Den elektroniske næse: en hurtig objektiv måling af frø-kvalitet?

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Agenda

Baggrund – ekspertiser på DTU

Hurtigmetoder til mykologisk kvalitets måling

Hvad er en elektronisk næse?

Eksempler på anvendelser

Korn

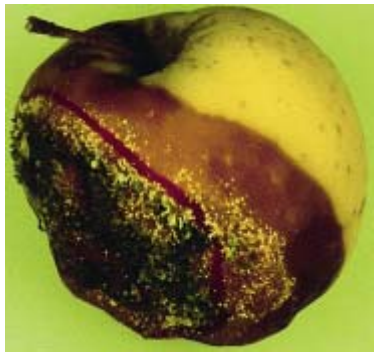
Ost

Mykotoxiner

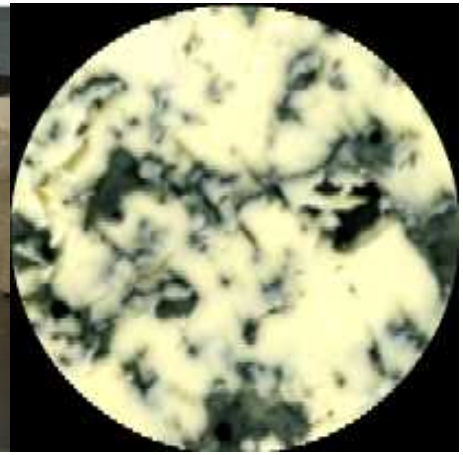
Konklusion / diskussions punkter

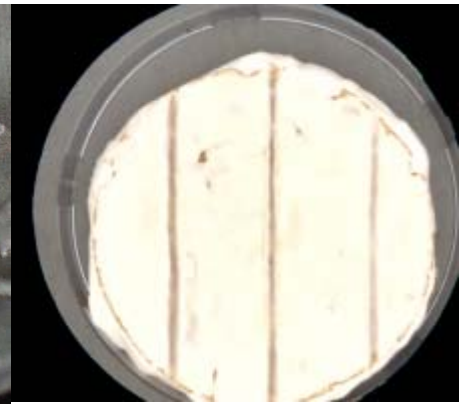
Food Ecology: All food products have their own specific group of microorganisms associate to them – The Microbiota

Spoilage



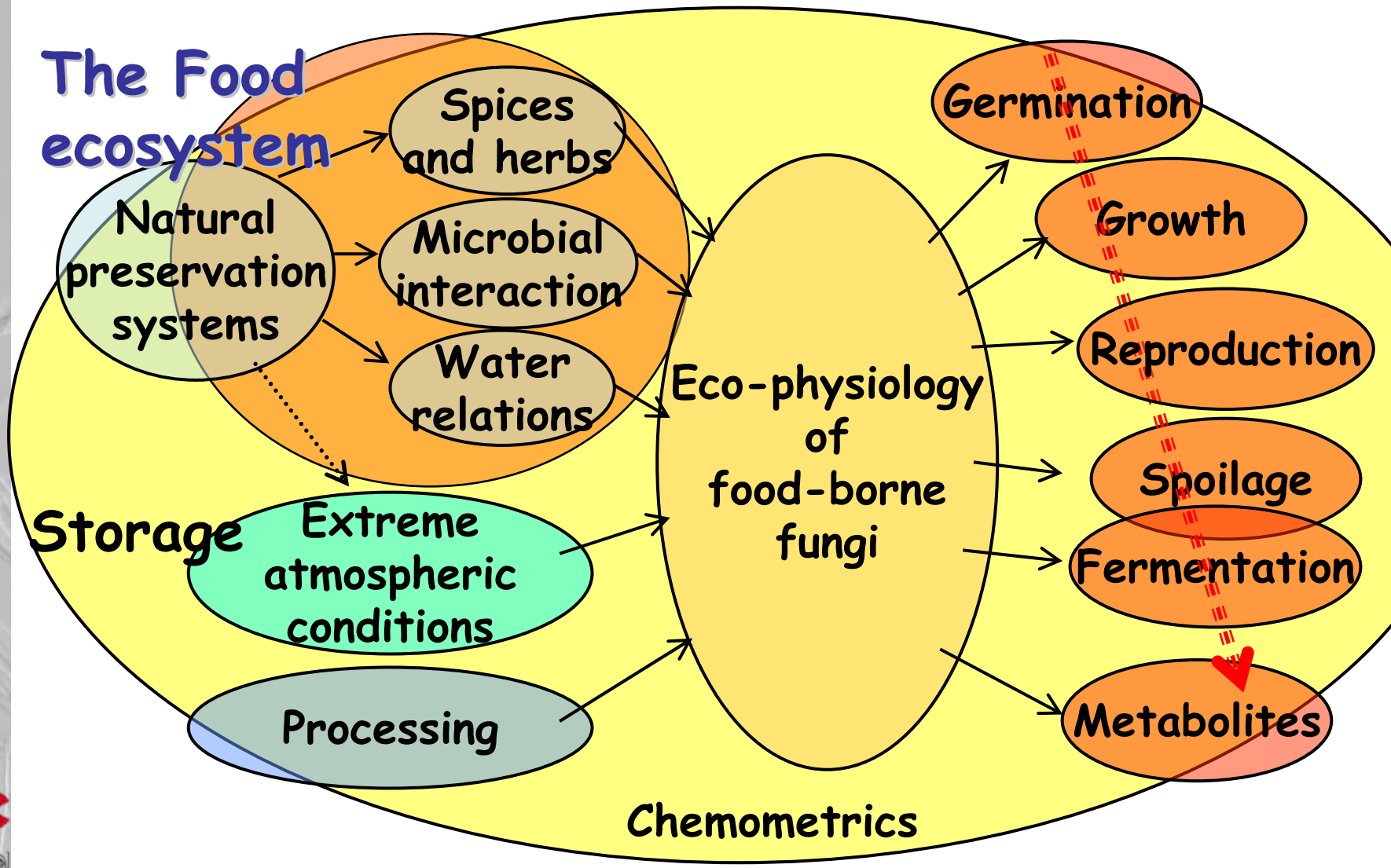
Fermentation





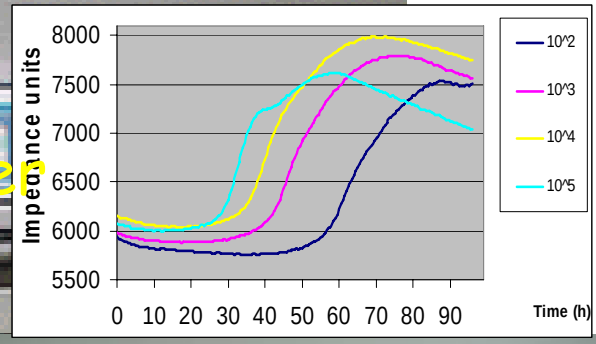
Ecophysiology of Food-borne Fungi

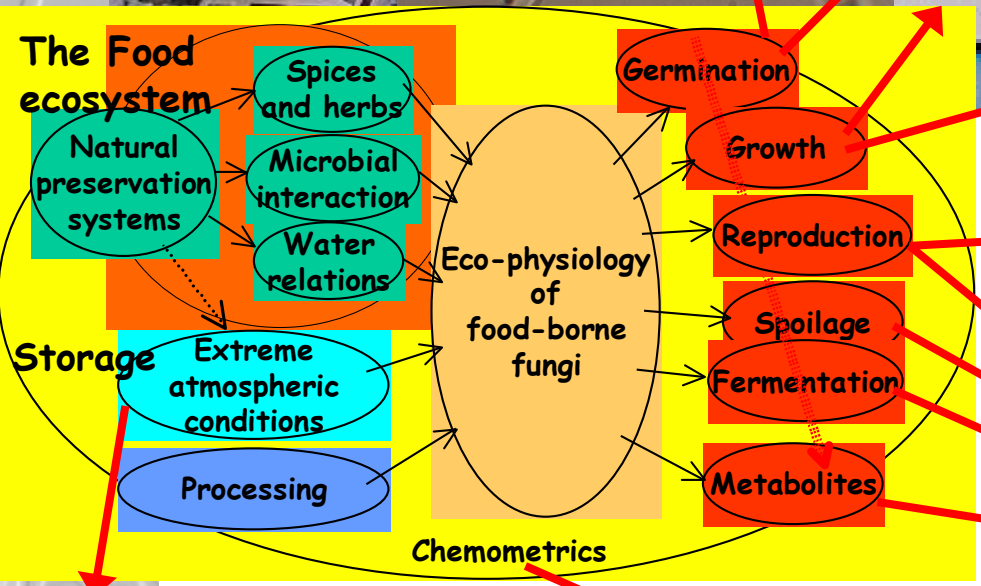
environmental conditions and stress



Microscopy

Bactometer

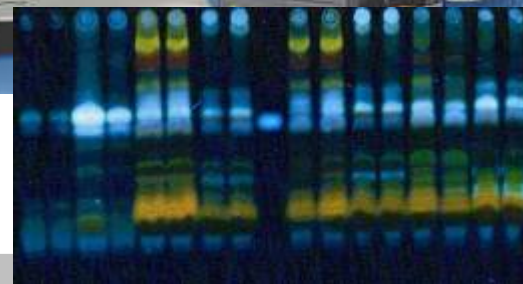




Morphology (Visual, Colour, NIR, Video meter)

Visual,
NIR,
E-nose

Metabolism: E-nose, flavour/ off-flavour, (TLC, HPLC, GC-MS, respiration)



Simulation (CA-incubators, MA-Pack equipment, respirometer)

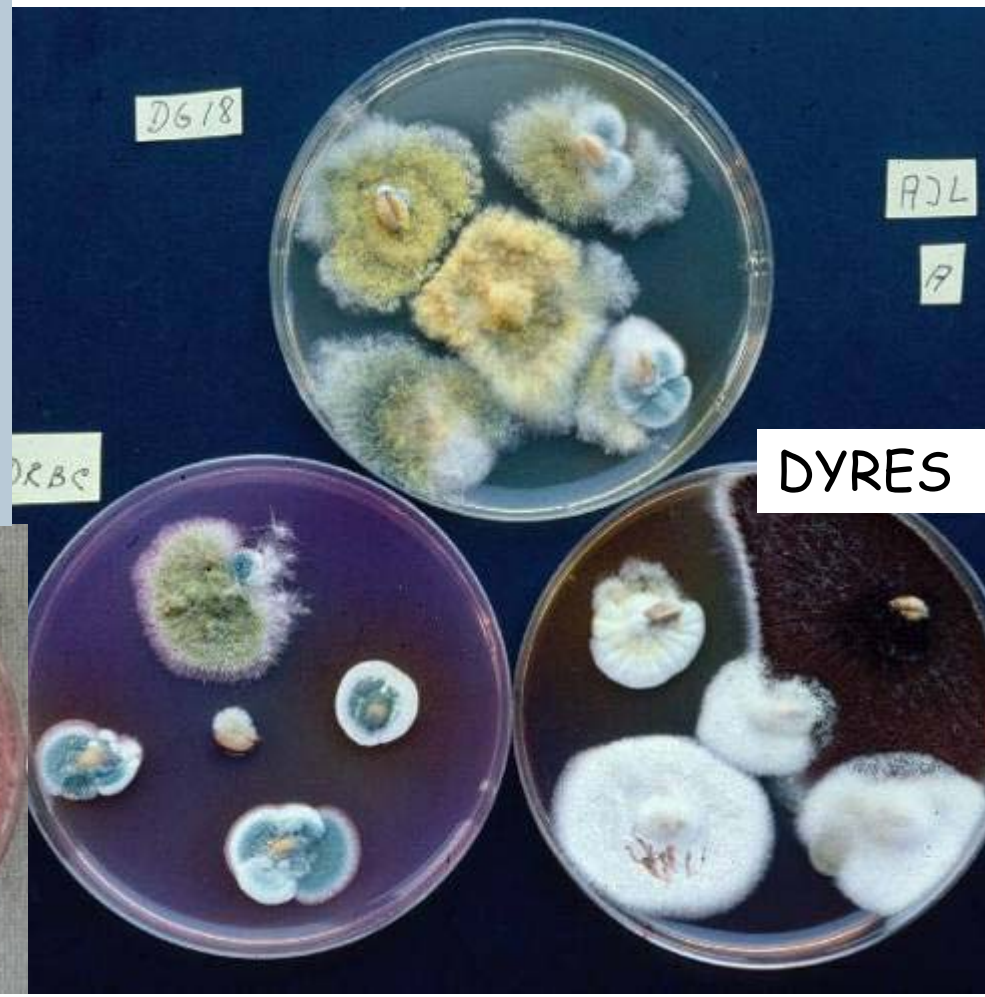
Design and Analysis (Exp. design and Chemometrics)

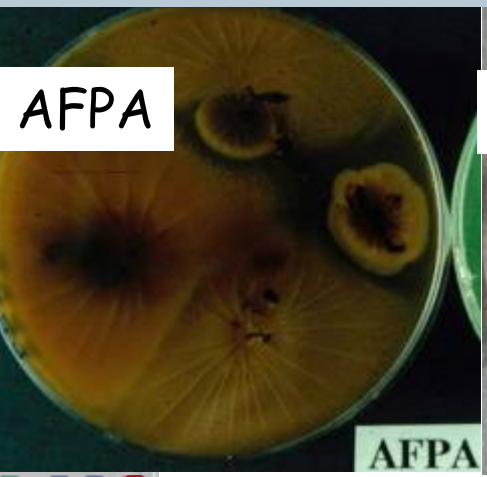
Konventionelle- / Hurtigmetoder
Nogle overvejelser

- **Hvad ønsker vi at opnå ved brug af vores metoder?**
- **Kvantitative metoder detekterer kontaminationsniveauet (CFU) eller biomasse**
- **Kvalitative metoder detekterer specifikke arter eller grupper af arter.**

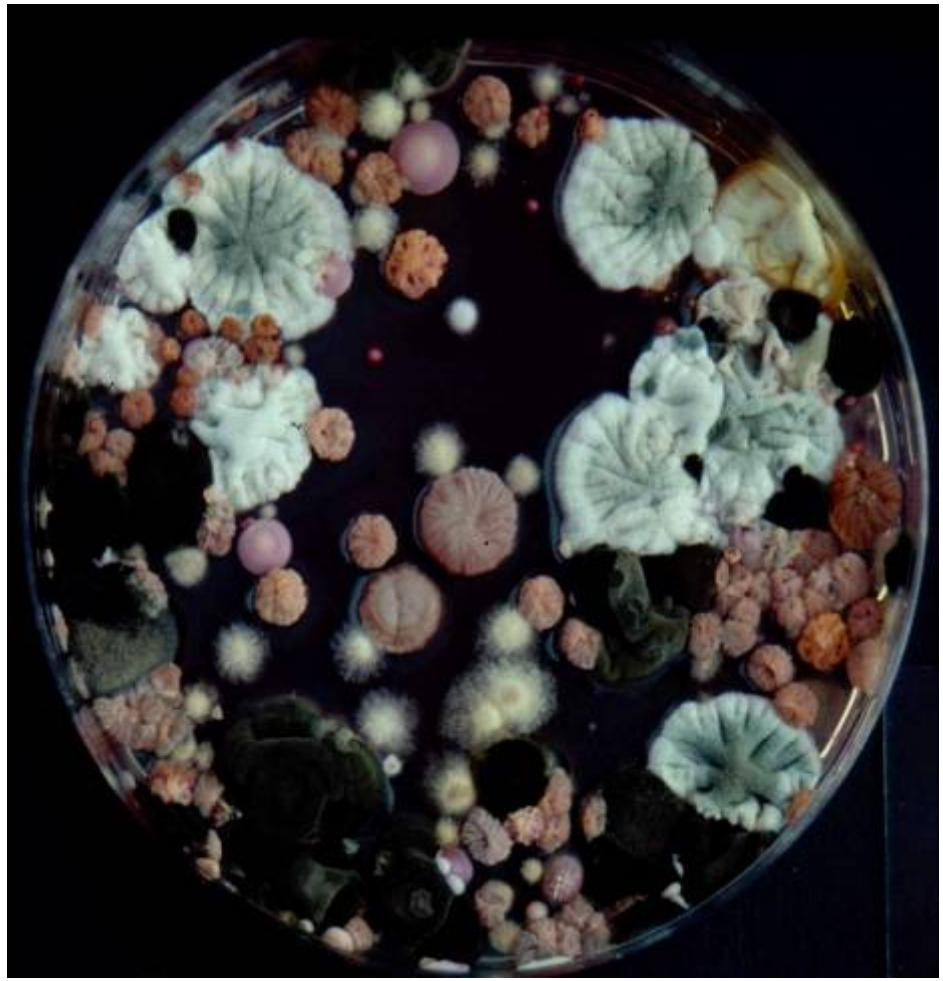
Accelererede konventionelle metoder

- **Mykotoksin dannende cerialie associerede svampe** **DRYES/DG18**
- **Xerophilie svampe** **DG18/MY50G**
- **Fusarium** **CZID**
- ***Aspergillus flavus* og *A. parasiticus*** **AFPA**
- **Syre tolerante svampe (*P. roqueforti*)** **ADYS**





Direkte udlæg eller suspension



Byg i lufttæt silo I 18 måneder

%		*Pen. roqueforti*	*Paecilomyces* sp.	*Eurotium* sp.	*Candida* sp.
DRBC	Direkte	100	0	0	0
	Fort.	17	0	0	68
AFPA	Direkte	0	100	0	0
	Fort.	21	0	0	62
DRYES	Direkte	0	0	0	100
	Fort.	11	0	0	64
DG18	Direkte	99	0	83	0
	Fort.	22	0	0	57

Mykologiske hurtigmetoder

- **Direct methods**
  - **Howard mould count**
  - **DEFT (direct epifluorescens filter technique)**
  - **Turbidimetrc measurement – combined with 2 phase separation**
- **Chemical and Biochemical methods**
  - **Bioluminescens - ATP**
  - **Chitin**
  - **Ergosterol**
  - **Enzymes**
  - **Secondary metabolites**
  - **Volatile organic compounds (VOC)**
- **Immunological methods**
- **Molecular biological methods**
- **Impedimetri**

What are the characteristics of an fungal isolate?

- **Morphology**
  - Microscopic structures
  - Macroscopic structures
  - Color
- **Growth characteristics**
  - Growth rate on specific substrates / growth conditions
  - Resistance / tolerance
- **Secondary metabolites**
  - Mycotoxins
  - Volatiles
- **Molecular characters**

De vigtigste svampe metabolitter

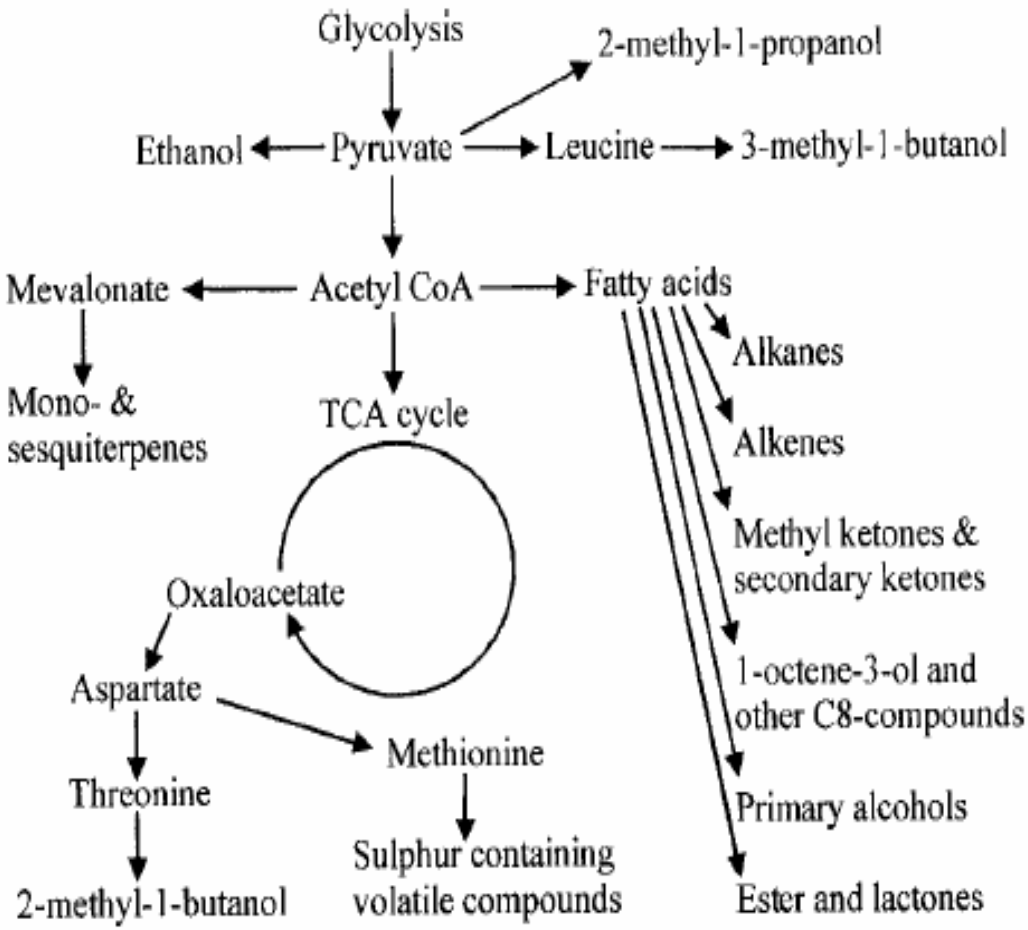


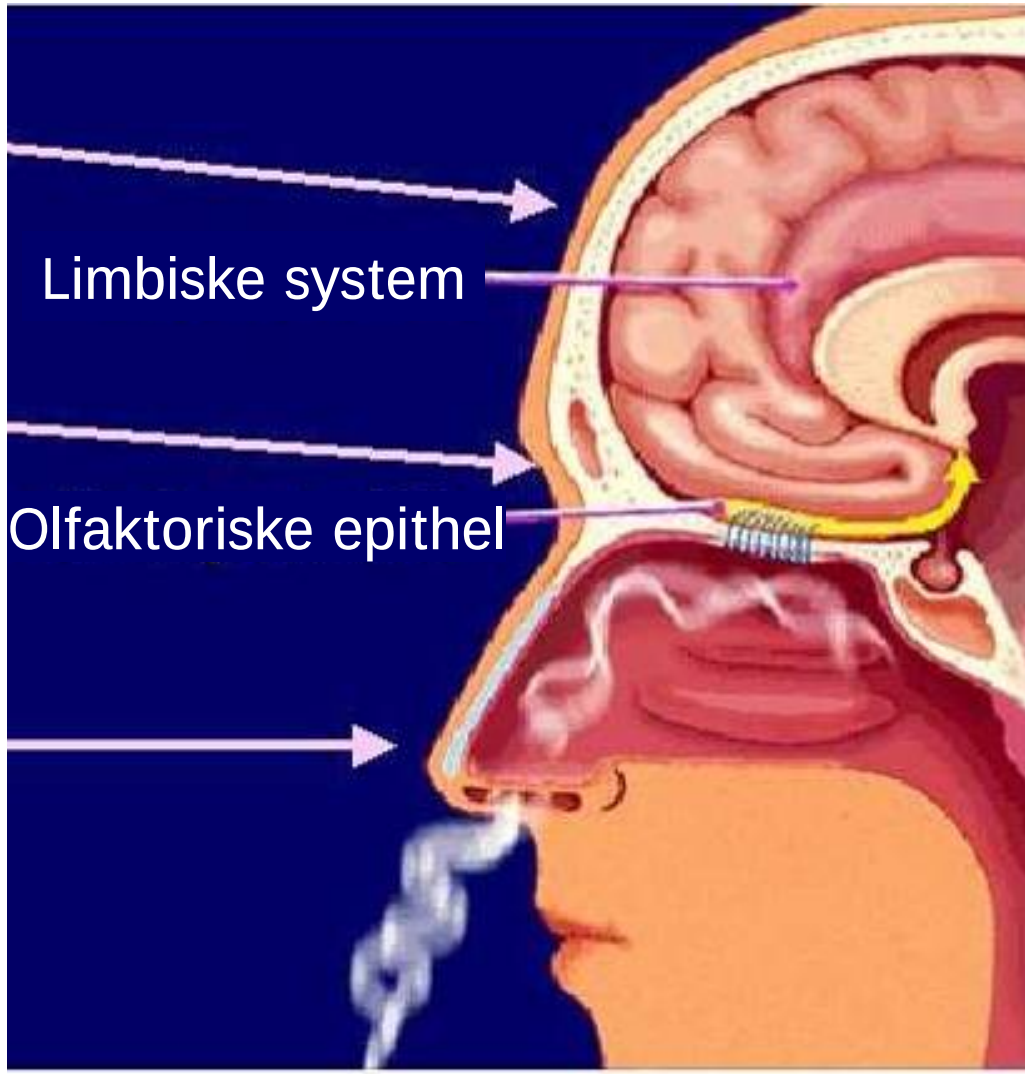
FIG. 1. Overview of metabolic pathways for biosynthesis of the main fungal volatile metabolites (Börjesson, 1993; Larsen, 1994).

Lugte sansen - det olfaktoriske system

Mønstergenkender (rigtig neural netværk)

Sensor array: 10^7 celler med ~100 forskellige receptor typer

Prøveudtagning og prøveforberedelse (filtrering, justering af temperatur, fugtighed, tryk og flow hastighed)

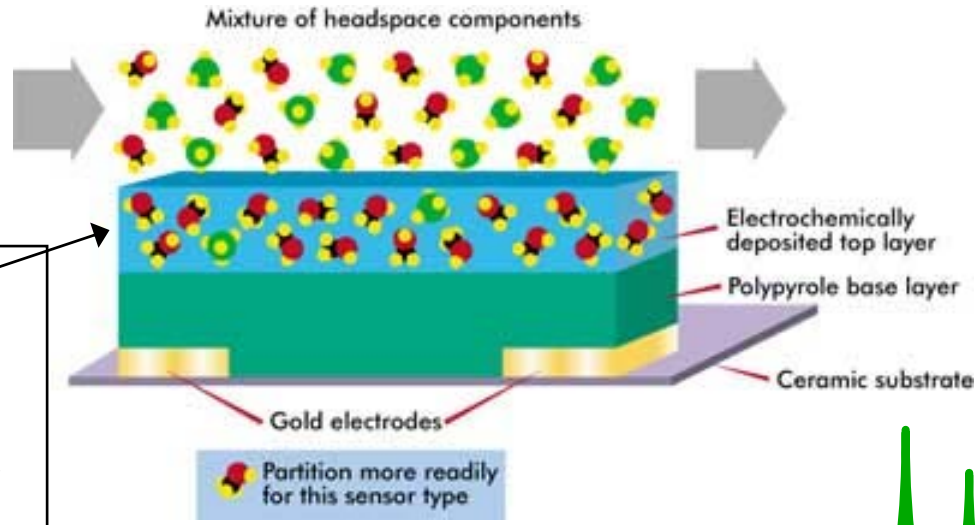


Den elektronisk næse

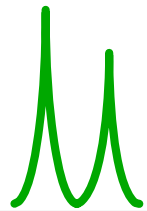
Opbygning

- **Gas opsamling og transportsystem (flow injection)**
- **Uspecifikke kemiske sensorer (8 – 48 stk.)**
- **Multivariat-kalibrering og -dataanalyse (neurale netværk / PCA, cluster analyse)**

Bloodhound bygger på conducting polymer sensor teknologi

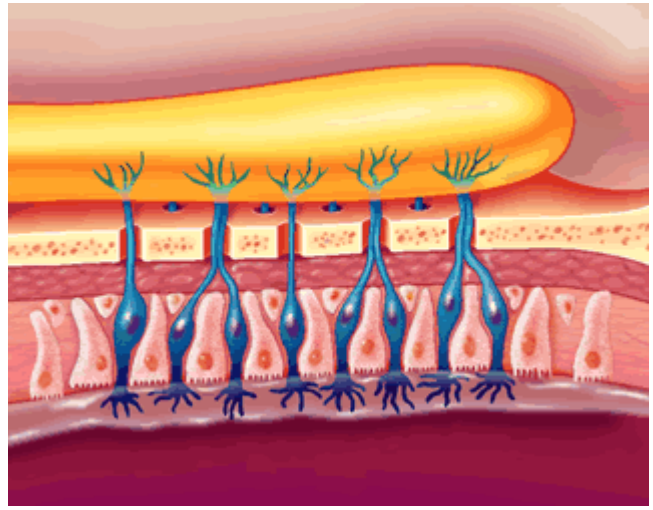


Adsorbtionen i toplaget svarer til interaktionen mellem componenter i en gasstrøm og den stationære fase i en GC-kollonne



Essentielle fysiske og kemiske egenskaber ved lugtstoffer

- **Tilstrækkelig høj damp tryk**
- **Lav polaritet**
- **Overflade aktiv**
- **Delvis opløselige i vand og i fedt**
- **Molekylevægt under 300 (der er ikke nogen kendt lugt stof der har en molekylevægt på over 294)**

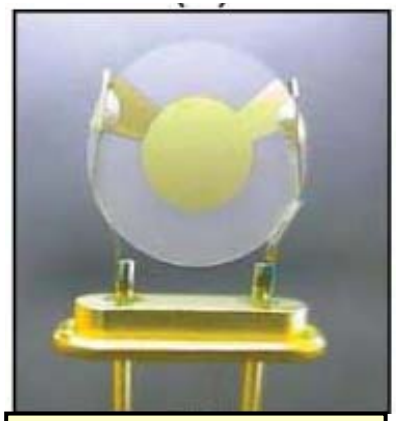


Mennesket kan skelne ca. 900 lugte

E-næse sensors

Metal oxide sensors (MOS)

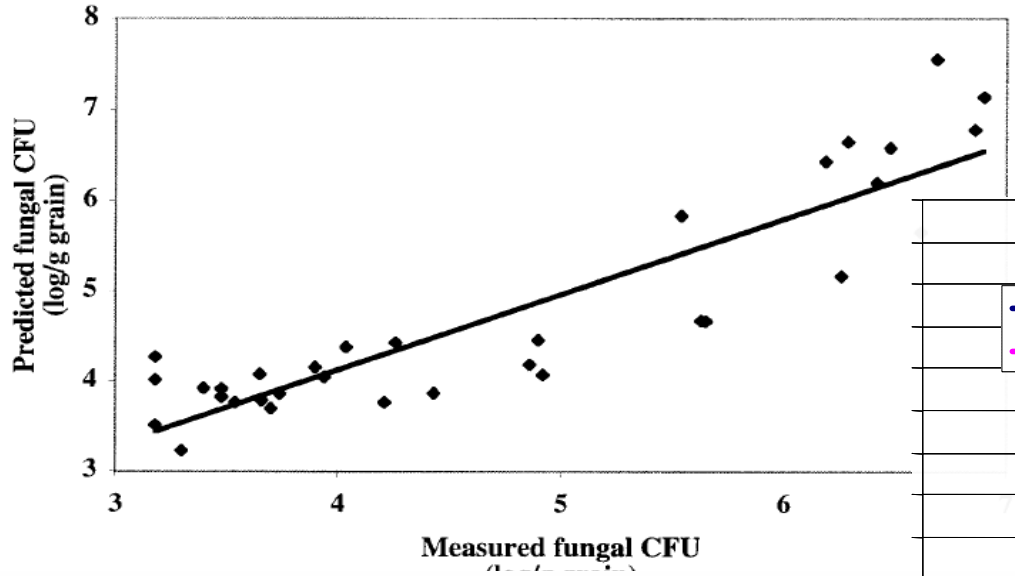
Surface acoustic wave sensors

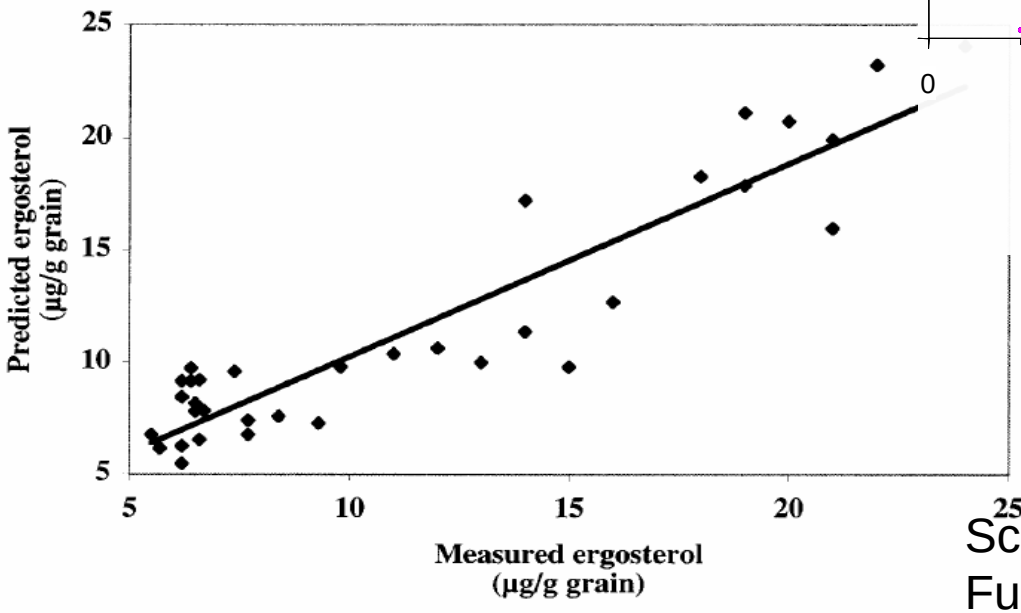


Quartz crystal microbalance

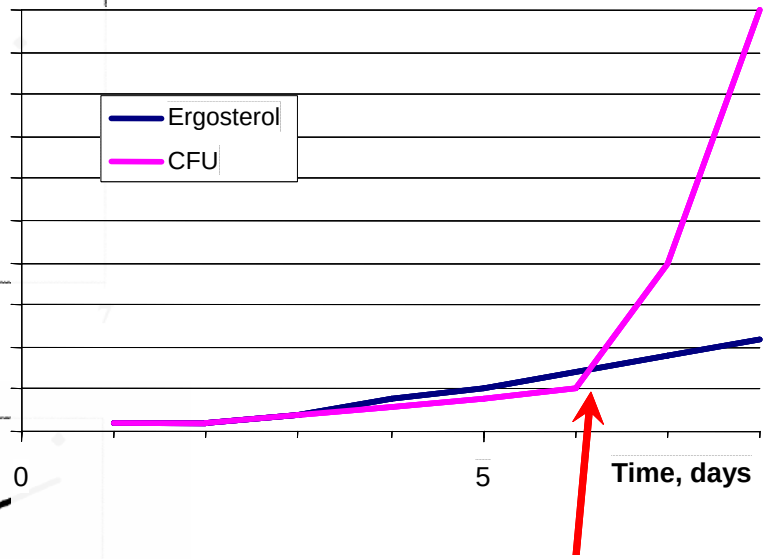
Conducting polymer sensor

Ergosterol





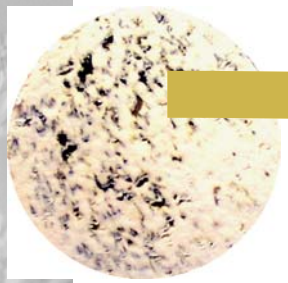
Hvede korn: Aw = 0,94
~26 - 30% vand

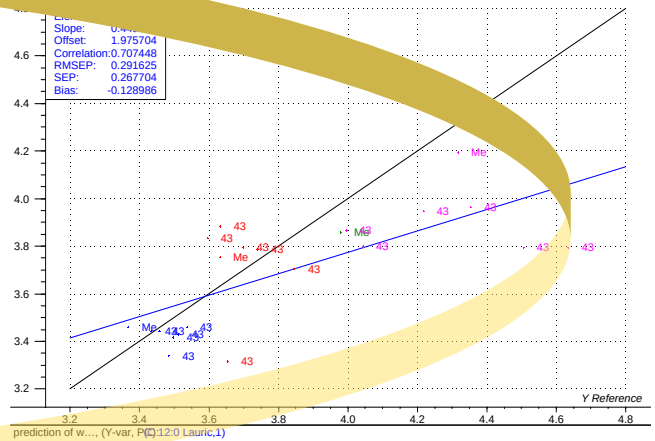


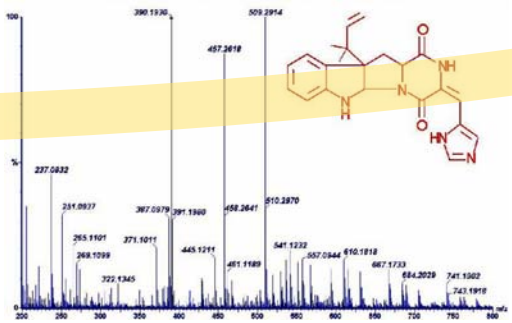
Sporulation

Schnürer, Olsson, Börjesson, 1999
Fungal Genetics and Biology

Applying Electronic Nose (e-nose) technology for the Prediction of Danish Blue Cheese Quality

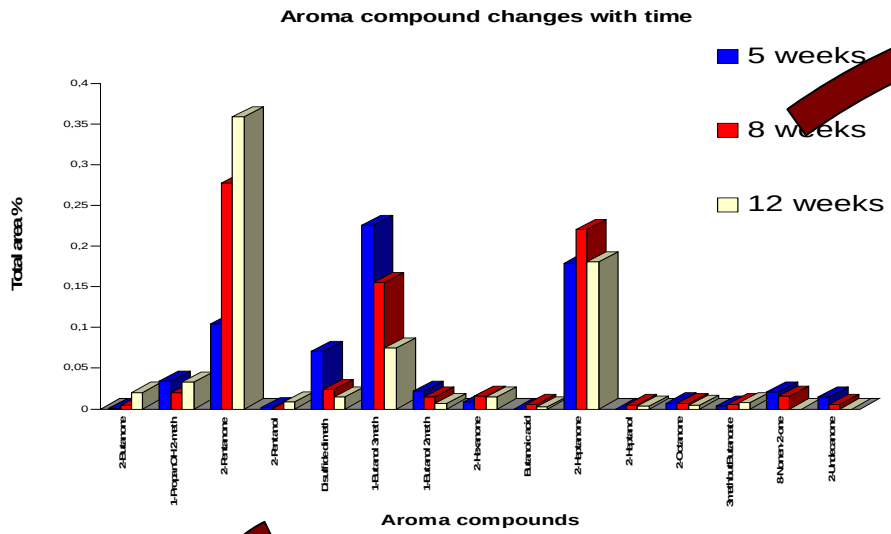






Opbygning af karakter

GC-MS

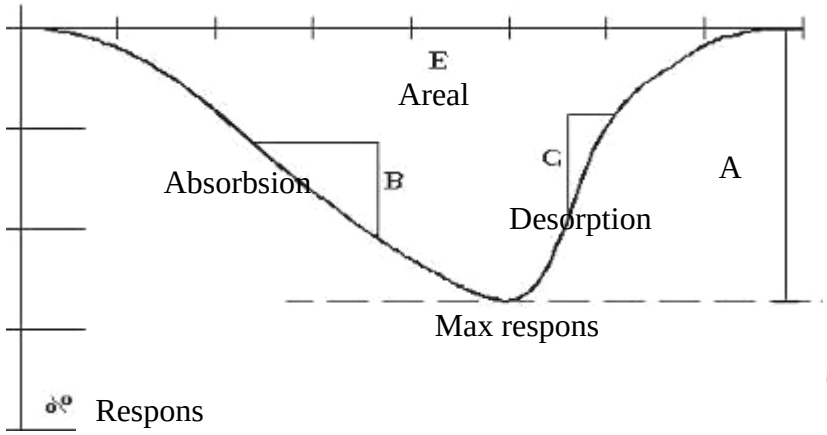


Sensorik

Lugt	Smag	Teksture
River i næsen	Sødme	Modstand
Valle	Svampeagtig	Flager
Syrlig	Salt	Cremet
Skimmel	Frugtagtig	Smeltet
Smørsyre	Fløde	Smuldre
Frugtagtig	Syrlig	
	Skimmel	
	Bitter	
	Eftersmag	

?

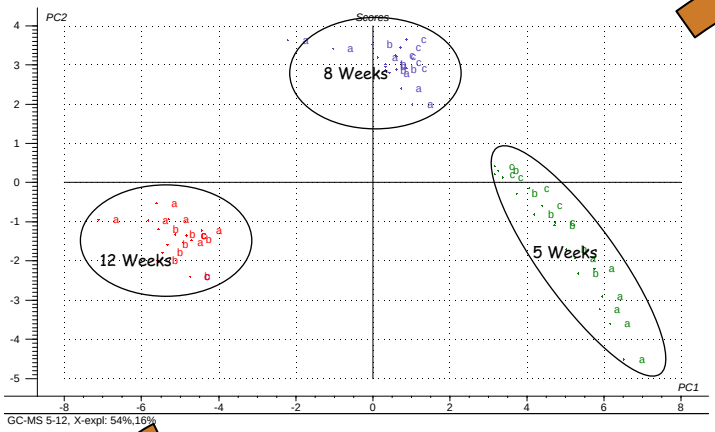
?



E-næse

Opbygning af karakter: Kombination af metoder

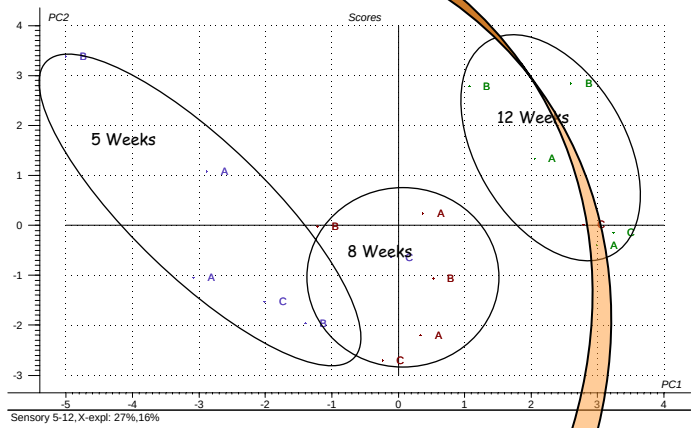
GC-MS



PCA results fra GC-MS data. 70% variation af dataene forklaret

?

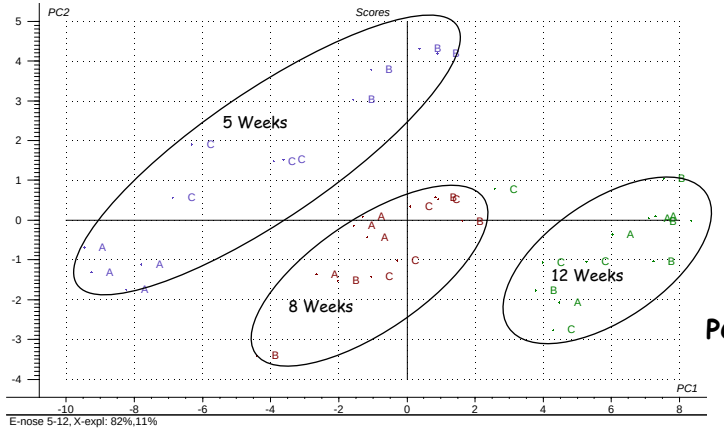
Sensorik



PCA results fra sensoriske data. 43% variation af dataene forklaret

?

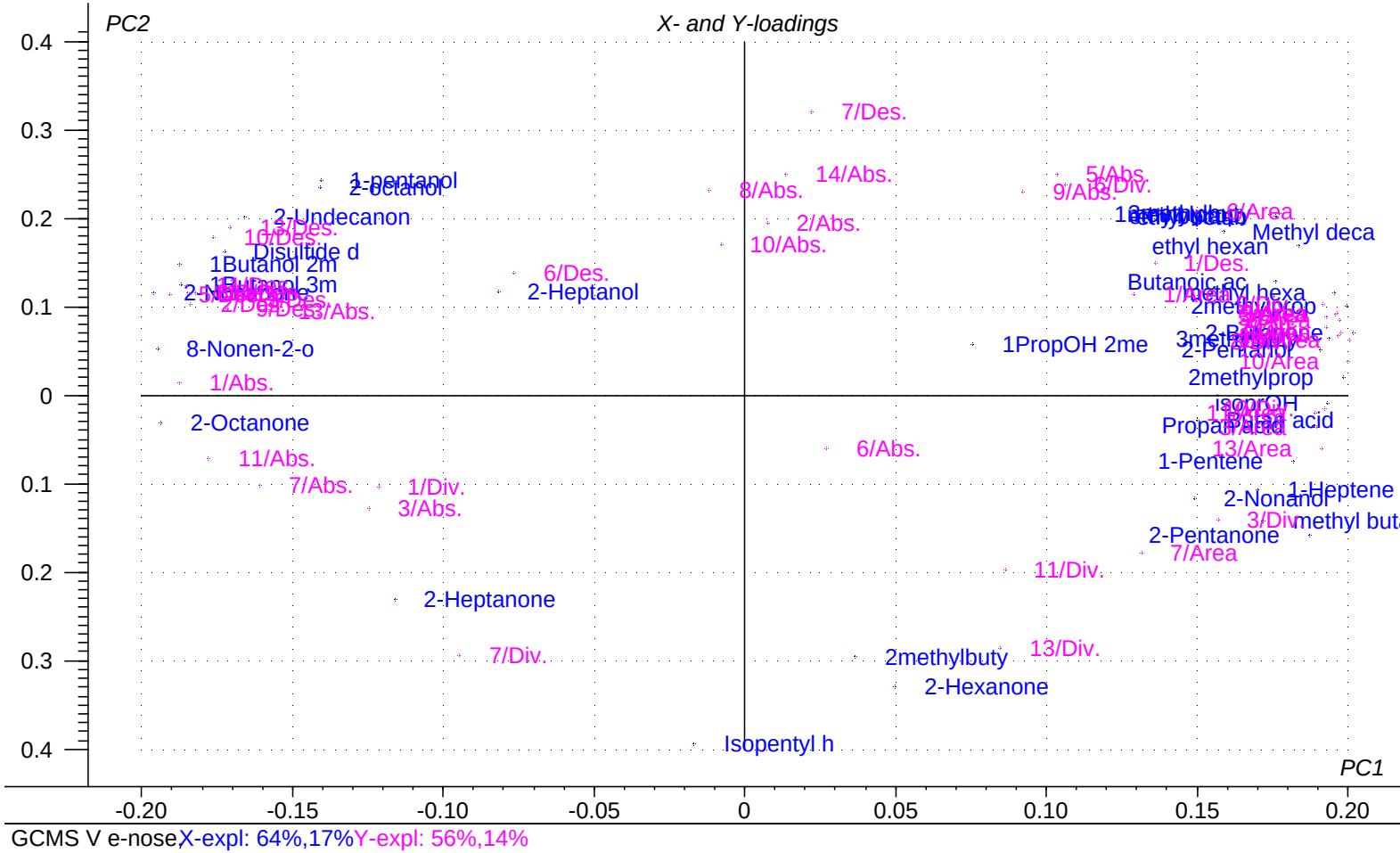
E-næse



PCA results fra BH 114 data. 93% variation af dataene forklaret

Sammenhæng mellem e-næse data og GC-MS

Kombination af data fra E-næse og GC-MS fra de samme oste gjorde det muligt at optimere brugen af den elektroniske næses sensorer til blåskimmelos te prøver.



Loadings plot fra PLS analyse. GC-MS data (X variabler) med E-næse data (Y variabler).

Quality control in the food industry

E-nose readings of a good and a not so good batch of Black Castello cheese on day 6 to day 25 after production.

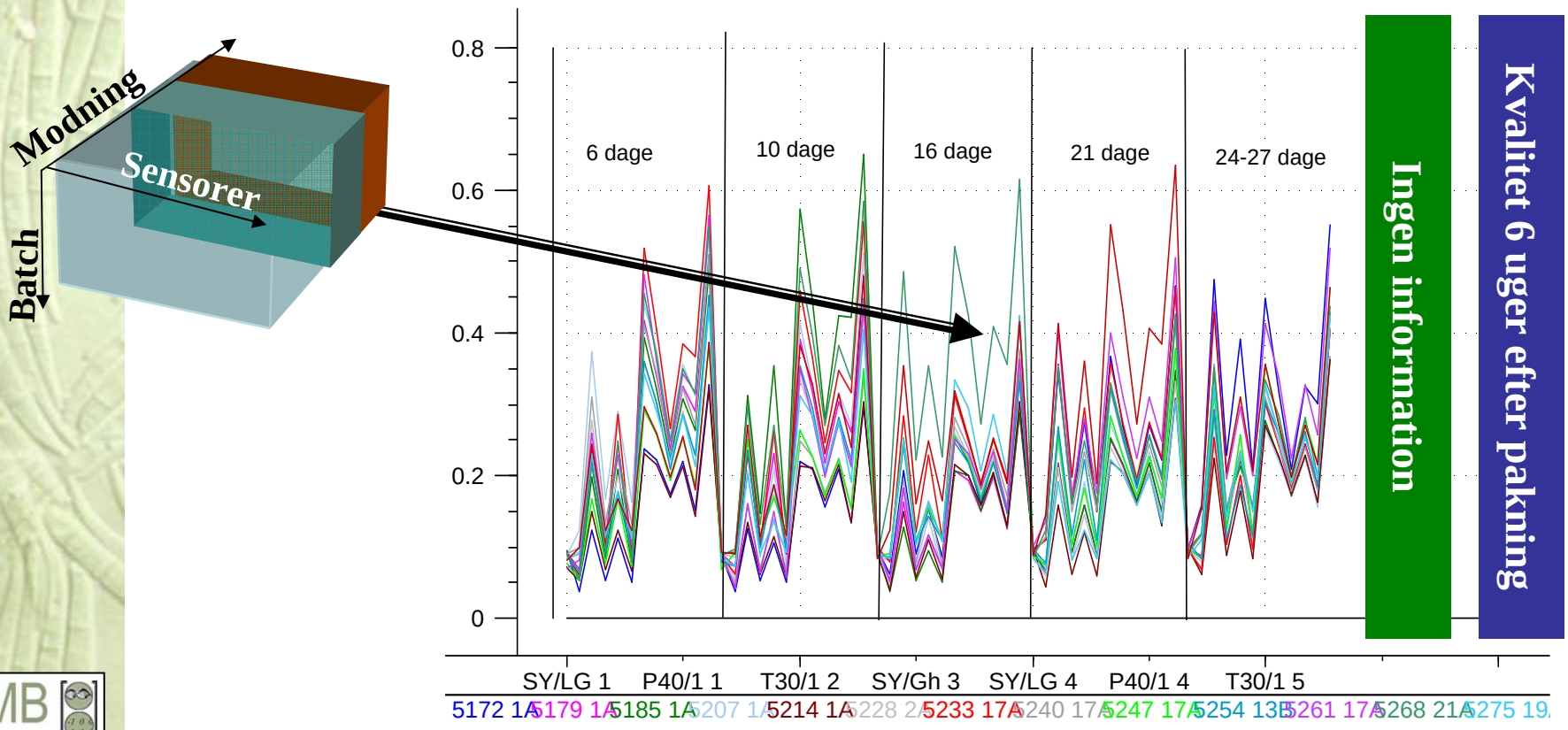
13-good

9-flowery taste

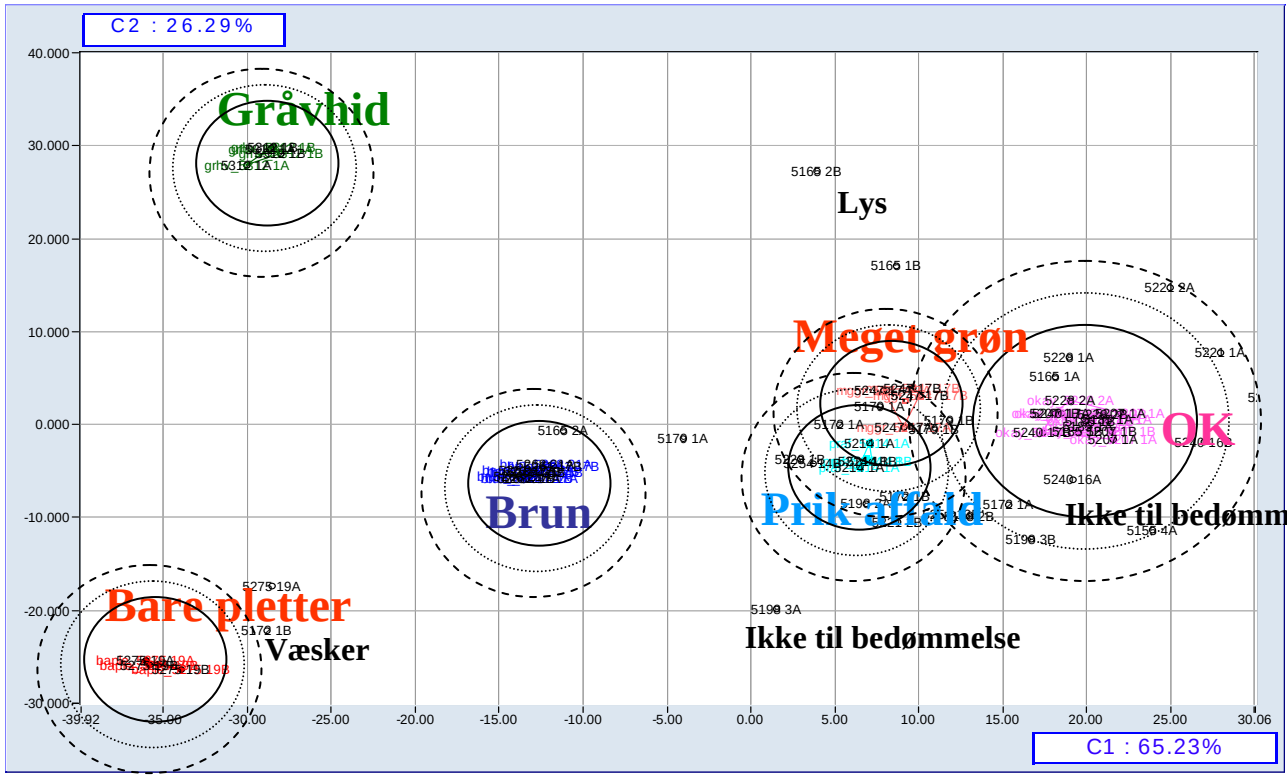
PC1

two batches X-expl: 82%,13%

Måle på lager, prediktere senere kvalitet

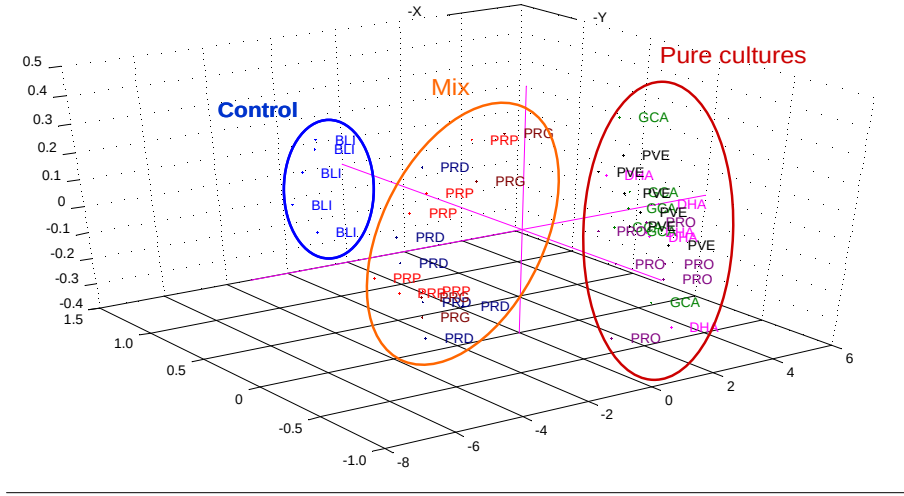


Brugen af kvalitetskort model



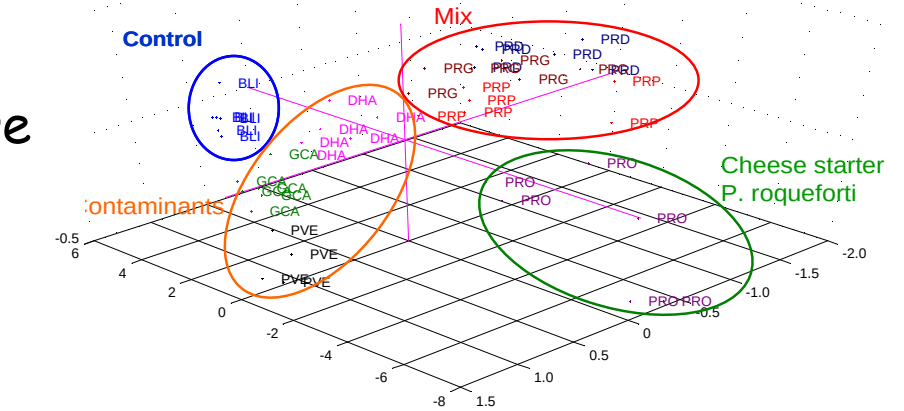
Graf taget fra α M.O.S. software. DFA model over kvalitet "ydre" uanset alderen.

Contaminants can be detected already after 3 days, which is before growth is visible



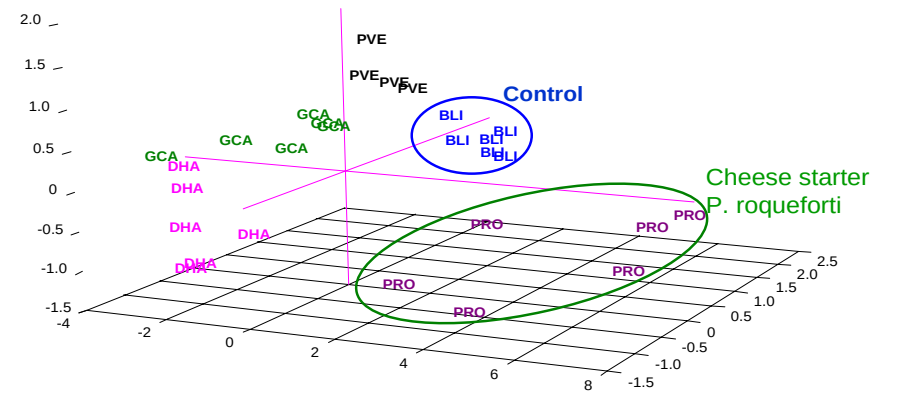
Differentiation between contaminants and mixtures is possible after 7 days (just before visible growth).

Center points 3..., X-expl: 96%,3%,0%



Differentiation between contaminants is more clear when samples with mixtures are taken out. (day 7)

Center points 7..., X-expl: 88%,11%,1%



Growth conditions

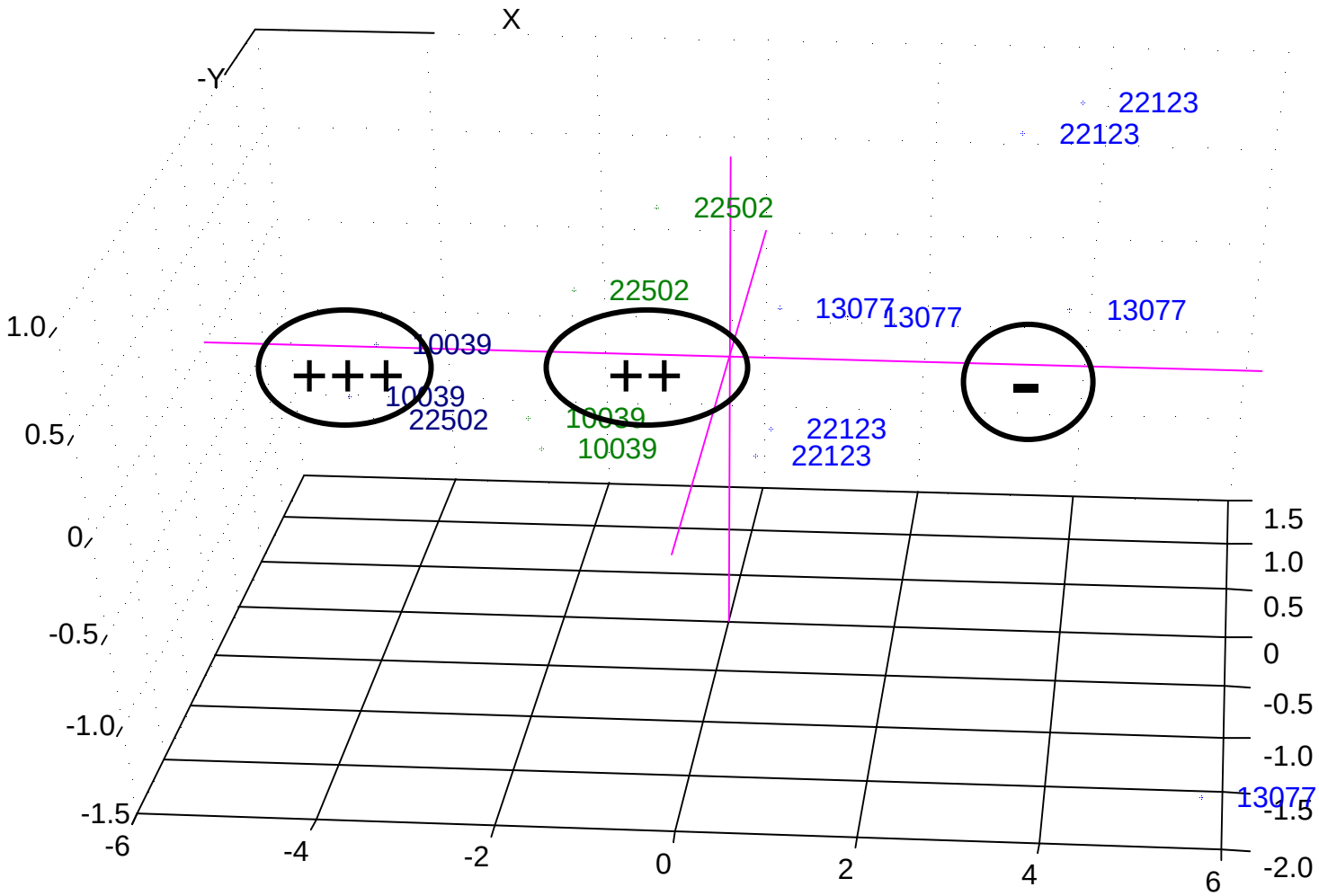
11% O_2 **25% CO_2**

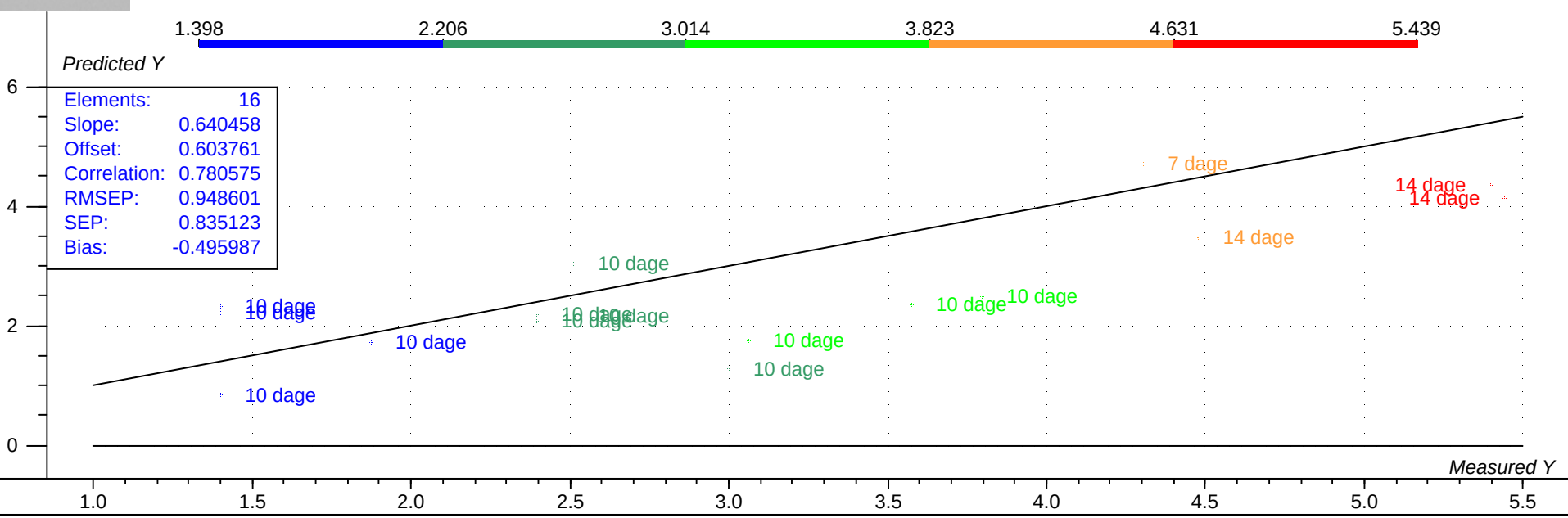
0,965 a_w **5.25 pH**

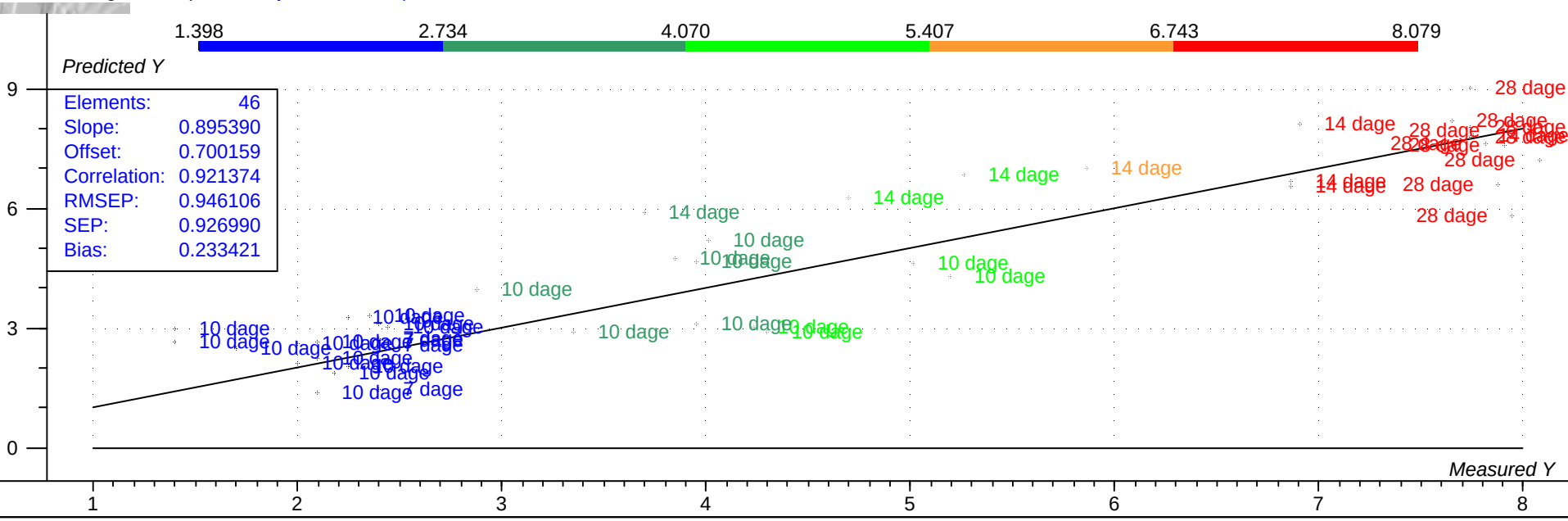
100 conidia per sample (5 ml cheese substrate)

P. verrucosum (4 isolates) on cheese and PDA measured by e-nose 2 days after inoculation. Citrinin production is measured 11 days after inoculation.

Scores




1.398
2.206
3.014
3.823
4.631
5.439
Predicted Y
Elements: 16
Slope: 0.640458
Offset: 0.603761
Correlation: 0.780575
RMSEP: 0.948601
SEP: 0.835123
Bias: -0.495987
Measured Y
maxVmikrlog ude..., (Y-var, PC) (G.candidum,5)


1.398
2.734
4.070
5.407
6.743
8.079
Predicted Y
Elements: 46
Slope: 0.895390
Offset: 0.700159
Correlation: 0.921374
RMSEP: 0.946106
SEP: 0.926990
Bias: 0.233421
Measured Y
maxVmikrlog ude..., (Y-var, PC) (P.roqueforti,5)

Diskussions punkter

- **Hvad ønsker vi**
  - **Kvalitativ eller kvantitativ**
  - **Pris**
  - **Tilgængelighed**
  - **Hastighed**
- **Total cfu giver sjælden det svar vi søger**
- **Identifikation på artsniveau er oftest vigtig for at vurdere sikkerhed – måske ikke mulig med E-næse?**
- **Andre kvalitets parametre, oxidation, spiring**
- **Hvad kendes om produktet: lagring og behandling**

Hvis du ikke kan finde den rigtige vej….

….brug næsen!

Gandalf fra Ringenes Herre