Instrumental methods for biological samples analysis without necessity of their separation

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Plan of presentation

Infrared spectroscopy as non-invasive analytial method

- Current and previous applications
- Statistical background for experimenatal data management
  PCA, PLS, modelling

Area of application

Examples of application

Comparing NIR and MIR

Advantages and disadvantages of FT-IR

The application of FT-IR spectroscopy for biological mixture analysis

Analysis with sample separation

- Distillation
- Crystallization
- Extraction
- Chromatography

- Elemental analysis
- Solubility test
- Chemical formula
- NMR spectrum
- IR spectrum

Analysis without sample separation

- The FT-IR technique
- FT-IR applications
- Statistical methods
- Study of foods

- Characteristic features
- Chemical composition of mixture
- Microbiological approaches
- GMO products determination
- Verification of process conditions

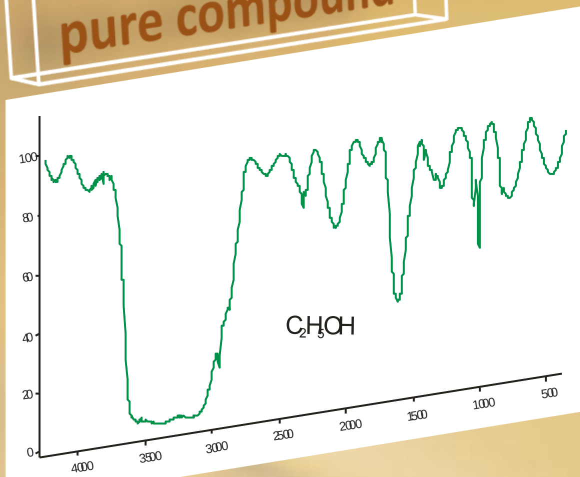
- MIR and NIR
- Advantages and disadvantages of spectral methods

IR spectroscopy

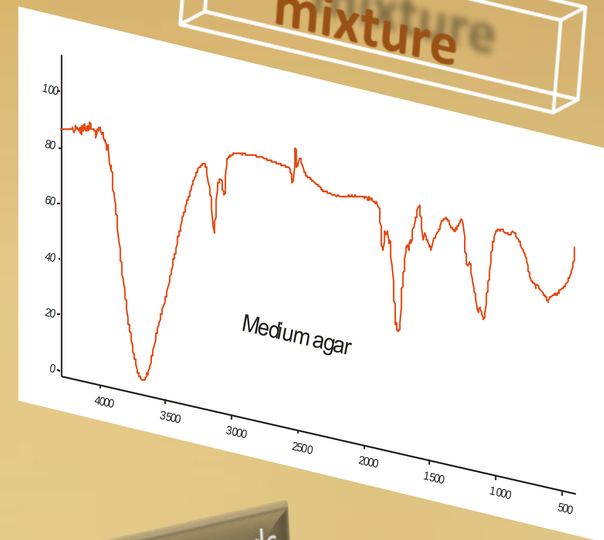
classic

modern

pure compound



mixture



From the mthematical point of wiev the spectrum is a plot of intensity against frequency (vawenumber) Independent variable

Characteristic bands

Wavenumber

Bands intensity

Pettern recognition

Statistical model

Characteristic bands

Wavenumber

Bands intensity

Compound identification

„Fingerprint" of the compound

Mixture composition determination

„fingerprint" of the mixture

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IR – REFERENCE METHOD RELATIONSHIP. STATISTICAL MODEL

Intensity or frequency of some dozen bands or IR ranges

PLS

PCA

component 1
component 2
component 3
or
feature 1
feature 2
etc....

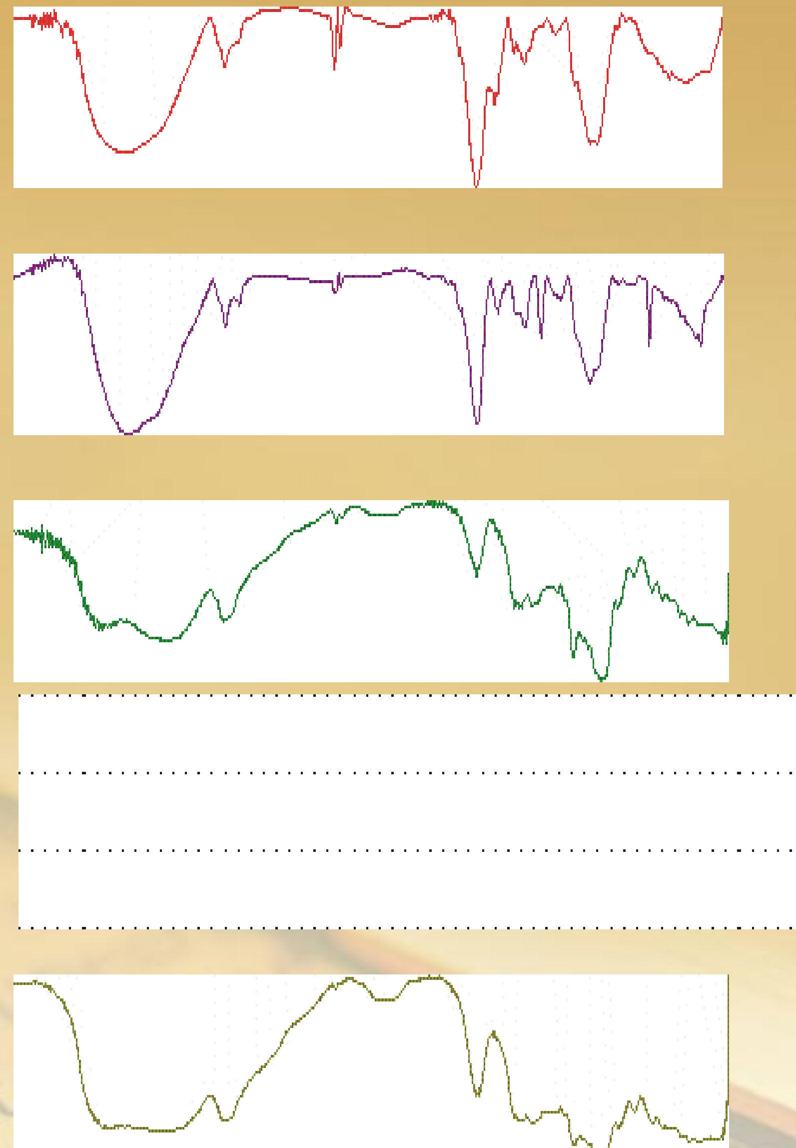
Linear combination of spectral (IR) data and correlation of obtained this way statistical numbers with analytical data from reference method

Linear model mono- or multi- elemental

PCA – principal component analysis

1. Registration of spectra series for samples with similar properties
2. Detection of spectral ranges of highest variability
3. Selection of spectral ranges for analysis
4. Calculation of „avarege” spectrum
5. Principal component calculation - the spectra of highest variability
6. The reconstruction of original spectrum – the number of principal components and the level of variability

RESULT		SAMPLE	SPECTRUM	PROCEDURE
$1PC_1$; $1PC_2$; …. $1PC_n$	←	1		
$2PC_1$; $2PC_2$; …. $2PC_n$	←	2		
$3PC_1$; $3PC_2$; …. $3PC_n$	←	3		PCA
nPC_1; nPC_2; …. nPC_n	←	n		



PLS – partially least square

1. Registration of spectra series for samples with similar properties
2. Detection of spectral ranges of highest variability
3. Determination of a given component or pattern preparation
4. Determination of principal components correlated with variability of the level of a given compound
5. Mathematical description of the model

$$y = k \sum_{i=1}^{n} x_i$$

The x variable denotes the principal component, while y denotes value of given parameter determined by a reference method , k is proportional coefficient for given varible in linear equation

Principal components

Sample equation:

$$\mathbf{C = 0.4PC_1 + 0.2PC_2 + 0.03PC_3}$$

Characteristics of the model

The higher value the better model

R^2 – the correlation coeffcient between actual and modeled data

PRESS – Prediction Residual Error Sum of Squares, the definition is

The lower value the better model

$$\sum_{i=1}^{n} \sum_{j=1}^{m} (Cp_{i,j} - C_{i,j})$$

SECV - standard error of cross validation

SEP – standard error of prediction

n – number of samples (number of spectra)

i – number of components in a model

Cp – matrix of values predicted by a model

C – matrix of real values

What is IR spectrum

Spectrum is a simply graph of certain x value versus y value

This relationship can be presented as a plot

This relationship can be presented as a table

In the second case differences are clerly seen

Two types of analysis

DISCRIMINANT ANALYSIS → **Samples differentiation**

REFERENCE ANALYSIS → **Component content determination**

Analysis of groceries

- Analysis of chemical composition of mixtures
- Features of a given product
- Determination of number of microorganisms
- Adulteration determination
- Verification of technological process conditions

Range of applications

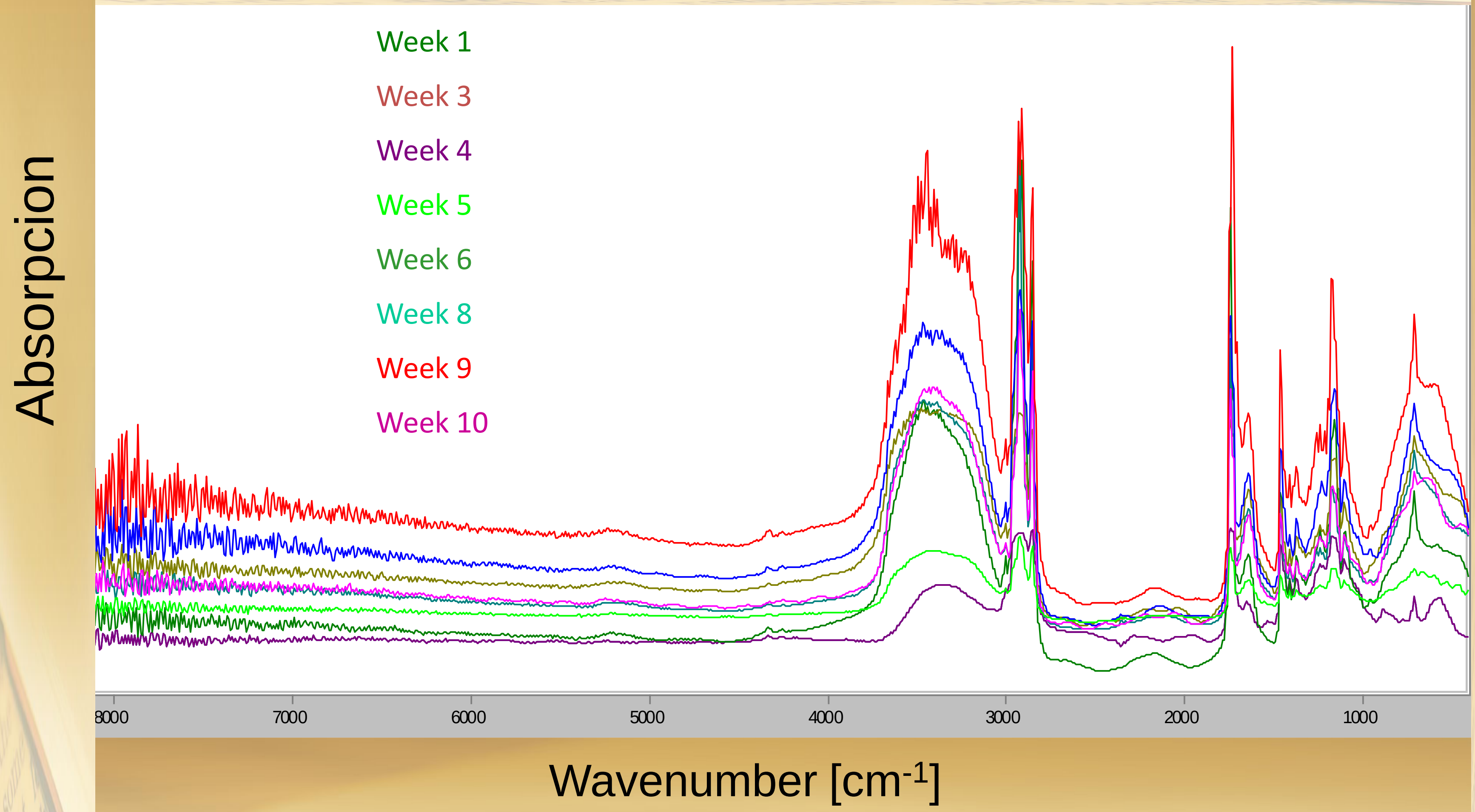
Alcoholic beverages
Fish
Meat
Eggs
Vegetables and fruits
Dairy products

Food mixtures

Other mixtures

Oils
Fuels
Cosmetics
Other

The IR spectra of samples of light butter „Dobre” registered during 10 weeks of storage at room temperature (25 ^{O}C)



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Results obtained for butters

Model for light butter „Dobre”:

18 samples were used to construct the model. Samples were stored at room temperature for 10 weeks

R^2 = 0.97

PRESS = 2.91

Factors number = 5

Spectral range: 1040 – 1020 cm^{-1}

Diagnostic: PLS, cross validation

The model relates acid number data with spectral data . Based on this model, one can determine acid number of an unknown sample and therefore evaluate freshness of butter.

Acid values for selected butter samples stored at room temperature for different lenghts of time. Values determined by classical method are in black while those modeled in green

Butter type	WEEK 1		WEEK 3		WEEK 4		WEEK 5	
	Accepted value	model	Accepted value	model	Accepted value	model	Accepted value	model
„Dobre" light	0.7	0.8	1.3	1.1	1.6	1.7	2.7	2.7
Butter type	**WEEK 7**		**WEEK 8**		**WEEK 9**		**WEEK 10**	
	Accepted value	model	Accepted value	model	Accepted value	model	Accepted value	model
„Dobre" light	3.9	3.9	5.2	5.3	7.3	7.1	10.6	10.3

Sample results obtained for light butters

The application of FT-IR to wine parameters determination

Quantities to be determined by FT-IR

- Titration acidity
- Acetic acid content
- Tartaric acid content
- Lactic acid content
- Glycerol content
- Polysaccharides content
- Malic acid content
- Sugars content (glucose + fructose)
- Polyphenols content
- Anthocyanins content
- SO_2 content
- Methanol content
- Ethanol content
- pH
- Volatile acidity

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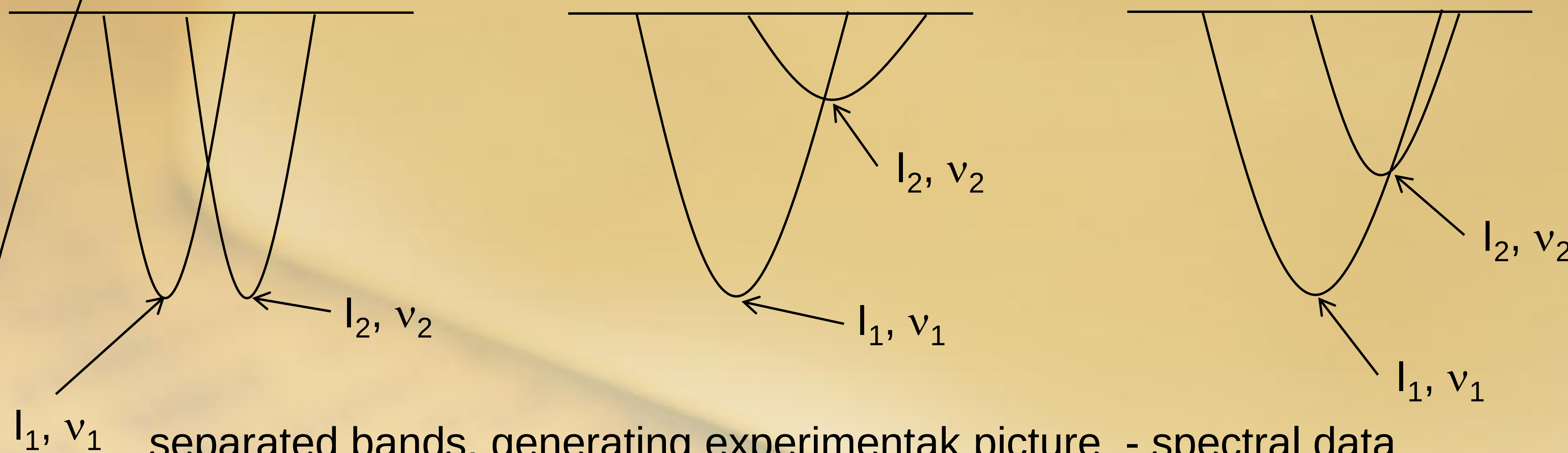
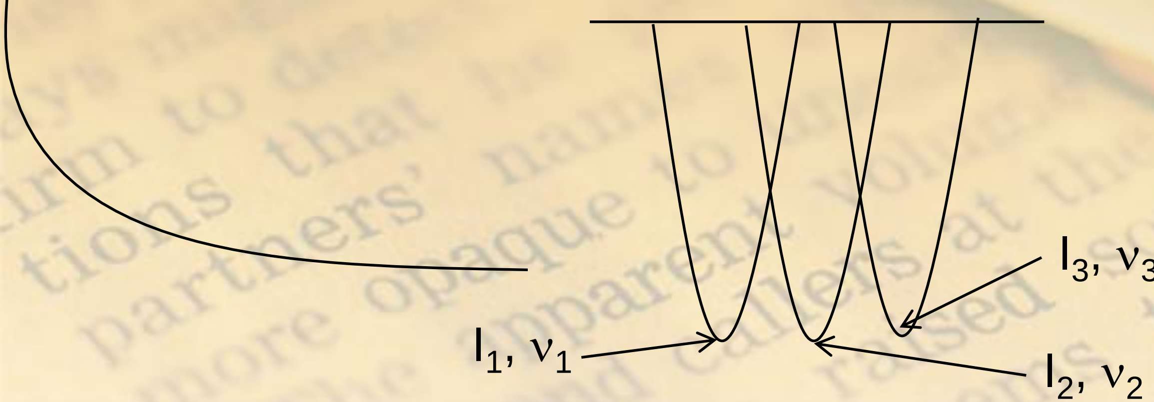
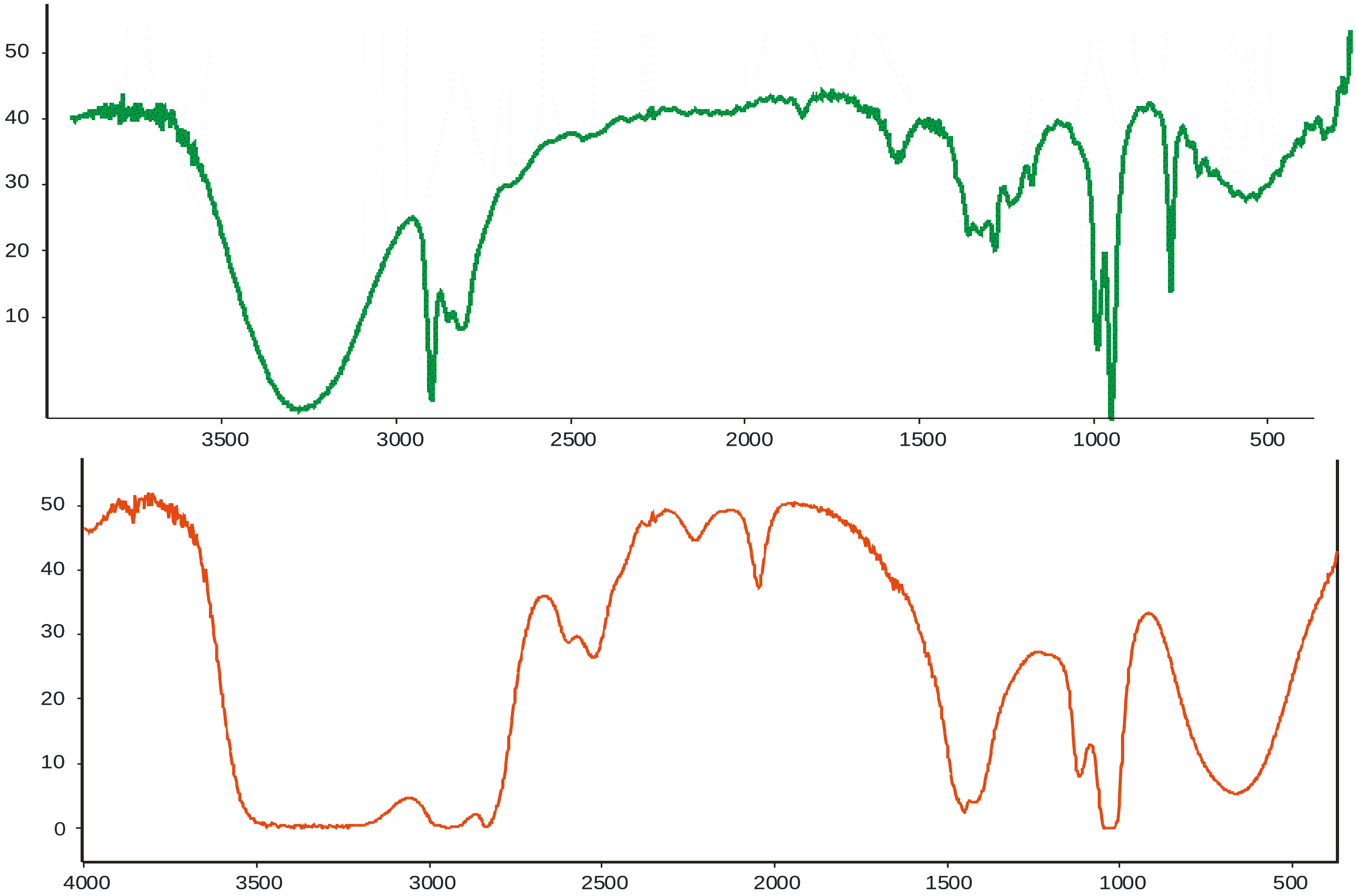
Overlaping bands present in spectrum



separated bands, generating experimentak picture - spectral data




50
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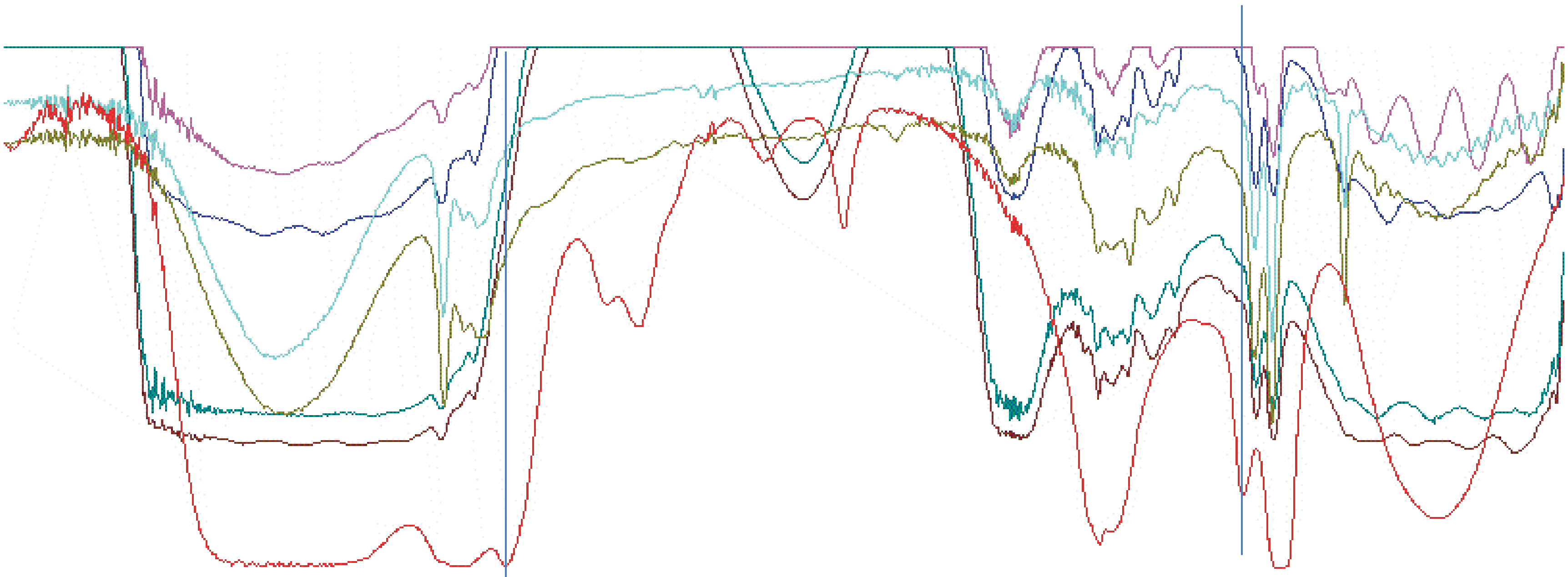
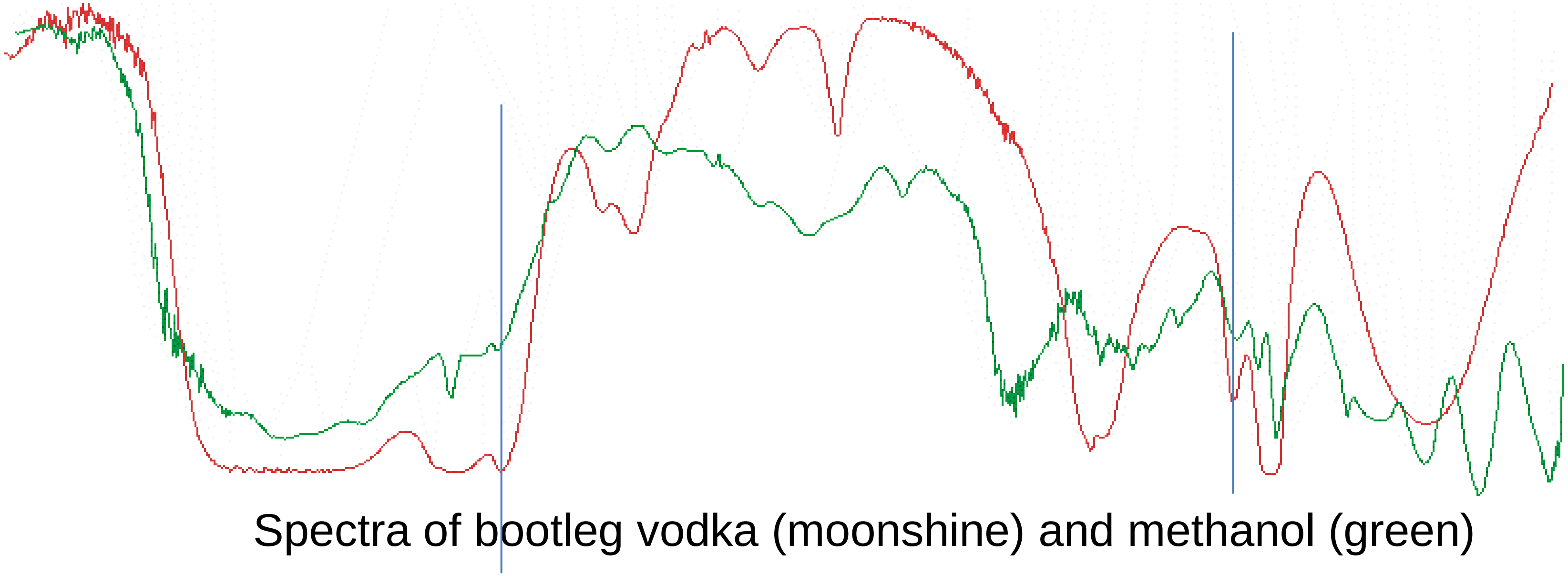


Spectra of different commercial vodkas and methanol (bottom)



Spectra of bootleg vodka (moonshine) and methanol (green)

Alcoholic beverages parameters determination

Sample results:

ethanol	R^2 = 0.98; SEP = 0.34%
pH	R^2 = 0.81; SEP = 0.07%
Milk acid	R^2 = 0.81; SEP = 0.41%
Reducing sugars	R^2 = 0.71; SEP = 0.33%;

SEP standard error of prediction

Data from Urbano-Quadrado iet al., 2004, 2005

Sample results obtained for Cabernet Sauvignon and Shiraz wines:

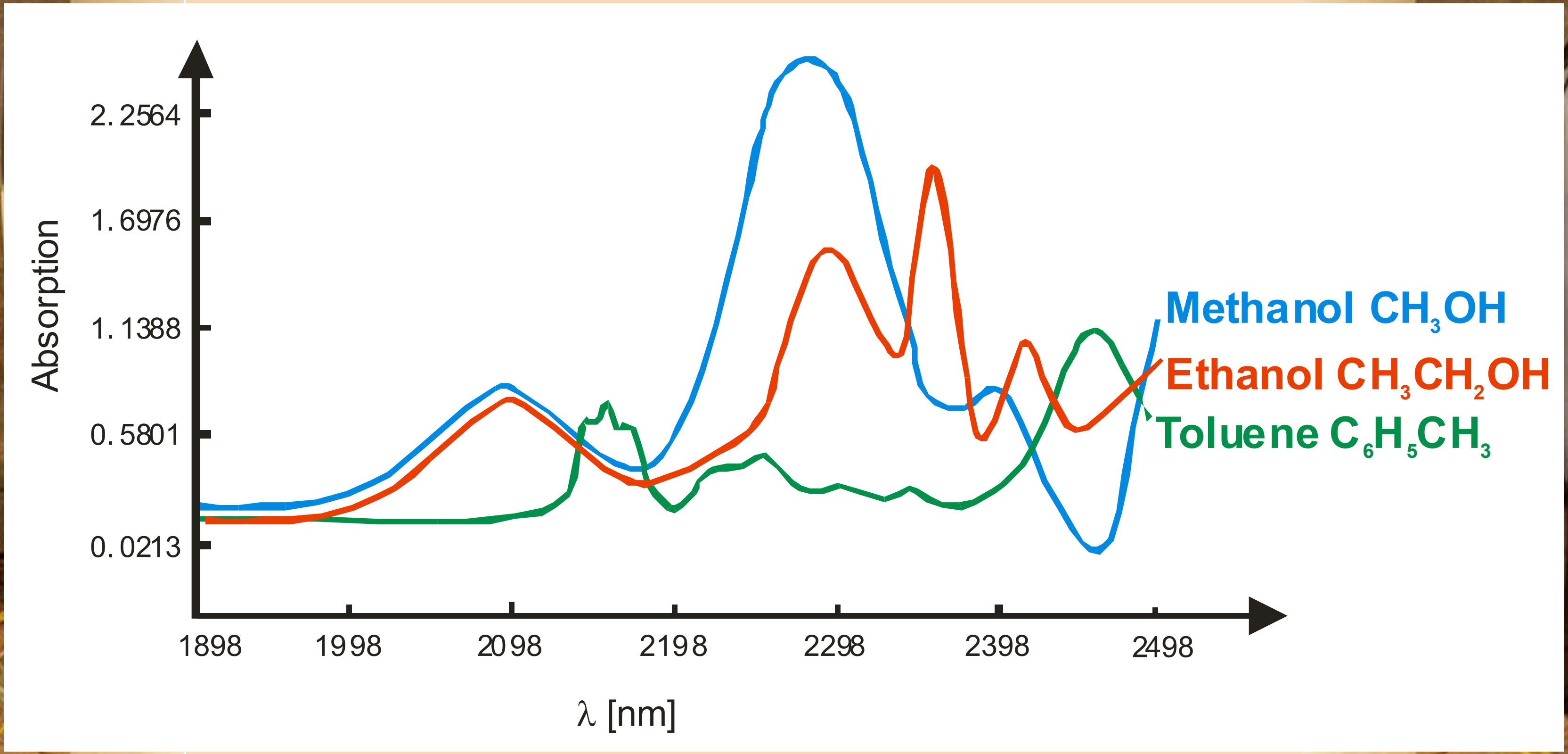
malvidin-3-glucoside	R^2 = 0.91; SECV = 28 mG/dm^3
colorants	R^2 = 0.87; SECV = 5.9 mG/dm^3
tannin agents	R^2 = 0.81; SEP = 0.41%

Data from: Cozzolino et al., 2004, 2006

SECV standard error of cross validation

The content of ethanol and methanol in wine distillates

The content of methnol in distillates can be relatively high due to activity of yeasts in raw materials



Studing fish composition

Chemical composition → **freshness** } Main parameters for fish quality assesment

The content of water and fat

Tuna

Fish fillets

Downey (1996)
Wold et al. (1996)

Fish with 5.7 – 17.5% fat content and weight of 1.0 – 1.54 kg.

Fat:

PLS – NIR

$R^2 = 0.97$

RMSECV = 0.75%

Whole fish

Wold i Isaakson (1997)

Fat:

$R^2 = 0.87$

(RMSECV = 1.12%)

Water:

$R^2 = 0.86$

RMSECV = 0.98%

Live fish

Solberga et al. (2003)

100 fish weighing 1.0 – 11.0 kg were tested

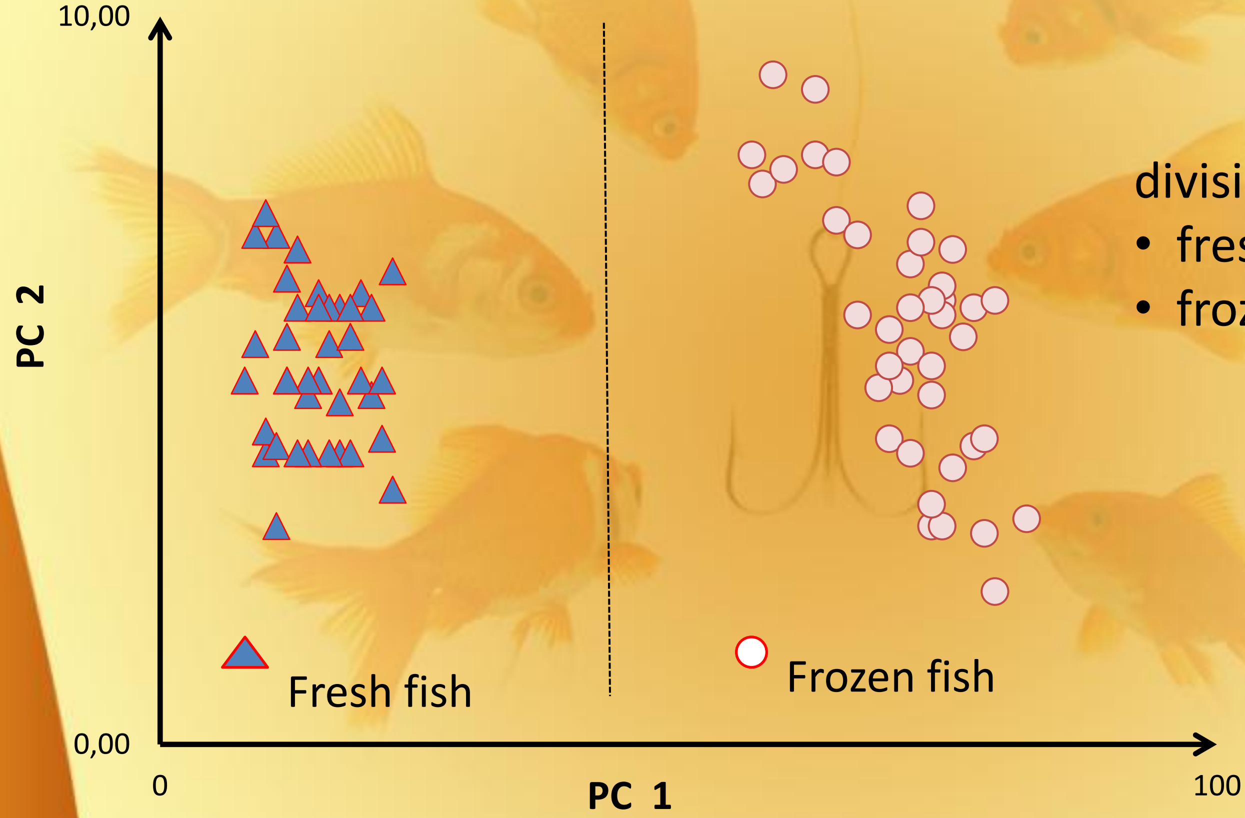
Fat:

PLS – NIR $R^2 = 0.90$

RMSECV = 14 g/kg to fat

Differetiation of fresh and frozen fish (authenticity of the product)

Sample of discriminant analysis



division

- fresh
- frozen and defrost

In the case of fresh fishes experimantal points are located closer to each other which shows better similarity of those samples

Number of microorganisms in beef

Ellis et al., Analytica Chimica Acta (2004) 514, 193 – 201.

Starting number of microorganisms	Final number of microorganisms
↓	↓
2 x10^5 CFU/cm^2 (5.31 log TVC)	2 x10^7 CFU/cm^2 (7.31 log TVC)

Model FTIR/PLS forecast number of microorganisms with accuracy of:

0.4 – 0.6 log TVC

Bands generated by molecule deformations located at 1413 cm^{-1} and 1405 cm^{-1} were sensitive to microbial contamination

This is C-N deformation band (amide band) arising due to degradation of proteins by microorganisms

Number of microorganisms in poultry meat

Measurements were conducted every 1 hour during 24 hours

Starting number of microorganisms

$7x10^6$ CFU/g (6.85 log TVC)

→

Final number of microorganisms

$2x9^6$ CFU/g (9.31 log TVC)

Amide band I (1640cm^{-1})

Amide band II (1550 cm^{-1})

Amine band (1240 cm^{-1} i 1088 cm^{-1})

Model allowed to determine microorgnisms level with accuaracy:

0.15 – 0.27 log$_{10}$TVC

Verification of technological process conditions

Verification of heating temperature of fish meat gels (kamaboko)
Uddin et al., 2006)

Temperature treatment

Incorrect

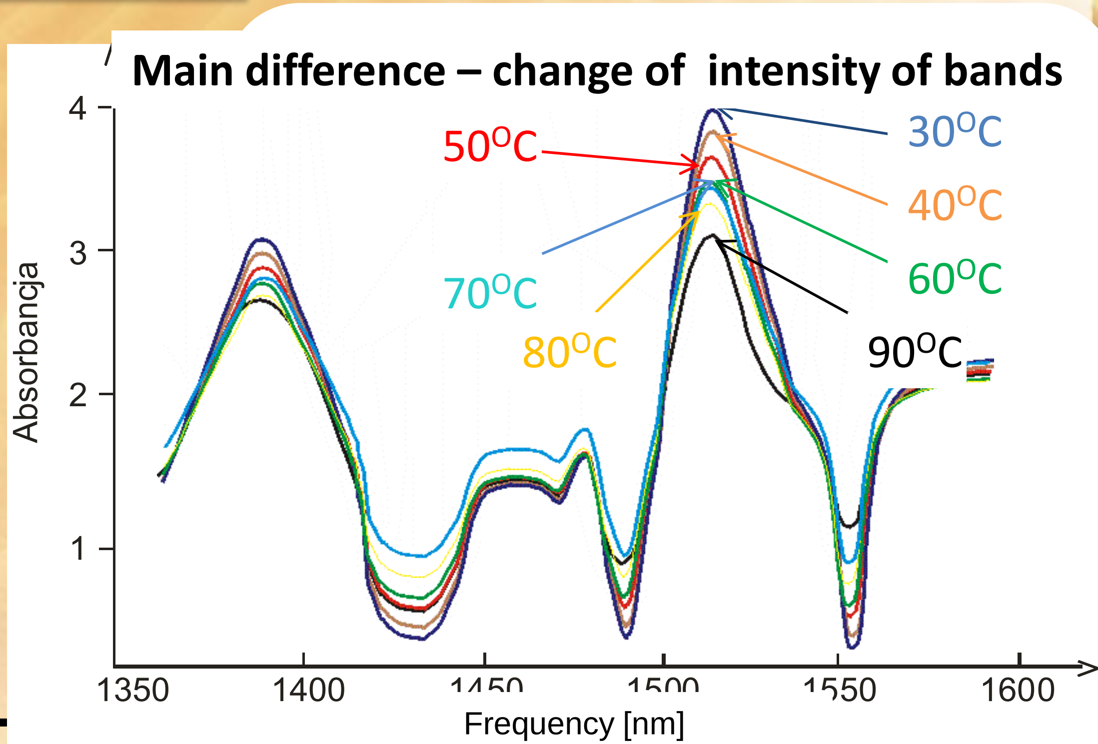
Correct

Ilnesses developing related to food-borne microorganisms

Tasty, durable product of proper nutritional value

KAMABOKO:

1. Fish meat was placed in polivynyl chloride packigings (circumference - 480 mm, lenght 100 mm)
2. Heating for 10 minutes in water bath: from 30 do 90^{o}C with 10^{o}C intervals
3. Fast cooling to 4^{o}C
4. Stored for one night
5. Spectral range : 1100 – 2498 nm



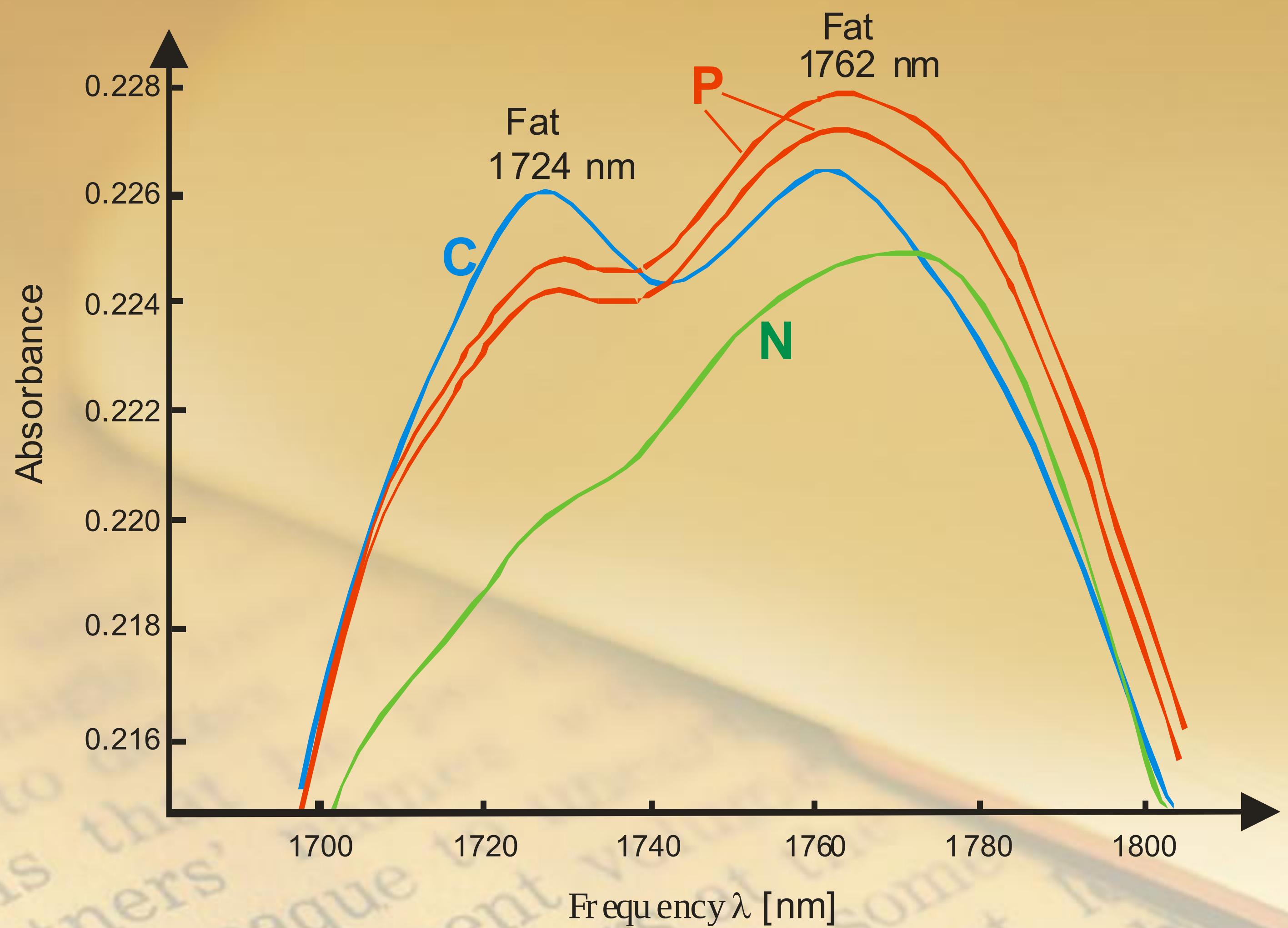
GMO study as modeled on barley

Munck et al., (2007) A new holistic exploratory aproach to systems biology by near infrared spectroscopy evaluated by chemometrics and data inspection **Journal of Chemometrics, 21, 406-426**

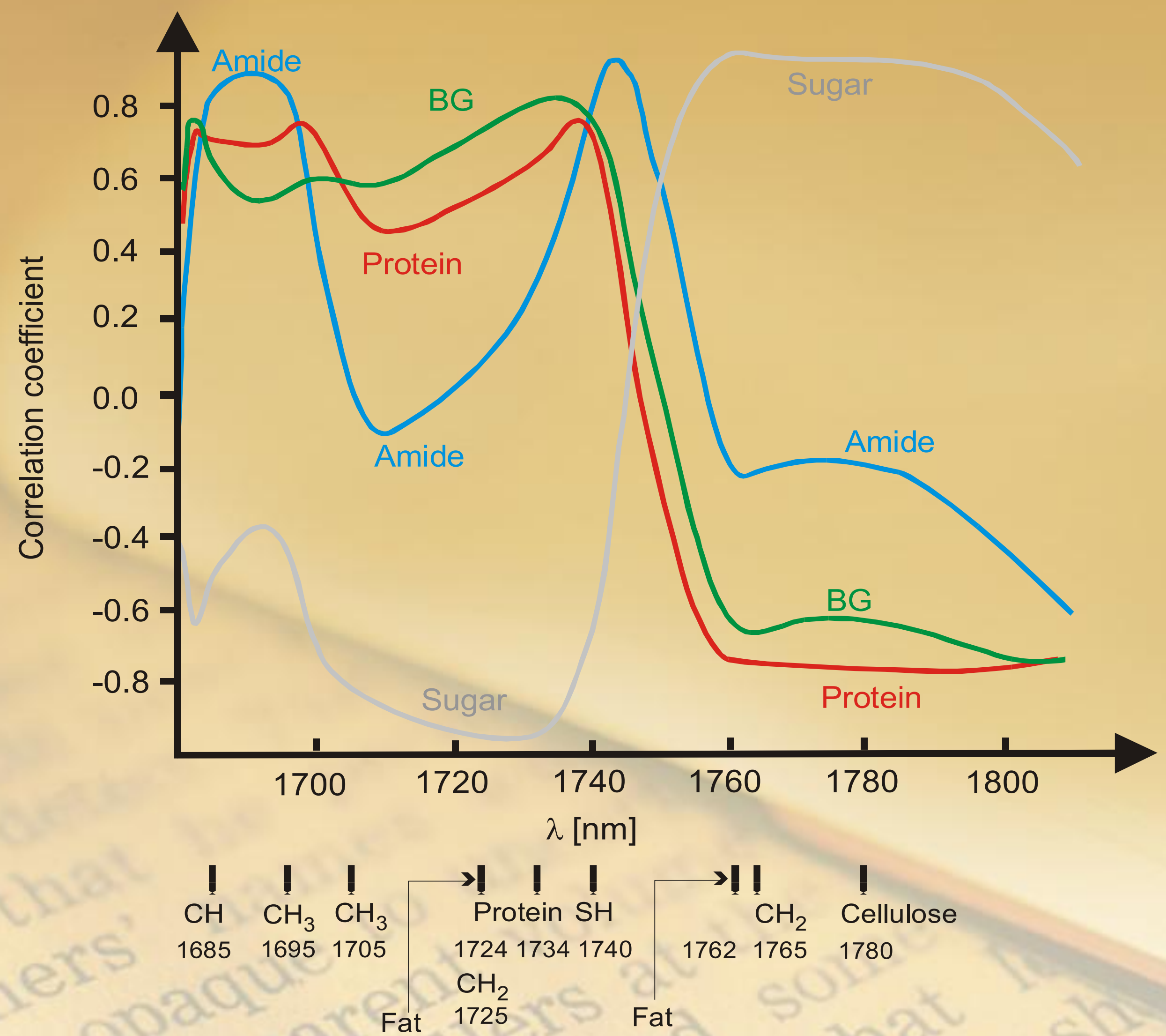
C type (lys5f) - (high β-gluten, content low sugar content)
P type (lys3a) - (low β-gluten, high lysine content)
N - native barley

Type	Dry matter	Protein	Amides	BG	Fat	Sugars
C	**91.62** ± 0.75	**16.98** ± 2.13	**0.42** ± 0.06	**14.21** ± 2.9	**2.71** ± 0.76	**32.1** ± 5.8
P	**90.18** ± 0.49	**15.77** ± 2.33	**0.29** ± 0.05	**3.79** ± 1.36	**3.5** ± 0.06	**44.75** ± 4.81
N	**90.22** ± 0.79	**15.64** ± 2.09	**0.42** ± 0.07	**5.72** ± 0.99	**1.91** ± 0.16	**52.16** ± 4.25

GMO study modeled on FT-IR NIR range spectra of barley



GMO study modeled on barley - correlation



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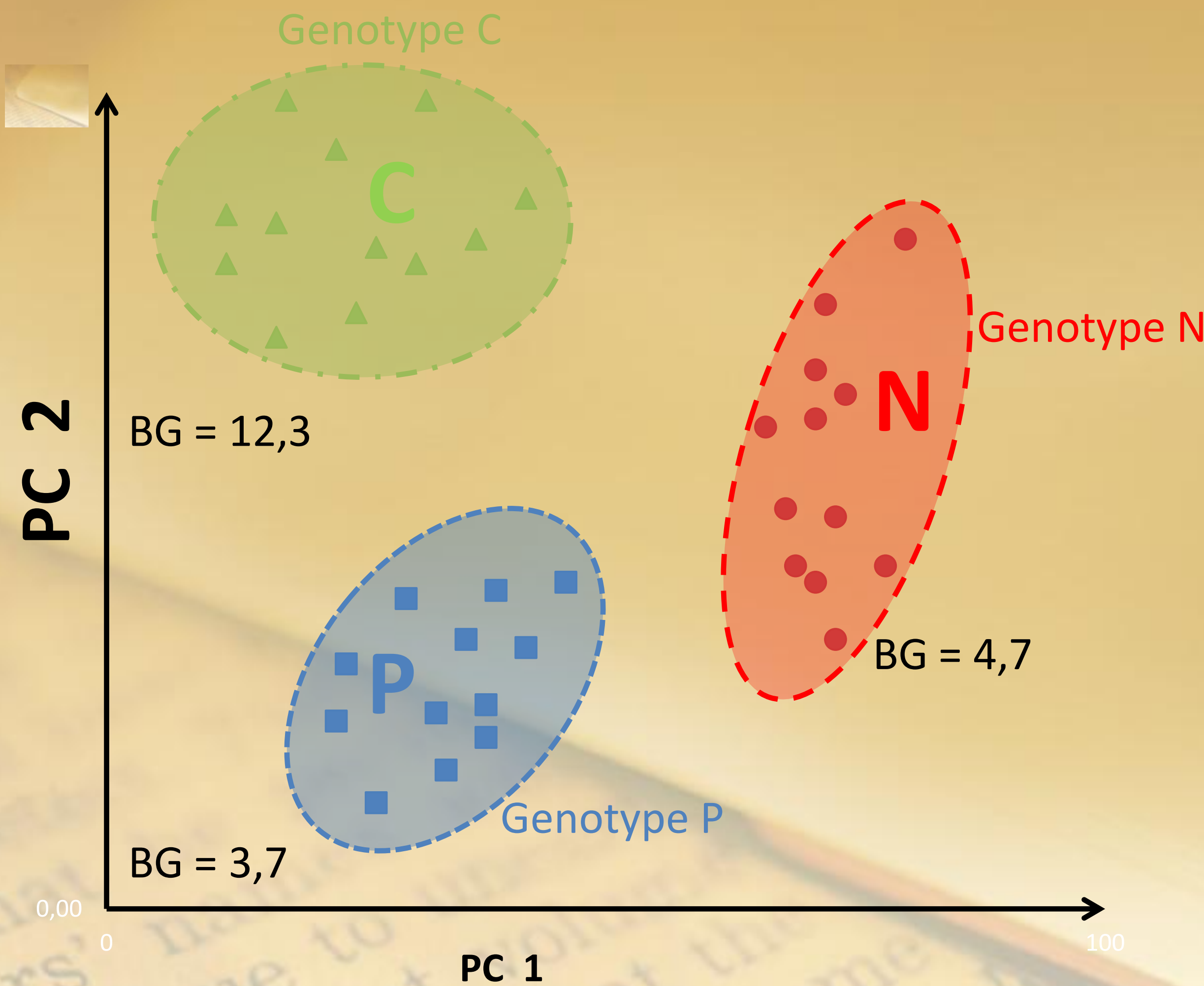
GMO study modeled on barley



BG - the percentage of β-gluten in dry matter

Application of discriminant analysis of IR spectra for dairy product analysis

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MIR and NIR

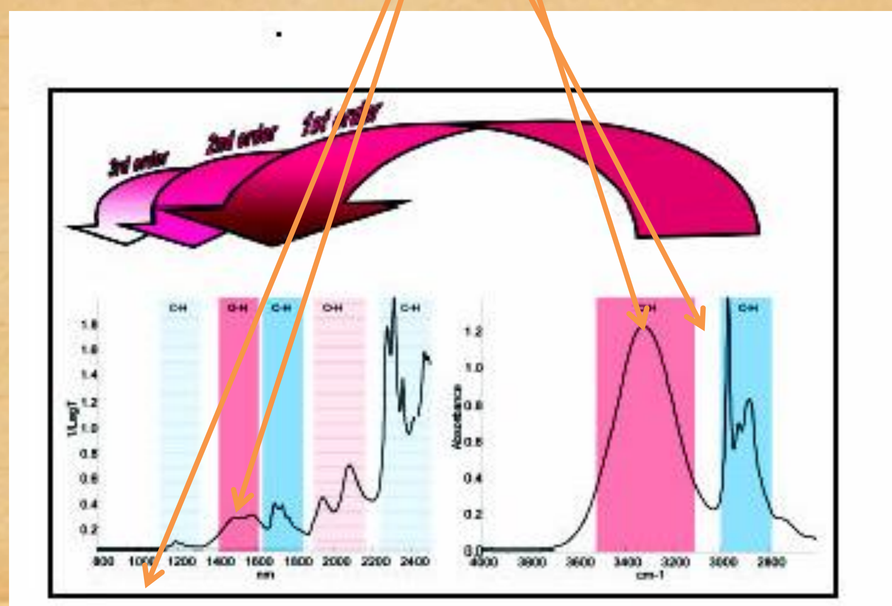
The same sample the same technique different spectral range

NIR Near infrared spectroscopy

MIR Midle infrared spectroscopy

Overtones and composed tones of CH, NH, CO, OH groups and other

Fundamental vibrations of CH ,NH, CO, OH and other groups



Base range bands
Are repeated in NIR
(1 ,2 or3 overtones
respectively)

Advantages and disadvantages of FT-IR spectroscopy

ADVANTAGES

- Short time of measurement
- No sample pre-preparation
- No chemicals used
- Ability to apply on-line and in-line

DISADVANTAGES

- IR is not independent method - correlation with data from oher methods in necessary
- Long time of model preparation

Selected literature

Ellis DI, Broadhurst D, Goodacre R **(2004**) Rapid and quantitative detection of the microbial spoilage of meat by Fourier transform infrared spectroscopy and machine learning. *Analytica Chimica Acta* , **514**, 193 – 201

Al-Jowder O, Defernez M, Kemsley EK, Wilson RH (1999) Midi – infrared spectroscopy and chemometrics for the authentication of meat products. *Journal of Agricultural and Food Chemistry*, **47**, 3210 – 3218

McElhinney J, Downey G, Fearn T (1999) Chemometric processing of visible and near infrared reflectance spectra for species identification in selected raw homogenised meats. *Journal of Near Infrared Spectroscopy*, **7**, 145 – 154

Gangidi RR, Proctor A, Pohlman FW (2003) Rapid determination of spinal cord content in ground beef by attenuated total reflectance Fourier transform infrared spectroscopy. *Journal of Food Science*, **68**, 124 0 127

Ding HB, Xu RJ (2000) Near – infrared spectroscopic technique for decetion of beef hamburger adulteration. Journal *of Agricultural and Food Chemistry*, **48**, 2193 – 2198

Prieto N, Andrés S, Giráldez FJ, Mantecón AR, Lavín P (2007) Discrimination of adult steers (oxen) and young cattle ground meat samples by infrared reflectance spectroscopy (NIRS). *Meat Science*, **79**, 198 – 201

Andrés S, Silva A, Soares – Pereira AL, Martins C, Bruno – Soares AM, Murray I (2008) The use of visible and near infrared reflectance spectroscopy to predict beef M longissimus thoracis et lumborum quality attributes. *Meat Science*, **78**, 217 – 224

Liu Y, Lyon BG, Windham WR, Realini CE, Dean T, Pringle S, Duckett S (2003a) Prediction of color, texture and sensory characteristics of beef steaks by visible and near infrared reflectance spectroscopy. A feasibility study. *Meat science*, **65**, 1107 – 1115

Downey G, Beauchêne D (1997) Discrimination between fresh and frozen – then – thawed beef m. longissimus dorsi by combined visible – near infrared reflectance spectroscopy: A feasibility study. *Meat Science*, **45**, 353 – 363

THANKS FOR YOUR ATTENTION