Prediktion av luktklass, svamp- och myktoxin-innehåll i spannmål med multivariata tekniker

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I samverkan mellan:
SLU, Uppsala universitet,
Landstinget och
Länsstyrelsen i Uppsala län

Disposition

Introduktion till luktproblematiken

Beskrivning av elektronisk näsa, GC-MS

Prediktion av ergosterol i artificiella spannmålsprover

Prediktion av ergosterol, ochratoxin A, DON i naturligt bra/dåliga spannmålsprover

Vilken utveckling sker idag?

Slutsatser

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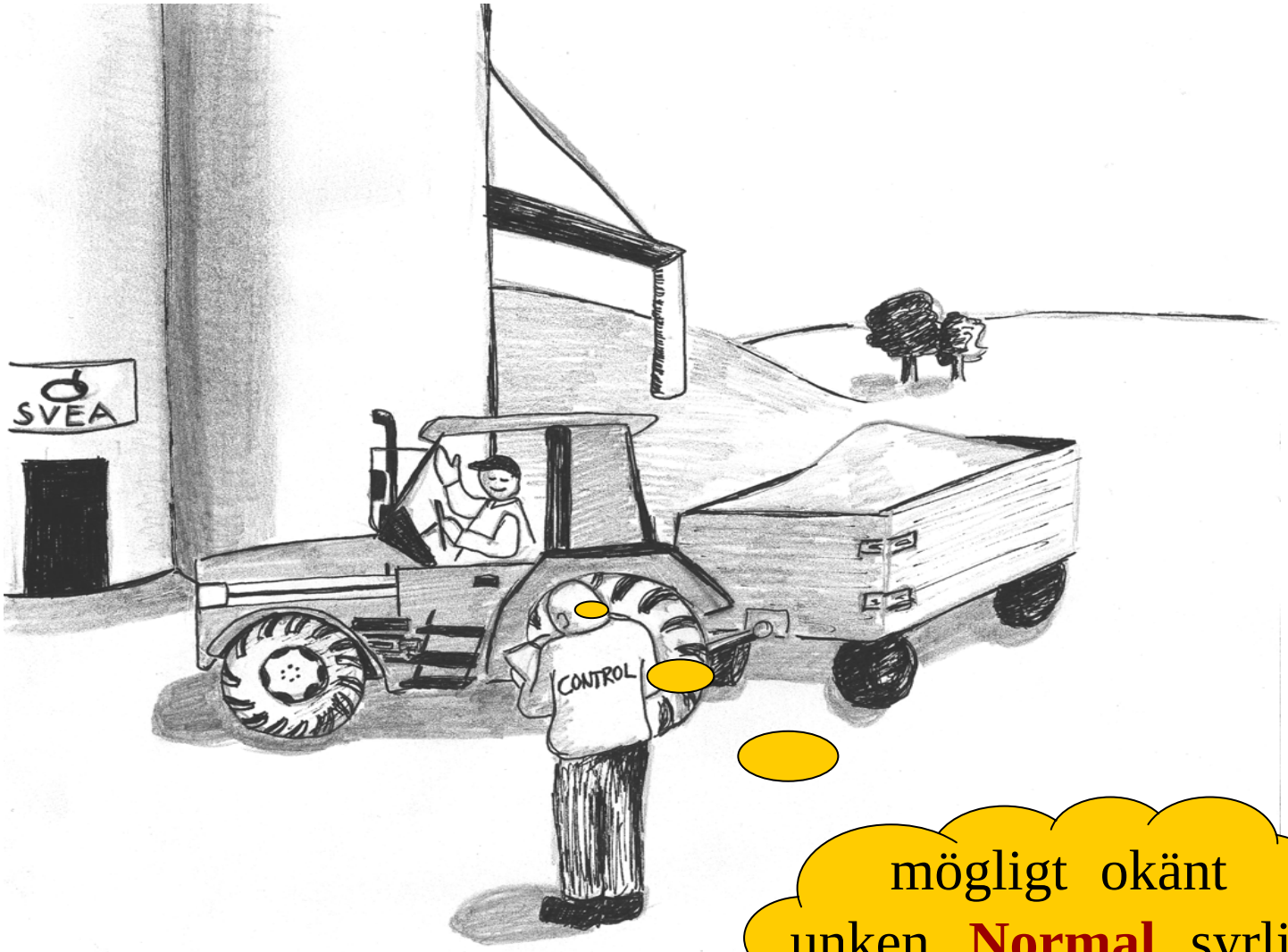
Spannmålshanteringen i Sverige



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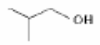
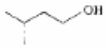
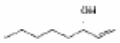
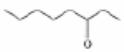
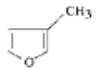
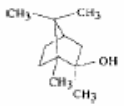
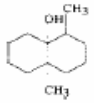
Exempel på vanliga flyktiga svampmetaboliter

Class	Compound	Structure	Producing fungus	Reference
Alcohols	2-Methyl-1-propanol		*Geotrichum candidum* *Penicillium roqueforti*	(1) (2)
	3-Methyl-butanol		*Fusarium graminearum* *Penicillium aurantiogriseum*	(3) (4)
	1-Octene-3-ol		*Penicillium glabrum* *Penicillium verrucosum*	(2) (5)
Ketones	3-Octanone		*Fusarium sporotrichoides* *Penicillium commune*	(5) (4)
Esters	Ethyl acetate		*Penicillium digitatum* *Pichia anomala*	(4) (6)
Furans	3-Methyl furan		*Aspergillus flavus* *Penicillium brevicompactum*	(2) (2)
Monoterpenes	2-Methyl-isoborneol		*Aspergillus niger* *Penicillium solitum*	(7) (8)
Sesquiterpenes	Geosmin		*Penicillium discolor* *Penicillium expansum*	(4) (9)

Note. A more complete tabulation of fungi and volatile compounds is given by Jelen and Wasowicz (1998).

References: (1) Jacobsen and Hinrichsen (1997); (2) Börjesson *et al.* (1992); (3) Jelen *et al.* (1997a); (4) Larsen and Frisvad (1995b); (5) Pasanen *et al.* 1996; (6) Lanciotti *et al.* (1998); (7) Börjesson *et al.* (1993); (8) Hocking *et al.* (1998); (9) Matheis and Roberts (1992).

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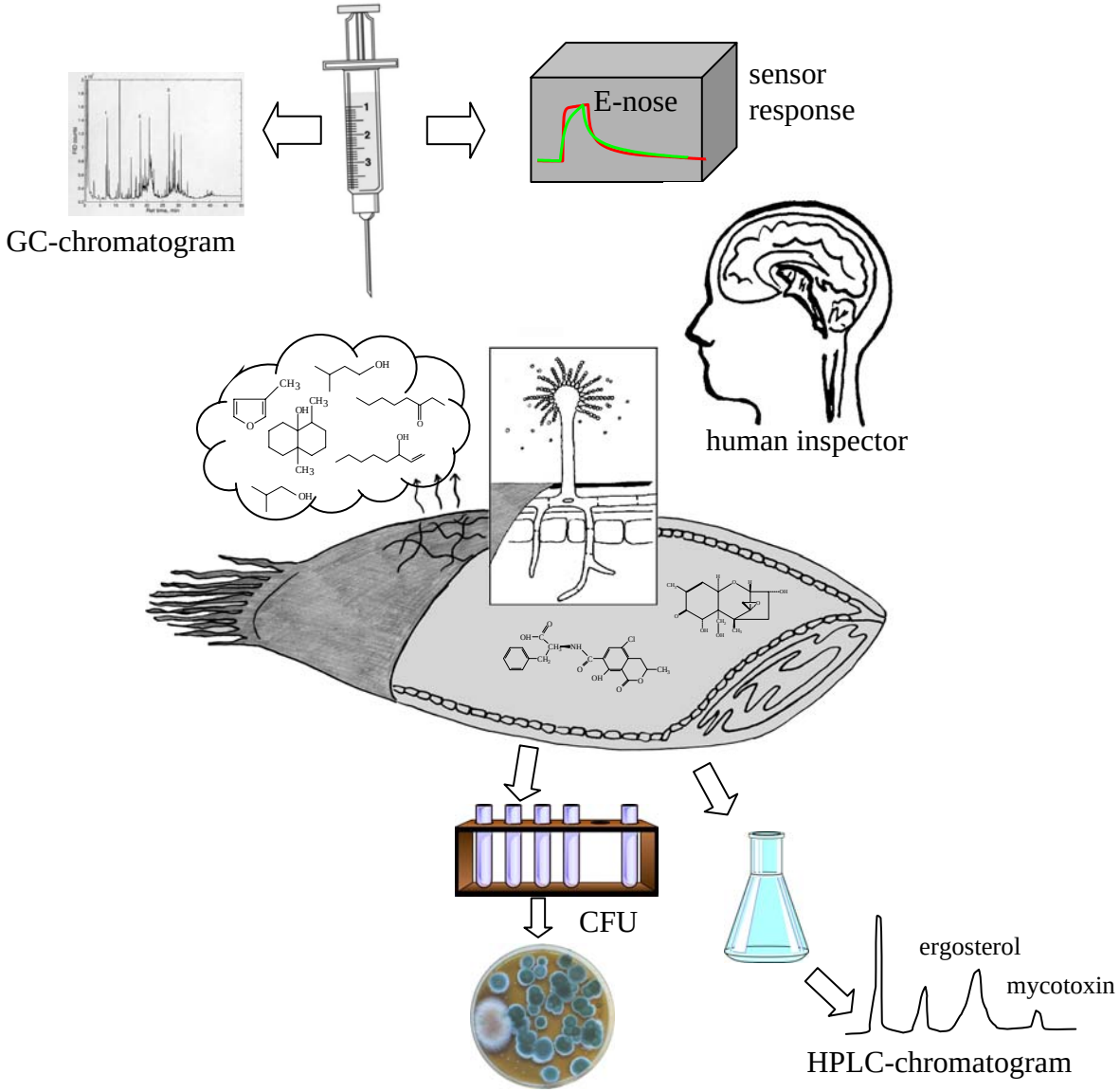
Forskningssyfte



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Board

2000

Möjligheter till:

- individuell värmning
- ingen provbearbetning

Hela systemet är uppvärmt

- ingen kondensering

Provtid

- 1995 ca 20 min
- 2000 ca 5 min

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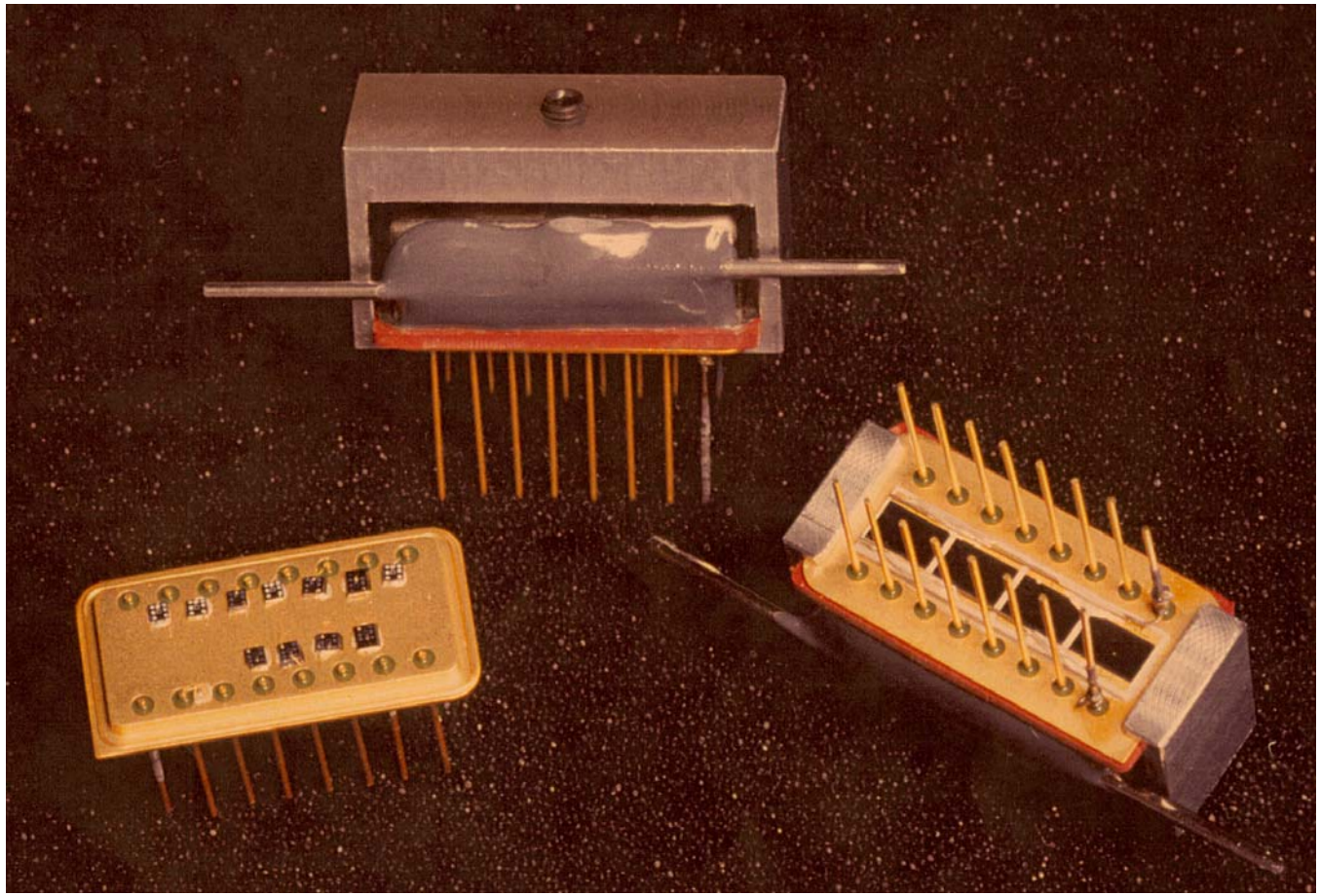
MOSFET sensorer



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Artificiella prover

Vete av god kvalité

Fukta upp vattenhalten till a_w 0,94

Ympa 10^3 sporer av Penicillium roqueforti per gram vete

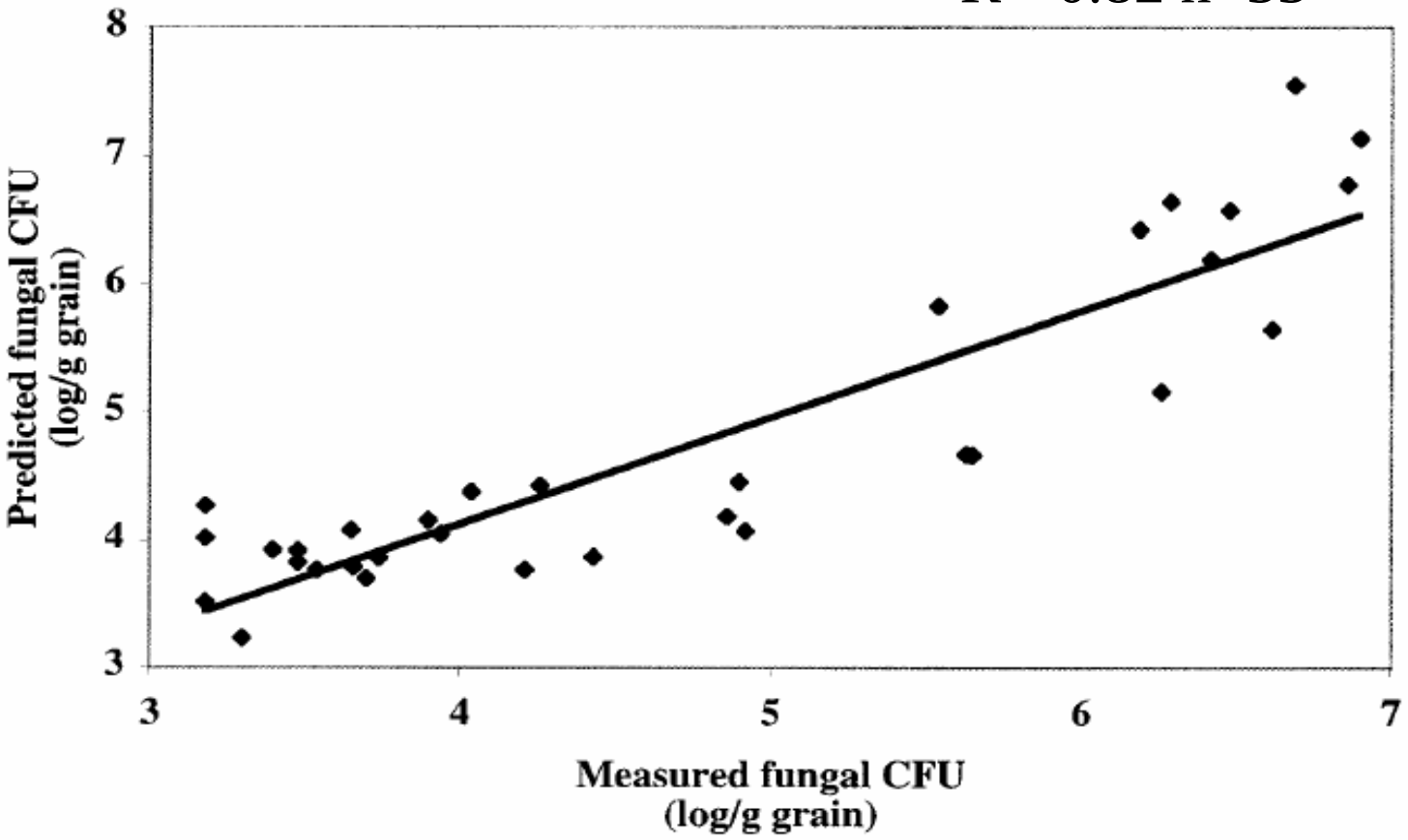
Inkubering vid 2, 4, 10, 15, 20, 25°C i sex replikat under 7 dagar

CFU räknat på DG 18 (NMKL metod 98)

Halten ergosterol bestämdes

Prediktion av CFU och ergosterol med en elektronisk näsa

R^2=0.82 n=33



Schnürer et al., (1999) Fungal Genetics and Biology 27, 209–217.

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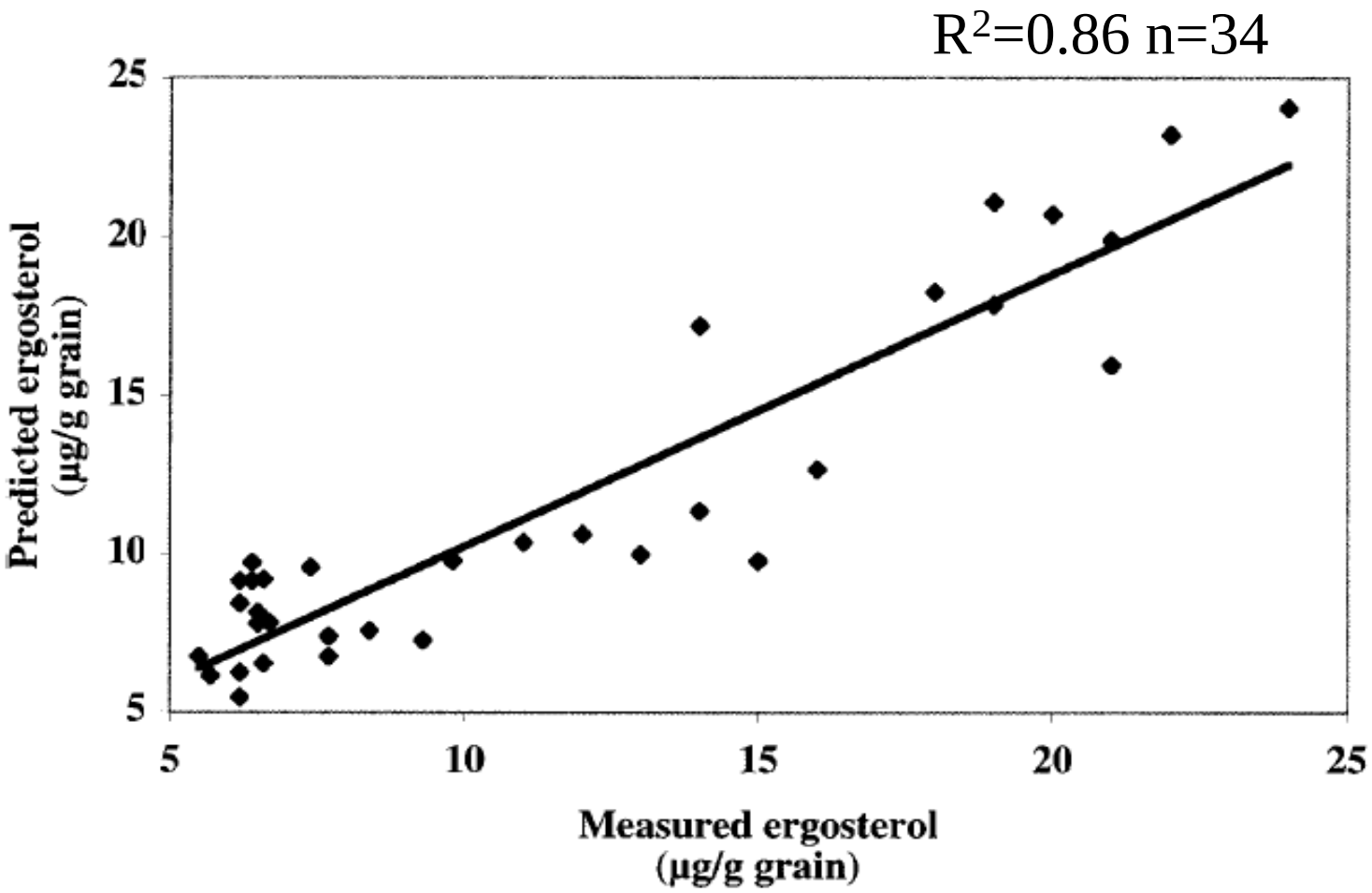
Prediktion av CFU och ergosterol med en elektronisk näsa



Schnürer et al., (1999) Fungal Genetics and Biology 27, 209–217.

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Spannmålsprover

10 naturligt bra
30 naturligt dåliga

1. Vattenhalt, CFU, ergosterol, mykotoxin
2. Elektronisk näsa
3. GC-MS

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Luktklass, vattenhalt och mikrobiologiskt innehåll

Sample	Odour	Moisture (%)	OA (μg kg^{-1})	DON (μg kg^{-1})	Ergosterol (μg kg^{-1})	CFU (log g^{-1})					Fusarium	Int. Inf. (%)[b]	
						Total	Pen	Asp	Eur	Fus	#[a] on CZID	DG18	CZID
590	normal	13.5	0	0	10.5	2.2	<3	<3	<3	4.0	4	90	50
591	normal	10.3	0	60	5.8	4.0	<3	<3	3.6	n.d.[c]	n.d.	n.d.	n.d.
592	normal	7.9	0	5	18.0	5.5	<3	4.0	4.9	3.0	0	70	64
593	normal	11.4	0	0	8.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
594	normal	10.4	0	80	4.1	2.7	<2	<2	<2	<2	0	20	10
595	normal	10.1	0	5	3.0	2.0	<2	<2	<2	<2	0	22	4
596	normal	10.1	0	0	2.6	4.7	4.7	4.4	n.d.	<2	1	80	20
597	normal	9.9	0	0	4.2	3.3	2.6	2.0	2.9	<2	0	92	6
598	normal	9.6	0	0	3.7	4.6	4.5	<3	<3	<2	0	80	20
599	normal	9.6	0	5	4.1	2.0	<2	<2	2.0	<2	0	50	12
471	acid A	19.8	346	97	35.0	6.2	6.0	n.d.	5.0	<2	n.d.	n.d.	n.d.
491	acid A	17.3	9	58	27.0	6.6	n.d.	n.d.	6.6	<2	0	100	54
492	mouldy A	15.1	3	48	7.7	4.4	3.8	n.d.	3.8	<2	5	100	20
291	mouldy B	13.9	274	42	15.0	4.4	3.7	n.d.	4.0	<2	1	95	96
292	mouldy B	13.8	11	48	7.8	5.6	n.d.	4.5	n.d.	<2	0	50	8
531	mouldy B	18.0	289	37	39.0	6.6	5.9	n.d.	6.5	<2	5	84	50
533	mouldy B	16.8	11	50	31.0	6.0	3.8	4.0	4.8	<2	0	100	40
550	mouldy B	14.4	0	0	11.0	4.5	3.3	4.5	<3	<2	0	100	96
553	mouldy B	11.9	0	0	11.0	4.7	3.3	3.5	3.4	<2	0	100	2
555	mouldy B	12.4	3	25	12.5	5.6	4.7	<3	<3	3.6	6	64	18
558	mouldy B	13.9	0	0	10.0	4.0	3.8	4.0	<3	<2	0	92	20
564	mouldy B	14.3	6	0	8.6	4.2	4.0	3.0	<3	<2	0	100	50
568	mouldy B	14.7	0	12	11.0	4.7	4.0	4.0	4.1	<2	2	100	6
572	mouldy B	13.3	4	14	6.0	4.0	3.5	<3	3.3	<2	4	80	40
588	mouldy B	16.6	3	26	10.0	5.1	4.3	<2	<2	3.3	1	86	70
573	mouldy C	16.7	178	37	49.0	7.1	4.7	5.8	4.5	<2	0	100	60
580	mouldy C	9.9	934	35	64.0	7.4	6.2	<3	<3	4.0	9	100	0
582	mouldy C	9.8	4	857	55.0	7.3	6.3	<3	<3	6.2	20	100	96
587	mouldy C	16.8	81	14	11.0	5.3	4.0	<3	<3	<2	1	100	60
600	mouldy C	17.2	26	110	25.0	6.0	5.3	5.4	<4	<2	7	98	86
602	mouldy C	16.5	43	0	14.0	4.4	6.0	<4	<4	<2	1	100	78
603	mouldy C	14.5	0	0	6.5	4.4	3.0	<3	4.4	<2	0	100	80
569	musty B	13.8	0	281	11.4	2.0	<2	<2	2.3	<2	1	56	30
574	musty B	15.4	5	16	6.0	4.7	4.0	4.0	3.8	3.3	0	980	30
589	musty B	17.3	13	60	10.0	4.6	4.6	3.7	<3	<2	0	96	2
6	musty C	13.0	10	89	13.0	4.7	3.0	n.d.	3.5	<2	0	98	93
552	sour B	15.9	0	80	13.5	5.3	4.4	4.3	4.5	<2	0	100	30
561	sour B	14.3	0	18	6.5	5.1	4.0	3.6	4.2	<2	0	100	52
565	sour B	15.9	14	10	25.0	5.9	4.6	4.3	4.7	<2	0	100	92
601	sour B	15.4	0	0	9.1	3.9	3.2	<2	3.1	<2	0	100	60

A = weak; B = pronounced; C = strong; Pen = *Penicillium* species; Asp = *Aspergillus* species; Eur = *Eurotium* species; Fus = *Fusarium* species.

[a] # = number of *Fusarium* colonies on CZID.

[b] Int. Inf. = % internal infected kernels.

[c] n.d. = not determined.

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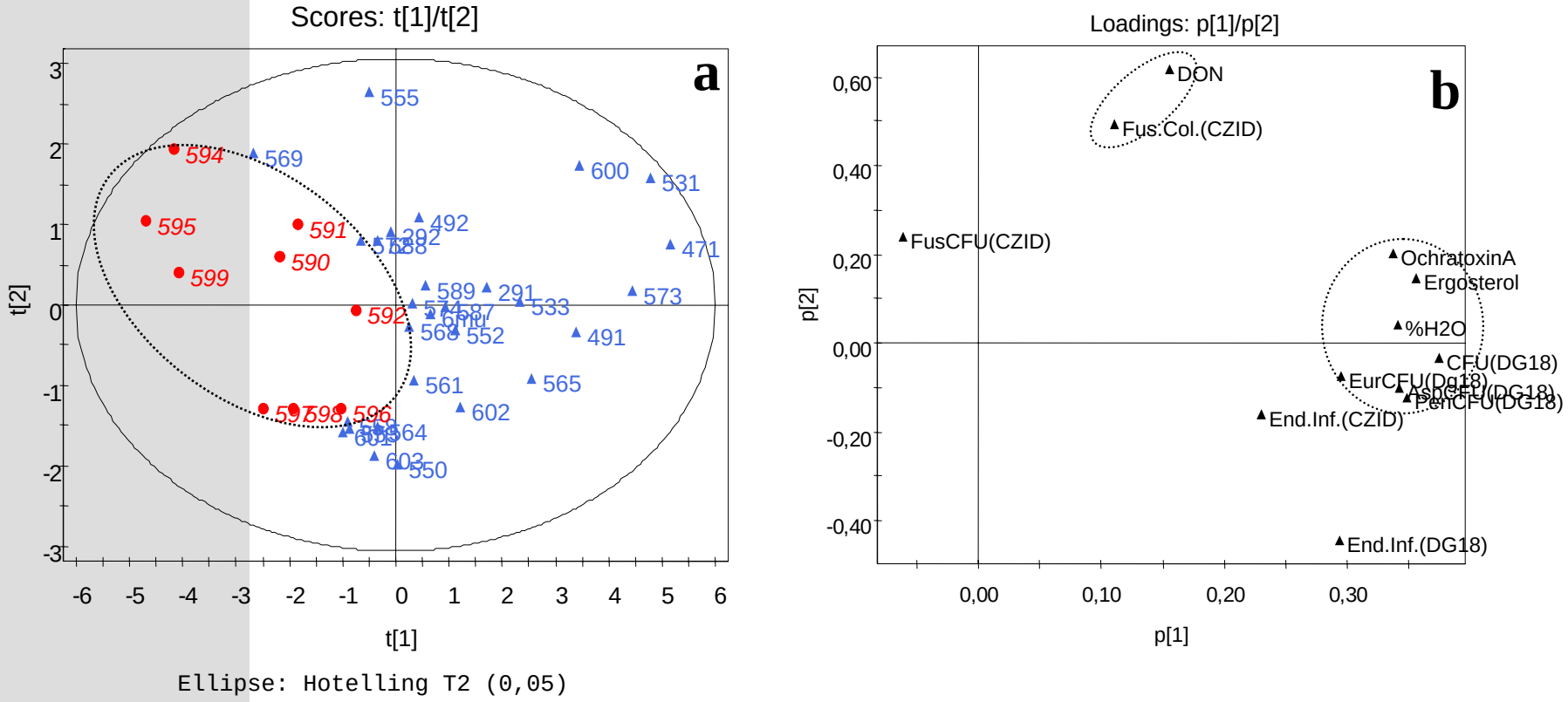
PCA mikrobiologiskt innehåll



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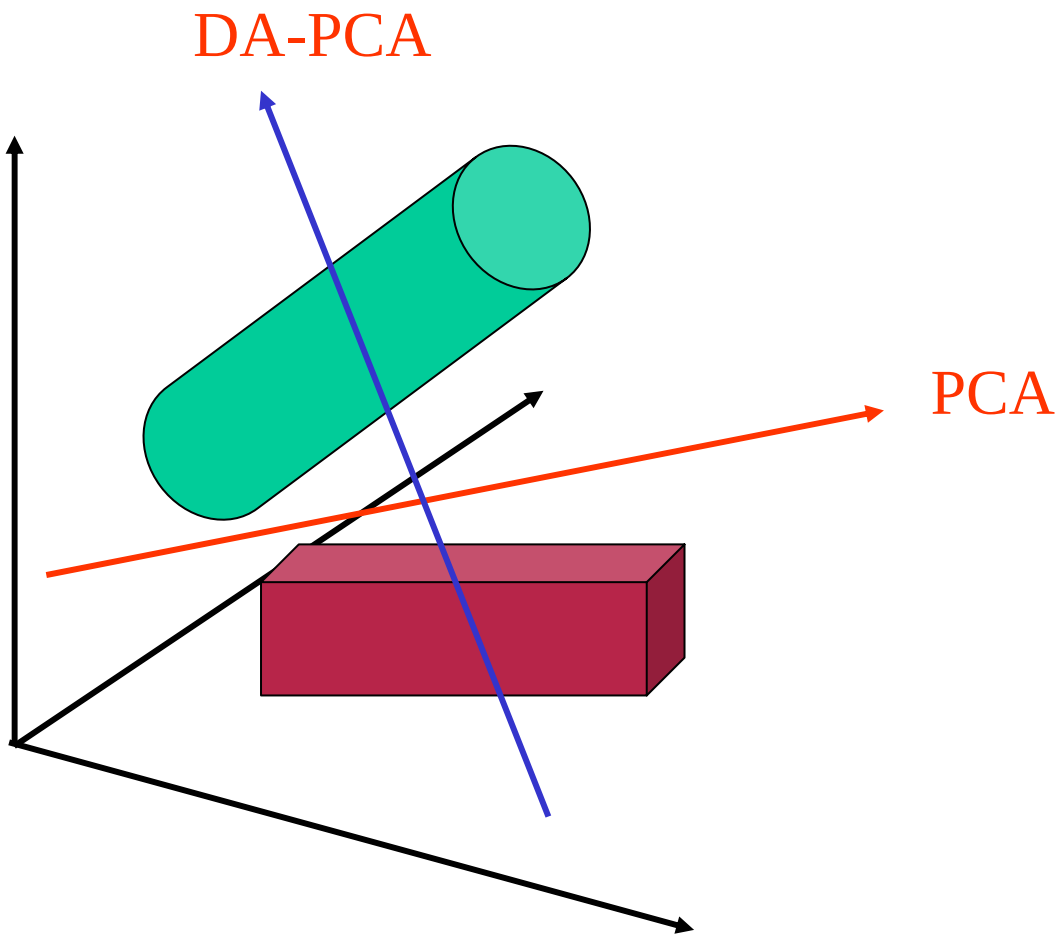
Principal Component Analysis PCA



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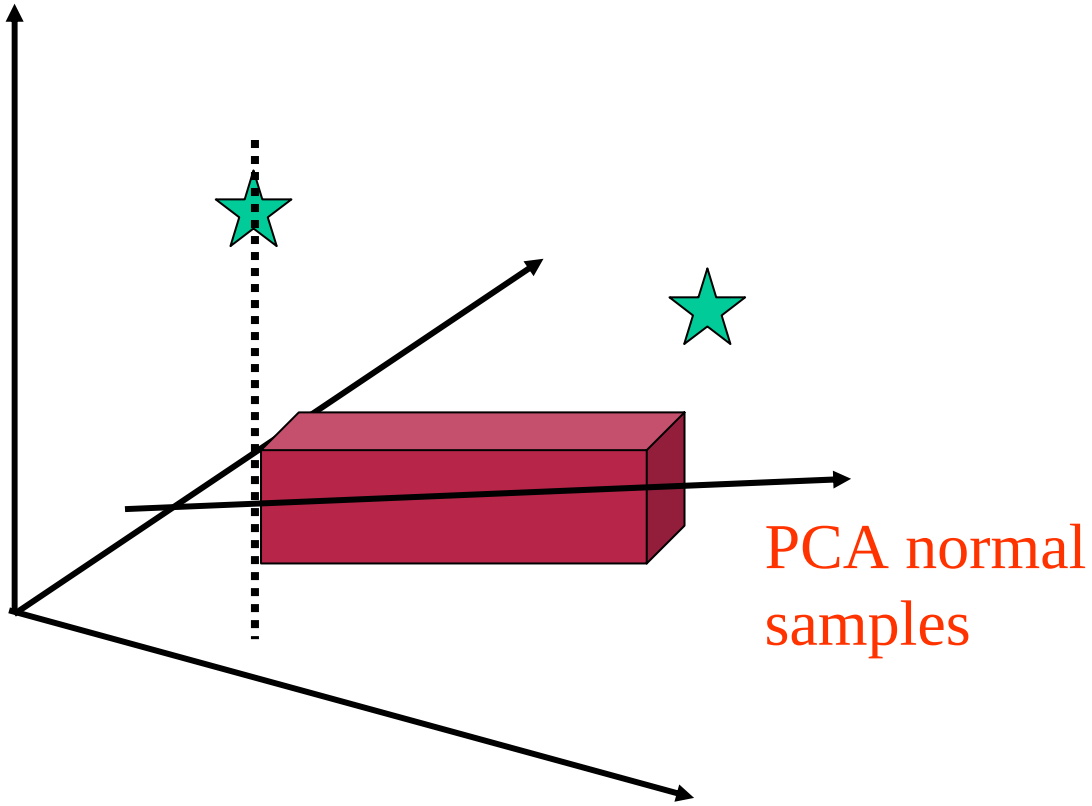
DMod X



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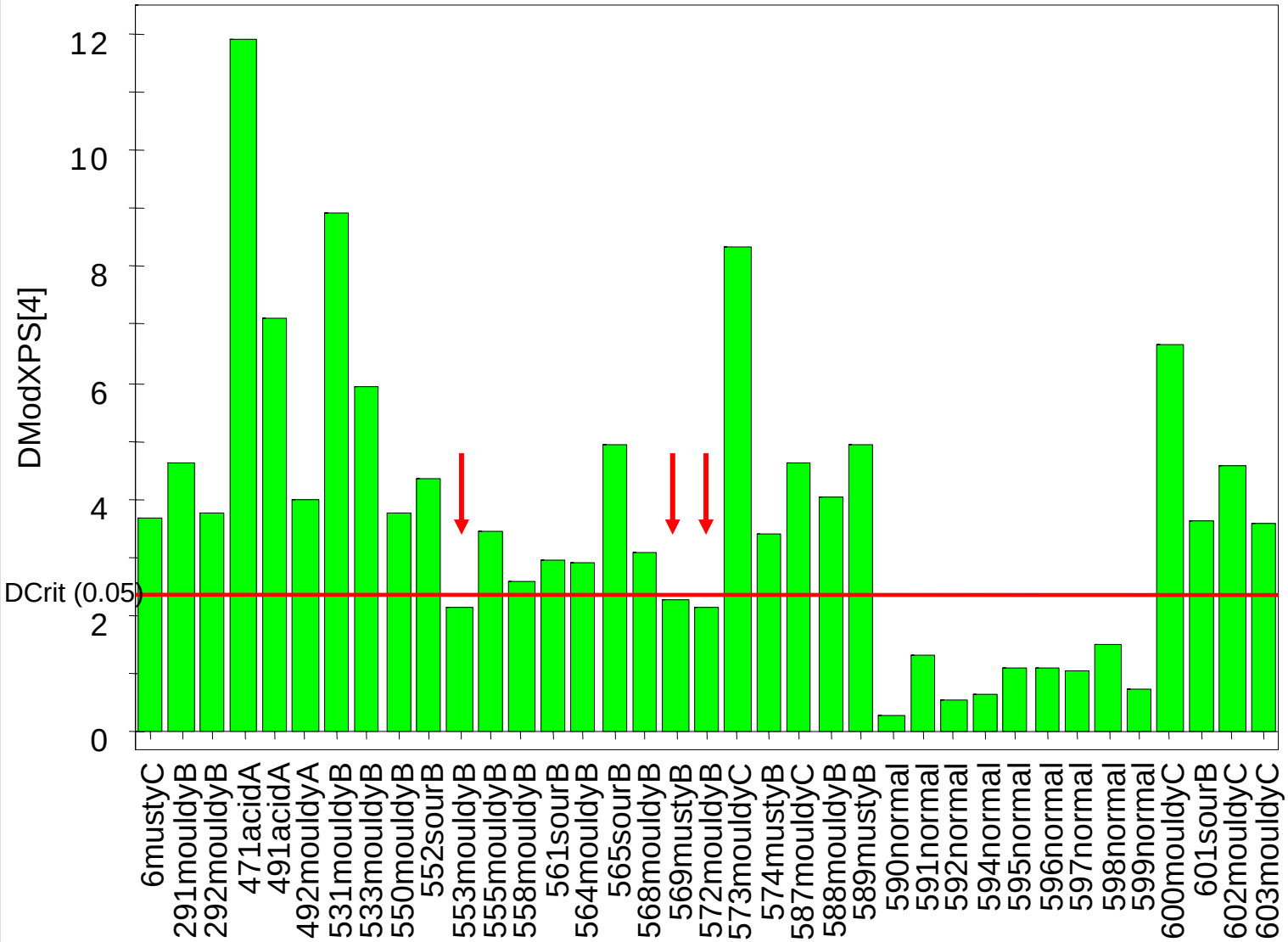
Prediction of odour class (normal or off-odour) using mycological data



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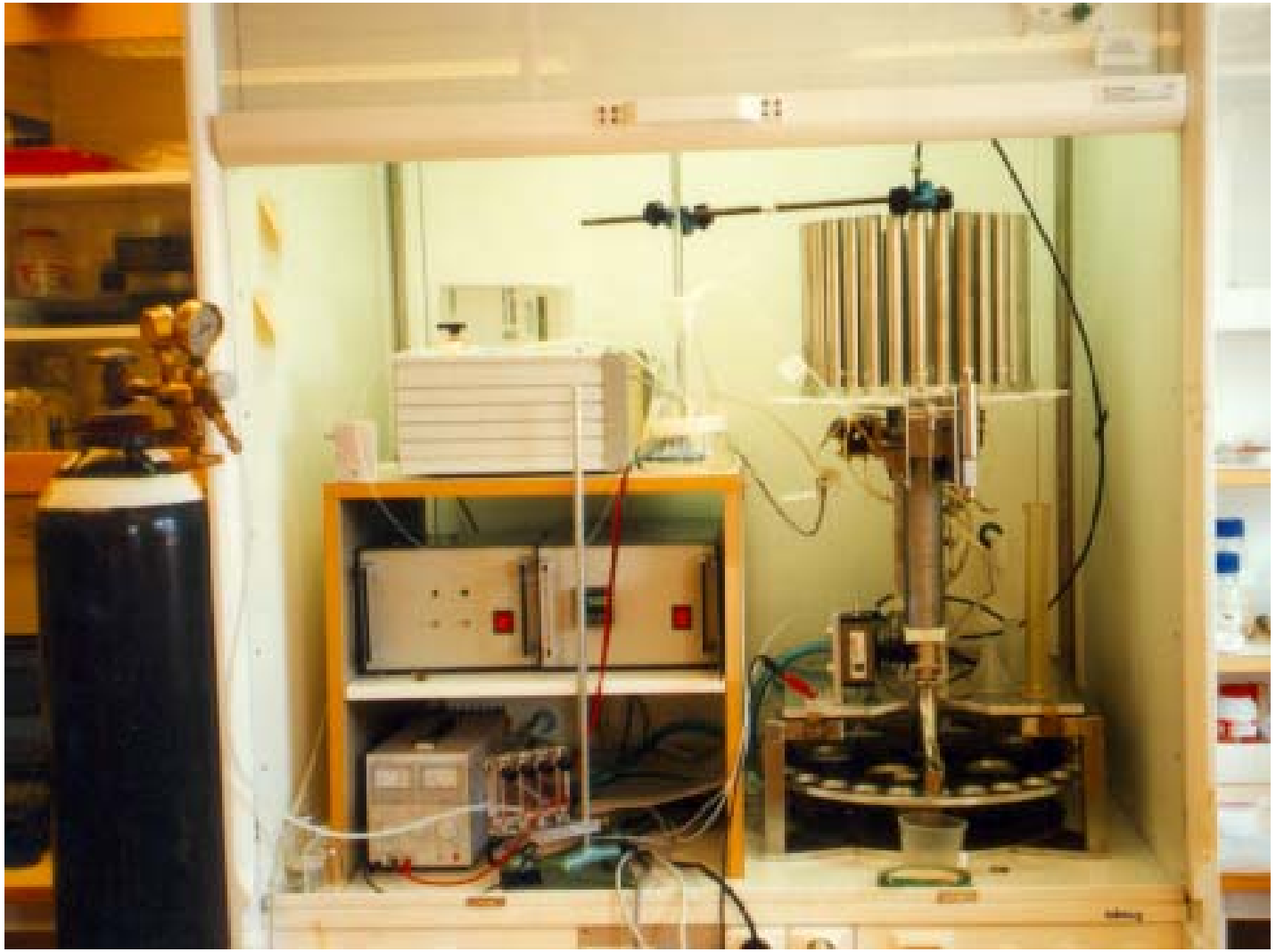
Electronic nose



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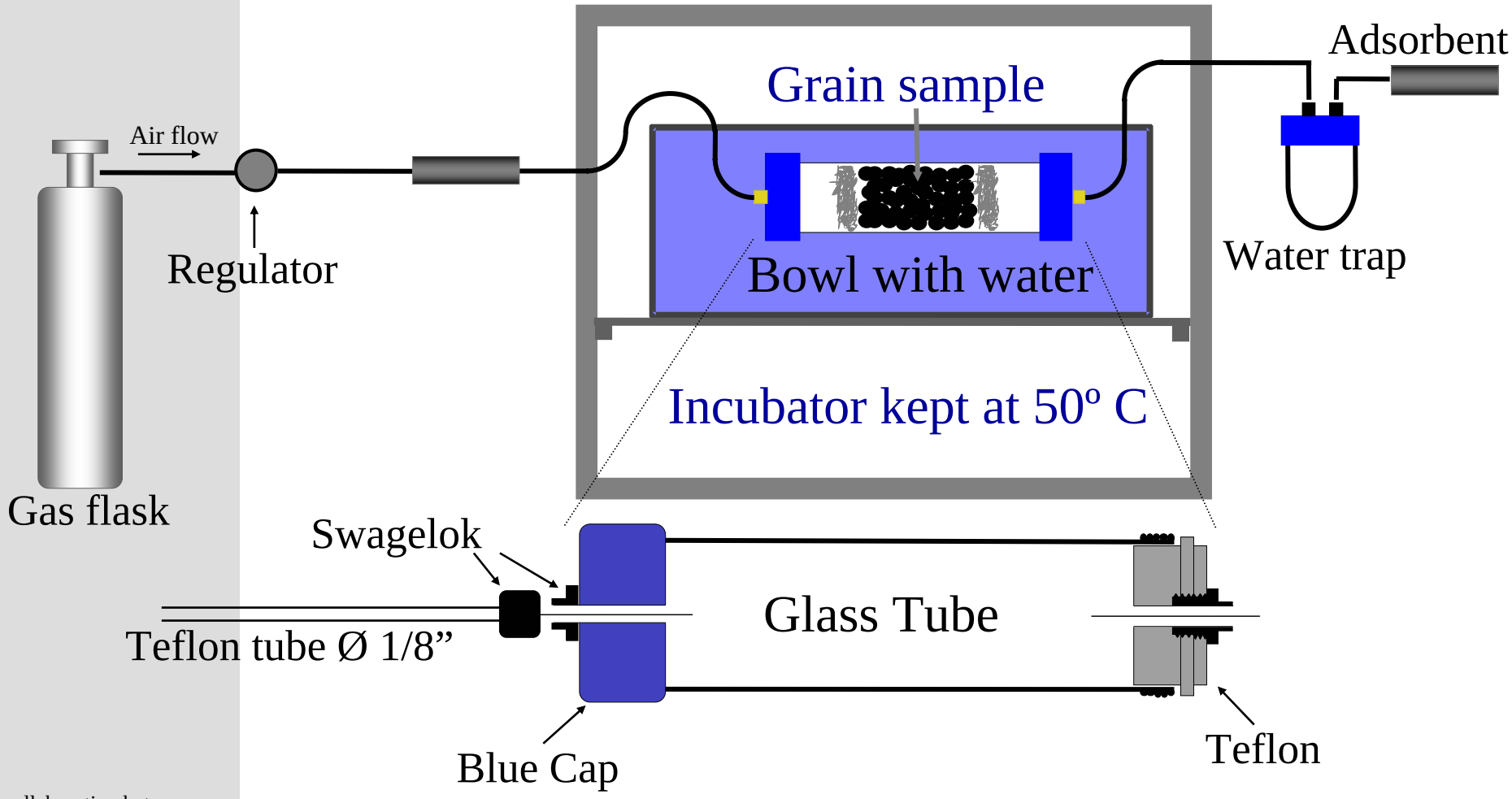
Uppsamling av flyktiga metaboliter för GC-MS analys



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Data set

Electronic nose	GC-MS
1 => 3 subsamples - analysed 3 times - totaly 850 measurements were done 85 sensor signals	40 samples - over 200 volatiles detected -103 identified and used
850 x 85 matrix	40 x 103 matrix

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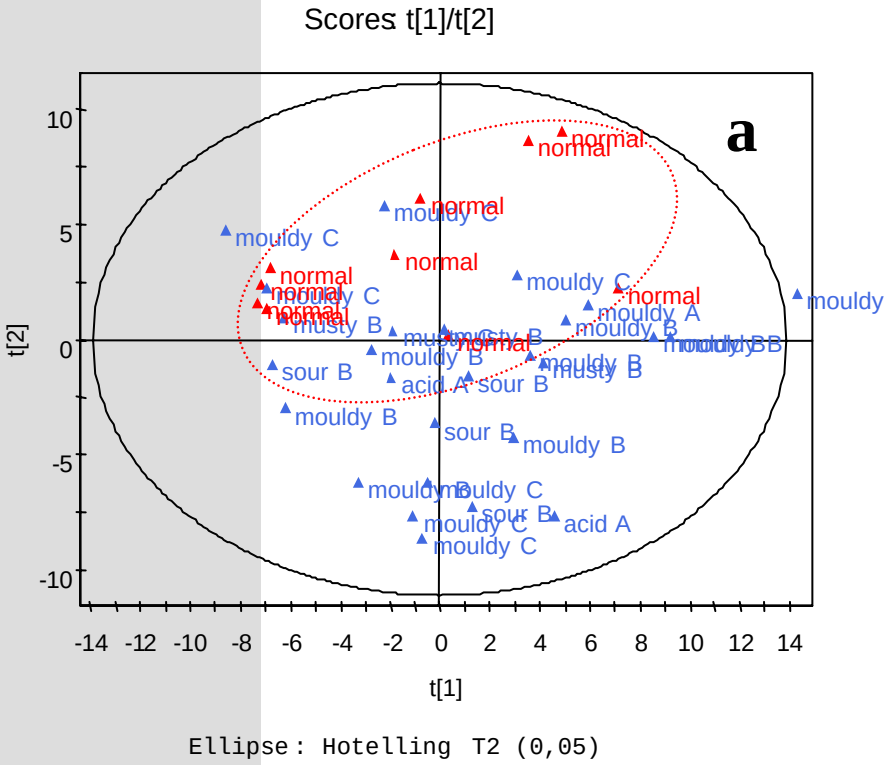
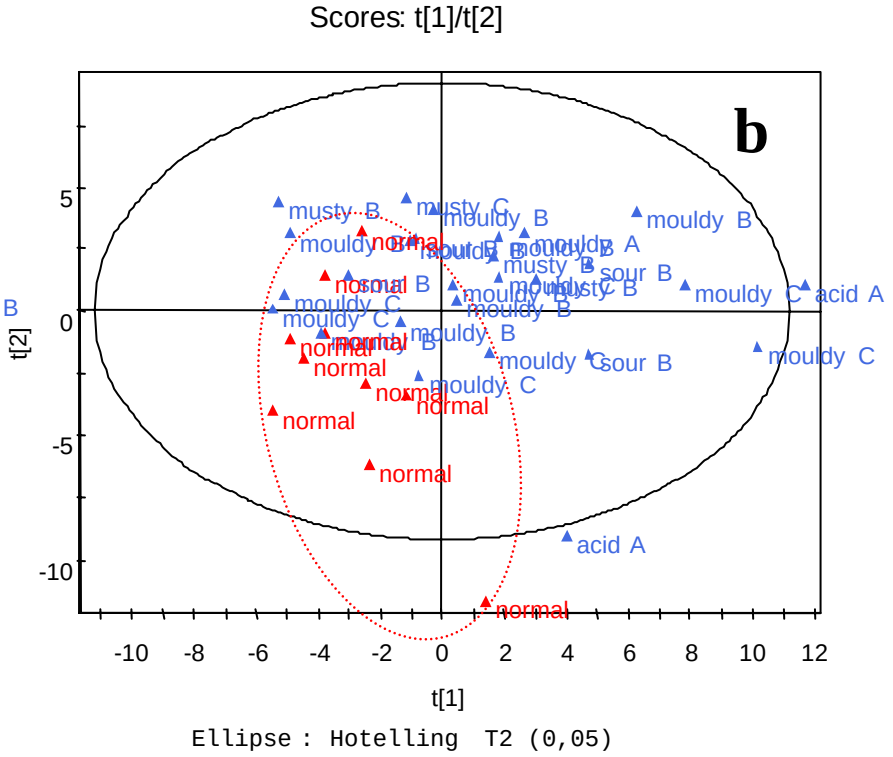
Separation of odour class
a) electronic nose b) GC-MS





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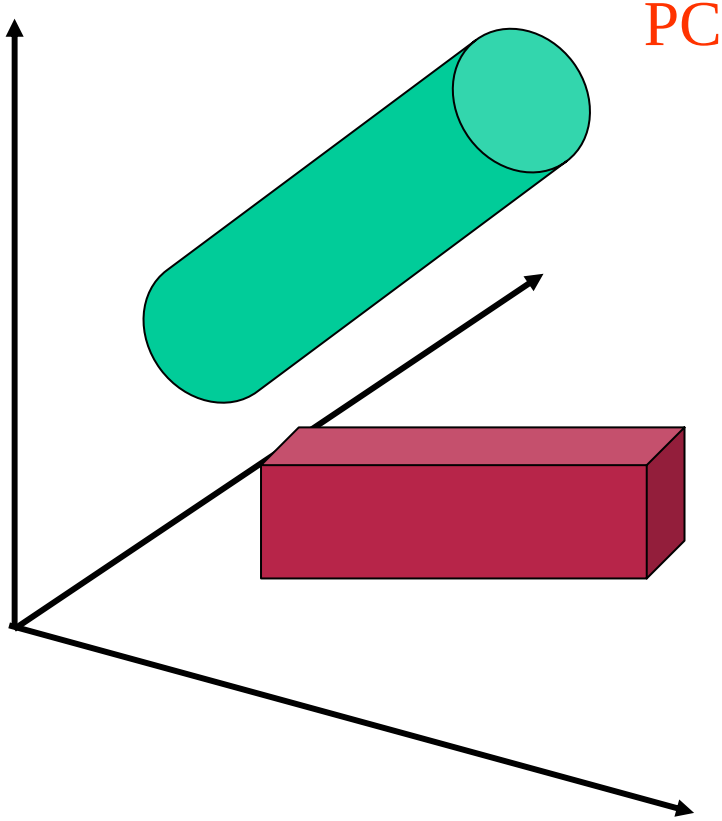
Principal Component Analysis PCA



PCA bad samples

PCA normal samples

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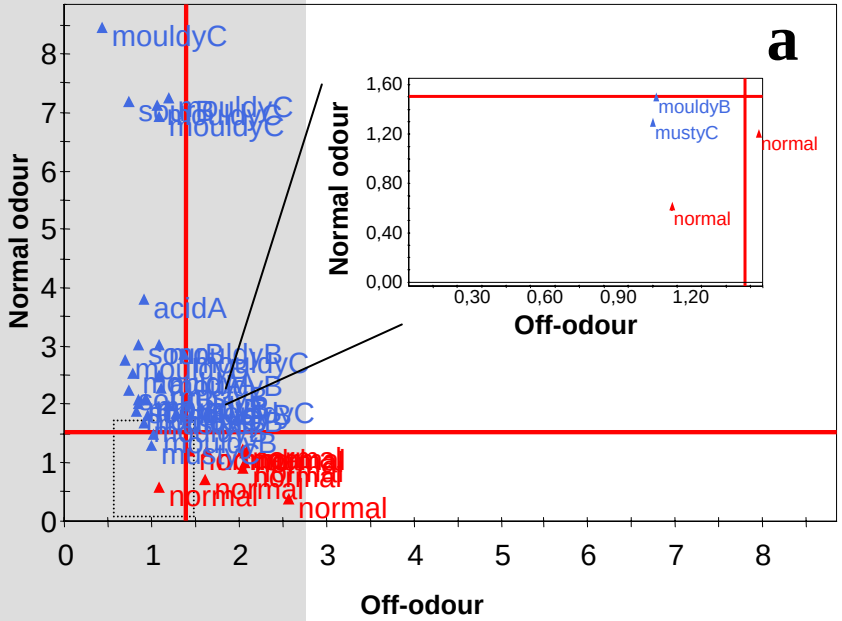
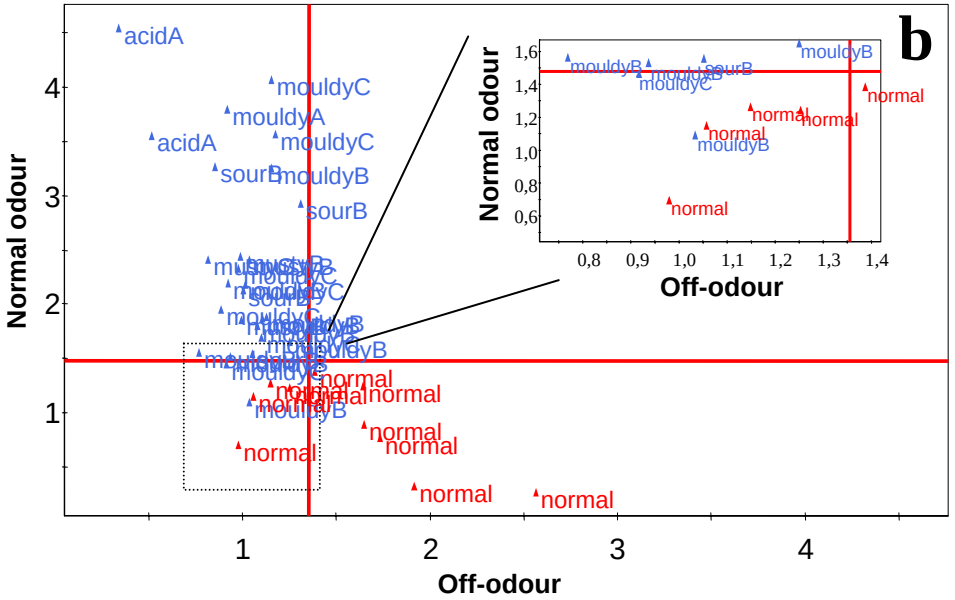
Cooman´s plot
a) electronic nose b) GC-MS





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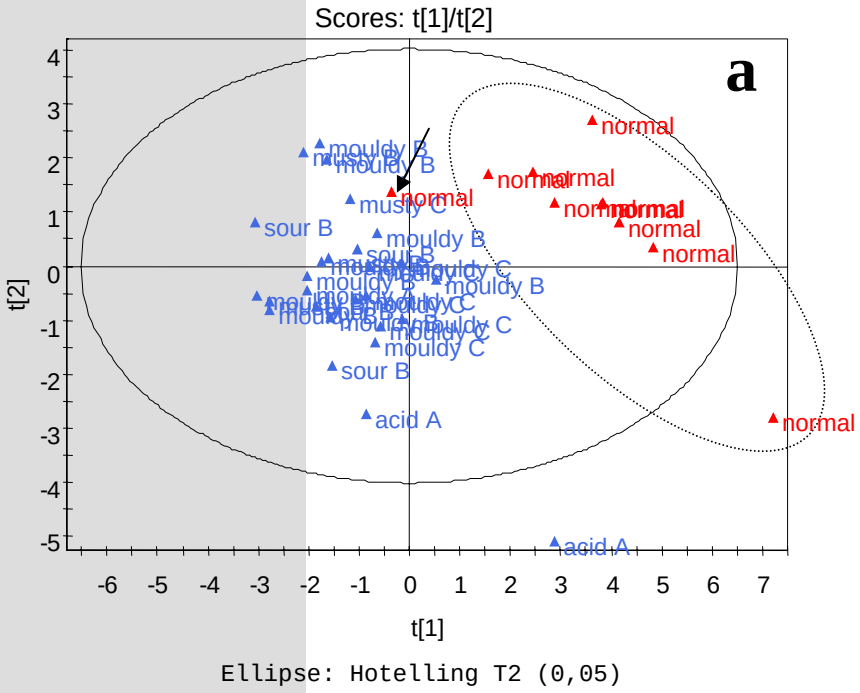
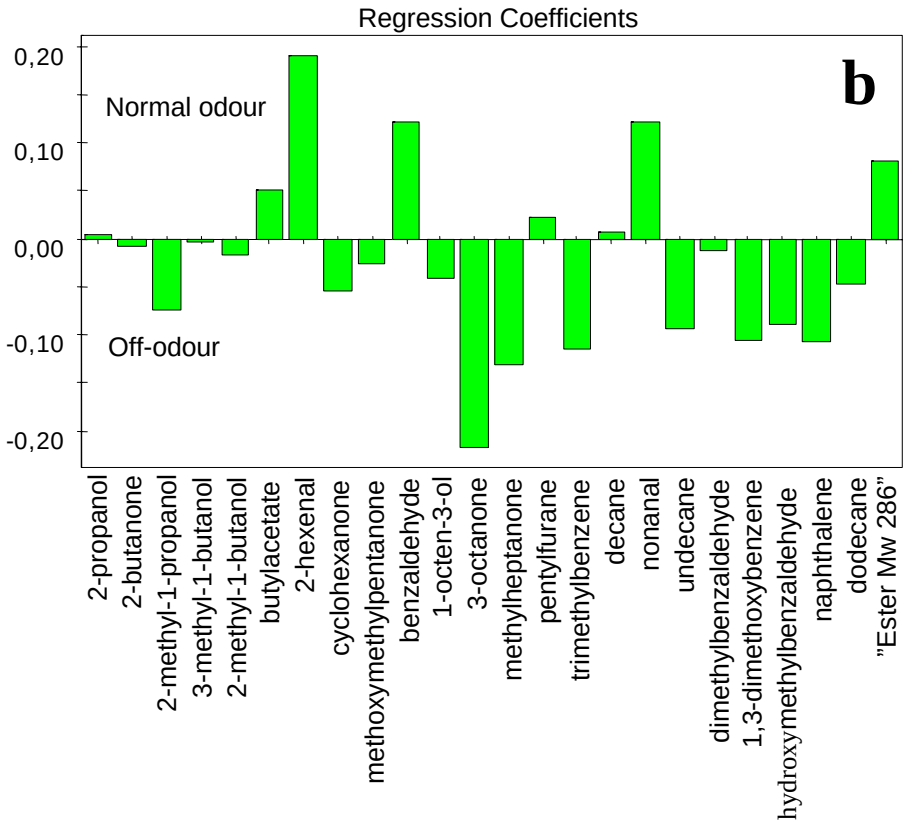
DA-PLS using GC-MS data





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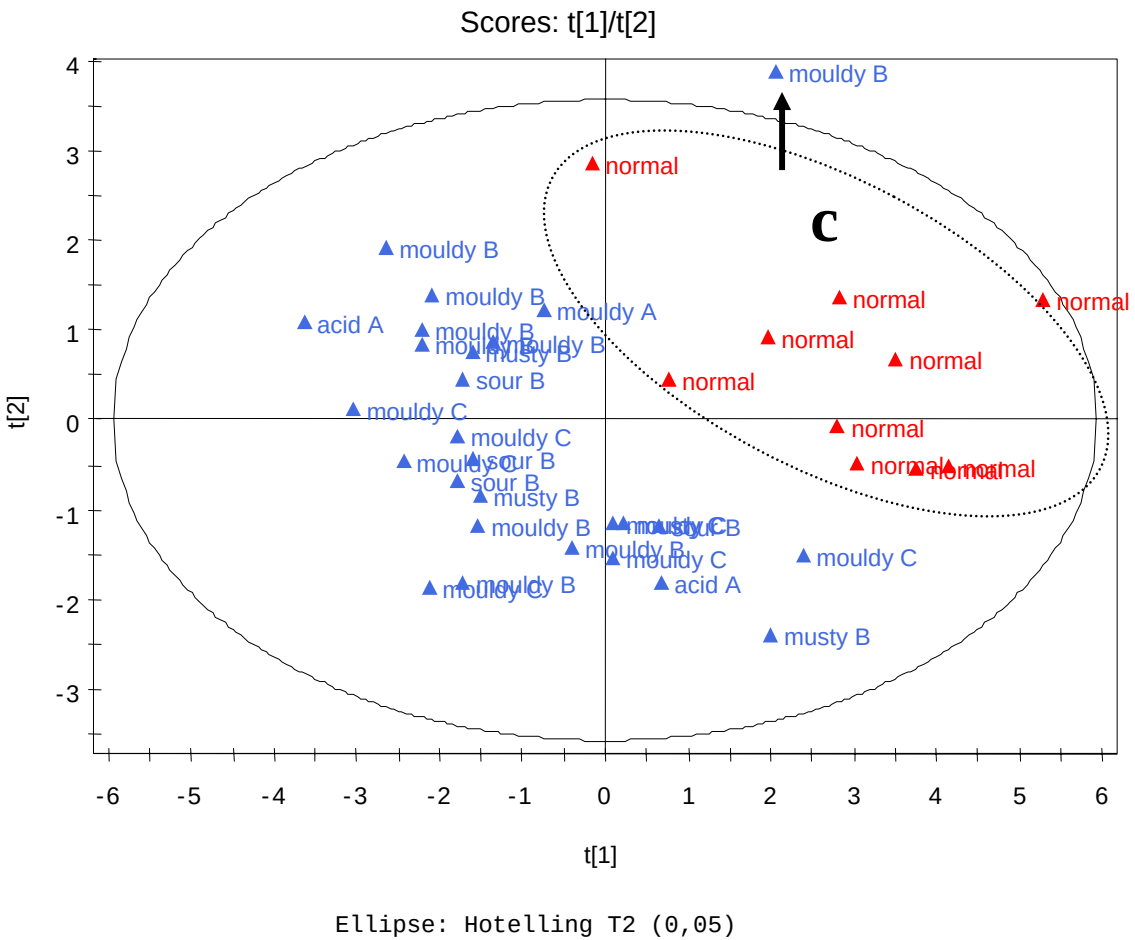
DA-PLS using electronic nose



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Prediktion of ergosterol with a PLS model based on electronic nose or GC-MS data

Overview of the PLS models that were used for ergosterol prediction in the interval 0–30 mg/kg and the OSC-PLS models that were used for CFU prediction in the interval log 2–7.4: RMSEE and RMSEP values for these models are also given

Method	Instrument	PC	$R^2_{X(\text{cum})}$	$R^2_{Y(\text{cum})}$	$Q^2_{(\text{cum})}$	RMSEE	RMSEP
Ergosterol	GC–MS	2	0.43	0.88	0.61	1.22	1.13
Ergosterol	Electr. nose	3	0.81	0.90	0.70	1.24	1.62
CFU	GC–MS[a]	2	0.22	0.89	0.50	0.51	1.21
CFU	Electr. nose[a]	3	0.73	0.77	0.55	0.70	1.45

[a] One OSC component was subtracted.

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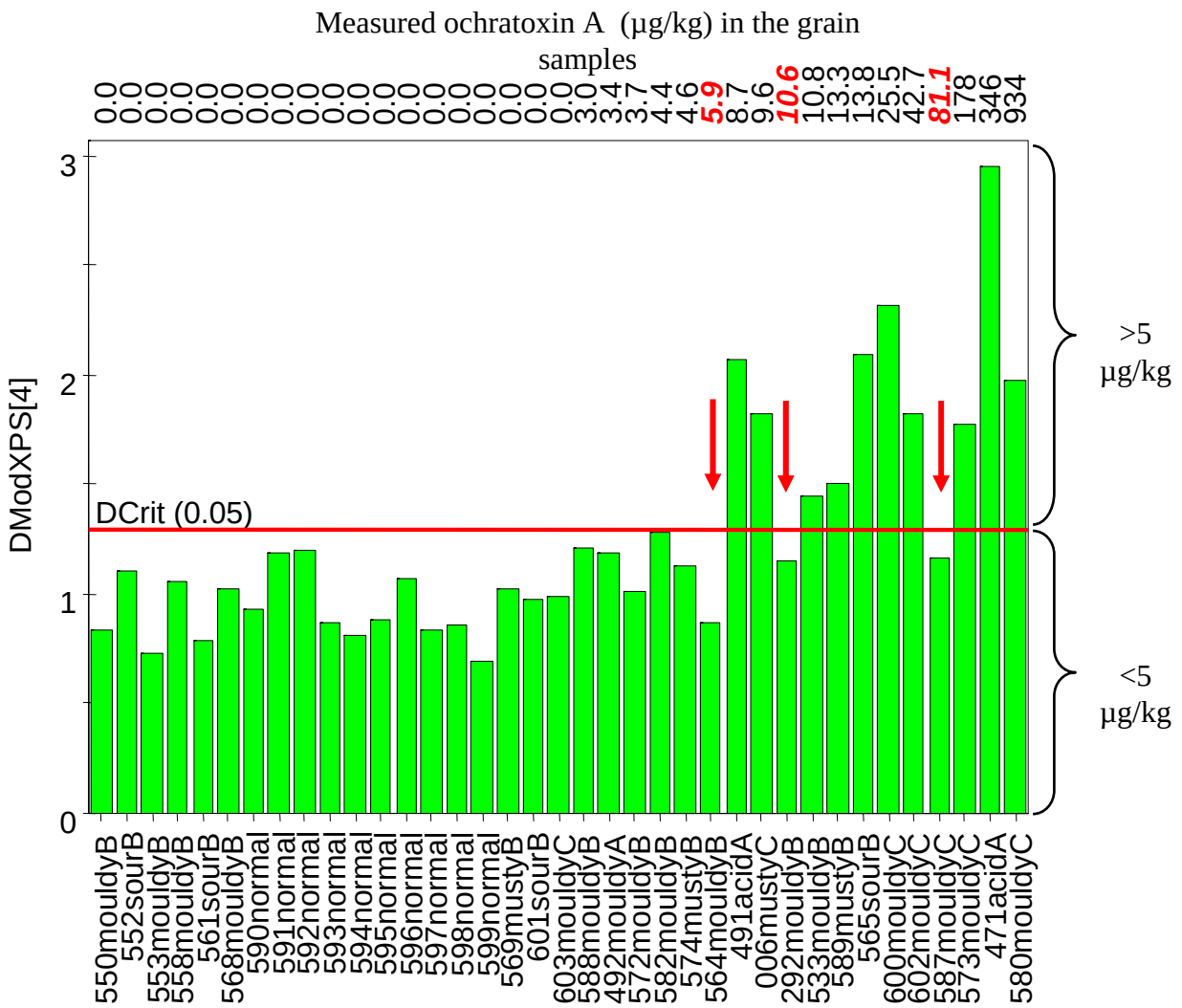
Prediction of OA class using GC-MS data



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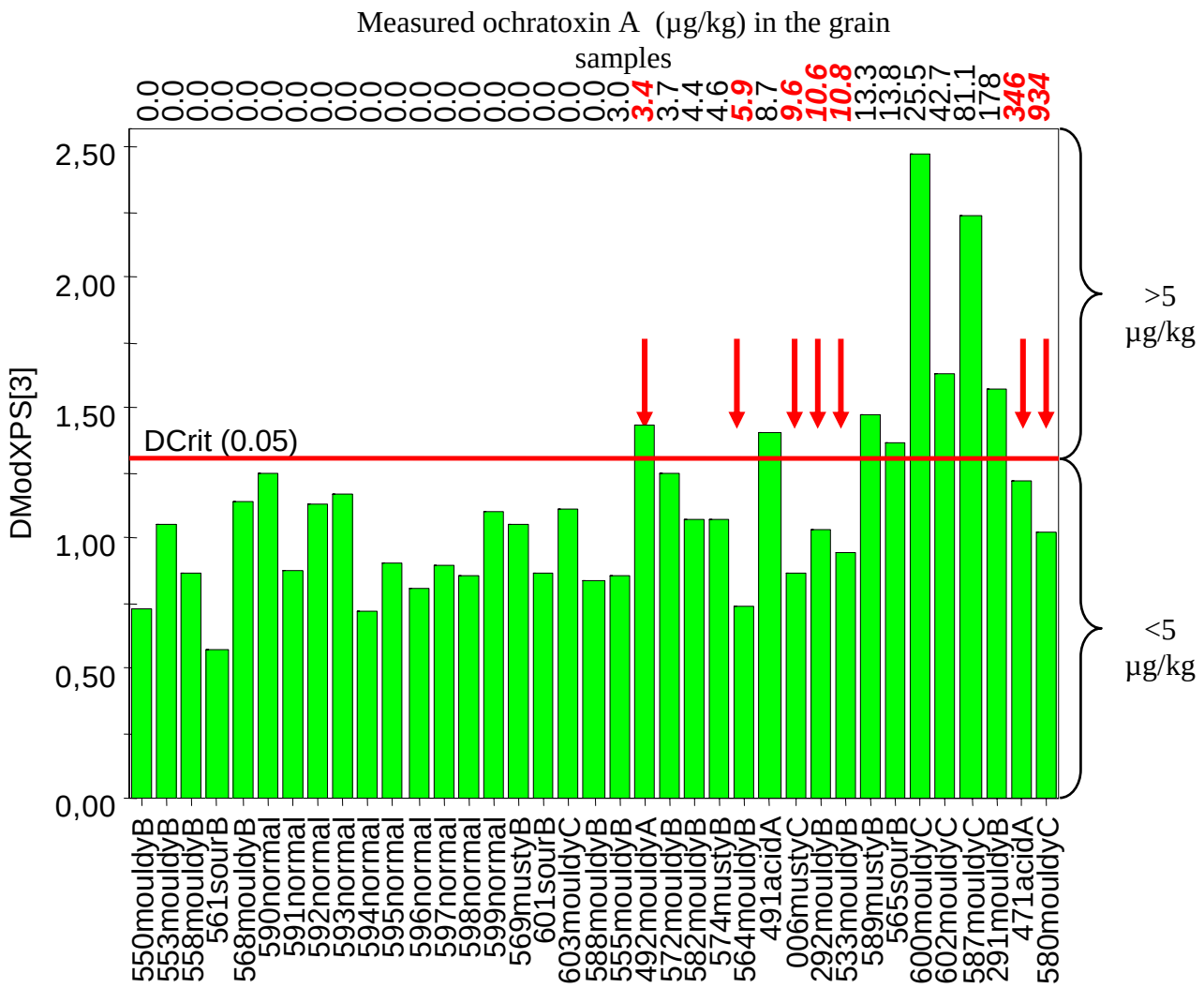
Prediction of OA class using electronic nose data



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Most important volatiles

3-octanone, trimethylbenzene, undecanal, 1-octanol viktigaste prediktera ergosterol

1,3-dimethoxybenzene, phenol, dodecane pos. korr. 1-butanol, 1-pentene-3-ol, 1-heptanol, nonanal neg. korr. CFU

Prover med lågt innehåll av ochratoxin A innehöll andra metaboliter än de prover som hade högre halter OA

Pentane, methylpyrazine, 3-pentanone, 3-octene-2-ol positiv korr DON medan ethylhexanol, pentadecane, toluene, 1-octanol, 1-nonanol, 1-heptanol neg korr.

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Infratec NIT equipment

FOSS

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Future perspectives

•2006: Focus on wheat for feed purposes:

- ELISA-tests (DON and ZEA) above certain levels
- Separation above certain levels
- If high levels found:

  Extend to other cereals

  Separate low levels also.

•2007:

- Included in normal routine at all elevators.
- Separation at certain levels.

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Sammanfattning

- Elektronisk näsa och GC-MS kan prediktera luktklass
- Lättare prediktera ergosterol än CFU
- Komplexa data som är multifaktoriella kräver multivariata utvärderings tekniker

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Finansiärer

Stiftelsen Cerealia
Svenska lantmännen
SJFR
SLU
S-SENCE

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