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## Detection of Spoilage Markers in Food Products using a Mass-Spectrometry Based Chemical Sensor

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### **KEYWORDS**

Headspace sampling, spoilage markers, chemical sensor, multivariate analysis

### **ABSTRACT**

It is known that specific compounds are produced when foods spoil. For example, commonly recognized spoilage markers include dimethyl sulfide for chicken and eggs, diacetyl for orange juice, and trimethylamine for fish and milk. As the entire headspace of the spoiled food changes it is possible to detect the spoilage degree by measuring the amount present of these markers. A fast and accurate technique using a mass spectrometry based chemical sensor is examined for the above food products with different spoilage markers' concentration levels.

Multivariate statistics were used to create models that detect the spoilage markers. Exploratory analysis such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) indicated the viability of the data set for classification models. Soft-independent-modeling-class-analogy (SIMCA) and K Nearest Neighbors (KNN) were used to create two classification models. Regression models

were developed using partial least squares (PLS).

Both SIMCA and KNN provided a quick and accurate identification of the above foods with and without spoilage markers. In both cases, testing sets were correctly classified with over 95% accurate prediction rates. PLS models detected spoilage markers' concentrations at the low to medium ppm levels. Overall, the positive and fast identification of spoilage indicators demonstrates the usefulness of the MS chemical sensor detecting samples with close chemical composition.

## INTRODUCTION

Detection of food spoilage needs to be fast and accurate. Recent studies using electronic noses have shown this type of technology to be effective in detecting spoilage markers in several food products [1, 2]. Analysis times are normally on the order of a few minutes making this type of technology ideal for the detection of food spoilage.

Mass spectrometry based e-noses use robust mass spectrometry technology that is unaffected by moisture in the sample, ambient humidity, or ambient temperature fluctuations. It is also immune to sensor poisoning. Ions associated with dominant sample components, such as acetic acid in vinegar, can be ignored and ions that model only the critical factors that differentiate samples are used. Also, ions in a suspect sample that are not present in a good sample can be monitored.

In this study, chicken and eggs were spiked with dimethyl sulfide [3] and the entire headspace was analyzed using a mass spectrometry based sensor. Similar studies were carried out with diacetyl in orange juice [4] and trimethylamine in milk and fish [5].

## EXPERIMENTAL

**Materials.** Chicken, eggs, orange juice, milk and flounder fish were purchased at a local store. Dimethyl sulfide 1000 ppm in water was obtained from Restek (Bellefonte, PA). 97 % diacetyl and 40% trimethylamine were obtained from Sigma-Aldrich (Allentown, PA). Solutions of diacetyl in water at 10,000 and 2,000 ppm were used to spike orange juice at levels specified in Table 1. Trimethylamine solutions in water at 500 and 5,000 ppm were used to spike milk and fish (Table 1). The 1,000 ppm solution of dimethyl sulfide was used to spike chicken and eggs at the levels described in Table 1.

**Table 1.** Concentration of spoilage markers in spiked food products.

Product	Spoilage Marker [ppm]		
	Dimethyl sulfide	Trimethylamine	Diacetyl
Egg	10, 50, 100		
Chicken	10, 50, 100		
Milk		10, 50, 100	
Fish		10, 50, 100 100, 500, 1000	
Orange juice			50, 500, 2000 10, 50, 100

**Instrumentation.** The chemsensor used was a Gerstel ChemSensor 4440 (Figure 1) that includes a headspace sampling unit (7694, Agilent Technologies) with a mass selective detector (5973, Agilent Technologies). This instrument integrates chemometric software from Infometrix (Pirouette 3.02 and Instep 2.0). The instrument was used in the scan mode from 35 to 250 amu for the chicken and egg experiments and from 41 to 180 amu for the milk, fish and orange juice. All the MSD runs were 1.20 min.



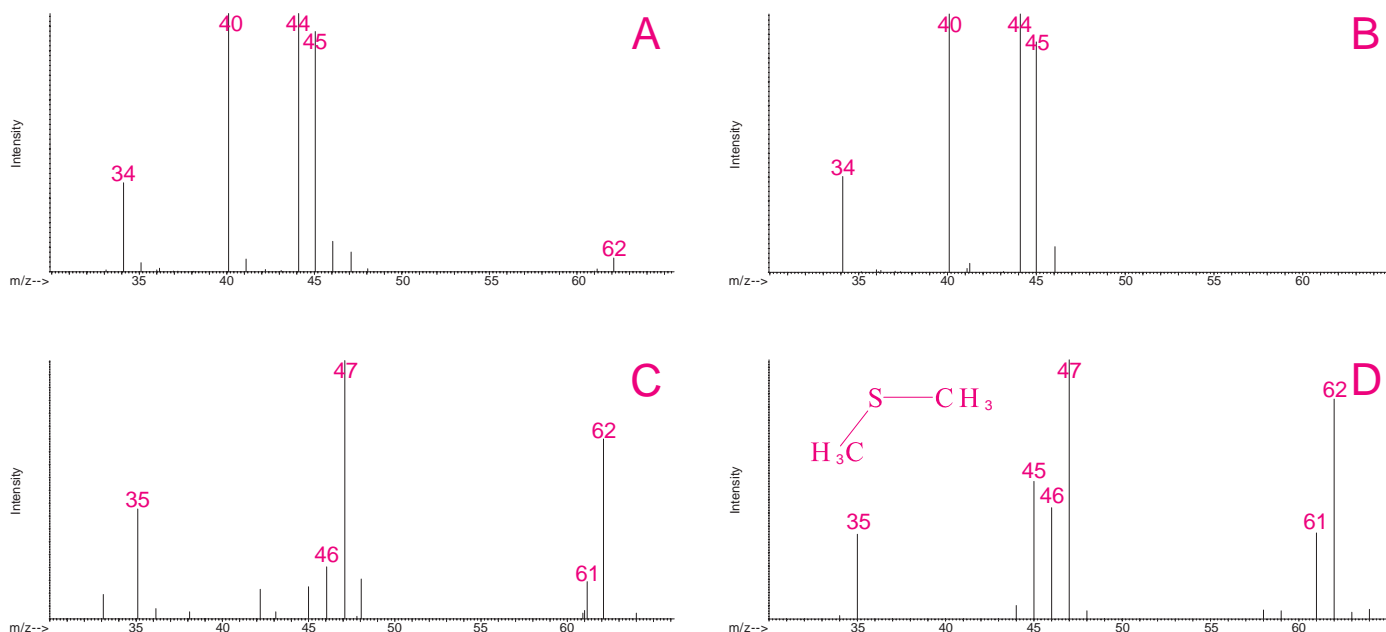
**Figure 1.** Gerstel ChemSensor 4440.

**Sample preparation & headspace sampling.** 5 ml aliquots of each liquid (orange juice, milk, and whisked eggs) were placed into 10 mL vials, which were crimped and equilibrated for 20 minutes at 80 °C before headspace sampling. Since the Gerstel 4440 ChemSensor does not use a column for a separation prior to the mass selective detector (MSD), the entire headspace of each sample is introduced into the MSD. 1 gram of solid material, previously pureed in a food processor, was used in the analysis of fish and chicken samples. The presence of the markers at 10 ppm was detected in all samples except for fish.

## RESULTS AND DISCUSSION

The first step of any chemometric analysis is to examine the raw data. In this case, mass spectra of each of the food products were examined using the Data Analysis program of ChemStation software (Agilent Technologies). The presence and identification of the spoilage markers at the lowest concentration was corroborated by subtracting the mass spectrum of a spiked sample

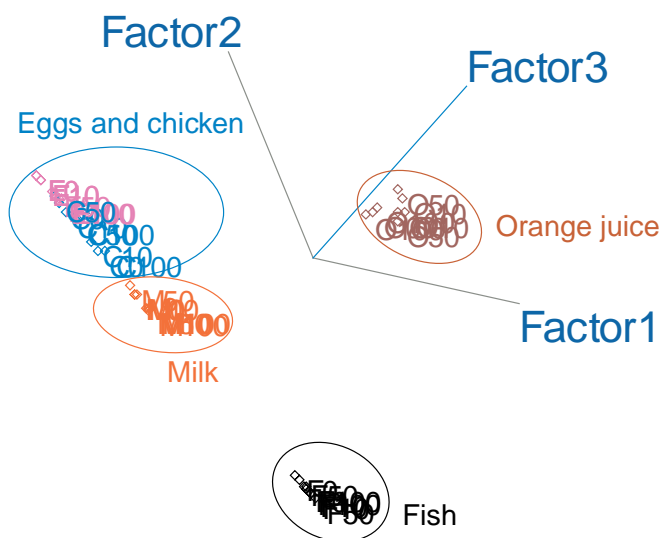
minus the spectrum of the non-spiked sample. Figure 2 illustrates this approach for the egg samples in which 10 ppm of dimethyl sulfide can be detected. Similar analyses were performed for the other products at the 10 ppm level. A second set of analysis (Table 1) was carried out for fish with higher marker concentrations since the identification of trimethylamine was not possible at 10 ppm.



**Figure 2.** Mass spectra of egg headspace. A) Egg with 10 ppm of dimethyl sulfide; B) pure egg; C) subtraction of pure egg from egg with dimethyl sulfide; D) dimethyl sulfide standard.

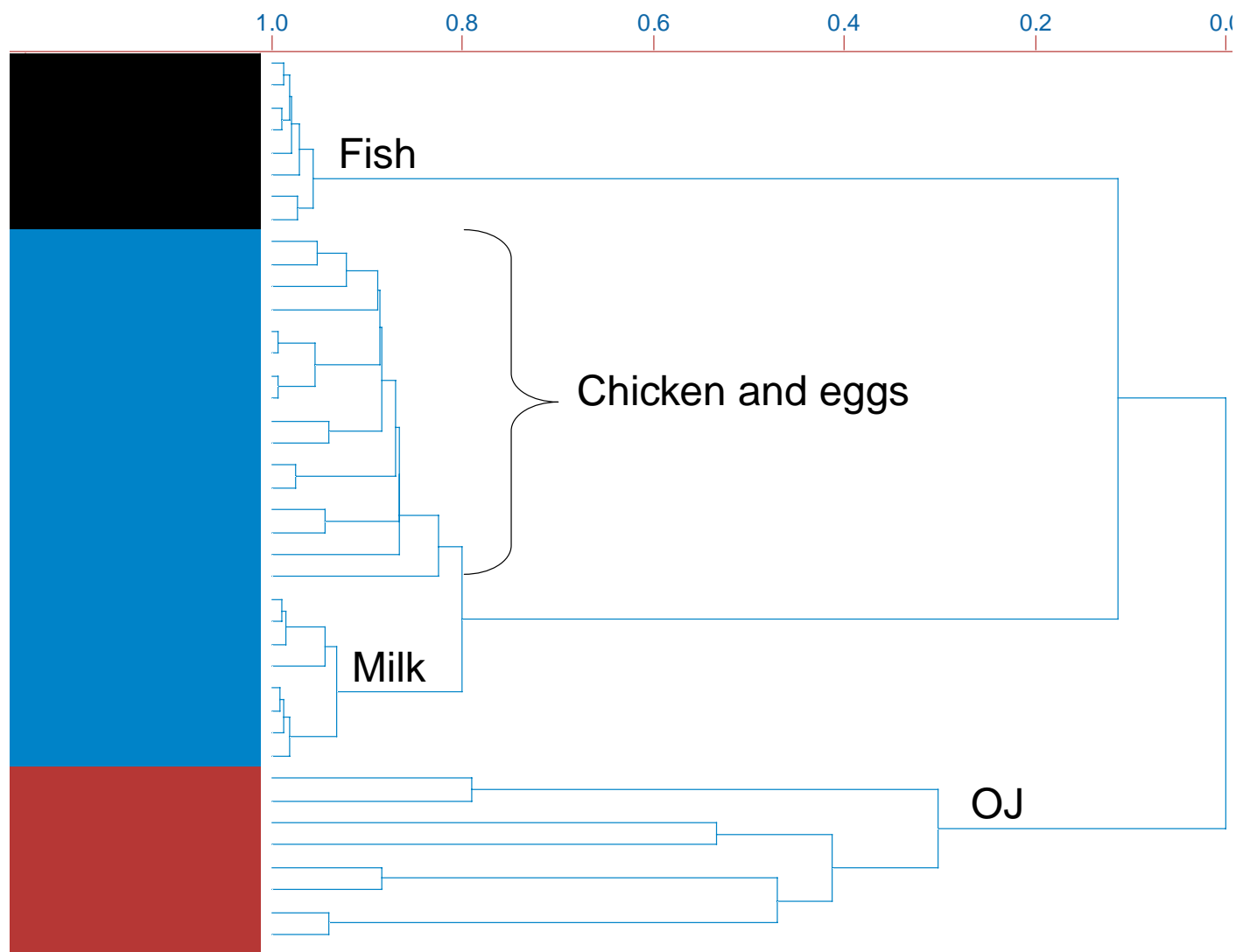
A principal component analysis (PCA) that classifies the samples according to food product indicates four distinctive clusters (Figure 3). The chicken and egg samples cluster together indicating very similar headspace volatiles. The total variance captured with the

first three principal components is over 92% (PC1, 51.5%; PC2, 36.3; PC3 9.4%) and a good indication that the systematic variability of the data set was captured within the first three PCs.



**Figure 3.** Projections of the food products' mass spectra into the space of the first three principal components. Variance captured was PC1, 51.5%; PC2, 36.3; PC3 9.4% for a total of 92.2%.

Figure 4 is a dendrogram obtained with hierarchical cluster analysis (HCA) using the Euclidean distance and single linkage algorithm. The orange juice and fish clusters appear to be different than the milk, chicken and eggs samples. These results are in agreement with the PCA plot (Figure 3) in which the chicken and egg samples clustered together with the milk cluster close-by.



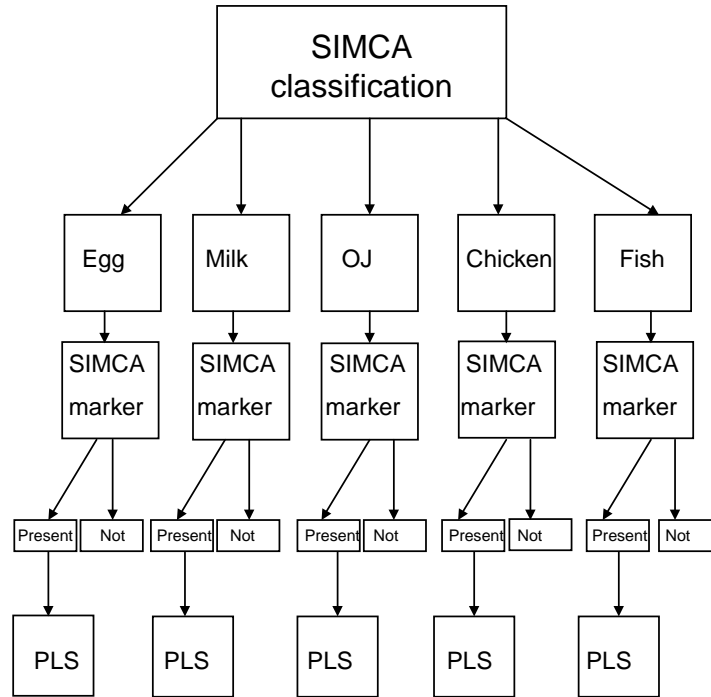
**Figure 4.** Cluster analysis using the Euclidean distance and single linkage method.

As indicated by the results of the exploratory analysis (PCA, HCA), enough differences present in the samples suggest the possibility of a good classification model, except for the chicken and eggs.

A cascading model (Figure 5) was used to integrate SIMCA and PLS models for food spoilage prediction. The integration of models is easily accomplished

using InStep 2.0 software (Infometrix) available in the Gerstel ChemSensor. Figure 6 is a screen capture of this software in which the models are integrated in a user-friendly folder based mode. KNN models for all samples (not shown) resulted in similar classification for all products.

### Cascading multivariate models



**Figure 5.** Diagram of cascading model integrating classification (SIMCA) and regression (PLS) algorithms.

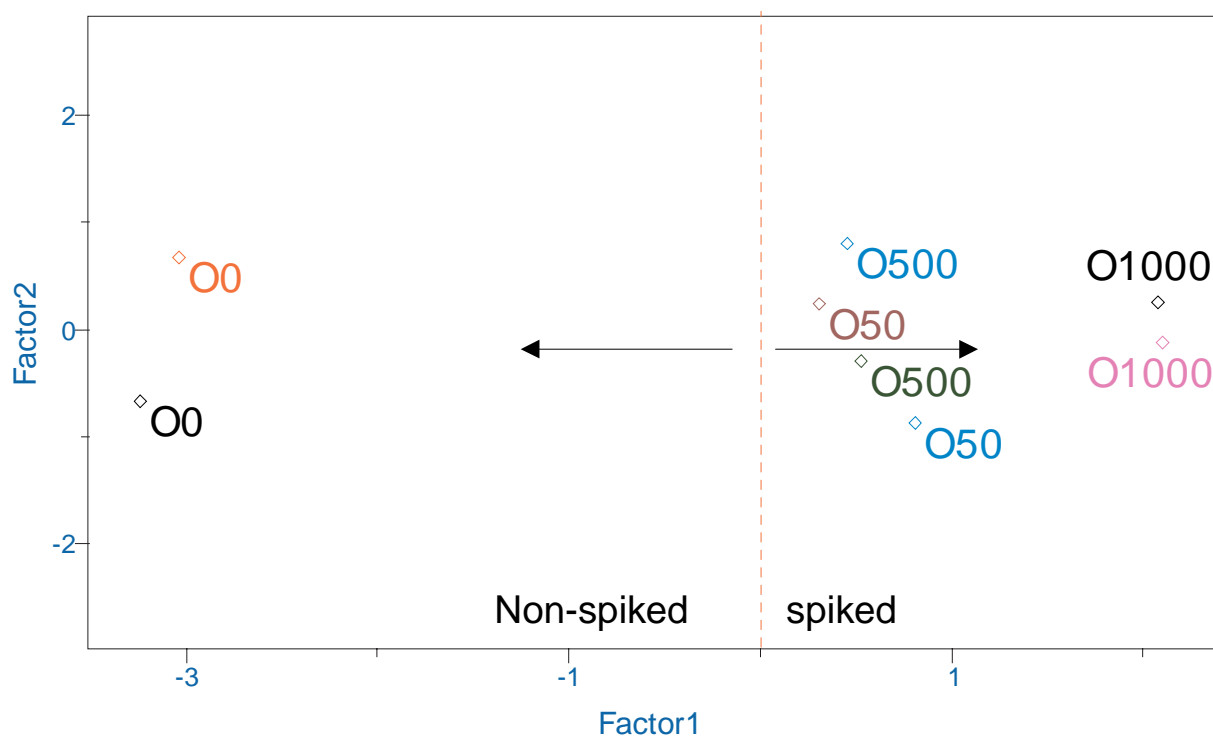
The screenshot shows the 'Logic Flow' tab in InStep 2.0. A file explorer on the left shows the 'Models' folder. A list of model files is shown in the center: chikpls.MOD, eggpls.MOD, fishpls.MOD, milkpls.MOD, and ojpls.MOD. Below is a table with 15 rows, each representing a model configuration.

Row #	Model	Type	YVar	Compare?	Compare Item	Compare Operator	Compare Value	If True, go to row #	If False, go to row #
1	SIMCA1.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	1	2	4
2	SIMCAOJ.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	1	3	0
3	ojpls.MOD	PLS	Amount	<input type="checkbox"/>					
4	SIMCA1.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	2	5	7
5	SIMCAchik.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	1	6	0
6	chikpls.MOD	PLS	Amount	<input type="checkbox"/>					
7	SIMCA1.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	3	8	10
8	SIMCAegg.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	1	9	0
9	eggpls.MOD	PLS	Amount	<input type="checkbox"/>					
10	SIMCA1.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	4	11	13
11	SIMCAchik.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	1	12	0
12	chikpls.MOD	PLS	Amount	<input type="checkbox"/>					
13	SIMCA1.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	5	14	0
14	SIMCAfish.MOD	SIMCA		<input checked="" type="checkbox"/>	Predicted value	=	1	15	0
15	fishpls.MOD	PLS	Amount	<input type="checkbox"/>					

**Figure 6.** Screen capture of InStep 2.0 software (Infometrix) that integrates models created using Pirouette 3.02.

The model created was used to predict the sample type first, then the presence of a marker and lastly the amount of marker present. The models predicting the presence of markers were examined using PCA scores. As seen in Figure 7, PCA scores for the orange juice

samples indicate that the first PC (horizontal axis in Figure 7) describes the difference between samples containing marker (positive scores on the first PC) and no marker (negative scores).



**Figure 7.** Projections of the orange juice mass spectra into the space of the first two principal components.

The model (Figure 5) was validated using a testing set. Results of these unknowns are shown in Table 2. The 1st classification into 5 product categories was accurate, even air blank samples were not classified, as expected, since they were not part of the model. The

second classification of food products into spoiled or not was 100% accurate. Regression models predicted the amount of marker more accurately for high marker concentrations in the 100 ppm range for all food products with the exception of fish.

**Table 2.** Prediction of testing set using cascaded model.

Sample	SIMCA 1st	SIMCA 2nd	PLS
air blank	unidentified	na	na
milk-50 ppm	milk	TMA-positive	46.944
milk no-marker	milk	TMA-negative	na
air blank	unidentified	na	na
OJ-10 ppm	OJ	Diacetyl-positive	8.075
OJ-no-marker	OJ	Diacetyl-negative	na
OJ-100 ppm	OJ	Diacetyl-positive	104.585
egg- no-marker	Egg	DMS-negative	na
egg-100 ppm	Egg	DMS-positive	102.599
egg-10 ppm	Egg	DMS-positive	7.601
chicken-100 ppm	Chicken	DMS-positive	102.068
chicken- no marker	Chicken	DMS-negative	na
Fish-1000 ppm	Fish	TMA-positive	1485.307
Fish-no marker	Fish	TMA-negative	na
Fish-500 ppm	Fish	TMA-positive	690.812
air blank	unidentified	na	na

## CONCLUSIONS

These preliminary results indicate the possibility of using a mass-spectra based chemical sensor to predict the level of spoilage in foods. More research with more markers and more levels is necessary. Also, the possibility of using the Chemical sensor in the single ion-monitoring mode (SIM) could improve the sensitivity below 10 ppm. Since off-flavors are normally present at low concentrations, other sample introduction techniques such as stir bar sorptive extraction (SBSE) and solid-phase microextraction (SPME) need to be investigated in future studies.

## REFERENCES

- [1] K. Goodner, R. Russell, J. Agric. Food Chem. 2001, 49, 250-253.
- [2] R.T. Marsili, J. Agric. Food Chem. 1999, 47, 648-654.
- [3] L.R. Freeman, G.J. Silverman, P. Angelini, C. Merritt, W.B. Esselen, Appl. Environ. Microbiol. 1976, 32, 222-231.
- [4] W.S. Hatcher. Special Report, New York State Agricultural Experiment Station. 1979, 31(8) 1-4.
- [5] D. Natale, G. Olafsdottir, S. Einarsson, E. Martinelli, R. Paolesse, A. D'Amico. Sens. Actuators, B. 2001, 77, 572-578.



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