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ScienceDirect

Procedia Engineering 87 (2014) 1302 – 1305

Procedia Engineering

www.elsevier.com/locate/procedia

EUROSENSORS 2014, the XXVIII edition of the conference series

Fast identification of microbiological contamination in vegetable soup by electronic nose

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Abstract

Microbial contamination, either before or during food production phases, is one of the major concerns of food manufacturers. In this work we present the EOS507C Electronic Nose (EN) for early screening of *Enterobacter hormaechei* type strain (ATCC 49162) contamination in vegetable soup. The EOS507C, based on an array of metal oxide semiconductor (MOX) sensors, is a rather innovative system equipped with dynamic headspace autosampler and new functionalities such as real-time sample humidity compensation, sensor response linearization and automated periodic calibration. The EOS507C has provided excellent results in terms of screening capabilities: *E.hormaechei* contamination was detected in 24 hours.

Peer-review under responsibility of the scientific committee of Eurosensors 2014

Keywords: Electronic Nose; MOX; contamination; food; bacteria;

1.Introduction

Electronic Nose EOS507C (EN) (Sacmi Imola scarl, Italy) is an abundant studied in recent years to be used in different applications. In this work the electronic nose is utilized for identification of food quality and in particular microbiological spoilage.

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Peer-review under responsibility of the scientific committee of Eurosensors 2014
doi:10.1016/j.proeng.2014.11.686

Microbial contamination of food is cause of economic losses for the food industry and may often lead to emerging disease risks for the consumers. Today microbial contamination is screened by the industries through post-production storage of the food packages in large incubators for two weeks. For this reason, simpler, faster and low cost analytical techniques, like the EN, can be a big advantage for the food industry. Previous works [1,2] have demonstrated that the EN is capable of early diagnosing microbial contamination in different food matrices through the detection of volatile secondary metabolites produced by the organisms during their growth.
The objective of this work was to investigate whether the EN is capable of performing reliable detection of *Enterobacter hormaechei* in vegetable soups in at least 24 hours at low concentrations. If so, the benefit that could bring the electronic nose within the industry is huge considering the time of analysis commonly used for the screening of products.

2.Material and methods

2.1 Vegetable soup

The matrix utilized in this work is vegetable soup, was provided by Consorzio Casalasco del Pomodoro (CCDP), Italy. Soup, mix of vegetable (carrots, potatoes, onions, leeks, broccoli, celery, green beans), was inoculated with monoculture of *Enterobacter hormaechei*, type strain (ATCC 49162). Suspension cell (10 µl) were inoculate in 100 ml of vegetable soup and incubated at 35 °C for a variable time: 3, 5, 7, 12, 15, 17 and 21 hours, in order to identify the best detection time. The concentration of inoculated cells ranges from 10 to 10^2 CFU. Subsequently the sample was liquated in 10 ml crimped vials and incubate at thermostatic at the same temperature for 3 hours before the measurement for headspace production. Control samples (uncontaminated) followed the same procedure. After the incubation, all samples are immediately measured with Electronic Nose EOS507C.

2.2 Electronic Nose

The EOS507C (see Fig. 1) [3], based on an array of metal oxide semiconductor (MOX) sensors [4]. The advantage of having a pattern of six different sensors is the response, since the set of multiple sensors increases the sensitivity to more volatile compounds present in to the headspace of the vials.
This Electronic Nose are equipped with dynamic headspace autosampler and new functionalities such as real-time sample humidity compensation, sensor response linearization and automated periodic calibration. This electronic nose detects the moisture content of the sample (DP) and try drying it before flushing environmental air dehumidified. In this way, you can minimize the influence of humidity on the response of the sensor.
Another important advantage of this device is to obtain periodic automatic calibration of sensors in comparison with a fix concentration of n-butanol, used has internal reference standard. For each sensor it is possible to obtain a response by the extraction of one feature, called “EOS unit, that is a value normalized against the calibrant.
For the measures have been used vials of 100 ml, placed in a dynamic headspace autosampler, which is equipped to the nose. It has 10 positions for vials and connected to the nose by PTFE tubes, in which pass the volatile compounds to be analyzed. The classification of bacterial contamination was performed using 5-fold CROSS-Validation Linear Discriminant Analysis (5CV-LDA)

2.3 Microbiological control

Vials were analyzed at the same time by classical microbiological analysis using electronic nose. One milliliter of each contaminated sample was taken and inoculated, into a plate containing microbiological compatible soil, Nutrient Agar (OXOID), according to *Enterobacter hormaechei* growth. After 1 day incubation at 35 °C we checked growth of the microorganism. In this way we can calculate the bacteria concentration present in each sample. By this comparative method it was possible to validate the electronic nose response.

Fig 1: Electronic Nose EOS 507C (Sacmi Imola scarl, Imola, Italy) equipped with a custom HT autosampler for dynamic headspace (HTA srl, Brescia, Italy)

3.Result and discussion

As shown in the PCA plot (Figure 2a) the EN is able to discriminate the vegetable soups contaminated (C) by *E.hormaechei* from the control samples uncontaminated (NC) after 24 hours of incubation. All contaminated samples for less than 24 hours, are positioned together with the NC samples in the right part of Figure 2a. The large range of EN patterns of contaminated samples is due to different final concentrations depending on different inoculum concentration and microorganism grow. As can be seen the EN can perfectly detect the spoilage at 24h of growth time. The classification 5CV-LDA classification performance is 98.8%.

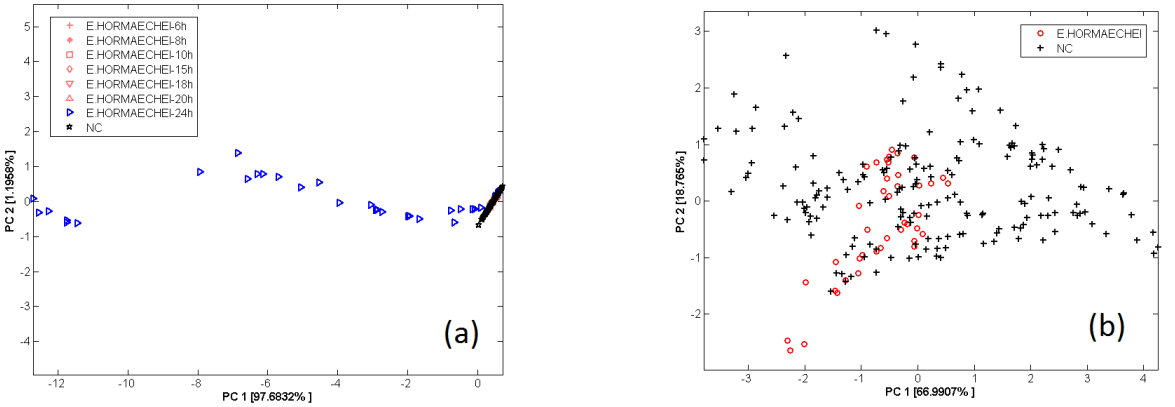

Fig 2: (a) PCA score plot of EN patters of samples not contaminated (NC) and contaminated by *E.hormaechei*. (b) PCA score plot of EN patters of samples not contaminated (NC) and contaminated by *E.hormaechei* incubated for 6h to 20h (zoom of previous PCA reported in fig. (a)).

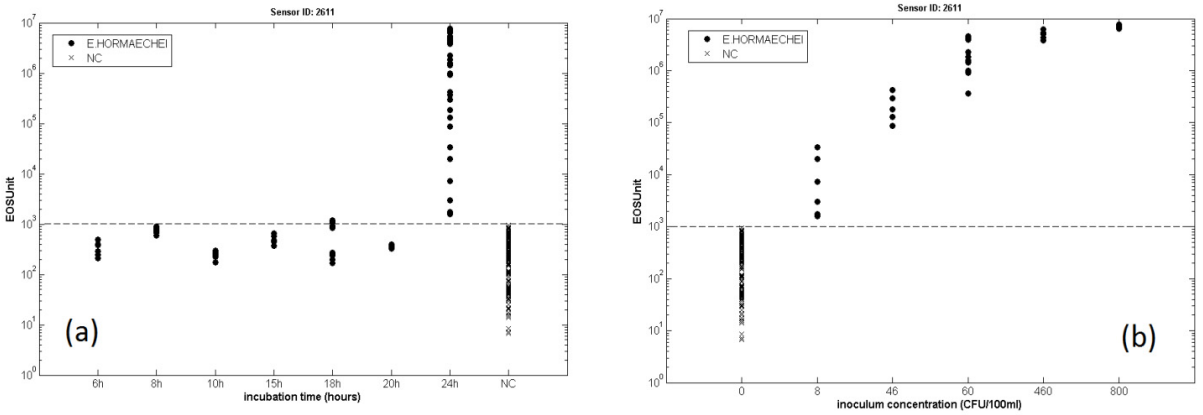

Fig 3: (a) plot of the sensor 2611 EOS units against the sample incubation time from 6h to 24h. C and NC samples are labeled in different ways (dots vs crosses). (b) plot of the sensor 2611 EOS units as a function of the inocula concentration (only C samples incubated for 24h).

NC samples have been incubated for the same time as contaminated ones. Although the mean values of NC samples is significantly lower with respect to the mean values of C samples incubated for less than 24h, in Figure 3a it is evident that all those samples can be considered to be under the detection threshold of the EN (dotted line). All the samples contaminated can be correctly classified while the minimum detection threshold looks to be around 10 CFU/100ml (Figure 3b) which is compatible with typical contamination values that may occur during actual production. The plots show that the sensor detection threshold is about 1000 EOS units, corresponding to 8 CFU for 100 ml inoculum of soup. The sensor response also correlates quite well with the inoculum concentration showing a saturating trend at very high inocula (above 400 CFU per 100ml).

4.Conclusion

The EOS507 has proven to be an excellent instrument for microbiological screening, although the diagnosis is limited by the production of volatile metabolites that can occur after few hours of growth. For these reasons, the electronic nose could be a useful tool for food companies. Would allow them to lower the time of detection and reduce the costs.
Future improvements of the technology which are currently under investigation, the incorporation of much more sensitive sensors, can lead to reduction of detection limits and hence of the detection time.

Acknolegments:

This work has been supported by Consorzio Casalasco del Pomodoro, Cremona (Italy)

5.Reference

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