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MOS-based artificial olfactory system for the assessment of egg products freshness

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Abstract

The determination of egg freshness as a fundamental aspect directly related to consumers' perception of food product quality is a major target of the egg industry. An artificial olfactory system (AOS) based on common metal oxide sensors is presented here as an interesting tool to carry out, in a simple and rapid way, the freshness assessment of industrial egg products. Contemporaneously, the correlation between AOS responses and the correspondent chemical (lactic and succinic acids) and microbiological (total viable mesophilic bacteria and Enterobacteriaceae counts) parameters, which are legal references to attest the quality of the egg products, has been demonstrated.

Keywords: Egg products; Freshness; Organic acids; Colony count; Artificial olfactory system; Electronic nose; Metal oxide sensors

1. Introduction

Eggs can be considered a milestone for human and animal diets thanks to the large content of high quality proteins and vitamins. For this reason, eggs are ingredients largely used in the industrial preparation of different types of foods (foams, emulsions, pastry and bakery products) under the mixture forms which denominate egg products. The concept of egg products is related to all the forms of presentation of the egg: yolk, albumen or a mix of them.

In particular, industry is interested in high quality egg products in a liquefied form, obtained from eggs shelled within 4 days and subjected to homogenization and pasteurization processes: their use is mainly related to the preparation of egg pasta and bakery products [1].

Immediately after the laying phase, the contents of the egg with its entire shell are practically sterile and can be contaminated from environmental microorganisms only if the shell is broken. In general the significant microbial growth that occurs in a shelled egg, before the pasteurization process, causes the formation of different microbial metabolites and leads to a significant alteration of the original enzymatic properties. The egg contains a series of organic acids like succinic acid and lactic acid, whose presence is directly correlated to the microbial quality and which cannot be altered through thermal restoration actions [2]. The amounts of lactic and succinic acids in high quality liquid fresh egg products are usually not higher than 200 and 5 mg kg^{-1} dry egg, respectively [3]. Currently, the legal European Union limits [4–6] are: lactic acid $\leq$1000 mg kg^{-1} dry egg; succinic acid $\leq$25 mg kg^{-1} dry egg.

Non-destructive methods to determine egg freshness, including optical and spectroscopic measurements on shell or yolk colors, have been proposed in the past [7]. At the same time, researchers have attempted to identify volatile flavor components that contribute to the egg's unique flavors and aromas, working with different extraction and analytical techniques (steam-distillation, solvent extraction, purge and trap, etc.): several aldehydes, aromatic compounds and sulfur compounds were identified in greatest concentrations [8]. In particular, methyl-sulfide compounds are strictly related to deterioration and perception of unacceptable odors in whole eggs [9]. In most cases these methods are interesting to demonstrate all the potential compounds that can be emitted from eggs, but often the correspondent necessary heating procedures may produce an excess of volatiles which is not representative of the real situa-

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tion of an egg product which is refrigerated and evaluated from a sensory panel at room temperature or after a short treatment at 30–40 °C.

An alternative strategy to sensing the global profile of organic volatiles emitted by eggs can potentially be achieved by using artificial olfactory systems (AOS), also called "electronic noses" [10]. Currently, the application of AOS has been encouraged thanks to the outstanding developments which sensor technology and data processing systems have undergone over the last 10 years.

The aim of the present work is to evaluate the potential of AOS, based on metal oxide semiconductors (MOS) sensor technology, in the solution of the following points regarding the quality of egg product lots, subjected to acceptance procedures in the food industry quality control laboratories: (1) comparison between AOS and the classical analytical and sensory quality control methods, promoting possible support and eventual replacement of expert panelists dedicated to this routine control and (2) AOS determination of the freshness level of egg products compared with chemical and microbial markers. The purpose of this work is to show that AOS can replace panel test evaluation and partners with traditional quality control methods (organic acid determination/microbial analysis) in the freshness evaluation of egg products.

2. Quality control and sensory characteristics of food products

The term "flavor" is referred to the complex perception of olfactory-gustative, tactile and kinesthetic stimulus that allow us to identify a food and establish a criteria to assess it. Flavor is directly related to the quality of food products: this fact explains the countless efforts to characterize and identify it. Fragrant molecules present some common characteristics: (1) high volatility, essential to get into the nasal cavity, melting in the mucus and stimulating the olfactory receptors by forming weak bonds with their proteins and (2) liposoluble behavior that helps absorption of the molecules into the membrane of the sensorial cells. The amount of volatile substances present in food is in general extremely low (10–15 mg kg^{-1}), but the number of such compounds is very high. In foods which have been subjected to thermal treatments, alone (like coffee) or in association with a fermentation process (like beer), it is possible to characterize more than 500 volatile products. Compounds responsible for the flavor cover all the classes of chemical products: neutral, acids, bases, high volatiles, low volatiles, etc. Furthermore, they are susceptible to various types of chemical transformation.

One of the main aspects of the quality is the so called "perceived quality" which exceeds the analytical chemistry aspect and concerns the pure sensorial field. Nowadays, in food companies it is recognized that consumers' approval strongly depends on sensorial properties.

To better define the sensorial characteristics and understand how they influence the global food quality, it is essential to consider all the information that could arise either from the analytical or from the "hedonistic" point of view. The analytical techniques dedicated to the analysis of fragrant molecules are mainly those based on chromatography, which allow us to dissect an aroma in its molecular components, determining and quantifying them. However, even if they allow the identification and quantification of the volatile substances, they do not allow to establish which of the volatile components are really fragrant and to quantify their contribution to the final aroma perceived by the human sense of smell. Besides, such techniques require a preliminary sample treatment to obtain a volatile extract in a sufficient quantity for a significant analysis and often the data interpretation does not result foreseeable [11]. On the other hand, sensory analysis techniques are encoded procedures which result from the demand to solve practical problems in food production. The studies on the sensorial perception have indicated that the human sensorial organs, after an appropriate training, could supply reliable results.

Characteristic sensory changes occur in the appearance, odor, taste and texture of eggs during ageing or modified storage conditions [12]. The sensory panel is progressively educated and trained to memorize the quality and the intensity of the feelings, that each panelist, in an isolated and autonomous way, perceives when tasting the food using the relative evaluation scheme. Despite this, the judgment is always subjective and not fully reproducible; in fact, the response could be influenced by physical factors within the same food commodity (e.g. samples temperature, interaction between the constituents), by each panelist's experience (e.g. culture, level of training, knowledge of the products), by psychological (e.g. prejudices, distractions) and physiological (e.g. sensation, sex, age) factors. Furthermore, in the case of continuous tasking, the panelists suffer from olfactory fatigue with the obvious consequence that only a low number of analyses can be carried out per day.

3. Artificial olfactory systems

AOS are instruments created and structured to mimic the human olfactory responses and represent an ideal tool for an objective analysis of odors, without the need of sample treatments. The purpose and the work principle of AOS can be differenced from those of instruments typically used in the analysis of volatile compounds (GC, GC–MS, etc.): in fact an AOS does not decompose the volatile fraction of food in its constitutive components, but supplies a global evaluation. The application field of this type of instrumentation is vast and heterogeneous, from environmental monitoring to medicine; the most foreseeable range of interest is in the food industry; in particular, concerning the analysis of packaging, quality and freshness of foods. It is necessary to develop a specific protocol for each application, with the individuation of the sensor setup for the best selectivity/sensitivity compromise, the generation of a congruent database and the selection of adequate pattern recognition techniques for the final data elaboration [13,14].

4. Eggs—enzymatic alterations and bacterial contaminations

Albumen and yolk contain enzymes, and if eggs are not stored at a sufficiently low temperature, the proteins can alter. The opti-

Table 1
Flavor descriptors for egg products olfactory evaluation

Olfactory evaluation	Results
Satisfying	Accepted
Strong egg odor	Accepted with reserve
Slightly pungent	New sampling for a second evaluation
Pungent	Rejected

mal temperature for a correct egg storage is normally about 6–8 °C. The enzymatic alterations of the albumen modify its viscosity, which allows us to recognize the freshness: in fact when the egg is not fresh the albumen tends to liquefy and the yolk easily breaks.

The microbial contamination of eggs could be due to: (1) endogenous factors, due to the contact with microorganisms present in the cloacae which go up in the oviduct and contaminate the egg during its own formation process, and (2) exogenous factors, that is, microorganisms in certain conditions could enter through the shell which is highly porous. This contamination frequently happens in pasteurized and hulled eggs.

Egg microbial contamination could be due to pathogen and/or alterative microorganisms responsible for the organoleptic changes (color and odor). Among the most well known pathogens there are: *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Salmonella Enteritidis*; the last cited one is the most frequent and feared. Amongst the most well known alterative microorganisms there are: *Pseudomonas* spp., *Aeromonas* spp., *Alcaligenes* spp., *Escherichia coli*, *Proteus* spp. and *Serratia* spp.

Egg products that have been restored through a thermal pasteurization treatment must respect in particular these two microbial parameters: total viable mesophilic bacteria, max 5 log CFU g^{-1}; Enterobacteriaceae count, max 2 log CFU g^{-1}.

The eventual microbial growth which occurs before the thermal treatment causes the formation of different microbial metabolites and a qualitative decay of the food product from an organoleptic and functional point of view. Clearly all these changes cannot be evidenced in a simple microbiological analysis once the thermal treatment has been carried out. Fortunately, chemical markers (lactic acid and succinic acid) could give information on the original microbial quality of shelled raw egg before the pasteurization.

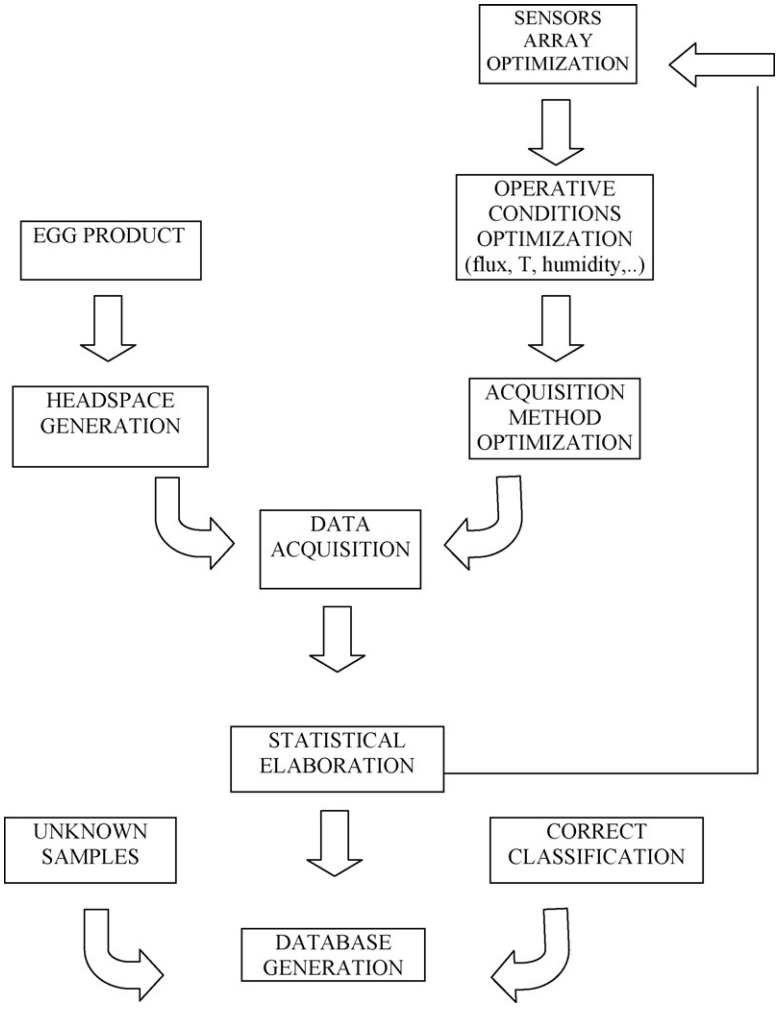

Fig. 1. Scheme which represents the methodology approach followed to optimize AOS performances for the discrimination of egg products at different freshness stages.

A logical conclusion is that a good functional quality always coincides with a good microbial quality, but the opposite is not valid [15].

5. Experimental methods and setup

5.1. Olfactory evaluation

To increase the development of volatile substances, samples (50 mL) were exposed in a glass container (500 mL volume) in hot water for a few minutes until the temperature reached around 40 °C. A trained panel of the Quality Control Lab (five members at least) of a food company individually sniffed the samples, then reached a consensus evaluation classifying them following the scheme reported in Table 1, in which the chosen flavor descriptors were inspired from United States Department of Agriculture guidelines [4,16]. No more than three samples were evaluated during a session.

5.2. Organic acids determination

Boehringer Mannheim enzymatic tests (R-Biopharm AG, Darmstadt, Germany) were carried out to determine the level of lactic and succinic acid in the egg products. Sample preparation and analysis were done using an UV–vis spectrophotometer and following Boehringer Mannheim kit instructions/recommendations and other correspondent works reported in literature [17].

5.3. Microbiological analysis

Microbiological analysis were performed following specifications reported in ISO and AOAC official documents [18–20]. Total viable mesophilic bacteria were enumerated using spread plates of plate count agar incubated at 30 °C for 72 h. Enterobacteriaceae counts were determined by using violet red bile glucose agar with a double layer, incubated at 37 °C for 24 h.

5.4. Artificial olfactory system setup

5.4.1. Calibration samples preparation

A 400 mL of egg product with the following organic acid content was taken from an initial amount of 2 L: lactic acid 299 mg kg^{-1} and succinic acid 9 mg kg^{-1}. This aliquot (aliquot A) was closed in a glass container and put in incubation at a constant temperature of 25 °C. The other 1600 mL (aliquot B) of this egg product sample was immediately frozen at −18 °C.

Table 2
Calibration samples preparation scheme

	Group 1	Group 2	Group 3	Group 4
Lactic acid concentration (mg kg^{-1})	299	500	1000	1500
Succinic acid concentration (mg kg^{-1})	9	14	25	35

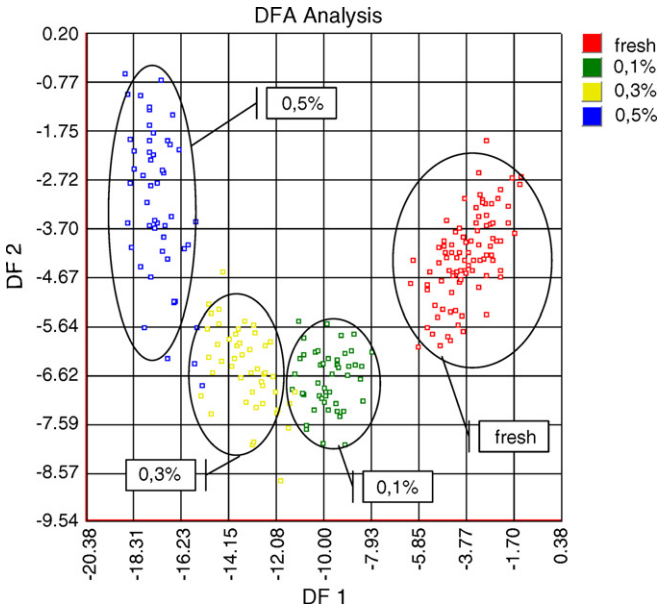

Fig. 2. AOS discrimination of four groups with a different adulteration level: fresh product, 0.1–0.3–0.5% addition of a degraded one. Data set (sample): 236 measurements. Variables: eight sensors, two features (acquisition points at 50 and 75 s after injection for each sensor trace), four classes to be discriminated. Ratio sample/variables = 3.69. Cross-validation: train 98.5%, test 99.09%, DFA recognition 98.3%.

After 3 days, organic acids were controlled again on the incubated aliquot (aliquot A): lactic acid 4531 mg kg^{-1} and succinic acid 776 mg kg^{-1}. After this, aliquot B was put at a refrigeration temperature (4–6 °C) for 24 h and then divided in four aliquots of 400 mL. Three of them were spiked and homogenized with different percentages of the aliquot A to obtain at the end the four sample groups reported in Table 2.

5.4.2. Samples preparation with different "adulteration levels" for AOS performance test

A 800 mL (aliquot 1) of a fresh egg product sample was immediately frozen and another 200 mL (aliquot 2) was placed in incubation at a temperature of 25 °C: this second aliquot was monitored daily from an olfactory point of view, until a satisfying state of degradation was achieved, which was obtained at the end of the third day. Then, the aliquot 1 was put at a refrigeration temperature (4–6 °C) for 24 h and divided in four aliquots of 200 mL. Three of them were spiked with different percentages (0.1–0.3–0.5%) in volume of degraded aliquot 2 to obtain four sample groups with different "adulteration levels".

Table 3
AOS performances—chemical parameters correlation scheme

AOS responses determinations plan		Organic acids determinations plan	
4 °C	25 °C	4 °C	25 °C
Initially	Initially	Initially	Initially
*	After 8 h	*	*
*	After 24 h	*	After 24 h
After 168 h	*	After 168 h	*

5.4.3. Artificial olfactory system setup

The diagram reported in Fig. 1 schematizes in a simplified way the complete method followed. In the analyses an AOS manufactured by SOATEC S.r.l, was used, being formed of an array of 12 Figaro thick layer MOS sensors. The digital fingerprint $\Delta R/R_0$, where ΔR is the difference $R - R_0$ (R is the instant resistance and R_0 is the resistance at the beginning of the acquisition), is recorded as a function of time for each sensor. Samples were analyzed with the following procedure:

- 10 mL aliquots of the calibration sample groups (see Section 5.4.1) were carefully maintained at a constant temperature of 40 °C;
- 2 mL of them were introduced in 25 mL headspace vials, repeating the sampling four times for each aliquot;
- the four vials were closed with Teflon corks and left in the thermostatic oven of the autosampler at 40 °C to generate the volatile components headspace, simulating the same temperature conditions used by the expert panel in the organoleptic tests; then they were consecutively injected in the sensors chamber under the following operative conditions: carrier gas, chromatographic air in cylinder (80% N_2, 20% O_2); gas flow, 300 mL min^{-1}; temperature, 40 °C; gas humidity, stabilized at 12 g m^{-3}; headspace generation time, 300 s; baseline acquisition time, 10 s; injection time, 20 s; data acquisition time, 300 s; time delay between samples, 600 s.

Each analysis cycle was completed by fluxing chromatographic air for at least 50 s to avoid a contamination phenomena related to the possible absorption of aromatic molecules on the injection valves and tubes.

Three data sets were collected, composed respectively by 236, 226 and 483 measurements. In each data set the $\Delta R/R_0$ traces belonging to the most discriminating sensors were chosen

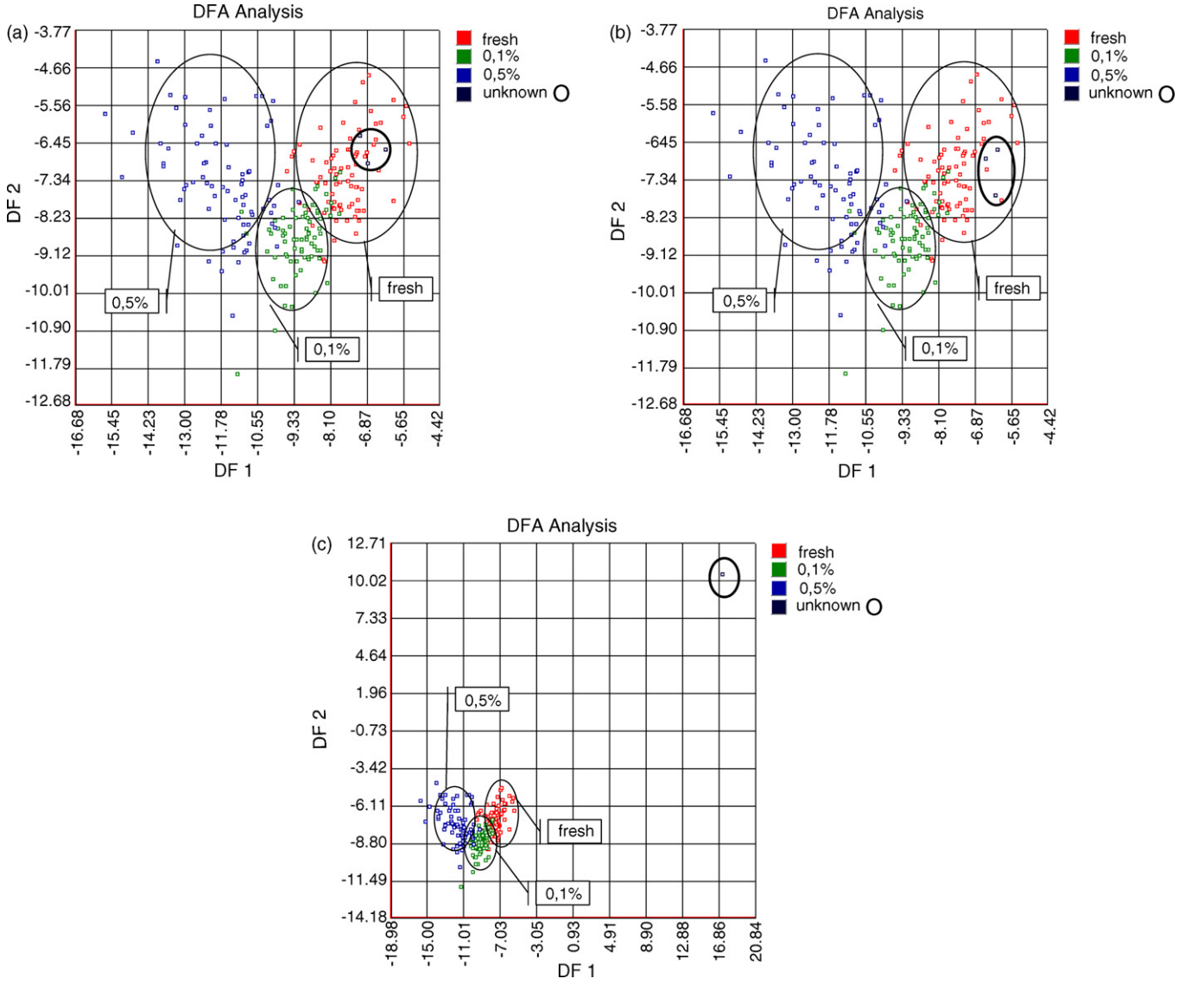

Fig. 3. Chemical parameters correlation study. Data set (sample): 226 measurements. Variables: eight sensors, three features (acquisition points at 50, 75 and 135 s after injection for each sensor trace), three classes to be discriminated. Ratio sample/variables = 3.13. AOS response (a) to a fresh egg product illustrated in its collocation in the database, (b) after 8 h storage at 25 °C, and (c) after 24 h storage at 25 °C.

(8, 8 and 9 traces out of the 12 recorded, respectively). Then, from each $\Delta R/R_0$ trace two or three features were extracted, being either the $\Delta R/R_0$ values at a given time or the first three coefficients of the Fourier transform fitting the trace (see captions to the figures). All the features were implemented in the discriminant function analysis (DFA) for the classification. The pattern recognition technique chosen was the canonical DFA, coupled with a "cross validation" process in which the minimum accepted recognition percentage was set at 90% [21].

Misclassification due to data over-fitting was avoided by keeping the ratio samples to variables above three in all data treatments, as suggested in literature [22]. The exact values are reported for each DFA treatment in the captions to the figures.

6. Results

6.1. AOS performances—chemical parameters correlation

Calibration samples were prepared following the sequence reported in Section 5.4.1 and were then analyzed. To compare the potentialities of AOS with those of a quality control panel specifically for acceptance of egg product lots, the various sample groups prepared as reported in Section 5.4.2 underwent an olfactory evaluation which gave the followings results: fresh sample, *approved*; 0.1% adulterated sample, *approved with reserve*; 0.3% adulterated sample, *newly sampled for a repeated evaluation*; 0.5% adulterated sample, *rejected*.

AOS analysis coupled with DFA pattern recognition technique permitted a high discrimination degree among the various groups, as reported in Fig. 2.

AOS responses and the panelist evaluations are in line. Therefore, in this phase the ability of AOS to discriminate fresh samples from those under degradation with an impartiality of judgment was demonstrated. Then, with a new database, contemporaneous AOS and organic acids content determinations were done to find a correlation among the response changes of the instrument and the increase in microbial metabolites directly associated to a freshness change.

The analysis scheme is reported in Table 3; the sample analyzed initially was characterized by the following organic acids and pH values: lactic acid, 261 mg kg^{-1}; succinic acid, 9 mg kg^{-1}; pH 7.4. If this fresh sample is introduced as an unknown sample in the AOS analysis protocol (three consecutive repetitions), the AOS correctly classified it in the "freshness cluster" of the database (Fig. 3a). After 8 h the same sample, kept at 25 °C, was analyzed again: AOS (Fig. 3b) placed the sample in the same "freshness cluster" as previously at zero time.

This means that the microbes present had not developed to such levels to cause appreciable organoleptic changes in the product. In the time period between 8 and 24 h the situation drastically changed. In fact, in the last analysis, the same sample kept at 25 °C after 24 h was projected straight out of all the recognition clusters of the database (Fig. 3c). Contemporaneously, pH and organic acids content were determined again, giving the following results: lactic acid, 784 mg kg^{-1}; succinic acid, 26 mg kg^{-1}; pH 6.8.

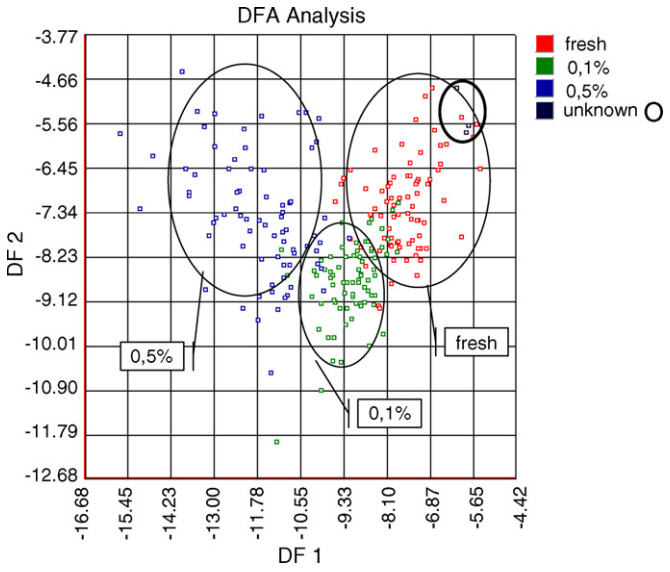

Fig. 4. Chemical parameters correlation study – AOS response to an egg product after 1 week – storage at 4 °C. Data set (sample): 226 measurements. Variables: eight sensors, three features (acquisition points at 50, 75 and 135 s after injection for each sensor trace), three classes to be discriminated. Ratio sample/variables = 3.14.

Therefore, a reduction of the pH value together with a clear increase of the organic acid content was verified (with the succinic acid level above the permitted legal limit). There was therefore a unanimous response by the AOS on one side and by the chemical indicators on the other. This monitoring was concluded with the last tests carried out on another aliquot preserved for 1 week at a storage temperature of 4 °C in a refrigerator. In this case the organic acid content remained practically unmodified and in effect AOS, as could be observed in Fig. 4, once again placed the sample in the so called "freshness cluster".

6.2. AOS performances—microbiological parameters correlation

To confirm the effectiveness of the chosen approach, a second database was created thanks to other 330 useful measurements, analyzed with a DFA statistical approach using the first three coefficients of Fourier transform that allowed a recognition score of 92.7%. This new database was then used again to monitor two fresh aliquots of egg products stored at different temperatures (25 and 4 °C): this time the idea was to individuate a possible link among the AOS response changes and the increase of the microbial population present in the sample (the analysis scheme is reported in Table 4). Initially, the sample stored at 25 °C presented legal microbial parameters with values well under the permitted limits: mesophyll aerobics colony count, 2.9 log CFU g^{-1}; Enterobacteriaceae colony count, *absent*.

AOS correctly introduced it in the "freshness cluster" (Fig. 5a). The response did not change even after 8 h of storage at 25 °C, indicating no significant proliferation of the microbial populations present in that period. Similarly to what already happened in the chemical parameters (organic acids) linked study, the situation changed when the 24 h of stor-

Table 4
AOS performances—microbiological parameters correlation scheme

AOS responses determinations plan		Microbial parameters determinations plan	
4 °C	25 °C	4 °C	25 °C
Initially	Initially	Initially	Initially
*	After 8 h	*	*
*	After 24 h	*	After 24 h
After 48 h	*	After 48 h	*
After 168 h	*	After 168 h	*

age at 25 °C was reached: in fact, in this case AOS placed the sample in a totally different region that is again out of all the recognition clusters of the database (Fig. 5b). The microbial indicators supported the AOS response: mesophyll

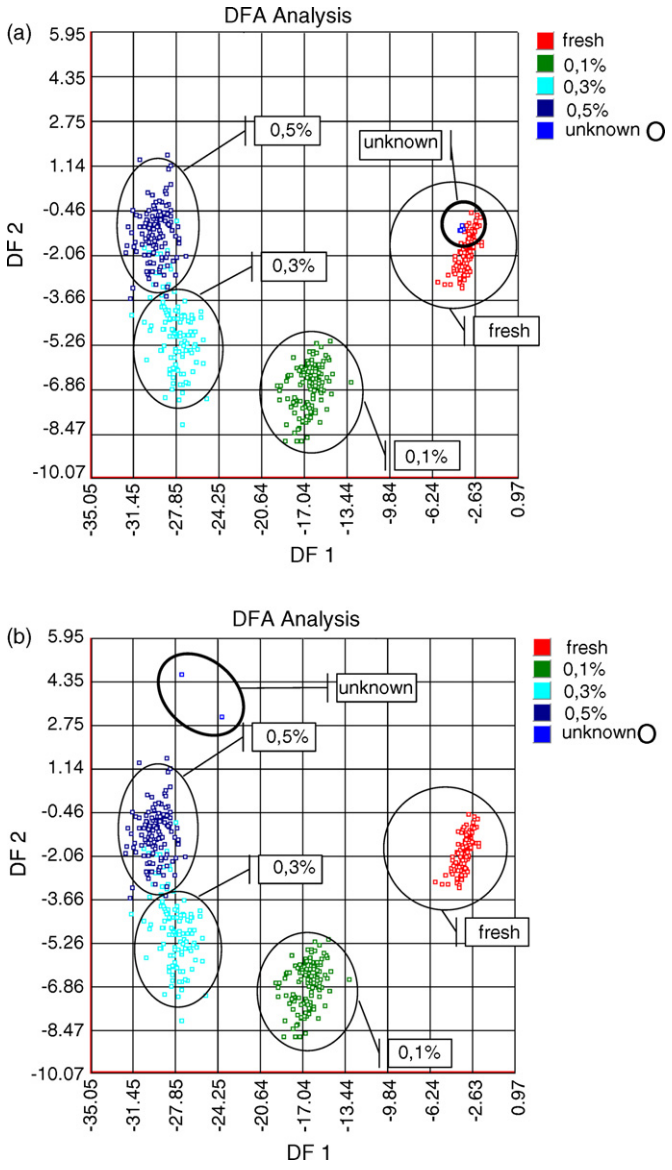

Fig. 5. Microbial parameters correlation study. Data set (sample): 483 measurements. Variables: 9 sensors, three features (the first three coefficients of the Fourier transform for each sensor trace), four classes to be discriminated. Ratio sample/variables = 4.47. AOS response (a) to a fresh egg product illustrated in its collocation in the database and (b) after 24 h storage at 25 °C.

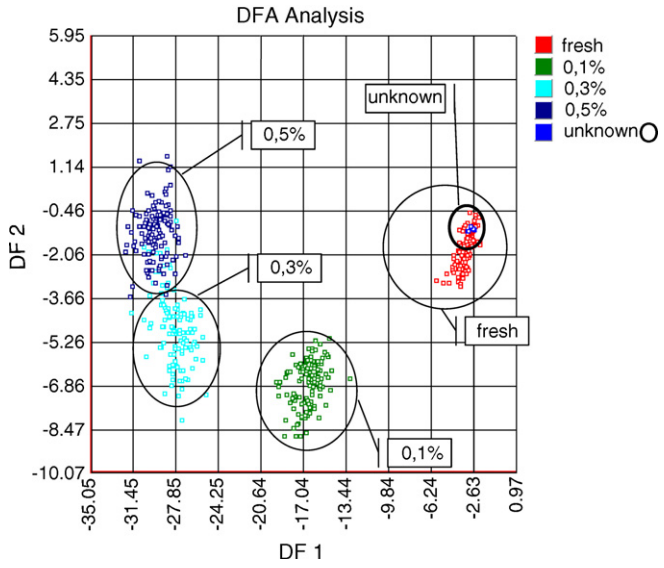

Fig. 6. Microbial parameters correlation study. AOS response to an egg product after 1 week storage at 4 °C. Data set (sample): 483 measurements. Features (variables): 9 sensors, three coefficients of the Fourier transform, four classes to be discriminated. Ratio sample/variables = 4.47.

aerobics colony count, 8.0 log CFU g^{-1}; Enterobacteriaceae colony count, 2.6 log CFU g^{-1}.

Also after 1 week of storage at 4 °C microbial and organoleptic characteristics of the sample did not change and this fact was confirmed again either from the AOS response (Fig. 6) or from the microbial analysis: mesophyll aerobics colony count, 3.7 log CFU g^{-1}; Enterobacteriaceae colony count, 0.1 log CFU g^{-1}.

7. Conclusion

An artificial olfactory system (AOS) equipped with MOS sensors was applied to tackle the problems regarding the different freshness state of egg products. Database generation was obtained by adulterating a fresh product with progressive aliquots of a degraded one. AOS demonstrated a high discrimination ability related to the chemical (change of the organic acid contents) and microbiological (growth of the present microbial populations) evolution of the samples during their degradation process, even if they are still within the corresponding legal limits. Additionally, AOS quickly supplies practical responses (in around 30–40 min with three repetitions for a single sample against the hours necessary to complete traditional chemical and microbiological analysis) without requiring highly trained staff. AOS also gives the possibility of continuous sample analysis allowing to check all the raw materials that will then be used for industrial production, without reducing the monitoring range; furthermore, concerning the economic impact, the use of AOS is cheaper compared to the enzymatic kit analysis.

Concluding, this feasibility study has confirmed that AOS demonstrates the ability to supply a concrete support to quality control laboratories of food companies, for the acceptance procedures of egg products, thanks to the absence of sample

pretreatments and the possibility to express the results in a simple, objective and easily interpretable way.

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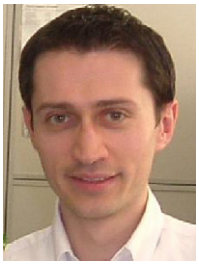
Biographies



Michele Suman obtained his analytical chemistry degree, Summa Cum Laude, at University of Ferrara in 1997; Master in science, technology and management from University of Ferrara in 1998. In 2005, he received the PhD in science and technology of innovative materials from University of Parma in the Organic and Industrial Chemistry Department. His current research activity is conducted in Barilla Spa Company, firstly studying food contact plastic materials and artificial olfactive systems for packaging and food applications; since the middle of 2003, he is the analytical chemistry responsible in Central Research Labs of the same company, working on research projects in the field of food chemistry, sensing and mass spectrometry applications for food products.

Gabriele Riani received the degree in food science and technology in 2005 from the University of Parma. His actual research activity deals with the development of dedicated applications for food analysis and monitoring based on artificial olfactory systems technology.

Enrico Dalcanale received the degree in industrial chemistry (Cum Laude) from the University of Bologna, Bologna, Italy, in 1981. After working as a research scientist at the Donegani Research Institute of Montedison, Novara, Italy, from 1982 to 1990, he joined the Faculty of the Department of Organic and Industrial Chemistry of the University of Parma, Parma, Italy, where he is currently an associate professor. His research interests include supramolecular sensors and self-assembly of nanostructures (coordination cages, mesogenic porphyrins).