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Rapid diagnosis of *Enterobacteriaceae* in vegetable soups by a metal oxide sensor based electronic nose

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ABSTRACT

A rapid protocol for the early diagnosis of microbial contamination of commercial food products by Electronic Nose is presented. Mixed vegetable soup samples are artificially contaminated by *Enterobacter hormaechei* and *Escherichia coli* and a large dataset of 584 samples, over two experimental campaigns, was analyzed by the electronic nose EOS507C based on a four metal oxide sensors array. Diagnosis of the contamination is obtained after 21 h and 18 h from the inoculation of *E. hormaechei* and *E. coli* respectively. EOS detection thresholds at 24 h were as little as 8 cells/100 ml for *E. hormaechei* and 3 cells/100 ml for *E. coli*. The achieved LDA classification performance of contaminated samples was 98%. Also a significant correlation between the sensors responses and the inocula concentrations was obtained. Good long-term repeatability and reliability was demonstrated by comparing the results of the two experimental campaigns spanning for 14 months. The EOS resulted to fulfill all the main requirements of an ideal industrial screening system: specificity, sensitivity, early diagnosis, operational simplicity, reproducibility and cost effectiveness.

1. Introduction

Foodborne diseases have become a significant public health problem worldwide as outbreaks linked to food-borne pathogens account for millions of deaths and hospitalizations; causing colossal economic losses each and every year [1–4].

In addition, also food spoilage draws rising attention: 25% of the world's food supply is lost as a result of microbial spoilage [5] with food loss arising from manufacturing representing 39% of total food losses [6].

Food safety is therefore fundamental to food company ongoing business. Consequently, the food industry is concentrated in preventing contaminations along the food chain, during the food processing operations [7].

In this context, technologies for food safety control play a pivotal role in preventing food contamination and as such, food and beverage producers have been continually searching for reliable rapid analytical methods for the detection of microbial presence at the earliest stage.

Process Analytical Technology (PAT) is the new paradigm in the food industry. PAT initiative is "designing, analyzing and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality" [8]. PAT is a system developed for the pharmaceutical industry and widely adopted within the pharmaceutical and petrochemical process industries but still poorly adopted within the food industry [9,10].

Recently, there have been significant advances in process sensors and in model-based monitoring and control methodologies whose potentiality can allow a larger expansion of PAT in food safety industrial monitoring.

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Devices based on sensor arrays represent a powerful platform for a broad range of applications [11] where detection of gases is desired such as in healthcare and life sciences [12–16]. Gas sensors offer many advantages in such applications for their direct, label free and quasi real-time chemical signal transduction, low-power consumption and high sensitivity [17].

The association between the production of VOCs and the microbial life is well known [18,19] and analyses of the volatile profile of food, depending both on the nature and the relative amount of VOCs fingerprint of the product can be used to assess food quality and to detect adulteration, including microbial spoilage [20–25].

Among gas sensors, electronic noses (ENs), instruments based on an array of semi-selective gas sensors and pattern-recognition methods [26], are gaining strength as a diagnostic tool. Recent works have demonstrated the possibility to employ ENs in various food contexts such as process monitoring, freshness evaluation, shelf-life investigation, authenticity determination and product traceability [27–29] and to diagnose microbial contamination in various food products [30–33].

Today the quality control systems, traditionally applied in the food industry, allow verifying the presence of contamination only after several days from filling. This period, called "quarantine", has an average duration of 2 weeks.

A reduction of the interval between the filling and the detection of a microorganism from several days to hours would be critical to the food company allowing to promptly act and thus limit the damage to a part of the entire batch with considerable savings in terms of food, resources and money, and obviously with an increase of safety of the consumers.

Most of the common bacterial detection methods today are either slow (classic microbial) or technically demanding (antibody, PCR, spectrophotometry and chromatography). They often require well-trained operators for the sample pre-treatment steps, and cost-effective analysis. Some systems, a few years on the market, reduce the quarantine period to "only" few days but require heavy investment in equipment and have a substantial cost per analysis.

Fast and precise analytical methods are essential to ensure product quality and safety.

In this study the feasibility of the electronic nose EOS507C (Sacmi Imola scarl, Italy) dedicated to environmental monitoring, as a PAT for food industry was verified. Volatile compounds from the headspace of vegetable soup samples, inoculated with two members of the *Enterobacteriacea* family, *Enterobacter hormaechei* and *Escherichia coli*, and incubated for different times, were collected and analyzed by the EOS507C in the attempt to develop a rapid method for the reliable diagnosis of microbial contamination that could reduce the required time for from days to hours.

The *Enterobacteriaceae* are a large family of Gram-negative, non-spore-forming bacteria. Although strains of some species are harmless commensals, such as some strains of *E. coli*, others are important human and animal pathogens such as *Salmonella* spp., *Yersinia enterocolitica*, pathogenic *E. coli* (including *E. coli* O157:H7), *Shigella* spp. and *Cronobacter* spp, and some are pathogenic to plants and insects. Their ubiquitous distribution means that it is inevitable that some members of the *Enterobacteriaceae* will enter the food chain. Thus *Enterobacteriaceae* demand particular attention both in perishable food and in processed foods with a long shelf-life such as the already-made mixed vegetable soup chosen for this study. Moreover already made food is gaining importance in our world among all the food products in fact today the food products processed by aseptic filling in bricks have acquired a large commercial and industrial impact.

ENs offer other major advantages over the current analytical technologies applied in food industry, including good sensitivity and correlation with data from traditional microbiological tests and with sensory panels. Besides their good selectivity and low cost, they are portable to be used in working sites, sufficiently rapid to be used at-line or on-line, and can directly screen products with no sample preparation. Another significant advantage is the easiness of use: the procedure is straightforward and, once trained, the EN can work standalone.

2. Material and methods

2.1. Vegetable soup samples production

Mixed vegetable soups in 500 ml and 1l bricks of three different batches were provided by Consorzio Casalasco Del Pomodoro (CCDP), Italy, and inoculated with monocultures of *E. hormaechei*, previously isolated as food contaminant by CCDP and *E. coli*, isolated, characterized and provided by the Clinical Microbiology Laboratory of the Vittorio Emanuele Hospital in Catania, Italy. Not inoculated vegetable soup samples were also prepared and treated identically as negative controls.

Fresh bacterial cultures were maintained on Nutrient agar. Ten microliter bacterial cell suspension at the presumed concentrations of 10 or 10^2 colony forming units (cfu) were inoculated in 100 ml aliquot of the food product. All the samples were incubated at 35 °C. *E. hormaechei* inoculated samples were evaluated at 6, 8, 10, 15, 18, 20, 21 and 24 h while the *E. coli* samples at 10, 15, 18, 20, 21 and 24 h. Ten ml aliquots were then transferred into 100 ml glass vials and incubation was prolonged for other 3 h at the same temperature, for headspace production. The samples were immediately analyzed by electronic nose. For each incubation time, a minimum of six replicates were prepared and measured. A total of 584 measurements were done with the EOS507C and the two experimental campaigns, 2013 and 2014, lasted (discontinuously) for over 14 months as shown in Table 1.

2.2. Electronic nose

The commercial EOS507C (Sacmi Imola scarl, Imola, IT) [34] has been used in this study.

This was equipped with four metal oxide gas sensors, namely: (1) SD0610 (thin film mixed metal-oxide SnO_2 and MoO_3 oxide, WT = 400 °C), (2) TGS2611 (Figaro sensor, WT = 400 °C), (3) ST0608

Table 1
The datasets are reported with the indication of the two experimental campaigns (2013 and 2014), of the used batches and of the respective incubation times of the samples (NC = negative controls).

Sample	Campaign 2013				Campaign 2014				Total
	Batch	Incubation time (hour)	Duration of experiments	No of measurements	Batch	Incubation time (hour)	Duration of experiments	No of measurements	No of measurements
E. hormaechei	1, 2	6, 8, 10, 15, 18, 20, 24	5 months 04/2013–09/2013	76	3	21, 24	1 month 04/2014–05/2014	35	111
E.coli	1, 2	10, 15, 20, 24		108	3	15, 18, 21, 24		69	177
NC	1, 2	6, 8, 10, 15, 18, 20, 24		265	3	15, 18, 21, 24		31	296
Total				449				135	584