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## Cheese Flavour: Formation and analysis

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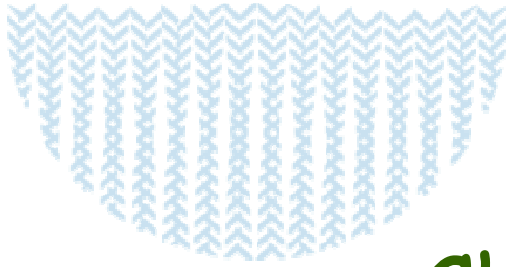
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# Cheese Flavour: Formation and Analysis

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STLO, INRA Rennes  
**John Hannon**

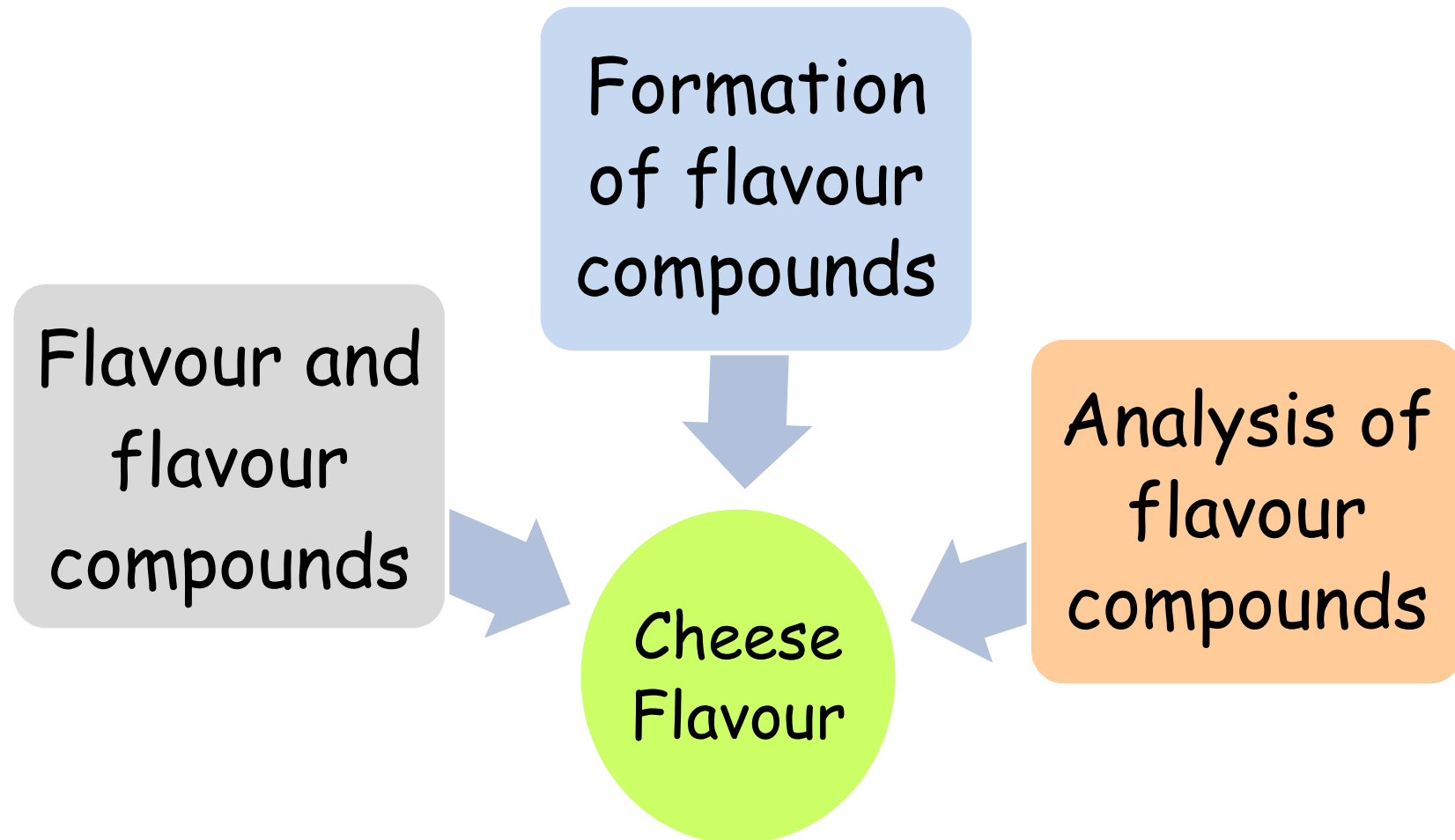
Teagasc Food Research Centre, Moorepark, Cork



ALIMENTATION  
AGRICULTURE  
ENVIRONNEMENT



# Outline



# Flavour: definition and perception

**Definition:** Complex combination of the **olfactory**, **gustatory** and **trigeminal sensations** perceived during tasting

(ISO 5492:2008)

Flavour

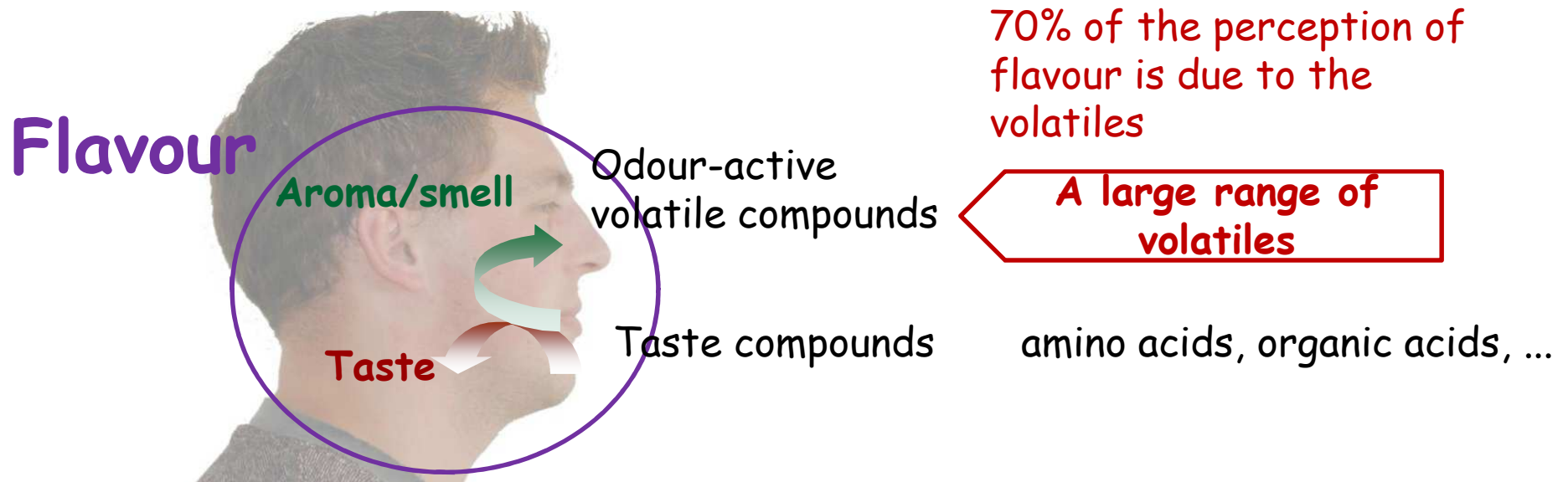


# Component balance Theory

Flavour is the result of the correct balance and concentration of a wide variety of volatile flavour compounds

*Mulder (1952)*

*Kosikowski and Mocquot (1958)*




# Main features of volatile flavour compounds

- ◆ MW < 400 Da
- ◆ Many different chemical functions
- ◆ concentration in foods:  
from < 1 ppb to 1000 ppm
- ◆ threshold odour value:  
from < 0.001 ppb to > 1000 ppb

Not all volatiles are odour active !

## Threshold odour value in water, $\mu\text{g/L}$

2-methoxy-3-isopropyl pyrazine:	0.002
furaneol:	0.16
dimethylsulfide:	0.33
diacetyl:	7
ethanol :	53 000



## In summary...

**Flavour** results from the perception of taste and odour-active volatile compounds

The assessment of flavour by sensory analysis does not tell us the compound(s) responsible, their concentration or give us clues regarding their origin

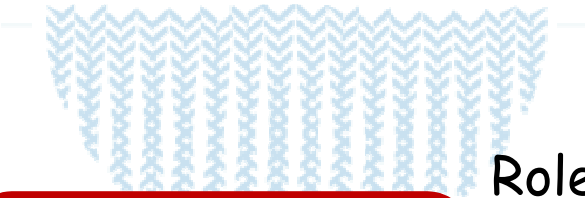
Only **some** of the volatile compounds are odour-active

Most flavour compounds are common to widely different cheese types

Their contribution to flavour depends on their threshold value, their relative proportion and their interactions with food matrix

The same compound can positively contribute to cheese flavour or be responsible for flavour defects





Role of micro-organisms  
Origins of flavour compounds  
Bottlenecks

Formation of flavour compounds

Flavour and flavour compounds

Analysis of flavour compounds

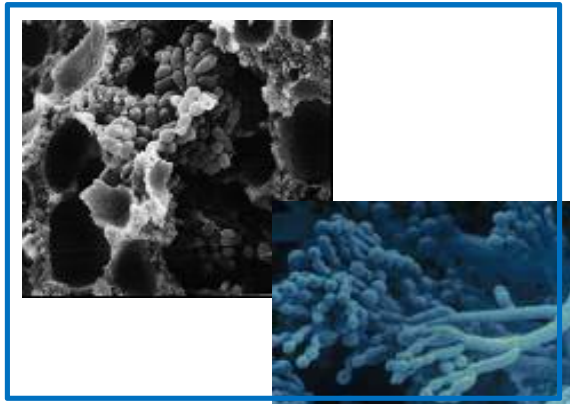
Cheese Flavour





# Micro-organisms are the main actors

- Microbial enzymes



cheese curd components



- indigenous milk enzymes and coagulants
- a few non-enzymatic reactions

flavour compounds



cheese flavour

## Cheese ecosystem

- in constant evolution
- starters and non-starter flora
- culturable, non culturable cells, permeabilised and lysed cells
- strain-dependent activities

# Origin of flavour compounds



Ex: Emmental cheese

**NSLAB**

Citrate

Lactose

Casein

plasmin

Lipo  
protein  
lipase

Milk Triglyceride

*Str. thermophilus*  
*Lb. helveticus*  
*Lb. delbrueckii*

Fermentation

Lactic Acid

Oligopeptides

Free Fatty Acids

*Propionibacterium  
freudenreichii*

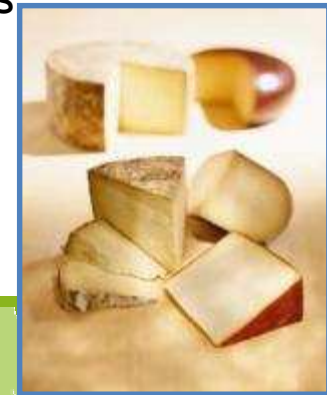
Peptides & Free  
Amino Acids

volatiles

Acetic &  
propionic  
acids

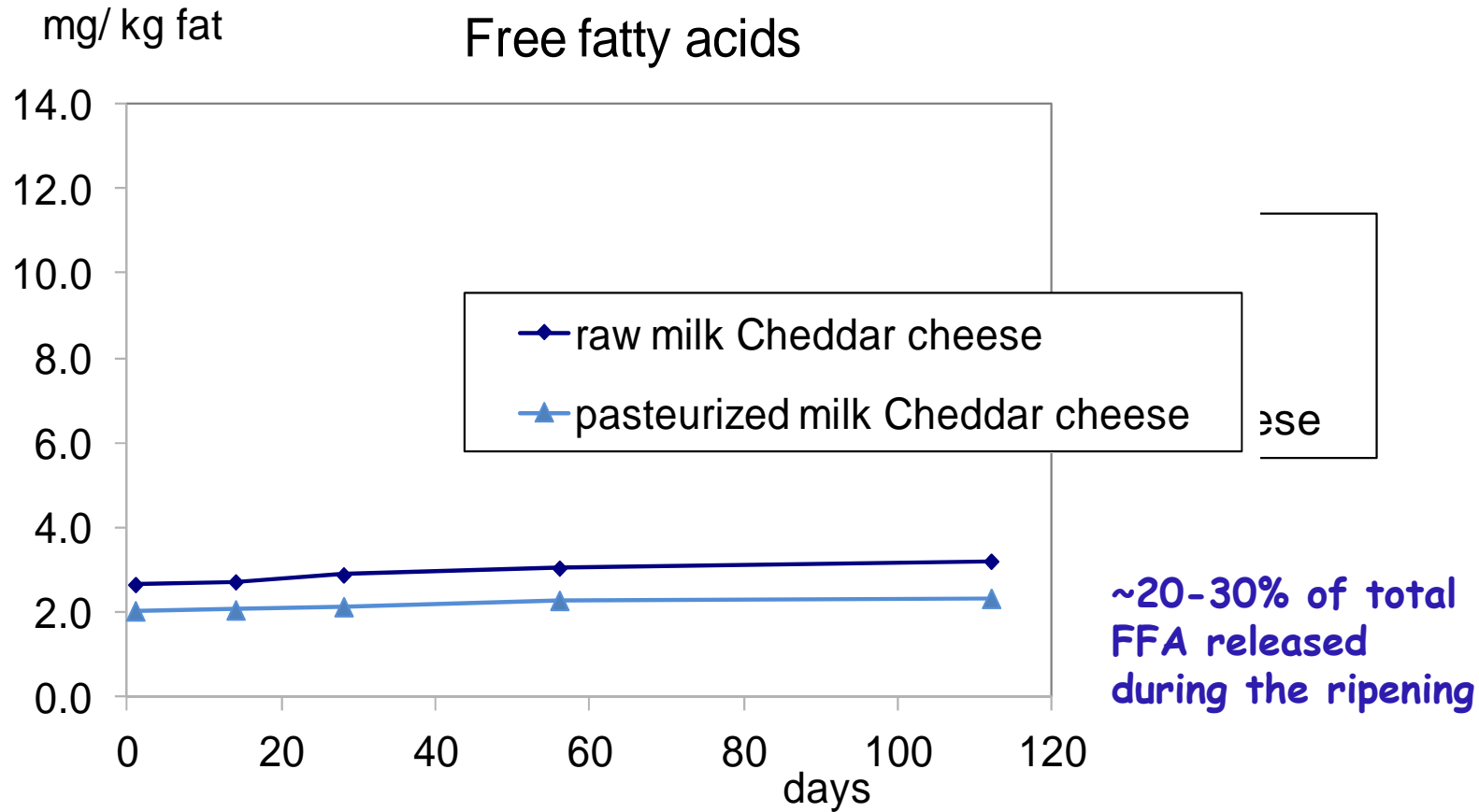
Branched-chain  
acids

A variety of volatile  
flavour compounds



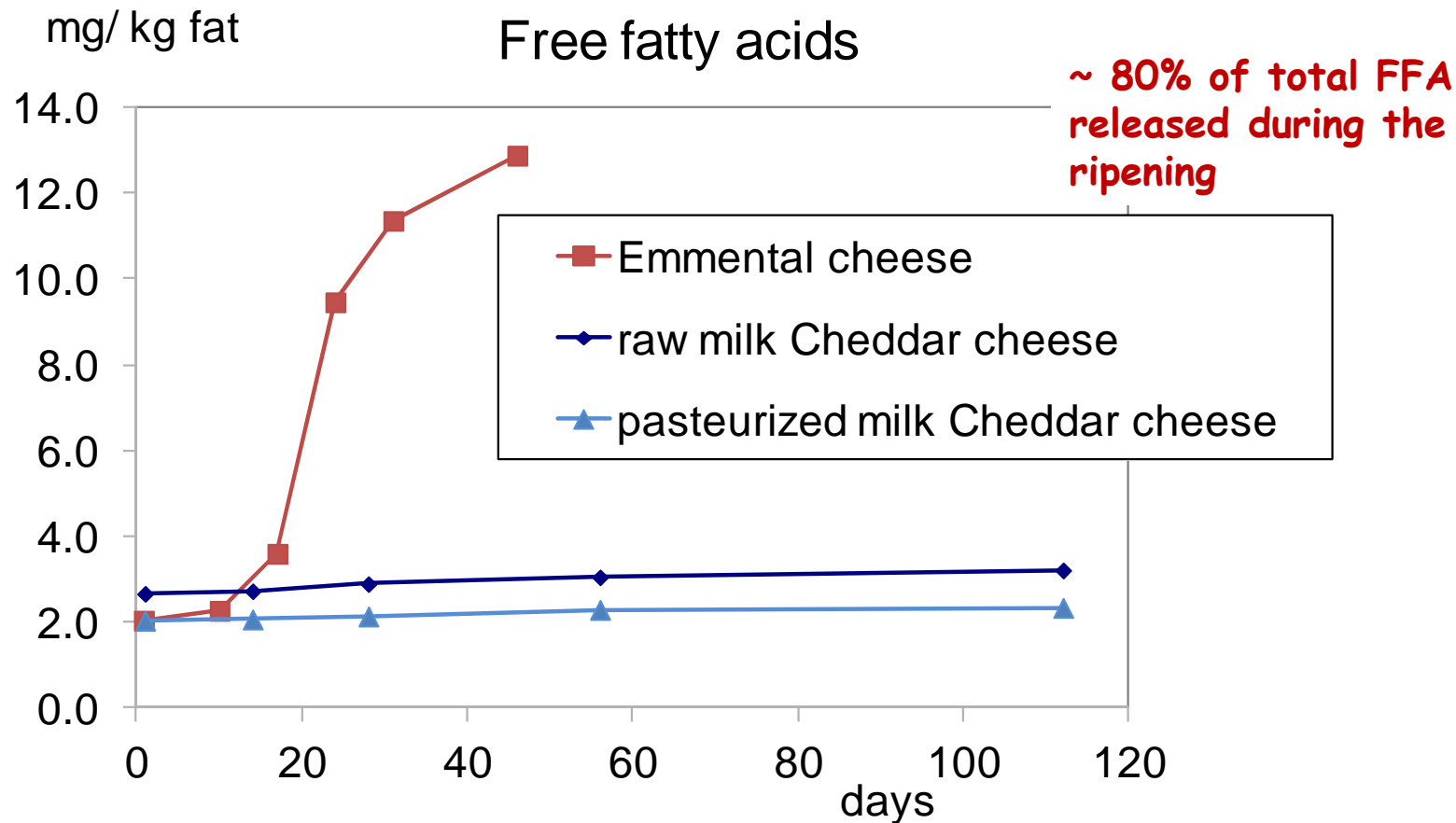
# Time course of lipolysis in Cheddar and Emmental cheeses

*Hickey, 2007*  
*Dherbecourt, 2010*

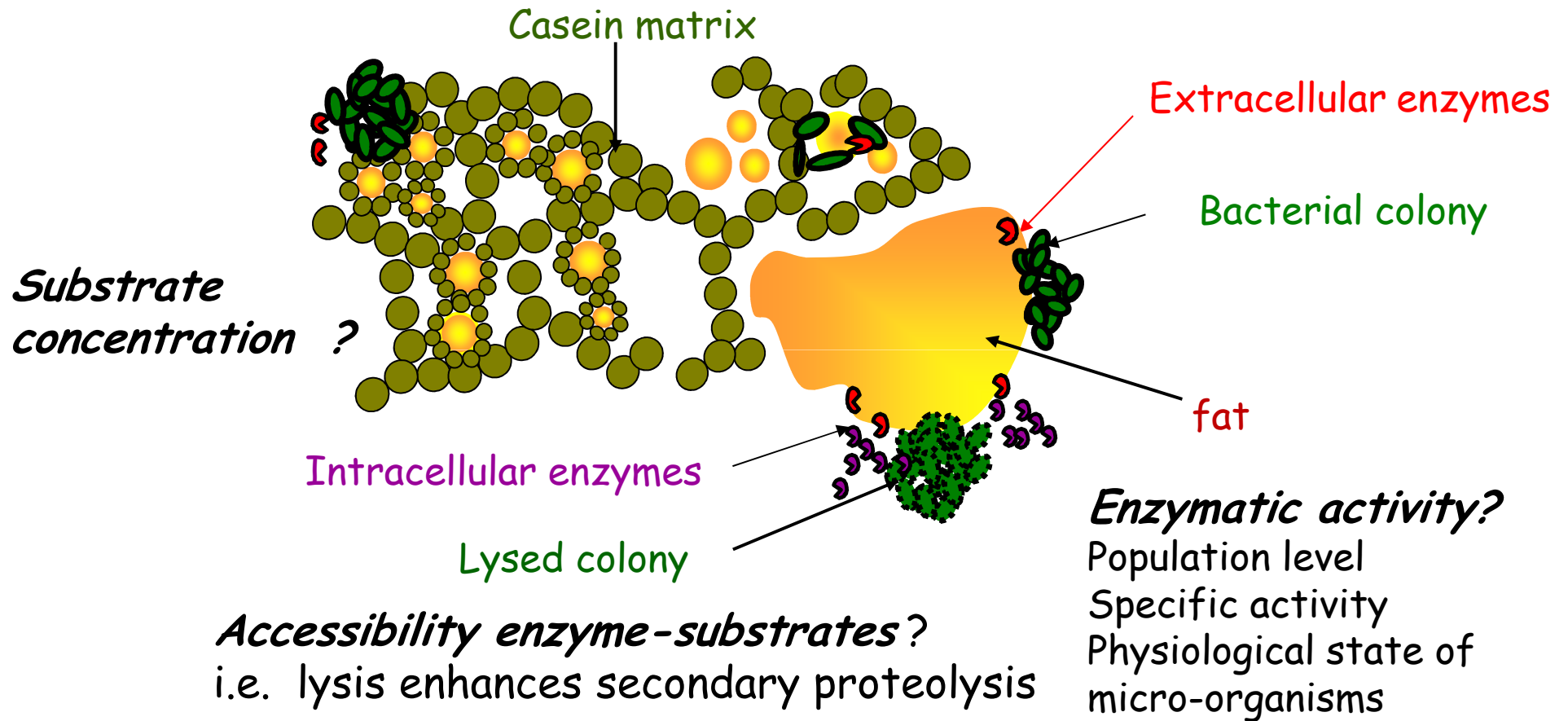


# Time course of lipolysis in Cheddar and Emmental cheeses

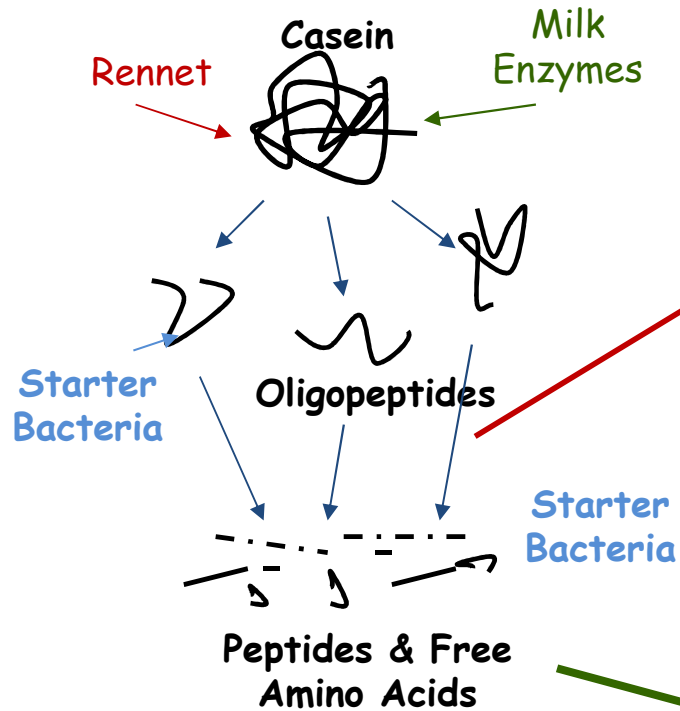
Hickey, 2007  
Dherbecourt, 2010



# Factors that may limit the rate of flavour compound formation



# Example 1: factor limiting the rate of flavour compound formation from casein



Increasing free amino acids by either adding amino acids or enhancing peptidolysis...

does not always modify cheese flavour

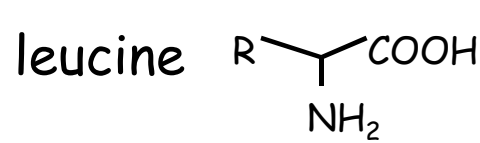
*Yvon et al, 1998*

The rate-limiting step is the conversion of amino acids rather than their release

Volatile Flavour Compounds

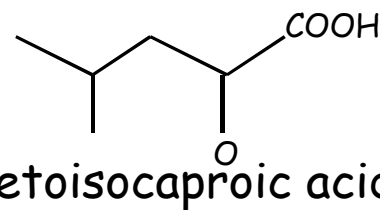
# Example 1: factor limiting the rate of flavour compound formation from casein

## Transamination



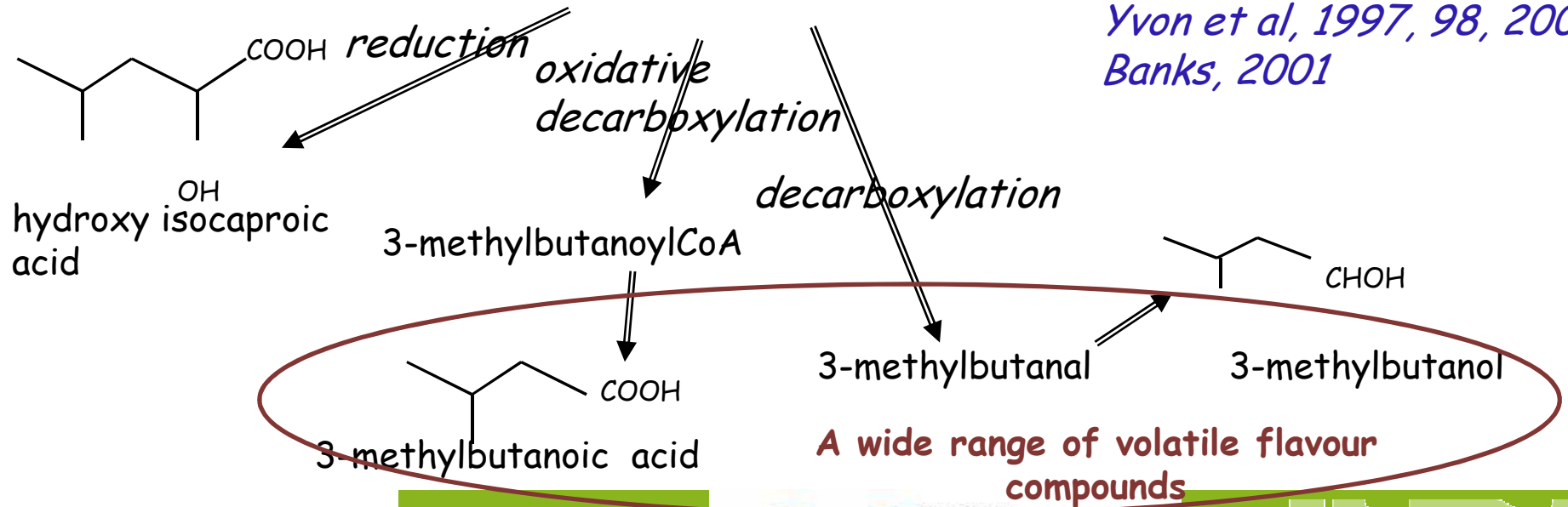
$\alpha$ -ketoglutarate

The  $\alpha$ -keto acid is the limiting factor for the amino acid conversion



glutamate

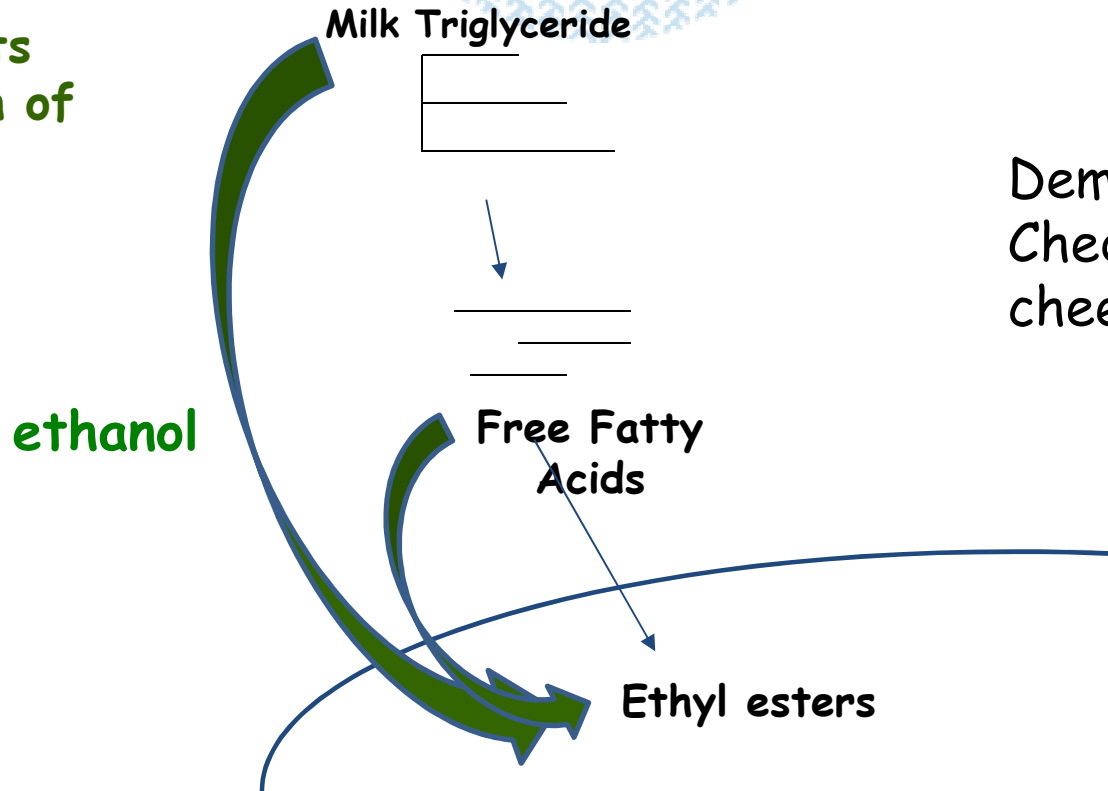
*Yvon et al, 1997, 98, 2001  
Banks, 2001*





# Example 2: factor limiting the rate of ethyl ester formation

[ethanol] limits the formation of ethyl esters (not esterase activity)



Demonstrated in Cheddar and Swiss cheeses



## Reactions of formation of ethyl esters



*Urbach, 1993*  
*Liu et al., 2004*  
*Thierry et al, 2006*

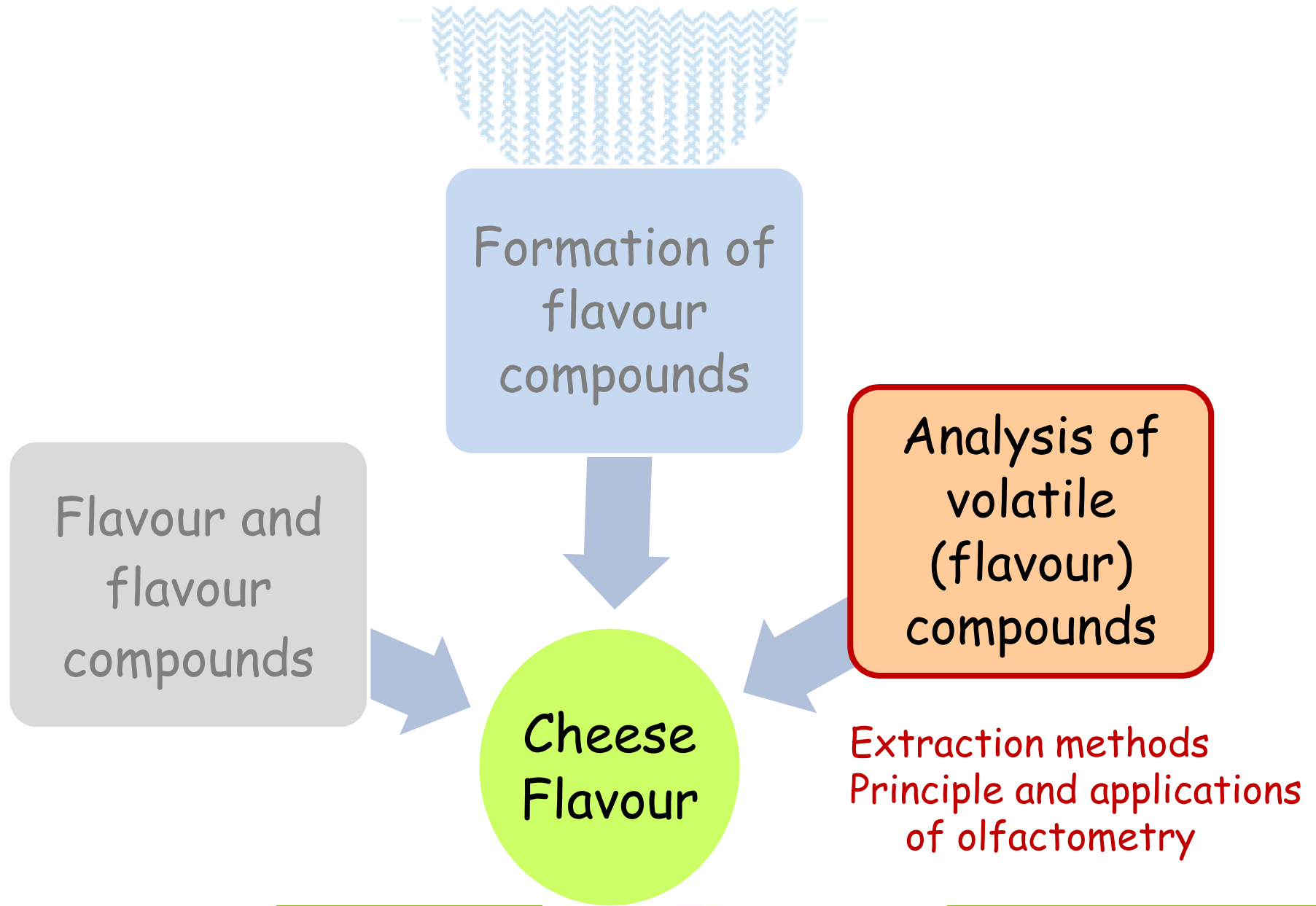
## In summary...

**Micro-organisms** play a major role in the formation of volatile flavour compounds

Culturable, non culturable cells, permeabilised and lysed bacteria contribute to the production of flavour compounds

The evolution of volatile compounds varies according to the volatile, the variety of cheese, as well as the technological parameters employed

Better knowledge about the pathways of formation of flavour compounds and some bottlenecks have been identified in these pathways



# Methods of extraction and analysis

## 1. **Extraction:** isolate volatile compounds from the food matrix

Methods based on the polarity and/or volatility of flavour compounds: solvent extraction, **head space techniques**

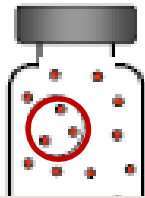
A delicate and crucial step !  
Objective: to extract "relevant" volatiles,  
representative of what is released in the mouth

## 2. **Analysis: Separation - detection - identification**

by gas chromatography - mass spectrometry (GC-MS)

# Head space-related extraction methods

## Static headspace (SH)



aliquot of head space injected



sample heated (40-60°C) until equilibrium of volatiles in the headspace

- ☺ simple, reliable and easily automated
- ☹ poor sensitivity



## Other head space-related methods

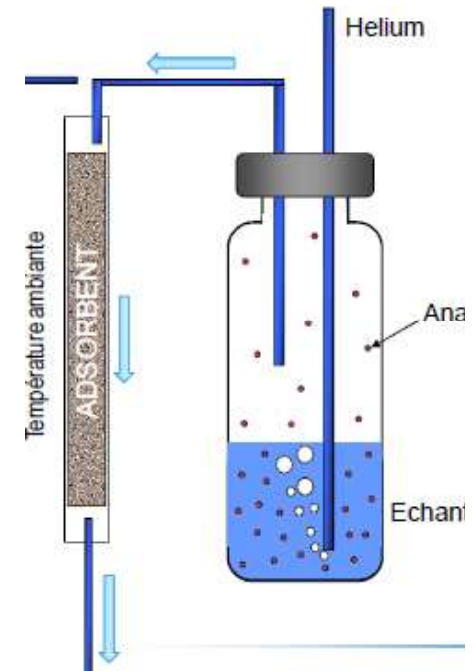
SPME: Solid Phase Microextraction

ITEX: in tube extraction

Micro chamber thermal extraction system and thermal desorption (Markes International)

HS Trap (Perkin Elmer)

## Dynamic headspace (DHS) Purge & trap



- Volatiles removed from the headspace by a flow of He and concentrated in a trap by adsorption
  - ☺ great sensitivity
  - ☹ not fully automated



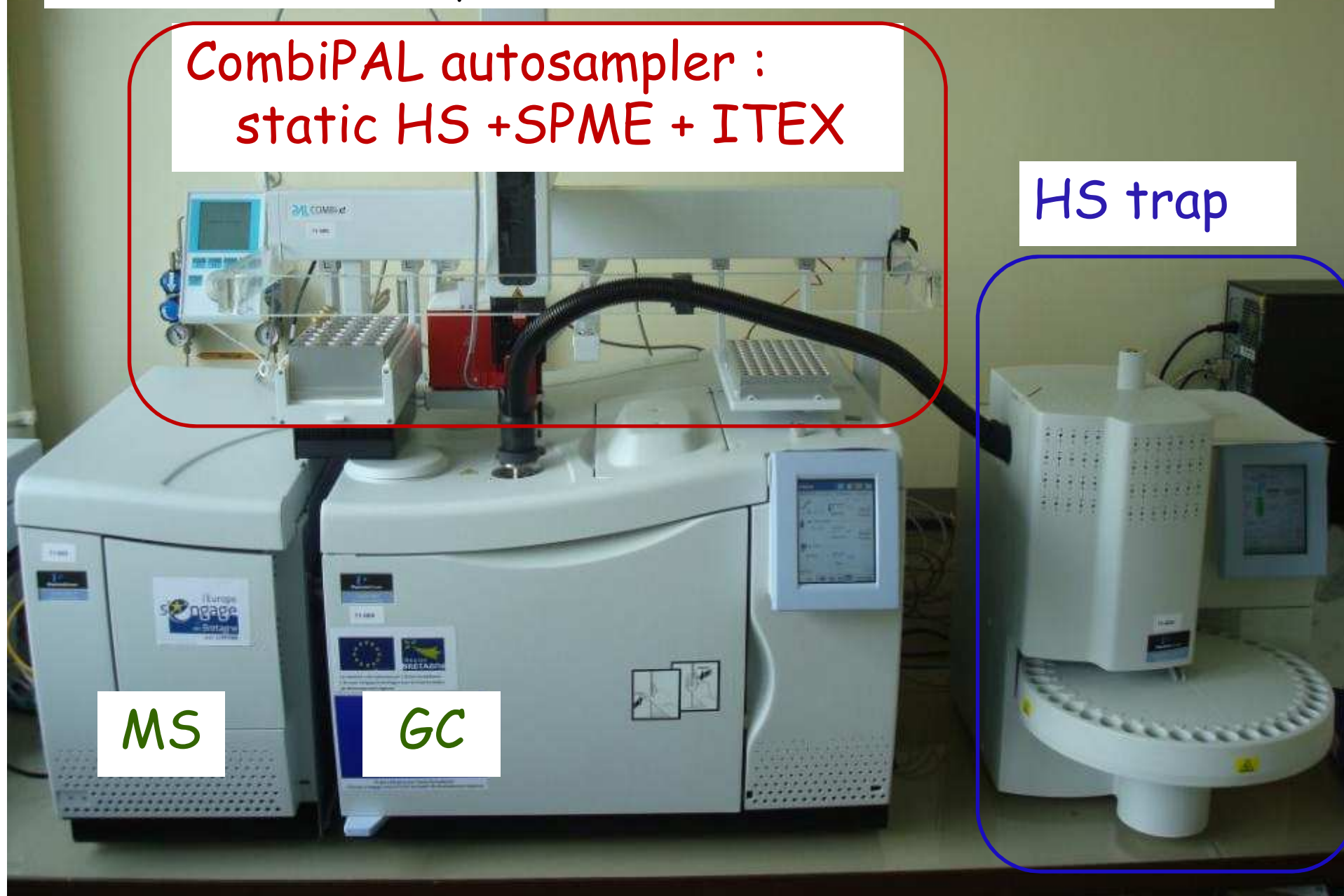
Different extraction systems can be attached to the same GC-MS

CombiPAL autosampler :  
static HS + SPME + ITEX

HS trap

MS

GC



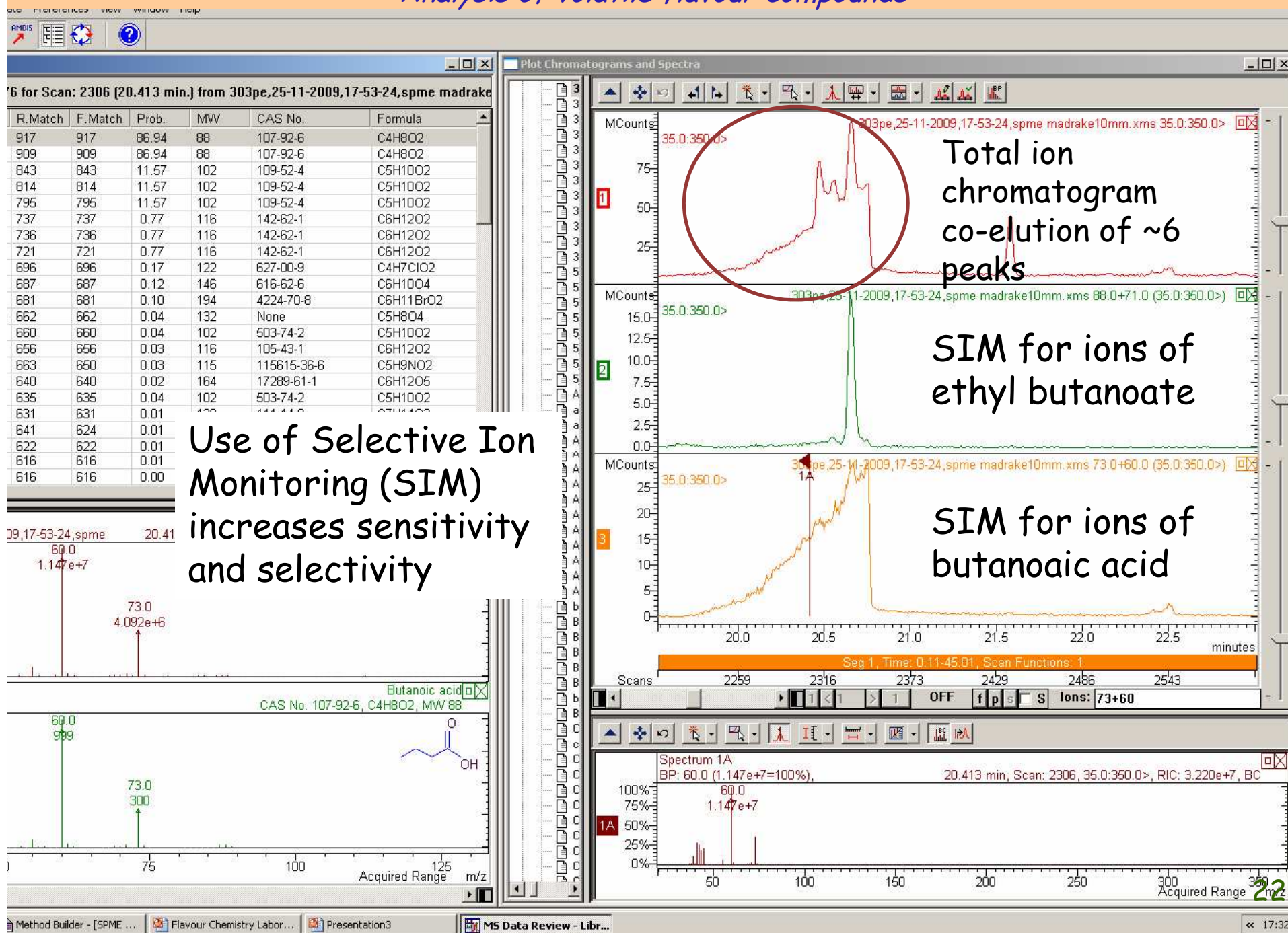


# Analysis of volatiles

Advances in the sensitivities of mass spectrometers (quadrupole, triple quadrupole or Time-Of-Flight), improvements in the databases, improvements in the deconvolution software are all helping to make analysis more accurate and user-friendly



# Analysis of volatile flavour compounds



# Comparison of DHS extraction methods

Barron et al. , 2005

3 DHS methods routinely used in 3 laboratories applied to a Spanish cheese (La Serena)

→ 78-85 individual compounds identified by each method

The volatiles analysed are the ones extracted !

Percentages of different classes of volatiles

	Lab. A Rektorik graphitised carbon	Lab. B O.I. Analytical tenax	Lab. C Tekmar tenax
acids	5.5	2.4	0.01
alcohols	15.1	84.6	84.8
aldehydes	58.4	0.6	1.1
ketones	4.5	8.1	2.3
esters	14.2	3.0	11.4
others	2.3	1.2	0.3

# Comparison of different extraction methods

Ring test in progress to assess different systems

Five labs



Belfast  
Linda Farmer



MFRC  
John Hannon



Gareth Roberts

Rennes  
Anne Thierry



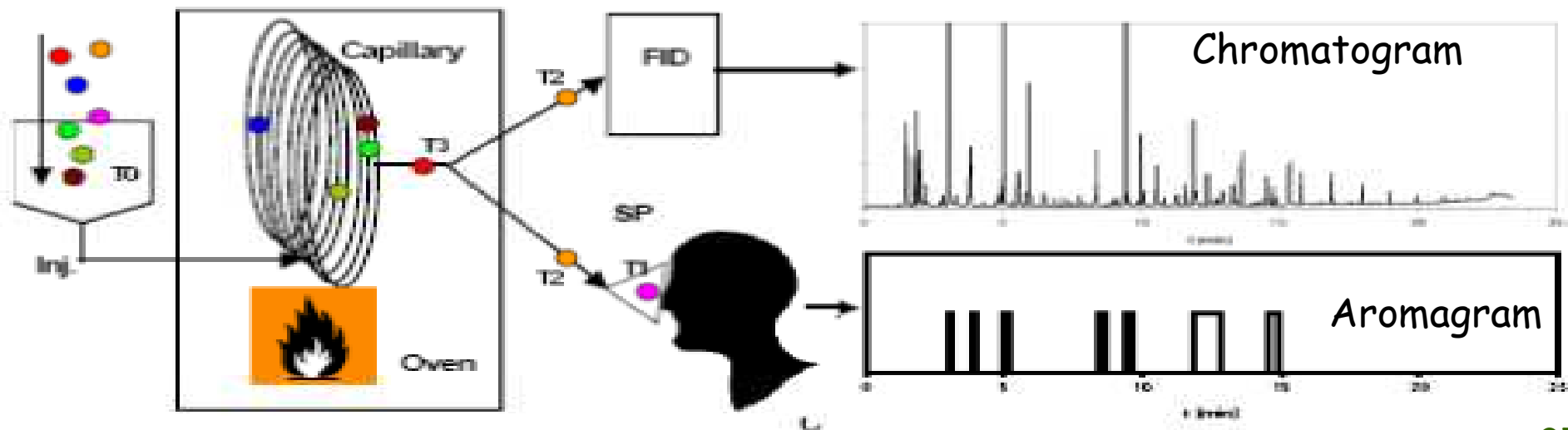
INRA Poligny  
Solange Buchin

Four cheese samples



# Principle and interest GC-Olfactometry

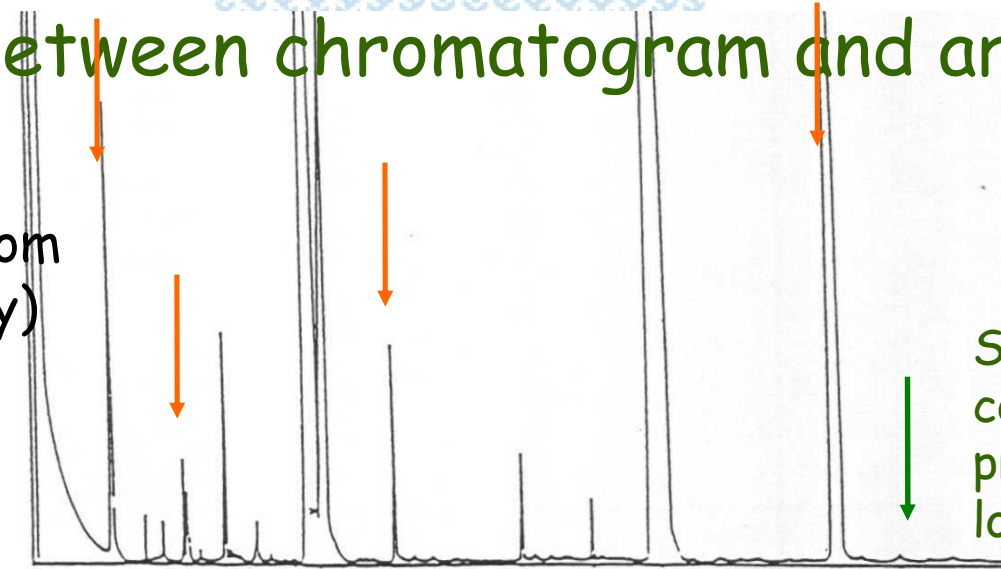
- Olfactometry: essential to identify the most potent flavour-active volatiles
- Column split in 2, 1 to the sniffing port and 1 to the detector
- As an odorant appears, the sniffer presses a button during the perception and generates an aromagram





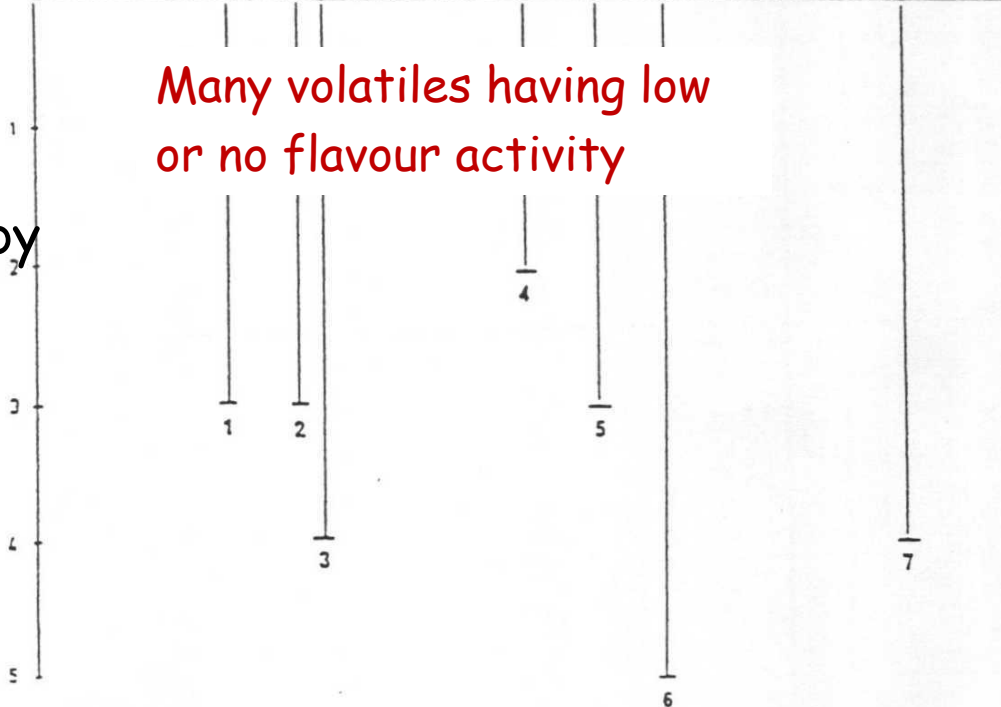
# Comparison between chromatogram and aromagram

Chromatogram (from mass spectrometry)



Some key flavour compounds are present at a very low concentration

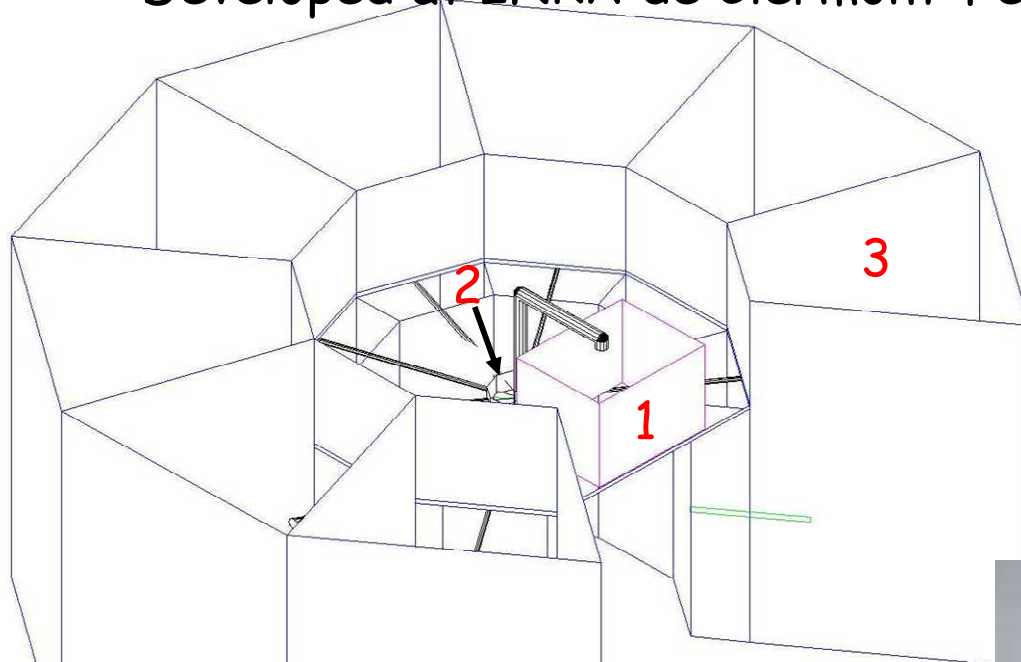
Aromagram (from perception by the human nose)



Many volatiles having low or no flavour activity

## 8 ways GC-Olfactometry system

Developed at INRA de Clermont-Ferrand-Theix (JL Berdagué)



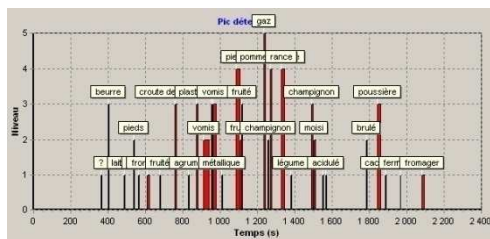
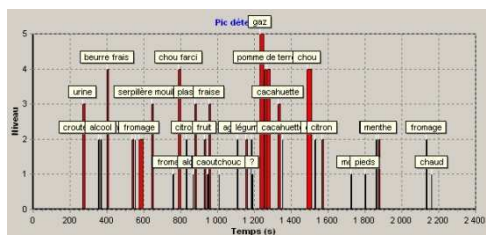
- 1 - Gas chromatograph
- 2 - Synchronous division of gas flux
- 3 - 8 olfactometry stations

Allows 8 judges to smell in parallel at 8 olfaction ports

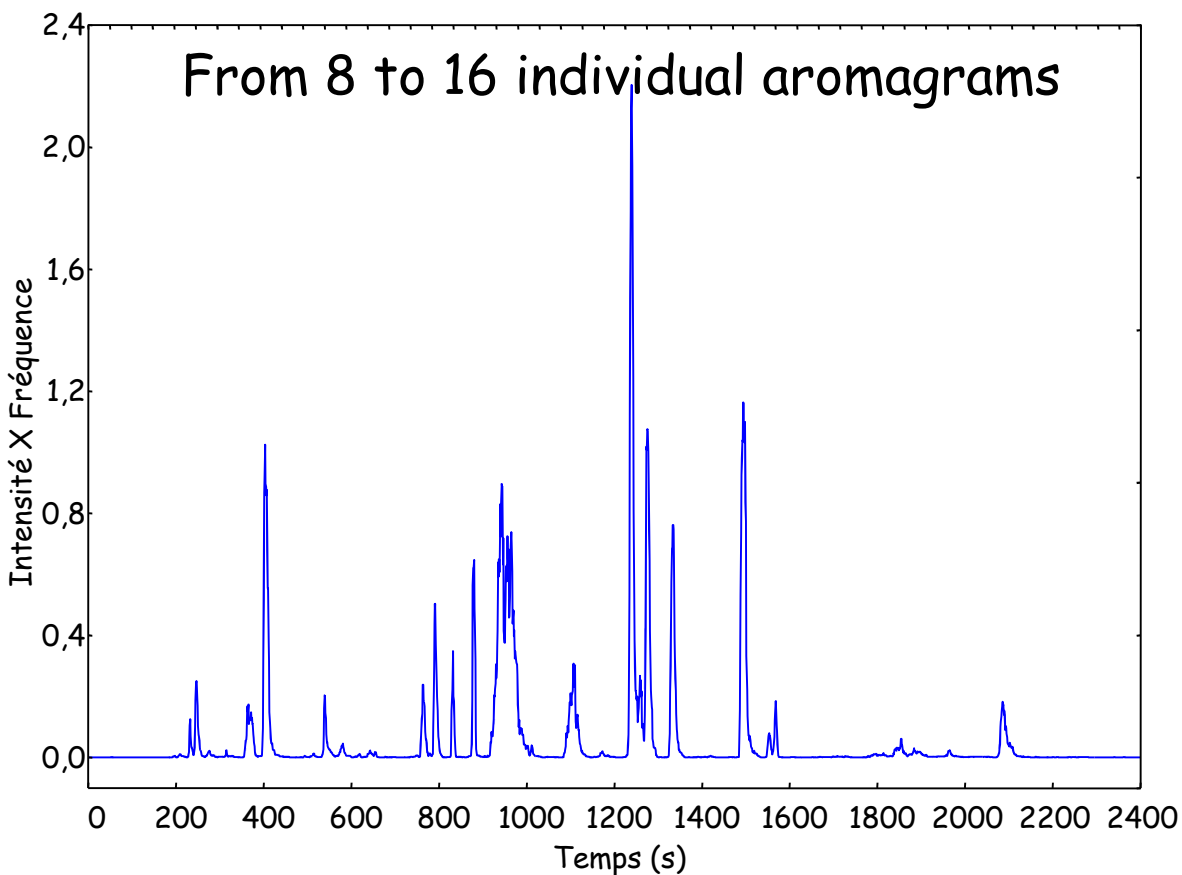
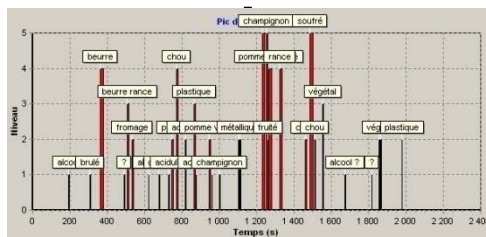
→ more robust aromagrams 😊



# Obtention of a global aromagram

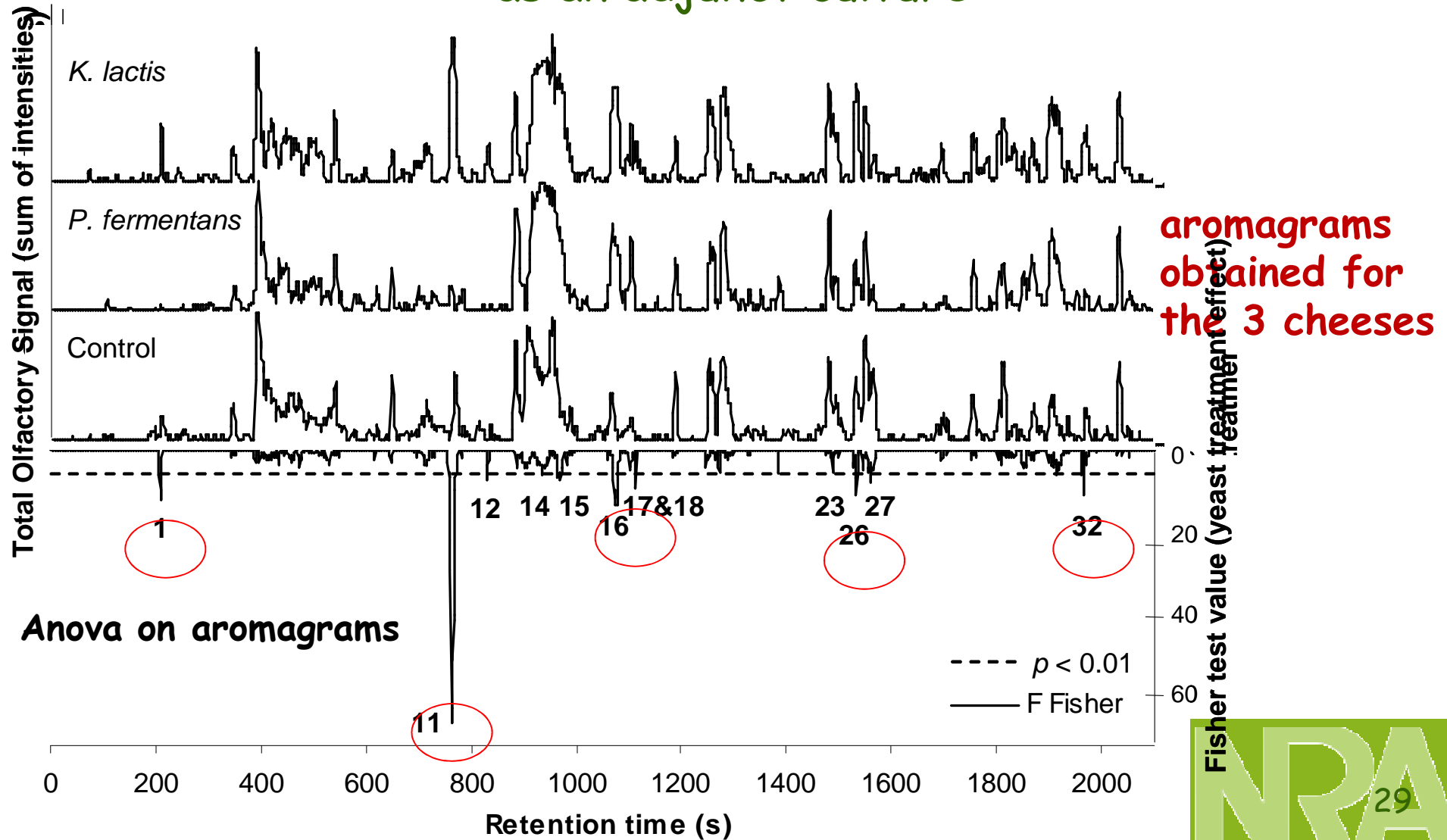


2 X 8 acquisitions...





# Example of use of 8 ways-GC-olfactometry to differentiate Cantal cheeses added or not with yeasts as an adjunct culture (De Freitas et al., 2008)



## In summary

### Extraction is a crucial step

A variety of head space-related methods are now available to extract volatile compounds

However, as each method generates a specific volatile profile, to obtain a comprehensive view several extraction should be combined - not practical!!

Essential that key flavour active compounds are extracted by whichever method employed

**GC-olfactometry/MS** helps in identifying key (new) flavour compounds

# Conclusions

Further work needed:

To further elucidate **pathways of formation of aroma compounds and identify key enzymes**

- intracellular ? (requirement of lysis)
- enzymatic activity requires intact bacteria cells ?

To better exploit the synergies between species and strains

To identify **bottlenecks** in the succession of reactions leading to the formation of flavour compounds

The ability to produce flavour compounds is highly strain-dependent.  
Therefore, **strain selection is essential**.

Screening tests have to be defined based on knowledge of the metabolic pathways and in particular their limiting steps

# Conclusions

Further work is needed to investigate:

- the contribution of viable non culturable populations in cheese,
- the metabolic activity of bacteria under the conditions of cheese ripening

(low temperature, sugar starvation,...)

Some advances expected:

- from the use of culture-independent methods to characterize populations and activity of cheese ecosystems (pyrosequencer/RT-qPCR)
- from meta-approaches addressing the global functions of the cheese ecosystem