

Electronic nose: Smelling the microbiological quality of cheese

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Abstract

The volatile compounds produced by microorganisms involved in cheese production often vary in a very characteristic way for a species or a group of species. Measurements were made on the headspaces of Danish blue cheese, Camembert cheese and cheese models with electronic noses. An explorative data analysis on the signals derived from the chemical sensors was performed in order to define the best profile parameters for estimating the microbiological quality.

Keywords

E-nose, Danish blue cheese, Camembert cheese, cheese models and multivariate analyses

INTRODUCTION

The quality control of intermediate and final food products especially with regard to undesirable components is of great importance for the food industry. It is important that the consumer has confidence that there are no microbial contaminants, toxins, off flavors and other odors.

The development of innovative rapid detection systems with potential for early detection is therefore required by the food industry.

The aim of this study was to investigate the possibility of implementing electronic nose technology in order to evaluate the microbiological quality of mould cheeses. In the experiments performed, electronic nose technology has been applied directly on representative Danish blue cheese samples, which were contaminated by *G. candidum*. Additionally Camembert cheeses made with different strains of *P.camemberti* were evaluated as well as cheese models simulating fresh cheese curd for Danish blue cheese inoculated with pure mould and yeast cultures. This was done in order to get an objective characteristic of the aroma profile concerning different contaminants and different strains used in cheese manufacturing.

MATERIALS AND METHODS

Danish blue as well as Camembert cheeses were collected from major manufacturers in Denmark. Danish blue cheeses were measured 5 weeks after brining. Contamination at that ripening stage was identified in great numbers (10^7).

Camembert cheeses were manufactured with 20 different commercial isolates of the mould *P.camemberti* and were analyzed on 6 consecutively weeks. Model cheeses were made with 4 of these strains and were similar in terms of physiological, sensorial and e-nose profiles. Cheeses produced with other strains, were evaluated for their maturity based on their e-nose aroma profile models.

Cheese models (cheese agar medium in 9 cm Petri dishes) were manufactured from fresh Danish blue cheese taken before brining (inactivation of *P.roqueforti* was achieved by melting the cheese mass). These models were inoculated with 0,1 ml spore suspension (10^4 spores/ml) of cultures from the house collection as well as others (see table 1). All models were placed at 10 °C before profiling.

“Table 1”. Microorganisms used for the cheese model experiments

IBT ¹ number	Microorganism
12845	<i>P. roqueforti</i>
20911	<i>P. caseifulvum</i>
20 different strains	<i>P. camemberti</i>
10253	<i>P. commune</i>
9285	<i>G. candidum</i>
From KVL ² collection	<i>D. hansenii</i>
From KVL collection	<i>C. colicullosa</i>

An e-nose (model BH-114: Bloodhound Sensors Ltd. Leeds, UK), which employed 14 conducting polymer (polyaniline) sensors, was used in the experiments with Danish blue cheeses and cheese models. Samples were taken from the headspace of cheese slices. Slices were put in glass Petri dishes in self-sealing plastic bags (20X15cm). Bags were filled with dry air (humidity<0.5%), secured and equilibrated at 20 °C for 1 h. All experiments were performed in triplicate.

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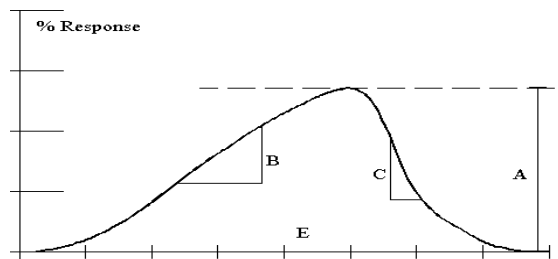
The model aFOX-3000: Alpha M.O.S. (Multi Organoleptic Systems), France, employs 12 sensors and includes 6 Metal Oxide Semiconductor sensors (MOS), 4 Conductive Polymer (CP) sensors and 2 Quartz Crystal Microbalance (QCM) sensors. This instrument was used in the experiments with the Camembert cheeses. All samples were 1cm cylindrical cuts from sample. Cuts were sealed in 10 ml headspace vial (46x22.5 mm) and were put in an HS-100 autosampler (CTC Analytics AG, Switzerland). The samples were incubated at 50 °C for 10 min. A 500 µl from the headspace was injected into the sensor chambers. Dry filtered airflow through the sensors was adjusted to 150 ml min⁻¹.

Principal Component Analysis (PCA), Discriminant Partial Least Square (PLS-DISCR), Partial Least Squares Regression (PLS-R) and Soft Independent Modeling of Class Analogy (SIMCA) methods were performed using the software package Unscrambler (CAMO) version 7.61 SR1.

RESULTS AND DISCUSSION

Danish blue cheeses

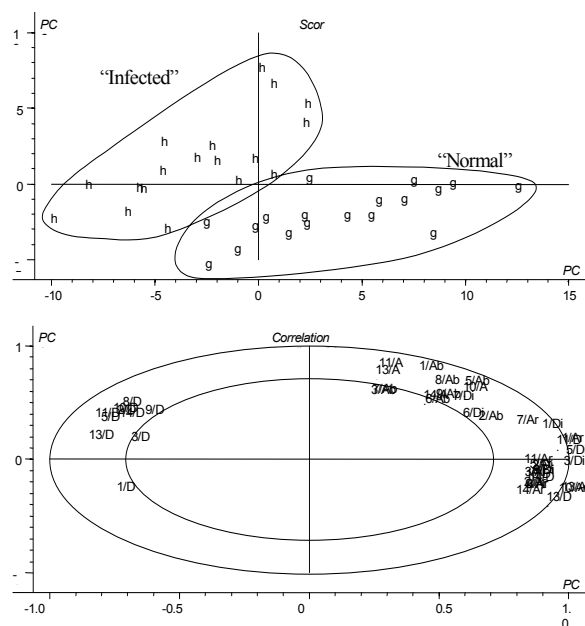
Four characteristic parameters of the response curves from the BH-114 were exported from the control software (see figure 1). These characteristics were absorption (B: maximum rate of change of resistance), desorption (C: maximum negative rate of change of resistance), divergence (A: maximum step response) and area (E: area under the actual sensor curve).



“Figure 1”. Response curves from e-nose and parameters used for multivariate analysis

PC analysis carried out with all variables (see figure 2) found that 2 PC's accounted for 80 % of the experimental variance. PC1 (61% of the total explained variance) was dominated by the difference between the good and contaminated samples. PC 2 (19%) is dominated by the variation between batches of cheeses and this is due to the day-to-day variations of the cheeses, which can be very high.

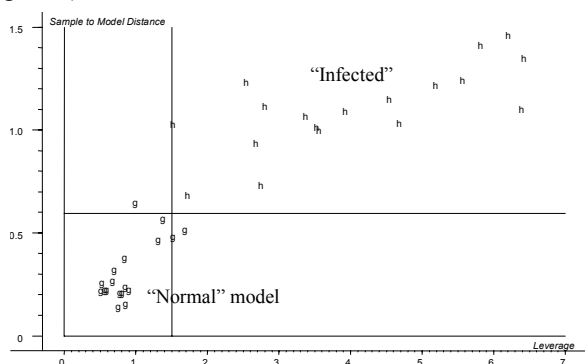
Selection of sensor features with help of PCA helped improve the discrimination between cheeses. In a new PCA model only 10 sensors were used.



“Figure 2”. PCA score and loadings plot from good and contaminated cheeses. All variables used

The new PCA model could describe 90% of the total variation within 2 PC's. The first PC accounted for 84% of the total variance and describes the differences between good and contaminated cheeses while PC2 accounts for 6% of the total variance and describes again the differences between production days.

Two classes of samples were chosen and used for SIMCA analysis. These models (“normal” and “infected”) were able to classify unknown samples very successfully (see figure 3).



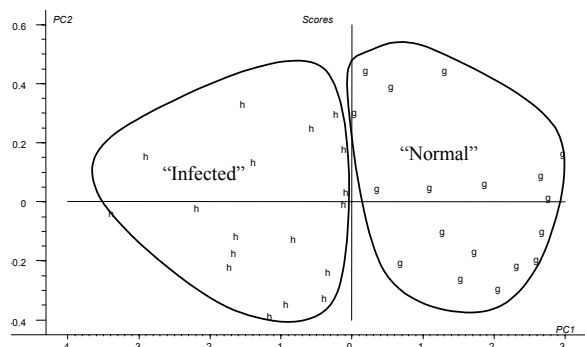
“Figure 3”. SIMCA classification of unknown samples at 25% significance level (5-week-old cheeses).

The discrimination power between these models indicated the individual sensor variables that discriminate best between the two cheese classes.

A final PC analysis based on these three variables gave the best discrimination model where PC 1 accounted for the differences between “normal” and “infected” cheeses with a 97% of the total variance (see figure 4). The sec-

ond PC (2%) was not accounted for any differences between the “normal” and “infected” samples.

PLS-Regression analysis based on these 3 variables (Y) and 8 sensorial characteristics (X) that describe the “normal” and the “infected” cheeses in terms of taste and smell yielded high correlation coefficients.



“Figure 4”. PCA score plot from “normal” and “infected” cheeses. Data from 2 sensors

Microbiological findings were positively correlated to the e-nose variables, describing very well the growth of the “normal” fungi and the existence of other fungi in the cheeses. “Normal” cheeses had higher pH than the “infected”. This difference can be attributed to the differences in mould growth [1].

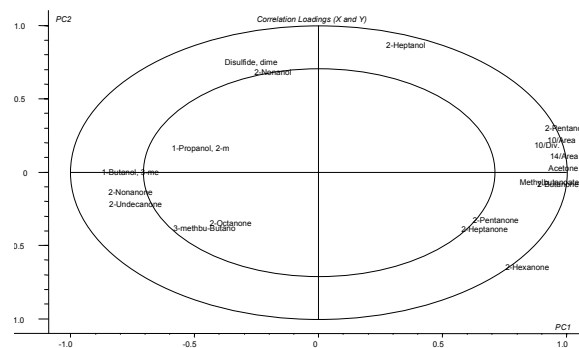
The main differences between the two cheese classes in terms of aroma detected in their headspace can be seen in table 2.

Table 2. Total area % of 14 compounds from Danish blue cheese headspace

Compound	“Normal”	“Infected”
Acetone	7.86	15.34
2-Butanone	0.00	0.55
1-Propanol, 2-methyl-	3.37	2.39
2-Pentanone	10.23	20.49
Methyl Butanoate	0.00	0.13
2-Pentanol	0.00	0.63
1-Butanol, 3-methyl-	24.41	12.90
2-Hexanone	0.87	1.01
2-Heptanone	17.77	19.45
2-Heptanol	0.83	1.20
2-Octanone	0.74	0.62
3methylbutyl Butanoate	0.41	0.10
2-Nonanone	21.51	15.24
2-Undecanone	1.53	0.62

PLS-R analysis based on the 3 e-nose variables (Y) and the aroma compounds (X) showed highly positive correlation coefficients for many of the compounds measured. Reverse analysis (e-nose X variables and aroma compounds Y variables) gave valuable information concerning the relationship of the sensors with the

ing the relationship of the sensors with the compounds in the headspace (see figure 5). In this way the changes in sensor signals associate to aroma compound changes in the headspace.

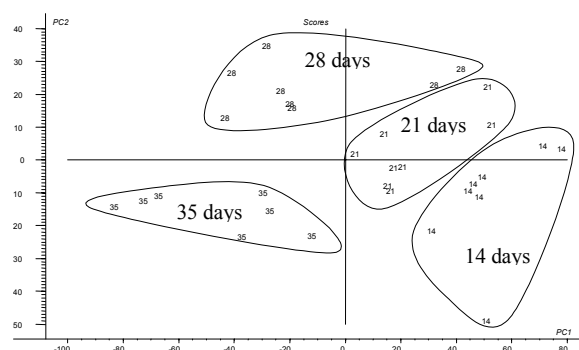


“Figure 5”. Loading plot from PLS-R analysis. (X = log₁₀ aroma compounds, Y = e-nose variables).

Camembert cheeses

The response curves of the 12 different sensors from this experiment were used for analysis. Data used for the models was from 14 to 35 days old cheeses.

PC analysis carried out with all sensors (see figure 6) found that 2 PC’s accounted for 78% of the experimental variance.



“Figure 6”. PCA score plot of “model” cheeses. Whole response signals used.

PC1 (67% of the total explained variance) was dominated by the time changes occurring to smell with maturation. PC2 (11%) is dominated by the variation of the different cheeses between 28 and 35 days. These 2 PC’s accounted for 83 % of the experimental variance. More PC’s were not accounted for any characteristics of the cheeses changes.

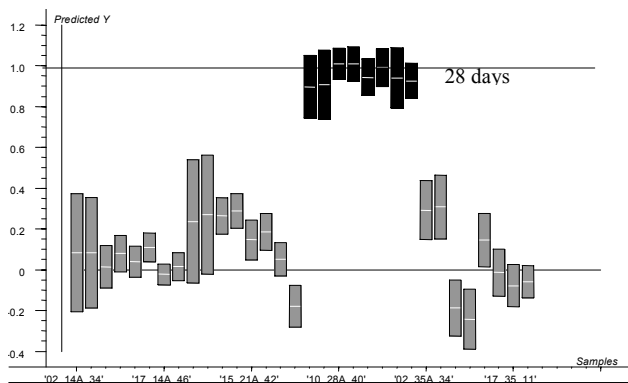
With the help of PLS-DISCR analysis a model based on the time changes (discrete variables) was made. Selection of variables significant on a 10% level was made. Based on these variables a new simpler PCA model was introduced increasing the total explained variance. SIMCA analysis was performed based on these last models.

“Similar” cheeses made with different isolates to the model cheeses were identified.

The area of interest in this particular type of cheeses is between the age of 21 and 28 days. After 28 days the cheeses were matured and ready for consumption having all the characteristics of a Camembert cheese [2].

Discrimination power model of the model of 21 days on the model of 28 days for these cheeses helped to choose the important variables, which were exported in a new reduced matrix for further analysis. This model was improved slightly and described the maturity changes using less than 20% of the original variables. Discrimination between 21 and 28 days cheeses was improved in this model.

Based on these results a prediction model of cheese maturity (Y) was created based on e-nose responses (X). Results from this analysis showed that the model was very good in predicting the age of other strains, already judged as similar to the ones used for the model (see figure 8). The prediction performance was better when the cheeses were reaching their ready-to-eat age (28 days).



“Figure 7”. Prediction of 28 days old unknown Camembert cheeses by the e-nose responses.

The cheese models

Models (0 and 4% NaCl) were made from fresh Danish blue cheese. Average values for a_w and pH for this type media are:

- Water activity: 0.990
- pH: 4.7

Addition of salt (4% NaCl) made the gel unstable and very soft. Neither the increase of agar from 1,5% to 2% in the media nor the addition of glycerol resulted to a better gel.

Another serious problem of this type media was that they have high percentage of fat (~13% of total weight) and big surface therefore exposed to oxidation. For this reason they are not stable over time. Differences measured by e-nose though, were only detected three weeks from fabrication day.

Growth of microorganisms

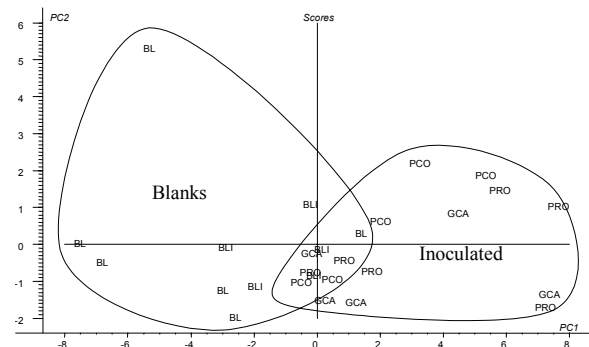
All samples were controlled for visible growth for a period of 8 days. Yeasts were visible prior to fungi (5-6 days on the 0% model at 10 °C).

Fungi showed visible growth only after 7-8 days (on the 0% model at 10 °C). Minor contaminations of some plates were observed only after 10 days at 10°C with *P. roquefortii*.

PC analysis was performed with data from the BH 114 after 2 and 4 days of incubation at 10 °C.

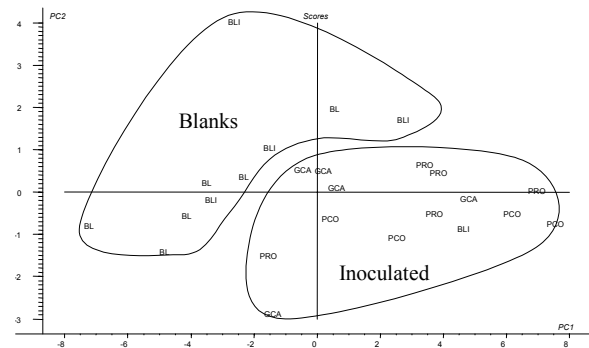
0% salt model

The 2 days model explained 75% of the total variation on 2 PCs (see figure 8). The first PC (65%) described the existence of microorganisms or not.



“Figure 8”. PCA score plot from 0% plates (2 days old).

The 4 days model (see figure 9) explained 70 % (PC1: 63%, PC2: 7%) of the total variation. The variation described by PC2 is attributed to the various fungi used as cultures. Blank plates that were contaminated were clearly placed near to the inoculated plates.



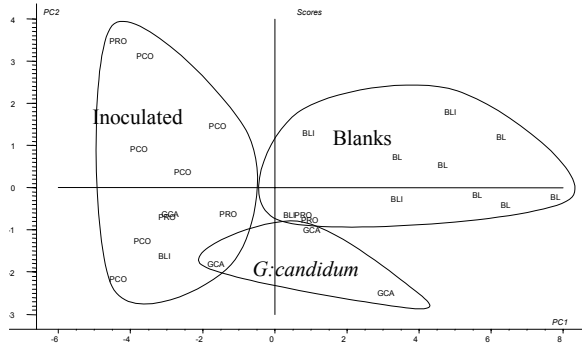
“Figure 9”. PCA score plot from 0% plates (4 days old).

4% salt model

Results were similar to the ones with the 0% model with the exception of the samples inoculated with *G.candidum*. These samples were more like the blanks and this can be

explained by the high sensitivity of that particular fungus to salt [3].

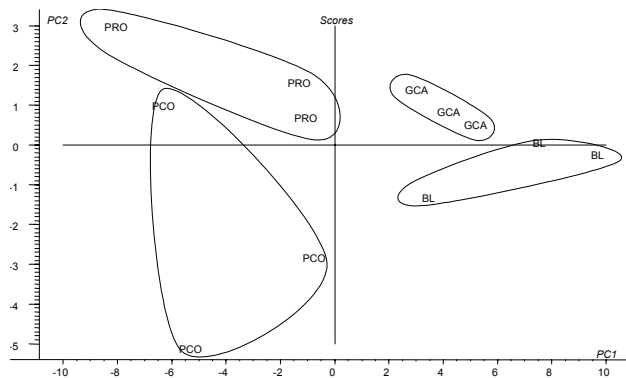
The model 4% salt after 4 days (see figure 9) explains 73% of the variation on 2 principal components (PC). PC1 64% describes again the main difference between blanks and inoculated plates while PC2 (9%) is characteristic for the fungi *G.candidum*.



“Figure 9”. PCA score plot 4 days 4% salt model at 10 °C after inoculation

Identification of contaminants

Based on PCA analysis the e-nose was able to differentiate between fungi (see figure 10) and yeasts mainly on the 0% media after 48h incubation at 10 °C. Media with 4% salt were better for the discrimination of the salt tolerant moulds like *P.camemberti* and *P.caseifulvum*.



“Figure 10”. PCA score plot 4 days 4% salt model at 10 °C after inoculation

CONCLUSIONS

The BH 114 E-nose system was successfully used in detecting the origin and the microbiological quality of Danish blue cheeses. Cheeses with contamination were identified by e-nose with the use of only few variables. Results were positively correlated to other analyses (GC-MS, microbiological and chemical). Selection of these variables was possible by using multivariate analyses methods as PCA, SIMCA and PLS-R.

The aFOX-3000 model provided the means to model the smell of Camembert cheese made by specific microbial strains. The application of multivariate analyses methods

as PCA, SIMCA and PLS-R helped to locate the best features from the response signals. Simplification of the model increased its effectiveness and lead to the successful prediction of the maturity of other “unknown” cheeses.

Media simulating cheese have been successfully used within a short period of time due to rapid degradation. Microorganisms growing on cheese media were detected after 2 days at 10 °C and verified after 4 days at the same temperature before visual growth of the microorganisms.

Differentiation of yeasts, and filamentous fungi on cheese media with the use of electronic nose technology was possible after 4 days at 10 °C and for some of the species studied. Identification models could not be used in order to identify the contaminant growing on the plates. This was due to the changes that occurred to the media making the long-term identification impossible.

Optimizing the use of sensor arrays with the help of multivariate analysis methods was investigated with the help of the software package Unscrambler (CAMO) version 7.61 SR1.

More stable media appropriate for long-term identification use of spoilage microorganisms are required. Next stage in this investigation will be performed with a “new age” cheese media that is more stable.

ACKNOWLEDGMENTS

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