

# MOX-NW Electronic Nose for detection of food microbial contamination

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**Abstract**— Gas chemical sensors were identified in the past few years as valuable candidate for food safety controls, e.g. early diagnosis of microbial contamination. Microbial management is a crucial task in food processing industry all along the entire food production chain; residual contamination may lead to loss of quality and, if the microorganisms are pathogens, can bring about severe risks for consumers' health. For this reason an accurate and fast control of production using, possibly, at-line sensor systems is highly demanded. In this work we present the industrial application of an Electronic Nose based on metal oxide nanowire (MOX-NW) sensors for the screening of two food matrices (tomato paste and vegetable soups) contaminated with different microorganisms (yeasts and bacteria). The EN was able to detect microbial contamination in about 24 hours at very low inocula concentration, less than 1 CFU/ml.

**Keywords**— *electronic nose, metal oxide sensors, nanowires, food spoilage screening, tomato, vegetable soups, bacteria, yeasts*

## I. INTRODUCTION

New versatile and affordable Process Analytical Technologies (PAT) are required to be integrated with existing food processing systems. Indeed, food procedures are strongly demanding for sensitive, rapid and reliable sensors that should enable in at-line and (quasi) real-time monitoring of food microbial contamination. Today microbial contamination is screened by the industries through post-production storage (*quarantine*) of the food packages in large incubators for two or three weeks.

Recent publications have reported the possibility to exploit gas sensor systems, or Electronic Noses (EN), in various food contexts and many different applications such as process monitoring, freshness evaluation, shelf-life investigation, authenticity determination, and product traceability [1]. EN technology has been also identified as valuable candidate for rapid and affordable spoilage screening [2], [3]. EN can provide many advantages over conventional analytical technologies applied in food industry, including: good sensitivity and correlation with data from traditional microbiological screenings and with sensory panels. EN can be

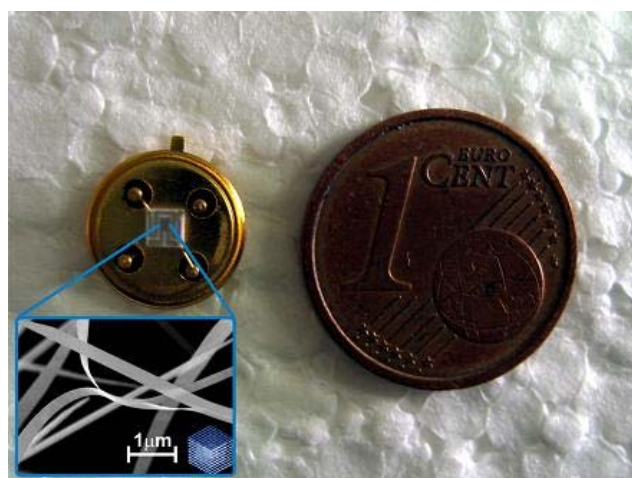


Fig. 1. Picture of MOX-NW sensor on TO5 electronic case. The size of alumina substrate is 2x2 mm while the sensing area is about 200x200  $\mu\text{m}$ . The inset shows an SEM image of SnO<sub>2</sub> MOX nanobelts.

directly used near to the food processing sites/facilities and it is sufficiently rapid to be used at-line. Another significant advantage is the easiness of use: the procedure is straightforward and, once trained, the EN can work standalone.

In this work, the EOS507 EN (Sacmi Imola scarl, Italy) was exploited for the rapid screening of tomato paste and vegetable soups artificially contaminated with yeasts and bacteria. The EOS sensor array was modified by including new tin oxide MOX-NW sensors (Fig. 1) which show finer sensitivity to VOCs [4], [5], and therefore can improve the system detection performance.

## II. MATERIALS AND METHODS

### A. Artificially contaminated food samples

Tomato paste and mixed vegetable soups were provided in 500 ml bricks by Consorzio Casalasco Del Pomodoro (CCDP), Italy, and inoculated with monocultures of *Candida milleri*

(YAB 15), *Enterobacter hormaechei* (ATCC 49162) and *Escherichia coli*. Presumed concentrations of 10 or 10<sup>3</sup> colony forming units (CFU) were inoculated in 100ml aliquot of the food product. The inoculated samples were then incubated at the optimal growth temperature of the microorganism. The samples were measured at different growth times. Not inoculated vegetable soup samples were treated identically and used as negative controls. The experimental conditions are summarized in Table I.

TABLE I. FOOD SAMPLES AND CONTAMINATION CONDITIONS

Food matrix	Microorganism	Inoculum (CFU per 100ml)	Incubation T (°C)	Growth time (hours)
Tomato paste	<i>C. milleri</i>	10-1000	28	24-28
Vegetable soup	<i>E. hormaechei</i>	8-800	35	6-24
	<i>E. coli</i>	3-360	35	10-24

### B. Electronic Nose EOS507

The EOS507 (Fig. 2) is a rather innovative system equipped with a dynamic headspace autosampler and new functionalities such as: a) real-time sample humidity compensation, b) sensor response linearization and c) automated periodic calibration for drift compensation [6]. The EOS was equipped with four conventional MOX gas sensors, namely: SD0610 (thin film mixed metal-oxide SnO<sub>2</sub> and MoO<sub>3</sub> oxide, WT<sup>1</sup> = 400 degC), TGS2611 (Figaro sensor, WT = 400 degC), ST0608 (thin film SnO<sub>2</sub> catalyzed with Mo, WT= 400 degC), STN130 (tin oxide, WT= 400 degC) and one MOX-NW based on SnO<sub>2</sub> (WT = 300degC).

The sensor response processing involves the extraction of one feature for each sensor. This is called “EOS Unit” (E.U.), which is calculated by normalizing the point wise sensor resistance during the measure with respect to the measurement performed against the calibrant. The processed features were analyzed by classical exploratory analysis tools (univariate

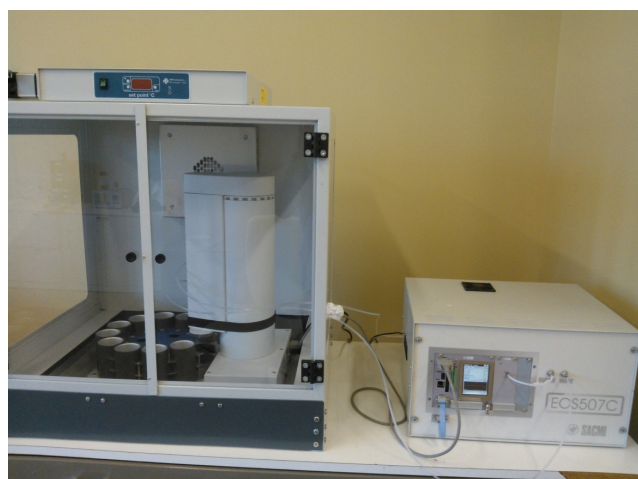


Fig. 2. Electronic Nose EOS507C (Sacmi Imola scarl, Imola, Italy) equipped with a custom HT autosampler for dynamic headspace (HTA srl, Brescia, Italy)

<sup>1</sup> WT = Working Temperature of the sensing layer

feature plots and Principal Component Analysis (PCA)). In order to test whether the sensor responses were related to the inocula concentration we have performed a correlation analysis by taking the magnitude of the linear correlation coefficient and its p-value to test the null hypothesis of no correlation. Supervised classification of contamination was carried out by 5-fold Cross-Validated Linear Discriminant Analysis (CV-LDA).

### C. SPME-GC-MS

DVB/carboxen/PDMS stable flex (50/30 μm) (Supelco Co. Bellefonte, PA, USA) SPME fibers were exposed to the headspace of the samples at room temperature. The volatile compounds were subsequently thermally desorbed and transferred into the GC system. GC-MS analysis of the vegetable soup headspace was carried out using the HP 6890 series GC system, 5973 mass selective detector with a DB-WAX capillary column (Agilent Technologies Italia S.p.A., Cernusco s/N, Italy). The following GC oven temperature program was applied: 40°C for 3.5 min, 5 C°/min to 90°C, 12°C/min to 220°C, 220°C hold for 7 min. The injection was verified in splitless mode at 240°C using helium as gas carrier with a setting flow of 1.5 ml/min. In order to evaluate semi-quantitative differences in the aromatic profile of the samples investigated, GC peak areas (normalized to 100% GC peak areas) were calculated for the detected compounds.

## III. RESULTS AND DISCUSSION

### A. Tomato paste contaminated by *C. milleri*

The EOS507 was able to correctly classify tomato samples spoiled by *C. milleri* after 24 hours of incubation at 28°C and initial concentration lower than 100 CFU per 100 ml of product (< 1 CFU/ml). Samples contaminated with 10 CFU per 100 ml were very close to uncontaminated controls, thus we argued this could be the minimum detection threshold for this type of contaminant.

These results were confirmed by GC-MS analysis. The GC-

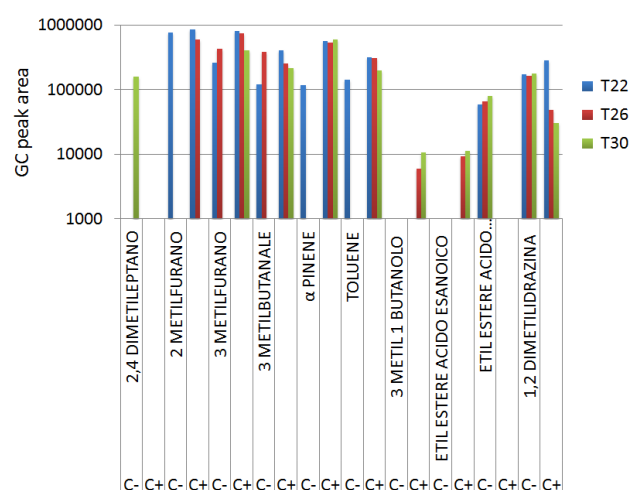


Fig. 3. SPME-GC-MS results obtained on contaminated tomato samples. The bar chart shows the semi-quantitative difference between the profile of contaminated (C+) versus uncontaminated (C-) samples. Legend: T=incubation time.

MS spectra showed a complex pattern (bouquet) of volatile compounds; about one hundred volatile compounds were identified in the tomato fingerprint.

The presence of *C. milleri* was found to alter the global volatile profile of the tomato sample either in terms of relative abundance of some volatiles, which can be related with the metabolism of the yeast, or because the appearance (or disappearance) of certain compounds (Fig. 3). These semi-quantitative differences were observed starting from 22 hours after the inoculation and the difference between the two profiles are increasing with the growth time, e.g. some compounds emerge only after 26 or 30 hours of growth.

### B. Vegetable soups contaminated by enterobacteria

The detection of *enterobacteria* contamination in vegetable soups was achieved in 24 hours, a lower incubation time was leading to detection failure and misclassification of the contaminated samples (Fig. 4). This was attributed to the bacteria metabolism that leads to release of featured volatiles only around the 24 hours of growth. This was confirmed by GC-MS analysis (data not shown).

Conversely, after 24 hours of incubation, almost all the samples contaminated by *E. hormaechei* were correctly classified by the EOS. The CV-LDA classification gives 98.9% of correct classification. Some misclassifications were still present and occurred at the lowest concentration (8 cfu per 100 ml) as shown in Fig. 5. Similar results have been achieved for *E. coli* contaminated samples; in this case the CV-LDA model returns 100% correct classification for the samples incubated for at least 24 hours.

As shown in Fig. 5 the sensors response correlate very well with the initial inoculum concentration, although the correlation coefficient value depends of the bacterial specie. The Pearson correlation coefficients are comprised between 0.84-0.92 for *E. hormaechei* contaminated samples while they are between 0.57-0.62 for the *E.coli*. This result supports the hypothesis that it is not only possible to detect and classify the

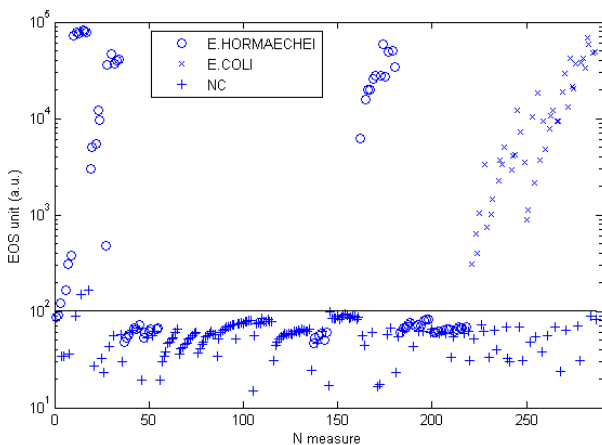


Fig. 4. Feature plot (EOS units) of tin oxide MOX sensor of contaminated and uncontaminated vegetable soup samples. Data are marked according to the different type of spoilage organism. Misclassified measurements are related with contaminated samples which were incubated for less than 24 hours.

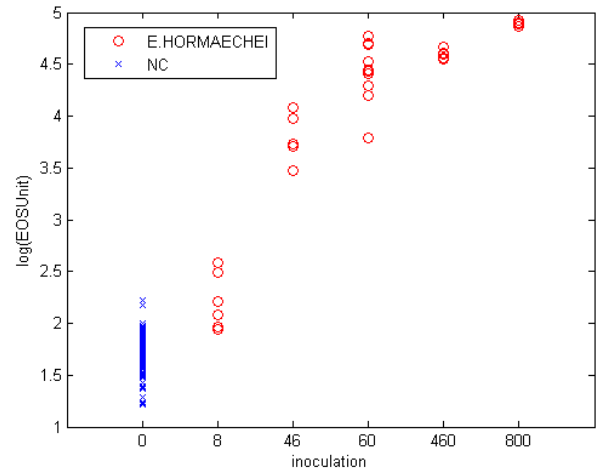


Fig. 5. Feature plot (Log(EOS units)) of tin oxide MOX sensor versus the initial inoculum concentration (CFU per 100ml). The plot shows that the sensor detection threshold can be estimated to be around 10 CFU/100ml and a good correlation with increasing concentration.

contamination but also to quantify the initial amount of contaminant.

## IV. CONCLUSIONS

Ensuring the safety of consumers and preventing expensive waste of food products is a priority of food producers and a critical issue. In this work we addressed early detection and screening of microbial contamination of two common food products by an innovative EN machine. The diagnostic capability of the EOS has been proven to be very effective, being capable to achieve an excellent classification rate (close to 100%) in a relatively short time (24h from inoculation) compared to the two/three weeks of quarantine that are commonly required. Compared to traditional approaches, this would mean a time saving of 90% and then a comparable saving of overall costs. These results encourage the rapid exploitation of EOS technology at the industrial level.

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