obtained analysing a pure compound, 2-heptanone, which is a known volatile present in butter headspace, and the other signal obtained after the fibre being exposed to the butter headspace. Both signals show fast and reversible responses.

Butter samples were analysed in triplicate or quadruplicate and the values presented along the paper are always the median of those results. As the work proceeded for quite a long period, butter samples were from 5 different packages, all of the same brand. Although five months elapsed between the first and last sample analysis, no differences were found between the results

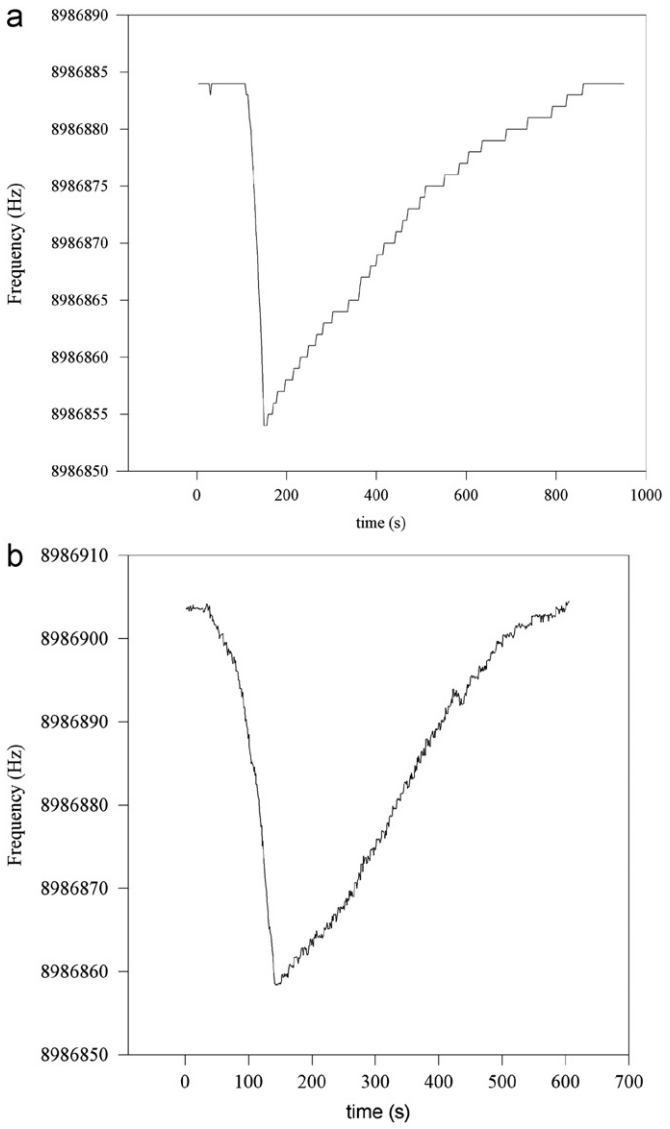

Fig. 2. Responses of the system SPME/sensor to headspace samples of (a) 2-pentanone and (b) butter.

of the analysis performed by the SPME/sensor system for the different butter packages. For this comparison 12 samples of each package, taken from the oven after exactly the same period of storage at 45 °C, ranging from 2 to 43 day, were analysed. Those results showed there was no difference between samples but, most important, showed the roughness of the sensor, and its long life. However, different symbols were used in the graphics for samples of different packages.

Fig. 3 shows the responses of the sensor, expressed as frequency decrease vs. time of storage of butter at 45 °C. A few chromatograms show a perspective of the evolution of the volatile compounds of butter along time. Several compounds contribute to the sensor response, and the chromatograms can give an insight into the process. Fig. 4 shows the median of the areas of the chromatographic peaks vs. storage time. Although the SPME fibre used in the extraction of butter headspace was the same for the analysis by GC/MS and the acoustic wave sensor, sensitivity of the methodologies to the compounds may differ. Table 1 shows the sensitivity of both systems of analysis to the major compounds present in butter headspace. From the table, it can be seen that both the system SPME/GC/MS and SPME/sensor respond to all the 13 compounds identified

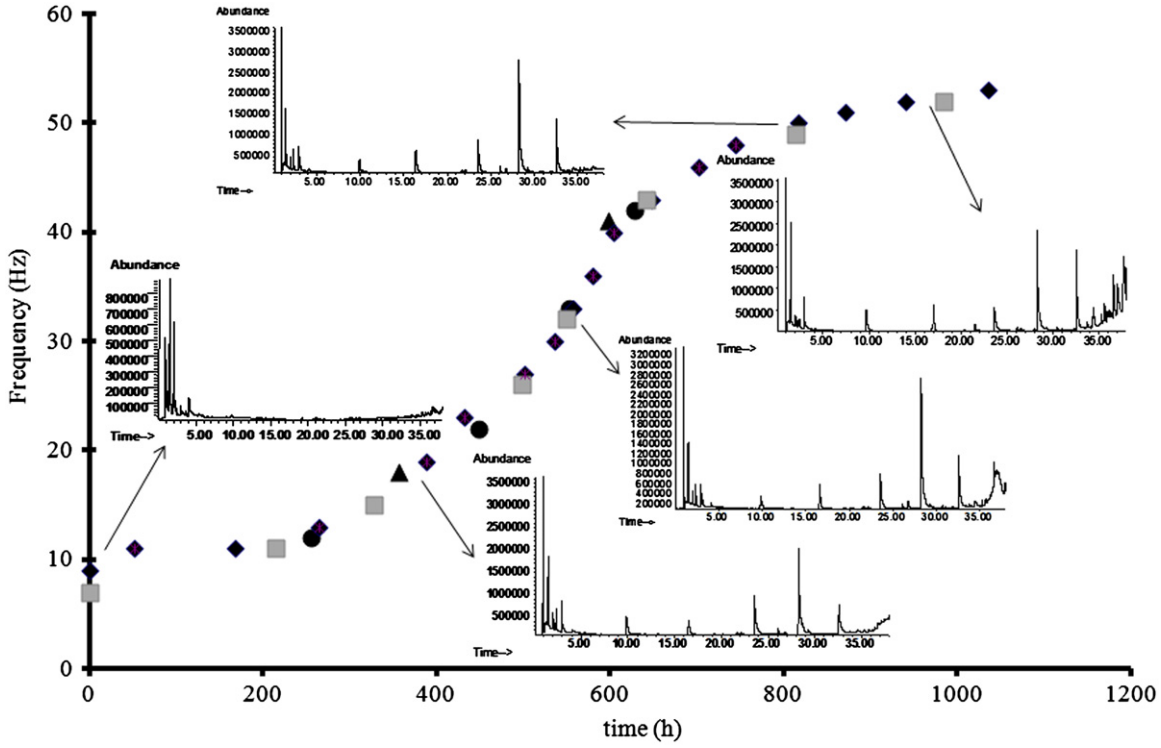

Fig. 3. Responses of the sensor to butter headspace along time (different symbols for samples from different packages). A few chromatograms are shown, with arrows pointing to the position those samples would occupy in the sensor response zone, according to the elapsed time.

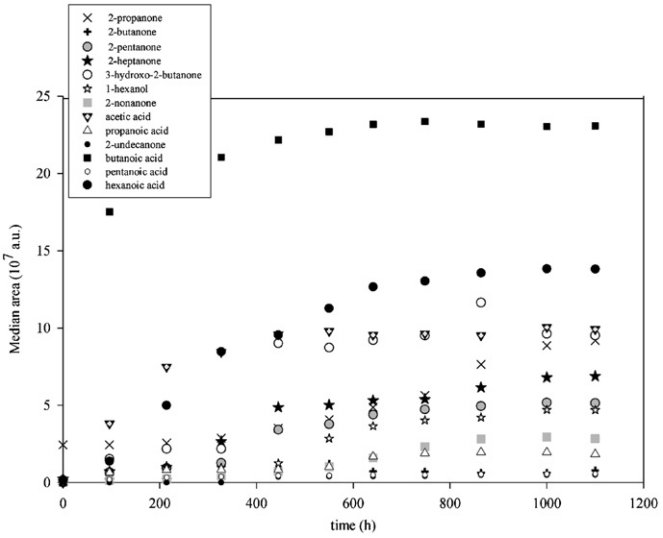

Fig. 4. Median of the areas of the chromatographic peaks vs. storage time.

Table 1
Sensitivities of the analytical systems SPME/GC/MS and SPME/sensor to the main compounds present in the butter headspace.

Compound	Sensitivity	
	SPME/GC/MS (mol^{-1} L)	SPME/sensor (Hz mol^{-1} L)
2-propanone	0.74 ± 0.04	2.78 ± 0.07
2-butanone	0.87 ± 0.05	3.08 ± 0.09
2-pentanone	1.03 ± 0.05	3.847 ± 0.003
2-heptanone	4.2 ± 0.2	4.2033 ± 0.0001
3-hydroxi-2-butanone	0.33 ± 0.02	1.3217 ± 0.0004
1-hexanol	1.10 ± 0.01	2.260 ± 0.004
2-nonanone	3.5 ± 0.3	3.5 ± 0.1
acetic acid	0.19 ± 0.03	1.138 ± 0.002
2-undecanone	1.9 ± 0.1	3.22 ± 0.07
Propanoic acid	0.24 ± 0.01	1.790 ± 0.003
Butanoic acid	1.79 ± 0.05	2.54 ± 0.08
Pentanoic acid	0.109 ± 0.002	2.347 ± 0.009
Hexanoic acid	0.038 ± 0.004	2.257 ± 0.002

in the butter headspace. However, important differences in terms of sensitivities do exist. While the system SPME/sensor presented the highest sensitivity to the methylketones and the lowest sensitivities to fatty acids, the SPME/GC/MS system of analysis showed the highest sensitivities to 2-heptanone and 2-nonanone, followed by 2-undecanone and butanoic acid.

Fig. 5 shows not only the response of the sensor and the sum of the areas of the 13 identified chromatographic peaks, but also the points where the panel that performed the sensory analysis identified a change in the odour. As already expected, the shape of the curves obtained from the chromatographic analysis and the sensor are quite different.

Both the systems SPME/GC/MS and SPME/sensor detected with high sensitivity the 2-heptanone, which is a good marker to follow butter oxidation tilt the 500 h, after which the rate at which its concentration increases slowed down. 2-nonanone was the second compound, in terms of sensitivity of the system SPME/GC/MS and the third regarding the analysis by the SPME/sensor system, but cannot be regarded as a good marker to follow butter oxidation, because the levels detected are very low. The levels of 2-undecanone are also low.

Butanoic acid dominates the chromatograms, in terms of quantity, and evolution in the first days, but the sensitivity of the sensor to detect this compound is low, it is the seventh in the ranking of sensitivity. Therefore, the moderate sensitivity of the sensor to butanoic acid is, at the first glance, the greatest weakness of the

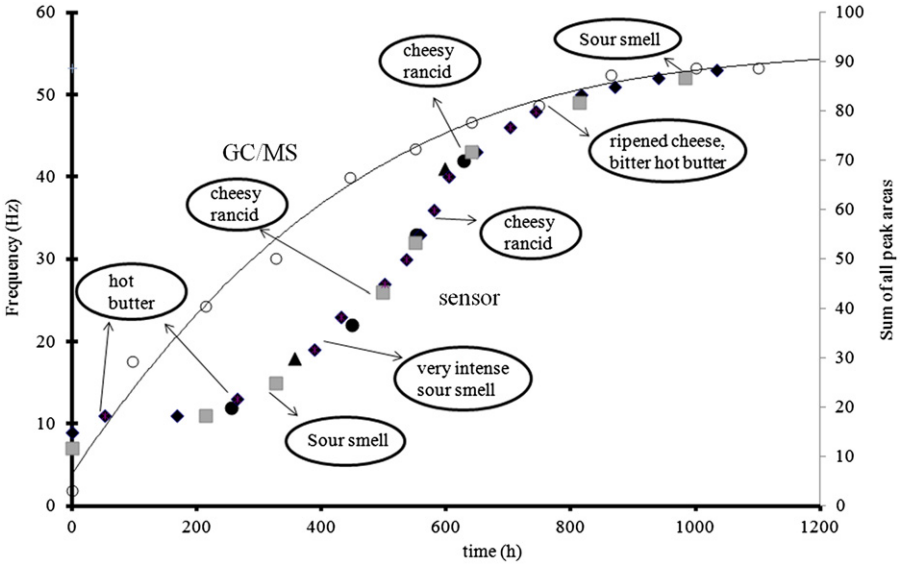

Fig. 5. Responses of the sensor to butter headspace along time (different symbols for samples from different packages), and the sum of the areas of the 13 identified chromatographic peaks (open circles). Descriptions of the odour by the sensory panel are also displayed.

sensor. However, as the concentration of butanoic acid experiences a step increase in the first days, sensitivity of the sensor is enough to register a significant change in response after the first 2 day. On the other hand, the high quantity of butanoic acid hides the evolution of the other compounds in the curve representing the sum of responses of the SPME/GC/MS analytical system and this is one of the reasons for such a difference in the shape of the curve for total signals in GC/MS and in the sensor, displayed on Fig. 5. At last, the moderate sensitivity of the sensor to butanoic acid turns to be an advantage, allowing to keep tracking of the odour evolution, which otherwise, would be completely dictated by a single compound.

Other compounds, as 2-pentanone, are detected by the system SPME/sensor with high sensitivity, but another compound that evolves along time in a similar way, 1-hexanol, and is detected with similar sensitivity by the system SPME/GC/MS, is detected by the sensor with a much lower sensitivity.

2-propanone is also a significant compound in terms of quantity, and the system SPME/sensor is capable of detecting it with a sensitivity that is in the middle of the ranking (6th place), while the system SPME/GC/MS detects it with a lower relative sensitivity (8th place). 3-hydroxy-2-butanone is detected by the system SPME/sensor with lower sensitivity (it occupies the 12th position in term of sensitivity).

Results displayed by the electronic nose are highly dependent on the sensor sensitivity to the several compounds present in the aroma, but this is no different for other analytical methodologies, and for human nose. Therefore, results of sensory analysis performed by volunteers are also displayed on Fig. 5. The panel was not able to detect the sour smell before 294 h, which corresponded to the beginning of the rising in the responses of the sensor, after the initial two days period. Meanwhile, the GC/MS total responses have already experienced a significant increase, which had no equivalence in the panel registers. The inflection point, after which a steep increase on the sensor response was observed, was coincident with a change on the panel description. From this point on, which corresponds to the 498 h, the smell was no longer characterized as sour but as cheesy and rancid. This description can be related to a slower acid production and to an increase in ketones. This phase may be related with the end of the induction period of oxidation, and with the beginning of the second phase, where acceleration of oxidation rate does occur [2]. By the 800 h, when the evolution of the sensor responses levels off, the description of the panel changed to ripened cheese and hot bitter butter, and returned to sour smell by the end of the experiment.

4. Conclusion

A single piezoelectric quartz crystal coated with1.10-decanedithiol was proved to respond to the evolution of the headspace composition along butter degradation. The sensor responded to all the 13 major volatile compounds identified by GC/MS, although with different sensitivities. It is more sensitive to methylketones than to free acids, which allowed detecting continuous butter evolution, starting at the second storage day, without losing its ability to sense the changes after the concentration of butanoic acid became predominant. The analytical system SPME/sensor showed a response pattern where inflection points and significant changes were coincident with alterations perceived by a sensory panel.

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