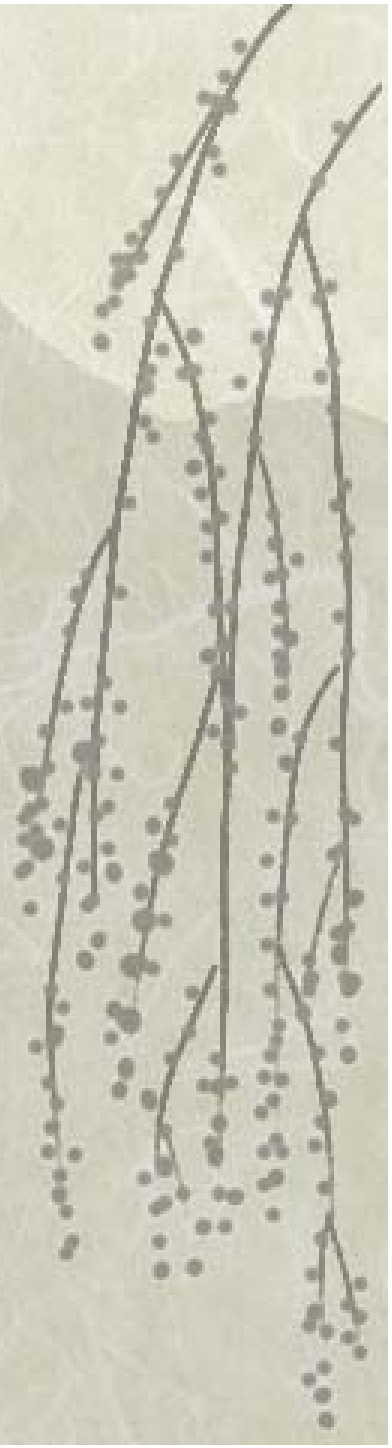
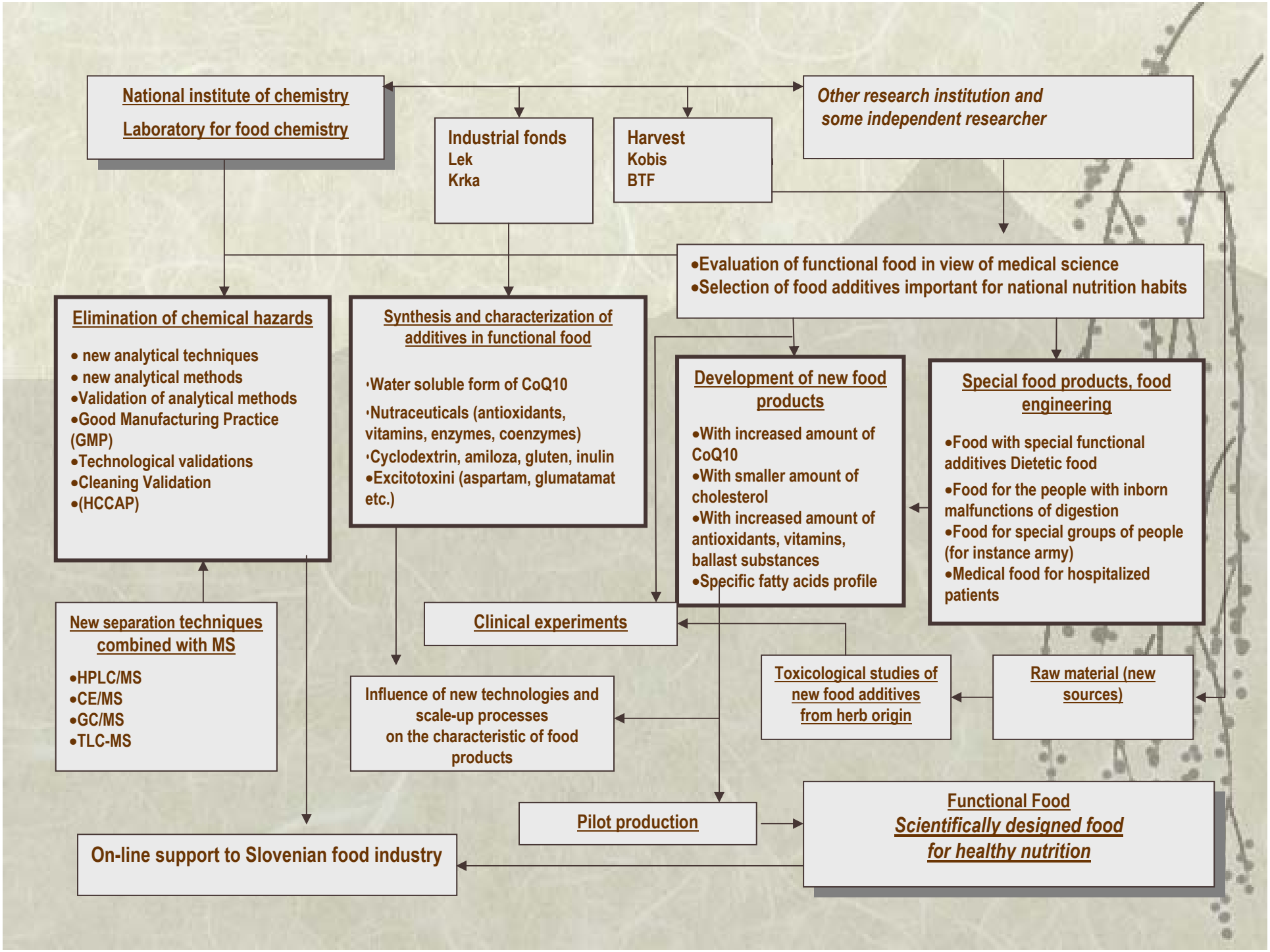
QTLC characterisation of components in food

Prošek Mirko

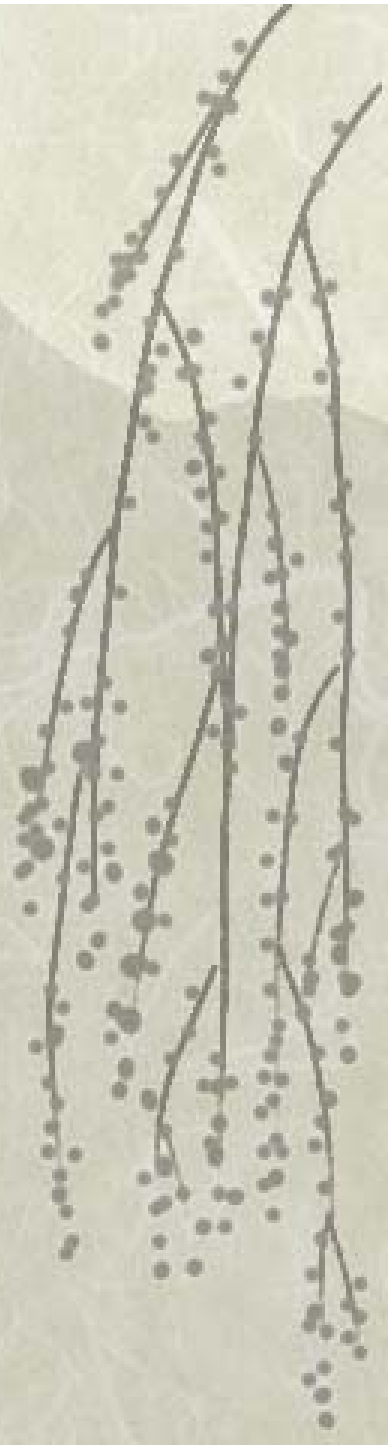
Laboratory for Food Chemistry
National Institute of Chemistry,
Ljubljana (SLO)




National institute of chemistry
Laboratory for food chemistry
Industrial fonds
Lek
Krka
Harvest
Kobis
BTF
Other research institution and some independent researcher
•Evaluation of functional food in view of medical science
•Selection of food additives important for national nutrition habits
Elimination of chemical hazards
• new analytical techniques
• new analytical methods
•Validation of analytical methods
•Good Manufacturing Practice (GMP)
•Technological validations
•Cleaning Validation
•(HCCAP)
Synthesis and characterization of additives in functional food
·Water soluble form of CoQ10
·Nutraceuticals (antioxidants, vitamins, enzymes, coenzymes)
·Cyclodextrin, amiloza, gluten, inulin
•Excitotoxini (aspartam, glumatamat etc.)
Development of new food products
•With increased amount of CoQ10
•With smaller amount of cholesterol
•With increased amount of antioxidants, vitamins, ballast substances
•Specific fatty acids profile
Special food products, food engineering
•Food with special functional additives Dietetic food
•Food for the people with inborn malfunctions of digestion
•Food for special groups of people (for instance army)
•Medical food for hospitalized patients
New separation techniques combined with MS
•HPLC/MS
•CE/MS
•GC/MS
•TLC-MS
Clinical experiments
Toxicological studies of new food additives from herb origin
Raw material (new sources)
Influence of new technologies and scale-up processes on the characteristic of food products
Pilot production
Functional Food
Scientifically designed food for healthy nutrition
On-line support to Slovenian food industry

Functional Food

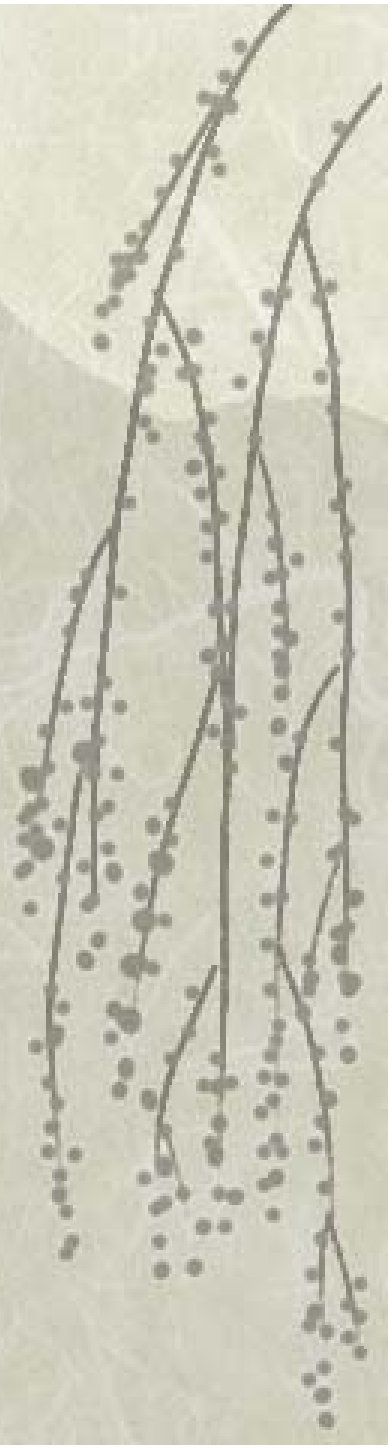
- Food is a delight



Functional Food

Food scientists have to focus on developing products with the best:

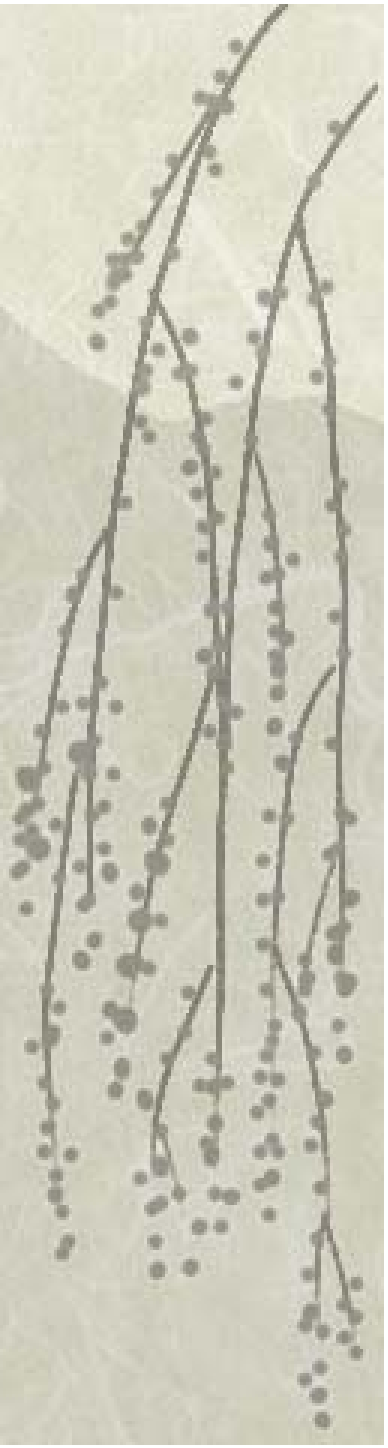
- appearance
- excitement
- freshness
- convenience
- texture
- flavour
- maximum indulgence
- contaminant free
- long shelf life
- Etc.



FUNCTIONAL FOOD

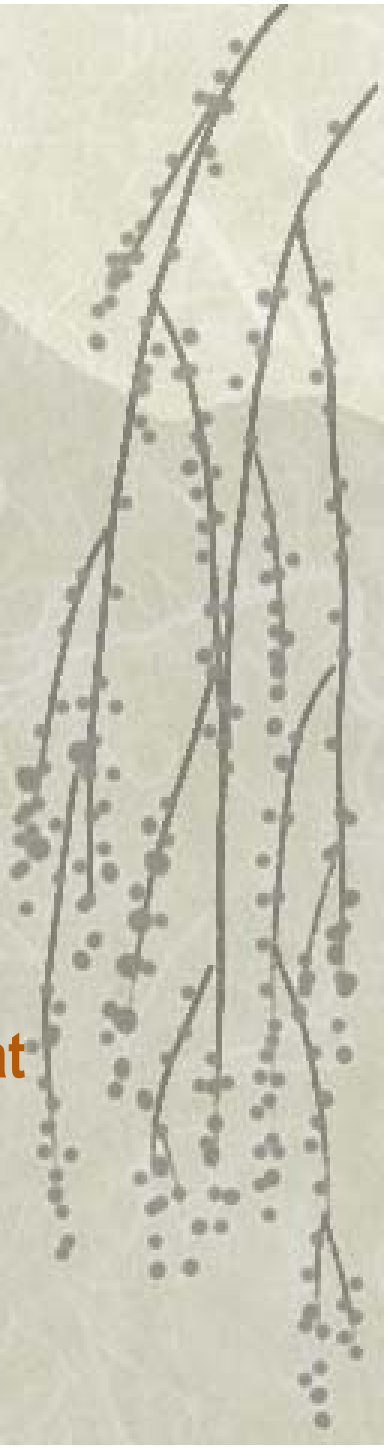
Nowadays, consumers are much more aware of the importantce of food contribution to;

- **improve quality of life**
- **health**
- **reduction of risk factors of chronic diseases**



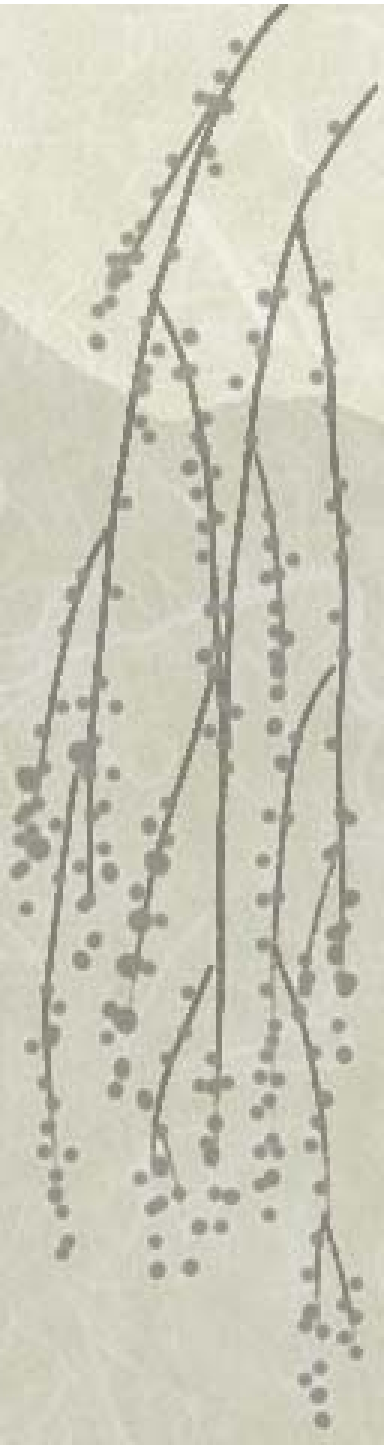
Confidence in analytical reports

- Everyday, millions of analyses are carried out in industry, hospitals, research institutes, control laboratories etc.
- The results of chemical analysis are important factors for correct decision-making in many fields of human activities.
- Users need to be sure about the reported values.
- We have to demonstrate the quality in our work that inspire confidence in analytical reports.

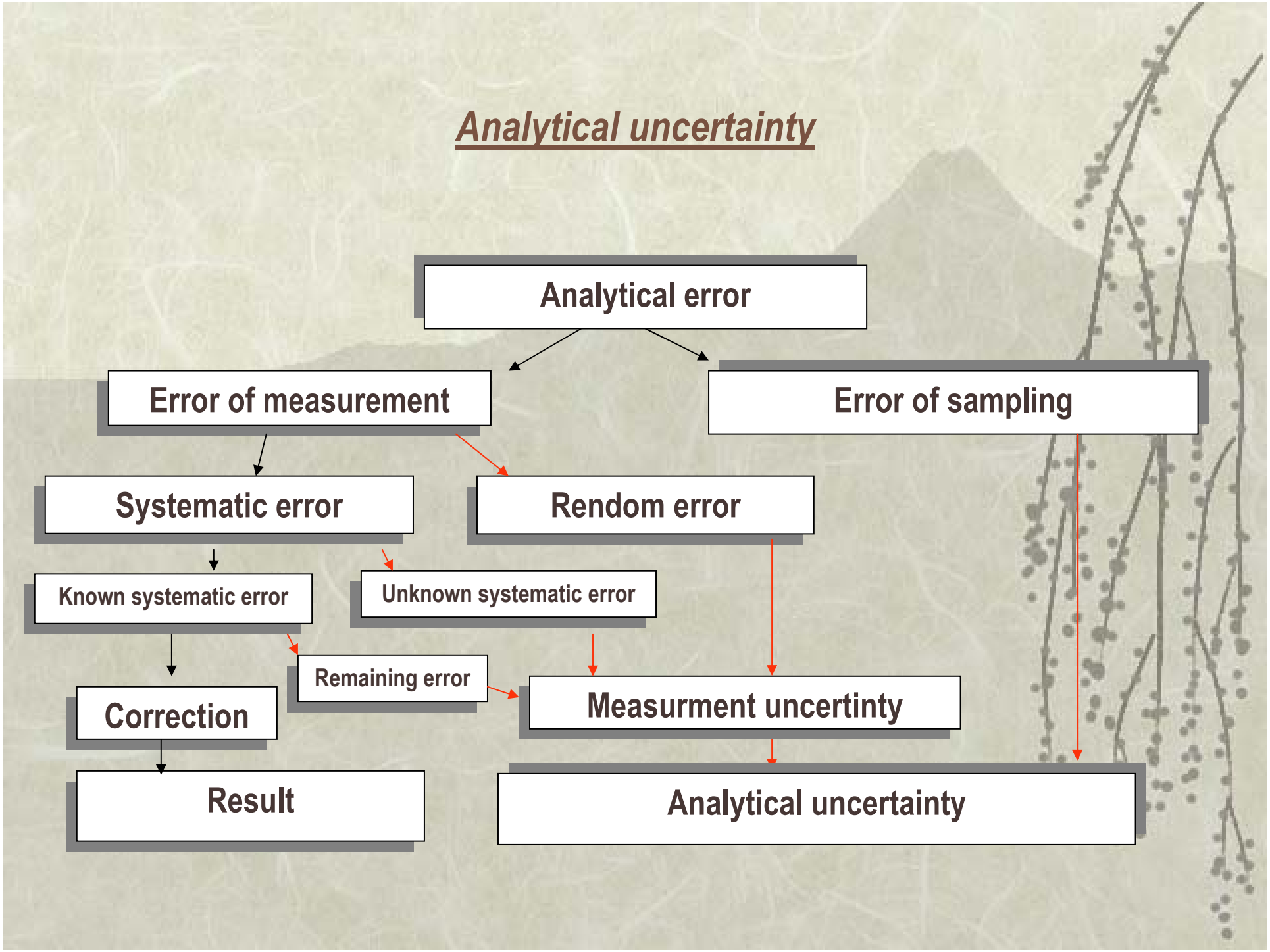


Analytical measurements

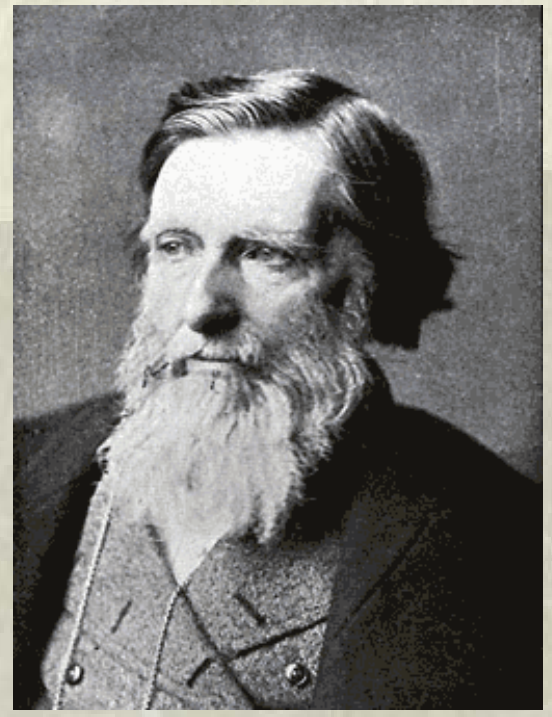
- Reliable analytical result is not an easy request today.
- It was not easy thirty or more years ago, when analysts had no instruments, computers, and metrological standards.
- But analytical results were sound and decisions were correct. Where was the miracle? The answer is simple.
- In that time, analyst were familiar with analytical management. Analytical knowledge was oriented towards the understanding and the elimination of sample uncertainty and not into formalistic solutions of measurement uncertainty that are so popular today.



Analytical uncertainty



Basic motto of Laboratory for Food Chemistry is



“Quality is never an accident.

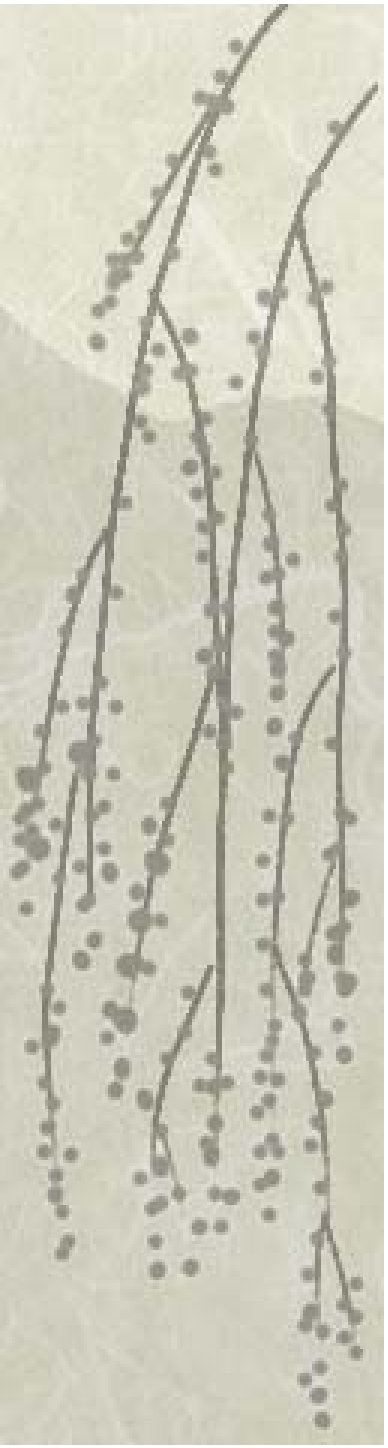
It is always the result of intelligent effort.

There must be the will to produce a superior thing”

John Ruskin

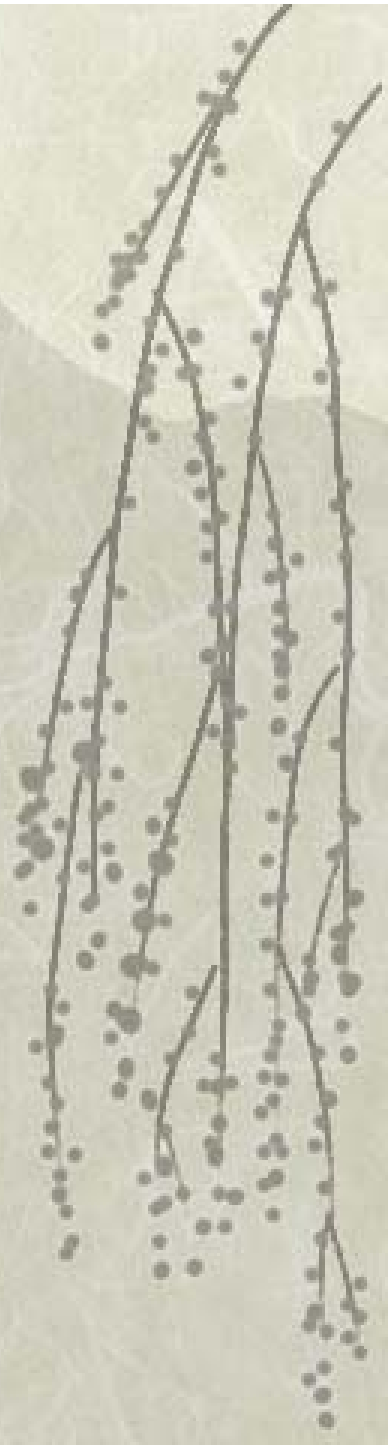
Elimination of chemical hazards

- New analytical techniques
- New analytical methods
- Validation of analytical methods
- Technological validations
- Cleaning Validation
- Good Manufacturing Practice (GMP)
- (HCCAP)



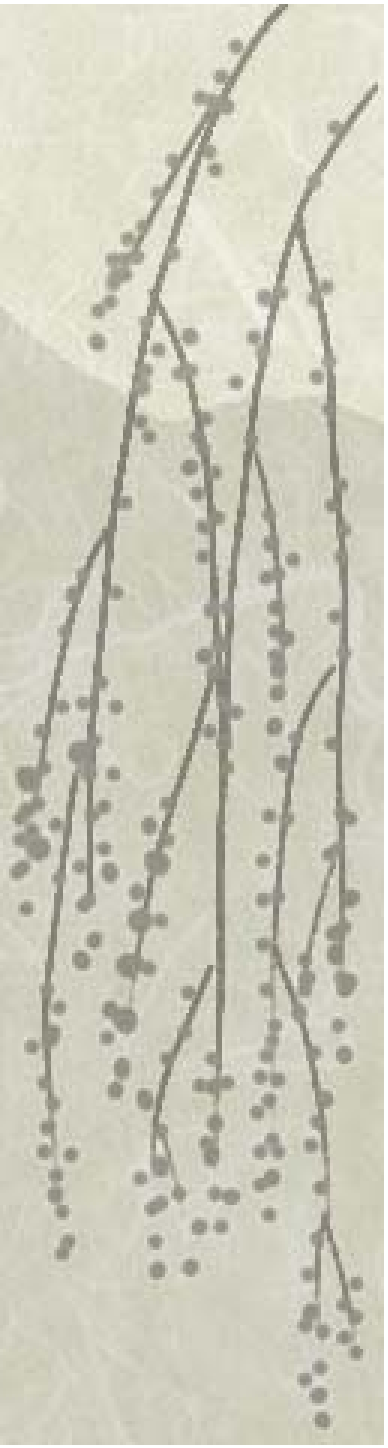
Synthesis and characterization of additives in functional food

- **Water soluble form of CoQ10**
- **Nutraceuticals (antioxidants, vitamins, enzymes, coenzymes)**
- **Excitotoxini (aspartam, glumatamat etc.)**
- **Cyclodextrin, starch, amilose, gluten, inulin**



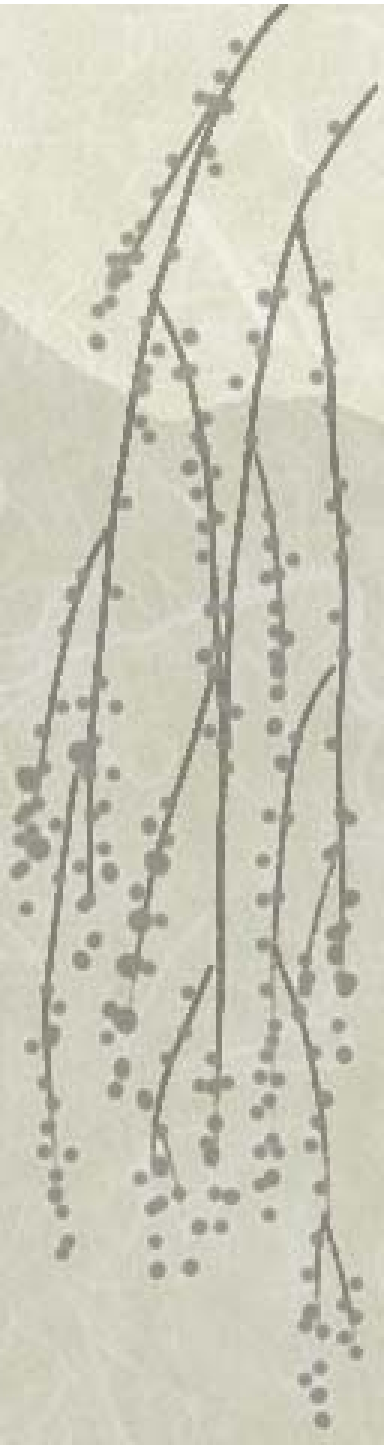
Development of new food products

- With increased amount of CoQ10
- With smaller amount of cholesterol
- With increased amount of antioxidants, vitamins, ballast substances
- Specific fatty acids profile

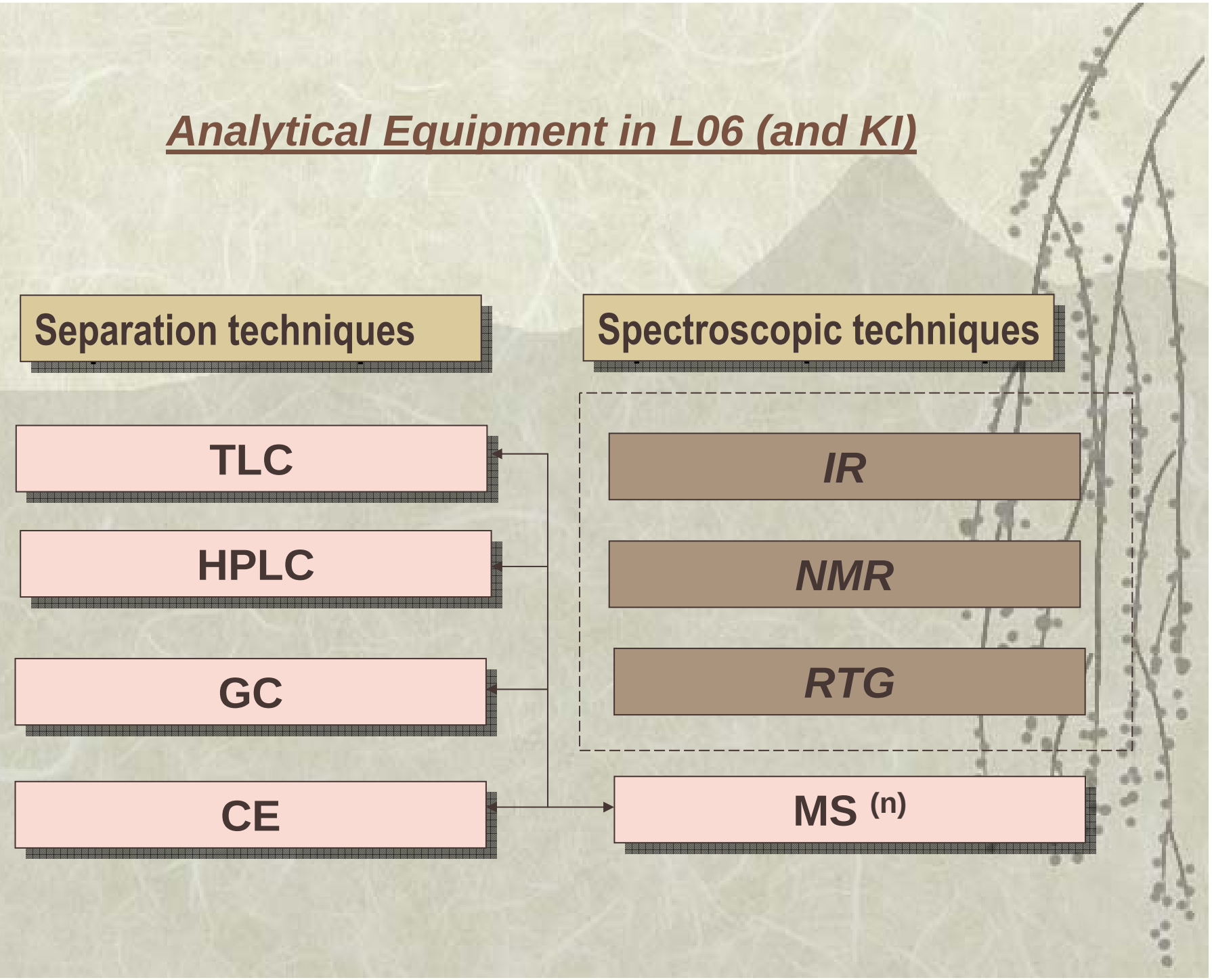


Special food products, food engineering

- Food with special functional additives
- Food for the people with inborn malfunctions
  Ataxia, PKU
- Food for special groups of people (for instance army)
- Medical food for hospitalized patients
- Dietetic food



Analytical Equipment in L06 (and KI)



Video scanner

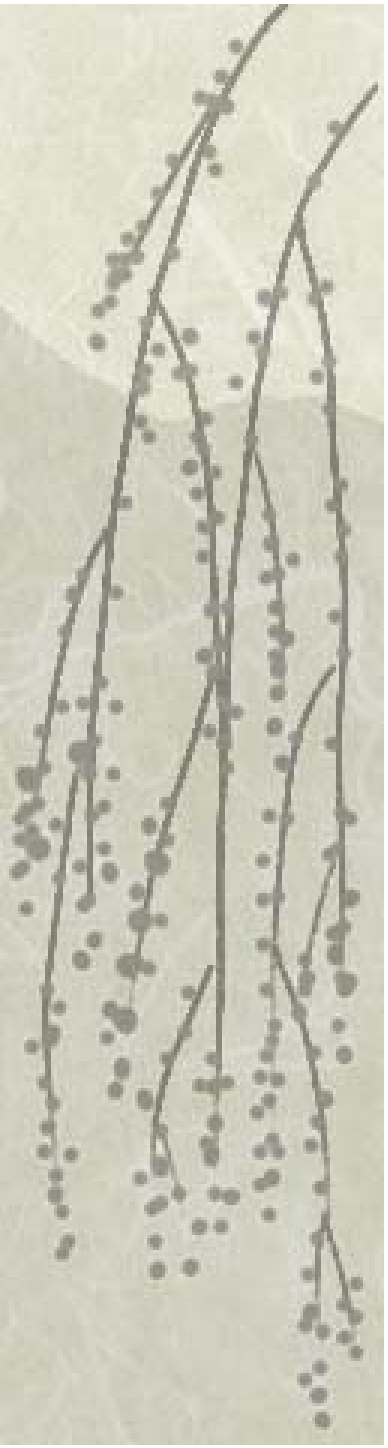
TLC dryer developed and constructed in KI

HPLC-MS system in L06

Analysis of food additives

Quantitative determination of

- **CoQ10**
- **Inulin**
- **Aloin**
- **phenylalanine and tyrosine -** *Phenylketonuria (PKU)*
- **Fatty acide profile**
- **Glutamine**
- **etc**



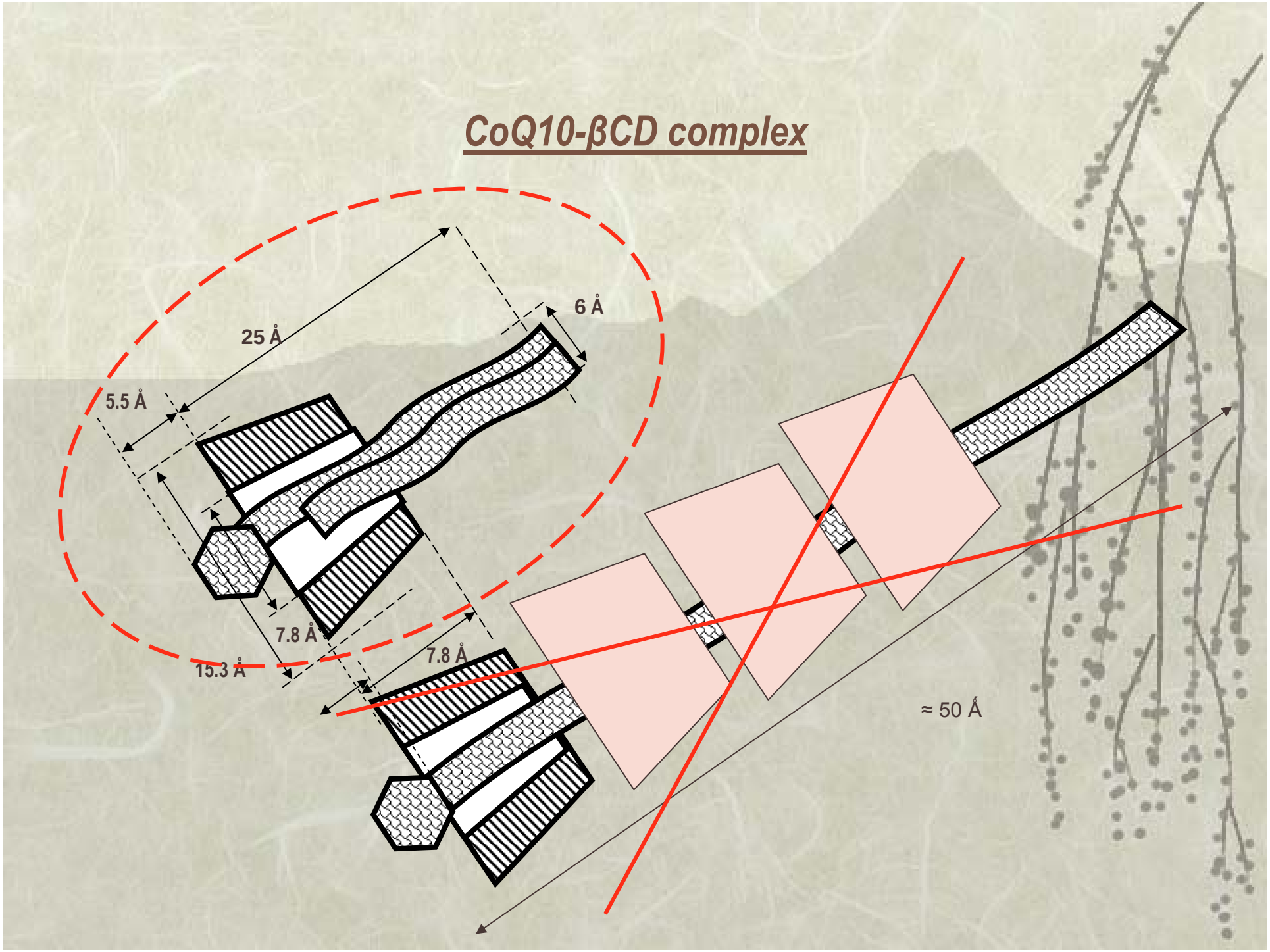
Coenzyme Q10 as a Food Additive

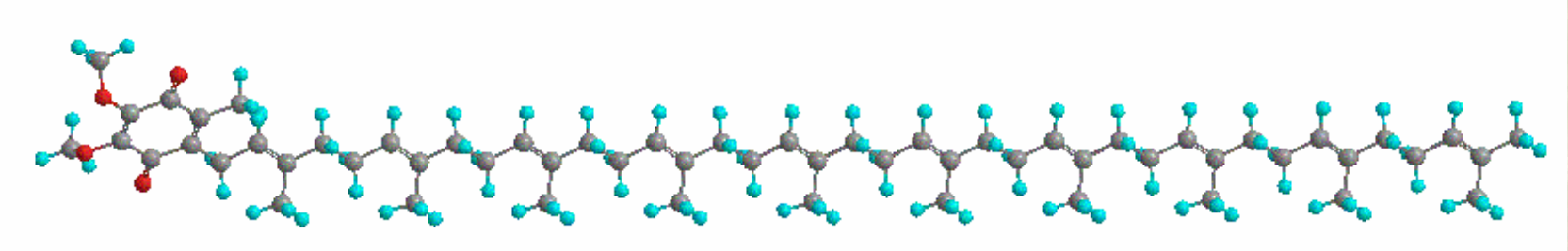
- CoQ10 is essential compound for normal body function .
- The electron and proton transfer functions of the quinone ring are of fundamental importance to all life forms.
- CoQ10 is also in its reduced form a potent antioxidant.
- Normal levels in a body are maintained both by CoQ10 intake and by the body's synthesis.
- The biosynthesis of CoQ10 is a 17 step process
- This process is highly vulnerable as we become older. We don’t manufacture enough CoQ10 and we have to increase the intake of ubiquinone.
- levels of supplementation are usually 30–90 mg per day.

Preparation of CoQ10 - β-cyclodextrin complex

- We prepared many inclusion complexes of different molecules with antioxidants activities with different types of cyclodextrins.
- One of our most succesful product is fater soluble complexof CoQ10 with β-cyclodextrin (βCD).
- Our results show that CoQ10 molecule must be foded if we want to prepare stable inclusion complex with βCD molecules.

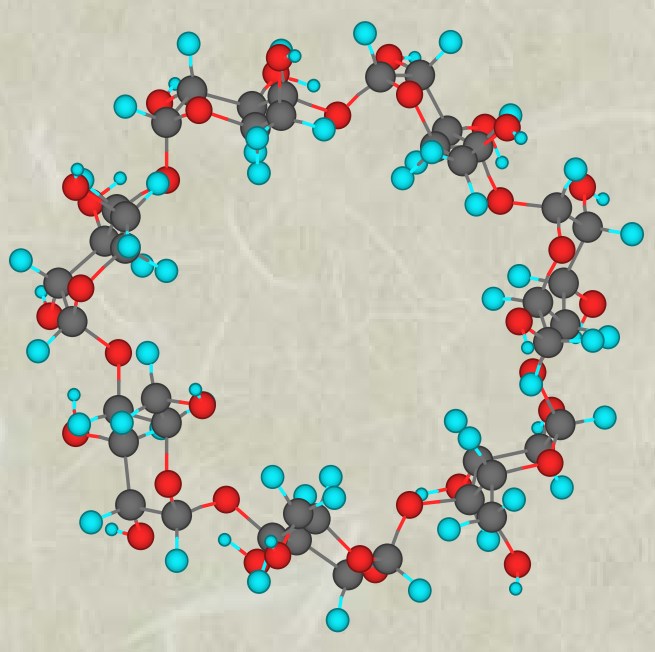
CoQ10-βCD complex

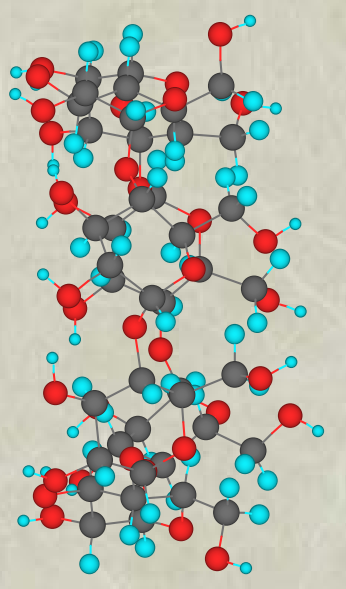


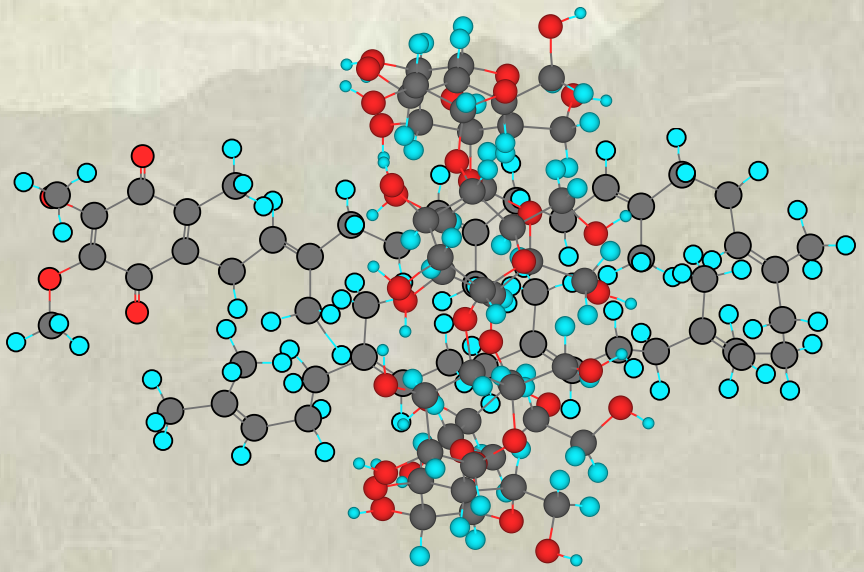


CoQ10 is long lipofilic molecule (50 Å)

β-cyclodextrin (modified starch) is a ring with water soluble outern side and lipofilic inner side.





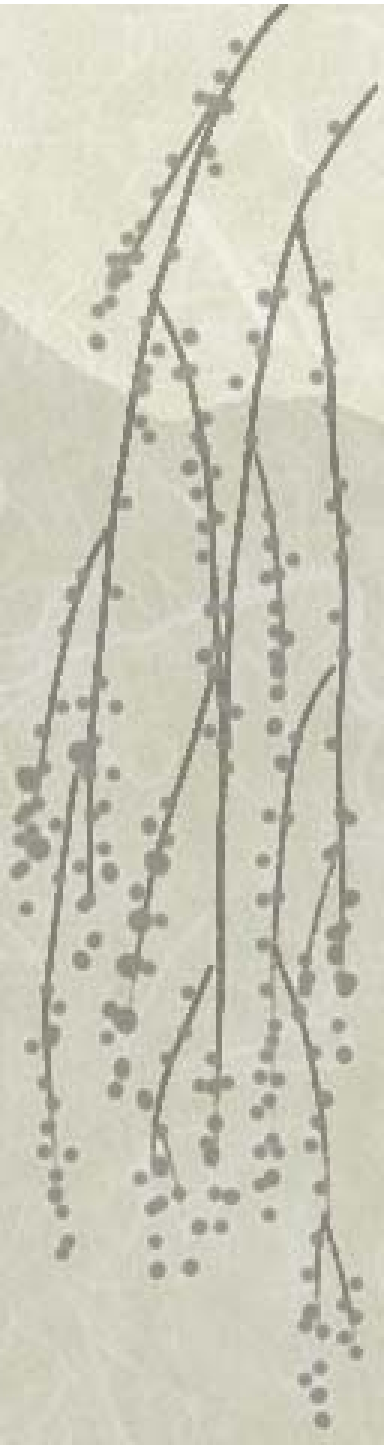


Inclusion complex

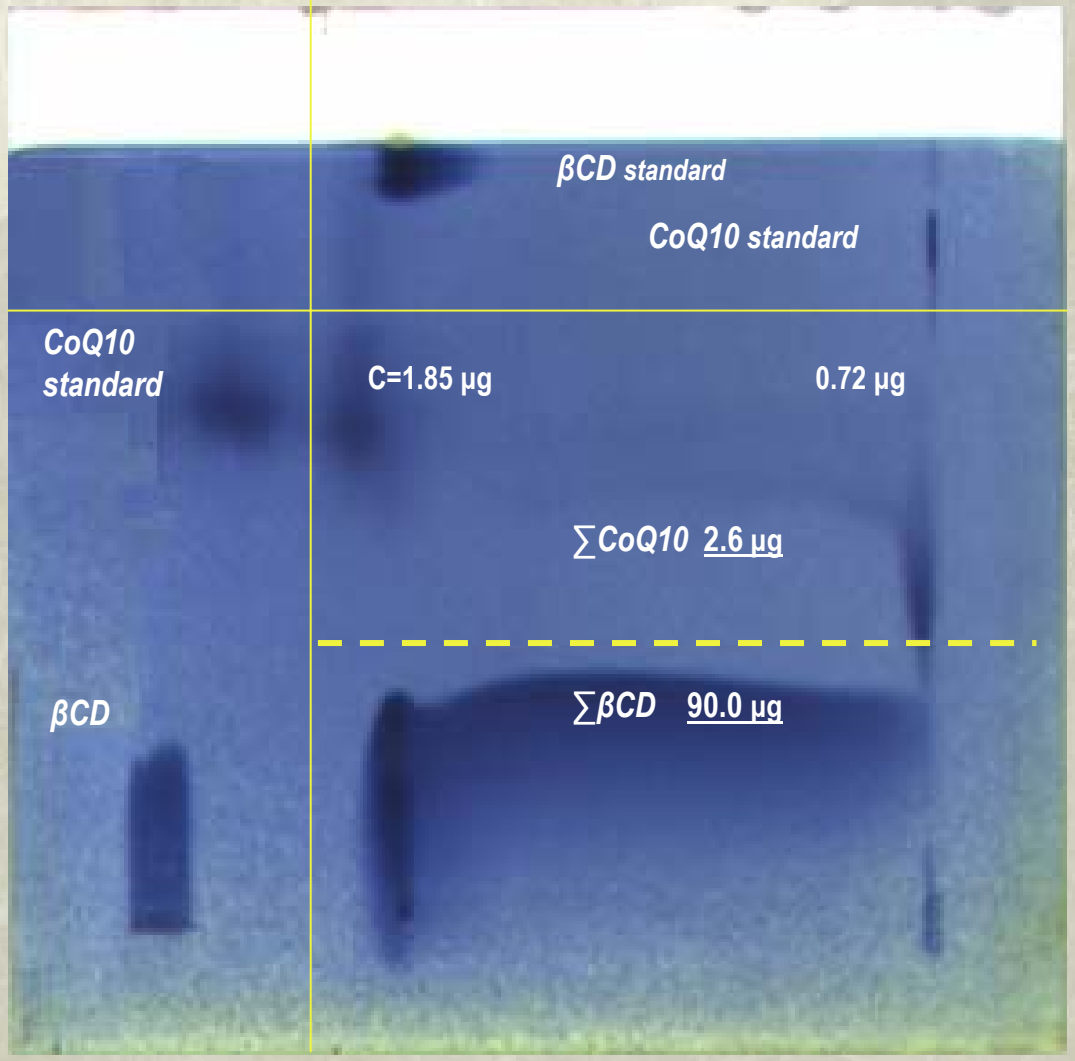
CoQ10-βCD complex

- Volume of the cavity inside βCD is about 373 A^3
- Volume of CoQ10 molecule with folded tail is 351.7 A^3 , length is 21 A, and calculated diameter is 4.6 – 5.0 A.
- Volume of not folded CoQ10 molecule is 682 A^3 , length 50 A, and diameter 4.0 – 4.5 A .
- Volume of head of CoQ10 is about 60 A^3 .

Values are calculated from the data taken from the literature



Two dimensional TLC separation of CoQ10-βCD complex



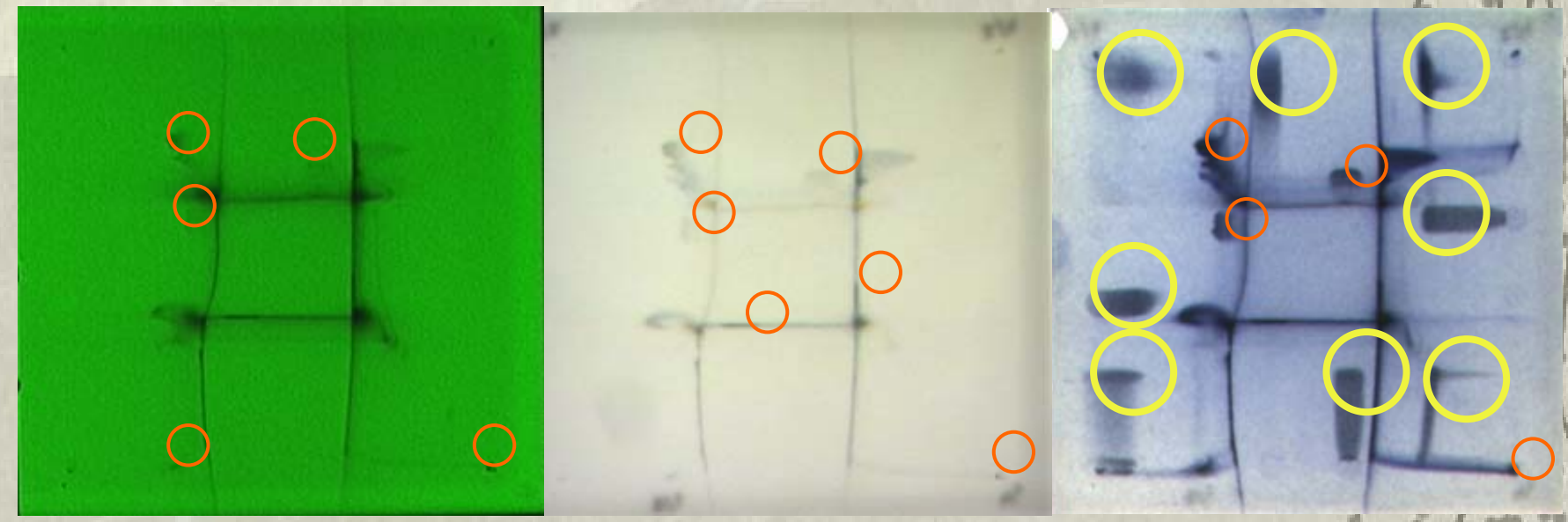
Two dimensional TLC separation of CoQ10-βCD

- In the corner 1, 8 µL of complex was applied.
- Standard of ßCD 6µL, (5 mg/mL) and standard of CoQ10, 4µL, (0.37 mg/mL) were applied on both side of the plate.
- In upper part on right side CoQ10 eliminated from β-CD during the chromatographic process
- on the left side CoQ10 forming stable complex with the β-CD are seen.
- In lower part of the plate is located β-CD. The spot in start position is β-CD and not CoQ10.

Multidimensional TLC separation

- figure B is scanned in UV
- figure C is scanned in VIS after visualization with first reagent
- figure D after second reagent.
- In figure A application positions of sample (corners) and standards (edges) are shown together with positions of developed spots, position of CoQ10 is marked with red circles.

Multidimensional TLC separationof CoQ10



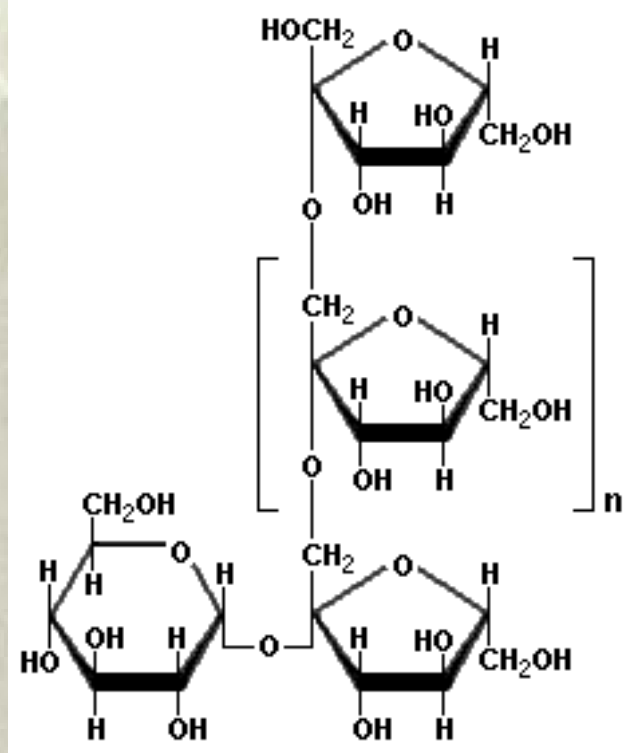
Quantitative determination of inulin

- Quantitative analysis of inuline in food samples is not a simple task, it can be a real nightmare.
- It is possible to use indirect method. In which free sugars are quantyfied after the hydrolysis of inuline. Sugars are quantyfied with GC, HPLC or TLC. This method is not very accurate.
- We developed new straight methods for semiquantitative evaluation of inulina with TLC and quantitative evaluation with HPLC-MS

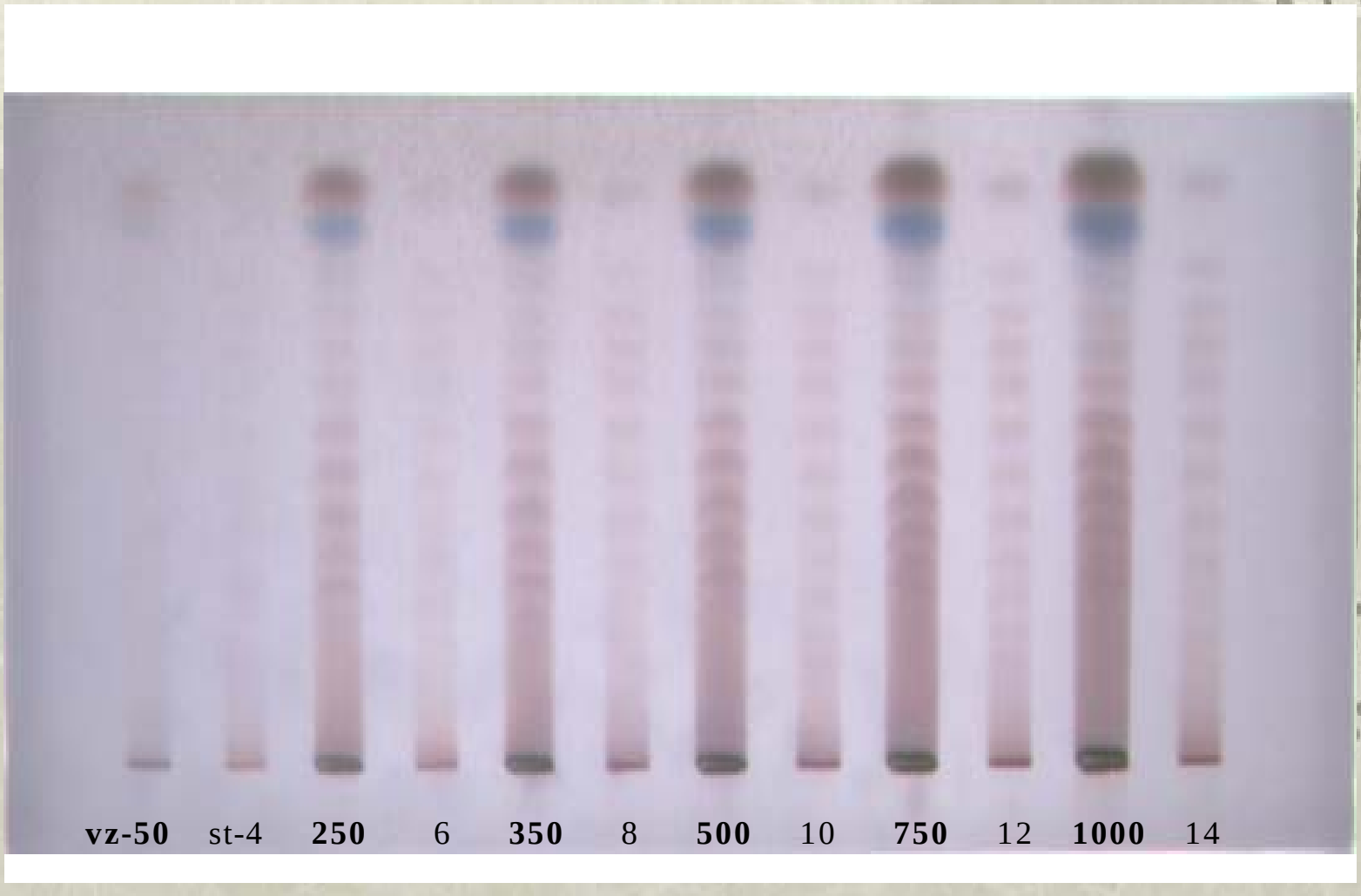
Inulin

Inulins are polymers consisting of fructose units that typically have a terminal glucose.

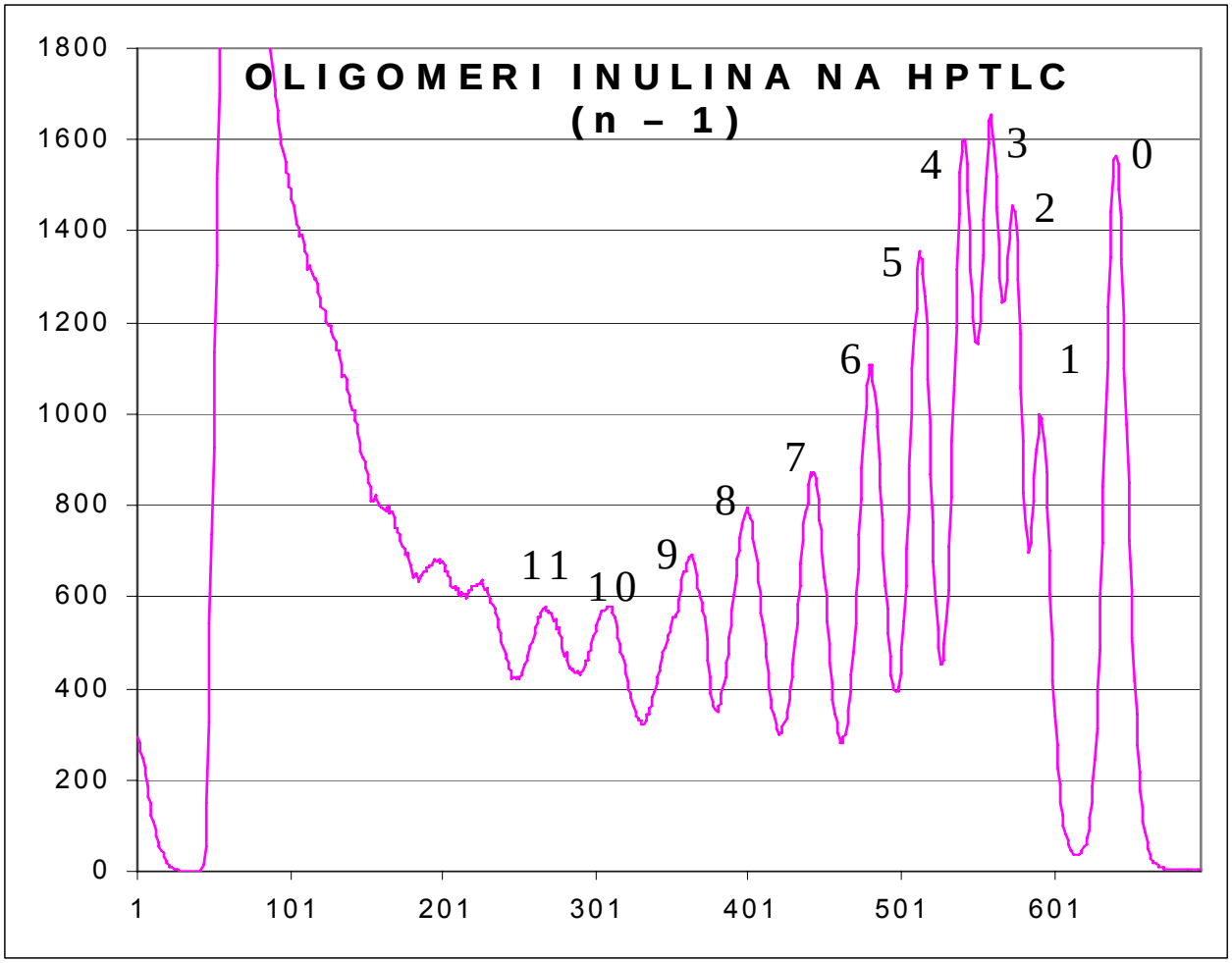
Inulins have a sweet taste and are present in many vegetables and fruits, including onions, leeks, garlic, bananas, asparagus, chicory, and Jerusalem artichokes.



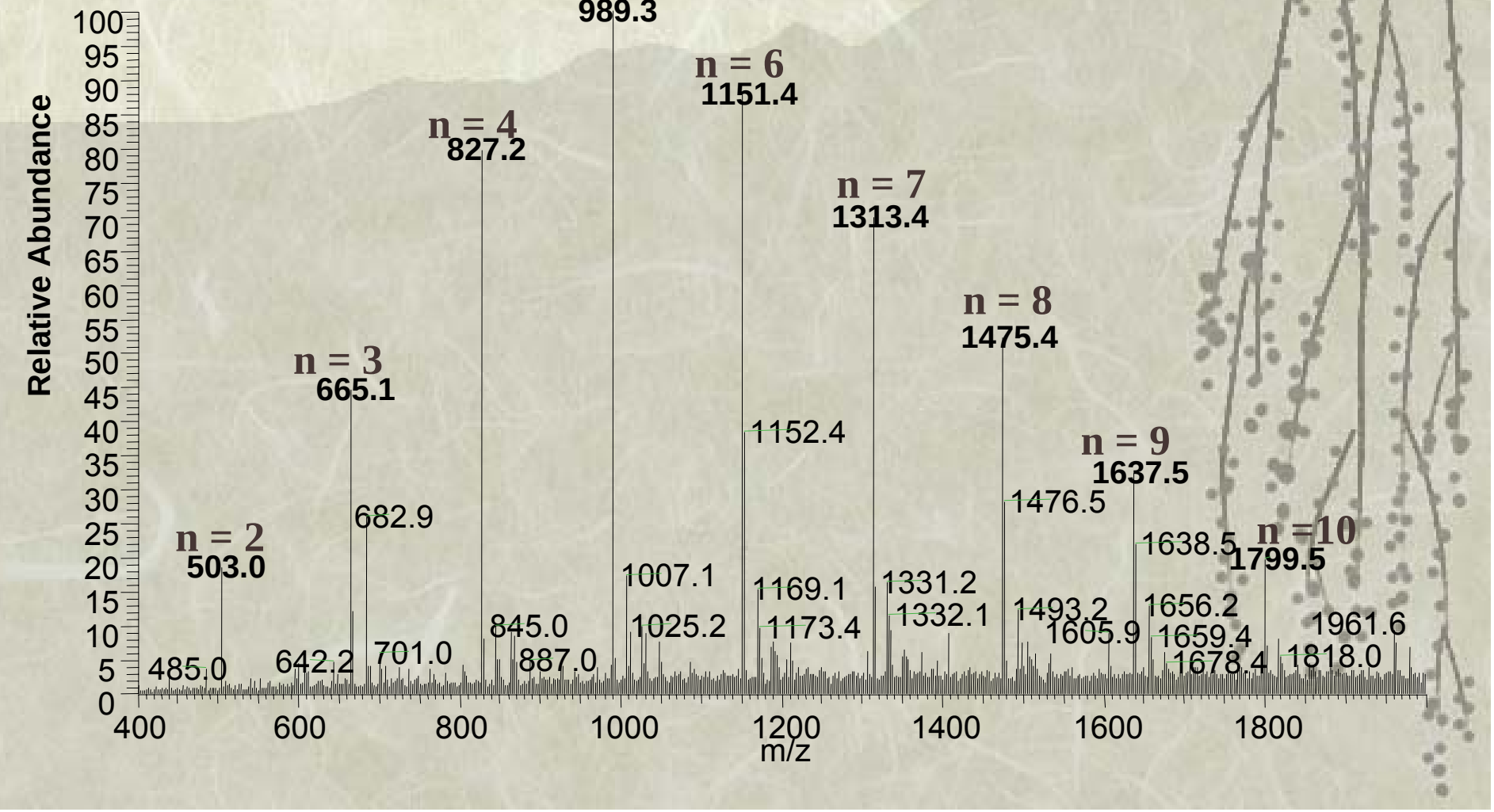
TLC anaysis of standards and samples



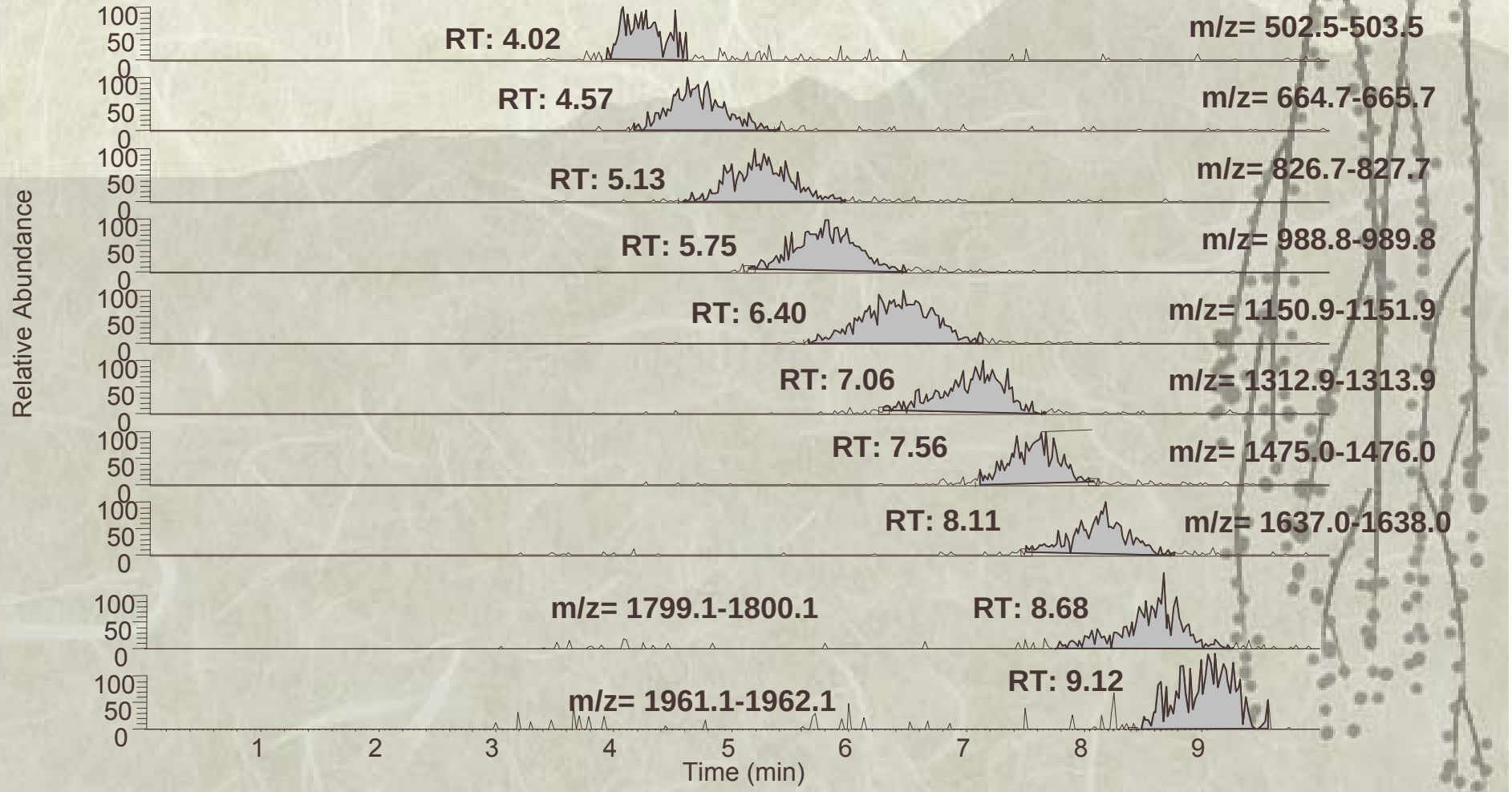
Densitogram of separated oligomers in an inulin sample



ESI-MS SPECTRUM OF INULIN OLIGOMERS $[M-H]^-$



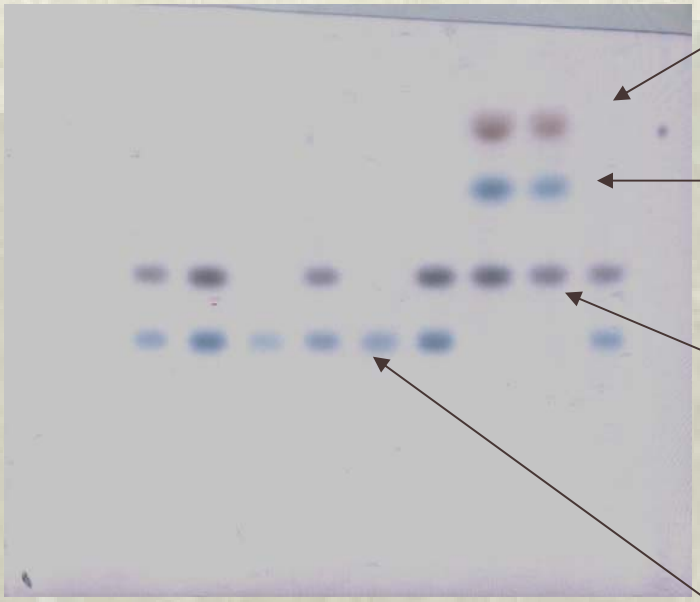
HPLC-ESI-MS ANALYSIS OF INULIN



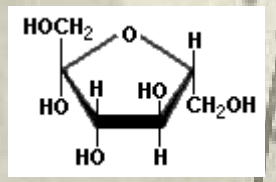
Quantitative determination of inulin in chocolate

mean	RSD %	declaration	chocolatete
24.1	12.5	22.7	With nuts
23.8	15.2	25.8	Milk
23.5	4.3	22.0	Rice

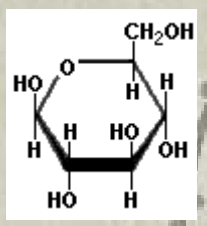
Determination of sugers



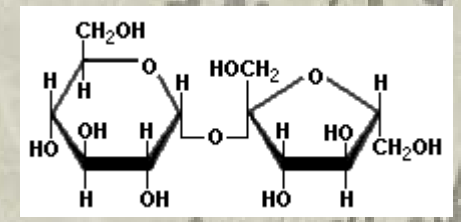
Fructose



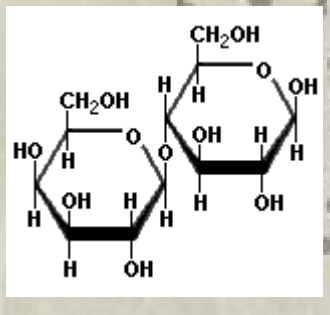
Glucose



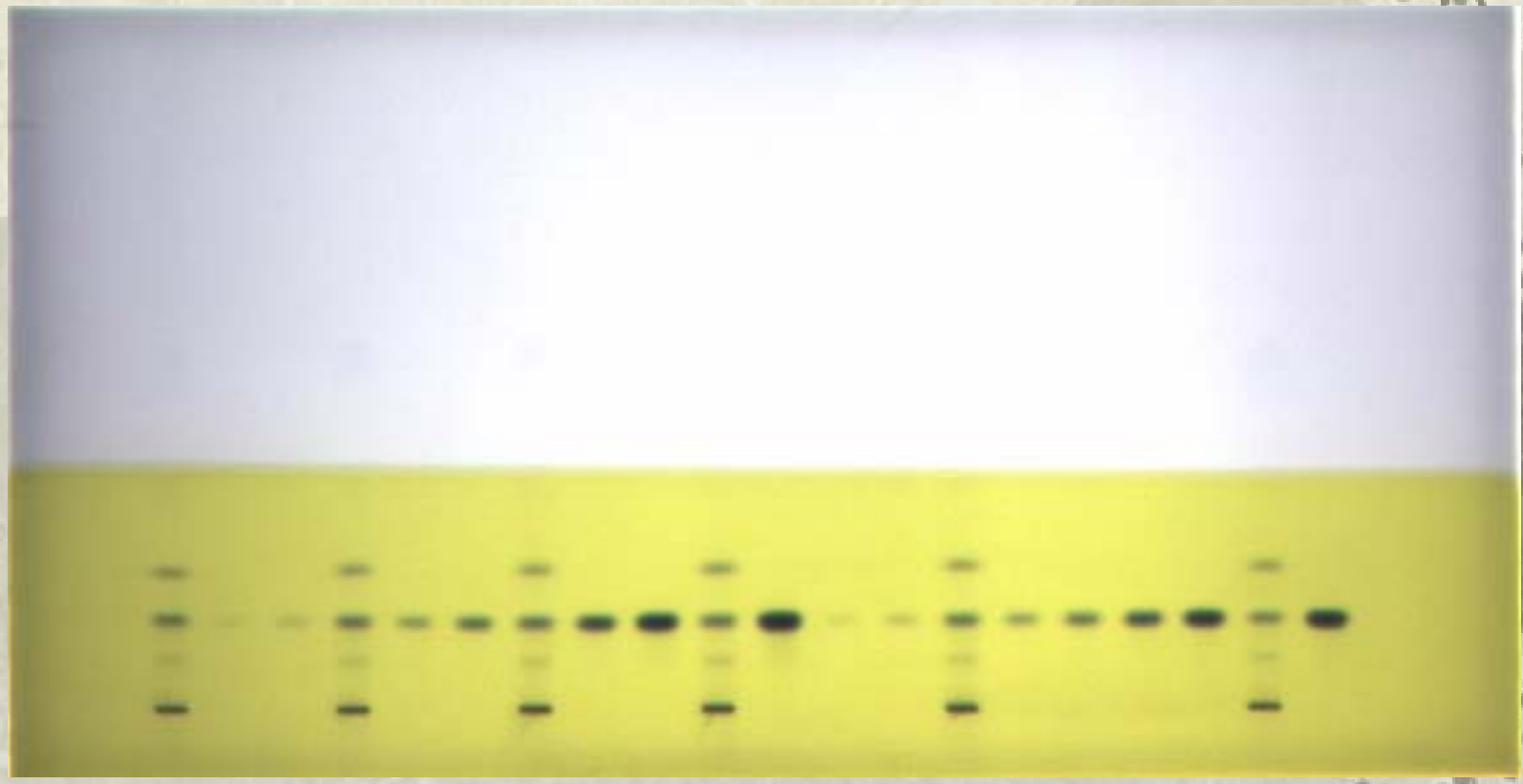
Sucrose



Lactose



Determination of cholesterol



Determinaton of alliin in commercial capsulas

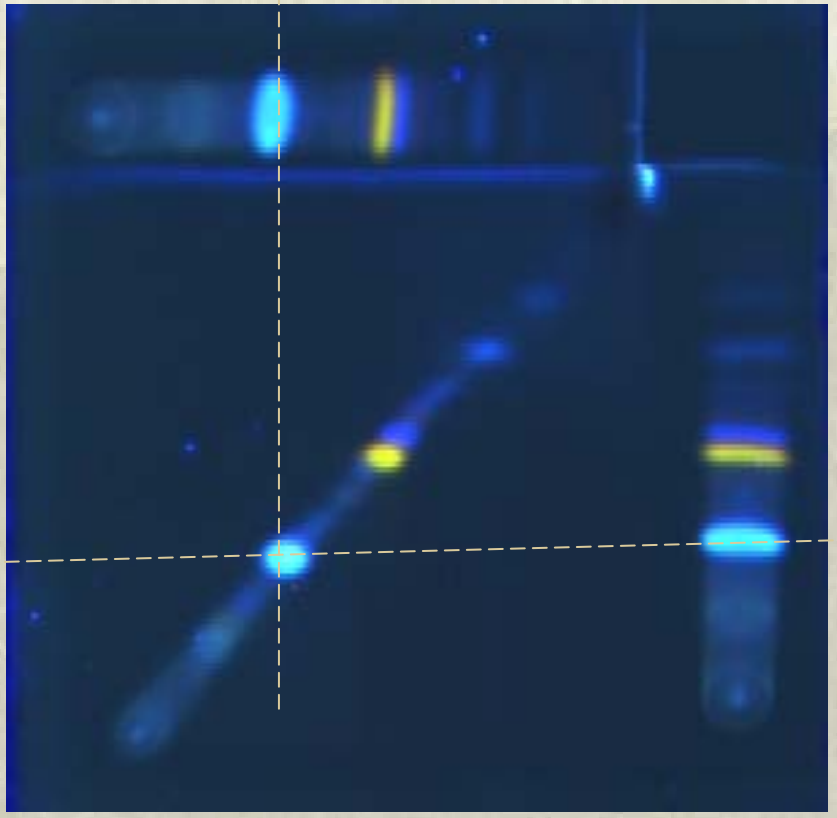
1 2 3 4 5 6

Extract from fresh garlic 1,4

Extract from dry garlic 2,5

Commercial capsulas 3,6

Two dimensional separation of aloin



Standard 5 ppm

Conclusion

- Quantitative evaluation of components in food aditives and food samples is very important task.
- Different type of analytical methods can be used. From a simple spot tests to the most sophisticated combinations of separation and spectroscopic techniques.
- Unfortunately in food industry there is often not enough money for regular chemical inspection.
- Selected examples show how simple TLC can be succesfuly used in real life. It is cheap, not complicated and very informative analytical technique.