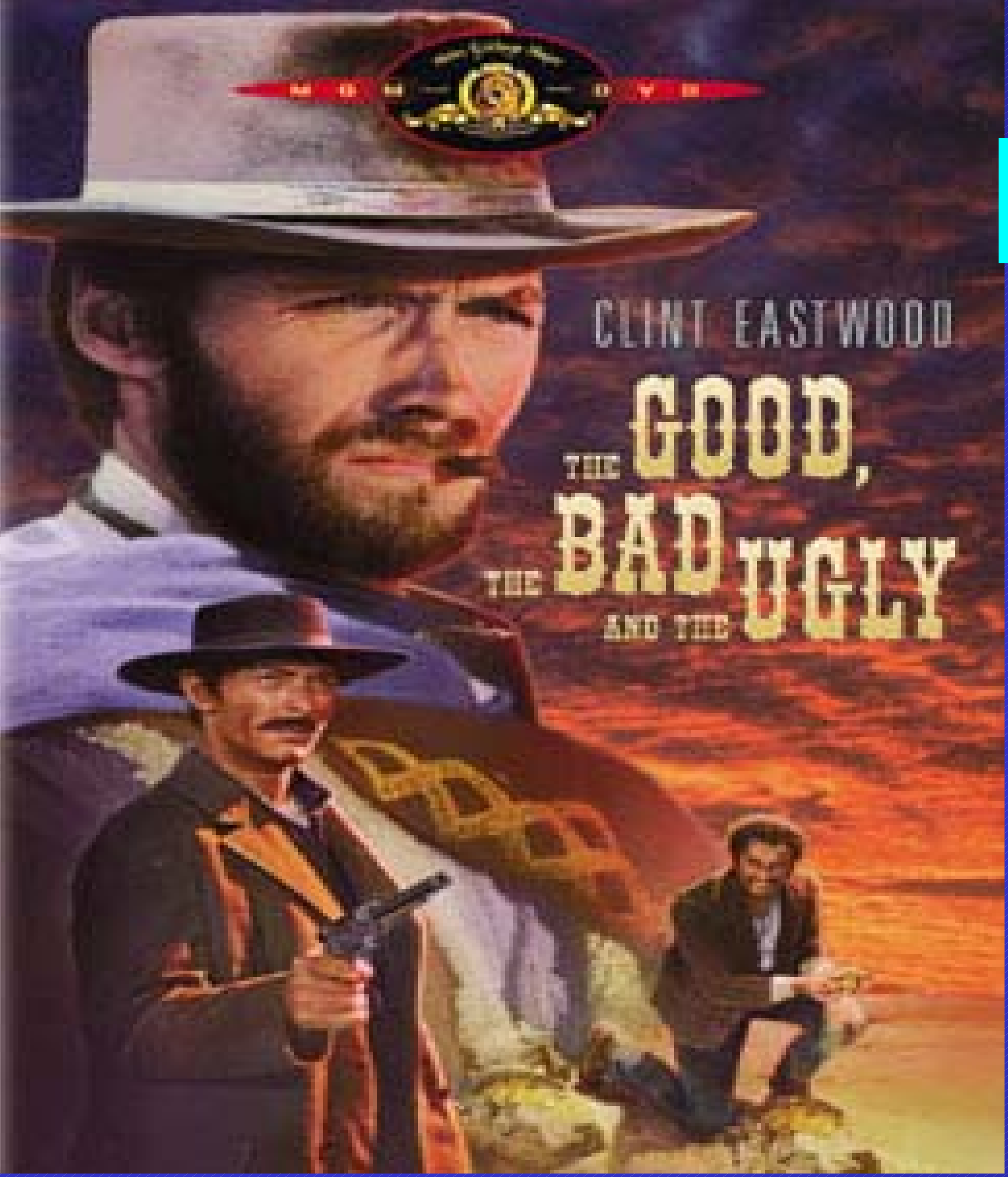


Meat Microbiology

The GOOD (technological flora)

The BAD (Pathogens) &

The UGLY (spoilage flora)

Agricultural University of Athens

Dept. Food Science & Technology

Laboratory of Microbiology & Biotechnology of Foods

Microbiology of spoilage of chilled products

Microbiology of Spoilage; spoilage of chilled food products; spoilage ecology as a function of process and packaging; Quantitative evaluation of spoilage

George – John E. Nychas

Microbiology of Spoilage;

Spoilage of chilled food products;

Ecology of Spoilage as a function of process and packaging;

Quantitative evaluation of spoilage

What is spoilage

Who is causing spoilage

How we can evaluate/measure spoilage

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied

What is (meat) spoilage

Who is causing spoilage

How we can evaluate/measure spoilage

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied

Spoilage of meat can be considered as an ecological phenomenon that encompasses changes of the available components (e.g. low molecular compounds), during proliferation of bacterial present in the microbial association of the stored meat.

Food (meat) spoilage can be considered as any change which renders a product (meat) unacceptable for human consumption (Hayes 1985)

What it makes Meat Unacceptable

Color defects

Changes in texture

Development of off-flavor, off-odor and slime as a result of microbial growth

What is spoilage

Who is causing spoilage

✓General aspects of spoilage organisms

✓Factors affecting their development

✓spoilage ecology as a function of process and packaging

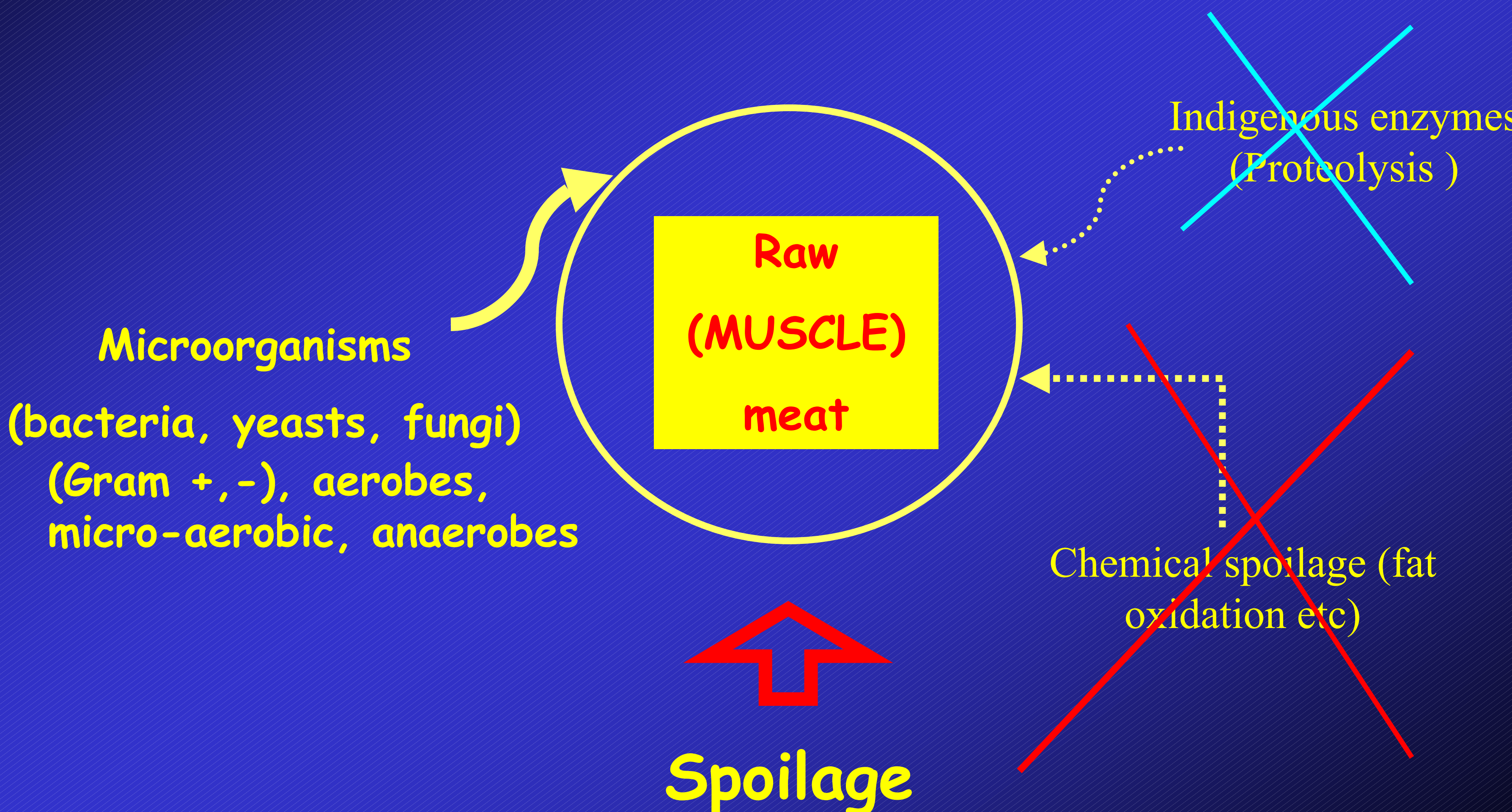
How we can evaluate/measure spoilage

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied

Gill 1976, 1986; Dainty & Mckey 1992; Nychas et al. 2006



Meat Microbial Ecology

A small fraction will spoil the meat

Association [Westerdijk 1949]

Specific association & Dynamic Spoilage Association [Mossel 1992 & Mossel et al.1996]

Spoilage association [Gram & Huss 1996]

Specific Spoilage Organisms [Dalgaard 1993]

Ephemeral Spoilage Organisms (Nychas & Skandamis 2005; Nychas, Marshall, Sofos 2006]

Facultative anaerobes	**Catalase reaction-negative**
Shewanella putrefaciens *S. baltica* *S. oneidensis* *Photobacterium phosphereum* *Enterobacteriaceae* *Serratia* spp. *S. marcencens* *S. liquefaciens* *Citrobacter* spp. *C. freundii* *C. koseri* *Providencia aerogenes* *Enterobacter* spp. *E. aerogenes* *E. cloacae* *E. agllomerans* *Hafnia alvei* *Kluyera spp.* *Morganella morganii* *Pantoea agglomerans* *Raoultella spp.* *R. planticolla (Kl. pneumonieae)* *Vibrionaceae*	*Lactobacillus* spp. *L. sake* *L. curvatus* *L. bavaricus* *Carnobacterium* spp. *C. divergens* *C. piscicola* *Leuconostoc* spp. *L. carnosum* *L. gelidum* *L. amelibiosum* *L. mesenteroides* subsp. *mesenteroides* *Pediococcus* spp. *Weissella* spp. *W. hellenica* *Lactococcus raffinolactis*

Gram-negative bacteria	Gram-positive bacteria
Aerobes	Catalase reaction-weak
Neisseriaceae	
Psychrobacter immobilis	*Brochothrix thermosphacta*
P. phenylpyruvica	*Kutrhia zophii*
Acinetobacter spp.	*Staphylococcus* spp.
A. twoffii	*Clostridium estertheticum*
A. Johnsonii	*Clostridium frigidicarnis*
Pseudomonadaceae	*Clostridium casigenes*
Pseudomonas rRNA homology	*Clostridium algidixylanolyticum* sp. nov
Group 1	
Pseudomonas fluorescens	
Biovars I, II, III, IV,V (includes 7 clusters)	
P. lundensis,	
P .fragi	
P. putida	

	Meat/Fish			
Cardinal Members of the	South EU		North EU	
Microbial association of meat	air	Vp/ map	air	Vp/ map
Pseudomonas spp.	**		**	†
Br. thermosphacta	*	*/*		nd
Lactic acid bacteria		**		**
Sh. putrefaciens	†	†	NO data	NO data
Enterobacteriaceae	†		†	
Phot. Phosphereum			†	†

Table 1.1: Minimum (min), maximum (max) and average (mean), of microbiological flora of ground pork from Athens open market.

Microorganisms	**Samples**	**Min**	**Max**	**Mean**
			log cfu/g	
TVC	50	4.60	8.49	6.74
Pseudomonads	50	3.30	8.47	6.33
Br. thermosphacta	50	3.47	7.60	5.82
Lactic acid bacteria	50	2.60	6.74	4.81
Shewanella putrefaciens	**50**	**1.00**	**6.48**	**4.08**
Enterobacteriaceae	50	1.70	6.48	4.03
E.coli	50	<1	4.48	1.21
Salmonella	50	<2	<2	<2
Listeria	50	<2	2,00	<2
pH	50	5.49	6.42	5.92

	Meat		Finfish	
Component	***Pre***	***Post***	***Pre***	***Post***
Creatine Phosphate	300	nf	9.3*	0.2*
Creatine	450	650	nd	nd
Betaine	nf	nd	nad	100**
ATP	300	nad	6.5*	0.2*
IMP	20	300	nd	nd
Glycogen	1000	100	220	40
Glucose	500	100	220	40
Glucose-6-Phosphate	100	200	21	32
Lactic acid	100	900	100	400
pH	7.2	5.5	7.3	6.5
Free Amino acids	200	350	nad	250
TMAO	nd	nd	nd	350-1000
Carnosine & Anserine	300	300	nd	100***

Substrates used for growth of major meat spoilage microorganisms

	A	B	C	D
Glucose	1	1	1	1
glucose-6-P,	2			2
D-L Lactic acid	3	2		3
Pyruvate,	4	3		
Gluconate,	5	4		
Gluconate 6-P,	6			
proprionate		5		
ethanol		6		
acetate		7		
Amino acids,	7	8	2	4
creatine	8			
creatinine	9			
citrate,	10			
aspartate,	11			
glutamate	12			
ribose			3	
glycerol			4	

A: *Pseudomonas* spp.

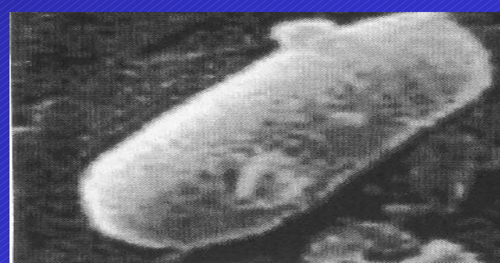
B *Sh. putrefaciens*

C. *Br. thermosphacta*

D. Enterobacter spp.

Peptides, proteins, fats

Glucose-6-P, GLUCOSE, Lactate, amino acids



Short term inoffensive end-products

In Short Time very offensive end-products (off-odours & off-flavours)

What is spoilage

Who is causing spoilage

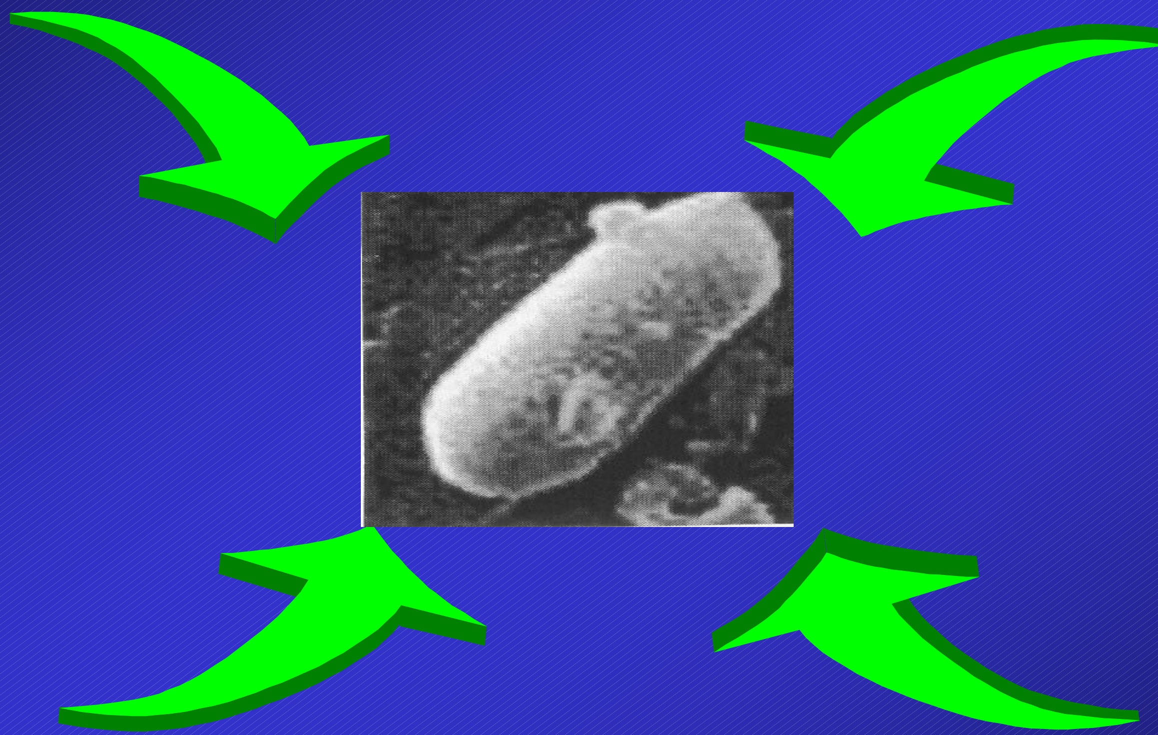
- ✓General aspects of spoilage organisms
- ✓Factors affecting their development
- ✓spoilage ecology as a function of process and packaging

How we can evaluate/measure spoilage

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied


Intrinsic factors
Extrinsic factors
Implicit factors
Processing factors

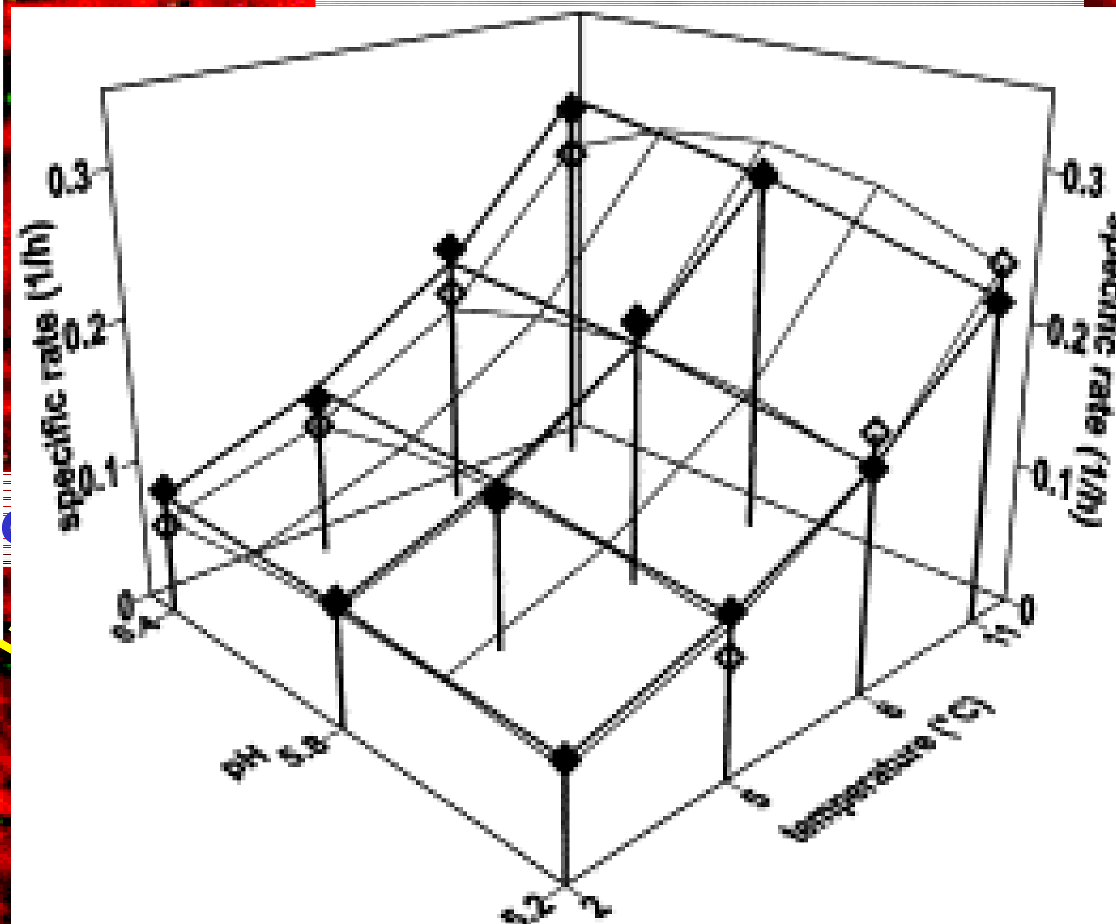
Niche / Domain; Y= Growth X=pH Z=T°C

Gram et al. 2002

N=available nutrients

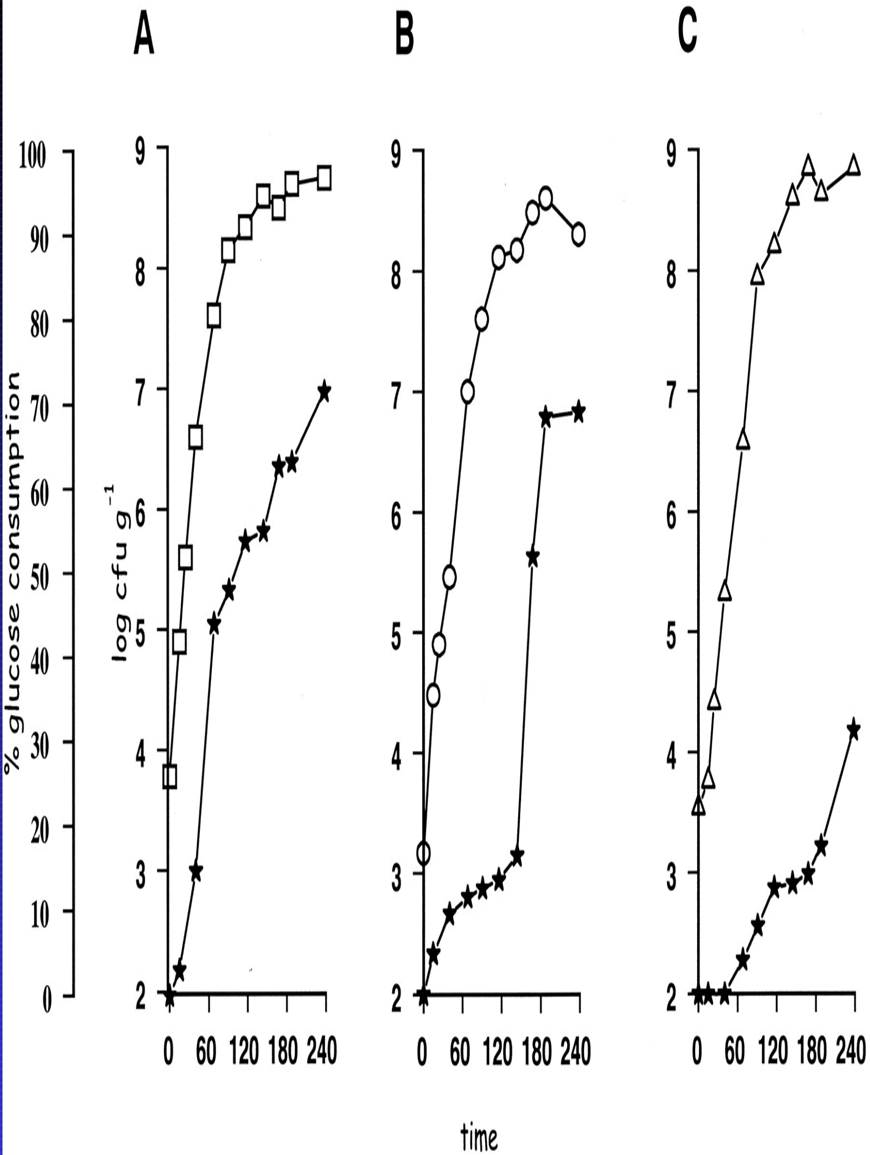
N+1=interacti

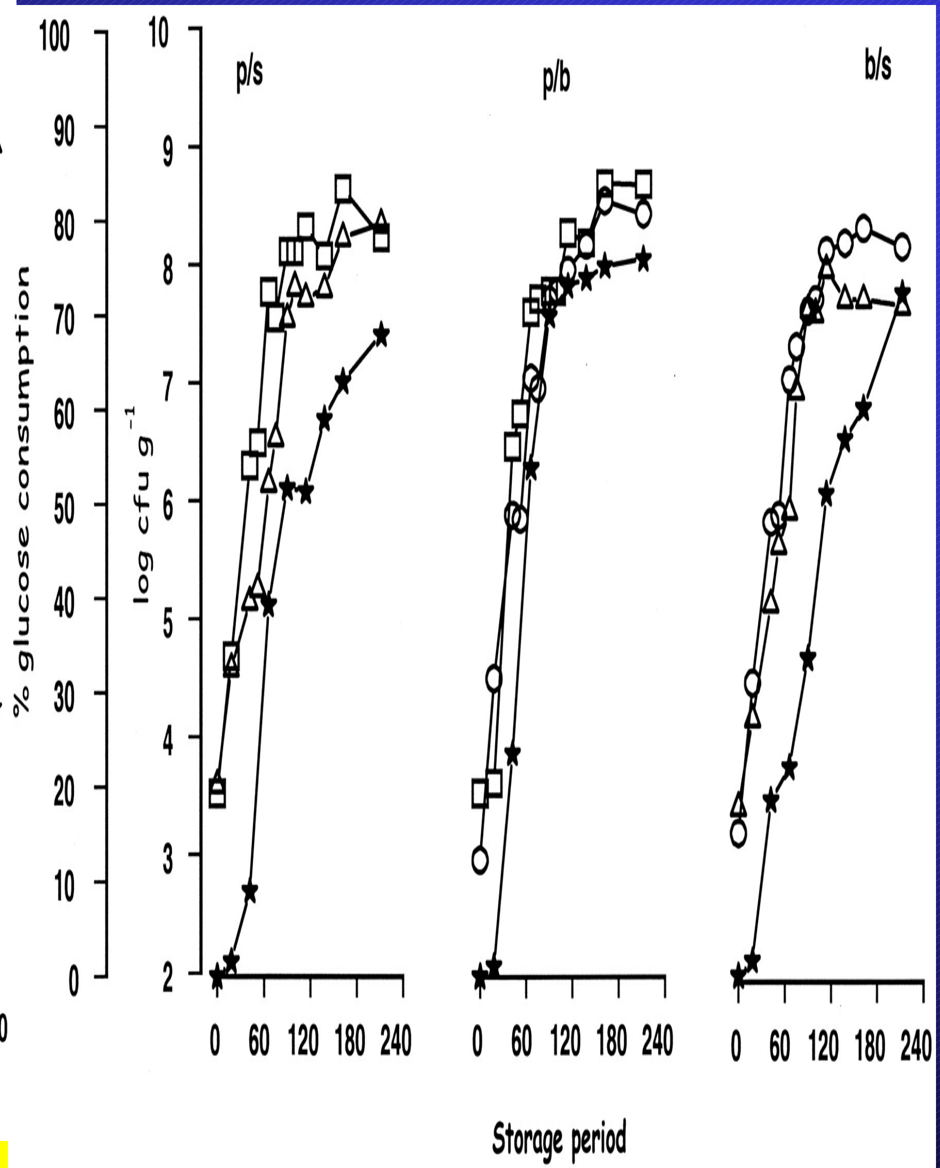
N+1



100 µm

Z





Appl. Environ. Microbiol. 69, 204 Tsigarida et al. (2003)

Growth curves of control cultures of *Pseudomonas* sp. strains (A), *B. thermosphacta* strains (B), and *S. putrefaciens* strains (C) and changes in glucose concentration (☆).

Appl. Environ. Microbiol. 69, 204 Tsigarida et al. (2003)

	rate (hrs^{-1})
C_P	
Pseudomonas sp.	0.057±0.005[a]
C_B	
Br. thermosphacta	0.036±0.002
C_S	
Sh. putrefaciens	0.053±0.003
M_{PB}	
Pseudomonas sp.	0.060±0.005
Br. thermosphacta	0.048±0.003
M_{PS}	
Pseudomonas sp.	0.055±0.004
Sh. putrefaciens	0.037±0.004
M_{BS}	
Br. thermosphacta	0.042±0.004
Sh. putrefaciens	0.047±0.005
M_{PBS}	
Pseudomonas sp.	0.058±0.006
Br. thermosphacta	0.039±0.002
Sh. putrefaciens	0.045±0.005

[a] Standard deviation

The type and rate of metabolic products in co-cultured samples of this organism with *B. thermosphacta* suggested that other responses (negative or positive) might also account for this inhibition.

Despite the extremely high levels of formic acid formation and the unknown peaks a and b, evident in singly cultured samples of *S. putrefaciens*, the corresponding levels were significantly lower in samples in which this orgamism was co-cultured with pseudomonads or *B. thermosphacta*. The lower production of these metabolic products was found in the latter case. In our opinion, competition for glucose may also be a key factor for this negative response.

Appl. Environ. Microbiol. 69, 204 Tsigarida et al. (2003)

	rate (hrs^{-1})
C_P	
Pseudomonas sp.	0.057±0.005[a]
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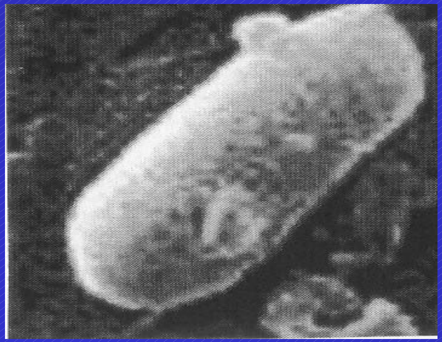
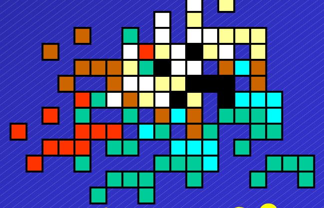
[a] Standard deviation

A negative response (antagonistic) can be regarded also as a factor governing the selection of spoilage flora, particularly of meat. This is the case with pseudomonads and *S. putrefaciens*. It is well established in the literature that the inhibitory effect of the former bacterium on the latter is attributed to the ability of the *Pseudomonas* sp. to produce siderophores



Processing factors

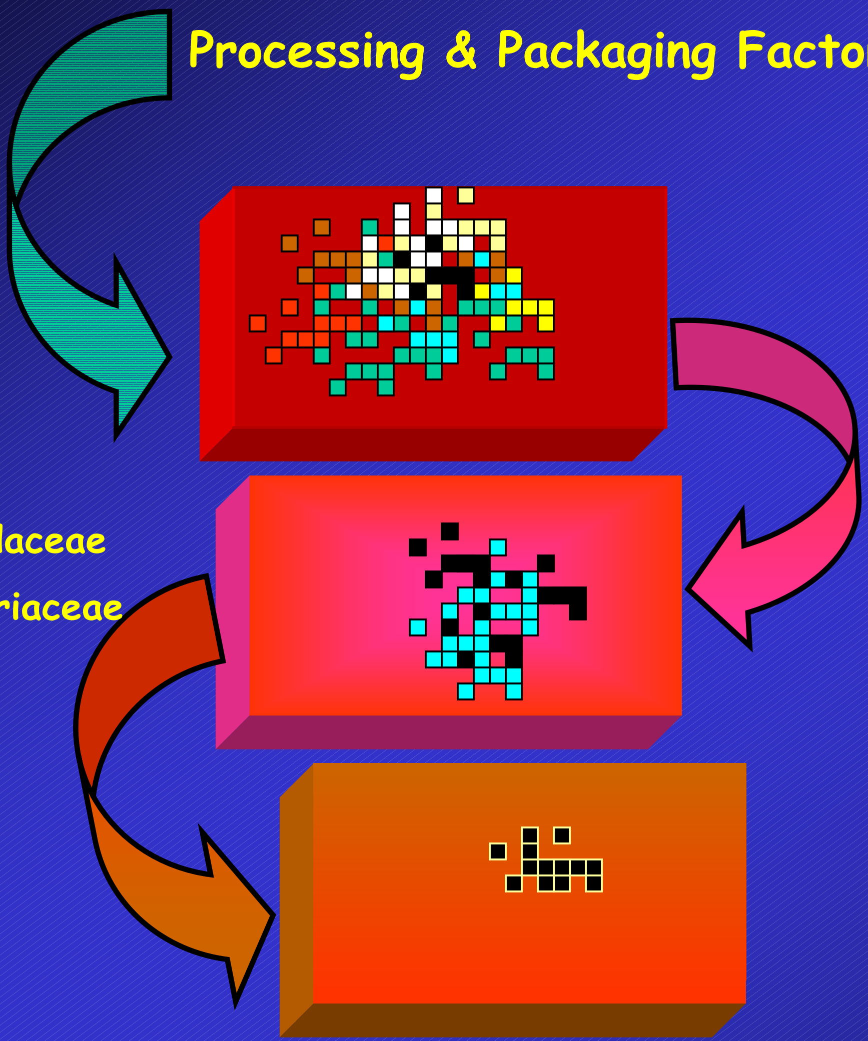
Microbial Association



Processing & Packaging Factors e.g. air at 3° C

Community Development

in space and time



Pseudomonadaceae

Enterobacteriaceae

Ps. fragi

Dynamic Microbial Association

Selected Microbial Association

Specific Spoilage Organisms

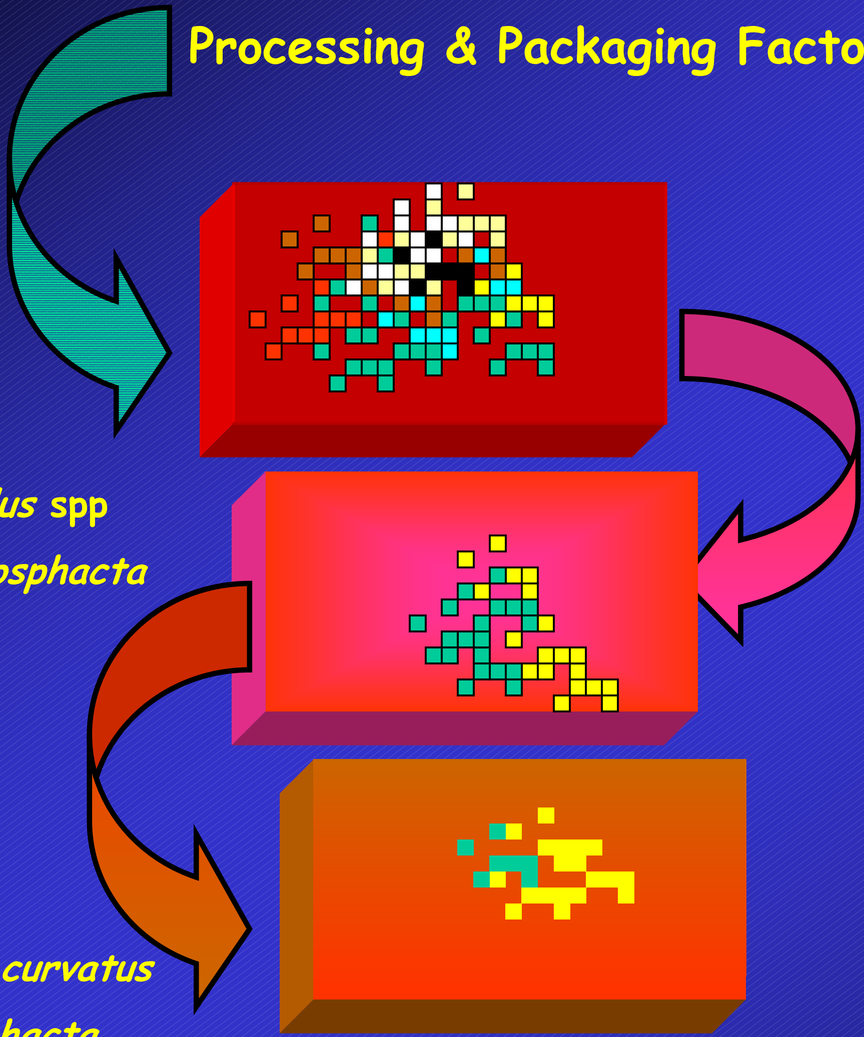
Ephemeral Spoilage Organisms

Microbial Association

Processing & Packaging Factors e.g. map

Community Development

in space and time



Lactobacillus spp

Br. thermosphacta

L.sake & L. curvatus

Br.thermosphacta

Dynamic Microbial Association

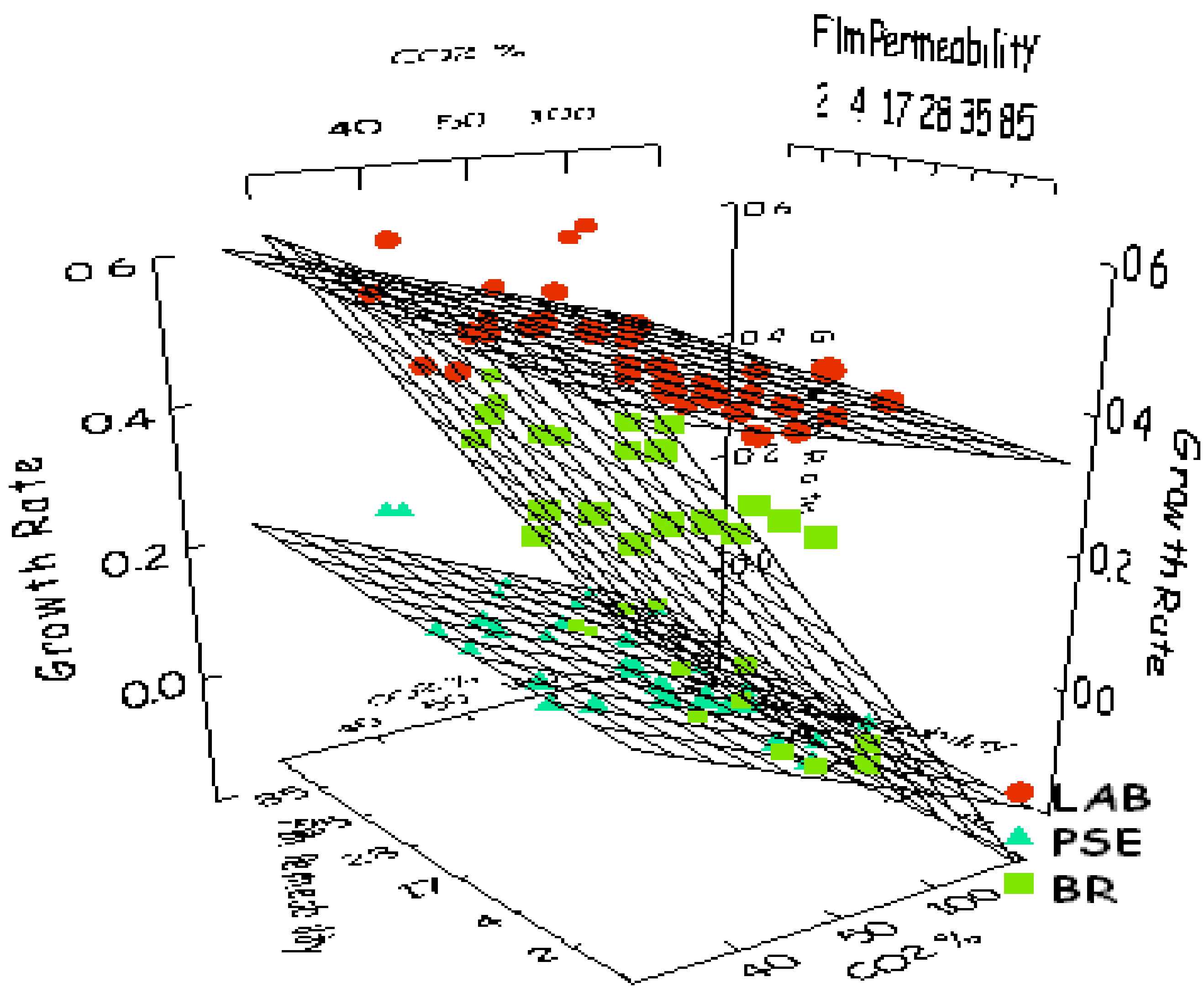
Selected Microbial Association

Specific Spoilage Organisms

Ephemeral Spoilage Organisms

Gas Composition	Specific spoilage flora meat and poultry	Specific spoilage flora fish
Air	*Pseudomonas* spp.	*Shewanella putrefaciens, Pseudomonas* spp.
>50% CO_2 with O_2	*B. thermosphacta*	*B. thermosphacta, S. putrfaciens*
>50% CO_2 ?	Enterobacteriaceae	*Photobacterium phosphereum*
<50%CO_2 with O_2	*Brochothrix thermosphacta*, lactic acid bacteria	*Photobacterium phosphereum,* Lactic acid bacteria, *B. thermosphacta*
>50% CO_2 ?	lactic acid bacteria	Lactic acid bacteria
100% CO_2	lactic acid bacteria	Lactic acid bacteria
Vacuum packaged	*Pseudomonas* spp., *B. thermosphacta*	Pseudomonads

Effect of film characteristics (permeability vs % CO_2) on the growth rate of main spoilage bacteria



What is spoilage

Who is causing spoilage

✓General aspects of spoilage organisms

✓spoilage ecology as a function of process and packaging

How we can evaluate/measure spoilage

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied

Organoleptic analysis

Ca. 50 physico-chemical methods

Microbiological methods

Chemical methods

Physical Methods

Their use in the Meat Industry is under consideration since their disadvantage are related with

Time consuming,

Retrospective results,

Few samples,

Limited reproducibility,

Detection limits (e.g. insufficient for the reliable detection of low levels of organisms)

The time consuming microbiological analyses should be replaced by the chemical changes which associated with microbial growth on meat (e.g. microbial metabolites and/or substrates)

What is spoilage

Who is causing spoilage

✓General aspects of spoilage organisms

✓spoilage ecology as a function of process and packaging

How we can evaluate/measure spoilage

✓Available (?) Software

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied

- GrowthPredictor (UK) - www.ifr.ac.uk/Safety/GrowthPredictor/ (Based on data previously used in the FoodMicromodel software; 18 models for growth of pathogenic bacteria; Available free of charge since 2003)

- Sym'Previus - www.symprevius.net (French predictive microbiology application software under development)

- Seafood Spoilage and Safety Predictor (Dalgaard et al. 2003) [Shelf-life of seafoods and growth of specific spoilage organisms; *Listeria monocytogenes* in cold-smoked salmon]

- Food Spoilage and *E.coli* Predictors (under development by Tom Ross)

- **Greek predictive microbiology application software GRI-GRI (Koutsoumanis and Nychas Spin- off [under development]; The software is based on kinetic data of spoilage bacteria derived from fish, meat and milk in situ;**

- Pathogen Modeling Programme (USA) - www.arserrc.gov/mfs/pathogen.htm (37 models of growth, survival and inactivation; Frequently updated (version 7.0); Available free of charge during the last 15 years; ~ 5000 downloads per year

What is spoilage

Who is causing spoilage

- ✓General aspects of spoilage organisms
- ✓spoilage ecology as a function of process and packaging

How we can evaluate/measure spoilage

- ✓Organoleptic (sensory evaluation)

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied

Organoleptic analysis

<u>Ca. 50 physico-chemical methods</u>

Microbiological methods

Physical Methods

Chemical methods

Organoleptic analysis

Ca. 50 physico-chemical methods

Microbiological methods

Chemical methods

Physical Methods

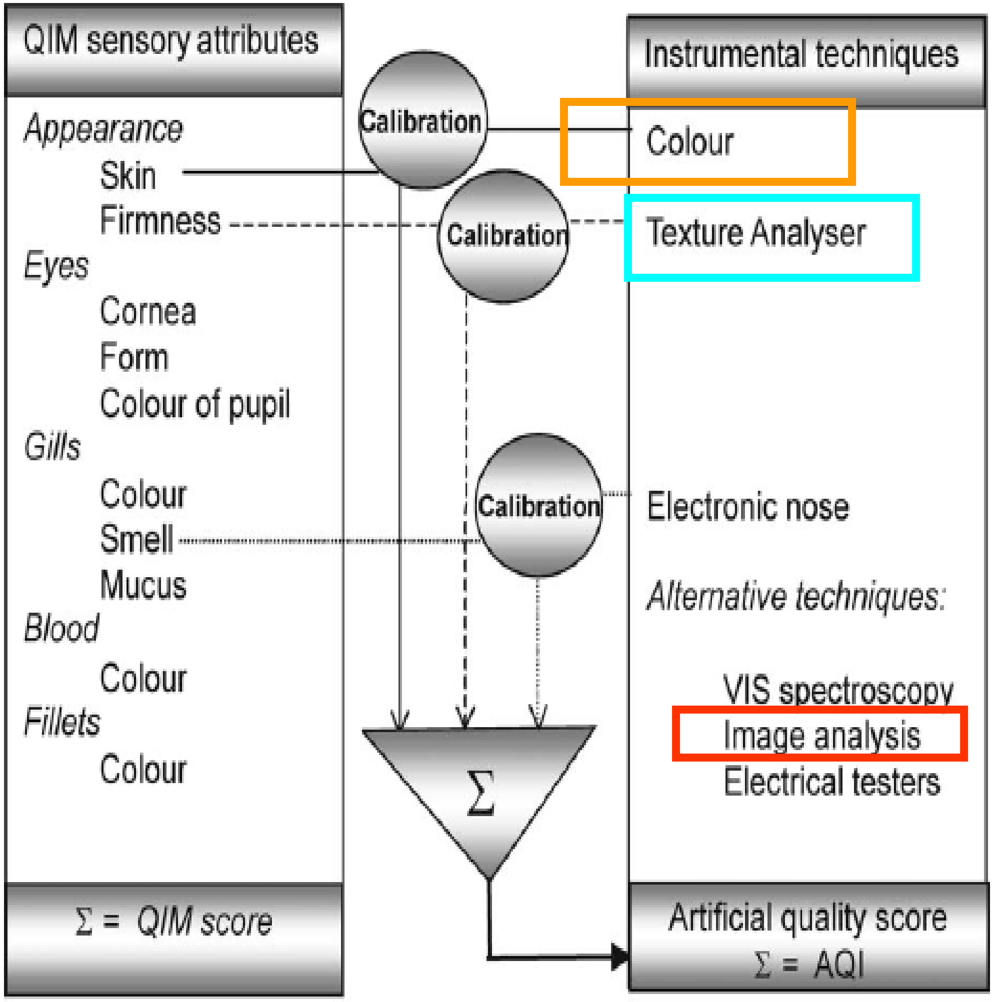


Fig. 1. Construction of the Artificial Quality Index (AQI). After calibration with sensory data (Quality Index Method (QIM)) the instrumental readings are combined into Artificial quality score giving the AQI (adapted from Di Natale, 2003).

What is spoilage

Who is causing spoilage

✓General aspects of spoilage organisms

✓spoilage ecology as a function of process and packaging

How we can evaluate/measure spoilage

✓**Microbial metabolites / BioChemical indices**

Why this valuation/measurement is needed

Where these measurements will be used

When and from whom these measurements will be applied

Organoleptic analysis

Ca. 50 physico-chemical methods

Microbiological methods

Chemical methods

Physical Methods

Compound	Test	Tested Storage conditions	Red meat & poultry	Finfish
Glucose	Enzymatic kit	Air, vp, map	✝	✝
Acetate	Enzymatic kit, HPLC	vp-map	✝	✝
gluconate	Enzymatic kit	Air, vp-map	✝	
Total lactate	HPLC	vp-map	✝	
D-lactate	Enzymatic kit	vp-map	✝	✝
Ethanol	Enzymatic kit, GLC	vp-map	✝	
Free amino acids	chromatometric	Air	✝	
Ammonia	Enzymatic, colorimetric	Air	✝	✝

Compound	Test	Tested Storage conditions	Red meat & poultry	Finfish
Acetone, methyl ethyl ketone, dimethyl sulphide, dimethyldisulphide Hydrogen sylfide	GLC GC/MS Sulphur selective detector	vp-map	✙	✙
diacetyl, acetoin	colorimetric	vp-map	✙	
Biogenic amines	HPLC, sensors, enzymic test, GLC, Enzyme electrodes, test strips	Air, vp, map	✙	✙
Diamines	Amperometric electrodes (enzymatic systems)	Air	✙	
Microbial activity	Enzymic/Resazurin	air	✙	✙
Volatiles (odours)	Electronic noses, PTR-MS (chemical sensors)	Air-vp-map	✙	✙
Proteolysis (Amides, amines, etc.)	FT-IR, NIR, MIR	Air-vp-map	✙	✙

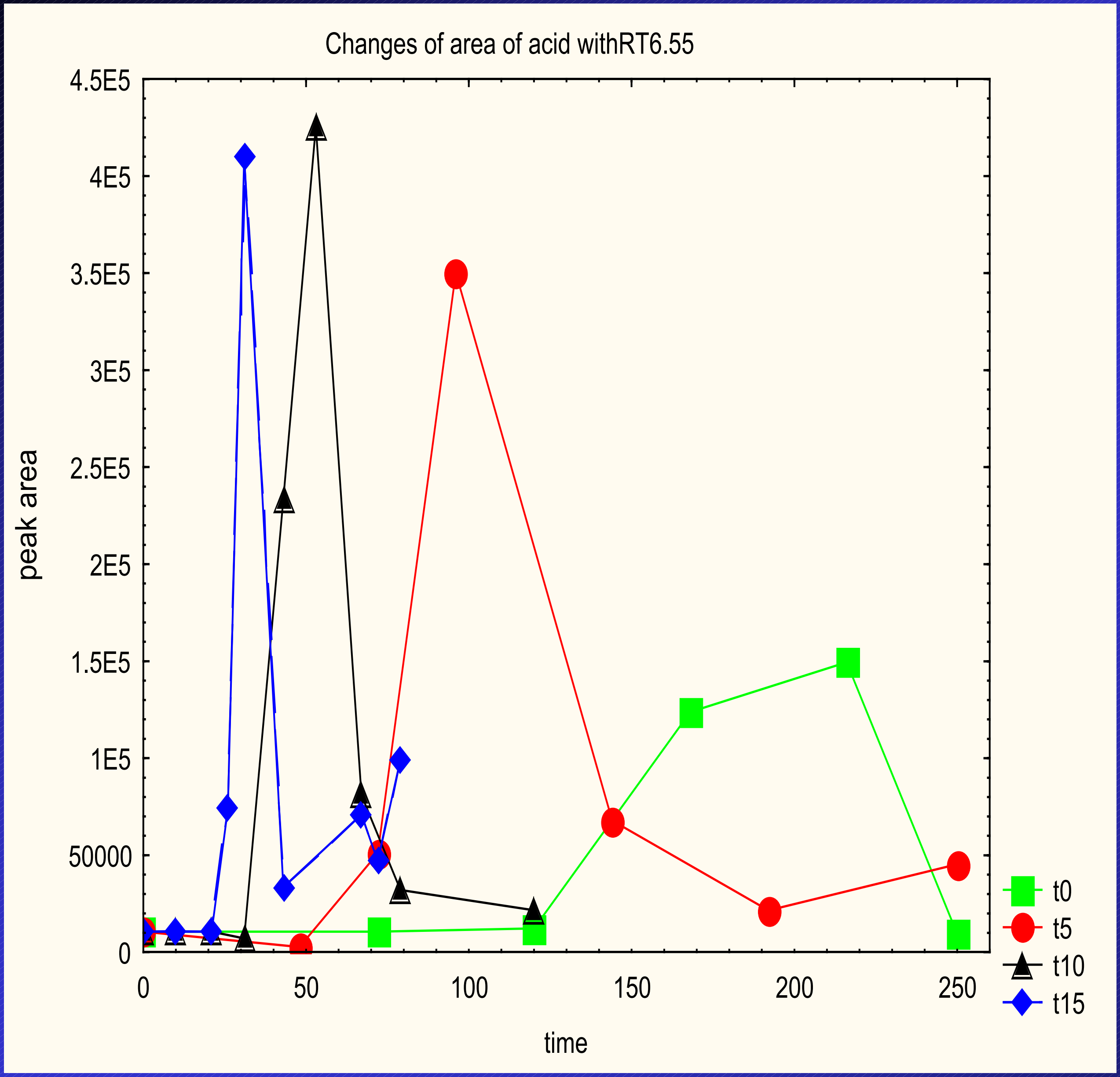
Compound	Test	Tested Storage conditions	Red meat & poultry	Finfis h
Torymeter				✞
ATP/IMP	HPLC	air		✞
K-values	HPLC	air		✞
Lipid oxidation	HPLC, GC, spectroscopy	Air,vp		✞
TMA	HPLC, GC, sensors, enzymatic	air		✞

The spoilage indicators or microbial metabolites should meet among others the following criteria (Jay, 1986a):

(i) the compound should be absent or at least at low levels in meat

(ii) should increase with the storage

(iii) should be produced by the dominant flora and have good correlation with organoleptic test.


Changes of area of acid withRT6.55
peak area
4.5E5
4E5
3.5E5
3E5
2.5E5
2E5
1.5E5
1E5
50000
0
0
50
100
150
200
250
time
t0
t5
t10
t15

As far as the spoilage indicators or microbial metabolites concern it is general accepted that these should meet among others the following criteria (Jay, 1986a):

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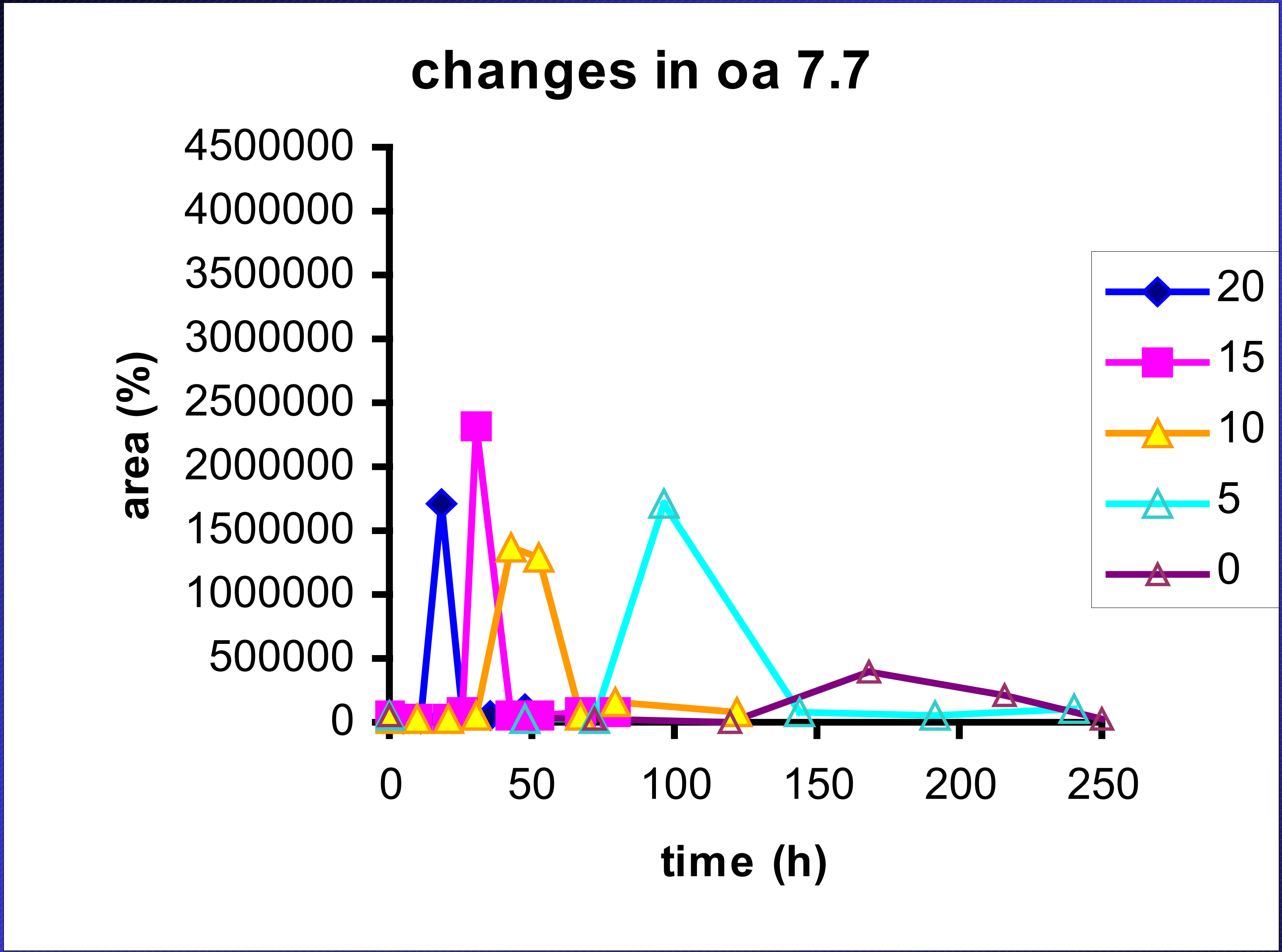
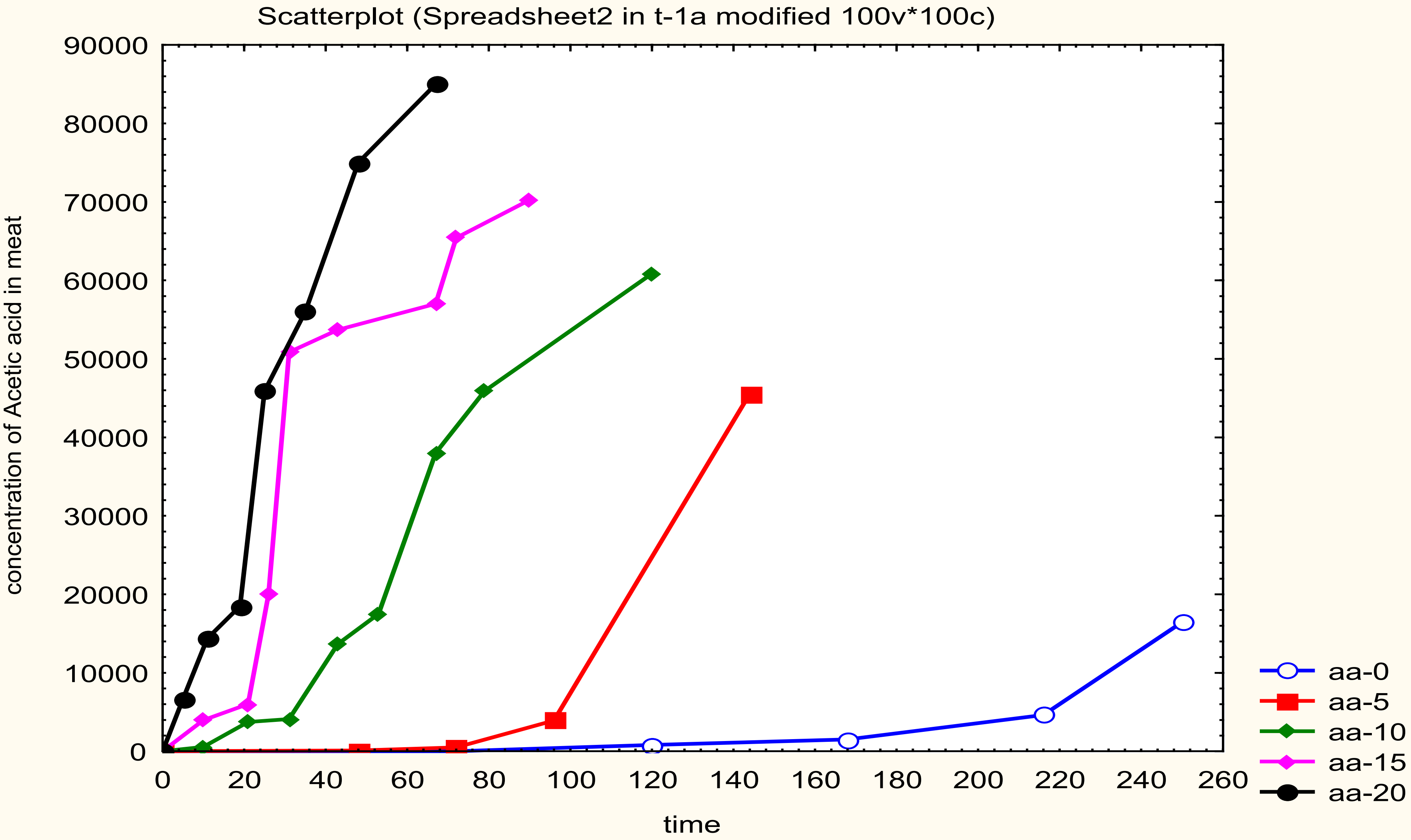

changes in oa 7.7
area (%)
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
50
100
150
200
250
time (h)
20
15
10
5
0



Acetic acid Production in pork meat stored aerobically at different T° C

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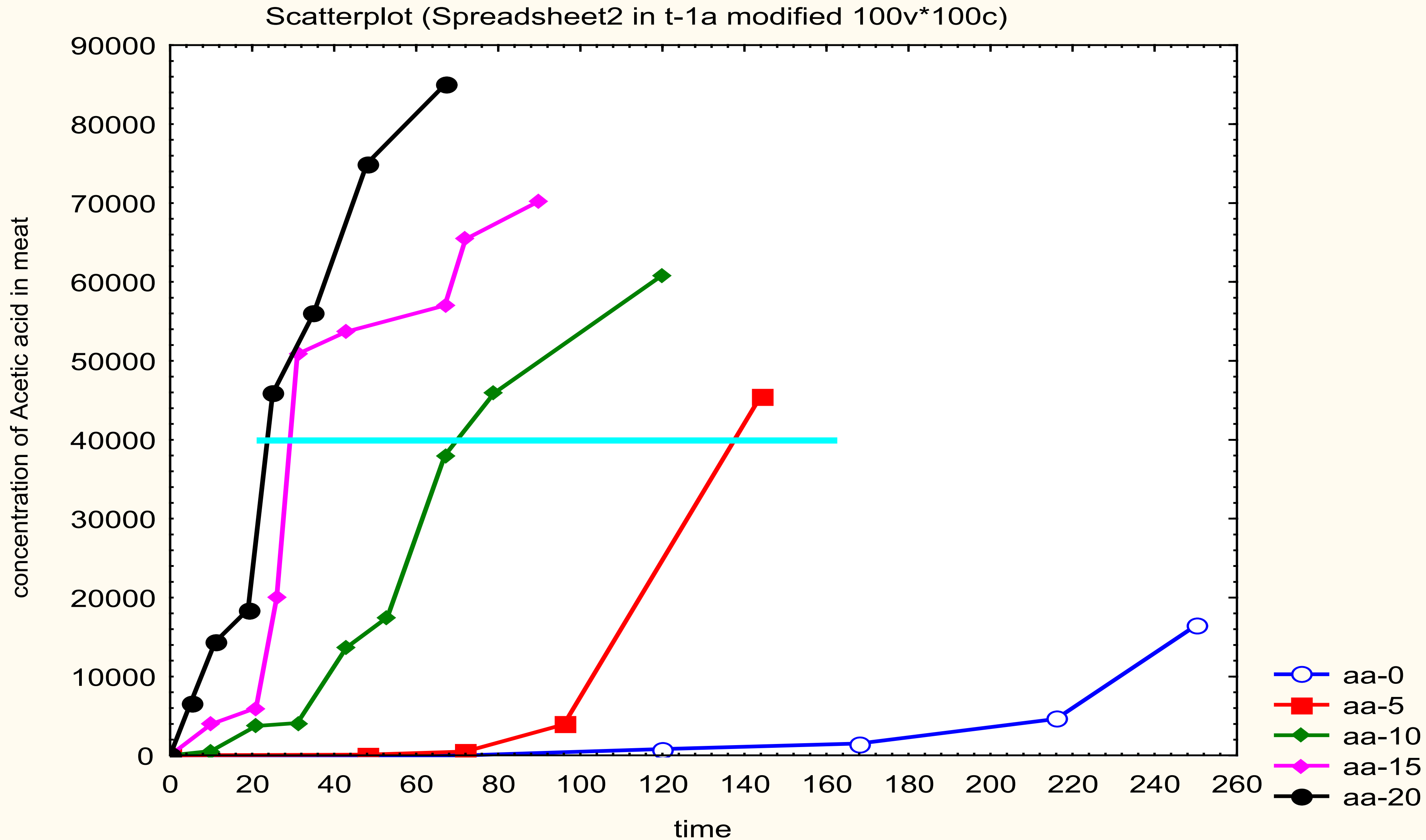


Acetic acid Production in pork meat stored aerobically at different T° C

The identification of the ideal metabolite that can be used for spoilage assessment has proved a difficult task:

a) most metabolites maybe specific to certain organisms (e.g. gluconate to pseudomonads) and when these organisms are not present or inhibited by the natural or imposed from man, food ecology, this provides incorrect spoilage information,

b) the metabolites are the result of the consumption of a specific substrate but the absence of the given substrate or its presence in low quantities does not preclude spoilage,

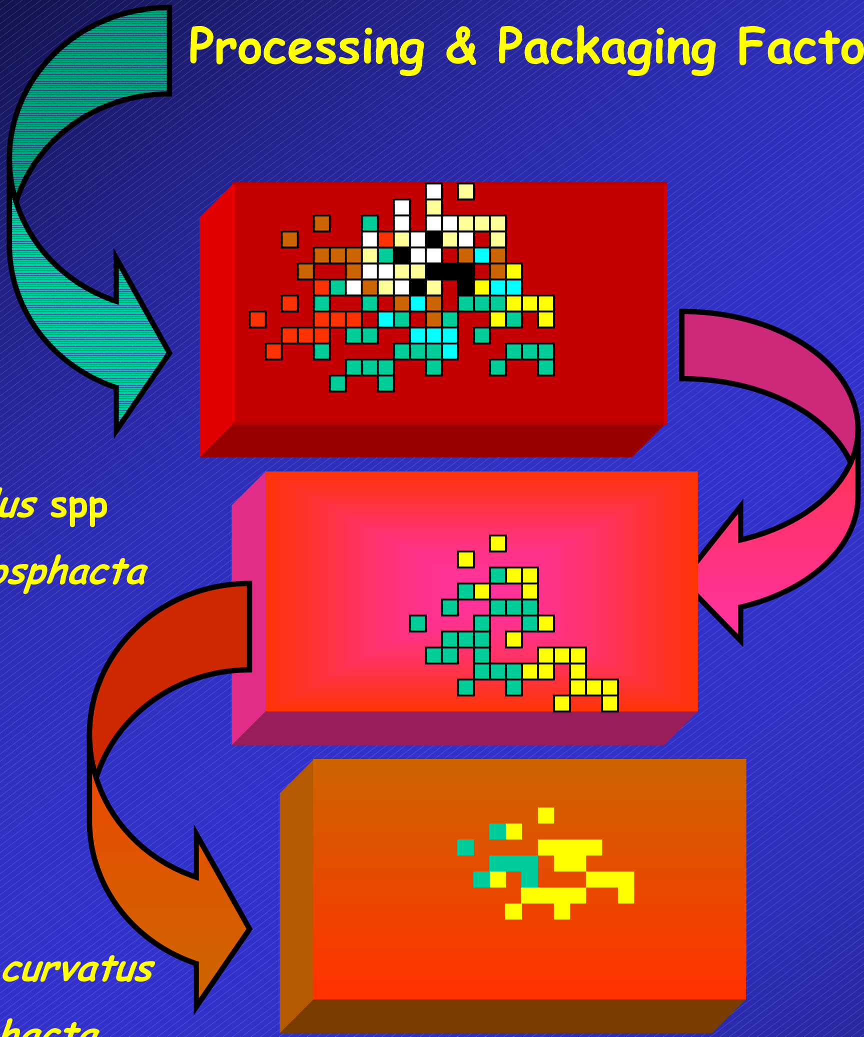
c) the rate of microbial metabolite production and the metabolic pathways of these bacteria are affected by the imposed environmental conditions (e.g. pH, oxygen tension, temperature etc.),

d) the accurate detection and their measurements requires sophisticated procedures, high educated personnel, time and equipment and

e) many of them give a retrospective information which is not satisfactory.

Microbial Association

Processing & Packaging Factors e.g. map



Lactobacillus spp

Br. thermosphacta

L.sake & L. curvatus

Br.thermosphacta

Community Development

in space and time

Dynamic Microbial Association

Selected Microbial Association

Specific Spoilage Organisms

Ephemeral Spoilage Organisms

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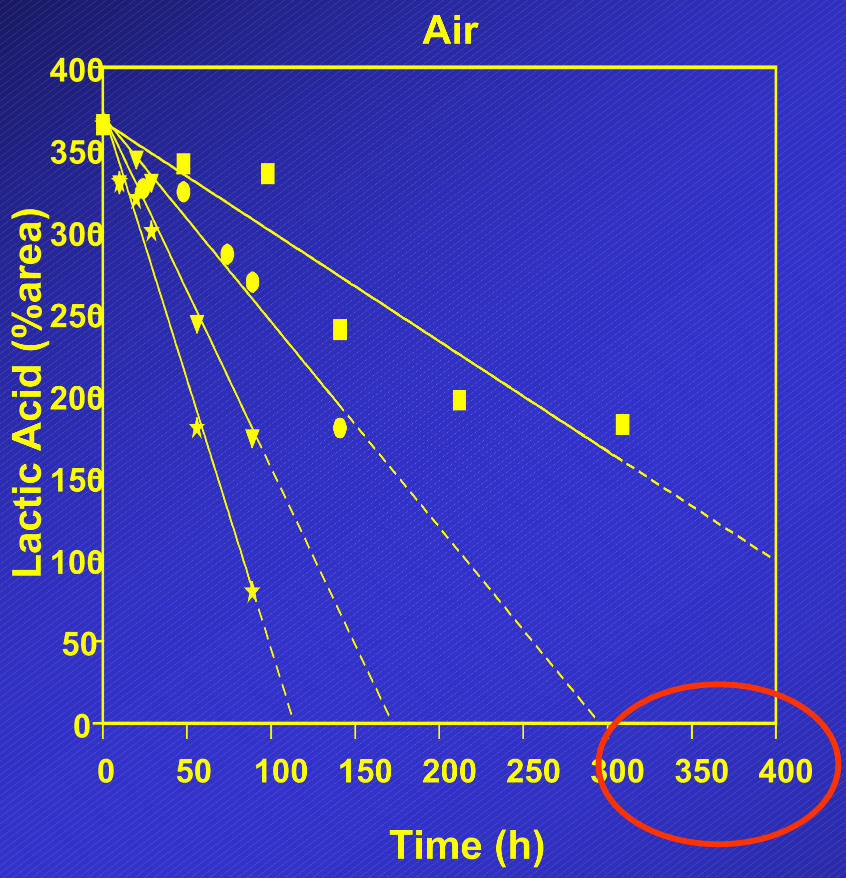
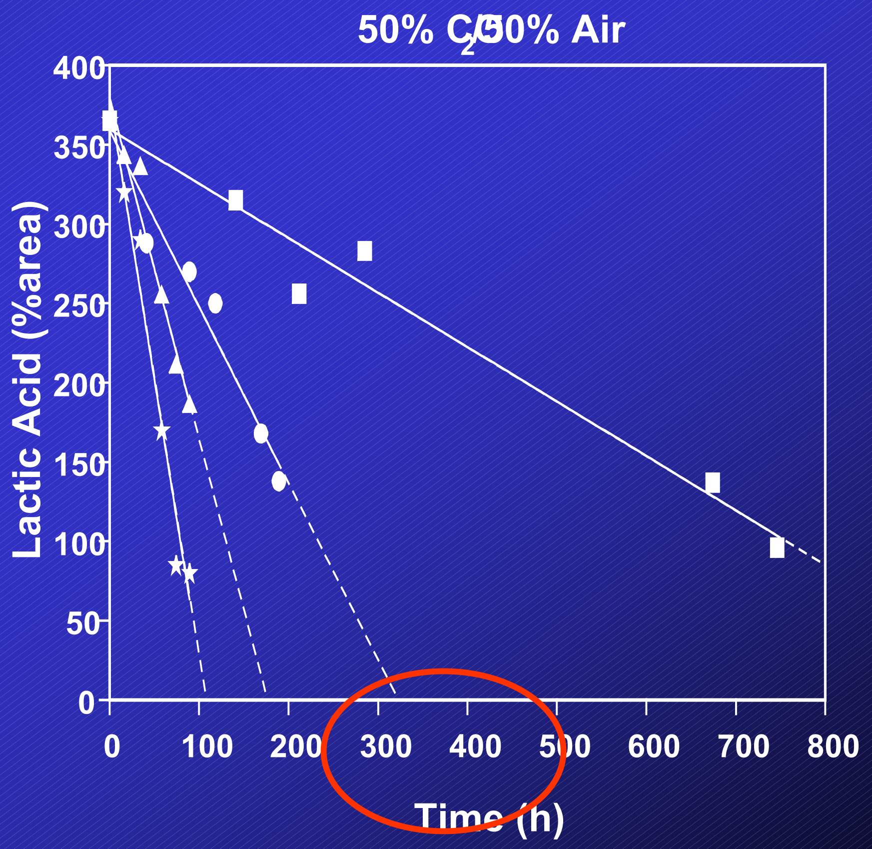
Linear regression of changes of lactic acid in meat stored under different T° C (■: 0 ºC, 10 ºC, ♦: 15 ºC, ☝: 20 ºC)





The identification of the ideal metabolite that can be used for spoilage assessment has proved a difficult task:

a) most metabolites maybe specific to certain organisms (e.g. gluconate to pseudomonads) and when these organisms are not present or inhibited by the natural or imposed from man, food ecology, this provides incorrect spoilage information,

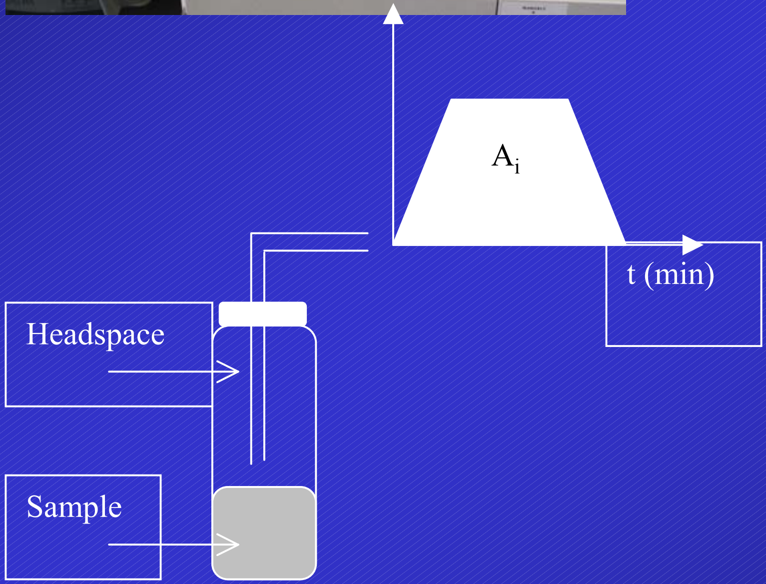
b) the metabolites are the result of the consumption of a specific substrate but the absence of the given substrate or its presence in low quantities does not preclude spoilage,

c) the rate of microbial metabolite production or consumption and the metabolic pathways of these bacteria are affected by the imposed environmental conditions (e.g. pH, oxygen tension, temperature etc.),

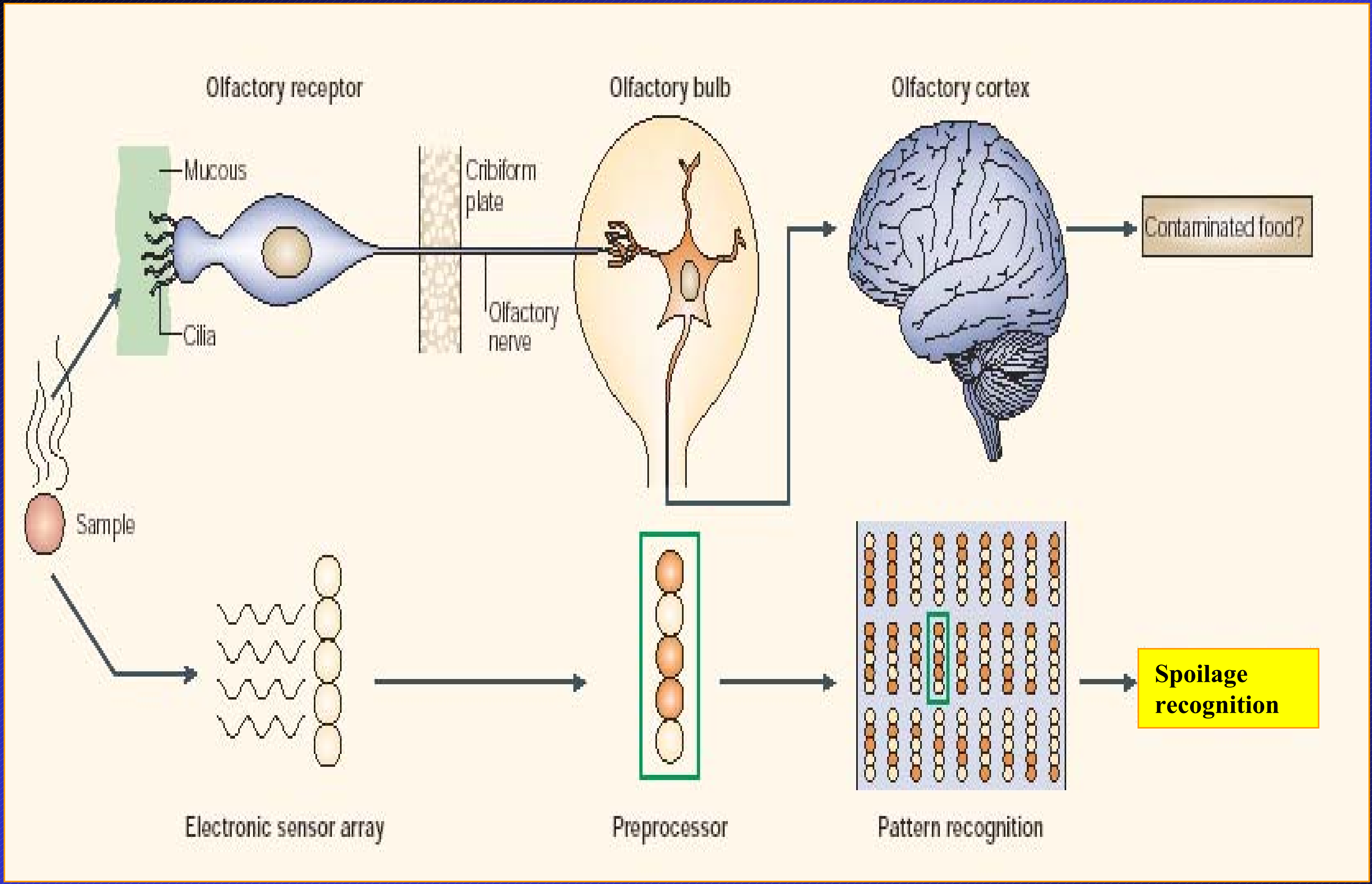
d) the accurate detection and their measurements requires sophisticated procedures, high educated personnel, time and equipment and

e) many of them give a retrospective information which is not satisfactory.

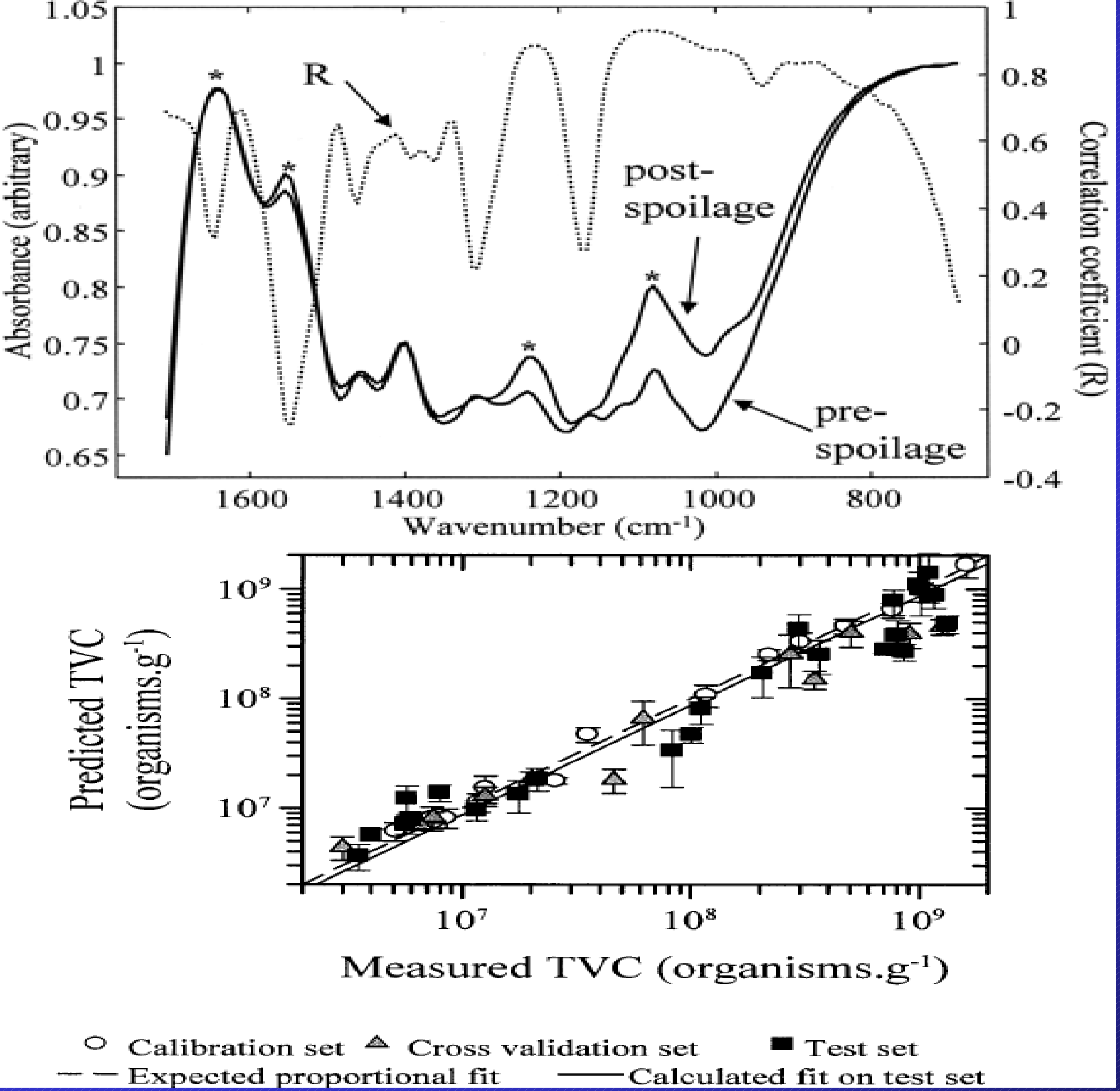
Bloodhound BH114 Electronic nose



GASP Instrument

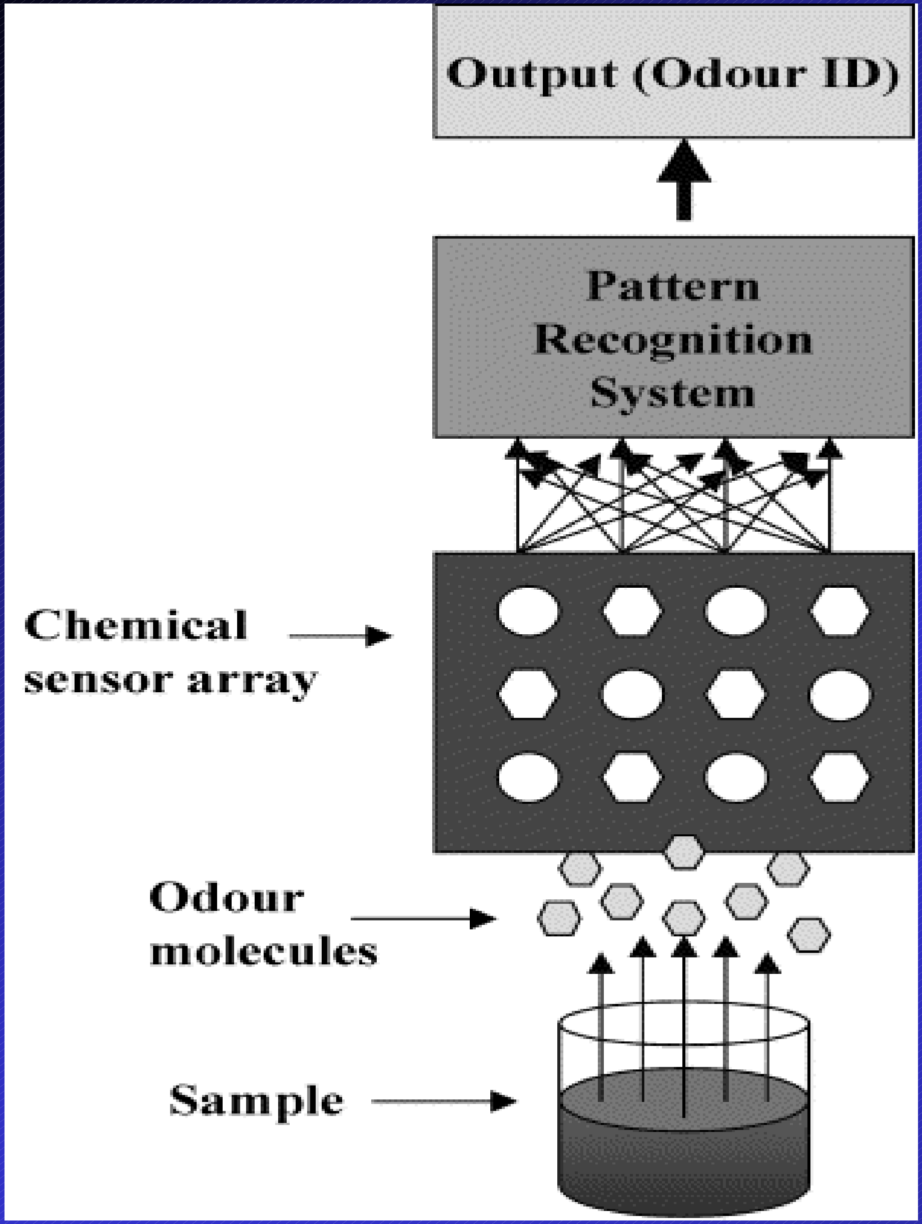

Olfactory receptor
Olfactory bulb
Olfactory cortex
Mucous
Cribiform
plate
Cilia
Olfactory
nerve
Contaminated food?
Sample
Spoilage
recognition
Electronic sensor array
Preprocessor
Pattern recognition

sensors



Typical FT-IR absorbance spectra from pre- and postspoilage chicken. Also shown is the Pearson correlation coefficient (R) between

the FT-IR absorbances and the log10(TVC). The asterisks indicate peaks that are attributable to amide I (1,640 cm1), amide II (1,550 cm1), and amine (1,240 and 1,088 cm1) vibrations.



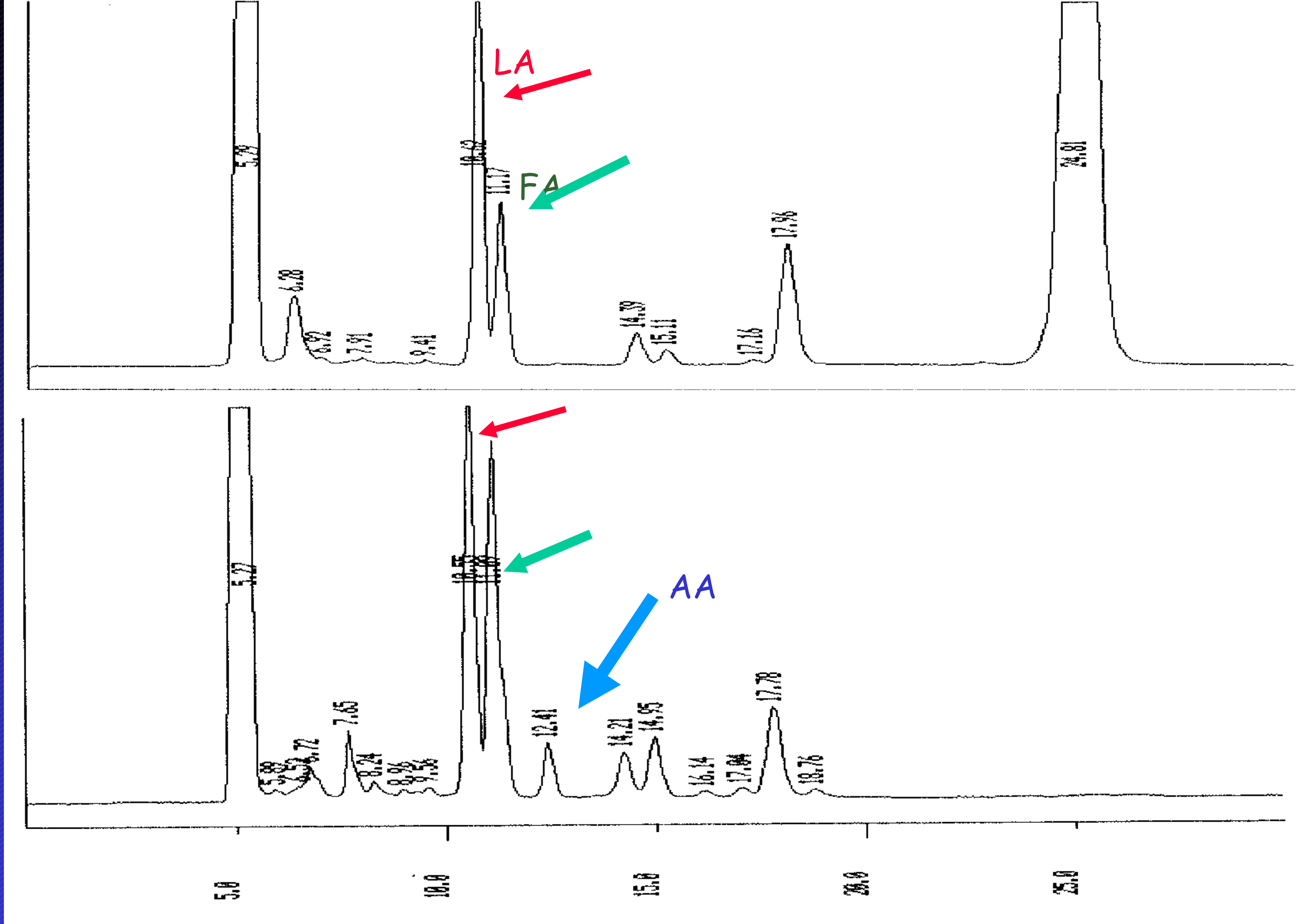
Generalised schematic of an electronic nose employing chemical sensors.

The pattern-recognition system, is based on neural networks (machine learning)

Monitoring the end-products formation and/or substrate changes occurring during storage of meat (pork) at different T° C was analyzed with HPLC

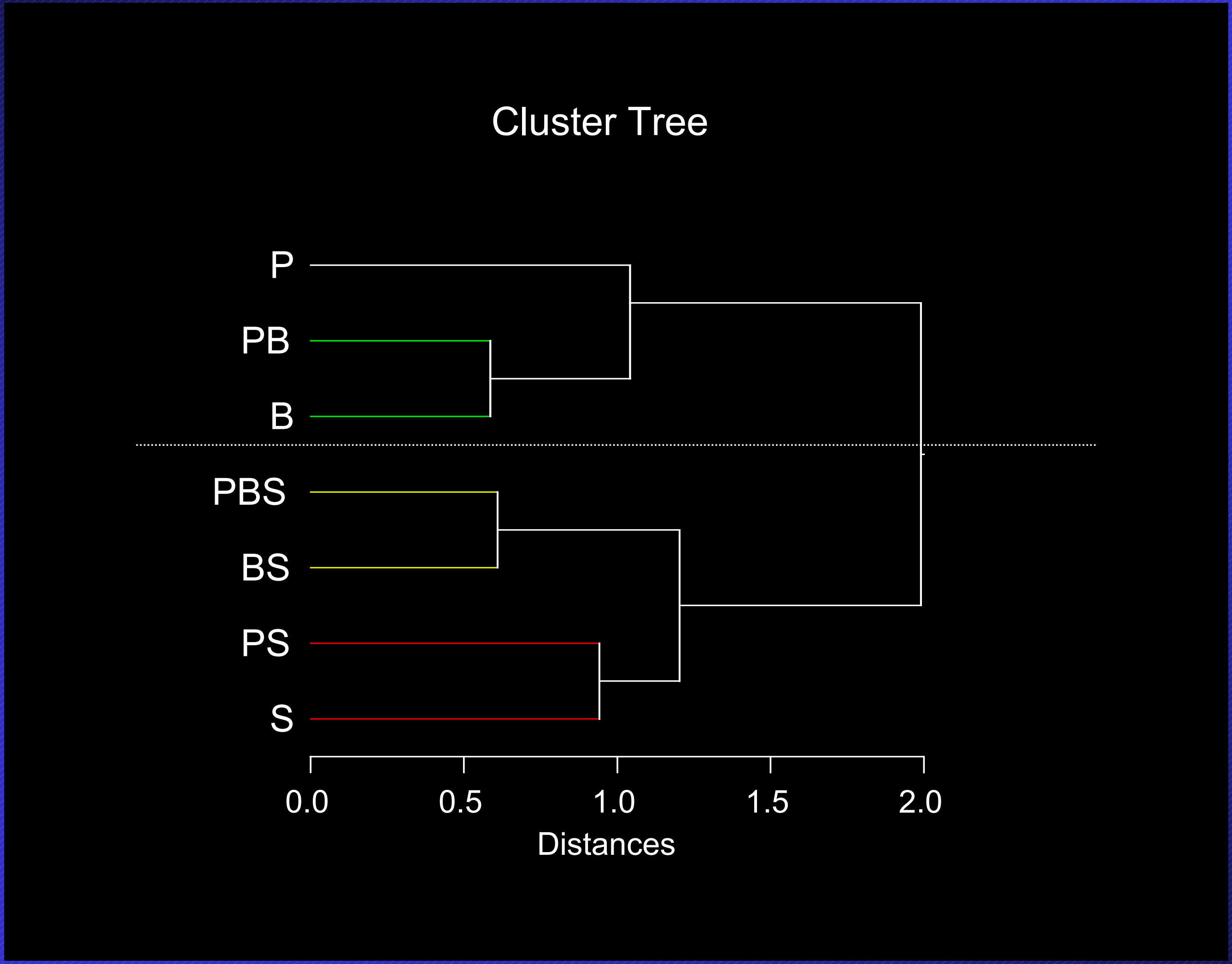
Cluster analysis

Factor Analysis

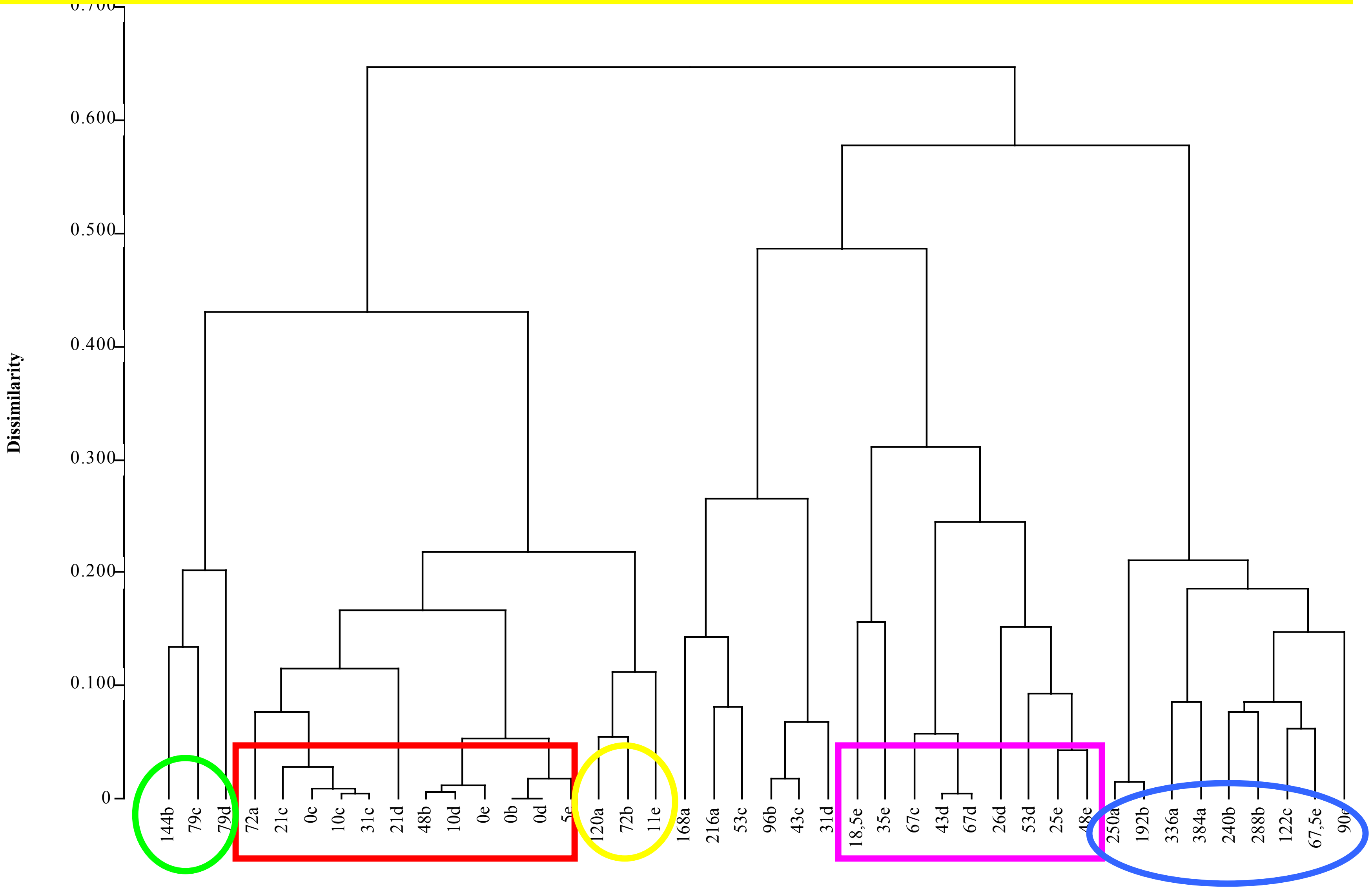


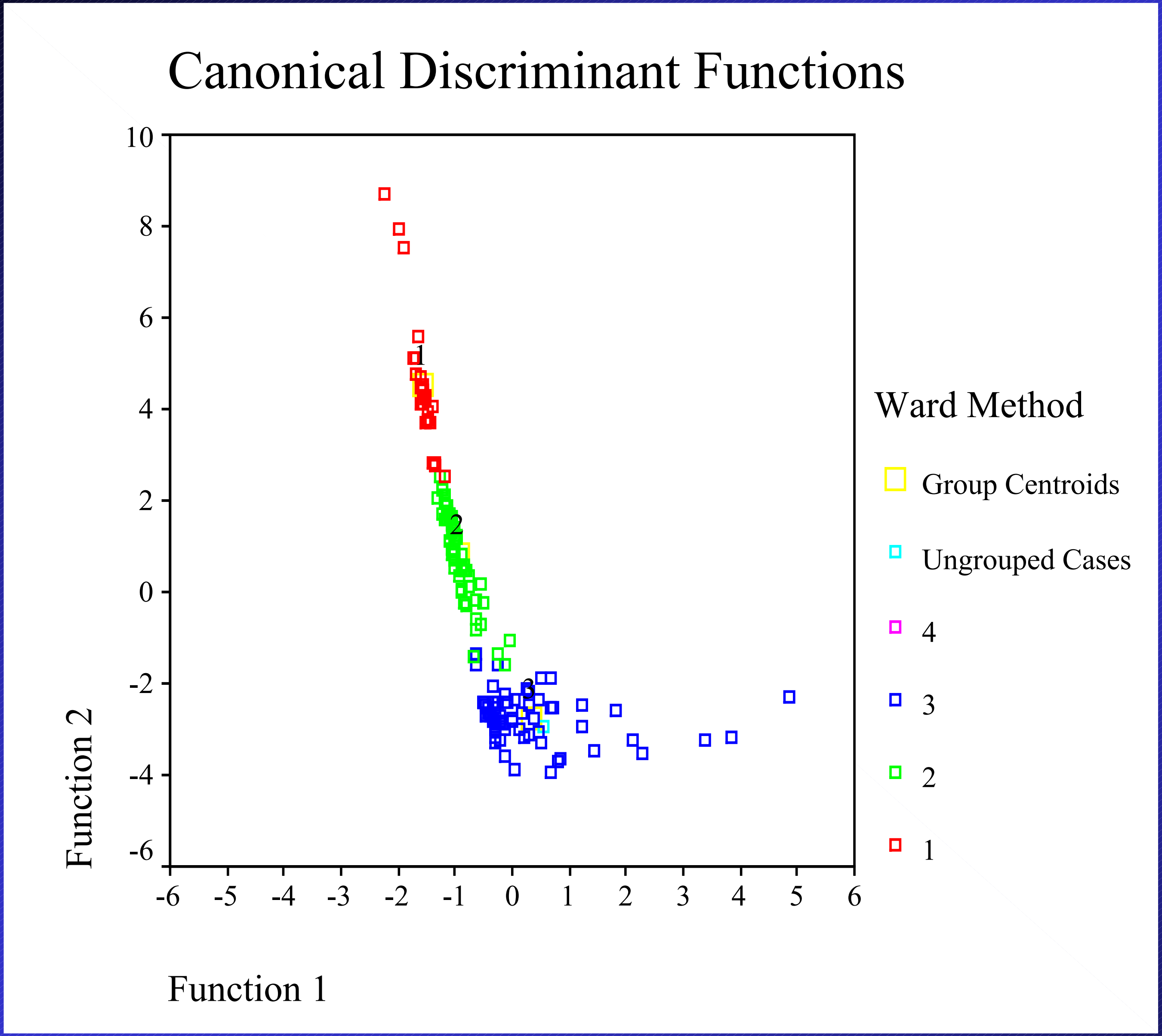
Typical organic acid profile from meat

Dendrogram using Average Linkage (Within Group)



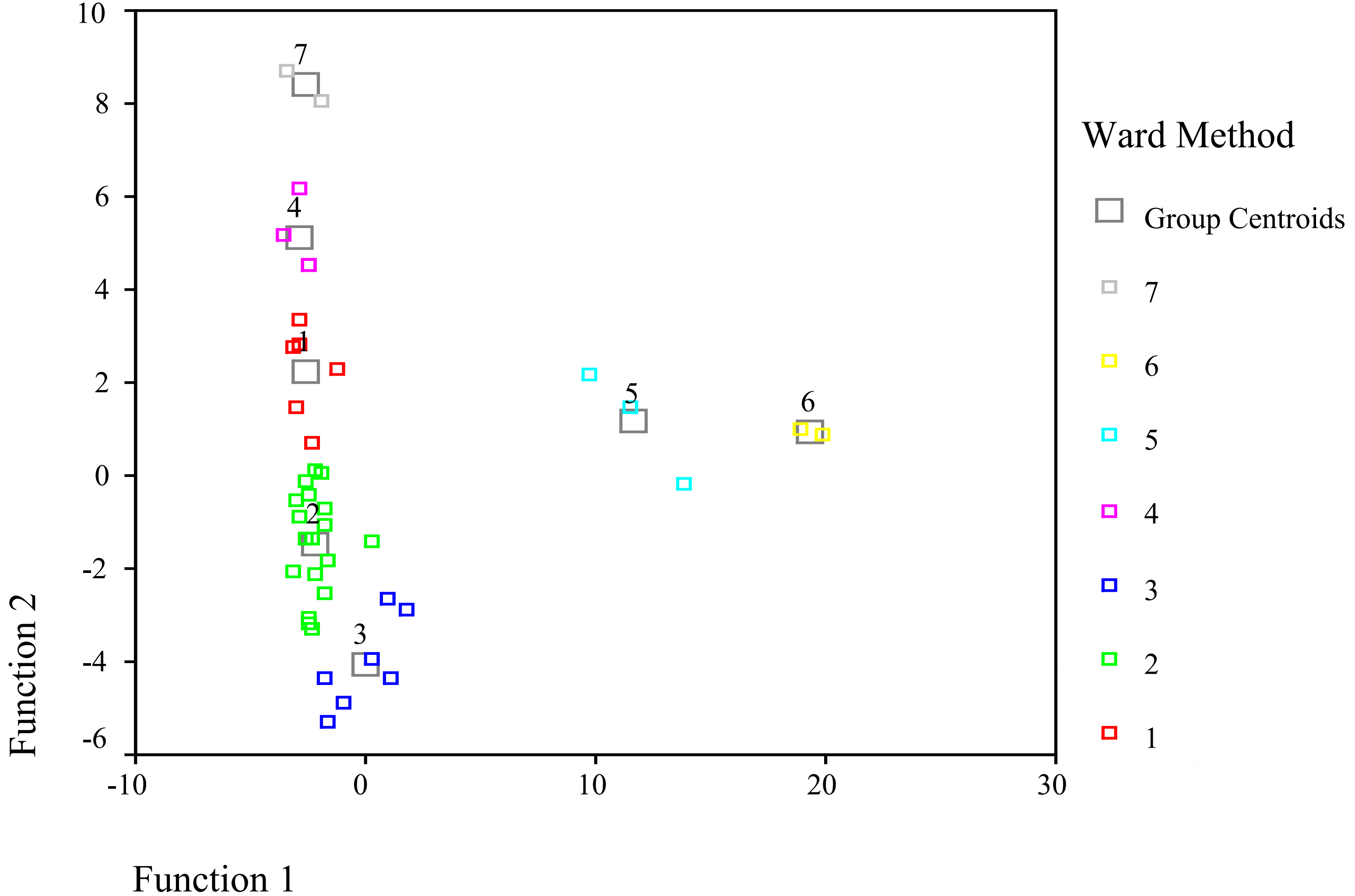
Dendrogram using Average Linkage (Within Group)

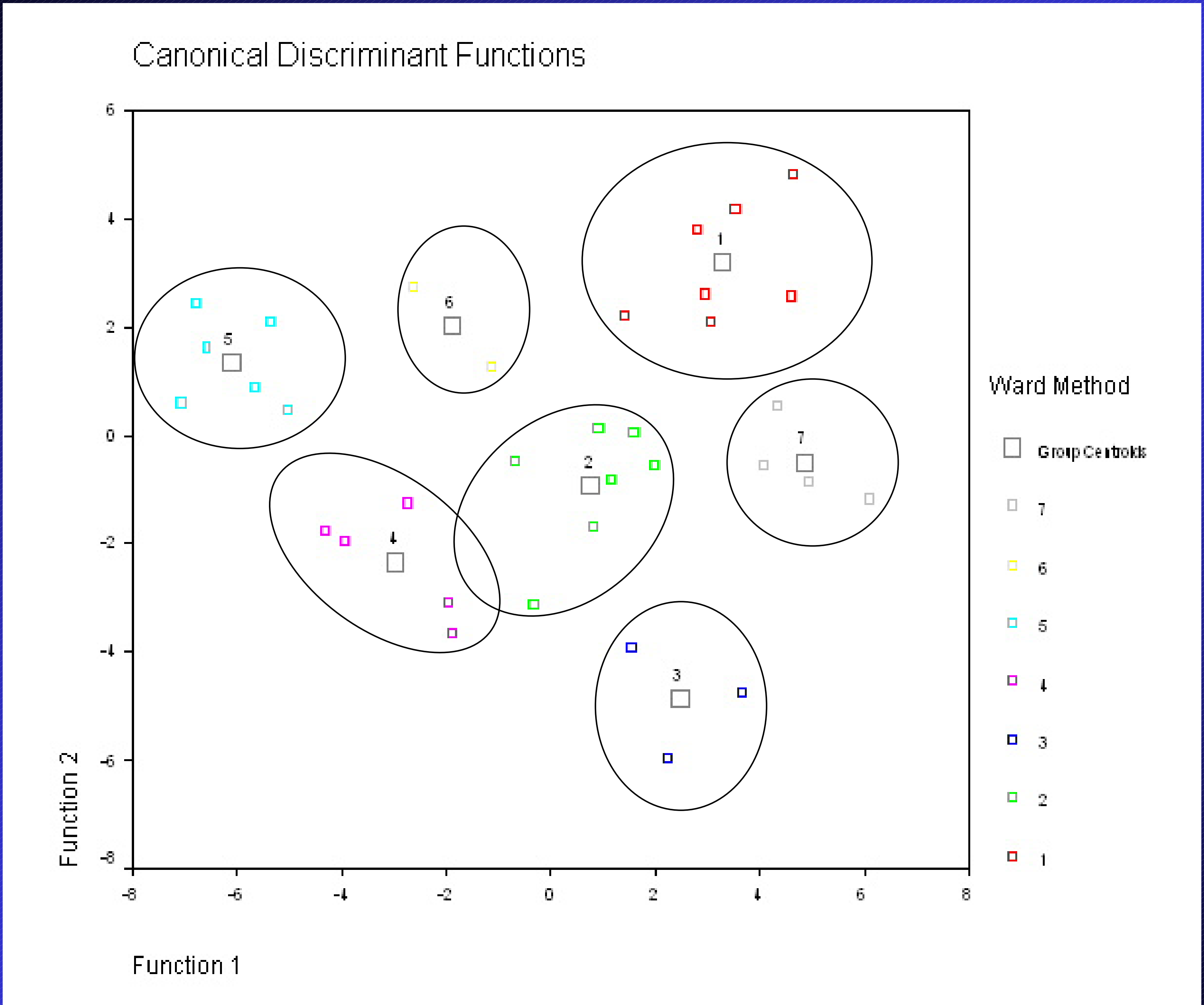


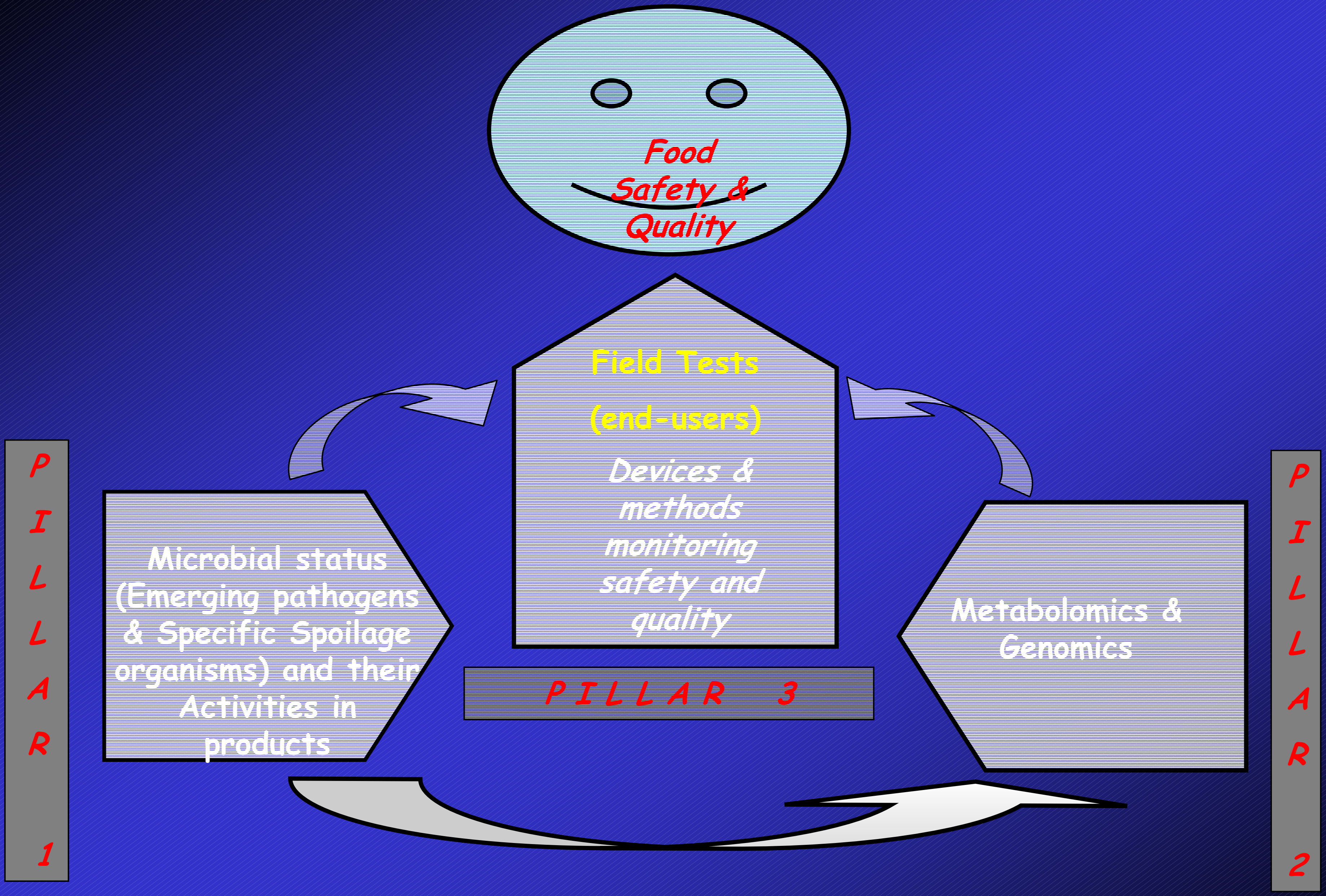


Canonical Discriminant Functions
10
8
6
4
2
0
-2
-4
-6
-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6
1
2
3
Function 2
Function 1
Ward Method
Group Centroids
Ungrouped Cases
4
3
2
1

Canonical Discriminant Functions




Canonical Discriminant Functions
Function 2
Function 1
Ward Method
Group Centroids
7
6
5
4
3
2
1


Food Safety & Quality
Field Tests
(end-users)
Devices & methods monitoring safety and quality
P I L L A R 3
P I L L A R 1
Microbial status (Emerging pathogens & Specific Spoilage organisms) and their Activities in products
P I L L A R 2
Metabolomics & Genomics

THANK YOU