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Research Article

Rapid Screening of *Alicyclobacillus acidoterrestris* Spoilage of Fruit Juices by Electronic Nose: A Confirmation Study

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Early screening of *Alicyclobacillus spp.* in fruit juices is a major applicative goal for the food industry, since juice contamination can lead to considerable loss of quality, and subsequently, to economic damages for juice producers. This paper presents an accurate study to assess and confirm the EOS507 electronic nose's (EN) ability of diagnosing *Alicyclobacillus acidoterrestris* spoilage in artificially contaminated fruit juices. The authors experimental results have shown that the EOS507 can early identify, just after 24 hours from inoculation, contaminated orange and pear juices with an excellent classification rate close to 90% and with a detection threshold as low as 10^3 cfu/ml. In apple juice the detection threshold was about 10^5 cfu/ml, thus requiring longer incubation times (72 hours). PLS regression of EOS507 data can be also used to predict with fair accuracy the colony-forming units concentration of the bacteria. These results were supported by the GC/MS/MS measurements of specific chemical markers, such as guaiacol.

1. Introduction

Aroma is one of the most significant parameters among the sensory properties of fruit derivates. The volatile compounds of any food product are not only able to give information about its typical flavour, but also to act as product and process markers. Indeed, some of compounds can be the outcome of chemical processes involving the main compounds of the food, as result of technological treatments or caused by the product storage. Unwanted smells, the so-called "off-flavours" may also include substances originating from the metabolism of spoilage microorganisms such as moulds and bacteria which may accidentally contaminate the fruit products [1]. The presence of microbial contaminants is still a severe problem heavily striking several aspects of the food chain: first of all, they are related to health risks for customers but also they cause organoleptic alterations of final products with resulting economic damages for producers.

Trained human sensory panels are often employed for evaluating quality parameters, but this approach suffers from some known drawbacks, such as lack of reliability due to human fatigue or stress and demanding training time and costs; that make them unsuitable for routine industrial controls. The development of alternative or supporting methods for sensory panels for objective quality and safety control of food products in a rapid and consistent manner is very attractive to food industry [2, 3].

Electronic noses (ENs) are instruments based on an array of semi selective gas sensors and pattern recognition methods [4, 5]. As reported in topical review papers [6–9], the EN technology emerged in the last decade as a valid approach for evaluating food aroma due to its simplicity of use, low cost, good correlation with sensory panel, and the ability, once trained, to be used for continuous at-line quality control of products.

At present ENs still present some downsides. With respect to classical analytical techniques, EN is typically less sensitive and can not identify specific volatile compounds. Besides, the training procedure can be lengthy and laborious, and finally, the lack of sensor stability and reproducibility over time can put at risk the use of previously collected databases, which are compulsory for data comparison purposes and for the classification of new unknown samples. These problems are currently approached on one hand by improving the sensors' performances with novel sensing materials [10], on the other hand by adopting (either univariate or multivariate) calibration approaches for compensating sensor drift [11].

Very recently, several works have evidenced the possibility to exploit the EN capabilities to screen microbial contamination by analyzing the pattern of volatile compounds (also called "fingerprint") produced during the metabolism of microorganisms. ENs have already been used to detect microbial spoilage of grains [12–14], bakery products [15], meat [16, 17], fish [18], milk [19], and more recently tomatoes [20]. Just in few cases [20–22], the EN analysis was also coupled with GC/MS characterization of food volatile profile.

In the last year we addressed the challenging problem of using an electronic nose based on a metal oxide (MOX) sensors array, then called EOS, to early diagnose the contamination by *Alicyclobacillus spp.* of commercial flavoured drinks [23] and fruit juices [24].

In early 80s new spore-forming acidophilic and thermophilic bacteria, then classified into *Alicyclobacillus* genus, emerged as one of the most significant food-spoilage organisms for the fruit juice industry [25, 26].

It is known that *Alicyclobacillus* spores can persist for long periods in fruit concentrates, though more diluted environments are required for growth. Strains grow from pH 2.5 to pH 6 and at a temperature higher than 25°C [27, 28]. Due to spores' ability to survive pasteurisation treatments, spores may germinate and grow by giving rise to strong incidence of spoilage on the end product. Moreover, since *Alicyclobacillus* microorganisms do not produce gas or modify the pH level, bacterial spoilage may not be visibly detectable, and hence, difficult to be diagnosed. A priority issue for juice manufactures is, therefore, the availability of quality control tools allowing for early detection of the bacterium and rapid identification of juice spoilage.

A. acidoterrestris is the most common specie able to produce taints in juice and similar products, although other species can also produce unpleasant smells in products fortified with minerals with low juice content. The presence of *A. acidoterrestris* in fruit juices causes off-flavours mainly because of production of 2-methoxyphenol (guaiacol) [29, 30], 2-6-dibromophenol [31], and 2-6 dichlorophenol [32], and as light sediment [33].

Traditionally, gas-chromatography (GC) and mass-spectrometry (MS) are used to determine the aroma components of food samples. These methods provide accurate measurements of the volatile fraction and allow defining the exact chemical nature of aroma compounds. These hyphenated techniques are also useful for identification of off-flavours compounds caused by chemical or microbiological contamination of foods. Much research work has been done in this area to identify specific substances related to the presence of microorganisms in various food environments. Nevertheless, these methods still remain rather complex and expensive, being more suitable for laboratory quality control than for routine industrial analyses, which often require faster, simpler, and massive screening of large product batches.

In our previous work [24], we tested the EOS electronic nose towards different types of fruit juices (orange, peach and apple) that were artificially contaminated by *Alicyclobacillus spp.* Though that work was essentially based on a limited number of trials, nevertheless the exploratory analysis of EN data showed important achievements (1) the EOS system was able to early detect the presence of *Alicyclobacillus spp.* in fruit juices, (2) the system could detect (for orange juice) bacterial concentration as low as 100 colony-forming unit/ml, and (3) the intragenus specificity of EOS was much lower than the genus specificity.

The present work is primarily a confirmation study. Replication studies are scientifically very important, although in the sensors field they are not frequent, and often, EN tests are limited to the preliminary exploratory phase due to sensors reliability and stability troubles, and to the difficulty in replicating the experimental conditions. Therefore, the main determinants of this study include (i) assuring the validity of former investigations and the reliability of results, (ii) applying previous results to slightly different (improved) experimental situations, (iii) determining the eventual role of external variables (e.g., sample preparation), and (iv) inspiring new research by combing findings from related studies.

Noticeably, with respect to the previous experiments, we have introduced a number of significant improvements.

(i) In [24], the juices were autoclaved before *Alicyclobacillus* inoculation to ensure the absence of other living microorganisms (bacteria or fungi). In this work, in order to mimic real working conditions, we did not sterilize the juice. This allowed us to test whether the EOS was able to diagnose *Alicyclobacillus* contamination even in presence of natural microbial flora of the juice.

(ii) In the previous work, we observed quite long analysis times (about 40 minutes) due to the use of static headspace sampling. Here we have implemented dynamic headspace sampling that allows shortening the sensors response and recovery (going down to about 10 minutes). We also used an upgraded version of the EOS equipment, then called EOS507.

(iii) In contrast with [24], we investigated the effect of microorganisms' growth on the EOS response over a longer incubation time (up to 96 hours of incubation), and then, over correspondently higher colony-forming units (cfu) concentration. This also made possible to determine the detection limit of contamination in apple juice; that was not done before. Moreover, it allowed to evaluate the EOS507's

ability to predict the contamination level (cfu per ml).

(iv) Finally, in [24] we argued that an appropriate amount of measurements and suitable statistical models were required to achieve more robust conclusions. In the present work we collected a statistically significant number of measurements, and we also implemented both classification and regression models for corroborating our claims with quantitative information.

In addition to former points, GC/MS/MS analyses were also carried out on the same samples, in parallel with the EOS tests, to identify and quantify potential chemical markers (guaiacol and/or others) responsible of such contamination. The relationship with the EN results was then investigated. In our former study, the trend of specific volatiles was not monitored, instead only the variations of the whole chromatographic patter were taken into account.

2. Materials and Methods

2.1. Samples Preparation. Commercially available apple, pear, and orange juices were used. For all experiments, identical brands of juice were considered.

In contrast with [24], the fruit juices were not autoclaved before *Alicyclobacillus* inoculation.

Spores of *A. acidoterrestris* SSICA 278/B were used in this study. The strain has been isolated from spoiled juice, identified, and then kept in culture in our institute (SSICA, Parma, Italy).

Alicyclobacillus cfu counting was carried out on YSG agar with the following composition (per g/1000 mL of distilled H_2O): 2.0 yeast extract, 1.0 glucose; 2.0 soluble starch, 15.0 agar bacteriological, pH 3.7 modified with HCl 1N. Petri dishes were incubated at 50°C for 48 hours.

Ten sterile glass jars (200 ml of volume) were filled with 150 ml of juice; before inoculum, spores were activated at 80°C for 10 minutes. Then, 8 jars were inoculated with <10 spores/ml while two other jars were kept as reference.

All jars were incubated at 37°C for a variable time (up to 96 hours). It is known that *Alicyclobacillus acidoterrestris* has a thermophilic character, thus slightly higher incubation temperature (i.e., 45°C) should guarantees better growth. However, in order to prevent possible organoleptic alterations of the juice matrix, we preferred to use a lower incubation temperature, which still guarantee good growth of bacterium for most of fruit juices by leaving us the opportunity to increase the temperature if the EN technology failed to detect contamination.

Every sample has been then analyzed at a 24-hour interval of incubation. In parallel aliquots were inoculated onto YSG agar in Petri dishes for cfu counting.

2.2. Electronic Nose. The electronic nose EOS507 (SACMI IMOLA scarl, Imola, Italy) was used in this study. It comprises a dynamic sampling unit, a semiconductor metal oxide (SMO) sensor array with its own read-out electronics, and software for data acquisition and signal processing. The EOS507 model is an upgrade of the EOS835 used in the former work on *Alicyclobacillus* [24]. The evolution mainly consists in the baseline humidity controller and on more accurate sensor read-out electronics.

The sensor array was equipped with six sensors: two commercial Taguchi sensors (TGS2611, TGS2442) and four home-made thin film sensors (see [10] and references therein for device preparation) among which tin oxide (catalyzed with Ag and Mo) and tungsten oxide (see Table 1 in [24]).

In our previous work [24] the use of static headspace sampling strongly limited the carrier flow rate to low values (10 ml/min), due to the small amount of available headspace (4 ml) that cannot be too much diluted without lacking of sensitivity. Consequently, this configuration impaired the analysis time, that was 32 minutes, because of very long sensors recovery time (28 min). The use of dynamic sampling can help to overcome this problem.

The dynamic sampling unit consists of a pump and a flow controller that conveys the air sample containing the odorant under investigation into the sensor array chamber. A Peltier cell allows one to set and control the baseline relative humidity (due point). Juice samples were analysed by dynamic headspace sampling technique: 15 ml of juice were taken from the jar and filled into a 100 ml vial; samples were conditioned for 1 hour at 25°C prior submitting to the measurement cycle.

The analysis cycle starts with a 10-second exposure of the sensors to baseline air at a flow rate of 50 ml min^{-1}, sensors were then exposed for 2.5 min to the sample headspace. Finally the sensors were exposed again to baseline air for 9 minutes in order to recover the baseline before the next analysis. Hence the analyses were much quicker than in earlier experiments with a net gain of 20 minutes.

2.3. GC/MS/MS. The detection of off-flavour compounds was performed by Varian 450 gas chromatograph coupled with Electron Impact Varian 300 mass spectrometer (GC/MS/MS) after headspace solid-phase micro extraction (SPME fiber 85 μm, with polyacrylate coating) using CTC Combi Pal autosampler. Column Varian Factofour VF-5 MS 30 m × 0.25 mm internal diameter, 0.25 μm film thickness. Temperature program: 40°C for 2 min, to 120°C (7.5°C/min), to 270°C for 7 min (20°C/min). Injector temperature, 250°C. Carrier Helium flow was constant at 1 ml/min. Transfer line temperature, 300°C and Ion Source temperature 250°C.

Analysis was focused on the presence of the following compounds: o, p, m-cresol (MS/MS transitions 108/78, 108/80, 108/89), guaiacol (124/81, 124/109), 2,6-dibromophenol (252/63, 252/143, 252/145), 2,6-dichlorophenol (162/63, 162/98, 162/126), and 2,4,6 trichloroanisole (195/83, 195/107, 195/167).

2.4. Data Analysis. The data were analyzed by Exploratory Data Analysis (EDA), a written-in-house software package based on MATLAB [34]. The EDA software includes the usual (univariate or multivariate) descriptive statistics functions among which principal component analysis (PCA) [35], with the additional utilities for easy data manipulation (e.g., data subsampling, dataset fusion) and plots customization.

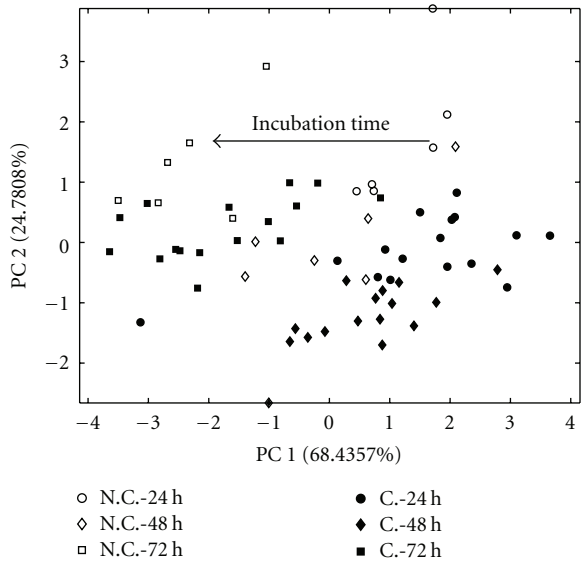


Figure 1: PCA score plot of orange juice samples projected on 1st and 2nd components: open markers refer to uninoculated not contaminated samples (N.C.) while full markers refer to contaminated ones (C.).

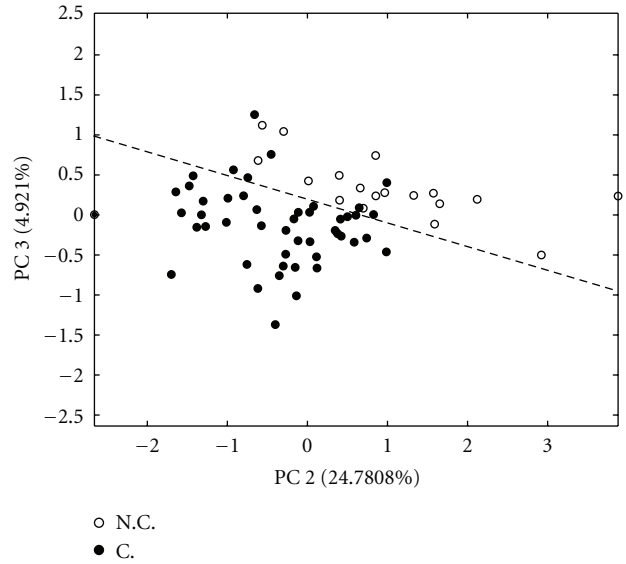


Figure 2: PCA score plot of orange juice samples projected on the 2nd and 3rd components with the following labels of samples: not contaminated (N.C.) and contaminated (C.). The dashed line represents the linear classification boundary as determined by SVM algorithm.

EOS data preprocessing followed the procedure reported in [24].

Supervised classification was carried out by two different pattern-recognition algorithms, namely, support vector machines (SVM) with linear kernel [36] and (for comparison) the more classical 1-nearest neighbour (1NN) classifier. Five-fold cross-validation (CV) was implemented to get more robust classification results.

Partial least squares (PLS) regression [37] was used for predicting the cfu concentration (cfu/ml). For computing the PLS regression components, that is, latent variables (LVs), the standard nonlinear iterative partial least squares (NIPALS) algorithm was used. The original matrices (true cfu/ml values and EOS prediction values) have been first transformed to have zero means and unit variance (*zscore* normalization). Finally, the EOS data were randomly split into two sets: the first to estimate the regression coefficients (training set) and the second for PLS model assessment (validation set).

3. Results and Discussion

3.1. Electronic Nose

3.1.1. Orange Juice. Exploratory data analysis, via PCA score plots, evidenced that the main source of variance in the dataset is the difference between incubation times (ranging from 24 to 72 hours) which gives rise to three clusters distributed along the 1st PC (Figure 1) for both contaminated and uncontaminated samples. It is worthwhile to observe here that the same effect was also evidenced for pear and apple juice, and so independently of the juice type.

The clustering effect related with the incubation is more evident for contaminated samples. This is an expected result because it follows directly from the microbial growth of the bacterium which gives rise to different headspace composition and higher microbial concentration with increasing the incubation time.

Table 1: Classification results for fruit-juice contamination by *A. acidoterrestris*.

Type of juice	Classification rate		Detection thresholds	
	SVM	1NN	Growth time (hours)	cfu/ml
Orange	86%	78%	24	10^3
Pear	90%	84%	24	10^2-10^3
Apple	60%	63%	72	10^5

The observed difference for uninoculated samples can be attributed to the natural microbial load of fruit juices that can lead to some headspace changes in consequence of the thermal incubation of samples at 37°C. Indeed, this phenomenon was not observed in earlier experiments [24] because the fruit juice was autoclaved before inoculating the *Alicyclobacillus*, and hence the intrinsic microbial load was eliminated *a priori*.

Nevertheless, the difference between contaminated and uncontaminated samples can be evidenced on higher order principal components (Figure 2). These are, by definition, orthogonal to the former variation, and therefore the drift of juice matrix does not affect too much the possibility to diagnose samples contamination at different times by the EOS.

Supervised samples classification was performed by performing PCA first and then by eliminating the 1st PC which does not account for discriminating the two classes. Table 1

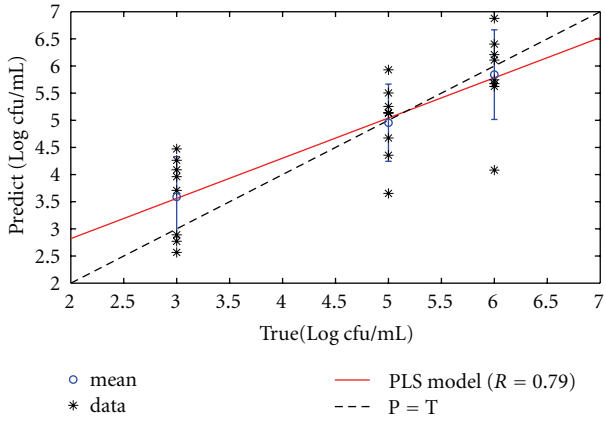


Figure 3: PLS regression of Log(cfu/ml) for orange juice samples contaminated by *A. acidoterrestris*. *X* axis reports true values output by microbiological analysis, *Y* axis gives the value predicted by the EN.

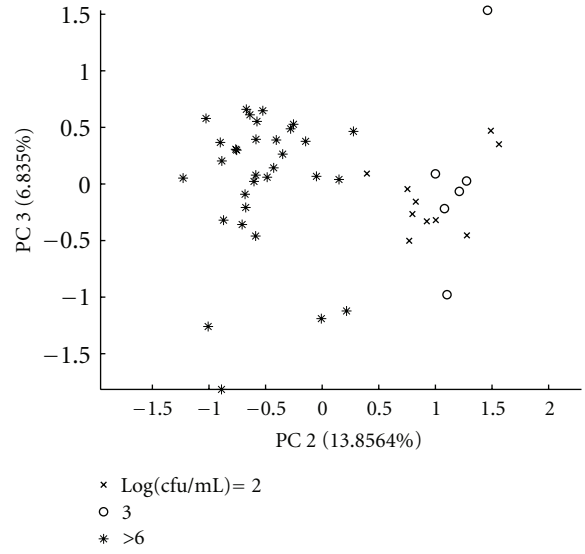


Figure 4: PCA score plot of pear juice samples projected on the 2nd and 3rd components, different markers were used to label the different Log(cfu/ml) values.

reports the classification results: 5-fold CV classification by SVM gives 86% which is a rather good results for comparison kNN ($k = 1$) scores only 78%. It is very important to note that contaminated samples could be correctly identified just after 24 hours of growth time when the cfu concentration was as low as 10^3 cfu/ml.

Once proven the possibility to diagnose *Alicyclobacillus* contamination, PLS regression has been applied to build a multivariate regression model for predicting the cfu concentration of contaminated samples. Since the number of counts increases with the incubation time of samples, we again eliminated the bias due to the intrinsic drift of juice samples by first subtracting the PC1 value before performing PLS.

Results are shown in Figure 3 for the contaminated orange juice. We observed a good agreement (the correlation coefficient scores 0.79) between the predicted values of counts by the EN and the actual value of Log (cfu/ml) given by the microbiological essays. Minor mismatch in the correlation value can be due to two factors: first, the EOS tends to overestimate low-count values, being the predicted mean value 3500 cfu/ml versus 1000 cfu/ml, which is related to a lack of EOS accuracy in the lower range; second, microbiology tests are typically not very accurate and give rise to an order of magnitude of microbial counts which is an average on several cultured Petri plates, accordingly the PLS model has been built on the basis of that mean Log(cfu/ml) value whilst the EOS measurements refer to the actual concentration of individual samples.

3.1.2. Pear Juice. By investigating PCA plots we first observed that pear juice samples behave similarly to orange juice samples. The main source of variance in the data (1st PC)—both for contaminated and uncontaminated samples—accounts for the headspace variation induced by the juice incubation.

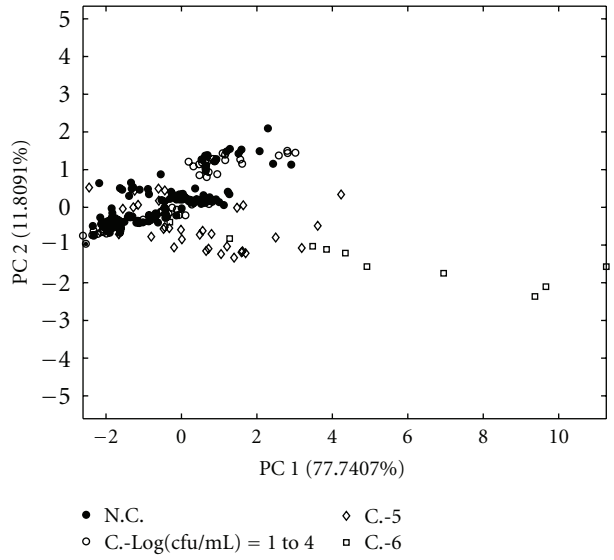
Again, inoculated samples can be separated from uninoculated ones on higher order PCs, hence by allowing the identification of samples containing *A. acidoterrestris*. CV-SVM classification performed after feature extraction by PCA provides 90% of correct classification (Table 1). Also in this case, contaminated samples were correctly identified after 24 h with a threshold even lower that 1000 cfu per ml.

Contaminated samples can be in turn clustered into two sub groups on the basis of their cfu concentration (Figure 4) either low (up to 10^3) or very high (above 10^6). In this case, intermediate values were not present due to missing microbial counts and then PLS regression was not feasible. However, the data suggested that the EOS system can be used to perform a semiquantitative analysis of *A. acidoterrestris* content in fruit juice.

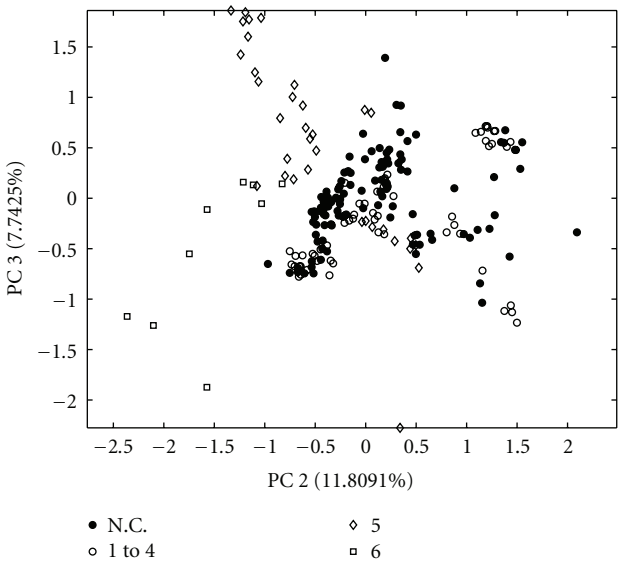
3.1.3. Apple Juice. By incubating the apple juice samples at 37°C we could not evidence any discrimination between contaminated and uncontaminated samples due to the too low growth rate of the bacterium in this type of juice.

Experiments were repeated a second time by changing incubation conditions to facilitate microbial growth. In the second experimental session the inoculated jars were incubated at 45°C instead of 37°C. Though this temperature is higher than that of typical juice storage condition, it accelerates the growth of microorganisms, hence we could achieve higher cfu concentrations at the same incubation time.

PCA analysis (Figure 5) shows a discrimination threshold of about 10^5 cfu/ml which corresponds to an incubation time of at least 72 hours. Indeed all contaminated samples having Log(cfu/ml) up to 4 overlap completely to uninoculated samples, whereas above this value the data are clustered apart except for a little overlap of class "5" (this class is better separated on PC2-PC3 score plot—Figure 5(b)). Sample with 10^6 cfu/ml are perfectly recognized by the EN on the lowest order PCs (Figure 5(a)).



(a)



(b)

Figure 5: PCA score plots of apple juice samples either not contaminated (N.C.) or artificially contaminated by *A. acidoterrestris*—different markers are used to distinguish different values of Log(cfu/ml). The same data are plotted against PC1-PC2 (a) or PC2-PC3 (b).

Classification scores were about 60% (Table 1). This follows from the fact that all contaminated samples under the threshold of 10^5 cfu/ml are misclassified by the EOS507.

These results corroborated those achieved in the previous study [24] where the discrimination between contaminated and uncontaminated apple juice samples was not evident at all, also because of the limited amount of collected data. By contrast, here we have been able to estimate the detection threshold and the classification capability of the EOS.

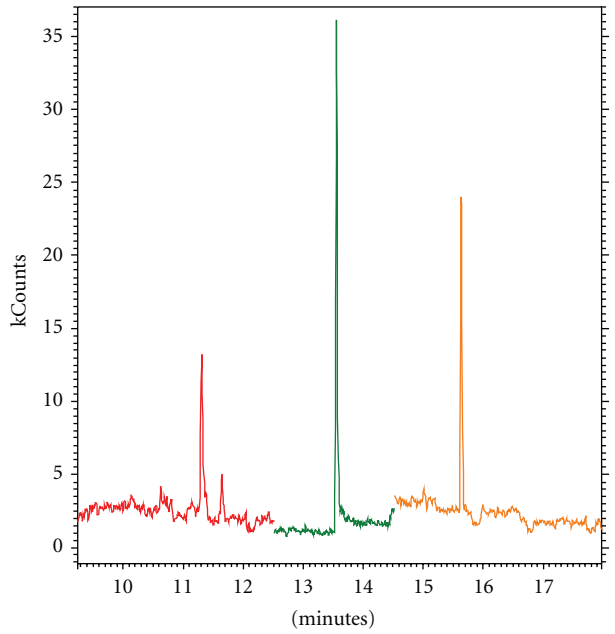


Figure 6: GC/MS/MS chromatographic profiles of reference samples (uninoculated apple juice containing guaiacol, 2,6-dichlorophenol, and 2,6-dibromophenol at 2 μg/Kg) aiming to determine the L.O.Q. of our analysis.

3.2. GC/MS/MS Analysis. In our earlier work, headspace GC/MS analyses were performed on juice samples after 24 hours from inoculum (see [24, Section 3.3]). However, in the contaminated samples, we could not evidence any characteristic chemical marker, but only global fingerprint variations (of some terpenic alcohols and hydrocarbons, aldehydes and ketones) which were associated to the Alicyclobacilli presence.

Here, we explicitly monitored over the whole sample incubation time (up to 96 hours) the trend of specific compounds, such as Guaiacol, 2,6-dichlorophenol, and 2,6-dibromophenol, that are retained to be featuring markers of *A. acidoterrestris* presence. For such compounds the GC/MS/MS analyses showed a limit of quantitation (L.O.Q.) (signal-to-noise ratio $S/N > 10$.) around 1 ppb (Figure 6 displays the GC/MS/MS chromatographic profiles of the three phenols added to apple juice at 2 μg/kg).

The three phenols were totally absent in all uninoculated juice samples. In contrast with our hypothesis, 2,6-dichlorophenol and 2,6-dibromophenol were also never found in the inoculated juice samples whereas guaiacol was found there, and, then, it is confirmed to be a signature of *Alicyclobacilli* contamination.

Guaiacol content was also found to vary with the individual type of juice and with the incubation time. Results obtained by GC/MS/MS for guaiacol are shown in Figure 7. For orange and pear juices it was observed that production

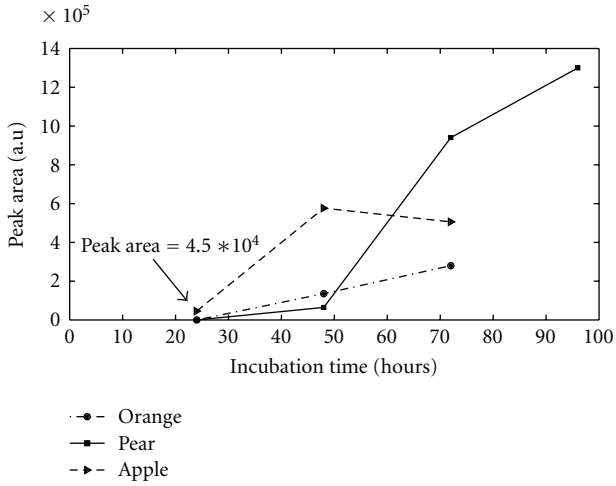


Figure 7: Trend of guaiacol content versus incubation time as determined by GC/MS/MS analysis.

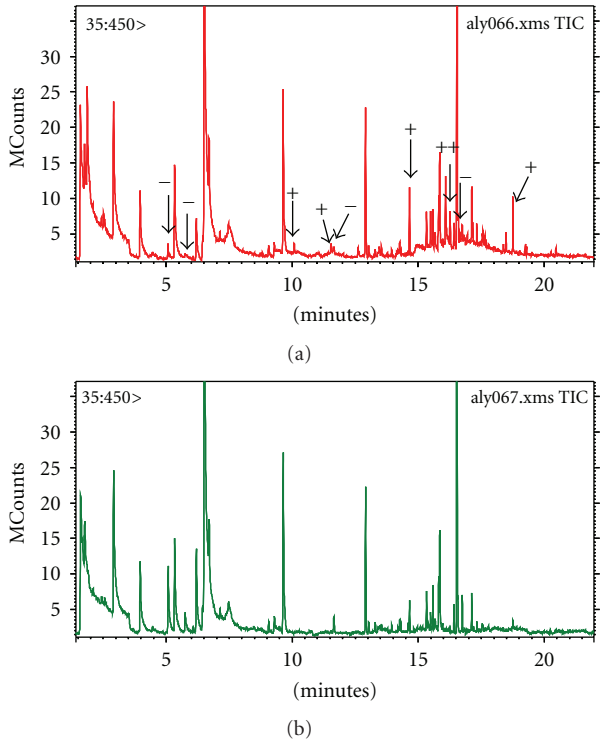


Figure 8: Comparison of full-scan GC/MS/MS chromatograms (35–450 a.m.u.) of inoculated (a) and uninoculated (b) apple juices samples at 48h of incubation.

of guaiacol started after 48 h of incubation at 37°C. For apple juice, guaiacol became detectable and quantifiable after 24 h of incubation at 45°C.

These findings explained why we did not observe guaiacol presence in former experiments; in fact, the analyses were limited to 24 hours and the incubation temperature of apple juice was too low for guaranteeing a suitable microbial growth.

When contrasting GC/MS/MS outcomes with EOS507 results we found only a partial agreement. As regards orange and pear juices, the EOS507 was able to reveal the contamination after 24 h of growth (when counts were as low as 10^3 cfu/ml), that means before the guaiacol production was detectable by GC/MS/MS. Conversely, for the apple juice GC/MS/MS analysis did not confirm the EN results. Indeed, EOS507 could not discriminate between contaminated and uncontaminated samples at early stages of growth, whereas GC/MS/MS revealed guaiacol just after 24 hours from inoculation.

To better understand the peculiar behaviour of the EN on apple juice, an additional GC/MS/MS analysis was performed by monitoring a wider mass range (35–450 a.m.u.) aiming to investigate whether there was any global change of fingerprint (for volatile and semivolatile compounds) between uninoculated and contaminated samples. Through the comparison of the chromatograms, we observed statistically significant differences that are clearly imputable to the presence of *A. acidoterrestris* in terms of arising and reduction of some peaks, as shown in Figure 8.

Merging all results together we argued that the gas sensors are sensitive to the change of the global olfactory fingerprint induced by *A. acidoterrestris* presence more than to the guaiacol content of the samples.

4. Conclusions

In this work, we have presented a laboratory study of the EOS507 electronic nose's capacity to perform a rapid and reliable screening of *Alicyclobacillus* spoilage in fruit juices which is an open and attractive problem at industrial level.

The EOS507 demonstrated excellent detection skills, accurate classification performance of contaminated samples and the potential of enabling rough, but very fast, quantification of colony forming units.

Some features like the ability of evidencing juice contamination at very early stage of microbial growth, the possibility of performing a coarse, but quick, quantification of microbial load, and the simplicity and rapidity of analysis, are certainly important for opening the possibility to transfer the EN technology to the industrial level for routine at-line quality control of fruit juices.

With regard to previous experiments, several new achievements were obtained thanks to the wider culture and measurements campaign:

(i) the assessment of *Alicyclobacillus* spoilage detection by the EOS in natural (not sterilized) fruit juices, and hence the possibility to evidence contamination independently of other microbial interferences;

(ii) shorter analysis time, down to about 10 minutes, which makes the technology far more interesting for industrial applicability;

(iii) more robust statistical sampling that allowed the implementation of classification and regression models to extract quantitative information from the data;

(iv) better assessment of detection limits and of detection time for the different types of fruit juices;

(v) deeper GC/MS investigation of chemical markers.

Concerning the last point, in some cases such as apple juice, the lack of correlation between the EOS response and the chemical markers characteristic of the contamination can be recognized as a limitation of EN technology. In fact, the development of sensors targeted to guaiacol detection, which has been shown to be a rather specific marker of *A. acidoterrestris* occurrence, would certainly help to reduce EN detection threshold and to improve its classification performance. For this reason, the sensing technology requires further improvements in order to obtain more sensitive and more specific chemical devices.

Acknowledgment

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